Twelfth Annual Technical Report 1994

Pond Dynamics/Aquaculture Collaborative Research Support Program

Title XII
Collaborative Research Support Program

Twelfth Annual Technical Report

1 September 1993 to 31 August 1994

This report addresses program accomplishments of the Pond Dynamics/Aquaculture Collaborative Research Support Program during the reporting period of 1 September 1993 to 31 August 1994. Program activities are funded in part by the United States Agency for International Development (USAID) under Grant No. DAN-4023-G-00-0031-00. The Egypt Project of the PD/A CRSP is funded by USAID Grant No. 263-0152-G-00-2231-00.

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Dedication

Since April of 1993, when the downing of President Juvenal Habyarimana's plane fanned the civil unrest in Rwanda into civil war, the PD/A CRSP has been deeply affected by the events taking place in Rwanda. An evacuation of U.S. citizens was effected almost immediately after the violence began. CRSP researchers Jean-Damascene Bucyanayandi, Lieven Verheust, and Anaclet Gatera were en route to Rwanda after the PD/A CRSP Annual Meeting, when their flight was re-routed to Bujumbura, Burundi. Although Research Associate Joyce (J-J) Newman was still in the U.S, her husband, as well as the spouses and families of the other researchers, had remained in Rwanda. Newman and Verheust's spouses were able to escape through Bujumbura, Burundi. Bucyanayandi and Gatera crossed back into Rwanda to find their families. Both men were killed. Bucyanayandi's wife and three children escaped.

Reports issuing from Rwanda since April have been sporadic and sketchy; nevertheless, we have pieced together enough information to know that other CRSP associates, such as Valens Ndoreyaho, and other research station workers met untimely and tragic ends.

We join their families and their country in mourning the loss of these talented individuals, who were promising, individually and collectively, to make great contributions to their communities, their country, and the world of aquaculture. We dedicate this annual report to their memory.

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I. CRSP RESEARCH PROGRAM BACKGROUND

The current period was overshadowed by the terrible war that engulfed Rwanda in April 1994 and which destroyed or displaced most of its people. The CRSP grieves together with the families of the Rwandan CRSP participants for these irreplaceable losses. This report is dedicated to the memory of the Rwandan CRSP Co-PIs and their co-workers.

Work Plan Seven experiments were conducted as scheduled on CRSP sites. Experiments were conducted at established CRSP research facilities as well as in farmers' ponds. A number of Special Topics Research activities were also performed.

The CRSP team also participated in two evaluations. Oregon State University and the Central Laboratory for Aquaculture Research, Abbassa, Egypt were two sites that were selected for a technical review of the CRSP Egypt Project. This review had been commissioned by USAID/Cairo and the Egyptian National Agricultural Research Project (NARP). A second review had been ordered by USAID/Washington as part of the overall review of the CRSP programs. In both cases the PD/A CRSP researchers were lauded for their efforts and successes.

The Egypt Project was scheduled to end in September 1994; however, due to the positive review, USAID/Cairo and NARP agreed to extend the project six months to allow completion of ongoing research efforts. However, due to a change in priorities, research activities ceased by 31 December 1994. Preliminary results of the studies still underway at the end of this reporting period are presented in this issue. Data collected in the period from 1 September to 31 December 1994 will be summarized in the final report of the Egypt Project.

The CRSP 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 understand and improve the efficiency of pond culture systems.

In 1978, a technical plan proposing this strategy was developed under a planning study funded by USAID. The technical plan reviewed and synthesized literature on state-of-the-art pond aquaculture. Overseas sites were surveyed to 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 those ponds might be adapted to different sets of environmental conditions to optimize production. Hence, a common set of experiments was implemented globally, following a standardized experimental protocol at a number of research sites around the world.

The initial technical design for the Global Experiment involved monitoring environmental and fish production variables at seven geographical locations in six countries. Observations specified in biennial (originally annual) work plans are made on twelve or more ponds of similar size at each location. The variables observed, frequency of observation, and materials and methods used are uniform for all locations. Two brackish water and five freshwater research 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 termination of research activities in Panama and funding constraints had caused a hiatus in brackish water research. The outbreak of civil war in Rwanda in the spring of 1994 caused the cessation of all research activities on this site.

The first cycle of experiments aimed to develop a set of baseline data on ponds at the various sites. Subsequent Global Experiment studies have focused on investigations of the effects of different fertilizer regimes on pond productivity and yield. This series 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.

PD/A CRSP RESEARCH LOCATIONS AROUND THE WORLD

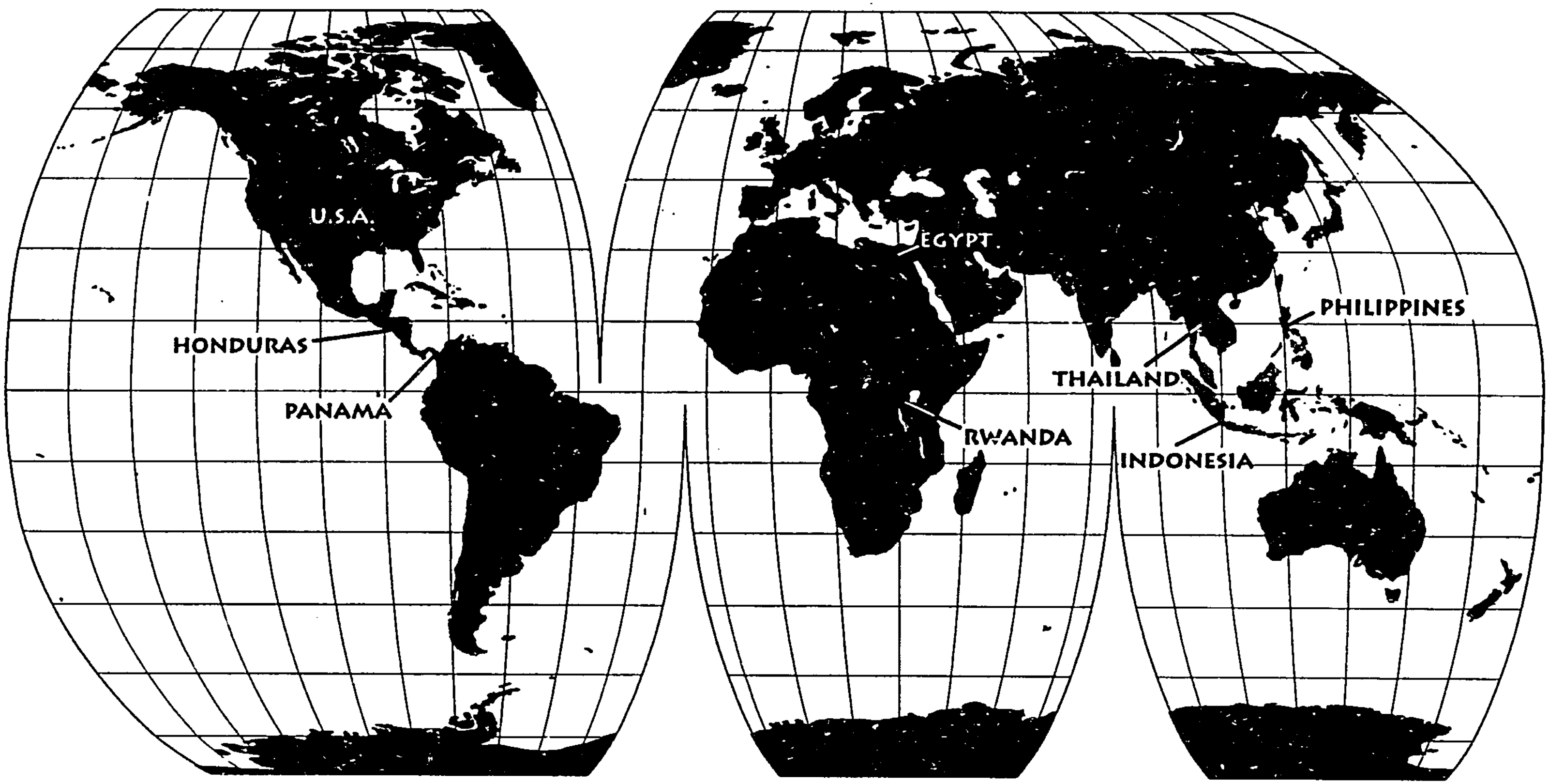

Figure 1: Past and present PD/A CRSP research locations in Central America (Honduras, Panama), Africa (Egypt, Rwanda), Asia (Thailand, Indonesia, and the Philippines), and U.S.A.

Furthermore, 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. As a result, a predatory fish is sometimes stocked together with tilapia in Global Experiment studies. 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 U.S. 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 attempts to gain direct information on the practical applicability of CRSP technologies under everyday farm conditions, and on the extent of economic returns that the new management strategies may generate 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 originally maintained by the Management Entity until Spring of 1993 when it was transferred to the University of Hawaii at Hilo.

Standardized data are tabulated at each research location in accordance with CRSP work plans. At the individual sites, data on physical variables (e.g., solar radiation, temperature, and rainfall) and chemical variables (e.g., water and soil characteristics) are collected concurrently with biological measurements (e.g., primary productivity, fish growth, and fish production). Over 160 physical, chemical, and biological variables (approximately 90,000 observations per site and year) are observed. Whereas the resulting sets of data are useful for site-specific studies, the compilation of all the individual data sets into the Central Data Base provides opportunities for many kinds of global analyses. Detailed standardized records such as those found in the CRSP Central Data Base are rare in the aquaculture literature. 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 through the inclusion of new data generated under the Sixth and Seventh 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 U.S. 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 U.S. 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 to be of low priority. Consequently, administrators in the host countries sometimes have difficulty justifying participation in the CRSP. The CRSP support for the Special Topics Research activities helps them to see the value of their institutions' participation in the CRSP.

CRSP Work Plans

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

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

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

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 U.S. and in host countries. The Seventh Work Plan covers the remaining period of the current grant.

This reporting period consists of the first year of the Seventh Work Plan for all sites but Egypt, and concludes studies scheduled under the Sixth Work Plan. Technically the Seventh Work Plan covers the period from 1 September 1993 to 31 August 1995. 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, even though experiments of the Egypt Project started in Spring of 1993. These activities ceased in December 1994.

The companion volume to this Technical Report is the Administrative Report which reports on administration, research, and outreach activities, as well as program history, personnel, financial status, publications and abstracts of all technical reports included in this volume. (See Appendix B for the table of contents to the Administrative Report.)


SAMPLE

II. RESEARCH PROGRAM ACCOMPLISHMENTS

Global Experiment and Related Investigations

This reporting period covers the first year of the Seventh Work Plan experiments in Honduras, Rwanda, Thailand, and the Philippines, as well as the second year of experiments carried out in Egypt, and concludes research described in the Sixth Work Plan. The theme of the Global Experiment was the validation of CRSP fertilizer recommendations. Results from the Egypt experiments indicated that the highest yields were obtained if ponds were fertilized only initially and tilapia were fed in the second part of the experiment. The application of purely chemical fertilizer resulted in the lowest yields. Part two of the Egypt Project Global Experiment was a comparison of the production characteristics of Nile tilapia *(Oreochromis niloticus)* and blue tilapia *(O. aureus)*, both species endemic in Egypt. Initial results indicated that Nile tilapia, the test species of most of the CRSP experiments, performed consistently better than blue tilapia.

The effects of fertilizer applications according to recommendations generated by the decision support system PONDCLASS were compared to the effects of local management practices in Honduras, Thailand, and the Philippines. Research in the Philippines is still ongoing and results are not yet available. Thailand ponds which were receiving a weekly application of fertilizer produced similar yields as ponds which were managed according to PONDCLASS recommendations. However, less fertilizer was used under the PONDCLASS management system. PONDCLASS recommendations therefore resulted in equivalent yields produced with less input. The Honduras team, however, obtained different results. They observed that PONDCLASS recommended fertilizer applications which resulted in a nitrogen overfertilization of treatment ponds. Mean fish weight and production of the control treatment were 15% and 28% greater, respectively, than in PONDCLASS treatment. Researchers believed that yield was limited by primary productivity which in turn was carbon-limited. The carbon limitation prevented phytoplankton from using all of the supplied nitrogen.

Accompanying the above-mentioned Global Experiment studies, each research team conducted additional experiments which also have global relevance. The Egypt Project has added biotechnology research, a new line of inquiry in the family of CRSP studies. Several of the studies focused on the use of sex hormones, mainly 17α-methyltestosterone (MT), as a masculinizing agent in aquaculture. An Oregon State University study characterized a binding site for sex-reversing hormones in gonadal tissue of adult Nile tilapia. This research team also developed a receptor assay, a new and fast tool to screen newly developed sex inversion agents for efficacy. Auburn University, the American Tilapia Association, an U.S. feed producer, and the CRSP collaborated in another MT study. The U.S. Food and Drug Administration has granted a "compassionate" Investigational New Animal Drug exemption (INAD) for the use of MT, which has not yet been approved for use on animals. A field trial was started in July 1994 at the Central Laboratory for Aquaculture Research at Abbassa, Egypt. Initial results indicate high survival rates. The sex-reversing effects of MT were separated from the growth-promoting effects of this drug in a study conducted at the University of Hawaii. It was found that the growth rate of *O. aureus* was nearly twice that of *O. mossambicus* at each dose level. In almost all treatments, MT-treated animals grew significantly better. 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. So far, four possible YY supermales have been identified. However, males which produced more than 95% male progeny in the first mating did not consistently produce such frequencies of male offspring in consequent spawns.

Bioconversion and polyculture research, another focus of the Egypt Project, aims to build a polyculture system suited to the conditions found in Egypt by utilizing currently unused pond system components like aquatic weeds or snails as fish food. Initial tests indicated the effectiveness of grass and black carp as control agents, however, considerable contamination of the treatment ponds with common carp and mullet somewhat restrict interpretation of these results.

The effect of shrimp farming on environmental quality was the concern of two studies performed in Honduras. Water quality research in the Gulf of Fonseca revealed that estuaries influenced by rivers had a considerably lower capacity to assimilate waste loads than those estuaries not influenced by rivers. Eutrophication of riverine estuaries was greater with increased distance from the gulf and was more severe in the dry than in the wet season. Results of a second study will help shrimp farmers to reduce waste input into estuaries. Researchers found that the standard shrimp feeding rate could be halved during the dry season without negatively effecting shrimp yields. The Honduran team also conducted polyculture research at its freshwater site in El Caro. Different stocking rates of tilapia and tambaqui (*Colossoma macropomum*) were tested. The best growth rates were obtained with a 75% tilapia and 25% tambaqui mixture. However, tambaqui growth was thought to be hindered by cool water temperatures.

Research efforts in Rwanda were abandoned after the outbreak of civil war. Unfortunately, all data that had been collected up to this point were lost when Rwasave station was vandalized and looted. A supplemental study to the Rwanda work plan was carried out at the University of Arkansas at Pine Bluff. Two different forms of defatted rice bran, an agricultural by-product, were evaluated for use as fish food. Ponds receiving pellets had a higher fish yield than ponds receiving "loose" (unprocessed) rice bran, even though they were receiving less fertilizer. Defatted rice bran pellets were also easier to handle than the loose rice bran.

The Thailand research team investigated intensified farming practices for Nile tilapia. Supplemental feeding has been shown to produce larger, more valuable tilapia. In the first study researchers attempted to determine the best time to commence supplemental feeding. Supplemental feeding was found to be most effective and efficient at growing larger tilapia, if commenced after fish had grown to approximately 150 g. A companion study investigated the relationship of different stocking density and carrying capacity when providing supplemental feeding. Initial results indicated that growth rates were similar among treatments and that carrying capacity had not been reached.

Global Experiment

Centerpiece of PD/A CRSP research is the Global Experiment, an activity in which all sites participate. The Global Experiment of the Seventh Work Plan for Honduras, Thailand, and Rwanda is an organic/inorganic fertilization experiment which compares a fixed fertilization regime with recommendations obtained from the decision support system PONDCLASS. However, because of the war in Rwanda, all data were lost from this site and thus no report is presented in this section. The Global Experiment in Egypt compares yields obtained with traditional Egyptian methods to those produced using PD/A CRSP guidelines. A second study compares the yield characteristics of Nile tilapia, the species of choice for most of the PD/A CRSP research, with those of blue tilapia, another species endemic to Egypt.

Validation of PD/A CRSP Pond Management Strategies

Work Plan 7, Egypt Study 1A

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Introduction

Fish is an important source of animal protein and is a component of the traditional Egyptian diet. Consumption of fish and fish products was estimated at 350,000 MT in 1989, of which 100,000 MT were imported and 33,000 MT were produced through aquaculture, with the remainder supplied by domestic capture fisheries. While aquaculture has been practiced in Egypt for thousands of years, systematic aquacultural research to increase fish pond yields in Egypt is relatively new.

Pond aquaculture of tilapia is an important component of Egyptian aquaculture, yet management practices and fish yields are variable. Traditional Egyptian aquaculture involves a polyculture of mixed-sex, young-of-the-year tilapia, common and silver carp, and mullet. Ponds are fertilized with organic and chemical fertilizers, and fish are offered a commercial ration. Results of PD/A CRSP Global Experiment research in other countries have identified a number of tilapia pond management strategies that have high fish yields and positive economic returns. However, these pond management systems were developed in countries located in the tropics, and Egypt is located in the sub-tropics and has an arid climate. Thus, the objectives of Year I Global Experiment research in Egypt were to test the performance of established PD/A CRSP pond management systems under local climatic, edaphic, and water quality conditions, and to compare these systems to Egyptian pond management systems.

Materials and Methods

This research was conducted in twenty 0.1-ha earthen ponds located at the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou Hammad, Sharkia, Egypt. A completely randomized design was used to test five pond management practices (Table 1). Young-of-the-year Nile tilapia (*Oreochromis niloticus*) averaging 1 to 3 grams in weight were stocked in ponds at 20,000 fish/ha on 1 July 1993.

Fingerling African catfish (*Clarias gariepinus*) averaging 59.2 g/fish were stocked at 60 fish/ha on 13 September 1993 to prey upon any tilapia reproduction that occurred. Ponds were harvested in late November, at an average of 145 days after stocking. At harvest tilapia were separated according to established market size classes: 1st class (1 to 5 fish/kg); 2nd class (6 to 12 fish/kg); 3rd class (13 to 25 fish/kg); 4th class (26 to 40 fish/kg).

Chemical fertilizers were dissolved in buckets prior to dispersal over pond surfaces. Chicken litter was purchased from a local layer operation and was comprised of bedding (rice or wheat chaff), manure, feathers, and waste feed. Chicken litter was applied on a dry matter (DM) basis; DM was determined weekly before application from weight loss of a weighed sample after drying 24 h at 60°C. Water was added to ponds periodically to replace evaporation and seepage.

Water quality variables were analyzed weekly for 17 weeks according to PD/A CRSP standard methods. Primary productivity was determined on six occasions according to the free-water diel curve method. Dissolved oxygen was measured with a polarographic dissolved oxygen meter at 4-hour intervals beginning at 0600 hours and continuing until 0600 hours the following day; measurements were made at depths of 5, 25, 50, and 75 cm. Community respiration was estimated as two times the decrease in dissolved oxygen between 1800 hours and 0600 hours the following day. Dissolved oxygen measurements were not corrected for diffusion because wind data were not available.

Data were analyzed by ANOVA followed by Student-Newman-Keuls post-hoc comparison. Means were declared significantly different at alpha level 0.05.

Table 1. Nutrient inputs and frequency of application by treatment for 0.1-ha ponds stocked with Nile tilapia (*O. niloticus*).

Treatment	Tilapia stocked	Chicken litter (dry matter)	Urea	Super-phosphate	Pelleted feed
			(kg/ha)		(25% protein)
Traditional Egyptian	Mixed sex	715 (initial) 238 (monthly)	23.8 (2-wk intervals)	212.2 (2-wk intervals)	3% fish biomass daily
Enhanced Egyptian	Mixed sex	1,000 (weekly for first 60 days)	***	***	3% fish biomass daily beginning day 61
Feed only	Monosex (males)	***	***	***	3% fish biomass daily
Fertilization then feed	Monosex (males)	1,000 (weekly for first 60 days)	***	***	3% fish biomass daily beginning day 61
Chemical fertilization (N:P of 4:1)	Monosex (males)	***	54.4 (weekly)	92.4 (weekly)	***

Results and Discussion

Nile tilapia gross yields ranged from 1,278 to 2,877 kg/ha per 145 days (Table 2). Unfortunately, screened pond water inlets did not completely exclude wild tilapia (*Oreochromis aureus, Sarotherodon galilea, Tilapia zilli*) or African catfish present in Egyptian waterways. Total tilapia yield represented 79% to 96% of gross fish yield (Table 2). Significantly greater quantities of tilapia offspring were produced in the *Traditional Egyptian* treatment than in remaining treatments (Table 2). African catfish comprised from 4% to 13% of gross fish yield.

The lack of a significant difference in total tilapia yield between the *Traditional Egyptian* and *Fertilizer then Feed* treatments indicated that the addition of the commercial ration was not necessary during the first two months of culture. Natural productivity stimulated by pond fertilization was sufficient to permit rapid fish growth during the first two months of culture (Green, 1992). The pelleted fish ration was probably not consumed efficiently by tilapia during the initial 60 days of culture and acted more as an expensive pond fertilizer. Fertilizer applications beyond day 60 in the *Traditional Egyptian* treatment did not appear to affect tilapia yield significantly when compared to the *Fertilizer then Feed* treatment where only feed was applied. Thus, fertilizer application beyond day 60 in the *Traditional Egyptian* treatment was probably not necessary.

Application of 3.6 kg N/ha per day at a 4N:1P ratio in the *Chemical Fertilizer* treatment yielded 9.7 kg tilapia/ha per day (net tilapia yield). Net tilapia yield of 10 kg/ha per day was obtained in ponds fertilized with 3.8 kg N/ha per day in Rwanda (Newman et al., 1994). In Thailand, net tilapia yields of 30.5 kg/ha per day

Table 2. Summary of mean harvest data for 0.1-ha earthen ponds that received different nutrient inputs during a 145-day production period.

Treatment	Gross fish yield[1]	Tilapia yield: Nile	Tilapia yield: Other[2]	Tilapia yield: Total	Tilapia reproduction	Catfish yield
	(kg/ha per 145 days)					
Traditional Egyptian	4,074 a	2,877 a	346 a	3,223 a	634 a	201
Enhanced Egyptian	2,881 ab	2,674 a	81 a	2,755 ab	0 b	121
Feed Only	2,467 ab	1,770 ab	218 a	1,989 ab	170 b	308
Fertilizer then Feed	3,895 a	2,851 a	686 a	3,537 a	99 b	253
Chemical Fertilizer	1,526 b	1,278 b	129 a	1,407 b	21 b	97

[1] includes tilapia reproduction and extraneous fish harvested.
[2] includes *O. aureus, S. galilae, T. zilli.*
abc means within columns followed by same letter are not significantly different (P>0.05).

(Knud-Hansen et al., 1992) and 21.5 kg/ha per day (Diana et al., 1994) were reported for ponds fertilized with 4 kg N/ha per day at a 4N:1P ratio. Ponds in Honduras fertilized with 3.6 kg N/ha per day resulted in a net tilapia yield of 11.5 kg/ha per day (Teichert-Coddington et al., 1993). Net tilapia yields from ponds fertilized with a high rate of chemical fertilizer appear to be similar in Egypt, Honduras, and Rwanda, and less than those obtained in Thailand. Higher mean water temperatures in Thailand may be responsible for the observed differences.

The poor performance of fish in the *Feed only* treatment most likely resulted from the inability of young fish to consume the commercial fish feed pellet, rather than because of feed quality. Feed for small fish must be of a small particle size, yet commercially available fish feeds in Egypt generally have pellet sizes 3 to 4 mm in diameter. The small quantity of feed initially offered fish is of little value even as fertilizer to stimulate natural productivity, so fish growth is limited by food availability. This conclusion is supported by the observation of lower mean chlorophyll *a* concentration and significantly lower net primary productivity in the *Feed Only* treatment (Table 3). Natural productivity that results from heavy fertilization is sufficient to maintain fast fish growth during the first two months of grow-out, as observed in the *Fertilization then Feed* treatment. In fact, fish growth during the first two months did not differ between the *Fertilization then Feed* and the *Traditional Egyptian* treatments, even though fish were offered feed in the latter treatment. Once natural productivity can no longer maintain fast growth, the use of fish feed once again permits fast growth of fish as seen the *Fertilization then Feed* treatment.

While PD/A CRSP pond management systems did not produce significantly greater tilapia yields than the *Traditional Egyptian* system, examination of tilapia size distribution revealed some differences among treatments (Table 4). A larger percentage of tilapia in the *Enhanced Egyptian* and *Fertilization then Feed* treatments were in the first and second size classes compared to *Traditional Egyptian* treatment. A full economic analysis of these pond management systems is presented separately (see report by Hebicha et al. in this volume). Results of that research indicate that tilapia ponds managed according to the methodology used for the *Fertilization then Feed* treatment will yield more favorable results than ponds managed according to *Traditional Egyptian* methodology. The *Fertilization then Feed* management strategy results in a greater percentage of harvested tilapia classified as first- or second-class and in significantly less reproduction during grow-out. The economic implications of this are important: first-class-sized fish are worth about 30% more than second-class-sized fish and about 130% more than third-class-sized fish. Tilapia offspring produced during grow-out compete with initial stock for food, result in lower yield of marketable fish and have little economic value. Therefore, pond management strategies should attempt to achieve efficient utilization of nutrient inputs and to minimize the potential for production of tilapia offspring during grow-out. In conclusion, results indicate that ponds stocked with young-of-the-year monosex tilapia and managed according to the tested systems are feasible in Egypt.

Table 3. Water quality variable means in 0.1-ha earthen ponds stocked with 20,000 Nile tilapia/ha for a 145-day grow-out period. Management systems are described in detail in the text.

Treatment	Total Alkalinity (mg/l $CaCO_3$)	Ammonia (mg/l NH_3-N)	Nitrite (mg/l NO_2-N)	Nitrate (mg/l NO_3-N)	Total Phosphorus (mg/l PO_4-P)	Chlorophyll *a* (mg/m^3)	Net Primary Productivity (g $O_2/m^3/d$)	Gross Primary Productivity (g $O_2/m^3/d$)	Community Respiration (g $O_2/m^3/d$)
Traditional	347.32 b	0.74 a	0.005 a	0.120 a	3.02 b	154.1 a	10.44 b	18.66 bc	16.44 bc
Enhanced	374.28 bc	0.84 a	0.020 a	0.125 a	2.63 b	234.0 a	12.64 b	23.67 c	22.06 c
Fertilizer then feed	412.56 c	0.80 a	0.015 a	0.125 a	2.12 b	241.0 a	9.39 b	16.50 b	14.22 b
Feed	347.29 b	0.84 a	0.007 a	0.110 a	0.52 a	125.5 a	5.87 a	10.20 a	8.66 a
Chemical fertilization	283.40 a	0.86 a	0.150 b	0.803 b	2.04 b	202.8 a	10.68 b	19.37 bc	17.39 bc

Note: abc means within columns followed by same letter are not significantly different ($P>0.05$).

Table 4. Mean yield of tilapia, by size class, from 0.1-ha ponds managed according to different nutrient input regimes during a 145-day grow-out period.

	Tilapia Yield by Size Class			
	1st Class	2nd Class	3rd Class	4th Class
Treatment	kg/ha per 145 days			
Traditional Egyptian	228 ab	1,658 a	1,130 a	203 a
Enhanced Egyptian	1,066 a	1,413 a	190 b	87 a
Feed Only	148 ab	880 a	646 ab	315 a
Fertilization then Feed	844 a	1,584 a	604 ab	506 a
Chemical Fertilization	4 b	516 a	721 ab	167 a

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Yield Characteristics of Two Species of Tilapia under Two Different Pond Environments

Work Plan 7, Egypt Study 1B

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Introduction

PD/A CRSP research designs have been based on the use of Nile tilapia (*Oreochromis niloticus*) as the test species because this species was common to all research sites. In Egypt, Nile and blue (*Oreochromis aureus*) tilapia, both good culture species, are endemic. Assurance of adequate Nile tilapia fingerling availability in the delta region of Egypt requires greenhouse over-wintering facilities for fingerlings and brood fish. Severe cold weather occurs periodically in the Egyptian delta during the winter months of December through February, and can decimate unprotected stocks of Nile tilapia, as happened during the 1991/92 winter. *O. aureus*, much more tolerant of cold temperatures, survives winters in Egypt without having to be over-wintered indoors. In addition, production ponds may be able to be stocked earlier in the season with *O. aureus* than with *O. niloticus*. The greater cold tolerance of *O. aureus* would provide this species with a competitive advantage over *O. niloticus* for pond culture in the Egyptian delta, assuming all other production characteristics were similar. Thus, the objective of this experiment was to compare production characteristics and production economics of *O. niloticus* and *O. aureus* reared in ponds managed under two different nutrient input regimes.

Materials and Methods

Eighteen 0.1-ha ponds at the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou Hammad, Sharkia, Egypt, were used for this study. A completely randomized design, in 3 x 2 factorial arrangement, was used. The factors were tilapia species (Nile, blue, or co-stocked) and pond nutrient input regime (chemical fertilization or fertilization then feed). Weekly applications of nitrogen at 25 kg/ha and sufficient phosphorus to maintain an N:P ratio of 4:1 were made in the *Chemical*

Fertilizer treatment. In the *Fertilizer then Feed* treatment, chicken litter was applied weekly at 1,000 kg dry matter/ha for the first eight weeks followed by feed (25% protein commercial fish feed) only. Chemical fertilizers were dissolved in buckets prior to dispersal over the pond surface. Chicken litter was purchased from a local layer operation and was comprised of bedding (rice or wheat chaff), manure, feathers, and waste feed. Chicken litter was applied on a dry matter (DM) basis; DM was determined weekly before application from weight loss of a weighed sample after drying for 24 h at 60°C. All ponds were full by mid-June and received two initial applications of chicken litter (945 kg DM/ha) on 19 and 26 June 1994. Water was periodically added to ponds to replace evaporation and seepage losses.

Ponds were stocked with sex-reversed tilapia fingerlings on 1 July 1994. At stocking, Nile and blue tilapia each averaged 0.5 g/fingerling. Past experience indicated a mean survival of 75% for tilapia fingerlings weighing ≤1-g stocked in nursery ponds. Therefore, ponds were stocked with 25,000 fingerlings/ha to provide an effective stocking rate of approximately 20,000 fingerlings/ha. Equal numbers of Nile and blue tilapia were stocked in co-stocked treatment ponds. African catfish (*Clarias gariepinus*) fingerlings weighing 20.8 g/fish were stocked at the rate of 340 fish/ha on 28 August 1994 to prey upon any tilapia reproduction that occurred. Tilapia were sampled by seine net to monitor growth on 22 September 1994, 83 days after stocking.

Results

Average mean individual fish weights after 83 days of growth, based on seine sample and estimated fish biomass and assuming 20,000 fish/ha, are shown in Table 1.

Table 1. Summary of average fish weight and estimated biomass of tilapia in 0.1-ha earthen ponds after 83 days of growth. Ponds management strategies are described in the text.

Treatment	Tilapia species	Average weight (g/fish)	Estimated biomass (kg/ha)
Chemical fertilization	*O. niloticus*	94.1	1,863
Chemical fertilization	*O. aureus*	75.4	1,507
Chemical fertilization	Co-stocked	70.8	1,415
Fertilization then feed	*O. niloticus*	151.4	3,027
Fertilization then feed	*O. aureus*	99.4	1,988
Fertilization then feed	Co-stocked	123.5	2,469

Ponds are scheduled to be harvested after completion of 150 days of grow-out. Fish production, water quality, primary productivity, and economic data will be analyzed upon completion of the experiment.

Anticipated Benefits

Results of this experiment will provide Egyptian aquacultural scientists and fish culturists with data to support selection of the most appropriate tilapia species to culture in the delta region of Egypt. In addition, this experiment will provide a second year of fish production, water quality, and economic data for production ponds stocked with Nile tilapia and managed according to the *Chemical Fertilization* or *Fertilization then Feed* pond management strategies. Availability of these latter data will permit an estimation of year-to-year variability for these production systems.

Nutrient Input Management by the Computer Program, PONDCLASS, and by Concentration of a Key Nutrient

Work Plan 7, Honduras Study 4A

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Introduction

The computer program, PONDCLASS, was developed by the Data Analysis and Synthesis team of the Pond Dynamics/Aquaculture CRSP to assist aquaculturalists with pond management. It had been issued for use, but needed to be verified by field testing.

Previous studies in Honduras consistently demonstrated that total ammonia nitrogen (TAN) concentrations greater than 0.15 mg/l did not result in greater primary production (Teichert-Coddington et al., 1992). Total ammonia represents the majority of dissolved inorganic nitrogen available to phytoplankton in Honduran ponds, because concentrations of combined nitrates and nitrites are usually an order of magnitude lower than ammonia. Regulation of nitrogen inputs according to ammonia concentrations should result in lower nitrogen inputs, lower ammonia levels, and possibly lower pHs.

Tilapia stocking rates in PD/A fertilization experiments were initially 1 fish/m^2. Higher nutrient inputs through the combination of nitrogen with organic fertilizers allowed stocking rates to be raised to 2 fish/m^2. Densities higher than 2 fish/m^2 have not been well tested under current management schemes.

The objectives of these studies were:

1) to test the utility of PONDCLASS as a pond management tool;
2) regulate nitrogen fertilization according to total ammonia concentrations;
3) evaluate the effect of raising stocking rates from 2 to 3 fish/m^2.

Materials and Methods

Three experiments were carried out simultaneously in earthen ponds that were 0.1 ha in area and 0.9 m deep. Each experiment consisted of a completely randomized design replicated three times. The control treatment in all experiments consisted of weekly applications of chicken litter at 250 kg dry matter/ha, urea to maintain weekly total N input at 28 kg/ha, and diammonium phosphate to supply 7 kg/ha of total P input (1P:4N). Ponds in all experiments were stocked with *Oreochromis niloticus* at 2 fish/m^2 and guapote tigre (*Cichlasoma managuense*) at 500 fish/ha unless otherwise noted. Ponds were stocked on 5 May 1993 and harvested after 141 days on 10 October 1993.

Experiment 1: Management of ponds using PONDCLASS (PCLASS)

A one-factor experiment evaluated the effectiveness of the PONDCLASS pond management computer program for producing fish. Ponds were fertilized weekly with chicken litter, nitrogen (urea), and phosphorus (diammonium phosphate) as dictated by the computer program.

Experiment 2: Regulation of nitrogen input by ammonia concentration (AMMON)

A treatment was designed to maintain TAN at about 0.15 mg/l by adjustment of nitrogen (urea) input. Phosphorus input was regulated to achieve a P:N ratio of 1:4. The control treatment management regime was followed initially until total ammonia concentrations rose above 0.18 mg/l. Urea was then reduced by at least 25%. If TAN fell below 0.12 mg/l, urea input was increased again.

Experiment 3: Effect on tilapia production of increasing the stocking rate from 2 to 3 fish/m^2(HDEN)

The control management regime was followed with the exception that tilapia were stocked at 3 fish/m^2 instead of 2 fish/m^2. This experiment was carried out instead of the programmed experiment with nitrate fertilizer as a nitrogen source, because no pure nitrate source (not combined with phosphate) could be located in Honduras at the study start-up date.

Water quality measurements followed the PD/A CRSP protocol. Early morning dissolved oxygen (DO) and total ammonia-nitrogen were usually determined once a week. Primary productivity, chlorophyll *a*, total phosphorus, total nitrogen, total hardness, and total alkalinity were determined three times during the study.

Data were analyzed by 1-factor ANOVA. Each experiment was analyzed separately. Differences were declared significant at an alpha level of 0.05.

Results and Discussion

Experiment 1: Management of ponds using PONDCLASS (PCLASS)

Mean fish weight and production of the control treatment were 15% and 28% greater, respectively, than the corresponding means from PCLASS treatment (Table 1), but the differences were not significant. The mean weight gain of fish in the PCLASS treatment was minimal after 16 weeks (Figure 1).

Total phosphorus and filterable phosphate were significantly higher in the control treatment (Table 2). However, mean filterable phosphate levels were high in the PCLASS treatment (> 0.5 mg/l), so phosphorus was unlikely limiting to primary productivity. Chlorophyll *a* was not significantly regressed on filterable phosphate concentrations.

Total nitrogen and TAN were significantly higher in the PCLASS treatment. However, mean dissolved inorganic nitrogen concentrations were relatively high in the control treatment (> 0.35 mg/l), indicating that nitrogen was not limiting to primary productivity. Chlorophyll *a* was not significantly regressed on dissolved inorganic nitrogen concentrations. The PONDCLASS computer program recommended high rates of nitrogen fertilization even when dissolved inorganic nitrogen concentrations were already high (Figure 2), causing total ammonia levels to rise excessively. Low guapote tigre survival and relatively low tilapia survival in some PCLASS ponds (Table 1) may have been related to high ammonia levels.

The mean chlorophyll *a* concentration was 24% higher in the control treatment than in the PCLASS treatment, but the difference was not significant. Chlorophyll *a* was low in both treatments relative to potentials realized in past studies (Teichert-Coddington et al., 1992; Teichert-Coddington et al., 1993). Because high concentrations of nitrogen and phosphorus were available, phytoplankton must have been limited by another factor. In a prior study, primary productivity and fish production decreased as inorganic nitrogen and phosphorus were substituted for chicken litter (Teichert-Coddington et al., 1993). It was hypothesized that carbon was limiting in the treatments receiving low levels of organic fertilizers where pH was high. The results of the current study are similar. Fish production in the control treatment was similar to fish production of similar treatments in the prior study. Production was comparatively low relative to treatments that received proportionately more chicken litter and less inorganic fertilizer. Hot season yields in

Table 1. Summary of fish production and comparisons of treatment means with the control.

Pond	Treatment	Tilapia Mean weight (g)	Tilapia Production (kg/ha)	Tilapia Survival (%)	Guapote tigre Mean weight (g)	Guapote tigre Production (kg/ha)	Guapote tigre Survival (%)	Total production (kg/ha)
1	CONTROL	126.5	2496.6	98.65	56.7	15.9	56	2512
2	PCLASS	81.7	1268.7	77.65	-	0.0	0	1269
3	AMMON	139.1	2707.4	97.35	47.7	10.5	44	2718
4	HDEN	108.2	3019.2	93	17.5	2.3	26	3021
5	CONTROL	155.0	3060.9	98.75	45.4	15.9	70	3077
6	PCLASS	138.3	2348.2	84.9	17.7	1.4	16	2350
7	AMMON	111.2	2172.0	97.7	53.8	20.4	76	2192
8	PCLASS	147.0	2841.6	96.65	-	0.0	0	2842
9	CONTROL	137.9	2697.7	97.8	41.1	11.9	58	2710
10	AMMON	139.2	2711.4	97.4	39.1	11.3	58	2723
11	HDEN	72.2	2056.3	94.9	40.7	15.9	78	2072
12	HDEN	117.2	3309.1	94.1	51.2	15.9	62	3325
	Treatment means **							
	CONTROL	140a	2752a	98.4a	47.7a	14.6a	61.3a	2766a
	PCLASS	122a	2153a	86.4a	17.7a	0.5b	5.3b	2153a
	AMMON	130a	2530a	97.5b	46.9a	14.1a	59.3a	2544a
	HDEN	99a	2795a	94.0b	36.5a	11.3a	55.3a	2806a

** Means, within a column, followed by different letters are different from the control ($P < 0.05$).
Comparisons only valid between control and each other treatment.

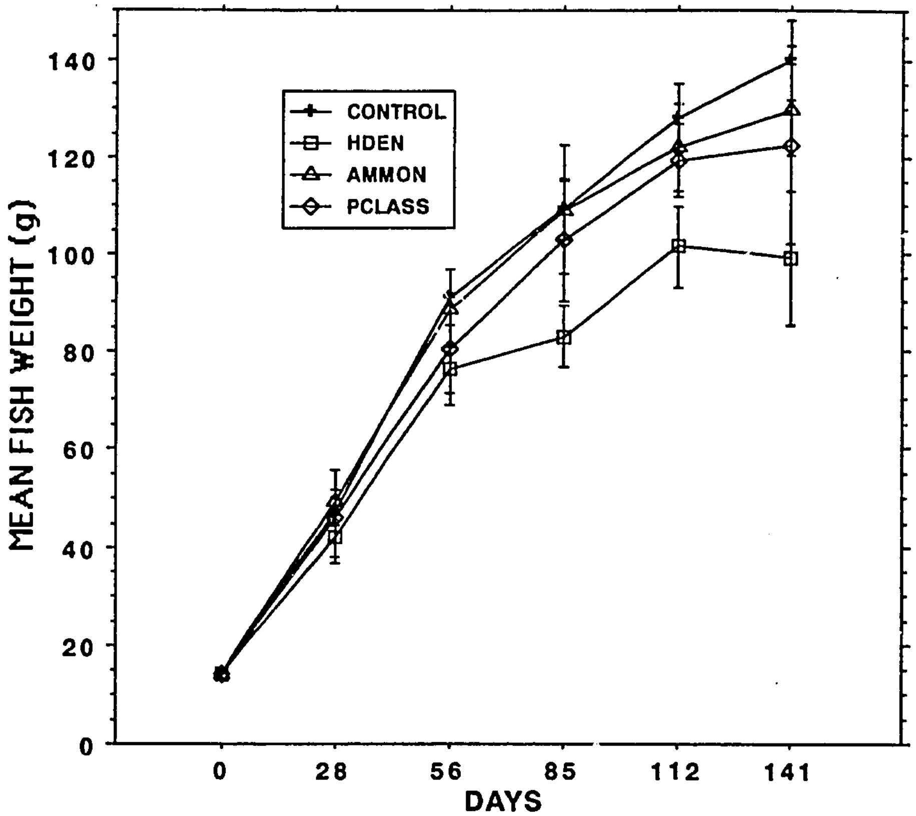

Figure 1. Tilapia growth in ponds managed according to total ammonia concentrations (AMMON), PONDCLASS, or control protocols. One treatment of ponds was stocked at 3 instead of 2 fish/m^2 and managed according to control protocol (HDEN).

excess of 3500 kg/ha and higher profitability are consistently achieved if at least 500 kg chicken litter and total nitrogen input of 28 kg/ha are applied to ponds weekly (Teichert-Coddington et al., 1992; Teichert-Coddington et al., 1993; Green et al., 1994). PONDCLASS recommended weekly chicken litter applications of about 200 kg/ha, which was lower than control pond applications. Although the correlations were not significant, both chlorophyll *a* and fish production tended to be lower in PONDCLASS than in control ponds.

The PONDCLASS program performance was mediocre relative to a control treatment that was itself mediocre compared to prior studies. The control treatment in this experiment was not appropriate for Honduras, because higher fish yields and greater profits were achievable under a different fertilization regime (Teichert-Coddington et al., 1992; Teichert-Coddington et al., 1993; Green et al., 1994). The control treatment was stipulated in this experiment by the technical committee, based primarily on results from the Thailand CRSP project. Control treatments in future global experiments should be more carefully selected, perhaps by site, to reflect the best demonstrable management regime.

Table 2. Summary of water quality variables and comparisons of treatment means with the control.

POND	Treatment	Secchi disk (cm)	Total P (mg/l)	Filterable phosphate (mg/l)	Total N (mg/l)	Ammonia nitrogen (mg/l)	$NO_3 + NO_2$ nitrogen (mg/l)	Total alkalinity (mg/l $CaCO_3$)	Total hardness (mg/l $CaCO_3$)	Volatile solids (mg/l)	Chorophyll *a* (µg/l)
1	CONTROL	9.7	2.32	1.538	3.01	0.287	0.141	53.7	48.1	110	380
2	PCLASS	14.1	1.13	0.563	3.83	0.817	0.059	87.5	55.2	102	308
3	AMMON	15.4	1.90	1.281	2.68	0.337	0.058	67.3	45.5	102	408
4	HDEN	12.5	2.02	1.373	2.97	0.570	0.080	87.1	56.0	111	412
5	CONTROL	13.7	1.88	1.327	3.22	0.271	0.026	66.9	49.1	114	402
6	PCLASS	14.4	1.11	0.587	3.66	0.818	0.086	78.5	55.2	87	260
7	AMMON	14.5	2.25	1.722	3.23	0.329	0.071	55.3	39.8	88	343
8	PCLASS	13.7	1.36	0.623	4.08	0.660	0.080	85.6	58.3	120	430
9	CONTROL	14.8	2.15	1.363	3.55	0.345	0.063	84.5	57.6	97	455
10	AMMON	12.3	2.10	1.303	3.77	0.396	0.055	82.9	55.7	114	455
11	HDEN	12.1	1.95	1.775	3.66	0.290	0.182	57.0	40.8	112	551
12	HDEN	14.5	2.90	2.247	3.80	0.336	0.114	64.3	46.7	97	498
						Treatment means **					
	CONTROL	12.7a	2.12a	1.409a	3.26a	0.301a	0.077a	68.4a	51.6a	107a	412a
	PCLASS	14.1a	1.20b	0.591b	3.86b	0.765b	0.075a	83.9a	56.2a	103a	333a
	AMMON	14.1a	2.08a	1.435a	3.23a	0.354a	0.061a	68.5a	47.0a	101a	402a
	HDEN	13.0a	2.29a	1.798a	3.48a	0.399a	0.125a	69.5a	47.8a	107a	487a

** Means, within a column, followed by different letters are different from the control ($P < 0.05$). Comparisons only valid between control and each other treatment.

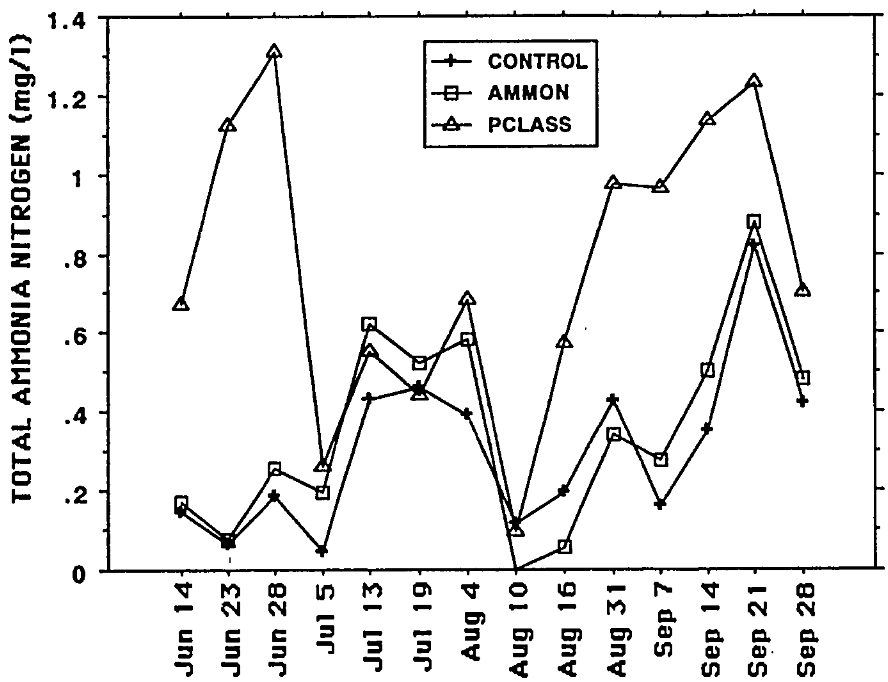

Figure 2. Mean total ammonia concentrations determined for ponds managed according to total ammonia concentrations, PONDCLASS, or control protocols.

Current revision of the PONDCLASS program to include data acquired during CRSP research should improve its management performance. Program revision should allow for greater organic inputs, and put a cap on inorganic fertilizer input when certain key variables, like TAN or filterable phosphate, exceed concentrations known to be adequate for high primary productivity.

Experiment 2: Regulation of nitrogen input by ammonia concentration (AMMON)

There were no significant treatment differences for fish production or water quality (Tables 1 and 2; Figure 1). Mean AMMON total ammonia was not different from control TAN. Moreover, AMMON total ammonia was more than twice as high as it should have been according to treatment specifications. A mean of 0.15 mg/l should have been maintained, but a mean of 0.35 mg/l was actually observed. Total urea input was less in the AMMON treatment, but not sufficiently less to make a difference.

Long distance management can be blamed for the results. Water samples were being analyzed in Choluteca. It usually took more than a week to process a set of samples and transmit the information back to El Carao. Communication with El Carao was uncertain because of station managerial difficulties and poor telephone connections. Many times, it took two to three weeks to implement procedures resulting from water analysis. By that time, the analyses had changed and new procedures were required. Inferences concerning the regulation of nitrogen input by ammonia concentration cannot be made from these data.

Experiment 3: Effect on tilapia production of increasing the stocking rate from 2 to 3 fish/m^2 (HDEN)

Increasing the tilapia stocking rate from 2 to 3 fish/m^2 had no significant affect on fish production or water quality (Tables 1 and 2). The mean average fish weight of the control treatment was 41% greater than that for fish from the HDEN treatment, but total production in the two treatments was almost identical. Fish of the HDEN treatment were too small to market, so the profitability of that treatment would have been low. Carrying capacity was reached in the HDEN treatment after about 16 weeks, when fish growth ceased (Figure 1). Primary productivity at the tested fertilization regime was too low to support more than 2 fish/m^2. Fish growth in ponds stocked at 2 fish/m^2 practically stopped after 16 weeks in ponds fertilized with similar levels of inorganic N and P during this study (Teichert-Coddington et al., 1993). Chicken litter applications of at least 500 kg/ha with enough supplemental nitrogen to equal total N input of about 28 kg/ha is necessary to achieve high production of marketable size fish at El Carao.

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

Work Plan 7, Thailand Study 6

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Introduction

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

Because of the importance of pond soil conditions in TA management, the best practical approach is to analyze and condition the soil before ponds are filled, as extensively discussed by Boyd (1990). However, TA can change substantially during growth cycles in ponds which have received appropriate soil conditioning. CRSP observations in Thailand have documented that ponds fertilized at high rates with purely chemical fertilizers often exhibit decreasing TA during five-month growth cycles, sometimes to levels well below 20 mg/l $CaCO_3$. In recent trials in Honduras, TA decreased in chemically-fertilized ponds (as observed in Thailand), whereas increases in TA were observed in manured ponds (Green et al., 1990). Green et al. (1989) during Cycle II experiments in Honduras found final TA to be only slightly higher than initial levels in chemically-fertilized ponds, whereas marked increases (>400%) were observed in heavily manured ponds. Manured ponds also exhibited increasing TA during Cycle III in Honduras.

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

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

Therefore, methods for interim management of TA are of both theoretical and practical interest. This study addressed the question, "How can decreasing TA best be managed in fertile ponds during a growth cycle?" This experiment documented the temporal trends of TA in 15 ponds, and quantified the effect of interim addition of soluble carbonate on trendsin alkalinity.

For the CRSP Global Experiment, one of the five triplicated treatments consisted of management of inputs by means of the PONDCLASS decision-support system software; another treatment consisted of regular inputs according to the specified control protocol (PD/A CRSP, 1993).

