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This administrative report addresses the management and technical accomplishments of the Pond Dynamics/Aquaculture Collaborative Research Support Program during the reporting period of 1 September 1992 to 31 August 1993. Program activities are funded in part by the United States Agency for International Development under grants DAN-4023-G-00-0031-00 and 263-0152-G-00-2231-00.

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DISCLAIMER

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I. CRSP RESEARCH PROGRAM BACKGROUND

The current period is characterized by the following accomplishments: improvements to the PONDCLASS decision support system, successful and smooth transfer of the CRSP Data Base management function to the University of Hawaii at Hilo, re-establishment of brackish water research at a new site in Honduras, initiation of freshwater aquaculture research in Egypt, and completion of activities scheduled under the Sixth and Seventh Work Plans. Experiments were conducted at established CRSP research facilities as well as in farmers ponds. A number of Special Topics Research activities were also performed. As always, efforts to disseminate research results continued through a variety of avenues.

Global Experiment and Related Investigations: Historical Overview

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 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 determine 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 these 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 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. Observations specified in biennial (originally annual) work plans were made on twelve or more ponds of similar size at each location. The variables observed, frequency of observation, and materials and methods used were uniform for all locations. Two brackish water and five freshwater research projects were begun in Central America (Panama and Honduras), Africa (Rwanda), and Southeast Asia (Indonesia, Thailand, and the Philippines) in 1983 (Figure 1). All of the sites were within a zone 15 degrees north or 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 three of the six countries originally selected (Thailand, Rwanda, Honduras) to maintain sites in the three major regions of the tropics. Since 1991 the CRSP program has been 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. Also, in 1993, brackish water research was resumed with the addition of a coastal site in Honduras.

The first cycle of experiments was designed to develop 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. This series of experiments 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.

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. The findings gained from these studies have worldwide practical application. Further, these studies are resulting in a better understanding of pond dynamics. Two examples illustrate this point: polyculture and on-farm research.

A recurrent problem of tilapia culture is tilapia's high reproductive potential. The Honduras, Thailand, and Rwanda teams studied different aspects of polyculture in an attempt not only to reduce unwanted tilapia offspring, but also to improve yield. Polyculture attempts not only to solve a recurrent management problem but to also add another economically important species. This concept has been further developed by the Egypt team which was able to profit from initial CRSP research in this area. The Egypt team added a new twist to the polyculture studies by exploring the possibility of bioconverting as yet unused pond system components, like aquatic weeds and snails, into fish food.

After the first few years of Global Experiment research, it became evident that rigorous economic analyses of pond aquaculture systems must be part of the aquaculture development strategy in both the US and host countries. In order to determine if contemporary pond management practices are the most efficient approach to fish production, it became necessary to develop quantitative production functions to facilitate analyses of various strategies or combinations thereof. It was not possible to develop these functions without making numerous and often tenuous assumptions, because the dynamic mechanisms regulating the productivity of the ponds were poorly understood and the existing data base, until now, had been inadequate.

On-farm research is an attempt to gain direct information on the practical applicability of CRSP technologies under everyday farm conditions, and on the extent to which the new management strategies may generate economic returns for the local population. An added benefit of this type of work is the direct communication established between researchers and farmers. Such communication facilitates the dissemination of information and provides a mechanism for immediate feed-back of information relevant to subsequent research needs.

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. Therefore, provisions were made for the establishment and maintenance of a central data base. This Central Data Base was managed 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. All data from research activities conducted under the First through the Fourth Work Plans are already in the data base. The Central Data Base has continued to expand during this reporting period, through the inclusion of new data generated under the Fifth and Sixth Work Plans.

CRSP participants also decided that the comprehensive analysis and interpretation of global data would be greatly enhanced through the formation of an independent team, composed 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 models that reflect our growing understanding of pond systems. The DAST members are not just 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 allow 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 US researchers to collaborate on original research directed toward the needs and priorities of each host country. The intent is to strengthen linkages within the host country institution and to contribute to the development of research capabilities within 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 US scientists. The proposals are endorsed by the host country institution and are reviewed by the CRSP Technical Committee and Board of Directors for technical merit and relevance to the general goals of the CRSP. The Board also requires that the projects be consistent with USAID and host country development strategies and priorities.

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 highest priority on the long-term basic research defined as the Global Experiment. Host country institutions and USAID Missions, however, often consider basic research activities such as the Global Experiment 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 initially introduced as a part of the First Work Plan has been improved with each subsequent work plan and has 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 in 1992.

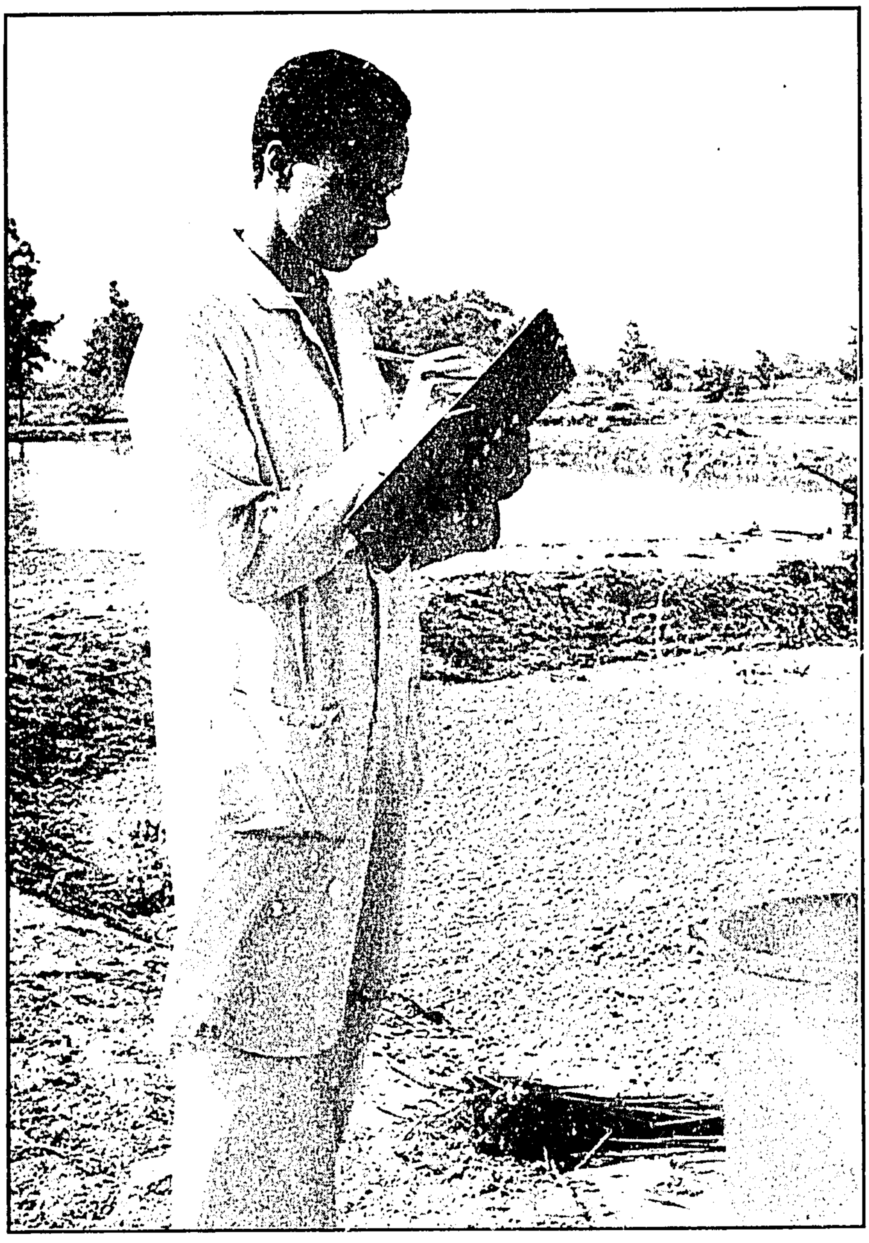
The Fourth Work Plan included tests of specific hypotheses formulated after review of the first three cycles of CRSP research. Special attention was paid to the economic aspects of CRSP pond management procedures. Further, the Data Analysis and Synthesis Team (DAST) started to systematically use the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

This reporting period consists not only of the second year of the Sixth Work Plan but also of the beginning of the Seventh Work Plan. While technically the Seventh Work Plan covers the period from 1 September 1993 to 31 August 1995, several events necessitated the inclusion of Work Plan Seven studies during this reporting period. Egypt joined the CRSP in Fall 1992, one year after activities delineated in the Sixth Work Plan had begun. Hence, the description of Egyptian research activities was included in the Seventh Work Plan. Further, the war in Rwanda prevented the expatriate US researcher from entering the host country as planned. In order to best use the available time, a Seventh Work Plan study was conducted in the United States.

The Seventh Work Plan is characterized by several changes. The CRSP has 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 introduces a new research focus, biotechnology, which has the potential to greatly aid the aquaculture industries in the US and in host countries. The Seventh Work Plan covers the remaining period of the current grant. A coordinating committee of CRSP researchers is currently working on new proposals for a continuation grant.



II. RESEARCH PROGRAM ACCOMPLISHMENTS

Global Experiment and Related Investigations

This reporting period covers the second year of research conducted under the Sixth Work Plan in Honduras, Rwanda, Thailand, and the Philippines, as well as the first year of experiments carried out in Egypt, which are described in the Seventh Work Plan. At all sites, the major theme of the Global Experiment is the investigation of fertilizer effects on pond systems. The Thailand project team examined the efficacy of CRSP fertilizer guidelines for small-scale, low-intensity aquaculture. They found that using only inorganic nitrogen and phosphorus fertilizers resulted in consistently high tilapia yields averaging around 8,000 kg/ha/yr. In the Philippines, scientists tested PONDCLASS fertilization guidelines. Preliminary results from these experiments suggest that tilapia can be produced at rates of more than 4000 kg/ha/yr by using only fertilization. Egypt project personnel compared PD/A CRSP management strategies with traditional Egyptian aquaculture methods. Ninety days after stocking, mean fish weight was highest in the "enhanced Egyptian" treatment and lowest when only feed was supplied to the ponds. In Rwanda, researchers studied the maximum levels of pond productivity under non-limiting fertilizer conditions. The highest rate of fish production achieved in this experiment was 3850 kg/ha/yr—the best rate yet reported for Rwanda. A study conducted in Honduras focused on the effect of different fertilizer types on soil respiration. This experiment revealed possible reasons why total alkalinity and total hardness levels often increased in organically fertilized ponds at El Carao.

Accompanying the above-mentioned Global Experiment studies, each research team conducted additional studies which also have global relevance. The Egypt project has added biotechnology research, a new line of inquiry in the family of CRSP studies. In one biotechnology study, Oregon State University researchers characterized the binding site for sex hormones in tilapia testes for the purpose of developing a fast screening method to estimate the masculinization potential of synthetic steroids. The U.S. Food and Drug Administration has granted a "compassionate" Investigational New Animal Drug (INAD) exemption for the use of 17α-methyltestosterone, a drug not yet not approved for use in animals, as a masculinization agent. Researchers in Egypt are participating in the field trials required under the INAD. Another biotechnology study, undertaken by Auburn researchers, is concerned with the development of a YY tilapia breeding program to generate monosex tilapia offspring that have not been treated with hormones. Scientists at the University of Hawaii are investigating the growth-promoting effects of 17α-methyltestosterone which they aim to separate—under hatchery conditions—from its masculinization effects.

Bioconversion, another research focus of the Egypt project, aims to ascertain which as yet unused pond system components like aquatic weeds or snails can be utilized as fish food. A test of the effectiveness of grass and black carp as control agents is currently underway.

In Honduras a new brackish water site at Choluteca was established in addition to the freshwater research facility at El Carao. Start-up activities at Choluteca were difficult; they entailed remodeling the facilities, developing adequate laboratory techniques for brackish water research, and training local shrimp producers on proper sample collection procedures. One of the experiments conducted at Choluteca last year was a study on the effect of humidity on soil respiration. It was found that wetting a dry pond bottom to subsaturation humidity levels (≈35%) would beneficially increase soil respiration and the mineralization of carbon, which would normally decrease drastically after the first week or so of pond drying.

Three related studies focused on the effect of elevation on pond systems in Rwanda. In an on-farm study, mono-sex tilapia were raised in five different high-elevation zones. Fish yield decreased at elevations above 1700 m, probably as a result of reduced food consumption or utilization on the part of tilapia rather than because of decreased primary productivity, because chlorophyll *a* levels remained very high even at the highest elevations. In another study, researchers tried to ascertain if dietary energy/dietary protein ratios reported in the literature were applicable under Rwandan conditions. The findings suggest that the ratio may be different for tilapia reared at higher elevations, because the ratio observed seems to be lower at lower temperatures. In a third study, researchers investigated the effects of temperature on appetite, growth, feed conversion efficiency, and body composition of tilapia. Growth and feed conversion were better at higher temperatures and greater feed availability. However, final body composition (crude protein and lipid content) was affected only by feeding rate, not by temperature.

Part of the Rwanda project effort was an economic analysis of small-scale aquaculture in comparison with other farming activities. While soybean production provided the least expensive source of protein, fish farming yielded the highest net returns to land, labor, and management. Hence, fish-farming is mainly a cash generating endeavor in Rwanda.

The Thailand research team investigated intensified farming practices. The experiment attempted to evaluate the benefits of supplementing fertilized ponds with feeds. The results indicated that farmers can save on feed costs by feeding at the 50% rather than the full *ad libitum* rate. A second experiment investigated alternative methods of destratifying pond systems by comparing the performances of a submersible pump, an air lift tube, and a vertical fan blade aerator. Each of these was more cost-effective than active aerators in preventing pond stratification in ponds up to 1600 m^2 if mixing is initiated early in the day.

Validation of PD/A CRSP Pond Management Strategies

Work Plan 7, Study 1A

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Introduction

Successful pond management systems for tilapia production result not only in high fish yields but also in positive economic returns. A variety of pond nutrient input regimes, which range from chemical fertilization to combinations of organic fertilization and supplemental feed, have been developed under the auspices of the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP). The PD/A CRSP has been conducting research in Honduras, Rwanda, Philippines, Thailand, and, now, Egypt.

Implementation of the PD/A CRSP Project in Egypt began in October 1992, and the first research ponds were stocked in the Spring of 1993. Egypt is located in the subtropics and has an arid climate. Air and water temperatures are cool enough during winter (December through February) to require some type of over-wintering facility, especially for *Oreochromis niloticus* brood stock and fingerlings. Such over-wintering facilities are not widespread in Egypt, and the majority of fish farmers stock their ponds with young-of-year tilapia fingerlings.

In Egypt, fish ponds usually are managed as a polyculture of young-of-year, mixed-sex tilapia, common carp, silver carp, and mullet. Nutrient inputs vary, but a "traditional" nutrient input regime involves periodic applications of organic and chemical fertilizers and daily provision of a formulated feed.

The objectives of the present research (Global Experiment) were:

1. To quantify tilapia yields of established PD/A CRSP pond nutrient input strategies under the climatic, edaphic, and water quality conditions found in Egypt.
2. To compare yields obtained with traditional Egyptian management practices to those obtained with PD/A CRSP production practices.
3. To determine the economics of each production system tested.

Materials and Methods

Twenty 0.1-ha earthen ponds located at the Central Laboratory for Aquaculture Research, Abbassa, Egypt, were used. Treatments were randomly allocated to ponds; there were four replicates per treatment. Young-of-year sex-reversed (monosex) or mixed-sex *Oreochromis niloticus* fingerlings (average weight 2 g) were stocked into ponds at 20,000 fish/ha on 1 July 1993. Treatments tested were:

1. "Traditional" Egyptian system
   Initially chicken litter was applied at 300 kg/feddan (1 feddan = 0.42 ha), followed by monthly applications of 100 kg/feddan. Triple superphosphate was applied at 30 kg/feddan every two weeks. Urea was applied at 10 kg/feddan every two weeks. A commercial fish ration (25% protein) was fed daily at 3% of fish biomass. Mixed-sex tilapia were stocked.
2. "Enhanced" Egyptian system
   Chicken litter was applied at 1,000 kg/ha per week for the first eight weeks. A commercial fish ration (25% protein) was fed daily at 3% of fish biomass beginning on day 61 of the experiment. Mixed-sex tilapia were stocked.
3. Feed only
   A commercial fish ration (25% protein) was fed daily at 3% of fish biomass. Monosex tilapia were stocked.
4. Fertilization then feed
   Chicken litter was applied at 1,000 kg/ha per week for the first eight weeks. A commercial fish ration (25% protein) was fed daily at 3% of fish biomass beginning on day 61. Monosex tilapia were stocked.
5. Chemical fertilization
   Nitrogen, as urea, was added at 25 kg N/ha per week. Phosphorus, as triple superphosphate, was added at 14.3 kg/ha per week (N:P of 4:1). Monosex tilapia were stocked.

Chicken litter was applied on a dry matter basis; dry matter content was determined periodically by measuring weight loss after drying for 24 h at 60°C. Fish were sampled monthly by seine net to monitor growth. Feed was offered to fish daily; the daily ration was adjusted monthly, based on results of seine samples. Six African catfish (*Clarias gariepinus*) were stocked into each pond on 13 September 1993.

Results

After 90 days of growth, mean individual fish weights based on seine samples were 89.5 g for the chemical fertilization treatment, 107.5 g for the enhanced Egyptian system treatment, 97.1 g for the fertilization-then-feed treatment, 68.2 g for the feed-only treatment, and 72.5 g for the traditional Egyptian system treatment. This experiment is scheduled to continue for an additional 60 days, after which all ponds will be harvested.

Water quality, primary productivity, and economic data will be analyzed upon completion of the experiment.

Anticipated Benefits

The performance of PD/A CRSP pond nutrient input regimes relative to the traditional Egyptian and the modified Egyptian pond management systems will be determined. In addition, the economics of each production system, quantified by enterprise budgets, will be determined. If PD/A CRSP production systems result in superior fish yields and in greater positive economic returns, modification of the existing tilapia pond management system in Egypt would be recommended.

This research will also determine whether PD/A CRSP-developed pond management strategies, applied to Egyptian ponds, produce tilapia yields similar to yields obtained at other PD/A CRSP sites, and serve as an indication of the applicability of PD/A CRSP pond management strategies for additional sites.

Soil Respiration: Effects of Chicken Litter and Urea

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Introduction

Supplementation of organic fertilizer with inorganic nitrogen has resulted in high primary productivity, fish yields, and pH. The use of inorganic fertilizers alone has resulted in reduced primary production and fish yields when compared with similar N and P inputs contributed by a combination of organic fertilizer and inorganic nitrogen. Organic fertilization is correlated with an increase in total alkalinity and total hardness, presumably because CO_2 released from decomposition of organic matter combines with free $CaCO_3$ in the soil to form soluble bicarbonate. Primary production consumes CO_2, raising pH and lowering concentrations of pH-dependent free CO_2 in the water column. Primary production at high fertility levels has become dominated by blue-green algae, which out-compete green algae under low-CO_2 conditions. It is hypothesized that lower primary productivity and fish production with sole inputs of inorganic fertilizers resulted from carbon limitation caused by high pH and reduced CO_2 input of decomposing organic matter. An experiment to test this hypothesis was conducted in laboratory microcosms.

Objective

The objective of this experiment was to observe the effect of chicken litter and urea fertilization on respiration, total alkalinity, total hardness, and pH in microcosms containing pond soil and water.

Materials and Methods

Five-gallon plastic buckets with tight fitting lids were used as microcosms in this study. Buckets had a bottom area of 603 cm^2. Soil, water, chicken litter, and urea were combined in different treatments as follows:

1. Soil, water, chicken litter, urea (SCLU)
2. Soil, water, chicken litter (SCL)
3. Soil, water (S)
4. Water, chicken litter (CL)
5. Water only (control)

Soil was taken from the top 10 cm of fish pond bottom, sun dried, broken up by hand, and well mixed. Soil contained in a 3085-cm^3 can was added to buckets to achieve a depth of about 5 cm. Ten liters of deionized water and 0.2 L of pond water were added to all buckets. Chicken litter was ground and dried, and applied to buckets at a rate of 12 g per week, which was equivalent to 2000 kg/ha. Urea was applied weekly at 0.66 g, which was equivalent to 109 kg/ha. After two weeks, chicken litter and urea fertilization rates were halved. Three buckets containing 10.2 L of water were used as controls. The experiment began on 16 November 1992 and ended 16 days later on 2 December 1992. After 1, 2, 4, 10, and 16 days, respiration, total alkalinity (TA), total hardness (TH), and pH were determined. Total ammonia nitrogen (TAN) was measured on Day 16.

During the experiment, water was stirred once a day. Buckets were tightly covered except for about one hour a day, when covers were removed to allow for gas exchange. Replacement water was added after each sampling day.

Soil respiration was ascertained from the quantity of CO_2 trapped in an alkali solution during a 24-h period. The methodology described by Anderson (1982) was followed, with these modifications: concentrations of alkali and acid solutions were reduced from 1.0 to 0.6 N and the ratio of CO_2 collecting jar area to area under incubation was decreased.

Results and Discussion

Total ammonia nitrogen concentrations (± SE) at the end of the experiment were 6.98 ± 0.646, 4.98 ± 0.516, 0.17 ± 0.015, and 4.53 ± 0.448 mg/L for SCLU, SCL, S, and CL treatments, respectively. Urea fertilization raised TAN levels about 47% higher than the application of chicken litter alone. Urea fertilization appeared to inhibit respiration, because respiration was consistently lower in the SCLU than the SCL treatment (Figure 1 and Table 1). High urea and/or ammonia concentrations may have reduced bacterial activity.

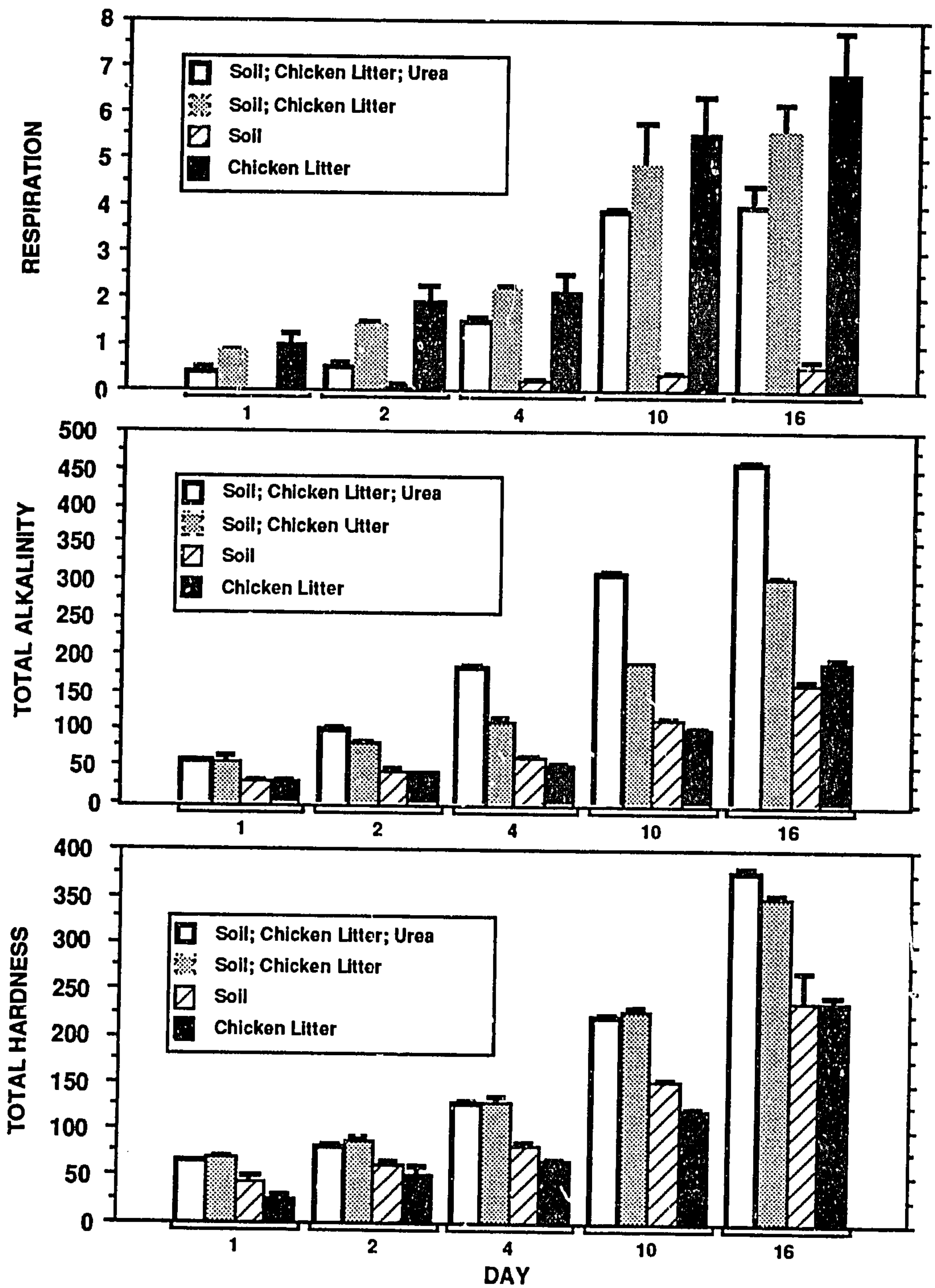

Figure 1. Mean respiration (g $CO_2/m^2/d$), total alkalinity (mg $CaCO_3$/L, and total hardness (mg $CaCO_3$/L) in 5-gallon bucket microcosms determined 1, 2, 4, 10, and 16 days after filling with water and combinations of soil, chicken litter, and urea. Bars represent 1 SE.

Table 1. Respiration, total alkalinity, total hardness, and pH in 5-gallon buckets, determined 1, 2, 4, 10, and 16 days following filling with water. Buckets contained soil (S); chicken litter (CL); soil and chicken litter (SCL); or soil, chicken litter, and urea (SCLU).

	Day					
Treatment	1	2	4	10	16	Mean
			Respiration (g $CO_2/m^2/d$)			
SCLU	0.42	0.50	1.44	3.89	3.96	2.04
SCL	0.84	1.40	2.16	4.87	5.62	2.98
S	0.00	0.07	0.21	0.37	0.54	0.24
CL	0.99	1.94	2.13	5.58	6.86	3.50
			Total alkalinity (mg $CaCO_3/L$)			
SCLU	55.6	96.1	180.8	306.9	456.3	219.1
SCL	56.5	79.7	110.6	190.1	302.1	147.8
S	28.7	42.5	61.9	113.6	162.3	81.8
CL	27.3	39.4	49.8	97.8	189.9	80.8
			Total hardness (mg $CaCO_3/L$)			
SCLU	64.3	81.1	127.2	220.8	376.2	173.9
SCL	68.8	89.5	129.5	228.6	349.6	173.2
S	45	60.2	83.4	150.8	237.6	115.4
CL	26.5	51.7	67.4	120.6	238.2	100.9
			pH			
SCLU	7.49	8.17	8.20	8.28	8.24	7.95
SCL	7.57	7.50	7.46	7.53	7.98	7.57
S	7.40	7.58	7.58	7.69	8.00	7.61
CL	7.39	7.08	7.11	7.36	7.85	7.28

Respiration in the CL treatment was apparently higher than that of SCLU and SCL treatments, despite the equal application of chicken litter to all. It is probable that respiration was actually similar, but that some of the CO_2 evolved in buckets containing soil reacted with $CaCO_3$ in the soil to make bicarbonate. That is why TA and TH were higher in buckets containing soil and CL than in those receiving solely CL.

Respiration of flooded soil (S) was low relative to those receiving allochthonous organic matter. Respiration was similar to that previously measured for saturated soils in bucket microcosms and *in situ* in flooded ponds (Boyd and Teichert-Coddington, in review).

Total alkalinity and pH were both consistently higher in the SCLU than in the SCL treatment (Figure 1 and Table 1). Hydroxide, formed from the reaction of NH_3 and H_2O, probably contributed to increased TA in the urea-fertilized treatment, because TH was similar in both treatments. If bicarbonate had been responsible for the increased alkalinity, then TH would have been higher in the SCLU compared with the SCL treatment.

This experiment illustrated the processes involving alkalinity, hardness, and pH that occur in organically fertilized ponds. Degradation of organic matter releases CO_2. If free $CaCO_3$ is present in the soil, then it will combine with CO_2 to form bicarbonate that is measured as increased concentrations of total alkalinity and total hardness. Organically fertilized ponds in all El Carao experiments have always shown a substantial increase in TA and TH during the growth cycle. Ponds receiving inputs comprised solely of inorganic fertilizers, however, have shown little change in TA and TH over time.

If chicken litter applications are supplemented with urea, then higher pH values can be expected as a result of the formation of hydroxide from ammonia and water. In the presence of sufficient oxygen, ammonia will eventually be mineralized to NO_3. This reaction is acid forming, theoretically destroying the alkalinity formed by ammonia and water. However, nitrate formation in our ponds is low, probably because dissolved oxygen concentrations are not maintained at a high enough level for a sufficient period of time.

This experiment was not entirely analogous to the processes that occur in a pond, because it did not take into account the effects of primary production. Primary producers consume CO_2, thereby moderating its concentrations. In the bucket microcosm, TA became very high in buckets containing soil and CL, partly because there was no consumption of excess CO_2. In El Carao ponds that receive high quantities of organic and nitrogen fertilizers, there has been an increase in early morning pH, from low 8's to low 9's. It is believed that a combination of hydroxide from ammonia fertilizer and high primary production has been responsible for increased pH. High primary productivity can result in net removal of CO_2 from the alkalinity system, thereby raising the pH. Ponds receiving inputs consisting solely of inorganic fertilizers have limited primary production, apparently because of carbon limitation.

Literature Cited

Anderson, J.P.E. 1982. Soil respiration. Pages 830-871 *in* A.L. Page, editor. Methods of soil analyses, Part 2. Chemical and microbiological properties, Second edition. Number 9 (Part 2) in the series Agronomy. American Society of Agronomy, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

Boyd, C.E., and D.R. Teichert-Coddington. In review. Pond bottom soil respiration during fallow and culture period in heavily fertilized tropical fish ponds. Manuscript submitted to the Journal of the World Aquaculture Society.

Intensive Fertilization of Tilapia Ponds in the Philippines

Work Plan 6, Experiment 10

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Introduction

The Freshwater Aquaculture Center (FAC) at Central Luzon State University and the Collaborative Research Support Program in Pond Dynamics/Aquaculture (CRSP) are leaders in the development of fertilization strategies for tropical fishponds and the scientific investigation of pond dynamics. In 1991, these organizations established a collaboration for the testing and further development of fertilization guidelines for Philippine tilapia farms.

The CRSP has developed three strategies for fertilizing fishponds. The simplest is fixed fertilizer input at rates empirically determined from numerous yield trials (Knud-Hansen et al 1993). The second strategy uses regular water quality analysis to determine nutrient limitation. This strategy is the basis of the CRSP expert system for pond fertilization called PONDCLASS (Lannan 1993). The third strategy utilizes bioassay to determine nutrient limitation (Guttman 1991).

Two experiments were conducted during 1992 to test, under Philippines conditions, several fixed fertilizer input rates. These rates were based on the most productive rates developed by the CRSP in Thailand. A third experiment was conducted in 1993 to evaluate PONDCLASS in the Philippines and to compare the yields of sex-reversed FAC-strain *O. niloticus* to yields attained with *O. niloticus* produced by an FAC/University of Wales Swansea project using YY males (Mair et al 1991).

Objectives

1. Test under Philippine conditions, the high nitrogen fertilization recommendations which were developed by the CRSP in Thailand;
2. Determine the partial substitutibility of chicken manure for inorganic fertilizer;

3. Test PONDCLASS under Philippines conditions; and
4. Compare the response of male *O. niloticus* produced from YY male broodstock.

Methodology

Three experiments were conducted in 500 m^2 ponds at the FAC, located 150 km north of Manila. Experiment 1 was conducted from November 1991 to April 1992 (152 days). Experiment 2 started in July 1992 and was completed in November (126 days). Experiment 3 was conducted from January to May 1993 (125 days).

Stocking density for the experiments was 2 fingerlings per m^2. *O. niloticus* fingerlings which had been treated with testosterone to produce all-male populations were used in all treatments except for one Experiment 3 treatment in which genetically produced male (GMT) offspring from YY broodstock were used.

There were three treatments and three replicates during each experiment. Chicken manure, urea and ammonium phosphate were the available nutrients. The treatments were:

<u>Experiment 1.</u>

Treatment 1.	High rate inorganic fertilizer
Treatment 2.	Moderate rate chicken manure
Treatment 3.	Moderate chicken manure supplemented with inorganic

<u>Experiment 2.</u>

Treatment 1.	High rate inorganic fertilizer
Treatment 2.	High rate chicken manure
Treatment 3.	Moderate rate inorganic fertilizer

<u>Experiment 3.</u>

Treatment 1.	High rate inorganic fertilizer
Treatment 2.	High rate inorganic fertilizer using GMT *O. niloticus*
Treatment 3.	PONDCLASS

High rate fertilization was set at 4 or 5 kg/ha/d with manure amounts calculated from table values during Experiments 1 and 2. After proximate analyses of manure were conducted during Experiment 3, the actual fertilizer input rates were determined (Table 1).

Water quality parameters were monitored once every two weeks. The parameters were: water temperature, dissolved oxygen, ammonia-N, soluble-reactive P, pH, alkalinity, and secchi disk visibility. Analytical methods followed procedures prescribed by the CRSP (Piedrahita et al. 1992). Fish samples were collected monthly.

Results

Average yields attained during the three experiments are summarized in Table 2. The typical CRSP Thailand fertilizer recommendation of 4-5 kg N/ha/d gave average yields 4 to 5 t/ha/yr. Interestingly, 2 kg/ha/d gave similar yields.

Table 1. Average nitrogen and phosphorus inputs.

Exp.	Treatment	Chicken Manure kg dry matter/ha/d	Average Organic Input kg N/ha/d	Average Organic Input kg P/ha/d	Average Inorganic Input kg N/ha/d	Average Inorganic Input kg P/ha/d	Total Input kg N/ha/d	Total Input kg P/ha/d
1	High Inorganic	0	0.0	0.0	5.0	1.0	5.0	1.0
1	Organic	36	1.1	0.1	0.0	0.0	1.1	0.1
1	Inorganic & Organic	36	1.1	0.1	4.0	0.9	5.1	1.0
2	High Inorganic	0	0.0	0.0	4.0	0.8	4.0	0.8
2	High Organic	116	3.6	0.4	0.0	0.0	3.6	0.4
2	Medium Inorganic	0	0.0	0.0	2.0	0.4	2.0	0.4
3	High Inorganic	0	0.0	0.0	4.0	0.8	4.0	0.8
3	High Inorganic w/ GMT	0	0.0	0.0	4.0	0.8	4.0	0.8
3	PondClass	0	0.0	0.0	4.2	0.5	4.2	0.5

Note: Proximate analyses conducted during Experiment 3 indicated chicken manure composition of 3.1% N and 0.32% P.

Table 2. Average fish yields (kg/ha/yr) during fertilizer trials in the Philippines.

Exp.	Treatment	Yield of Market Size Fish Average	Range	Recruits	Total Yield
1	High Inorganic	4691	4274 - 5091	0	4691
1	Organic	2145	1729 - 2786	0	2145
1	Inorganic & Organic	4755	4466 - 5091	81	4835
2	High Inorganic	3986	3888 - 4116	2201	6187
2	High Organic	3390	0 - 5510	1750	5140
2	Medium Inorganic	4151	4061 - 4248	1863	6014
3	High Inorganic	3941	2922 - 5232	1639	5580
3	High Inorganic w/ GMT	5324	4612 - 5988	260	5584
3	PondClass	4262	3512 - 4843	2314	6576

Approximately equivalent nitrogen loading totally from chicken manure gave a lower average yield because of a plankton crash and subsequent mass mortality in one of the replicates. Incomplete sex-reversal during Experiments 2 and 3 lead to substantial production of recruits which appears to have decreased yields of market-size fish by approximately 600 kg/ha/yr. Using GMT fish almost eliminated uncontrolled recruitment. The growth parameter Phi Prime for FAC *O. niloticus* averaged 3.32 which is less than the 3.40 to 3.50 usually attained with comparable nutrient inputs in Thailand.

Difficulties with instrumentation caused numerous problems with water quality determinations during the first experiment. Alkalinities started at 200 mg/L or higher and tended to decrease by approximately 100 mg/L over the culture period in all ponds receiving only inorganic fertilizer. Alkalinities in ponds receiving manure remained relatively constant or increased.

A preliminary analysis of returns to labor and capital is presented in Table 3. Data from a simultaneous feed trial was included for comparative purposes. Although fed ponds had an average yield of 6600 kg/ha/yr, the cost of the feed was much higher than the cost of fertilizer. Thus, the returns per ha using fertilizer alone were just as profitable as using feed. As, the farmer will require a much lower operating capital using fertilizer, return to investment will be increased and risk reduced. Also, the lower capital requirement for fertilized system will allow more farmers to use the technology.

Conclusions

The following conclusions can be drawn from these preliminary experiments:

1. Tilapia yields in excess of 4000 kg/ha/yr can be reliably produced using inorganic or a combination of inorganic/manure fertilization with N input levels of 2 - 4 kg/ha/d.
2. Manure only inputs at 4 kg N/ha/d may increase the chances of fish kills.
3. Further experiments are need to verify that 2 kg N/ha/d does indeed provide equivalent results to 4 kg/ha/d.
4. The FAC *O. niloticus* appears to have a lower growth potential than *O. niloticus* from Thailand.
5. Fertilized ponds are probably more profitable than fed ponds under Philippine conditions.

Table 3. Preliminary analysis of returns to labor & capital.

Exp.	Treatment	Average Yield kg/ha/yr	Gross Income Peso/ha/yr	Nutrient Cost Peso/ha/yr	Fingerling Cost Peso/ha/yr	Returns to Labor & Capital (Peso/ha/yr)
1	High Inorganic	4691	211095	37727	15000	158368
1	Organic	2145	96525	11670	15000	C9855
1	Inorganic & Organic	4755	213975	42860	15000	156115
2	High Inorganic	3986	179370	30200	15000	134170
2	High Organic	3390	152550	29659	15000	107891
2	Medium Inorganic	4151	186795	30200	15000	141595
3	High Inorganic	3941	177345	30200	15000	132145
3	High Inorganic w/ GMT	5324	239580	30200	15000	194380
3	PondClass	4262	191790	20663	15000	156127
*	Feed	6600	297000	130680	15000	151320

* Based on unpublished M.S. research conducted simultaneously at FAC.

Literature Cited

Guttman, H. 1991. Assessment of nutrient limitation in fertilized fish ponds by algal assay. M.Sc. Thesis, Asian Institute of Technology, Bangkok, Thailand. 87 p.

Knud-Hansen, C. F., C.D. McNabb, and T.R. Batterson. 1993. The role of chicken manure in the production of Nile tilapia (*Oreochromis niloticus*). Aquaculture and Fisheries Management.

Lannan, James. 1993. User's Guide to PONDCLASS Guidelines for fertilizing Aquaculture Ponds Version 1.1. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon. 60 p.

Mair, G.C., A.G. Scott, D.J. Pennan, J.A. Beardmore, and D.O.F. Skibinski. 1991. Sex determination in the genus *Oreochromis* 1. Sex reversal, gynogenesis and triploidy in *O. niloticus* (L.). Theor Appl Genet 82:144-152.

Piedrahita, Raul, Claude Boyd, and James Szyper. 1992. Handbook of Analytical Methods. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon. 60 p.

Field Testing Least Intensive Aquaculture Techniques on Small-Scale Farms in Thailand

Work Plan 6, Study 9

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Introduction

Research at the Asian Institute of Technology (AIT) in small-scale farmer aquaculture systems began with a survey of fish farms in Pathumthani Province, Central Thailand in 1979 (Edwards et al. 1983). A major conclusion was that farmers were constrained by a shortage of suitable fertilizers and feed inputs. Large-scale commercial farms in the province were integrated with feedlot livestock and/or used waste food from canteens or agro-industrial factories as fish feed. As the latter are seldom available in rural areas, it was recommended that small-scale farmers culture fish in integrated systems with feedlot livestock.

Since 1989, AIT has also conducted an Outreach program involving practical, low-risk fish farming strategies for resource-poor farmers in selected agro-ecological zones representative of Northeast Thailand. The program uses an interdisciplinary approach together with a rolling planning model. This model requires that project management decisions and methodological development be based on field data, farmer consultation, and discussions with field staff, rather than on assumptions made solely from on-station research results. The main objective is to test pond management recommendations under real farm conditions, and farmers who participate in the project are regarded as co-experimenters. Extension materials appropriate for the locale are being produced and evaluated. Results to date have been encouraging. Using project recommendations, farmers have been able to increase annual yields three-fold, and their examples have stimulated considerable interest among neighboring communities. Even higher yields are projected for the future.

The AIT Outreach project involves farmers who already culture fish. An important aspect of the project's philosophy is to avoid the creation of artificial markets by not providing free inputs such as larval fish seed and feed and nets. This also includes not financing the construction of new fish ponds, as work will be undertaken only with farmers who already possess one or more ponds. As no financial assistance is given to farmers as an incentive to adopt project recommendations, project staff must be confident that their recommendations are both technically feasible and socio-economically viable.

Global and site-specific experiments during the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) have resulted in the development of guidelines for fertilizing and stocking fish ponds to obtain high yields (Knud-Hansen et al. 1993, Knud-Hansen and Lin, in press). On-station work has been conducted at facilities of the Royal Thai Department of Fisheries (DOF) and at AIT. Tilapia yields have increased to >10,000 kg/ha/year, and a partial budget analysis has indicated that commercial fertilizer alone or in combination with manure is economically viable (McNabb et al. 1990, Knud-Hansen et al. 1993). The PD/A CRSP project in Thailand has advanced to the point where such recommendations can be implemented within the outreach infrastructure that has been established in Northeast Thailand by AIT. Research results presented here deal with a combination of on-farm and on-station trials utilizing fertilization and stocking strategies developed by PD/A CRSP researchers at AIT for the production of Nile tilapia (*Oreochromis niloticus*).

Objectives

The objectives of this work were to assess the efficacy of several PD/A CRSP guidelines for fertilization and stocking of Nile tilapia under field conditions. A further objective was to test a method devised at AIT to assess nutrient limitation of algal production on farm ponds.

Materials and Methods

Growout field trials were conducted at Romsai Farm (Central Thailand), Udorn Patana Farm (Northeast Thailand) and at the Thai Department of Fisheries Huay Luang Station (Northeast Thailand). Two surveys were conducted in Northeast Thailand. The first examined nutrient limitation of farmers' ponds using algal bioassays. The second survey examined inorganic carbon availability in farmers' ponds as evidenced by alkalinity concentrations. Regression analyses presented below were done according to Steel and Torrie (1980) using the Statgraphics 4® statistical software package (Manugistics, Inc. 1992).

Growout field trials

Romsai Farm: This field trial examined both the PD/A CRSP fertilization strategy and the intermittent stocking and harvesting strategy described by Knud-Hansen and Lin (in press) at Romsai Farm, located near Bang Sai in Central Thailand from 3 June 1991 to 6 March 1992. Six ponds of approximately 0.12 ha received the same

Table 1. Pond area and inputs for the growout and partial harvest field trial at Romsai Farm (3 June 1991 to 6 March 1992).

		Pond Area		Inputs (kg/ha/wk)	
Site	Pond	rai	m²	Urea-N	TSP-P
Romsai	B10	0.75	1,198	33.0	16.6
Romsai	B11	0.74	1,176	33.6	16.9
Romsai	B13	0.79	1,271	31.1	15.6
Romsai	B14	0.83	1,335	29.6	14.9
Romsai	B16	0.82	1,314	30.0	15.1
Romsai	B17	0.81	1,302	30.3	15.2
	Total:	4.74	7,596		

Note: All ponds received same amounts of urea and TSP

Table 2. Fish stocking data for the three stockings the partial harvest field trial at Romsai Farm (3 June 1991 to 6 March 1992).

			Stocking		Stocking		
Site	Date	Day	No.	fish/pond	fish/m²	kg/pond	g/fish
Romsai	3 Jun 91	0	1	1600	1.3	0.9	0.5
Romsai	19 Aug 91	77	2	1600	1.3	1.0	0.6
Romsai	20 Dec 91	200	3	1600	1.3	0.5	0.3

total fertilization inputs, but as the pond sizes differed slightly so did the per-unit-area inputs. Inputs to the six ponds ranged from 29.6 to 33.6 kg/ha/wk of urea-N (0.42-0.48 g $N/m^2/d$) and phosphorus (P) as TSP (45% P_2O_5) added to give a N:P ratio of 2:1 by weight (Table 1). Sex-reversed Nile tilapia (about 1 g each) were stocked three times at 1.3 fish/m² on days 0, 77, and 200 (Table 2). Partial harvesting occurred on days 179 and 255, with complete harvesting taking place on day 277 after initial stocking. Water depths were maintained at about 1.0 m.

Initial and routine water quality measurements were made in al' ponds. Surface water dissolved oxygen (DO) and temperature were measured *in situ*, using a Model 57 Yellow Springs Instrument meter (pre-dawn and at 0900, respectively). Surface water pH was measured in the field using a Model Sp-5A Suntex digital meter. Integrated water column samples were collected at 0900 hours by vertically lowering and capping a pre-rinsed 5-cm (i.d.) PVC tube. Initial water quality measurements on these samples included total alkalinity, analyzed potentiometrically using 0.02 N HCl to titrate to pH 5.1 (APHA 1985), total ammonia-N, using phenolhypochlorite (Solórzano 1969), nitrate-nitrite-N, using cadmium reduction (APHA 1985), Kjeldahl N (APHA 1985), soluble reactive phosphorus (SRP) and total P by acid molybdate and ascorbic acid (APHA 1985), chlorophyll *a* corrected for pheophytin *a* by extraction in 90% acetone followed by acidification (APHA 1985), and total suspended

(TSS) and total volatile solids (TVS) (APHA 1985). Total alkalinity, SRP, and ammonia-N were monitored along with field measurements of DO, temperature, and pH every two weeks throughout the field trial.

Huay Luang: This field trial compared ponds managed according to the PD/A CRSP fertilization strategy (inorganic fertilizer only) with ponds receiving inorganic fertilizer plus chicken manure. It was conducted at the Department of Fisheries Huay Luang Field Station, near Udorn Thani in Northeast Thailand, from 16 September 1991 to 22 February 1992. Three ponds of approximately 0.16 ha (1 rai) received total fertilization inputs of 29.6 kg/ha/wk of urea-N (0.42 g $N/m^2/d$) and TSP added to give a N:P ratio of 3:1 by weight (Table 3). Three other 0.16 ha ponds received 29.0 kg/ha/wk of urea-N (0.41 g $N/m^2/d$) and TSP added to give a N:P ratio of 3:1 by weight, as well as fryer chicken manure (12% moisture, 1.03% N, and 0.64% P dry weight) at 300 kg dry weight/ha/wk (Table 3). Sex-reversed Nile tilapia (about 5 g each) were stocked three times at 2.6 fish/m^2 (Table 4). Complete harvesting took place 159 days after stocking. Pond water depths were maintained at about 1.0 m.

Water quality in ponds was monitored every two weeks. Pond water DO and temperature were measured at 0630 and 1500 hours *in situ* (at depths of 25, 50 and 75 cm) using a Model 57 Yellow Springs Instrument meter. Integrated water column samples were also collected at 0630 and 1500 hours by vertically lowering and capping a pre-rinsed 5-cm (i.d.) PVC tube. The water quality measurements made on these samples were total alkalinity, using methyl orange indicator (APHA 1985), pH, using a Model Sp-5A Suntex digital meter, total ammonia-N, using phenolhypochlorite (Solórzano 1969), and SRP, by acid molybdate and ascorbic acid (APHA 1985). Net primary productivity (NPP) was estimated from mass balance changes in DO measured at pond depths of 25 and 50 cm before dawn (≈0630 hours) and at 1500 hours according to the method of Hall and Moll (1975). Secchi depth was also determined every two weeks.

Table 3. Pond area and inputs for growout field trial at the Huay Luang Department of Fisheries Field Station at Udorn Thani (16 September 1991 to 22 February 1992).

			Inputs (kg/ha/wk)				
	Pond Area				Chicken Manure		
Pond	rai	m^2	Urea-N	TSP-P	dry weight	N	P
20	1.0	1,600	29.6	12.3			
22	1.0	1,600	29.6	12.3			
24	1.0	1,600	29.6	12.3			
21	1.0	1,600	29.0	11.7	300	3.1	1.9
23	1.0	1,600	29.0	11.7	300	3.1	1.9
25	1.0	1,600	29.0	11.7	300	3.1	1.9

Note: Chicken Manure: 1.03% N (dry weight); 0.64% P (dry weight); and 11.8% moisture

Table 4. Fish stocking data for the growout field trial at the Huay Luang Department of Fisheries Field Station at Udorn Thani (16 September 1991 to 22 February 1992).

	Growout	Stocking			
Pond	days	fish/pond	fish/m^2	kg/pond	g/fish
20	159	4,080	2.6	27.3	6.7
22	159	4,080	2.6	21.6	5.3
24	159	4,080	2.6	26.1	6.4
21	159	4,080	2.6	19.6	4.8
23	159	4,080	2.6	9.0	2.2
25	159	4,080	2.6	15.9	3.9

Udorn Patana Farm: This field trial utilized the PD/A CRSP fertilization strategy (inorganic fertilizer only) in three ponds at the Udorn Patana Farm near Udorn Thani in Northeast Thailand from 29 July 1991 to 17 January 1992. The three ponds had traditionally given low fish yields because of difficulty in getting the ponds green. The ponds ranged from 4,704 to 5,840 m^2 in area (Table 5), and received the same total fertilization inputs per unit area – 28.7 kg/ha/wk of urea-N (0.41 g N/m^2/d) and TSP added to give a N:P ratio of 2.3:1 by weight. Sex-reversed Nile tilapia (about 10 g each) were stocked between 2.5 and 3.6 fish/m^2, and harvested between 141 and 159 days after stocking (Table 5). Pond water depths were maintained at about 1.0 m.

Water quality in ponds was monitored approximately every 10 days. Pond water DO and temperature was measured at 0600 and 1500 hours *in situ* (at depths of 25, 50 and 75 cm) using a Model 57 Yellow Springs Instrument meter. Secchi depth and surface water pH were also measured at this time. Surface water was collected from each pond for further analysis five times during the growout. Water quality measurements made on these samples were total alkalinity, using methyl orange indicator (APHA 1985), total ammonia-N, using phenolhypochlorite (Solórzano 1969), and SRP, by acid molybdate and ascorbic acid (APHA 1985). Net primary productivity (NPP) was estimated from mass balance changes in DO measured at pond depths of 25 and 50 cm before dawn (≈0600 hours) and at 1600 hours, according to the method of Hall and Moll (1975).

Surveys

Bioassay: Sub-surface water from twenty farm or village ponds around the Udorn Thani area (Northeast Thailand) was collected for algal nutrient limitation analysis using the algal bioassay method (Guttman 1992). These ponds are also included among those monitored by the AIT Outreach Program. In addition to identifying carbon, nitrogen, and/or phosphorus nutrient limitation, water was analyzed for concentrations of total alkalinity, using methyl orange indicator (APHA 1985), total ammonia-N, using phenolhypochlorite (Solórzano 1969), and SRP, by acid molybdate and ascorbic acid (APHA 1985). These analyses were conducted between October and November 1991.

Table 5. Pond characteristics and fish stocking data for growout field trial at the Udorn Patana Farm (29 July 1991 to 17 January 1992).

	Pond Area		Growout	Stocking	
Pond	rai	m^2	days	fish/pond	fish/m^2
51	3.58	5,728	141	14,112	2.5
56	3.65	5,840	159	17,520	3.0
57	2.94	4,704	158	17,088	3.6

Carbon: Seventeen farmers with a total of 34 ponds in the Udorn Thani area were interviewed between October and November 1991. The questionnaire asked for information regarding each pond's age, source of water, whether or not it had been drained, and if mud had been removed from the pond. Each pond's apparent surface color was evaluated with the AIT Outreach pond color chart with values ranging from brown (1) to increasing intensities of green (6 being the most green). Sub-surface water was collected from these 34 ponds, as well as 21 other ponds, and measured for concentrations of total alkalinity using methyl orange indicator (APHA 1985) and conductivity and salinity using a WTW conductivity meter.

Results and Discussion

Growout field trials

Romsai Farm: Table 6 summarizes harvest data for the three individual stockings. Total percent survival for the first-stocked fish was 86.8%, but for the second and third stockings survival rates decreased to 23.8% and 39.1%, respectively. The low survival rates for the latter two stockings (Table 6) was probably due to the consumption of tilapia fry by carnivorous snakehead (*Channa striata*), which were found in four ponds at harvest. For all ponds and all stockings the average gross fish yield was 4,713 kg, or an extrapolated yield of 8,176 kg/ha/yr (Table 7). Individual pond yields could not be determined because fish from the first harvest were grouped together. The input (urea and TSP) cost to attain the total yield was nearly Baht 32,000 [US$1 = 25 Baht (THB)], or approximately THB 6.7 per kg fish (Table 8). Although 8,176 kg/ha/yr was a reasonable yield, it probably would have been higher if the snakehead had not eliminated most of the fry in the second and third stockings. This high fry mortality probably also increased overall relative input costs per kilogram of harvested fish.

Periodic sampling revealed that the growth rate of the first-stocked fish remained linear through Day 240 after stocking, even though the second stocking occurred 77 days after the first (Figure 1). Fish growth varied depending on the age of the ponds. Ponds B10, B11, B13, and B14 were older ponds, and mean weights after 240 days for the first-stocked fish ranged from 650 to 747 g/fish. In contrast, mean weights of fish from new ponds B16 and B17 were only 440 and 407 g/fish after 240 days growth. In the four old ponds, the growth rates for the first-stocked fish and the second-stocked fish were nearly identical at about 3 g/fish/d, suggesting that neither stocking had an effect on each other with regard to fish growth (Figure 2). The exact significance of this is not clear because on Day 179 about 30% of

Table 6. Summary of harvest data for the three stockings ofthe partial harvest field trial at Romsai Farm (3 June 1991 to 6 March 1992) (NIL = *O. niloticus*).

Date	Day	Pond	Species Code	Stocking No.	Harvest Fish kg	Harvest Fish No.	% Survival
29 Nov 91	179	all	NIL	1	1,273	2,742	
13 Feb 92	255	all	NIL	1	1,099	2,712	
6 Mar 92	277	all	NIL	1	1,603	2,882	86.8
6 Mar 92	277	all	NIL	2	603	2,288	23.8
6 Mar 92	277	all	NIL	3	107	3,751	39.1
6 Mar 92	277	B10	snakehead		4.4	3	
6 Mar 92	277	B11	perch		0.4	77	
6 Mar 92	277	B11	catfish		7.4	76	
6 Mar 92	277	B11	snakehead		3.5	11	
6 Mar 92	277	B13	snakehead		0.2	1	
6 Mar 92	277	B14	snakehead		3.8	11	
6 Mar 92	277	B16	perch		6.1		

Table 7. Total fish harvest data (GFY = gross fish yield; NFY = net fish yield) after 277 days for all ponds and all harvests for tilapia, predator fish, and non-predator fish for the partial harvest field trial at Romsai Farm (3 June 1991 to 6 March 1992).

Ponds	Stocking (kg)	Tilapia GRY (kg)	NFY (kg/ha)	Pred. GFY (kg)	Non-pred. GFY (kg)	Total GFY (kg)	Total GFY (kg/ha/d)	Total GFY (kg/ha/yr)
All	14.0	4,685	22.2	14.2	13.9	4,713	22.4	8,176

the first-stocked fish were removed, and final harvest data indicated only 24% of the second-stocked fish survived (Table 6). Since it is not known when mortalities of the second-stocked fish occurred, it is impossible to estimate actual fish densities or possible density effects after the second stocking.

Water quality remained fairly consistent both over time (Table 8) and between ponds (Table 9). Ammonia-N averaged <1.0 mg/L and SRP remained <0.5 mg/L for all ponds. Total alkalinity averaged between 63 and 108 mg $CaCO_3$/L, and pH ranged from 7.2 to 10.0. Pond water temperatures ranged from 22.0 to 31.9°C, and pre-dawn DO concentrations averaged 3.8 mg/L for all ponds during the growout period.

