Pond Dynamics/Aquaculture Collaborative Research Support Program

Eighteenth Annual Technical Report

1 August 1999 to 31 July 2000

Acknowledgments
The Program Management Office of the Pond Dynamics/Aquaculture CRSP gratefully acknowledges the contributions of all the CRSP researchers and the support provided by the Universidad Juárez Autónoma de Tabasco, Villahermosa, Mexico; Escuela Agrícola Panamericana El Zamorano, Honduras; Instituto de Investigaciones de la Amazonia Peruana, Iquitos, Peru; Universidad Nacional de la Amazonia Peruana, Iquitos, Peru; Fisheries Department, Ministry of Agriculture and Rural Development, Nairobi, Kenya; Moi University, Eldoret, Kenya; University of Nairobi, Kenya; Central Luzon State University, Muñoz, Nueva Ecija, Philippines; and Asian Institute of Technology (AIT), Pathumthani, Thailand.

This report addresses program accomplishments of the Pond Dynamics/Aquaculture Collaborative Research Support Program during the reporting period of 1 August 1999 to 31 July 2000. Program activities are funded in part by the United States Agency for International Development (USAID) under Grant No. LAG-G-00-96-90015-00.

Edited by Anu Gupta, Kris McElwee, Deborah Burke, Jeff Burright, Xena Cummings, and Hillary Egna. Assistance provided by Danielle Clair, Heidi Furtado, Kristen Lewis, and Steve Sempier.

OREGON STATE UNIVERSITY

Pond Dynamics/Aquaculture CRSP Management Office
Oregon State University
418 Snell Hall
Corvallis, Oregon 97331-1643 USA

Contents

Appropriate Technology Research

III. SOCIAL AND ECONOMIC ASPECTS

Marketing and Economic Analysis Research

Adoption/Diffusion Research

Decision Support Systems Research

PD/A CRSP annual technical reports are the compiled reports of annual technical progress by PD/A CRSP researchers in addressing the objectives for which the various experiments, studies, and activities were funded. These technical reports address program accomplishments during the period 1 August 1999 to 31 July 2000.

The corresponding work plans for reports included appear in the CRSPs Eighth Work Plan and addenda and in the Ninth Work Plan and addendum. Work plans are available at the CRSP website (<pdacrsp.orst.edu>), by email request (claird@ucs.orst.edu), or by regular mail request by writing to Pond Dynamics/Aquaculture Collaborative Research Support Program, 418 Snell Hall, Oregon State University, Corvallis, OR 97331-1643, USA.

With a few exceptions, reports in this publication have been edited for clarity and consistency by the PD/A CRSP Information Management and Networking Component. Reports received after the submission deadline have not been edited and appear with the words “Printed as Submitted” on the first page.

Program activities are funded by USAID under Grant No. LAG-G-00-96-90015-00 and by participating US and host country institutions.

Pond Soil Characteristics and Dynamics of Soil Organic Matter and Nutrients

Ninth Work Plan, Pond Dynamics Research 2 (9PDR2)
Progress Report

Claude E. Boyd
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

C. Wesley Wood
Department of Agronomy and Soils
Auburn University, Alabama, USA

Taworn Thunjai and Martha Rowan
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Karen Dube
Central Institute of Fisheries Education
Verosa, Mumbai, India

Abstract

Pond soil cores were obtained from six ponds in Thailand. Cores were segmented into 2-cm-long segments, which were analyzed for texture, bulk density, pH, organic carbon, and major and minor nutrients. The resulting information increases the data base for use in presenting a concept of pond soil development and for preparation of a pond soil classification system. The six ponds in Thailand had profiles with discernible layers (horizons). Bulk density tended to increase with depth in the profile. Concentrations of carbon, nitrogen, and phosphorus decreased with depth, but sulfur concentration increased with depth. Concentrations of mineral nutrients were within the ranges of values found at other sites sampled during this project. There was considerable variation in bottom soil pH estimates made by several methods used in aquaculture. The simplest and most reliable method, direct insertion of a standard pH probe into a 1:1 mixture of dry soil (dried at 40°C and pulverized to pass through a 2.36-mm screen) and distilled water, should be adopted as a standard procedure.

Introduction

This report contains data on physical and chemical properties of pond soils from freshwater fish ponds at the former PD/A CRSP site at the Thailand Department of Fisheries Ayutthaya Fisheries Station and from shrimp ponds at Banggachi, Thailand. In addition, results of comparisons of different methods of measuring pond soil pH are included. This work was done because we became aware during conduct of this research that many different methods of soil pH measurement were being used in aquaculture. Therefore, our objective was to obtain background data for use in recommending a standard pond soil pH procedure.

During June 2000 we were able to collect additional pond soil cores for physical and chemical analyses from ponds in Brazil and from the PD/A CRSP site in the Philippines. However, it will take several months to complete all of the analyses.

Methods and Materials

Three ponds at the Ayutthaya Fisheries Station were sampled on 6 September 1999, and three ponds on a shrimp farm at Banggachi were sampled on 9 September 1999.

Analyses of Pond Soil Cores from Thailand

Soil cores were taken with a hand-operated, 5-cm-diameter core sampler (Wildlife Supply Company, Saginaw, Michigan, USA, Model No. 242A15). Procedures for separating the cores into successive 2-cm-long core segments were described by Munsiri et al. (1995). Core segments were dried at 102°C (moisture content and dry bulk density) or 60°C (other analyses) and transported to Auburn University for analyses.

Samples were analyzed for moisture content (gravimetry), dry bulk density (gravimetry), color (Munsell color chart), dry soil pH (1:1 slurry of dry soil and distilled water, glass electrode), exchangeable acidity (Adams-Evans buffer method), total carbon and nitrogen (Leco CHN Analyzer), total sulfur (Leco Sulfur Analyzer), acid-extractable phosphorus and metal ions (extraction in a 0.075 N acid solution of 0.05 N HCl plus 0.025 N H_2SO_4 followed by plasma spectrophotometry), and distilled-water-extractable phosphorus. Particle size analyses of samples were made by the pipette method. All methods followed details provided by Munsiri et al. (1995) and Boyd and Munsiri (1996).

Soil pH Measurement

Many soil samples used in this study had been retained from earlier research projects on soils from the United States, Honduras, Thailand, Indonesia, Madagascar, and Ecuador. Samples were taken from the upper 5-cm layers of pond bottoms of both brackishwater ponds (saline soils) and freshwater ponds (non-saline soils). All samples had been dried at 60°C in a forced-draft oven and pulverized to pass a screen with 0.85-mm openings. Additional samples for pH measurement were obtained during routine soil sampling at PD/A CRSP sites in Thailand, Honduras, Peru, Kenya, and the United States.

A series of 23 freshwater pond samples and 24 brackishwater pond samples were analyzed for pH (1:1 dry pulverized soil:distilled water) with a combination electrode and with dual electrodes. The pH was measured in two ways with dual electrodes: 1) the dual electrodes were inserted into the mixture and pH measured while stirring, or 2) the reference electrode was held in the supernatant and the glass electrode inserted into the sediment. The pH of these samples was also determined in soil:distilled water ratios of 1:2.5, 1:5, and 1:10; in 1:2 mixtures of soil:0.01 M $CaCl_2$; and in 1:2.5 mixtures of soil:1.00 M KCl using the combination electrode only. In addition, seven replicates of three samples from each series (low, medium, and high pH) were analyzed using the different soil:liquid mixtures and the combination electrode in order to estimate precision of measurements. Stirring was applied intermittently with a glass rod for periods of 10 to 15 s at 4- to 5-min intervals for 30 min before measuring pH. Stirring also was applied during pH measurement except where the soil was allowed to settle so that the reference electrode could be held in the supernatant and the glass electrode held in the sediment.

Table 1. Concentrations of moisture, dry bulk density, pH, and exchangeable acidity in pond soil profiles from Ayutthaya and Banggachi, Thailand.

Depth (cm)	Moisture (%)	Dry Bulk Density (g cm^{-3})	pH	Exchangeable Acidity (meq (100 g)$^{-1}$)
AYUTTHAYA				
0–2	417.9 ± 187.8	0.34 ± 0.02	6.80 ± 0.09	5.05 ± 0.72
2–4	239.6 ± 67.3	0.44 ± 0.03	6.53 ± 0.07	5.60 ± 0.46
4–6	240.0 ± 84.9	0.45 ± 0.03	6.35 ± 0.10	5.87 ± 0.53
6–8	279.2 ± 140.8	0.50 ± 0.02	6.43 ± 0.14	5.60 ± 0.46
8–10	159.5 ± 32.6	0.52 ± 0.01	6.28 ± 0.13	6.40 ± 0.46
10–12	148.7 ± 27.2	0.52 ± 0.02	6.28 ± 0.12	7.63 ± 0.69
12–14	141.6 ± 19.7	0.53 ± 0.02	6.40 ± 0.15	6.67 ± 0.27
14–16	152.8 ± 30.2	0.47 ± 0.03	6.45 ± 0.16	6.40 ± 0.00
16–18	143.9 ± 17.6	0.52 ± 0.03	6.47 ± 0.16	6.40 ± 0.80
18–20	160.0 ± 40.1	0.54 ± 0.01	6.60 ± 0.23	6.40 ± 0.92
20–22	137.2 ± 29.1	0.57 ± 0.02	6.52 ± 0.22	5.07 ± 0.27
22–24	132.6 ± 20.9	0.56 ± 0.01	6.57 ± 0.26	6.13 ± 0.71
24–26	143.0 ± 29.6	0.56 ± 0.02	6.42 ± 0.31	5.76 ± 0.49
26–28	110.1 ± 5.0	0.54 ± 0.02	6.40 ± 0.25	6.03 ± 0.23
28–30	106.3 ± 5.2	0.58 ± 0.02	6.42 ± 0.37	5.76 ± 0.49
30–32	101.2 ± 6.9	0.63 ± 0.03	6.43 ± 0.32	5.76 ± 0.49
32–34	94.6 ± 7.5	0.66 ± 0.02	6.37 ± 0.35	6.93 ± 0.53
34–36	85.8 ± 3.3	0.76 ± 0.01	6.52 ± 0.31	5.87 ± 0.53
36–38	86.1 ± 2.1	0.74 ± 0.03	6.73 ± 0.10	6.13 ± 0.71
38–40	79.9 ± 5.5	0.64 ± 0.05	6.67 ± 0.08	6.13 ± 0.27
40–42	81.7 ± 12.0	0.73 ± 0.06	6.55 ± 0.05	5.87 ± 0.71
42–44	84.0 ± 2.0	0.71 ± 0.02	6.57 ± 0.12	5.87 ± 0.71
44–46	113.0 ± 43.5	0.71 ± 0.07	6.52 ± 0.07	5.87 ± 0.71
BANGGACHI				
0–2	183.9 ± 26.2	0.44 ± 0.03	6.57 ± 0.20	7.53 ± 0.71
2–4	112.2 ± 13.7	0.59 ± 0.07	6.58 ± 0.19	7.20 ± 1.22
4–6	108.4 ± 15.1	0.63 ± 0.09	6.32 ± 0.24	9.33 ± 2.37
6–8	113.7 ± 10.4	0.60 ± 0.07	6.08 ± 0.28	11.20 ± 2.77
8–10	114.4 ± 16.0	0.60 ± 0.08	5.95 ± 0.22	9.17 ± 2.74
10–12	121.7 ± 5.5	0.54 ± 0.03	5.77 ± 0.35	8.91 ± 2.02
12–14	120.2 ± 0.8	0.54 ± 0.02	5.87 ± 0.39	8.53 ± 2.18
14–16	111.8 ± 3.6	0.60 ± 0.02	6.00 ± 0.43	8.00 ± 3.03

Fresh, wet samples from ponds at Auburn University and PD/A CRSP sites in Thailand, Honduras, Peru, and Kenya were subjected to direct pH measurement by inserting a combination electrode into the wet soil (upper 5-cm layer) and stirring gently with a glass rod. Samples were then dried at 60°C in a forced-draft oven and pulverized for pH determination in a 1:1 soil:distilled water mixture with a combination electrode.

To determine the influence of time on the pH measurement, triplicates of non-saline and saline soil samples were mixed at a 1:1 ratio with distilled water, and pH was measured with the combination electrode at 2-min intervals.

The influence of drying temperature and particle size on pH was evaluated. Samples of bottom soils from ponds on the Auburn University Fisheries Resource Unit heated to 40°C, 60°C, and 102°C in a forced-draft oven were pulverized to pass screens with the following United States Standard Sieve Series numbers and opening sizes: Number 8, 2.36 mm; Number 20, 0.85 mm; Number 40, 0.425 mm; Number 60, 0.25 mm; Number 100, 0.149 mm; and Number 270, 0.053 mm. The pH measurements were made by a combination electrode in 1:1 soil:distilled water mixtures.

The influence of stirring during the pH measurement was compared to not stirring on a series of soil samples. Also, for selected samples the soil:water mixtures were filtered through fine filter paper (Whatman Number 42), and the pH measured in the mixture was compared to the pH of filtrate.

A new Kelway® Soil Acidity and Moisture Tester, Model HB-2 was obtained. Dry pulverized soils (500 g) from nine brackishwater ponds and eleven freshwater ponds were placed in plastic containers and thoroughly mixed with enough water to provide a 25% moisture content. The soil acidity tester was inserted into the soil, and the soil was pressed around the tester to assure close contact. The pH was measured according to the manufacturer's instructions. Another portion of each soil sample was used for pH determination by a combination electrode in 1:1 soil:distilled water mixtures. In another trial, dry pulverized soil from a freshwater pond at Auburn University was treated by filling the pore space in different aliquots with water of 0, 0.17, 0.34, 0.68, 1.36, 2.72, 5.44, 10.2, 15.0, and 30‰ salinity. Soils were dried and pH was measured by the soil acidity tester and the standard pH meter with the combination electrode as described above.