The objectives of this experiment were:

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

2. To examine the potential for managing decreasing TA levels by interim additions of soluble carbonate.

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

4. To perform the Global Experiment for the Thailand project.

Materials and Methods

Twelve 0.04-ha ponds at the Asian Institute of Technology were stocked with male Nile tilapia fingerlings at a rate of 20,000 fish/ha. Water depth was adjusted weekly to 1.0 m. The experiment lasted 150 days. Six ponds were fertilized with urea and phosphates at 28 kg N/ha/week with N/P = 4; six others were fertilized with an isonitrogenous (and of equivalent ratio) combination of 250 kg/ha/week (dry matter basis) chicken manure and chemical fertilizers. Within each of these two treatments, three of six ponds received no inputs of carbonate (except that contained in manure, which was analyzed) during the trial, while three of six received additions of sodium carbonate (Na_2CO_3) every two weeks in proportion to observed TA decreases, when they occurred. Three additional ponds were managed by PONDCLASS, using an initial program input of 3 g $C/m^3/d$ daytime net primary production (dNPP). This figure was adjusted upwards when necessary, based on observational data, but never downwards. This was done because 1) the input dNPP should be interpreted as the potential of ponds at a particular site; and 2) if a high input dNPP became unrepresentative, then nutrients should remain in the water at the next sampling interval, and these values will cause the program to specify lower inputs.

Soil pH was analyzed before stocking and after harvest in each pond. Analysis of chlorophyll *a* concentrations and total alkalinity levels was performed every two weeks on depth-integrated samples from each pond; samples were taken two days before scheduled fertilization, with inputs then based on the analytical results. Daytime net primary production and nighttime community respiration (nR) were assessed monthly by diel sampling of dissolved oxygen (DO) and pH (at 0600, 0900, 1200, 1500, 1800, 2200, and 0600 hours) at three depths (25, 55, 75 cm). Other analyses were performed according to recent standard protocols.

Results and Discussion

The results of this experiment are currently undergoing analysis; preliminary results are summarized and briefly interpreted here.

The five treatments produced no significant differences in final mean individual fish weight, survival, or net yield ($P > 0.05$, Single-factor ANOVA). This result is reasonable in view of the similar lack of significant treatment differences in parameters of food production, namely mean concentration of chlorophyll *a*, and dNPP, defined as the difference in concentration of dissolved oxygen between that at 1600 hours and the previous 0600 value.

Inputs determined by PONDCLASS are expected to be, and in this case were, less than the regular inputs for the control and all other treatments, because the software takes account of nutrients remaining in ponds from previous inputs. The treatment in which inputs were determined using PONDCLASS (Work Plan 7 Global Experiment) produced yields with numerically greater efficiency (not yet tested statistically) than did treatments involving regular weekly fertilizer inputs in constant amounts. That is, similar yields were produced with less input.

The actual net yields, extrapolated to units of t/ha/y, ranged from 3.3 to 6.5, and approximately twice as great as yields previously obtained at this site in the same season (wet) during earlier experiments (Szyper et al., 1991), but are substantially less than yields obtained with the same methods at a nearby site (Knud-Hansen et al., 1993). Seasonal differences in potential yields have not been observed for these sites; the PD/A CRSP Data Base should be used to examine the results of past experiments at each site for seasonal differences.

It is likely that rainfall affected the attempt to examine effects of soluble carbonate inputs on alkalinity of pond water. During the 150-day experimental period, a total of 43.6 cm of rain fell at the site, none of it during the first 50 days, 30% during the second 50-day period, and 70% during the third. Rain water falling directly into the ponds is expected to have little alkalinity. However, much of it enters ponds as runoff containing solutes and soil particles. During the first 50 days, alkalinity tended to decline between sampling intervals in all ponds, although concentrations could be effectively stabilized in ponds receiving carbonate additions. After the first 50-day period, alkalinity showed little decline in ponds of any treatment. Temporal patterns of alkalinity will be examined for relationships with dNPP, particularly during the first 50 days, and for time-related coefficients of variation, hypothesizing that the carbonate addition protocol provides at least a reliable and efficient means to ensure stable alkalinity values. If this protocol did, as expected, effectively stabilize alkalinity values, C in this form might be considered for addition to N and P as inputs which can be specified by PONDCLASS, either whenever decreased alkalinity is observed during a sampling interval, or when a C-limitation threshold is approached.

Daytime net primary production ranged (pond-by-pond) from -0.1 to 19.4 mg O_2/l/d, with means of 7.0 for the entire experiment and 7.6 for the PONDCLASS treatment. Using the conversion factor of 0.288 g C/g O_2 specified in the description of the Global Experiment (PD/A CRSP, 1993), the experiment mean dNPP in terms of C fixed per unit surface area is 7.0 x 0.288 = 2.0 mg C/l/d, which is equivalent to 2.0 g C/m^3/d. Mean dNPP figures in this form are needed as required starting points for operation of PONDCLASS. Far higher values have been observed on this site, particularly during seasons without rain and/or cloud cover. The Data Base should be used to characterize sites and seasons for this purpose.

Anticipated Benefits

If completed analyses suggest that addition of soluble carbonate is effective in stabilizing TA, such inputs can readily be incorporated into the PONDCLASS and POND software systems and incorporated into transferred CRSP protocols in other ways. This will effectively eliminate the potential for decreasing alkalinity due to photosynthetically-mediated pH dynamics during production cycles in ponds, leading to more stable blooms and production rates, and thus more reliable fish production protocols.

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We appreciate the collaboration of the University of Michigan, the Royal Thai Department of Fisheries, and the Asian Institute of Technology in CRSP endeavors. Thanks are due to the AIT Aquaculture Laboratory for technical support, and to Messrs. Yang Yi and Arun Patel for management and execution of field and laboratory work and data handling.

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Global Studies and Activities

The different global aspects of PD/A CRSP research are evident in several other CRSP studies and activities. Honduras, Rwanda, Thailand, and the Philippines participated in an investigation to gain information about the reasons why some farmers adopt CRSP guidelines but not others. Initial results of this study describe the differences in resource availability and their consequences for management practices. The future comparison of PD/A CRSP adoption successes and failures across all sites will provide important inside information which will be used to fine-tune PD/A CRSP recommendations.

Release of the expert system POND is another example of PD/A CRSP research with global implications. This decision support system provides valuable information for aquaculture facility managers. Managers can explore different management options by projecting yield and economic return for each option before committing to a particular production regime.

Development of the models which are the building blocks of such decision support systems would not have been possible without the information available from the PD/A CRSP Central Data Base. Data stored in the Central Data Base are not only used for model construction but also for model output validation. But the global nature of the PD/A CRSP's Central Data Base is not limited to this activity. The information of the Central Data Base is available to researchers worldwide who wish to explore aspects of pond aquaculture.

Minding the Pond: Feeding, Fertilization, and Stocking Practices for Tilapia Production in Rwanda, Thailand, The Philippines and Honduras

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(Printed as Submitted)

Introduction

Social and economic dimensions interact with biological variables to determine the conduct of fish culture in various locales (Harrison, 1991). This report provides basic descriptive information concerning the way aquaculture is practiced in four CRSP countries. Subsequent analyses will consider family, community, and farming system considerations as they affect the process of learning about and using tilapia production technologies (Green et al., 1994).

Socioeconomic factors are framing constraints – the conditions or circumstances that determine the possibilities and incentives for tilapia production in a particular locale (Cernea, 1991; Molnar et al., 1991; Roling, 1988). Socioeconomic framing constraints include market incentives, consumer preferences, household commitment to fish culture, and the level of security in the farming environment. This report details survey findings on four central aspects of tilapia culture; pond management, feeding, fertilization, and stocking practices.

Material and Methods

Data were collected from tilapia farmers in four CRSP countries; Rwanda, Honduras, Thailand, and the Philippines. The following sections detail the procedures employed in each country and the use of the data for this report.

Rwanda

Data were obtained from a sample of 121 active Rwanda fish farmers randomly selected from National Fish Culture Service (SPN) rolls in eight local administrative districts (communes) during the winter and early spring of 1992. The 141 communes are the basic units of administration in Rwanda. Several districts were chosen to represent diversity in the nation's regions; others were selected randomly. Interviews were conducted with 115 active fish farmers 45 percent of whom were women. Each survey instrument was developed, translated into Kinyarwanda, pretested with members of each category, and accordingly adjusted.

The interview schedule used in Rwanda in 1992 was revised and adapted for each of the three CRSP countries surveyed in 1993-94. Additional questions obtained information about production practices, particularly feeding and fertilization, as these are the central CRSP technologies examined in the study.

Honduras

Data were obtained from a sample of 51 active Honduran fish farmers in five of 15 Honduras departments during the fall 1993. Tilapia farmers were identified through referrals made by Peace Corps volunteers working in fish culture, Honduran extension personnel, and by fish farmers identifying neighbors raising tilapia. The departments were chosen to represent the major tilapia production regions in the country. Interviews were conducted with 51 active fish farmers.

Thailand

Data were obtained from a sample of 51 active Thai fish farmers in four of 75 Thai provinces during winter 1994. Tilapia farm operators in Ayutthaya, Nakhom Pathom, and Suphanburi provinces Central Thailand were interviewed. The survey was revised and adapted, then translated into the Thai language.

Tilapia farmers were identified through referrals made by Department of Fisheries extension personnel, knowledgeable local individuals, and by fish farmers identifying neighbors raising tilapia. The provinces were chosen to represent major tilapia production regions in south central Thailand, the major aquaculture region in the country. Interviews were conducted with 51 active fish farmers.

Philippines

Data were obtained from a sample of Philippine fish farmers in four of 15 provinces on the main island of Luzon during winter 1994. Tilapia farm operators in Bulacan, Nueva Ecija, Pampanga, and Tarlac provinces were interviewed. The survey was revised and adapted, then translated into the Tagalog language. Tilapia farmers were identified by sampling lists of farmers purchasing fingerlings at the Freshwater Aquaculture Center at Central Luzon State University in Munoz. Sample farmers were asked to identify neighbors raising tilapia who also were approached for interviews. The provinces were chosen to represent the major tilapia production region in the country. Interviews were conducted with 51 active fish farmers.

Table 1 shows the sample jurisdictions in each country and the number of interviews obtained in each. The number of farmers in each sample segment is roughly proportional to the corresponding population. In Rwanda, the 121 farmers in the sample represent 3.9 percent of the 3,102 (1,950 group and 1,152 individual) ponds in the country in 1990. Women are 24 percent of the fish farmers in Rwanda and 43 percent of the sample. Women were oversampled in the 1992 study. Molnar et al. (1994) previously examined the Rwanda data in detail, but the aggregate findings are presented here allow comparative analysis across four CRSP sites.

Table 1. Sample regions and number of respondents, four CRSP countries, 1994.

Region	Number	Percent
Rwanda Communes		
Gishamvu	9	7
Karago	17	14
Kayove	14	12
Kigembe	16	15
Ndusu	9	7
Nyamabuye	17	14
Tumba	14	12
Mugambazi	16	19
(Total)	(121)	
Honduras Departments		
Cortes	9	18
Santa Barbara	3	6
Copan	9	18
Comayagua	9	18
Atlantida	5	10
Colon	4	7
Francisco Morazon	1	2
Olancho	4	7
El Paraiso	5	10
Yoro	2	4
(Total)	(51)	
Thailand Provinces		
Ayuttha	17	33
Pathum Thani	20	39
Nakorn Prathom	14	28
(Total)	(51)	
Philippine Provinces		
Nueva Ecija	9	16
Pampagna	17	30
Bulacan	14	25
Tarloc	16	29
(Total)	(56)	

In each country, the interview schedules were edited to reconcile missing data, ambiguous answers, and exceptional cases. The data were keypunched according to precoded numerical response categories on the printed questionnaire that did not require translation.

The data for each country sample are tabulated in parallel to facilitate comparisons across the four sites (Casley, 1988). The objective is to document the nature and circumstances of practicing tilapia farmers who might adopt CRSP technologies.

Results

This section describes the practices and production regimes employed by tilapia farmers. The technologies to be considered include: the use of monosex fingerlings; chemical fertilizers, commercial feeds; and alternative harvesting strategies.

Pond Management

More than 80 percent of the Rwandan farmers had but a single pond, as shown in Table 2. In contrast, more than 70 percent of the Philippine and Honduran farmers had more than one pond. Most Honduran farmers had more that a hectare of ponds.

Table 2. Pond management practices, tilapia farmers in four CRSP countries, 1994.

	Rwanda	Honduras	Philippines	Thailand
	Pct.	Pct.	Pct.	Pct.
How many ponds do you have on your land?				
One	84	16	9	39
Two	11	12	20	26
Three or more	5	72	71	35
What is the area of the ponds on farm?				
< .25 hectare	--	0	27	91
.25 to 1 hectare	--	2	32	7
> 1 hectare	--	98	41	2
Have you had problems getting enough water to keep pond filled?				
No				
Yes	76	82	66	45
	24	18	34	55
Where are ponds in relation to house?				
Next to house	--	66	36	79
< 1 kilometer	--	12	35	6
1 to 3	--	2	22	9
More than 3	--	20	7	6
Water source for the ponds on this farm?				
Well	--	2	9	0
Spring	--	8	7	0
River or stream	--	18	14	2
Lake - reservoir	--	48	0	2
Irrigation canal	--	14	13	64
Collected runoff	--	0	16	0
Combination	--	10	41	32
Water pumped or gravity flow to pond?				
Pumped	0	16	42	96
Gravity flow	100	82	38	2
Combination	0	2	20	2
(Number)	(136)	(51)	(50)	(56)

Most Rwandans obtained water for their ponds from streams or springs. Honduran farmers supplied their ponds from a variety of sources, most frequently identifying lakes or reservoir sources. Thai farmers depended most on irrigation canals, while Philippine farms indicated the least dependence on any single source. All Rwandan ponds used gravity flow, but most Thai farmers had pumps.

More than half the Thai sample reported problems getting enough water to keep ponds full. A third of the Philippine farmers said so, as did a quarter of the Rwandans.

The location of the fish pond relative to the household is significant. Ponds near households are easier to monitor. Family members can attend to the pond as well give regular surveillance to deter theft. About 79 percent of the Thai fish ponds were located next to the house, as were two-thirds of the Honduran ponds and a third of the Philippine farms.

Although the question was not asked of the Rwanda sample, fish ponds are always located in the marshy valley bottom lands (marais). Consequently, ponds usually are some distance from the houses built on the hills in that mountainous nation. In some areas, group or family members take turns guarding harvestable fish at night. Some farmers hire watchmen to protect the ponds and other crops.

Fish Feeding

Farmers in the four countries fed their tilapia a variety of different items reflecting differences in the intensity of aquaculture practice in each nation, Table 3. Leaves and manure were the most common items in Rwanda. Chicken litter and commercial feed were most frequently used in Honduras. Figure 1 suggests that Honduran farmers were most likely to make exclusive use of fish feed. In Thailand, farmers most often utilized compost, rice bran, commercial feed, and chicken litter. A similar pattern was noted in the Philippines, although rice bran was used more often.

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

Honduran and Rwandan farmers were most likely to report inadequacies in feed availability on their farms. About seven percent of the Rwandan farmers said that they never had enough.

Honduran farmers also reported a high level of attentiveness to their ponds. About a quarter of the Rwandans fed their fish several times a week or less often. Feeding in the Rwanda case refers primarily to the provision of manure and other inputs. Some items are directly consumed by the fish; others mainly serve as nutrients to foster primary productivity in the ponds.

Table 3. Feeding practices, tilapia farmers in four CRSP countries, 1994.

	Rwanda	Honduras	Philippines	Thailand
	Pct.	Pct.	Pct.	Pct.
What are tilapia fed most of the time?				
Termites	6	0	0	0
Beeswax	2	0	0	0
Leaves	87	0	0	0
Manure	67	0	0	0
Sorghum waste	32	0	0	0
Kitchen waste	0	14	2	12
Fresh vegetation	0	16	6	34
Rice bran	0	14	61	34
Dead animals	0	0	0	8
Slaughter waste	15	4	0	2
Commercial feed	0	41	32	42
Chicken litter	0	0	37	45
Other	8	57	0	2
Grass cuttings	28	0	0	0
Compost	28	0	0	0
Inorganic N	0	8	48	61
Chicken feed	0	0	2	0
Fish feed	0	0	0	0
What proportion of feed is purchased and what proportion is other inputs?				
Use no feed	--	61	40	20
Only purchased	--	21	10	7
Mainly purchased	--	10	24	20
Both equally	--	6	2	0
Other inputs	--	2	24	53
What commercial feed is usually purchased?				
None purchased	100	43	25	0
Rice bran	0	14	35	36
Rabbit pellets	0	8	0	0
Chicken feed	0	2	3	2
Fish feed	0	21	33	28
Other	0	12	0	34
(Number)	(136)	(51)	(50)	(56)

Fertilization

Table 4 shows the use of fertilizer in the four samples. In Rwanda, commercial fertilizer is very expensive and not applied to fish ponds. Hondurans typically use cattle and chicken manure. Chicken manure is the most frequent pond fertilizer in Thailand and the Philippines.

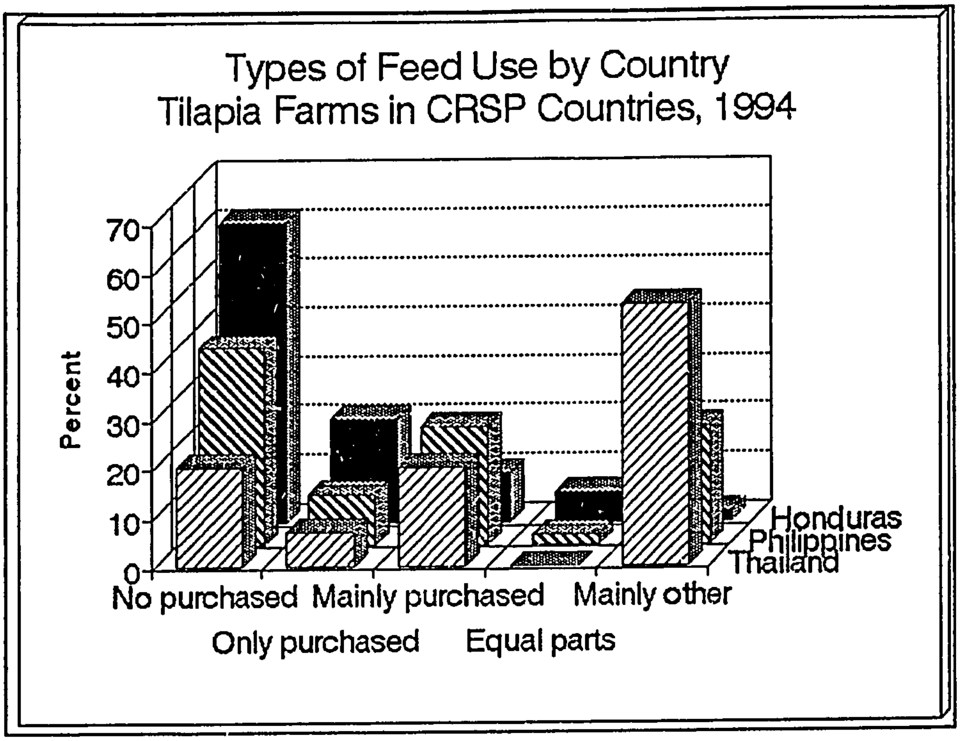

Figure 1.

Figure 2 shows the types of poultry and animals raised by tilapia farmers in the four countries. Cattle and goats were most often reported in Rwanda, pigs in Honduras, and chickens in the Philippines, and ducks were more frequent on Thai farms. In the Philippines, water buffalo were recorded in the category labeled "other."

Given the pervasive use of integrated systems in Thailand, ponds are most frequently fertilized by animal manure in that country. Thai farmers also are more likely to apply lime to improve the alkaline balance of the pond and foster primary productivity.

Almost two-thirds of the Thai farmers fed their fish several times a day. The high incidence of integration with poultry and duck production that require multiple daily feedings allows feed to literally spill over to the tilapia crop. Poultry houses are typically located directly over the fishpond, so feed and litter are nearly continuously deposited into the pond.

More intensive pond management tends to require more frequent visits by the operator. Rwandan farmers indicated the most passive approach to fish farming – about half said they visited every day. Philippine farmers spent the most time with their ponds when they visited them; Thai farmers the least.

Table 4. Fertilization practices, tilapia farmers in four CRSP countries, 1994.

	Rwanda	Honduras	Philippines	Thailand
	Pct.	Pct.	Pct.	Pct.
What pond fertilizer do you use?				
No chemical	--	0	86	91
Urea	--	0	14	0
0-46-0	--	0	0	9
18-46-0 (dap)	--	10	2	0
Chicken manure	--	29	70	53
Cattle manure	--	37	4	6
Compost	--	0	2	25
Other fertilizer	--	24	79	49
How often do your fertilize your ponds?				
Several weekly	--	27	0	69
Weekly	--	18	11	10
Several monthly	--	14	19	2
Monthly	--	10	21	0
Less often	--	21	43	4
Never	--	10	6	15
Put lime in ponds in the past year?				
No	--	57	95	26
Yes	--	43	5	74
How often do you visit your ponds?				
Several daily	0	39	34	73
Every day	53	37	36	19
Almost every day	2	14	25	0
Several weekly	32	2	5	6
Once a week	13	6	0	0
Several monthly	0	2	0	2
How much time do you usually spend when you visit your pond?				
Hour or less	34	18	4	79
About an hour	48	30	5	11
Two or 3 hours	14	20	16	4
More than 3	5	32	75	6
(Number)	(136)	(51)	(50)	(56)

Fingerlings

Table 5 profiles the fingerling sources used by fish farmers in the four countries. Rwandan farmers are dependent on government hatcheries for fingerlings. No commercial fingerling production has yet developed in the country, although fingerling sales were reported between neighboring farmers. Similarly, few private farm dealers have evolved in Honduras. The private sector provided fingerling to

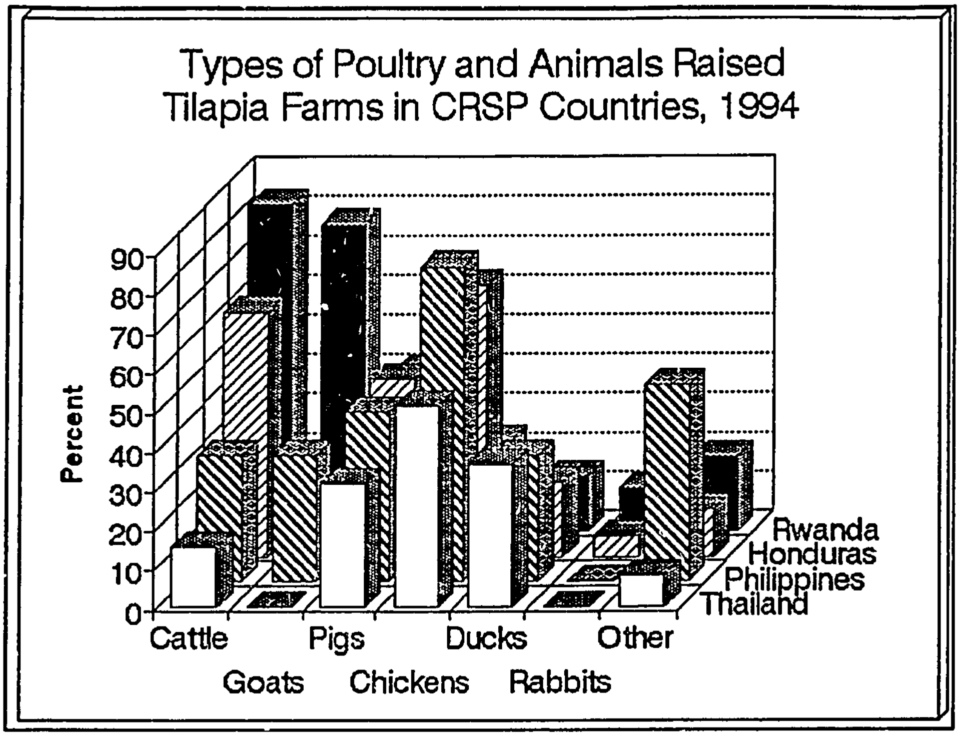

Figure 2

more than 80 percent of the Thai farmers and about 37 percent of the Philippine operators. In each country, most farmers were using *Oreochromis niloticus.*

Figure 3 suggests that Honduran farmers tended to stock somewhat larger fish.. Thai and Philippine farmers tend to densely stock the smallest fingerlings available. All-male tilapia were stocked in each country, although Rwandan patterns tended to be more heterogeneous. In all sites, chemical sex-reversal techniques were most common method of producing fingerlings.

Fingerling availability was a problem for 30 percent of the Philippine respondents, less than 25 percent in Rwanda and Honduras, and a concern for only 4 percent in Thailand.

Stocking and Grow-Out Practices

Table 6 suggests that most farmers are growing but single crop of tilapia each year in Rwanda and Thailand. In Honduras, almost half reported two or more crops, but in the Philippines two-thirds obtained two crops per year.

In Rwanda, cold water slows fish growth and lengthens the crop cycle to eight months or more. Warmer water in Honduras allowed more than a quarter of the sample to report growing tilapia in less than 180 days.

Table 5. Fingerling sources, tilapia farmers in four CRSP countries, 1994.

	Rwanda	Honduras	Philippines	Thailand
	Pct.	Pct.	Pct.	Pct.
Where do you obtain tilapia fingerlings?				
Govt. hatchery	--	57	40	4
Research station	--	18	0	0
Hatchery&station	--	6	0	0
Private dealer	--	2	37	82
From a neighbor	--	2	7	9
From own ponds	--	16	9	9
What fingerlings do you usually use?				
Wild or natural	--	63	7	100
Red colored	--	6	0	0
Other (nilotica & red)	--	31	93	0
What types of tilapia do you usually use?				
Nilotica	--	100	96	100
Mossambica	--	0	0	0
Aureas	--	0	2	0
Hybrids	--	0	2	0
Do you stock all-male or mixed-sex tilapia?				
Mixed sex	100	4	0	0
All-male	0	88	93	100
Both		8	7	0
What method produced all-male fingerlings?				
Hybridization	--	2	11	0
Sex reversal	--	76	78	75
Hand-sexing	--	20	11	13
Some combination	--	2	0	0
Don't know	--	0	0	12
(Number)	(136)	(51)	(50)	(56)

Polyculture, or raising other species of fish in the same pond, was practiced by nearly all the Thai respondents. A third of the Honduras farmers reported stocking other species, but only 11 percent of the Philippine operators did so. Polyculture was even less common in Rwanda. In most cases, the other stocked species tended to be a predator fish. The presence of a predator eliminates small fish and reduces the impact of unwanted reproduction in a crop of fish. Small fish are undesirable because they compete for feed and oxygen with the market-size fish.

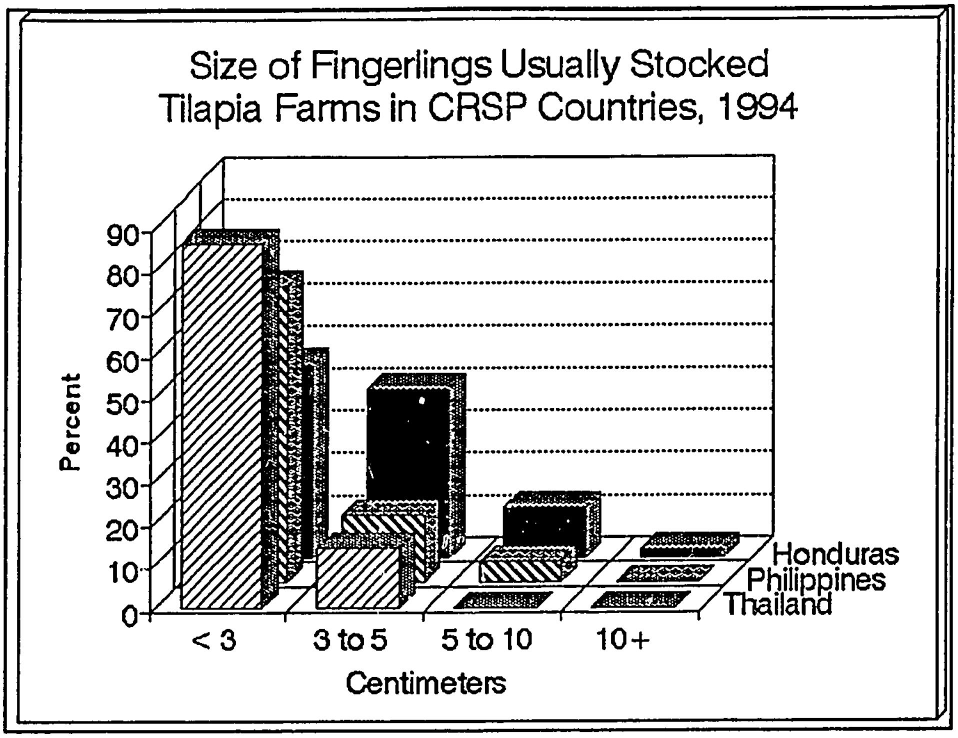

Figure 3.

Discussion

In all four countries, the purchase of fingerlings is a significant opportunity for contact with producers. When government stations are the main source of seed stock for fish farmers, fingerling sales present a distinct opportunity to directly communicate with farmers. Posters, publications, and organized events can reach many producers who travel to stations to obtain seed stock.

In Thailand, however, the private sector infrastructure for fingerling production has matured to the point that many dispersed fingerling suppliers have regular contacts with local sets of farmers. The fingerling suppliers themselves then become an important target group for extension because of their regular, direct interactions with farmers. Private hatchery managers often provide informal advisory and diagnostic services to their customers, if only to maintain the reputation of their seed stock against competing sources. In Rwanda, similar albeit less formal processes were at work. Fingerling sales by farmers to their neighbors also were mechanisms for transmitting basic information and instruction to new producers.

Aquaculture succeeded in Rwanda because it was properly supported during the initial diffusion stage (Huisman, 1990). The technology was appropriate to the setting and it met critical needs for cash income, nutrition, food security, and enterprise diversity. Producers found ready markets for their fish among their friends and neighbors, as well in organized markets in towns and urban centers.

Table 6. Stocking and grow-out practices, tilapia farmers in four CRSP countries, 1994.

	Rwanda	Honduras	Philippines	Thailand
	Pct.	Pct.	Pct.	Pct.
How many crops each year from each pond?				
One	--	54	25	95
Two	--	40	66	5
Three or more	--	6	9	0
How long does it usually take to grow a crop of tilapia?				
< 180 days	--	50	94	10
180 to 240 days	--	14	6	6
More than 240	--	36	0	84
Raise other species of fish with tilapia?				
Yes	--	36	11	98
No	--	64	89	2
Stock a predator species with tilapia?				
No	--	6	71	2
Guapote tigre	--	94	23	98
Yes, other	--	0	6	0
Had problems finding enough fingerlings to restock your pond?				
No	76	78	70	96
Yes	24	22	30	4
How many tilapia do you usually stock?				
Less than $1/m^2$	--	8	68	9
$1/m^2$ or more	--	92	32	91
How large are the fingerlings that you usually stock?				
Less than 3 cm.	--	46	70	86
3 to 5 cm.	--	40	16	14
5 to 10 cm.	--	12	5	0
More than 10 cm.	--	2	0	0
Mixed sizes	--	0	9	0
(Number)	(136)	(51)	(50)	(56)

Anticipated Benefits

The data emanating from this study present a comparative perspective on tilapia culture in four CRSP countries. The similarities and differences suggest different patterns of technology utilization and need in each setting. In Thailand, tilapia is primarily a complementary activity to chicken and duck production. In the Philippines, high prices provide a high level of producer incentive to generate farm income. In Honduras, difficult terrain and distances to fingerling suppliers and output markets tend to discourage producers. In Rwanda, tilapia provide a cash crop with discretionary harvest to meet family food and monetary needs. The benefits of these understandings should help shape research directions and enhance the development impacts of CRSP technologies.

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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 Central 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 a relational database, consolidated, and stored. Access to the consolidated files is currently done interactively requiring both knowledge and ownership of the database management program. Thus, distribution of the data usually requires the CRSP Data Base manager to export specifically requested data into spreadsheet 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. To rectify these limitations, an RFP was issued in 1992. After review of the proposals, database management was awarded to the University of Hawaii and the CRSP Central Data Base was transferred to Hilo in April 1993.

In the past year, the database has been consolidated and storage requirements have been reduced by approximately 50%. In order to increase speed, utility, and user-friendliness of the database, the database was transferred to a different relational database management program, FoxproTM. An additional advantage of Foxpro™ is its ability to work with different platforms. A menu-driven interface for Macintosh and IBM-type computers is being developed which will allow for the distribution of the entire database as a self-contained unit.

Efforts to continue the integration of the CRSP Central Data Base with ICLARM's FISHBASE are continuing. An analysis of growth data has been conducted and a method has been developed to ensure compatibility of CRSP data format with FISHBASE data format. The transformation of these data is almost complete.

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POND: A Decision Support System for Pond Aquaculture

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Introduction

Decision support systems have emerged as viable and powerful tools for capturing expert knowledge about particular domains and providing that knowledge in a friendly, easy-to-use package to end users. Such end users may include pond operators, scientists, and strategic planners. The power of decision support systems results from their capability for representing and manipulating both quantitative and qualitative knowledge that describe objects in the domain of interest and their inter-relationships. In agriculture, expert systems have been developed for providing decision support in the diagnosis of plant diseases (Michalski et al., 1982), production of crops (Smith et al., 1985), analysis of marketing alternatives (Uhrig et al., 1986), monitoring and control of greenhouses (Jacobsen et al., 1987), selection of crop cultivars (Lodge and Frecker, 1989; Bolte et al., 1991), the application of irrigation and fertilizer (Cao, 1993), and many others.

Two decision support systems have been developed by the Oregon State University DAST to address various issues relevant to decision-making processes in pond aquaculture. The first of these (PONDCLASS Versions 1.1 and 1.2) deals with issues relating to freshwater pond fertilization and the determination of lime requirements (Lannan, 1993; Nath and Lannan, 1993). The second one (POND Version 2) expands PONDCLASS functionality to include consideration of fundamental processes controlling pond dynamics as well as enterprise-level analysis of production facilities. POND Version 2 was released in Fall 1994 (Bolte et al., 1994a) and about 80 copies of the software have been distributed to a variety of potential users including actual producers, extension agents, educators, and researchers. This report focuses on the overall structure of POND Version 2, and the simulation models that serve as analysis tools in the software.

Objectives

POND Version 2 was developed with the following objectives:

1) To develop and implement simple models describing facility- and pond-level dynamics with minimal user inputs for rapid analyses of pond performance,

2) To develop and implement more sophisticated models of fish bioenergetics and pond dynamics for the exploration of fundamental processes that control pond performance,

3) To provide capabilities for conducting enterprise-level economic analyses, and

4) To provide a highly configurable, user-friendly interface for specifying pond inputs and interpreting results.

Overall Structure of POND

Software Architecture

The overall structure of POND is summarized in Figure 1. POND focuses on providing a view of pond dynamics at both the individual pond level as well as at the facility level. This involves providing capabilities for simulating processes within a pond, as well as allowing the definition of multiple ponds and multiple fish lots (i.e., a population of fish stocked in a pond), each with its own characteristic data. The simulation of dynamic pond processes requires expertise from a number of domain areas including aquatic biology, aquatic chemistry, fish biology, fish culture,

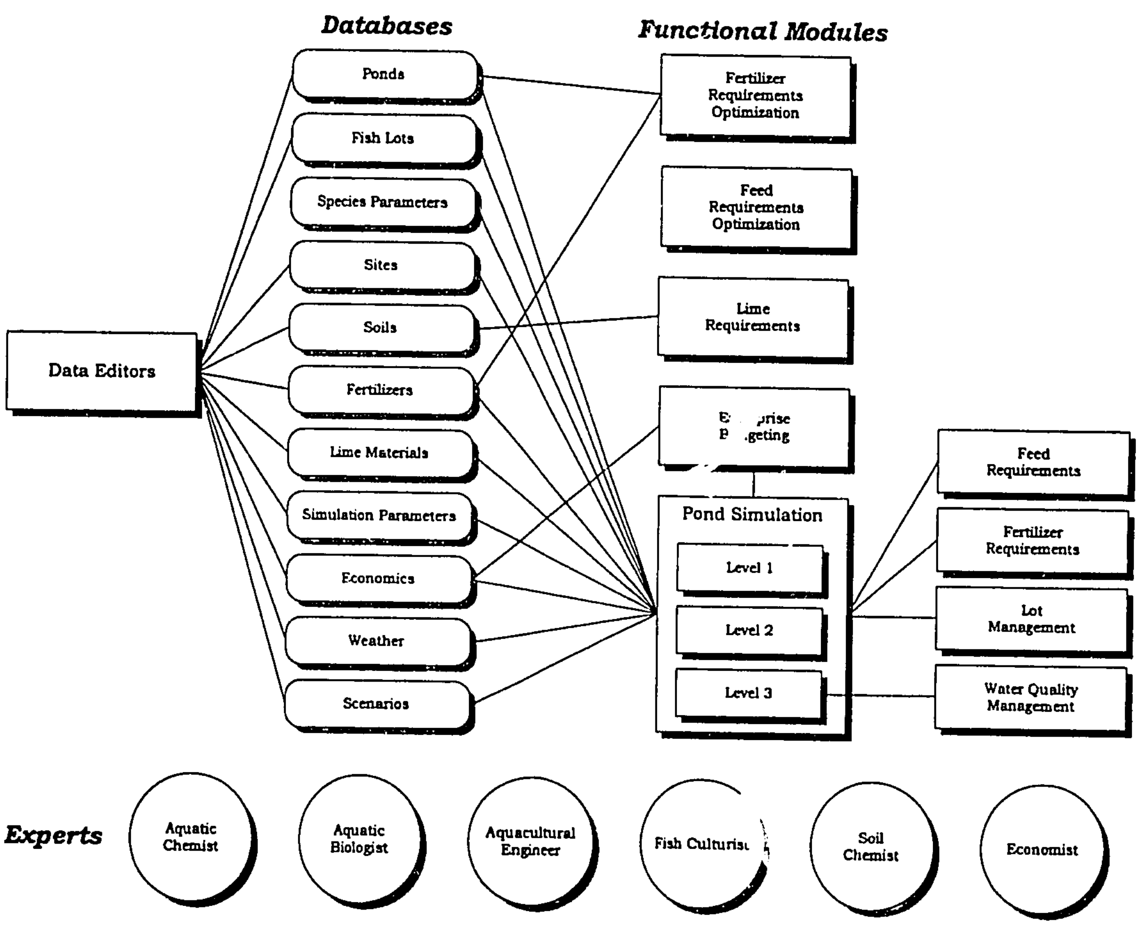

Figure 1. General architecture of POND.

aquacultural engineering, and economics. In an aquacultural facility, each of these domain areas is typically represented by well-defined entities; a facility is a collection of these entities, operating under a particular management context to allocate resources and produce fish.

The POND simulator represents a production facility in a very similar manner to that described above. Using an *object-oriented programming* (OOP) paradigm, POND defines a series of object classes, with each class representing one of the entity types described above. Each class maintains data and capabilities appropriate to its function. A POND simulation consists of creating an appropriate collection of class *instances*, each of which represents one of the objects specified. A typical simulation might be conducted by creating a series of experts (e.g., an aquatic chemist, an aquatic biologist, an aquatic engineer, a fish biologist, and a fish culturist) and a collection of facility entities (e.g., one or more fish POND instances, each representing a pond in the facility, and one or more fish lot instances, each representing a single population of fish of the same species). The simulation then proceeds by 'asking' the fish culturist to manage the fish lot; it does so with the assistance of its associated experts according to the conditions specified for each pond by the user.

POND simulations are dynamic, providing a time series analysis for a range of variables that are dependent on the level of resolution requested prior to simulation. During the simulation, POND accumulates time series data for each variable; these data may be viewed in plots or tables at the end of the simulation run. Because POND encapsulates an economist in its collection of supported object classes, economic analysis of the facility may be conducted at any time. Such analysis may use simulation data if available, as well as other fixed and variable costs and income streams as defined by the user. This approach to facility-level simulation has proven to be very flexible and effective for accomplishing POND's design goals. Thus, it is possible to rapidly explore 'what-if' questions regarding fundamental assumptions and operational criteria used for designing and managing real pond aquaculture facilities.

Further, the partitioning of expertise into well defined components allows reuse of these components in other simulation contexts.

POND contains a series of databases, that are accessible to the various objects in the software. Databases are maintained for each lot and pond in a facility, as well as for simulation scenarios, economic information, soil types, fertilizers and liming materials, site information, and weather characteristics.

POND is implemented in the C^{++} programming language and requires an IBM-PC[1] compatible personal computer running the Microsoft Windows[2] (version 3.1 or higher) operating environment. The program needs about 1.5 MB of available hard

1 IBM-PC is a registered trademark of IBM Corporation.

2 Microsoft Windows is a registered trademark of Microsoft Corporation.

disk space and a minimum of 4 MB RAM. An 80386 CPU is required, and an 80486 or greater CPU is recommended.

Economics

POND supports economic analyses of facilities in the form of enterprise budgets. Enterprise budgets allow for the accumulation of various types of costs and incomes, summarized and coupled with interest and depreciation expressions, to assess the overall economic viability of a particular production enterprise. POND supports three cost categories: (i) fixed, (ii) depreciable, and (iii) variable costs. Fixed costs are those costs which do not change over the course of facility operation (e.g., the construction cost for a pond, a one-time cost which does not vary over time). Related to fixed costs are depreciable costs, which typically are used for items which require up-front expenditures, but which may have some back-end redeemable value after a given period of time. POND incorporates depreciation schedules describing the loss of value of the depreciable asset over time. An example of a depreciable cost is the cost of a tractor, which has an initial cost as well as a resale value after some period of use at the facility. Variable costs are those costs that are not fixed or depreciable, and which typically vary according to the scale of production (e.g., labor costs, fertilizer and feed costs, and fuel and electricity costs).

To generate an enterprise budget, income sources are also required. POND allows the specification of any number of income sources, based on either a per unit area, per unit of production, or per facility basis. Income sources relating to fish production are provided by the facility simulator. Additionally, interest rates used for calculating fixed and variable investment costs are required. After specifying each cost by an amount, a cost type (fixed, depreciable or variable), basis (per unit area, per unit of production, or per facility), and other related information, the economics module in POND summarizes costs on an areal, per unit of production, or facility basis, balances those costs against income, and reports the results in a tabular form. By including and/or excluding particular costs/incomes, or adjusting cost/income details, one can quickly 'experiment' to determine possibilities for the economic viability of various facility and management configurations.

Future work in the economics analysis package of POND will focus on the development of techniques for optimizing pond inputs (e.g., fertilizer and feeding levels) based on the different models in the program. Such optimization techniques can be coupled with expert system approaches to enhance the support for decision-making processes.

Simulation Models in POND

Various experts in POND (Figure 1) have capabilities for simulating different aspects of production. This is accomplished by the use of simulation models (see section on Model Descriptions below).

Model Organization

To provide users with the capability of performing different kinds of analyses based on data availability and output resolution requirements, the various models in POND are organized hierarchically into two levels:

Level 1: Level 1 models are fairly simple, require minimal data inputs, and are intended for applied management and rapid analysis of pond facilities. At this level, the variables simulated are fish growth (based on a bioenergetics model) and water temperature. Consumption of natural food by fish is assumed to be a function of fish biomass and appetite. Fertilizer application rates are user specified; the model optionally generates supplementary feeding schedules.

Level 2: Level 2 models provide a substantially more sophisticated view of pond dynamics, allowing prediction of phytoplankton, zooplankton, and nutrient dynamics (carbon, nitrogen, and phosphorus) in addition to fish growth and water temperature. This modeling level is intended for detailed pond analysis, management optimization, and numerical experimentation. Fish can feed from natural and/or artificial food pools. Consumption of natural food (phytoplankton and zooplankton pools) by fish is predicted on the basis of a resource competition model, and also depends on fish appetite. At this level, a constant, user-specified concentration of pond nitrogen, phosphorus, and carbon is assumed. Mass balance accounting for each of these variables is maintained, allowing estimation of fertilizer requirements necessary to maintain steady state levels. Both fertilization and feeding schedules are generated by the models.

Model Descriptions

POND includes models to describe the following *state variables* (i.e., variables that describe the state of a system at any given time): fish growth, water temperature, carbon, nitrogen and phosphorus, phytoplankton, and zooplankton. These state variables are described by the use of differential equations, which are solved by a fourth order Runge-Kutta integration technique. In this report, we discuss the fish growth/bioenergetics model in detail. Other POND models are also briefly discussed; detailed documentation on these models is available elsewhere (Bolte et al., 1994b).

For pond aquaculture, management tasks that may be addressed by the use of fish growth models include (1) the prediction of fish yields under conditions where natural food is the only source of nutrition to fish, (2) the generation of supplementary feed schedules based on natural food resources and fish production targets, (3) the analysis of observed fish production relative to expected levels, (4) the management of fish densities and harvest schedules to maximize overall conversion efficiency of natural and supplemental food resources, and (5) the assessment of water quality management as a potential tool for increased production.

Variables that significantly influence fish growth are size (or weight), food availability, photoperiod, temperature, dissolved oxygen, and unionized ammonia concentrations (Fry, 1947; Winberg, 1960). These variables affect fish growth via their impacts on food intake (Brett, 1979). Further, the development and maturation of reproductive structures reduces growth because energy that might otherwise have been used for tissue build-up is diverted to these functions (Brody, 1945).

A bioenergetics model developed by Ursin (1967), and subsequently modified by Liu and Chang (1992) has been used to model Nile tilapia (*Oreochromis niloticus*) growth in warmwater ponds. This modified model has since been expanded into a more generalized version called the BE model, which is implemented in POND.

BE Model Structure: The BE model has been developed primarily as a tool that can be used to forecast fish growth and yields under different management and environmental conditions. Therefore, the use of traditional parameter estimation procedures which require actual growth data (e.g., non-linear regression as in Liu and Chang, 1992) has been minimized. The BE model has been parameterized for Nile tilapia using information from bioenergetic studies and by calibration.

The growth of Nile tilapia in the model developed by Liu and Chang (1992) was assumed to depend on body size, food availability (a function of the stocking density of fish in the pond and the amount of fertilizers added), and gametogenetic activities. Because of difficulties in predicting the onset of maturity and the proportion of intake energy that may be diverted for gametogenetic activities, the BE model assumes that energy losses due to gametogenesis are negligible (e.g., as might be the case in a monosex culture of Nile tilapia). Besides size and food availability, the BE model includes the effects of variables such as photoperiod, temperature, dissolved oxygen (DO), and unionized ammonia (UIA) concentrations on fish growth (Tables 1 and 2). The BE model assumes that such effects occur via the influence of these variables on food consumption, and that they are of an interactive nature (Cuenco et al., 1985). The latter assumption implies that the effect of any variable on food intake is not independent of the other variables (equation 3, Table 1).

Effects of size: Fish growth rate generally increases at a declining rate with size or weight and is often described by the use of a power function (e.g., von Bertalanffy, 1938; Winberg, 1960). However, the Ursin model assumes that anabolism and catabolism may be paced at different rates in relation to fish weight, with subsequent effects on fish growth. This is achieved by use of the exponents m and n (equation 1, Table 1). Because m cannot be easily estimated, it is assumed to equal 0.67 in the BE model (Hepher, 1988; Liu and Chang, 1992; Table 3). However, n can be estimated if oxygen consumption data are available for starving fish (Ursin, 1967). Analysis of such data on Nile tilapia (Farmer and Beamish, 1969) result in n = 0.81, which is similar to Ursin's (1967) estimate of n = 0.83 (from data on 81 fish species).

Effects of photoperiod: any cultured fish, including tilapias, tend to feed only during daylight hours (Caulton, 1982). Estimates of daily photoperiod for different sites can be obtained from sunrise and sunset hour angle calculations (Hsieh, 1986). The BE model uses a daylight scaler p based on such calculations to adjust daily food intake (equation 3, Table 1). For example, a photoperiod of 12 h would result in p = 0.5.

Table 1. Listing of equations for the BE model in POND (notations given in Table 2).

$$\frac{dW}{dt} = HW^m - kW^n \quad (1)$$

$$\frac{dW}{dt} = b(1-a)\frac{dR}{dt} - kW^n \quad (2)$$

$$\frac{dR}{dt} = h \ p \ f \tau \ \delta \ \upsilon \ W^m \quad (3)$$

$$\frac{dR_{max}}{dt} = h \ p \ \tau \ \delta \ \upsilon \ W^m \quad (4)$$

$$\tau = \exp\left\{-4.6\left[(T_{opt} - T)/(T_{opt} - T_{min})\right]^4\right\}, \quad \text{if } T < T_{opt}$$

$$\exp\left\{-4.6\left[(T - T_{opt})/(T_{max} - T_{opt})\right]^4\right\}, \quad \text{if } T \geq T_{opt} \quad (5)$$

$$k = k_{min} \exp\left[s(T - T_{min})\right] \quad (6)$$

$$\delta = 1.0, \quad \text{if } DO > DO_{crit}$$
$$(DO - DO_{min})/(DO_{crit} - DO_{min}), \quad \text{if } DO_{min} \leq DO \leq DO_{crit}$$
$$0.0, \quad \text{if } DO < DO_{min} \quad (7)$$

$$\upsilon = 1.0, \quad \text{if } UIA < UIA_{crit}$$
$$(UIA_{max} - UIA)/(UIA_{max} - UIA_{crit}), \quad \text{if } UIA_{crit} \leq UIA \leq UIA_{max}$$
$$0.0, \quad \text{if } UIA > UIA_{max} \quad (8)$$

$$f = 1.0, \quad \text{if } FB < CFB$$
$$CFB/FB, \quad \text{if } FB \geq CFB \quad (9.1)$$

$$\mu_{FS}^{max} = \frac{dR_{max}}{dt} W^{-1} \quad (9.2.1)$$

Table 1 (continued).

$$\mu_{FS} = \sum_{i=1}^{N} \mu_{FS}^{max} \left(\frac{\frac{F_i}{c_i}}{1 + \sum_{j=1}^{N} \frac{F_j}{c_j}} \right) \quad (9.2.2)$$

$$f = \frac{dR / dt}{dR_{max} / dt} = \frac{\mu_{FS} W}{dR_{max} / dt} \quad (9.2.3)$$

Effects of temperature: Food consumption tends to increase with temperature from a lower limit below which fish will not feed (Tmin) until the optimum temperature (Topt) for the given fish species is reached. Beyond T_{opt}, consumption decreases rapidly to zero until an upper limit (T_{max}) is reached above which fish will not feed (Brett, 1979). Because many fish species (including tilapias) (Caulton, 1978) tend to have a maximum food consumption rate within a temperature range rather than at a single optimum temperature, a function like the one used by Svirezhev et al. (1984) which is somewhat flat around a known optimal temperature may be appropriate to describe the effects of temperature on food consumption (equation 5, Table 1).

The above temperature function appears to be appropriate for the effects of temperature on food consumption, and therefore on anabolism. However, catabolism increases exponentially with temperature within the tolerance limits for a given species (Ursin, 1967). In the BE model, the exponential function of the Ursin model has been modified to include the lower temperature tolerance limit for a given species (equation 6, Table 1), which is assumed to be equivalent to T_{min}.

Pond temperature data (T) for the functions given by equations 5 and 6 (Table 1) are obtained from the water temperature component of the systems model in POND. Available literature for Nile tilapia suggests values for T_{min}, T_{max}, and T_{opt} as listed in Table 3. The parameters k_{min} and s have been estimated by model calibration (Table 3). Using values for k_{min}, s, and T_{min} from Table 3, and assuming $T = T_{opt}$, equation 6 predicts $k = 0.0327$. This predicted value is within the range of $k = 0.0319$-0.0468 estimated by Liu and Chang (1992).