This experiment clearly illustrated two difficulties of the partial harvest and intermittent stocking strategy. First, by not draining ponds for extended periods, mobile carnivorous fish like snakehead can remain undetected in ponds and feed on additionally stocked fry. Second, the 30% capture rate of the first-stocked fish reflected the difficulty in harvesting tilapia from a partially full pond. The equally good growth rate of the second-stocked fish as compared to the first, however, suggests that this management strategy still merits further study.

Table 8. Summary of mean water quality data for all ponds (n=6) in the partial harvest field trial at Romsai Farm (3 June 1991 to 6 March 1992).

Date	Total Alkalinity (mg/L)	Soluble Reactive P (mg/L)	Total P (mg/L)	Total Ammonia-N (mg/L)	NO_3-NO_2-N (mg/L)	Kjeldahl-N (mg/L)	Chlorophyll *a* (mg/m^3) uncorrected	Chlorophyll *a* (mg/m^3) uncorrected	Total Susp. Solids (mg/L)	Total Volatile Solids (mg/L)	DO at Dawn (mg/L)
18 Jun 91	114	0.35	0.32	0.06	0.03	4.05	189	183	30	19	
2 Jul 91	93	0.46		1.00			61	63	21	9	1.9
1 Aug 91	56	0.37		0.19							3.2
28 Aug 91	58	0.22		0.04							4.6
11 Oct 91	91	0.35		0.07			352	371			3.0
30 Oct 91		0.29		0.10			229	224			4.4
20 Nov 91		0.51		0.65			183	213			
18 Dec 91	87	0.33		0.78			492	528			
08 Jan 92	88	0.12		1.15							4.2
22 Jan 92	83	0.56		1.68							5.0
5 Feb 92	79	0.31		1.63							4.2

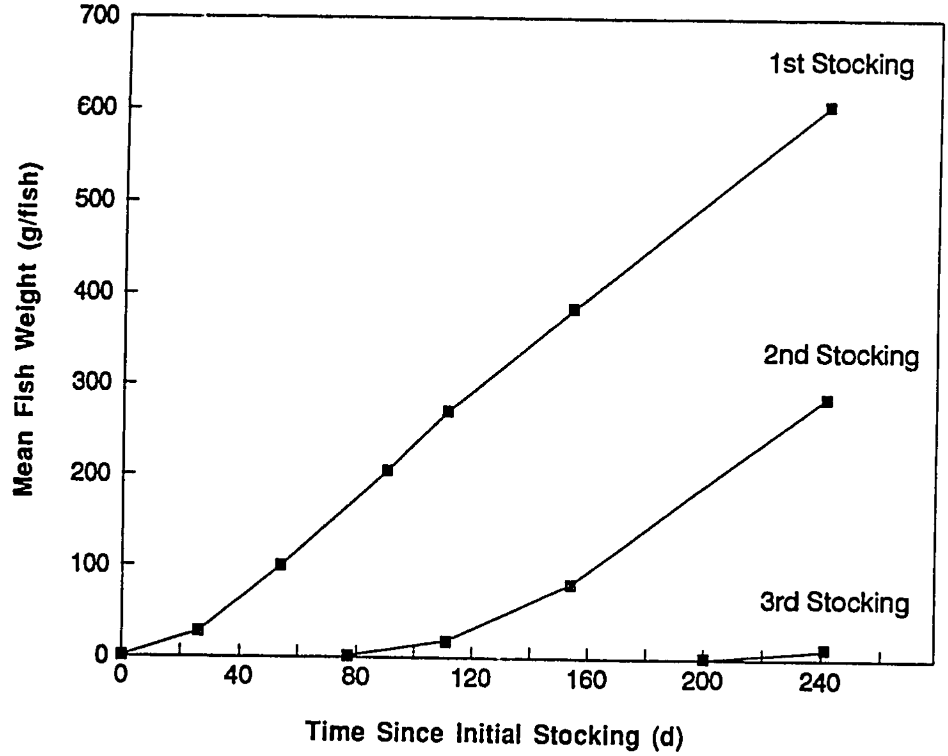

Figure 1. Mean growth of tilapia (g/fish, n = 6 ponds) from three different stockings at the Romsai Farm.

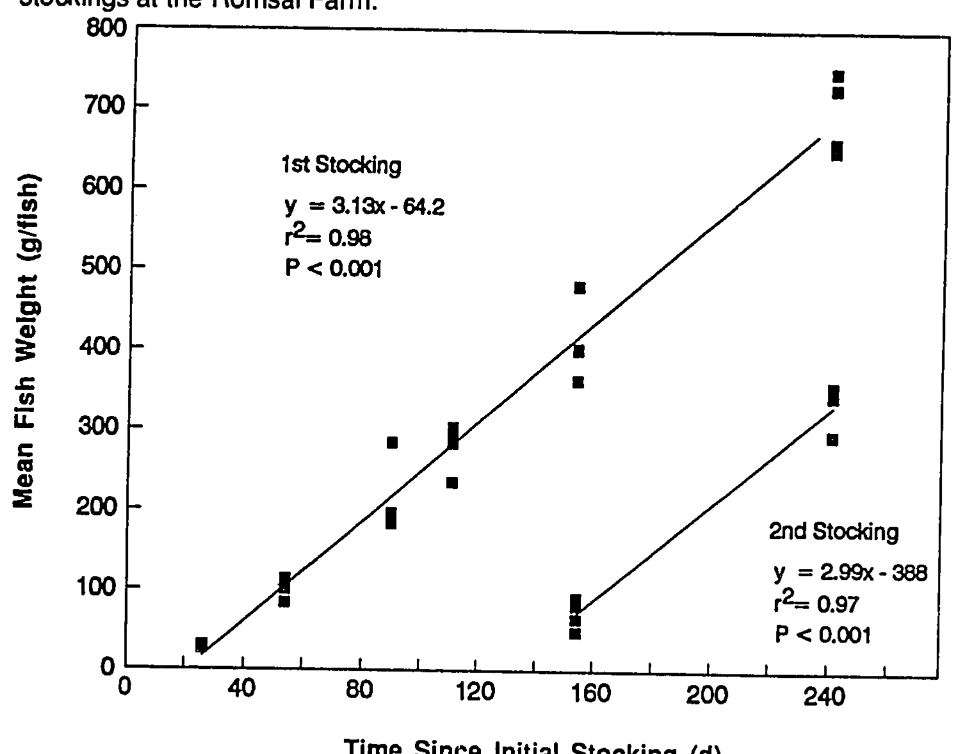

Figure 2. Linear relationships between mean fish weight (g/fish) and time (d) for first and second stockings for fish grown in the four older ponds at Romsai Farm.

Table 9. Mean water quality data for each pond the partial harvest field trial at Romsai Farm (3 June 1991 to 6 March 1992).

Pond	Total Alkalinity (mg $CaCO_3$/L)	SRP (mg/L)	Total Ammonia-N (mg/L)	Chlorophyll *a* (mg/m^3) uncorrected	corrected
B10	63	0.32	0.76	468	461
B11	108	0.43	0.54	322	328
B13	86	0.29	0.95	192	209
B14	88	0.33	0.55	163	176
B16	78	0.28	0.56	139	175
B17	73	0.27	0.47	220	234

Huay Luang: Extrapolated fish yields ranged from 4,673 to 7,911 kg/ha/yr, with the three highest yields coming from the ponds that were fertilized with urea, TSP, and chicken manure (Table 10). Mean fish weights at harvest ranged from 114 to 158 g/fish with a significant difference between the two treatments. Differences in fish yields could not be explained by differences in net primary productivity (Figure 3). The more probable reason for observed differences in fish yields was due to increased mortality in the non-manured ponds. Tilapia survival ranged from 49.6% to 70.3% in the non-manured ponds, but ranged from 81.4% to 88.2% in ponds receiving chicken manure (Table 10). The linear relationship between percent survival of stocked tilapia and tilapia net fish yield gave an r^2 value of 0.63, but was not statistically significant ($P < 0.10$) because of the small number (4) of degrees of freedom (Figure 4). Percent survival was also linearly related to the weight of predator fish (snakehead and a few *Notopterus* spp.) captured in each pond at harvest (Figure 5). This relationship is also not statistically significant ($r^2 = 0.65$, $P < 0.06$), but certainly suggests biological significance. When percent survival is included in the ANOVA, there is absolutely no significant difference ($P > 0.5$) between the two fertilization treatments with regard to tilapia yields. Why there were more predator fish in the non-manured ponds remains unclear.

The input cost for the entire experiment was THB 7.7 per kg fish (Table 11). This table also suggests that fertilizing with manure is more cost effective than fertilizing only with inorganic materials. But this difference again is due to the increased mortality observed in the non-manured ponds. The three manured ponds had a mean survival rate of 84.5% and an average yield of 7,091 kg/ha/yr. This gave an input cost of THB 6.5 per kg fish, very similar to the THB 6.7 per kg fish noted at the Romsai Farm. The yields were slightly less at Huay Luang than at Romsai Farm, partly because the latter had a higher rate of nutrient input (31.2 kg N/ha/wk versus 29.6 kg N/ha/wk). The lower rate of individual fish growth at Huay Luang (about 150 g/fish at 159 days, Table 10) compared to Romsai (about 400 g/fish at 159 days, Figure 1) was probably a function of the higher stocking density used (1.3 fish/m^2 versus 2.6 fish/m^2).

With regards to field measurements, temperature ranged from 20.4°C to 37.1°C and pH ranged from 6.6 to 10.0 during the experiment. Low DO was never a problem during the experiment. DO at dawn averaged 3.7 mg/L in non-manured ponds, and 2.7 mg/L in manured ponds.

Table 10. Fish harvest data (tilapia, and predator and non-predator recruits) for the growout field trials at the Huay Luang Department of Fisheries Field Station at Udorn Thani (16 September 1991 to 22 February 1992). GFY = gross fish yield; NFY = net fish yield.

Pond	Harvest (fish/pond)	% Survival	Tilapia Harvest (g/fish)	GFY (kg/pond)	NFY (kg/ha/d)	Predator GFY (kg/pond)	Non-predator GFY (kg/pond)	Total GFY (kg/ha/d)	Total GFY (kg/ha/yr)
20	2,864	70.3	126.0	360.9	13.1	4.5	9.3	14.7	5,376
22	2,675	64.8	127.9	342.0	12.6	8.5	23.7	14.7	5,369
24	2,052	49.6	150.0	307.7	11.1	11.0	7.0	12.8	4,673
21	3,597	88.2	114.0	410.0	15.3	1.5	10.5	16.6	6,055
23	3,352	81.4	146.3	490.3	18.9	3.4	15.7	20.0	7,309
25	3,414	84.0	157.5	537.6	20.5	7.5	6.3	21.7	7,911

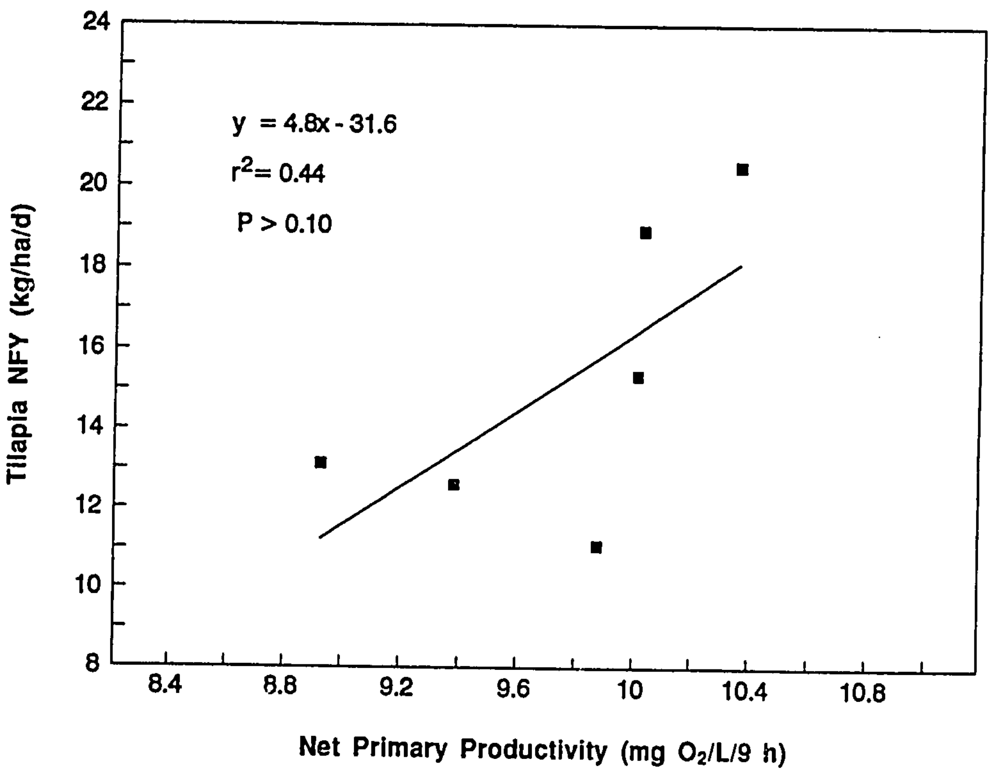

Figure 3. Relationship between net primary productivity (mg O_2/L/9 h) and tilapia net fish yield (NFY, kg/ha/d) at the Huay Luang Fisheries Station.

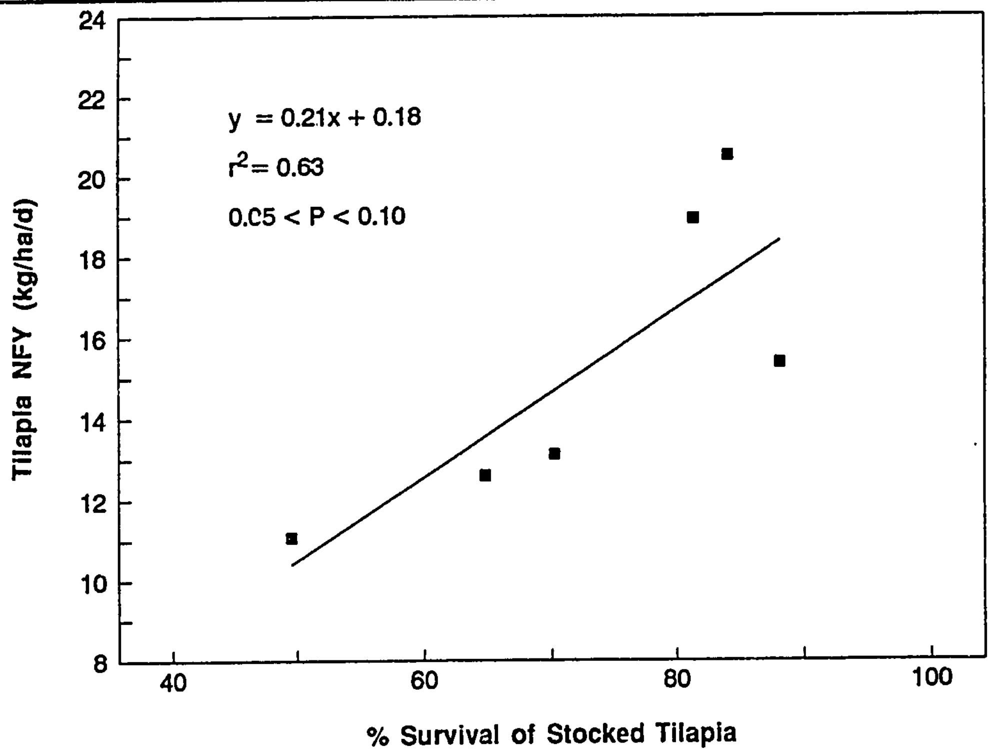

Figure 4. Linear relationship between percent survival of stocked tilapia and tilapia net fish yield (NFY, kg/ha/d) at the Huay Luang Fisheries Station.

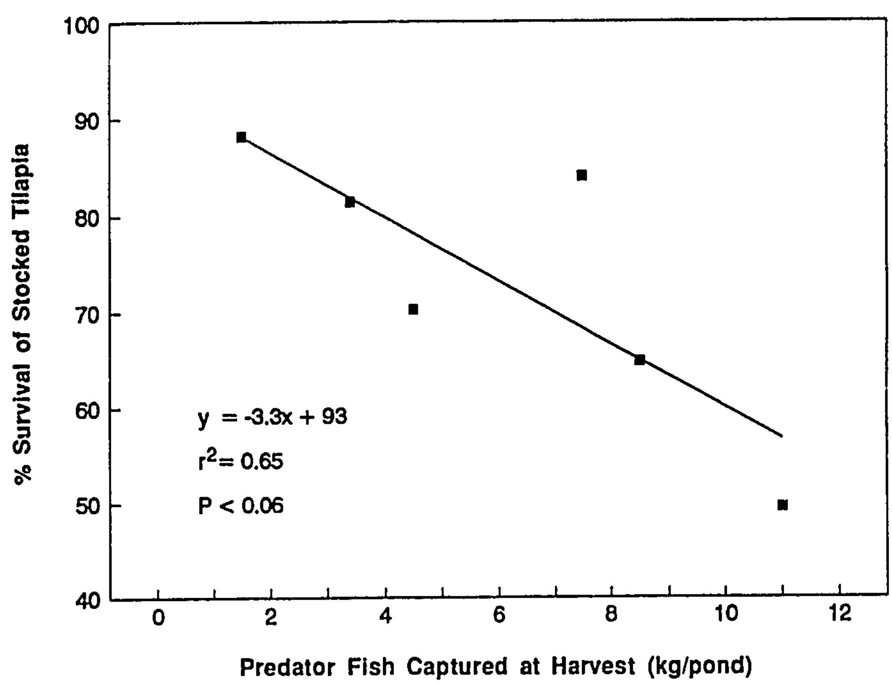

Figure 5. Linear relationship between predator fish captured at harvest (kg/pond) and percent survival of stocked tilapia at the Huay Luang Fisheries Station.

Table 11. Economic analysis based on total ponds inputs and gross fish yield (GFY) in the growout field trials at the Huay Luang Department of Fisheries Field Station at Udorn Thani (16 September 1991 to 22 February 1992). US$1 ≈ 25 Baht; Baht = THB; THB/kg = Thai Baht/kg harvested fish.

	Input						
Pond	Urea (kg/pond)	TSP (kg/pond)	Manure (kg/pond)	Cost (THB/pond)	GFY (kg/pond)		Cost (THB/kg)
20	230	230		3,220	360.9		8.9
22	230	230		3,220	342.0		9.4
24	230	230		3,220	307.7		10.5
21	226	217	1,104	3,086	410.0		7.5
23	226	217	1,104	3,086	490.3		6.3
25	226	217	1,104	3,086	537.6		5.7
Total	1,368	1,341	3,312	18,918	2,448.5	Mean	7.7

cost of urea: THB 250/50 kg
cost of TSP: THB 450/50 kg
cost of chicken manure: THB 20/50 kg

Water quality measurements made throughout the growout period showed accumulations of ammonia-N and SRP. Ammonia-N concentrations reached nearly 5 mg/L in both non-manured ponds (Figure 6) and manured ponds (Figure 7). Mean ammonia-N concentrations ranged from about 1.5 to 2.5 mg/L for all ponds (Table 12). SRP concentrations generally increased throughout the experiment for both non-manured ponds (Figure 8) and manured ponds (Figure 9), exceeding 6 mg/L in four of the six ponds. Mean SRP concentrations ranged from about 2.5 to 5.1 mg/L for all ponds (Table 12). The accumulation of both soluble inorganic N and P probably occurred because algal productivity in the ponds were limited by carbon and light and not N and P (Knud-Hansen and Batterson, unpublished data). These ponds were relatively new and occasionally exhibited substantial inorganic turbidity.

Alkalinities also tended to be relatively low, with pond means ranging from 49 to 71 mg $CaCO_3$/L (Table 12). Alkalinities in non-manured ponds tended to remain constant during the growout period (Figure 10). On the other hand, ponds receiving chicken manure showed a steady increase in alkalinity (Figure 11). This increase can be attributed to the lime chicken farmers put on the manure to minimize odors. It is possible that increased carbon availability in the manured ponds caused the small but consistent differences in NPP noted between treatments (Table 12). ANOVA suggested that treatment differences in percent survival were responsible for observed differences in net fish yield (NFY), but increased NPP due to inadvertent lime inputs may also have contributed to fish yield variability between treatments.

Udorn Patana Farm: Harvest data at Udorn Patana Farm gave a three-pond mean extrapolated fish yield of 8,687 kg/ha/yr (Table 13). The percent survival of stocked fish was not determined. Mean fish weights at harvest ranged from 141 g/fish to 175 g/fish; this is more similar to Huay Luang than to Romsai Farm because the stocking densities were similar (Figure 12, Table 13). At Udorn Patana Farm, the

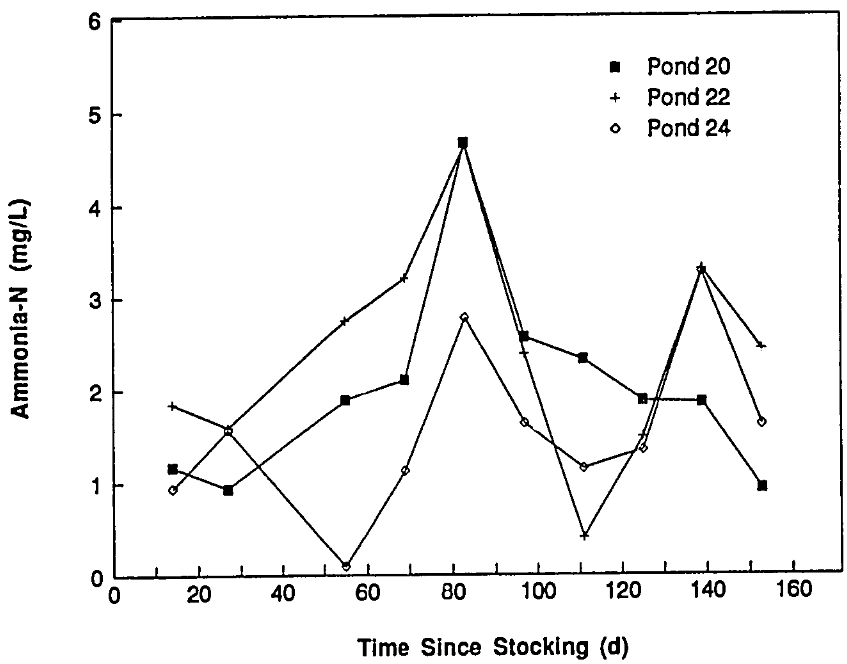

Figure 6. Variations of ammonia-N concentrations (mg/L) over time (d) in non-manured ponds receiving only inorganic fertilizers (urea and TSP) at the Huay Luang Fisheries Station.

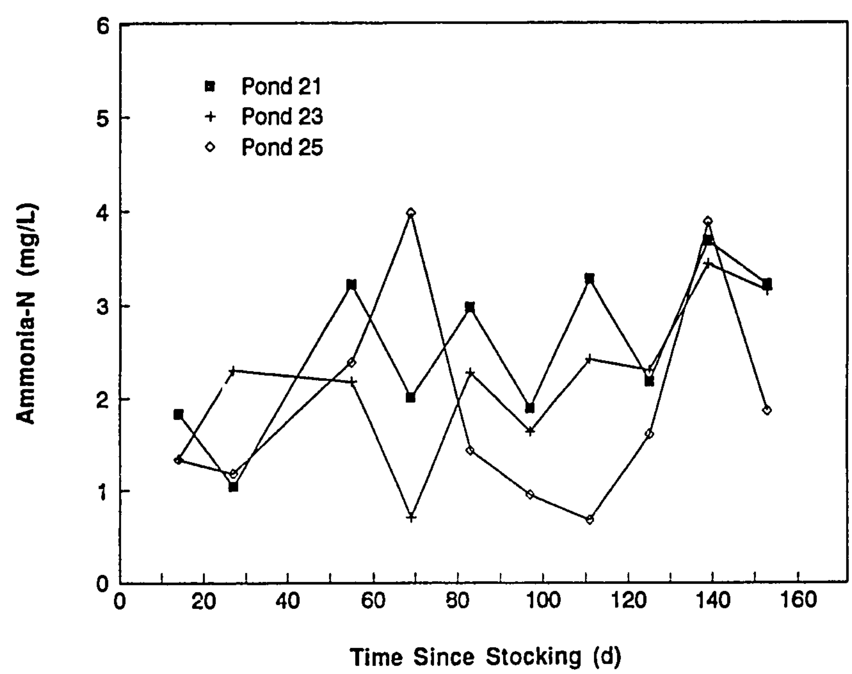

Figure 7. Variations of ammonia-N concentrations (mg/L) over time (d) in ponds receiving chicken manure, urea, and TSP at the Huay Luang Fisheries Station.

Table 12. Mean water quality and field data for the growout field trials at the Huay Luang Department of Fisheries Field Station at Udorn Thani (16 September 1991 to 22 February 1992). NPP = net primary productivity.

Pond	Total Alkalinity (mg $CaCO_3$/L)	SRP (mg/L)	Total Ammonia-N (mg/L)	NPP (O_2/9 hr)	Dawn DO (mg/L)	Secchi depth (cm)
20	50	3.39	2.02	8.9	3.8	22
22	49	4.04	2.39	9.4	3.0	24
24	55	2.46	1.54	9.9	4.4	21
21	55	4.21	2.53	10.0	2.3	21
23	57	5.08	2.18	10.0	2.5	23
25	71	3.43	1.93	10.4	3.3	24

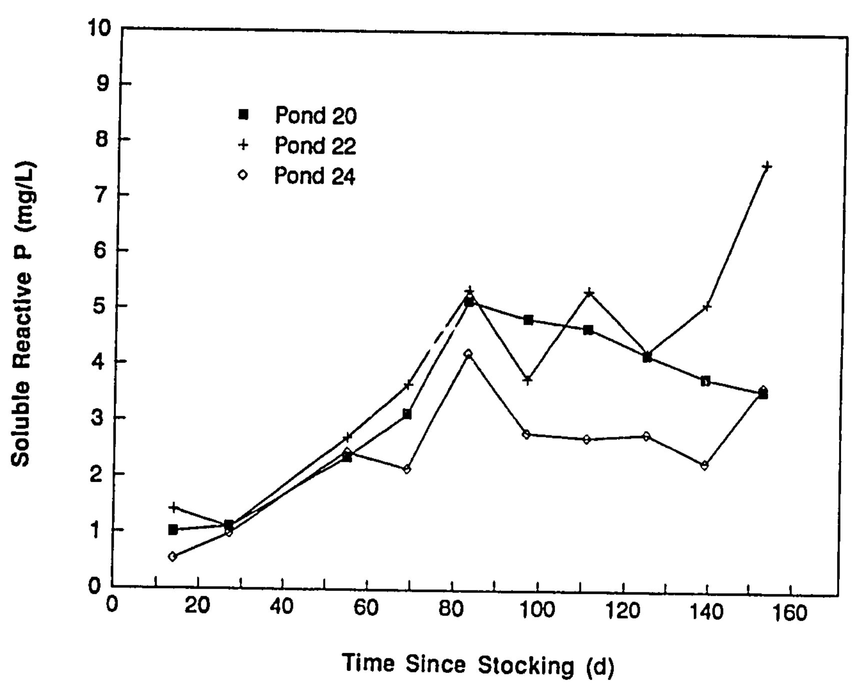

Figure 8. Variations of soluble reactive P concentrations (mg/L) over time (d) in non-manured ponds receiving only inorganic fertilizers (urea and TSP) at the Huay Luang Fisheries Station.

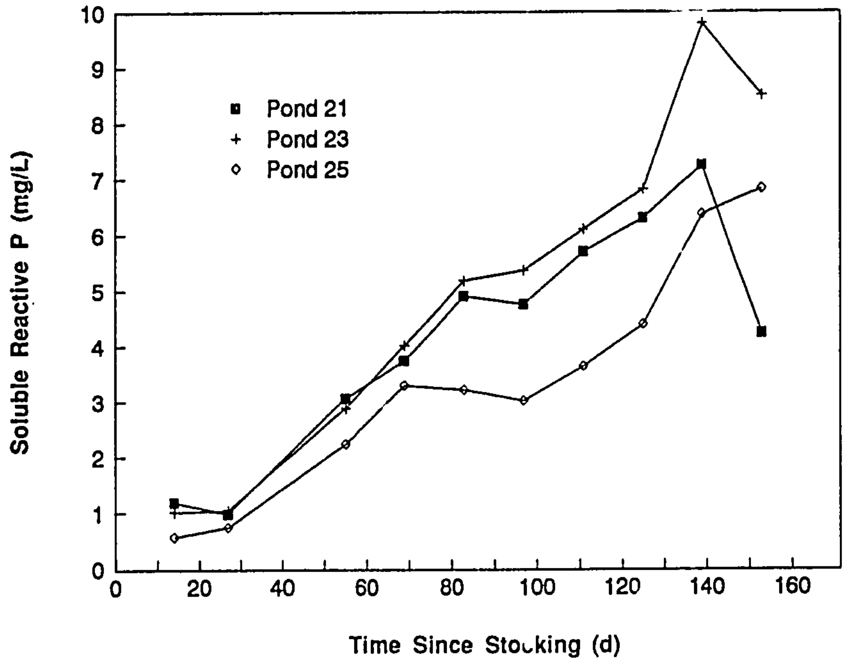

Figure 9. Variations of soluble reactive P concentrations (mg/L) over time (d) in ponds receiving chicken manure, urea, and TSP at the Huay Luang Fisheries Station.

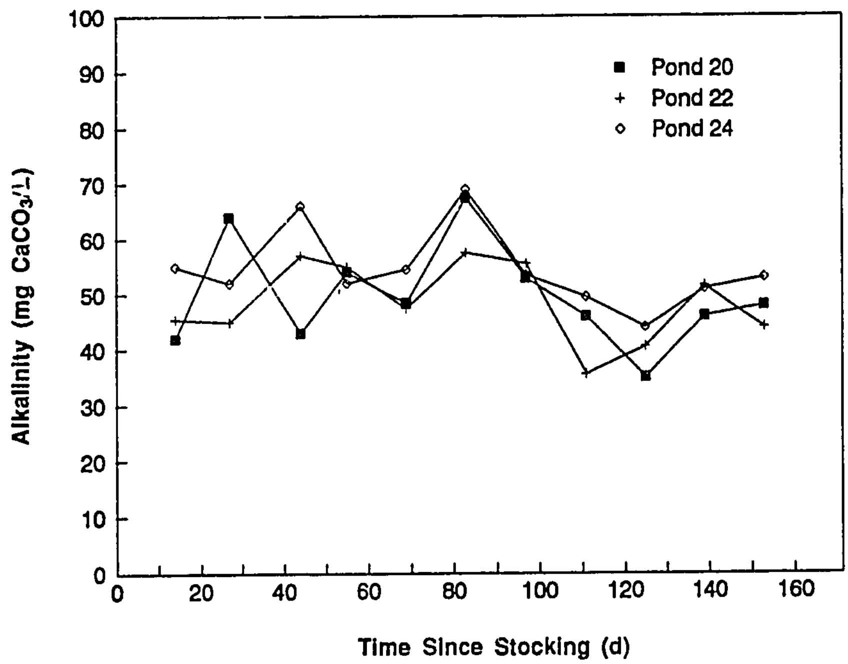

Figure 10. Variations of total alkalinity concentrations (mg $CaCO_3$/L) over time (d) in non-manured ponds receiving only inorganic fertilizers (urea and TSP) at the Huay Luang Fisheries Station.

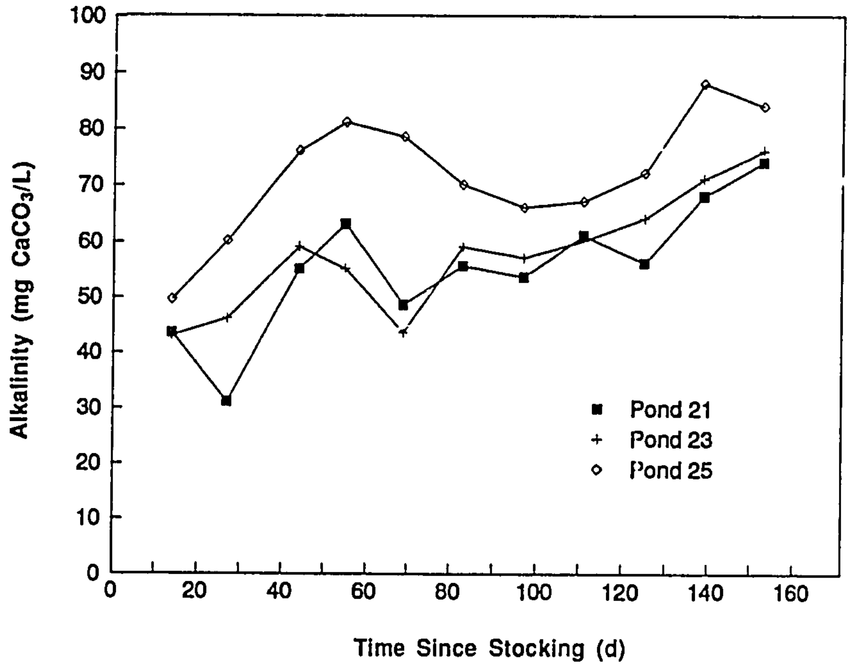

Figure 11. Variations of total alkalinity concentrations (mg $CaCO_3$/L) over time (d) in ponds receiving chicken manure, urea, and TSP at the Huay Luang Fisheries Station.

Table 13. Fish harvest data for the growout field trial at the Udom Patana Farm (29 July 1991 to 17 January 1992). Percent survival not deter-mined because of recruitment. GFY = gross fish yield.

Pond	Harvest (g/fish)	GFY (kg/pond)	GFY (kg/ha/d)		GFY (kg/ha/yr)
51	175	2,233	27.6		10,091
56	141	2,000	21.5		7,863
57	152	1,651	22.2		8,108
				Mean	8,687

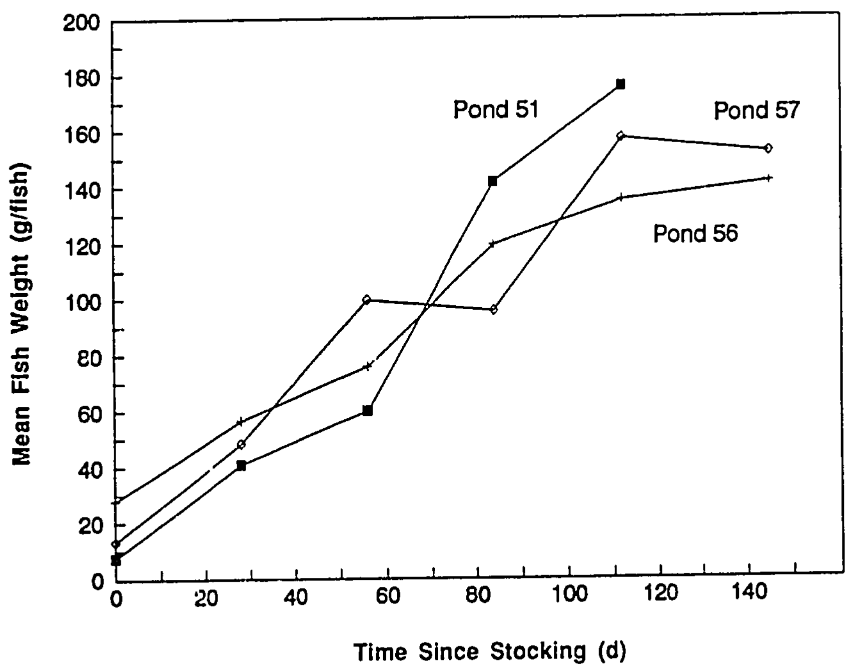

Figure 12. Growth of tilapia from three ponds at the Udom Patana Farm.

larger fish came from Pond 51, which had the lowest stocking rate, even though the growout period was more than two weeks shorter than that of the other two ponds (Table 5).

The input economic efficiency was the best at Udorn Patana with an average of THB 5.4 per kg fish compared to the other two sites (Table 14). One possible reason is that the ponds at Udorn Patana are considerably older (>5 years) than at Huay Luang or Romsai Farm. Older ponds tend to be more productive (see survey below), an observation also suggested by the harvest results at Romsai Farm described above. Increased fertilization efficiency in ponds with greater historical fertilization is discussed in detail by Knud-Hansen (1992).

Field measurements gave a temperature range from 15.0°C to 37.0°C and average Secchi depths of 20 to 23 cm for the three ponds (Table 15). Similar to the other two growout experiments, low DO was never a problem during the experiment. The lowest mean DO at dawn was 2.9 mg/L, and all ponds together averaged 3.5 mg/L.

Water quality varied among the three ponds with few notable trends. The highest tilapia yield (>10,000 kg/ha/yr) was found in the pond with the highest NPP (Pond 51 – Tables 14 and 15, respectively). But mean pond SRP and ammonia-N concentrations varied between about 0.3 and 2.2 mg/L with no relationship with either fish yield or NPP (Table 15). Mean pond alkalinity ranged from 72 to 103 mg $CaCO_3$/L, with the lowest mean in the pond which had the highest productivity (Pond 51). This result is consistent with Knud-Hansen et al. (1993) which suggests that alkalinities of about 70 mg $CaCO_3$/L or greater are sufficient to satisfy algal carbon

Table 14. Economic analysis based on total ponds inputs and gross fish yield (GFY) for the growout field trials at the Udorn Patana Farm (29 July 1991 to 17 January 1992). US$1 ≈ 25 Baht; Baht = Bt; Bt/kg = Thai Baht/kg harvested fish.

Pond	Urea (kg/pond)	Inputs TSP (kg/pond)	Cost (Bt/pond)	GFY (kg/pond)	Cost (Bt/kg)
51	751.8	751.8	10,525	2,233	4.7
56	847.3	847.3	11,862	2,000	5.9
57	683.5	683.5	9,569	1,651	5.8
Total	2,282.6	2,282.6	31,956	5,884	Mean 5.4

cost of urea: Bt 250/50 kg
cost of TSP: Bt 450/50 kg

Table 15. Mean water quality and field data for the growout field trial at the Udorn Patana Farm (29 July 1991 to 17 January 1992). NPP = net primary productivity.

Pond	Total Alkalinity (mg $CaCO_3$/L)	SRP (mg/L)	Total Ammonia-N (mg/L)	NPP (O_2/10 hr)	Dawn DO (mg/L)	Secchi depth (cm)
51	72	0.59	2.19	9.1	3.7	23
56	73	1.83	1.09	7.8	4.0	23
57	103	1.44	0.34	7.7	2.9	20

requirements even in highly productive fish ponds. Since all ponds were fertilized at the same rate and all demonstrated N and P accumulation, differences in algal and fish productivities may have been due to differences in inorganic turbidity (Knud-Hansen and Batterson, unpublished data).

Surveys

Bioassay: Of the twenty farm ponds around Udorn Thani surveyed for algal nutrient limitation (Figure 13), N and P were equally deficient in 15 ponds (75%) relative to algal needs (O'Brien 1974) (Figure 14). Water chemistry analyses supported bioassay results. The two ponds limited by P had ammonia-N concentrations >0.7 mg/L and SRP concentrations >0.05 mg/L (Table 16). Similarly, the one N-limited pond where nutrients were measured showed 0.25 mg/L ammonia-N and a SRP concentration of 2.12 mg/L. Ponds exhibiting N and P limitation were relatively deficient in both ammonia-N and SRP. Interestingly, seven of the ponds with N and P limitation had alkalinities of 22 to 47 mg $CaCO_3$/L, values low enough to suspect carbon limitation (Knud-Hansen et al. 1993). It could be that the algal populations in the pond water incubated for algal assays were too small to exhaust available inorganic carbon. In more productive ponds these alkalinities may be sufficiently low to limit carbon availability (Guttman 1992).

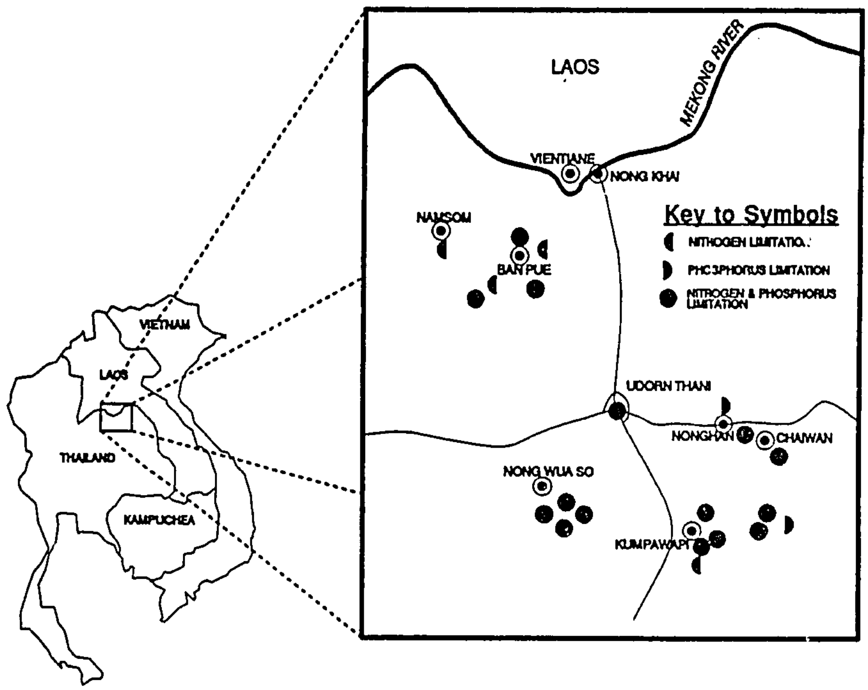

Figure 13. Map of the Udorn Thani area showing the location and nutrient limitations of ponds surveyed using the algal bioassay procedure.

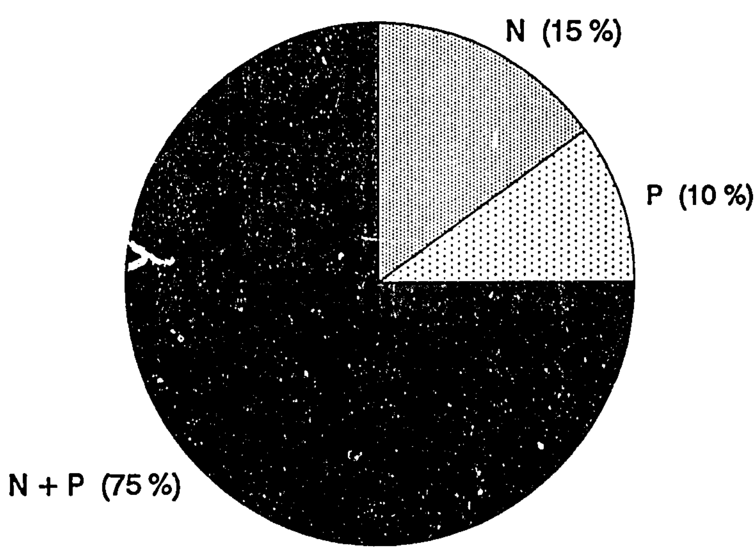

Figure 14. Diagram showing the percentages of ponds surveyed in the Udorn Thani area found to be limited by nitrogen (N), and/or phosphorus (P).

Table 16. Algal bioassay survey for AIT outreach farmers' aquaculture ponds around Udorn Thani (October-November 1991).

		Nutrient Concentration		
Farmer/village (name or AIT code)	Limiting Nutrient	Ammonia-N (mg/L)	SRP (mg/L)	Total Alkalinity (mg $CaCO_3$/L)
Kamloh	P	0.75	0.03	44
Nong Ham	P	1.10	0.04	87
Pho Sa Nga	N	0.25	2.12	70
Ban jarenchai	N			80
Dong way	N			22
2516	N + P	0.00	0.00	65
Ban Pue	N + P	0.05	0.03	28
Chaiwan	N + P	0.05	0.03	22
2107/2	N + P	0.07	0.00	43
Pho Sa Nga	N + P	0.08	0.00	47
Kam Muang	N + P	0.09	0.00	25
Huai Yang	N + P	0.09	0.01	113
2103	N + P	0.11	0.00	100
Pho Sa Nga	N + P	0.18	0.00	177
2107/1	N + P	0.25	0.00	55
Pho Sa Nga	N + P	0.41	0.00	47
Ban Muong	N + P			199
Ban Muang pan	N + P			36
Ban Nalom	N + P			158
Ban Nongwaeng	N + P			58

Carbon: The survey of 17 farmers showed that about 83% of the total number of ponds (34) were rainfed, with klong (canal) and groundwater contributing to the remainder of the ponds (Figure 15). Twenty-two of the ponds had been previously drained, while only four of the ponds had ever had mud removed.

Pond age ranged from six months to twenty years. Interestingly, older ponds seemed to have less alkalinity than newer ponds (Figure 16). Using Spearman's rank correlation coefficient (r_S), however, this relationship was not significant ($r_S = 0.27$, $P > 0.1$). There was a significant relationship between pond age and color ($r_S = 0.36$, $P < 0.05$), with older ponds tending to be greener (Figure 17). This result was consistent with results from Romsai Farm, where the four older ponds yielded larger fish than the two newer ponds (ponds B16 and B17).

An objective of the carbon survey was to look at the range of alkalinities in ponds of Udorn Thani province to determine the extent to which carbon limitation may be a potential problem in tilapia production. Figure 18 shows that most of the ponds sampled had alkalinities of >50 mg $CaCO_3$/L. A second objective was to establish the relationship between alkalinity and conductivity in order to provide a quick estimation of alkalinity using a conductivity meter. When only upland ponds (i.e., not in valleys) were examined, there was a strong linear relationship between

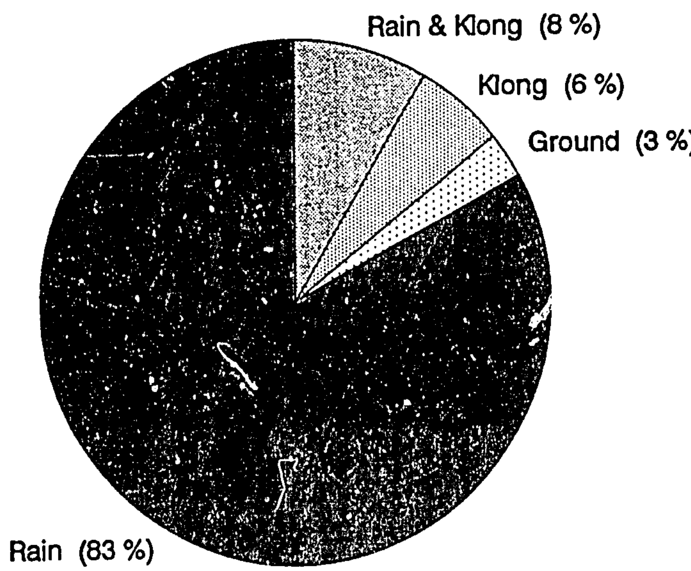

Figure 15. Diagram showing the percentages of ponds surveyed in the Udorn Thani area receiving rainwater, klong (canal) water, or groundwater as their source of water.

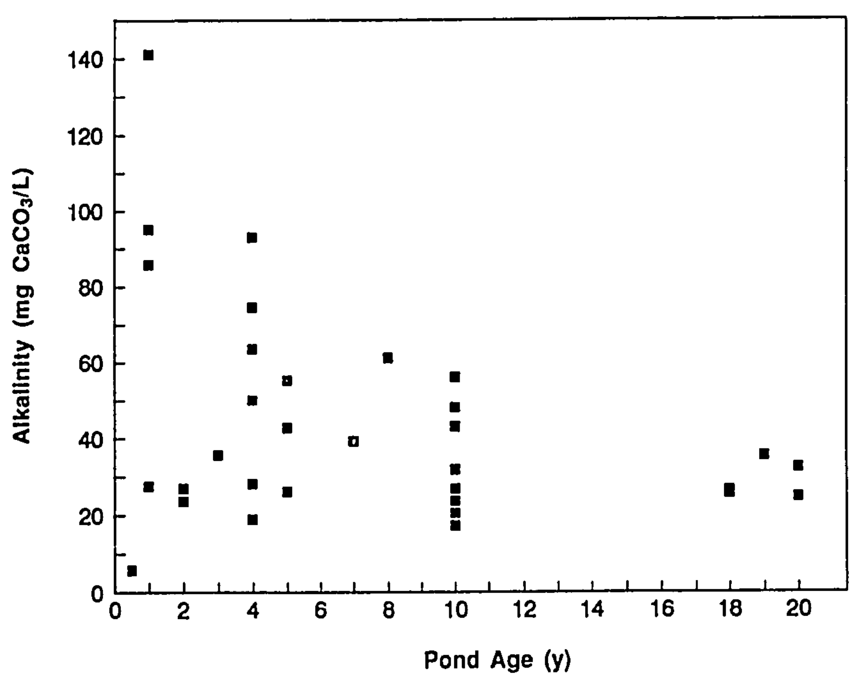

Figure 16. Relationship between pond age (yr) and alkalinity (mg $CaCO_3$/L) in the carbon survey of ponds in the Udorn Thani area.

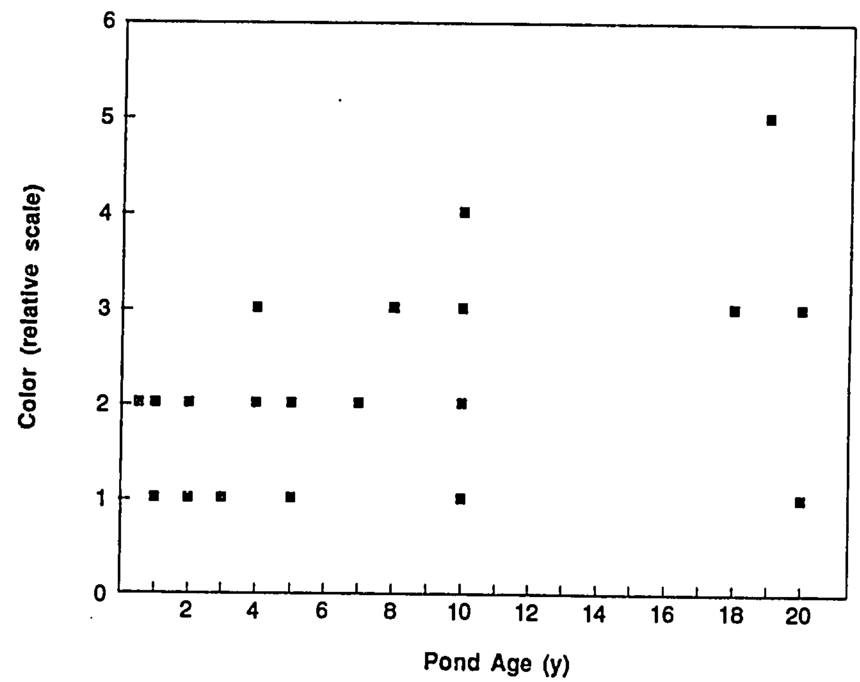

Figure 17. Relationship between pond age (yr) and relative pond color found in the carbon survey of ponds in the Udorn Thani area [color chart scaled from brown (1) to deep green (6)].

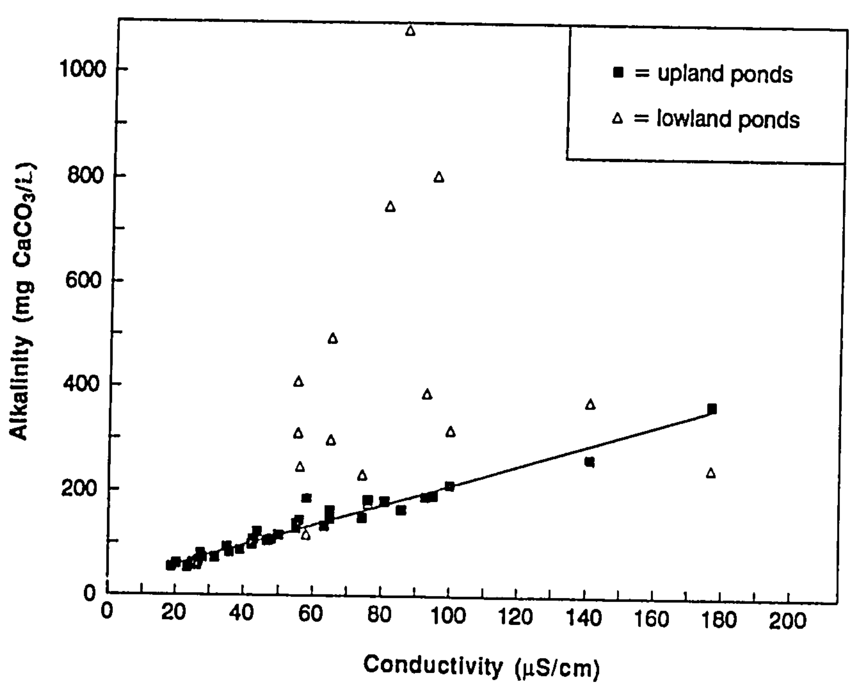

Figure 18. Relationship between conductivity (μS/cm) and alkalinity (mg $CaCO_3$/L) in the carbon survey of ponds in the Udorn Thani area.

conductivity and alkalinity ($r^2 = 0.96$, $P < 0.001$, where alkalinity (as mg $CaCO_3/L$) = 19.3 + 1.92 x conductivity (as $\mu S/cm$)). This relationship totally failed for ponds located in valley areas where salts from human activities may accumulate. So the alkalinity/conductivity relationship may be quite useful, but only for upland ponds.

Anticipated Benefits

The growout trial results presented here supported previous investigations demonstrating the importance of algal-based pathways for tilapia production (Almazan and Boyd 1978, Colman and Edwards 1987, Schroeder et al. 1990, Knud-Hansen et al. 1993). Yields at all three sites consistently averaged around 8,000 kg/ha/yr at a fertilizer input cost of about THB 6/kg harvested fish. Since farm-gate prices for tilapia are around THB 15 to 20/kg, using only TSP and urea to fertilize ponds and stimulate algal production proved economical. Other noted benefits were the relative ease of application and reduced transportation costs as compared to manures or pelleted feeds, and the consistently high DO concentrations (>2.5 mg/L) at dawn even in highly productive green ponds.

The multiple harvesting and stocking trial at Romsai Farm revealed two important management concerns not discussed by Knud-Hansen and Lin (in press). One is the difficulty of harvesting adult tilapia from a partially filled pond, and the other is the importance of keeping predator fish out of ponds. Just a few large snakehead can effectively eliminate additionally stocked tilapia if the fingerlings are not sufficiently large (Kaewpaitoon and Edwards, unpublished data).

The bioassay survey demonstrated the importance of fertilizing farm ponds in Northeast Thailand with both N and P. The correlation between bioassay results and ambient nutrient concentrations demonstrates the utility of the bioassay survey as an extension or fisheries station tool for assisting local farmers in implementing nutrient-efficient fertilization strategies. First suggested as a management tool by Yusoff and McNabb (1989), algal bioassays are both conceptually and technically simple to use. A growout trial using algal bioassays as the only indicator of fertilization requirements improved input cost efficiency to less than THB 4/kg harvested tilapia (Hopkins and Knud-Hansen, submitted).

Perhaps the most important benefit of the carbon survey was the empirically determined linear relationship between alkalinity and conductivity for upland farm ponds. Because conductivity can be easily determined either on site or back at a fisheries station, upland farmers can immediately tell if their ponds are deficient in alkalinity. Identification of ponds requiring additional alkalinity (e.g., lime) will reduce the likelihood of carbon limitation and therefore promote more efficient utilization of nitrogen and phosphorus inputs.

Literature Cited

Almazan, G., and C.E. Boyd. 1978. Plankton production and tilapia yields in ponds. Aquaculture 15:75-77.

APHA (American Public Health Association). 1985. Standard Methods for Examination of Water and Wastewater. 16th ed., American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C. 1268 pp.

Colman, J., and P. Edwards. 1987. Feeding pathways and environmental constraints in waste-fed aquaculture: Balance and optimization. Pages 240-281 *in* D.J.W. Moriarty and R.S.V. Pullin, editors. Detritus and microbial ecology in aquaculture. ICLARM Conference Proceedings 14, International Center for Living Aquatic Resources Management, Manila, Philippines.

Edwards, P., K.E. Weber, E.W. McCoy, C. Chantachaeng, C. Pacharaprakiti, K. Kaewpaitoon, and S. Nitsmer. 1983. Small-scale fishery project in Pathumthani Province, Central Thailand: A socio-economic and technological assessment of status and potential. AIT Research Report No. 158, Asian Institute of Technology, Bangkok, Thailand. 256 pp.