Statistical analyses included t-tests, Duncan's multiple range test, regression analysis, and tests to determine if slopes and intercepts of regression lines differed from 1.0 and 0.0, respectively. The statistical package SigmaStat was used (SPSS, 1997).

Table 2. Profile for color in soil cores from bottoms of aquaculture ponds from Ayutthaya and Banggachi, Thailand. Color values are given as standard Munsell Color Chart Units. Each entry is the value of a particular pond.

Depth (cm)	**Ayutthaya**			**Banggachi**		
	Pond 9	*Pond 10*	*Pond 11*	*Pond 1*	*Pond 2*	*Pond 3*
0–2	5Y 4/1	5Y 4/1	5Y 4/1	2.5Y 2.5/1	2.5Y 2.5/1	2.5Y 2.5/1
2–4	5Y 4/1	5Y 2.5/1	5Y 4/1	2.5Y 4/2	2.5Y 4/1	2.5Y 4/1
4–6	5Y 4/1	5Y 2.5/1	5Y 4/1	2.5Y 4/2	2.5Y 5/1	2.5Y 4/1
6–8	5Y 4/1	5Y 4/1	5Y 4/1	2.5Y 2.5/1	2.5Y 5/1	2.5Y 5/1
8–10	5Y 4/1	5Y 4/1	5Y 4/1	2.5Y 3/1	2.5Y 5/1	2.5Y 4/2
10–12	5Y 4/1	5Y 4/1	5Y 4/1	2.5Y 5/1	2.5Y 5/1	2.5Y 4/2
12–14	5Y 4/1	5Y 4/1	5Y 4/1	2.5Y 5/1	2.5Y 5/1	2.5Y 2.5/1
14–16	5Y 4/1	5Y 2.5/1	5Y 4/1	2.5Y 5/1	2.5Y 5/1	2.5Y 4/1
16–18	5Y 4/1	5Y 2.5/1	5Y 4/1			
18–20	5Y 4/1	5Y 2.5/1	5Y 5/1			
20–22	5Y 3/1	5Y 2.5/1	5Y 5/1			
22–24	5Y 3/1	5Y 2.5/1	5Y 5/1			
24–26	5Y 2.5/1	5Y 2.5/1	5Y 5/1			
26–28	5Y 2.5/1	5Y 2.5/1	5Y 5/1			
28–30	5Y 2.5/1	5Y 2.5/1	5Y 5/1			
30–32	5Y 2.5/1	5Y 2.5/1	5Y 5/1			
32–34	5Y 2.5/1	5Y 2.5/1	5Y 5/1			
34–36	5Y 2.5/1	5Y 2.5/1	5Y 5/1			
36–38	5Y 2.5/1	5Y 2.5/1	5Y 4/1			
38–40	5Y 2.5/1	5Y 2.5/1	5Y 4/1			
40–42	5Y 2.5/1	5Y 3/1	5Y 4/1			
42–44	5Y 2.5/1	5Y 3/1	5Y 4/1			
44–46	5Y 4/1	5Y 4/1	5Y 4/1			

Note: 5Y 2.5/1—black; 5Y 3/1—very dark gray; 5Y 4/1—dark gray; 5Y 5/1—gray; 2.5Y 2.5/1—black; 2.5Y 3/1—very dark gray; 2.5Y 4/1—dark gray; 2.5Y 4/2—dark grayish brown; 2.5Y 5/1—gray

RESULTS AND DISCUSSION

Characteristics of Cores

The moisture content of soil was highest in the 0–2 cm layer at Ayutthaya and Banggachi (Table 1). At Ayutthaya, soil moisture content decreased rapidly with depth, but at Banggachi there was little difference in soil moisture between the 2–4 cm layer and deeper layers. Ponds at both sites were in low-lying areas, and the groundwater level was apparently contiguous with the water-saturated bottom soil. Thus, although dry bulk density of soil increased with depth (Table 1), we were unable to core to depths with bulk density of 1.0 g m^{-3} and above as has been the case in ponds at other sites sampled during this research effort (Munsiri et al., 1995; Boyd et al., 1998, 1999, 2000). Soil color did not change appreciably with depth in the cores (Table 2), so the soils apparently were not as highly reduced as most of the other pond soils sampled in this project (Munsiri et al., 1995; Boyd et al., 1998, 1999, 2000).

Because of the variation that occurs by using different measurement techniques for soil pH, we decided against measuring wet soil pH. The dry soil pH appears to be a more reliable method for soil pH (see section on Pond Soil pH Measurement, p. 6), so we only made this measurement. The dry soil pH (Table 1) was highest in the surface 0–2 and 2–4 cm layers and tended to decrease with depth in the cores. However, there was some variation within this pattern. For example, in the cores from ponds at Ayutthaya, the pH was 6.80 in the 0–2 cm layer, 6.28 in the 8–10 cm layer, 6.57 in the 22–24 cm layer, and 6.37 in the 32–34 cm layer. The soils at both sites did not have particularly low pH; most values exceeded 6. Therefore, it was not surprising that exchangeable acidity was rather low and usually below 7 meq $(100\ g)^{-1}$ and 10 meq $(100\ g)^{-1}$ in samples from Ayutthaya and Banggachi, respectively (Table 1).

Total carbon concentrations were between 0.72 and 1.40% at Ayutthaya and 2.34 and 4.49% at Banggachi (Table 3). The ponds at Banggachi were operated for intensive shrimp production, and they had much higher inputs of nutrients and organic matter than ponds at Ayutthaya. This probably is the reason for the difference in carbon concentration. There was a distinct decrease in carbon concentration with depth in the soil profiles at both sites. Nitrogen concentrations at Ayutthaya were between 0.12 and 0.23% (Table 3), and carbon:nitrogen ratios were approximately 6. At Banggachi, nitrogen concentrations were 0.23 to 0.28% (Table 3), and carbon:nitrogen ratios were approximately 10 to 16. Nitrogen concentrations declined with depth in the soil profile at Ayutthaya but not at Banggachi.

Total sulfur concentration ranged from 0.07% in the 0–2 cm layer to a maximum of 0.32% in the 20–22 cm layer in ponds at Ayutthaya (Table 3). Sulfur concentrations tended to be higher below a depth of 20 cm than in upper layers. Soils in ponds at Ayutthaya had fairly high concentrations of sulfur for a freshwater site, but they were much lower in sulfur concentration than the brackishwater ponds at Banggachi (Table 3). Sulfur concentrations in pond cores at Banggachi exceeded 0.5% at all depths, and the soils below 10 cm depth can be considered potential acid-sulfate soils because they contain more than 0.75% sulfur (Soil Survey Staff, 1994).

Acid-extractable phosphorus concentrations in the samples from Ayutthaya ranged from 18.7 to 28.7 ppm with no clear indication of increases or decreases with depth (Table 3). At Banggachi, acid-extractable phosphorus concentrations were between 38.3 and 47.0 ppm (Table 3), and no trend in concentration with depth was apparent. Concentrations of water-extractable phosphorus often varied considerably between adjacent layers in soil cores from Ayutthaya (Table 3), but there was a general tendency for concentrations to decline with depth. The 0–2 cm layer contained 0.92 ppm water-extractable phosphorus. This is a high concentration, which probably resulted from recent applications of phosphate fertilizer to ponds. Boyd and Munsiri (1996) demonstrated that fertilizer phosphorus recently adsorbed by pond soil is more readily released than other forms of soil phosphorus. Water-extractable phosphorus concentrations were low at all depths in samples from Banggachi.

Concentrations of calcium and magnesium are provided in Table 4. The high concentrations of calcium and magnesium in soil samples from freshwater ponds at Ayutthaya are surprising. Soil pH is less than 7 at this site, suggesting that free carbonates are not present in the soil, so the source of the calcium and magnesium is not due to naturally occurring calcium and magnesium carbonates. Also, the increase in calcium with depth suggests that applications of calcium to the water via liming materials or gypsum is not a likely source of the calcium. Magnesium concentrations tended to decline with increasing depth in the soil profile at Ayutthaya. The soil cores from the ponds at Banggachi were higher in calcium and magnesium than those from Ayutthaya (Table 4). Of course, brackishwater ponds typically are higher in calcium and magnesium because these two ions have high concentrations in brackish water.

Potassium and sodium concentrations also were relatively high in soil cores from ponds at Ayutthaya (Table 4) as compared to many freshwater pond soils. For example, most soils from freshwater ponds contain less than 100 ppm each of sodium and potassium (Boyd et al., 1994). Sodium and potassium concentrations tended to be higher in upper layers of soil than in deeper layers at Ayutthaya. Brackish water has high concentrations of sodium and potassium, so it is not surprising that these two elements were more concentrated in soils at Banggachi than in soils at Ayutthaya (Table 4).

Concentrations of iron, manganese, zinc, and copper in pond soils at Ayutthaya and Banggachi (Table 5) were within ranges of minor element concentrations normally found in freshwater and brackishwater pond soils (Boyd et al., 1994, 1998, 1999,

Table 3. Concentrations of total carbon, total nitrogen, total sulfur, acid-extracted phosphorus, and water-soluble phosphorus in pond soil profiles from Ayutthaya and Banggachi, Thailand.

Depth (cm)	**Total Carbon** (%)	**Total Nitrogen** (%)	**Total Sulfur** (%)	**Acid-Extracted Phosphorus** (ppm)	**Water-Soluble Phosphorus** (ppm)
AYUTTHAYA					
0–2	1.40 ± 0.19	0.23 ± 0.04	0.07 ± 0.01	28.7 ± 0.7	0.92 ± 0.46
2–4	1.37 ± 0.16	0.20 ± 0.01	0.15 ± 0.04	24.7 ± 3.5	0.05 ± 0.02
4–6	1.27 ± 0.12	0.19 ± 0.02	0.11 ± 0.04	25.0 ± 4.6	0.18 ± 0.15
6–8	1.17 ± 0.05	0.19 ± 0.01	0.12 ± 0.01	29.3 ± 3.0	0.42 ± 0.37
8–10	1.08 ± 0.11	0.18 ± 0.03	0.10 ± 0.01	25.6 ± 5.5	0.85 ± 0.45
10–12	1.06 ± 0.09	0.17 ± 0.02	0.12 ± 0.02	27.3 ± 0.3	0.16 ± 0.12
12–14	1.04 ± 0.08	0.18 ± 0.02	0.09 ± 0.00	27.0 ± 0.6	0.06 ± 0.03
14–16	1.04 ± 0.04	0.17 ± 0.01	0.08 ± 0.02	24.3 ± 3.2	0.15 ± 0.14
16–18	1.04 ± 0.10	0.18 ± 0.04	0.09 ± 0.02	21.0 ± 0.0	0.51 ± 0.48
18–20	1.06 ± 0.11	0.19 ± 0.02	0.09 ± 0.03	19.7 ± 0.3	0.32 ± 0.29
20–22	1.12 ± 0.14	0.19 ± 0.02	0.32 ± 0.20	26.3 ± 1.5	0.08 ± 0.04
22–24	0.95 ± 0.05	0.16 ± 0.00	0.16 ± 0.09	19.7 ± 3.7	0.46 ± 0.42
24–26	0.95 ± 0.12	0.17 ± 0.03	0.16 ± 0.08	20.3 ± 1.9	0.04 ± 0.01
26–28	0.92 ± 0.11	0.16 ± 0.02	0.22 ± 0.06	20.7 ± 0.3	0.05 ± 0.01
28–30	0.95 ± 0.13	0.16 ± 0.02	0.19 ± 0.05	24.7 ± 3.9	0.05 ± 0.01
30–32	0.93 ± 0.09	0.16 ± 0.02	0.19 ± 0.06	25.0 ± 2.1	0.04 ± 0.01
32–34	0.92 ± 0.06	0.17 ± 0.02	0.18 ± 0.04	22.3 ± 3.5	0.04 ± 0.01
34–36	0.93 ± 0.02	0.16 ± 0.01	0.15 ± 0.04	27.7 ± 6.4	0.05 ± 0.01
36–38	0.85 ± 0.05	0.15 ± 0.02	0.16 ± 0.03	26.0 ± 4.7	0.04 ± 0.01
38–40	0.86 ± 0.04	0.15 ± 0.03	0.16 ± 0.03	21.7 ± 5.7	0.12 ± 0.08
40–42	0.95 ± 0.03	0.19 ± 0.00	0.23 ± 0.01	23.0 ± 3.8	0.02 ± 0.00
42–44	0.76 ± 0.16	0.14 ± 0.03	0.15 ± 0.04	26.3 ± 4.7	0.03 ± 0.01
44–46	0.72 ± 0.11	0.12 ± 0.01	0.23 ± 0.01	18.7 ± 4.5	0.03 ± 0.00
BANGGACHI					
0–2	4.49 ± 0.77	0.28 ± 0.03	0.71 ± 0.19	47.0 ± 9.6	0.04 ± 0.01
2–4	4.08 ± 0.84	0.24 ± 0.01	0.64 ± 0.16	43.7 ± 4.3	0.07 ± 0.03
4–6	3.89 ± 0.83	0.26 ± 0.01	0.68 ± 0.21	46.0 ± 4.0	0.07 ± 0.04
6–8	3.62 ± 0.78	0.27 ± 0.02	0.78 ± 0.30	45.3 ± 7.4	0.08 ± 0.05
8–10	3.13 ± 0.47	0.25 ± 0.01	0.53 ± 0.30	46.3 ± 8.0	0.06 ± 0.03
10–12	2.77 ± 0.37	0.23 ± 0.00	0.78 ± 0.27	45.0 ± 10.5	0.07 ± 0.04
12–14	2.74 ± 0.71	0.23 ± 0.02	0.97 ± 0.38	42.0 ± 2.5	0.06 ± 0.03
14–16	2.34 ± 0.58	0.24 ± 0.01	0.92 ± 0.49	38.3 ± 11.4	0.05 ± 0.04

Table 4. Concentrations of calcium, magnesium, sodium, and potassium in pond soil profiles from Ayutthaya and Banggachi, Thailand.