Effects of dissolved oxygen and unionized ammonia: Dissolved oxygen typically does not affect food consumption if its concentration is above a critical limit that is species dependent, but further decreases in DO levels reduce food consumption more or less linearly until lethal concentrations of DO are reached (Cuenco et al., 1985; equation 7, Table 1). The effects of UIA are similar with the exception that food

Table 2. Description of notations used in the BE model.

Notation	Description	Units
	State Variables	
T	Water temperature	°C
W	Fish weight	g
	Rate terms	
dR/dt	Food intake rate or daily ration	$g\ d^{-1}$
dR_{max}/dt	Maximum food intake rate	$g\ d^{-1}$
μ_{FS}	Overall specific food intake for fish	d^{-1}
μ_{FS}^{max}	Maximum specific food intake rate for fish	d^{-1}
	Parameters and constants	
a	Fraction of food assimilated used for feeding catabolism	None
b	Efficiency of food assimilation	None
c_i	Half-saturation constant for the feeding of fish on the ith natural food resource (e.g., phytoplankton)	*
CFB	Critical fish biomass (or critical standing crop)	$kg\ ha^{-1}$
DO	Dissolved oxygen level	$gO_2\ m^{-3}$
DO_{crit}	Critical DO level above which food intake is not affected	$gO_2\ m^{-3}$
DO_{min}	Minimum DO level below which fish will not feed	$gO_2\ m^{-3}$
f	Relative feeding level	None
F	Concentration of a natural food resource (e.g., zooplankton)	*
FB	Fish biomass (or standing crop)	$kg\ ha^{-1}$
h	Coefficient of food consumption	$g^{1-m}\ d^{-1}$
k	Coefficient of fasting catabolism	$g^{1-n}\ d^{-1}$

* Depends on the particular resource. For example, units for phytoplankton and zooplankton are $gC\ m^{-3}$ and $g\ m^{-3}$ respectively.

Table 2 (continued). Description of notations used in the BE model.

Notation	Description	Units
	Parameters and constants	
k_{min}	Coefficient of fasting catabolism at T_{min}	$g^{1-n}\ d^{-1}$
m	Exponent of anabolism	None
n	Exponent of catabolism	None
N	Total number of natural food resources	None
p	Daylight scaler	None
s	Constant to describe temperature effects on catabolism	$°C^{-1}$
t	Time	d
T_{min}	Minimum water temperature below which fish will not feed	°C
T_{max}	Maximum water temperature above which fish will not feed	°C
T_{opt}	Optimum water temperature for fish feeding	°C
UIA	Unionized ammonia level	$gNH_3\text{-}N\ m^{-3}$
UIA_{crit}	Critical UIA level below which food intake is not affected	$gNH_3\text{-}N\ m^{-3}$
UIA_{max}	Maximum UIA level above which fish will not feed	$gNH_3\text{-}N\ m^{-3}$
δ	Function to describe the DO effects on food intake	None
τ	Function to describe temperature effects on food intake	None
υ	Function to describe the effects of UIA on food intake	None

consumption is affected if UIA levels *exceed* a certain critical concentration (Cuenco et al., 1985; equation 8, Table 1). These authors assumed that if both DO and UIA concentrations are in the range where food consumption is reduced, the overall effect would be determined by the lower of the two concentrations (i.e., as predicted by equation 7 *or* 8). However, this assumption is not consistent with earlier observations that the effects of high UIA concentrations are more pronounced when DO is low (Merkens and Downing, 1957; Thurston et al., 1981). The BE model assumes that the effects of UIA and DO are interactive (equation 3, Table 1). Feasible DO and UIA parameters (expressed in g m^{-3}) for tilapia are as follows: DO_{min} = 0.5-1.0, DO_{crit} = 3 (Boyd, 1990; Stickney, 1994), UIA_{max} = 1.40, and UIA_{crit} = 0.06 (Abdalla, 1989).

Table 3. Parameter values used for simulations and data sources from which they were estimated. Values in parentheses denote the range of the estimates. Some parameter values were estimated by model calibration.

Parameter	Value used in simulations	Data source
a	0.256	Brett, 1979
b	0.625 (0.53-0.70)	Meyer-Burgdorff et al., 1989
c_1 (Phytoplankton A)	2	Calibration
c_2 (Phytoplankton B)	10	Calibration
c_3 (Zooplankton)	1	Calibration
h	0.8	Calibration
k_{min}	0.025	Calibration
m	0.67 (0.50-0.83)	Hepher, 1988
n	0.81	Farmer and Beamish, 1969
T_{min}	15	Gannam and Phillips, 1992
T_{max}	41	Denzer, 1967
T_{opt}	33 (30-36)	Caulton, 1982
s	0.015	Calibration

Effects of food availability: The Ursin model includes a relative feeding level parameter f (0-1), which is the ratio of the actual food intake rate (dR/dt; equation 3, Table 1) to the maximum possible intake rate or food intake rate at satiation (dR_{max}/dt; equation 4, Table 1). The parameter f was modeled by Liu and Chang (1992) on the basis of a function developed by Ivlev (1961), who observed that food intake by fish tends to increase asymptotically towards a maximum intake level. The Ivlev function depends on the quantity of food resources available and the number of fish present in the pond. Liu and Chang (1992) used a fertilizer richness parameter as a measure of available food resources. This parameter was assumed to correspond to the amount of manure added to the pond.

However, such a function for f cannot be easily extended for use in ponds that receive various levels of fertilizer inputs or a mixture of organic and synthetic fertilizers because an estimate of the fertilizer richness parameter would be required. Moreover, Liu and Chang (1992) assumed natural food availability to be function of the number of fish, instead of fish biomass or standing crop as suggested by Hepher (1978). This assumption implies that food availability in a pond is constant for a particular fertilization regime and culture period, unless the number of fish is substantially altered due to mortality or partial harvest. However, natural food availability in ponds that are fertilized identically usually varies over time (Hepher, 1978).

During the initial phase of fish production, adequate natural food is produced in the pond to sustain fish at their maximum feeding level (i.e., $f = 1$), but once the biomass of fish in the pond exceeds the "critical standing crop" or critical fish biomass (CFB), natural food availability declines with fish biomass until the carrying capacity of the pond is reached (Hepher, 1978). Although natural food availability can potentially decline to zero, this situation does not appear to occur in ponds, perhaps because there is adequate food to meet maintenance requirements of fish, even at relatively high biomass levels, as a result of adaptation to conditions of poor food availability (Hepher et al., 1983). A simple expression is used to approximate the relationship between food availability and fish biomass in the BE model for Level 1 simulations (equation 9.1, Table 1). This expression overcomes some of the drawbacks in the function used by Liu and Chang (1992).

Use of equation 9.1 (Table 1) requires estimates of the CFB of a pond, which can be obtained from fish growth data because the short-interval growth rate of individual fish tends to increase initially, reach a maximum at the CFB, and decrease thereafter (Hepher, 1978). In practice, the first step in estimating the CFB is to calculate the average growth rate for different sampling intervals. It is then necessary to determine when the growth rate begins to deviate from a maximum value. Our analysis of PD/A CRSP data indicates that this deviation occurs between two time intervals, in which case the average fish biomass for that time period is taken to be the CFB. Because sampling in these ponds was usually conducted on a monthly basis, significant errors in CFB estimation may be introduced by averaging fish biomass. Finally, the CFB calculated by this method accounts for losses of fish due to mortality by applying an average survival rate (calculated from harvest data) to the number of fish remaining in the pond.

Growth data from PD/A CRSP experiments suggest that CFBs vary substantially according to climate, water, and soil characteristics of a site, as well as with management practices; CFBs among identically treated ponds also vary substantially. As a result of such variability, prediction of pond CFBs has not been very successful. In addition, the CFB-based function for f (equation 9.1, Table 1) does not directly account for the effects of pond fertilization on natural food availability.

A more involved approach of predicting f would be to actually simulate the dynamics of individual natural food resources in ponds (e.g., as in Svirezhev et al., 1984). These authors assumed that each fish species in a carp polyculture system had a high probability of uptake of a *preferred* food until it was depleted to some critical concentration (due to over-grazing), at which point the fish would switch to another resource designated as the *substituting* food. Thus, for silver carp, phytoplankton was designated as the preferred food, and detritus the substituting food. However, many fish (including different species of carp and tilapia) feed on a variety of food resources (Schroeder, 1980; see review by Colman and Edwards, 1987). Therefore, the modeling approach of Svirezhev et al. (1984), which considers only two resources at any given time and is not easily extensible to multiple resources, appears to be inappropriate. An alternate feeding function is the *resource substitution* model (Tilman, 1982; O'Neill et al., 1989), which is essentially an extension of Monod uptake kinetics to multiple resources. This model is advantageous in that an unrestricted number of resources can be considered, food preferences are inherently captured in the half-saturation constants, and fewer parameters are required compared to the function used by Svirezhev et al. (1984).

As an alternative to the CFB-based feeding function, the resource substitution model (equation 9.2.1-9.2.3, Table 1) is used for Level 2 simulations. The model currently considers three different food resources (two pools of phytoplankton designated as A and B, and one of zooplankton). Half-saturation constants to describe the feeding of fish on the two phytoplankton and one zooplankton pools have been estimated by model calibration (Table 3). Because the effects of nutrients (carbon, nitrogen, and phosphorus) on phytoplankton growth are also considered, these tools will likely be useful for examining the effects of fertilizer practices on natural food availability, and ultimately on fish growth.

Bioenergetic parameters: Besides the above variables, the BE model includes three bioenergetic parameters, *a*, *b*, and *h* from the Ursin model. The parameter *a* is the fraction of the food assimilated that is used for feeding catabolism, *b* the efficiency of food assimilation, and *h* the coefficient of food consumption (Table 2). Liu and Chang (1992) determined $h = 0.997$ by non-linear regression. Calibration of the BE model suggests a value of $h = 0.80$ to be more appropriate. The term $b(1 - a)$ in equation 2 (Table 1) represents energy that is available for growth and fasting catabolism. In energetic terms, *b* is the proportion of gross energy or food intake that is available as metabolizable energy. Liu and Chang (1992) assumed $b = 0.53$, which is the mean value for the range of 0.478 to 0.587 reported by Caulton (1982) for *Tilapia rendalli*. Although *b* actually decreases with food intake (Caulton, 1982; Meyer-Burgdorff et al., 1989), it is assumed to be constant in the BE model (Table 3). The mean value of 0.625 for *b* implies that excretory losses are about 37.5% of the gross energy intake.

Liu and Chang (1992) estimated $a = 0.435$ by non-linear regression. However, *a* can be indirectly estimated by assuming 16% of the gross energy intake to be lost via heat increment and urinary wastes (Brett and Groves, 1979). Thus, overall energy losses associated with the processing of food (i.e., excretion, heat increment, and urinary wastes) are about 53.5%. Alternately, the term $b(1 - a)$ is about 46.5% of the gross energy intake, which results in $a = 0.256$.

Results of simulations with the BE model: The BE model has been used to simulate the results of PD/A CRSP experiments with Nile tilapia at different sites. These simulation trials include:

Simulation I: Comparison of fish growth at different stocking densities in ponds located at Ayutthaya, Thailand (data from Diana et al., 1990; Figure 2),

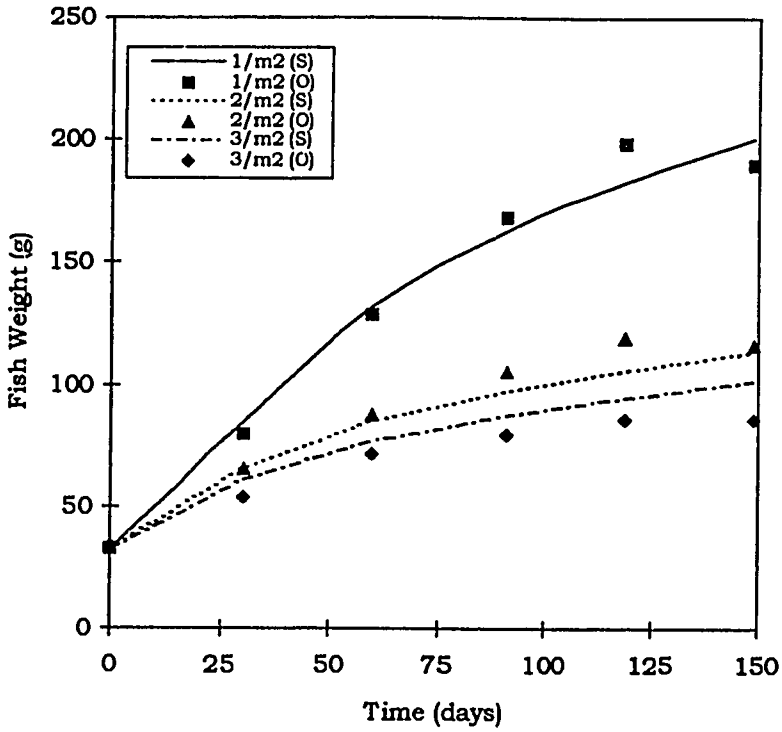

Figure 2. Simulated (S) and observed (O) fish weights (g) for a 150-day stocking density experiment in Ayutthaya, Thailand. Mean CFB's for the three replicates per treatment were estimated to be about 791, 790, and 1050 kg ha^{-1} for the 1, 2 and 3 fish m^{-2} treatments, respectively. All the ponds were fertilized with chicken manure at 500 kg ha^{-1} wk (DM basis).

Simulation II: Fertilization trials in Bang Sai, Thailand (Knud-Hansen et al., 1991; Fig. 2) and El Carao, Honduras (data from Teichert-Coddington et al., 1990; Figure 3), and

Simulation III: A fertilization experiment in El Carao (data from Green et al., 1989; Figure 4).

All the simulations used the CFB-based function (equation 9.1, Table 1) to estimate the relative feeding level *f*. In addition, Simulation III involved use of the resource substitution model (equations 9.2.1-3, Table 1). CFBs were estimated from fish growth data reported for the above experiments using the procedure outlined earlier. A daily time step was used for all three simulations. Daily water temperature was predicted by use of a model that assumes completely mixed conditions in the pond (Fritz et al., 1980; Nath and Lannan, 1993). Solar radiation, daily minimum and maximum air temperatures, and wind speed data recorded in the PD/A CRSP database for the above experiments were used as inputs to the water temperature model. The effects of DO and UIA were not considered in the simulations (i.e., d = u = 1) because their inclusion is of limited use unless the dynamics of DO and UIA in the pond water are modeled simultaneously.

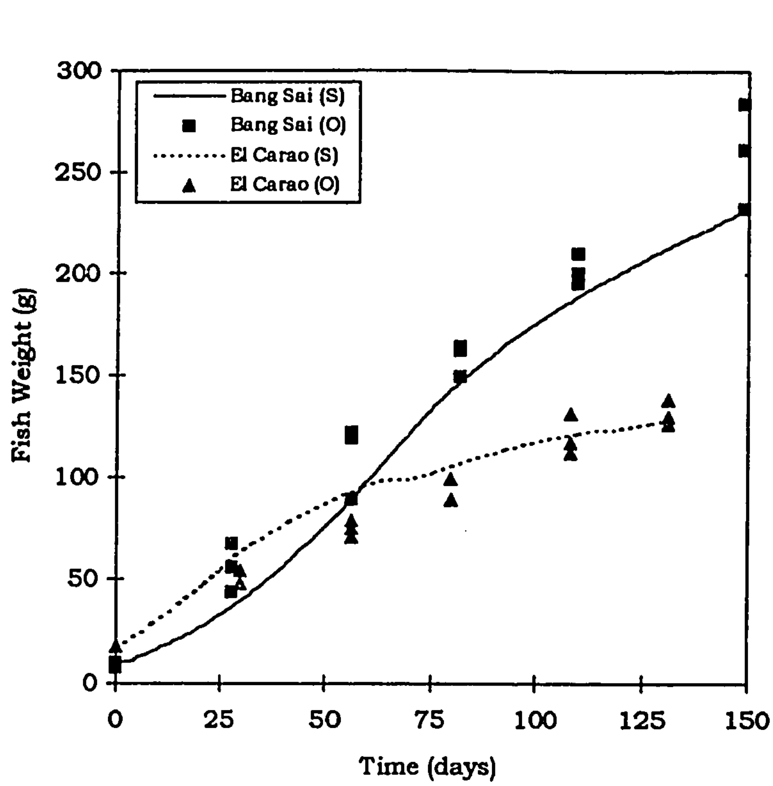

Figure 3. Simulated (S) and observed (O) fish weights (g) for a 150-day experiment in Bang Sai, Thailand, and a 132-day experiment in El Carao, Honduras. The Bang Sai ponds were stocked at 2 fish m^{-2} and received chicken manure at 75 kg ha^{-1} wk^{-1} (DM basis), supplemented with adequate inorganic nitrogen and phosphorus inputs to provide a total of 5 kg ha^{-1} wk^{-1} of nitrogen and 1.2 kg ha^{-1} wk^{-1} of phosphorus. The mean CFB for the three ponds at this site was estimated to be 1975 kg ha^{-1}. The El Carao ponds were stocked at 1 fish m^{-2} and fertilized with chicken manure at 500 kg ha^{-1} wk^{-1} (DM basis). The mean CFB was estimated to be about 500 kg ha^{-1}.

Results of Simulation I indicate that the BE model predicted fish growth quite well except for the highest stocking density (three fish m^{-2}) (Figure 2). The discrepancy between observed and simulated growth for this treatment may be due to errors in the prediction of the CFB. The mean CFB estimated for ponds stocked at lower densities was about 790 kg ha^{-1} compared to 1050 kg ha^{-1} for the three fish m^{-2} treatment.

Fish weight was consistently under-predicted by the BE model for the fertilization trial in Bang Sai, Thailand (Simulation II; Figure 3). This discrepancy may also have occurred because of errors in the estimation of the CFB. However, model results for the fertilization experiment conducted in 1986 at the El Carao site were quite similar to observed values (Figure 3).

Results for the fertilization experiment conducted in 1988 at El Carao using both the CFB-based (Level 1) and the resource substitution function (Level 2) were comparable to observed values (Simulation III; Figure 4), although the sigmoidal

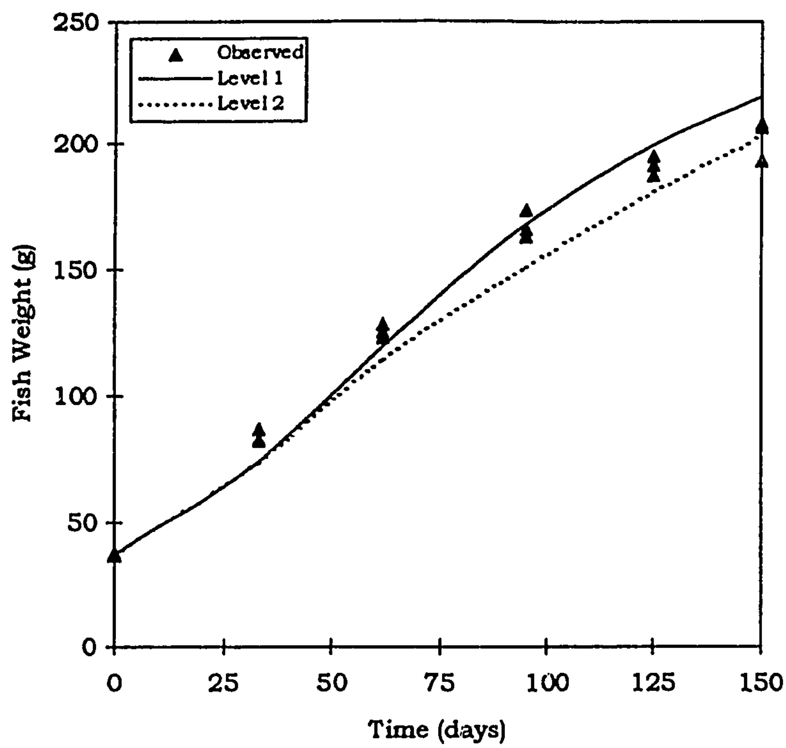

Figure 4. Simulated and observed fish weights (g) for a 151-day fertilization trial in El Carao, Honduras. The two sets of simulated values reflect model predictions using the CFB-based (Level 1) and the resource substitution functions (Level 2). The mean CFB used for the Level 1 simulation was estimated to be 970 kg ha^{-1}. The ponds were stocked at 1 fish m^{-2} and fertilized with chicken manure at 500 kg ha^{-1} wk^{-1} (DM basis).

nature of fish growth appears to be better captured by the CFB-based function. The final fish weight predicted by use of the latter function exceeded observed values. This may either be a result of inappropriate parameter values or because of environmental effects (e.g., low DO or high UIA concentrations) that are not considered in the current version of the BE model. Models for DO and UIA dynamics will likely be implemented in future versions of POND to enable exploration of the effects of these variables on fish growth.

PD/A CRSP data indicate that fish growth varies substantially even within ponds that are treated identically (e.g., Figures 3 and 4). Some of this variability may be a function of prior pond history (Knud-Hansen, 1992). It is also possible that part of the variability is purely stochastic in nature. This implies that it may be beneficial to add a stochastic component to the BE model, in which case a distribution of model outcomes can be examined, as opposed to a single prediction.

However, the BE model in its current form does appear to be a useful tool for predicting fish growth under different management and climatic conditions, with the caveat that re-calibration may occasionally be necessary for some sites, as indicated earlier. Re-calibration will also be required to describe the growth of fish species other than Nile tilapia. Further, although the CFB-based function for the relative feeding level can be used · predict growth, there are two primary drawbacks that limit its use. Firstly, CFB ι ,timates may vary substantially, even for identically treated ponds at the same site. Secondly, the effects of fertilization cannot be directly described by use of the CFB-based function. The resource substitution function overcomes these drawbacks to some extent, but adds considerable complexity to the growth model.

The BE model can also be used to address supplementary feeding requirements in ponds. For example, if a target feeding level such as satiation feeding (i.e., feeding rate equal to dR_{max}/dt, as estimated by equation 4, Table 1) is specified, the model can be used to estimate the proportion of this requirement satisfied by natural food. The difference between the target requirement and the proportion met by natural food indicates the amount of supplemental feed to be added to the pond.

Another potential application of the BE model relates to scheduling tasks such as partial harvesting, where the goal may be to ensure that fish biomass is thinned so that fish growth is not limited by food. Partial harvesting should therefore occur when the CFB of the pond is reached. The culture period required to reach the CFB can be predicted by the BE model. Such uses of the BE model are particularly relevant in situations where reasonably accurate estimates of CFB are available.

Other POND models: The basic differential equations that describe other state variables (water temperature, carbon, nitrogen and phosphorus dynamics, phytoplankton and zooplankton) in POND have been adapted from previously published models. For example, the phytoplankton model is adapted from the work of Steele (1962), Di Toro et al. (1971), Eppley (1972), Smith (1980), Svirezhev et al. (1984), and Piedrahita and Giovannini (1991). It includes the effects of light, temperature, and nutrients on gross primary productivity, and it includes terms for phytoplankton respiration and death, and the consumption by fish and zooplankton. The zooplankton model is structured similarly, and includes terms for zooplankton growth, respiration and death, as well as for consumption by fish (Di Toro et al., 1971; Svirezhev et al., 1984).

The water temperature model assumes completely mixed conditions and is adapted from Fritz et al. (1980; see also Nath and Lannan, 1993). The models for nutrient (carbon, nitrogen, and phosphorus) dynamics assume steady state conditions, and include terms for losses to or gains from processes associated with phytoplankton, zooplankton, and fish metabolism and mortality. Gains from fertilizer application (calculated within POND) and miscellaneous losses/gains that are user-defined are also considered in the nutrient models.

Experience with the various models in POND suggest that the interaction between variables controlling pond dynamics may be considerable. Therefore, ecosystem-level validation of these models is difficult. However, specific components of POND (e.g., fish growth, as discussed earlier) have been validated with favorable results. Future work on the simulation models in POND will focus on more comprehensive validation in field situations and sensitivity analyses of specific model components to various parameters and operating criteria. As validation proceeds, refinement of the model algorithms will likely occur. This will allow new knowledge of fundamental pond processes to be synthesized and presented in POND for use in conducting more rigorous analyses of pond aquaculture facilities.

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Binding Sites for the Masculinizing Steroid Mibolerone in the Gonadal Tissue of Adult Nile Tilapia (*Oreochromis niloticus*)

Work Plan 7, Egypt Study 4C1

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Introduction

Efficient sex control of tilapia species has much potential for increasing the profitability of aquacultural operations. Tilapia in mixed populations may begin reproductive behavior prior to reaching market size. Expenditure of energy for gamete production and reproductive behaviors can significantly lower the growth rate. Mono-sex populations avoid the loss of energy associated with reproduction. Male populations are particularly attractive because males grow larger and faster than females.

Four methods are currently utilized for producing mono-sex populations of tilapia (Pandian and Varadaraj, 1990). First, juvenile tilapia may be sexed by visual examination of secondary sexual characteristics. This technique is very labor intensive, has a high degree of error, and requires that fish be grown to a sufficient size for visual examination of sexual differences. Second, hybrid crosses between species of tilapia have been employed. However, hybridization is a method with several problems, e.g., difficulty in obtaining genetically pure parental stocks or incompatibility of interspecific breeders. Third, certain synthetic steroids, when administered to undifferentiated fry, can act as sex inverting agents, effectively masculinizing or feminizing an entire population (reviews in: Schreck, 1974; Hunter and Donaldson, 1983). The last technique is still in the developmental stages, and involves the use of YY males for broodstock (Scott et al., 1989). The YY male broodstock (O. *niloticus*) can be produced by feminizing genetic males, which are considered heterogametic (XY) (Trombka and Avtalion, 1993). The genetic male/ phenotypic females are crossed with normal males to obtain the following ratio of progeny genotypes: 1 XX female: 2 XY males: 1 YY male. Since XY and YY males are indistinguishable by external characteristics, the YY males are identified by progeny testing, i.e., a YY male crossed with a normal XX female should produce a progeny of all XY males. This technique circumvents the need to use steroid treatment in fish destined for market, although it is still necessary for broodstock production. However, one drawback is the time required to identify YY individuals, which involves testing several generations of progeny.

Many synthetic androgens are potent masculinizing agents, including 17α-methyltestosterone (17-hydroxy-17-methylandrost-4-en-3-one; MT) and mibolerone (17-hydroxy-7,17-dimethylestr-4-en-3-one; Mb). Much less study has been done on the feminization of genetic males. However, 17a-ethynylestradiol (17α-ethynyl-1,3,5(10)-estratrien-3,17β-diol; EE), a synthetic estrogen, has been used successfully as a feminizing agent in many teleost species, including *O. aureus* (Hopkins et al., 1979). The majority of sex-inverting steroids are structurally similar in that they are synthetic steroids with an alkyl group at position 17. Although procedures are well established for using steroids as sex inverting agents, the physiological mechanism of action of these steroids is still unknown. The classical model of steroid action is through an intracellular receptor that once bound to a steroid acts as a transcription factor (O'Malley and Tsai, 1992; Carson-Jurica et al., 1990). The net effect of steroid receptor action is the activation or inhibition of transcription of certain genes, thus controlling mRNA production, and, subsequently, protein translation.

Receptors that bind sex-inverting agents in the target organs of tilapia have yet to be identified. Studies have examined the elimination and distribution of MT in tilapia after oral administration. Goudie et al. (1986a) and Johnstone et al. (1983) demonstrated that tritiated MT was sequestered for the most part in viscera of undifferentiated fry of *O. aureus* and *O. mossambicus*. Curtis et al. (1991) examined the distribution of tritiated MT fed to sex-inverted juvenile tilapia (*O. niloticus*). Similarly, the viscera were primarily responsible for accumulating MT, with the liver and digestive tract containing the majority of MT. The gonadal content of tritiated MT was not reported. Goudie et al. (1986b) demonstrated that tritiated MT fed to adult tilapia (*O. aureus*) was primarily concentrated in the liver, digestive tract, kidney, and gonadal tissues. The presence of MT in tissues associated with digestion and clearance is not surprising. The appearance of MT in gonadal tissues suggests that they may be target organs for masculinizing steroids.

One mode of steroid action is through a receptor that acts as a transcription factor. Furthermore, the potent masculinizing agent MT accumulates in the gonads after administration to adult tilapia. Thus, it is plausible that a receptor exists in gonadal tissue which binds specifically with sex inverting agents. A receptor specific for masculinizing androgens has been characterized in ovarian cytosol of adult coho salmon (*Oncorhynchus kisutch*) (Fitzpatrick et al., 1994). The receptor met all of the criteria for a model steroid receptor (Clark and Peck, 1977): it had high affinity and low capacity for ligand binding, was specific for androgens, was specific for gonadal tissue, and was correlated with a physiological response. The purpose of this study was to determine if a binding site for sex-inverting agents is present in the testicular tissue of adult Nile tilapia (*O. niloticus*). Adults were chosen because of the difficulties in obtaining a sufficient amount of tissue from differentiating fry. Characterization of a binding site will provide a useful means of screening newly developed sex-inverting agents. Although presence of a binding site is not absolutely indicative of its involvement in steroid-induced sex inversion, it may reflect persistence of the molecule responsible for mediating steroid-induced sex inversion.

Materials and Methods

Animals

Nile tilapia (Ivory Coast Strain) were obtained from Wayne Seim at Oregon State University, Corvallis, OR. Fish were reared in recirculating systems, which included filters for particle separation and ammonia conversion. Temperature was maintained at 24-29°C. Water quality was monitored weekly for ammonia, nitrite, alkalinity, oxygen, carbon dioxide, and pH. Fish were fed to satiation once daily, using either a commercial catfish diet or a commercial trout diet.

Tissue Collection and Processing

Testicular cytosol was prepared in the following manner. Mature male tilapia were killed by an overdose of the anesthetic tricaine methanesulfonate (300 mg/l) buffered with sodium bicarbonate. The testes were dissected into ice-cold homogenization buffer, TEMS (Tris-HCl 10 mM, EDTA 1 mM, Na_2 molybdate 20 mM, α-Monothioglycerol 12 mM, and Glycerol 10% v/v; pH=7.4), weighed, and then homogenized with a motor-driven Teflon homogenizer in 2 x vol TEMS. The homogenate was then centrifuged at 1500 x g for 20 min at 4°C. The pellet was held on ice for extraction of the nuclear fraction. To remove endogenous steroids the supernatant was incubated on ice for 10 min with 0.5 x vol of 5.0% charcoal (w/v) and 0.5% dextran (w/v) in TEMS. The suspension was then centrifuged at 1500 x g for 25 min at 4°C. To isolate the cytosolic fraction from the mitochondrial and endoplasmic reticulum, the supernatant was further centrifuged at 100,000 x g for 60 min at 4°C.

Cytosolic fractions were collected from white muscle, liver, heart, gill, and ovary samples using the same procedure. Ovarian cytosol was prepared using ovaries from immature females.

The pellet for nuclear extraction was washed three times with nuclear wash buffer (Tris-HCl 10 mM, $MgCl_2$ 3 mM, α-Monothioglycerol 2 mM, and Sucrose 250 mM; pH=7.5). The nuclei were then extracted with extraction buffer (Tris-HCl 50 mM, EDTA 1 mM, α-Monothioglycerol 12 mM, KCl 700 mM, and Glycerol 30% v/v; pH=7.5). All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Immediately after preparation, all samples were stored at -80°C.

Binding Assays

Protein concentrations of cytosol and nuclear fractions were determined by the method of Bradford (1976). Prior to dilution with steroid, the protein concentration of cytosol was adjusted to 5 mg/ml. Mibolerone [17α-methyl-^{3}H] (^{3}H-Mb) and radioinert mibolerone (Mb) were obtained from DuPont NEN Research Products (Boston, MA); all other steroids were obtained from Sigma. All steroids were stored in stock solutions of 100% ethanol. Steroids were dried under N_2, and dissolved in TEMS for use in experiments. In all experiments, unless otherwise noted, the final volume of cytosol and steroid solutions was 250 ml. In all cases, total binding was the amount of binding observed in the absence of Mb, and nonspecific binding was determined by addition of 100 fold excess Mb. Total and nonspecific bindings were determined in all binding experiments. Specific binding was calculated as the difference between total and nonspecific binding observations.

Association Parameters

Equilibrium binding conditions were determined by incubating 150 ml of cytosol with 1 nM ^{3}H-Mb for varying amounts of time up to 24 h at 17°C. Observations of both nonspecific and total binding were included in the experiment. At sampling times, the reaction was stopped by placing the incubation tubes on ice. To remove unbound steroid the tubes were incubated on ice with 500 ml 2.5% (w/v) charcoal-0.25% (w/v) dextran in TEMS for 10 min. The tubes were then centrifuged at 1500 x g for 20 min at 4°C. The supernatant was decanted into liquid scintillation cocktail and counted on a liquid scintillation counter (counts were corrected for machine efficiency).

Saturation Analysis

Saturation analysis was employed to determine the binding characteristics of the steroid/binding site interaction. Varying concentrations of ^{3}H-Mb were incubated with 150 ml of cytosol in the presence or absence of 100 x radioinert Mb. The ^{3}H-Mb concentrations varied from 0.05 nM to 6.0 nM. The reaction was halted, unbound steroid removed, and radioactivity determined as previously described. The dissociation constant (K_d) and the maximum number of binding sites (B_{max}) were determined by nonlinear regression using the computer program Inplot 4.0 (Graph Pad Software Inc., San Diego, CA).

Tissue Specificity

To assess the distribution of Mb binding sites, 1 nM ^{3}H-Mb was incubated at 17°C for 12 h with 150 ml of cytosolic extract from white muscle, liver, heart, gill, and ovaries. The reaction was stopped and radioactivity determined as previously described.

Ligand Specificity

Testicular cytosol (150 ml) was incubated for 12 h at 17°C with 1 nM ^{3}H-Mb and 1, 10, 100, or 500 fold excess concentration of competitor. The reaction was halted as previously described. Competitors used were: EE; testosterone (17β-Hydroxyandrost-4-en-3-one; T); 11-ketotestosterone (4-Androsten-17b-ol-3,11-dione; KT); 5α-dihydrotestosterone (5α-androstan-17β-ol-3-one; DHT); 17α-methyltestosterone (MT); 17β-estradiol (17β-estra-1,3,5(10)-triene-3,17-diol; E_2), progesterone (Pregn-4-ene-3,20-dione; P_4); 11β-Hydroxyandrostenedione (4-androsten-11β-ol-3,17-dione; 11OH-An); 17α,20β-dihydroxyprogesterone (4-pregnen-17α,20β-diol-3-one; 17,20-DHP); and Mb. Binding was expressed as the percentage specific binding displaced by 500-fold Mb.

To assess the ability of various masculinizing steroids to displace ^{3}H-Mb binding the following assay was employed. A single concentration (100 nM) of the following masculinizing steroids: ethynyltestosterone (4-androsten-17α-ethynyl-17β-ol-3-one; ET); 17α-methyl-androstan-17α-ol-3-one (MeAna); norethindrone acetate (4-estren-17α-ethynyl-17β-ol-3-one acetate; NEA); fluoxymesterone (4-androsten-9α-fluoro-17α-methyl-11β,17β-diol-3-one; Fx); and 17α-methyl-androstene-3β,17β-diol (MeAne) were incubated with testicular cytosol (150 ml) and 1 nM ^{3}H-Mb. The steroids were incubated with cytosol at 17°C. The incubation was halted after 12 h as previously described. Binding was expressed as the percentage of specific binding displaced by 100-fold Mb.

Nuclear Binding

To determine the ability of ^{3}H-Mb to bind to nuclear extracts of testicular tissue, nuclear extract (150 ml) was incubated for 12 h at 17°C in the presence of varying concentrations of ^{3}H-Mb (.0625-6.0 nM). The reaction was halted and radioactivity determined as previously described.

Results

Association Parameters

At 17°C equilibrium binding was achieved by 4 h and remained stable for up to 16 h (Figure 1). Due to the stability of binding, all subsequent incubations were performed at 17°C for 12 h.

Saturation Analysis

Saturation analysis revealed a typical hyperbolic binding curve (Figure 2). The K_d was determined to be 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 3).

Tissue Specificity

Testicular and ovarian cytosol showed the greatest ability to specifically bind ^{3}H-Mb (Figure 4). Specific binding was also seen in liver and heart cytosol, but it was considerably less than binding observed in the gonadal tissues. Muscle and gill cytosol demonstrated no specific binding.

Ligand Specificity

Various steroids were assessed for their ability to displace ^{3}H-Mb binding (Figure 5A, 5B). The synthetic androgen MT was most effective at displacing bound ^{3}H-Mb. Other compounds displaying strong competitive tendencies were DHT, KT, and EE. Ineffective at displacing Mb binding were P_4, E_2, T, 17,20-DHP, and

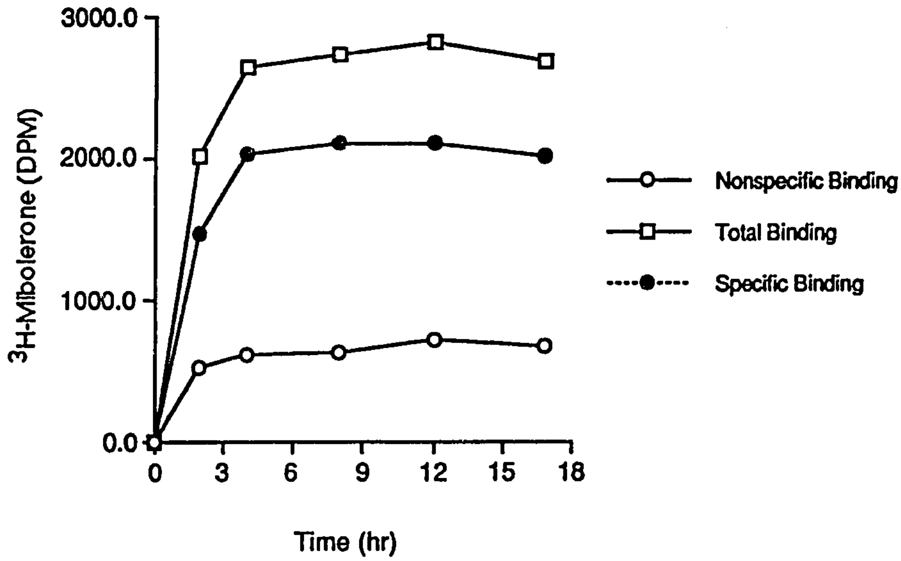

Figure 1. Association through time of ^{3}H-mibolerone and testicular cytosol at 17°C. Each point is the mean of three observations. Specific binding was the difference between total and nonspecific binding.

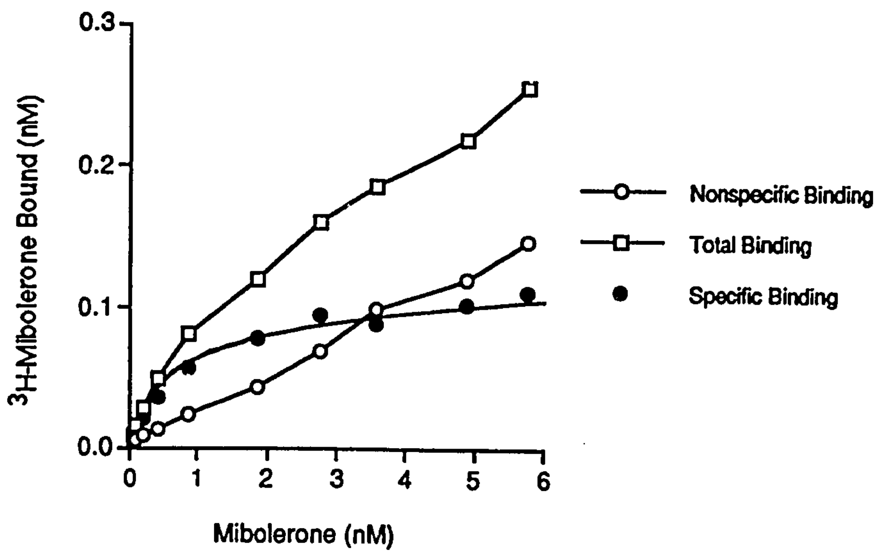

Figure 2. A representative saturation curve for ^{3}H-mibolerone binding in testes. Testicular cytosol was incubated for 12 h at 17°C with a range of ^{3}H-mibolerone concentrations. Total and nonspecific binding data are presented as the mean of three observations. Specific binding was calculated as the difference between total and nonspecific binding. The K_d for this particular assay was 1.1 nM and the B_{max} was 6.25 fmol/mg protein.

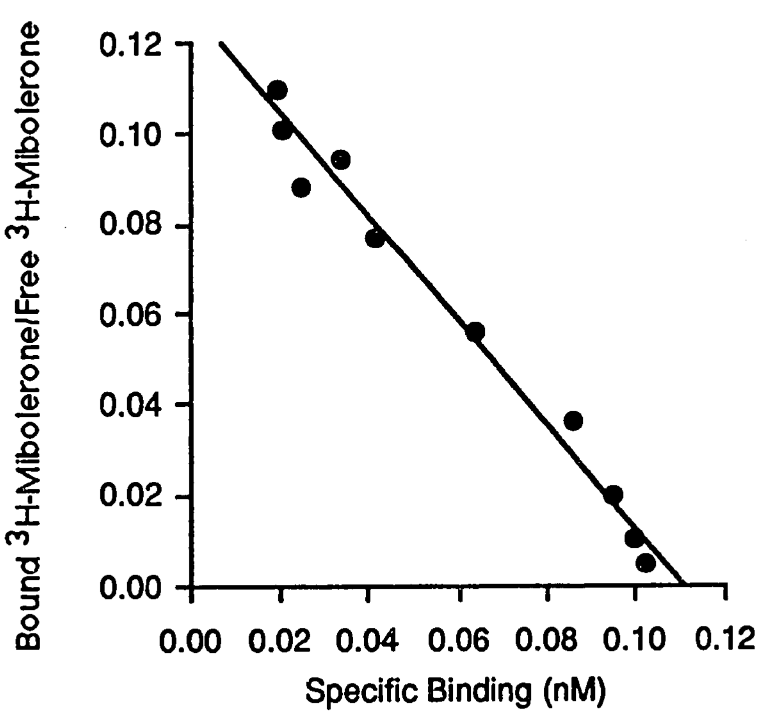

Figure 3. A representative Scatchard plot of transformed specific data from Figure 2.

11-OHAn. Several masculinizing steroids were also assessed for their ability to displace bound ^{3}H-Mb in a single concentration (100-fold excess) assay (Figure 6). The most effective was MeAna and MeAne which displaced about 90% and 70% of the specific ^{3}H-Mb binding, respectively. Norethindrone acetate and Fx displaced about 50% and 40%, respectively.

Nuclear Binding

No specific binding was observed in nuclear extract prepared from testes (data not shown).

Discussion

Our results support the hypothesis that a steroid receptor specific for sex inverting steroids exists in the gonadal cytosol of adult Nile tilapia. Furthermore, the binding characteristics met the established criteria for steroid receptors (Clark and Peck, 1977). Saturation analysis revealed a saturable binding site, which had high affinity for ^{3}H-Mb (K_d = 1.03 ± 0.11 nM) and low capacity (B_{max} = 5.65 ± 0.42 fmol/mg protein). Furthermore, the saturation data revealed that a single class of binding sites was responsible for the observed specific binding. The binding was tissue specific, as more specific ^{3}H-Mb binding was observed in gonads than in other tissues. If steroid-induced sex inversion acts through a steroid receptor, then genetic females should

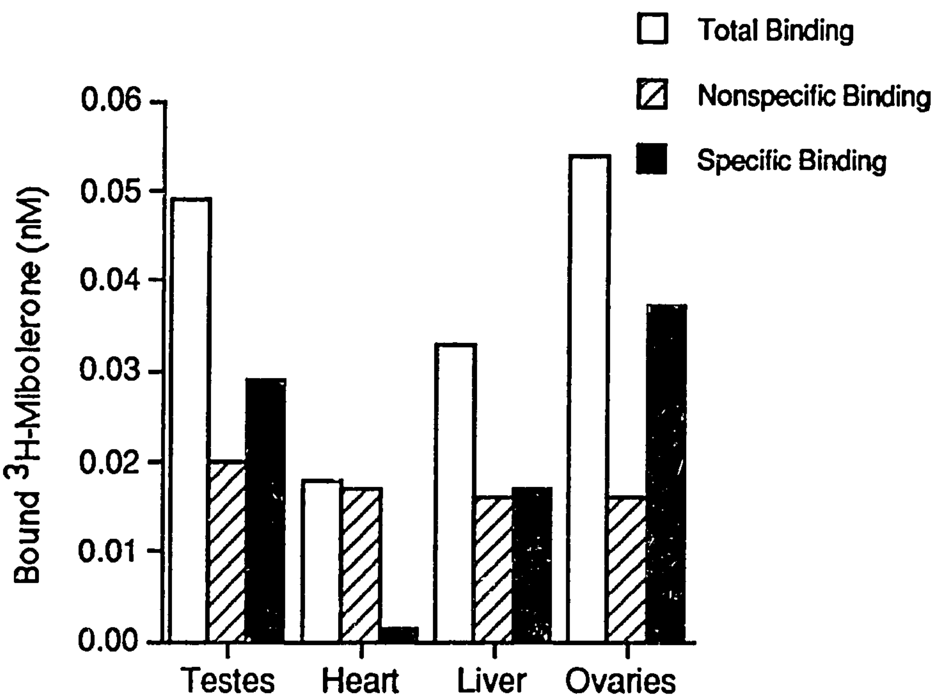

Figure 4. Binding of 1 nM ^{3}H-mibolerone to cytosolic extracts from different tissues. Cytosol was incubated with steroid for 12 h at 17°C. Nonspecific and total binding was calculated as the difference between total and nonspecific binding. Muscle and gill samples were also examined but did not display specific binding and hence are not shown.

possess binding sites for masculinizing agents. Indeed specific binding of ^{3}H-Mb was observed in ovarian cytosol. The lack of binding in the nuclear preparations is unexpected, since the majority of steroid receptors studied in other species have been localized in the nuclear fraction of the cell (Carson-Jurica et al., 1990). The absence of nuclear binding may be due to low protein levels (the highest protein concentration obtained was less than 2 mg ml^{-1}) or ineffective preparation of the nuclear extract.

Androgen receptors have been found in tissues of other fish. Binding sites with a similar K_d to the tilapia ^{3}H-Mb site have been described in the brain of goldfish (*Carrassius auratus*; Pasmanik and Callard, 1988), the skin of brown trout (*Salmo trutta*; Pottinger, 1987, 1988), and in the testes of the dogfish (*Squalus acanthias*; Cuevas and Callard, 1992). The K_d for the site in tilapia is lower than that described for the other androgen receptors, which reflects a site with higher affinity for androgens than previously described in other fish species. The K_d reported for the site in tilapia is for a synthetic steroid, whereas the K_d for the other receptors are for naturally occurring steroids, which may explain the differences in ligand affinity. However, in the other studies synthetic steroids demonstrated a lower affinity for the receptor than the naturally occurring ligands, which is the opposite of the results for tilapia. The differences in affinity between natural and synthetic steroids may explain the greater sex-inverting potency of synthetic steroids. Furthermore, the receptors described in other fishes were shown to bind T with a higher affinity than KT. In

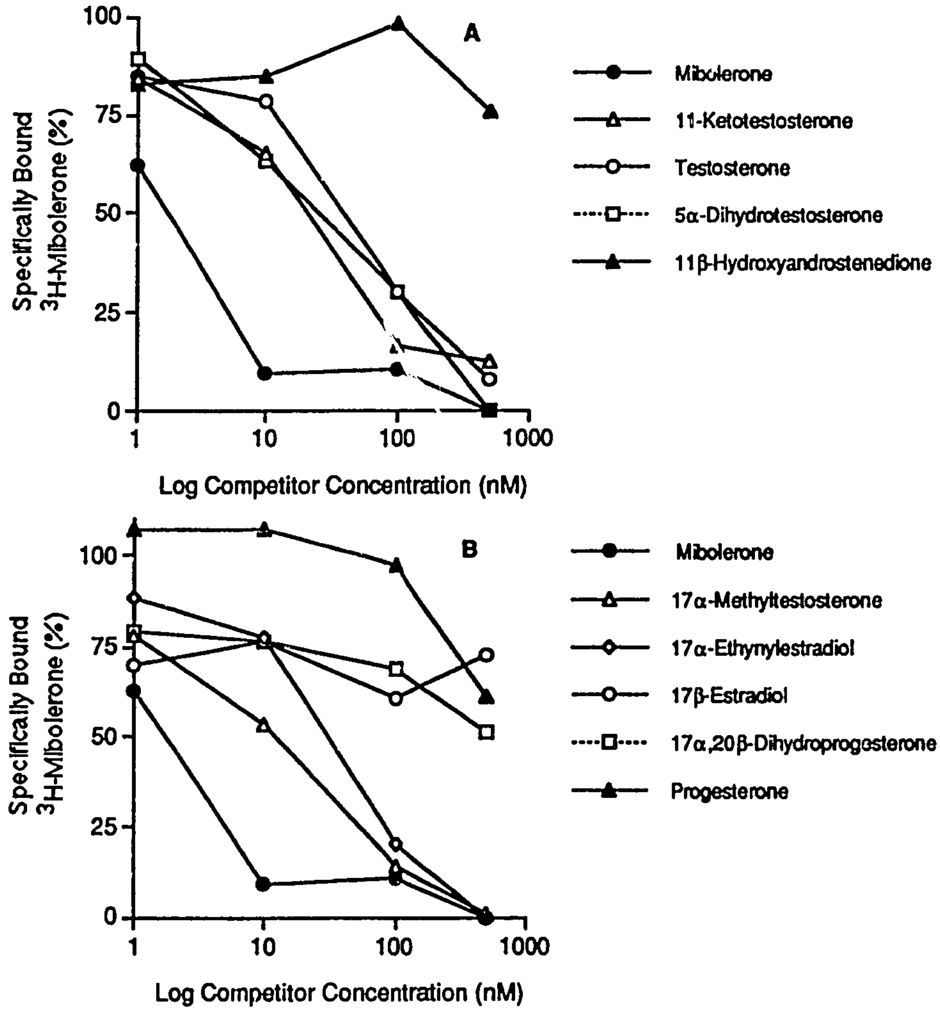

Figure 5. Competitive displacement of 1 nM ^{3}H-mibolerone binding to testicular cytosol by selected steroid competitors. Cytosol was incubated for 12 h at 17°C. Binding was expressed as the percentage of specific binding displaced by 500 x concentration of Mb. Each point is the mean of three observations. Naturally occurring androgen competitors are depicted in (A), synthetic steroid and non-androgen competitors are seen in (B).

tilapia, the mibolerone site exhibited a higher affinity for KT, and little affinity for T. Thus, the mibolerone site in tilapia is markedly different in affinity and specificity from androgen receptors in other fish species.