Guttman, H. 1992. Assessment of nutrient limitation in fertilized fish ponds by algal assay. Page 56 *in* H. Egna, M. McNamara, and N. Weidner. Ninth Annual Administrative Report. Pond Dynamics/Aquaculture CRSP. Oregon State University, Corvallis, Oregon.

Hall, C.A.S., and R. Moll. 1975. Methods of assessing aquatic primary productivity. Pages 19-53 *in* H. Leith and R.H. Whittaker, editors. Primary Productivity of the Biosphere. R.H. Springer-Verlag, New York.

Hopkins, K., and C.F. Knud-Hansen. Submitted. Comparison of three fertilization strategies: algal assay, nutrient concentration, and empirically determined constant rate. Journal of the World Aquaculture Society.

Kaewpaitoon, K., and P. Edwards. Unpublished manuscript. Live tilapia (*Oreochromis niloticus*) as forage fish for snakehead (*Channa striata*).

Knud-Hansen, C.F. 1992. Pond history as a source of error in fish culture experiments: A quantitative assessment using covariate analysis. Aquaculture 105:21-36.

Knud-Hansen, C.F., T.R. Batterson, and C.D. McNabb. 1993. The role of chicken manure in the production of Nile tilapia (*Oreochromis niloticus*). Aquaculture and Fisheries Management 24:483-493.

Knud-Hansen, C.F., and C.K. Lin. In press. Strategies for stocking Nile tilapia (*Oreochromis niloticus*) in fertilized ponds. Proceedings of the Third International Symposium on Tilapia in Aquaculture held in Abidjan, Côte d'Ivoire from 11-16 November 1991.

Manugistics, Inc. 1992. Stratigraphics statistical software. Manugistics, Inc. Rockville, Maryland.

McNabb, C.D., T.R. Batterson, B.J. Premo, C.F. Knud-Hansen, H.M. Eidman, C.K. Lin, K. Jaiyen, J.E. Hanson, and R. Chuenpagdee. 1990. Managing fertilizers for fish yield in tropical ponds in asia. Pages 169-172 in R. Hirano and I. Hanyu, editors. Proceedings of the Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

O'Brien, J.W. 1974. T he dynamics of nutrient limitation of phytoplankton algae: A model reconsidered. Ecology 55:135-141.

Schroeder, G.L., G. Wohlfarth, A. Alkon, A. Halevy, and H. Krueger. 1990. The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219-230.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14:799-801.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics, Second Edition. McGraw-Hill, New York. 633 pp.

Yusoff, F.M., and C.D. McNabb. 1989. Effects of nutrient availability on primary productivity and fish production in fertilized tropical ponds. Aquaculture 78:303-319.

Nitrogen Requirements for Maximum Fish Production in Rwandan Ponds

Work Plan 6, Experiment 2

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Introduction and Objectives

Nutrient input rates and resulting fish production have been lower in Rwandan ponds than in ponds in other Pond Dynamics/Aquaculture CRSP countries such as Thailand and Honduras. Maximum primary and fish production, as well as the nutrient levels required to achieve maximum production in Rwandan ponds, have not yet been determined for Rwanda. This experiment was designed to determine maximum primary and fish production at Rwasave Station (1625 m elevation) when neither nitrogen, phosphorus, nor inorganic carbon were limiting.

Materials and Methods

Sixteen 660-m^2 ponds at Rwasave Station were stocked in April, 1993, with three *Oreochromis niloticus* juveniles (3/m^2) from a population having a sex ratio of six males to four females. Starting ten days before fish stocking, nitrogen (N) was added weekly as urea at rates of 5.1, 3.8, 2.4, and 1.0 kg N/ha/d; phosphorus was added as triple superphosphate (TSP) at a 1:1 nitrogen:phosphorus ratio. Four replicates were used for each treatment in this 153-day study.

Fish were sampled monthly. Pond dissolved oxygen (DO), temperature, pH, total ammonia nitrogen (TAN), and secchi disk visibility were measured weekly, and chlorophyll *a* was measured every two weeks. Other measurements followed standard CRSP protocol as described in the Work Plan. Total alkalinity was maintained above 50 mg/L as $CaCO_3$ by frequent applications of agricultural limestone.

Data were analyzed by regression and analysis of variance. Significant differences between treatment means were determined with the Newman-Keuls test.

Results

Dissolved orthophosphate remained between 0.6 and 7.9 mg/L even after lime applications. Mean total ammonia nitrogen (TAN) values were significantly different among treatments (Table 1). TAN values were variable during the study but were proportional to urea input levels; TAN values remained high throughout the experiment in all ponds except those that received the lowest nitrogen input. Individual pond TAN concentrations reached as high as 7.3 mg/L; the unionized portion reached as high as 2.1 mg/L on afternoons with high pH during the final month of the experiment. TAN did not accumulate in ponds during the experiment (Figure 1). Although a urea-N disappearance rate of 3.5 kg/ha/d has been reported for aquarium experiments conducted at warmer temperatures (Knud-Hansen and Pautong 1993), some accumulation was expected in the cooler temperatures at Rwasave Station (<22°C) at the high fertilization levels used in this experiment. Knud-Hansen and Pautong (1993) suggested that contact with bacteria-rich sediments may further increase decomposition rates.

Mean uncorrected chlorophyll *a* was significantly greater for ponds at the two highest input rates than for ponds at the lowest input rate (Table 1). Polynomial regression of chlorophyll *a* against TAN (r^2=0.62) suggests that increases in TAN concentrations above about 1.5 mg/L were not accompanied by increased chlorophyll *a* concentrations (Figure 2).

Table 1. Total ammonia nitrogen, morning surface dissolved oxygen and corrected and uncorrected chlorophyll *a* by treatment for ponds fertilized weekly with urea and TSP at nitrogen input rates as shown and phosphorus at an N:P ratio of 1:1.

Treatment (kg N/ha/d)	Total ammonia (mg/L)	Dissolved oxygen (mg/L)	Chlorophyll *a* (mg/m^3)	Corr. chlorophyll *a* (mg/m^3)
5.1	2.5a*	2.3a*	351a*	270a*
3.8	1.3b	3.2b	349a	270a
2.4	0.8c	4.5c	313ab	253a
1.0	0.4d	6.5d	252b	215a

* Means followed by the same letter are not different by Newman-Keuls test ($p<0.05$).

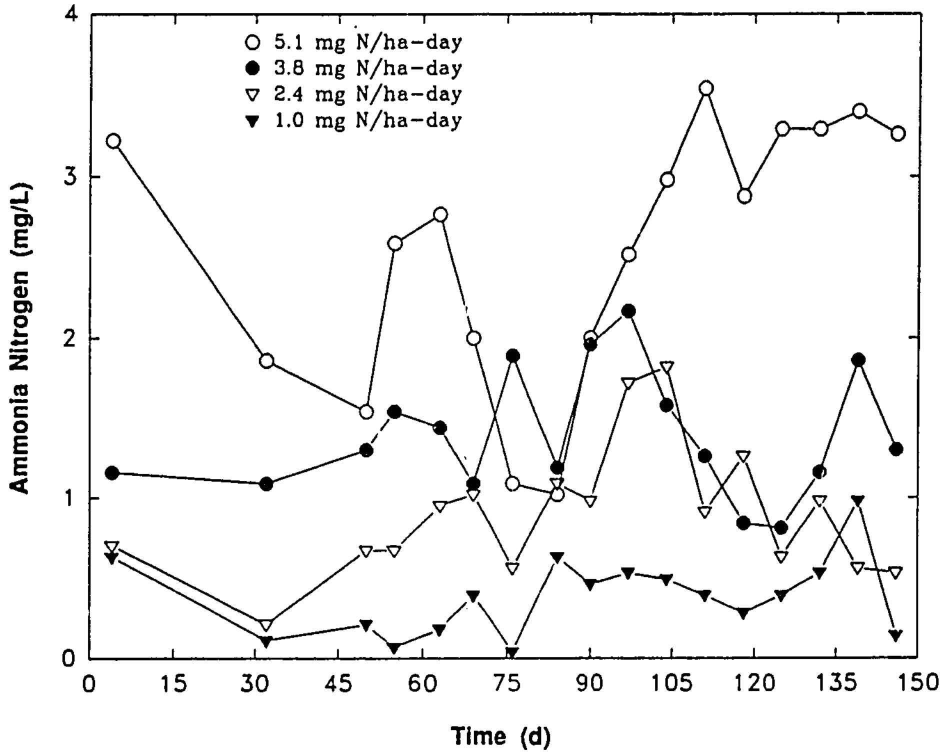

Figure 1. Concentration (mg/L) of ammonia through time in ponds receiving 5.1, 3.8, 2.4, or 1.0 kg N/ha/d.

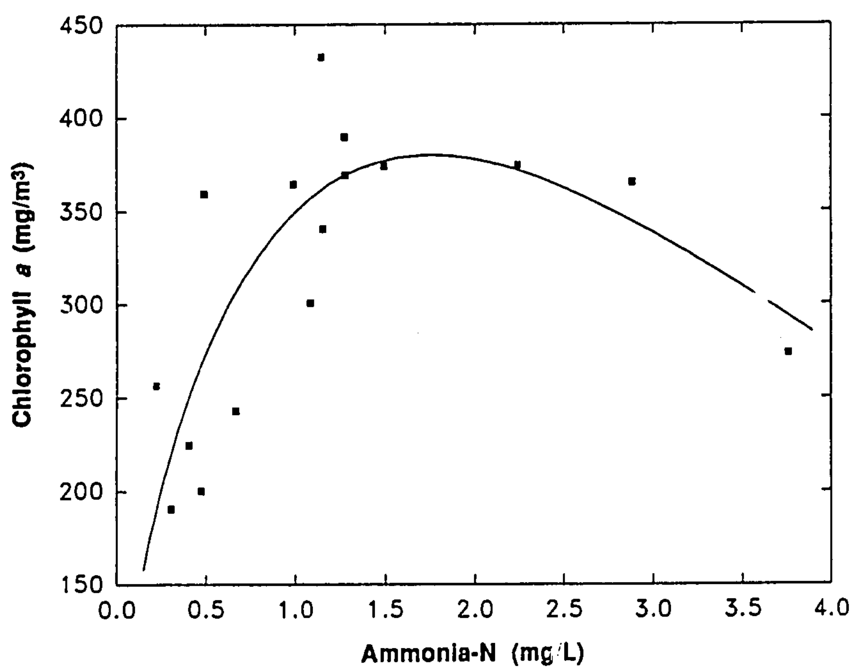

Figure 2. Regression of uncorrected chlorophyll *a* concentrations (mg/m^3) versus ammonia concentrations (mg/L). $r^2 = 0.64$.

Linear correlation of chlorophyll *a* and fish growth was poor (r^2=0.02). Fish growth was depressed at the highest nitrogen input rates even though chlorophyll levels were high. The growth rate of fish in ponds receiving 3.8 kg N/ha/d was significantly greater ($P<0.10$) than that of fish in ponds receiving 1.0 kg N/ha/d, but average growth declined at the highest fertilization level (Table 2). Although there were no significant differences in net fish production among treatments, the greatest annualized net fish production (3850 kg/ha/yr) occurred in ponds receiving the second highest input rate, 3.8 kg N/ha/d.

Depression of tilapia growth and production was attributed to combined stresses resulting from exposure to high unionized ammonia and low dissolved oxygen (DO) concentrations. The maximum unionized ammonia levels recorded ranged from 1.7 to 2.1 mg/L, and occurred at both low and high input levels. These concentrations are assumed to have severely depressed growth (Abdalla et al. 1992). Mean morning DO (measured 25 cm below the surface) declined significantly with increasing nutrient inputs (Table 1), but did not appear to affect survival. Survival was not significantly different between treatments and ranged from 81% to 96% in all ponds except one of those receiving 2.4 kg N/ha/d, in which survival was 73%.

Net profits, calculated as the estimated market value for net fish production minus the costs of urea and TSP, were negative for all fertilization levels (Table 2). Costs for nutrients, especially phosphorus, may be excessive for the higher input rates, because they were purposely added to be in excess. The lowest loss occurred at an intermediate input rate (2.4 kg N/ha/d). The greatest loss, 1740 RWF/are (US$ 1.00=RWF 140) occurred at the highest input (5.1 kg N/ha/d) nd the second greatest loss occurred at the lowest input rate (1.0 kg N/ha/d).

Anticipated Benefits

High rates of N and P fertilization (5.1 kg/ha/d) in Rwanda produced average corrected and uncorrected chlorophyll *a* concentrations of 270 and 351 mg/m^3, respectively, exceeding levels reported for similarly fertilized ponds in Thailand (Diana et al. 1993). Net fish production using the above described methods resulted in higher yields than were previously achieved in Rwanda when input combinations of grass, inorganic fertilizer, chicken litter, and cassava were used (Remy et al. 1993,

Table 2. Mean fish production, final fish weight, growth, and survival, as well as profit for four ponds per treatment.

Treatment (kg N/ha/d)	Net prod. (kg/ha/yr)	Final wt. (g)	Growth (g/d)	Survival (%)	Profit (RFW/are)[1]
5.1	2810a[2]	58.2ab[2]	0.27ab[2]	90a[2]	-1740
3.8	3850a	74.8a	0.38a	88a	-240
2.4	2840a	67.4ab	0.34ab	78a	-160
1.0	1260a	37.5b	0.15b	86a	-260

[1] US$ 1.00 = RWF 140.

[2] Values with the same letter are not different by Newman-Kuels test ($P<0.10$).

Rurangwa et al. 1992, Veverica et al. 1991a, 1991b, Veverica et al. 1990, Rurangwa et al. 1990). However, the highest average net fish production in this experiment (in the 3.8 kg N/ha/d treatment) was only 55% of the amount reported for Thailand (10 vs. 18 kg fish/ha/d).

Physiological stress from low DO and high ammonia may account for some reduced growth, but Diana el al. (1993) also cite these factors as constraints to growth in ponds in Thailand. Lower water temperatures are probably the primary cause of the lower growth rates observed in Rwanda. Gannam and Phillips (1993) reported that the growth of tilapia fed to satiation in a daily min-max temperature cycle of 16 to 24°C was only 41% as high as the growth in a temperature cycle of 20 to 28°C.

The growth rates observed in this study should not be considered maximal for Rwanda, however. Verheust et al. (1992), using the same stocking density as in this study, reported net production levels of 20 kg/ha/d, approximately twice the maximum of the present study (10 kg/ha/d), by fertilizing with green grass and chicken litter and feeding rice bran at 5 g/fish/d.

Our results suggest that under Rwandan environmental conditions, maximum chlorophyll densities and fish production in ponds receiving adequate P and no supplemental organic carbon are attained when inorganic nitrogen inputs do not exceed 4 kg/ha/d. Excessive costs and poor water quality associated with the use of urea and TSP in Rwanda indicate that the use of other inputs may result in higher biological production and economic returns.

Literature Cited

Abdalla, A.A.F., C. McNabb, C. Knud-Hansen, and T. Batterson. 1992. Growth of *Oreochromis niloticus* in the presence of unionized ammonia. Page 98 *in* H. Egna, M. McNamara, J. Bowman, and N. Weidner, editors. Ninth Annual Administrative Report. PD/A CRSP 1991. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Diana, J.S., C.K. Lin, and K. Jaiyen. 1993. Supplemental feeding of tilapia in fertilized ponds. Pages 58-63 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin, editors. Tenth Annual Administrative Report. PD/A CRSP 1992. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Gannam, A., and J. Phillips. 1993. Effect of temperature on growth of *Oreochromis niloticus*. Pages 136-143 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin, editors. Tenth Annual Administrative Report. PD/A CRSP 1992. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Knud-Hansen, C.F., and A.K. Pautong. 1993. On the role of urea in pond fertilization. Pages 173-184 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin, editors. Tenth Annual Administrative Report. PD/A CRSP 1992. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Remy, N., K.L. Veverica, T.J. Popma, and W.K. Seim. 1993. Supplemental dietary energy to enhance utilization of natural food organisms for growth by tilapia. Pages 48-51 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin, editors. Tenth Annual Administrative Report. PD/A CRSP 1992. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Rurangwa, E., K.L. Veverica, W.K. Seim, and T.J. Popma. 1992. On-farm production of mixed sex *Oreochromis niloticus* at different elevations. Pages 35-40 *in* H. Egna, M. McNamara, J. Bowman, and N. Weidner, editors. Ninth Annual Administrative Report. PD/A CRSP 1991. Office of International Research and Development. Oregon State University, Corvallis, Oregon.

Rurangwa, E., L. Verheust, and K. Veverica. 1990. Fertilizer input method influences pond productivity. Pages 25-26 *in* H.S. Egna, J. Bowman, and M. McNamara, editors. Seventh Annual Administrative Report. PD/A CRSP 1989. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Verheust, L., E. Rurangwa, and K.L. Veverica. 1992. Production and growth of supplementally fed Oreochromis niloticus males stocked at three densities in fertilized ponds. Pages 34-35 *in* H. Egna, M. McNamara, J. Bowman, and N. Weidner, editors. Ninth Annual Administrative Report. PD/A CRSP 1991. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Veverica K.L., T.J. Popma, A. Gatera, E. Karangwa, W.K. Seim, and R.A. Tubb. 1991a. The influence of composting regimes and stocking density on mixed-sex tilapia production. Pages 37-40 *in* H.S. Egna, J. Bowman, and M. McNamara, editors. Eighth Annual Administrative Report. PD/A CRSP 1990. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Veverica, K.L., T. Popma, P. Rwalinda, and W. Seim. 1991b. Production of *Oreochromis niloticus* as a function of organic matter application rates and supplementation with inorganic nitrogen and phosphorus fertilization. Pages 42-46 *in* H.S. Egna, J. Bowman, and M. McNamara, editors. Eighth Annual Administrative Report. PD/A CRSP 1990. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

erica, K.L., R. Rurangwa, L. Verheust, T. Popma, W. Seim, and R. Tubb. 1990. Tilapia production in Rwandan ponds is influence by composting method. Pages 24-25 *in* H.S. Egna, J. Bowman, and M. McNamara, editors. Seventh Annual Administrative Report. PD/A CRSP 1989. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Optimization of Gender Control Techniques for Tilapia

Work Plan 7, Study 4C1

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Introduction

Sex control in fish offers many potential commercial benefits, including enhanced growth, prevention of early maturation, production of large numbers of female broodstock, and culture of the sex with highest market value. Therefore, separation of the sexes may be a good strategy for optimizing efficiency in many aquaculture situations. However, secondary sex characteristics often do not appear until later in development, making segregation of the sexes labor intensive and inefficient in terms of achieving production goals (e.g., optimal growth). As a result, methods for generating monosex fish populations are in demand.

Sex control can be achieved by hormone therapy or genome modification. Although hormone therapy 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.

Objectives

1. To determine androgen binding characteristics of tilapia testicular cytosol.
2. To determine if short-term immersion of tilapia fry in 17α-methyltestosterone causes masculinization.
3. To determine if short-term immersion of tilapia fry in 17α-ethynylestradiol causes feminization.

Materials and Methods

Animals

Tilapia (*Oreochromis niloticus*), Ivory Coast strain, were obtained from Wayne Seim at Oregon State University, Corvallis, Oregon. For the binding site studies, fish were maintained in a 3.7-m circular tank with a recirculating water system. The temperature was maintained at 24 to 29°C. Water quality was monitored weekly. Fish were fed a commercial dry catfish diet *ad libitum.*

For steroid immersion studies, adult tilapia were placed in breeding families consisting of one male and four to six females. Each family was housed in a 208-L aquarium equipped with an undergravel filter and a power filter. The temperature was maintained at 27 to 30°C. Water quality was monitored weekly. Fish were fed a commercial dry trout diet *ad libitum*. After spawning, the female was removed to a separate 79-L aquarium until release of the fry occurred.

Receptor Studies

Tissue Collection and Processing. Mature male tilapia were killed by overdose of the anesthetic tricaine methansulfonate (20 mg/L) buffered with sodium bicarbonate. The gonads were dissected into ice-cold homogenization buffer (Tris-HCl 10 mM, EDTA 1 mM, Molybdic acid-disodium salt 20 mM, Monothioglycerol 12 mM and Glycerol 10% (v/v), pH = 7.4), weighed, and then homogenized with a motor-driven piston in 2x volumes of homogenization buffer. The homogenate was centrifuged at 1700 x *g* for 20 min at 4°C. The pellet was held on ice for extraction of the nuclear fraction and the supernatant was incubated with 0.5 volumes of 5.0% charcoal-0.5% dextran in homogenization buffer for 10 min on ice and then centrifuged at 1700 x *g* for 15 min to remove any endogenous steroids that could interfere with the binding assay. The resulting supernatant was then centrifuged at 100,000 x *g* for 1 h at 4°C to separate mitochondria and endoplasmic reticulum (pellet) from the cytoplasm (supernatant). The supernatant (cytosol) was stored frozen at -80°C. The pellet for the nuclear extraction was washed three times with wash buffer (Tris-HCl 10 mM, $MgCl_2$ 3 mM, Monothioglycerol 2 mM, and Sucrose 250 mM, pH = 7.5) and then the nuclei were extracted with extraction buffer (Tris-HCl 50 mM, EDTA 1 mM, Monothioglycerol 12 mM, 700 mM KCl, and glycerol 30% (v/v), pH = 7.5). The nuclear fraction was stored frozen at -80°C. All chemicals were obtained from Sigma Chemical Company (St. Louis, Missouri).

Binding Assays. Protein concentrations of cytosol and nuclear fractions were determined by the method of Bradford (1976). When possible, protein content was adjusted to 5 mg/mL in 0.150 mL. Mibolerone [17α-methyl-^{3}H] (^{3}H-Mb) and radioinert mibolerone (Mb) were obtained from Du Pont NEN (Boston, Massachusetts); other steroids were obtained from Sigma. All steroids were stored in stock solutions of 100% EtOH and dissolved in homogenization buffer for use in binding assays.

Equilibrium binding conditions were determined by incubating 0.150 mL of cytosol, 0.05 mL of 1 nM ^{3}H-Mb, and 0.05 mL of either buffer (Total Binding) or 500-fold excess radioinert mibolerone (Non-Specific Binding) at 17°C for varying amounts of time up to 24 h. At sampling times the reaction was stopped by placing the tubes on ice. The tubes were incubated with 0.5 mL of 2.5% charcoal-0.25% dextran in homogenization buffer for 10 min on ice and then centrifuged at 1700 x *g* for 20 min at 4°C to separate receptor-bound from free steroid. Samples were decanted into liquid scintillation cocktail and counted on a liquid scintillation counter (counts were corrected for machine efficiency). Specific binding was determined as the difference in radioactive counts between total and nonspecific binding.

Once equilibrium conditions were established, saturation binding characteristics were determined by incubating 0.150 mL of cytosol with 0.05 mL of ^{3}H-Mb at concentrations between 0.05 and 5.77 nM and 0.05 mL of either buffer or 500-fold excess radioinert mibolerone for 12 h at 17°C. Separation of bound and free mibolerone was the same as described above. The dissociation constant (K_d, a measure of the affinity of the binding site for the steroid) and the number of binding sites (B_{max}) were determined by nonlinear regression using the computer program Inplot 4.0 (Graph Pad Software Inc., San Diego, California).

Specificity of receptor binding was determined by competition assays in which 0.150 mL of cytosol was incubated with 1 nM ^{3}H-Mb in the presence of 1-, 10-, 100-, or 500-fold excess of a number of other steroids for 12 h at 17°C. Separation of bound and free mibolerone was the same as described above.

Immersion Studies. Stock solutions of 17α-methyltestosterone (MT) and 17α-ethynylestradiol (EE) were made up by dissolving dry steroid into 100% ethanol at a concentration of 10 mg/mL. Tilapia fry were collected after release from brooding females and separated into control and treatment groups. At 12, 24, 25, or 27 days after fertilization, treatment groups were immersed in 500 mL of tank water that contained 500 µg/L MT or EE for 2 h. Control groups were immersed in 500 mL of tank water that contained 25 µL of 100% ethanol for 2 h. Each group was placed in a separate tank for holding until identification of gender. Gender will be identified by examination of gonads using the aceto-carmine squash method (Guerrero and Shelton 1974) from a subsample of fish (the gender of the remaining fish will be identified by examination of the genital pore once external sex characters develop). A portion of each group will be maintained through maturity to assess the impact of steroid treatment on fertility.

Results

Binding Assays

Equilibrium binding for testicular cytosol at 17°C was reached by four hours, and remained stable for up to 16 h (Figure 1). Due to the stability of the binding, all subsequent incubations were performed for 12 h at 17°C. Incubation of cytosol with increasing amounts of ^{3}H-Mb displayed a characteristic saturation binding curve (Figure 2A). The K_d was 1.03 ± 0.11 nM (n=2) and the B_{max} was 5.65 ± 0.42 fmol/mg protein (n=2). The Scatchard plot of the transformed data was linear (Figure 2B), which suggests a single binding site.

The competition assay demonstrated that the binding site was specific for Mb (Figure 3). Of the other steroids examined, MT was the most effective at displacing ^{3}H-Mb binding, followed in order of potency by EE and then 5α-dihydrotestosterone. Estradiol and progesterone were ineffective at displacing ^{3}H-Mb.

Binding of ^{3}H-Mb could not be demonstrated in either ovarian cytosol or in nuclear fractions prepared from testes.

Immersion Studies

The immersion trials are still underway and the results will be forthcoming once the fish reach sufficient size for identification of gender.

Anticipated Benefits

We have demonstrated the presence of a high-affinity, low-capacity binding site for mibolerone in the testicular cytosol of adult tilapia. Mibolerone has been used successfully to masculinize tilapia (*O. aureus*) (Torrans et al. 1988). The presence of a gonadal binding site for this steroid in differentiated testes and the steroid specificity results are consistent with the idea that this binding site may be the receptor responsible for mediating the masculinizing effects of MT. The results of further

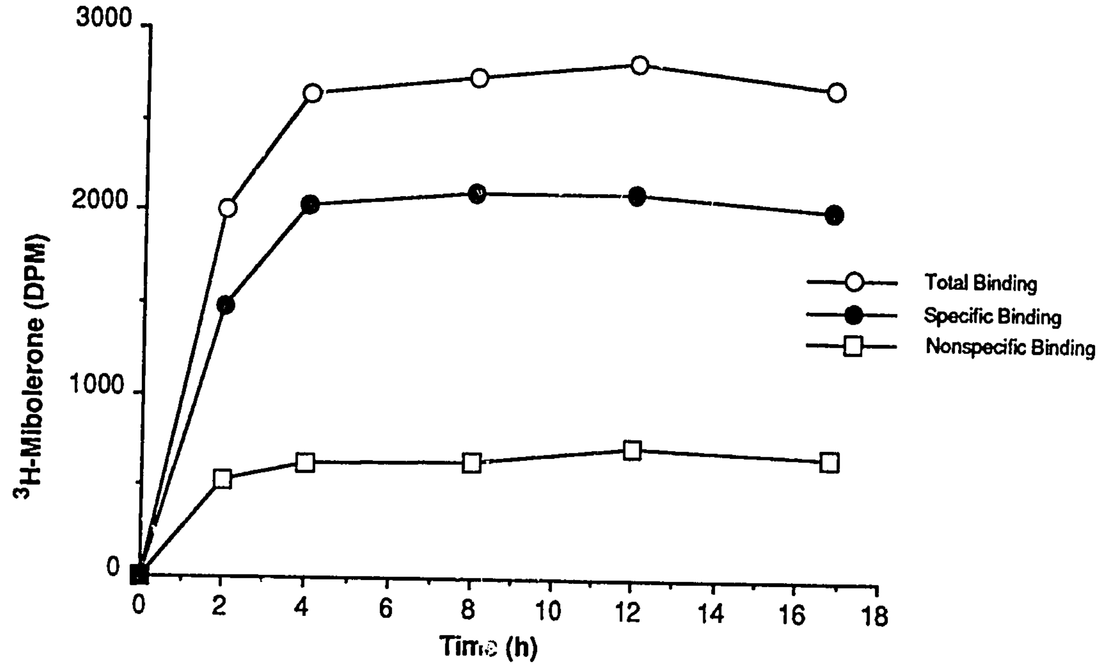

Figure 1. Time course ^{3}H-Mibolerone binding using tilapia testicular cytosol at 17°C. Each point represents the mean of three observations. DPM = distintegrations per minute.

competition assays utilizing other masculinizing steroids may support this contention. Interestingly, the feminizing steroid EE also competes for this binding site, which may indicate that feminization by steroid treatment occurs through competition for binding with some masculinizing chemical. The lack of binding in the ovaries is contrary to the results obtained in our laboratory with coho salmon (*Oncorhynchus kisutch*), but may have been due to the advanced maturity of the gonads, which caused dilution of the binding site below detection by large quantities of yolk protein.

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

Demonstration of the presence of a receptor for masculinizing steroids in the gonads of tilapia represents the first step towards understanding the mechanism of how steroids cause sex inversion. Understanding this process may lead to more efficient and safer techniques for controlling gender in important aquaculture species.

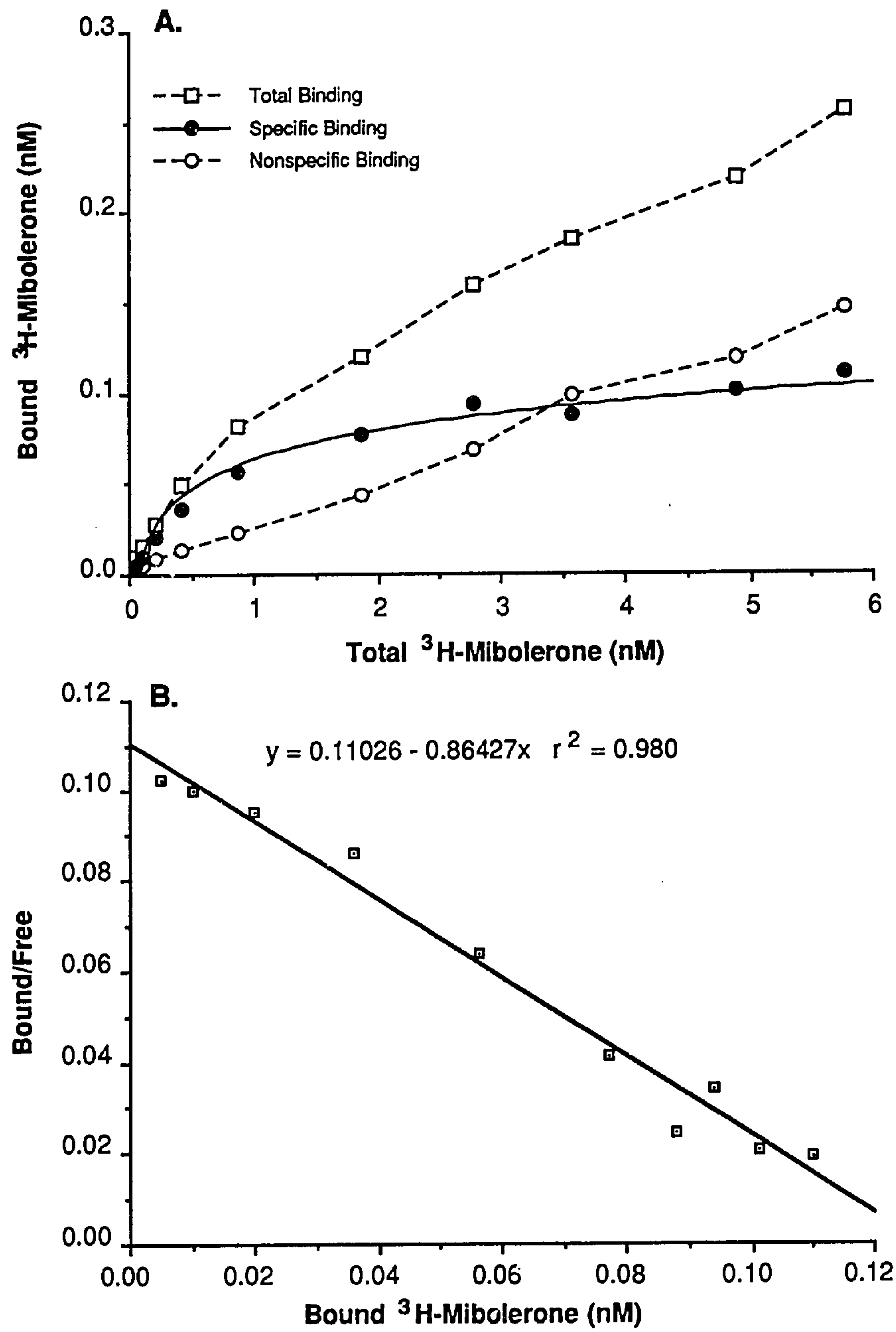

Figure 2. Representative saturation plot (A) of ^{3}H-Mibolerone binding in testicular cytosol of tilapia; and Scatchard plot (B) of the specific binding in (A). Each point represents the mean of three observations. K_d = 1.1 nM and B_{max} = 6.25 fmol/mg protein.

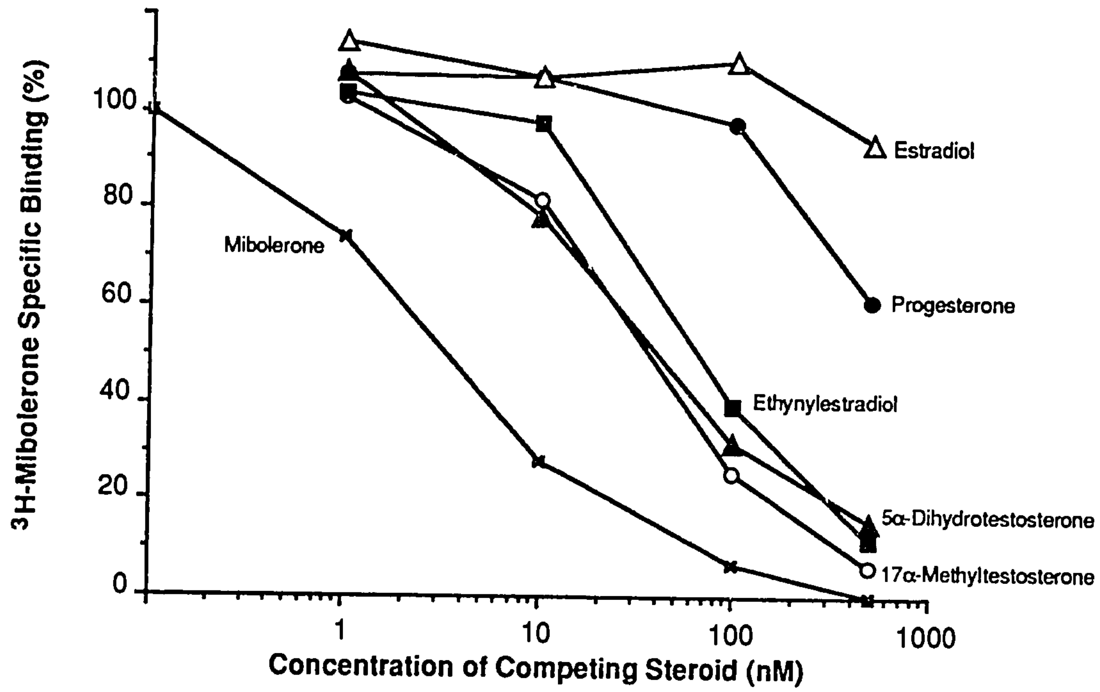

Figure 3. Specificity of ^{3}H-Mibolerone binding in testicular cytosol of tilapia. Data are expressed as percentage of ^{3}H-Mibolerone specifically bound. Each point is the mean of three observations.

Literature Cited

Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254.

Guerrero, R.D., and W.L. Shelton. 1974. An aceto-carmine squash method of sexing juvenile fishes. Progressive Fish-Culturist 36(1):56.

Torrans, L., F. Meriwether, F. Lowell, B. Wyatt, and P. Gwinup. 1988. Sex-reversal of *Oreochromis aureus* by immersion in mibolerone, a synthetic steroid. Journal of the World Aquaculture Society 19(3):97-102.

Progeny Testing to Identify "YY" Male Tilapia

Work Plan 7, Study 4A1

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Introduction

Sex determination in *Oreochromis niloticus* has been described as a XX female:XY male system, where the presence of Y establishes the male sex (Jalabert et al. 1974). Tave (1990) outlined a breeding program in which offspring of the YY genotype can be produced by sex reversing a genotypic male to a phenotypic female and crossing that female with a normal male. Fish of the YY genotype would give only Y sperm and produce only male offspring. There are no readily apparent genetic markers to identify possible XY females or YY males. These genotypes are recognized by the sex ratios of their progeny. An XY female crossed with a XY male should produce a 3:1 male:female sex ratio (XX+2XY+YY). Subsequently, the YY male produced from the XY female *x* XX male cross will sire 100% male offspring. This report describes a study to identify possible YY males in particular populations of tilapia. Results through 1 November 1993 are presented.

Materials and Methods

Ten groups of tilapia were produced by mating of 10 sex-reversed females with 10 normal males. A minimum of ten males from these groups (which had skewed sex ratios) were mated with normal XX females. Individual males were stocked with one or more females into 2 m^2 hapas suspended in outdoor concrete tanks. Each hapa was examined at 14-day intervals and the fry of individual females removed. Each spawn was reared separately in hapas or concrete tanks until the fish had a minimum length of 5 cm. One hundred fish of each spawn were preserved and their sex determined by removing the gonads and examining them microscopically. A total of 82 spawns were obtained and to date the sex ratio of 24 has been determined.

Results and Discussion

In populations with 75% males—i.e. with YY males present—mating of individual males should have resulted in offspring sex ratios of 50:50 or 100:0 with a 2-to-1 frequency. Among twenty-four sets of offspring that have been examined, however, only two have had sex ratios that were significantly skewed toward males. To date an adequate number of spawns from each parent population have not been examined to conclude whether a YY breeding program is feasible.

Anticipated Benefits

The development of a YY tilapia breeding program has the benefit of being able to produce male tilapia that has not been treated with androgenic hormones for consumption by the public. The results of the current study will also provide additional insight into the mechanisms of sex inheritance in tilapia.

Literature Cited

Jalabert, B., J. Moreau, D. Planquette, and R. Billiard. 1974. Determinisme du sexe chez *Tilapia macrochir* et *Tilapia nilotica*: action de la methyltestosterone dans l'alimentation des alevins sur la differentiation sexuelle; proportion des sexes dans la descendance des males "inverses." Annales de Biologie Animale Biochimie Biophysique 14:729-739.

Tave, D. 1990. Supermale tilapia. Aquaculture Magazine 16:69-71.

Growth-Promoting Action of 17α-Methyltestosterone on Two Species of Tilapia, *Oreochromis mossambicus* and *Oreochromis aureus*

Work Plan 7, Study 4B1

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Introduction

Hormones have been used with obvious success in agriculture to enhance livestock production. By contrast, there has been no systematic application of hormones in finfish aquaculture in the United States, due largely to the absence of basic information on the actions, effective doses, and clearance of hormones in fishes. While the situation is being redressed in salmonids and other cold-water species, there is little information on growth-promoting effects of hormones in warmwater fish species best suited to aquaculture in Hawaii, Egypt, and many warmer climates.

It is widely accepted that most fish species grow better on diets formulated with fishmeal than on those based on plant meal. Most fishmeals are derived from herring and herring-like fishes that are caught while in spawning aggregations. In this condition, ovaries and testes may comprise 70% and 30%, respectively, of the total weight of the fish. Such fishmeals contribute considerable amounts of anabolic steroids to commercial feeds. These integral hormones may account for the increased growth performance of fish on fishmeal-based diets as much or more than the quality of nutrients.

The results of our laboratory studies clearly show that 17α-methyltestosterone (MT), when administered as a feed additive, enhances growth performance in the tilapia, *Oreochromis mossambicus* (Kuwaye et al. 1992, and Howerton et al. 1992). Under laboratory conditions, MT consistently increases growth by 3 to 10 times that observed in control fish during the critical period between hatching and 50 g. This effect is related to dose and extends well beyond the effect of sex reversal. Tilapia treated continuously with MT outgrow sex-reversed fish by 30 to 40%. We have found that MT also substantially reduces the heterogeneity of growth among individual tilapia, thereby leading to a more uniformly sized and easily handled product. Our findings have shown that MT does not measurably affect proximate body composition.

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

Materials and Methods

A factorial design with replicate treatments was used for the experiments. The factors are species, sampling dates, and either dose of MT (Experiment I) or feeding regime of MT (Experiment II).

Methyltestosterone is administered orally as a feed additive. The dose levels in Experiment I are 0 (control), 1, 10, and 25 mg MT/kg of feed. The feeding regimes in Experiment II are control treatment (animals fed control feed over the entire experiment), continuous treatment (animals fed MT-treated feed over the entire experiment), delayed treatment (non-masculinizing–animals are fed control food for the first two months and then fed MT-treated food for the remainder of the experiment), and early treatment (masculinizing–animals fed MT-treated feed for the first two months and then fed control feed for the remainder of the experiment). Since male tilapia tend to grow faster than females, this study separates the growth-promoting effects of MT from its masculinizing effects.

Tilapia fry, *O. mossambicus* and *O. aureus*, of mixed sex were distributed equally among treatment groups at 100-300/group in 700-liter tanks (~1.0 m H by 1.0 m L by 0.7 m W). Animals are fed a commercial feed (Purina Trout Chow). 17α-methyltestosterone, administered in the feed, is dissolved in 20 mL of 95% ethanol and sprayed on the surface of 1 kg of the feed pellets. The control diet is sprayed with ethanol only. The ethanol is allowed to evaporate before the feed is used. Feed pellet size will range from ground feed to 10 mm to accommodate the size of the animals. Initially, fish will be fed at a rate of 3% of the body weight twice daily (6% total). This will be reduced in all tanks simultaneously to 2% twice daily as food consumption decreases.

Growth performance is evaluated from the bi-monthly length and weight measurement of all animals. Residual MT levels in the animals will be determined from blood, muscle, and liver samples collected at the end of the experiment. Feed conversion efficiency, condition factor, hepatosomatic index, and gonadosomatic index are also being evaluated.

Results

Data collection for fish weight, length, sex ratio, and gonadosomatic index for Experiment I are ongoing.

Anticipated Benefits

Insofar as hormones increase growth, they can significantly improve the economic viability of any aquaculture enterprise. Although the potential economic gain of MT has yet to be completely evaluated, the application of MT under laboratory conditions can reduce the time required to reach the critical 50 g size by 70%. The cost/benefit ratio for MT in tilapia culture appears to be similar to that of the grouper, *Epinepherus salmoides*, in which MT reduced the time required to reach market size (500 g) from 139 days to 93 days, a 33% reduction (Chua and Teng 1980). This reduced production costs by more than 25% even though treatment costs were increased by 12%.

Literature Cited

Chua, T.E., and S.K. Teng. 1980. Economic production of estuary grouper, *Epinephelus salmoides* Maxwell, reared in floating cages. Aquaculture 20:187-228.

Howerton, R.D., D.K. Okimoto, L.M.H. Helms, and E.G. Grau. 1992. The effect of orally administered 17α-methyltestosterone and triiodothyronine on growth and proximate body composition of seawater-adapted tilapia (*Oreochromis mossambicus*). Aquaculture and Fisheries Management 23:123-128.

Kuwaye, T.T., D.K. Okimoto, S.S. Shimoda, R.D. Howerton, Hao-Ren Lin, P.K.T. Pang, and E.G. Grau. 1992. Effect of 17α-methyltestosterone on the growth of the euryhaline tilapia, *Oreochromis mossambicus*, in fresh water and in seawater. Aquaculture 113:137-152.

Use of 17α-Methyltestosterone for Tilapia Sex Reversal: Participation in the 1993 Clinical Field Trial under U.S. Food and Drug Administration Investigational New Animal Drug Exemption (INAD 8479 C-002 and C-003)

Work Plan 7, Study 4A2

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Introduction

For more than a decade tilapia producers worldwide have relied upon the oral administration of 17α-methyltestosterone to newly hatched tilapia fry as the most cost-effective, efficient method for producing populations of male fish for growout. This technology has been utilized by commercial tilapia producers in the United States. Sex-reversed tilapia currently are being produced commercially in many countries for export to the United States and Europe. Commercial tilapia culture, based on sex-reversed fish, is predicted to continue to expand both overseas and in the United States. However, the use of 17α-methyltestosterone for sex reversal of newly hatched tilapia was not approved officially by the U.S. Food and Drug Administration (FDA), and, in fact, 17α-methyltestosterone was not approved for use in any animals.

Given the concerns for the safety of the human food supply, the U.S. FDA is initiating an inspection process to ensure that unapproved drugs and chemicals are not used in aquaculture. In an effort to secure FDA approval to use 17α-methyltestosterone for sex reversal of newly hatched tilapia fry, Auburn University, the American Tilapia Association, and a commercial feed producer applied for and were granted a "compassionate" Investigational New Animal Drug (INAD) exemption in order to collect data to support the New Animal Drug Application. One activity contemplated under this exemption was the implementation of clinical field trials by research institutions and commercial tilapia growers throughout the United States and overseas. In order to further this process, participation by scientists of the Egypt Project of the Pond Dynamics/Aquaculture Collaborative Research Support Program in the clinical field trials was planned.

Materials and Methods

Research was conducted at the Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt. Newly hatched tilapia fry 9 to 11 mm in length (approximately 7 to 12 days old) were stocked into 2-m^2 hapas suspended in a 3.8-m^2 circular fiberglass tank or directly into the tank. Fifty-liter aquaria were also used as experimental units. *Oreochromis niloticus* or *O. aureus* fry were used in the trials. Fry in hapas, tanks, or aquaria were randomly assigned to the control or androgen-treated feed treatments. Preliminary unreplicated trials were conducted. Fry were fed a powdered diet that contained 60 mg 17α-methyltestosterone/kg at a daily rate of 100 g of feed per kilogram of fish biomass. The same formulation was used for the androgen-treated and control feeds, except that the latter did not contain androgen. The daily feed ration was divided into at least three meals. The treatment duration was 28 days. Upon completion of treatment all fry were harvested and weighed en masse, and the average individual weight of a counted sample was determined gravimetrically. The total number harvested was estimated by dividing the total weight by the average individual weight. Fry were then stocked into 20-m^2 concrete tanks or earthen ponds of varying size for further growth. When the fingerlings had attained a size of approximately 5 g, a random sample of 100 fish per treatment was analyzed for sex by the aceto-carmine gonadal squash technique.

Results

Preliminary, unreplicated trials were initiated on 25 August 1993. To date, four trials each for *Oreochromis niloticus* and *O. aureus* have been initiated. Fingerlings from the first trial have completed treatment and were stocked into a concrete tank for further growth. Approximately 25,000 *O. niloticus* fry and 16,000 *O. aureus* fry have been harvested from reproduction ponds and submitted to androgen treatment. Data collection continues and data analyses will be initiated when all data become available. While preliminary trials will continue as long as water temperatures permit, the formal experiments described in the work plan likely will be postponed until next year.

Anticipated Benefits

Data to support the New Animal Drug Application process will be obtained. U.S. FDA approval of 17α-methyltestosterone for sex reversal of newly-hatched tilapia will greatly benefit commercial tilapia production in the United States and overseas.

Interaction of Plant/Snail Bioconversion by Grass Carp and Black Carp in Egyptian Fish Culture Ponds

Work Plan 7, Study 2C1

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Introduction

The bioconversion of plants by grass carp can be considered as a component of a polyculture system, but the consumption of snails by black carp must take into account the status of plants because of their role as food and cover for snails. Gastropod production may diminish by virtue of grass carp feeding on plants; thus, the relationship between snails and black carp is less predictable in the presence of grass carp. Abundant vegetation may provide protection for snails and their biomass may increase even in the presence of black carp, rather than being suppressed by predation. Thus, it is appropriate and necessary to consider the potential synergism between black and grass carps, and to consider this combination as a unit of polyculture rather than simply as the contributions of two individual species.

Materials and Methods

Ponds were prepared for this study in March and April of 1993; vegetation was cut to the soil surface and the ponds were filled. The ponds were stocked in late June. Four 4000 m^2 ponds were stocked (Table 1).

Results

Results will be based on the population status of the fish, snails, and plants at the time of harvest. Ponds will be cleaned and restocked immediately after harvest according to the plan of work for year two.

Anticipated Benefits

Late stocking lowered the effectiveness of grass carp control of plants and the impact of black carp predation on the snail populations. Nevertheless, the results will provide an improved basis for anticipating efficacy of the year-two studies.

Table 1. Data for grass carp and black carp stocked in June in four ponds with different levels of aquatic plant coverage.

Vegetation coverage	Grass carp		Black carp	
	Size class 1	Size class 2	Size class 1	Size class 2
Medium* (20-60%)				
Number/ha	379	286	115	229
Kg/ha	113	159	5	5
Average (g)	300	600	37	45
High (>60%)				
Number/ha	286	286	76	240
Kg/ha	270	355	3	9
Average (g)	900	1200	42	38

* Predominant macrophytes were *Typha* and/or *Phragmites*; some ponds had *Potamogeton* or *Ceratophyllum*, and a few were dominated by *Azolla*. A control pond was established at each level of vegetation.

Variation Of Soil Respiration With Humidity Using Pond Soil From Honduras

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Introduction

Field work indicated that a sharp rise in soil respiration after pond drainage was short lived. A couple of days of high respiration rates were followed by a steady decrease with time to a low steady state value. Soil moisture also decreased with time, which suggested that the respiration rate might be partially controlled by soil moisture. During one season, soil respiration increased following a couple days of light rains, thereby lending support to the hypothesis that soil moisture was a controlling factor in pond bottom respiration.

Boyd and Pippopinyo (in press) demonstrated in subsequent laboratory tests that respiration was directly related to soil moisture as well as organic matter content. Their tests indicated that the optimum soil moisture for respiration was between 12 and 20% for soils taken from east-central Alabama. However, field data at El Carao, Honduras, indicated that optimum productivity took place at soil moisture levels between 28 and 45%, or close to saturation.

Objective

The objective of this study was to examine the influence of soil humidity on soil respiration in laboratory microcosms, and to compare the results with field data.

Materials and Methods

Five-gallon plastic buckets with tight-fitting lids were used as microcosms for this study. Buckets had a bottom area of 603 cm^2. Soil was taken from the top 10 cm of fish pond bottom, sun dried, broken up by hand, and mixed well. On 15 October 1992, 3085 cm^3 of soil was added to each of 18 buckets (about 5 cm depth). Water was added to buckets in varying amounts according to Table 1.

Each treatment was replicated three times. Buckets were tightly covered except for one hour a day, when covers were removed to allow for gas exchange. Water was added once a week to all buckets to replace moisture lost to evaporation. Treatments 1 to 5 received 20, 18, 15, 10, and 5 mL, respectively, per application.

Table 1. Amounts of water added and mean initial humidities for the six treatments of the pond soil respiration study.

Treatment	Water added (mL)	Mean initial humidity (%)
T1	1425	34.5
T2	1200	29.9
T3	1000	28.5
T4	650	22.7
T5	300	16.7
T6	1600	35.0

Soil respiration was determined by capturing CO_2 using the methodology described by Boyd and Teichert-Coddington (in review), with one exception: the area of soil under incubation was increased. Three empty buckets were used as controls.

Soil for humidity determination was sampled from the buckets with a 3.8-cm diameter plastic tube and dried at 95°C for 24 hr. Humidity was determined four times. Organic carbon was determined at the beginning and end of the experiment. Soil samples were pulverized, passed through a 35-mesh screen, and analyzed by the Walkley-Black method, as described by Boyd and Tucker (1992). No correction factor was used.

It was intended that one of the soil treatments would be sufficiently saturated with water to reduce respiration. It was quickly obvious that this was not achieved with the first five treatments; therefore, Treatment 6 was started on day 20 with a higher quantity of water. An additional 300 mL of water was added on day 26 so that water could be seen just covering the soil surface. On day 26, 1100 mL of water was also added to the buckets of Treatment 5 so that soil humidity would be raised to values similar to that of Treatment 1. Measurements from Treatments 2, 3, and 4 were terminated on day 25. Treatment 1 was continued as a reference point for Treatments 5 and 6.

Results and Discussion

Soil humidity in each treatment is summarized in Table 2. As Figure 1 and Table 3 show, respiration decreased with diminishing soil moisture. Soil respiration and moisture were linearly correlated on each sampling date on which both measurements were taken (Figure 2). Slopes of the regression curves revealed that moisture level had a greater effect on respiration initially than it did later. For every unit rise in moisture, respiration increased 0.28 on the first sampling day, compared with an increase of 0.14 on the last day.

Respiration was highest on day four, and decreased to a generally steady level by day eight. Moisture levels were unchanging, so the initially high rates of respiration must have been generated from the decomposition of labile organic material. Boyd and Pippopinyo (in press) demonstrated similar effects in Alabama soils held at

Table 2. Initial, final, and mean soil humidity in buckets with soil added to 5 cm depth and wetted with varying quantities of water.

Treatment	Initial	Humidity (%) Final	Mean
T1	34.5	31.0	32.8
T2	29.9	28.8	29.4
T3	28.5	25.1	26.8
T4	22.7	19.6	21.2
T5 (Before day 26)	16.7	11.4	14.1
T5 (After day 26)	32.7	-	-
T6 (Before day 26)	35.0	33.6	34.3
T6 (After day 26)	37.5	-	-

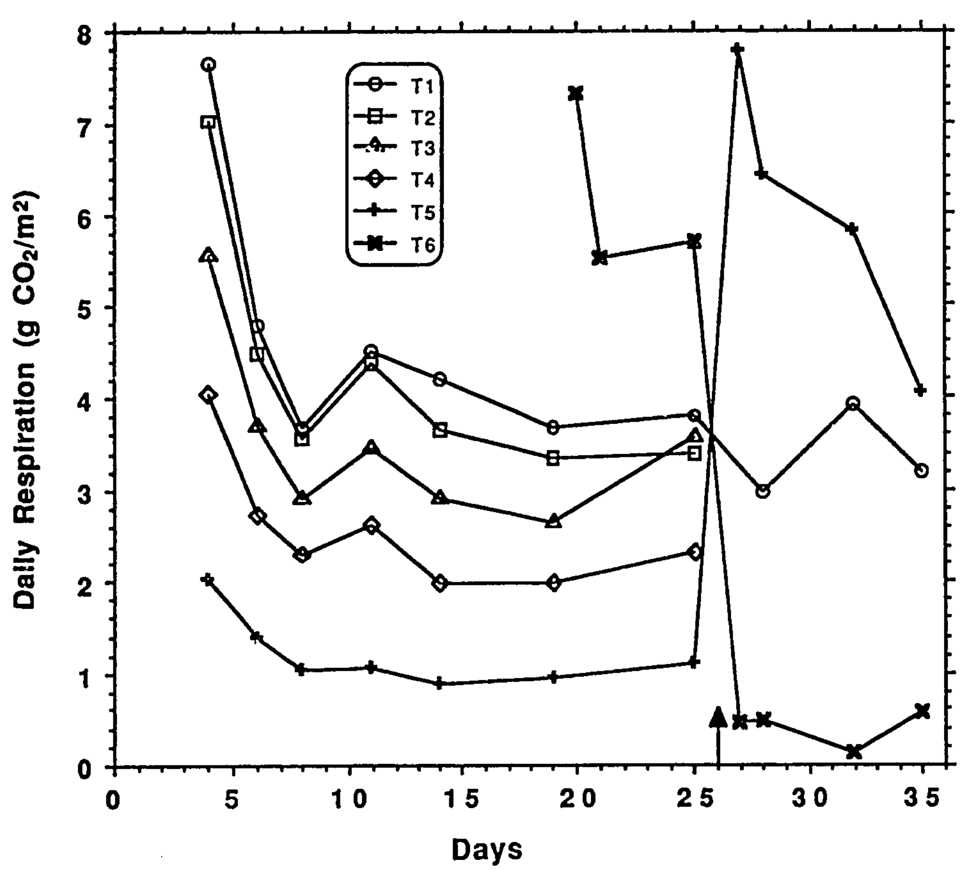

Figure 1. Mean daily soil respiration (g CO_2/m^2) at different soil humidity levels starting four days after water was added to soil. Mean soil humidity to day 25 was 32.8, 29.4, 26.8, 21.2, 14.1, and 34.3% for treatments 1 to 6, respectively. Arrow indicates when water was added to treatments 5 and 6 to raise soil humidity to 32.7 and 37.5%, respectively.

Table 3. Soil respiration in buckets filled to a depth of 5 cm with soil and various amounts water to establish humidities ranging from 35 (T1) to 17% (T5).