Depth (cm)	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Potassium (ppm)
AYUTTHAYA				
0–2	5,404 ± 736	2,539 ± 190	906 ± 167	354 ± 21
2–4	5,334 ± 614	2,474 ± 259	659 ± 91	357 ± 27
4–6	5,570 ± 914	2,434 ± 217	727 ± 105	348 ± 15
6–8	5,543 ± 640	2,309 ± 205	597 ± 65	336 ± 10
8–10	5,164 ± 1137	1,933 ± 128	565 ± 109	299 ± 33
10–12	5,827 ± 457	2,126 ± 110	547 ± 50	314 ± 3
12–14	5,897 ± 480	2,119 ± 83	667 ± 82	314 ± 13
14–16	6,037 ± 363	2,119 ± 96	562 ± 47	309 ± 16
16–18	6,714 ± 713	2,117 ± 114	531 ± 51	294 ± 13
18–20	6,817 ± 571	2,084 ± 132	689 ± 83	292 ± 11
20–22	7,967 ± 1,106	2,414 ± 390	4,834 ± 4,304	411 ± 115
22–24	7,539 ± 1,000	2,041 ± 144	545 ± 63	279 ± 14
24–26	7,203 ± 1,023	2,064 ± 142	626 ± 10	281 ± 17
26–28	7,396 ± 1,346	2,071 ± 132	528 ± 73	272 ± 25
28–30	7,752 ± 1,473	2,014 ± 120	570 ± 52	262 ± 33
30–32	8,053 ± 1,046	2,009 ± 148	547 ± 138	259 ± 22
32–34	8,114 ± 940	1,990 ± 125	484 ± 75	248 ± 24
34–36	7,988 ± 845	1,994 ± 153	486 ± 88	248 ± 21
36–38	7,804 ± 994	1,934 ± 122	479 ± 80	250 ± 22
38–40	9,029 ± 304	1,916 ± 113	590 ± 132	233 ± 22
40–42	8,490 ± 369	1,980 ± 107	529 ± 100	247 ± 24
42–44	8,105 ± 363	1,863 ± 195	512 ± 132	222 ± 47
44–46	9,528 ± 151	1,885 ± 140	558 ± 157	209 ± 26
BANGGACHI				
0–2	5,521 ± 356	4,519 ± 186	16,004 ± 1,866	1,120 ± 97
2–4	3,808 ± 164	3,958 ± 81	14,637 ± 1,864	1,172 ± 76
4–6	3,484 ± 346	3,868 ± 267	15,779 ± 2,772	1,279 ± 67
6–8	3,804 ± 748	3,984 ± 528	16,750 ± 3,790	1,260 ± 118
8–10	3,712 ± 1,014	3,874 ± 536	17,965 ± 4,214	1,318 ± 113
10–12	4,917 ± 1,990	4,067 ± 640	19,773 ± 5,150	1,349 ± 124
12–14	5,575 ± 2,247	4,132 ± 251	21,566 ± 5,249	1,300 ± 73
14–16	5,751 ± 1,821	3,695 ± 445	15,645 ± 2,543	1,145 ± 279

2000). There are no interesting points to discuss about the minor elements.

Soils at both sites were categorized as clays or silty clays (Table 6). At Ayutthaya, clay concentrations were generally between 50 and 60% at all depths, and there was no distinct layering of clay concentrations (Table 7). Most of the remaining particles were silt-sized. At Banggachi, clay content was 40 to 45% in the upper 12-cm layer of the profile and about 30% in the deeper layer (Table 7). Silt content tended to increase as clay content decreased. Sand comprised 8 to 12% of particles in soils at Banggachi.

This project on pond soils has yielded a large amount of information on the characteristics of pond soil cores. Samples have been collected as follows: Thailand, four sites (15 ponds); Philippines, one site (3 ponds); Honduras, two sites (6 ponds); Kenya, one site (3 ponds); Brazil, two sites (6 ponds); Peru, two sites (6 ponds); and the United States, five sites (18 ponds). We have completed all analyses except those on samples from Brazil and the Philippines. These analyses should be completed by October or November 2000.

Pond Soil Profile Development

We have obtained cores from ponds that range from 2 to 52 years in age. Even the youngest ponds developed profiles with distinct layers (horizons). The thicknesses of the S and M horizons (Munsiri et al., 1995) tend to increase with pond age. Concentrations of organic matter are usually low in new ponds and quickly increase to 2 to 4% in the S horizon and 1 to 3% in the M horizon. Afterwards, there seems to be little change in organic matter concentration with increasing pond age, but the thicknesses of the S and M horizons tend to increase over time. Aquaculture activities also influence bottom soil profiles, as upper layers of soil tend to have greater concentrations of phosphorus as a result of fertilization and higher pH and calcium and magnesium concentrations as a result of liming. The dominant processes in pond soil development appear to be external inputs of mineral sediment (soil particles), internal input of organic sediment (dead plankton), internal resuspension and resedimentation, and changes in soil properties resulting from nutrient and lime inputs. Soil profile development appears to be much faster than in terrestrial soils, because the soil was already developed before ponds were constructed. Thus, the parent material for pond bottom soil development is the soil in the pond bottom and soil particles entering as suspended solids from the water supply. The particle size distribution, the nature of the clay minerals, and the acidity of this material influences the characteristics of pond soil profiles and horizons, but the major factor leading to formation of profiles and horizons in pond soils is sedimentation.

Examination of the data suggests that the factors around which to develop a pond soil classification system are:

- Thicknesses of S and M horizons;
- Bulk density of S horizon;
- pH and acidity of S horizon;
- Organic carbon and C/N ratio;
- Total sulfur concentration;
- Cation exchange capacity; and
- Texture (particle size distribution).

As soon as all of the chemical analyses have been completed, we will begin preparation of the pond soil classification system.

Pond Soil pH Measurement

Values for pH obtained from samples by both dual electrodes and combination electrodes with electrodes inserted into the soil-water mixture were highly correlated for both non-saline and saline soil (Figure 1). The slopes and intercepts of the regression lines did not differ from 1.0 and 0.0 ($P > 0.05$). Means of pH measured for replicate samples by the two methods did not differ for the six samples (Table 8). There were no differences in the precision of the two methods based on F-tests of variances ($P > 0.05$). Thus, a combination electrode and dual electrodes should provide comparable pH readings when inserted into a mixture of soil and distilled water. The small differences of 0.05 to 0.1 pH units sometimes observed in this study between the dual electrodes and the combination electrode were attributed to experimental error.

Table 5. Concentrations of iron, manganese, zinc, and copper in pond soil profiles from Ayutthaya and Banggachi, Thailand.

Depth (cm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)
AYUTTHAYA				
0–2	388 ± 83	688 ± 29	13.67 ± 0.88	7.00 ± 0.58
2–4	464 ± 73	657 ± 21	14.67 ± 2.19	7.67 ± 0.33
4–6	511 ± 97	666 ± 29	14.00 ± 1.53	7.33 ± 0.33
6–8	523 ± 99	687 ± 49	14.33 ± 0.67	7.67 ± 0.33
8–10	507 ± 146	588 ± 103	13.33 ± 1.20	7.33 ± 0.33
10–12	565 ± 67	635 ± 64	16.33 ± 0.67	8.33 ± 0.33
12–14	652 ± 91	614 ± 66	17.67 ± 1.20	8.33 ± 0.33
14–16	688 ± 92	631 ± 67	19.00 ± 0.58	8.67 ± 0.67
16–18	667 ± 90	636 ± 71	19.33 ± 0.88	8.33 ± 0.88
18–20	660 ± 119	614 ± 75	18.67 ± 0.88	8.33 ± 0.88
20–22	540 ± 105	574 ± 81	16.33 ± 3.18	5.33 ± 2.67
22–24	557 ± 194	585 ± 69	16.33 ± 1.45	6.00 ± 2.08
24–26	709 ± 128	573 ± 84	17.00 ± 1.53	6.67 ± 1.86
26–28	703 ± 244	589 ± 79	16.33 ± 1.76	6.00 ± 3.06
28–30	661 ± 274	546 ± 55	15.67 ± 3.84	6.00 ± 3.06
30–32	669 ± 129	544 ± 25	16.00 ± 1.73	6.33 ± 1.67
32–34	469 ± 212	544 ± 29	15.33 ± 2.33	5.00 ± 2.00
34–36	516 ± 121	532 ± 19	14.67 ± 2.19	5.67 ± 2.19
36–38	647 ± 161	547 ± 34	15.67 ± 1.67	6.33 ± 2.03
38–40	504 ± 92	529 ± 45	13.67 ± 1.76	3.00 ± 1.73
40–42	600 ± 94	538 ± 28	16.67 ± 2.67	6.33 ± 1.67
42–44	869 ± 127	801 ± 168	15.33 ± 5.78	5.67 ± 2.96
44–46	511 ± 87	566 ± 48	13.00 ± 3.21	3.00 ± 1.53
BANGGACHI				
0–2	345 ± 66	167 ± 63	12.33 ± 2.19	0.00 ± 0.00
2–4	590 ± 113	103 ± 35	11.33 ± 1.86	0.33 ± 0.33
4–6	760 ± 194	91 ± 27	17.00 ± 5.51	0.67 ± 0.67
6–8	796 ± 146	98 ± 35	18.67 ± 7.45	1.33 ± 0.67
8–10	794 ± 149	106 ± 47	12.67 ± 5.78	1.33 ± 0.67
10–12	713 ± 71	132 ± 58	12.33 ± 5.04	1.00 ± 0.58
12–14	534 ± 208	142 ± 49	14.00 ± 10.54	0.67 ± 0.67
14–16	747 ± 154	236 ± 95	18.67 ± 9.68	1.67 ± 0.33

There was a strong correlation between soil pH determined with the dual electrodes and the combination electrode when the dual electrodes were arranged with the indicating electrode in the sediment and the reference electrode in the supernatant (Figure 2). However, at $P = 0.05$ the slope of the regression line did not equal 1.0 and the intercept did not equal 0.0 for non-saline soil, and while the intercept was 0.0 for saline soil, the slope did not equal 1.0. The difference in pH measured by the combination electrode and that measured by dual electrodes with the indicating electrode in the sediment and the reference electrode in the supernatant ranged from –0.25 to 0.35 (average = 0.23) for non-saline soil and from –0.80 to 0.39 (average = 0.17) for saline soil. By use of regression equations, it was found that the difference in pH by the two methods decreased from –0.38 pH units at pH 4 to –0.28 pH units at pH 8 for non-saline soils and from 0.22 pH units at pH 4 to –0.06 pH units at pH 8 for saline soils.

Although the method of inserting the indicating electrode in the sediment and the reference electrode in the supernatant theoretically minimizes liquid junction and suspension effects on soil pH measurements, the differences in pH values obtained by the two methods do not appear large enough to justify the greater difficulty in positioning the two electrodes at different positions in the soil:distilled water medium. The high correlation between the two procedures also justifies the use of the easier method of inserting either a combination electrode or the dual electrodes directly into the sediment and measuring pH while stirring.

Table 6. Profile for soil texture in soil cores from bottoms of aquaculture ponds from Ayutthaya and Banggachi, Thailand. Each entry is the average of three ponds.

Depth (cm)	Ayutthaya	Banggachi
0–2	Silty clay	Silty clay
2–4	Clay	Silty clay
4–6	Clay	Silty clay
6–8	Silty clay	Silty clay
8–10	Clay	Silty clay
10–12	Clay	Silty clay
12–14	Silty clay	Silty clay
14–16	Silty clay	Silty clay
16–18	Silty clay	
18–20	Silty clay	
20–22	Silty clay	
22–24	Silty clay	
24–26	Silty clay	
26–28	Silty clay	
28–30	Silty clay	
30–32	Silty clay	
32–34	Silty clay	
34–36	Clay	
36–38	Silty clay	
38–40	Clay	
40–42	Silty clay	
42–44	Silty clay	
44–46	Silty clay	

Table 7. Profile for particle sizes in soil cores from bottoms of aquaculture ponds from Ayutthaya and Banggachi, Thailand. Averages and standard errors are given as percentages. Each entry is the average of three ponds.