The binding site exhibited specificity for sex-inverting steroids. Mibolerone masculinized *O. aureus* (Torrans et al., 1988) at such low doses that the authors suggested that Mb may be one of the most potent masculinizing steroids yet tested. The strong masculinizing potency of Mb may explain its strong association with the binding site in comparison with other masculinizing steroids. Of the steroids tested, MT was the most effective competitor of ^{3}H-Mb binding, and has long been used to masculinize undifferentiated fry of many teleosts (Schreck, 1974; Hunter and

Donaldson, 1983). 11-Ketotestosterone also demonstrated an ability to displace ^{3}H-Mb binding. This naturally occurring steroid has been used to masculinize Chinook salmon (*Oncorhynchus tshawytscha;* Piferrer et al., 1993), and when implanted into ovariectomized adult female goldfish, caused the growth of testicular tissue (Kobayashi et al., 1991). 17α-Ethynylestradiol, a synthetic feminizing steroid, was also capable of displacing ^{3}H-Mb binding, which suggests that steroid induced feminization and masculinization may act through a common receptor in differentiating fry; i.e., feminizing agents may bind to the receptor and inhibit male development, or masculinizing agents may act by inhibiting female development. 5α-Dihydrotestosterone, which also competed for Mb binding sites, has been shown to feminize channel and blue catfish (Davis et al., 1992). Paradoxically, in catfish, administration of synthetic androgens such as MT causes feminization. Thus, two scenarios are possible to explain the displacement of binding by DHT. First, it is possible that DHT, acting as a feminizing agent, is binding to the site in a manner similar to EE. Secondly, since very little research has been done using DHT as a sex-inverting agent in species other than catfish, it may be that DHT binding is indicative of its potency as a masculinizing agent in other fish.

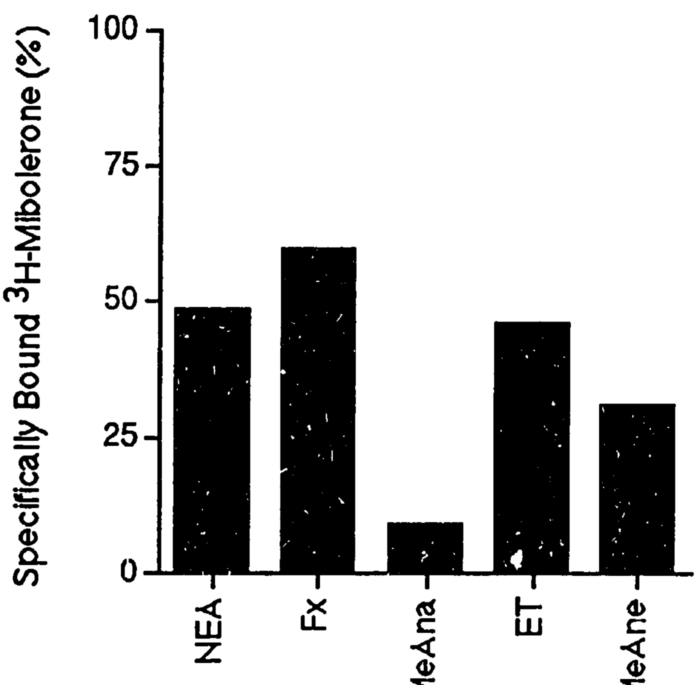

Figure 6. Binding of ^{3}H-mibolerone in the presence of 100 nM competitor. All competitors shown are masculinizing steroids (NEA:norethindrone acetate, Fx:fluoxymesterone, MeAna:17α-methyl-androstan-17β-01-3-one, ET:ethynyltestosterone, MeAne:17α-methyl-androstene-3β,17β-diol). Steroids were incubated with testicular cytosol for 12 h at 17°C. Binding is expressed as the percentage of specific binding when displaced by 100-fold mibolerone.

A binding assay using an excess concentration of masculinizing steroid to displace ^{3}H-Mb binding represents an important tool for the screening of untested sex-inverting agents. Indeed, all of the sex-inverting steroids assayed were capable of displacing at least 40% of specific ^{3}H-Mb binding. The range in percent displacement may correspond to the relative potency of the individual sex-inverting steroids.

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Effects of Form of Defatted Rice Bran Offered on Nile Tilapia Production in Ponds

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Introduction

Agricultural by-products are a major source of supplemental feeds used by cash-poor farmers and in regions without commercially prepared feeds. The yield of tilapia has been shown to increase with the addition of supplemental feed in Rwanda (Ndikumwami et al., 1993). In tropical regions, especially Southeast Asia, rice bran is one of the most common by-products used in carp culture (Jhingran and Pullin, 1985). Recent developments in rice bran processing allow oil to be extracted economically, thus producing a defatted rice bran that is higher in protein and carbohydrate and lower in fat and fiber than full-fat rice bran. One factor affecting the utilization of rice bran by fish may be the form presented. Unconsolidated rice bran is a fine dust, susceptible to dispersal losses by wind and in water. The availability of pelleted defatted rice bran allowed testing of defatted rice bran as a supplemental feed for tilapia as well as the effects of pelleted versus loose rice bran on production and water quality.

Materials and Methods

The study was conducted at the Aquaculture Research Station, UAPB. Mixed-sex Nile tilapia (*Oreochromis niloticus*), with an average individual weight of 9.8 g were stocked at 2.3 fish/m^2 into each of six 0.040-ha earthen ponds on 20 April 1994. Hardness was 130 mg/l and the soil was a fine clay. All ponds were fertilized with inorganic fertilizers on an as-needed basis to maintain a secchi disk visibility of 20 cm by adding 0.45 kg of liquid fertilizer (11-37-0) and 1.5 kg of urea (45-0-0). Later, due to clay turbidity interferences in secchi readings, fertilizer additions were made to maintain chlorophyll *a* levels of 100-150 mg/m^3 (Smith, 1994). After one month fish were sampled to record growth. Then fish were fed for 10 days with a catfish feed supplemented with corn meal at 2% body weight per day. The corn meal diet was designed to alter the carbon isotope ratio in the flesh, so that the influence of natural foods could be assessed by later carbon isotope analysis. Following the corn meal diet, commercial unconsolidated or pelleted defatted rice bran (Riceland Foods Inc.) was fed at 2% body weight daily, divided into a morning and afternoon feeding. During this period, fish were sampled every two weeks to adjust feeding rates. Tilapia were fed unconsolidated rice bran in three ponds and pelleted rice bran in three ponds. Initially, the pellets were crumbled to adjust pellet size to the small fish size. Proximate analyses are presented in Table 1.

Largemouth bass (*Micropterus salmoides*) were obtained from a local hatchery as young-of-the-year fingerlings (3.8 cm, 220/kg) and stocked into the ponds at 0.15 fish/m^2 on 3 June 1994 for tilapia reproduction control, following Wurts et al. (1993).

Table 1. Proximate composition of full-fat rice bran and defatted rice bran as analyzed by Riceland Foods, Inc.

Nutrient	Full-fat	Defatted
Moisture	9.7%	12.9%
Fat	15.8%	2.2%
Protein	13.3%	18.0%
Carbohydrate	39.3%	46.4%
Ash	10.4%	13.1%
Fiber	11.5%	8.1%

Water quality measurements were conducted daily and weekly. Daily measurements were taken at daybreak (0600 hours) and late afternoon (1600-1700 hours) and included water temperatures and dissolved oxygen levels. Weekly measurements were taken at 0800-0900 hours and included secchi disk, corrected chlorophyll *a*, pH, nitrite, and total and unionized ammonia. Ponds were aerated when morning dissolved oxygen levels were below 3 mg/l or afternoon levels were below 5 mg/l to insure survival of the bass.

Ponds were drained and all fish harvested after 159 days.

Paired treatment means were compared by Student's t test. Significance was established at the 0.05 level.

Results

Partial analysis of data will be presented at this time. Carbon isotope determinations are in progress and analyses of water quality differences by treatment are unfinished.

Total tilapia biomass was not significantly different between treatments at harvest (Table 2). The annualized gross and net yields were 6356 and 6128 kg/ha in pellet-fed ponds and 6544 and 6316 kg/ha in loose rice bran-fed ponds. The weight of stocked males averaged 148.6 g at harvest and that of females averaged 52.2 g, with an estimated average survival of 48% in pellet-fed ponds. In the loose bran treatment the weight of stocked males averaged 133.8 g and that of females averaged 46.1 g, with an estimated average survival of 50%. Tilapia reproduction was classed into 40 g, 17 g, and 4 g sizes. In pellet-fed ponds stocked fish and offspring classes comprised an average 52%, 31%, 16%, and 1% by weight, respectively. In loose-bran fed ponds these groups comprised 39%, 40.5%, 20%, and 1%, respectively. Although the mean composition of the treatments was not significantly different, differences between the percentages of stocked fish approached significance (P=0.20). The maximum feeding rate was 46 kg/ha/d. Fish eagerly accepted the two forms of defatted rice bran and were observed at the surface consuming floating unconsolidated rice bran particles, but were not observed at the surface when fed the sinking pellets.

Bass yields were not significantly different. Average net yields and survivals were 154.1 $\pm$ 35.8 kg/ha and 39% in loose-bran fed ponds and 131.6 $\pm$ 51.5 kg/ha and 38.6% in pellet-fed ponds. Average sizes were 236.5 g and 198.0 g, respectively. On an annual basis bass yields were 454 and 387 kg/ha, respectively.

Chlorophyll *a* averaged 114.9 µg/l in the pellet treatment and 87.5 µg/l in the loose rice bran treatment (Table 2). Fertilizer applications averaged 15.3 and 23.0 in the pellet treatment and loose-bran treatment, respectively. This is a significant difference at the 0.05 level. Fish yields were not related to chlorophyll *a* (r=0.62).

Table 2. Tilapia harvest results and mean chlorophyll *a* levels in ponds receiving inorganic fertilizer and two forms of defatted rice bran.

Treatment/ Pond	Gross yield (kg/ha)	Fish yield (kg/ha)	survival (%)	Mean chlorophyll (mg/m^3)	Fertilizer application (No.)
Pelleted rice bran + fertilization					
38	3570	1326	45	142.4	14
41	2823	1853	57	114.5	14
43	2437	1297	42	87.7	18
Mean ±	2943 ±	1492 ±	50 ±	114.9 ±	15.3 ±
S.D.	576	313	25	27.3	2.3
Loose rice bran + fertilization					
37	3421	1082	45	95.0	23
39	3019	1176	56	87.1	22
45	2651	1221	50	80.3	24
Mean ±	3030 ±	1160 ±	50 ±	87.5 ±	23 ±
S.D.	305	71	5	7.3	1.0

Discussion

Supplemental feeding of tilapia with defatted rice bran in ponds receiving inorganic fertilizers resulted in a 45% higher yield than previously obtained at this site with mixed-sex tilapia and inorganic fertilizer only. These yields were higher than those reported for mixed-sex tilapia fed cassava meal in Rwandan ponds receiving inorganic fertilization (Veverica et al., 1994). Similar yields were obtained in fertilized ponds stocked with monosex tilapia in Thailand (Diana et al., 1994). In Honduras, feeding monosex tilapia stocked at 1 fish/m^2 and fed a 25% protein supplemental feed at 1.5% body weight daily resulted in a similar yield. The authors reported an increase in gross yield of approximately 500 kg/ha due to feeding (Teichert-Coddington et al., 1991).

No significant differences in yield and feed conversion can be attributed to the form of defatted rice bran offered. Significantly less fertilizer was required when pellets were fed, however. This is attributed to increased clay turbidity resulting from fish disturbing the sediments in the loose-rice bran treatment. The increased yield of stocked fish in the pellet-fed treatment was not statistically significant, but appears to be biologically and economically significant. This 13% difference, if real, would be economically significant because it would increase income by producing relatively greater quantities of the largest fish.

The addition of a predator was successful in reducing tilapia reproduction, and had the added benefit of producing 160 kg/ha of a marketable, high-value predator species. The young-of-the-year predators grew at a rapid rate and attained a larger size (average 200 g, up to 430 g) than the stocked tilapia. Stocking larger predators may reduce yield from tilapia reproduction by more than 50%. Increasing the stocking rate from 1729 predator fish/ha may aid in further controlling tilapia reproduction. However, the 50% bass survival rate observed in all ponds possibly indicates cannibalism, which would increase as stocking density increases. Bird, reptile, and amphibian predators may also have contributed to the low survival of bass and stocked tilapia.

Anticipated Benefits

This study documents the effects of defatted rice bran on tilapia yield and the effects of the form of bran offered. The advantages of pelleting (to be weighed against cost of pelleting) include a reduced need for fertilizer, easier handling and perhaps a greater yield of stocked fish. Local rice oil production could reduce cooking oil imports and thus the availability of defatted rice bran may increase. Pelleting machines may also increase in availability as animal production modernizes.

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Effect of 17α-Methyltestosterone on the Growth of Two Tilapia Species, *Oreochromis aureus* and *Oreochromis mossambicus*, in Fresh Water

Work Plan 7, Egypt Study 4B1

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Introduction

Most fish species grow better on diets formulated with fishmeal than on those based on a plantmeal (Rumsey, 1993). Most fishmeals are derived from herring and herring-like fishes that are caught while in spawning aggregations. In this condition, testes and ovaries may comprise 30% and 70%, respectively, of the total weight of the fish. Such fishmeals may contribute considerable amounts of anabolic, growth-promoting steroids to commercial feeds (Feist and Schreck, 1990). These integral hormones, as much or more than the quality of nutrients, may account for the increased growth performance of fish fed fishmeal-based diets.

The anabolic steroid 17α-methyltestosterone (MT) augments the growth of a variety of fish, including tilapia (for discussion and citations see Donaldson et al., 1979; Matty, 1986; Ostrowski and Garling, 1987; Ridha and Lone, 1990; Killian and Kohler, 1991). The results of our previous studies (e.g., Kuwaye et at., 1993) show that MT, when administered as a feed additive, enhances growth performance in the tilapia, *Oreochromis mossambicus*. In freshwater-reared animals, MT consistently increases growth by 200-300% of that observed in control fish without measurably affecting proximate body composition (Howerton et al., 1992). The effect of MT is related to dose and extends well beyond the effect of sex reversal. Tilapia treated continuously with MT outgrow sex-reversed fish by 30-40% (Kuwaye et al., 1993). We have found that MT also reduces substantially the heterogeneity of growth among individual tilapia, thereby leading to a more uniformly sized and easily handled product.

Studies both in Egypt and in the United States are extending these findings to other tilapia species. These studies are critical. Before MT can be applied safely in aquaculture, its effects must be evaluated in other tilapia species/strains. Different species do not necessarily respond equally to the same level of hormone (McBride and Fagerlund, 1976; Degani and Dosoretz, 1986; present study). The magnitude of the response to a hormone depends on the dose employed, the species (or even strain) treated and the circumstances of hormone application as well as on the season, temperature, and developmental stage of the animal. These variables and others make it impossible to predict with accuracy the dose-response relationship until the hormone is actually applied. In the present study, we examined the effect of

MT on the blue tilapia, *Oreochromis aureus,* and compared its response to that of *O. mossambicus* under the same experimental treatments in fresh water.

Objectives

The central goal of our work is the development of inexpensive feed supplements that will maximize growth and food utilization efficiency. The objective of this study was to characterize the growth-promoting effects of 17α-methyltestosterone on the blue tilapia, *O. aureus* and to compare its response to that of the tilapia, *O. mossambicus.*

Materials and Methods

Animals

Tilapia, *O. mossambicus,* were obtained from the State of Hawaii Department of Land and Natural Resources, Anuenue Fisheries, Honolulu Hawaii. Blue tilapia, *O. aureus,* were obtained from the United States Fish and Wildlife Service, Southern Fish Cultural Laboratory, Marion, Alabama. Fish were 3 months of age with a mean weight of 1.85 g at the start of the study.

Design

The study was conducted at the Mariculture and Research Training Center, Hawaii Institute of Marine Biology, University of Hawaii. To ensure that the fish of each species were the same size prior to starting, they were weighed and statistically compared by Students "t" test. The initial weights were not significantly different between species (p=.51). Eighteen identical 700-liter tanks were then divided into two groups of nine tanks each. The fish of each species were randomly distributed among nine tanks within a group to yield 45 fish per tank. The treatments 0, 1, 10, and 25 mg of MT/kg of feed were randomly assigned within each group of nine tanks, with the 0 mg MT/kg of feed (control) treatment replicated twice. Water quality was maintained by a water replacement system at approximately 60 liters per hour. Animals were exposed to ambient temperature and photoperiod. The study was conducted over 237-day period from December 8, 1993, to August 2, 1994.

Sample Collection

The fish from each tank were weighed in groups of 4-5 animals each at monthly intervals beginning one month after the start of the study. They were not weighed on day 0; the fish had been weighed the previous day prior to placement in treatment groups. At the end of the study, samples were collected and the gonadosomatic index (GSI= [gonad weight/body wt] x 100) and hepatosomatic index (HSI = [liver weight/ body wt] x 100) were determined. Twenty-four hours after the last feeding of MT, plasma and muscle samples were collected for evaluation of residual MT levels in these tissues. The sex ratios of both species in each treatment group were also determined.

Diet and Hormone Application

Animals were fed commercial Purina trout chow. Feed pellet size ranged from ground feed to 10-mm pellets to accommodate the size of the animals. Fish were fed initially at a rate of 2% of the body weight twice daily (4% total). At weekly intervals over the last quarter of the study, the ration level was altered simultaneously in all tanks between 1 and 2% of body weight twice daily as food consumption varied. MT-treated feed was prepared by diluting 1, 10, or 25 ml of MT stock solution (1 mg of MT/ml of 95% ethanol) to 50 ml with 95% ethanol. Each solution was then sprayed onto 1 kg of the feed. The control diet was prepared by spraying 1 kg of feed with 50 ml of 95% ethanol only. The ethanol was allowed to evaporate from the feed before use.

Statistical Analysis

Weight data were evaluated using a three factorial analysis of variance on species, dose level, and sampling date. Species and dose level were used in a two factorial analysis of variance to evaluate gonad and liver weight and the gonadosomatic and hepatosomatic indices. Mean values were compared using the "Least Significant Difference" test (Steele and Torrie, 1980). Chi square was used to evaluate the ratio of males to females in each treatment group.

Results

Growth Performance

Figure 1A and 1B show the effect of MT on the growth of *O. aureus* and *O. mossambicus*, respectively. The growth rate of *O. aureus* was nearly twice that of *O. mossambicus*. This difference between species was significant ($p<0.01$) by the fourth weighing period. The affect of MT on either species was not significant until the final weighing period. At this time, with the exception of *O. aureus* treated with 1 mg of MT/kg of feed, MT treatment significantly increased ($p<0.01$) growth in both species over their respective control groups. In *O. mossambicus*, growth performance increased with increased levels of MT. By contrast, the 10 and 25 mg of MT/kg of feed treatments stimulated growth equally well in *O. aureus*.

Gonadosomatic Index

Figure 2A illustrates the gonadosomatic index (GSI) measured in *O. aureus* and *O. mossambicus* within each treatment at the end of the study. Significant differences were not found among treatments within each species. The GSI was, however, significantly lower ($p<0.0001$) in *O. aureus* compared with that in *O. mossambicus*. Figure 2B shows absolute gonad weight. The mean values, while somewhat smaller in *O. aureus*, were not significantly different between species. This suggests that the smaller GSI in *O. aureus* was due, for the most part, to the larger somatic mass of *O. aureus* compared with that of *O. mossambicus*.

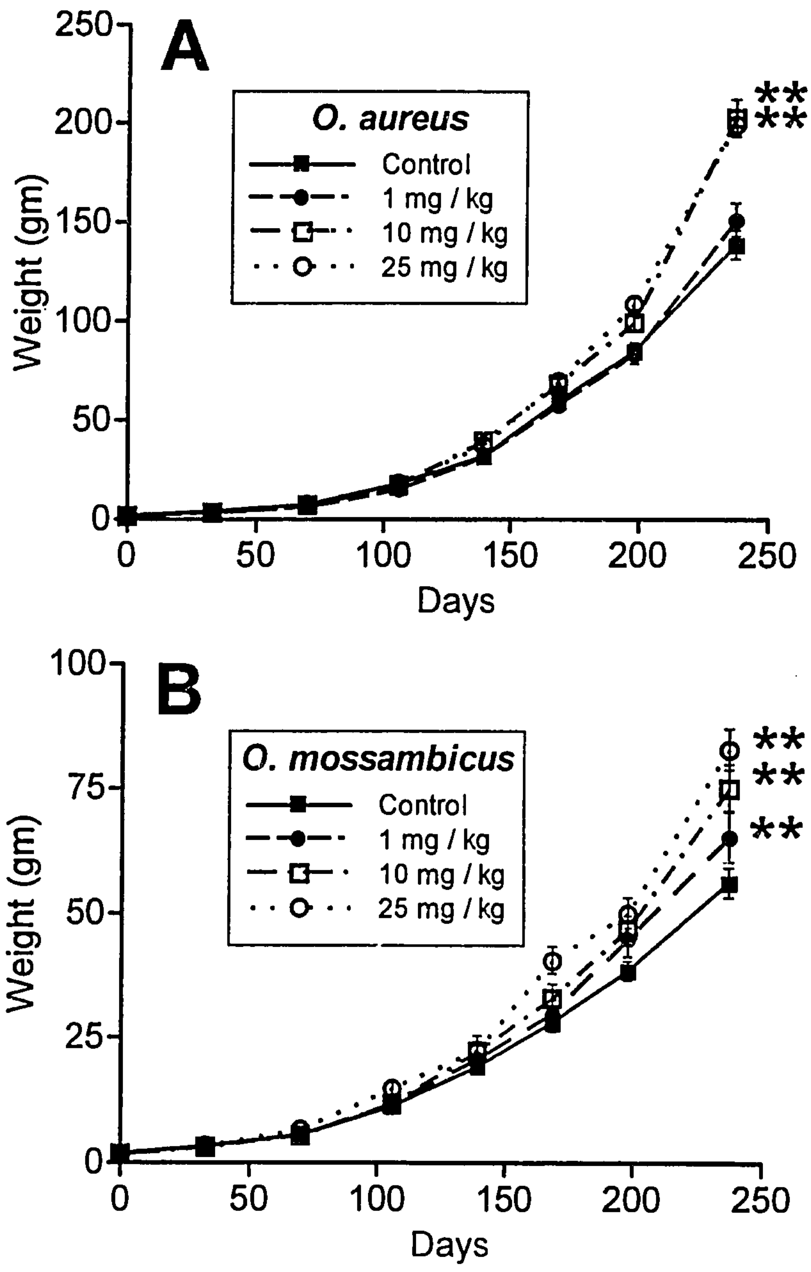


Figure 1. The effect of 0, 1, 10, and 25 mg MT/kg of feed on the growth of *O. mossambicus* (panel A) and *O. aureus* (panel B). With the exception of *O. aureus* at 1 mg MT/kg of feed, MT significantly increased the growth of fish in both species by the end of the study (** $p<0.01$). Although not illustrated, the growth rate of *O. aureus* was significantly greater ($p<0.01$) than that of *O. mossambicus* by the fourth sampling period.

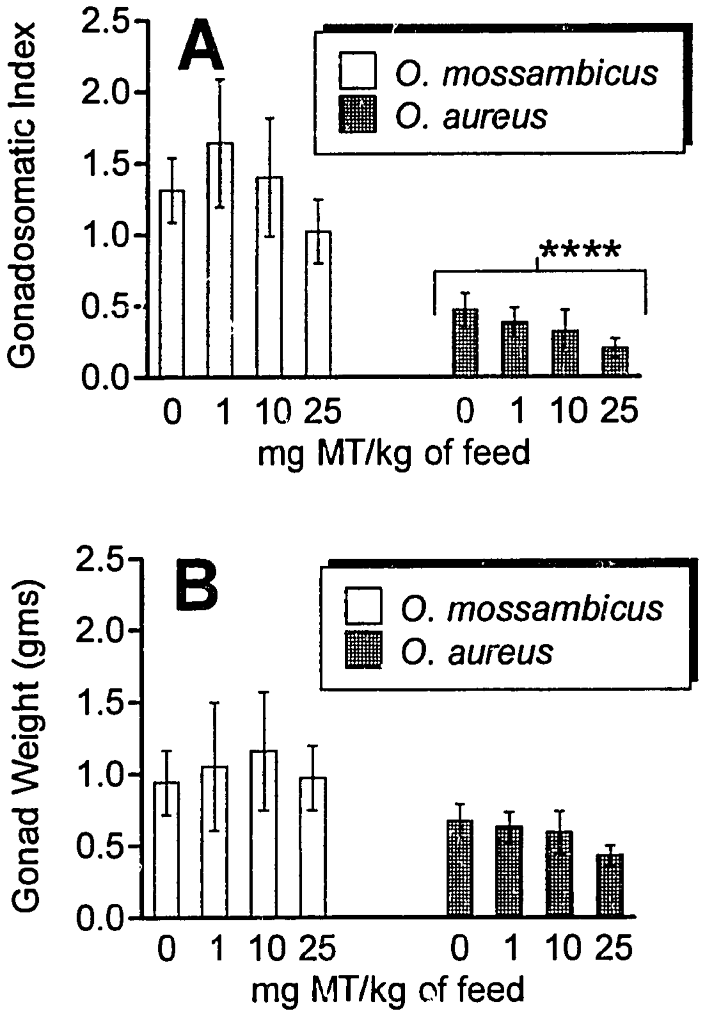
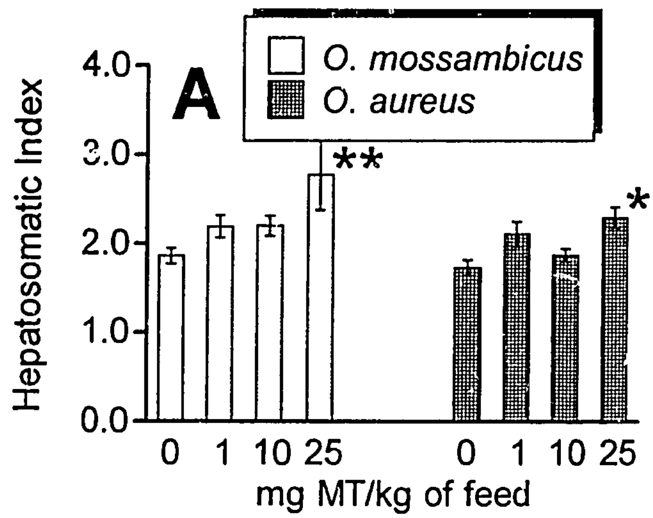


Figure 2. Panel A. The effect of 0, 1, 10, and 25 mg MT/kg of feed on the gonadosomatic indices of *O. mossambicus* and *O. aureus*. Significant differences in the gonadosomatic index between treatment groups within species were not found. The values were, however, significantly lower (****$p<0.0001$) in *O. aureus* than in *O. mossambicus* at all dose levels. Panel B. The effect of 0, 1, 10, and 25 mg MT/kg of feed on absolute gonad weights for *O. mossambicus* and *O. aureus*. The absolute gonad weight was not significantly different among treatments or species, which suggests that the lower gonadosomatic index in *O. aureus* was due to its larger somatic mass.

Hepatosomatic Index

Figure 3A illustrates the hepatosomatic index (HSI) values obtained from each species in each treatment at the end of the study. In both species, the HSI tended to increase with increased levels of MT and was significantly higher in the 25 mg/kg treatment groups than in controls. This same trend is also observed in absolute liver weight (figure 3B).



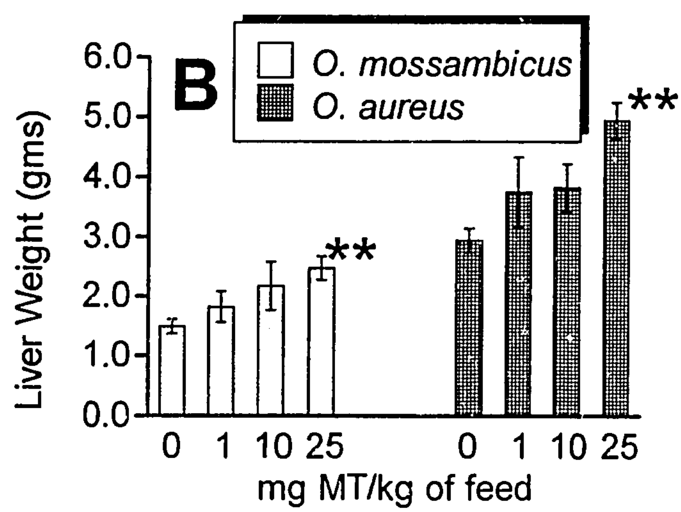


Figure 3. Panel A. The effect of 0, 1, 10, and 25 mg MT/kg of feed on the hepatosomatic indices of *O. mossambicus* and *O. aureus.* Within each species, the hepatosomatic index tended to increase with increased levels of MT and was significantly different from control values at 25 mg MT/kg of feed. Panel B. The effect of 0, 1, 10, and 25 mg MT/kg of feed on the absolute liver weights *O. mossambicus* and *O. aureus.* Like the hepatosomatic index, liver weight within each species tended to increase with increased levels of MT, and was significantly different from the control group at 25 mg MT/kg of feed. (* is $p < 0.05$, ** is $p < 0.01$.)

Sex Ratios

Table 1 shows the number of males and females in each treatment group. The sex ratio in all cases was not significantly different from 1:1. Treatment with MT failed to induce sex reversal because the animals were, at the start to the study, beyond the stage where MT is effective in masculinizing females. Older fish of both species were used because the older *O. aureus* fish were better able to withstand shipment from the Southern Fish Cultural Laboratory in Marion Alabama.

Table 1. The number of male and female tilapia of each species by treatment group.

Treatment	*O. mossambicus* Male	*O. mossambicus* Female	*O. aureus* Male	*O. aureus* Female
0 mg MT/kg feed	64	62	55	59
1 mg MT/kg feed	33	34	24	34
10 mg MT/kg feed	43	42	18	19
25 mg MT/kg feed	43	47	35	40

Residual MT Analysis

Determinations of residual MT levels in the serum and muscle tissue samples are currently ongoing.

Anticipated Benefits

The results of our study clearly show that MT significantly increases the growth rate of *O. aureus* and *O. mossambicus*. The effective dose for *O. aureus* appears to lie between 1 and 10 mg of MT/kg of feed as the response of the animal to MT was similar at 10 and 25 mg of MT/kg of feed. By contrast, the effective dose of MT in *O. mossambicus* is equal to or greater than 25 mg of MT/kg of feed. In addition, our studies show that, in fresh water, *O. aureus* grows faster than *O. mossambicus*. Studies in our laboratory with *O. mossambicus* confirm that its growth performance is significantly reduced in fresh water compared with that of fish grown in seawater (Howerton et al., 1992; Kuwaye et al., 1993).

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Use of 17α-Methyltestosterone for Tilapia Sex Reversal

Work Plan 7, Egypt Study 4A2

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Introduction

For more than a decade tilapia producers world-wide have relied upon the oral administration of 17α-methyltestosterone to newly hatched tilapia fry as the most cost-effective, efficient method of producing all-male populations of fish for grow-out. 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, use of 17α-methyltestosterone for sex reversal of newly-hatched tilapia has not been approved officially by the U.S. Food and Drug Administration (U.S FDA), and, in fact, 17α-methyltestosterone is not approved for use in 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 New Animal Drug approval from the U.S. FDA for 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 INAD was the implementation of a clinical field trial by research institutions and commercial tilapia growers throughout the United States and overseas. In order to further this process, participation by the Egypt Project of the Pond Dynamics/Aquaculture Collaborative Research Support Program scientists in the clinical field trial was planned.

Materials and Methods

Research was conducted at the Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt. Newly hatched tilapia fry of 9 to 11 mm total length (approximately 7 to 12 days old) were stocked into 2-m^3 hapas suspended in 28-m^3 concrete tanks. Number of fry stocked per hapa was estimated by visual comparison of fry samples to a counted standard. *Oreochromis niloticus* or *O. aureus* fry were used. Fry were randomly assigned to the androgen-treated or control feed treatments, which were conducted in separate concrete tanks. Fry were fed a powdered diet that contained 60 mg 17α-methyltestosterone/kg; the daily feed ration was calculated based on average fry total length as specified in the INAD protocol for clinical field trials. 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. 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. A sub-sample of harvested fry were then stocked into 2-m^3 hapas suspended in 28-m^3 concrete tanks for further growth. Once fingerlings attained a size of approximately 5 g, a random sample of 100 fish per treatment were analyzed for sex by the acetocarmine gonadal squash technique.

The first trial was initiated on 10 to 12 July 1994 when *O. niloticus* and *O. aureus* fry were stocked into treatment hapas. A second trial was initiated on 18 to 20 September 1994. Fry availability allowed for stocking of two control and two MT-treatment hapas per species. Fish in Trial I completed treatment on 8 to 11 August; sub-samples of control and treated fry were restocked into hapas for nursery growth. Trial II was in progress, with an expected treatment completion date of 16 to 18 October 1994.

Results

Available data for Trials I and II are presented in Table 1. Sub-samples of fingerlings from Trial I were stocked into hapas for further growth. Unfortunately, the water level in the control tank inadvertently submerged the hapas allowing fish to mix. Fingerlings can be separated according to species, but it is not possible to separate fish according to replicate hapas. Restricted availability of water because of equipment malfunction has been limiting ability to manage water quality effectively in Trial II tanks.

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.

Table 1. Data from Trials I and II where newly hatched Nile (*Oreochromis niloticus*) and blue (*O. aureus*) tilapia were fed a control or androgen-treated ration for 28 days. Methyltestosterone was incorporated into the treated ration at 60 mg/kg. Trial II was in progress at this reporting. Fry were stocked into 2-m^2 hapas suspended in 28-m^2 concrete tanks.

Treatment	Hapa	Initial weight (g/fry)	Initial length (mm/fry)	Numbers tocked	Final weight (g/fry)	Final length (mm/fry)	Number harvested	Survival (%)
				Trial I - Nile Tilapia				
Control	1	0.02	9.6	4,000	0.70	32.1	3,857	96
Control	2	0.02	8.3	5,000	0.64	31.8	1,969	39
MT	1	0.02	9.6	4,000	0.58	30.6	4,241	106
MT	2	0.02	8.3	5,000	0.62	31.9	3,097	62
				Trial I - Blue Tilapia				
Control	1	0.02	9.7	6,000	0.38	24.7	6,421	107
Control	2	0.02	9.6	5,000	0.34	27.0	4,235	85
MT	1	0.02	9.7	6,000	0.36	24.9	5,444	91
MT	2	0.02	9.6	5,000	0.30	30.1	4,533	91
				Trial II - Nile Tilapia				
Control	1	0.02	8.9	7,250				
Control	2	0.02	11.3	2,000				
MT	1	0.02	8.9	7,250				
MT	2	0.02	11.3	2,000				
				Trial II - Blue Tilapia				
Control	1	0.02	8.7	4,800				
Control	2	0.02	8.5	1,500				
MT	1	0.02	8.7	4,800				
MT	2	0.02	8.5	1,500				

Progeny Testing to Identify "YY" Male Tilapia

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Introduction

Sex determination in *Oreochromis niloticus* has been described as an XX female:XY male system in which the presence of a Y chromosome establishes the male sex (Jalabert et al., 1974). Tave (1990) outlined a breeding program to obtain offspring of a "YY" genotype sex reversing a genotypic male to a phenotypic female and crossing that female with a normal male. Fish of the resulting YY genotype would give only Y sperm and produce only male offspring. Currently, there are no known readily apparent genetic markers to identify possible XY females or YY males. Rather, the presence of these genotypes is inferred from the sex ratios of their progeny. An XY female crossed with an XY male should produce a 3:1 male:female sex ratio; a YY male should produce 100% male offspring. This report describes the results of efforts to identify YY males and to determine the male:female ratios of their progeny during fiscal year (FY) 1994.

Objective

Identify male tilapia *O. niloticus* which may produce offspring having a sex ratio highly skewed towards males.

Materials and Methods

Nine populations of tilapia having skewed sex ratios were obtained by mating a sex-reversed female with a normal male. A minimum of ten males from each population were then mated with normal XX females. Each potential YY male was stocked with one or more females into a 2-m^3 hapa suspended in an outdoor concrete tank. Each hapa was examined every 14 days for fry. Fry produced by each female were removed and reared separately in a hapa or concrete tank until the fish reached a minimum length of 5 cm. One hundred fish of each spawn were killed and preserved and the sex was determined by removing the gonad and examining it microscopically. In FY 94 a total of 51 spawns were obtained. The sex ratios of 32 spawns produced in FY 93 and of 17 spawns produced in FY 94 have been studied this year.

Two males that produced > 95% male progeny in FY 93 were mated with normal females in FY 94 to determine if they would consistently produce predominantly male progeny.

Results and Discussion

Nine populations of potential YY males were established from pair matings of non-hormone-treated males with estrogen-treated females. It was hoped that such mating would result in sex ratios of 25% XX female:50% XY male:25% YY male. Three of the nine populations established had sex ratios differing from 1:1, i.e., having approximately 75% males. Males from the first of these three populations were mated with females and 13 spawns were obtained. Only two of these males produced 100% male offspring; however, in the second population, only one of nine males produced all-male offspring. None of the eight males selected from the third population produced all-male offspring. A fourth male; one of five from a parent population with a sex ratio of 63% males:37% females, also produced all-male offspring.

Of the four brood males that gave > 95% male offspring, two were spawned again with other females. Three spawns produced by the first male consisted of 51, 51, and 55% males; the other male produced 60, 83, and 100% male offspring.

The lower-than-expected frequencies of males producing all-male progeny and the variability in progeny sex ratios from males which gave all-male progeny in the first mating, suggest that inheritance is not strictly a male-determined characteristic, but may be influenced by the female and/or by environmental factors. The lack of a simple XX:XY sex inheritance increases the difficulty in breeding a form of *Oreochromis niloticus* that will consistently produce male progeny.

The Ivory Coast strain of tilapia used in this study is a highly inbred line. Abdelhamid (1988) compared the Auburn Ivory Coast strain of *O. niloticus* to six others and found 13.2% of the 38 loci to be polymorphic, whereas in the other strains more than 26% of the loci were polymorphic. He found a mean heterozygosity of 0.018 for the Ivory Coast strain, whereas the others ranged from 0.037 to 0.069. Tave and Smitherman (1980) established a predicted heritability of 0.04 ±0.14 for the Auburn Ivory Coast strain. Teichert-Coddington and Smitherman (1988) found the realized heritability to be -0.1 ±0.02 for this strain. Inbreeding should have reduced any variability in sex ratio contributed by either female or male.

The matings in this study were one in a fertile outdoor environment with diurnal fluctuations in temperature, pH, and dissolved oxygen. The variations in these parameters were within the ranges normally associated with tilapia culture and were of similar magnitude throughout the breeding season. No inference can be made as to the effect of environment on the sex ratios found in this study.

The results to date are preliminary and additional sets of progeny from the nine brood populations and the four possible YY males have yet to be examined.

Anticipated Benefits

The development of a YY tilapia breeding program has the potential benefit of producing male tilapia from consumption that have not been treated with androgenic hormones. The results of the current study suggest that the development of a YY tilapia breeding program will be more difficult than originally anticipated.

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Bioconversion of Gastropods by Black Carp in Egyptian Fish Culture Ponds

Work Plan 7, Egypt Study 2B

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Introduction

The bioconversion studies focus on transformation of underutilized resources in Egyptian fish culture ponds while simultaneously resolving or reducing a management problem. Snails are abundant in Egyptian aquatic systems and several species are significantly related to human health problems. The cause of at least three serious medical conditions have snails as intermediate hosts. This study aims to evaluate the black carp's potential to reduce or irradicate snails and to become a component of a polyculture system. Snails are the main food of black carp; conversion ratios range from as high as 40 to 75 (Hickling, 1971; Wu, 1985). Abundant plants may provide food and shelter to snails (Covich and Knezevic, 1978). Thus, full consideration of potential polyculture systems must include combined stocking of grass carp and black carp. However, this will be examined in a separate study; the present experiment focuses on the effects of black carp on snail population under different levels of plant infestation.

Materials and Methods

Twelve 4000 m^2 ponds were prepared for the black carp studies in March and April 1993. Aquatic plants were cut by hand to near the mud line prior to refilling the ponds. Vegetation coverage was visually estimated. Fish were held in quarantine until inspected for disease and parasites, and checked for residual radiation levels. These samples and the mortality which occurred during holding reduced numbers available for the study. The originally planned stocking rates were modified based on remaining stocks. Table 1 lists the rates and average sizes of black carp stocked in

June 1993. Fish growth samples were not taken during the growing season because the late stocking of grass carp and plant regrowth hampered seining operations.

Several methods of sampling snails have been attempted. Quadrant-substrate, trapping, and area-effort sampling have been tried. The latter method came closest to producing a good estimate of abundance. Snails were identified to genus and measured, and vector species were examined for cercariae. Food habit data for black carp were collected from a pond population separate from the primary study ponds. Food items were identified and counted when possible.

Table 1. Data for black carp stocked in June in 12 Egyptian fish ponds with different levels of aquatic plant coverage.

	Low stocking		High stocking	
Vegetation coverage	Rate (#/ha)	Average (g)	Rate (3/ha)	Average (g)
Low* (<20%)	65	40	229	47
	115	44	229	47
	115	44	–	–
Medium (20-60%)	115	41	229	41
	115	49	229	43
High (>60%)	115	46	229	42
	–	–	229	41

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

Results

Growth was variable between ponds, not as a result of differential stocking rates, but apparently because of contamination by other species. Wild fish entered all ponds, but black carp growth was lowest in ponds which were contaminated by common carp and/or mullet (Figure 1). These data are being more thoroughly analyzed. Snail sampling was done by quadrant picking in freshly drained ponds, but few snails were found, even in the control ponds.

Black carp examined for food habits were clearly eating snails, even though snail sampling revealed a low abundance of snails. Snails are crushed during feeding and thus are not easily identified; however, all three of the species that are disease vectors have been found in stomachs. Some fish contained large numbers of snails; one fish of 177 mm TL had at least 60 recognizable snails and one carp of 220 mm had parts of over 100 individual snails.

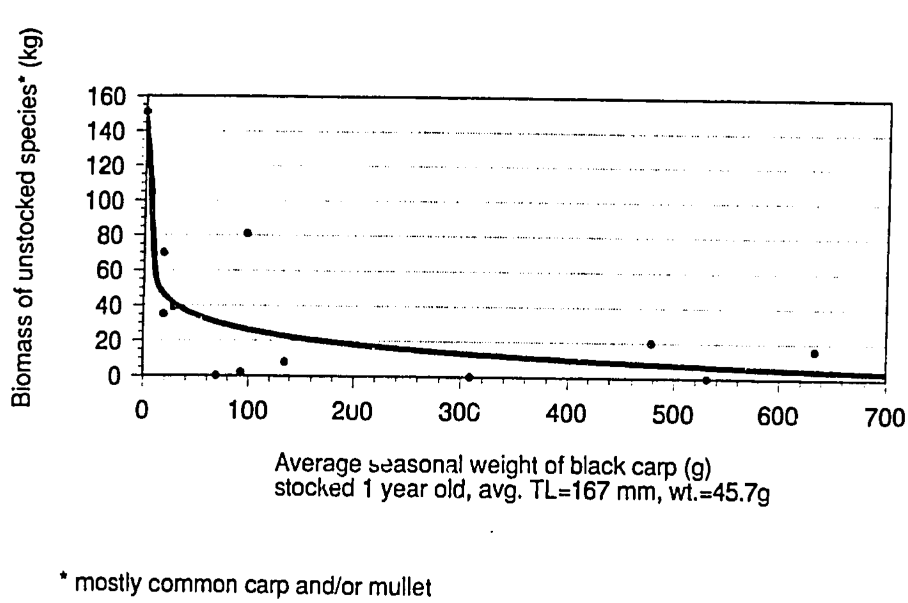

Figure 1. Average seasonal weight of black carp in relationshp to the biomass of unstocked species.

Anticipated Benefits

Draining and harvest provided data on growth of the fish, but was confounded by the presence of unstocked competing species. Snail population levels were not well quantified, but black carp are clearly targeting this food source. The tremendous rate of consumption described and documented for the black carp should have a major impact on the snail populations. The potential for disease reduction must await further evaluation.

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Bioconversion of Nuisance Aquatic Plants by Grass Carp in Egyptian Fish Culture Ponds

Work Plan 7, Egypt Study 2A

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Introduction

A series of studies has been initiated involving bioconversion of underutilized resources which also relate to a management problem. These bioconversion units will be integrated as components of a polyculture system. Grass carp, *Ctenopharyngodon idella*, will not only be one of the primary building blocks for this polyculture system, but will also reduce dependence on mechanical plant control, thus transforming a management problem into a fish production resource. Initial mechanical control of the plants will facilitate plant utilization by grass carp because most of the nuisance plants in this system are fibrous. The grass carp should be able to readily eat the tender regrowth and maintain clean ponds, as reasonable control has been reported for these plants even without mechanical pre-treatment (ElGharably and Khattab, 1982; Michewicz et al., 1972; Opuszynski, 1972; Van Zon, 1977; Zonneveld and Van Zon, 1985).

Materials ands Methods

Twelve 4000-m^2 ponds were prepared in March and April 1993. Macrophytes, primarily *Typha* and/or *Phragmites*, were hand cut to near the soil surface. The area of plant development was visually estimated by predominant species. Vegetation coverage was classified as low (<20%), medium (20-60%), or high (>60%). Then ponds were refilled. Stocking was originally planned for 500-g grass carp. However, modified stocking was necessary after delays in obtaining stocks and because of variable size (Table 1). The modified work plan established low and high (approximately 280 and 600 kg/ha, respectively) stocking rates across three general levels of plant coverage.

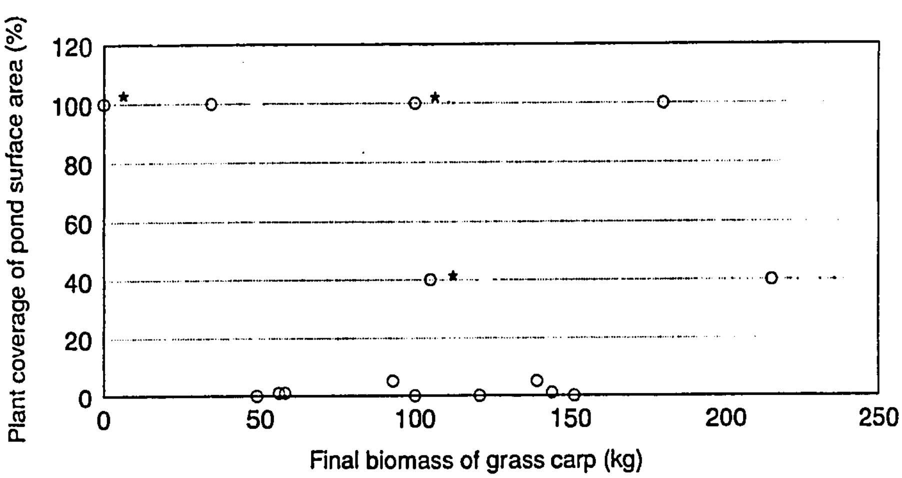
Table 1. Data for gras carp stocked in June in 12 Egyptian ponds with various levels of aquatic plant coverage.

	Low Stocking			High stocking		
Vegetation coverage	Rate (#/ha)	Biomass (kg/ha)	Average (g)	Rate (#/ha)	Biomass (kg/ha)	Average (g)
Low* (<20%)	286	57	200	608	319	500
	310	115	400	649	387	600
Medium (20-60%)	286	165	580	597	425	700
	286	207	700	916	401	400
High (>60%)	286	172	600	586	398	700
	286	286	1000	859	431	500

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

Results

Stocking was not completed until late June. Biomass and average size were more variable than proposed (Table 1), but limited fish availability made these changes necessary. Regrowth of vegetation in most ponds during the period prior to stocking interfered with planned sampling of fish by seine. Harvest was deferred until December 1993 to provide at least a 6-month growing season. Data are still being analyzed, but preliminary plant control data are depicted in Figure 1. Grass carp were effective in maintaining ponds virtually free of emergent vegetative regrowth. However, the delayed stocking permitted the development of noxious plant populations in excess of what the grass carp could control in some ponds. The problem plants were *Azolla* (floating) and *Ceratophyllum* (submergent), which had greater than 40% coverage in several ponds.



* Ponds that were stocked late in the season

o Individual pond

Figure 1. Grass carp biomass in relationship to plant coverage on pond surface area.

Anticipated Benefits

Water temperature usually does not cool below 10°C in the study area, which permits active feeding and year-round growth of grass carp throughout most of the year (Buckley, 1981; Prowse, 1971; Shireman and Smith, 1983). The demonstration of effective plant control and the bioconversion of the plant "resource" will provide a basis for using the grass carp as a polyculture component thus grass carp will simultaneously be a management tool and an additional crop.

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Interaction of Grass Carp and Black Carp in Egyptian Fish Culture

Work Plan 7, Egypt Study 2C1

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Introduction

Bioconversion of plants by grass carp and of snails by black carp can be considered as separate components in a polyculture system, but they are also interrelated because the plants provide food and cover to snails and thus affect black carp predation. Gastropod production might diminish by virtue of grass carp feeding on plants, but it is unclear to what extent the predation of black carp on snails is influenced by grass carp feeding. Abundant vegetation could provide protection for snails, so that their biomass might increase 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 in polyculture ponds.

Materials and Methods

Ponds were prepared in March and April 1993 for this study; vegetation was cut near the soil surface and the ponds refilled. Delays in obtaining fish stocks resulted in a late stocking date. Sufficient stocks were available to develop only some of the planned combinations. Only four 4000-m^2 ponds were stocked (Table 1).

Results

Ponds were drained and harvested in December 1993. Ponds were cleaned and restocked immediately, according to the work plan for year two, which reduced the problems with late stocking, plant regrowth, and species contamination encountered previously. Data are being analyzed.

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 1	Grass carp 2	Black carp 1	Black carp 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/Phragmites*; some ponds had *Potamogeton/Ceratophyllum*, and a few were dominated by *Azolla*. A control pond was established at each level of vegetation.

Anticipated Benefits

The delayed stocking lowered effectiveness of grass carp control of some plants, and may have had an impact on snail populations. Nevertheless, the data from this year will provide some preliminary information on possible grass carp/black carp synergism.

Estuarine Water Quality and Sustainable Shrimp Culture in Honduras

Work Plan 7, Honduras Study 1

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Introduction

Shrimp sales from 11,000 ha of shrimp ponds in Honduras generated about $78,000,000 in 1993. It is estimated that 0.3 to 0.5 persons are directly employed for every hectare cultivated and that up to three persons are employed per ha when all supporting industries are included. Shrimp farming therefore has great impact on the country's economy. A total of 15,000 to 18,000 ha of additional land may be developed into shrimp farms in Honduras.

There has been much interest in Honduras and the rest of Latin America in the related effects of shrimp farm effluents, estuarine water quality, reduced shrimp growth and survival rates. These concerns have been fueled by accounts of industry difficulties due to water quality problems in the eastern hemisphere (Phillips et al., 1993; Rosenberry, 1994). Rosenberry (1994) attributed a 16% drop in world shrimp production between 1992 and 1993 to environmental problems. Most problems occur where intensively managed shrimp farms are operated in congested estuarine and bay areas that are also polluted by municipal wastes. However, large scale production losses have also been experienced in Ecuador, South America, where extensive and semi-intensive farming techniques are employed. The "sea gull syndrome" in the Guayas River estuary during 1989-1990 was attributed to higher than normal salinities and nutrient buildup in the river because of abnormally low river flows (Lightner, 1994). In 1992-1993, farms in the Guayas River delta began experiencing high mortalities of juvenile shrimp. A causative agent has not been confirmed, but a pesticide residue unrelated to shrimp farming is suspected (Lightner, 1994). As of 1993, Honduras had not experienced environmental problems, but shrimp producers were concerned about auto-pollution of the more heavily developed estuaries.