Treatment	Soil respiration (g $CO_2/m^2/d$) Days after filling												
	4	6	8	11	14	19	20	21	25	27	28	32	35
T1	7.65	4.79	3.67	4.50	4.21	3.66	-	-	3.78	-	2.98	3.92	3.18
T2	7.02	4.50	3.55	4.38	3.64	3.34	-	-	3.37	-	-	-	-
T3	5.58	3.69	2.92	3.44	2.91	2.65	-	-	3.56	-	-	-	-
T4	4.06	2.74	2.29	2.63	1.98	1.97	-	-	2.32	-	-	-	-
T5	2.03	1.38	1.04	1.06	0.88	0.95	-	-	1.10	7.79	6.41	5.80	4.05
T6	-	-	-	-	-	-	7.32	5.54	5.70	0.46	0.46	0.13	0.57

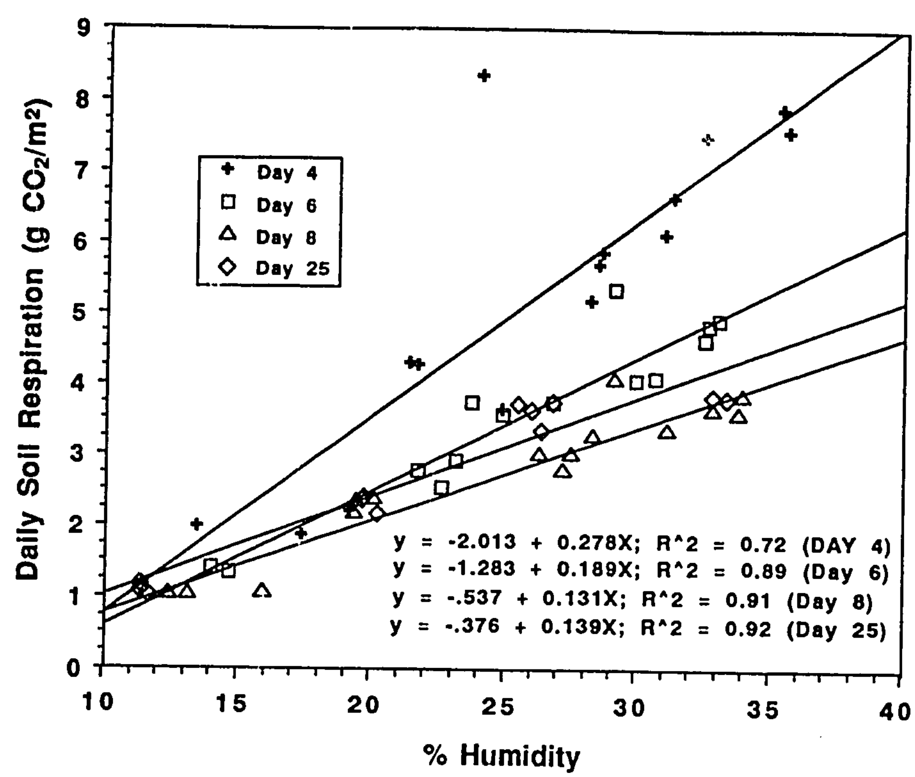

Figure 2. Relationship between soil respiration and soil moisture content in laboratory containers after 4, 6, 8, and 25 days of incubation.

constant moisture levels. According to Gale and Gilmore (1986), the breakdown of manures incorporated into soil shows a similar pattern. Of the total carbon (C) applied as chicken litter on a soil surface, 25% was evolved within the first 7 days, 10% was evolved during the second 7-day period, and the remainder during the next 138 days (Gale and Gilmore 1986). Bitzer and Sims (1988) reported that the majority of nitrogen (N) mineralization from various chicken manures mixed with soils occurred within the first 14 days. The sharp rise in soil respiration observed in the field upon pond drainage was probably related to the same cause. High amounts of organic matter accumulated during low rates of respiration while in hypoxic conditions under water. Upon exposure to air, the labile organic matter was quickly decomposed. The subsequent decrease in respiration was probably related both to a lack of labile organic matter and to decreasing soil moisture. The rate of decrease was more closely related to labile organic material, but the level at which the respiration stabilized was more closely related to soil moisture. The laboratory data showed that maintenance of a higher level of soil moisture will result in higher steady state rates of respiration.

Determination of CO_2 evolution from Treatment 6 began the day after the buckets were filled. Respiration was high because the soil was still not saturated despite greater additions of water than in Treatment 1. Therefore, more water was added to achieve complete saturation, i.e., until water could be seen covering the surface of the soil. The effect on respiration was dramatic. Respiration immediately fell to about half the level of Treatment 5, which had a mean humidity of 14%. Coverage of soil by water clearly decreased the transfer of gases, namely oxygen, to the soil for use by mineralizing bacteria. Saturation inhibited respiration more than the low moisture content in Treatment 5. However, there was a fine line between completely saturating the soil and maintaining a high moisture content appropriate for bacteria growth. There appeared to be a linear decrease in respiration as soil moisture decreased from subsaturation humidity (35%) to 14%. These data are different from those reported for Alabama soils. Boyd and Pippopinyo (in press) demonstrated a reverse relationship: when humidity was increased from 20% to saturation at 30-45%, soil respiration decreased.

Respiration in Treatment 5 increased dramatically when soil moisture was increased from 14 to 33%. Wetting of a dry pond bottom could therefore have an immediate impact on organic matter decomposition. These data indicate that in the absence of rains or frequent wettings, ponds will efficiently mineralize carbon only during the first week of drying. High steady state respiration rates can be maintained on soils of high organic C content for several weeks in the presence of sufficient soil moisture to satisfy the needs of bacteria.

Literature Cited

Boyd, C.E., and C.S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Boyd, C.E., and S. Pippopinyo. In press. Factors affecting respiration in dry pond bottom soils. Aquaculture.

Boyd, C.E. and D.R. Teichert-Coddington. In review. Pond bottom soil respiration during fallow and culture period in heavily fertilized tropical fish ponds. Submitted to the Journal of the World Aquaculture Society.

Bitzer, C. C., and J.T. Sims. 1988. Estimating the availability of nitrogen in poultry manure through laboratory and field studies. Journal of Environmental Quality 17:47-54.

Gale, P.M., and J.T. Gilmore. 1986. Carbon and nitrogen mineralization kinetics for poultry litter. Journal of Environmental Quality 15:423-426.

On-farm Production of Monosex *Oreochromis niloticus* in Rwandan Farm Ponds at Altitudes above 1300 Meters

Work Plan 6, Experiment 1

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Introduction

Highland portions of Africa, including Rwanda, have been considered too cool for tilapia culture, yet farmers actively culture these fish at elevations from 1200 to over 2200 m above mean sea level (Hanson et al. 1988). The Rwanda CRSP has capitalized on this natural laboratory with its extensive environmental gradient to conduct a series of experiments to examine the influence of elevation-related factors, such as temperature, on tilapia growth and production. The goal of this stuy was to determine if tilapia growth is depressed at higher elevations because temperature declines to the physiological limits for this species or because pond productivity is reduced. These considerations have management implications for farmers in highland areas around the globe. If reduced food consumption and utilization are the primary reasons for depressed growth at higher elevations, increased stocking densities would be appropriate. If depressed primary pond productivity is the major factor, fish production might still be enhanced by supplemental feeding.

Previous research with mixed-sex *Oreochromis niloticus* in Rwanda (Work Plan 5, Experiment 3) revealed that net productivity declined with increasing elevation but that growth rates in adult fish were similar at different altitudes. It was hypothesized that growth rates might have been depressed at lower elevations because of higher reproduction and greater competition from offspring. This experiment was designed to eliminate reproduction effects by stocking ail-male *O. niloticus* fingerlings.

Objectives

The primary objective of this experiment was to quantify the relationships of elevation with tilapia growth, production, and survival, using only one level of fertilizer input for both farmer-owned and Rwasave Station research ponds. The second objective of this study was to determine water quality changes resulting from additions of fresh grass and inorganic fertilizers to ponds at different elevations.

Materials and Methods

A total of 28 ponds (five ponds at Rwasave Station and four to five ponds at each of five altitude zones) were stocked with 1 male *Oreochromis niloticus*/m^2. Elevations ranged from 1370 m (Zone 1) to 2180 m (Zone 5). The average weight of fingerlings stocked ranged from 19 to 28 g. Ponds were fertilized with grass (*Cyperus* sp.) at 10 kg wet weight/are/wk (1 are=100 m^2) and with inorganic fertilizer at 256 g urea and 178 g TSP/are/wk). This supplied 100 kg of carbon, 16 kg of nitrogen, and 4 kg of phosphorous/ha/wk. Fish were sampled for growth every four to six weeks. The culture period was five months or until average fish weight reached 150 grams. Prior to fish sampling, pH, chlorophyll *a*, total alkalinity, total hardness, dissolved oxygen, Secchi disk transparency, conductivity, and maximum/minimum temperatures of each pond were measured. The CRSP standard sampling protocol was followed for the five ponds at Rwasave Station (Pond Dynamics/Aquaculture 1992). In addition, temperature was continuously recorded in one pond per elevation zone. These recorders sometimes failed; consequently only maximum/minimum temperature data are available for some periods.

Rural farmers were participants in this study. Farmers weighed and added the fresh grass inputs along with preweighed packages of chemical fertilizer (supplied by the researchers) to the ponds. Farmers also took weekly Secchi disk readings, managed water levels, recorded fish mortalities, and made weekly visual color evaluations using a color comparator chart.

Data were analyzed by ANOVA using Statgraphics Statistical Graphics Program version 6.0 (Manugistics, Inc. 1992) and Duncan's Multiple Range Test (Ott 1977) with alpha=0.05, and by non-linear regression using Tablecurve Curve Fitting Software®, version 3.1 (Jandel Scientific 1992).

Results

At the beginning of the experiment ponds showed the following characteristics: 30-90 mg/L total alkalinity, 20-90 mg/L hardness, 100-300 µmoles/cm conductivity, 200-700 m^2 surface area, and 30-120 cm depth. In Zone 1, the lowest elevation, mean alkalinity increased beyond this range to 143 mg/L during the experiment.

Temperature data showed a decline in mean, minimum, and maximum values as elevation increased about 800 m from the lowest to highest ponds (Table 1). Ponds at Rwasave Station (1625 m) were warmer than those at lower elevations, probably because of better water management than at farmer-operated sites. In the highest zone (mean elevation 2175 m) mean morning temperatures were only 15.1°C and mean afternoon values reached 19.2°C (Table 1).

Growth rates at higher elevations (Zones 3 and 5) were significantly lower (0.55 to 0.61 g/d) than in Zones 1 and 2 and at Rwasave Station (0.81 to 0.86 g/d) (Table 2). Fish growth rates for each pond and elevation zone were correlated with elevation, with r^2=0.41 (Figure 1). Mean growth per zone correlated more closely with elevation than did the rates for each pond, with r^2=0.72 (not shown).

Temperature appears to have been the major factor determining fish growth in the range of elevations tested. Mean daily, mean morning, and mean afternoon temperatures all correlated highly with fish growth. Temperature data from Zone 4 were incomplete at the time of this report.

Net extrapolated annual fish production was significantly less at the highest elevation than in ponds at all lower elevations (Table 2). Both lower survival and reduced growth at the highest elevation contributed to the low production. Mean fish production per zone was less well correlated with elevation than was growth rate (r^2=0.52, not shown), apparently because of lower survival at the lowest and highest elevations. Variation in survival may be a function of extraneous factors such as the distance fish were transported for stocking. Therefore, no conclusions regarding the relationship of elevation to survival were drawn at this time.

The means of the monthly chlorophyll measurements indicate that the decline in growth at the highest elevation was not due to lack of phytoplankton, because uncorrected chlorophyll *a* concentrations were 1.4 to 2.4 times greater than in lower elevation ponds (Table 3). Thus the low growth in Zone 5 likely resulted from a temperature-mediated restriction on food consumption or utilization by tilapia.

Table 1. Elevation above sea level (m), mean, mean daily afternoon and morning surface water temperatures (°C) for 4 or 5 Rwandan ponds per zone.

Zone	Elevation (m)	Mean Temperature (°C)	Mean Afternoon Temperature (°C)	Mean Morning Temperature (°C)
1	1375	23.4	26.1	20.9
2	1575	22.7	24.9	20.5q
Rwasave	1625	23.0	25.8	21.1q
3	1775	21.1	23.0	19.5
4	1975	19.5 *	**	**
5	2175	17.2	19.2	15.1

* Estimated from incomplete data.
** Data not yet available.

Table 2. Elevation, tilapia growth, net production, and percent survival for ponds at Rwasave Station and in the five elevation zones (n=4 or 5 per zone).

Zone	Elevation (m)	Growth* (g/d)	Net Prod. (kg/are/yr)	Survival* (%)
1	1375	0.82 bc	20.4 a	71.2 a
2	1575	0.81 bc	20.7 a	78.5 ab
Rwasave	1625	0.86 c	25.2 a	83.6 bc
3	1775	0.60 a	19.3 a	88.5 c
4	1975	0.66 ab	21.0 a	89.9 c
5	2175	0.55 a	12.7 b	72.6 ab

* Values followed by the same letter are not different by Duncan's multiple range test (alpha = .05).

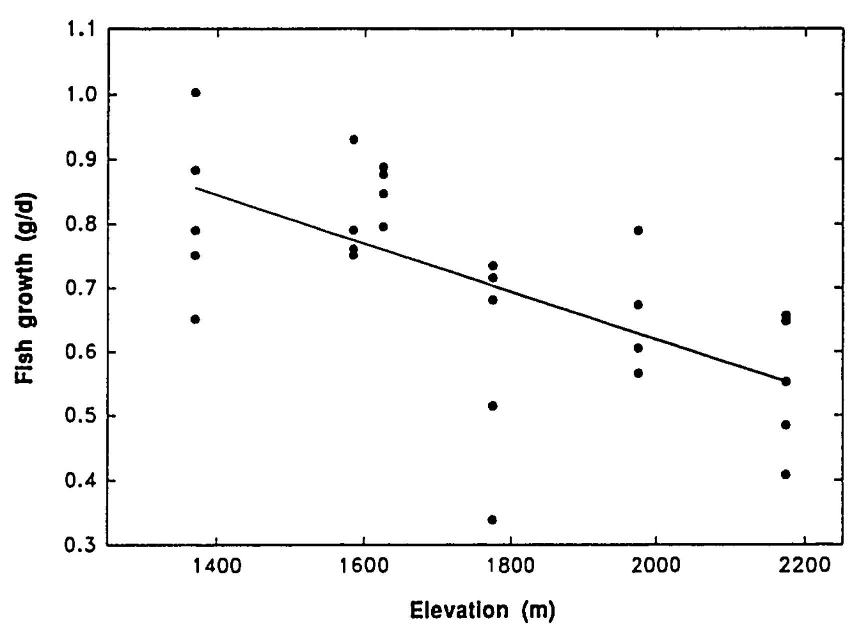

Figure 1. Regression of elevation (meters above sea level) and mean fish growth per pond (g/d). For the function y=1.37-0.00037x, $r^2 = 0.41$.

Table 3. Corrected chlorophyll *a*, uncorrected chlorophyll *a*, hardness, and alkalinity of samples taken about monthly from the experimental ponds. At least four ponds were sampled in each zone.

Zone	Elevation (m)	Corr.* Chl *a* (mg/m³)	Chl *a** (mg/m³)	Hardness* (mg/L)	Alkalinity* (mg/L)
1	1375	132 a	166 a	89 c	143 b
2	1575	228 ab	294 bc	23 a	55 a
Rwasave	1625	183 ac	227 ab	61 bc	64 a
3	1775	151 a	180 ab	31 a	42 a
4	1975	129 a	189 ab	31 a	43 a
5	2175	306 b	399 c	34 a	46 a

* Values followed by the same letter are not significantly different by Duncan's Multiple Range Test (alpha=0.05).

In the following on-farm experiment (Work Plan 6, Study 3) we will reduce nitrogen input to 6 kg/ha/wk and phosphorus to 1.5 kg/ha/wk to minimize potential water quality problems, and compare ponds receiving only fertilizer with those receiving both fertilizer and supplemental feeding. This will allow us to determine if fertilizer was added in excess during the present experiment and whether growth slows with increasing elevation because of reduced pond productivity or because of physiological limitations directly affecting the fish.

Anticipated Benefits

This experiment forms the beginning of our effort to provide basic data on tilapia culture in relation to factors associated with elevation, such as temperature. Coupled with our laboratory experiments and additional on-farm studies (Work Plan 6, Study 3; Work Plan 7, Study 1), an understanding of the capacity to produce tilapia above optimum elevations will emerge. Broad areas of Africa and other global locations have highland areas that may be suitable for tilapia culture if elevation-dependent management strategies are applied. Both resource use planners and individual farmers would benefit from knowledge developed through this series of experiments.

Literature Cited

Hanson, B., J. Moehl Jr., K. Veverica, F. Rwangano, and M. Von Speybroeck. 1988. Pond culture of tilapia in Rwanda, a high altitude equatorial African country. Pages 553-559 *in* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J.L. Maclean, editors. The Second International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings 15. Department of Fisheries, Bangkok, Thailand, and ICLARM, Manila, Philippines.

Jandel Scientific. 1992. Tablecurve curve fitting software, version 3.1. Jandel Scientific, Inc. Corte Madera, California.

Ott, L. 1977. Introduction to statistical methods and data analysis. Duxdury Press, Boston, Massachusetts.
Pond Dynamics/Aquaculture CRSP. 1992. Sixth Work Plan. Program Management Office, OIRD, Oregon State University, Corvallis, Oregon.
Statgraphics. 1992. Statgraphics statistical software, Version 6.0. Manugistics, Inc., Rockville, Maryland.

Benefits of Supplemental Dietary Energy in Tilapia Ponds Enriched with Fresh Grass and Chemical Fertilizer

Work Plan 6, Experiment 4

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Introduction

Non-protein dietary energy, available as carbohydrates or fats, is added to practical fish rations to "spare" dietary protein for growth. Practical diets for channel catfish are generally formulated to contain 8.8 kcal of digestible energy (DE) per gram of crude protein (Lovell 1988); the optimum energy/protein ratio for common carp feeds was estimated at 8.3 kcal DE/g protein (Takeuchi et al. 1979). Assuming a protein digestibility coefficient of 0.85 for practical feeds (Smith and Lovell 1973), the optimum protein/energy ratios for catfish and carp feeds are 10.4 and 9.8 kcal DE/g digestible protein (DP), respectively. Similarly, the optimum protein/energy ratio for tilapia feeds was calculated at 9.7 kcal DE/g DP (NRC 1993). Hanley (1987), feeding tilapia practical rations with ratios of 8.6 to 11.1 kcal DE/g DP, reported that the greatest growth occurred at the intermediate level of 10.1 kcal DE/g DP. Foods consumed by tilapia would therefore be considered energy-deficient if they contain less than approximately 10 kcal DE/g DP.

In an earlier study in Rwanda (Work Plan 5, Experiment 6), natural food organisms consumed by tilapia in ponds enriched with fresh grass were found to be slightly energy-deficient (9.7 kcal DE/g DP), suggesting that fish growth would be enhanced by the addition of non-protein energy. In a subsequent study (Ndikumwami et al. 1993), increased fish production resulted when a high-energy feedstuff, cassava meal, was added to ponds fertilized with grasses only. It was not clear, however, whether the primary mechanism was a direct nutritional benefit to the fish or an indirect benefit as an energy source for heterotrophic organisms subsequently consumed by the fish.

Objectives

This experiment was designed to separate the direct and indirect nutritional benefits of supplemental energy by allowing fish to consume it directly in some ponds while preventing direct access by fish in other ponds. Cassava, the energy supplement, consists mostly of carbohydrates and contains very little protein (<2%). Chemical fertilizers were added to all ponds to stimulate phytoplankton production, thus preventing the chronically low dissolved oxygen levels observed in a previous experiment in which only grasses were used as fertilizer. Earlier research suggested that the natural food resulting from a grass plus chemical fertilizer is somewhat energy deficient, with DE/DP ratio of about 8. Treatments in the present study were as follows:

1. No supplemental energy
2. Supplemental energy as feed
3. Supplemental energy inaccessible to fish

Materials and Methods

Fifteen 6.6-are ponds at the Rwasave fish culture station in Butaré, Rwanda, were stocked with 13 g *Oreochromis niloticus* at 2/m^2. All ponds were fertilized weekly with fresh grasses at 350 kg dry weight per hectare, 6.75 kg N/ha as urea and 1.6 kg P/ha as triple superphosphate. Five ponds received 450 g ground cassava daily (6.8 kg/ha) mixed with a small amount of water and formed into a large patty that was set on a submersed feeding platform. Five other ponds received the same amount of cassava prepared the same way but placed in a small fiberglass mesh cage. Three cages were suspended in a larger cage constructed of 1 cm mesh metal screen, which prevented the cassava from being consumed directly by the fish. The remaining five ponds received no supplemental cassava (control treatment).

Fish were sampled monthly to determine average weight by sex. Dissolved oxygen concentrations and temperatures were measured weekly at 25, 50, and 75 cm below the surface in the morning and afternoon. Chlorophyll *a* concentrations and secchi disk visibility were measured every two weeks. Other water chemistry parameters were measured according to standard CRSP protocol as described in the Sixth Work Plan. Ponds were drained after 164 days, and all adult fish were separated by sex, counted, and weighed. Fingerlings were graded into two size groups and the total and average weights of each group were measured.

Results and Discussion

Weekly morning dissolved oxygen concentrations at 25 cm averaged greater than 3 mg/L for all ponds. Average net annualized fish yield was 3,055 kg/ha. There were no significant differences in fish growth, survival, or net production between treatments (Table 1), indicating that there was little benefit from the addition of energy-rich cassava meal in ponds with nutrient inputs of chemical fertilizer and fresh grass.

In an earlier study (Work Plan 5, Experiment 7; Ndikumwami et al. 1993), total fish yield from grass-enriched ponds had increased by 37% when cassava meal was added. An even greater growth increase was expected in the present study with both cassava and chemical fertilization because the results of Experiment 6 of Work Plan 5 (Veverica et al. 1992) indicated that chemical fertilization of grass-enriched ponds produced natural food that was more energy-deficient than food organisms from ponds enriched only with grass (8.2 versus 9.7 kcal DE/g DP). The reason for failure to respond to supplemental energy in the present study is unclear, but two possible explanations are offered: first, increased abundance of a phytoplankton food source may have masked the benefits of the cassava meal; and second, the optimum energy/protein ratio for tilapia may be lower at cooler temperatures than previously estimated in warmer environments.

In the present study the fish growth rate in control ponds was 25% higher than in the previous study (Ndikumwami et al. 1993) without chemical fertilization even though fish density was twice as high. The increased abundance of a phytoplankton food source in the present study may have masked the benefit of supplemental energy, especially if the benefits of cassava meal were indirect, functioning primarily as an energy source for heterotrophic food organisms consumed by tilapia. Similar growth among fish that had or were denied access to the cassava meal suggested little direct nutritional benefit from the supplemental energy.

Optimum energy/protein ratios for tilapia have been estimated at approximately 10 kcal DE/g DP for water temperatures of at least 25°C (Hanley 1987, NRC 1993). If the energy needs of tilapia are depressed more than their protein growth potential in cooler environments, optimum energy/protein ratios would be reduced at lower temperatures. Consequently, food organisms consumed by tilapia in fertilized ponds in Rwanda, which have energy/protein ratios of 8.5 to 9.7 kcal DE/g DP, may not be energy deficient at an average water temperature of 21°C. Little information is

Table 1. Water quality and fish production averages for 5 ponds per treatment. There were no significant differences between treatments (p>.20 for all results).

Treatment	Morning DO (mg/L)	Corrected chl. *a* mg/m^3)	Final wt (g)	Survival (%)	Net annualized yield (kg/ha/yr)	
					Adults	Adults & offspring
No cassava	3.9	106	93	78	2670	2970
Accessible cassava	4.1	105	91	77	2542	3014
Inaccessible cassava	3.4	84	100	77	2824	3162

available on optimum protein-energy balance as a function of temperature. If optimum energy/protein ratios are lower in cooler climates, this fact may have little practical significance because it may be compensated by a proportional reduction in digestive efficiency. Popma (1982) reported that carbohydrate digestibility by tilapia was 17% lower at 22°C than at 30°C, but that protein digestibility was similar at both temper–atures, thus reducing energy/protein ratios of diets at lower temperatures.

Anticipated Benefits

Until additional information is available, we conclude that, in ponds enriched with chemical fertilizer and fresh grass, the food organisms consumed by tilapia contain digestible energy at or near levels required for maximal utilization of dietary protein for fish growth. Supplemental energy without additional dietary protein likely provides little direct nutritional benefit to the fish but may increase production of natural food organisms in the pond. Energy/protein requirements may need to be revised in relation to temperature before general application to global environmental conditions.

Literature Cited

Hanley, F. 1987. The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determination in tilapia, *Oreochromis niloticus* (L). Aquaculture 66:163-179.

Lovell, T. 1988. Nutrition and feeding of fish. Van Nostrand Reinhold, New York.

National Research Council. 1993. Nutrient requirements of fish. National Academy of Sciences. National Academy Press. Washington, D.C.

Ndikumwami, R., K.L. Veverica, T.J. Popma, and W.K. Seim. 1993. Supplemental dietary energy to enhance utilization of natural food organisms for growth by tilapia. Pages 48-51 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin, editors. Tenth Annual Administrative Report, Pond Dynamics/Aquaculture Collaborative Research Program, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Pond Dynamics/Aquaculture. Fifth Work Plan. 1989-1991. Pond Dynamics/ Aquaculture Collaborative Research Program, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Pond Dynamics/Aquaculture. Sixth Work Plan. 1991-1993. Pond Dynamics/ Aquaculture Collaborative Research Program, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Popma, T.J. 1982. Digestibility of selected feedstuffs and naturally occurring algae by tilapia. Ph.D. Dissertation, Auburn University, Auburn, Alabama.

Takeuchi, T., T. Watanable, and C. Ogino. 1979. Optimum ratio of energy to protein for carp. Bulletin of the Japanese Society of Scientific Fisheries 45:983-987.

Smith, B.W., and R.T. Lovell. 1973. Determination of apparent protein digestibility in feeds for channel catfish. Transactions of the American Fisheries Society 102(4):831-835.

Veverica, K., E. Rurangwa, T. Popma, W. Seim, and R. Tubb. 1992. Digestibility of food consumed by *Oreochromis niloticus* in fertilized ponds. Pages 41-44 *in* H. Egna, M. McNamara, and N. Weidner, editors. Ninth Annual Administrative Report, Pond Dynamics/Aquaculture Collaborative Research Program, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Temperature Affects Appetite, Growth, Feed Conversion Efficiency, and Body Composition of Tilapia

Workplan 7, Study 4

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Introduction

Nile tilapia, *Oreochromis niloticus*, tolerate water temperatures up to 42°C (Denzer 1968) but prefer temperatures between 28 and 30°C (Beamish 1970). Gui et al. (1989) reported that an average temperature of 28°C was optimal for growth of Nile tilapia fry. For most commonly cultured tilapias feeding activity is minimal below 20°C (Popma 1982, Pruginin 1983) and ceases completely at approximately 16°C (Kelly 1956, Popma 1982). Few biologists dispute that appetite and growth of tilapia are progressively depressed as temperatures fall below the optimum, but little is known about feed conversion efficiency as a function of temperature. Most controlled temperature studies with tilapia have been conducted at constant temperatures, rather than daily fluctuating temperatures as occur in nature. However, Gui et al. (1989) reported that at near-optimum average temperatures of 28° to 30°C, growth of tilapia fry was greater in daily fluctuating temperature regimes than in constant temperatures. The effect of fluctuating temperatures at or near the practical minima for tilapia has not been reported.

Average pond water temperatures in Rwanda are 21 to 22°C (Verheust et al. 1994). Afternoon temperatures reach 24 to 25°C, but fall to 18 or 19°C by morning (Hishamunda and Moehl 1989). These average and maximum temperatures are above the reported 20°C minimum for feeding and growth by tilapia, but morning temperatures in Rwanda are below the critical value. Do daily minimum temperatures severely depress the physiological processes of food consumption, digestion, and assimilation, or is tilapia growth in cool climates primarily a function of average or maximum water temperature? Is reduced feeding activity at low temperatures a less serious constraint in food-limited environments? These and related questions motivated this research.

Objectives

The objective of the study was to determine, in a controlled laboratory environment, maximum feed consumption, growth rate, feed conversion efficiency, and body composition of Nile tilapia fingerlings as a function of feed availability under constant or fluctuating temperatures that were near minimum or optimum for tilapia. Feed consumption was measured under conditions of relatively constant food supply during daylight hours; our purpose was to approximate the food availability conditions of low-input management practices where natural food organisms constitute a significant fraction of total ingesta, rather than of intensive practices where most nutrients are provided as feed in one or two meals daily.

Materials and Methods

Research was conducted in a laboratory at Auburn University. Water supply was from an embankment pond filled by rainwater runoff. The experimental design of the study was 2x2x3 factorial with 3 replicates per treatment: two average water temperatures (22 and 26°C), each of which was either constant or variable (4°C below average at 0800 hours to 4°C above average at 1700 hours), with a nutritionally complete ration being offered at 30%, 60%, or 100% of satiation at each temperature regime.

Each battery of the four temperature regimes included nine 45-L aquaria from which water was recirculated through a common 300-L fiberglass reservoir containing 21 L of 3-cm flexrings for biofiltration. Recirculated water was continuously pumped from the reservoir to the aquaria to achieve one water exchange every two to three hours in all aquaria. During the night hours fresh water was added to reservoirs at a rate equivalent to one complete water exchange daily for the system. All aquaria received continuous aeration. Fish biomass never exceeded 8 g/L in any aquarium or 4 g/L in any system. Temperature regimes were maintained by submerged aquarium heaters in reservoirs and aquaria, by refrigerated cooling coils in the reservoirs where needed, and by nightly addition of cooler water to reservoirs. Water temperatures were monitored and adjusted at two-hour intervals during daylight hours.

Eleven overwintered, predominantly male *Oreochromis niloticus* fingerlings were randomly distributed to each aquarium, and were acclimated to the appropriate temperature regime for one week. During acclimation the fish in each aquarium received a daily maintenance ration of 32% protein, 3-mm diameter, floating catfish feed. Fish were then fasted for one day before being weighed individually. Average initial weights varied from 11.2 to 13.8 g in all aquaria. At 0800 hours the next day, fish that were to be fed to 100% satiation were offered an excess of feed. Pellets remaining after 20 minutes were removed and counted. Weight of feed consumed was determined by subtracting the weight of excess feed (number of pellets remaining multiplied by previously determined average weight of pellet) from the initial weight of feed offered. This process was repeated at two-hour intervals until 1800 hours. Average total daily satiation consumption, expressed as percent of body weight (BW) per day, was determined for each temperature regime, and daily rations for groups to be fed at the restricted rates of 60% and 30% satiation were calculated accordingly. Day 1 for fish on restricted rations began the following day. During the remainder of the week daily rations were divided into six approximately equal meals that were offered at two-hour intervals from 0800 to 1800 hours. Fish were weighed individually after the final meal on the last day of each week, and the procedure for estimating satiation feeding rates was repeated at the beginning of weeks 2, 3, and 4.

The experiment was concluded after 28 days. Fish were weighed individually, fasted for one day, and re-weighed. Three fish were taken from each aquarium, dried at 90°C for four days to determine moisture content, finely ground, and stored for subsequent analysis of crude protein and lipid content.

Statistical analyses included ANOVA and simple and polynomial regression.

Results

Results are summarized in Table 1. A description of the factors affecting appetite, growth, feed conversion efficiency, and protein and lipid content of tilapia follows.

Maximum feed consumption
As expected, temperature had a significant impact on maximum feed consumption ($P<0.01$). Average maximum feed consumption was 37% greater at 26°C (4.5% BW/d) than at 22°C (3.3% BW/d). When fish were fed to satiation, average daily feed consumption under the cool-variable temperature regime (22±4°C) was not significantly different from feed consumption at a constant 22°C, even though morning temperatures were only 18°C ($P>0.20$). There was no evidence of reduced appetite at the first meal of the day when the water temperature was only 18°C, especially for fish on limited feeding schedules. At an average water temperature of 26°C the average maximum daily feed intake was 4.5% BW, regardless of whether temperatures were variable or constant.

At 26°C fish that fasted the previous day consumed 6.6% of BW on day 1, whereas they consumed only 5.0% BW/d after one week of feeding to satiation. Likewise, at 22°C fasted fish consumed 4.6% of BW on day 1, while one week later fish fed to satiation the previous day consumed only 2.7% BW/d.

Factors related to age and size also influenced feeding rates at satiation. At average temperatures of 22°C, maximum feed consumption remained relatively stable during the final three weeks of the study, varying from 2.6 to 3.4% BW/d. At an average temperature of 26°C, maximum feed consumption dropped from 5.0% BW/d on day 8 to only 2.8% BW/d on day 28 (1.3 to 4.3% BW/d, CV=44). This was apparently caused by the territorial behavior of a few maturing fish which affected the feeding ability of the remaining fish.

Weight gain
Average weight gains for fish fed to satiation, expressed as percent increase during 28 days at 22 and 26°C were 81 and 142%, respectively. Temperature-related differences in weight gain were greatest at high feeding rates. The weight gain of full-fed fish was 75% greater at the warmer temperature than at the cooler temperature. The relative superiority in weight gain at warmer temperatures was 69% when fed at 0.6 satiation and only 47% at 0.3 satiation.

At feeding rates between 2.0 and 3.3% BW/d fish grew significantly faster at 26°C than at 22°C. However, at feed consumption rates below 1.5% BW/d, weight gain was similar in both temperature regimes. At lower feeding rates fish held at 22°C could be shown to grow faster than those at the warmer temperature based on extrapolation of the relationships in Figure 1. At these restricted feeding levels (1.5% BW/d, which correlates to 46% satiation at 22°C, and to 33% satiation at 26°C), fish in warm water apparently utilized a proportionally greater fraction of the diet for maintenance instead of growth. Thus, only in commercially undesirable food-limited situations would tilapia in cool climates grow faster than in warmer waters.

Table 1. Maximum feed consumption, growth rate, feed conversion efficiency, and body composition of tilapia at four temperature regimes and three feeding levels.

Temperature regime	Statistical Signif. (footnote)	Cool Constant 22 ± 0	Cool Variable 22 ± 4	Warm Constant 26 ± 0	Warm Variable 26 ± 4
Feed consumption at satiation, %BW/d	1				
day 1 (fasted previous day)		4.5	4.8	6.3	6.9
day 8 (fed to satiation previous day)		2.6	2.7	5.0	4.9
day 22 (territoriality at 26°C)		2.9	2.2	2.3	3.3
4-week average		3.2	3.4	4.5	4.5
Average weight gain, % increase in 28 d	2				
at 100% satiation feeding		78	84	152	133
at 60% satiation feeding		44	46	82	75
at 30% satiation feeding		18	17	28	27
FCR, 28-d, g feed/ g weight gain	3				
at 100% satiation feeding		1.47	1.46	1.29	1.39
at 60% satiation feeding		1.39	1.43	1.23	1.23
at 30% satiation feeding		1.70	1.96	1.58	1.50
Protein conversion efficiency, P gain/P fed	4				
at 100% satiation feeding		0.34	0.34	0.38	0.36
at 60% satiation feeding		0.35	0.35	0.41	0.39
at 30% satiation feeding		0.28	0.24	0.30	0.30
Final body composition, % live weight					
Moisture	5				
at 100% satiation feeding		72.9	72.3	72.6	73.4
at 60% satiation feeding		76.3	74.9	74.3	76.4
at 30% satiation feeding		77.2	77.2	76.3	78.4
Lipids	6				
at 100% satiation feeding		7.1	7.2	6.7	6.8
at 60% satiation feeding		3.8	4.4	5.2	3.8
at 30% satiation feeding		3.0	3.0	3.3	2.0
Crude protein	7				
at 100% satiation feeding		16.3	16.5	16.7	16.4
at 60% satiation feeding		16.1	16.5	16.7	15.6
at 30% satiation feeding		15.7	14.8	15.8	15.0

Parameters where treatment differences were significantly different by ANOVA at $P<0.05$:

1 Average temperature, time/size, temperature-time interaction; not stability of temperature
2 Average temperature, feeding rate, temperature-feeding rate interaction; not stability of temperature
3 Average temperature, feeding rate; not stability of temperature
4 Average temperature, feeding rate; not stability of temperature
5 Feeding rate; not average temperature or stability of temperature
6 Feeding rate; not average temperature or stability of temperature
7 Feeding rate; not average temperature or stability of temperature

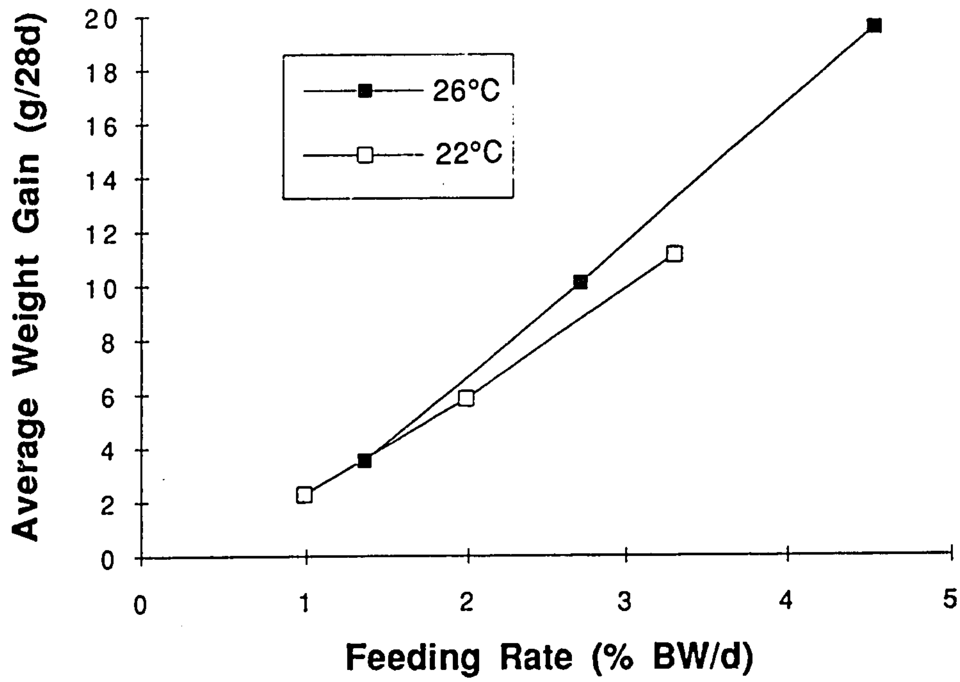

Figure 1. Average weight gain as a function of feeding rate for tilapia fed to 30, 60, or 100% satiation at average temperatures of 22° or 26°C.

Feed conversion

Feed conversion ratios (FCR), expressed as g feed/g gain, were a function of average temperature and feeding rate ($P<0.01$) but not of daily variation in temperature ($P>0.20$). At a given temperature, feed conversion ratios were similar when feed consumption was 60 to 100% of satiation; however, 20 to 28% more feed was required per unit weight gain when feed consumption was only 30% of satiation levels (Table 1) because a higher percentage of the ration was partitioned to maintenance.

At 26°C, feed conversion was most efficient at feed consumption rates of approximately 3.5% BW/d, while at 22°C feed conversion was most efficient at feeding rates of 2.5% BW/d. At feeding rates less than 2% BW/d, FCR was similar for both temperatures, but at higher feeding rates fish were most efficient at the warmer temperature (Figure 2).

Protein conversion efficiency

Protein conversion efficiency, expressed as g protein gain/g protein fed, averaged 0.34, ranging from 0.26 (for fish fed at 30% of satiation at 22°C) to 0.40 when fish were fed at 60% satiation. Conversion of dietary protein to fish protein was affected by average temperature and feed consumption rates ($P<0.01$), but not by daily variation in temperature ($P>0.20$). Fish fed at 30 to 100% of satiation converted dietary protein more efficiently at the warmer temperatures, but the improvement was less dramatic than the FCR improvements. FCR was 20 to 28% better than in cool water, whereas protein conversion was only 9 to 15% superior at the warmer temperature (Table 1). Temperature-related differences in protein conversion efficiency are not as pronounced because protein digestion by tilapia is not as strongly affected by declining temperatures as carbohydrate conversion (Popma 1982).

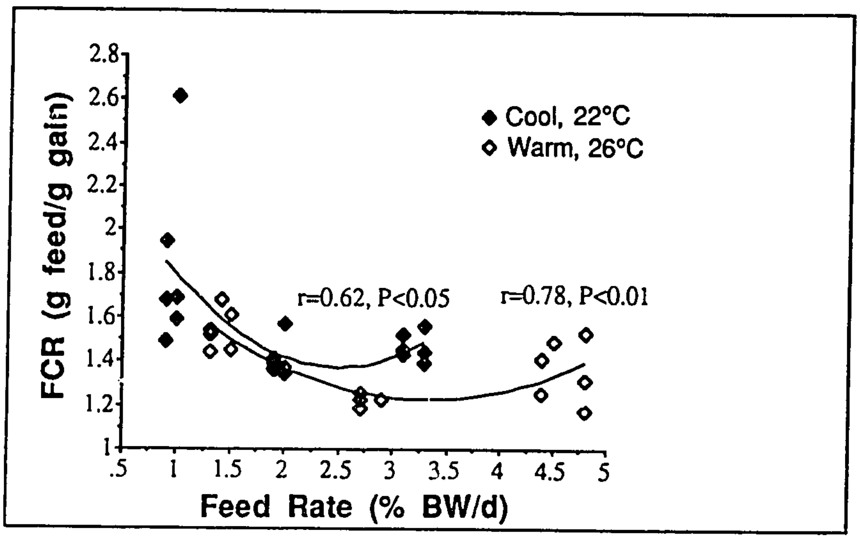

Figure 2. Feed conversion ratios for tilapia fed to 30, 60, or 100% satiation at average temperatures of 22° or 26°C.

Body composition of tilapia

Dry matter, protein, and lipid content of tilapia were affected by feeding rate ($P<0.01$) but not by temperature ($P>0.20$). Differences in the protein content of whole fish, although statistically significant ($P<0.05$), were not great, ranging from a low of 15.3%, when fish were fed to 30% satiation, to 16.5% at full satiation. Differences in lipid content, however, were more marked. The average lipid levels in fish fed to 30% satiation were only 2.8% of live weight whereas the levels in fish fed to full satiation were 7.0%.

Anticipated Benefits

Gui et al. (1989) reported that tilapia fry held at near optimum average temperatures grew better in a fluctuating temperature regime than in a constant temperature regime. We found no effect of temperature fluctuation on growth or related factors. At suboptimal average temperatures of 22 to 26°C, the benefits of fluctuating temperatures are apparently greatly reduced or eliminated. This suggests that under these conditions controlled-temperature studies can be conducted at constant temperature regimes, which are logistically easier to maintain than fluctuating temperatures.

Both the growth and the feed conversion efficiency of tilapia improve as optimum water temperatures are approached, especially when fish are fed at rates near satiation. Only at feeding levels below 1.5% BW/d are fish at 22°C thought to convert more efficiently than at 26°C. However, such low feeding rates would not be desirable on commercial farms because fish production and feed conversion efficiency are substantially reduced. Cool temperatures retard spawning and overpopulation in growout ponds in Rwanda, but the only other apparent advantage of low water temperatures might be in subsistence aquaculture situations where poverty and isolation prevent fish farmers from managing their ponds more intensively. At commercially desirable feeding rates above 1.5% BW/d, tilapia convert less efficiently at cooler temperatures than at warmer temperatures. This conclusion, however, does not imply that tilapia culture in cool climates is not a profitable activity that surpasses the alternative uses of the resources invested. Enterprise budgets (Hishamunda et al. 1994) are needed to support conclusions on the financial and economic appropriateness of a production practice.

Literature Cited

Beamish, F.W.H. 1970. Influence of temperature and salinity acclimation on temperature preferenda of the euryhaline fish *T. nilotica*. Fisheries Research Board of Canada 27(7):1209-1214.

Denzer, H.W. 1968. Studies on the physiology of young *Tilapia*. FAO Fisheries Reports 44(4):356-366.

Gui, Y., Z. Wang, Y. Chen, W. Zheng, and F. Li. 1989. Use of fluctuating temperature to promote growth of *Tilapia nilotica*. Journal of Fisheries of China, 13(4):326-331.

Hishamunda, N., and J.F. Moehl. 1989. Rwanda national fish culture project. Research and Development Series No. 34, Alabama Agricultural Experiment Station, Auburn University, Alabama.

Hishamunda, N., M. Thomas, D. Brown, and C. Engle. 1994. A comparative economic analysis of small-scale fish culture in Rwanda. Pages 87-94 *in* H. Egna, J. Bowman, B. Goetze, and N. Weidner, editors. Eleventh Annual Administrative Report of Pond Dynamics/Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Kelly, H.D. 1956. Preliminary studies on *Tilapia mossambica* Peters relative to experimental pond culture. Proceedings of the Annual Conference of the Southeast Association of the Game Fish Commission 10:139-149.

Popma, T.J. 1982. Digestibility of selected feedstuffs and naturally occurring algae by tilapia. Ph.D. Dissertation, Auburn University, Alabama. 78 pp.

Pruginin, Y. 1983. Planning aquaculture projects in developing countries. Pages 544-549 *in* L. Fishelson and Z. Yaron, editors. First International Symposium on Tilapia in Aquaculture, Nazareth, Israel, 1983. Proceedings. Tel Aviv University, Tel Aviv, Israel.

Verheust, L, F. Ollevier, K. Veverica, T. Popma, A. Gatera, and W. Seim. 1994. High elevation monoculture and polyculture of *Oreochromis niloticus* and *Clarias gariepinus* in Rwandan ponds. Pages 163-170 *in* H. Egna, J. Bowman, B. Goetze, and N. Weidner, editors. Eleventh Annual Administrative Report of Pond Dynamics/Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

A Comparative Economic Analysis of Small-Scale Fish Culture in Rwanda

Work Plan 6, Study 5

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Introduction

The main objective of small-scale fish farming in developing nations is to supply protein-rich food to the rural people at reasonable prices and to provide them with limited but steady income and employment (Belsare 1986). However, surveys conducted in Rwanda have shown that many small-scale fish farmers consider fish to be a cash crop. Findings by Engle et al. (in press) indicated that fish farming provided cash to the family in addition to supplementing the diet of the Rwandan farmer. Molnar et al. (1991) and Engle et al. (in press) both showed that fish production represents the main cash crop for over 50% of private pond holders and members of cooperative farms. Past studies used partial farm analyses and economic engineering techniques to assess costs and returns to fish production (Moehl 1993, Engle et al. in press). However, no data exist to compare fish farming with other crops in Rwanda in terms of profitability or in terms of generating protein for household consumption.

Objectives

The objective of this study was to compare returns from fish production with those from other crops commonly produced in the Rwandan marais (valley bottom land), both in terms of net cash income and net contribution to animal protein production.

Materials and Methods

A cost of production survey was conducted in September 1991. A random sample was drawn from the universe of fish farmers (the 1250 cooperative and 1050 individual farmers listed with the Rwandan National Fish Culture Service), using a cluster sampling technique (Weisberg et al. 1989). The original sample included 167 cooperative and 113 individual farmers. Of these, 93% of the respondents from cooperatives and 98% of the respondents from individual farms completed the survey, resulting in an overall response rate of 95%.

The survey instrument was developed originally in English and then translated into Kinyarwanda. It was divided into several sections, including farm family characteristics (age, training level, marital status), farm status (land tenure and allocation), farm location and characteristics (size, equipment, and facilities), and farm management. Basic information on livestock and production levels was collected. Information regarding off-farm employment and income, distribution and use of farm output, market outlets, prices, and cash farm income was also collected.

The survey instrument was pre-tested for applicability to each type of farmer and modified according to pre-test results. The pre-test covered approximately 5% of the expected respondents in each category of farmers in three different communes. Farmers interviewed during the pre-test were not included in the final sample.

Enumerators (Rwandan extension agents) were then given a two-day training course at the Kigembe Fish Culture Training Center. They were briefed on the purpose of the survey and the objectives of each question. Emphasis was placed on methods of initiating interaction with potential respondent farmers and the methodology for administering the questionnaire. Role play techniques were used as part of the training sessions. To standardize units of measure for quantitative variables such as "handfuls," "piles," "baskets," and "buckets," enumerators were requested to record the unit of measure and weigh or estimate the weight of the unit in kilograms.

Data collected through direct interviews were supplemented with individual pond records maintained by extension agents. All data were entered on a LOTUS 1-2-3® spreadsheet and cross-tabulation techniques were used to summarize results. Two-way and three-way frequency tables were generated using the SAS-PC tabulation program. ANOVA and chi-square tests were used to test for significance and for relationships between variables.

Enterprise budgeting techniques were used to estimate costs and returns for each of the 12 marais production alternatives for both individual and cooperative farms. These crops included fish (*Oreochromis niloticus*), sweet potatoes (*Ipomoea batatas*), Irish potatoes (*Solanum tuberosum*), cassava (*Manihot esculenta*), taro (*Colocasia esculenta*), sorghum (*Sorghum vulgare*), maize (*Zea mays*), sweet peas (*Pisum sativum*), red beans (*Phaseolus vulgaris*), soybeans (*Glycine max*), rice (*Oryza sativa*), and cabbage (*Brassica oleracea*). Protein, energy, and carbohydrate production for each of these crops were also estimated and compared.

Results

Fish Production Characteristics

The principal field crop of cooperative farmers was sweet potatoes, which occupied 29% of the total cooperative land under cultivation (Table 1). Areas as large as six hectares were sometimes allocated to this crop. Taro, sorghum, soybeans, and fish ponds occupied 9%, 8%, and 8% of the total land area farmed by cooperatives, respectively. Individual farmer respondents raised more fish (80%), beans (6%), sorghum (6%), sweet potatoes (3%), taro (3%), and maize (3%) than cooperative farmers.

Pond construction is a major capital investment item for fish production in many parts of the world. However, in Rwanda all fish ponds are built using human labor. In fact, 74% of the farmers responding made exclusive use of family labor and only

Table 1. Area cultivated and percentage of total cropped area for all respondents in Rwandan study, Survey of Rwandan Fish Farmers, 1991.

Crop	Area cultivated (are)			Total cropped area (%)		
	Min[1]	Max[2]	Mean	Min	Max	Mean
Fish Pond	1	15	3	1	100	11
Sweet Potato	0	600	9	0	99	29
Irish Potato	0	200	2	0	94	7
Cassava	0	100	2	0	74	5
Taro	0	475	4	0	99	13
Sorghum	0	100	3	0	96	9
Maize	0	70	2	0	61	6
Peas	0	1	0[3]	0	17	0
Beans	0	80	2	0	49	7
Soybean	0	305	3	0	79	8
Peanuts	0	0[3]	0[3]	0	5	0
Rice	0	0[3]	0[3]	0	10	0
Cabbage	0	100	1	0	63	4
Total	1	603	31	100	100	100

[1] Minimum
[2] Maximum
[3] Less than 0.1

26% hired some or all of the labor used to construct ponds. Although only 25% of the respondents had records adequate for recording the quantity of labor utilized to construct ponds, the average for these respondents was 391 person-days/are (1 are = 100 m^2), with a range of from 26 to 2,464 person-days.

O. niloticus was by far the leading species cultured. It was grown in monoculture by 95% of all farmers interviewed. Small percentages of responding farmers raised *O. macrochir* in monoculture (2%), *Tilapia rendalli* in monoculture (7%), or *T. rendalli* in polyculture systems (1%).

The overall stocking density varied from 15 to 210 fish/are with a mean of 84 fish/are. Government fish stations or development projects produced 36% of the fingerlings stocked in the ponds of responding farmers, whereas 64% were supplied by private farms (both cooperatively and individually owned).

The average price of *O. niloticus* fingerlings was RWF 330/100 fingerlings (80 RWF= US $1.00). Government-produced *O. niloticus* fingerlings were RWF 320/100, whereas private farmers sold them for an average price of RWF 350/100. The lowest price was RWF 100/100 fingerlings and the highest was RWF 800/100 fingerlings.

During the growing cycle studied, eighty percent of the farmers responding to the survey did not feed their fish. For those who did feed, 4% used brewer wastes, 4% used cereal wastes, 3% used plant leaves, and 3% used other miscellaneous wastes. Most of the farmers (89%) applied fertilizer to the ponds in the form of animal manures, green manures, or compost from household wastes.

The farmers who applied feed and fertilizer to ponds did so in limited quantities. On the average, feed was offered at a rate of 2 kg/are/month. Generally fish were fed once a week and manure was applied once a month.

Water was added to fish ponds weekly. Grass was cut three times a month to feed fish and once a month to fertilize ponds. Farmers tended their fish ponds twice a week and spent 44 minutes a week for this activity. Although some farmers did not manage their fish ponds at all, there were farmers who tended their ponds daily for about three hours a day.

On the average, farmers harvested ponds once a year, generally 11 months after stocking. The overall average weight of fish at harvest was 173 grams. An overall mean annual yield of 16 kg/are/yr was obtained; yields ranged from 1 to 49 kg/are/yr. The overall average proportion of marketable fish was 82%, with a range of 40 to 100%.

Ninety-two percent of the respondents consumed some of the fish they produced and only 8% of the farmers never consumed any fish produced in their ponds. Ninety percent of the farmers marketed some of their fish and 73% gave away part of their harvest. Fifty-six percent of the fish harvested was sold and 28% was consumed by producers. Only small quantities were given away (11%) or used to restock ponds (6%).

Seventy-four percent of the farmers sold fish to non-wage earning neighbors, 24% to wage earning neighbors, 18% in market places, 3% to restaurants, and 6% to bars. Fish were primarily sold by the kilogram (92% of respondents). Seven percent of the farmers responding to the survey sold fish by the piece and 1% sold fish by the bucket.

The overall mean price of food fish was RWF 147/kg; the price ranged from RWF 100 to RWF 257/kg. Most of the respondents (98%) sold fish for cash, 10% sold fish on credit, and none bartered.

Costs and Returns
All enterprises except Irish potatoes showed positive income above variable costs and positive net returns to land, labor, and management (Table 2). Fish farming yielded the highest income above variable costs and the highest net returns to land, labor, and management. The cabbage enterprise ranked second.

When opportunity costs were charged for family labor in the analysis, the cabbage enterprise, managed by individual farmers, was the only profitable enterprise. Fish farming by cooperatives yielded the lowest (highest negative) net returns to land and management. This is because cooperatives allocated labor inefficiently. For example, it was found that 43 person-days per year were used to maintain a one-are fish pond, 11.5 person-days per year were allocated to harvesting 16 kg of fish, and 4.6 person-days per year were used to market 16 kg of fish. The situation indicates a surplus of farm labor, which signifies a condition of disguised unemployment (available work tasks are split among labor resources in such a way that they all seem fully employed, but, in reality, much of their time is spent on unproductive activities).

Table 2. Estimated cost and returns (RWF) for marais agricultural enterprises, Rwanda, 1991.

Crop	Gross Receipts	Variable Cost	Income Above Variable Cost	Total Cost	Net Returns Above Land, Labor & Management	Family Labor	Net Returns to Labor & Management
Fish							
Coop.[1]	3,076	279	2,797	496	2,580	10,404	-7,824
Ind.[2]	3,408	337	3,071	503	2,905	6,228	-3,323
Sweet Potato							
Coop.	1,294	520	774	1,093	201	5,972	-5,771
Ind.	1,471	388	1,083	826	645	2,265	-1,620
Irish Potato							
Coop.	1,275	1,607	-332	1,745	-470	6,260	-6,730
Ind.	2,103	1,789	313	1,895	207	2,113	-1,906
Cassava							
Coop.	1,080	365	715	464	616	7,190	-6,574
Ind.	1,160	955	205	1,031	129	1,810	-1,681
Taro							
Coop.	855	288	567	545	310	7,140	-6,830
Ind.	960	403	557	600	360	1,960	-1,600
Sorghum							
Coop.	810	325	485	502	308	4,870	-4,562
Ind.	540	154	386	332	208	1,350	-1,142
Corn							
Coop.	1,175	407	768	525	650	5,220	-4,570
Ind.	925	424	501	515	410	1,884	-1,474
Sweet Pea							
Coop.	–	–	–	–	–	–	–
Ind.	400	302	98	302	98	440	-342
Beans							
Coop.	1,360	393	967	531	829	5,370	-4,541
Ind.	920	414	506	690	230	1,530	-1,300
Soybean							
Coop.	1,193	674	518	832	360	4,190	-3,830
Ind.	864	412	452	533	331	1,340	-1,009
Peanuts							
Coop.	–	v	–	v	–	–	–
Ind.	1,968	148	1,820	148	1,820	2,170	-350
Rice							
Coop.	–	–	–	-	–	–	v
Ind.	1,325	366	959	369	956	1,530	-574
Cabbage							
Coop.	2,380	429	1,951	508	1,872	7,570	-5,698
Ind.	3,120	551	2,569	611	2,509	1,320	1,189

[1] Cooperative respondents
[2] Individual respondents

In terms of carbohydrates, sweet potatoes gave the highest yield (36 kg/are/yr) (Table 3). The highest amount of energy was obtained from maize production, which produced 131,846 kcal/are/yr. The lowest quantity of energy was obtained from the cabbage enterprise (3,194 kcal/are/yr), and fish farming yielded the second lowest quantity of energy (15,330 kcal/are/yr). This is because fish and cabbage contain only small amounts of fat and carbohydrates.