Depth (cm)	Ayutthaya			Banggachi		
	Sand (%)	*Silt* (%)	*Clay* (%)	*Sand* (%)	*Silt* (%)	*Clay* (%)
0–2	1.47 ± 0.53	43.49 ± 1.53	55.04 ± 2.05	9.88 ± 3.62	47.96 ± 1.79	42.16 ± 4.09
2–4	0.81 ± 0.34	39.35 ± 2.35	59.84 ± 2.36	10.85 ± 4.75	44.60 ± 1.33	44.55 ± 4.16
4–6	0.71 ± 0.06	39.87 ± 2.12	59.43 ± 2.13	8.09 ± 3.41	47.34 ± 1.30	44.57 ± 2.54
6–8	1.34 ± 0.33	43.09 ± 1.42	55.57 ± 1.41	9.27 ± 4.23	48.62 ± 0.79	42.11 ± 4.19
8–10	1.18 ± 0.54	39.38 ± 0.63	59.44 ± 0.73	10.10 ± 1.79	45.09 ± 1.42	44.81 ± 3.03
10–12	1.08 ± 0.30	39.83 ± 1.28	59.09 ± 1.16	10.42 ± 3.96	48.95 ± 2.23	40.64 ± 6.16
12–14	1.04 ± 0.31	42.15 ± 3.17	56.81 ± 2.95	11.62 ± 5.10	56.05 ± 6.91	32.33 ± 9.50
14–16	0.78 ± 0.07	40.88 ± 1.81	58.33 ± 1.73	11.98 ± 5.07	56.72 ± 4.48	31.30 ± 8.57
16–18	0.70 ± 0.05	40.86 ± 3.80	58.44 ± 3.83			
18–20	0.92 ± 0.17	42.70 ± 4.58	56.37 ± 4.70			
20–22	0.86 ± 0.47	43.01 ± 5.72	56.13 ± 6.19			
22–24	0.73 ± 0.17	44.11 ± 2.79	55.16 ± 2.95			
24–26	0.97 ± 0.39	41.82 ± 3.84	57.21 ± 3.91			
26–28	0.80 ± 0.20	41.91 ± 3.81	57.29 ± 4.01			
28–30	0.48 ± 0.06	44.29 ± 2.92	55.23 ± 2.91			
30–32	0.76 ± 0.10	43.86 ± 2.60	55.37 ± 2.68			
32–34	0.80 ± 0.23	41.73 ± 1.15	57.47 ± 1.35			
34–36	0.85 ± 0.14	35.35 ± 6.54	63.80 ± 6.44			
36–38	1.19 ± 0.11	48.19 ± 5.29	50.61 ± 5.23			
38–40	1.21 ± 0.31	37.60 ± 5.91	61.19 ± 6.14			
40–42	1.34 ± 0.45	42.24 ± 1.32	56.43 ± 1.37			
42–44	0.95 ± 0.10	44.10 ± 1.52	54.95 ± 1.61			
44–46	1.97 ± 0.41	44.35 ± 1.64	53.68 ± 1.38			

Table 8. Means ± SD for the pH of seven replicates of three saline and three non-saline soil samples. The pH values were measured with a combination electrode and dual electrodes with electrodes inserted into 1:1 mixtures of soil and distilled water.

Electrode Type	Low[1]	Medium[1]	High[1]
Non-Saline			
Dual	5.31 ± 0.04 [a]	7.24 ± 0.04 [a]	7.84 ± 0.02 [a]
Combination	5.31 ± 0.02 [a]	7.19 ± 0.02 [a]	7.81 ± 0.02 [a]
Saline			
Dual	4.08 ± 0.03 [a]	7.35 ± 0.04 [a]	8.67 ± 0.03
Combination	4.10 ± 0.03 [a]	7.34 ± 0.04 [a]	8.47 ± 0.05 [a]

[1] Means indicated by the same letter do not differ at $P = 0.05$; vertical comparisons within either non-saline or saline blocks only.

Table 9. Means ± SD for the pH of seven replicates of three non-saline bottom soil samples. The pH values were measured by the combination electrode in different dilutions of distilled water and in 0.01 M $CaCl_2$ and 1.00 M KCl.

Method	Low[1]	Medium[1]	High[1]
1:1 (water)	5.17 ± 0.02 [a]	7.16 ± 0.06 [a]	8.01 ± 0.02 [a]
1:2.5 (water)	5.44 ± 0.07 [b]	7.41 ± 0.02 [b]	8.16 ± 0.02 [b]
1:5 (water)	5.51 ± 0.06 [b]	7.59 ± 0.08 [bc]	8.26 ± 0.03 [b]
1:10 (water)	5.58 ± 0.02 [b]	7.62 ± 0.06 [c]	8.40 ± 0.03 [c]
1:1 (0.01 M $CaCl_2$)	3.98 ± 0.02 [c]	6.44 ± 0.02 [d]	7.40 ± 0.02 [d]
1:1 (1.00 M KCl)	3.57 ± 0.02 [c]	7.16 ± 0.02 [a]	7.58 ± 0.04 [e]

[1] Means indicated by the same letter do not differ at $P = 0.05$, vertical comparisons only.

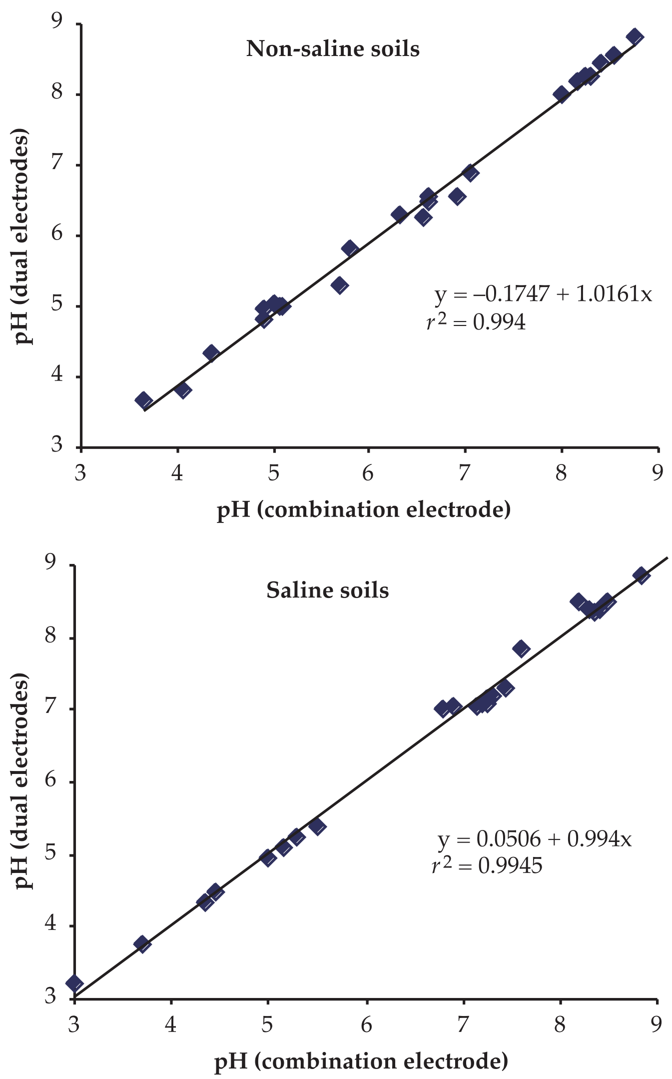

Figure 1. Relationship between pH values obtained with a combination electrode and with dual electrodes in saline and non-saline soil samples. The electrodes were inserted into 1:1 mixtures of distilled water and soil.

Values for pH measured at 1:1 soil:distilled water ratios were lower than those obtained at smaller soil:distilled water ratios (Figure 3; Tables 9 and 10) over the entire pH range considered. Average decreases in pH between the 1:1 soil:water ratios and the 1:2.5, 1:5, and 1:10 ratios were 0.16, 0.32, and 0.47 pH units, respectively, for non-saline soils. For saline soils, these decreases were 0.22, 0.41, and 0.58, respectively. Based on regression equations, differences in pH did not change appreciably with pH over the pH range of the samples. However, at $P = 0.05$ the slopes usually did not equal 1.0 and the intercepts did not equal 0.0 for the regression lines of pH in 1:1 soil:water ratios versus the other ratios. Variation in repeated measurements was usually less ($P < 0.05$) at the 1:1 soil:distilled water ratio than at other soil:distilled water ratios (Tables 9 and 10).

It seems prudent to use 1:1 soil:distilled water ratios in pond soil pH measurement because most of the recommendations regarding pond soil pH have been developed for 1:1 soil:water ratios; the higher soil:water ratios tended to give higher pH, and no improvement in precision resulted from greater dilutions. For some heavy clay soils, the 1:1 soil:distilled water ratio gives a stiff paste in which stirring during pH measurement is difficult. In such cases, enough distilled water may be added to provide a more fluid mixture. This usually can be accomplished at a 1:1.5 soil:distilled water ratio, but in some clays a 1:2 ratio may be necessary. In order to minimize the increase in pH reading, as little extra water as possible should be added. In practice, a 1:1 mixture can be made initially and then water added from a squeeze bottle as necessary.

Soil pH measured in 0.01 M $CaCl_2$ and 1.00 M KCl for non-saline soil samples was considerably lower than pH measured in a 1:1 dilution with distilled water (Tables 9 and 10; Figure 4). The pH values determined in distilled water and in electrolyte solution were highly correlated (Figure 4), but intercepts did not equal 0.0 and slopes did not equal 1.0 at $P = 0.05$. Soil pH values measured in 0.01 M $CaCl_2$ averaged 0.79 pH units lower than those for distilled water, while in 1.00 M KCl, values averaged 0.39 pH units lower than in distilled water.

Differences between pH measured in distilled water and in an electrolyte solution were less for saline soil than for non-saline soil (Tables 9 and 10; Figure 4) because the saline soil samples contained soluble salts that dissolved in the distilled water to provide electrolytes. The pH averaged 0.04 pH units higher in 0.01 M $CaCl_2$ than in distilled water, while pH averaged 0.32 pH units lower in 1.00 M KCl than in distilled water. There was a strong correlation between pH measured in electrolyte

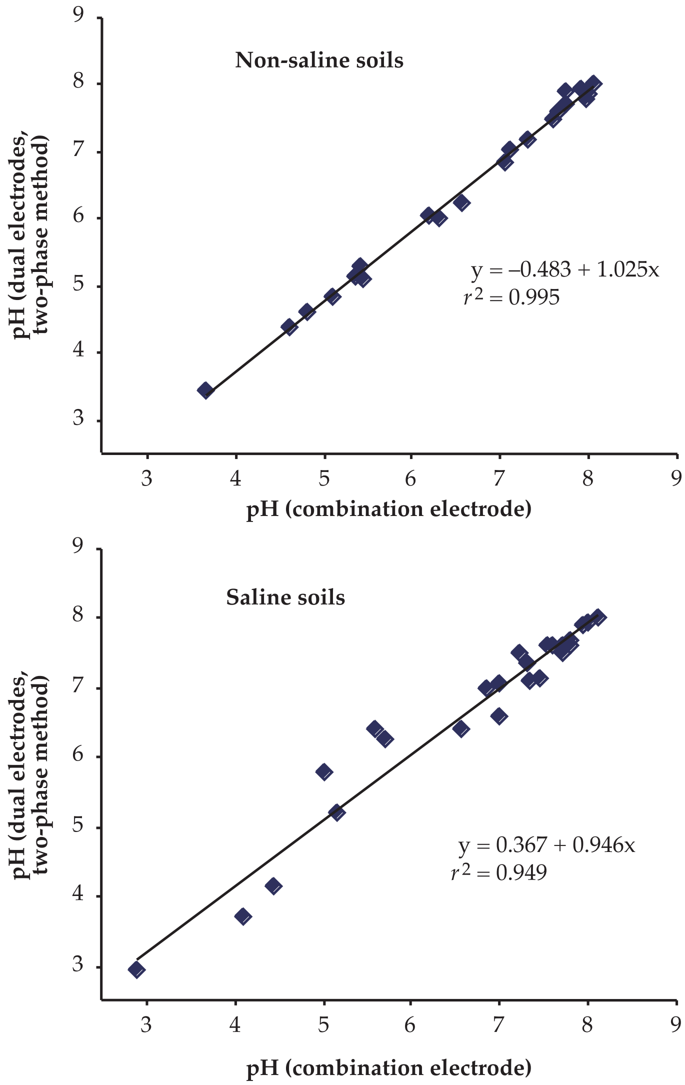

Figure 2. Relationship between pH values obtained with a combination electrode and with dual electrodes in saline and non-saline soil samples. The combination electrode was inserted directly into the 1:1 mixtures of distilled water and soil; with the dual electrodes, the glass (indicating) electrode was inserted into the sediment phase and the reference electrode was held in the supernatant phase (two-phase method).

solutions and in distilled water for saline soils (Figure 4) but slopes did not equal 0.0 and intercepts did not equal 1.0 at $P = 0.05$.

Electrolyte solutions and distilled water provided considerably different values for soil pH. Precision is not better using electrolyte solutions, and interpretations of results obtained with electrolyte solutions are difficult because most recommendations about pond soil pH are based on measurements made in 1:1 soil:distilled water mixtures. Thus, there does not appear to be any advantage to using electrolyte solutions.

Trends of difference in pH measured between or among methods were similar for saline and non-saline soil samples. Thus, the influence of stirring the soil:water mixtures versus not stirring during pH measurement in 1:1 dry soil:distilled water ratios and the difference in pH measured in filtrates versus mixtures were only evaluated for a series of non-saline soil samples. Even though there was a strong correlation between pH measured

Table 10. Means ± SD for the pH of seven replicates of three saline bottom soil samples. The pH values were measured by the combination electrode in different dilutions of distilled water and in 0.01 M $CaCl_2$ and 1.00 M KCl.

Method	Low[1]	Medium[1]	High[1]
1:1 (water)	3.99 ± 0.03 [a]	7.01 ± 0.01 [a]	7.93 ± 0.02 [a]
1:2.5 (water)	4.23 ± 0.04 [b]	7.31 ± 0.02 [b]	8.13 ± 0.03 [b]
1:5 (water)	4.42 ± 0.05 [b]	7.51 ± 0.09 [bc]	8.28 ± 0.04 [c]
1:10 (water)	4.62 ± 0.03 [c]	7.65 ± 0.03 [c]	8.41 ± 0.06 [c]
1:1 (0.01 M $CaCl_2$)	4.21 ± 0.06 [b]	6.92 ± 0.04 [a]	7.95 ± 0.02 [a]
1:1 (1.0 M KCl)	3.94 ± 0.07 [a]	6.36 ± 0.01 [d]	8.13 ± 0.02 [b]

[1] Means indicated by the same letter do not differ at $P = 0.05$; vertical comparisons only.