There were further concerns in Honduras that shrimp farm discharge could eventually pollute the Gulf of Fonseca, which is shared by Honduras, Nicaragua, and El Salvador. El Salvador has limited land available for shrimp farms in the Gulf, but Nicaragua is contemplating the use of approximately 25,000 ha of land for shrimp farm development. Most wastes would be discharged into the Gulf, should the development potential be realized. Potential problems confronting Honduras are therefore common to Nicaragua, and both countries could contribute to eutrophication of the Gulf of Fonseca.

Shrimp farm discharge, particularly from intensively managed farms, has been characterized in Hawaii (Ziemann et al., 1992) and to certain extents in other locations (Lin et al., 1993; Hopkins et al., 1993a; Hopkins et al., 1993b). Connections between farm effluents and environmental concerns have been drawn (Boyd and Musig, 1992; Pruder, 1992; Hopkins et al., in press). But there are no studies that characterize the receiving waters of shrimp farm discharge or correlate water quality of these waters to farming activity.

Sustainment of the shrimp industry requires regulation, which is impossible without a scientific basis. Characterization and monitoring of receiving waters are essential in order to correlate water quality changes to shrimp farm development. Differentiation between pollutants entering the estuaries from the rivers and those originating from the farms is desirable. Knowledge of the assimilative capacities of estuaries for farm wastes is essential to estimate the upper limits of farm development.

A research project was established in southern Honduras in 1993 to collect the water quality and hydrographic data necessary to estimate assimilative capacities and to investigate management techniques that would minimize environmental impact from farm discharge. The project is a collaborative effort of producers, a university, a trade organization, and the government of Honduras. This study reports on the water quality of the major estuaries in relation to estuary type, season, and farm location.

Materials and Methods

Study Area

The shrimp producing area of southern Honduras is located on the periphery of the Gulf of Fonseca in the Department of Choluteca at about 13°N latitude (Figure 1). Farms occupy estuaries dominated by rivers (riverine) or the Gulf (gulf embayments). All estuaries are fringed with dense stands of mangroves. Gulf embayments are branches of the Gulf of Fonseca, and do not receive direct outflow of rivers. Riverine estuaries are directly influenced by major rivers and comprise the majority of the shrimp farming area. La Jagua and El Pedregal estuaries receive the Choluteca River while the Estero Sn. Bernardo comprises the mouth of the Negro River. The rainy and dry seasons are very distinct in Honduras, so discharge volumes of rivers vary accordingly (Figure 2). The hydrologic year begins in May. During particularly prolonged dry seasons, discharge of the Negro River becomes negligible, while that of the Choluteca might be less than 2 m^3/s. Daily discharge of the Choluteca can exceed 1500 m^3/s, although mean annual flow is about 45 m^3/s.

Farmers generally stock juvenile shrimp at rates ranging from 5 to $10/m^2$. Stocking densities are generally lower during the dry season than in the wet season. Shrimp seed is acquired from both local wild catch and laboratories. Laboratory animals are

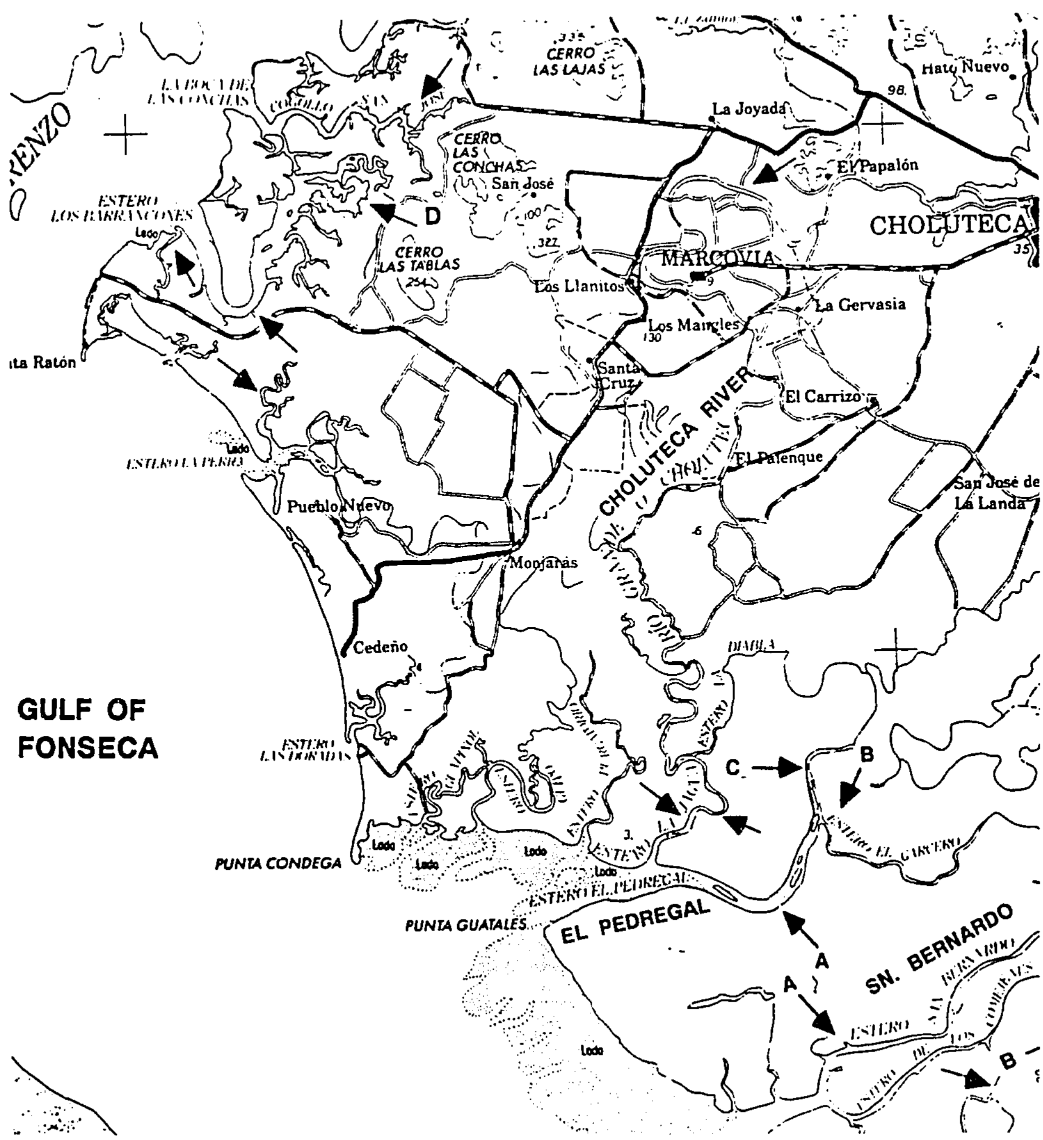


Figure 1. Map of the study area located in southern Honduras and bordering the Gulf of Fonseca. Arrows show approximate sampling points. Letters indicate locations of farms mentioned in the text.

Penaeus vannamei while wild catch is a mixture of *P. vannamei* and *P. stylirostris*, in proportions that vary with season. Culture periods generally range from 12 to 16 weeks, although shorter or longer periods are not uncommon, depending on shrimp growth. Shrimp yields vary greatly within a year and between years (Teichert-Coddington et al., in press). Seasonal affects are particularly large. Shrimp may not reach 10 g during the dry season, but may exceed 20 g during the wet season (Teichert-Coddington and Rodriguez, in review). Shrimp are fed a pelleted diet usually formulated to contain between 20 and 28% protein. Some farmers reduce protein as the shrimp increase in size, while others do the opposite. Most feed a fixed protein diet at standard rates that decrease with shrimp size. Organic fertilization is usually not used because of the adverse impact that bad press might have on

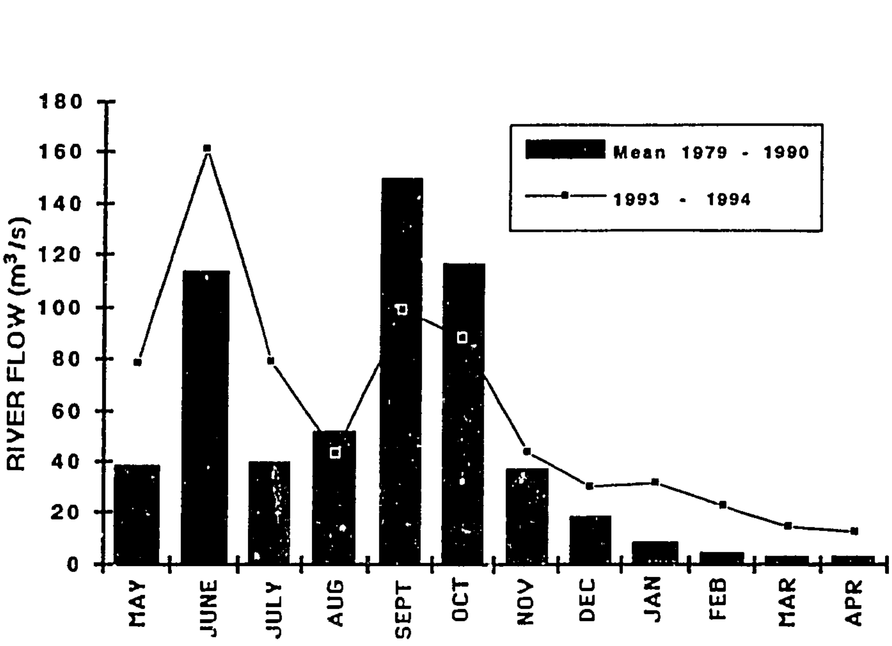


Figure 2. Mean monthly flow of the Choluteca River during 1979–1990, and river flow during the hydrologic year of 1993.

marketing. Inorganic fertilization is used occasionally, but rates are not standardized. Pond bottom liming is used occasionally, although it is unnecessary because acidic soils are rare if not non-existent. Water exchange usually ranges from 3 to 10%. It is generally perceived that more is better, but actual rates are usually limited by pumping capacity. Some farms exchange water based on water quality parameters, while others exchange at a set daily rate.

Sampling

Water quality was monitored weekly at 13 sites in 6 estuaries of the Gulf of Fonseca in Honduras (Figure 1). Arrows in Figure 1 indicate location of sampling sites. Data reported on in this study were collected between April, 1993 and July, 1994. Water samples were taken from pump intakes of shrimp farms during high tide. This sample represented a mixture of depth strata as indicated by a superficial vortex caused by the 60- to 90-cm diameter pump intakes. Samples were put on ice and transported to the laboratory where analyses commenced within 6 hours of sampling. The Choluteca River was sampled weekly at a site located downstream from the city of Choluteca and upstream from tidal influence. This sample was used as a basis for calculating discharge of river-borne nutrients. River flow data were collected at the Choluteca River bridge in the city of Choluteca by the Department of Hydrology and Climatology, Ministry of Natural Resources, Government of Honduras.

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

Data were grouped by estuary and season. Data from most of the farms on an estuary were included in the grouping. Some farms did not contribute samples regularly for analysis, or started to bring samples late in the season, and these farms were not included in analyses. Seasonal groupings corresponded mostly to river flow, which was directly monitored by river stage readings (Figure 2), and qualitatively by estuarine salinities. Salinity is a conservative measurement and can be used to monitor freshwater inflow and dilution of saline estuarine water. The 1993 wet season occurred between May and November, but in 1994 it was limited to May and June. The 1993 dry season started in December and ended in April in the next year, but in 1994 only the month of July was dry.

The relationship between farm location and water quality along a longitudinal transect was studied in the Pedregal and Sn. Bernardo estuaries (Figure 1). Lettered arrows in Figure 1 indicate farms involved in the longitundinal sampling. In the Pedregal, Farm A was located about 9 km from the confluence of the Choluteca River and the Gulf of Fonseca; Farm B was located about 7.5 km upstream from Farm A, and Farm C was located another 4.5 km upstream from Farm B. The Pedregal terminated several more km upstream from Farm C. In the Sn. Bernardo, Farm A was about 10 km from the Gulf and Farm B was about 24 km upstream from Farm A. Additional farms were located in the upper reaches of the Sn. Bernardo, but insufficient data were collected to allow for their inclusion in this analysis.

A rough estimate of the exchange rate of estuarine water at different farm locations within the Gulf was made by calculating the dilution rate of salinity during time periods when freshwater input was presumed to be minimal. Evaporation was not taken into account. Salinities measured on a large estuary with good access to the Gulf (Los Barrancones) were used as the reference for the Gulf. The equation used to make the calculations was:

$$C_3 = [(1-X)(C_1)] + [(C_2)X],$$

where C_1 = initial salinity,
C_2 = reference salinity,
C_3 = final salinity, and
X = proportion exchanged.

Data were analyzed using software by Haycock et al. (1992). Box plots were used to display the median, upper and lower quartile (75 and 25 percentile), and upper and lower extremes (90 and 10 percentile). Significant differences among data sets were determined by comparisons of 95%-confidence intervals around the median.

Results

Estuaries

Gulf embayments were pristine compared with riverine estuaries and the Choluteca River. Except for an occasional short-lived plankton bloom in small inland dendritions of the Gulf, little organic matter was encountered, and inorganic nitrogen and phosphorus concentrations were minimal (Figure 3; Table 1). By comparison, riverine estuaries were several times more fertile in organic nutrients, up to an order of magnitude more concentrated in dissolved inorganic nutrients (Figure 3; Table 1), and turbid with river-borne sediments. Mean total settleable solids of 0.9 and 1.2 ml/l were encountered in Sn. Bernardo and Pedregal estuaries, respectively.

Organic nitrogen, organic phosphorus, and chlorophyll *a* were significantly higher in the Pedregal than in the Sn. Bernardo. Freshwater input at the head of the Sn. Bernardo contributes to estuarine cleansing during the wet season. The Pedregal is not directly flushed by a river; the Choluteca River discharges into the mouth of the Pedregal near its confluence with the Gulf. However, rainfall runoff floods the lowlands bordering the upper reaches of the Pedregal, thereby contributing significant water inflow to the Pedregal.

The Choluteca River was higher in dissolved inorganic phosphorus and nitrogen but lower in organic nutrients than Pedregal or Sn. Bernardo estuaries. Wide box plots demonstrated high variability in dissolved nutrient concentrations. DIN ranged from 0 to 1.5 mg/l and filterable phosphate ranged from 0.07 to 0.43 mg/l. Dissolved nutrients spikes were recorded during the dry season, when river flow was low, and upon commencement of rainfall, because of agricultural runoff. High chlorophyll *a* concentrations were only encountered during the dry season.

The organic form of nitrogen was predominant in all water bodies (Figure 4; Table 2). Proportions of total nitrogen as DIN were greatest in the river (35%) and smallest in gulf embayments (6%). DIN comprised 19 and 13% of total nitrogen in Sn. Bernardo and Pedregal estuaries, respectively. Nitrates comprised the majority of DIN in the Choluteca River (87%) and Sn. Bernardo (59%), but was relatively less in the Pedregal (43%). Nitrites were low in all water bodies. Relatively high transitory nitrite spikes (0.12 to 0.28 mg/l) were recorded occasionally in the upper reaches of Sn. Bernardo and Pedregal estuaries during high tides when anoxic bottom sediments were disturbed. Total ammonia nitrogen (TAN), the first product of protein decomposition by bacteria, was generally low, never approaching levels dangerous to shrimp. Buildup in TAN was seen most often in the upper reaches of riverine estuaries during dry season months (Figure 5). In the absence of freshwater input, concentrations of TAN were controlled by phytoplankton absorption (Figure 6) as described by Tucker et al. (1984). Low TAN during February to April, 1994, (Figure 5) accompanied chlorophyll *a* values of almost 400 µg/l.

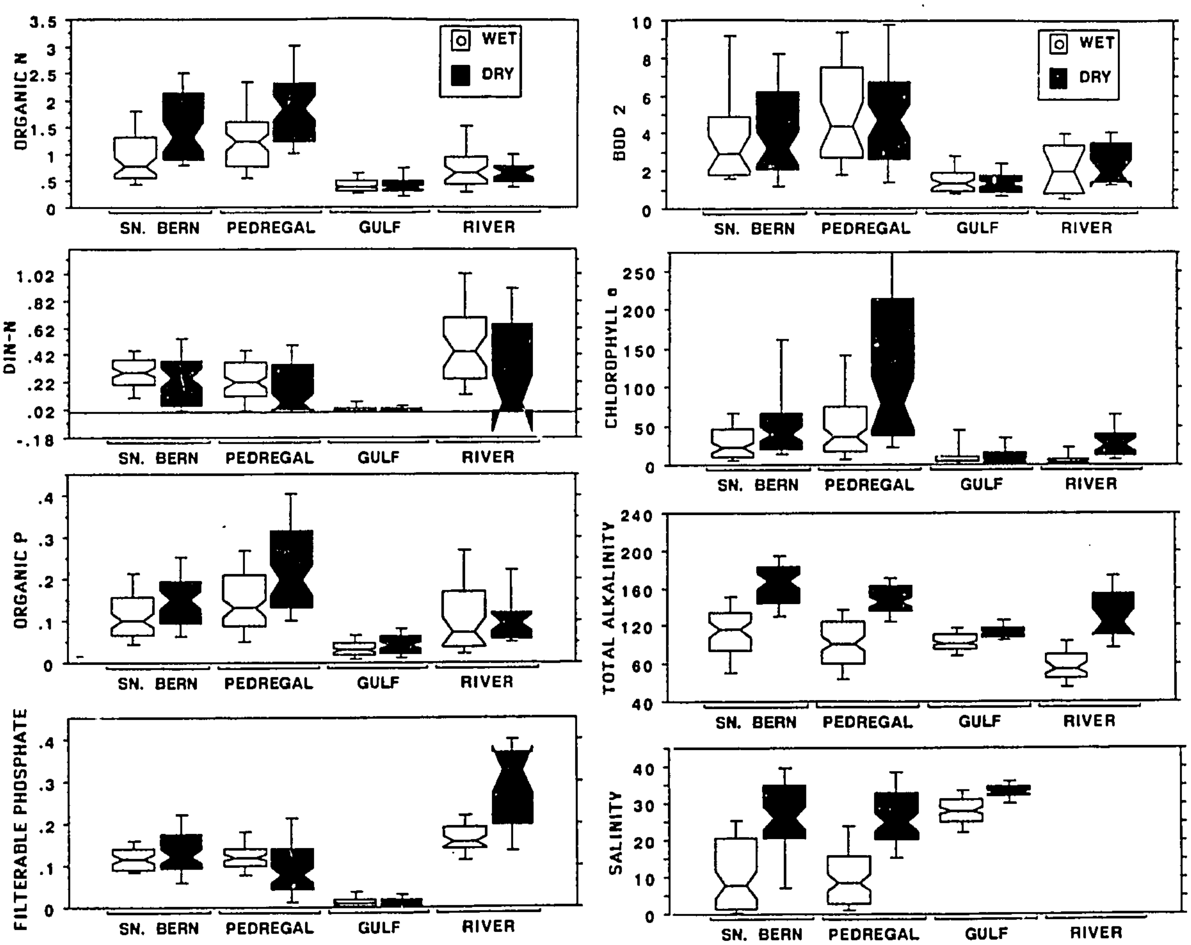


Figure 3. Box plots of water quality variables determined for the Choluteca River, for intake water of farms located on Sn. Bernardo and Pedregal estuaries, and for gulf estuaries not influenced by rivers. Data are grouped by dry and wet season.

Table 1. Water quality variables determined for the Choluteca River, for intake water of farms located on Sn. Bernardo and Pedregal estuaries, and for gulf embayments not influenced by rivers. Data are grouped by dry and wet season.

Water body	Para-meter	Total alkalinity ($mg\ CaCO_3/l$)		Organic nitrogen-N (mg/l)		Dissolved inorganic nitrogen-N (mg/l)		Total phosphorus-P (mg/l)		Filterable phosphate-P (mg/l)		Settleable solids (ml/l)	
		Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
	Mean	164	113	1.48	0.96	0.26	0.29	0.29	0.24	0.137	0.119	0.9	1.0
	SE	3.3	3.6	0.091	0.067	0.035	0.018	0.013	0.012	0.009	0.004	0.18	0.18
Sn.	Count	53	66	49	59	53	65	51	66	53	66	33	72
Bernardo	Min	107	41	0.59	0.24	0	0	0.14	0.12	0.030	0.027	0	0
	Max	203	161	2.80	2.30	1.23	0.74	0.50	0.71	0.370	0.183	4.5	8.0
	Median	167	120	1.32	0.79	0.25	0.29	0.30	0.22	0.123	0.117	0.6	0.2
	Mean	148	101	1.88	1.32	0.19	0.25	0.33	0.27	0.100	0.124	1.2	0.8
	SE	2.6	3.2	0.104	0.079	0.031	0.021	0.016	0.009	0.010	0.005	0.29	0.19
	Count	60	85	56	70	60	83	59	85	60	85	38	94
Pedregal	Min	103	30	0.37	0.35	0	0	0.13	0.08	0	0.007	0	0
	Max	209	164	3.83	2.84	1.04	1.15	0.70	0.53	0.390	0.291	7.5	13.0
	Median	150	100	1.86	1.23	0.07	0.22	0.31	0.26	0.079	0.120	0.4	0.05
	Mean	113	103	0.45	0.45	0.022	0.034	0.060	0.053	0.014	0.016	0.005	0.05
	SE	0.9	1.0	0.028	0.022	0.003	0.006	0.004	0.004	0.001	0.002	0.004	0.03
	Count	83	139	79	120	83	139	80	139	83	139	55	148
Gulf	Min	90	67	0.07	0.14	0	0	0	0.007	0	0	0	0
	Max	136	152	1.63	1.56	0.23	0.74	0.20	0.29	0.058	0.185	0.2	5.0
	Median	112	100	0.39	0.38	0.016	0.015	0.060	0.041	0.013	0.010	0.000	0.000
	Mean	129	76	0.68	0.78	0.31	0.50	0.40	0.27	0.286	0.165	0.02	0.5
	SE	5.7	2.9	0.073	0.092	0.082	0.055	0.028	0.016	0.021	0.008	0.02	0.13
River	Count	24	39	21	30	23	39	24	39	24	39	16	42
	Min	87	37	0.17	0.11	0.002	0.08	0.19	0.11	0.107	0.070	0	0
	Max	184	109	1.80	2.24	1.26	1.55	0.75	0.52	0.430	0.310	0.3	3.5
	Median	120	75	0.65	0.65	0.07	0.44	0.42	0.25	0.326	0.155	0.000	0.08

Table 1. Continued

Water body	Parameter	Chlorophyll *a*		BOD_2		Salinity (ppt)	
		Dry	Wet	Dry	Wet	Dry	Wet
Sn. Bernardo	Mean	62.3	29.6	4.2	3.9	26	11
	SE	8.13	3.23	0.43	0.52	1.7	1.3
	Count	59	61	51	25	51	67
	Min	0	2.5	0.9	0.9	2.5	0.5
	Max	259.2	114.4	16.0	10.2	49	36
	Median	39.1	21.7	3.2	3.0	26	8
Pedregal	Mean	128.1	58.1	5.0	5.2	26	11
	SE	13.94	6.57	0.40	0.51	1.2	0.9
	Count	66	81	55	35	58	84
	Min	12.6	0	0.7	0.3	10	0.5
	Max	396.1	266.2	11.8	12.8	51	34
	Median	77.9	35.3	4.7	4.4	25	9
Gulf	Mean	11.9	14.7	1.5	1.5	33	28
	SE	1.75	2.43	0.10	0.14	0.3	0.4
	Count	91	135	82	42	82	139
	Min	0	0	0.1	0	28	14
	Max	101.4	184.2	5.8	4.9	40	37
	Median	5.2	4.7	1.3	1.3	33	28
River	Mean	52.1	6.8	2.5	2.0	0	0
	SE	19.95	1.29	0.32	0.41	-	-
	Count	28	37	21	12	-	-
	Min	4.2	0	0.9	0.3	-	-
	Max	541.5	31.0	6.8	4.1	-	-
	Median	25.2	4.0	1.9	1.9	-	-

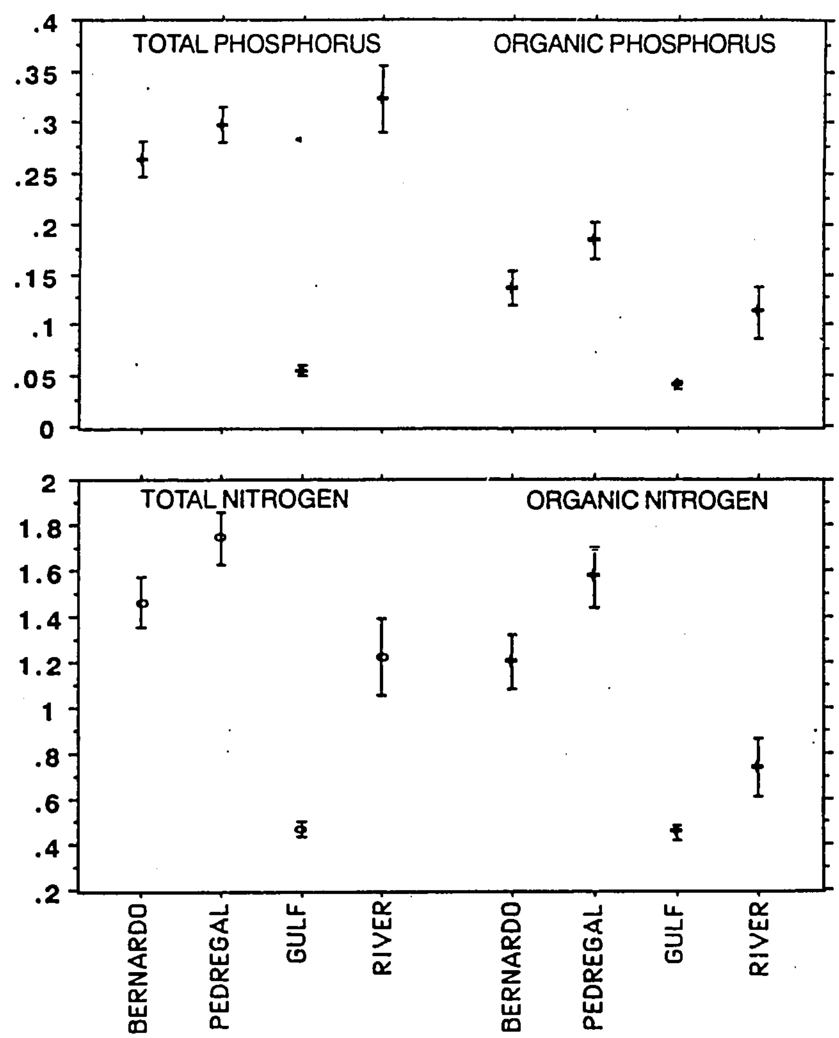


Figure 4. Mean (± 95% CI) phosphorus and nitrogen concentrations determined for the Choluteca River, for intake water of farms located on Sn. Bernardo and Pedregal estuaries, and for gulf estuaries not influenced by rivers.

Season

Large seasonal differences were discovered in riverine estuaries and in the Choluteca River, but little in gulf embayments (Figure 3). Total alkalinity and salinity were higher during the dry season in all estuaries. Rainfall and runoff maintained salinities at hypooceanic levels during the wet season. Salinities of zero ppt were recorded along the lengths of Pedregal and Sn. Bernardo estuaries during a particularly heavy storm in September 1993, as heavy freshwater inflow displaced estuarine water. Dry season salinities rose to hyperoceanic levels in all estuaries as evaporation exceeded freshwater input.

Table 2. Mean nitrogen concentrations in the Choluteca River and in the Sn. Bernardo, Pedregal, and gulf embayments (Gulf).

Water body	Mean concentration (mg/l)					
	Total nitrogen-N	Nitrate-N	Nitrite-N	Total ammonia -N	DIN-N	Organic nitrogen-N
Bernardo	1.46	0.166	0.041	0.079	0.28	1.20
Pedregal	1.74	0.098	0.027	0.119	0.23	1.57
Gulf	0.47	0.011	0.002	0.018	0.029	0.45
River	1.22	0.374	0.017	0.033	0.43	0.74

Major nutrients, mostly in the form of organic matter, were concentrated in riverine estuaries during the dry season as freshwater input diminished. High density algal blooms (396 and 259 μg/l chlorophyll *a* in Pedregal and Sn. Bernardo, respectively) were recorded. Dissolved inorganic phosphorus and nitrogen actually tended to decrease in the dry season as they were absorbed by plankton blooms.

The Choluteca River demonstrated significant increases in total alkalinity, filterable phosphate, and chlorophyll *a* during the dry season. Dissolved inorganic nitrogen was generally high also, except when plankton was dense. During the dry season, river flow decreased to only a couple of cubic meters per second, allowing nutrient concentrations to increase, total settleable solids to diminish, and phytoplankton to proliferate. At the end of January, 1994, for example, a buildup of filterable phosphorus and nitrate-N to over 0.4 and 1.2 mg/l, respectively, was followed by a 2-week-long phytoplankton bloom which resulted in chlorophyll *a* concentrations of over 540 μg/l.

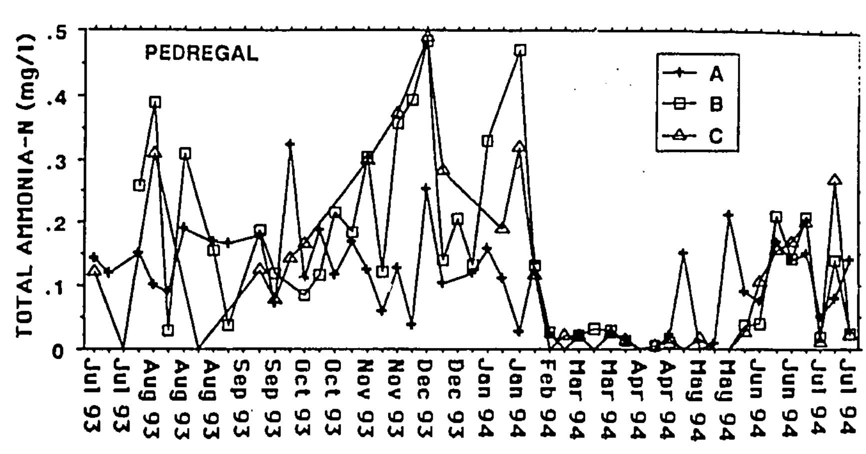
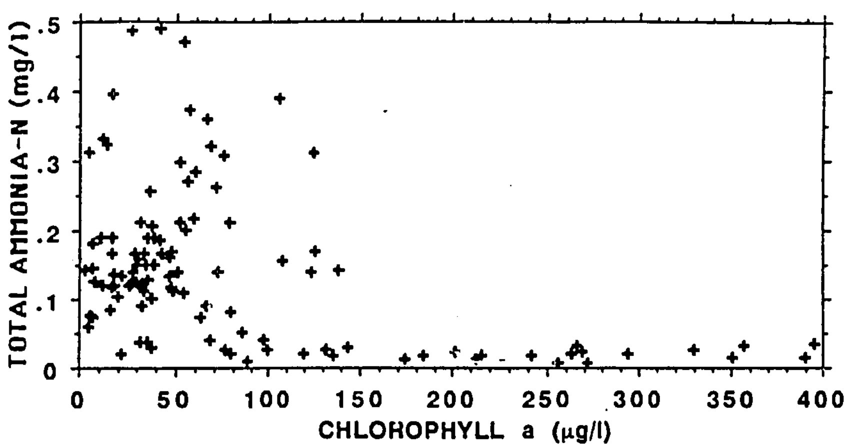


Figure 5. Total ammonia-N determined from intake water of three farms located on the Pedregal estuary. Farms A and C are located closest and farthest away, respectively, from the Gulf.

Figure 6. Relationship between total ammonia-N and chlorophyll *a* in the Pedregal estuary.



Location

Organic matter concentrations tended to increase with longitudinal distance from the Gulf of Fonseca in both Pedregal (Figure 7) and Sn. Bernardo (Figure 8) estuaries, especially during the dry season. Variability of measurements was high, particularly for farms located in the upper reaches. High variability made large differences insignificant; however, the occurrences of nutrient and organic matter buildup in estuaries were unequivocally greater in the upper reaches of the estuaries.

Pedregal Estuary – There were no significant water quality differences between upstream Farms B and C, but at least one of the upstream farms was significantly higher in organic-N and BOD_2 than Farm A during both seasons. Organic-P and chlorophyll *a* were significantly higher at the upstream farms than at Farm A. Inorganic phosphorus and dry season DIN were not significantly different among farms. Wet season DIN was significantly lower at upstream farms B and C. Inorganic nutrients tended to diminish when phytoplankton increased.

Sn. Bernardo Estuary – Upstream BOD_2 was significantly higher at Farm B than at Farm A during both seasons. Organic nitrogen, organic phosphorus, and filterable phosphate were significantly higher at Farm B during the dry season.

Calculations of estuarine exchange rates with the Gulf indicated that estuarine water at Farm A on both estuaries was totally exchanged about once every 4 weeks, while upstream water was exchanged about once every 7 to 10 weeks (Table 3). Exchange rates increased with greater tidal fluctuation at most points sampled (Figure 9). Organic matter became more concentrated as water exchange with the Gulf diminished.

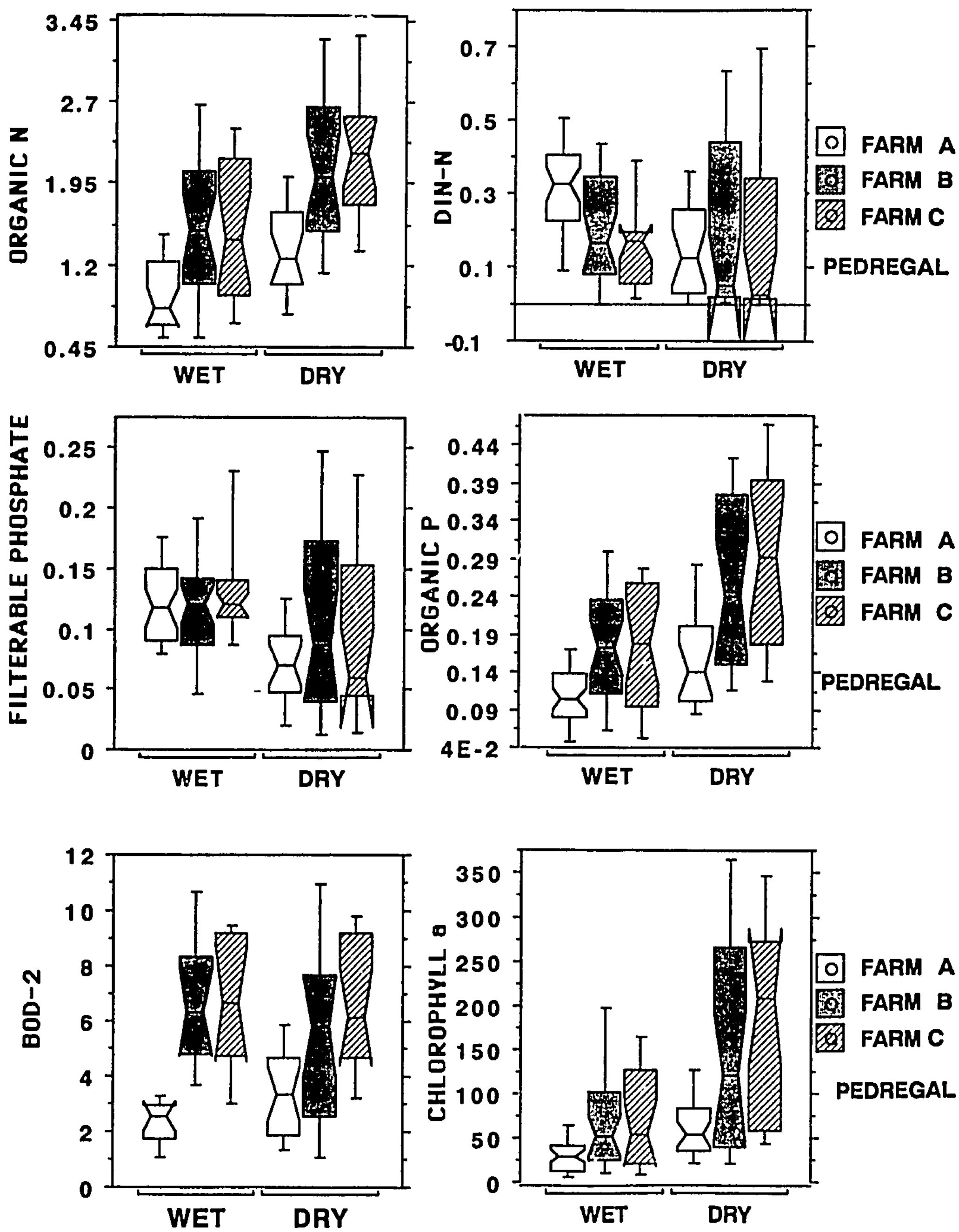


Figure 7. Box plots of water quality variables determined for intake water of farms located along a longitudinal axis of the Pedregal estuary. Farms A and C are located closest and farthest away, respectively, from the Gulf. Data are grouped by dry and wet season.

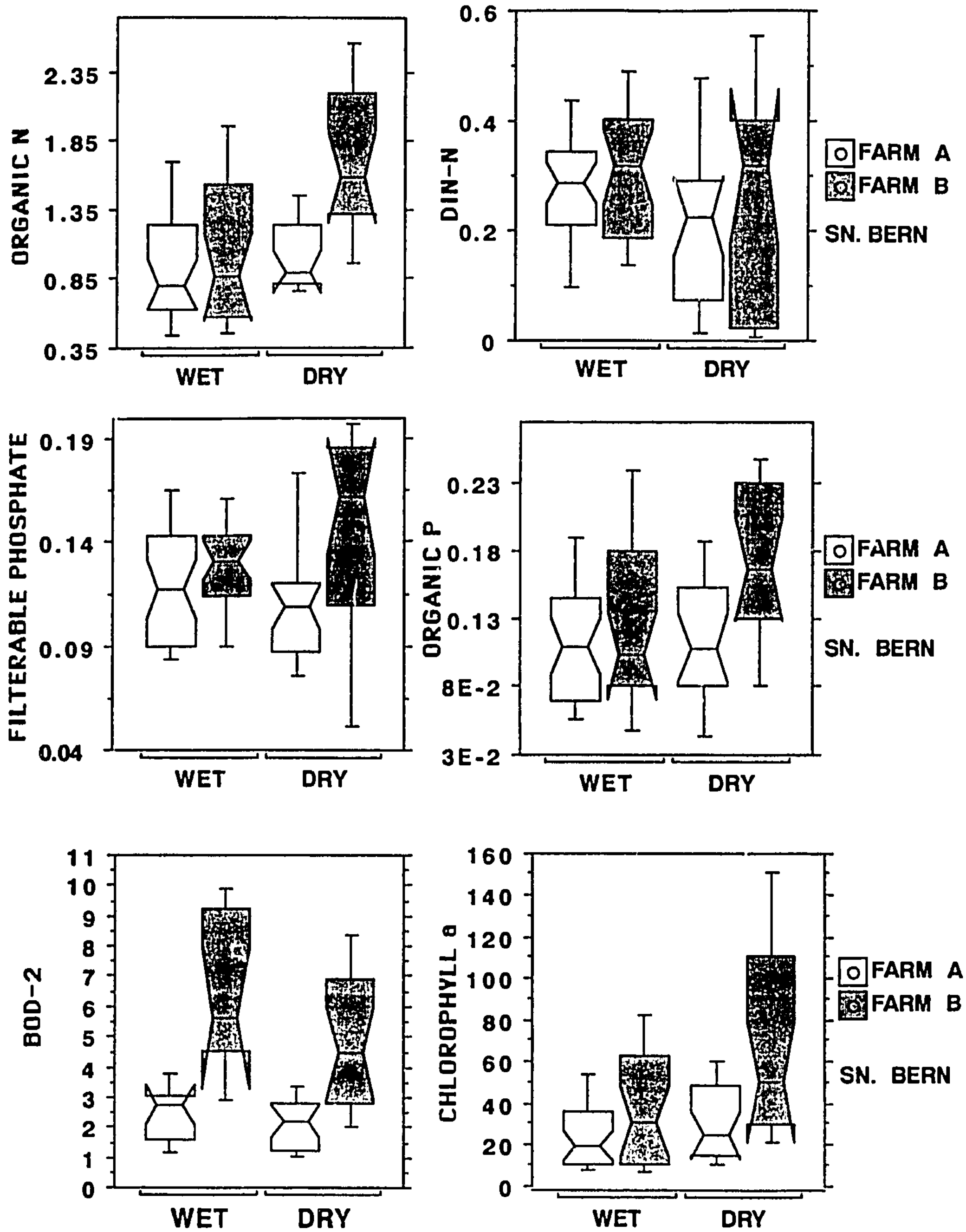
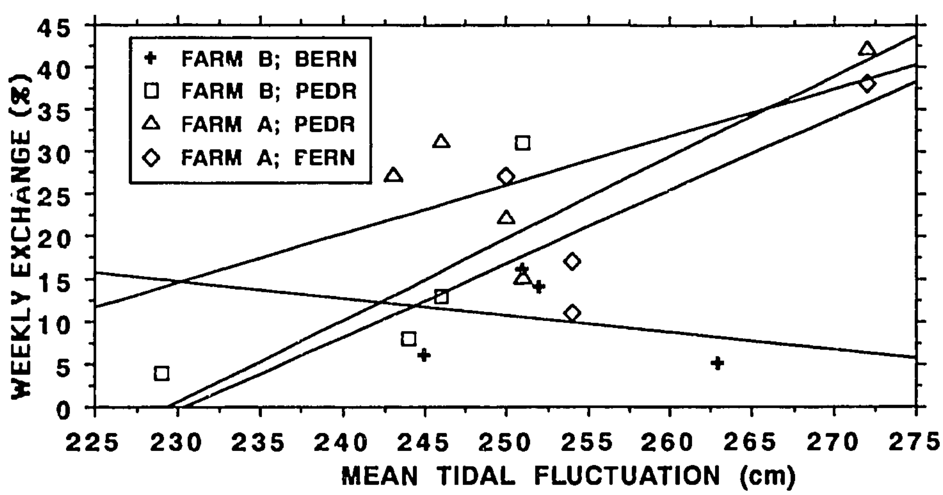


Figure 8. Box plots of water quality variables determined for intake water of farms located along a longitudinal axis of the Sn. Bernardo estuary. Farms A and B are located closest and farthest away, respectively, from the Gulf. Data are grouped by dry and wet season.

Table 3. Exchange rates with the Gulf of Fonseca of Pedregal and Sn. Bernardo estuaries at different points along their longitudinal axes.

		Date		Salinity (ppt)			Exchange (%)	
Farm	Estuary	Initial	Final	Initial	Final	Reference	Weekly	Mean
A	Pedregal	6-Jul-93	27-Jul-93	0.5	17.5	26.5	22	
A	Pedregal	5-Oct-93	26-Oct-93	1	11.5	25	15	
A	Pedregal	2-Nov-93	16-Nov-93	8	24	27	42	
A	Pedregal	23-Nov-93	14-Dec-93	8	25	29	27	
A	Pedregal	18-Jan-94	1-Feb-94	22.5	29	33	31	27
B	Pedregal	6-Jul-93	10-Aug-93	0.5	11	27.9	8	
B	Pedregal	26-Oct-93	9-Nov-93	2	4	26	4	
B	Pedregal	23-Nov-93	11-Jan-94	8.5	19.5	28.9	8	
B	Pedregal	18-Jan-94	1-Feb-94	19	22.5	32.7	13	
B	Pedregal	15-Feb-94	8-Mar-94	22	32.5	33.4	31	13
A	Bernardo	6-Jul-93	27-Jul-93	1.5	21.5	26.5	27	
A	Bernardo	28-Sep-93	26-Oct-93	0.5	11.5	24.9	11	
A	Bernardo	2-Nov-93	16-Nov-93	11	23	27	38	
A	Bernardo	30-Nov-93	4-Jan-94	15	28	30	17	23
B	Bernardo	6-Jul-93	10-Aug-93	0.5	9	27.9	6	
B	Bernardo	2-Nov-93	23-Nov-93	1	5	27	5	
B	Bernardo	14-Dec-93	4-Jan-94	5.5	18	31	16	
B	Bernardo	18-Jan-94	8-Mar-94	22	33.5	33.5	14	10



Y = -220.1 + 0.96 X; R^2 = 0.55 (FARM B; PEDREGAL)
Y = -116.5 + 0.57 X; R^2 = 0.41 (FARM A; PEDREGAL)
Y = -198.4 + 0.86 X; R^2 = 0.51 (FARM A; SN BERNARDO)
Y = 60.8 - 0.2 X; R^2 = 0.07 (FARM B; SN BERNARDO)

Figure 9. Relationships between weekly percentage estuarine water exchange with the gulf and mean tidal fluctuation during the same period.

Discussion

A baseline of water quality data was established for the major estuaries supporting the shrimp culture industry of Honduras. The data revealed differences between seasons, among estuaries, and with distance from the Gulf within riverine estuaries. Gulf embayments were less fertile than riverine estuaries, because they were comparatively larger and deeper, they had better access to the Gulf, and they were not affected by river waste load. Mean concentrations of inorganic P and N during this study were 0.25 and 0.12 mg/l, respectively, for riverine estuaries, and 0.029 and 0.015 mg/l, respectively, for gulf embayments. These concentrations fell within the range of values given by Kennish (1992) for a variety of temperate, river-dominated estuaries and embayments. Ranges of 0.008 to 0.84 mg/l N and 0.003 to 0.25 mg/l P were given for estuaries, and 0.006 to 0.015 mg/l N and 0.002 to 0.077 mg/l P for embayments. Autotrophic productivity in the gulf was limited by both N and P, while productivity in riverine estuaries was probably limited by the light-inhibiting effects of mud turbidity. Primary productivity in heavily fertilized fish ponds was limited in a similar manner (Teichert-Coddington et al., 1992). When estuarine phytoplankton blooms occurred, inorganic N and P were quickly reduced.

Carrying capacities for shrimp production are expected to differ by estuarine type, particular location within an estuary, and season of the year. An area's shrimp production carrying capacity is directly proportional to the capacity of an estuary or section of estuary to assimilate farm waste discharge without becoming eutrophied beyond a given limit. The assimilative capacity is related to an estuary's hydrography, which is characterized by morphology and bathymetry, hydrology, tides, meteorology, and density currents (Ward, 1994). These hydrographic characteristics are only partially understood for the estuaries described in this study, but qualified conclusions can be drawn from present evidence.

Gulf embayments in this study can assimilate a higher waste load than riverine estuaries, primarily because of nutrient dilution by a larger water mass. However, circulation patterns within the Gulf may allow faster accumulation of nutrients along inshore areas than thought. Small fronts along density, temperature, or nutrient gradients may be created between farm discharge and receiving waters (Largier, 1993), thereby inhibiting mixing. Discharge plumes tend to hug shorelines and not disperse unless disrupted by headlands (Wolanski, 1992). If localized currents and winds are weak, nutrients may concentrate despite large tidal excursions. There is evidence that this concentrating affect does occur. For example, high nutrient concentrations were periodically recorded for intake water of Farm D, located on a small branch of a large gulf embayment (Figure 1); total nitrogen and chlorophyll *a* values of over 1.6 mg/l and 180 µg/l, respectively, were registered. During the same time span, a neighboring farm on the main estuarine channel (Los Barrancones) had maximum values of 0.7 mg/l total nitrogen and 48 µg/l chlorophyll *a*. Dry season salinities of gulf embayments become hyperoceanic (> 40 ppt). This indicates that water exchange with the main Gulf body is slow enough to allow for hypersalinization through evapotranspiration. If water exchange is slow, then undesirable eutrophication of the estuaries is conceivable if stocking rates on existing farms were to be significantly increased.

Rivers discharge large loads of nutrients to the estuaries. No data on the Negro River are available, but the Choluteca River is by far the largest of the two rivers influencing the major shrimp producing area. It drains 7550 km^2 of land, runs 380 km, and has 8 main tributaries (MNR, 1987). Drainage includes the municipal waste discharge of Tegucigalpa, the Capital city of Honduras, Choluteca, and numerous other smaller towns. Total ammonia and nitrate concentrations of up to 11.9 and 10.6 mg/l, respectively, were recorded in 1989 in the Choluteca River just downstream from Tegucigalpa (Myton et al., 1992). Nutrient concentrations were much lower downstream from Choluteca in this study, but the river was nevertheless higher in dissolved nutrients than were the estuaries.

Total quantities of total N and total P discharged from the Choluteca River were up to an order of magnitude greater than N and P discharged from Pedregal shrimp farms. Mean total N and total P were 1.13 and 0.34 mg/l, respectively in the river, and mean annual river flow is 45 m^3/s. The mean estimated annual discharges of N and P would be 1,603,600 and 480,400 kg/yr, respectively. In comparison, 4200 ha of shrimp farms would annually discharge about 253,000 kg of N and 28,200 kg of P to the Pedregal. Farm calculations were based on pond chemical budget studies that indicated mean increases of 0.24 and 0.04 mg/l of N and P, respectively, as water passed from intake to discharge points in the ponds, and mean pond volume exchanges of 21 per year (Unpublished data). If the farm discharge rates were extrapolated to 11,000 ha of farms, then total annual N and P discharged from all current farms would be about 663,000 and 74,000 kg, respectively. These data indicate that the Gulf of Fonseca is receiving at least twice as much nitrogen and six times as much phosphorus from the Choluteca River than it is from the current shrimp industry. The Choluteca River is only one of 5 rivers that discharges into the Gulf, and nutrient discharge from the others is unknown. But there is little danger that unacceptable Gulf eutrophication will be caused by current shrimp farm waste loads. However, the shrimp industry could quickly put itself in danger from estuarine eutrophication, because the estuarine assimilation capacity is very small relative to that of the Gulf.

Tidal range within the Gulf of Fonseca is from 1 to 4 meters, occurs semi-diurnally, and is among the highest along the Pacific coastline (Ward, 1994). Tidal fluctuation is propagated with apparently little attenuation to the middle reaches of the estuaries. It is a common misconception that large tidal excursions imply an equal magnitude of estuarine exchange with the Gulf. Retention time in mangrove fringed estuaries during the absence of fresh water input is in fact directly proportional to the square of estuary length (Wolanski, 1992), according to the relationship

$$T_0 = L^2/B$$

where L is the length of mangrove-fringed creek and B is the longitudinal diffusion coefficient (flushing rate). Distance from the Gulf and retention time increase together. Empirical estimates of exchange rates indicated that total exchanges occurred on the order of once every 4 weeks at Farm A and once every 7 to 10 weeks at Farms B of Pedregal and Sn. Bernardo estuaries.

The tangible effects of greater retention time were increased salinities from evapotranspiration and the accumulation of nutrients discharged from shrimp ponds. The quality of farm supply water in riverine estuaries was dependent on location along the estuary because of varying estuarine retention times. Estuaries serve as both water supplies and depositories for farm wastes. Farms along a common estuary recirculate each other's wastes as they pump from, and discharge into, the same estuary. However, the comparative disadvantage of upstream versus downstream location along an estuary had not been previously quantified. Downstream farms intake wastes from upstream farms during ebb tide. The flood tide redistributes wastes to all farms including a fresh supply of discharge to upstream farms from their downstream neighbors. This study clearly demonstrated that mean water quality was better gulfward. Upstream producers recirculated more wastes through their ponds than did their downstream neighbors.