When opportunity costs for family labor were not included in the analysis, the least expensive source of protein produced by cooperatives was maize (RWF 56/kg, Table 4). The cost of fish protein (RWF 310/kg) was almost six times higher than the cost of maize protein. Peanuts were the least expensive source of protein for individual farmers (RWF 77/kg). Fish ranked seventh in this regard.

When the opportunity cost of family labor was charged in the analysis, soybeans emerged as the most cost-effective way of producing protein for both groups of producers. A kilogram of soybean protein costs cooperatives an average of RWF 610, whereas it costs an individual farmer RWF 314. The production costs of one kilogram of fish protein were RWF 3941 for cooperatives and RWF 2372 for individual producers.

When the quality of protein was accounted for in the analysis, it was found that soybeans remained the cheapest source of protein. One kilogram of soybean protein cost individual farmers RWF 38 when the opportunity cost of family labor was not accounted for in the analysis, and RWF 135 with family labor costs included. For cooperatives, costs were RWF 45/kg without labor costs and RWF 263/kg with labor costs. Peanuts ranked second for individual producers, at RWF 47/kg (family labor not accounted for) and RWF 730/kg (family labor valued at RWF 100/day). Fish ranked third for individuals and fourth for cooperatives, with costs of RWF 76/kg

Table 3. Average annual quantity of protein, carbohydrates, and energy per marais farm activity in Rwanda, 1991.

	Protein (kg/are/yr)			Carbohydrates (kg/are/yr)			Energy (kcal/are/yr)		
	All	Coop.[1]	Ind.[2]	All	Coop.	Ind.	All	Coop.	Ind.
Fish	2.905	2.865	2.963	0	0	0	15,330	15,120	15,640
Sweet Potato	1.976	1.845	2.104	35.928	33.550	38.246	49,376	139,491	159,015
Irish Potato	1.281	1.007	1.668	18.302	14.386	23.830	61,796	48,575	80,461
Cassava	0.296	0.280	299	21.505	20.354	21.750	26,190	24,788	26,489
Taro	0.841	0,788	838	15.739	14.735	16.611	47,823	44,772	50,471
Sorghum	1.511	1.960	1.264	15.002	19.460	12.549	64,190	83,269	53,697
Maize	3.471	9.356	3.103	29.026	33.080	25.952	131,846	150,256	117,879
Peas	1.095		1.095	3.040	•	3.040	16,651	•	16,651
Beans	5.197	6.658	4.506	4.390	5.645	3.820	80,164	103,064	69,754
Soybean	7.016	8.235	5.962	4.516	5.301	3.838	82,873	97,266	70,424
Peanuts	1.923	•	1.923	2.782	•	2.782	45,491	•	45,591
Rice	2.115	•	2.115	40.810	•	40.810	109,710	•	109,710
Cabbage	2.085	1.783	2.339	5.555	4,754	6.235	3,194	2,734	3,586

[1] Cooperative farms
[2] Individual farms

Table 4. Cost of protein (RWF/kg)[a] by enterprise in Rwanda, 1991.

	Protein Efficiency Ratio Not Considered				Protein Efficiency Ratio Considered			
	Cooperatives		Individuals		Cooperatives		Individuals	
	w/out Labor	with Labor	w/out Labor	w/Labor	w/out Labor	w/Labor	w/out Labor	w/Labor
Fish	310	3,941	270	2,372	87	1,110	76	668
Sweet Potato	592	3,829	393	1,469	n.a	n.a	n.a	n.a
Irish Potato	1,733	7,949	1,136	2,403	n.a	n.a	n.a	n.a
Cassava	1,657	27,336	3,448	9,502	n.a	n.a	n.a	n.a
Taro	692	9,753	676	2,883	n.a	n.a	n.a	n.a
Sorghum	256	2,741	263	1,331	144	1,540	148	748
Maize	56	614	166	773	50	548	148	690
Peas	n.a	n.a	276	678	n.a	n.a	178	432
Beans	80	886	153	493	54	599	103	333
Soybeans	101	610	89	314	45	263	38	135
Peanuts	n.a	n.a	77	1,205	n.a	n.a	47	730
Rice	n.a	n.a	174	898	n.a	n.a	80	412
Cabbage	285	4,531	261	826	n.a	n.a	n.a	n.a

[a] 80 RWF= $1.00 (U.S.)

and RWF 87/kg, respectively, when the value of family labor was not included. When family labor was included in the analysis, the fish enterprise ranked sixth, at RWF 668/kg.

In conclusion, enterprise budget analysis showed that fish production yielded the highest net returns to land, labor, and management; sweet potatoes produced the highest yield of carbohybrates; and soybeans were the least expensive source of protein. These results explain why fish is used mostly as a source of cash income, while sweet potatoes remain the major staple source of carbohydrates for household consumption.

Anticipated Benefits

The results of this study demonstrate clearly that fish production is superior, in terms of cash income per unit of land, to other alternative crops that can be raised in the Rwandan marais. This is important because prevailing thought considers aquaculture in subsistence economies to be primarily a source of animal protein for household consumption. In the case of Rwanda, however, the higher net returns to land, labor, and management from fish production compared to other crops explain why farmers sold over half of the fish they produced. Government and international donor policies related to fish farming should take into consideration the importance of fish farming in generating cash income for Rwandan small-scale farmers.

Literature Cited

Belsare, D. K. 1986. Tropical fish farming. Environmental Publications, Karad, India.

Engle, C. R., M. Brewster, and F. Hitayezu. In press. An economic analysis of fish production in a subsistence agricultural economy: The case of Rwanda. Journal of Aquaculture in the Tropics.

Moehl, J. F. 1993. Aquaculture development in Rwanda: a case study of resources, institutions, and technology. Ph.D. dissertation, Auburn University, Auburn, Alabama.

Molnar, J. J., A. Rubagumya, and V. Adjavon. 1991. Sustainability of aquaculture as a farm enterprise in Rwanda. Journal of Applied Aquaculture 1(2):37-62.

Weisherg, H. F., J. Krosnick, and B. D. Bowen. 1989. An Introduction to Survey Research and Data Analysis. Second edition. Scott, Foresman, and Co., Glenview, Illinois.

Pond Dynamics Under Semi-Intensive and Intensive Culture Practices

Work Plan 6, Study 5

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Introduction

Tilapia are commonly grown in Thailand using semi-intensive culture with fertilization to increase primary production and fish food. Size at harvest under such systems usually averages 200 g in five months, and it may take as long as five more months to rear fish to 450 g under semi-intensive culture. Market prices in Thailand and many other areas are much higher for large tilapia than small ones, and the demand for larger tilapia can be met either by extending the growout period or by adding feed to the ponds. A previous study conducted under the CRSP showed that although fish growth increased with supplemental feeding, final size at harvest did not reach 450 g and growth appeared to be limited by poor water quality (Diana et al. 1993). That study used three treatments: fertilization alone, *ad libitum* feeding alone, and fertilization and feeding combined. Better water quality may be maintained by using smaller amounts of feed to supplement the basic fertilization regime. Such a system could also be more economical because the addition of feed would be limited to the amount eaten, which would result in less food wastage.

Objectives

The purpose of this study was to evaluate the effects of incremental increases in supplemental feeding rates on the production of tilapia in fertilized ponds. To accomplish this, five treatments were tested:

1. Ponds with triple superphosphate and urea supplements only;
2. Ponds with *ad libitum* feeding and no fertilization;
3. Ponds with 75% *ad libitum* feeding and fertilization;
4. Ponds with 50% *ad libitum* feeding and fertilization;
5. Ponds with 25% *ad libitum* feeding and fertilization.

Materials and Methods

The experiments were conducted in 250-m^3 ponds at Bang Sai in Thailand. Each treatment was triplicated. All ponds were initially fertilized on 5 June 1992. Sex reversed *Oreochromis niloticus,* averaging 10.1 g per fish, were stocked at 3 fish per m^2 (750 fish per pond) on 11 June 1992. Stocking and harvest results for each pond are given in Table 1. Treatment A consisted of inorganic fertilization only at 60 kg/ha/wk of urea and 35 kg/ha/wk of triple superphosphate. Treatment B included *ad libitum*

Table 1. Stocking and harvest data for the 15 experimental ponds. Treatment A=fertilizer only, B=*ad libitum* feeding only, C=75% *ad libitum* feeding and fertilizer, D=50% *ad libitum* feeding and fertilizer, E=25% *ad libitum* feeding and fertilizer.

Pond	At Stocking Number	Size (g)	Biomass (kg)	At Harvest Number	Size (g)	Biomass (kg)
A1	750	11.5	8.6	673	149.2	100.4
A2	750	11.7	8.8	588	195.0	114.7
A3	750	11.4	8.6	580	104.1	60.4
B1	750	12.0	9.0	607	370.3	224.8
B2	750	11.3	8.4	583	479.6	279.6
B3	750	11.5	8.7	638	398.3	254.1
C1	750	11.4	8.6	657	452.1	297.0
C2	750	12.1	9.1	697	427.6	298.1
C3	750	11.8	8.9	670	329.3	220.7
D1	750	11.4	8.6	605	376.0	227.5
D2	750	10.9	8.2	626	372.8	233.4
D3	750	11.8	8.8	605	415.0	251.1
E1	750	12.0	9.0	658	239.5	157.6
E2	750	11.3	8.5	646	282.2	182.3
E3	750	11.3	8.9	566	228.3	129.2

feeding only, with no fertilization. The *ad libitum* ration was determined weekly using a floating feed at controlled rates and determining the total amount of feed taken in one feeding. Initial maximum feeding rates were 480 g per day (4.7% BW/d) and these rates gradually increased to 3000 g per day (1.2% BW/d) by harvest. Treatments C, D, and E consisted of fertilization supported by feeding at 75%, 50%, and 25% of the *ad libitum* ration, respectively. Water chemistry analyses were conducted monthly. Ponds were harvested on 13 November 1992 (155 days). Treatment effects were tested statistically by analysis of variance (ANOVA) and multiple regression. Results were considered significant if $p<0.05$. For multiple regression, values were included in the regression if $p<0.10$.

Results

Growth, survival, and yield in each pond are shown in Table 2. Growth differed significantly between treatments (ANOVA, $p<0.05$), with ponds fed at levels of at least 50% *ad libitum* having the highest growth rate, ponds fed at 25% *ad libitum* having an intermediate growth, and fertilized ponds having the lowest growth (Figure 1). The mean weights of fish increased steadily in each treatment (Figure 1).

The final net yield (kg/ha) in each pond differed significantly among treatments (ANOVA), with trends similar to growth results among treatments. There were no significant differences in survival among treatments (ANOVA).

The results of this experiment were much more consistent among replicates than many of our previous experiments, which have exhibited high variability among ponds in a treatment for fish growth, survival, and yield. However, the growth of fish was not only significantly related to feed input rate and presence of fertilization,

Table 2. Growth, survival, and yield of fish from each of 15 experimental ponds. Treatments as in Table 1.

Pond	Growth (g/fish)	Survival (%)	Yield (kg/ha)
A1	137.68	89.7	3671
A2	183.29	78.4	4235
A3	92.70	77.3	2073
B1	358.35	80.9	8632
B2	468.34	77.7	10846
B3	386.74	85.1	9818
C1	440.61	87.6	11537
C2	415.49	92.9	11558
C3	317.49	89.3	8471
D1	364.54	80.7	8756
D2	361.96	83.5	9010
D3	403.28	80.7	9691
E1	227.53	87.7	5944
E2	270.88	86.1	6952
E3	216.46	75.5	4814

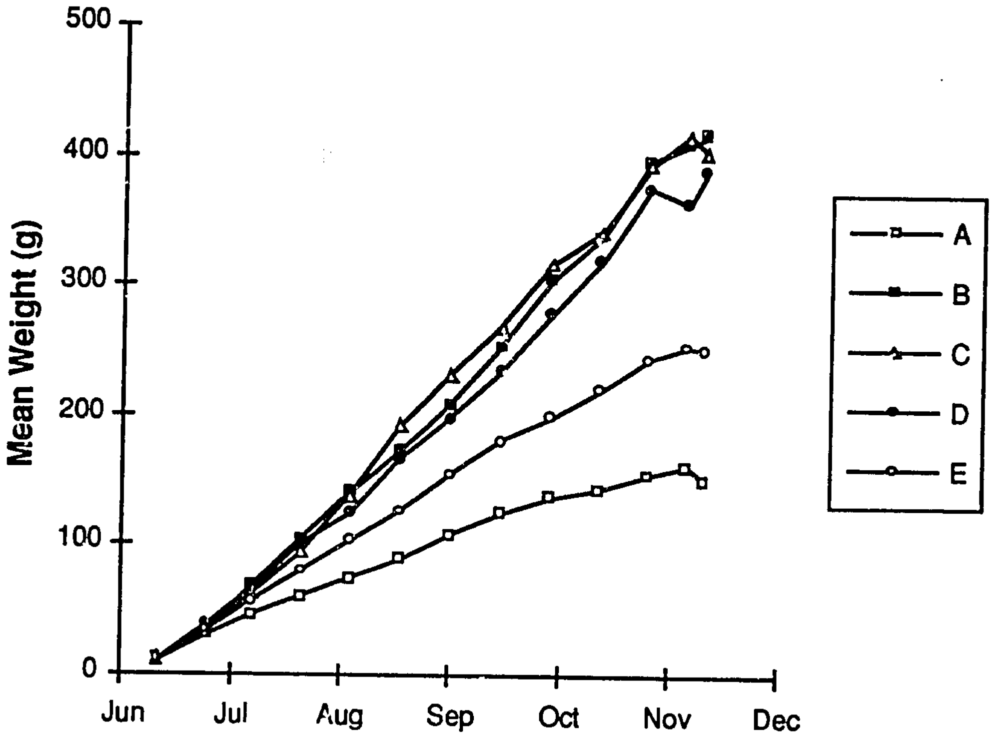

Figure 1. Changes in average weight of tilapia for all ponds per treatment. Treatment A=fertilizer only, B=*ad libitum* feeding only, C=75% *ad libitum* feeding and fertilizer, D=50% *ad libitum* feeding and fertilizer, E=25% *ad libitum* feeding and fertilizer.

but also to ammonia levels, which varied among both, ponds and treatments (Table 3). These variables produced a strong multiple regression (R^2=0.887, $p<0.001$). High ammonia levels (>1 mg/L) occurred in all ponds, and 18% of all ammonia measures exceeded 1 mg/L. Ammonia concentrations were negatively correlated with fish growth, indicating that water quality was deteriorating in later stages of the growout, although it did not affect survival.

Fish size exceeded 450 g at harvest in only 2 of the 15 ponds, although it approached 450 g in two others. All treatments with at least 50% *ad libitum* feeding levels had similar growth. Because the growth was similar in these three treatments, the best production system would be to feed at 50% *ad libitum* and fertilize, since input costs would be considerably less than for complete feeding. While the final weight did not reach 450 g in many treatments, an extension of the growout period by one month would at most result in all three of the high feed treatments reaching 450 g, because the ponds did not appear to reach carrying capacity in the experimental period.

Ammonia levels in the ponds were negatively correlated with fish growth. Ammonia concentrations increased somewhat during the growout period, and were highest in the three treatments with highest feed inputs (Figure 2). The levels became particularly high in Treatment C. It is difficult to understand why that treatment would become so high in ammonia, when Treatment B had higher nitrogen input rates.

Table 3. Multiple regression values for the relationship between growth, the main input variables, and ammonia concentrations.

Variable	Coefficient	Partial r	P
Feed	274.515	0.872	0.001
Fertilization	106.182	0.551	0.006
Ammonia	-118.335	-0.569	0.042
Constant	55.546		0.307

Dissolved oxygen levels in the morning were initially high in all ponds, then became low in all treatments for most of the experiment, returning to high levels just prior to harvest (Figure 3). While dissolved oxygen did not differ significantly among treatments and was not significantly correlated to fish growth, it may have been low enough in all ponds to limit growth overall. However, it did not affect between-pond differences in growth. The reason for the return of high oxygen levels just prior to harvest is unclear, considering that the ponds had their highest fish biomass and highest feed input rates at that time.

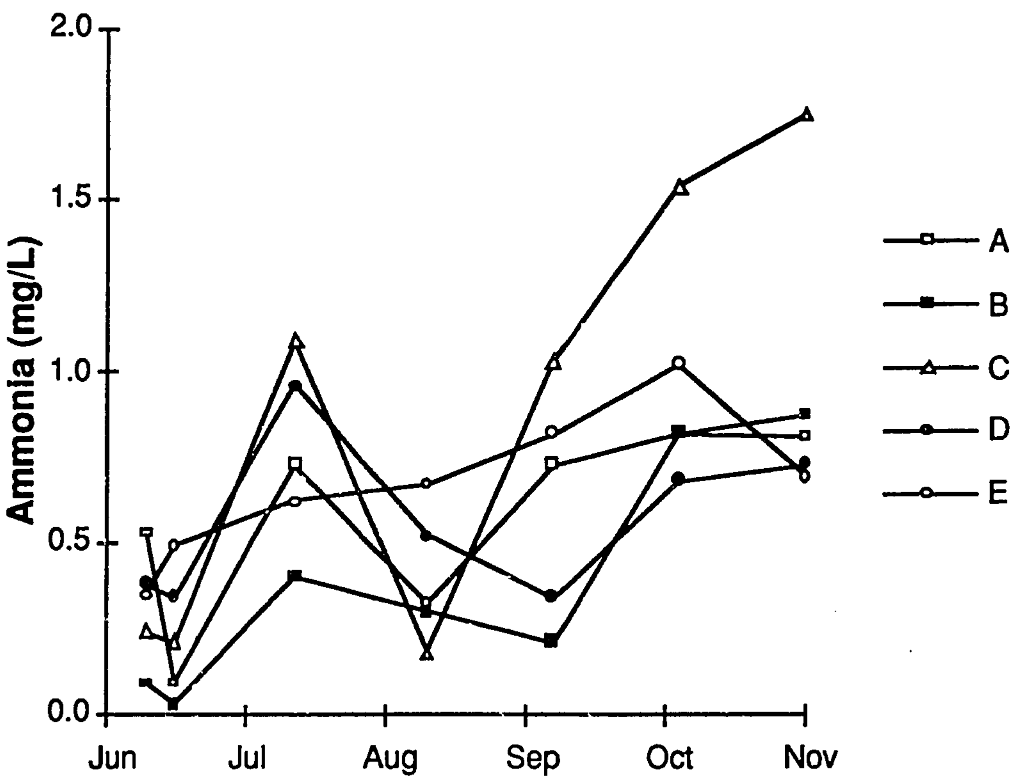

Figure 2. Average monthly changes in ammonia concentration in each treatment. Treatments as in Figure 1.

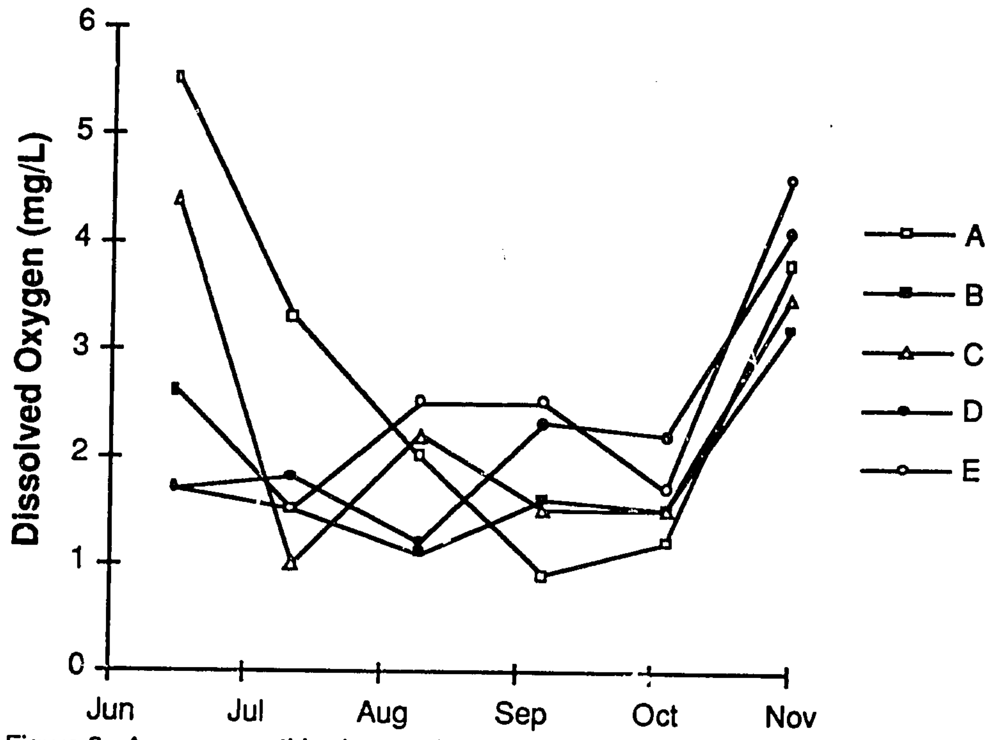

Figure 3. Average monthly changes in morning dissolved oxygen in each treatment. Treatments as in Figure 1.

Anticipated Benefits

Supplemental feeding resulted in more rapid growth of tilapia, particularly in the last three months of culture, and significant increases in market size (from about 180 to 400 g). There were no significant differences in fish growth between ponds not fertilized but fed *ad libitum* and fertilized ponds supplemented with feed at 75 to 50% of the *ad libitum* feeding rate, indicating that fertilization was also effective in producing natural feeds in intensive tilapia ponds. Hence, fish farmers can save up to 50% of feed cost, by supplementing the basic fertilization regime with 50% *ad libitum* feeding rather than with 100% *ad libitum* feeding.

Literature Cited

Diana, J., C.K. Lin, and K. Jaiyen. 1993. Supplemental feeding of tilapia in fertilized ponds. Pages 58-62 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin. Tenth Annual Administrative Report. Pond Dynamics/Aquaculture Collaborative Research Support Program. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Evaluation of Low Cost Methods for Destratification and Oxygen Conservation in Tropical Ponds

Work Plan 6, Study 8

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Introduction

Earthen ponds in warm climates characteristically exhibit intense density stratification during daytime. This condition isolates bottom water, allowing it to become depleted of dissolved oxygen (DO) through community respiration without replenishment from upper photosynthetic layers. Depletion often persists for much of the night as well, and is only relieved by vertical transport due to convective overturn, when modest amounts of DO are mixed into the bottom layers. Oxygen depletion typically occurs again within a few hours. If the oxygen-poor hypolimnion is sufficiently large compared with the oxygenated photic zone, mixing by convective overturn may be detrimental to the pond as a whole, with the final uniform DO concentration being below that required for the cultured animals. A further potential ill effect of bottom isolation is the prevention of regenerated nutrients from reaching the upper layers, thus potentially limiting production of DO even in the lighted zone.

Mechanical devices (mixers or aerators) are often used to destroy density stratification and to increase DO concentrations in ponds. Fish or shrimp production improves under night-long or continuous active aeration when compared with ponds that receive only emergency aeration. Active aeration is generally avoided in the daytime, however, to conserve the typical supersaturated surface DO levels so that this oxygen will be available for use after dusk. Mixing without active aeration has the potential to redistribute DO through space and time to enhance conservation of DO. Such conservation has the obvious potential of sparing more costly active aeration for emergency use during critical parts of the diel cycle.

Szyper and Lin (1990) have shown that properly-timed mixing relieves bottom DO depletion during daylight and early evening hours, and conserves surface supersaturation levels from loss to the atmosphere by diffusion. In their investigation they employed only one mixing device (an electric submersible pump which brought water from 80 cm depth to the surface) in two ponds of similar surface area (approximately 400 m^2), and compared effects of mixing during two different subsets of the daytime period. Stratification was more effectively destroyed (and bottom conditions improved earlierin the day) by mixing during two one-hour intervals (1200-1300 and 1500-

1600 hours) than during one two-hour interval (1500-1700 hours). It was also observed that destratification by this means was energetically inefficient, in the sense that the energy involved in maintaining stratification was far less than 0.01% of the electrical energy consumed by the pump.

In order to develop design criteria for cost-efficient pond mixing, I compared the performance of three mixing devices in ponds of four sizes. Water depth was maintained at 100 cm in all ponds, and all mixing treatments were performed between 1300 and 1600 hours each day; this time period was chosen to optimize differences between mixing treatments by working against the day's most intense stratification.

Objectives

1. To compare the capabilities of three devices which use different mixing strategies.
2. To compare the performances of the mixing devices, at fixed energy consumption levels, in ponds of different sizes.
3. To examine the results for trends and parameters which may guide the development of efficient destratification strategies or of future experiments.

Materials and Methods

Observations of diel temperature cycles were made at the Asian Institute of Technology during a two-week period (24 February to 11 March 1992) within the five-month experimental period of Work Plan 5 Study 9, "Effects of Pond Size." Sets of two adjacent ponds were chosen from arrays of ponds of four different sizes; surface areas were approximately 200, 400, 800, and 1600 m^2. One pond from each pair was designated the "permanent control," and was never mixed artificially. The other member of each pond pair was first examined without mixing ("double control") for comparison to the permanent control, and then mixed from 1300 to 1600 hours on each of the three succeeding days with one of the three devices described below. The double control observation was repeated at the end of the trials with the largest ponds only. In the mixed 1600 m^2 pond only, temperature observations were made simultaneously at three locations.

Temperature was recorded at 8 depths (2, 10, 20, 30, 40, 50, 70, and 90 cm) at each monitored location every 15 minutes from thermocouples mounted on plastic pipes embedded in pond bottoms. Installations were tended daily, and water depths were well maintained. Each monitored location generated approximately 800 temperature recordings per day. Ponds were characterized qualitatively by the patterns in their isotherm diagrams (Figures 1 and 2) and quantitatively by a stratification index (S) commonly used in limnological studies and detailed by Szyper and Lin (1990). This index consists of the energy per unit area stored in the unequal distribution of density in stratified ponds, and, when multiplied by the pond area, provides an estimate of the minimum energy theoretically required to mix the pond to uniform temperature. The stratification indices are not yet available; results will be discussed in terms of the isotherm diagrams.

The three mixing devices were 1) a Rule 2000 submersible 12 VDC pump (SP), which was deployed at 80 cm depth and discharged water horizontally just below the surface; 2) an air lift tube (AL) of 10 cm diameter which took in water at 80 cm depth and discharged it at the surface, the air being driven by a 12 VDC pump on the pond bank; and 3) a small (1/6 hp, 110 VAC) vertical fan blade aerator called the "Ice-Eater" (IE) which was designed to throw surface water up into the air, but which was deployed sufficiently deep to take in water at about 40 cm depth and discharge it near the surface without a splashing effect. The power consumption of each device was measured with portable meters during typical operation. The SP was not used in 800 m^2 ponds due to time constraints, however.

Results

The observed power consumption of each mixing device was close to that expected from the manufacturer's specifications and the state of battery charge. The SP operated at 86.1 W, the AL's air pump at 63.5 W, and the IE at 173.6 W (1/6 hp = 162.2 W). Thus the IE consumed twice the power of the SP, which in turn consumed slightly more than the AL. The AL displaced water at zero head (into buckets at water surface) slightly more efficiently than the SP. The AL pumped 75.8 L/min (liters per minute) and the SP at 90.0 L/min, showing efficiencies of 1.19 and 1.04 L/min/W, respectively. Water displacement by the IE was not estimated, but appeared to be much greater than that of the other devices.

Since pairs of unmixed ponds showed very similar isotherm patterns they are expected to have similar values of S. Isotherm patterns varied more strongly on different dates for the same pond than for different ponds on the same date.

The IE proved to be the most powerful mixing device in ponds of all sizes, which was expected due to its power consumption and a qualitative estimate of its water displacement. Figures 1 and 2 compare the effects of IE operation from 1300 to 1600 hours in the 800 and 1600 m^2 ponds, respectively. In Figure 1, the nearly vertical isotherms between 1200 and 1600 hours show the effect of mixing in an 800 m^2 pond (lower plot) compared with the permanent control (upper plot). This pattern was similar to that produced by the IE in ponds of 200 and 400 m^2 ponds, except that the mixing process was slightly faster in the smaller ponds. In 1600 m^2 ponds, the IE's effect is slower and less complete. Data from the supplemental temperature observations at two additional locations in the mixed 1600 m^2 pond were similar to each other and to those at the primary location.

Discussion

All devices under investigation were able to modify typical intense midday stratification in tropical ponds. The most powerful of the devices (IE), which at 1/6 hp was in fact much less powerful than the smallest commercially available aerator, was able to mix a 1600 m^2 pond to uniformity during the hours of most intense solar irradiance. The 1600 m^2 pond, however, was large enough (contained enough energy in its vertical density distribution) to indicate limits to the mixing ability of the IE. However, any of the mixers could prevent development of stratification in ponds up to 1600 m^2 if mixing is initiated earlier in the day, as discussed by Szyper and Lin (1990).

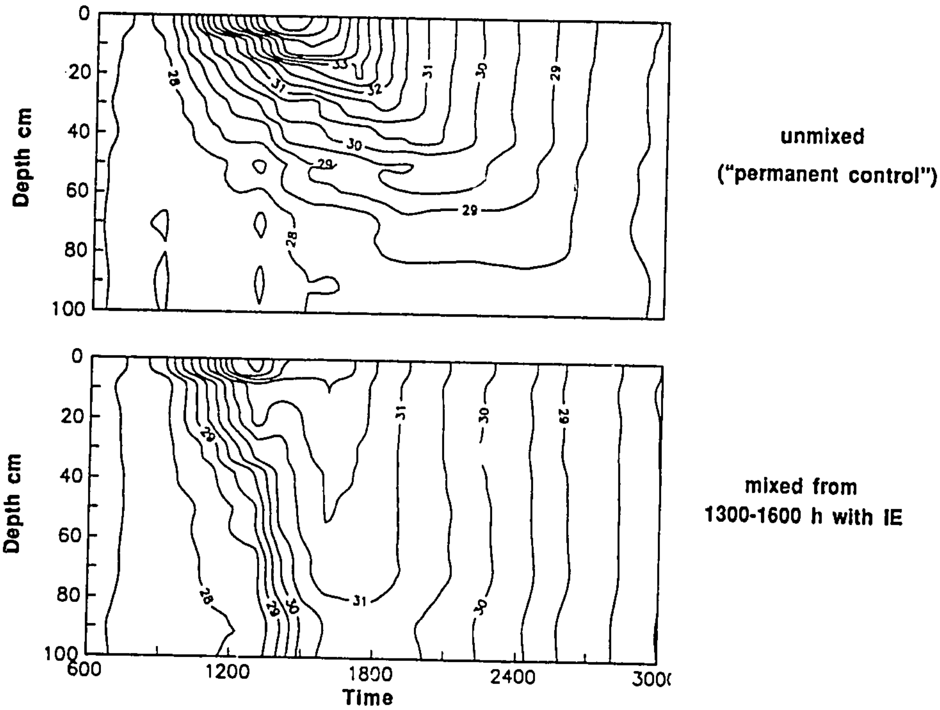

Figure 1. Isotherm diagrams showing a single diel temperature cycle in two neighboring ponds of 1 m depth and 800 m^2 area. The upper plot represents observations made in an unmixed control pond; the lower plot represents observations made in a pond mixed from 1300 to 1600 hours with the "Ice Eater" device discussed in the text.

Comparisons of the onset of isothermal conditions within ponds of a given size suggest that the SP was the least effective mixing device. It was also the least efficient in terms of electrical consumption per unit water displacement. The AL was approximately as effective as, or only slightly less efficient than, the IE in ponds smaller than 1600 m^2. In these largest ponds, the AL appeared to be less effective, which is consistent with its lower water displacement rate. The performance of the AL suggests that it was more effective at "S-reduction" per unit power consumption than the IE, which is consistent with the suggestion of Szyper and Lin (1990) that slower and more diffuse application of mixing energy would be more efficient than rapid point-source application. Both systems cost approximately the same, but considerably less than active aerators.

Final analysis of this data will entail calculation of the numerical index of the energy of stratification (S). Analysis will include quantification of the variation observed between neighboring ponds under control (unmixed) conditions, and of the variation observed among sites within a single pond using ANOVA techniques.

Anticipated Benefits

This work provides information which might in some cases directly indicate a mixing strategy for a particular pond system. More generally, this information can be used to design definitive replicated experiments aimed at developing optimal mixing strategies for pond systems with different characteristics.

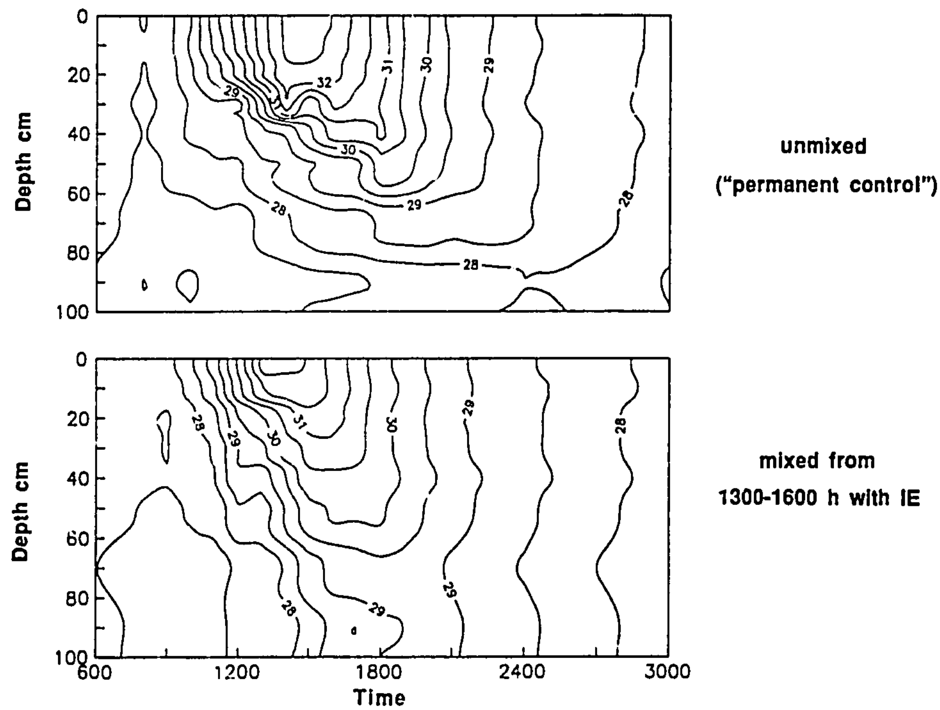

Figure 2. Isotherm diagrams showing a single diel temperature cycle in two neighboring ponds of 1 m depth and 1600 m^2 area. The upper plot represents observations made in an unmixed control pond; the lower plot represents observations made in a pond mixed from 1300 to 1600 hours with the "Ice Eater" device discussed in the text.

Literature Cited

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

Data Analysis and Synthesis

Introduction

Title XII of the International Development and Food Assistance Act of 1975 implies that CRSP research activities should be mutually beneficial to developing countries and the United States. In planning this CRSP, the consensus among CRSP participants was that this requirement would be met through collaborative research involving both the US and developing country institutions. However, subsequent to awarding the CRSP grant, USAID interpreted "mutually beneficial" to mean that the CRSP should fund research activities both in the developing countries and in the US and instructed the CRSP to support research activities at the US institutions. While various studies related to the Global Experiment and biotechnological research are conducted at US universities, all research performed by the Data Analysis and Synthesis Team and the maintenance of the CRSP Central Data Base are US-based activities.

The U.S. research component was also established in response to the needs of the CRSP participants themselves. CRSP scientists soon became aware that the enormous amount of data their research generated created a specific problem. In order to make all the data accessible to not only other CRSP researchers, but also to the aquaculture community at-large, the establishment of a standardized, central data storage and retrieval system became an inevitable necessity. Hence, in 1986 a Central Data Base was installed at Oregon State University under the auspices of the Program Management Office.

In April 1993, the CRSP Central Data Base was moved to the University of Hawaii at Hilo. With this move, the Central Data Base is now treated as a project and is no longer administered by the Program Management Office. The University of Hawaii at Hilo was the winning proposal among three that were submitted by CRSP principal investigators in response to a Request For Proposals. Data entry from the Fourth Work Plan had been completed at Oregon State University prior to the transfer. Data generated from studies proposed in the Fifth and Sixth Work Plan are currently being entered.

The function of the Data Analysis and Synthesis Team (DAST) which was formally established in 1986 is to provide a comprehensive analysis and interpretation of the global data available through the CRSP Central Data Base. During this reporting period, the DAST has been focusing on the refinement of two important pond processes: respiration and dissolved oxygen dynamics. They also are in contact with the host country teams regarding the verification of CRSP management guidelines.

Data Base Management

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A centralized database for storage and dissemination of data collected from CRSP experiments was established as part of the Program Management Office (PMO) in the early 1980s (Hopkins et al. 1988). The database facilitates data analysis by the CRSP Data Analysis and Synthesis Team (DAST) and provides access to CRSP data for outside researchers. Because much of the data collection and reporting is standardized, comparisons of the various CRSP sites and global data synthesis are enhanced.

The CRSP Data Base was designed for ease of data entry by field personnel. Spreadsheets, primarily Lotus 1-2-3™ and Excel™, are used for data entry. These spreadsheets are then imported into RBase™, consolidated, and stored. Access to the consolidated RBase™ files is currently done interactively and requires both a knowledge of RBase™ and ownership of the RBase™ program. Thus, distribution of the data usually requires the CRSP Data Base manager to export specifically requested data into spreadsheets or ASCII files for distribution.

The operating procedures described above served the CRSP well when the primary user of CRSP data was the DAST. However, the power of the database for browsing, combining, and comparing has not been easily available for other researchers. Cognizant of this limitation and desirous of a more widespread distribution of the entire CRSP Data Base, the CRSP Management Entity and Board of Directors ordered an examination of means to improve accessibility to the database. This directive resulted in plans for transferring the database from the PMO to another location upon the resignation of Hilary Berkman, longtime database manager at Oregon State University. An RFP was issued by the PMO in 1992 and three proposals were submitted (by University of Hawaii at Hilo, Oregon State University Department of Fisheries and Wildlife, and University of Michigan). After the proposals were reviewed by the Technical Committee and Board of Directors, the Management Entity decided to transfer the Central Data Base to University of Hawaii at Hilo. Work Plan 4 data were entered and verified at OSU prior to the transfer of the Central Data Base in April 1993.

Data from the Fifth and Sixth Work Plans are still being processed and entered. The RBase™ files currently require in excess of 30 megabytes for storage. Efforts are currently underway to reduce the data storage requirements, possibly by as much as 50%, and to write a menu-driven interface which will not require users of the database to be familiar with the RBase™ query language. Once the interface is compiled, it will allow the distribution of the entire database as a self-contained unit. Additionally, changes in the database structure are being implemented to allow the submission of data from both the global and site-specific experiments.

Literature Cited

Hopkins, K.D., J.E. Lannan, and J.R. Bowman. 1988. Managing a database for pond research data – the CRSP experience. Aquabyte 1(1):3-4.

Data Analysis and Synthesis Team

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During this reporting period, the Data Analysis and Synthesis Team (DAST) consisted of three principal investigators, Drs. John P. Bolte and James E. Lannan at Oregon State University (OSU) and Dr. Raul H. Piedrahita at University of California, Davis (UCD). Their efforts are focused on the following areas of activity:

1. Improvement of PONDCLASS operation, refinement of existing fertilization and liming routines, and addition of new functionality in the form of a fish growth simulation utility (OSU).
2. Development of simple models for long-term simulation of fish growth and water temperature (OSU).
3. Development of software architecture suitable for the next generation of decision support systems, and implementation of existing PONDCLASS functionality in the new environment (OSU).
4. Development of improved methods for modeling and optimizing oxygen regimes in ponds (UCD).
5. Development of models for stratified ponds (UCD).

During this period, the IBM-PC version 1.1 of PONDCLASS was modified considerably to simplify data entry and editing. The lime requirement utility was modified to enable users to change variables like liming depth and soil density, to make selections from a list of possible liming materials, and to compare costs for the different materials. New functionality was implemented in the form of a fish growth simulation utility. This utility allows users to simulate fish growth and water temperature in ponds over long periods of time. Simplified methods of approximating the data requirements for these models are also available in the growth simulation utility. An object-oriented software architecture suitable for the next generation of decision support systems is being developed. The new software will provide two levels of functionality: (a) detailed short-term analysis of pond dynamics, with the intent of providing users with a tool for diagnosing possible production problems and prescribing strategies to deal with them, and (b) lower resolution views of pond dynamics for long-term simulations in order to monitor resource inputs and generate enterprise budgets for whole facilities. A version of this software that includes much of the original functionality of PONDCLASS as well as the fish growth and water temperature models has been completed.

Improvements in techniques for the measurement and analysis of diel changes in respiration rates in ponds have been one of the areas of emphasis during this period. Models were developed for predicting temperature and dissolved oxygen concentrations at three depths in stratified ponds, using data from the PD/A CRSP Data Base and from other research.

The reports below describe in detail the activities of the DAST during this period. Information derived from the various models and experiments continues to be presented to other CRSP participants by means of a newsletter.

Decision Support Systems for Pond Aquaculture

Work Plan 6, Study 1

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Introduction

Research by the Oregon State University DAST has emphasized development of adequate descriptions of the pond ecosystem from a management perspective, and translatation of these system descriptions into computer-based analysis tools. Such computerized tools are often called "decision support systems." The term *system description* is used in this paper in a generic sense to indicate a mathematical model or an analogous abstraction of the pond ecosystem.

Within the broad areas of emphasis indicated above, we have concentrated both on the improvement of software already developed by the Oregon State University DAST, resulting in the completion of PONDCLASS version 1.2 (Work Plan 6, Study 1), and on the implementation of a completely new approach to the decision support system (Work Plan 7, Studies 3 and 5).

Objectives

The objectives of the Oregon State University DAST during the current reporting period are:

1. To enhance the user-friendliness and improve the existing functionality of PONDCLASS version 1.1.
2. To develop and adapt existing models for aquaculture pond systems and implement the models in the form of an analysis tool.
3. To develop a software architecture suitable for the next generation of pond aquaculture decision support systems, and to transfer the functionality of the original version of PONDCLASS to the new software environment.

Revisions to PONDCLASS

A common criticism from users of PONDCLASS version 1.1 was the limited user-friendliness of the program. This concern has been addressed in version 1.2 (programmed in True Basic, and available only for the IBM-PC), and new functionality has been added in the form of a utility that allows long-term simulation of fish growth and pond water temperature (described separately in Section 2 below). Minor modifications have also been made to the fertilization and lime requirement routines of the program.

Operational Details
The tasks of data entry and editing in PONDCLASS have been considerably simplified by the inclusion of data entry screens, which allow users to enter or edit data in different fields on a single screen. On-line help, which indicates the kind of data needed for each field, is available in association with the data entry screens. The display screens for program output have also been improved. Other operational refinements include the addition of computer code that optimizes program operation based on the available hardware, and direct printer control for graphics output.

Modifications to Fertilization and Lime Requirement Calculations
In the earlier version of PONDCLASS, the assumption was made that all of the nitrogen (N) and phosphorus (P) from the fertilizer(s) was available for algal uptake following fertilizer application. There is evidence from CRSP research to invalidate this assumption, particularly for animal manures (Nath 1992, Knud-Hansen et al. 1991). However, the proportions of N and P that actually become available (availability coefficients) are likely to vary among different fertilizers. We have addressed this variability in PONDCLASS version 1.2 by requesting users to enter N and P availability coefficients for each of the selected fertilizers. The total N and P contents of the fertilizers weighted by their respective availability coefficients are then used in fertilizer calculations.

The utility for estimating the lime requirement of pond soils in PONDCLASS has been revised to allow users to vary the liming depth (the depth of pond soil within which the liming reaction is expected to occur), select liming materials, and compare the costs of different materials.

In PONDCLASS version 1.1, lime requirement (L) was defined as the amount (kg/ha) of calcium carbonate, $CaCO_3$, required to neutralize the exchange acidity of a pond soil following Peech (1965) as:

$$L = E (B_{des} - B_0) \omega \quad (1)$$

where E = cation exchange capacity, B_{des} = desired percent base saturation, B_0 = initial percent base saturation, and ω = a correction term required to convert soil acidity (meq/100g) to the amount of lime (kg $CaCO_3$) applied to a mass of soil. Bowman (1992) expanded the correction term in Equation 1 into its component parts as follows:

$$L = E (B_{des} - B_0) CF \, D \, D_b \quad (2)$$

where CF = correction factor for converting soil acidity units into lime application units (kg $CaCO_3$/ha-cm soil) which actually takes on a value of 50 (the equivalent weight of $CaCO_3$), D = liming depth (cm) and D_b = soil density (g/cm^3). The value of 750 for ω in PONDCLASS version 1.1 was based on the assumption that the liming depth was 15 cm (Boyd 1979) for a soil with a density of 1 g/cm^3. This assumption may not be valid for all soil groups. Therefore, computations in PONDCLASS 1.2 are based on Equation 2, and users can edit all the terms except the correction factor (CF) which is a constant.

The soil lime requirement estimated by PONDCLASS 1.1 was expressed as kg pure $CaCO_3$/ha. Users were required to manually multiply this requirement by the lime coefficients for other liming materials (ratio of the neutralizing value of pure $CaCO_3$ to that of the liming material) to estimate the quantity of the material actually required. In PONDCLASS 1.2, however, users can select from some common liming materials and the program will calculate the equivalent amount required together with the cost of the liming material based on data entered by the user.

Models for the Pond Ecosystem

When fish ponds are examined from a production point of view, the questions often asked are "How much yield can I expect given a particular site and a certain set of management practices?" or "What is the expected average size of fish after a given number of days?" Such questions are difficult to answer because of the complex nature of fish ponds and their inherent variability. However, because we are beginning to gain an increased understanding of such systems and have access to improved analysis tools, it is possible to make certain generalizations about fish growth and translate them into appropriate models, which users can use as analysis tools to explore different scenarios. A systems approach is required to address the overall problem because fish growth results from exposure to a complex pond environment, implying that variables influencing growth have to be simultaneously estimated and used in the growth model.

Most models require considerable data input to generate meaningful results. Because users of decision support systems may not often have access to some or all of the required data, it is necessary to provide them with simplified means of either approximating or generating such data. The status of two models (for fish growth and water temperature) and their data requirements is discussed below. Both models use a time step of one hour, and the equations are solved using a 4th-order Runge-Kutta numerical integration method. The fish growth model, which shares some features with a previously developed CRSP model (Liu and Chang 1992), is primarily intended to describe the growth of Nile tilapia (*Oreochromis niloticus*), although the rationale may be applicable to other fish species as well.

Fish Growth Model

Ursin (1967) expressed the rate of change of body weight of fish as the difference between anabolism and catabolism:

$$dW/dt = H\,W^m - k\,W^n \quad (3)$$

where W = weight of fish (g), t = time (d), H = coefficient of anabolism (g^{1-m}/d), k = coefficient of catabolism (g^{1-n}/d), m = exponent of body weight for anabolism, and n = exponent of body weight for catabolism. Because catabolism comprises feeding and fasting components (Ursin 1967), Equation 3 can be written as:

$$dW/dt = [\,b\,(\,1 - a\,)\,dR/dt\,] - k\,W^n \quad (4)$$

where b = efficiency of food assimilation (dimensionless), dR/dt = rate of food consumption or daily ration (g/day), and a = fraction of the food assimilated that is used for feeding catabolism (dimensionless). From an energetic point of view, the parameter b refers to the proportion of the gross energy or food intake that is available as metabolizable energy, and is typically not constant, but decreases with increasing food availability for most fish, including tilapias (Caulton 1982). The parameter a accounts for further losses of metabolizable energy via heat increment and urinary excretion. Thus, net energy available for maintenance and growth is represented by the first term on the right hand side of Equation 4, whereas the second term represents only maintenance requirements.

It is possible to describe certain types of feeding behavior mathematically. For instance, most tilapia species, including *O. niloticus*, tend to feed during daylight hours (Caulton 1982). This implies that food consumption is zero at night, so that the first term on the right hand side of Equation 4 also reduces to zero, assuming a similar pattern for digestion.

The daily food consumption rate can be described by the function (Ursin 1967, Cuenco et al. 1985):

$$dR/dt = h\,c\,W^m \quad (5)$$

where h = coefficient of food consumption (g^{1-m}/d), and c = parameter that describes the overall effects of different factors on food consumption (dimensionless).

Food consumption is influenced primarily by temperature, food availability, dissolved oxygen, and unionized ammonia concentrations (Brett 1979, Cuenco et al. 1985). Assuming that food intake is not limited by low dissolved oxygen or high unionized ammonia concentrations, temperature and food availability become the primary variables that determine food consumption. Therefore, Equation 5 can be expressed as (Ursin 1967, Cuenco et al. 1985):

$$dR/dt = h\, f\, \tau\, W^m \quad (6)$$

where f = relative feeding level, and τ = parameter describing the relative effects of temperature on food consumption, both of which are dimensionless.

The relative feeding level indicates the quantity of food available for consumption, and takes on a value of one under conditions of non-limiting food availability. According to Hepher (1978), the amount of food available to pond fish depends on fertilization practices (and supplemental feeding, if used) and the standing crop of fish. Typically, during the initial period of culture, fertilized ponds produce enough natural food to ensure non-limiting food availability, and natural food becomes insufficient to meet food requirements of fish feeding at their maximum consumption rate only at the critical standing crop (CSC) for the pond (Hepher 1978). We have not yet been able to identify a means of predicting CSC levels for ponds. However, data for a red variant of *O. niloticus* (Zonneveld and Fadholi 1991) suggest that the relative feeding level (parameter f in Equation 6) decreases more or less exponentially with stocking density even if the fish are fed to satiation (Figure 1), a relationship that can be expressed as:

$$f = \exp(-u\, S_d) \quad (7)$$

where S_d = stocking density of fish (no. of fish/m^2), and u = constant.

O'Neill et al. (1972) derived a generalized temperature function to describe the effects of temperature (T) on any biological activity such as food consumption. The function assumes an optimum temperature (T_{opt}) at which the rate of the activity reaches a maximum value of one, and also assumes that the rate reaches zero at a maximum or

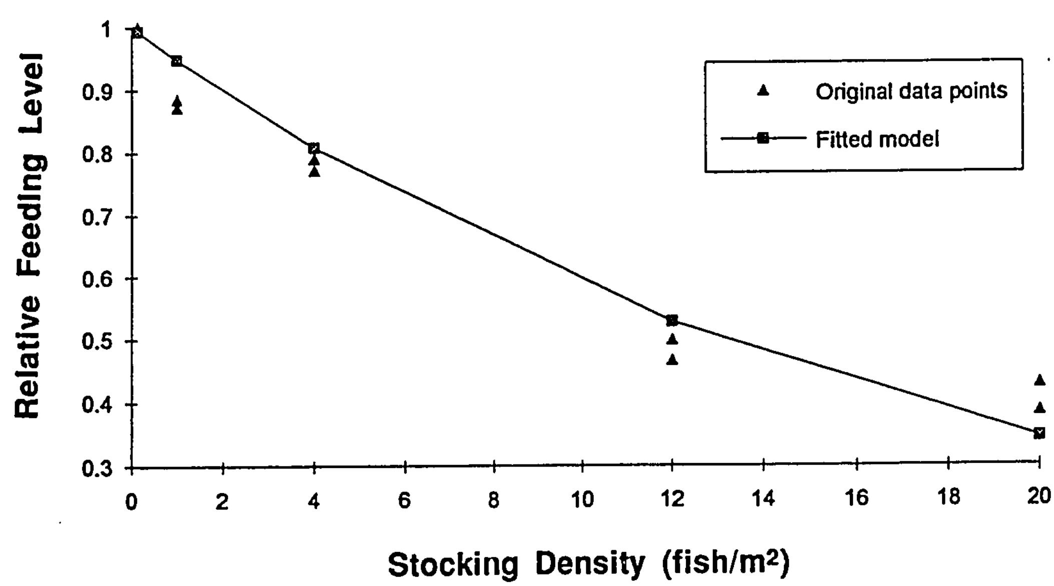

Figure 1. Relationship between relative feeding level and stocking density for a red variant of O. niloticus. Model fitted using Equation 7. Data source: Zonneveld and Fadholi (1991).

lethal temperature (T_{max}). Further, the function assumes that the activity responds to temperature in the form of a Q_{10} relationship. The O'Neill temperature function (τ) is of the form:

$$t = V^x \exp[\, x\,(1 - V)\,] \quad (8)$$

where: $V = (T_{max} - T) / (T_{max} - T_{opt})$
$x = [s_1^2\,(1 + (1 + 40/s_2)^{0.5})^2]/400$
$s_1 = \ln Q_{10}\,(T_{max} - T_{opt})$
$s_2 = \ln Q_{10}\,(T_{max} - T_{opt} + 2)$.

Ursin (1967) and Sperber et al. (1977) assumed that the coefficient of catabolism (k) increases exponentially with temperature. We modified this exponential form to include the minimum temperature below which the fish species cannot survive (T_{min}) as follows:

$$k = k_{min} \exp[\, j\,(T - T_{min})\,] \quad (9)$$

where k_{min} = coefficient of catabolism (g^{1-m}/d) at T_{min}, and j = constant (1/°C).

The overall fish growth model comprises Equations 3-9, and provides a means of predicting fish growth as a function of temperature and food availability over a period of time T_{max}) specified by the user of the model.

Parameters and Data Requirements for the Fish Growth Model
If we assume that the anabolic term in Equation 3 is a function of food intake rate, which is proportional to the surface area available for food assimilation, the exponent m approximates 2/3 (Ursin 1967). If it is assumed that food break-down takes place throughout the body, the exponent n theoretically approximates a value of one, although analysis of oxygen consumption data of fasting fish suggests that n is actually about 0.83 (Ursin 1967). We analyzed similar data for *O. niloticus* (from Farmer and Beamish 1969), and estimated the mean and standard deviation for n to be 0.806 and 0.0001, respectively. However, insertion of n = 0.8 during test runs of the model resulted in growth rates for tilapia far in excess of observed rates (data not shown), which is consistent with the results of Liu and Chang (1992). Therefore, based on their work, the values of 2/3 for m and and 1 for n were retained in the growth model.

In order to estimate the feeding period for the fish (i.e., daylight hours) and when net anabolism (gross anabolism minus feeding catabolism) approximates zero, it is necessary to calculate the onset of sunrise and sunset for each day of the simulation, which is accomplished using standard solar engineering calculations (Hsieh 1986).

Although studies indicate that the efficiency of food assimilation (b) decreases with increased food intake and ranges from 0.53 to 0.70 for *O. niloticus* (Meyer-Burgdorff et al. 1989), other factors such as temperature also influence b (Caulton 1982). In the absence of experimental data for efficiency at different temperatures for *O. niloticus*, we assumed b to be constant, with a value of 0.62 (the mean value from the above range). Meyer-Burgdorff et al. (1989) also reported that the proportion of net energy retention to metabolizable energy was independent of the feeding level for *O. niloticus* and approximated 0.47, implying that the parameter a is about 0.53.

For *O. niloticus*, T_{max} is about 41°C (Denzer 1967), and T_{opt} between 30 and 36°C (Caulton 1982). We assumed T_{opt} to be 36°C. Based on laboratory experiments with this species (Gannam and Phillips 1992), T_{min} appears to be about 15°C. Data were not available to estimate the Q_{10} value for *O. niloticus*. The Q_{10} value of 2.37 calculated from Caulton (1978) for another species, *O. mossambicus*, is used in the present version of the growth model. However, Caulton's data suggest that the Q_{10} value is not a constant, but tends to decrease with increasing temperature (from about 2.97 to 1.86 in the temperature range of 16-37°C). Therefore, it may be necessary to take a different approach to modeling temperature effects on growth at a later stage. The O'Neill temperature function with two different optimum temperatures is shown in Figure 2.