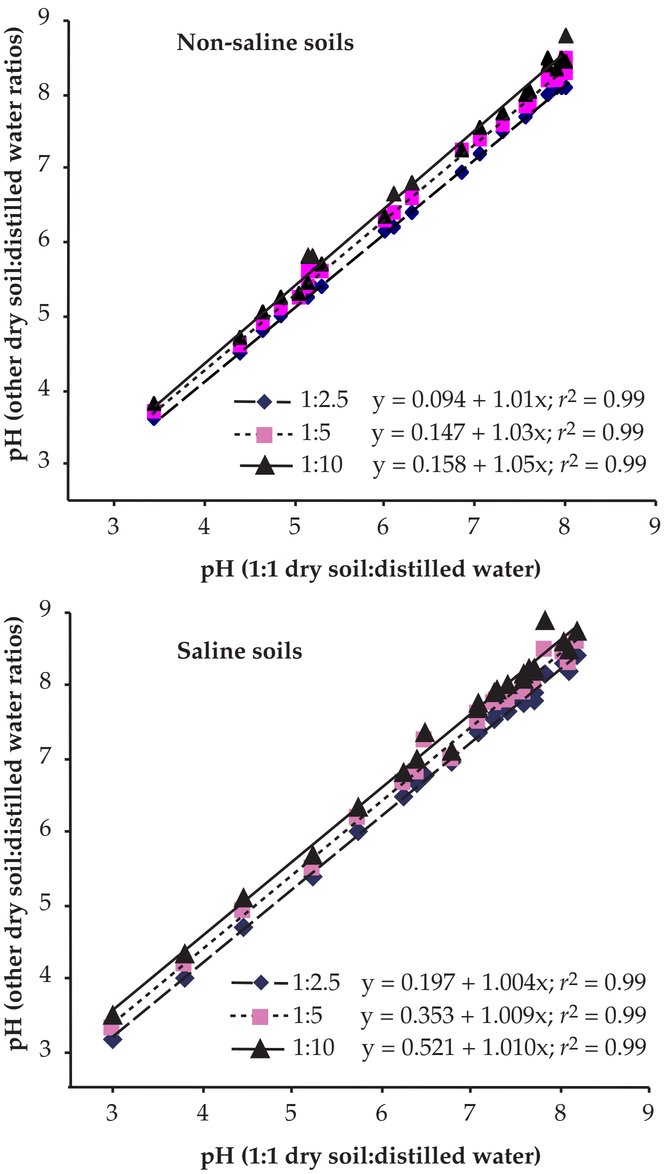

Figure 3. Relationship between pH measured in saline and non-saline soils with the combination electrode inserted into the 1:1 dry soil:distilled water mixture and pH measured with the combination electrode inserted into the other dry soil:distilled water ratio mixtures.

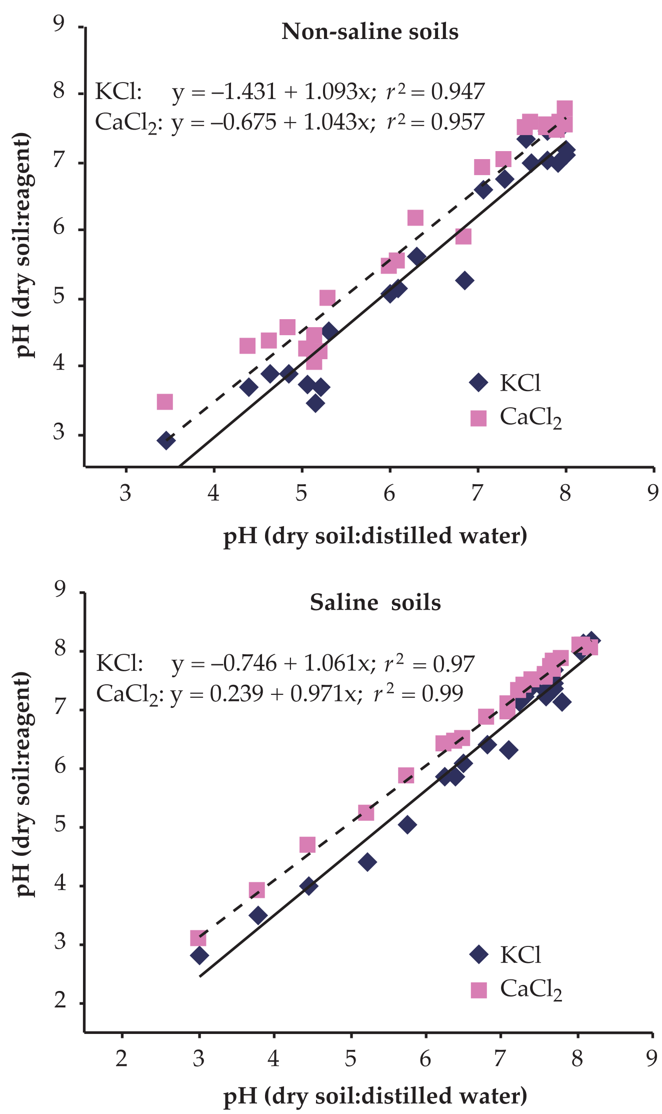

Figure 4. Relationship between pH measured in saline and non-saline soil with the combination electrode inserted into 1:1 dry soil:distilled water mixtures and pH measured with the combination electrode inserted into mixtures of dry soil and 0.01 M $CaCl_2$ or 1.00 M KCl.

with stirring and pH measured without stirring when the combination electrode was inserted into the soil:distilled water mixture (Figure 5), the non-stirring method always gave higher results than the stirring method. The differences ranged from 0.60 to 1.50 pH units (mean ± SD = 0.92 ± 0.27). In addition, the pH meter drifted badly without stirring, resulting in a longer time and greater difficulty in obtaining a reading. Thus, stirring is necessary to obtain accurate and precise pH measurements in a short period.

There was also a good correlation between pH measured by the combination electrode inserted into 1:1 dry soil:distilled water mixtures and that measured in the filtrate from these mixtures (Figure 6). However, the pH measured in the filtrate was always higher than the pH measured in the mixture. The values measured in the filtrate were 0.1 to 1.15 pH units higher than those in the mixture (mean ± SD = 0.46 ± 0.26). We do not understand why this difference occurred, but separation of the soil and aqueous phases does not seem appropriate because of the discrepancy. Also, filtration is time-consuming.

The particle size of samples did not have a significant effect at $P = 0.05$ on the pH measured in three samples dried at different temperatures. However, there were several differences in pH related to different drying temperatures (Table 11). The most striking difference was the lower pH ($P < 0.05$) of sample 3 when dried at 105°C as compared to 60°C or 40°C; other differences also occurred in the data. We cannot offer a good explanation as to why drying caused pH to change. However, in ponds, bottom soils seldom are exposed to temperatures above 40°C. It seems logical that samples dried at 40°C would be altered less in physical and chemical properties, including pH, than samples dried at higher temperatures. Drying at 40°C is slower than at higher temperatures, so a forced-draft oven should be used to accelerate drying and minimize biological changes during drying.

The effect of stirring time (intermittent stirring during the interval) for two samples is provided in Figure 7. The pH of one sample increased initially and then decreased to a constant value after 20 min. The pH of the other sample increased to a stable value after 20 min. This suggests that the usual intermittent stirring for 30 min should be adequate to reach a constant pH value.

The pH measured by inserting the combination pH electrode directly into fresh samples of waterlogged pond soil was lower than pH measured in 1:1 dry soil:distilled water mixtures of samples from the PD/A CRSP sites in Thailand, Honduras, Peru, and Kenya (Table 12). Wet soil pH and pH of dry soil in distilled water for samples from 22 ponds at the US site (Auburn University Fisheries Research Unit) were highly correlated (Figure 8). The slope of the regression line did not equal 1.0, and the intercept did not equal 0.0 at $P = 0.05$. The wet soil pH was greater than the dry soil pH for all samples and averaged 0.49 pH units higher. However, wet soil pH is not always greater than dry soil pH. For example, in three brackishwater shrimp ponds in Peru, pH averaged 7.20 ± 0.29 in wet soil and 7.60 ± 0.17 in 1:1 dry soil:distilled water mixtures.

The difference between pH measured in wet and dry soil is not consistent. Data in Table 5 revealed differences of 0.23 to 2.13 pH

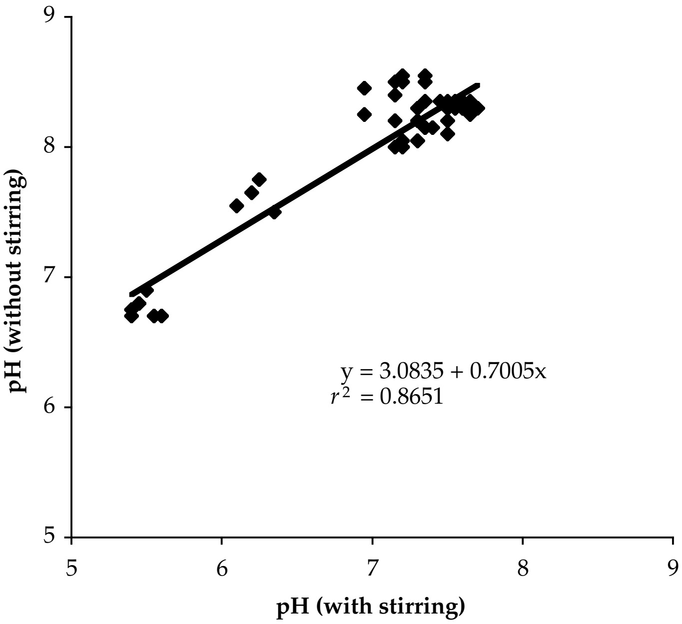

Figure 5. Relationship between pH measurements made by inserting a combination electrode into 1:1 dry soil:distilled water mixtures and either stirring or not stirring while taking the measurement.

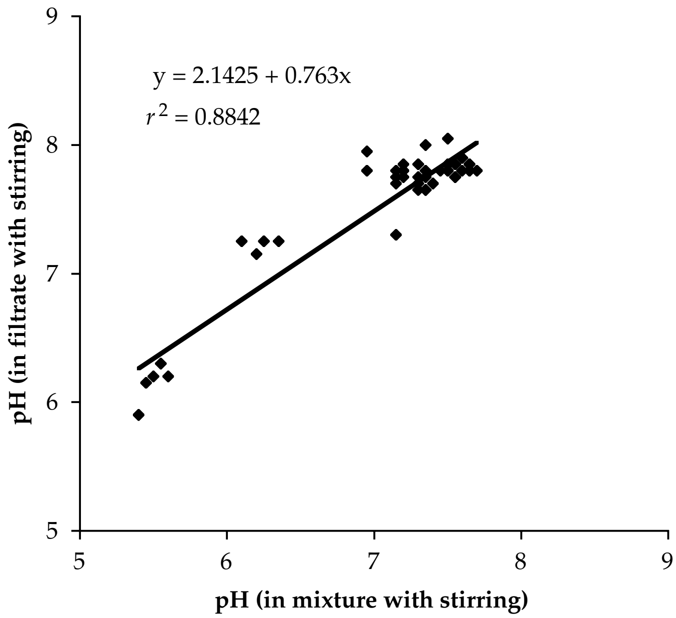

Figure 6. Relationship between pH measurements made by inserting a combination electrode into 1:1 dry soil:distilled water mixtures and by measuring the pH in filtrates of the mixtures.

Table 11. The pH ± SD of 1:1 mixtures of dry soil and distilled water in which soil samples had been dried at three temperatures and passed through sieves of different mesh sizes.

Temperature (°C)	**Sieve Mesh Size** (mm)					
	2.36	*0.85*	*0.425*	*0.25*	*0.149*	*0.053*
PH (SAMPLE 1)[1]						
40	7.98 ± 0.03 ^{a}	8.00 ± 0.00 ^{a}	7.73 ± 0.03 ^{a}	7.70 ± 0.00 ^{a}	7.68 ± 0.03 ^{a}	7.93 ± 0.08 ^{a}
60	7.90 ± 0.00 ^{a}	7.95 ± 0.09 ^{a}	7.98 ± 0.05 ^{b}	8.03 ± 0.03 ^{b}	8.02 ± 0.03 ^{b}	7.68 ± 0.03 ^{b}
105	7.91 ± 0.01 ^{a}	8.00 ± 0.00 ^{a}	7.95 ± 0.00 ^{b}	8.00 ± 0.00 ^{b}	7.97 ± 0.06 ^{b}	7.90 ± 0.00 ^{a}
PH (SAMPLE 2)[1]						
40	7.77 ± 0.03 ^{a}	7.70 ± 0.00 ^{a}	7.60 ± 0.00 ^{a}	7.55 ± 0.00 ^{a}	7.50 ± 0.00 ^{a}	7.58 ± 0.03 ^{a}
60	7.74 ± 0.08 ^{a}	7.42 ± 0.08 ^{b}	7.23 ± 0.06 ^{b}	7.20 ± 0.00 ^{b}	7.25 ± 0.00 ^{b}	7.22 ± 0.03 ^{b}
105	7.43 ± 0.41 ^{b}	7.12 ± 0.08 ^{c}	7.12 ± 0.03 ^{b}	7.03 ± 0.06 ^{b}	7.12 ± 0.06 ^{c}	7.07 ± 0.08 ^{c}
PH (SAMPLE 3)[1]						
40	6.72 ± 0.08 ^{a}	7.05 ± 0.05 ^{a}	7.30 ± 0.17 ^{a}	7.43 ± 0.20 ^{a}	7.10 ± 0.00 ^{a}	7.32 ± 0.03 ^{a}
60	6.87 ± 0.06 ^{a}	6.85 ± 0.06 ^{a}	5.93 ± 0.03 ^{b}	6.02 ± 0.03 ^{b}	6.20 ± 0.10 ^{b}	6.93 ± 0.03 ^{b}
105	5.48 ± 0.53 ^{b}	5.18 ± 0.03 ^{b}	5.13 ± 0.06 ^{c}	5.10 ± 0.00 ^{c}	5.18 ± 0.03 ^{c}	5.23 ± 0.08 ^{c}

[1]Means indicated by the same letter do not differ at $P = 0.05$, vertical comparisons only.

units. The samples from Thailand that differed in wet and dry pH by 2.13 pH units were known to contain iron pyrite. Pyrite oxidizes when dried to release sulfuric acid (Dent, 1986). Standard pH probes are so large that they cannot be positioned to contact only soil in the thin surface aerobic layer (Munsiri et al., 1995), and they indicate the pH in the pore water in the anaerobic soil near the soil-water interface. The lime requirement and other pond soil assessments requiring information on pH are based on the pH of aerobic soil. The pH of aerobic pond soils are controlled by the same factors controlling pH in dry soil–distilled water mixtures. Therefore, aquaculturists should not rely upon pH measurements made by inserting standard pH electrodes into samples of freshly collected soil. Soil samples from the upper 5-cm layer should be dried and pulverized and the pH determined in 1:1 mixtures of dry, pulverized soil and distilled water.