Boyd and Musig (1992) related the pollution of estuarine supply water to the eutrophication of a shrimp pond. The immediate result is early morning oxygen problems. Oxygen problems begin sooner in ponds receiving eutrophied estuarine water, because the water has less capacity to assimilate further organic and metabolic enrichment. In this study, two-day BOD at upstream farms was significantly greater during both seasons on both estuaries (Figures 7 and 8). Estuarine dissolved oxygen (DO) at upstream farm sites is regularly less than 2 mg/l, and often less than 1 mg/l during the dry season, although concentrations may approach saturation during the rainy season. Exchanging pond water for estuarine water may actually deteriorate pond water quality and promote chronic DO problems. Increasing the waste load by expanding land area under cultivation or increasing stocking densities could only result in more frequent periods of estuarine hypoxia and eventually, anoxia.

The wet season was very important for flushing estuaries with freshwater inflows. After heavy rains and floods in September 1993, salinities at all depths were zero to the mouth of both Pedregal and Sn. Bernardo estuaries, indicating that all brackish water had been expelled from the estuaries. Wet season organic nutrient concentrations were lower and showed less variation compared with the dry season concentrations because of the flushing action of freshwater inflows.

Recommendations

Sustainable shrimp culture in the riverine estuaries will require either the cooperation and goodwill of all producers along a common estuary or strong governmental regulations and enforcement. The potential for eutrophication of an estuary beyond its assimilative capacity would be reached first in its upper reaches, but producers downstream would be subsequently affected.

There are some immediate steps that could be taken to minimize estuarine pollution and promote industry sustainability. Restrictions on stocking densities should be implemented immediately to prohibit high-intensity culture in riverine estuaries and to limit stocking rates in gulf embayments. The use of intensive culture techniques should be restricted, because of the disastrous effects that such systems have historically had in some areas. Semi-intensive culture techniques that allow for maximum digestion of materials within a pond should instead be promoted.

Saltflats in the upper reaches of riverine estuaries or on minor branches of the major estuaries should not be developed. The probability of developing severe water quality problems is high because of reduced water exchange with the gulf and nutrient buildup. Current environmental regulations in Honduras prohibit construction of ponds on land that contains a dense cover of mangrove, i.e., non-saltflats. However, licensing of farms should be based as much on water quality and the chance of farm success as on mangrove destruction. It doesn't serve the environment or economy well to allow farm construction on saltflats supplied with water that would be degraded beyond usefulness within a short time span.

Fertilization of ponds on riverine estuaries should be discouraged for two reasons. First, there is usually a sufficient supply of nutrients to promote good phytoplankton blooms without fertilization. Second, several fertilization trials on a riverine farm in southern Honduras indicated that fertilization had absolutely no affect on shrimp production during the dry season and little affect during the wet season (Unpublished data). Unabsorbed inorganic nutrients or excessive phytoplankton from over-fertilization are merely discharged into the estuaries adding to the waste load. Fertilization of embayment farms may actually be beneficial because of the poverty of nutrients in water supplies. Ultimately, if fertilization doesn't increase shrimp yields, regardless of its benefits to primary productivity, then it shouldn't be used. Reduction of material discharge from ponds could also be accomplished by reducing water exchange rates, because greater digestion occurs within the culture pond (Hopkins et al., 1993a; Hopkins et al., in press). Shrimp have been grown experimentally at high stocking and feeding rates with aeration and minimal water exchange (Hopkins et al., 1993a).

Low protein feeds should be used on semi-intensive farms in Honduras unless it is proven that a higher protein feed actually promotes growth. Garcia-Casas (1990) demonstrated in southern Honduras that a high quality 35%-protein feed had a significant effect on shrimp growth only during the wet season, and only with *P. stylirostris*, a minor culture species in Honduras. Teichert-Coddington and Rodriguez (in review) demonstrated no growth improvement by using a 40% versus a 20% protein feed during either season in ponds stocked with *P. vannamei* at 5 to $11/m^2$. Higher protein feeds result in more nitrogenous wastes upon decomposition.

Finally, there are indications that feeding rates could be reduced on some farms, especially during the dry season, without affecting shrimp production. Correct feeding rates are essential to wasteload management.

Conclusions

The southern Honduras shrimp industry was used as a case study of the relationship between shrimp farming and estuarine water quality. The data were site specific, but the ecological and hydrographic processes described herein are presumably similar in other shrimp producing estuaries and embayments of the world. This study was possible only because the shrimp producers had sufficient vision to cooperate and share data with each other. This project is an example of how a minimal financial investment (< $165,000/year) can result in long-term usefulness to, and possibly survival of, an entire industry. Savings in costs through more efficient management as a result of the project could more than pay the investment costs. There is no substitute for real data.

Water quality differences were noted among estuaries, along longitudinal transects of estuaries, and between rainy and dry seasons of the year. Riverine estuaries were more fertile than gulf embayments, and had less potential capacity to assimilate greater waste loads. Eutrophication of riverine estuaries was greater with distance upstream from the Gulf. Eutrophication was greater in riverine estuaries during the dry season, when freshwater inflows diminished.

These data will be used to help estimate carrying capacities of estuaries and to recommend management techniques that promote sustainment of the shrimp culture industry. The industry, with governmental enforcement, will ultimately have to decide to adopt sustainable measures or not.

Acknowledgments

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Varying the Proportion of *Colossoma macropomum* and *Oreochromis niloticus* in Polyculture

Work Plan 7, Honduras Study 4B

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Introduction

Information on the comparative growth characteristics of tilapia (*Oreochromis niloticus*) and tambaquí (*Colossoma macropomum*) is limited, especially given the importance of each as an aquaculture species. The tambaquí is native to the Orinoco and Amazon rivers, where fish weighing up to 30 kg can be found (Goulding and Carvalho, 1982). Tambaquí grows rapidly on a variety of food products (Martínez, 1984), but a large fish size is usually needed at market, because the intramuscular bones characteristic of characids are particularly bothersome in small fish. The tambaquí can reach a much larger size than tilapia, but tilapia are marketable at smaller sizes and can achieve high areal yields. The economic advantage of growing one species over the other has not been well defined.

Peralta and Teichert-Coddington (1989) compared the growth of tambaquí and tilapia stocked at 0.25 or 1 fish/m^2. Sizes at stocking were 31 and 22 g, respectively. Fish were offered a 25% protein pellet. After 129 d, production was not significantly different at either density ($P > 0.05$). Mean tambaquí weight was significantly greater than tilapia, because tilapia reproduction had competed with adult fish for feed. Tambaquí competed well with the tilapia during growth from fingerling to marketable size for tilapia. Growth to a larger size would be expected to favor the tambaquí which appears to gain weight faster at larger sizes.

Semi-intensive fish culture in many developing countries depends on efficient use of natural pond productivity, which is stimulated by fertilization. Feed use is usually justifiable only as a nutrient supplement, or as a finisher (Green, 1992). In Honduras, feed use was not profitable unless the tilapia stocking rate was at least 2 fish/m^2 (Green et al., 1994), whereas fertilization of tilapia ponds was profitable at lower stocking rates.

Experiments in Central America demonstrated that tilapia grew well on natural pond production (Green et al., 1990; Teichert-Coddington et al., 1992; Teichert-Coddington and Green, 1993; Green et al., 1994) or feeds (Green, 1992). Tambaquí, especially juveniles up to about 0.5 kg, derive a large part of their nutrition in natural waters by consuming zooplankton (Goulding and Carvalho, 1982). However, tambaquí grow poorly in organically fertilized ponds (Ferrari et al., 1987; van der Meer and Martínez, 1993) which are thought to be rich in zooplankton. A diet containing 23-30% protein is usually used for tambaquí culture (Saint-Paul, 1986). A trial in Honduras illustrated the necessity of an artificial diet for tambaquí growth (Teichert-Coddington et al., 1991a). Earthen ponds were stocked with male tilapia fingerling (13-g mean weight) at 1 fish/m^2, and tambaquí (40-g average weight) at 0.15 fish/m^2. Three ponds were fertilized weekly with chicken litter (CL) at 1,000 kg dry matter/ha for the first six weeks, and then at 750 kg dry matter/ha for the remainder of the study. Three other ponds were fertilized weekly with CL at 500 kg dry matter/ha, and fish were offered a pelleted ration (25% protein) at 1.5% of tilapia biomass per day, six days per week. After 126 days, there were no significant tambaquí survival differences ($P > 0.05$), but the mean tambaquí weight difference between treatments was large. Tambaquí were 86 g and 447 g in organically fertilized and supplementally fed treatments, respectively. Tambaquí doubled their weight with organic fertilization, but grew more than 10 times their initial weight with supplemental feeding. Many semi-intensive farmers in Honduras were interested in stocking tambaquí, but were not prepared to feed ponds adequately. Others were interested in co-stocking tambaquí with tilapia, but were unsure of stocking proportions or necessary feeding rates. The use of feed would have to be justified by higher fish yields or better market price for the tambaquí. Feed might be more efficiently utilized if planktivorous tilapia were co-stocked with fast growing tambaquí (Lovshin, 1980).

The optimum stocking rate of tambaquí for semi-intensive pond culture was not known. Studies reviewed by Martínez (1984) and Saint-Paul (1986) did not include pond stocking rates higher than 1 fish/m^2. Much higher densities were tested in cages; yields could be high, but average daily gain was usually less than 1.5 g. Lovshin (1980) reported that tambaquí average harvest weight was significantly less at 1 fish/m^2 than at 0.5 fish/m^2, but that annual production was significantly higher at the higher density (9391 kg/ha) than at the lower density (6683 kg/ha). Fish were offered a 17%-protein chicken pellet in that study. Peralta and Teichert-Coddington (1989) demonstrated that individual growth rate of tambaquí fed a 25%-protein pelleted ration in earthen ponds was not significantly different when stocked at 0.25 or 1 fish/m^2; stocking less than 1 fish/m^2 would result in lower yields while not producing a significantly larger fish. Most semi-intensive commercial tilapia farmers stock ponds at 2 to 3 fish/m^2.

This study was designed to demonstrate the effect of species stocking proportions on polyculture of tambaquí and tilapia when cultured at a fish density commonly used by tilapia farmers.

Materials and Methods

A completely randomized design was used to test four stocking combinations, each replicated three times:

1. tilapia (100%);
2. tilapia (75%) plus tambaquí (25%);
3. tilapia (25%) plus tambaquí (75%);
4. tambaquí (100%).

Earthen ponds, 0.90 m deep and 0.1 ha in area, were stocked on 2 December 1993 with male *Oreochromis niloticus* and *Colossoma macropomum* (tambaquí) at a total density of 3 fish/m^2. In addition, fingerlings of guapote tigre (*Cichlasoma managuense*), a native piscivore, were stocked at 750/ha to prey on tilapia reproduction. Tilapia had been hormonally sex-reversed by oral administration of 17α-methyltestosterone (Green and Teichert-Coddington, 1994), but reversal is rarely 100% effective.

During the first month, ponds were fertilized weekly with chicken litter (500 kg dry matter/ha) and urea to bring total N input to 30 kg/ha. No feed was added because previous studies had demonstrated no increase in initial growth for fed ponds (Green, 1992; Teichert-Coddington et al., 1991b). After one month, fertilization was stopped and fish were fed a 28%-protein pellet at 3% of mean tambaquí biomass 6 days a week in treatment 4. Equal amounts of feed were applied to all ponds. When daily early morning temperatures dropped below 23°C, the feeding rate was reduced to 2% of biomass. The daily feed allotment was divided into two meals.

Water was added to replace evaporation and seepage. During the last month of growth, water was exchanged once a week at 10% of pond volume. The pond level was first dropped, and then the pond was refilled.

Early morning dissolved oxygen (DO) and water temperature were measured weekly with a YSI polarigraphic oxygen sensor. Dissolved oxygen was monitored more frequently when concentrations became regularly lower than 2 mg/l. Chlorophyll *a* was determined weekly according to Boyd and Tucker (1992). Total ammonia nitrogen and filterable phosphate were determined according to Boyd and

Tucker (1992) three times during the cycle. Total nitrogen and total phosphorus were measured three times during the cycle using a combined oxidation procedure (Grasshoff et al., 1983). Water samples for chemical analyses were drawn shortly after daylight with a 90-cm column sampler from various locations within the ponds.

Ponds were drained and fish harvested on 2 June 1994, 182 days after stocking. Results were statistically analyzed by one-way ANOVA (Gagnon et al., 1989). Linearity of response to proportion of tilapia stocked was tested by orthogonal comparisons. Results were declared significant at alpha level = 0.05.

Results

Mean treatment production ranged from 2478 to 5120 kg/ha (Table 1). Feed efficiency ranged from 1.15 to 2.78. Total production increased and feed efficiency decreased curvilinearly as the percentage of stocked tilapia increased (Figure 1). Mean tilapia and tambaquí weight ranged from 187 to 325 g and from 122 to 270 g, respectively (Table 1). Tilapia mean weight decreased curvilinearly and tambaquí mean weight increased linearly as the percentage of stocked tilapia increased. Survival was not significantly affected by the percentage of stocked tilapia. However, mean tambaquí survival (65%) was 30% lower than mean tilapia survival (93%).

Table 1. Orecchromis niloticus (Til) and Colossoma macropomum (Tam) production in ponds communally stocked at proportions varying from 0 to 100% of each species.

		Tilapia			Tambaqui				
POND	Treatment	Weight (kg/ha)	Mean wt. (g)	Survival (%)	Weight (kg/ha)	Mean wt. (g)	Survival (%)	Production (kg/ha)	Feed efficiency
1	Til100/Tam0	4766.2	170.0	93.4	–	–	–	4766.2	1.30
2	Til0/Tam100	–	–	–	2089.2	115.2	60.5	2089.2	2.98
3	Til25/Tam75	2178.8	315.3	92.1	2252.6	164.5	60.8	4431.5	1.40
4	Til0/Tam100	–	–	–	1697.7	78.3	72.3	1697.7	3.66
5	Til75/Tam25	4293.0	207.7	91.9	1449.2	288.7	66.9	5742.2	1.08
6	Til100/Tam0	5337.0	197.2	90.2	–	–	–	5337.0	1.17
7	Til75/Tam25	3968.5	185.3	95.2	1175.7	253.9	61.7	5144.1	1.21
8	Til75/Tam25	3990.0	196.8	90.1	1416.3	267.2	70.7	5406.3	1.15
9	Til0/Tam100	–	–	–	3647.3	172.7	70.4	3647.3	1.70
10	Til25/Tam75	2294.6	340.4	89.9	2466.0	162.4	67.5	4760.6	1.31
11	Til100/Tam0	5256.5	193.0	90.8	–	–	–	5256.5	1.18
12	Til25/Tam75	2479.6	319.9	103.3	2452.3	189.1	57.6	4931.9	1.26
		Treatment means							
	Til100/Tam0	5120	186.7	91.5	–	–	–	5120	1.22
	Til75/Tam25	4084	196.6	92.4	1347	269.9	66.4	5431	1.15
	Til25/Tam75	2318	325.2	95.1	2390	172	62	4708	1.32
	Til0/Tam100	–	–	–	2478	122.1	67.7	2478	2.78
		Orthogonal contrasts							
	Linear	hs	hs	n	n	hs	n	hs	hs
	Quadratic	n	hs	n	n	n	n	hs	s

hs = highly significant (P < 0.01); s = significant (P<0.05); n = not significant (P > 0.05)

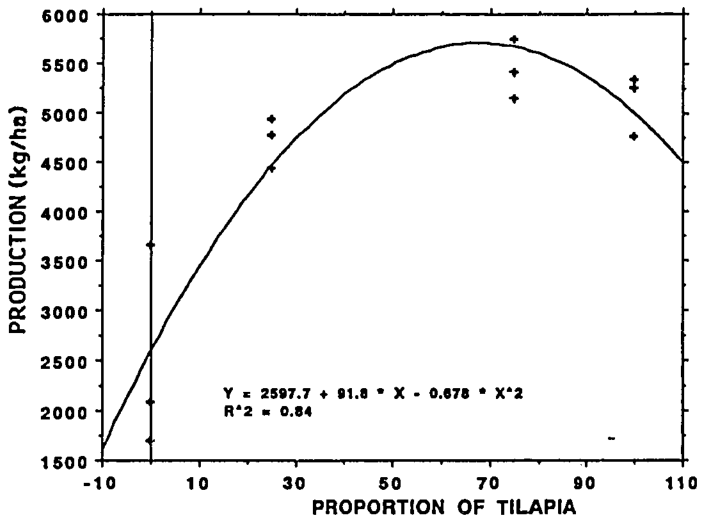

Figure 1. Relationship between total fish production and the percentage of tilapia stocked in polyculture with tambaquí.

Average daily weight gain (ADG) among treatments was little different during the first two months (Figures 2 & 3). Thereafter, tilapia gained weight faster, first in the low percentage tilapia treatment (Til25/Tam75), and later in the other two tilapia treatments (Figure 3). Tambaquí growth was low in all treatments until after 3.5 months (17 March) when ADG suddenly increased, especially in the low tambaquí density treatment. Tambaquí growth rate in the two lowest tambaquí density treatments eventually surpassed tilapia ADG; ADG during the last 21 days was over 5 g in the low tambaquí density treatment.

Water quality is summarized in Table 2. Significant treatment differences were noted for total nitrogen and chlorophyll *a* concentrations. Values of both variables increased linearly as percentage of stocked tilapia decreased. Total phosphorus and total volatile solids tended to be highest at the lowest tilapia density, but the differences were not significant. Early morning temperatures ranged from 19 to 27.5°C, with a mean of 24.4°C. Temperatures generally increased during the course of the experiment (Figure 4).

Discussion

The best stocking proportions appeared to be a 75% tilapia and 25% tambaquí mixture. Stocking only tambaquí resulted in the lowest production. Stocking 100% tilapia appeared less optimal than including 25% tambaquí, because tambaquí growth at that density was particularly high during the last 6 weeks of culture. Extending the culture period would have increased the advantage conferred by stocking tambaquí.

Chlorophyll *a* and total nitrogen decreased with increasing tilapia density, indicating that tilapia was harvesting plankton. Particularly efficient feed conversion in the

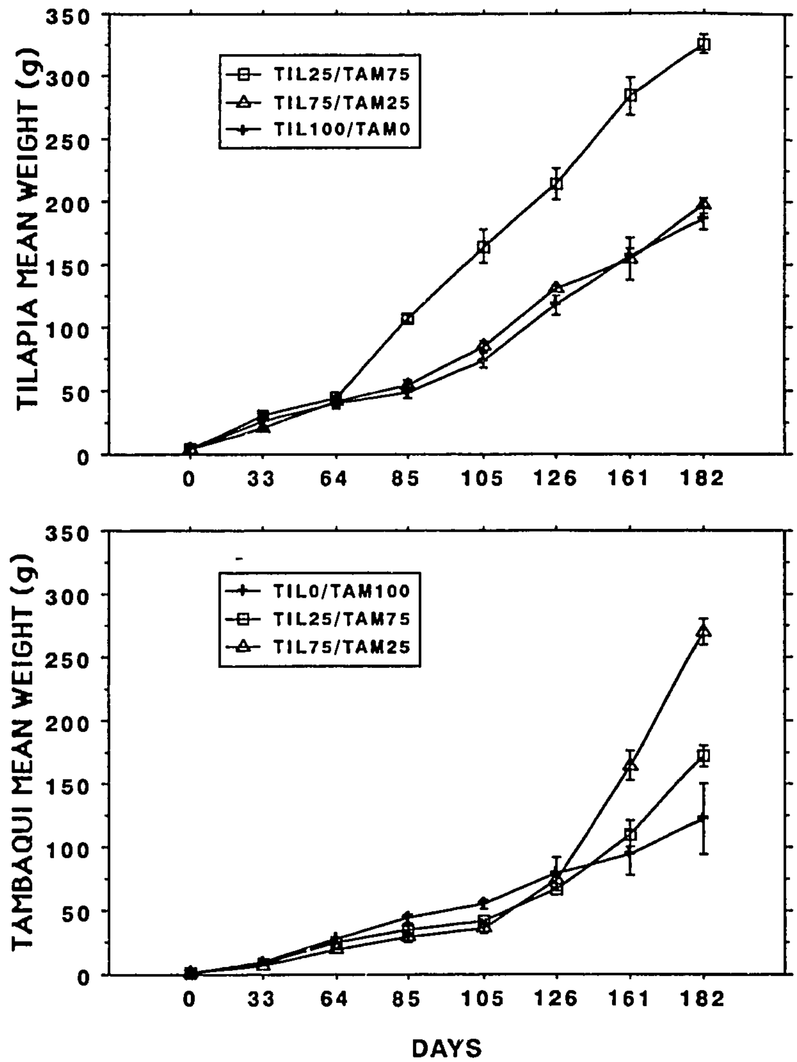

Figure 2. Growth of tilapia and tambaquí co-stocked at various proportions.

tilapia treatments confirms that tilapia were obtaining nutrition from natural pond biota. Blue-green surface films developed only in the 100% tambaquí treatments because of a lack of grazing by tilapia. Lovshin (1980) demonstrated that feeding efficiency of a tambaquí culture could be improved tremendously by co-stocking a tilapia hybrid. Annual production and feed conversion of tambaquí stocked at 1 fish/m^2 were 9391 kg/ha and 2.8. When a tilapia hybrid was included as 50 percent of the total number stocked, yield and feed conversion were 8939 kg/ha and 1.8. Feed rates had been calculated according to the tambaquí biomass, so tilapia production was realized without additional feed.

Production was lower than expected in all treatments, especially during the first three months, for two reasons. First, the trial was carried out during the dry, winter months when temperatures are lower (Figure 4). In Honduras tilapia production loses during a 150-d growth cycle can be over 25% more during the cold season than

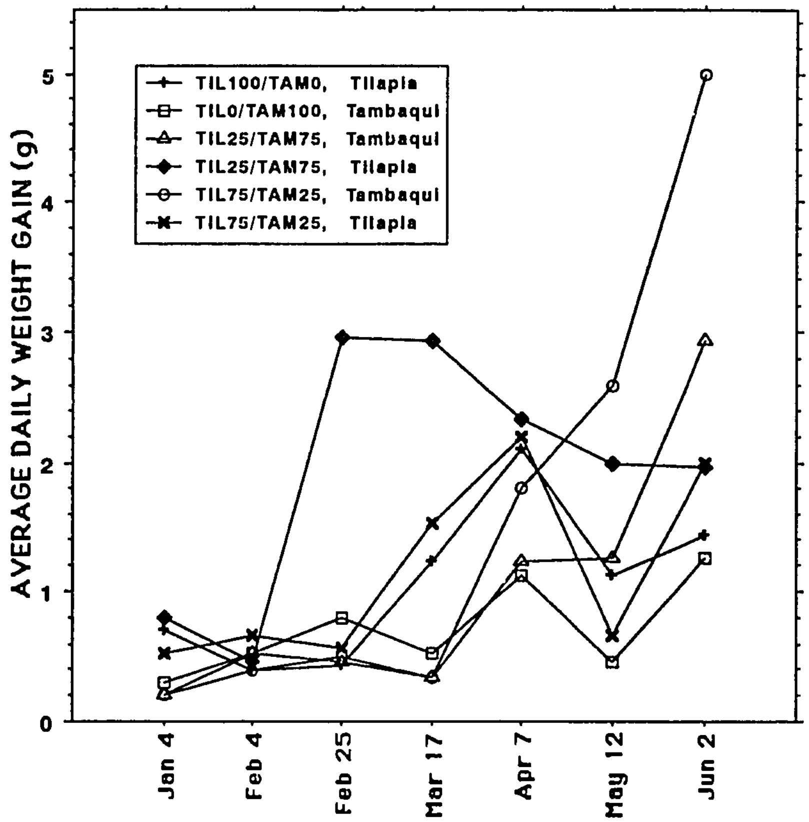

Figure 3. Average daily weight gain between sampling dates for tilapia and tambaquí co-stocked at various proportions.

in the hot season (Green et al., 1994). Second, the fish were fed according to the calculated biomass of the 100%-tambaquí treatment, which was the slowest growing. Therefore, feed input was probably suboptimal for high fish yield in the fastest growing treatments. Low feed conversion values ranging from 1.15 to 1.32 for the highest tilapia density treatments confirm that feed inputs were low. By comparison, Green (1992) reported production at El Carao of 5305 kg/ha in 150 d, with a feed conversion of 1.8 when only feed (24% protein) was offered to tilapia stocked at 2 fish/m^2.

Tilapia growth was clearly superior to tambaquí growth at all densities until the last 1 to 2 months, when tambaquí growth at the two lowest tambaquí densities became superior. Tambaquí daily weight gain was minimal for the first 3.5 months, probably because of suboptimal temperatures. Tambaquí are appare.ıtly less tolerant of low water temperatures than tilapia. Although a threshold temperature was not defined, the adverse affect of cool water temperatures on tambaquí growth was reported by Martínez (1984) in a review of tambaquí culture in Latin America. Characids other than tambaquí are stocked in the cool waters of southern Brazil, because tambaquí growth is inferior at the lower temperatures (Newton Castagnolli, personal communication). Tambaquí yields would probably have been higher if this experiment had been carried out entirely during the hot season months. Very slow

Table 2. Water quality in ponds communally stocked with varying proportions of *Oreochromis niloticus* (Til) and *Colossoma macropomum* (Tam).

POND	Treatment	Total P (mg/l)	Filterable phosphate (mg/l)	Total N (mg/l)	Total ammonia (mg/l)	Total alkalinity (mg $CaCO_3$/l)	Total hardness (mg $CaCO_3$/l)	Total solids (mg/l)	Total volatile solids (mg/l)	Dissolved oxigen (mg/l)	Chlorophyll *a* (µg/l)
1	Til100/Tam0	1.39	0.940	3.55	0.130	111.7	83.0	352	139	3.0	162.8
2	Til0/Tam100	1.07	0.617	4.55	0.087	99.4	72.7	269	121	3.5	256.0
3	Til25/Tam75	1.55	0.940	4.32	0.050	124.9	91.1	344	140	3.0	203.9
4	Til0/Tam100	1.73	0.693	6.40	0.070	103.2	68.4	355	174	3.0	376.9
5	Til75/Tam25	1.26	0.723	4.61	0.117	120.2	86.4	352	143	2.8	229.5
6	Til100/Tam0	0.78	0.383	3.82	0.040	100.8	79.8	307	140	2.1	232.3
7	Til75/Tam25	1.94	1.533	3.20	0.107	119.9	95.1	343	123	3.0	137.5
8	Til75/Tam25	1.46	1.033	3.46	0.043	141.1	103.8	354	114	3.1	141.8
9	Til0/Tam100	1.87	1.013	5.29	0.023	115.3	85.8	361	180	2.3	253.7
10	Til25/Tam75	1.49	0.877	4.32	0.137	117.5	79.3	348	158	2.5	328.7
11	Til100/Tam0	1.37	0.983	3.22	0.083	135.4	93.3	319	105	2.9	151.8
12	Til25/Tam75	1.56	0.900	4.90	0.043	88.8	65.3	344	112	2.4	315.2
						Treatment means					
	Til100/Tam0	1.18	0.769	3.53	0.084	116.0	85.4	326	128	2.6	182.3
	Til75/Tam25	1.55	1.096	3.76	0.089	127.1	95.1	350	127	3.0	169.6
	Til25/Tam75	1.53	0.906	4.51	0.077	110.4	78.6	345	137	2.6	282.6
	Til0/Tam100	1.56	0.774	5.41	0.060	106.0	75.6	328	158	2.9	295.5
						Orthogonal contrasts					
	Linear	n	n	s	n	n	n	n	n	n	s

s = significant (P<0.05); n = not significant (P > 0.05)

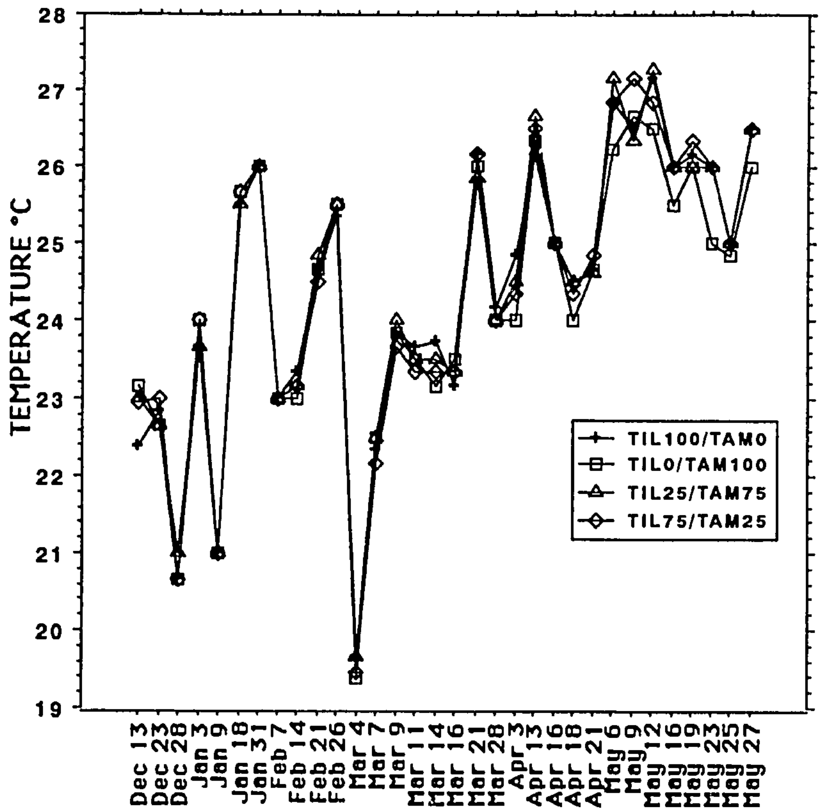

Figure 4. Early morning water temperature in ponds co-stocked with tilapia and tambaquí at various proportions.

initial tambaquí growth was not discovered in other reported experiments where a good quality diet was used. Particularly slow growth at the highest tambaquí densities suggested a density-growth interaction. Relatively low tambaquí growth rates in high density cage culture may arise from this interaction.

This study indicated that tilapia had a comparative advantage over tambaquí, especially during the cold season, when stocked at densities normally used by semi-intensive tilapia farmers. Monoculture of tambaquí for local markets is not recommendable. However, stocking a low percentage of tambaquí with tilapia could be economically advantageous, especially if the growing season is extended to allow the tambaquí to express their natural growth capacities at larger sizes. Alternatively, tambaquí could be stocked into tilapia ponds at larger initial sizes in order to contribute more quickly to production. More research on stocking size, density-production relationships, and practical feeding are needed before tambaquí can be fully integrated into tilapia-dominated fish culture systems.

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Inorganic Fertilization and Feed Reduction in Commercial Production of *Penaeus vannamei* during Wet and Dry Seasons in Honduras

Work Plan 7, Honduras Study 3A

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Introduction

Low dry season yields (Teichert-Coddington et al., 1994; Teichert-Coddington and Rodriguez, in review), high feed costs, low correlations between feed input and shrimp yields (Garcia-Casas, 1990; Teichert-Coddington and Rodriguez, in review), and concern about estuarine pollution from organic discharge (Teichert-Coddington, in press) have motivated Honduran shrimp farmers to investigate techniques for increasing feed efficiency. Fertilization to increase natural pond productivity has long been used to increase fish production (Hepher, 1962; Boyd, 1990; Teichert-Coddington et al., 1992). Organic fertilization has been used beneficially in shrimp culture (Lee and Shleser, 1984; Wyban et al., 1987; Teichert-Coddington et al., 1991), but its use in Honduras is discouraged because of the bad image that the practice bestows on the product. Inorganic fertilizers are often recommended for use in manuals on semi-intensive shrimp culture, but little systematic research on inorganic fertilizers and brackish water has been accomplished. Rubright et al. (1981) demonstrated that inorganic fertilization of fed shrimp ponds increased natural pond fertility, but resulting shrimp yields were little different from ponds receiving only feed. Inorganic fertilization of commercial ponds in southern Honduras has resulted in greater primary productivity, but not in greater shrimp yields (Green and Teichert-Coddington, 1990; Rodriguez et al., 1991). The Honduran trials were conducted mostly during the dry season when shrimp grow poorly regardless of input (Teichert-Coddington et al., 1994). Wet season trials were needed to test fertilization during the time of year when shrimp might make better use of natural pond productivity.

Low feeding efficiencies during past experiments with semi-intensively cultured shrimp in southern Honduras (Teichert-Coddington et al., 1991; Teichert-Coddington and Rodriguez, in review) indicated that reduced feeding levels might be employed without negatively impacting shrimp yields. This possibility was particularly great in the dry season when feeding efficiency is especially low. Feed inputs may be further decreased during both seasons through the use of cheaper inorganic fertilizers, which would increase natural food production.

The objectives of this study were:

1. to compare yields of *Penaeus vannamei*, primary productivity, and water quality in ponds receiving feed or combinations of feed and inorganic fertilizers; and

2. to obtain preliminary data on the impact of feed reductions on shrimp growth.

Materials and Methods

A completely randomized design was used to test 4 treatments, each replicated three times. The experiment was repeated during the wet and dry seasons of the year. The four treatments follow:

1. feed offered at the normal rate (normal-ration);

2. feed offered at half the normal rate (1/2-ration);

3. feed offered at half the normal rate, and inorganic fertilization (1/2-ration & fertilizer);

4. inorganic fertilization during the first eight weeks followed by the normal feeding rate (8-wk fertilization).

P. vannamei juveniles were stocked at 7.5 shrimp/m^2 in earthen ponds ranging from 0.7 to 1.0 ha in area. Shrimp larvae were received from hatcheries and nursed in earthen ponds.

Diammonium phosphate (18-46-0) and urea were applied to give 20 kg N/ha and 4 kg of P/ha per week to inorganic fertilizer treatments. These rates resulted in high primary productivity when used in Honduran freshwater ponds (Teichert-Coddington and Green, 1993). Prior fertilization trials in Choluteca had indicated that higher applications of N (32 kg/ha/wk) were excessive (unpublished data).

Shrimp were fed with a locally fabricated 20% crude protein pellet, which contained sufficient binder to remain stable for a couple of hours in water (Table 1). The total daily food ration was divided into two meals. Shrimp were sampled every week. Feeding rates were adjusted weekly based on average shrimp size. The normal shrimp feeding rate decreased with size and was calculated according to the equation Y = 11.74 - 6.79X, where Y = % of biomass and X = the log of mean individual shrimp weight. All ponds within a treatment were fed the same quantity of feed.

Water was exchanged at 10% of pond volume once a week before fertilization. Water was first discharged and then the pond refilled. If the early morning dissolved oxygen in a particular pond fell below 2.5 mg/l for two days in a row, then another 10% of the water in that pond was exchanged.

Dissolved oxygen (DO) and temperature were measured daily before sunup and in the late afternoon. Water was analyzed weekly for salinity and Secchi disk visibility. The following measurements were taken every two weeks: combined nitrate and nitrite by cadmium column reduction (Parsons et al., 1992), total ammonia (Parsons et al., 1992), chlorophyll *a* Parsons et al., 1992), filterable reactive phosphate (Grasshoff et al., 1983), total alkalinity by titration to 4.5 pH endpoint, total phosphorus, and total nitrogen. Total nitrogen and total phosphorus were determined by nitrate and phosphate analysis, respectively, after simultaneous persulfate oxidation (Grasshoff et al., 1983). Dissolved inorganic nitrogen (DIN) was the sum of combined nitrate-nitrite and total ammonia nitrogen (TAN). Organic nitrogen was calculated as the difference between total nitrogen and DIN. Net primary productivity (NPP) was estimated from the difference between morning and late afternoon DO. This estimate was uncorrected for oxygen diffusion across the air-water interface (Boyd and Teichert-Coddington, 1992); estimates become increasingly conservative as late afternoon DO increased above saturation because of oxygen loss from the pond.

Table 1. Ingredients of 20%-protein pelleted diet.

Ingredient	Proportion of ingredient (%)
Fish meal 65%	15.5
Soy bean meal 48%	-
Middlings	44.8
Poultry by product	-
Corn	31.2
Fish oil	3.5
$CaCO_3$	1.5
Binder	3.2
Vitamins & minerals	0.3
Stay "C"	0.06

Data were analyzed by ANOVA (Haycock et al., 1992). Means were separated by Fisher's PLSD test. This study was carried out under farm conditions, so experimental error was more difficult to control than under experiment station conditions. Differences were therefore declared significant at alpha level 0.10.

Results

Yield, survival, and mean shrimp size were 294%, 36%, and 177% greater during the wet than dry season (Table 2). These seasonal differences were similar to those recorded in past studies (Teichert-Coddington et al., 1994; Teichert-Coddington and Rodriguez, in review).

Wet season

Salinity ranged from 2 to 18 ppt, with a mean of 7.7. Mean alkalinity was 92 mg $CaCO_3/l$. Mean morning and late afternoon water temperatures were 29.2°C and 31.6°C, respectively. One replicate of the normal-ration treatment had very low survival (45%), probably related to stocking error or mortal:' · No stressed or dead shrimp were discovered in the pond during the experimental period, and all other ponds had good survival (>74%). Data from this pond were eliminated from statistical analysis because its low survival was probably not related to treatment.

Table 2. Yields and profitability of *P. vannamei* stocked at 7.5/m^2 in earthen ponds. Shrimp were fed normally (Feed); fed half the normal rate (1/2 Feed); fed half the normal rate and ponds fertilized inorganically (1/2 Feed & Fertilizer); or ponds fertilized inorganically for 8 weeks and then shrimp fed at normal rate (Fertilizer then Feed).

Treatment	Yield (kg/ha)	Mean size (g)	Survival (%)	Feed efficiency (feed/yield)	Costs ($/ha)			Net ** income ($/ha)
					Feed	Fertilizer	Total	
Wet season								
Feed	1258ab	18.1a	96a	2.34a	813	0	813a	3999ab
1/2 Feed	1075a	15.1a	95a	1.37bd	397	0	397b	3173b
1/2 Feed & Fertilizer	1470b	20.0a	98a	1.02c	411	189	599c	5335a
Fertilizer then Feed	1269ab	19.8a	87a	1.34d	470	101	570c	4628a
Dry season								
Feed	360a	6.7a	74a	4.44a	441	0	441a	283a
1/2 Feed	314a	6.8a	67a	2.97b	227	0	227b	444a
1/2 Feed & Fertilizer	314a	7.0a	67a	2.80b	237	151	388c	309a
Fertilizer then Feed	299a	6.0b	68a	2.82b	233	88	321d	254a

** Calculated from difference of income at the processing plant and feed and fertilizer costs.

a,b Within season means followed by the same letter are not different (P < 0.10).

There were no significant treatment differences for survival or mean size. Mean yield for the 1/2-ration & fertilizer treatment was significantly greater than the 1/2-ration treatment. Otherwise, there were no significant treatment differences for yield. Feed efficiency was significantly lower in the normal-ration treatment than in the other treatments. The most efficient treatment was 1/2-ration & fertilizer, where feed efficiency approached 1.0.

The duration of this study was 16.5 weeks, but shrimp apparently stopped growing in all treatments by the 12th week (Figure 1). Growth in the 1/2-ration treatment became notably lower than the other treatments after the 8th week. At the same time, growth in the 8-wk fertilization treatment appeared to increase as feeding commenced in that treatment.

Water quality treatment differences were modest (Table 3). Filterable phosphate and DIN were significantly greater in fertilizer treatments than in treatments receiving no fertilizer. However, chlorophyll *a* and organic N were not significantly different among treatments. Chlorophyll *b* was significantly highest in the 1/2-ration & fertilizer treatment, but mean treatment differences were low; chlorophyll *b* was usually 10% or less of chlorophyll *a*. Total P was significantly greater in the fertilizer treatments, but differences are attributable to higher filterable phosphate levels, not to organic matter. Net primary productivity was significantly higher in the 1/2-ration & fertilizer treatment (5.8 mg O_2/l/d) than the normal-ration (4.4 O_2/l/d) or 1/2-ration (4.8 O_2/l/d) treatments.

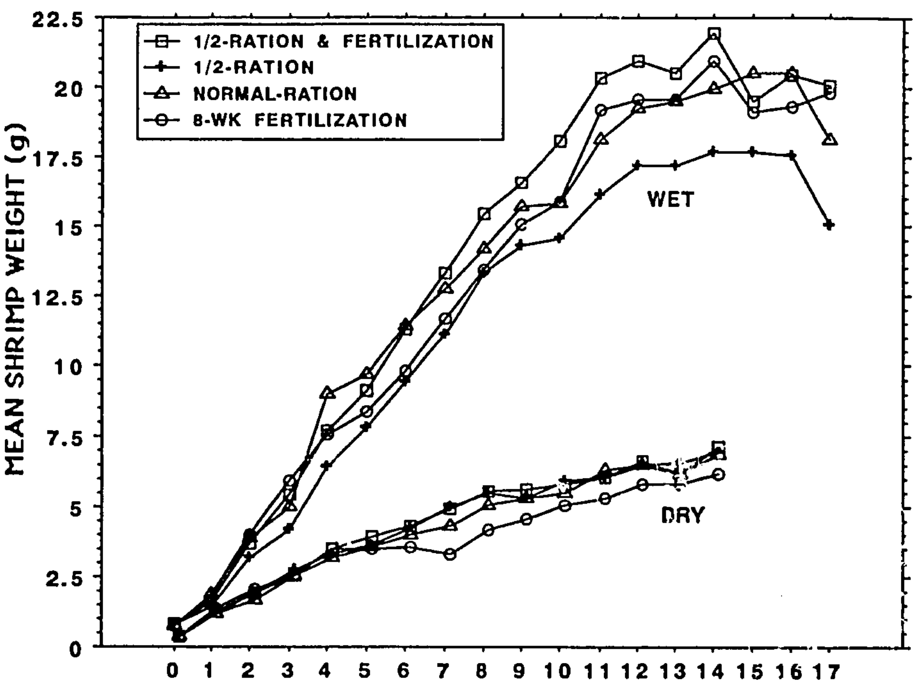

Figure 1. Mean treatment shrimp weights during wet and dry season culture in Honduras.

Table 3. Wet and dry season means of water quality variables in ponds stocked with *P. vannamei* at 7.5/m^2. Shrimp were fed normally (Feed); fed half the normal rate (1/2 Feed); fed half the normal rate and ponds fertilized inorganically (1/2 Feed & Fertilizer); or ponds fertilized inorganically for 8 weeks and then shrimp fed at normal rate (Fertilizer then Feed).

Treatment	Morning DO (mg/l)	Afternoon DO (mg/l)	Secchi disk (cm)	Chlorophyll *a* (µg/l)	Chlorophyll *b* (µg/l)	Total P (mg/l)	Filterable phosphate-P (mg/l)	Organic N (mg/l)	Dissolved inorganic N (mg/l)	Total water exchange (% volume)
Wet season										
Feed	4.7a	9.1a	43ab	86a	8.5a	0.26a	0.090a	1.46a	0.025a	340a
1/2 Feed	5.2ab	10.0b	39a	109a	10.8a	0.22a	0.042a	1.60a	0.037a	343a
1/2 Feed & Fertilizer	5.7b	11.4c	52c	116a	16.4b	0.34b	0.158b	1.59a	0.082b	340a
Fertilizer then Feed	5.6b	10.4bd	49bc	79a	11.2a	0.36b	0.180b	1.48a	0.095b	350a
Dry season										
Feed	5.8a	9.3b	54b	108ab	14.3ac	0.28a	0.124a	1.32a	0.004a	130a
1/2 Feed	5.9a	8.8a	55b	79a	11.8a	0.27a	0.117a	1.22a	0.005a	130a
1/2 Feed & Fertilizer	5.7a	11.0c	47a	182c	24.2b	0.59b	0.277b	2.45c	0.040b	173b
Fertilizer then Feed	6.4b	11.1c	46a	136b	18.8bc	0.40c	0.143a	2.00b	0.015ab	130a

a,b Within season means followed by the same letter are not different ($P < 0.10$)

Dry season

Salinity ranged from 22 to 34 ppt, with a mean of 27.4 ppt. Salinities were lower than usual for this time of year because of unusually late rainfall. Mean alkalinity was 155 mg $CaCO_3$/l. Mean morning and late afternoon water temperatures were 27.3°C and 29.6°C, respectively. There were no significant treatment differences for yield, mean shrimp size, or survival. Feed efficiency was significantly less for the normal-ration treatment compared with the other treatments. Shrimp growth was similar in all treatments until after the 5th week, when the 8-wk fertilization treatment started to slow. Commencement of feeding after the 8th week in that treatment appeared to stimulate growth. The study was terminated after the 14th week because of low weekly weight gains.

Primary productivity was higher in the fertilized treatments. Mean chlorophyll values, organic nitrogen, and organic phosphorus were significantly higher, and Secchi disk visibilities were significantly lower in the 1/2-ration & fertilizer treatment (Table 3). Net primary productivity was significantly higher in the 1/2-ration & fertilizer treatment (5.3 mg O_2/l/d) than in the normal-ration (3.6 O_2/l/d) or 1/2-ration (2.9 O_2/l/d) treatments.

Discussion

Lower yields and mean size were obtained in this study than in a study conducted one year earlier with the same feed and shrimp stocking rates ranging from 5 to 11 shrimp/m^2 (Teichert-Coddington and Rodriguez, in review). In the earlier study, yields averaged 1782 kg/ha and 508 kg/ha during the wet and dry seasons, respectively, and mean sizes averaged 23.2 g and 9.6 g during the wet and dry seasons, respectively. Yield differences were likely due to climatic variation.

Fertilizers are applied to augment the supply of nutrients for phytoplankton growth. Phosphorus is more often limiting in freshwater than nitrogen, but the opposite may be true for brackish water (Boyd, 1990). Insufficient research in brackish water fertilization has been conducted to recommend specific fertilization regimes. Boyd (1990) reviewed several regimes in use by Ecuadorian shrimp farmers. Equivalent weekly application rates ranged from 2.6 to 21 kg N/ha, and from 0.5 to 2.6 kg P/ha. The proportion of diatoms, a preferred algal species by shrimp culturists, may be increased by silicate fertilizers and N:P ratios exceeding 15:1 (Daniels and Boyd, 1993). In previous shrimp pond fertilization experiments at Choluteca, combined weekly applications of nitrogen and phosphorus ranging from 0 to 8 kg N/ha and 0 to 3 kg P/ha had no significant effect on either pond fertility or shrimp growth during the dry season (Green and Teichert-Coddington, 1990). In a subsequent dry season pond trial (unpublished data), nitrogen as diamonium phosphate and urea were applied weekly at 0, 4.5, or 32/ha, and phosphorus was applied at 0, 4.5, and 4.5 kg/ha, respectively. Primary productivity was significantly higher at the high N rate, but there were no significant shrimp yield differences among treatments. Total ammonia rose to levels higher than 0.6 mg/l at the high N treatment, indicating that excessive N was being applied. Weekly nitrogen application was therefore reduced to 20 kg/ha for this study and P was applied to maintain an N:P ratio of 5:1.

The effects of fertilization on water quality were manifested principally in higher concentrations of filterable phosphate and DIN in fertilized treatments. Mean DIN was relatively low (< 0.10 mg/l), especially during the dry season, indicating that a higher N application rate would have been tolerated. On the other hand, mean filterable phosphorous was high, especially during the dry season, indicating that the application rate should have been lower. A more ideal fertilization regime may have been to apply 25 kg N/ha/wk and 2.5 kg P/ha/wk to maintain an N:P ratio of about 10:1.

Algal productivity during the wet season was significantly higher in fertilized treatments only in terms of net primary productivity and chlorophyll *b*. Secchi disk visibility was actually greater in fertilized than in non-fertilized treatments. The effects of fertilization on primary productivity were more notable during the dry season. The basis for different seasonal responses to fertilization is unclear. They might have been related to the quality of incoming estuarine water, although examination of estuarine water quality data (Table 4) does not support this hypothesis. Total P in these waters was similar during both seasons, and organic N and chlorophyll *a* were higher during the dry season; in ponds, however, non-fertilized treatments had similar chlorophyll *a* values during both seasons and higher organic N levels during the wet season. An alternative explanation is that greater rainfall and lower evaporation rates during the wet season resulted in higher mean pond water levels, thereby diluting nutrient concentrations.

Fertilization had no affect on shrimp production during the dry season despite increasing primary productivity. An earlier experiment on different dietary protein levels (Teichert-Coddington and Rodriguez, in review) had demonstrated that there was no difference in shrimp growth due to dietary protein level during the dry season. Natural pond production would be expected to supply shrimp with missing nutrients if the offered diet was deficient. Apparently neither natural pond productivity nor applied nutrition limited dry season shrimp growth. Much of the feed applied at the normal rate was wasted, as indicated by the poor feed conversion (4.44). Teichert-Coddington et al. (1994) hypothesized that temperature was a primary factor limiting dry season shrimp growth and that nutritional improvement would consequently be ineffectual in improving growth. The results of this study support that contention.

Table 4. Mean water quality values for incoming estuarine water. The number of measurements taken during wet and dry seasons were 28 and 20, respectively.

Season	Total N (mg/l)	Organic N (mg/l)	DIN (mg/l)	Total P (mg/l)	Filterable phosphate (mg/l)	Chlorophyll *a* (µg/l)	Chlorophyll *b* (µg/l)	Settleable solids (ml/l)
Wet	1.07	0.75	0.32	0.22	0.115	22.7	4.4	1.7
Dry	1.33	1.13	0.19	0.23	0.101	52.6	7.3	1.7

Reducing the feeding rate by half resulted in insignificant yield reductions during both seasons. Although it was statistically insignificant, a mean yield reduction of 15% during the wet season resulted in an income reduction of 21% ($826/ha) after subtracting feed costs (Table 2). Rations should therefore not be reduced by half during the wet season. On the other hand, feeding rates could be reduced by at least half during the dry season with comparatively higher profitability (Table 2). Relatively poor dry season feed conversion at the 1/2-ration (2.97) indicated that even further feed reductions might be implemented.

Feed efficiency could be improved during both seasons without resorting to drastic universal reductions in feeding rate. Similar initial growth during both seasons in all treatments indicated that feed inputs could be reduced or eliminated during the first 4 to 6 weeks in fertile ponds when shrimp are small and have adequate natural pond production on which to graze. Alternatively, fertilizers might be substituted for feed to augment natural pond fertility during the initial growing period. Teichert-Coddington et al. (1991) demonstrated that moderate organic fertilization (220 kg chicken litter/ha/wk) during the first 8 to 9 weeks, or low organic fertilization during the first 4 weeks, instead of feeding, had no detrimental affect on shrimp yields, but increased feed efficiency. In this study, application of inorganic fertilizer during the first 8 weeks followed by normal feeding resulted in shrimp yields that were similar to those in the normal-ration treatment. This study was not designed to demonstrate an optimum fertilizing regime, so it is unclear how best to apply fertilizers. Empirical data from some shrimp farms indicate that fertilization would initially be unnecessary to maintain pond fertility for good growth. Incoming estuarine water is sufficiently fertile to maintain good plankton blooms.

Reduction of waste discharge is a concern of Honduran shrimp producers. Organic wastes could be reduced by reducing feed inputs. Use of inorganic fertilization to reduce feed inputs may actually increase effluent BOD, P, and N, in the forms of algae and waste nutrients. Fertilization should not be used if it does not demonstrate a clear benefit to shrimp production. Wet season yields were increased by decreasing feed by half and fertilizing. More research should be done to refine fertilizer usage on farms receiving fertile estuarine water, as well as on those that are supplied with clear embayment or marine water. Fertilization should be avoided during the dry season, because shrimp production was not increased by fertilizer application. Water quality of riverine estuaries in Choluteca becomes degraded during the dry season as freshwater inputs diminish (Teichert-Coddington, 1995). Avoiding the use of fertilizers during the dry season would help maintain good estuarine water quality in addition to reducing input costs.