An exponential regression model was fitted to the stocking density (range of 0.125 to 20 fish/m^2) and feeding level data of Zonneveld and Fadholi (1991) (Figure 1), under the assumption that fish stocked at 0.125 fish/m^2 or less were not food limited (f = 1). From this regression, the value of u was estimated to be 0.05338.

We used data on fasting *O. niloticus* from Satoh et al. (1984) to estimate the fasting catabolism parameters, k_{min} and j, to be 0.00133 and 0.0132, respectively. However, during model testing, growth rates continued to increase even after Day 200 of the simulations, which is inconsistent with CRSP reports of tilapia growth patterns. Hepher et al. (1983) indicate that fish appear to adapt to prolonged starvation periods, so that estimates of maintenance requirements made from starvation data may underestimate actual requirements. Further, the value of 0.997 for the coefficient of food consumption (h) estimated by Liu and Chang (1992) may have also caused the

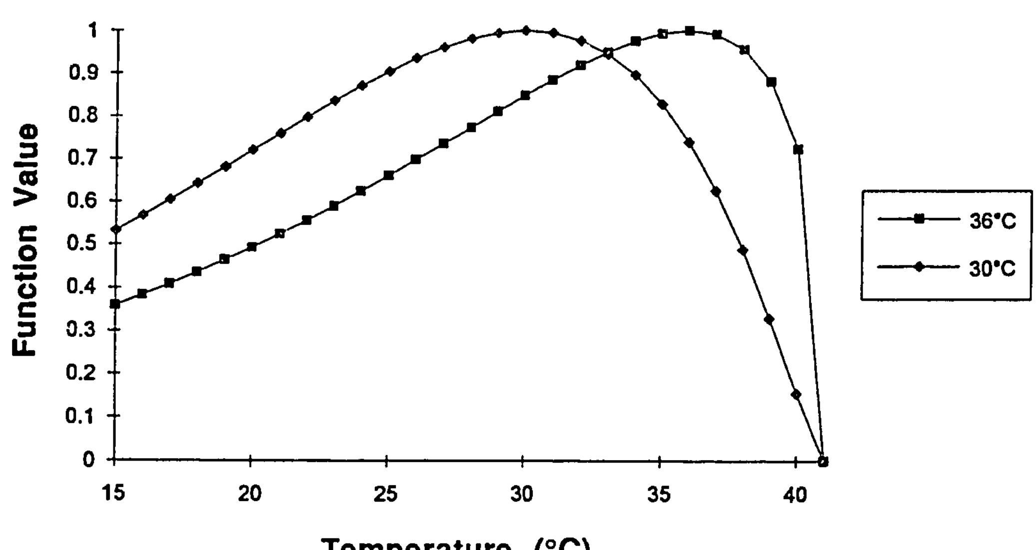

Figure 2. Temperature function for the fish growth model fitted using Equation 8 for two different optimum temperatures (30 and 36°C), and assuming a constant maximum temperature of 41°C).

high growth rates observed during our simulation runs. Based on repeated model testing, reasonable starting values for h, k_{min}, and j appear to be 0.75, 0.005, and 0.02, respectively.

It should be mentioned here that all the fish growth model parameters can be edited by the user, and that the default values used in the model may not be appropriate under all conditions. Other data requirements which users must enter to use the model include the Julian Day on which the simulation should commence, site location details, length of the simulation, stocking density, stocking weight, and expected survival rate (used to compute expected yields). Default values, which can be edited, are available for these variables. Users can choose to use water temperature data required for the simulation from the pond data files in PONDCLASS, or use a separate model to predict water temperature.

Water Temperature Model

Several models, varying from simplified ones that assume completely mixed conditions (e.g., Fritz et al. 1980) to more complex ones that simulate stratified conditions (Losordo and Piedrahita 1991, Culberson and Piedrahita 1992), have been developed to predict pond water temperature. Although the stratified models simulate pond water temperature fluctuations more realistically, they are very computation intensive and require a large number of data inputs. Moreover, the effects of pond stratification on fish behavior and growth are poorly understood. We therefore assume that the ponds are completely mixed. The temperature model that has been implemented in PONDCLASS is largely derived from the Fritz model developed for small waste stabilization ponds (Fritz et al. 1980), and a complete listing is available in the documentation for PONDCLASS version 1.2 (Nath and Lannan 1993).

Data Requirements for the Water Temperature Model

A considerable amount of data is needed for the original version of the Fritz model. In PONDCLASS, depth data are automatically read from the data file for the pond, and it is assumed that the depth will remain constant for the period of simulation. In addition, site location (latitude and longitude), cloud cover, wind speed, and air temperature data are required. Users are likely to have easy access to site location data. However, weather data vary widely and are more difficult to procure. A model that addresses stochastic weather behavior is under development by the UC Davis component of the DAST, but the model has not advanced to a point where it can be included in PONDCLASS. Until such a model becomes available, we provide users with the options of either using typical values for cloud cover and wind speed observed at the site or generating these data based on simple rules without any particular physical interpretation of the problem. Use of these 'weather generators' is strictly optional, and the sole purpose of their implementation is to provide users with a tool to begin to examine weather variability and how it might influence pond performance. However, air temperature data are predicted in a slightly different manner, as described below.

Cloud cover information. The temperature model requires values for the fraction of the sky that is covered by clouds (C_c) for each day of the simulation. Ryan and Stolzenbach (1972, cited in Fritz et al. 1980) recognize four general categories of cloud conditions, and provide values for certain empirical factors (x and y) that are used in the model for each of the cloud cover categories (Table 1).

Table 1. Cloud categories, values for cloud cover (C_c), and estimates of empirical factors (x and y) from Fritz et al. (1980).

Cloud conditions	C_c	x	y
Clear	0.0	1.18	-0.77
Scattered	0.1-0.5 (0.3)[1]	2.20	-0.97
Broken	0.6-0.9 (0.75)	0.95	-0.75
Overcast	1.0	0.35	-1.45

[1] Numbers in parentheses are mean values for the range.

PONDCLASS users can opt to simulate water temperature and fish growth for extended periods of time (e.g., a full season) by selecting one of the categories that best describes local weather conditions. The program will then use the corresponding values for the selected cloud category (or mean values, for scattered and broken conditions) in the simulation. However, the degree of cloudiness at any given site tends to vary both seasonally and diurnally, and is a function of air humidity, cloud altitude, and cloud density (Straskraba and Gnauck 1985). In addition, the degree of cloudiness also varies stochastically. Therefore, use of a single cloud category for a full season may not adequately reflect changing weather conditions.

Alternately, users may choose to have PONDCLASS generate cloud cover data. Essentially, the process involves computer generation of random numbers, which are converted to cloud cover values that are used as inputs into the overall water temperature model. Prior to starting the simulation, users are required to specify whether the season to be simulated is predominantly wet or dry. We assume that cloud cover can take on a random value in the range of 0 to 0.75 for the dry season or a value of 0.25 to 1 for the wet season. Based on this value, the empirical factors (x and y) are determined simultaneously (Table 1). For example, if the season is dry and the generated value for C_c is less than 0.1, we assume clear conditions. The corresponding values of x and y are 1.18 and -0.97, respectively. Similarly, if the season is wet and C_c is greater than 0.9, overcast conditions are assumed ($x = 0.35$, $y = -1.45$). This process is automatically repeated by the computer for each day of the simulation in the selected season, under the additional assumption that C_c is a constant for a given day.

Wind speed information. Wind speed data are handled in a manner similar to cloud cover data generation. Users may either enter an average value of the wind speed (m/s) for the site, which is subsequently used in the simulation, or enter the typical range of wind speeds encountered at the site. In the latter case, PONDCLASS uses the random number generator to produce a value of the wind speed that is within the user-specified range for each day of the simulation.

Air temperature data. As with the other weather parameters, air temperature is difficult to predict because of seasonal and diurnal trends. However, Straskraba and Gnauck (1985) provide the following sets of polynomial equations that can be used to predict daily mean air temperatures (°C) based on the latitude of the site and the Julian Day (JD) of the year:

$$T'_{am} = 25.92 + (0.4893\,L'_t) - (0.02739\,L'^2_t) + (0.0001782\,L'^3_t) \quad (10)$$

$$T'_{aa} = 1.536 + (0.05735\,L'_t) - (0.01296\,L'^2_t) + (0.0001312\,L'^3_t) \quad (11)$$

$$T'_{dm} = T'_{am} + T'_{aa}\sin[\pi/180\,(JD + P_a)] - (T_r * z) \quad (12)$$

where T'_{am} = annual air temperature range (°C), T'_{aa} = semiamplitude of annual air temperature variations (°C), T'_{dm} = daily mean air temperature (°C), T_r = temperature change with each 100m rise in elevation above mean sea level (°C), L'_t = correction factor applied to the site latitude, P_a = phase angle, and z = site altitude (m).

The correction factor for latitude is given by:

$$L'_t = |\,L_t - 3.4\,| \quad (13)$$

where L_t = latitude (°; negative for sites in the Southern hemisphere).

Temperature decreases at a rate of approximately 0.5-0.6°C for each 100 m rise in elevation above mean sea level (Straskraba and Gnauck 1985). The phase angle is about 220° for the Northern hemisphere and 100° for the Southern hemisphere (note: the phase angle was erroneously reported to be 60° in the June 1993 issue of the DAST newsletter). Mean daily air temperature data predicted using Equation 12 is shown for three PD/A CRSP sites in Figure 3.

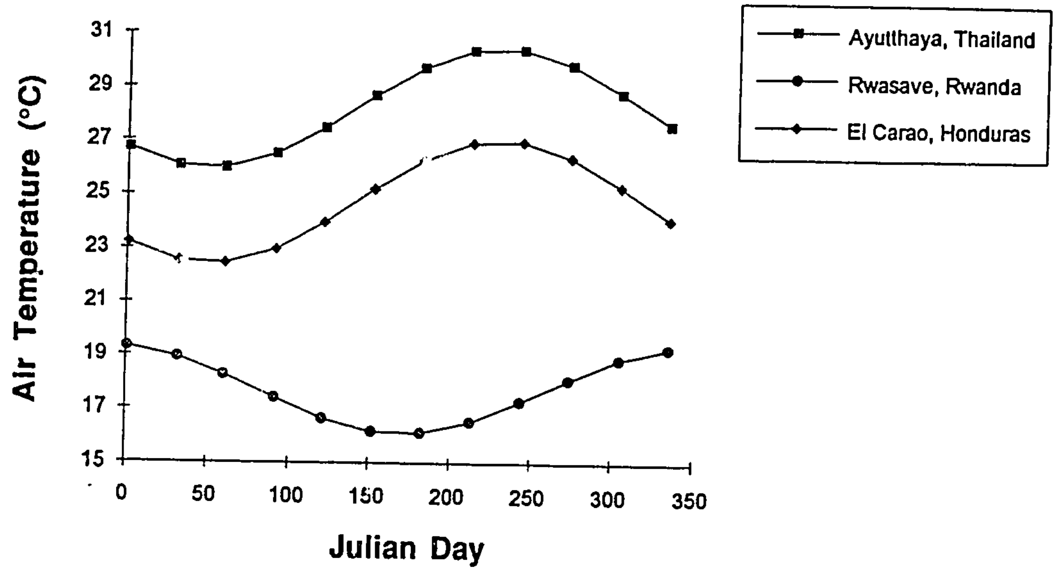

Figure 3. Mean daily air temperature predicted for three PD/A CRSP sites using equations from Straskraba and Gnauck (1985).

Besides seasonal trends in air temperature which might be described by Equations 10-13, air temperature also varies on a diurnal basis. If the maximum (T_{amax}) and minimum (T_{amin}) air temperatures are known, and if it is assumed made that they occur at 0600 and 1500 hours, respectively, it is possible to estimate air temperature on an hourly basis as follows (Culberson and Piedrahita 1992):

$$T_a = T'_{dm} + (T_{amax} - T_{amin}) * 0.4484 * \{ \sin(p/12 * t_L - 2.7489) + 0.2706 * \sin[2 * (p/12 * t_L - 2.7489] \} \quad (14)$$

where t_L = local time of the day, calculated according to Hsieh (1986). A convenient means for estimating T_{amax} and T_{amin} is not available. However, if PONDCLASS users are able to provide an estimate of the typical daily temperature amplitude, (T_{amp} = difference between maximum and minimum air temperatures), it is possible to estimate T_{amax} and T_{amin} as follows:

$$T_{amax} = T'_{dm} + (T_{amp}/2) \quad (15)$$

$$T_{amin} = T'_{dm} - (T_{amp}/2) \quad (16)$$

Use of Equations 15 and 16 assumes a constant temperature amplitude for each day of the simulation. Thus, the hourly air temperature (T_a) for each day of the simulation is predicted in PONDCLASS by the use of equations 10-16.

Simulation Results from the Water Temperature and Fish Growth Models
Results from simulation runs of the water temperature model over 24-hour periods for three CRSP sites are shown in Figures 4-6. In general, the model follows diurnal profiles reasonably well, although discrepancies of up to about 2°C between observed and predicted values are evident. Long-term simulation results of water temperature for Rwasave, Rwanda, and El Carao, Honduras as shown in Figure 7.

Results of similar runs with the fish growth model (Figure 8) demonstrate some of the differences users may observe during model execution in deterministic and stochastic modes (Rwanda Runs #1 and #2), and also possible effects of stocking density on growth (Honduras Runs #1 and #2).

Use of the models in PONDCLASS is optional, and their primary purpose is to illustrate the types of variables that might influence fish growth and pond water temperature. However, these models can be used as analysis tools and for guiding decisions about pond site selection, fish stocking density and stocking weight, and the length of culture period required for the fish to reach a desired harvest size.

New Generation of Decision Support Systems
Our experience suggests that potential users of computerized tools like PONDCLASS may include production managers, planners, economists, educators, and research scientists. The objectives of different users are often in conflict. For example, production managers may be interested in tools that would help them manage one or more ponds over short-term periods typically ranging from one day to one week, planners and economists would like tools that enable a facility-level analysis of several ponds over a long-term period typically ranging from a season to a few years,

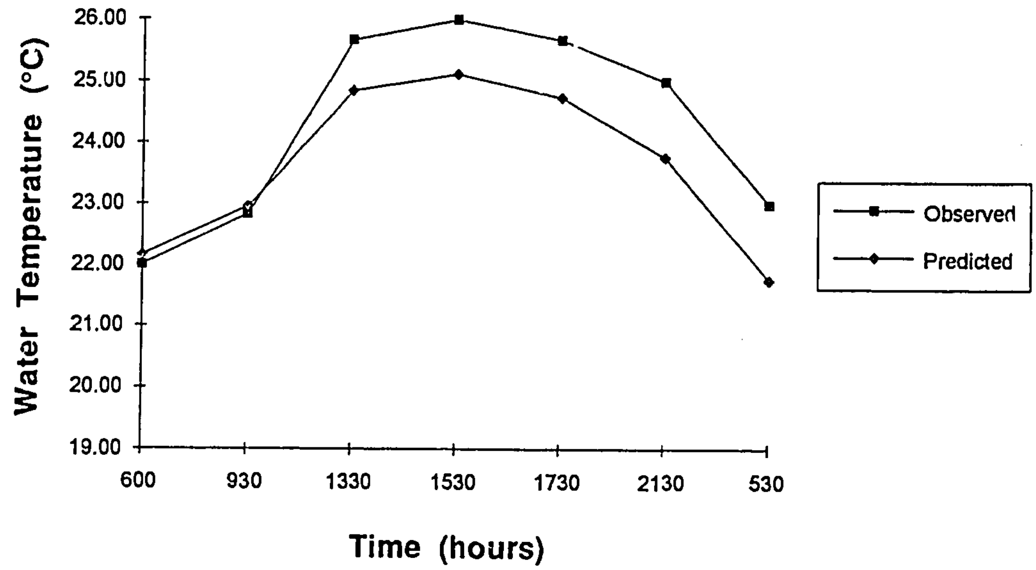

Figure 4. Observed and predicted diurnal water temperature profiles for Pond C2 at Rwasave, Rwanda, on JD 323-324, 1989. Simulation run assumed scattered cloud conditions and an initial temperature of 24°C. CRSP data used were pond depth (1.2 m), air temperature amplitude (14°C) and wind speed (4.08 m/s).

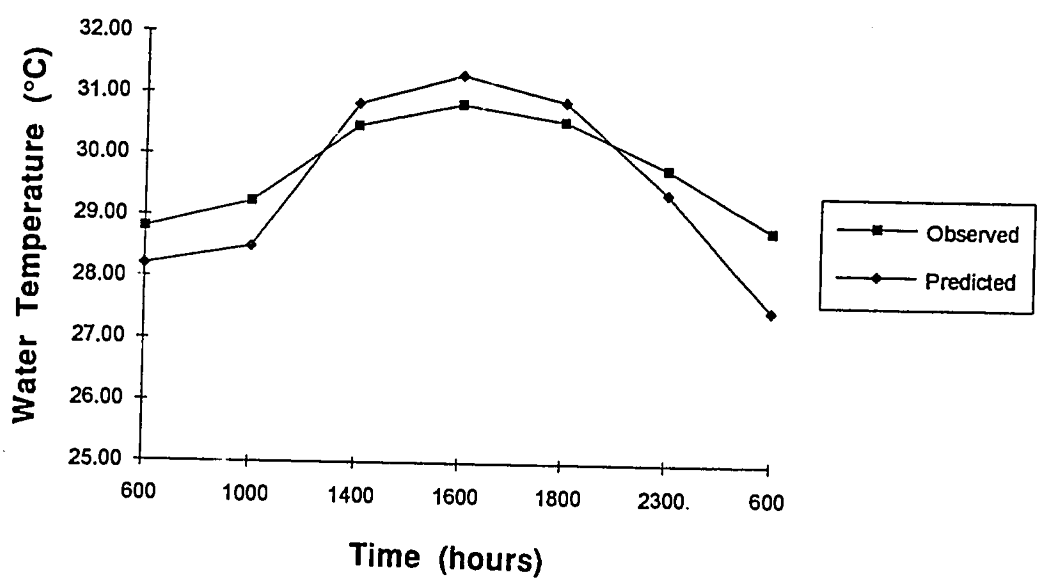

Figure 5. Observed and predicted diurnal water temperature profiles for Pond #3 at Ayutthaya, Thailand, on JD 40-41, 1988. Simulation run assumed scattered cloud conditions and an initial temperature of 30°C. CRSP data used were pond depth (0.9 m), air temperature amplitude (8.5°C) and wind speed (3.5 m/s).

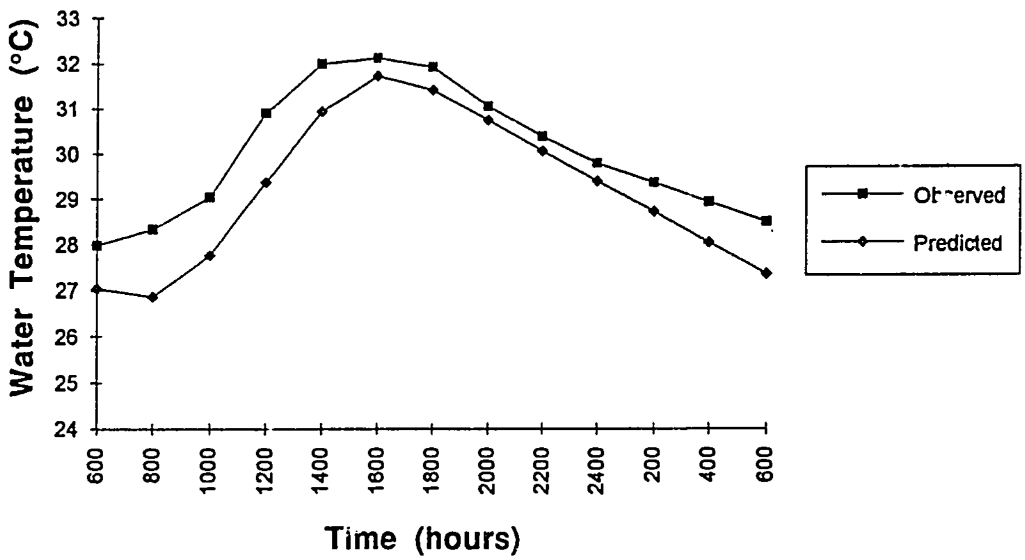

Figure 6. Observed and predicted diurnal water temperature profiles for Pond #5 at El Carao, Honduras, on JD 229-230, 1988. Simulation run assumed scattered cloud conditions, an initial temperature of 30°C, and a wind speed of 3 m/s. CRSP data used were pond depth (0.75 m) and air temperature amplitude (15°C).

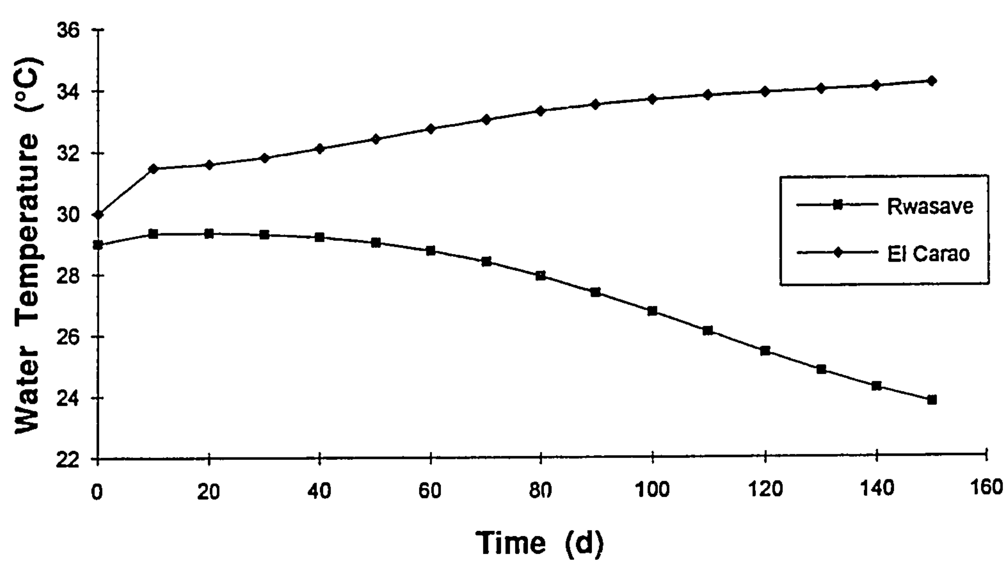

Figure 7. Long-term simulated water temperature profiles for Rwasave (assuming an initial temperature of 29°C, air temperature amplitude of 15°C, pond depth of 1.2 m, scattered cloud conditions, and a constant wind speed of 1.5 m/s) and El Carao (assuming an initial temperature of 30°C, air temperature amplitude of 12°C, pond depth of 0.7 m, clear conditions, and a constant wind speed of 1.5 m/s).

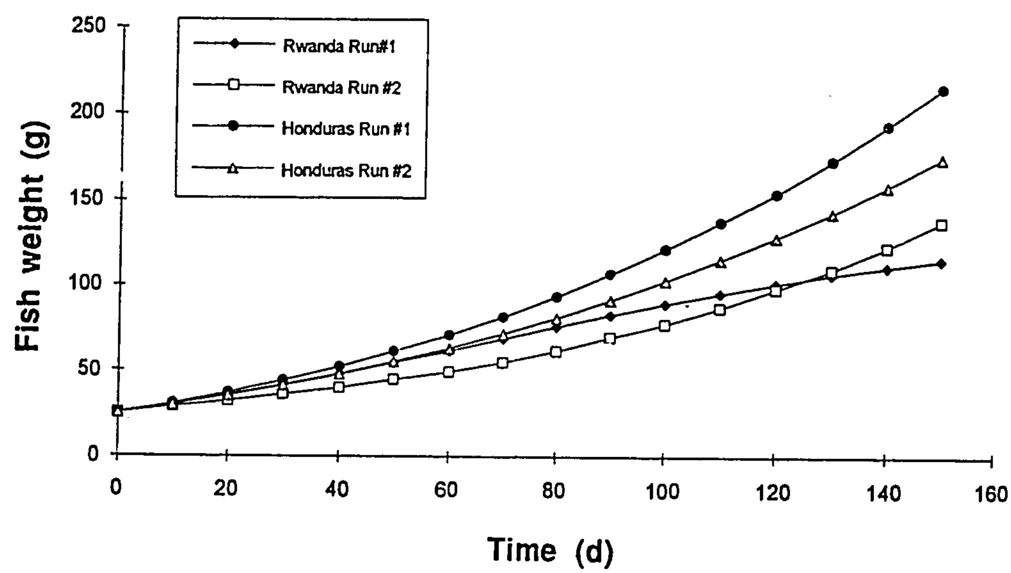

Figure 8. Simulation results from the fish growth model for Rwanda and Honduras. Conditions assumed were: *Rwanda Run #1* – scattered clouds, wind speed 1.5 m/s, S_d = 10000/ha; *Rwanda Run #2* – wet season, cloudiness assumed to be stochastic, wind speed generated stochastically in the range of 0-2 m/s, S_d = 10000/ha; *Honduras Run #1* – clear skies, wind speed 1.5 m/s, S_d10000/ha; and *Honduras Run #2* – identical to *Run #1*, except that S_d = 30000/ha.

and educators and research scientists might want to examine individual ponds over very short-term periods (a few hours, for instance). Therefore, it is necessary to develop a software architecture in the decision support system that would address the needs of these different users.

Planning, design, and implementation of a major upgrade to the PONDCLASS program has been inititated. The primary focus of this effort is to provide a robust, flexible, and easy-to-use framework for analyzing individual pond dynamics with a high level of resolution as well as performing enterprise-level economic and production analyses and optimizations. To accomplish this, efforts to develop a robust underlying simulation framework, graphical analysis tools, and simulation constructs for major pond and enterprise components were initiated. Because of constraints in dealing with the construction and maintenance of sophisticated analysis frameworks using the current PONDCLASS approach, the new version is being implemented in an object-oriented architecture using the C++ programming language. To ensure ease of use and to provide graphical representation of input data and analysis results, a graphical user interface using the Microsoft Windows operating environment is being adopted.

Existing functionality of PONDCLASS that has been implemented in this new environment includes the user interface for entering site, water, and soil data, the fertilizer and soil classification databases, a linear programming unit for computing least-cost functions for fertilizer combinations (which is not restricted to a maximum number of three fertilizers at a time, but allows any number of fertilizers to be simultaneously tested), and the fish growth and water temperature models described earlier.

While incorporating the basic functionality of the existing PONDCLASS program, we are emphasizing two major additional levels of functionality in the new revision. The first is to provide a detailed short-term analysis of pond dynamics, considering diurnal fluctuations in weather inputs, detailed descriptions of water quality variables including oxygen, nitrogen, and phosphorus dynamics, primary productivity considerations, and effects on fish bioenergetics and yield. This view is intended to provide day-to-day management support for forecasting feed and fertilizer requirements, oxygen deficits, food utilization, and fish growth. The intent of this view is to allow the operator to take a detailed look at what is occurring in a pond or group of ponds, to diagnose potential production problems, and to prescribe appropriate management strategies to deal with these problems.

The second level of functionality will involve the addition of whole enterprise analysis capabilities. This approach will couple a lower-resolution, daily timestep view of pond dynamics with accounting of pond production resource requirements and the incorporation of enterprise budgets into the analysis framework. The goal of the modeling efforts here is to provide estimates of resource requirements and utilization under various facility management strategies, as well as fish yield estimates. This information, coupled with traditional enterprise budget accounting of other fixed and variable production costs, will be used to analyze the performance of particular facility layouts and management strategies. The enterprise-level view will necessarily initially involve the introduction of stochastic descriptions of climatic and production variables and relationships, and will ultimately be extended to multi-run analyses to perform risk assessment and management strategy optimization. Non-linear optimization strategies coupled with site-specific probabilistic measures to assess the efficacy of alternative facility layouts, production types, and management strategies will be used to develop recommendations for the most cost-effective approaches for facility management.

Literature Cited

Bowman, J.R. 1992. Classification and management of earthen aquaculture ponds, with emphasis on the role of the soil. Ph.D. Dissertation, Oregon State University, Corvallis, Oregon.

Boyd, C.E. 1979. Water quality in warmwater fish ponds. Agricultural Experiment Station, Auburn University, Auburn, Alabama.

Brett, J.R. 1979. Environmental factors and growth. Pages 599-675 *in* W.S. Hoar, D.J. Randall, and J.R. Brett, editors. Fish Physiology, Volume 8, Academic Press, New York.

Caulton, M.S. 1978. The effect of temperature and mass on routine metabolism in *Sarotherodon* (Tilapia) *mossambicus* (Peters). Journal of Fish Biology 13: 195-201.

Caulton, M.S. 1982. Feeding, metabolism and growth of tilapias: some quantitative considerations. Pages 157-180 *in* R.S.V. Pullin and R.H. Lowe-McConnell, editors. The biology and culture of tilapias. ICLARM Conference Proceedings 7, International Center for Living Aquatic Resources Management, Manila, Philippines.

Cuenco, M.L., R.R. Stickney, and W.E. Grant. 1985. Fish bioenergetics and growth in aquaculture ponds: I. Individual fish model development. Ecological Modelling 27:169-190

Culberson, S., and R.H. Piedrahita. 1992. Modification of stratified temperature model to accomodate reduced data inputs; identifying critical requirements. Presented at the Aquaculture 92 International Conference, Orlando, Florida. ASAE Paper No. AQUA-92-102.

Denzer, H.W. 1967. Studies on the physiology of young tilapia. FAO Fisheries Report 44:358-366.

Farmer, G.J., and F.W.H. Beamish. 1969. Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. Journal of the Fisheries Research Board of Canada 26:2807-2821.

Fritz, J.J., D.D. Meredith, and A.C. Middleton. 1980. Non-steady state bulk temperature determination for stabilization ponds. Water Research 14:413-420.

Gannam, A., and H. Phillips. 1992. Effect of temperature on growth of *Oreochromis niloticus*. Pages 136-142 *in* H. Egna, M. McNamara, J. Bowman, and N. Astin, editors. Tenth Annual Administrative Report, PD/A CRSP, Oregon State University, Corvallis, Oregon.

Hepher, B. 1978. Ecological aspects of warm-water fishpond management. Pages 447-468 *in* S. Gerking, editor. Ecology of fresh water fish production, Wiley Interscience.

Hepher, B., I.C. Liao, S.H. Cheng, and C.S. Hsieh. 1983. Food utilization by red tilapia–effects of diet composition, feeding level and temperature on utilization efficiencies for maintenance and growth. Aquaculture 32:255-275.

Hsieh, J.S. 1986. Solar energy engineering. Prentice-Hall Inc., New Jersey.

Knud-Hansen, C.F., T.R. Batterson, C.D. McNabb, I.S. Harahat, K. Sumantadinata, and H.M. Eidman. 1991. Nitrogen input, primary productivity and fish yield in fertilized fish ponds in Indonesia. Aquaculture 94:49-63.

Liu, K.M., and W.Y.B. Chang. 1992. Bioenergetic modelling of effects of fertilization, stocking density, and spawning on growth of Nile tilapia, *Oreochromis niloticus*. Aquaculture and Fisheries Management 23:291-301.

Losordo, T.M., and R.H. Piedrahita. 1991. Modelling temperature variation and thermal stratification in shallow aquaculture ponds. Ecological Modelling 54:189-226.

Meyer-Burgdorff, K.-H., M.F. Osman, and K.D. Gunther. 1989. Energy metabolism in *Oreochromis niloticus* Aquaculture 79:283-291.

Nath, S.S. 1992. Total and available nutrients in manures for pond aquaculture. M.S. Thesis, Oregon State University, Corvallis, Oregon. 67 pp.

Nath, S.S., and J.E. Lannan. 1993. Revisions to PONDCLASS. Guidelines for Fertilizing Aquaculture Ponds. 1993. Pond Dynamics/Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon.

O'Neill, R.V., R.A. Goldstein, H.H. Shugart, and J.B. Mankin. 1972. Terrestial ecosystem energy model. Eastern deciduous forest biome memo report #72-19.

Peech, M. 1965. Lime Requirement. Pages 927-932 *in* C.A. Black, editor. Methods of soil analysis. American Society of Agronomy, Inc., Madison, Wisconsin.

Satoh, S., T. Takeuchi, and T. Watanabe. 1984. Effects of starvation and environmental temperature on proximate and fatty acid compositions of *Tilapia nilotica*. Bulletin of the Japanese Society of Scientific Fisheries 50:79-84.

Sperber, O., J. From, and P. Sparre. 1977. A method to estimate the growth rate of fishes, as a function of temperature and feeding level, applied to rainbow trout. Meddelelser fra Danmarks Fiskeri-og Havundersogelsen 7:275-317.

Straskraba, M., and A.H. Gnauck. 1985. Freshwater ecosystems: Modelling and simulation. Developments in Environmental Modelling 8, Elsevier, Amsterdam.
Ursin, E. 1967. A mathematical model of some aspects of fish growth, respiration, and mortality. Journal of the Fisheries Research Board of Canada 24:2355-2453.
Zonneveld, N., and R. Fadholi. 1991. Feed intake and growth of red tilapia at different stocking densities in ponds in Indonesia. Aquaculture 99:83-94.

Simulation of Water Quality in Stratified CRSP Ponds: Dissolved Oxygen Concentration

Work Plan 6, Study 3

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Introduction

A dissolved oxygen (simulation) model for stratified aquaculture ponds has been developed. The model consists of two coupled sections, one to simulate temperature and the other to simulate dissolved oxygen concentration. Results from the temperature simulations are used in the dissolved oxygen model. The temperature component of the model has been described previously (Piedrahita et al. 1993, Culberson and Piedrahita 1992), and a brief description of preliminary results with the dissolved oxygen model has also been presented (Piedrahita et al. 1993). A complete description of the coupled models is available elsewhere (Culberson 1993), and a description of the dissolved oxygen component of the model is presented here.

Attempts at predicting pond dissolved oxygen levels to date have often assumed the water column to be homogeneous throughout its depth, and/or that a complete characterization of the entirety of factors contributing to oxygen levels in aquaculture ponds is not feasible (Boyd 1990, Smith and Piedrahita 1988, Romaire and Boyd 1979). This report describes the modification and simplification of a previously developed stratified dissolved oxygen model, hereafter referred to as the Losordo model (Losordo 1988). The Losordo model, while demonstrating accuracy in predicting dissolved oxygen levels for given atmospheric and pond ecosystem regimes in Northern California, has proven somewhat cumbersome for use at aquaculture sites other than those where Losordo worked, or for sites where sophisticated, electronic weather and water quality monitoring equipment is unavailable. The rates of oxygen production, consumption, and diffusion to and from the atmosphere (which are dependent in part upon pond water temperatures) are calculated using temperatures estimated by the above-mentioned revised temperature model; that is, the oxygen model has been incorporated into the temperature model, and outputs of temperature and dissolved oxygen levels are generated simultaneously.

Validation of the revised model and oxygen-balance modifications were conducted using data from the Northern California pond sites for which the Losordo model was developed. Demonstration runs for the revised model were then conducted using pond data from PD/A CRSP sites in Honduras, Rwanda, and Thailand, and the results of those simulations are presented below.

The dissolved oxygen model was implemented using a dynamic modeling language called STELLA™ (High Performance Systems, Inc. 1990). Brief descriptions of the language are provided in previous reports (Culberson 1993, Piedrahita et al. 1992), and in the developers' literature (High Performance Systems, Inc. 1990).

Objectives

The effort to develop models to simulate temperature and dissolved oxygen concentrations in stratified ponds has resulted in a simplified model that is suitable for execution with CRSP data. The development of this model addresses the following objectives, as stated in the Sixth Work Plan (Study 3):

1. To simplify the data requirements of existing temperature and dissolved oxygen stratification models and test the models with non-CRSP and CRSP data sets.
2. To incorporate into existing models the more accurate characterization of primary production and respiration rates identified in Study 2 of the Sixth Work Plan.
3. To calibrate and verify the modified/simplified models with CRSP data.

Materials and Methods

The basis for the model described here is the assumption that stratification in a pond can be represented by three volume elements or layers that are of uniform quality. That is, there is homogeneity in a horizontal direction and stratification is strictly a vertical phenomenon. As described earlier (Culberson and Piedrahita 1992), one of the most fundamental changes to the Losordo model is the reduction in the number of layers in the model from five to three, which consequently reduces the complexity of the model and reduces the amount of input data needed to initialize the simulation. In addition, the revised model can be used for ponds of any depth, and will redefine the thickness of the volume element layers automatically once the operator has input the pond depth to be studied.

Further revisions include the consolidation of input nodes into easy-to-read icon groupings and the construction of a separate mass-balance structure for those processes directly involved in the oxygen-balance equations (Culberson 1993).

Mass-balance Modifications

There have been important changes to several of the mass-balance equations from the Losordo model, due in part to improvements in the characterization of diurnal respiration changes among phytoplankton populations. In addition, some of the inputs required by the Losordo model were not routinely collected at the PD/A CRSP research sites, nor are they measurements that farmers unassociated with research facilities are likely to have access to; for this reason certain other modifications to the Losordo model were made. These changes will be discussed below, and justifications will be advanced for how the mass-balance characterizations were altered.

Photosynthetic Respiration Rate

The Losordo model characterizes phytoplankton respiration rate as a proportion of the rate of photosynthesis, as first suggested by Meyer (1980). The original ratio of respiration to photosynthesis (0.10) is retained in the revised model, but a time lag has been incorporated into the expression to account for the fact that there is a delay between sundown and the time at which the phytoplankton return to their baseline nighttime respiration rate. Based on a preliminary analysis of high-resolution, rapid-response oxygen probe studies of cultured phytoplankton populations, the revised model uses an estimate of three hours for the time lag between minimum phyto-plankton photosynthetic rate and minimum phytoplankton respiration rate (P. Giovannini, personal communication 1992). It is this feature of the revised model that most dramatically improves the simulated output of dissolved oxygen levels over those outputs generated by the Losordo model.

Water Column Respiration Rate

The water column respiration rate was defined by the Losordo model as the rate of respiration exhibited by the pond water column that is not explicitly considered separately (Losordo 1988). Estimates of the water column respiration rate are most conveniently made using dark bottle incubations of water sampled from the water column. For use in the revised model, however, a slight revision of the estimation was made due to the fact that different water column respiration rates could be calculated, depending on what time of day the samples were collected and on how long the dark bottles were incubated (Teichert-Coddington and Green 1993). In this revision, a regression equation was generated from Teichert-Coddington and Green's data which related four-hour, daytime-collected dark bottle measurements of water column respiration rates to those rates measured by four-hour, nighttime-collected dark bottle tests. In effect, the daytime-collected measurements overestimated the water column respiration rates seen during the nighttime hours, possibly because high oxygen consumption rates occurred in the incubation bottles during the course of the test. For the PD/A CRSP sites, dark bottle estimates (where available) of nighttime water column respiration were obtained from dark bottle measurements conducted during the daytime, and were adjusted using the regression equation outlined above. In general, however, the overall effect of this revision to the Losordo model is quite small.

More importantly, there are frequent cases where no dark bottle measurements of water column respiration rates were available in the CRSP Data Base. In these cases, water column respiration rates are estimated from the nighttime decline in oxygen levels within the pond. While criticisms will no doubt arise owing to the fact that a respiration term has been derived from *a posteriori* knowledge of the effect of this respiration on dissolved oxygen levels in the pond, the readers are reminded that the methodology and mechanistic accuracy of the revised model is what is being tested here, and not the absolute predictive accuracy. What is hoped is that the testing and validation of the model will reveal an overall robustness and generality of the characterizations of the physical, chemical, and biological processes contained within the model, and that then the extension of these characterizations to novel situations would seem to have logical, accurate underpinnings. Once the model has proved its ability to simulate current or historical aquaculture situations, it can be used to examine future or "imagined" situations.

Sediment Respiration Rates
The Losordo model initializes the sediment respiration rate term using measured sediment respiration rates and then adjusts that rate for ambient temperatures using a temperature correction factor (Losordo 1988). This rate was considered to be constant over the entire pond bottom, and was admittedly poorly defined. As was the case for the water column respiration estimates, many of the PD/A CRSP research sites do not routinely report sediment respiration rates. Where this is the case, the nighttime dissolved oxygen decline estimation for water column respiration rates is considered to include respiration by pond bottom sediments as well.

One slight modification to the estimation of sediment respiration was made in light of recent work by PD/A CRSP and other researchers (Szyper et al. 1992, Madenjian 1990). Where sediment respiration rates were measured and not included in the water column respiration term, and where dissolved oxygen levels fell below a certain threshold value (1.0 mg/L), sediment respiration was assumed to decline to zero. When this assumed threshold value was *not* included in the Losordo and revised models, the lowest layer of the pond volume element was frequently simulated as becoming anoxic, even when the measured values in the pond for this layer were 1.0 mg/L or more.

Carbon/Chlorophyll a Ratio
In the Losordo model, gross oxygen production per mass of chlorophyll *a* was estimated using the Steele equation (Meyer 1980) integrated over depth, as follows:

$$GP = \frac{P_{max}\, exp((exp[-(I/I_{max})exp(-\eta\, z_2)])-(exp[-(I/I_{max})exp(-\eta\, z_1)]))}{\eta(z_2-z_1)} \quad (1)$$

where:

- GP = oxygen production rate, mg O_2/mg Chl-*a*/s
- P_{max} = maximum O_2 production rate at light saturation, mg O_2/mg Chl-*a*/s
- I = surface PAR intensity, µmol/m^2/s
- I_{max} = underwater PAR intensity at which light saturation occurs, µmol/m^2/sec
- η = light extinction coefficient, m^{-1}
- z_1 = depth of the upper boundary of the volume element, m
- z_2 = depth of the lower boundary of the volume element, m
- PAR = photosynthetically active radiation

Losordo was able to estimate P_{max} using detailed primary productivity studies performed at each of his study sites (Losordo 1988). Because this kind of study is not routinely performed at the PD/A CRSP research sites, an alternate method of estimating P_{max} was needed. Eppley (1972) used an estimation for U_{max} (mg O_2/mg C/hr) to relate gross oxygen production to the rate of carbon fixation exhibited by the phytoplankton. The rate of carbon fixation by the phytoplankton was then determined from limits listed by Reynolds (1984), and from calibration of the model based on the known dissolved oxygen regime for a given pond. In the absence of detailed primary productivity studies and/or measures of carbon/chlorophyll *a* ratios, the authors could not find a way to eliminate the need for this calibration that would result in accurate simulations by the revised model. Nonetheless, once the calibration was made, the model showed considerable robustness over a range of pond depths, solar regimes, and local weather conditions. In summary, the following substitution was made for Losordo's measured P_{max} values:

$$P_{max} = \text{Carbon/Chlorophyll } a \; U_{max} \quad (2)$$

where:

P_{max} = mg O_2/mg Chlorophyll *a*/hr
Carbon/Chlorophyll *a* = 12.5 - 50 (from Reynolds 1984)
U_{max} = maximum specific primary production rate, mg O_2/mg C/hr (from Eppley 1972)

Underwater PAR Intensity At Which Light Saturation Occurs
The underwater Photosynthetically Active Radiation (PAR) level at which light saturation (photo-inhibition) of the phytoplankton occurs was determined by Losordo through detailed primary productivity studies. As with the P_{max} modification outlined above, this kind of study is not available within the PD/A CRSP Data Base. Because one can expect the phytoplankton populations to adapt to local conditions over time, the authors felt that an approximation of the saturation PAR intensity (I_{max}) could be made by setting the I_{max} value equal to the maximum PAR intensity at a particular site on a given day. In other words, this modification says that locally-adapted phytoplankton will not become photo-inhibited during a given day at a given site. While this approximation might not be wholly accurate, Losordo (1988) noted that only rarely did the local solar regime exceed his measured light saturation intensities, and even then only in the very top of the water column.

Light Extinction Coefficient
As was the case in the revised temperature model (Culberson and Piedrahita 1992), the revised dissolved oxygen model has had to rely upon substitute estimates for Losordo's light extinction coefficient values, which were based on measurements of underwater light intensity.

Because measurements of underwater light intensity are not part of the routine PD/A CRSP data collection protocol, the revised model relies on estimates of light extinction coefficients (η) derived from Secchi Disk Measurements (Boyd 1990, Poole and Atkins 1929):

$$\eta = 1.7/SDD \quad (3)$$

where :
SDD = Secchi Disk Depth, m

This estimation is a widely used approximation, and has proven satisfactory for use in the revised models.

Summary of Data Inputs for the Losordo and Revised Models
Reduction of data requirements was one of the primary objectives prompting the authors to revise Losordo's model. Much of what was required by the Losordo

model could only be collected using sophisticated, automated water and weather sampling equipment, which is not normally available to farmers at their ponds. The above discussion points out the ways in which the model has been modified to accommodate simpler and less-intensive data collection schemes, without significantly altering simulation outputs. Comparison of the data required by the Losordo model and the revised model reveals that:

a. the overall data input demand by the revised model is significantly reduced from the Losordo model, from 528 data points per day to 102 data points per day. While this may still appear to be a great deal of input, much of it describes initial pond and weather conditions – the only data which need to be collected throughout the day are those for solar radiation and wind speed;

b. replacement data for the revised model are more easily measured than the original data; i.e. Secchi Disk Depths replacing underwater PAR measurements, Umax replacing Pmax, and reference values of the Carbon/Chlorophyll *a* ratio replacing detailed primary production studies;

c. improved model structure allows for ease of data entry and more user-friendly icon groupings.

Results

After the detailed database gathered by Losordo for ponds in Northern California was modified to resemble the less-complex and less-intensive PD/A CRSP database (see Culberson and Piedrahita 1992 for explanation) the revised model was validated by comparing its predicted dissolved oxygen concentrations with those previously predicted by the Losordo model. While there are small differences between the generated outputs (Losordo vs. Revised), both models predict dissolved oxygen concentrations that are quite similar to measured oxygen levels throughout the twenty-four hour simulation period (Figure 1).

After these validation runs were made for the revised model, it was then initialized with data from the PD/A CRSP Global Data Base. The data used represent individual dates in 1988 from Thailand, 1989 in Rwanda, and 1990 in Honduras. Calibrations were made as described above, and the simulation results are displayed with the measured dissolved oxygen profiles in Figures 2, 3, and 4.

As was the case with the revised temperature model, the predictive accuracy of the revised dissolved oxygen model remains quite high in spite of the mass-balance modifications. There is little difference between the outputs from the Losordo model and the revised model for the validation runs in Northern California. While there are some slight differences between the measured and modeled dissolved oxygen concentrations from the PD/A CRSP experimental sites in terms of absolute values, the model simulates the observed qualitative dynamics of the ponds quite well: the timing of oxygen concentration peaks, the magnitude of the peaks, the extent of oxygen stratification, and the simulation of anoxic conditions near the bottom of the pond are all represented quite accurately.

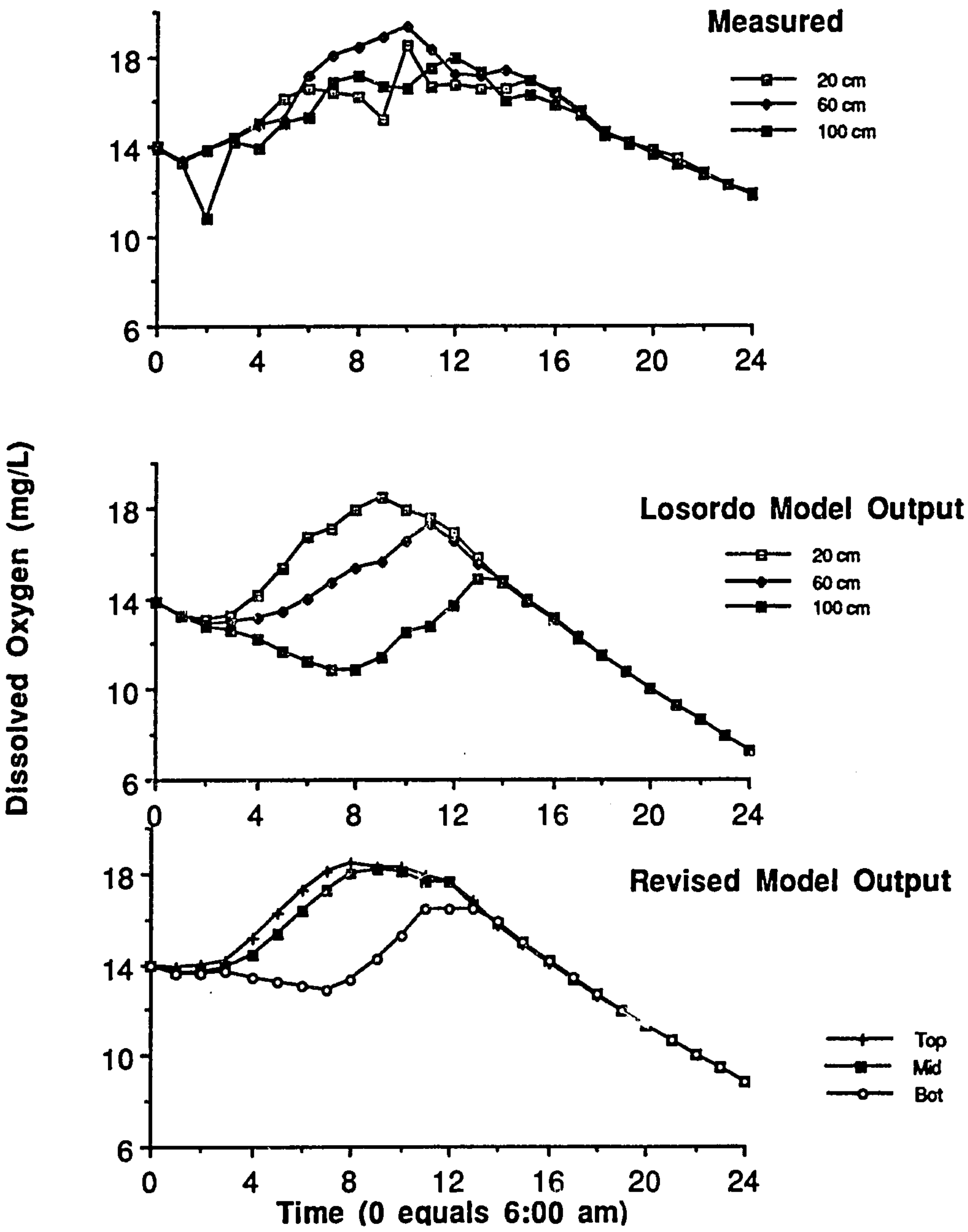


Figure 1. Comparison of measured dissolved oxygen with outputs from the Losordo and revised models; Northern California, Julian Day 224, 1987.

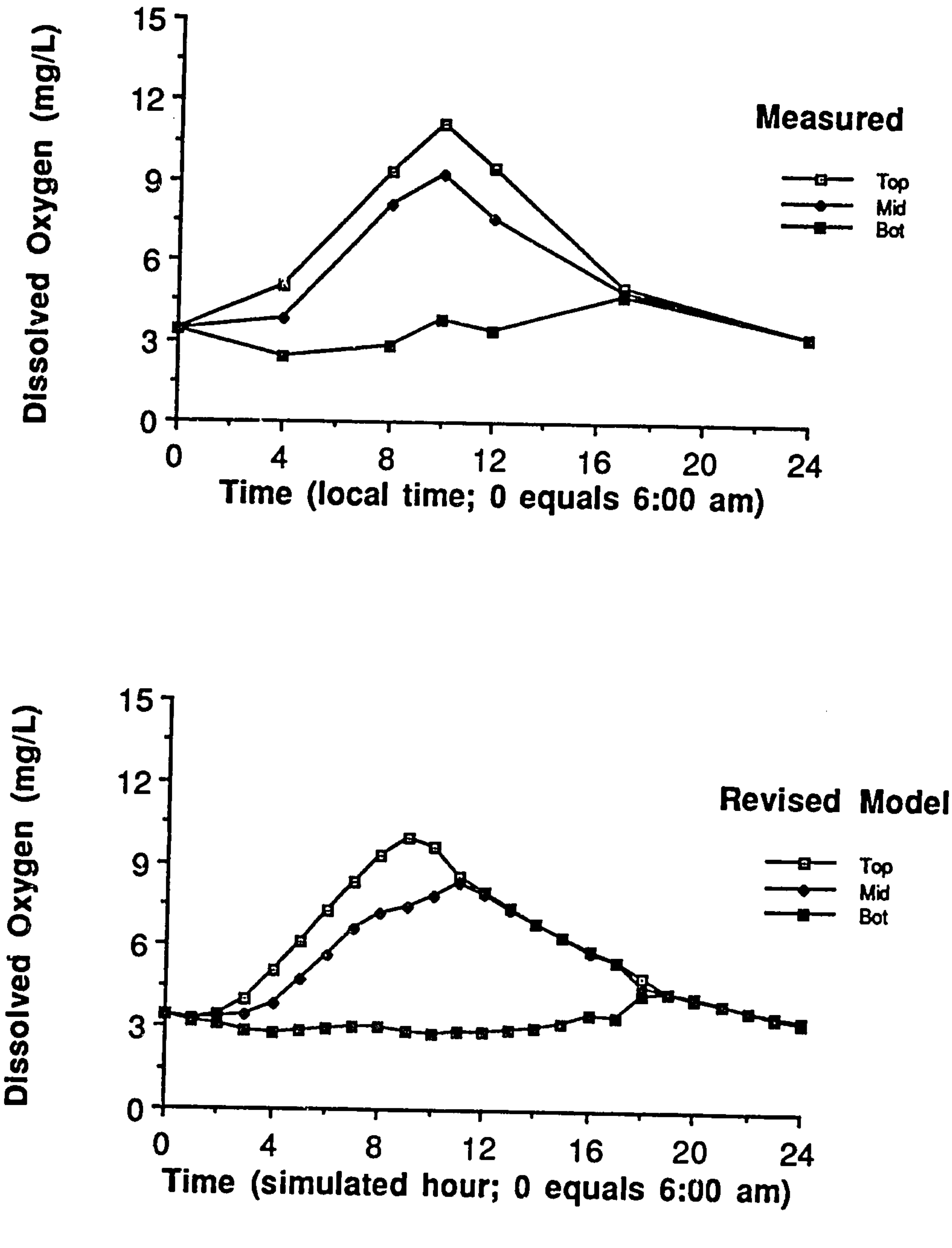


Figure 2. Comparison of measured dissolved oxygen with output from revised model; Thailand PD/A CRSP Site, Julian Day 96, 1988, Pond #12.