The portable soil acidity tester often is used to measure pH directly in bottoms of ponds that have been drained for harvest. Results of comparisons between the portable soil acidity tester and a standard pH meter with combination electrode are provided in Figure 9. The absolute differences between the two methods averaged 1.22 pH units for non-saline soils and 1.42 pH units for saline ones. The soil acidity tester gave lower pH values than the pH meter for all saline pond soil samples, but both lower and higher values were obtained for non-saline pond soils.

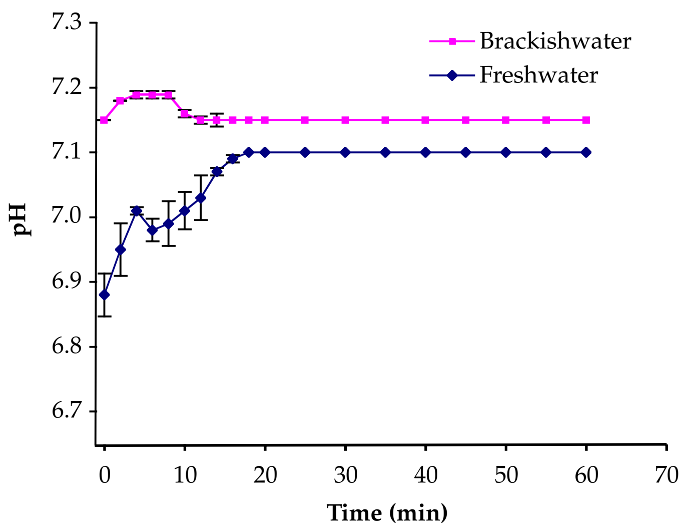

Figure 7. Influence of intermittent stirring time on the pH in a 1:1 mixture of dry soil and distilled water.

Table 12. Means ± SD for bottom soil pH from three ponds measured directly in freshly collected wet, non-saline soil and in 1:1 mixtures of dry soil and distilled water with the combination electrode.

Sample Description	Wet[1]	Dry[1]
Thailand	7.65 ± 0.19 [a]	6.37 ± 0.04 [b]
	7.28 ± 0.03 [a]	6.32 ± 0.05 [b]
	5.51 ± 0.29 [a]	3.38 ± 0.05 [b]
Honduras	7.48 ± 0.06 [a]	7.07 ± 0.03 [b]
Peru	6.53 ± 0.15 [a]	6.30 ± 0.26 [a]
Kenya	7.17 ± 0.04 [a]	6.90 ± 0.03 [b]

[1]Means indicated by the same letter do not differ at $P = 0.05$, horizontal comparisons only.

There were positive, though weak, correlations between the two methods for both types of soil samples, but the correlation coefficients were too low to be of much predictive value. Results reported in Figure 10 reveal that the presence of salt in the soil did not greatly affect the pH as measured with the combination electrode. The soil pH in the control soil (0‰ salinity) was 6.15, the pH at 0.68 and 1.36‰ salinity was 5.85, and the pH at 30‰ salinity was 6.05. The soil acidity tester measured a lower pH at 0‰ salinity than did the standard pH meter; values were 5.5 and 6.15, respectively. The pH difference between the two methods of measurement was much greater at salinities of 0.68‰ and more

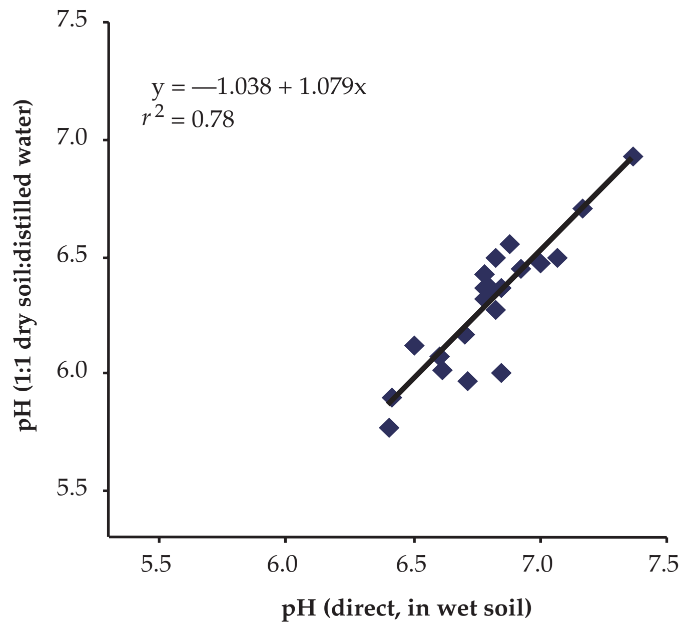

Figure 8. The pH of pond soil measured by insertion of the combination electrode directly into freshly collected, wet soil and in 1:1 dry soil:distilled water mixtures. Soil samples were taken from ponds on the Auburn University Fisheries Research Unit, Auburn, Alabama.

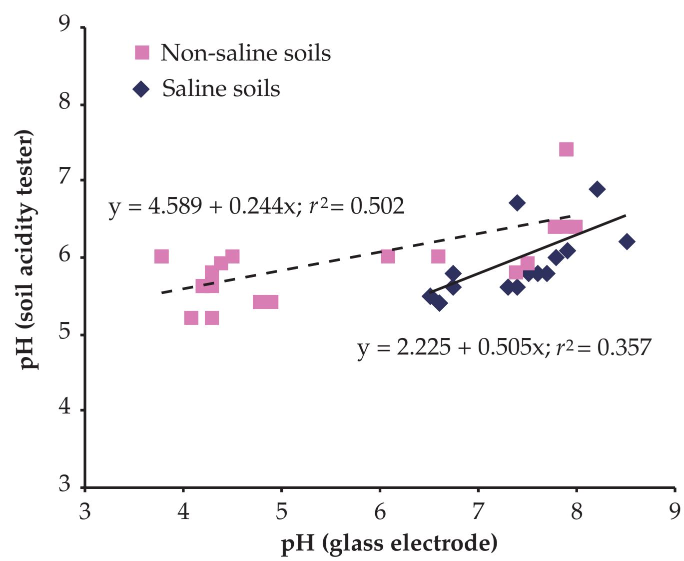

Figure 9. Relationship between pH measured in saline and non-saline bottom soils using either a portable acidity tester or a glass electrode inserted into a 1:1 mixture of dry soil and distilled water.

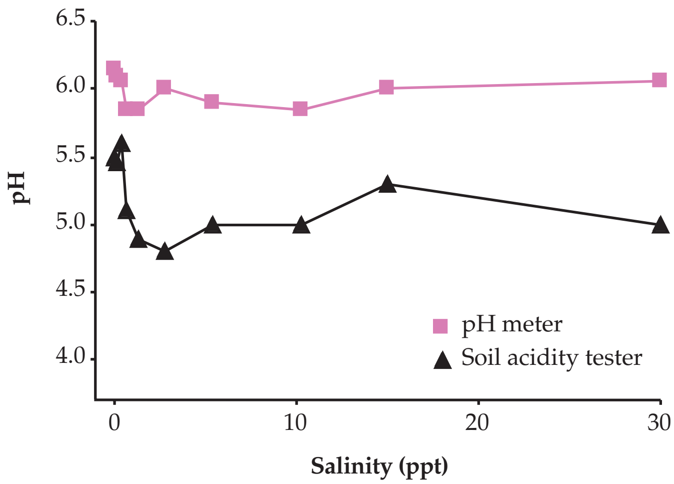

Figure 10. Influence of salinity concentration in soil pore water on pH measures made with a soil acidity tester and with the combination electrode.

than at lower salinities. For example, at 5.44‰ salinity, the glass electrode read 5.9 and the soil acidity tester read 5.0, while at 30‰ salinity the respective readings were 6.05 and 5.0. This suggests that increasing salt content in the soil causes the soil acidity tester to underestimate pH as determined by a standard pH meter. This is not surprising because the manufacturer's instructions for the soil acidity tester stated that soluble salts in soils can cause erroneous readings and the instrument should not be used for measuring the pH of saline or alkaline soils. Of course, results in Figure 9 suggest that the device does not agree well with a standard pH meter even in non-saline soils.

Results of this study of pH measurements clearly show that the method used for measuring pH can have a marked influence on the pH value obtained for a given sample. Many different methods are used to assess pH in practical aquaculture, and even researchers may use different methods. There is an obvious need to standardize pond soil pH measurements to avoid differences in pH being introduced through varying methodologies. Based on the findings of this study, pond bottom soil measurement should be standardized as follows:

- Dry soil at 40°C in a forced-draft oven;
- Pulverize soil to pass a 2.36-mm sieve;
- Mix dry soil and distilled water in a 1:1 ratio (slightly more distilled water can be added if the mixture is not fluid enough to stir);
- Stir the mixture intermittently for 30 min with a glass rod;
- Insert a dual electrode or a combination electrode directly into the soil-water mixture; and
- Gently stir the soil-water mixture with a glass rod while measuring pH.

ANTICIPATED BENEFITS

Research conducted under this project is anticipated to have several benefits to pond aquaculture. A data base has been accumulated that is sufficient to allow us to develop a concept of pond soil development and a system of pond bottom soil classification. The S horizon (upper few centimeters) is the most influential part of the pond soil profile affecting water quality. This layer has the highest concentration of reactive organic matter and the greatest respiration rate; phosphorus is also adsorbed by this layer. Thus, the recommendation to sample this soil layer (or the upper 5-cm layer of soil) should become a standard for future studies of pond soils.

Measurement of pond soil pH in 1:1 mixtures of dry soil (40°C) and distilled water also will become a standard procedure as a result of this research. Improved understanding and management of pond soils should result from the following conclusions of this research:

1) Pond soils do not accumulate organic matter as rapidly as often thought by practical aquaculturists;
2) Pond soils are sinks for phosphorus;
3) Microbial activity in many pond soils may be nitrogen-limited; and
4) Although pond bottom soils of this study exhibited a wide range in chemical and physical characteristics, it was still possible to successfully culture fish and shrimp.

LITERATURE CITED

Boyd, C.E. and P. Munsiri, 1996. Phosphorus adsorption capacity and availability of added phosphorus in soils from aquaculture areas in Thailand. J. World Aquacult. Soc., 27:160–167.

Boyd, C.E., J. Queiroz, and C.W. Wood, 1998. Pond soil characteristics and dynamics of soil organic matter and nutrients. In: D. Burke, J. Baker, B. Goetze, D. Clair, and H. Egna (Editors), Fifteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 11–25.

Boyd, C.E., J. Queiroz, and C.W. Wood, 1999. Pond soil characteristics and dynamics of soil organic matter and nutrients. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 1–7.

Boyd, C.E., M.E. Tanner, M. Madkour, and K. Masuda, 1994. Chemical characteristics of bottom soils from freshwater and brackishwater aquaculture ponds. J. World Aquacult. Soc., 25:517–534.

Boyd, C.E., C.W. Wood, T. Thunjai, and S. Sonnenholzner, 2000. Pond soil characteristics and dynamics of soil organic matter and nutrients. In: K. McElwee, D. Burke, M. Niles, X. Cummings, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 1–8.

Dent, D., 1986. Acid Sulphate Soils: A Baseline for Research and Development. Publication 39. International Institute for Land Reclamation and Improvement, Wageningen, The Netherlands. 200 pp.

Munsiri, P., C.E. Boyd, and B.J. Hajek, 1995. Physical and chemical characteristics of bottom soil profiles in ponds at Auburn, Alabama, and a proposed method for describing pond soil horizons. J. World Aquacult. Soc., 26:346–377.

Soil Survey Staff, 1994. Keys to Soil Taxonomy, Sixth Edition. United States Department of Agriculture, Soil Conservation Service, Washington, DC, 306 pp.