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Timing of Supplemental Feeding for Tilapia Production

Work Plan 6, Thailand Study 7

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Introduction

Tilapia culture in Thailand is commonly done in semi-intensive systems with fertilization. Additionally, feed may be provided for part or all of the grow-out period to increase carrying capacity and growth of fish. Earlier CRSP experiments indicated that supplemental feeding in fertilized ponds resulted in significantly higher growth rates and larger yields (Diana et al., 1995). These experiments indicated that feeding rates of 50 to 100% ad libitum, combined with optimal CRSP fertilization protocols, resulted in similar growth rates and yields (Knud-Hansen et al., 1991). Fish in such systems grew to 500 g in 160 days, and yields approached 10,000 kg ha^{-1}. Early tilapia growth differed significantly in these feeding treatments, and it was unclear whether critical standing crop was ever reached or whether it occurred in the first month of grow out.

Hepher (1978) proposed the concepts of critical standing crop and carrying capacity as mechanisms to regulate and understand fish production in aquaculture ponds. According to his data from carp ponds, fish growth was similar in early grow out stages regardless of inputs. However, once the critical standing crop was reached, growth declined. This growth could then be stimulated by increasing inputs, such as moving from fertilization alone to supplemental feeding. Data collected so far from CRSP experiments do not clearly show the existence or the timing of this growth decline for tilapia. The purpose of this experiment was to determine the optimal time for starting supplemental feeding for tilapia in fertilized ponds, as one means of further determining the relationship between growth and critical standing crop.

Materials and Methods

The experiment was conducted in 15 ponds at Bang Sai. Each feeding treatment was done in triplicate. The five treatments varied in the size of fish at first feeding, with first feeding at (A) 50 g, (B) 100 g, (C) 150 g, (D) 200 g, and (E) 250 g. These treatments resulted in different timing for first feeding and harvest for each treatment. Sex-reversed Nile tilapia were stocked on 15 January 1993. Stocking density was 3 fish/m^2 (750 fish per pond) and size at stocking averaged 11 g. In all treatments, fish were reared until the preset target weight was reached. All ponds were fertilized weekly with 1.5 kg urea and 0.85 kg triple super-phosphate (60 and 34 kg ha^{-1} wk^{-1}, respectively). Every two weeks, 40 fish from each pond were sampled, individually weighed (to 1 g), and measured in length (to 1 mm). The growth rate during each sampling period was calculated as the increase in weight per day. Once the average size of fish in a treatment approximated the target weight, feeding commenced. Floating feed was provided to each pond, and maximum food consumption was determined by the total amount of feed eaten during 4 h in the morning and 2 h in the afternoon. Initially, feeding rate data were collected for each pond. As the experiment progressed and more ponds reached the size for feeding, determination of individual feeding rates for each pond was too time consuming. Therefore, for the remainder of the experiment, maximum feeding rate was determined once a week for all three ponds in a treatment, and the average rate was subsequently applied to the three ponds. Feeding was continued until the fish in all ponds of a particular treatment averaged 500 g, then all the ponds of that treatment were harvested.

The chemical and physical conditions in the ponds were also monitored. All standard CRSP parameters were measured biweekly. Weather parameters were measured daily. Diel cycles of temperature, oxygen, alkalinity, and pH were measured monthly.

Statistical analyses were conducted using SYSTAT (Wilkinson, 1990). Overall growth (g per day), net yield (kg/ha), and daily yield (kg/d) were calculated for each pond. Average overall values for physical and chemical parameters, total days of culture, total days of feeding, total food input, and total fertilizer input were also calculated. Multiple regressions between growth and design variables (feed input, days of culture) were performed to test main effects. Because many of the chemical variables were interrelated, residuals of the above regressions were correlated to each physical or chemical variable. Significantly correlated variables were then examined for autocorrelation, and multiple regression analysis was performed with acceptable variables to evaluate determinants of fish growth. Variables were included in the regression if $p < 0.10$. The biweekly data set was used to determine treatment effects on fish or chemical variables using ANOVA and Tukey's multiple range test. Differences were considered significant at an alpha level of 0.05.

Results

Initial growth rate trajectories were similar among all treatments. Once feeding commenced, fish growth rate in each treatment increased dramatically (Figure 1). Most treatments showed parallel rates of growth under fertilization conditions, and also parallel rates of growth under feeding conditions. Fish in treatments A and B both reached similar weights at harvest, and both were harvested at the same time. Fish in treatment C had slightly slower growth than did fish in treatment D under fertilization conditions, but the growth of fish in treatment C rapidly exceeded that of those in treatment D once feeding was initiated. Fish in treatment E took a very long time to reach 200 g (their target weight for first feeding).

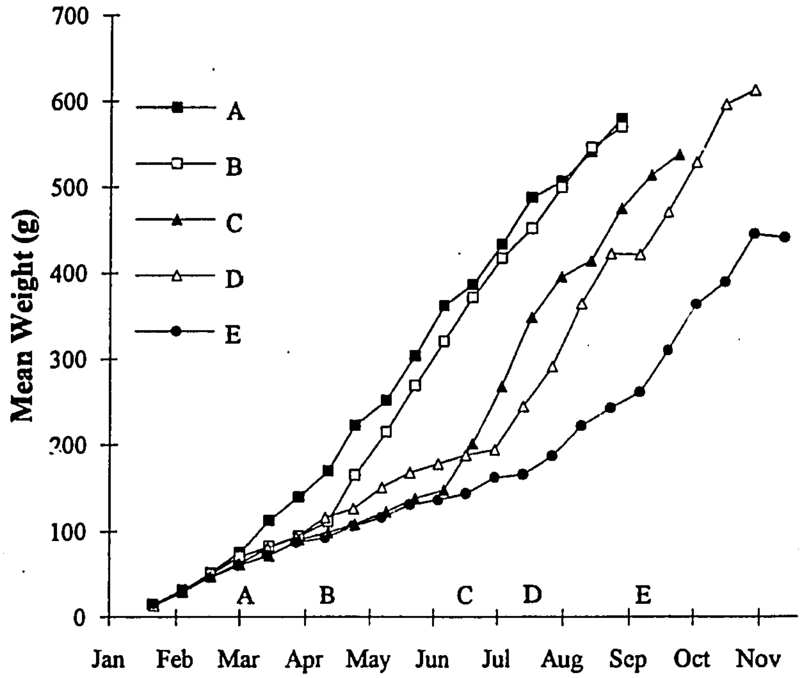

Figure 1. Weekly mean weight for each treatment. The letters A - E on the x axis indicate dates when supplemental feeding was initiated for treatment.

First feeding occurred between one and eight months after stocking for various treatments (Table 1). While fish were stocked at similar sizes, their sizes diverged early after the initiation of supplemental feeding, and both size and growth rate varied with treatment (Tables 2, 3). Growth rates were significantly different during the feeding stage and the fertilizer only stage of the experiments, but were not significantly different between treatments within a stage.

Table 1. Sizes and dates of first feeding and harvest in each experimental pond.

	Mean Size (g)			Number of Days		
Pond	Stocking	First feeding	Harvest	No feeding	Fed	Total
A1	14.0	64	547	38	198	236
A2	14.9	42	657	38	198	236
A3	15.1	50	574	38	198	236
B1	15.7	160	686	80	156	236
B2	15.3	193	523	80	156	236
B3	15.2	142	579	80	156	236
C1	15.9	140	534	153	112	265
C2	15.1	179	606	153	112	265
C3	14.3	124	462	153	112	265
D1	15.9	161	584	178	127	305
D2	14.0	202	640	178	127	305
D3	14.0	219	657	178	127	305
E1	14.8	273	502	234	94	328
E2	15.9	269	553	234	94	328
E3	14.0	107	410	234	94	328

Table 2. The biomass, number, and mean size of tilapia harvested from each pond.

Pond	Biomass (kg)	Number	Size (g)
A1	334.0	611	547
A2	427.4	651	657
A3	393.3	685	574
B1	455.7	664	686
B2	342.7	655	523
B3	354.4	612	579
C1	319.3	598	534
C2	371.2	613	606
C3	316.0	684	462
D1	373.3	639	584
D2	422.7	660	640
D3	223.9	341	657
E1	333.5	664	502
E2	377.2	682	553
E3	260.8	636	410

Table 3. Culture period (d), growth rate (g d^{-1}), survival (%), yield (kg ha^{-1}), and forecasted annual yield (kg ha^{-1} yr^{-1}) for tilapia from each pond.

Pond	Duration	Growth	Survival	Yield	Annual Yield
A1	230	2.32	81.5	12,940	20,535
A2	230	2.79	86.8	16,648	26,420
A3	230	2.43	91.3	15,280	24,249
B1	230	2.92	88.5	17,756	28,178
B2	230	2.21	87.3	13,248	21,024
B3	230	2.45	81.6	13,720	21,773
C1	259	2.00	79.7	12,296	17,328
C2	259	2.28	81.7	14,396	20,288
C3	259	1.73	91.2	12,212	17,210
D1	299	1.90	85.2	14,456	17,647
D2	299	2.10	88.0	16,488	20,127
D3	299	2.15	45.5	8,536	10,420
E1	322	1.51	88.5	12,896	14,618
E2	322	1.67	90.9	14,612	16,563
E3	322	1.23	84.8	10,012	11,349

Fish parameters were sometimes correlated with treatments. Absolute growth, survival, and yield were not significantly different among treatments (ANOVA, $p > 0.05$). However, both growth and yield rates (g d^{-1} and kg d^{-1}, respectively) were significantly higher in the two earliest fed treatments than in the latest fed treatment.

Multiple regression analyses indicated that growth rate (g d^{-1}) was significantly correlated with the main experimental variables of days of feeding and input of feed (Table 4). The regression explained 73.8% of the variation in growth rate. Residuals of this regression were significantly correlated with alkalinity ($r = 0.672$, $p = 0.006$), chlorophyll *a* content ($r = 0.734$, $p = 0.002$), total inorganic nitrogen ($r = -0.699$, $p = 0.004$), and dissolved oxygen ($r = 0.628$, $p = 0.012$). Alkalinity and chlorophyll *a* were strongly correlated to one another ($r = 0.862$, $p = 0.001$), so only chlorophyll *a* was used in the overall regression. The final regression with fish growth included feeding rate, days of culture, and chlorophyll *a* content, and explained 88.9% of the variance in growth (Table 4).

Some chemical parameters varied among treatments in this experiment. Chlorophyll *a* content and alkalinity both differed significantly among treatments (ANOVA, $p < 0.05$), with the latest-fed treatment (E) being significantly lower in both values than the next to last-fed treatment (D). Also, pH differed significantly, with the three earliest-fed treatments having lower pH values than the two last-fed treatments.

Table 4. Results of multiple regression analyses for fish growth (g d^{-1}).

Variable	Coefficient	P
	Main effects - $R^2 = 0.738$, $p = 0.001$	
Constant	2.799	0.008
Feed Input	0.000	0.028
Days	-0.006	0.009
	Overall Effects - $R^2 = 0.889$, $p = 0.001$	
Constant	3.117	0.001
Feed input	0.000	0.006
Days	-0.007	0.002
Chlorophyll *a*	0.001	0.097
Dissolved oxygen	0.074	0.290
Total inorganic nitrogen	-0.057	0.611

Discussion

The results of this experiment were close to expectations, with a slower growth rate during the fertilization only stage than after feeding commenced. Once fish were fed, the growth rates reached similar levels, regardless of the duration of prior fertilization. These results indicate that critical standing crop of tilapia must occur during the first month of culture. If critical standing crop had not occurred prior to the first initiation of feeding, then the fish should have maintained the same growth rate for some time after feeding in the first treatment started. Since growth increased dramatically in treatment A after feeding started, critical standing crop either does not exist for tilapia or had already occurred. No decline in early growth is evident over the first months of culture, so if critical standing crop had already occurred, it must have occurred before the first sampling period.

Supplemental feeding resulted in much more rapid fish growth in all treatments than did fertilization alone. However, the timing of supplemental feeding affected the overall growth rate. The first two treatments, fed after 38 and 80 days, respectively, ultimately resulted in the same size of tilapia at harvest. All subsequent feeding treatments required longer times to reach harvest size. Feed conversion rates averaged 1.033 for all treatments combined, and there were no significant differences among treatments in conversion efficiency. However, treatments fed earlier in the cycle had a larger portion of the fish growth occur under feeding regimes than under fertilization regimes, and reached harvest size sooner. The total amount of food applied differed significantly among treatments (ANOVA, $p < 0.05$), with treatment E requiring the least feed, then C, then B and D (which were not significantly different), and A requiring the most feed. In terms of overall efficiency including time, treatment C required limited feed input and only slightly longer time than A or B to reach harvest. Thus, it appears from this analysis that supplementally feeding fish once they reached 150 g was the most effective means to reach larger size at limited costs. However, this analysis of efficiency would benefit greatly from an economic analysis, since time to harvest, size at harvest, cost of feed, and cost of fertilizer all vary in this analysis and all affect efficiency.

Acknowledgments

Wattana Leelapatera aided in data collection and provided logistical support for this study.

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Stocking Density and Supplemental Feeding in Tropical Fish Ponds

Work Plan 6, Thailand Study 6

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Introduction

Pond carrying capacity is mainly determined by management practices. Earlier CRSP work on semi-intensive culture of tilapia using manure or inorganic fertilizers indicated that carrying capacity might reach 2,000 to 3,000 kg ha^{-1} (Diana et al., 1991a, b). As stocking density is increased in these fertilized ponds, carrying capacity remains nearly the same and density-dependent growth occurs (Diana et al. 1991b). Thus the ultimate size of fish at harvest is related mainly to density stocked in fertilized ponds, while biomass at harvest is more consistent regardless of stocking density. Maximum size at harvest for these fertilized ponds is approximately 250 g for fish grown five months.

Increasing the carrying capacity or size at harvest of tilapia requires more intensive management, which usually involves supplemental feeding. Earlier CRSP experiments with supplemental feeding indicated that fish can reach 500 g in 5 months when feed and fertilizer are provided in combination (Diana et al. 1995). Those experiments were done at fish densities of 3 fish/m^2, which would cause density related declines in fish growth in fertilized ponds. However, the addition of supplemental feeds can increase the growth rate of fish stocked at high density and result in a higher carrying capacity for the pond. The limit on such feeding and density increases would occur when conditions in the ponds reach limiting levels due to increased oxygen demand, build up of metabolites, or other factors which produce poor water quality. The purpose of this experiment was to determine the upper limits to tilapia production utilizing supplemental feeds.

Materials and Methods

Sex-reversed Nile tilapia were stocked into 9 ponds on 11 November 1993. The ponds were divided into three treatments, which received either 3, 4.5, or 6 fish/m^2 (750, 1125, or 1500 fish per pond). Fish were fed to satiation daily. The satiation ration was determined once a week using floating pellets. The average satiation ration for all ponds in a treatment was used for the remaining days of a week.

In addition to feeding, ponds were also fertilized weekly to bring N and P additions to 5 and 1.5 kg ha^{-1} d^{-1}, respectively. This required the addition of 54 kg of urea and 31 kg of triple superphosphate per hectare on a weekly basis.

Physical, chemical, and biological characteristics of the ponds were determined using standard CRSP protocols. Intensive samples were collected biweekly during the study, including diel studies. Ponds were harvested on 15 July 1994, after 242 days of culture.

Results and Discussion

This experiment failed to produce consistent numbers of fish at harvest. The ponds with poor fish returns were all located near the periphery of the station, and we strongly believe that poaching had occurred in these ponds. Evaluation of the growth, survival, and yield of each pond bears out this conclusion (Table 1). There was no evidence of mortality (which could have occurred sporadically in the ponds by disease) since no dead fish were found in the ponds. We will redo this experiment during 1994-95, and hope to use other ponds or increase security.

Growth of fish in all treatments was similar, and the only decline in growth occurred during the last time period when harvest data were used to estimate size (Figure 1). These declines were probably due to sampling differences, not due to carrying capacity. Due to intensive supplemental feeding, there were no density effects on growth rate of the fish.

During June and July, water conditions in the ponds which had expected survival rates (ponds A1, A2, B1, B2, and C3) were examined to determine if water quality deteriorated. Oxygen at the bottom (3.53 mg l^{-1}) and temperature (29.5°C) were normal, while pH (8.51), ammonia levels (1.02 mg l^{-1}) and nitrite levels

Table 1. Growth (g per fish), survival, biomass at harvest (kg), and net yield (kg ha^{-1}) for each of 9 ponds used in this experiment.

Treatment	Density	Growth	Survival	Biomass	Yield
A1	3	572	0.7000	304.4	11,928
A2	3	521	0.7053	279.8	10,952
A3	3	479	0.2387	87.4	3,232
B1	4.5	487	0.7333	408.8	15,960
B2	4.5	429	0.7138	351.3	13,668
B3	4.5	446	0.1467	74.9	2,636
C1	6	567	0.1160	100.2	3,504
C2	6	506	0.1333	103.0	3,568
C3	6	446	0.7033	481.0	18,672

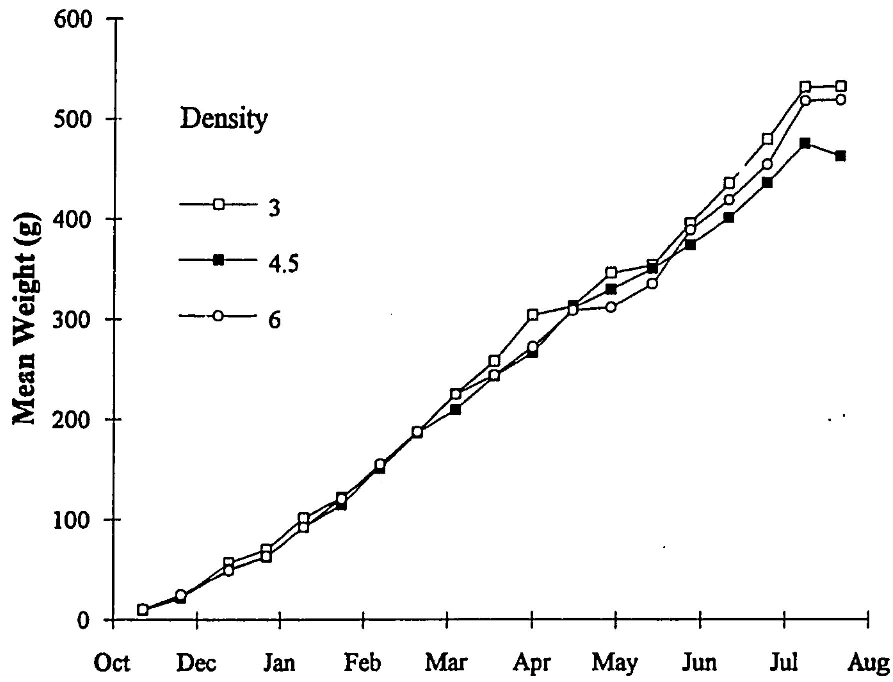

Figure 1. Weekly average weight of fish in each treatment.

(0.21 mg l^{-1}) were high. These latter conditions combined may be considered low water quality, especially in comparison with initial levels. For November, the latter 3 variables averaged 7.8, 0.5, and 0.1, respectively. However, pond water quality did not appear poor enough to cause mortality or reduced growth.

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Supplemental Feeding of Tilapia in Fertilized Ponds

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Abstract

The addition of feed to fertilized fish ponds was evaluated by adding feed alone, feed plus fertilizer, or fertilizer alone to nine ponds stocked with Nile tilapia Oreochromis *niloticus*. Two experiments were conducted. The first had 500 fish per 250 m^2 pond in 3-treatments: ad-libitum feeding; fertilizer only; or fertilizer and ad-libitum feeding. The second experiment had 5 treatments with 750 fish per pond: ad-libitum feed only; fertilizer only; or 0.25, 0.50, and 0.75 satiation ration plus fertilizer. Ponds in Thailand were maintained for 155-162 d, during which chemical and physical properties were monitored. In experiment 1 tilapia growth was highest in feed only ponds, and lowest in fertilizer only ponds. Net yield did not differ significantly among treatments, due to variation in survival. In experiment 2, tilapia growth was lowest in fertilizer only ponds, intermediate in 0.25 ration ponds, and highest in 0.50, 0.75, and ad-libitum ponds. The latter treatments were not significantly different. Multiple regressions for each experiment indicated only 47-87% of the variance in growth was explained by feed and fertilizer input, while 52-89% of the variance in yield was explained by those factors. For both experiments combined, 90.3% of the variance in growth was explained by feed input, fertilizer input, alkalinity, and total inorganic nitrogen concentration. For yield, R^2 was 0.888 and the regression included feed input, pH, and number of low dissolved oxygen events. Experiment 1 appeared to approach carrying capacity near the end, while no reduction in growth occurred in experiment 2 at higher fish density and biomass. Reductions in growth in experiment 1 were not correlated with declining water quality late in the grow out. Combinations of feed and fertilizer were most efficient in growing tilapia to large size (500 g) compared to complete feeding or fertilizing alone.

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UNITED STATES: DATA ANALYSIS AND SYNTHESIS

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 U.S. 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 U.S. and instructed the CRSP to support research activities at the U.S. institutions. While various studies related to the Global Experiment and biotechnological research are conducted at U.S. 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, Sixth, and Seventh 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 University of California DAST team has been developing a stochastic model of temperature in stratified ponds. They also field-tested a respirometer; however, they found that after successful initial data collection, fouling problems limited the use of the new equipment. The decision-support system POND, which has been the focus of the Oregon State University DAST team, includes simulation models to describe the dynamics of fish growth, water temperature, carbon, nitrogen and phosphorus, and phytoplankton, and zooplankton, as well as an economics program. A summary of current Central Data Base activities and a description of the decision support system POND are provided under the heading *Global Studies and Activities*.

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 of Oregon State University (OSU) and Dr. Raul H. Piedrahita at the University of California, Davis (UCD). Their efforts are focused on the following areas of activity:

1. Continued development and validation of simulation models for fish growth, water temperature, carbon, nitrogen and phosphorus, phytoplankton and zooplankton (OSU).

2. Implementation of expertise, an economics package, and simulation models for pond aquaculture analysis in the form of a decision support system POND (OSU).

3. Development of improved methods of modeling and optimizing oxygen regimes in ponds (UCD).

4. Development of models for stratified ponds (UCD).

During this period, work on the decision support system POND has been completed. POND was developed to provide the aquaculture community with a tool for rapidly analyzing warm water aquaculture systems, and to assist in developing optimal management strategies. Pond analysis is accomplished primarily by the use of simulation models combined with an economics package that can be used to generate enterprise budgets for a pond facility. POND can be used to set up pond facilities with different configurations and/or management strategies. Multiple simulations can be conducted to examine the effects of various pond management scenarios on fish yields and facility-level economics. The software features several easy-to-use databases for entering data specific to a facility/site and a graphics module for viewing the results of simulation runs. On-line help is also available. Approximately 80 copies of POND Version 2.0 have been distributed to a wide audience including extension agents, educators, producers, and researchers.

The UCD team concentrated its efforts on further improving existing models of oxygen dynamics and developing new models for stratified ponds. Current understanding of oxygen dynamics is hampered by insufficient information about diel respiration patterns. To collect the missing information, a respirometer has been developed for use in aquaculture ponds. Field tests showed that respiration rates change substantially over diel periods, with the highest rates occurring in the late afternoon. Model development for stratified ponds focused on water temperature behavior. A computer model using stochastic inputs of solar radiation, wind direction, and wind speed has been developed. Simulations, carried out for Thailand ponds, showed that surface temperatures exhibited the largest temperature fluctuations in response to stochastic inputs.

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.

Respiration Dynamics in Aquaculture Ponds

Work Plan 7, DAST Study 1

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Introduction

Modeling and prediction of dissolved oxygen (DO) in aquaculture ponds are based on estimates of oxygen production and consumption rates. There is a great deal of information on net photosynthetic oxygen production rates, but relatively little data are available on oxygen consumption rates by respiration, or on gross production rates. Estimating water column respiration rates (WCR) is particularly difficult during daylight hours. A respirometer has been designed and used to provide estimates of water column respiration at intervals of approximately 20 minutes. The respirometer consists of a chamber in which water samples are darkened and a device to measure the rate of dissolved oxygen concentration decline. The process of sample collection and analysis is fully automated and is linked to a computer for immediate data analysis. The respirometer as well as some results obtained with it, are described in this report.

Oxygen data collected from a pond provide only a measure of net photosynthetic production rates (the sum of gross production and water column respiration) and diffusion during daylight periods, and water column respiration and diffusion rates during the night. However, the estimation of gross primary production rates requires knowledge about the rates of oxygen consumption (respiration) in the pond. Normally, nighttime respiration rates, calculated from night DO decline corrected for diffusion, are used as estimates of phytoplankton respiration and are combined with measured net production rates to provide estimates of gross photosynthetic production rates (Weger et al., 1989). This is often done by averaging or extrapolating the dark respiration measurements, usually with the inclusion of a temperature compensation function (Zison et al., 1978; Groden, 1977; Riley, 1963).

Previous research (Giovannini and Piedrahita, 1990) has examined the accuracy of temperature functions in accounting for observed changes in rates of DO decline. Although a moderate correlation was observed between nighttime respiration rates and water temperatures, extreme change of the respiration rates with minor changes in observed temperature lead to the conclusion that respiration rates calculated with commonly used temperature dependent functions do not accurately portray actual respiration processes in these ponds. These results are consistent with those obtained by Teichert-Coddington and Green (1993) who noted that diurnal WCR was "primarily dependent on a factor other than temperature."

It is apparent that water column respiration rates cannot be described solely by empirical temperature dependent functions. Equally problematic is the common practice of extrapolating night time pond respiration rates to daytime periods. The most common method of determining respiration rates during the light period is by using dark bottles. Unfortunately, when long incubation times (> 1 h) are used, this results in an inability to observe enhanced respiration rates that may be present during the light period and shortly after darkening, and may significantly underestimate the actual respiration rate. The effect of incubation time has also been observed between incubations of two, six, and eight hours duration by Teichert-Coddington and Green (1993), who noted consistently higher rates in the shorter daytime incubations.

The recent development of a respirometer system for measuring water column respiration allows estimates of community respiration rates in aquaculture ponds continuously over diel periods by quickly darkening and incubating pond water samples containing actively photosynthesizing algal cells. By measuring the rate of O_2 consumption during a short period after darkening, it is possible to get a far more accurate estimate of the total community respiration rate during the light period than with techniques that require long incubation times. Preliminary results from this initial effort were published by Szyper et al. (1992).

Materials and Methods

The initial work on the design of the respirometer system was described by Piedrahita and coworkers in the Sixth Work Plan (PD/A CRSP, 1991). At that time the design criteria called for a very simple system with a high degree of accuracy that could be employed at field sites without external A/C power. Cooperators at the University of Hawaii showed the viability of the concept in a pond using a system based on measurements made with a conventional DO probe (YSI Model 5739) with sample stirrers and external pumps to supply periodic water samples. A complete description of this system is given in Szyper et al. (1992).

Building on the success of the Szyper system, a respirometer system was designed that addressed areas in need of improvement and added a control component for automatic processing of data. The resulting system (Figure 1) incorporates an external 12V centrifugal submersible pump (Rule Industries, Model 800) for supplying periodic water samples to the incubation chamber, and utilizes a small internal pump (Aquarium Systems Model IP67 Mini-Jet) to circulate water past a YSI (Model 5739) DO probe. The sample chamber is fairly large (2 liters) to reduce the effects on water quality of biological fouling on surfaces. One-way check valves (Hayward Model TC10075ST Check Ball Valve) are used to retain the water sample in the incubation chamber after pumping is stopped. The system is designed to be submerged in the pond to provide temperature stability.

Data processing is provided by a Macintosh SE computer running a program written in the Extend modeling software (Imagine That, Inc.) (the complete listing of the Extend model is available from the UC Davis DAST). The program was written to collect data at 30-s intervals from DO and temperature sensors in the respirometer chamber via a Campbell Scientific Model CR21X micrologger, which provides analog-to-digital conversion and some intermediate data processing (the program code for the CR21X is also available from the UC Davis DAST). The DO and temperature data from the respirometer system are processed along with auxiliary data from external quantum sensors (Li-Cor, Model Li-190SA and Li-192SA) which provide surface and underwater solar radiation (photosynthetically active radiation) data. The capability to process wind speed data is built into the system (Campbell Scientific, Model 014A Met One). All sensors, including the DO probe, are read by the micrologger every second. The DO, wind speed, and solar data are averaged for each 30-second interval before being sent to the serial port of the Mac SE.

The operation of the system is based on a 5-minute pumping cycle, during which time water from the body of water to be sampled is pumped through the sample chamber past the DO probe. The readings from the DO probe in the sample chamber during the pumping cycle effectively provide what is referred to as the free DO concentration reading. This is the DO concentration of the sample before incubation, and represents the DO concentration of the "free" water at the location of the sample pump. After the pumping cycle ends, the water sample is automatically sealed in the sample chamber from water or gas exchange from either the inlet or outlet by the pair of one-way check valves at the inlet and outlet of the chamber. The pumping

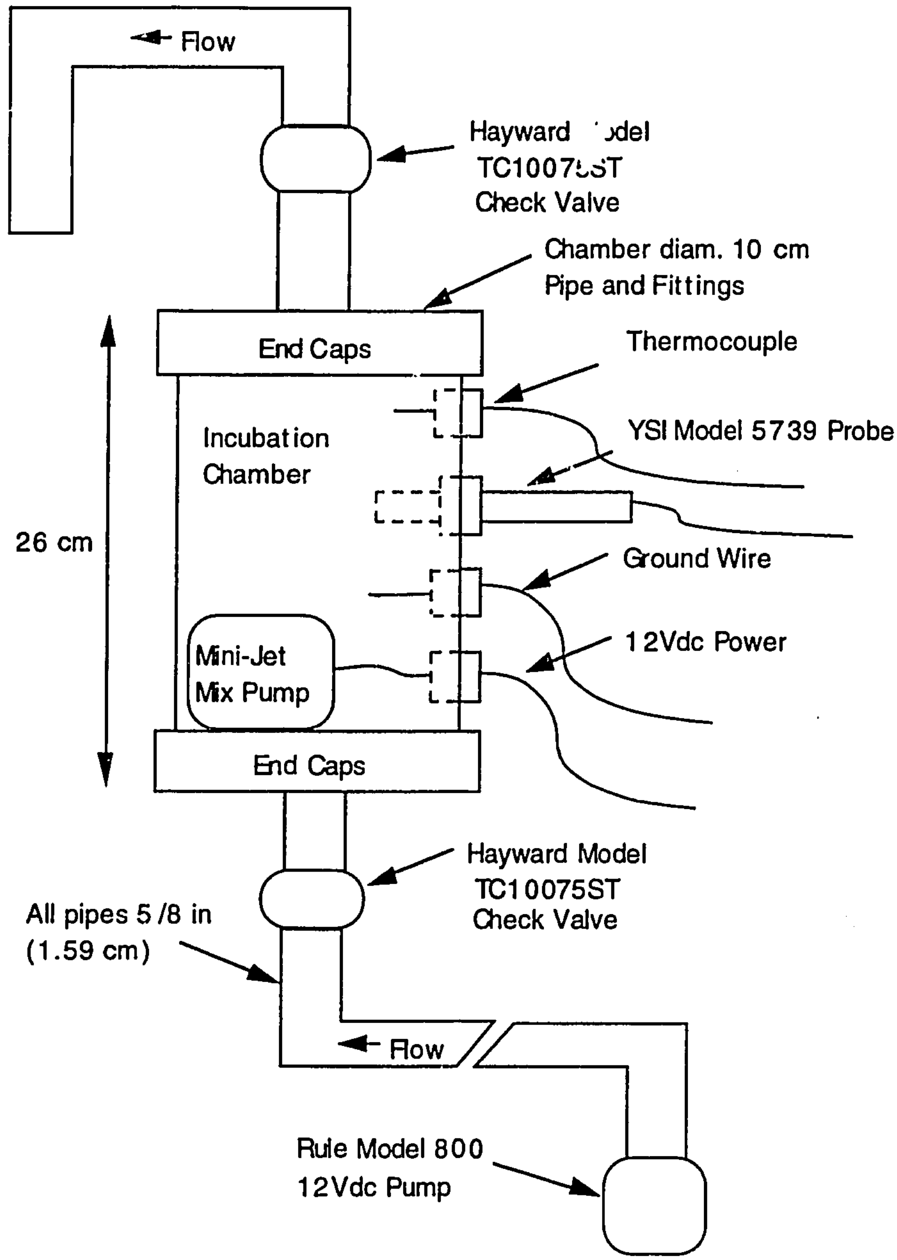

Figure 1. Schematic diagram of the respiration chamber.

cycle is followed by a 15-minute incubation cycle, during which time the water sample is isolated in the sample chamber in complete darkness. The darkened water sample is mixed inside the sample chamber by the internal mixing pump (flow rate of 300 l/h). The combination of the 5-minute pumping cycle and the 15-minute incubation cycle results in respiration measurements made every 20 minutes (72 measurements per diel period).

An estimate of the net primary production rate in the pond is obtained from the difference between free DO readings collected at the end of consecutive pumping cycles. This estimate of net primary production is adjusted by diffusion rate calculated from wind speed, temperature, and DO measurements (Boyd and Teichert-Coddington, 1992).

During the 15-minute dark incubation period, the Extend program receives 30 averaged DO readings from the micrologger. These data are processed into an array, and a linear regression line is plotted through them. The slope of this linear function represents the rate of oxygen consumption of the sample in the darkened chamber. This rate represents the "enhanced" respiration of the water sample, and includes all respiration processes occurring in the water sample during the incubation period.

The respirometer system was set up for initial testing with simulated pond conditions in tanks, both outdoors under natural light and indoors with controlled, high-intensity artificial lighting. Field testing was carried out at the Asian Institute of Technology in Bangkok, Thailand. Some results of the laboratory and field testing are presented below.

Results

Laboratory testing of the respirometers served to identify possible problems in their operation and to provide data for analysis. Several problems were noted, and some have been solved while others have only been partially solved as will be seen in the field data presented. The main difficulties that were found revolved around the measurements of dissolved oxygen concentrations and the processing of the electrical signal generated by the dissolved oxygen meter. Reasons for the lack of accuracy and for fluctuations in the dissolved oxygen measurements included fouling of the probe and chamber, pressure changes during the pumping cycle, electrical interference by external equipment and by the switching of the pumps, and insufficient flow past the DO probe. The fouling problem persists with the current version of the respirometer, although some compensation for it was performed on data collected in the field. The effect of pressure changes was eliminated by the use of a valve to regulate flow rate and pressure through the chamber. Problems created by electrical interference and noise were eliminated in the laboratory tests by the use of isolated circuits for the various functions (pump control, DO meter power, data logger power) and by grounding all equipment, including the sample chamber. Electrical problems persisted in the field experiments, however, and were not easily solved, as will be discussed later. The internal circulation pump currently in use maintains adequate flow past the probe.

Field testing was carried out in an experimental tilapia pond at the Asian Institute of Technology in January and February 1994. The pond used for these experiments was a (nominally) 1-m deep freshwater pond (approximately 400 m^2). Samples were collected from a depth of 10 cm. The pond was stocked one week prior to testing with 768 *Oreochromis niloticus* fingerlings weighing 10-15 g each. The pond was fertilized on 28 January 1994 with a combination of organic and inorganic fertilizers.

Several problems were encountered during the testing that affected the results obtained. The most prevalent problems were with electrical interference and auxiliary equipment malfunction. Despite using grounding procedures similar to those used during the laboratory testing, electrical interference occurred on several occasions and the source was never fully diagnosed. Because of these problems, there was some loss of utility of the data collected, and several diel data sets could not be used. Also, wind speed data were not recorded due to equipment malfunctions.

The results of one day of operation of the system (29 January 1994, Figure 2) are presented to illustrate the capabilities of the system and the respiration data that may be obtained, and to discuss procedures used to compensate for probe fouling. The data presented were recorded immediately after pond fertilization.

Figure 2 shows the DO data recorded from the chamber sensor and the calculated respiration rates for a 24-hour period from 0800 hours on 29 January 1994 to 0800 hours on 30 January 1994. These data show an increase in the DO concentration from 6 mg/l to almost 20 mg/l shortly before dusk, followed by a decline to 6 mg/l by dawn the next morning. The calculated respiration rates for this period rise during the daylight period from about 0.5 mg/l/h to a maximum of about 5.5 mg/l/h at sunset. Calculated respiration rates then decline rapidly during the dark period to a minimum value of about 1.5 mg/l/h.

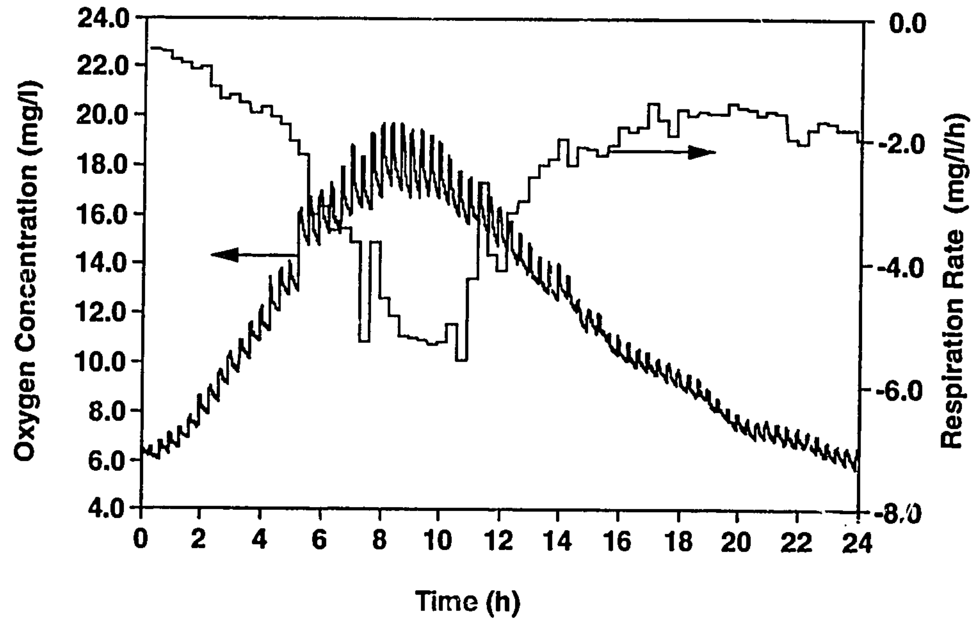

Figure 2. Dissolved oxygen data from the respiration chamber and calculated respiration rates during 24 hr diel cycle in field experiments (29 January - 30 January). Time zero on the diagram corresponds to 0800 hours.

Discussion

The results obtained show that respiration rates change in response to the light cycle and normally peak at or near the end of the light cycle. The data from 29 January 1994 (Figure 2) show a net change in DO concentration in the pond from 2000 hours to 0800 hours of about 10.0 mg/l (i.e., from 16.5 to 6.5 mg/l). This represents an average rate of about 0.83 mg/l/h for the 12-hour period. The measured short-term respiration rate data obtained with the respirometer system during this same period show a minimum respiration rate of about 1.5 mg/l/h. Another noticeable feature of these data is the presence of sudden rises in the chamber DO readings during the 5-minute main pumping cycle. The magnitude of these spikes changes during the diel period. A common pattern can be observed whereby the magnitude of the DO spikes increase progressively during the daytime period to a maximum value occurring at the time of the peak DO concentration in the pond.

A measurement of the DO concentration of the water flow exiting the chamber during the five-minute pumping cycle with a second, well calibrated YSI DO meter indicated that the top value of the spike was in fact an accurate DO reading. Therefore, the problem was that the DO values recorded with the chamber probe suddenly dropped at the end of the 5-minute pumping cycle. Several possible causes were investigated to explain the sudden drop in the chamber DO readings after the 5-minute pumping cycle, including electrical interference, rapid changes in the chamber pressure due to pumping, the possibility of gasses coming out of solution due to pumping effects and supersaturation, and biological fouling. No conclusive evidence was found to indicate any factor except the presence of biological fouling in the chamber. Regular examination and cleaning of the respiration chamber at 24-hour intervals showed that biological fouling was present, and that the fouling reoccurred very rapidly after cleaning. It is possible that if biological fouling is responsible for the rapid DO drop, then the algal respiration is gradually being obscured by the increasing magnitude of respiration by the fouling organisms.

The relationship between algal and bacterial metabolism may be a significant factor in explaining the DO drops caused by probe fouling. Photosynthesizing algae are known to release products of their metabolism that are utilized rapidly by bacteria in close association (Bell, 1983). Extracellular release of organic carbon by photosynthesizing algae and its subsequent utilization by heterotrophic bacteria has been studied in recent years (Coveney, 1982; Nalewajko, 1977; Hellebust, 1974; Fogg, 1971). Values of net extracellular release of carbon have commonly been reported in the range of 5 to 30% of total carbon fixation, and up to 95% release has been reported (Coveney, 1982; Fogg, 1971). The utilization of these extracellular products of algal metabolism are a significant source of bacterial nutrition (Bell, 1983) and some research suggests that specific species assemblages of bacteria are formed that are adapted for the uptake and metabolism of a specific algal extracellular product pool (Bell, 1983; Meffert and Overbeck, 1979). Up to 100% uptake of released products by bacteria was reported by Larsson and Hagström (1982), and Derenback and Williams (1974). In addition, relationships between bacterial production and net production of algae have been reported (White et al., 1991; Cole et al., 1988; Riemann et al., 1982), and diel patterns have been noted but not fully characterized (Coveney, 1982).

Because of the close association of bacterial assemblages with photosynthesizing phytoplankton, it is therefore postulated that the patterns of DO concentration seen in the individual pumping and incubation cycles in Figure 2 are the result of bacterial fouling of the surface membrane of the YSI DO probe in the respiration chamber. In addition, it is also suggested that the consistent diel variability in the magnitude of the DO drops in the pumping and incubation cycles may be due to the metabolic cycles of the bacterial populations, influenced by the availability of organic carbon released by the photosynthesizing phytoplankton. This hypothesis cannot be confirmed with the data available, but is supported by the fact that the DO concentration drops after pumping, increases during the diurnal period, peaks at the same time as pond DO concentration, and then rapidly diminishes after sunset. This pattern was seen in all the data collected from this field experiment.

The mechanism by which the bacterial fouling exerts such a significant effect on the DO readings is unclear. A possible explanation is that the metabolism of the bacterial populations is dependent upon the availability of extracellular products from phytoplankton in the water sample. Immediately after a fresh water sample is brought into the incubation chamber, the availability of extracellular products is highest. In a very short time however, the rapid metabolic rate of the bacterial populations reduces the availability of these nutrients. Because the DO probe membrane is covered with a microfilm of bacteria, their respiration demand, which is assumed proportional to their metabolic rate, prevents the DO in the chamber from diffusing across the DO probe membrane until the availability of extracellular products decreases.

To check this hypothesis, the relative magnitudes of the sudden DO drops over the diel cycle (Figure 2) were estimated and assumed to represent the relative degree of biological fouling on the probe membrane. Furthermore, since gross production must be zero when it is dark, positive darktime gross production calculated from the data in Figure 2 is assumed to be in error due to the overestimation in WCR. The reference magnitude of the error was assumed to correspond to the value at dusk, and was multiplied by the relative fouling value to yield a diel curve of the estimated error due to probe fouling (Figure 3), which was used to adjust the diel respiration values and arrive at estimates of gross production (Figure 4). Data recorded in an adjacent pond with a similar respirometer showed the same high rates of respiration, and were adjusted in a similar manner (Szyper, personal communication).

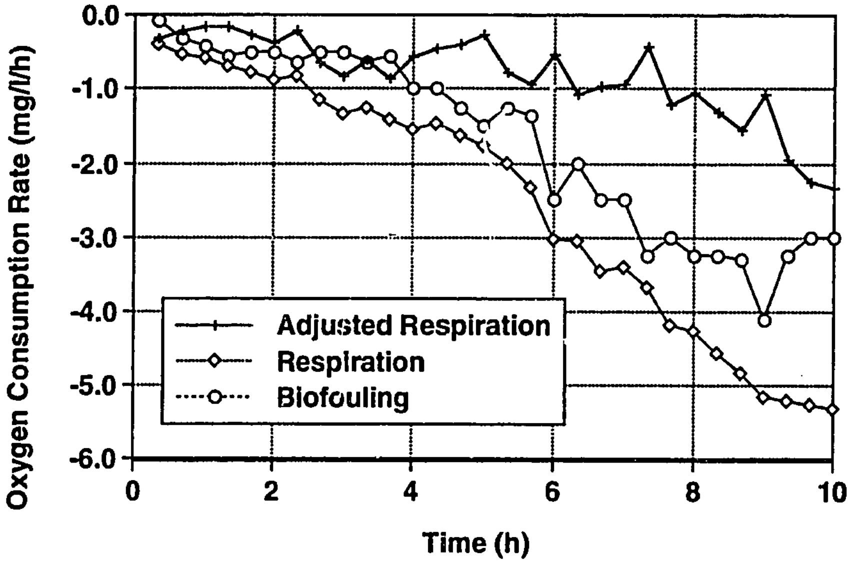

Figure 3. Estimated rate of DO consumption due to biofouling, with measured and adjusted respiration rates from field data (29 January). Time is defined as in Figure 2.

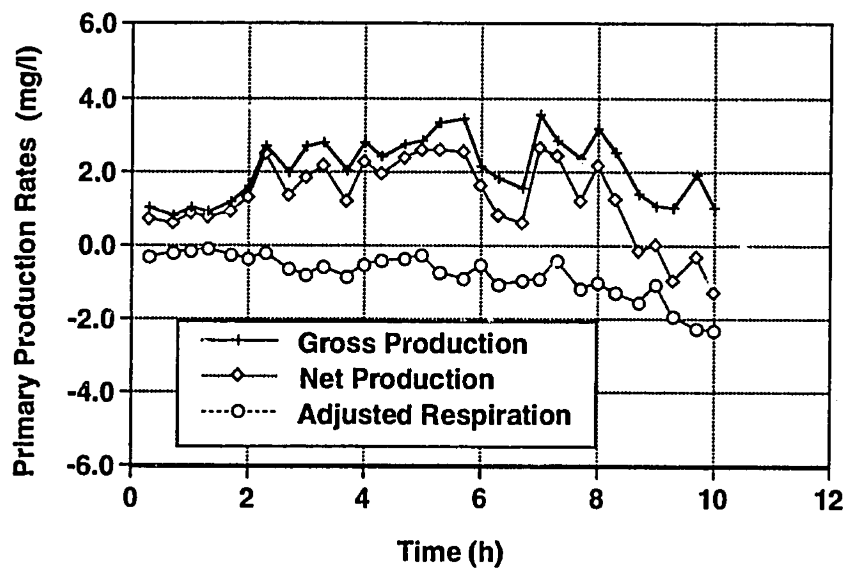

Figure 4. Adjusted respiration and gross production rates, with measured net production rates from field data (29 January). Time is defined as in Figures 2 and 3.

Anticipated Benefits

The development of this respirometer system has made possible the direct measurement of community respiration rates in ponds over diel periods and longer. These data have allowed a more accurate calculation of gross production, and in general provide a means of analysis of processes which require an accurate accounting of the dissolved oxygen balance in a pond.

The major problems in the development of this system have been identified through a combination of laboratory and field testing. The most significant problems concern: 1) fouling of the DO probe membrane, 2) electrical interference, and 3) instability of the DO probe and its susceptibility to pressure gradients. The primary benefit of this research has been the demonstration of the utility of automated frequent measurements of respiration in field conditions, using the technique of quickly darkening water samples containing photosynthesizing organisms. The use of supporting software described and direct microcomputer control allows instant determination of all three major rate processes occurring in primary production – gross production, net production, and respiration. The prototype respirometer system described and tested here represents the first step, along with the Szyper system (Szyper et al., 1992), towards developing a dependable field unit for accurately measuring these important parameters.

Acknowledgments

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Stochastic Modeling of Temperature in Stratified Aquaculture Ponds

Work Plan 7, DAST Study 2

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Introduction

Most current models of water quality and fish growth in aquaculture ponds are deterministic. In a deterministic model, the outcome of a simulation run is unique for a given set of input parameters. In a stochastic model, on the other hand, input parameters or model equations include a stochastic or probabilistic component such that slightly different results are obtained every time the model is run. The first efforts at developing aquaculture water quality models that incorporate some stochastic behavior have been initiated under DAST Study 2 of CRSP Work Plan 7. To make use of the water quality data collected from CRSP sites in testing the models, it has been necessary to develop tools for making stochastic predictions of solar radiation, wind speed, and wind direction that are in agreement with the observed weather data from the sites. This was an essential component of the development of the model because it allowed the use of extensive PD/A CRSP water quality data in the testing of the model simulations. The procedures followed to arrive at synthetic series of solar radiation, wind speed, and wind direction values will be described in some detail, followed by the results of temperature simulations over a 20-day period obtained using Monte Carlo techniques.

For the temperature simulations, the pond water column was assumed to consist of three layers of equal thickness as described by Culberson (1993). Heat balance equations for each of the three layers followed the formulations used by Losordo (1988) and Culberson (1993), with minor modifications.

Monte Carlo Simulation

A premise of stochastic models is that they contain an element of uncertainty and that a slightly different answer will be obtained every time the model is run. In the stratified temperature model, stochasticity is achieved by using stochastic values for weather inputs of solar radiation, wind speed, and wind direction. The range of predicted weather values used in the simulations must be such that the response of the model represents a realistic outcome for the site. If the input parameters (solar radiation, wind speed, and wind direction) are normally distributed, then a series of simulations can be carried out in which stochastic input values used are obtained using random sampling from the distributions. This procedure constitutes the basis of the Monte Carlo method (Shreider, 1964), which is often used in stochastic modeling and other applications. A complete discussion of the Monte Carlo method is beyond the scope of this report.

Generation of Synthetic Weather Parameters

There are various techniques for generating solar radiation and other weather projections from data for a particular site (e.g., CLIGEN and WGEN computer models). Unfortunately, the data requirements for these models are such that they could not be used with the CRSP data base. Typical requirements were for complete data sets (365 daily values) spanning 10 to 20 years. However, CRSP data sets span a maximum of eight to ten years, and have a large number of missing values. Different procedures were used to generate synthetic data for solar radiation, wind speed, and wind direction due to the relative availability of data for the different variables. The procedures for each variable are described below.

Solar Radiation

Solar radiation is the primary energy input factor determining the temperature in a pond. The model developed to simulate temperature in stratified aquaculture ponds (Culberson, 1993) is designed to generate hourly temperature values for the three layers into which the pond is partitioned. To achieve this degree of time resolution, hourly values for the input weather data are required. For example, in the case of solar radiation, the total solar radiation received over the day is needed as well as the distribution over the day for that radiation. The total solar radiation incident on a surface at the top of the atmosphere can be analytically calculated for any geographic location (e.g., Piedrahita, 1984). Calculation of the incident radiation at the surface of a pond must take into account energy absorbed by the atmosphere because of the presence of water vapor (clouds), aerosols, dust, etc. These factors are subject to geographical, seasonal, and stochastic variations.