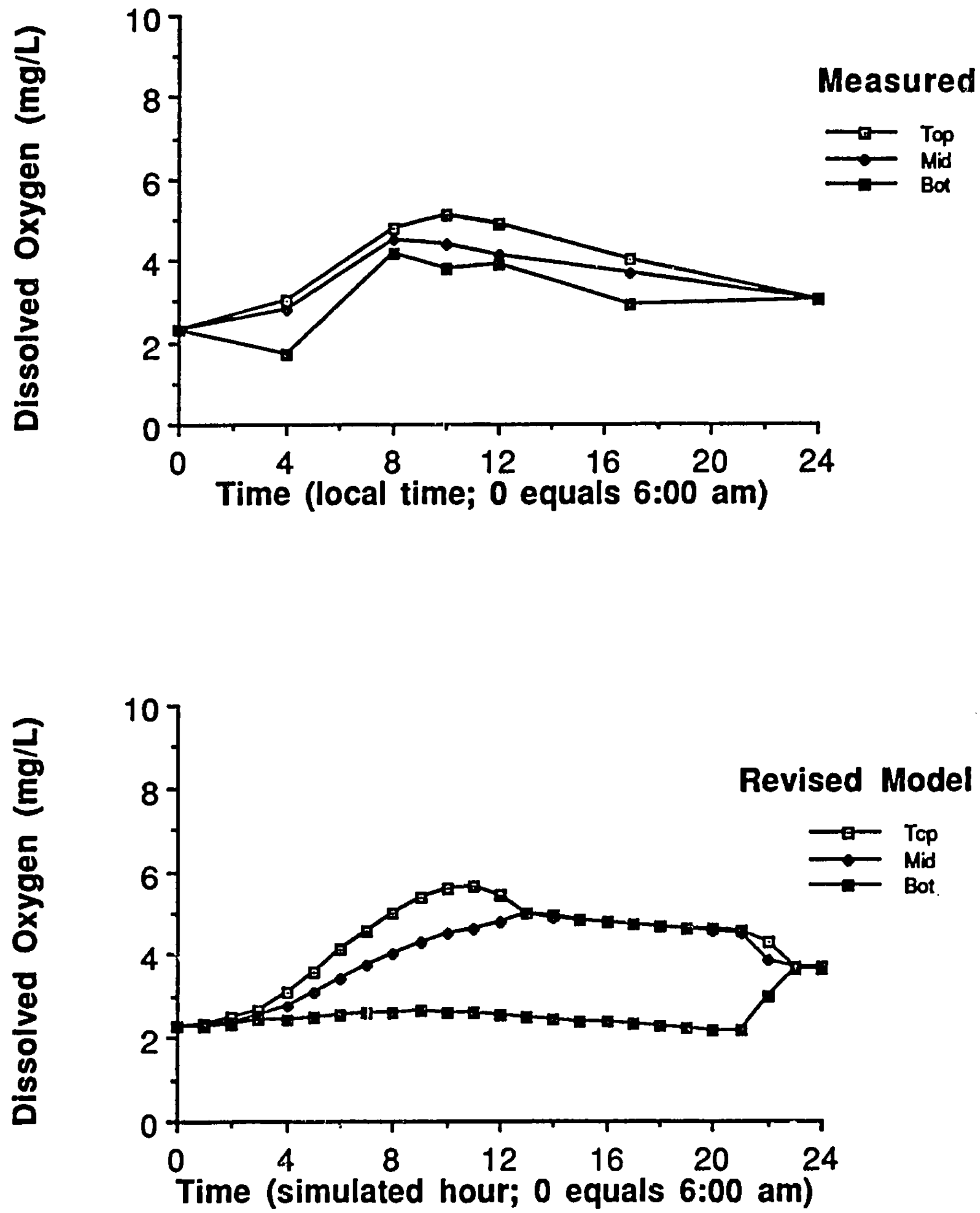
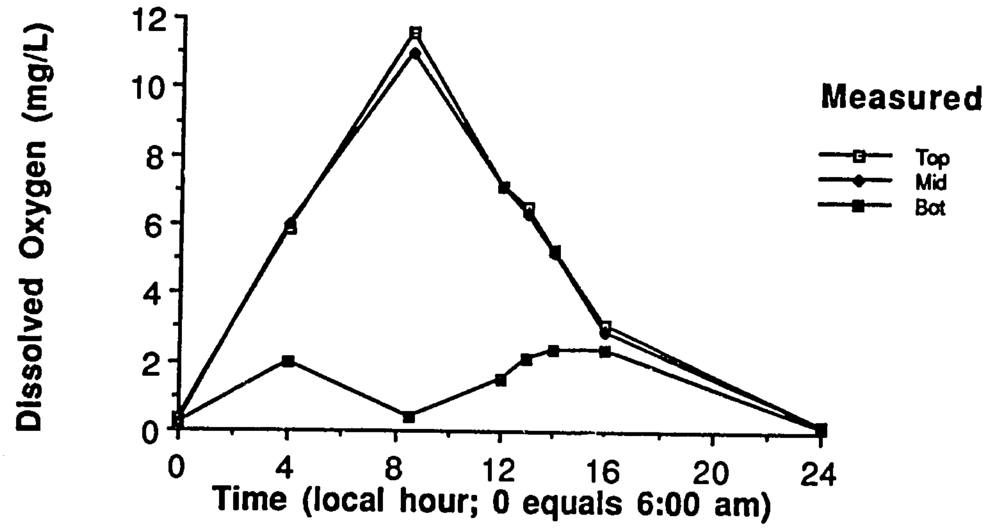


Figure 3. Comparison of measured dissolved oxygen with output from revised model; Rwanda PD/A CRSP Site, Julian Day 146, 1989, Pond #C1.



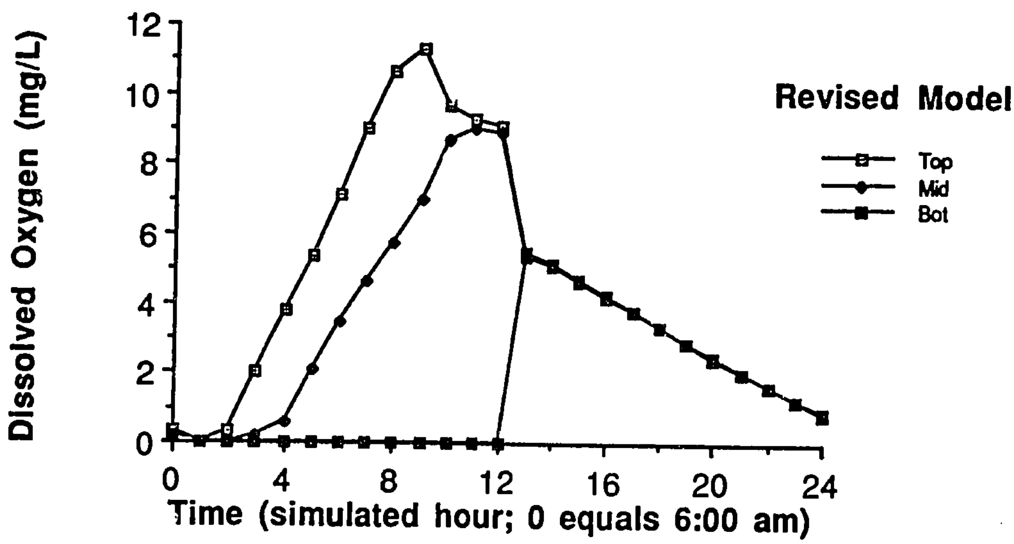


Figure 4. Comparison of measured dissolved oxygen with output from revised model; Honduras PD/A CRSP Site, Julian Day 351, 1990, Pond #B7.

We conclude that this revised model, initialized and calibrated with fairly simple and easily obtainable weather and water quality data, can be a reliable tool for use when trying to understand if changes in pond management or morphology will have qualitative and/or quantitative effects on dissolved oxygen levels within the pond. Also important to note is the robustness of the model when simulating ponds located in different parts of the world: the outputs presented here range from coastal to inland tropical regions, from tropical to temperate climates, and from elevations near sea level to 1700 meters.

Anticipated Benefits

The simulation results obtained with the revised dissolved oxygen model are very encouraging. The general trends in dissolved oxygen concentration changes at the various depths in a pond have been simulated with relatively modest data requirements. There are still differences between the measured and the simulated dissolved oxygen values, indicating weaknesses in our ability to model the processes of oxygen production and consumption in ponds. Continued improvements in the models are expected as the results of other CRSP research are incorporated. The revisions to the model described above now allow the user to ask a variety of "what if" questions which were not possible with the Losordo model. The revised model has been run for simulations describing ponds of depths from 45 cm to 120 cm, for ponds of surface areas ranging from 0.30 to 4.00 hectares, for ponds in sheltered areas and in exposed ones, and for ponds in both the southern and northern hemispheres. Simulation results have generally remained as consistently accurate for all these situations as for those described in this report.

The modified temperature and dissolved oxygen models retained their simulation accuracy even after implementing substantial reductions in data requirements, and after various changes in model structure. Given these results, the models can be very useful tools in the analysis of proposed aquaculture developments, and in the simulation of temperature and oxygen regimes in existing ponds. The models will be particularly useful in our continuing study of the effects of varying light extinction coefficients and pond depths on temperature and dissolved regimes in ponds.

Literature Cited

Boyd, C.E. 1990. Water quality in ponds for aquaculture. Birmingham Publishing Company, Birmingham, Alabama. 482 pp.

Culberson, S.D. 1993. Simplified model for prediction of temperature and dissolved oxygen in aquaculture ponds using reduced data inputs. M.S. Thesis. University of California at Davis.

Culberson, S.D., and R.H. Piedrahita. 1992. Modification of stratified temperature model to accommodate reduced data inputs: Identifying critical requirements. Presented at the Aquaculture 92 International Conference, Orlando, Florida. ASAE Paper no. AQUA-92-102.

Eppley, R.W. 1972. Temperature and phytoplankton growth in the sea. Fisheries Bulletin 70:1063-1085.

High Performance Systems, Inc. 1990. STELLA II® Software Program. Hanover, New Hampshire.

Losordo, T.M. 1988. The characterization and modeling of thermal and oxygen

stratification in shallow aquaculture ponds. Ph.D. Dissertation, University of California at Davis. 416 p.
Madenjian, C.P. 1990. Nighttime pond respiration rate: Oxygen or temperature dependent? Canadian Journal of Fisheries and Aquatic Science 47:180-183.
Meyer, D.I. 1980. Modeling diel oxygen flux in a simulated catfish pond. M.S. Thesis, University of California at Davis. 123 pp.
Piedrahita, R.H., S.D. Culberson, and P. Giovannini. 1992. Analysis and modeling of water quality in ponds. Pages 72-103 *in* Hillary Egna, Marion McNamara, Jim Bowman, and Nancy Astin, editors. Tenth Annual Administrative Report of the Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis, Oregon.
Poole, H.H., and W.R.G. Atkins. 1929. Photoelectric measurements of submarine illumination throughout the year. Journal of the Marine Biology Association United Kingdom, 16:297-324.
Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge. 384 pp.
Romaire, R.P., and C.E. Boyd. 1979. Effects of solar radiation on the dynamics of dissolved oxygen in channel catfish ponds. Transactions of the American Fisheries Society 108:473-478.
Smith, D.W., and R.H. Piedrahita. 1988. The relation between phytoplankton and dissolved oxygen in fish ponds. Aquaculture 68:249-265.
Szyper, J. P., J.Z. Rosenfeld, R.H. Piedrahita, and P. Giovannini. 1992. Diel cycles of planktonic respiration rates in briefly-incubated water samples from a fertile earthen pond. Limnology and Oceanography 37(6):1193-1201.
Teichert-Coddington, D.R., and B.W. Green. 1993. Influence of daylight and incubation interval on dark-bottle respiration in tropical fish ponds. Hydrobiologia 250:159-163.

Water Column Respiration in Aquaculture Ponds

Work Plan 6, Study 2

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Introduction

The measurement of pond respiration over diel periods provides an important tool for examining algal respiration dynamics. Current methods for estimating algal (or water column) respiration rates are usually limited to nighttime respiration. Measurements of respiration during daylight periods enable researchers to examine how respiration is affected by other processes and by physical changes in the pond such as light, net dissolved oxygen production, and water temperature. The UC Davis DAST has been cooperating with Dr. Jim Szyper, of the Thailand CRSP team, in the development of techniques and equipment for the measurement of respiration rates over diel cycles. The collaboration has extended to the analysis of results and the use of the newly available data in models of phytoplankton dynamics in ponds.

Objectives

The research described here addresses the research objectives presented in the Sixth Work Plan (Study 2), which call for:

1. The development of a consistent and convenient methodology for the determination of hourly rates of community light and dark respiration in aquaculture ponds over a diel period.
2. An examination of respiration rate dynamics over the diel period and the establishment of relationships between respiration rates and environmental factors.
3. The utilization of this knowledge to provide improved measurements of the pond primary production systems, for use in the CRSP Pond Efficiency and Optimization Models.

The first and third objectives have been addressed and discussed in detail in previous reports (Piedrahita et al. 1992). The focus of this report is therefore the second objective, which is discussed by presenting examples of diel water column respiration data compared to other pond water quality and environmental parameters.

Materials and Methods

The UCD DAST, in conjunction with Dr. Jim Szyper at the University of Hawaii, Mariculture Research and Training Center (MRTC), has developed and tested a prototype automated diel pond respiration measurement device. This device was used in September of 1991 to record respiration and other data from experimental tilapia ponds at MRTC. The data from these tests were analyzed to determine the potential for use of this type of apparatus for examining the respiration dynamics in shallow ponds. Modifications and improvements on the prototype tested at MRTC are continuing at UC Davis, under the Seventh Work Plan.

Results

Some examples of preliminary data collected at MRTC are presented to show the relationships between respiration rates and some of the processes mentioned. The data in Figures 1-4 show examples of commonly observed relationships between several variables and pond respiration rates from data collected during field research in MRTC experimental ponds during September 1991. These figures are representative of the patterns observed for a week of diel measurements under various cloud conditions.

Because respiration is a complex process that is considered to be a function of many variables, including DO concentration, temperature, light, and photosynthetic production, it is not possible to determine cause and effect relationships from simple correlations with other process variables, and no attempt is made to do so here. These results are presented to illustrate the dynamic behavior of pond respiration rates observed in some tests and to examine how these respiration rates change with respect to other observed variables under field conditions.

Solar Radiation
Figure 1 is an example of the relationship between solar radiation at the pond surface and the rate of water column respiration. The data show an increase in pond respiration coincident with increasing solar radiation. As solar radiation declines in the afternoon, respiration declines sharply as well, and remains low until solar radiation begins to increase again the next morning. This is consistent with results obtained from mass isotope studies, as reviewed by Graham (1980), which demonstrate the influence of light on respiration rates. More recently, Weger et al. (1989) reported similar results from laboratory studies that demonstrate the effect of light intensity on respiration rates in laboratory cultures of marine diatoms.

The effect of light intensity on phytoplankton respiration tends to persist even after the light source is removed or reduced. This post-illumination enhancement of respiration has been noted by many authors, as discussed by Falkowski et al. (1985). Although the post-illumination enhancement of respiration has been reported to be detectable for up to 2.5 hours (Falkowski et al. 1985), other researchers report a 60-80% decrease in respiration rates within five minutes after removal of the light source (Harris and Piccinin 1977). The examination of diel data from our field studies is consistent with the results reported by Harris and Piccinin (1977), and show that the maximum respiration rate usually tends to occur after the maximum solar radiation intensity. The relationship between pond water column respiration rate and solar intensity is the subject of continuing research by the UCD DAST.

Water Temperature
Respiration rates of phytoplankton and aquatic organisms are commonly modeled as functions of water temperature (Zison et al. 1978, Groden 1977, Riley 1963). Although the physiological basis for this relationship is well accepted with regard to

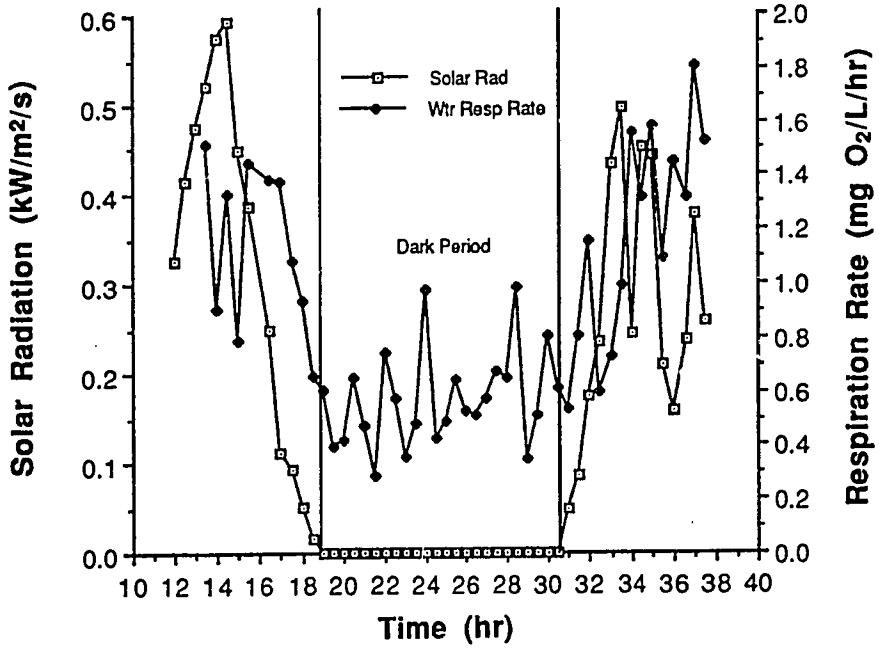

Figure 1. Solar radition and water column respiration rates measured over a 30-hour period starting at 1000 hours on September 14, 1991 (Time = 10), and ending at 1600 hours the following day (Time = 40). Sunset and sunrise are bracketed to indicate the dark period.

individual organisms, some studies suggest that the degree of temperature influence on community respiration rates decreases as the system approaches a "balanced ecosystem" (Beyers 1962). This observation is based on the postulate that highly integrated communities are characterized by a high interdependence, and that "the multiplicity of metabolic pathways assures the cyclic flow of energy regardless of... temperature extremes" (Beyers 1962). The data collected by the UCD DAST during this field study and previous studies have been consistent with this observation, in that they have not shown a significant correlation of water column respiration rates with temperature changes.

Figure 2 shows the same water column respiration data from Figure 1 plotted against water temperature. These data show no simple correlation between temperature and respiration rates. In addition to the possible counterbalancing effect of interrelated aquatic communities as noted above, another factor contributing to the lack of simple correlation between temperatures and respiration rates may be the simultaneous influence of endogenous respiration, which is temperature dependent, and photo-respiration, which is light dependent and which apparently dominates during the light period.

Dissolved Oxygen

As with temperature, it is generally accepted that respiration rates are influenced by dissolved oxygen concentrations. This is certainly true when dissolved oxygen

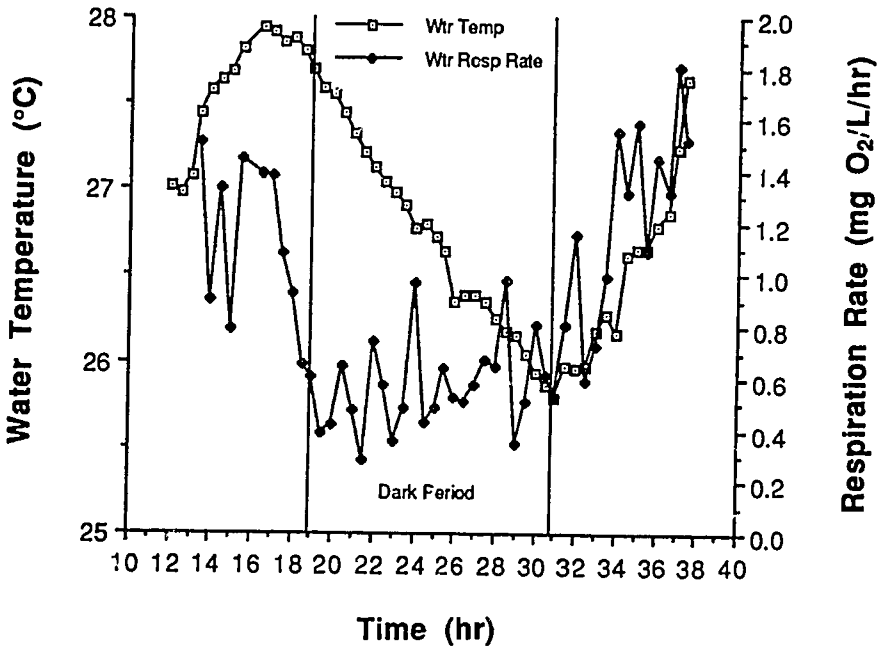

Figure 2. Water temperature and water column respiration rates measured over the same 30-hour period as in Figure 1.

concentrations decline below a threshold level and respiration becomes restricted by lack of oxygen. However, it is not clear at what point this effect becomes significant, and it is also unclear what influence dissolved oxygen concentrations have on respiration rates at higher concentrations. Two CRSP field studies were conducted which compared dissolved oxygen concentrations with diel water column respiration rates. The first (Szyper et al. 1992) showed that at dissolved oxygen concentrations below 8 mg/L, there was little correlation with observed respiration rates. At oxygen concentrations above 8 mg/L however, respiration rates increased with increasing dissolved oxygen concentrations (Szyper et al. 1992). Data from the more recent field study is shown in Figure 3. These data do not show a strong correlation during the dark period, but show a somewhat better correlation during the light period. In a recent study, Madenjian (1990) compared a model by Olah et al. (1986), which described nighttime respiration rates (endogenous respiration) as a function of DO concentration, against his own model, which described respiration rates as a function of temperature. Madenjian concluded that temperature was a more accurate predictor, but conceded that at lower DO concentrations the Olah model might have more validity. This is an area of investigation in which much more work needs to be done. The difficulties in this area are compounded by the fact that correlation does not indicate causality. This is especially true in that increasing dissolved oxygen concentrations are a result of increasing solar radiation which in turn is also responsible for increasing water temperatures.

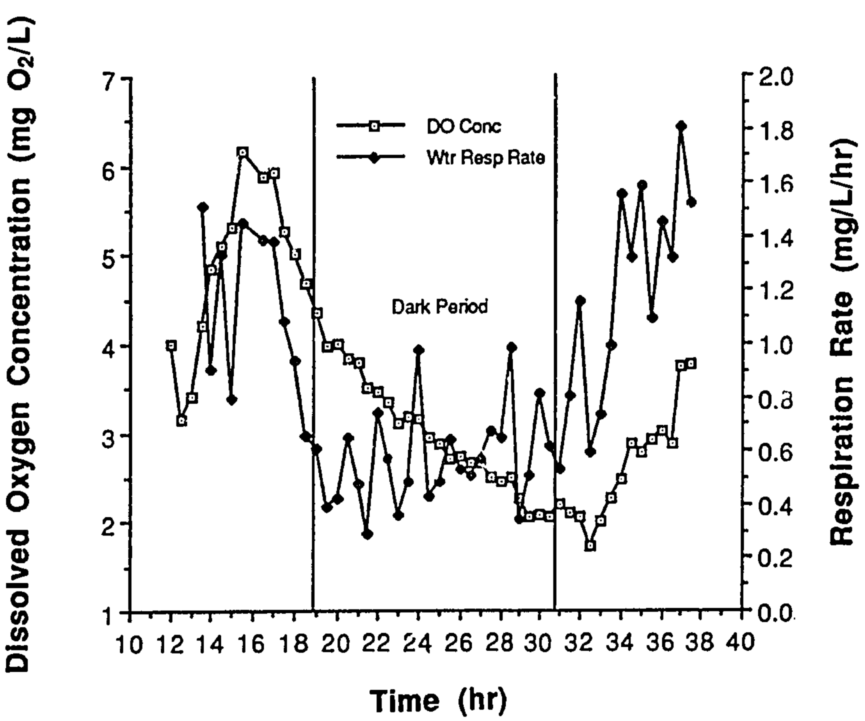

Figure 3. Measured water column dissolved oxygen concentrations and water column respiration rates measured over the same 30-hour period as in Figure 1.

Chlorophyll a Fluorescence

Chlorophyll *a* fluorescence is used in this study as an indicator of the light sensitivity of phytoplankton. In many cases, as phytoplankton are exposed to increasing levels of light intensity, their photosynthetic pigments tend to contract, resulting in a reduced level of light absorption and a consequent reduction in measured fluorescence (Harris and Piccinin 1977, Kiefer 1973a and 1973b). The relationship between respiration rates and chlorophyll *a* fluorescence is not usually described, although it has a physiological basis due to the effect of light and photosynthesis on both variables. This is an area which will be looked at more closely as further data are gathered.

An interesting correlation is seen in Figure 4, where respiration rates are plotted against chlorophyll *a* fluorescence. The inverse correlation appears to be fairly strong, but once again this does not necessarily imply a causative relationship.

Photosynthetic Production

Some empirical relationships have been proposed in the literature which describe pond respiration rates as a function of primary production. Groden (1977) gave a function for respiration as a percentage of photosynthetic production. The data recorded for primary production are not shown here because equipment malfunctions resulted in unusable data. The relationship was discussed in detail by Szyper et al. (1992). Culberson (1993) reported that good results can be obtained when modeling pond systems if daytime respiration is given as 10% of photosynthetic

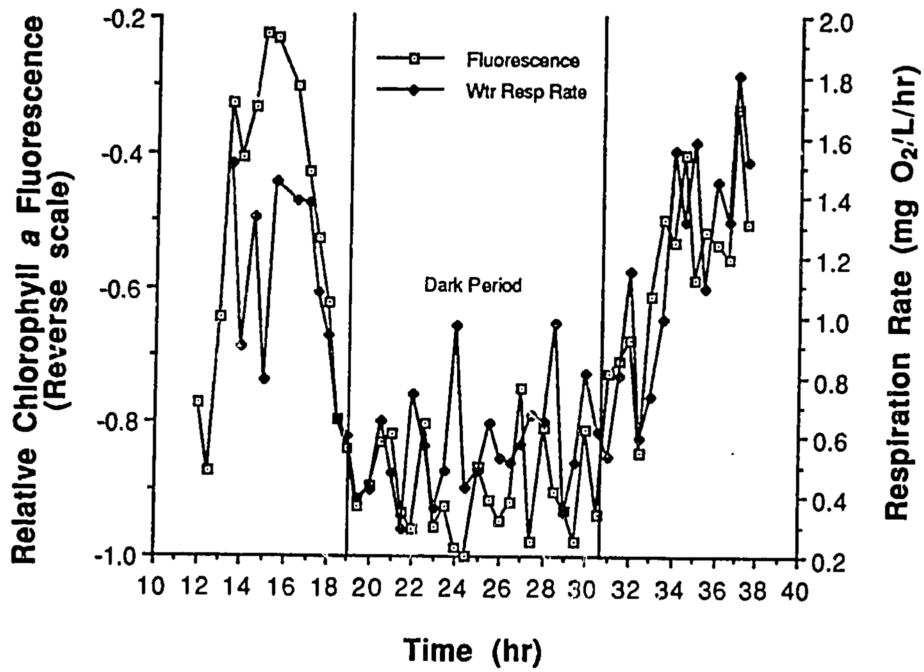

Figure 4. Measured chlorophyll *a* in vivo fluorescence and water column respiration rates measured over the same 30-hour period as in Figure 1. Fluorescence data are shown on a scale relative to the maximum millivolt output of the fluorometer. Fluorescence data are shown using a reverse scale to better show their relationship with respiration data.

production, and if respiration rate changes are offset in time by several hours from production rate changes. However, more research is needed to gain a better understanding of the relationships affecting this important pond process.

Anticipated Benefits

The results of this work have enabled an examination of how water column respiration rates change over the diel period, and of the relationships between diel respiration dynamics and other water quality, physiological, and environmental parameters. It is not possible to determine cause and effect from this type of analysis because of the interrelationships of many of the factors involved. However, it is possible to observe the effect of a particular parameter under field conditions, when the effect under controlled conditions is known to some degree. It is possible to examine, for example, how the known effects of solar radiation and temperature are expressed in field conditions. In this case, although temperature relationships at the organism and population level are well understood, the temperature effect was not readily apparent in the observed data. Conversely, a good correlation with solar radiation was apparent, the effect of which has not been as well documented in lab studies.

This research represents a beginning in the effort to describe diel pond primary production dynamics through the use of new tools and techniques for measuring water column respiration dynamics. It is expected that these tools will provide a continuing opportunity for detailed analysis of pond systems.

Literature Cited

Beyers, R.J. 1962. Relationship between temperature and the metabolism of experimental ecosystems. Science 136:980-982.

Culberson, S. 1993. Simplified model for prediction of temperature and dissolved oxygen in aquaculture ponds using reduced data inputs. M.S. Thesis. University of California at Davis, Davis, California.

Falkowski, P.G., Z. Dubinsky and G. Santostefano. 1985. Light enhanced dark respiration in phytoplankton. Verhandlungen des Internationalen Vereins für Limnologie 22:2830-2833.

Graham, D. 1980. Effects of light on "dark" respiration. Pages 526-575 *in* D.D. Davies, editor. The Biochemistry of Plants, Vol 2. Academic Press, New York.

Groden, T.W. 1977. Modeling temperature and light adaptation of phytoplankton. Report No. 2, Center for Ecological Modeling. Rensselaer Polytechnical Institute. Troy, New York.

Harris, G.P., and B.B. Piccinin. 1977. Photosynthesis by natural phytoplankton populations. Archiv für Hydrobiologie 80(4):405-457.

Kiefer, D.A. 1973a. Fluorescence properties of natural phytoplankton populations. Marine Biology 22:263-269.

Kiefer, D.A. 1973b. Chlorophyll-*a* fluorescence in marine centric diatoms: Responses of chloroplasts to light and stress. Marine Biology 23:39-46.

Madenjian, C.P. 1990. Nighttime pond respiration rate: oxygen or temperature dependent? Canadian Journal of Fisheries and Aquatic Sciences 47:180-183.

Olah, J., V.R.P. Sinha, S. Ayyappan, C.S. Purushothaman, and S. Radheyshyam. 1986. Primary production and fish yields in fish ponds under different management practices. Aquaculture 58:111-122.

Piedrahita, R.H., P. Giovannini, and J.P. Szyper. 1992. Procedures for estimating water column respiration rates. Pages 74-79 *in* H.S. Egna, M. McNamara, and N. Weidner, editors. Ninth Annual Administrative Report, PD/A CRSP 1991. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Riley, G.A. 1963. Theory of food-chain relations in the ocean. The Sea. 2:438-463.

Szyper, J.P., J.Z. Rosenfeld, R.H. Piedrahita, and P. Giovannini. 1992. Diel cycles of planktonic respiration rates in briefly incubated water samples from a fertile earthen pond. Limnology and Oceanography 37(6):1193-1201.

Weger, H.G., R. Herzig, P.G. Falowski, and D.H. Turpin. 1989. Respiratory losses in the light in a marine diatom: measurements by short-term mass spectrometry. Limnology and Oceanography 34(7):1153-1161.

Zison, S.W., W.B. Mills, D. Deimer, and C. W. Chen. 1978. Rates, constants, and kinetic formulations in surface water quality modeling. Tetra Tech Inc. Lafayette, California.

Special Topics Research in Host Countries

Introduction

The Global Experiment and related studies are the main emphasis of this CRSP. However, at times host country institutions or local USAID Missions have asked CRSP researchers to aid them by finding answers to more site-specific problems. Site-specific research though of a lower priority than the CRSP's core research nevertheless contributes significantly to the CRSP's overall success by further strengthening host country research institutions. Site-specific studies address immediate, practical concerns and thus demonstrate to administrators who may not always see the value of basic research in a developing country the benefit of being involved in this CRSP.

After the host country team has agreed to consider conducting a special topics study, it develops a proposal which is presented to the community of CRSP scientists. Proposals are reviewed for feasibility, scientific merit, contributions to the CRSP's research agenda, and consistency with host country development efforts. They are often funded by outside sources and thus are one of the avenues the CRSP uses to attract buy-ins.

During this reporting period, Special Topics Research reports have been contributed by CRSP workers at host country sites in Honduras, Rwanda, and Thailand. The host country institutions from these countries worked together with their colleagues from the University of Hawaii, the University of California at Davis, Auburn University, Oregon State University, and the Katholieke Universiteit Leuven in Belgium. Several private companies also cooperated in the two research studies conducted in Honduras.

One study examined the causes of cyclical variations in Honduran shrimp production, while a companion study investigated the effects of diet protein level on the growth of shrimp during wet and dry seasons. In Rwanda, researchers compared monoculture and polyculture practices in high elevation ponds. Studies in Thailand examined different aspects of plankton respiration in freshwater and brackish water ponds.

Causes of Cyclical Variation in Honduran Shrimp Production

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Introduction

Rainfall in central and southern Honduras is seasonal, starting in May and ending in November (Figure 1). Temperatures during this period are higher than during the dry season, which runs from November to April; the dry season largely corresponds to the North American winter season. Estuarine salinities vary from near zero ppt during the rainy season to hypersaline levels in the dry season. The proportion of *Penaeus vannamei* and *P. stylirostris* larvae caught in the estuaries also changes with time, although strict correlation with season is low. Yields of penaeid shrimp cultured in southern Honduras generally decrease during the dry season and increase during the wet season (Williams and Wong 1991). Low dry season yields have been observed in other shrimp producing regions of Central America as well. Variation in shrimp production between seasons is usually explained by differences in environmental factors, although neither the relative importance of individual environmental factors nor the effect that management strategies have on production has been quantified. The objectives of this study were to determine if cyclical production was predictable, and to evaluate the relationship between shrimp yields and data on key environmental and management variables collected over time on commercial farms.

Materials and Methods

Time series data on stocking, harvest, temperature, and salinity were evaluated for two commercial farms in southern Honduras. Both farms were located on estuaries of the Choluteca River. Data from Farm A were collected from 15 production ponds during consecutive production cycles between 1986 and 1991. For Farm B, data were collected from 26 ponds between 1988 and 1991. On Farm A, salinity and early morning and late afternoon temperatures were measured three times a week. Measurements were taken at about 30 cm depth. On Farm B, temperature was measured near the pond bottom once a week in all ponds, but only the maximum and minimum values for the farm were recorded. Average salinity for the farm was recorded once a week. Salinity was measured with a refractometer. Ponds were stocked with juvenile *P. vannamei* and *P. stylirostris* nursed from postlarvae that had been captured in the adjacent estuaries. The proportion of each species stocked

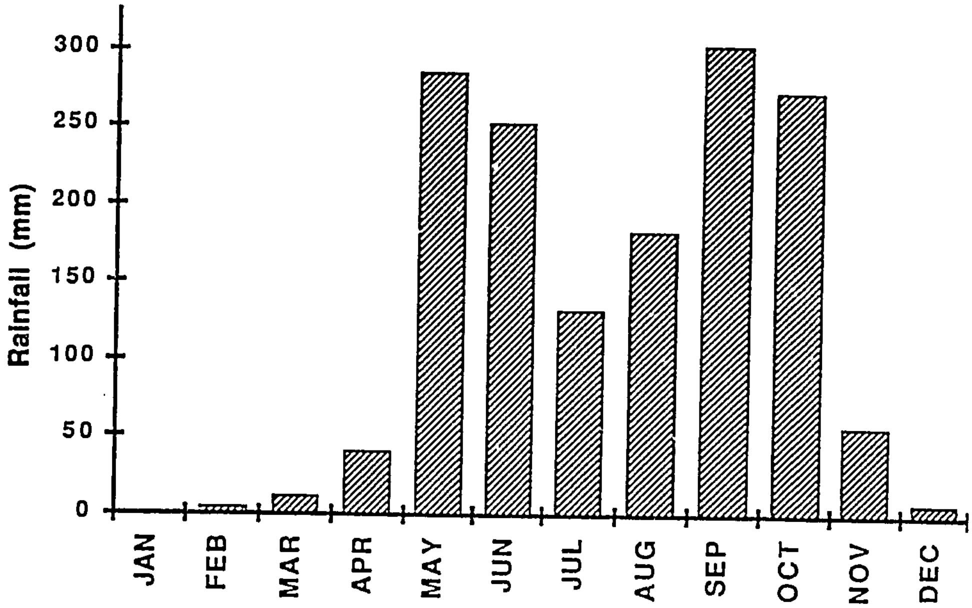

Figure 1. Thirteen-year average monthly rainfall recorded in La Lujosa, Choluteca, southern Honduras, by the Dirección General de Recursos Hídricos, Secretaria de Recursos Naturales, Government of Honduras.

varied according to the proportions of each species in wild-caught larvae. Mean weight and stocking density of shrimp were 0.6 g and 5.6/m^2, respectively. Ponds were managed similarly. Shrimp were left in ponds as long as they were growing. The mean length of the growth period was 16.6 to 17.6 weeks. Shrimp were sampled weekly for growth, and were fed a commercial diet usually consisting of 21% crude protein at rates that decreased as mean shrimp size increased (Teichert-Coddington et al. 1991). Ponds were usually fertilized with low quantities of chicken litter during the first month of growth.

Data from each farm were analyzed separately. Data for each growth cycle were averaged by pond, and for all cycles by month and year using the stocking date as the reference time. Box plots were used to represent data distributions by month of stocking. The box plot displays five parameters of the data distribution: the median (50 percentile), the upper and lower quartile (75 and 25 percentile), and the upper and lower extremes (90 and 10 percentile). Data were also analyzed by step-wise multiple regression. Data were analyzed using software by Haycock et al. (1992). Differences were declared significant at alpha level 0.05. Variables were not added to step-wise regressions unless their slopes were significantly different from zero ($P < 0.05$).

Results

The characteristics of each farm are described in Table 1. Time series production data at both farms revealed cyclical patterns in shrimp yield, temperature, and salinity (Figures 2 and 3). The cycles were more obvious on Farm A because data were collected over a longer period of time. The amplitudes of salinity cycles were higher than those of temperature cycles. Minimum and maximum salinity and early

Table 1. Means of production and environmental variables characterizing two commercial shrimp farms from which time series data were taken for analysis.

Variable	Farm A	Farm B
No. of cases	200	131
Pond area (ha)	22	16
Stocking Density (No./m^2)	5.6	6.2
% *P. vannamei* stocked	60	54
Growth cycle (wk)	17.6	16.6
Survival (%)	83	65
Mean shrimp weight (g)	16	14
Yield (kg/ha)	724	543
Salinity (ppt)	21	19
Max. temperature (°C)	31.4	30.5
Min. temperature (°C)	27.8	26.3

morning temperature for Farm A ranged from 2 to 43 ppt, and from 19 to 33°C, respectively. A notable exception to cyclical yields on Farm A occurred during 1989 (Figure 2), when yields remained low during the whole year. The mean yield for 1989 was 512 kg/ha, compared with 777 kg/ha during the other years sampled. Low production during 1989 was related to a low proportion of P. vannamei in wild-caught larvae. The mean percentage of *P. vannamei* during 1989 was 14%, compared with 72% during the other years.

Monthly averages indicated that yields were highest when ponds were stocked during the period from April to July and lowest when stocking occurred between November and February (Figures 4 and 5). Insufficient data were available for February and March to calculate box plots for Farm B. Data for July on Farm A (Figure 4) show low yields due to a low proportion of *P. vannamei*. A disproportionately high number of stockings occurred during July 1989, compared with June and August of 1989, thereby biasing yields and percentage *P. vannamei* downwards. Wide box plots for the proportion of *P. vannamei* stocked indicated high variation within months (Figures 4 and 5), and made it difficult to define cyclical variation during the year, especially for Farm A. However, median values on both farms were low during December and January and high during May through October.

Stepwise regression analyses were performed on all data and on monthly averages of each variable. Survival, stocking density, salinity, and early morning temperature accounted for 76% of the variation for all data on Farm A, while 80% of the variation on Farm B was explained by survival, stocking density, and minimum temperature (Table 2). Temperature, salinity, and percentage of P. *vannamei* stocked in ponds are environmental variables producers cannot directly control. The proportion of *P. vannamei* stocked in ponds was classified as an environmental variable because producers depend on wild-caught postlarvae and cannot control species composition of catch. When yield was regressed solely on environmental variables only 33% and 38% of the variation among yields for all data could be explained on Farms A and Farm B, respectively (Table 2).

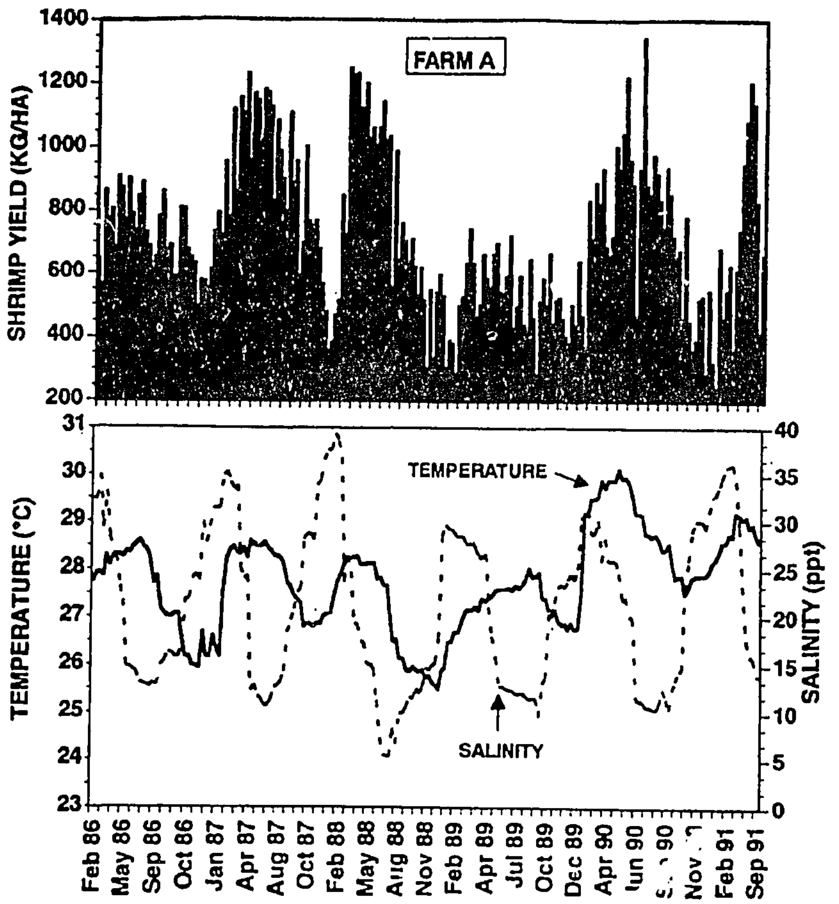

Figure 2. Shrimp yield, minimum water temperatu,.,, and salinity recorded in 15 production ponds of Farm A during 1°°6 to 1991. Temperature and salinity were averaged for each productio11 cycle.

Regression of mean monthly yield on monthly means of all variables accounted for 97% and 94% of the variation on Farms A and Farm B, respectively (Table 3). If only environmental variables were included as independent variables, then 88% and 78% of the monthly variation could be explained on Farms A and Farm B, respectively (Table 3).

Discussion

Total variation included inter-pond variability in addition to seasonal variability, whereas variation among monthly averages was attributed primarily to season (environment). Survival explained the greatest amount of total variation on both farms. Survival and yield have been highly correlated in other studies on Honduran shrimp ponds, regardless of treatment or stocking density (Teichert-Coddington et al. 1991, unpublished data). Survival is probably auto-correlated with yield; i.e., whatever induces poor yield also induces poor survival. Stocking density may similarly be partially autocorrelated with yield in that producers tended to stock higher densities of shrimp during the good growing seasons. It is not surprising that only about a third of total variation among pond data could be explained by

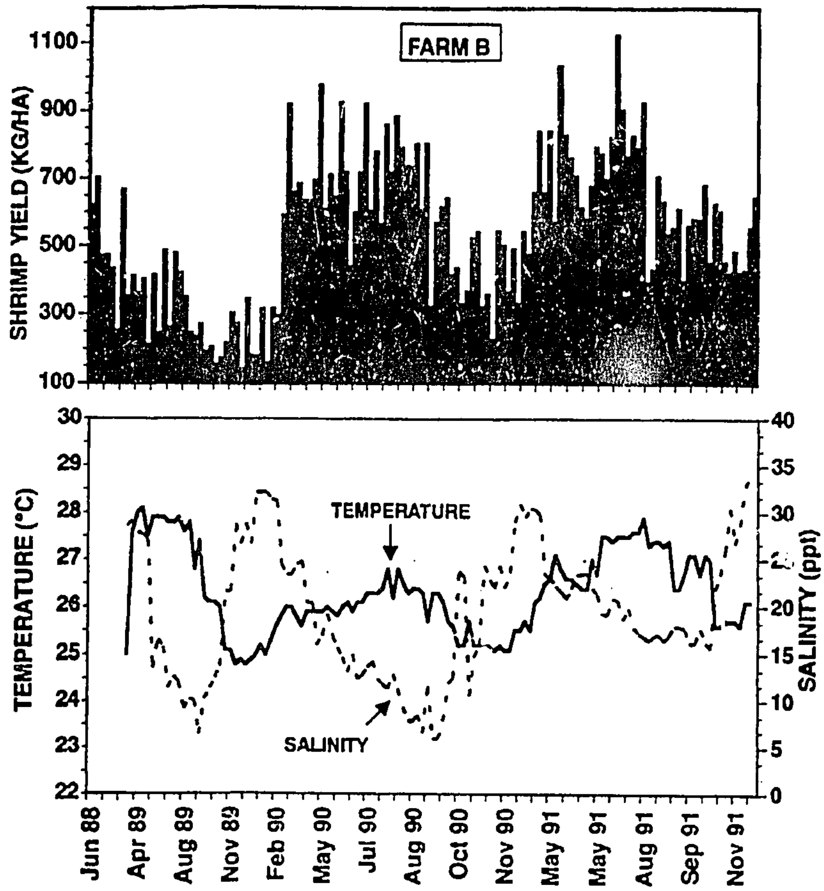

Figure 3. Shrimp yield, minimum water temperature, and salinity recorded in 15 production ponds of Farm B during 1988 to 1991. Temperature and salinity were averaged for each production cyc'

environmental variables, because environmental variation was small for ponds stocked within a relatively short period of time. Variation among ponds could be caused largely by other factors, such as stocking density, and variables not studied, such as dissolved oxygen, water exchange, feeding, size of juveniles at stocking, and growth period. Temperature and percentage of *P. vannamei* stocked in ponds explained all or most of the variation caused by environmental factors on both farms, while salinity apparently played a minor or insignificant role.

An objective of this study was to explain seasonal production differences. Seasonal variation is only a component of total variation and could therefore be obscured by inter-pond variation. Inter-pond variability was minimized and seasonal variability emphasized by averaging data by month. Regression of monthly shrimp yield on monthly means of other variables revealed that the periodicity of yields over a 12 month time span could be largely explained by temperature, and to a smaller extent, by percentage of stocked *P. vannamei*. Survival could still explain significant variation among yields, but temperature explained almost as much as survival on Farm A, and it explained more than survival on Farm B.

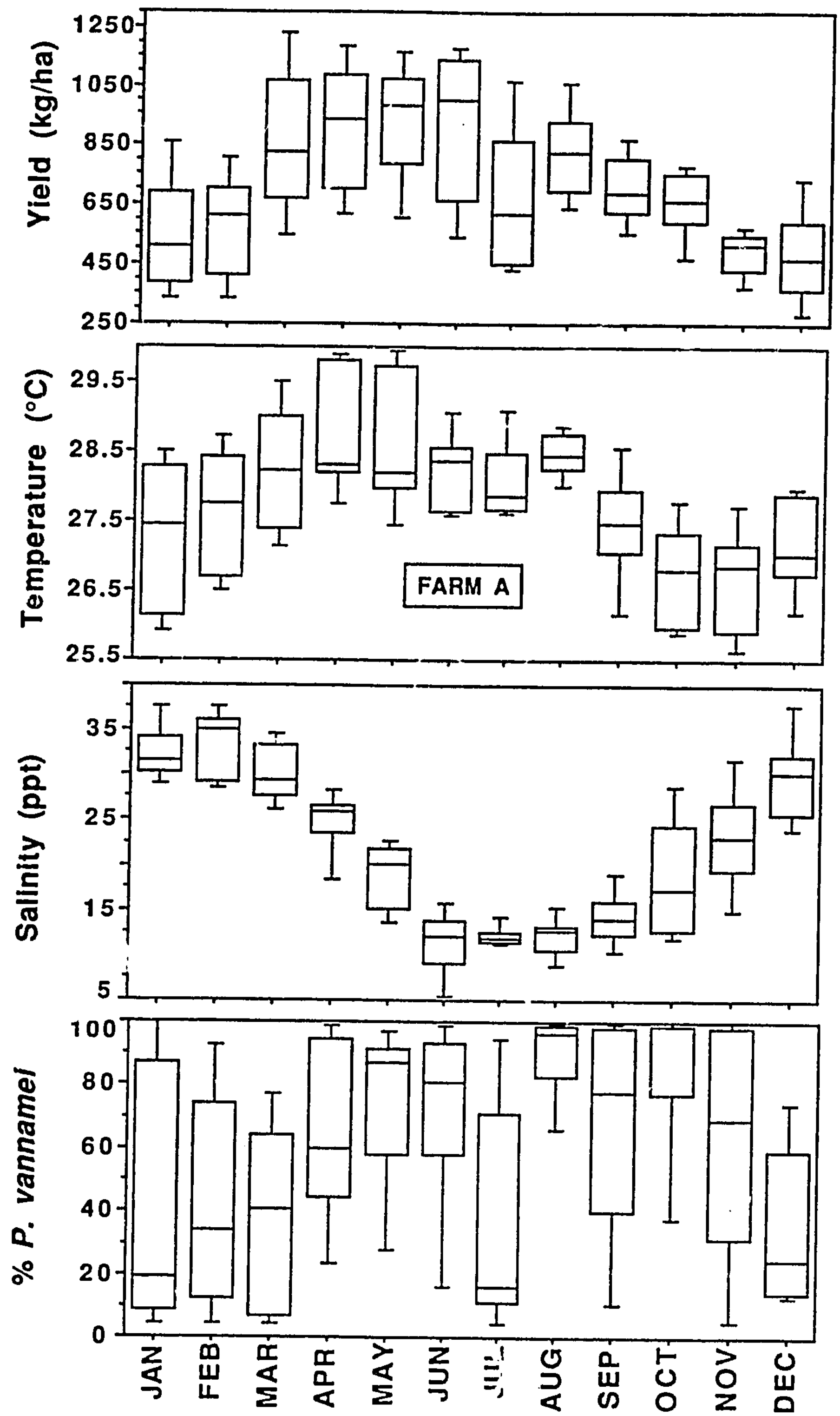

Figure 4. Box plots, by stocking month, of shrimp yield, minimum water temperature, salinity, and percent *P. vannamei* stocked in Farm A ponds during 1986 to 1991.

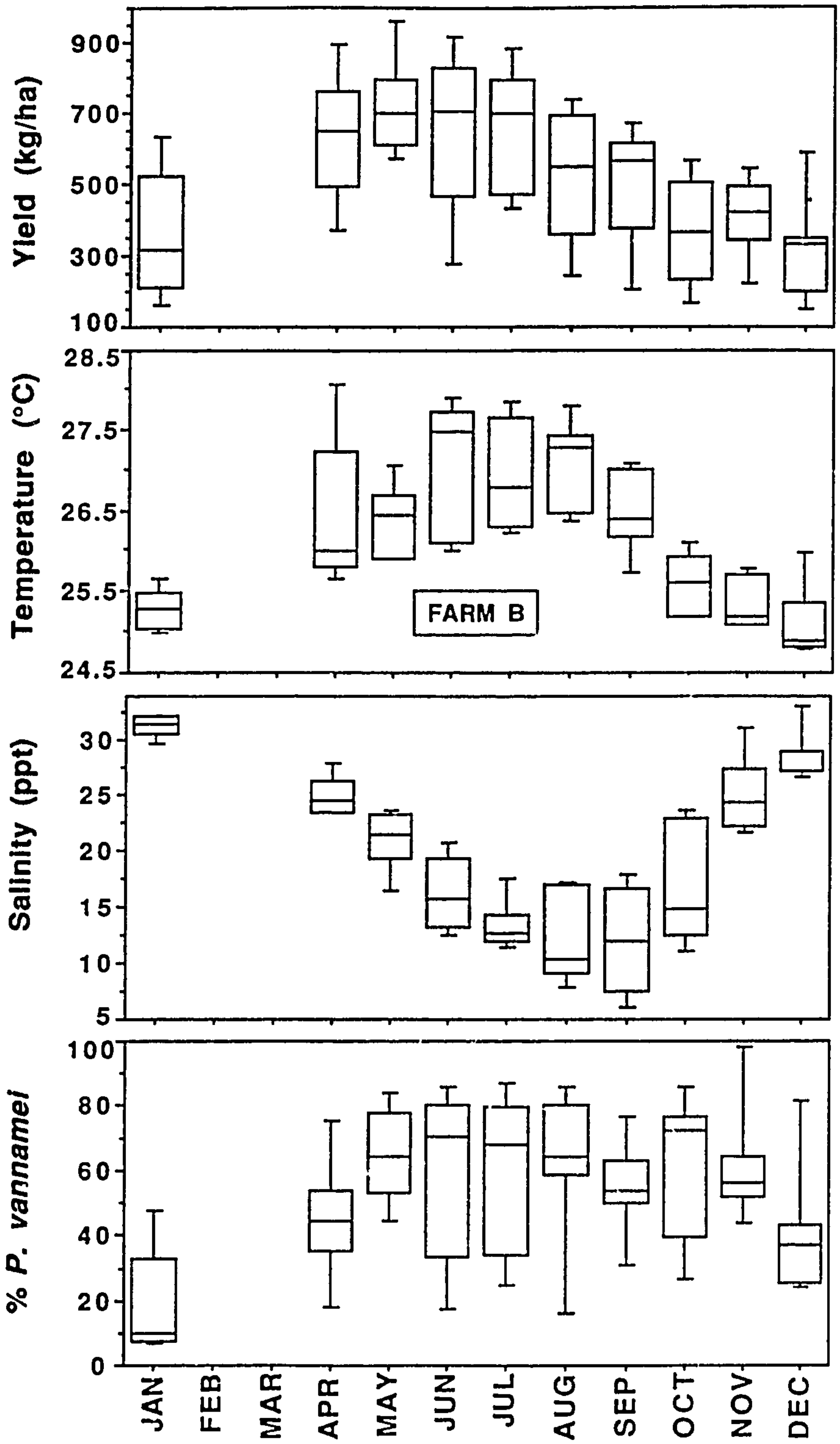

Figure 5. Box plots, by stocking month, of shrimp yield, minimum water temperature, salinity, and percent *P. vannamei* stocked in Farm B ponds during 1988 to 1991. Insufficient data were available for plots during February and March.

Table 2. Percentage of variation in shrimp yields from two farms that is explained by all variables or only by environmental variables, in step-wise multiple regression. n = 200 and 131 for Farms A and B, respectively.

Farm A		Farm B	
All variables			
Survival	48%		
Stocking density	15%	Survival	60%
Salinity	10%	Stocking density	19%
Min. temperature	3%	Min. temperature	1%
Total	76%	Total	80%
Only environmental variables			
Min. temperature	24%		
% *P. vannamei*	7%	% *P. vannamei*	27%
Salinity	2%	Max. temperature	11%
Total	33%	Total	38%

A high correlation between temperature and yield does not necessarily imply a cause and effect relationship. Low dry season temperatures may be associated with other dry season changes, for example, changes in water quality or plankton species, that are actually reducing shrimp growth. Temperature may directly affect shrimp physiology, because metabolic rates of poikilothermic animals decrease with declining ambient temperatures. The ratio of two rates separated by 10°C is customarily denoted as a Q_{10}. A review of Arrhenius Q_{10} values for metabolic rates of fish (measured as time-specific oxygen consumption) by Regier et al. (1990) revealed values ranging from 1.7 to 2.5. The Q_{10} for growth rate of common carp was 2.5 for a temperature range of 15.6 to 26.4°C. The ecological rate refers to the effects of temperature on the ecology of a system which give rise to the shrimp. The Q_{10} value for the ecological rate of penaeid shrimp capture yield in water temperatures ranging from 16.9 to 28.3°C is 5.3. Liao and Murai (1986) calculated a Q10 of $2.45 \times W^{0.036}$ for oxygen consumption by *P. monodon* between 25 and 30°C (W = weight of animal). Huang (1983) demonstrated the effects of relatively small temperature differences on growth of *P. vannamei* in the laboratory. After 30 days, the growth of 0.1-g postlarvae grown at 25.9°C and 25 ppt salinity was 45% less than that of postlarvae grown at 29°C and 25 ppt salinity. Results of these studies confirm that temperature can be expected to affect shrimp growth in the temperature range encountered during this study.

Mean monthly salinity did not correlate well with yield, although it was probably important during a month or two of the year when mean concentrations were near 40 ppt. The effects of high salinity on penaeid shrimp growth are less defined, partly because of the ability of penaeid shrimp to osmoregulate hemolymph over a wide range of salinities. Although it is assumed that shrimp grow best at iso-osmotic salinities, when energy would not be diverted to osmoregulation, Cheng and Liao (1986) found that salinity had little affect on the metabolic rates of penaeid shrimp.

Table 3. Percentage of variation among mean monthly shrimp yields from two shrimp farms that is explained by all variables or only by environmental variables, in step-wise multiple regression. n = 12 and 10 for Farms A and B, respectively.

Farm A		Farm B	
All variables			
Survival	75%		
Salinity	20%	Max. temperature	85%
Min. temperature	2%	Survival	9%
Total	97%	Total	94%
Only environmental variables			
Min. temperature	75%		
% P. vannamei	13%	Max. temperature	85%
Total	88%	Total	85%

Liao and Murai (1986) demonstrated that no increase in metabolic oxygen consumption of *P. monodon* was noted at ambient salinities ranging from 5 to 45 ppt. On the other hand, Huang (1983) observed a 21% reduction in the growth of *P. vannamei* postlarvae when the salinity of the water was increased from 25 to 45 ppt while maintaining the temperature at 29°C; the iso-osmotic salinity of *P. vannamei* hemolymph is about 25 ppt (Castille and Lawrence 1981). These data suggest that salinity may not affect growth until a threshold concentration is approached, and this threshold varies with animal size and species (Cheng and Liao 1986). The effects of temperature on growth were apparently greater over a smaller range, and probably masked the effects of hypersalinity, which tended to occur during months of low temperature (Figures 4 and 5). Farms located further upstream in the estuaries may experience salinities in excess of 50 ppt during the dry season, and observations indicate that their yields are indeed lower. Nonetheless, the effects of salinity on shrimp yields on Farms A and B were apparently less important than the effects of temperature.