SPSS, 1997. SigmaStat Statistical Software. SPSS Marketing Department, Chicago, Illinois.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Global Experiment: Optimization of Nitrogen Fertilization Rate in Freshwater Tilapia Production Ponds

Eighth Work Plan, Feeds and Fertilizers Research 1 (8FFR1K)
Final Report

Karen L Veverica
Resident Research Coordinator, Sagana Fish Farm
and
Department of Fisheries and Allied Aquacultures
Auburn University

Jim Bowman
Department of Fisheries and Wildlife
Oregon State University

Tom Popma
Department of Fisheries and Allied Aquacultures
Auburn University

Printed as Submitted

Abstract

Two experiments to determine the optimum nitrogen fertilization rates for freshwater tilapia production ponds at Sagana Fish Farm, Sagana, Kenya, were conducted during 1998 and 1999. Twelve 800m^2 earthen research ponds managed by the PD/A CRSP at Sagana were used for the experiments. Diammonium phosphate and urea were used to apply nitrogen to the ponds at rates of 0, 10, 20, and 30 kg N ha^{-1} wk^{-1}. Triple superphosphate or diammonium phosphate and sodium carbonate were applied to ponds to assure that phosphorus and carbon were not limiting. A completely randomized design was used, with three replicates for each of the four treatments. The experiment was conducted once during the 1998 cool season (May to October) and again during the warm season of 1998-1999 (November to March). In the cool-season experiment, ponds were stocked with sex reversed Nile tilapia, *Oreochromis niloticus*, averaging 16.9 grams at a rate of 1,000 kg ha^{-1} and with *Clarias gariepinus* fingerlings averaging 37 g at a rate of 37 kg ha^{-1}. In the warm season experiment, all ponds were stocked with sex reversed *O. niloticus* averaging 90 g at 1000 kg ha^{-1} and with *C. gariepinus* juveniles averaging 166 g at 125 kg ha^{-1}. Pond assignments were re-randomized prior to the second experiment. Ponds were drained when fish growth appeared to have stopped in all treatments. In both experiments, a highly significant ($p<0.01$) quadratic relationship best described gross fish yield (as well as net) as related to weekly nitrogen input. Presence of *Clarias* had little impact on the relationship but it appeared the high nitrogen input rates had no negative effect on *Clarias* production. Increasing nitrogen input beyond 20 kg N ha^{-1} wk^{-1} did not result in increased tilapia yields. Total nitrogen and all mineral forms of nitrogen increased with increasing nitrogen input, as did chlorophyll *a*. Partial budget analysis indicated that greatest marginal returns were at the calculated rates of 19.9 and 16.0 kg N ha^{-1} wk^{-1} for the cool- and warm-season experiments, respectively. A carryover effect from the first experiment is suggested. Results from this experiment are similar to those obtained at the CRSP site at El Carao, Honduras.

Introduction

Nitrogen (N), phosphorus (P), and carbon (C) availability are important considerations in the management of ponds for optimum fish production. Previous PD/A CRSP research has addressed increasing primary productivity through inorganic and organic nutrient additions to ponds, but findings on the optimum N, P, and C inputs required to improve fish yields at the PD/A CRSP sites appeared inconsistent and called for clarification. Higher nutrient inputs had resulted in increased fish production at all PD/A CRSP sites, but optimum input rates for N, P, and C had not been defined (see reports in Egna et al., 1990, 1991; Egna et al., 1992; Egna et al., 1993; Egna et al., 1994, 1995).

Fertilization rates in PD/A CRSP experiments were greater than rates reported for earlier pond fertilization research. In an often-cited series of fertilization experiments conducted in Malaysia, Hickling (1962) never used more than 1.1 kg P and 1.1 kg N/ha per week. In Israel, the standard fertilizer dose was 2.3 kg P and 6.5 kg N/ha per week (Hepher 1962a, b). The highest rates of P and N used in most experiments at Auburn University were 1.26 and 3 kg/ha per week, respectively (Swingle 1947; Boyd l976, l990; Boyd and Sowles 1978; Murad and Boyd 1987). Rates in Europe seldom exceeded 1 kg/ha per week for N and P (Mortimer 1954). Rates used in Malaysia, USA, Israel, and Europe were adequate to give dense phytoplankton blooms and good fish production. Also, in all of the studies cited above, P was the most important limiting nutrient.

This set of experiments was designed to determine the optimal application rates of N to attain the most profitable tilapia yields in tropical freshwater ponds at the PD/A CRSP site at

Sagana, Kenya and to provide data for the development of a full-cost enterprise budget for the most profitable fertilization rate. Trials were conducted during both the warm and cool seasons to determine seasonal effects.

MATERIALS AND METHODS

Cool Season Experiment

These experiments were conducted in twelve CRSP-managed 800m^2 earthen research ponds at Sagana Fish Farm, Sagana, Kenya. The cool season experiment was conducted between May and October 1998. Triple superphosphate (TSP) was applied to each pond at a rate of 250 kg P ha^{-1} prior to filling. Each pond was also limed at a rate of 2.5 tons ha^{-1}. The lime and TSP were raked into the bottom of the pond. Inorganic fertilizer, either as TSP or as diammonium phosphate (DAP) and urea, was applied weekly beginning two weeks prior to stocking (first application on 29 April 1998). Soda ash (97% sodium carbonate), obtained from Magadi Soda (the world's largest soda mine), was applied weekly to ponds with total alkalinity less than 75 mg/l as $CaCO_3$.

Dap and urea were used to apply N to the ponds at rates of 0, 10, 20, and 30 kg N ha^{-1} wk^{-1}. A completely randomized design was used, with three replicates for each of the four treatments. The final fertilizer and soda ash applications were on 6 October 1998. The total quantities of inputs used are shown in Table 1.

All ponds were stocked with sex-reversed tilapia averaging 16.9g at a rate of 1,000 kg ha^{-1} on 13 May 1998. This resulted in a stocking rate of about 60,000 fish ha^{-1}. Each pond also received 80 *Clarias* averaging 37 g, resulting in an additional 37 kg ha^{-1}. Ponds were sampled at bi-weekly intervals by seining. All fish caught in the seine were separated by species and counted and weighed. No attempt was made to obtain enough *Clarias* for a sample, so on some sampling dates no *Clarias* were caught, and growth curves were not made for this species. Ponds were drained on 16 October 1998 after it was determined that fish in all treatments had ceased to grow ("no growth" was defined as weight increases of less than 10% on two successive sampling occasions). Fish were separated by species, counted, and weighed. Of the fish harvested, 25,000 tilapia were sold to a cage culture operation for 120 KSh/kg, 10,640 tilapia and 720 *Clarias* were used to re-stock ponds for the next experiment, and 4,000 tilapia and the remaining *Clarias* were used to stock ponds in a supplementary carrying capacity experiment.

Temperature and dissolved oxygen (DO) were measured weekly at four depths (5, 25, 50, and 75 cm below surface) at the pier near the drain in the morning and the afternoon. Total alkalinity (TA) and pH were measured weekly using column samples collected at three places in each pond. Chlorophyll *a* (corrected and uncorrected), TAN, nitrite, nitrate, and soluble reactive-P were measured biweekly (a total of 11 measures for each parameter). Total N, total P, and total hardness (TH) were measured every four weeks, with a total of eight total N and total P measures being made during the experiment. Diurnal oxygen and temperature readings were also made to calculate primary productivity using the whole pond method. Gross and net primary production were calculated on three occasions: two weeks after stocking the ponds, six weeks after stocking, and during the week prior to pond draining. Methods used were as described in the CRSP Handbook of Analytical Methods (PD/A CRSP 1992), with the exception of nitrate analysis, which was performed using the NAS method. Water sampling and analysis was done on Mondays (all were usually finished the same day or by Tuesday), and fertilizers and soda ash were applied on Tuesdays. Fish sampling was done on Wednesdays.

Ponds water levels were recorded daily. Ponds were topped off to replace evaporation and seepage losses weekly (Mondays or Tuesdays). Night watchmen occasionally added water to some ponds when fish deaths occurred, but these ponds never overflowed, so effects on water quality are thought to have been negligible.

On occasions of fish death, dissolved oxygen, TAN, and nitrites were measured as soon as possible after fish deaths were reported. Most fish die-offs occurred on weekends (about four days after fertilization). Dead fish were counted and weighed. Only the ponds receiving the highest N input were affected.

Soil samples were taken from each pond two weeks after stocking and again during the week prior to pond draining. Nine cores of 2-cm depth (from the soil surface) were combined to make the soil sample for each pond at the beginning of the experiment; the core depth taken at the end of the experiment was 5 cm.

Warm Season Experiment

The warm-season experiment was conducted in the same twelve ponds between November 1998 and March 1999. At the

Table 1. Total quantities of inputs used during the cool-season (156 day duration) and warm-season (131 day duration) trials of the experiment on optimum nitrogen fertilization (CRSP global experiment) at Sagana Fish Farm (Sagana, Kenya).

Weekly Nitrogen Application	DAP (kg ha^{-1})	Urea (kg ha-1)	TSP (kg ha-1)	Lime (kg ha-1)	Soda Ash (kg ha-1)
Cool Season					
0 kg ha^{-1}	0	0	1585	2500	283
10 kg ha^{-1}	960	146	625	2500	492
20 kg ha^{-1}	960	668	625	2500	229
30 kg ha^{-1}	960	1190	625	2500	254
Warm Season					
0 kg ha^{-1}	0	0	840	1875	75
10 kg ha^{-1}	840	128	0	417	258
20 kg ha^{-1}	840	584	0	625	321
30 kg ha^{-1}	840	1041	0	2083	704

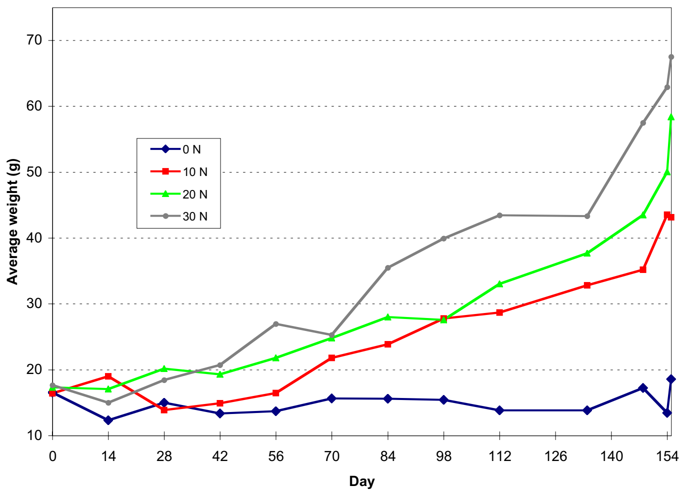

Figure 1. Growth of tilapia under four different nitrogen input rates during the cool season trial of the PD/A CRSP Global Experiment conducted at Sagana Fish Farm, Sagana, Kenya.

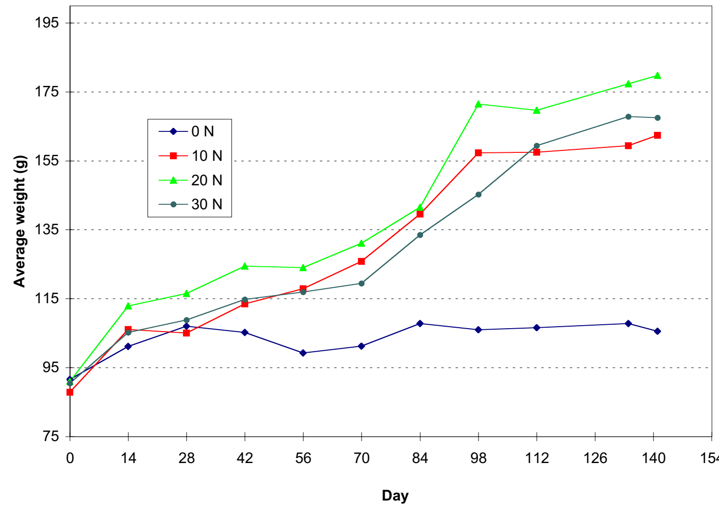

Figure 2. Growth of tilapia under four different nitrogen input rates during the warm season trial of the PD/A CRSP Global Experiment conducted at Sagana Fish Farm, Sagana, Kenya.

end of the first (cool-season) experiment, residual lime and TSP could be seen on the bottoms of most ponds. Prior to re-filling, ponds that had had TH less than 75 mg/l at the end of the cool season experiment were re-limed. Liming rates varied according to TH at the end of the first experiment. No further additions of TSP were deemed necessary because soluble P levels in all ponds had remained high in the first experiment. Treatments for the warm-season experiment were the same as those for the cool-season experiment: DAP and urea were again used to add N at rates of 0, 10, 20, and 30 kg N ha^{-1} wk^{-1}.

Ponds were re-randomized and the first fertilizer application was made on 4 November, 1998. Male tilapia averaging 90 g were used (left from the previous experiment) to stock all ponds at 1 ton ha^{-1} on 18 November. Sixty *Clarias* averaging 166g were added to each pond (125 kg ha^{-1}) on 23 November. This resulted in a stocking density of 11,000 tilapia and 750 *Clarias* per hectare. Two tilapia and two *Clarias* were removed from each pond once a month to examine stomach contents. The removed fish were counted as live when survival was calculated but their weight was not used in calculating gross fish yield, on the assumption that the fish remaining in the pond increased in weight to compensate for those that had been removed. Ponds were drained on 29 March 1999, after it was concluded that all treatments had less than 5% weight gain on two successive samplings. Fish were separated by

Table 2. Mean (± SE) tilapia yields, *Clarias* yields, average weights by species, and survival for cool- and warm-season trials of the experiment on optimum nitrogen fertilization (CRSP global experiment) at Sagana Fish Farm, Sagana, Kenya.