The procedure followed to generate hourly solar radiation values stochastically is carried out in two steps. The first step consists of projecting the total solar radiation for a particular day. The hourly distribution of the solar radiation over the day is generated in the second step of the calculations. The reason for this two step procedure is based on the nature of the CRSP data base in which there are only a few observations per year that include measured solar radiation values for different times of the day. Total daily solar radiation values were collected at much greater frequency, with values available for most days during some experimental periods.

A projected value for the solar radiation striking the pond at a given time (hourly average) is given by:

$$\text{RandomSolarRad}_{i,t} = \text{SolarDaily}_i \bullet \frac{\text{SolarHourly}_t}{\text{IntegratedSolarMean}} \qquad (1)$$

where:

$RandomSolarRad_{i,t}$ = Stochastically generated value for the average solar radiation over a one-hour period at time **t**, on day **i** (kJ m^{-2} s^{-1})

$SolarDaily_i$ = Stochastically generated value for the total solar radiation on day **d** (kJ m^{-2})

$SolarHourly_t$ = Stochastically generated value for the solar radiation over a one-hour period at time **t** during a "typical" day (kJ m^{-2} s^{-1})

IntegratedSolarMean = Total amount of solar radiation received by the pond during a "typical" day (kJ m^{-2})

Equation (1) makes use of the stochastically generated value for the total solar radiation striking the pond on a given day, and generates a set of hourly solar radiation values ($RandomSolarRad_{i,t}$) that are used as inputs to the pond temperature model. $SolarDaily_i$ values are obtained from the data set of total daily solar radiation measurements using a simple model that generates synthetic sequences of integrated daily values assuming a non-stationary sequence of residuals and a lag-one serial correlation of the historical values (Matalas, 1967; Amato et al., 1986). From a data base with integrated daily values for solar radiation recorded through **m** years with **n=365** possible valid records for each year, the values for the calculated residual time series are given by (Amato et al., 1986):

$$\chi(i,j) = \frac{x(i,j) - \bar{x}(i)}{\sigma(i)} \; ; \quad \text{for } i = 1,\ldots,n; \; j = 1,\ldots,m \tag{2}$$

where:

$\chi(i,j)$ = calculated residual for the **i** th day of the **j** th year

$x(i,j)$ = integrated daily value for solar radiation measured on the **i** th day of the **j** th year

$\bar{x}$ = average integrated daily value for solar radiation for the **i** th day, or

$$\bar{x} = \frac{\sum_{j=1}^{m} x(i,j)}{m - w} \tag{3}$$

where **w** represents the number of missing values for the measurement.

$\sigma(i)$ = standard deviation of the integrated daily value for solar radiation, or

$$\sigma(i) = \left[\frac{\sum_{j=1}^{m} \left(x(i,j) - \bar{x}(i) \right)^2}{m - w - 1} \right]^{\frac{1}{2}} \tag{4}$$

The estimated daily total radiation ($SolarDaily_i$) can be obtained from:

$$SolarDaily_i = \bar{x}(i) + \sigma(i) \bullet \hat{\chi}(i,j) \; ; \quad \text{for } i = 1,\ldots,n; \; j = 1,\ldots,m \tag{5}$$

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

$$\hat{\chi}(i,j) = \rho(j) \bullet \chi(i-1,j) + \left(1 - \rho^2(j)\right) \bullet \varepsilon(i,j) \tag{6}$$

where:

$\rho(j)$ = autocorrelation coefficient of lag one calculated for the complete data set

$\varepsilon(i,j)$ = random value sampled from a normal distribution with a mean of zero and a standard deviation of one

The calculations culminating in Equation (5) were used to generate solar radiation values for the Thailand site over a 20-day period for testing the temperature model.

The hourly distribution of solar radiation was generated from the available measurements of diel solar radiation. These measurements were used to calculate the average solar radiation at each hour for which values were available. In turn, these averages were used to generate a function describing the "typical" distribution of solar radiation over the day, which when integrated yields the value for IntegratedSolarMean that is used in Equation (1). Values for $SolarHourly_t$ are obtained by sampling from a distribution around the mean hourly values of solar radiation for the "typical" day (Naylor et al., 1966). As an example, the standard deviation for measured values at 1000 hours ($\sigma SR(10)$) is given by:

$$\sigma SR(10) = \frac{\sum_{j=1}^{m} \left(SR(10,j) - SolarMean(10) \right)^2}{m - k - 1} \tag{7}$$

and SolarMean(10) is the mean solar radiation at 1000 hours from historical data (SR(10,j) values).

Wind Speed

Wind plays an important role in determining the temperature and degree of stratification in a pond, and a stochastic function for wind estimation was developed. The procedure for generating synthetic wind values differed from that used for solar radiation due to the reduced data availability for wind. A time-dependent diurnal wind speed distribution was constructed from measurements collected during diel samplings. This distribution was used to generate wind speed values by adding a stochastic perturbation (Swartzman and Kaluzny, 1987):

$$W_t = WMean_t + \varepsilon_t(\sigma_t) \tag{8}$$

where:

W_t = generated value for wind speed at time t

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

$\varepsilon_t(\sigma_t)$ = stochastic variation around the mean wind value determined by sampling from a normal distribution with standard deviation σ_t obtained from historical data.

Wind direction

Wind direction was used by Losordo (1988) and Culberson (1993) to generate a wind speed adjustment for fetch length used in the calculations for evaporative cooling. The fetch adjustment was required because of changes in fetch as the direction of the wind changed with respect to the dimensions and orientation of the pond. Culberson (1993), in the absence of diel wind direction data, assumed a constant direction for the wind along the long axis of the ponds. In this way, any effects of wind fetch and wind shear are recognized in the model as having their maximum possible effect. The present work used a variate from a skewed normal distribution to generate wind direction based on historical records for a given site. In a simulation, wind direction is generated by sampling values from the skewed normal distribution obtained from (Nicks and Lane, 1989):

$$NORM = \frac{6}{g}\left\{\left[\frac{g}{2}\left(\frac{WINDIRECT - \mu}{\sigma}\right) + 1\right]^{\frac{1}{3}} - 1\right\} + \frac{g}{6} \tag{9}$$

where

NORM = standard normal variate

WINDIRECT = stochastically generated wind direction value μ, σ, and g — are respectively the mean, standard deviation, and skew coefficient of the wind direction distribution calculated from historical records.

To obtain a wind direction value, a random normal value is generated and WINDIRECT is calculated from Equation (9). The procedure is repeated for each time step during a simulation to arrive at hourly wind direction values.

Temperature Simulations

The temperature model used for the simulations was based on those of Losordo (1988) and Culberson (1993) with minor modifications. A period of 20 days was chosen for the trial simulations. The period was selected on the basis of data availability, and started on Julian day 54. Results of a total of 100 simulation runs are shown for each of the three depths in Figures 1 through 3. The curves in Figures 1 through 3 represent the mean, maximum, and minimum temperatures obtained for each time. The ranges (maximum minus minimum) for temperatures at the three depths and for the 20-day period are shown in Figure 4.

Temperatures at the three depths fluctuated during the 20 days of the simulation. The low daily values at the surface (Figure 1) did not change as much from day to

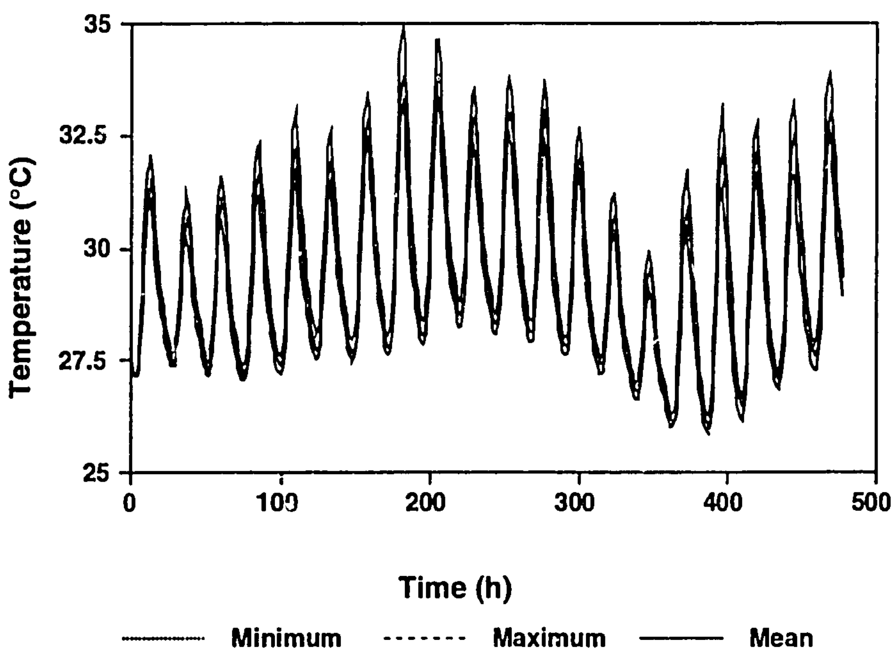

Figure 1. Simulated surface temperatures in a Thailand pond obtained after 100 simulation runs using stochastically generated series for solar radiation, wind speed, and wind direction. Results shown are the minimum, maximum, and mean values obtained at each hour of the simulation. The simulation was run for a period of 20 days, starting at 0600 hours on Julian date 54.

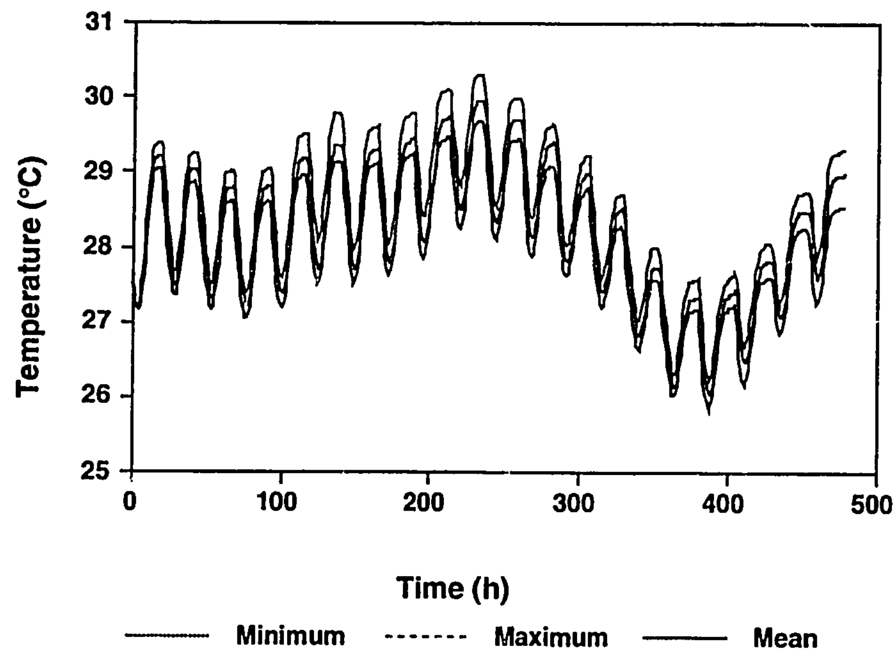

Figure 2. Simulated temperature in the middle layer of a Thailand pond obtained after 100 simulation runs using stochastically generated series for solar radiation, wind speed, and wind direction. Results shown are the minimum, maximum, and mean values obtained at each hour of the simulation. The simulation was run for a period of 20 days, starting at 0600 hours on Julian date 54.

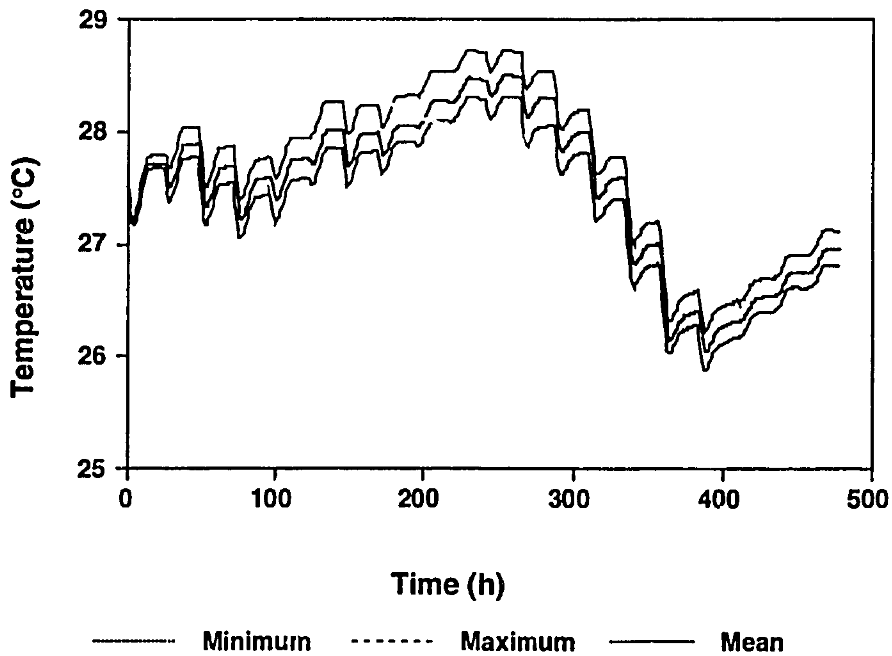

Figure 3. Simulated bottom temperature in a Thailand pond obtained after 100 simulation runs using stochastically generated series for solar radiation, wind speed, and wind direction. Results shown are the minimum, maximum, and mean values obtained at each hour of the simulation. The simulation was run for a period of 20 days, starting at 0600 hours on Julian date 54.

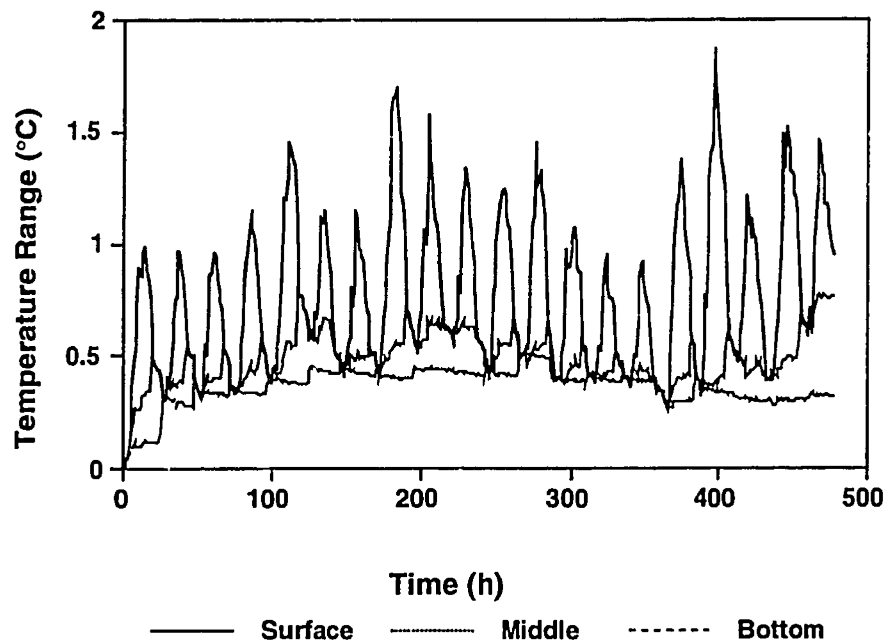

Figure 4. Difference between maximum and minimum simulated temperatures obtained after 100 simulation runs over a period of 20 days.

day as did the daily peaks. The surface temperature rose to almost 35°C during the 8th day of the simulation and dropped as low as approximately 26°C on the evening of the 16th day of the simulation. Some days showed a wide fluctuation between minimum and maximum daily surface temperatures (about 8°C for day 8), while the temperature fluctuation during other days was relatively small (about 3°C for day 15). Temperature was most stable in the bottom layer, where diel temperature changes were much smaller than in either of the other two layers (Figures 1 through 3). This can also be seen in Figure 4, where the range of values obtained in the simulations (difference between maximum and minimum value obtained for each hour for the 100 simulations run) are shown, indicating also that surface temperature was much more sensitive to changes in the stochastic inputs used.

Anticipated Benefits

Results of the temperature simulations obtained to date are very encouraging. The temperature model has been stable with long simulations, and the results obtained provide useful information on the variability in water temperature that can be expected at a particular site. Comparison of simulation results with temperature data collected from CRSP ponds will provide additional insights into the accuracy of the models. The techniques developed for obtaining synthetic series of solar radiation, wind speed, and wind direction data, together with the temperature model simulation results will serve as a basis for a stochastic water quality and fish growth model that is under development. Ultimately, results from stochastic simulations of fish growth and yield based on weather characteristics of a site and on management actions and pond design will provide information that can be used for planning and management that can have a greater degree of realism by accounting for probabilities of certain outcomes.

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Calculation of pH in Fresh and Sea Water Aquaculture Systems

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Abstract

A procedure for the calculation of pH in fresh and salt waters has been developed. The method is based on a fourth-order polynomial relationship between hydrogen ion concentration and other (conservative) water quality parameters. The method avoids trial and error estimations and results in a direct calculation procedure that can be implemented in models developed in various modeling environments, such as spreadsheets, conventional programming languages (BASIC, C, FORTRAN, PASCAL, etc.), or specialized modeling languages (Extend™, Stella™).

The method developed is based on the solution of the full alkalinity-pH equation. Because of the need to simplify the equations to yield explicitly solvable polynomial equations, the accuracy of the solutions depends on the simplification made and varies with water properties. Three simplifications are tested based on a second-, a third-, and a fourth-order polynomial equation for hydrogen ion concentrations. The equations have been tested for salinities ranging from 0 to 35‰ (fresh to sea water), for temperatures ranging from 0 to 35°C, for total carbonate carbon of 0.1 and 5.0 mmol/l, and for total ammonia nitrogen of 0 and 10 mg/l. Approximations are most accurate in waters of high total carbonate carbon and low ammonia concentrations, where the fourth-order approximation yields results that are within 0.05 pH units for the full range of pH values tested (5 to 10).

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Special Topics Research

Introduction

Special Topics Research includes additional research studies beyond the scope of Work Plans 6 and 7. 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 with the 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.

Special Topic Research was carried out in Egypt, Honduras, and Thailand. The CRSP Egypt team researched the effect of stocking rate on growth and yield of Nile tilapia. A second study aimed to develop methods for mass production of Nile tilapia and blue tilapia fry. In a third study, researchers evaluated the economic returns of different tilapia production systems. Management systems that combined fertilization with feeding had the highest margins between average price and break-even price to cover total cost. Using only chemical fertilizer applications as a management tool resulted in the lowest economic returns.

Hormonal sex-reversal is a commonly practiced method to obtain monosex fish populations. The Honduras team conducted two studies to further improve this technique. Determination of the optimal water temperature regime for mass production of tilapia fry for hormonal sex-reversal was the goal of the first investigation. Using a threshold temperature of 15°C, fry production did not occur below 140 degree-days; the upper boundary was given by 195 degree-days. In a companion study, researchers tried to determine if the administration of 17α-methyltestosterone improved the growth of treated fry and fingerlings, a side-effect of MT application which has been reported in the literature for other species. However, after 150 days no significant differences were observed in this experiment.

Determination of optimal phosphorus fertilization rates was the aim of two of the studies conducted in Thailand. The first of these investigated the relationship between phosphorous sediment concentrations and phosphorous fertilization rates. It was found that optimum phosphorous fertilization, which does not result in either under- or overfertilization, can be obtained if the fertilization rate is based on the phosphorous saturation level of the sediment. The second study reported a fast method to estimate phosphorous saturation level in sediment by using sediment clay content as the key variable. Management of carbon dioxide balance in order to maintain stable total alkalinity levels was the focus of another investigation. Fertilizer was applied according to recommendations generated by the decision-support system PONDCLASS or on a fixed schedule. Preliminary analysis of data did not reveal any significant differences in final mean individual fish weight, survival, or net yield.

Economic Analysis of Different Tilapia Pond Culture Systems in Egypt

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Introduction

Pond fish culture can be practiced at many levels of production intensity based on the quantity and quality of nutrients added to enhance, supplement, or replace natural pond productivity (Bardach et al., 1972). Aquaculture ponds also can be stocked with one species (monoculture) or several species (polyculture) of fish. Moreover, since growth may be affected by fish sex, the choice of a production system may involve monosex culture of the faster-growing sex. Economic considerations in selection of an appropriate aquacultural production system include its potential for economic returns, its economic efficiency, and the farmer's access to operating capital.

There are few reports on the economic evaluation of aquacultural production systems in Egypt. Soliman and Gaber (1988) compared production level, production variability, economic efficiency, and technology level for two different fish culture systems. El Hendy (1990) evaluated technical and economical aspects of tilapia cage culture in Domyatt, Egypt. Reports on evaluations of tilapia pond culture practices in Egypt have been lacking. Thus, the objective of the present study was to assess the economic potential of different tilapia pond culture systems as part of the Egypt Project of the Pond Dynamics/Aquaculture CRSP Global Experiment.

Materials and Methods

In order to evaluate economic potential and profitability of an enterprise, estimated expenses and returns associated with the enterprise are considered in the form of a budget (Kay, 1981; Boehlje and Eidman, 1984; Hatch and Hanson, 1991).

Five tilapia production systems were tested in 0.1-ha earthen ponds at the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou Hammad:

1) the traditional system with mixed-sex tilapia (TRAD), where urea, superphosphate, chicken litter, and 25%-protein pelleted feed were used;

2) an extensive chemical fertilization system using monosex tilapia (CHEM), where only urea and superphosphate were applied at an N:P ratio of 4:1;

3) feed only, using monosex tilapia (FEED), where a 25%-protein pelleted feed was used; and

4) a system in which chicken litter was followed by feed, using either mixed-sex or monosex tilapia (CLFEDMS and CLFEDSR, respectively), where chicken litter was used for the first 60 days, then 25%-protein pelleted feed was used for the remainder of the culture period.

All production systems were stocked with Nile tilapia (*Oreochromis niloticus*) at 20,000 fish/ha; the average individual weight at stocking was 1 to 3 g/fish. The daily feed allowance was based on 3% of fish biomass. The production cycle lasted an average of 145 days. Data on pond stocking, management, and harvest were used in the economic analyses. Data on labor and equipment requirements were obtained from available information from the production ponds at CLAR. Pond construction costs were obtained from the General Authority for Fish Resources Reclamation, Ministry of Agriculture and Land Reclamation. Enterprise budgets were developed for each production system based on a 2.1-ha pond stocked with 20,000 tilapia/ha for a 145-day grow-out period. No charge for land was included in the cost estimates. Land was assumed to be owned and previously used for aquaculture.

Results and Discussion

Net Returns and Costs

Net returns to land and management were greatest for the CLFEDSR treatment, followed in decreasing order by the TRAD, CLFEDMS, FEED, and CHEM systems (Table 1). Net returns to land and management for systems that combined fertilization and feeding (CLFEDSR, CLFEDMS, and TRAD) were, on average, 16.1 times the net returns for the extensive system (CHEM). The difference in net returns can be explained by production level, price received per unit of production, and total variable costs, since total fixed costs were similar for all production systems.

Table 1. Summary of economic returns and performance ratios for five Nile tilapia production systems in a 2.1-ha earthen pond for a 145-day grow-out period.

	Production systems*				
	CLFEDSR	TRAD	CLFEDMS	FEED	CHEM
Net returns (L. E.)	19,104	14,494	14,111	3,710	987
Total production (kg)	7,616	6,757	5,694	4,159	2,973
Average price (L. E.)	5.41	4.94	6.41	4.61	4.18
Total variable costs (L. E.)	18,100	14,900	18,370	11,461	7,430
AVC/kg produced (L. E.)	2.38	2.21	3.23	2.76	2.50
Return/kg above TVC (L. E.)	3.03	2.73	3.18	1.85	1.68
Value of production/man-h (L. E.)	104.05	56.50	94.76	55.90	56.31
Value of production/L.E. of TVC (L. E.)	227.62	224.03	198.71	167.30	167.28
Value of production/100 kg feed (L. E.)	486.34	368.62	358.25	245.34	NA
Break-even price to cover TC (L. E.)	2.90	2.80	3.93	3.72	3.84
Break-even yield to cover TC (kg)	3,830	3,873	3,506	3,269	2,569
Margin between average price and ATC (L. E.)	2.51	2.14	2.48	0.89	0.34
Rate of return to capital (%)	29.97	22.94	22.52	6.66	2.42
CV (%)	26.91	20.35	35.18	42.40	18.94

* Production systems described in text.
AVC: average variable cost
CV: co-efficient of variation
TC: total cost
TVC: total variable cost

Total production in the semi-intensive systems ranged from 7,616 kg for CLFEDSR to 4,159 kg for FEED. For the extensive system (CHEM), total production was estimated at 2,973 kg. A positive relationship between net returns and production level was observed.

Tilapia are marketed by size class in Egypt and the market price per kilogram increases with fish size. Tilapia size classes are: 1st class (1-5 fish/kg), 2nd class (6-12 fish/kg), 3rd class (13-25 fish/kg), and 4th class (26-40 fish/kg). Average prices were calculated according to percentage of fish classes produced (Table 1). There was a positive relationship between the degree of intensification of a production system and the average price received for fish. A high average price for a production system reflects the fact that a major part of the production consists of large fish.

Total variable costs (TVC) were higher for the semi-intensive systems than for the extensive system. The highest variable cost was for CLFEDMS and the lowest was for CHEM (Table 1). The high variable costs of CLFED systems resulted primarily from the cost of feed. Prior studies have shown that even though a positive relationship is observed between level of intensification and variable costs, the average cost may be lower than for extensive operations (Shang, 1981; Tucker et al., 1979). The average variable cost (AVC), in Egyptian pounds (L.E.), per kilogram produced for each system is shown in Table 1. For the semi-intensive systems the average variable cost per kilogram produced ranged from L.E. 2.21 for TRAD to L.E. 3.23 for CLFEDMS. The average variable cost per kilogram produced was estimated to be L.E. 2.50 for the extensive system (CHEM). These results indicated that not all intensified systems had lower average variable costs than the extensive system. However, in the case of uniform stocking rates, intensification should positively affect the final size of individual fish and increase fish production. Thus, considering the average price per kilogram, semi-intensive systems had higher average returns per kilogram above average variable cost than the extensive system (CHEM; Table 1).

Total variable costs and variable cost components as a percentage of total variable costs for each production system are given in Tables 2 and 3. The results of previous research indicated that feed and fertilizer were the most important cost items for intensive aquaculture (Shang, 1981; Hatch et al., 1987). For semi-intensive systems, feed costs ranged from 39% of TVC for CLFEDSR to 54% of TVC for FEED. Fertilizer costs ranged from 20% of TVC for TRAD to 31% of TVC for CLFEDSR. Together, feed and fertilizer comprised 54% to 75% of TVC of production. Cost of fingerlings and fertilizer represented 56% and 32% of TVC, respectively, for the extensive system (CHEM).

Performance Ratios

Performance ratios are summarized in Table 1. The value of production per man-hour ranged from L.E. 104 for CLFEDSR to L.E. 56 for FEED and CHEM systems. The highest value of production per L.E. of variable costs was for CLFEDSR followed, in decreasing order, by the TRAD, CLFEDMS, FEED, and CHEM systems. The value of production per 100 kg of feed ranged from L.E. 468 for CLFEDSR to L.E. 245 for FEED. Break-even prices and break-even yields to cover total costs also are shown in

Table 2. Estimated budgets for Nile tilapia pond management systems (see text for system descriptions) in 2.1-ha earthen ponds for a 145-day grow-out period.

		Price or	CLFEDSR			FEED			CHEM		
Item	Unit	cost/unit (L.E.)	Quantity	Value or Cost (L.E.)	% of total	Quantity	Value or Cost (L.E.)	% of total	Quantity	Value or Cost (L.E.)	% of total
					Gross Returns						
1st class tilapia	kg	7.85	1,843	14,467	35.11	307	2,410	12.57	7	55	0.44
2nd class tilapia	kg	6.00	3,413	20,478	49.70	1,832	10,992	57.33	1,099	6,594	53.10
3rd class tilapia	kg	3.40	1,290	4,386	10.65	1,355	4,607	24.03	1,516	5,154	41.51
4th class tilapia	kg	1.75	1,070	1,873	4.54	665	1,164	6.07	351	614	4.95
Total Returns				41,204	100.00		19,173	100.00		12,417	100.00
					Variable Costs						
Fingerlings	Thousand	100	42	4,200	23.20	42	4,200	36.64	42	4,200	56.53
Feed	Metric ton	800	8.797	7,038	38.88	7.814	6,251	54.54			
Superphosphate	50-kg bag	18							73.75	1,328	17.87
Urea	50-kg bag	25							43.34	1,084	14.58
Chicken litter	m^3	55.5	101.38	5,627	31.09						
Labor			396	298	1.64	343	261	2.28	221	183	2.46
Equipment repair				75	0.41		75	0.65		75	1.00
Pond maintenance				350	1.93		350	3.05		350	4.71
Interest on operating capital				513	2.83		325	2.83		211	2.83
Total Variable Costs				18,101	100.00		11,462	100.00		7,431	100.00
Income Above Variable Costs				23,103			7,711			4,986	
					Fixed Costs						
Depreciation pond equipment				1,949	48.70		1,949	48.70		1,949	48.70
Interest on investment				2,052	51.30		2,052	51.30		2,052	51.30
Total Fixed Costs				4,001	100.00		4,001	100.00		4,001	100.00
Net Returns to Land and Management				19,102			3,710			985	

Table 3. Estimated budgets for Nile tilapia pond management systems (see text for system descriptions) in 2.1-ha earthen ponds for a 145-day grow-out period.

Item	Unit	Price or cost/unit (L.E.)	TRAD Quantity	TRAD Value or Cost (L.E.)	TRAD % of total	CLFEDMS Quantity	CLFEDMS Value or Cost (L.E.)	CLFEDMS % of total
Gross returns								
1st class tilapia	kg	7.85	467	3,666	10.98	2,212	17,364	47.60
2nd class tilapia	kg	6.00	3,477	20,862	62.47	2,914	17,484	47.92
3rd class tilapia	kg	3.40	2,390	8,126	24.33	388	1,319	3.62
4th class tilapia	kg	1.75	423	740	2.22	180	315	0.86
Total Returns				33,394	100.00		36,482	100.00
Variable costs								
Fingerlings	Thousand	80	42	3,360	22.55	42	3,360	18.29
Feed	Metric ton	800	9.055	7,244	48.62	10.187	8,150	44.36
Superphosphate	50-kg bag	18	89.04	1,603	10.76			
Urea	50-kg bag	25	10.00	250	1.68			
Chicken litter	m^3	55.5	21.23	1,178	7.91	101	5,627	30.63
Labor			591	417	2.80	385	288	1.57
Equipment repair				75	0.50		75	0.41
Pond maintenance				350	2.35		350	1.91
Interest on operatingcapital				422	2.83		521	2.84
Total Variable Costs				14,899	100.00		18,371	100.00
Income Above Variable Costs				18,495			18,111	
Fixed costs								
Depreciation pond equipment				1,949	48.70		1,949	48.70
Interest on investment				2,052	51.30		2,052	51.30
Total Fixed Costs				4,001	100.00		4,001	100.00
Net Returns to Land and Management				14,495			14,111	

Table 1. TRAD had the lowest break-even price to cover total costs followed, in increasing order, by CLFEDSR, FEED, CHEM, and CLFEDMS. However, CLFEDSR had the highest margin between break-even price to cover total costs and average price, followed by, in decreasing order, CLFEDMS, TRAD, FEED, and CHEM.

Rates of return to capital were calculated as a measure of profitability for each system (Table 1). Land was valued at L.E. 32,640 for a 2.1-ha pond, other investments were estimated at L.E. 34,204, and return to management was assumed to be zero. The CLFEDSR system had the highest rate of return to capital (29.97%), followed by TRAD (22.94%), CLFEDMS (22.52%), FEED (6.66%), and CHEM (2.42%).

Sensitivity Analysis

The variation in yield, as indicated by coefficients of variation, was less in the extensive system (CHEM) than in the semi-intensive systems (Table 1). Similar results have been reported by other researchers (Tucker et al., 1979). Actual received prices were used to estimate total returns. However, these prices were slightly higher than local prices. Therefore, the impact of price reduction, output level reduction, and combinations of the two were estimated (Table 4).

At a 10 or 15% price reduction, all production systems except CHEM had positive net returns (Table 4). A 20% decrease in price level resulted in negative net returns for the CHEM and FEED systems. A decrease in production level by one or two standard errors with no reduction in sales price resulted in negative net returns for the FEED and CHEM systems. Results of the combined change in price and production level showed that: 1) a decrease in production level by one standard error and in price by 10%, 15%, or 20% resulted in negative net returns for the FEED and CHEM systems, and 2) a decrease in production level by two standard errors and in price by 10%, 15%, or 20% resulted in negative net returns for the FEED, CHEM, and CLFEDMS systems.

Conclusions

These analyses indicated sufficient incentive for the expansion of intensified pond culture of tilapia in Egypt. The analysis indicated that CLFEDSR, TRAD, and CLFEDMS systems, in decreasing order, were more economically viable than FEED and CHEM systems. Greatest total production, net returns, and average rates of return on capital were obtained with CLFEDSR, TRAD, and CLFEDMS systems, in decreasing order. These same systems had the highest values of production per man-hour, per kilogram of feed, or per L.E. of variable cost. Also, they had the highest margin between average prices and break-even prices to cover either total variable costs or total costs. CLFEDSR, TRAD, and CLFEDMS systems can tolerate up to 46%, 43%, and 39% reductions in average price, respectively, before net returns become negative. This indicated reduced risk to farmers in the event of an unexpected drop in market prices. Moreover, the above systems had the highest net returns when production levels were estimated at one or two standard errors below the mean. In the extreme case of the combined effects of production failure and a 20% drop in

Table 4. Sensitivity of net returns of five Nile tilapia pond management systems to changes in production and sales price. Net returns are based on 145-day grow-out period in 2.1-ha pond.

Production system*	Change insale price (%)	Net returns (L.E.) Production level estimated at: Mean	Mean - 1 S.E.	Mean - 2 S.E.
CLFEDSR	0	18,104	13,564	8,020
TRAD	0	14,494	11,120	7,719
CLFEDMS	0	14,111	7,697	1,280
FEED	0	3,710	(356)	(4,421)
CHEM	0	987	(188)	(1,365)
CLFEDSR	- 10	14,983	9,997	5,008
TRAD	- 10	11,154	6,617	5,057
CLFEDMS	- 10	10,463	4,690	(1,085)
FEED	- 10	1,793	(1,867)	(5,525)
CHEM	- 10	(255)	(1,312)	(2,371)
CLFEDSR	- 15	12,923	8,214	3,502
TRAD	- 15	9,485	6,512	3,726
CLFEDMS	- 15	8,639	3,187	(2,268)
FEED	- 15	835	(2,622)	(6,077)
CHEM	- 15	(875)	(1,874)	(2,875)
CLFEDSR	- 20	10,863	6,431	1,996
TRAD	- 20	7,815	5,116	2,395
CLFEDMS	- 20	6,815	1,684	(3,450)
FEED	- 20	(124)	(3,377)	(6,629)
CHEM	- 20	(1,496)	(2,437)	(3,378)

* Production systems described in text.

market prices, the TRAD and CLFEDSR systems maintained positive net returns to land and management. Finally, the CLFEDSR, TRAD, and CLFEDMS production systems had great potential for increasing fish yield as compared with monoculture and polyculture systems on private and governmental fish farms in Egypt as reported by Shelton (1989).

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Effect of Stocking Rate on Growth and Yield of Nile Tilapia

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Introduction

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

Materials and Methods

This study was conducted at the Central Laboratory for Aquaculture Research (CLAR), Egypt. Three replications of each treatment (stocking rates of 30,000 or 40,000 Nile tilapia/ha) were randomly assigned to 0.1-ha earthen ponds. Sex reversed Nile (*Oreochromis niloticus*) tilapia fry (mean weight: 0.5 g/fish) were stocked into ponds on 20 and 24 July 1994. Past experience indicated a mean survival of 75% for ≤1-g tilapia fingerlings stocked in nursery ponds. Therefore, each stocking rate was increased 25% to yield an effective stocking rate of 30,000 or 40,000 fingerlings/ha. Ponds received two applications of chicken litter (1,000 kg dry matter/ha/wk) during the two weeks prior to stocking. Chicken litter was applied weekly at 1,000 kg dry matter/ha for the first eight weeks of the production cycle followed by feed (25% protein commercial fish feed) only. Chicken litter was purchased from a local layer operation and was comprised of bedding (rice or wheat chaff), manure, feathers, and waste feed. The dry matter (DM) content of chicken litter was determined weekly, before application, from the weight loss of a weighed sample after drying for 24 h at 60°C. All ponds received two initial applications of chicken litter (1,000 kg DM/ha) on 10 and 17 July 1994. Water was added to ponds periodically to replace evaporation and seepage. Tilapia were sampled by seine net to monitor growth on 21 September 94, 61 days after stocking.

Results

Average mean individual fish weights based on seine samples were 69.9 and 79.4 g/fish for ponds stocked at 30,000 and 40,000 fish/ha, respectively. Estimated respective mean fish biomasses, assuming 100% survival at the effective stocking rates, were 2,098 and 3,178 kg/ha. Ponds are scheduled to be harvested after 150 days of grow-out. Fish production and economic data will be analyzed upon completion of the experiment and presented in the final technical report.

Anticipated Benefits

Results of this experiment should permit determination of pond carrying capacity for the *Fertilizer then Feed* management system as well as determination of Nile tilapia growth parameters. In addition, economics of pond culture at higher tilapia stocking rates will be estimated.

Mass Production of Nile (*Oreochromis niloticus*) and Blue (*O. aureus*) Tilapia Fry

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Introduction

A consistent, reliable supply of fingerlings of the desired species, sex, and size is critical to the success of any aquacultural enterprise. Tilapia farmers around the world generally stock monosex Nile tilapia (*Oreochromis niloticus*) in their production ponds; frequently, these monosex fingerlings have been produced by sex reversal, although populations of all-male tilapia fingerlings are still sometimes obtained by inter-specific hybridization and by manual separation of sexes based on visual examination of the genital papilla.

Four tilapia species are endemic to Egypt: Nile, blue (*O. aureus*), *Sarotherodon galilae* and *Tilapia zilli*. Aquaculture production ponds in Egypt are often stocked with mixed-sex, mixed-species assemblages of tilapia; sex ratio and species composition vary among stockings. It is well-established that male tilapia grow faster than female tilapia. Nile and blue tilapia are considered better species for pond culture than *S. galilae* and *T. zilli*. Therefore, monoculture of monosex Nile or blue tilapia would result in greater fish yields than polyculture of mixed-sex tilapia. However, such a production strategy requires large numbers of monosex fingerlings.

Hormonal sex reversal is the most efficient means of mass production of monosex tilapia fingerlings at present. Newly hatched fry of 9- to 11-mm total length are given an androgen-treated feed during a 28-day treatment period. Fry for sex reversal can be produced in earthen ponds, nylon hapas, or concrete tanks. Green et al. (1994) described mass production of Nile tilapia fry in earthen ponds in relation to water temperature. A similar technique may be applied in Egypt for production of Nile and blue tilapia fry.

Materials and Methods

Ten 0.01-ha earthen ponds located at the Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt, were used for this study. Ponds were equipped with a 6-m^2 concrete harvest sump located in the vicinity of the drain pipe. Average water depth in ponds was 50 cm; water was added periodically to replace losses to evaporation and seepage. Prior to pond filling a piece of 1.28-cm square mesh netting was draped over the harvest sump and held in place with rocks. Two to four ponds were simultaneously stocked with either Nile or blue tilapia broodfish. Broodfish were fed a 25%-protein commercial ration at 2% of fish biomass daily. One pond was generally drained after 17 days (range 13 to 17 days) and the other after 19 days (range 18 to 24 days). At draining, the pond water level was lowered until only the sump had water. The netting in the sump was lifted to remove the broodfish; broodfish were transferred to a hauling tank for transport to concrete holding tanks until restocking in reproduction ponds. Fry were harvested by dip net (1.6-mm ace nylon mesh) and graded through 3.2-mm square mesh plastic netting. The numbers of fry trapped in puddles on pond bottoms or caught on drain screens were not estimated. Fry that passed through the grader were stocked into 2-m^2 hapas (1.6-mm ace nylon mesh) suspended in 28-m^2 concrete tanks for sex reversal. Multiple air stones were used in each tank. The number of fry stocked in hapas was estimated by visual comparison to a counted standard. A sample of 100 fry were measured for total length (TL); 100 fry were also weighed en masse to determine average weight. The total weight of fry retained by the grader was determined, as was the weight of a 100-fish sample. The total number of fry retained by the grader was estimated by dividing total weight by average individual weight. One hundred retained fry were measured for total length. Trials were initiated on 17 April 1994; data collected through September 1994 were included in this report.

Water temperature at 5- and 50-cm depths in three reproduction ponds and the supply canal were monitored continuously by computerized data logger. Thermistors were mounted at fixed depths below the water surface on floats constructed of PVC pipe.

Fry were stocked into hapas at 1,200 to 10,000 fry/m^2, depending on fry availability, for sex reversal. Fry were fed androgen-treated feed (60 mg methyltestosterone/kg feed) at a rate of 0.15 kg/kg fish biomass; daily ration was divided into four meals. Treatment duration was 28 days, after which androgen was withdrawn permanently from the diet. Hapas were harvested upon completion of the androgen treatment. A counted sample of 1,000 fingerlings was weighed. Total weight of fingerlings harvested was obtained and total numbers calculated by dividing total weight by average individual weight.

Results

A total of 68 reproduction pond harvests have been completed since trials were initiated on 1 April 1994. To date, 858,300 fry have been harvested, of which 659,000, or 79%, were suitable for sex reversal. The performance of Nile and blue tilapia appeared similar (Table 1). The smaller size of female blue tilapia might explain the slightly higher fry productivity observed per gram of female. Grading fry through a 3.2-mm square plastic mesh grader consistently yielded fry of uniform size and weight; fry passing the grader were within the size range (9- to 11-mm TL) desired for sex reversal.

Forty-nine sex reversal trials have been completed and 16 were in progress at the time of this report (Table 2). The difference between the total number of fry suitable for sex reversal (Table 1) and the total number of fry stocked in sex-reversal trials (Table 2) resulted from fry used in other research and handling-induced fry mortality. Overall, fry survival during treatment was lower than that observed in Honduras. Fry were sex reversed in hapas suspended in 28-m^3 concrete tanks. Water quality deterioration, primarily due to high concentrations of ammonia caused by high feeding rates, was probably responsible for much of the fry mortality. Tank management was modified and survival improved. However, limited availability of water, because of equipment malfunction, continued to restrict the ability to manage water quality in tanks, even at moderate feed loading rates.

Anticipated Benefits

Results of this research will provide Egyptian tilapia farmers with pond management techniques for the mass production of Nile and blue tilapia fry for sex reversal. Timing of the of reproduction ponds to maximize the production of fry suitable for sex reversal should be possible based on an evaluation of water temperature in terms

Table 1. Results of sixty-eight fry production trials for Nile (*Oreochromis niloticus*) and blue (*O. aureus*) tilapia (34 trials each) conducted in 0.01 ha earthen ponds. Reproduction ponds were generally drained and harvested 17 to 19 days after stocking. At harvest, fry were graded through 3.2-mm square mesh plastic netting.

Variable	Total	Nile tilapia	Blue tilapia
Estimate of total fry harvested (No./0.01 ha)	858,300	430,400	427,900
Average number of fry per harvest (No./0.01 ha)	12,600	12,700	12,600
Mean total fry productivity (No. fry/g female)	1.83	1.59	2.06
Fry suitable for sex reversal			
Number per harvest (No./0.01 ha)	659,000	340,300	318,700
Percent of total harvest (%)	79	82	76
Mean weight (g/fry)	0.014	0.015	0.013
Mean total length (mm/fry)	9.2	9.3	9.1
Fry too large for sex reversal			
Mean weight (g/fry)	0.133	0.131	0.135
Mean total length (mm/fry)	14.5	14.6	14.5
Female broodfish			
Mean weight (g/fish)	125	146	102
Mean number stocked (No./0.01 ha)	64	61	67
Male broodfish			
Mean weight (g/fish)	202	212	191
Mean number stocked (No./0.01 ha)	41	39	44

Table 2. Results of sex-reversal trials conducted in 2-m^2 hapas suspended in 28-m^3 concrete tanks. Fry were fed an androgen-treated diet (60 mg methyltestosterone/kg feed) for 28-days.

Variable	Total	Nile tilapia	Blue tilapia
Trials completed	49	23	26
Trials currently in progress	16	8	8
Total number of fry stocked	544,600	269,000	275,600
Mean fry stocking rate (No. fry/m^2 of hapa)	4,200	4,300	4,050
Fry harvested from treatment hapas			
Mean weight (g/fry)	0.38	0.37	0.39
Mean total length (mm/fry)	23.8	23.0	24.4
Mean survival (%)	51	61	43

of degree-days. This technique will permit more precise management of tilapia reproduction ponds. In addition, the mass production of sex-reversed Nile and blue tilapia has been demonstrated. Implementation of this technology in Egypt will dramatically increase the availability of monospecies, monosex tilapia fingerlings.

Literature Cited

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Growth of Control and Androgen-Treated Nile Tilapia, *Oreochromis niloticus* (L.), during Treatment, Nursery and Grow-Out Phases in Tropical Fish Ponds

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(Printed as submitted)

Abstract

Masculinization of sexually undifferentiated tilapia fry is achieved by oral administration of the androgen 17-α methyltestosterone (MT). An anabolic response to androgen treatment of tilapia has been reported. Growth of control and MT-treated tilapia was evaluated during consecutive treatment, nursery and grow-out phases under conditions approximating commercial, semi-intensive tilapia farms in Central America. Oreochromis *niloticus* (L.) fry were fed a 0 or 60 mg/kg MT diet for 28 days. Growth curves for control and MT-treated fish did not have significantly different slopes. Mean harvest fry weights were similar, averaging 0.1 g/fry for both treatments. Fry were subsequently stocked into 0.2-ha nursery ponds for 94 days of growth. Slopes of control and MT-treated fish growth curves were significantly different. Mean final individual weights did not differ significantly between treatments. Control fish did not deviate from the 1.1 male:female ratio, but MT-treated fish were 97% males. Control-male and MT-treated male fingerlings were stocked for grow-out into 0.1-ha organically fertilized earthen ponds. No significant difference in growth was observed between control and MT-treated fish. Mean gross yields after 150 days and mean final individual weights were similar for both treatments.

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Production of *Oreochromis niloticus* Fry for Hormonal Sex Reversal in Relation to Water Temperature

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(Printed as submitted)

Abstract

Recently hatched tilapia 9 to 11 mm total length (TL) are preferred for hormonal sex reversal because they are most likely to be sexually undifferentiated. Thirty-three trials were conducted in Honduras between September 1998 (sic) and March 1990 to quantify the effect of water temperature on *Oreochromis niloticus* fry production in earthen ponds for hormonal sex reversal. Two 0.1-ha ponds were simultaneously stocked with brood fish in each trial; generally, one pond was harvested after 17 days, the other after 20 days (range 16 to 21 days). Fry production was evaluated in relation to degree-days from the threshold temperature of 15°C. Harvests averaged 86,000 fry/0.05 ha. A total of 4,897,000 fry were produced, of which 4,363,000 fry were of appropriate size for hormone treatment. No fry production occurred at less than 140 degree-days; fry production increased significantly with increased degree-days above this level. Above 195 degree-days percent of the population retained by a 3.2-mm vexar-mesh grader (too large for androgen treatment) increased significantly with increased degree-days. Fry retained by the grader averaged 14.2 mm TL, while fry not retained by the grader averaged 9.5 mm TL. No significant linear relationship between degree-days and number of fry not retained by the grader was observed between 140 and 280 degree-days. However, production appeared to peak at about 210 degree-days.

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Phosphorus Fertilization Strategy in Fish Pond Based on Sediment Phosphorus Saturation Level

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(Printed as submitted)

Abstract

Two experiments were conducted to determine effective phosphorus (P) fertilization strategy in fish ponds in relation to sediment P saturation level. Experiment 1 was conducted in cement tanks with five levels of P saturated sediments (5%, 24%, 44%, 60% and 79%) and with three P-fertilization rates (0.2, 0.1 and 0.05 g/m3/day, N:P ratio of 2:1, 4:1 and 8:1, respectively. Nile tilapia (*Oreochromis niloticus*) were cultured in those cement tanks for 57 days. Results showed that the mean concentration of soluble reactive phosphorus (SRP) in water column increased with increasing sediment P saturation and P fertilization rate. The maximum net fish yield (NFY), 4.2±0.3 g/m3/day, was obtained at SRP concentration of 0.3 mg/L and higher concentrations did not increase fish yield. This level of SRP and NFY were attainable with P fertilization rate of 0.2, 0.1 and 0.05 g/m^3/day and N:P ratio of 2:1, 4:1 and 8:1 in ponds were the level of sediment P saturation was below 10%, above 45% and above 60%, respectively. Experiment 2 was conducted in earthen ponds to test and verify the P fertilization rate based on cement tank experimental results. Three new and three old ponds with 8±1.7% and 88±7.3% sediment P saturated in top 5-cm mud were fertilized at a rate of 0.2 and 0.05 g/m^2/day and N:P ratio of 2:1 and 8:1, respectively. Nile tilapia were cultured at $2/m^2$ for 85 days. The mean NFY obtained in new and old ponds were 1.73±0.08 and 2.24±0.32 g/m^2/day, respectively, which were not significantly different ($P > 0.05$). Results conclude that P fertilization rate should be based on P saturation level in mud to overcome the problem of under supply or over supply of P in fish pond.

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Determination of Phosphorus Saturation Level in Relation to Clay Content in Pond Mud

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(Printed as submitted)

Abstract

An experiment was conducted to determine the amount of phosphorus (P) needed to saturate simulated fish pond sediments which were made up to contain six levels of clay at 0%, 30%, 41%, 64%, 73% and 81% by weight. A series of cylindrical cement tanks were filled to 20 cm depth with six sediment types and triple superphosphate (TSP) solution was added to reach P saturation in sediment. Results showed that all sediment types reached constant inorganic-P concentration in upper 5-cm after 12 weeks of TSP application, and P adsorption capacity of sediment increased with increasing clay content. Sediment P adsorption was slower and not significant ($P > 0.05$) below the 5-cm depth except that contained 0% clay. Regression analysis showed that rate and adsorption capacity of P in sediment are primarily governed by clay content and its dominant minerals. While organic-P and loosely bound-P are commonly deposited in sediment most inorganic P is absorbed by cations to form cation-P complex. The linear relationship between cation-P saturation level and percent clay in sediment is highly significant (r^2 0.34, $P < 0.001$) and therefore, the maximum adsorption capacity of cation-P in pond sediment can be calculated by $Y = 0.019 X$ (Y = 100% saturation level mg-P/g soil: X = % clay in sediment). In practice the level of P saturation (%) in sediment can be calculated by the initial cation-P and clay content (%) in top 5-cm of pond mud using the equation: P saturation (%) = initial cation-P (mg/g soil) x 100/P adsorption capacity (mg/g soil).

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Appendix A. 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	Lempiras, 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

Appendix B. Table of Contents, Twelfth Annual Administrative Report