Yields from ponds stocked predominantly with *P. vannamei* are greater than those stocked with *P. stylirostris* (Teichert-Coddington et al. 1991, Chamberlain et al. 1981). This study confirmed that yields increased as the proportion of stocked *P. vannamei* increased. Farms were sometimes constrained to stocking a low proportion of *P. vannamei* because of a lack of laboratory-produced animals. Recently, laboratory-reared *P. vannamei* have become more available, so yield variations due to low percentages of stocked *P. vannamei* juveniles will be minimized.

Climate cannot be controlled, but farm management can take its predictable effects into account. Ponds stocked during the period from April to July will predictably yield more than ponds stocked later in the year. Low dry season yields will probably not be offset by the currently employed strategies of increasing feeding rates and offering more expensive high quality feeds. On the contrary, if yields are lower because of decreases in temperature-dependent metabolic rates, then feeding rates should actually be reduced, because shrimp will consume less feed and will probably be unable to take advantage of higher quality diets. This contention is supported by a study in Choluteca (Garcia-Casas 1990) that demonstrated no advantage to using a high performance 35% protein diet instead of a standard 22% protein diet during the dry season. Current research is evaluating strategies for coping with dry season yield reductions.

Literature Cited

Castille, F.L., and A.L. Lawrence. 1981. The effect of salinity on the osmotic, sodium, and chloride concentrations in the hemolymph of euryhaline shrimp of the genus Penaeus. Comparative Biochemistry and Physiology 68A:75-80.

Chamberlain, G.W., D.L. Hutchins, and A.L. Lawrence. 1981. Mono- and polyculture of Penaeus vannamei and Penaeus stylirostris in ponds. Journal of the World Aquaculture Society 12(1):251-270.

Cheng, J.-H., and I.-C. Liao. 1986. The effect of salinity on the osmotic and ionic concentrations in the hemolymph of *Penaeus monodon* and *P. penicillatus*. Pages 633-636 *in* J.L. Maclean, L.B. Dizon, and L.V. Hosillos, editors. The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Garcia-Casas, I. 1990. Response of *Penaeus vannamei* and *Penaeus stylirostris* to various supplemental feeding regimes in semi-intensively managed earthen ponds in Honduras. Master's Thesis. Auburn University, Auburn, Alabama.

Haycock, K., J. Roth, J. Gagnon, W.R. Finzer, and C. Soper. 1992. Statview 4.0. Abacus Concepts, Inc., Berkeley, California.

Huang, H-J. 1983. Factors affecting the successful culture of *Penaeus stylirostris* and *Penaeus vannamei* at an estuarine power plant site: temperature, salinity, inherent growth variability, damselfly nymph predation, population density and distribution, and polyculture. Ph.D. Dissertation. Texas A&M University, College Station, Texas.

Liao, I.-C., and T. Murai. 1986. Effects of dissolved oxygen, temperature and salinity on the oxygen consumption of the grass shrimp, *Penaeus monodon*. Pages 641-646 *in* J.L. Maclean, L.B. Dizon, and L.V. Hosillos, editors. The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Regier, H.A., J.A. Holmes, and D. Pauly. 1990. Influence of temperature changes on aquatic ecosystems. Transactions of the American Fisheries Society 119(2):374-389.

Teichert-Coddington, D.R., B.W. Green, and R.W. Parkman. 1991. Substitution of chicken litter for feed in production of penaeid shrimp in Honduras. The Progressive Fish-Culturist 53:150-156.

Williams, C., and I. Wong. 1991. Manejo de granjas camaroneras semi-intensivas, Choluteca, Honduras. Memorias, Simposio Centroamericano Sobre Camaron Cultivado. FPX, P.O. Box 1442, San Pedro Sula, Honduras.

Effect of Diet Protein Level on Semi-Intensive Commercial Growout of *Penaeus vannamei* in Honduras During Wet and Dry Seasons

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Introduction

Most shrimp culture in Central America is semi-intensive, using *Penaeus vannamei* as a principal species and *P. stylirostris* as a secondary species. In Honduras, shrimp are generally stocked at densities of 5 to 10/m^2. Higher stocking densities are avoided to minimize water quality and disease problems. Shrimp are fed commercial diets formulated in-country or imported from other Central American countries or the USA (Meyers and Leyva 1993). The majority of the larger farms test various protein levels, types and sources of feed, but results are unclear.

Much research has been published on shrimp nutrition, but most has been conducted in aquaria, or in clear water and intensive systems. Little research about feeding semi-intensively cultivated shrimp has been published, yet feed is a major variable cost of production. Protein is a costly component of feed, so the reduction of protein content to required levels is essential reducing costs associated with feeds. Recommended protein levels for shrimp culture vary between 36 and 40% (Akiyama 1992). Protein contents in growout rations used in Honduras range from 20 to 40% (Meyers and Leyva 1993). It is suspected that lower protein levels would be adequate in semi-intensive production (Akiyama 1992), because semi-intensive pond culture includes natural foods that shrimp ingest and utilize for growth (Hunter et al. 1987, Anderson et al. 1987).

Some pond studies have indicated that protein levels could be lowered without decreasing semi-intensive shrimp production. Teichert-Coddington and Arrue (1988) demonstrated no differences in the yields of *P. vannamei* when dietary protein was increased from 29 to 37% at stocking rates of 4 and 8/m^2. Garcia-Casas (1990) tested the effects of 22 and 35% protein diets on yields of *P. vannamei* and *P. stylirostris* shrimp co-stocked at 10/m^2 in Honduras. An imported high-performance diet (35% protein) significantly improved yield in the wet season, but not in the dry season, when compared with locally formulated diets. Use of the high-performance diet did not significantly improve profitability, however.

The Garcia-Casas study (1990) provided preliminary information on the role of dietary protein in Honduran shrimp culture. However, the study was complicated because of different stocking rates for *P. vannamei* and *P. stylirostris*; only one set of stocking rates was used; and in the wet season shrimp were stocked too late to guarantee optimum growth. We therefore undertook a follow-up study to evaluate the effects of dietary protein levels on the production of 100% *P. vannamei* stocked at the range of densities used in Honduras and grown during the best and worst times of the year.

Materials and Methods

This study was carried out in ponds excavated in salt flats. Ponds were 0.7 m deep, 0.7 to 1.0 ha in area, and received water from an estuary near the mouth of the Rio Choluteca. The study was repeated during the wet and dry seasons. The wet season study was stocked on 3 July 1992 and harvested after 20 weeks, on 24 November 1992. The dry season study was stocked on 22 December 1992 and harvested after 16 weeks, on 14 April 1993. The methods were the same for each study except where noted.

A completely randomized block design in 2x2 factorial arrangement was used to test two levels of diet protein at two stocking densities. Treatments were replicated three times. Juvenile shrimp were stocked at 5.5 and $11/m^2$ during the wet season and 5 and $10/m^2$ during the dry season. A diet containing 20 or 40% crude protein was offered at each density. Initial shrimp sizes were 1.9 g for shrimp during the wet season and 0.3 g during the dry seasons. Shrimp were 100% *P. vannamei* that had been hatched in a laboratory and nursed in a single pond. Shrimp were fed, six days a week, with a pellet formulated by a local feed mill according to specifications shown in Table 1. The daily ration was divided into three meals. The daily feed ration offered was based on a relationship between percentage of biomass and mean individual shrimp weight: $Y = 11.74 - 6.79 \text{Log}_{10} X$, where Y = percentage of biomass and X = mean individual shrimp weight. A weekly mortality rate of 0.5% was assumed. Shrimp were sampled weekly for growth.

Early morning dissolved oxygen and temperature were determined daily using YSI oxygen meters. Secchi disk visibility, pH, and salinity (measured with a refractometer) were registered weekly. Chlorophyll *a* was measured weekly according to the method of Boyd and Tucker (1992) during the last half of the dry season. Twenty percent of pond water volume was exchanged once a week during the wet season and twice a week during the dry season. When early morning dissolved oxygen fell below 3 mg/L, an additional 10% of the pond water was exchanged. During the dry season water was exchanged more frequently to moderate high salinity; ponds exhibit higher salinities than estuaries because of evaporation. Water was exchanged by first draining and then refilling. Monks were used as water inlet and discharge structures, so water exchange was monitored by the number of boards removed to achieve a given exchange rate.

Ponds were completely drained and shrimp were harvested when weekly samples indicated that growth had stopped. A limited financial analysis was conducted using data on variable costs for feed, seed, and water, and on income received at the packing plant for the harvested product. Shrimp prices varied with size. Feed costs were 1.97 and 2.78 Lempiras (Lps.)/kg for low and high protein diets, respectively. Shrimp seed cost Lps. 69.60/1000 juveniles. Water costs were Lps. $0.095/m^3$, and included pump depreciation, fuel, and labor to maintain the pumps. The exchange

Table 1. Yields and profitability of *P. vannamei* stocked at 5 (low density) to 11/m^2 (high density) and offered a diet containing 20 or 40% crude protein.

Treatment	Yield (kg/ha)	Mean size (g)	Survival (%)	Efficiency (feed/yield)	Costs (Lps./ha)				Income (Lps./ha)	
					Larvae	Feed	Water	Total	Gross	Net
Wet season										
Low/20%	1437	24.8	99	2.07	3782	5698	3347	12827	50942	38115
Low/40%	1422	24.8	98	2.05	3782	7807	3259	14848	48410	33562
High/20%	2076	21.3	88	2.65	7736	10749	4256	22741	61592	38851
High/40%	2192	21.8	87	2.56	7736	15329	4212	27271	68418	41147
High vs. low density	**	**	*	*	**	**	**	**	*	ns
20% vs. 40% protein	ns	ns	ns	ns	ns	**	ns	**	ns	ns
Density X Protein	ns	ns	ns	ns	ns	**	ns	**	ns	ns
Dry season										
Low/20%	415	9.5	89	3.73	3438	3017	3657	10202	6675	-3528
Low/40%	423	9.6	89	3.44	3438	4039	3591	11158	6794	-4363
High/20%	596	9.7	62	4.92	6876	5750	3924	16727	10165	-6563
High/40%	599	9.8	61	5.16	6876	8368	4123	19544	10274	-9271
High vs. low density	**	ns	**	**	**	**	ns	**	**	**
20% vs. 40% protein	ns	ns	ns	ns	ns	**	ns	**	ns	ns
Density X Protein	ns	ns	ns	ns	ns	**	ns	*	ns	ns

* significant ($P < 0.05$); ** highly significant ($P < 0.01$); ns = not significant.

rate of Lempiras to the U.S. dollar was 5.8:1. Data were analyzed using statistical software by Haycock et al. (1992). Differences were declared significant at alpha = 0.05.

Results

Mean morning and afternoon temperatures were 29.0 and 32.0°C, respectively, during the wet season, and 27.8 and 30.7°C during the dry season. Salinity ranged between 3 and 26 ppt, with a mean of 14 ppt, during the wet season. During the dry season, salinity ranged from 26 to 46 ppt, with a mean of 36 ppt.

Protein Level
Protein level had no significant affect ($P > 0.05$) on yields, average weight of shrimp, or water quality variables at either density during either season (Tables 1 and 2). Mean shrimp yields for high and low protein levels were 1807 and 1756 kg/ha, respectively, during the rainy season, and 447 and 485 kg/ha during the dry season. Shrimp growth was curvilinear, stopping after 18 weeks during the wet season and 15 weeks during the dry season (Figure 1). Total costs were significantly higher for the high protein treatment, but net income was not significantly different.

Stocking Density
Higher stocking rates yielded significantly ($P < 0.05$) greater shrimp production during both seasons (Table 1). Mean shrimp yields for high and low density were 2134 and 1429 kg/ha, respectively, during the rainy season, and 547 and 384 kg/ha during the dry season. Net income was not significantly different between stocking densities during the wet season, but was significantly less for the high density during the dry season. Mean survival was significantly lower at the higher stocking rate during both seasons. The mean weight of shrimp stocked at the high density (24.8 g) was significantly less than the mean weight of shrimp stocked at the low density (21.5 g) during the wet season, but there were no significant differences between mean weights due to stocking density during the dry season. Mean shrimp weight during the dry season was 9.6 g. Although differences were small, mean early morning DO was significantly lower at the high stocking density during both seasons (Table 2). Water exchange, which was initiated on an emergency basis when DO fell below 3.0 mg/L, was significantly greater in the high density ponds during the wet season.

Mean yields and average shrimp weight were 380 and 242 percent greater, respectively, in the wet than in the dry season. Net income was negative for all treatments during the dry season. Primary production was apparently higher during the wet season as indicated by lower DO and Secchi disk visibilities (Table 2).

Discussion

Shrimp were grown under ideal management conditions during two distinct seasons of the year at the stocking densities used most often in Honduras with the objective of evaluating the usefulness of high-protein diets. Early morning oxygen concentrations were kept higher than 3.0 mg/L to minimize the deleterious effects of poor water quality. The daily ration was offered three times a day to maximize the accessibility of feed to the shrimp; Robertson et al. (1992) reported significant increases in shrimp growth as feeding frequency was increased up to four times a day. A monoculture of *P. vannamei* was used to avoid complicating the results with differing stocking

Table 2. Wet and dry season means of water quality variables in ponds stocked with *P. vannamei* at 5 (low density) to 11/m² (high density) and offered a diet containing 20 or 40% protein.

Treatment	Dissolved oxygen (mg/l)	Secchi disk (cm)	Chlorophyll *a* (µg/l)	Water exchange (ha-m)
		Wet season		
Low/20%	4.4	39.8	-	3.5
Low/40%	4.4	36.1	-	3.5
High/20%	3.7	33.4	-	4.5
High/40%	3.7	31.6	-	4.4
High vs. low density	**	*	-	**
20% vs. 40% protein	ns	ns	-	ns
Density X Protein	ns	ns	-	ns
		Dry season		
Low/20%	5.2	61	19.0	3.9
Low/40%	5.4	63	22.0	3.8
High/20%	4.7	62	16.6	4.1
High/40%	4.8	57	23.6	4.3
High vs. low density	*	ns	ns	ns
20% vs. 40% protein	ns	ns	ns	ns
Density X Protein	ns	ns	ns	ns

* significant ($P < 0.05$); ** significant ($P < 0.01$); ns, not significant

proportions and survival rates of *P. stylirostris*; high proportions of *P. stylirostris* will decrease yields (Teichert-Coddington et al. in review, Chamberlain et al. 1981). Seasonal effects on production were minimized by conducting wet and dry season studies during months that predictably resulted in good and poor yields, respectively (Teichert-Coddington et al. in review). Yields in this study were better than average for the wet season and average for the dry season (Teichert-Coddington et al. 1991, Teichert-Coddington et al. in review). Under these conditions, doubling the diet protein level from 20 to 40% had no effect on any parameter of shrimp production or water quality (Table 2) during either season.

The higher protein diet cost 41% more than the lower protein diet, so total variable costs for the use of that diet were higher. Net income was not significantly improved by the use of the lower protein diet, because feed was only one of several variables included in calculating net income. However, use of a lower cost input to achieve similar yields would reasonably increase long-term profitability. Economic analyses would be more realistic and more favorable to low protein feeds if an environmental cost were assessed for the feeds. Use of high protein feeds will result in higher

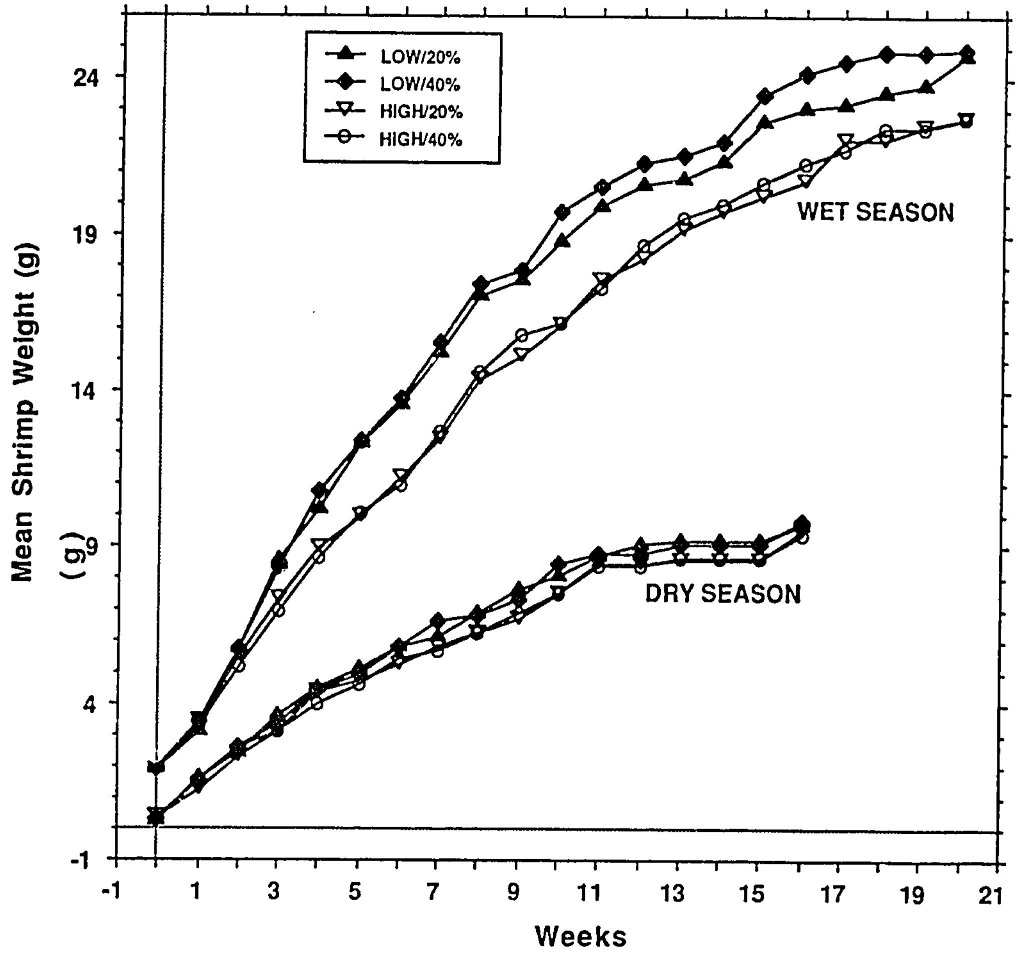

Figure 1. Dry and wet season growth of *Penaeus vannamei* stocked at 5 to 11/m^2 and offered a complete pelleted diet containing 20 or 40% protein.

concentrations of nitrogen (N) discharge, especially if the higher protein diets do not result in greater shrimp yields. Shrimp farm discharge generally enters the same estuary that supplies the farm. Unless there is significant freshwater inflow, or the farm is located near the mouth of the estuary where the discharge can be diluted and transported away from farm water inlet, eutrophication of supply water will probably occur. Severe shrimp production losses in Thailand, Taiwan, and Sri Lanka have been blamed on self-pollution of supply water by farm discharge (Phillips et al. 1993). Minimization of waste products at the farm level is desirable for sustainable production. During this study, an average of 3002 and 5513 kg/ha of feed were used during the wet season for low and high stocking rate treatments, respectively. Increasing the feed protein level from 20 to 40% increased total N input to 96 and 176 kg/ha for low and high densities, respectively, without increasing shrimp production. Excluding N volatilization and fixing by soils, extra N was discharged into the estuaries. A 40% protein diet would therefore have a higher environmental cost.

High protein feeds have been promoted in Honduras as a means to increase yields, especially during the dry season. Results of this study show these claims to be untrue at stocking rates of 5 to 11/m^2. Other studies in the region have demonstrated similar results. Garcia-Casas (1990) concluded that production of *P. vannamei* was unaffected by protein levels of 22 to 35% when stocked in ponds at 10/m^2; growth of *P. stylirostris* was apparently increased by higher protein, however. Teichert-Coddington and Arrue (1988) found no benefit from raising the diet protein level from 29 to 37% in pond culture of *P. vannamei* at densities of 4 to 8/m^2. Diet protein level would probably affect *P. vannamei* production at higher stocking rates when natural pond fertility could no longer provide sufficient supplementary nutrition to the shrimp. Stocking rates in excess of 10/m^2 are rarely used in Honduras or Central America, however.

Large yield differences were related to stocking density. Doubling the stocking rate increased yields by 49 and 42% during wet and dry seasons, respectively. Stocking rate had relatively little affect on individual shrimp size, however. When the stocking rate was doubled, mean shrimp size decreased by 13% during the wet season and remained unchanged during the dry season. The slight correlation between stocking rate and size suggests that nutrition was not limiting at either density. On the other hand, the sudden leveling off of growth at both densities and during both seasons, despite no known water quality problem, suggests a nutrient limitation or a suddenly unsuitable environment. Research is needed to clarify the relationship between stocking density, harvest size, and season in semi-intensive Central American shrimp culture.

Dry season mean harvest weights are usually 10 to 12 g. Monthly variation in yields has been well correlated with temperature (Teichert-Coddington et al. in review), but small shrimp and growth reduction in the dry season may also be related to differences in water quality and natural productivity. Estuarine water quality is greatly altered by the lack of freshwater inflow during the dry season. An obvious visible dry season difference is that the water is clearer. This study demonstrated that primary production was greater during the wet season, as indicated by lower Secchi disk visibility. Future work will evaluate the usefulness of fertilizers in augmenting natural fertility and shrimp production.

Shrimp are normally harvested when the growth curves level out. This study was extended by approximately two weeks to ensure that growth had stopped. Feed efficiency was therefore lower, and costs of feed and water were somewhat higher than under normal operation. Particularly low dry season efficiencies were from lower than expected survival, and probably from overfeeding. The feeding tables did not take into account the effect of the environment on shrimp consumption. Feeding rates should be lowered during the dry season, under the assumption that shrimp consume less food when stressed. Feeding tables could also be made more efficient if they accounted for higher mortality at the higher stocking densities. A surprisingly high cost was associated with pumping. Pumping costs were 27 and 19% of total costs during the dry and wet seasons, respectively. These costs did not include dredging, which involves significant costs on some farms, where up to 5 ml of sediment/L are discharged from estuaries into water supply canals (unpublished data). The amount of pumping could probably be reduced on some farms, as the following research indicates. Work during 1986 at the Ingeniero Enrique Enseñat Brackishwater Experiment Station in Aquadulce, Panama, demonstrated no significant ($P < 0.05$) differences in penaeid shrimp yields in ponds that had water exchanged at daily rates ranging from 0 to 20% of pond volume (unpublished data). Recent trials in Ecuador indicated that penaeid shrimp yields did not improve with daily exchange rates greater than 5% (C. E. Boyd, Auburn University, personal communication). More research on water exchange rates and methods is needed to reduce pumping costs and the sedimentation of water supply canals.

Net income did not rise significantly with stocking rate during the wet season, and it actually decreased significantly with stocking rate during the dry season. Like the higher protein feeds, the higher stocking rate treatments should have a greater theoretical environmental cost attached to it, because more waste per unit area would be discharged back into the estuaries. Such a cost would lower net incomes at higher stocking rates. Producers should consider the impact of higher densities on the long-term sustainability of their production systems, especially if profitability is only minimally increased by higher stocking rates. Lower stocking rates would also require fewer larvae, a large percentage of which are fished in the estuaries. Larvae fishing in Honduras has led to conflicts with environmentalists who claim that fishing is reducing natural stocks of shrimp and fish.

Acknowledgments

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Literature Cited

Akiyama, D.M. 1992. Future considerations for shrimp nutrition and the aquaculture feed industry. Pages 198-205 *in* J. Wyban, editor. Proceedings of the special session on shrimp farming. World Aquaculture Society, Baton Rouge, Louisiana.

Anderson, R.K., P.L. Parker, and A. Lawrence. 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. Journal of the World Aquaculture Society 18(3):148-155.

Boyd, C.E., and C.S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama.

Chamberlain, G.W., D.L. Hutchins, and A.L. Lawrence. 1981. Mono- and polyculture of *Penaeus vannamei* and *P. stylirostris* in ponds. Journal of the World Aquaculture Society 12(1):251-270.

Garcia-Casas, I. 1990. Response of *Penaeus vannamei* and *Penaeus stylirostris* to various supplemental feeding regimes in semi-intensively managed earthen ponds in Honduras. Master's Thesis. Auburn University, Auburn, Alabama.

Haycock, K., J. Roth, J. Gagnon, W.R. Finzer, and C. Soper. 1992. Statview 4.0. Abacus Concepts, Inc., Berkeley, California.

Hunter, B., G. Pruder, and J. Wyban. 1987. Biochemical composition of pond biota, shrimp ingesta, and relative growth of *Penaeus [illegible]ramei* in earthen ponds. Journal of the World Aquaculture Society 18(3):162-[illegible].

Meyers, D.E., and C.M. Leyva. 1993. Feeding strategies and penaeid shrimp culture in Honduras. Abstract of presented contributions at the International Conference World Aquaculture '93, Torremolinos, Spain, May 26-28, 1993.

Phillips, M.J., C.K. Lin, and M.C.M. Beveridge. 1993. Shrimp culture and the environment: lessons from the world's most rapidly expanding warmwater aquaculture sector. Pages 171-197 *in* R.S.V. Pullin, H. Rosenthal, and J.L. Maclean, editors. Environment and aquaculture in developing countries. ICLARM Conference Proceedings 31, International Center for Living Aquatic Resources Management, Manila, Philippines.

Robertson, L., A. Lawrence, and F. Castille. 1992. Feeding frequency and feeding time effects on growth of *Penaeus vannamei*. Page 296 *in* J. Wyban, editor. Proceedings of the special session on shrimp farming. World Aquaculture Society, Baton Rouge, Louisiana.

Teichert-Coddington, D. R., and M. Arrue. 1988. Efectos de dietas de proteinas y densidades de siembra sobre la producción de *Penaeus vannamei* en estanques de tierra. Revista Latinoamericana de Acuicultura 35:29-33.

Teichert-Coddington, D. R., B. W. Green, and R. W. Parkman. 1991. Substitution of chicken litter for feed in production of penaeid shrimp in Honduras. Progressive Fish-Culturist 53:150-156.

Teichert-Coddington, D. R., R. Rodriguez, and W. Toyofuku. In review. Causes of cyclical variation in Honduran shrimp production. Submitted to the Journal of the World Aquaculture Society.

High Elevation Monoculture and Polyculture of *Oreochromis niloticus* and *Clarias gariepinus* in Rural Rwandan Ponds

Work Plan 6, Special Topics Study

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Introduction

Since its introduction in African aquaculture in the seventies (De Kimpe and Micha 1974), culture of the African walking catfish, *Clarias gariepinus*, especially in polyculture with tilapia, has been studied in several African countries (Hecht et al. 1988, Huisman and Richter 1987, Egwui 1986, Viveen et al. 1985, Janssen 1985, Hogendoorn and Koops 1983, and Christensen 1981). The main advantage of stocking *Clarias* in polyculture with tilapia was that it would prey on excess tilapia fry, thereby reducing the overpopulation that would cause stunting in the tilapia population. In Rwanda, the effectiveness of *Clarias gariepinus* in reducing tilapia recruitment was demonstrated by Verheust et al. (1992). A more important reason for polyculture with tilapia in Rwanda is better utilizization of potential food organisms, especially tadpoles, frogs, and benthic organisms (Verheust et al. 1991).

Objective

The effect of elevation on the comparative advantages of tilapia monoculture vs. *Clarias* monoculture vs. tilapia-*Clarias* polyculture was the subject of the present study. This study was unique in that it was executed in rural farmers' ponds at the relatively high altitudes of 1570 m and 2180 m, both of which are above the normal distribution limit of 1500 m elevation and suggested tolerance limit for tilapia culture reported by Balarin (1988). A previous experiment (Rurangwa et al. 1992) with mixed-sex *Oreochromis niloticus* in Rwanda demonstrated that tilapia fingerling production did not occur above 2000 m, therefore reducing the utility of *Clarias* as a predator on tilapia fry at the higher elevation.

Materials and Methods

Earthen ponds between 243 and 707 m^2 in area were chosen at two elevations, 1570 and 2180 m. Criteria for pond selection were: initial total alkalinity of 30 to 90 mg/L, initial hardness of 20 to 75 mg/L, initial conductivity of 100 to 300 µmhos/cm, minimum water depth of 45 to 60 cm, and maximum water depth of 90 to 120 cm. Ponds were located at one site (Ndorwa, Gisenyi) at the higher elevation and at two sites (Kegera and K-zina, Butare) at the lower elevation.

Three stocking strategies were tested, all at 1 fish/m^2: 100% mixed-sex tilapia, with average weight of 6 g; 100% catfish, averaging 2 g at the low elevation sites and 5 g at the high elevation site; and 2/3 tilapia-1/3 catfish. Each treatment was replicated in five ponds at each elevation.

All ponds were to be fertilized with 250 kg total solids/ha/wk of freshly cut *Cyperus* sp. grass, plus urea and triple superphosphate to give total nitrogen and phosphorus inputs of 15 and 4 kg/ha/wk, respectively. However, fertilization was occasionally curtailed at the higher elevation due to logistical problems arising from the war, and at the lower elevation when pond water levels remained low following the dry season. Ponds at the lower elevation received 94% of the proposed fertilizer amount while ponds at the higher elevation received only 76%.

Average fish weight, conductivity, pH, alkalinity, hardness, and chlorophyll *a* were measured monthly. Secchi disk visibility was measured weekly by farmers and pond temperature was recorded continuously by thermograph in one pond at each altitude. The experiment continued for seven months or until an average minimum fish weight of 100 g was reached.

One and two-way ANOVA with 95% confidence intervals were used to compare the following variables: growth, net productivity, fingerling production, and survival rate. The main ANOVA factors included were the three stocking strategies and the two altitudes. Variables were analyzed after 130 to 150 days and at harvest. Ponds with less than 40% survival were excluded from the analyses at harvest.

Results and Discussion

Water quality and related management problems

Average water temperatures at a depth of 15 cm from March to November were 21.5°C at 1570 m and 17.9°C at 2180 m. A problem with the paper drive of the thermograph resulted in the recording of only minimum and maximum temperatures for the period from March to July at the lower elevation site. Later measurements revealed that daily min/max water temperature measurements averaged about 0.3°C higher than the average based on measurements made at two-hour intervals.

The mean difference between temperatures at the two sites was 3.6°C. The difference was greater in the first months of the experiment (March to June) than in later months (during and after the dry season, respectively, as shown in Figure 1). Typical daily fluctuations at the two altitudes are presented in Figure 2. Minimum temperatures were 14°C at 2180 m and 15°C at 1570 m whereas maxima were 27.8°C and 29.8°C, respectively.

Mean conductivity ranged from 60 to 120 µmhos/cm in all ponds. There were no significant differences among the three stocking strategies. Average secchi disk visibility decreased steadily from 20 cm in the beginning to 14 cm at the end of the culture period at both altitudes, with a mean of 18 cm for all treatments at both altitudes. Average monthly alkalinity remained between 36 and 89 mg/L, and average monthly hardness remained between 19 and 50 mg/L. Both values increased slightly during the culture period. Average monthly corrected chlorophyll *a* values increased from about 200 to 500 (mg/m^3) during the culture period. A dense blue-green algae bloom, consisting mostly of *Anabaena* and *Microcystis* spp., developed in most of the ponds after a few months.

Water levels at the 1570 m site were influenced by the dry season from July until September. Some ponds were harvested early because of insufficient water. After five months, low water level in some ponds increased the concentration of applied fertilizers to the extent that mortality resulted. Low water level also resulted in increased predation by birds.

Macrophyte growth (mainly *Azolla* spp. at 1570 and both *Azolla* and *Lemna* spp. at 2180 m) also disturbed production, especially in *Clarias* monoculture ponds. This management problem was observed particularly at 2180 m. *Lemna* growth was a problem only in catfish monoculture ponds, while *Azolla* covered even tilapia monoculture ponds when average fish weight was below 40 g.

Tadpoles and frogs, mostly *Xenopus laevis*, were abundant during sampling in tilapia monoculture ponds at both elevations. When average weight of *Clarias* reached 80 g, frogs and tadpoles became very rare in the samples in the polyculture and in the *Clarias* monoculture ponds.

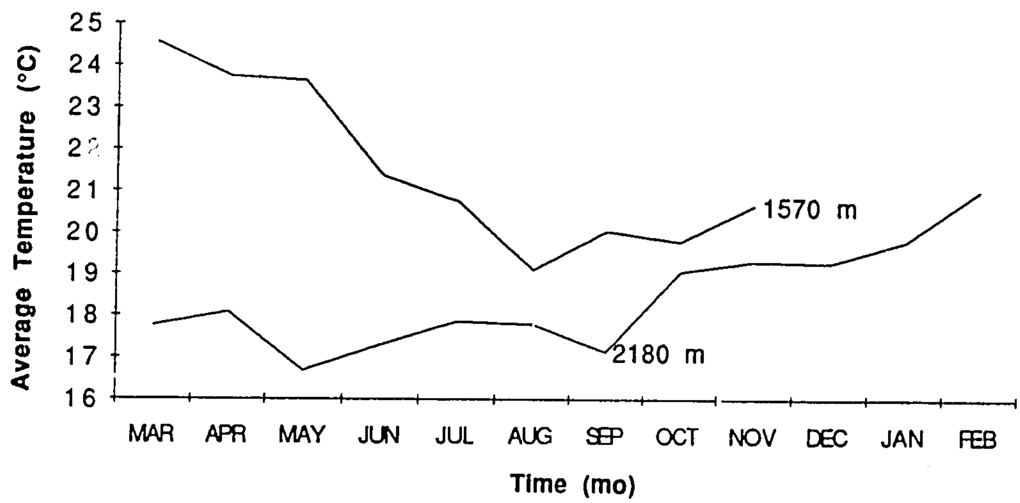

Figure 1. Average monthly water temperatures at 15 cm between March 1992 and February 1993 at 1570 m and 2180 m elevation.

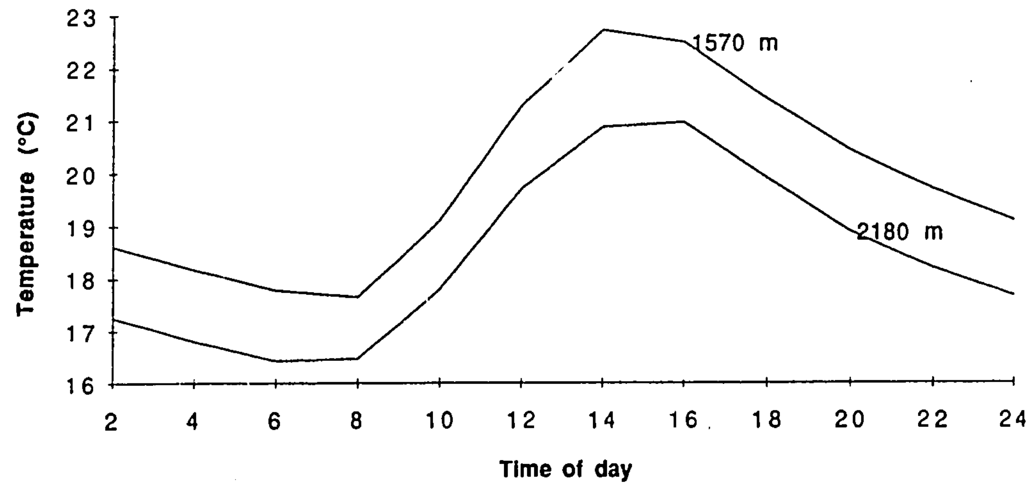

Figure 2. Typical daily fluctuations in pond water temperature at 15 cm at each site.

Growth and net productivity

Growth was significantly different (P<0.05) between the two altitudes after five months. The mean linear growth rates, for all three treatments combined, were 0.35 g/d and 0.72 g/d at 2180 and 1570 m, respectively (Figures 3 and 4). Catfish and tilapia growth were equally influenced by altitude: growth was twice as high at the lower altitude for both species in all treatments (Table 1). Significant differences in growth and estimated production after five months (Table 1) were found between the tilapia monoculture treatment (T) and the tilapia-catfish polyculture treatment (TC).

Differences among average net productivities of the three treatments at harvest were not significant. However, at 1570 m, the mean fish productivities of the C and TC treatments at harvest were 50% higher than that of the tilapia monoculture (T)

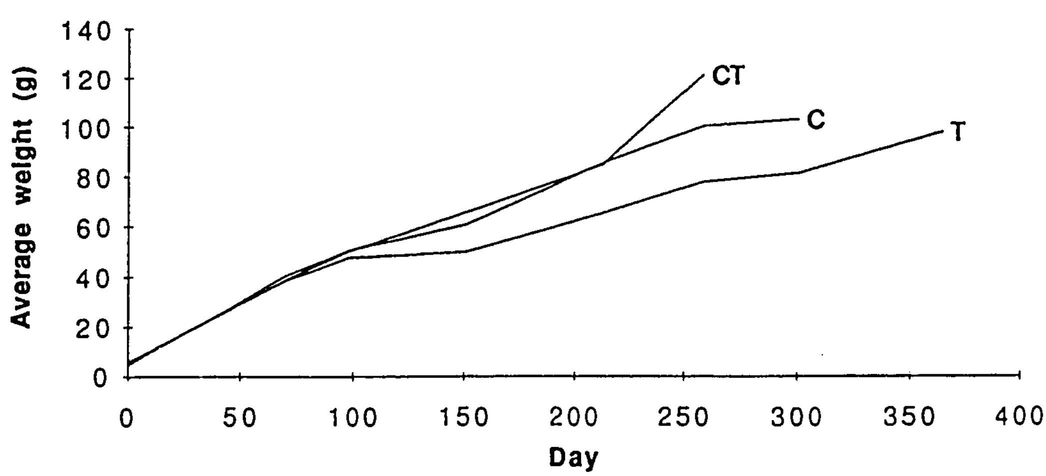

Figure 3. Growth of fish in the catfish monoculture (C), tilapia monoculture (T) and catfish/tilapia (TC) polyculture ponds at 2180 m. The TC average weight is calculated using the average weight of each species weighted for the percent composition in the pond.

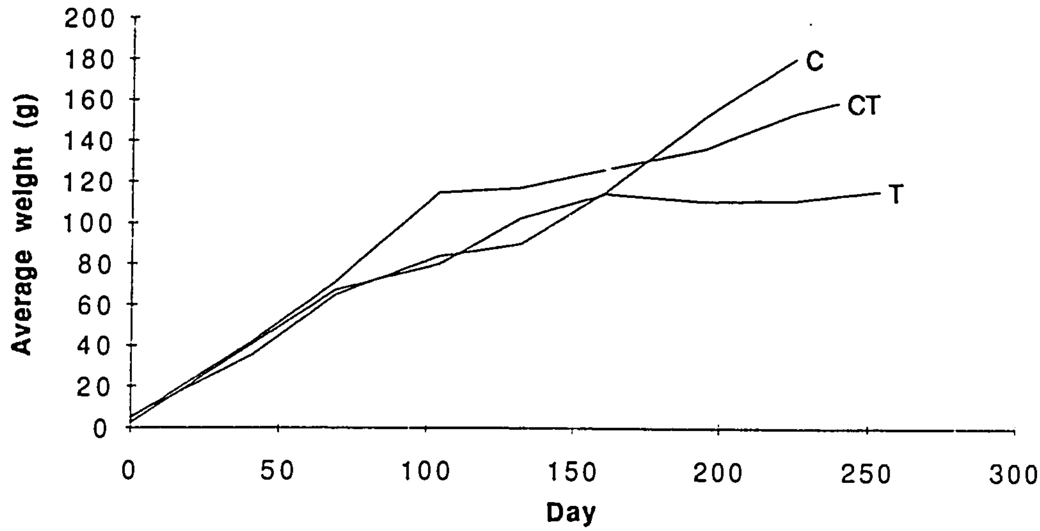

Figure 4. Growth of fish in the catfish monoculture (C), tilapia monoculture (T) and catfish/tilapia (TC) polyculture ponds at 1570 m. The TC average weight is calculated using the average weight of each species weighted for the percent composition in the pond.

treatment; at the higher altitude, the productivity of TC (polyculture) treatment was 50% higher than that of either the T or C monocultures (Table 2). Differences between results after five months and at harvest were caused by different management problems at the two altitudes. At the lower altitude, the dry season (beginning five months after stocking) influenced pond water level, while at the higher altitude, stress caused by dry season low temperatures was exacerbated by macrophyte growth (mainly *Azolla* and *Lemna* spp.) covering the entire pond surface in the C treatment. There was no significant difference in survival rate between the higher and lower altitudes nor between treatments (Table 3). Low water level at 1570 m caused poor tilapia survival.

Table 1. Average linear growth rate after five months for catfish monoculture (C), tilapia monoculture (T), and tilapia/catfish polyculture (TC) ponds at both elevations. Numbers in parentheses are the extrapolated annual net yield in kg/ha calculated from the average weights at 5 months.

Treatment	Average growth (g/d)		
	1570 m	2180 m	Both elevations
T	0.61 (2380)	0.29 (1202)	0.45 (1791) a
C	0.72 (2680)	0.37 (1448)	0.54 (2064) a b
TC	0.82 (3108)	0.40 (1604)	0.61 (2356) b
ALL	0.72 (2722) y	0.35 (1418) x	0.53 (2070)

Numbers followed by different letters are significantly different at the 95% confidence interval.

Table 2. Average extrapolated net annual fish yield for catfish monoculture (C), tilapia monoculture (T) and tilapia/catfish polyculture (TC) ponds at harvest. Ponds with more than 60% mortality were not included.

	Net fish yield (kg/ha/yr)		
Treatment	1570 m	2180 m	Both elevations
T	973 (n=3)	802 (n=5)	866 a
C	1582 (n=4)	831 (n=4)	1206 a
TC	1403 (n=3)	1232 (n=3)	1317 a
ALL	1346 y	919 x	1113

Numbers followed by different letters are significantly different at the 95% confidence interval.

Tilapia fingerling production
No fingerling production occurred in ponds at 2180 m even after a one-year culture period, which confirmed earlier observations for ponds at that altitude. A comparison of the TC and T treatments at the lower altitude showed that fingerling production was significantly different between these treatments. Average fingerling production after eight months was 17.0 fingerlings per are (1 are=100 m^2) in the T ponds and 1.4 per are in the TC ponds. Since farmers usually stock their ponds with at least 2 fish/3 m^2, fingerling production in the monoculture tilapia ponds was not enough for restocking. Low fingerling production was probably caused by the low temperature during the dry season at 1570 m (Figure 1).

Anticipated benefits

Estimated productivity as well as estimated compared benefits after five months were significantly higher in the polyculture ponds than in the catfish monoculture and the tilapia monoculture ponds (Table 4). In this experiment, only the polyculture treatments at both elevations and the *Clarias* monoculture at the low elevation provided enough revenue to cover costs of fingerlings (tilapia at 3 RFW/each and *Clarias* at 5 RFW/each) and fertilizers. Chemical fertilizers are expensive in Rwanda and the

Table 3. Average survival rate for catfish monoculture (C), tilapia monoculture (T) and tilapia/catfish polyculture (TC) ponds at both elevations.

	Average survival (%)		
Treatment	1570 m	2180 m	Both elevations
T	43	82	63 a
C	47	62	55 a
TC	43	50	46 a
ALL	44 x	65 x	55

Numbers followed by different letters are significantly different at the 95% confidence interval.

grass for this experiment was purchased at an abnormally high price of up to 3 RWF/kg.

The study clearly showed the potential advantage of catfish in Rwanda. Although the inputs used were not the best for catfish (mineral fertilizer and fresh, fibrous grass), *C. gariepinus* performed well in both polyculture and monoculture systems. It was also clear that *O. niloticus* and *C. gariepinus* have acceptable growth at elevations above 1500 m, even if reproduction is no longer possible above 2000 m. This may be due to the choice of well-adapted strains of both species in Rwanda.

Farmers at high elevations often cannot produce their own fingerlings and must therefore purchase fingerlings from a government-managed station or from other farmers at lower elevations. If they purchase from a government-owned station, they may have a choice of species. This experiment showed that tilapia-*Clarias* polyculture is an interesting option in fertilized ponds. At lower elevations, tilapia reproduction and stunting are usually a problem. This experiment supports evidence that fingerling numbers can be reduced by *Clarias*. *Clarias* can also help to reduce frog and tadpole numbers. Tilapia help keep macrophyte growth down, thereby indirectly aiding *Clarias* growth. The *Clarias* research project of the Catholic University of Leuven is currently examining *Clarias* mono- and polyculture in rural ponds using locally available inputs.

Acknowledgments

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Literature Cited

Balarin, J.D. 1988. Development planning for tilapia farming in Africa. Pages 531-538 in R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J.L. Maclean, editors. ICLARM Conference Proceedings 15. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Christensen, M.S. 1981. A note on the breeding and growth rates of the catfish *Clarias mossambicus* in Kenya. Aquaculture 25:285-288.

De Kimpe, P., and J. C. Micha. 1974. First guidelines for the culture of *Clarias lazera* in Central Africa. Aquaculture 4:227-248.

Egwui, P.C. 1986. Yields of the African catfish *Clarias gariepinus* (Burchell), from a low input, homestead, concrete pond. Aquaculture 55:87-91.

Hecht, I., W. Uys, and P.J. Britz. 1988. The culture of sharptooth catfish *Clarias gariepinus* in southern Africa. South African National Scientific Programmes Report no. 153. South African National Scientific Programs. Rhodes, Republic of South Africa.

Hogendoorn, H., and W.J. Koops. 1983. Growth and production of the African catfish, *Clarias lazera* (C. & V.). Effects of stocking density, pond size and mixed culture with Tilapia (*Sarotherodon niloticus* L.) under extensive field conditions. Aquaculture 34:253-263.

Huisman, E.A., and C.J.J. Richter. 1987. Aquacultural potential of the African catfish, *Clarias gariepinus* (Burchell, 1822). Aquaculture 63:1-14.

Janssen, J. 1985. Elevage du poisson-chat Africain *Clarias lazera* en République Centrafricaine. II. Alevinage en écloserie. FAO, Bangui, Central African Republic.

Rurangwa, E., K.L. Veverica, W.K. Seim, T.J. Popma. 1992. On-farm production of mixed-sex *Oreochromis niloticus* at different elevations (1370 to 2230 m). Pages 35-40 *in* H. S. Egna, M. McNamara, and N. Weidner, editors. Ninth Annual Administrative Report, PD/A CRSP 1991. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Viveen, W.J.A.R., C.J.J. Richter, P.G.W. Van Oordt, J.A.L.Janssen, and E.A. Huisman. 1985. Manuel pratique du poisson-chat (*Clarias gariepinus*). La Haye. Ministere des Affairs Extrangers La Haye, The Netherlands.

Verheust, L., E. Rurangwa, and K.L. Veverica. 1991. Comparative growth and mortality of *Oreochromis niloticus* and *Clarias gariepinus* fingerlings in earthen ponds (Rwanda). European Aquaculture Society Special Publication No. 14:318-319. Gent, Belgium.

Verheust, L., E. Rurangwa, and K.L. Veverica. 1992. Effect of *Oreochromis niloticus-Clarias gariepinus* polyculture on production of market-size fish and fingerling tilapia. Pages 88-91 *in* H.S. Egna, M. McNamara, and N. Weidner, editors. Ninth Annual Administrative Report, PD/A CRSP 1991. Office of International Research and Development, Oregon State University, Corvallis, Oregon.

Photosynthesis and Community Respiration at Three Depths During a Period of Stable Phytoplankton Stock in a Eutrophic Brackish Water Culture Pond

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Abstract

A 14-day period of dense but stable phytoplankton stock in a brackish water earthen pond (0.2 ha area, 0.7 m depth) was characterized to provide a baseline for study of instability. Results illustrate the potential of ponds to serve as microcosms of natural systems. Primary production and community respiration were assessed by diel curve analysis of oxygen and inorganic carbon sampled every 30 minutes at 3 depths. Neither stocks nor diel oxygen regimes were destabilized by two isolated days of low light, the first accompanied by heavy rainfall. Among nutrient elements, only inorganic nitrogen exhibited marginally limiting values. Daytime net production (dNPP) of oxygen ranged from 0 to 0.26 mol m^{-2} d^{-1}, carbon uptake from 0.01 to 0.22 mol m^{-2} d^{-1}. Nighttime respiration (nR) approximately matched dNPP, resulting in low mean diel net production (NPP). Minimal estimates of daytime respiration (dR) were substantially greater than nR and dNPP; minimal gross production (GPP = dR + dNPP) averaged 2.5 times dNPP. Estimated dR varied with dNPP in a stabilizing negative feedback, possibly mediated by photosynthetic products. Both dNPP and NPP varied with diel irradiance, but nR did not. Both dNPP and nR decreased with depth; positive NPP was concentrated in the upper layer. Stocks and oxygen cycles were more resistant to disturbance by low light than predicted by models assuming 1.0 m pond depth. We suggest for further examination that stability was related to the shallow depth of this pond, which permitted sufficient light penetration to the bottom layer for positive dNPP on most dates.

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Diel Cycles of Planktonic Respiration Rates in Briefly Incubated Water Samples from a Fertile Earthen Pond

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Abstract

Planktonic community respiration rates were assessed every 30 minutes through two 48-hour periods in near-surface water taken automatically from a fertilized earthen pond and incubated in a plastic chamber for 21 minutes of each sampling cycle. Parallel records of water temperature, air temperature, wind speed, and solar irradiance permitted calculation of gross and net primary production and photosynthesis-irradiance relationships. Nighttime respiration rates generally matched oxygen depletion rates in pond water, indicating that incubation-based rates were representative of a quickly darkened pond community throughout the day. Daytime rates averaged nearly 2 times the mean night rate, and 58% higher than the mean day rate determined by a typical interpolation used in free-water production calculations. Daily gross production ranged from 0.7 to 1.2 µmol O_2 liter^{-1} d^{-1}; respiration constituted 65 to 75% of gross rates. Gross oxygen production per unit Chl *a* during sampling intervals was light-saturated at irradiance values greater than 600 µEinst m^{-2} s^{-1}, with an asymptotic value of 1.58 µmol O_2 (µg Chl *a*)$^{-1}$ h^{-1}. This system and method were capable of resolving respiration and gross and net production when chlorophyll concentrations were near 40 mg liter^{-1}.

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Appendix. List of Acronyms and Definitions

AID	Agency for International Development
AIT	Asian Institute of Technology, Thailand
ANOVA	Analysis of Variance
AU	Auburn University
B_{max}	number of binding sites
Baseline Data	that information and data base in some sector or aspect of a developing country which is necessary to measure change in the future
BFAR	Board for Food and Agriculture Research
BIFADEC	Board for International Food and Agricultural Development and Economic Cooperation
Bilateral Programs	assistance programs involving arrangements between a single developing country and a single donor country
Board of Directors (for a CRSP)	an advisory body selected to assist, advise, and make policy recommendations to the ME in the execution of a CRSP; members represent the interests of the CRSP
BW	body weight
CGIAR	Consultative Group on International Agricultural Research
CIFAD	Consortium for International Fisheries and Aquaculture Development
Collaborating Institutions	institutions which form a partnership arrangement with a lead participating U.S. institution to collaborate on a specific research project
CRSP	Collaborative Research Support Program
d	day
DAST	Data Analysis and Synthesis Team

Data Analysis and Synthesis	the process of compiling and analyzing information about pond culture systems from diverse sources into a coherent, usable format that can be applied to the development of predictive models and to the improvement of the efficiency of these systems
DE	digestible energy
dNPP	diel net primary productivity
DO	dissolved oxygen
DOF	Royal Thai Department of Fisheries
DP	digestible protein
dR	daytime respiration
EE	17a-ethynylestradiol
EEP	External Evaluation Panel - senior scientists not involved in the CRSP and selected externally for their ability to evaluate objectively the scientific progress and relevance of a CRSP program on an ongoing basis
Experimental Protocol	a detailed plan of a field experiment which specifies experimental methods, sampling schedules, data collection, etc.
Experimental Treatment	fish cultural practices (e.g., fertilizer application, supplemental feeding, etc.) which modify the physical, chemical, and biological environment
Expert System	a computerized compilation of knowledge that is used to make "intelligent" decisions about the management or status of a process or system
FAC	Freshwater Aquaculture Center, Central Luzon State University, Philippines
FCR	feed conversion ratio
FDA	U.S. Food and Drug Administration
Field Experiments	controlled fish production experiments in which quantitative responses to different levels of treatments are measured
FTE	Full Time Equivalent

GFY	gross fish yield
Global Experiment	the overall plan of a CRSP for research on problems and constraints, global in nature, whose results are applicable and transferable regionally and globally (worldwide)
GOR	Government of Rwanda
Grant Agreement	the formal legal document which represents a binding agreement between AID and the ME institution for a CRSP; this is the legal document for the CRSP recognized as such by AID and the recipient institutions
Grant Proposal	the formal document submitted by an ME to AID, proposing a CRSP for receiving a grant outlining the manner of implementation of the program and showing the budgetary requirements
Host Country (HC)	a developing country in which a CRSP has formal activities
i.d.	inner diameter
INAD	Investigational New Animal Drug permit
INRP	International Research Project
Institutional Development	improvement in the capability of institutions in developing countries to conduct development programs for agriculture and other sectors, or for implementing educational/training, research, health, and other public programs. This may include improvements in physical facilities, equipment, furnishings, transportation, organization, but refers primarily to the development and training of a professional cadre.
JCARD	Joint Committee on Agricultural Research and Development (formerly Joint Research Committee), BIFADEC
JRC	Joint Research Council, USAID
LDC	Lesser Developed Countries
Lps	Lampiras, Honduran currency
Matching Requirement document	that sum of resources, financial or in-kind, which participating U.S. institutions must collectively contribute to a CRSP program as defined in the grant (also called "cost sharing")

mb	mibolerone
ME	Management Entity
MINAGRI	Ministere de l'Agriculture, de l'Elevage, et de l'Environement (Ministry of Agriculture, Livestock and Environment)
Mission	a formally organized USAID unit in a developing country led by a Mission Director or a country representative
MOU	Memorandum of Understanding
MRTC	Mariculture Research and Training Center, University of Hawaii
MSU	Michigan State University
MT	17α-methyltestosterone
NFY	net fish yield
NGO	Non Government Organization
NIFI	National Inland Fisheries Institute, Thailand
NMFS	National Marine Fisheries Service
NPP	net primary productivity
nR	nighttime respiration
NRP	National Research Project
OIRD	Office of International Research and Development
OSU	Oregon State University
PAR	photosynthetically active radiation
Participating Institutions	those institutions that participate in the CRSP under a formal agreement with the Management Entity which receives the AID grant
PD/A CRSP	Pond Dynamics/Aquaculture Collaborative Research Support Program

PI	Principal Investigators - scientists in charge of the research for a defined segment or a scientific discipline of a CRSP
PMO	Program Management Office
PPC	Program and Policy Coordination
Practices	fish cultural activities related to design, management, and operation of pond culture systems
Predictive Models	mathematical models used to simulate the processes occurring in pond systems; in the context of this CRSP, predictive models are used as analytical and management tools to improve the efficiency of pond systems
Principles	the physical, chemical, and biological processes occurring in pond systems and their interactions
PVC	polyvinyl chloride, common thermoplastic resin
RENARE	Department of Renewable Natural Resources, Honduras. Now known as Dirección General de Pesca y Acuicultura, Honduras
R&D Bureau (R&D/AGR)	(Formerly S&T/AGR Bureau of Science and Technology) central bureau of AID in Washington, charged with administering worldwide technical and research programs for the benefit of USAID-assisted countries
RWF	Rwandan franc
SPN	Service de Pisciculture Nationale (National Fish Culture Service)
SRP	soluble reactive phosphorus
Subgrant Agreement	a document representing a subagreement made between the ME and a participating institution under authority of the grant agreement by the ME and AID
TA	total alkalinity
TAN	total ammonia nitrogen
TC	Technical Committee - a group of scientists participating in the research of the CRSP as PI's, selected to help guide the scientific aspects of the research program of a CRSP
TH	total hardness

THB	Baht, Thai currency
Title XII	the Title XII Amendment to the International Development and Food Assistance Act of 1975 as passed by the United States Congress and subsequently amended
TSS	total suspended solids
TVS	total volatile solids
UAPB	University of Arkansas at Pine Bluff
UCD	University of California at Davis
UH	University of Hawaii
UM	University of Michigan
UNR	Universite Nationale du Rwanda
UO	University of Oklahoma
USAID	United State Agency for International Development
USAID Project Officer	an official AID employee designated to oversee a CRSP on behalf of AID
WID	Women In Development
yr	Year