Weekly Nitrogen Application	Yield ($kg\ ha^{-1}$)				Average Weight (g)		Tilapia Reprod ($kg\ ha^{-1}$)
	Tilapia	Clarias	Gross	Net	Tilapia	Clarias	
Cool							
0 kg ha^{-1}	1015 ±113	208 ±34	1297 ±116	251 ±117	23.1 ±1.6	156.1 ±2.9	74±20.6
10 kg ha^{-1}	2602 ±107	270 ±59	2949 ±120	1908 ±120	48.1 ±1.9	263.2 ±40.6	77±19.3
20 kg ha^{-1}	2953 ±296	250 ±22	3229 ±278	2191 ±280	60.4 ±3.8	275.1 ±28.2	30±3.5
30 kg ha^{-1}	2510 ±348	495 ±24	3043 ±371	2004 ±371	70.5 ±5.9	375.7 ±45.3	37±4.8
Best fit model	quadratic	linear	quadratic	quadratic	sq. root x	reciproc.-y	linear
R-squared	82%**	57%*	82%**	82%**	92%**	78%**	43%*
Warm							
0 kg ha^{-1}	1119 ±115	154 ±23	1272 ±137	296 ± 162	106 ± 8.3	251.3 ±29.5	43 ±7.4
10 kg ha^{-1}	1672 ±156	164 ± 6	1837 ±161	883 ± 169	162 ±12.4	262.9 ± 3.8	29 ±2.3
20 kg ha^{-1}	1720 ±100	181 ±89	1901 ±95	957 ± 91	181 ± 4.3	283.2 ± 6.7	34 ±11.5
30 kg ha^{-1}	1520 ±160	183 ±16	1703 ±45	761 ± 28	168 ± 6.5	283.1 ±16.4	17.5 ±0.9
Best fit model	quadratic	NS	quadratic	quadratic	quadratic	NS	exponen.
R-squared	68%**		67%**	67%**	86%**		41%*

** highly significant ($p < 0.01$)
* significant ($p < 0.05$)

models used in Tables 2 and 3:
linear: $y=a+bx$
exponential: $y=\exp(a+bx)$
sq. root y: $y=(a+bx)^2$
sq. root x: $y=a+bx^{1/2}$
reciprocal-y $y=(a+bx)^{-1}$
quadratic: $y=a+bx+cx^2$

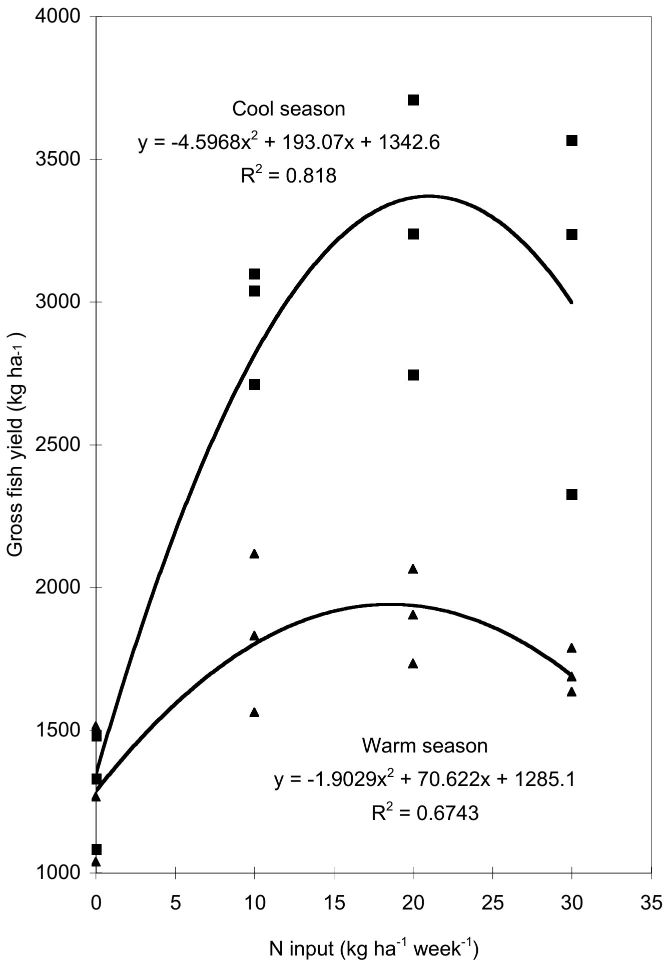

Figure 3. Gross fish yield (GFY) as a function of nitrogen (N) input for the cool and warm season trials of the PD/A CRSP Global Experiment conducted at Sagana Fish Farm, Sagana, Kenya.

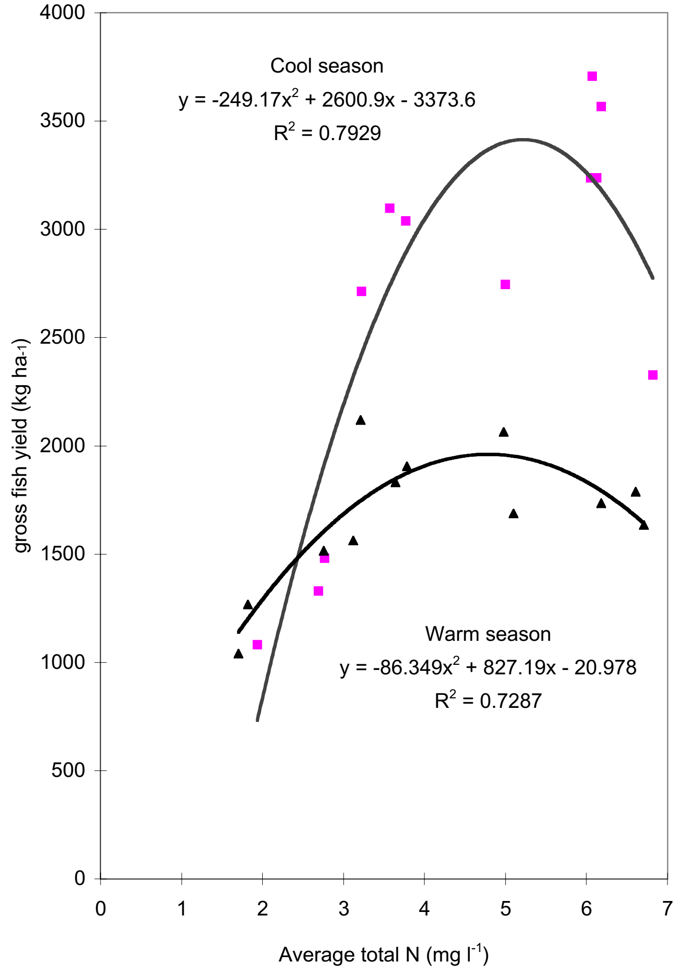

Figure 4. Relationship between gross fish yield (GFY) and average total nitrogen (N) content in pond waters during the cool and warm season trials of the PD/A CRSP Global Experiment conducted at Sagana Fish Farm, Sagana, Kenya.

species, counted and weighed. Tilapia were separated by sex and fingerling weight was noted. The tilapia and *Clarias* were sold at 120 KSh per kg to a Nairobi fish-out operator.

Water quality parameters were measured on the same frequencies as in the cool-season experiment. Fertilizers and soda ash were applied on Tuesdays. Some ponds in the experiment exhibited very high morning pH and their hardness was beginning to diminish so soda ash applications were suspended for ponds that had a morning column pH greater than 8.5. These ponds typically increased in pH to more than 10 before noon, so any additional carbonate would not have been available for photosynthesis. Chlorophyll *a* (corrected and uncorrected), TAN, nitrite, nitrate, and soluble reactive-P (a total of 9 measures for each parameter) were measured biweekly. Total N, total P, and TH were measured every four weeks. A total of five total N and total P measures were made during the experiment. Gross and net primary production were calculated by the whole pond method (PD/A CRSP 1992) on four occasions during the experiment, once at the beginning of the experiment (December 1998) and once each in January, February, and March of 1999.

Soil samples were taken from each pond two weeks after stocking and again during the week prior to pond draining. On each sampling occasion, nine cores of 2-cm depth from the soil surface were combined to make the soil sample for each pond.

Data for each experiment were analyzed statistically by regression analysis using Statgraphics Plus for Windows software (Statistical Graphics Corp. 1997). Models that resulted in the lowest p values (not necessarily the highest r^2 values) were used.

RESULTS

Site Observations

The Sagana site has very cool surface waters (usually 19 to 21°C) with low alkalinity and hardness compared to the El Carao station in Honduras and the AIT station in Thailand. TA runs about 16 to 28, and TH is 13 to 28, with higher values in dry months. There is not much difference in average air temperature in the so-called cool season. Minimum air temperatures tend to be the same but maximum temperatures are lower in cool season due to increased cloud cover. Solar radiation during the cool season is much lower than during the warm season. Average solar radiation during the first experiment was 24.64 Einsteins/m^2 (7.56 MJ m^2), whereas it was 41.91 Einsteins/m^2 (12.34 MJ m^2) during the warm-season experiment. The beginning and end of the cool season experiment actually fell into warm-season months. There was a slight difference in pond water temperatures between the cool and the warm season experiments.

Another seasonal difference has to do with winds. In the cool season, wind is less and the ponds mix only in the night to early morning. In the warm season, especially December through February, high winds occur every afternoon and the ponds mix twice, once in the evening and once in the early morning.

During the cool season, ponds in the 20 and 30 kg N treatments had dense blooms of euglenophytes (*Trachelomonas*, *Euglena*, and *Phacus* spp.) These blooms did not occur in the warm season experiment. The lower solar radiation tends to favor the euglenophytes during cool season (these blooms have occurred each cool season since the CRSP began work at Sagana). Surface blooms of any kind cannot withstand the intense solar radiation experienced at Sagana from December through February.

Fish Growth

Figures 1 and 2 show average fish weight by N input rate for each experiment. In the cool season, the high N input rate showed high growth after about 25 % of the fish died in two of the ponds.

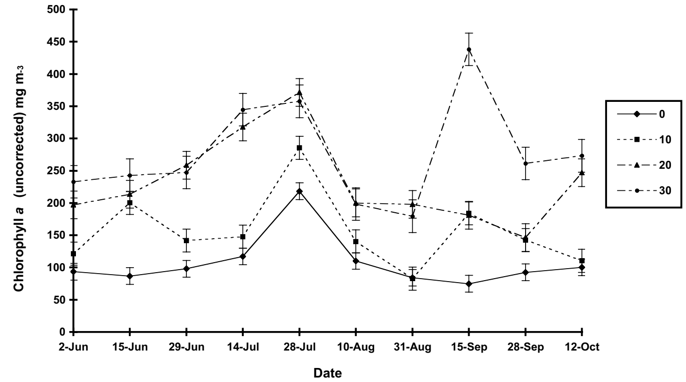

Figure 5. Trends in chlorophyll *a* (uncorrected) concentrations (treatment means and standard errors) during the cool season trial of the Global Experiment at Sagana Fish Farm, Sagana, Kenya.

The relationship between gross fish yield (GFY) and N input rate was best described by a quadratic model. It was highly significant ($p<0.01$) in both experiments but better correlated in the cool season experiment (Table 2 and Figure 3). Fish deaths occurred in the highest N application rate on at least two occasions during the cool season experiment, on 12-13 June in pond E09 and on 22 June in pond D04. Dead tilapia were

Table 3. Dissolved oxygen (DO) and temperature observations in ponds during the cool and warm season trials of the Global Experiment on optimum nitrogen fertilization conducted at Sagana Fish Farm, Sagana, Kenya.

		Dissolved oxygen (mg/l)							
	Weekly N	Morning				Afternoon			
Pond	**Input (kg)**	**5cm**	**25cm**	**50cm**	**75cm**	**5cm**	**25cm**	**50cm**	**75cm**
Cool									
D7, D8, E6	0	4.6	4.5	4.3	3.7	12.1	10.6	5.3	2.9
D6, E4, E5	10	4.3	4.1	4.0	3.7	14.7	13.6	6.9	3.7
D5, E7, E8	20	3.5	3.3	3.2	2.8	16.5	15.3	7.2	3.1
D4, E3, E9	30	2.5	2.2	2.0	1.9	15.9	13.7	7.0	3.3
Warm									
D4, D8, E9	0	3.0	3.0	3.0	2.9	9.5	9.3	6.5	3.3
D7, E3, E6	10	2.1	2.0	2.0	1.9	10.9	10.1	7.4	3.1
E4, E5, E7	20	2.0	1.9	1.9	1.9	11.9	10.5	7.1	2.9
D5, D6, E8	30	1.8	1.8	1.8	1.7	13.4	12.7	8.7	3.9

		Temperature							
	Weekly N	Morning				Afternoon			
Pond	**input (kg)**	5cm	25cm	50cm	75cm	5cm	25cm	50cm	75cm
Cool									
D7, D8, E6	0	22.5	22.5	22.5	22.5	26.4	25.9	24.0	23.4
D6, E4, E5	10	22.9	22.9	22.9	22.9	26.3	26.0	24.6	23.8
D5, E7, E8	20	22.8	22.8	22.8	22.8	26.4	26.0	24.5	23.8
D4, E3, E9	30	22.9	22.8	22.8	22.8	26.1	25.7	24.7	23.9
Warm									
D4, D8, E9	0	23.5	23.5	23.5	23.5	28.2	28.0	26.4	24.7
D7, E3, E6	10	23.5	23.5	23.5	23.5	27.8	27.6	26.5	25.1
E4, E5, E7	20	23.6	23.6	23.6	23.6	27.6	27.5	26.5	25.0
D5, D6, E8	30	23.6	23.3	23.3	23.3	28.0	27.9	26.6	24.7

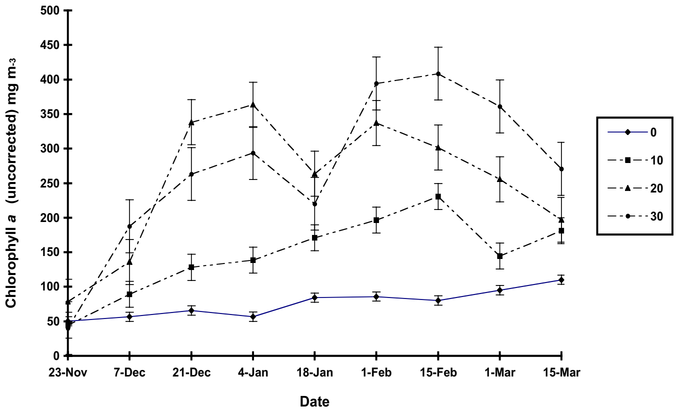

Figure 6. Trends in chlorophyll *a* (uncorrected) concentrations (treatment means and standard errors) during the warm season trial of the Global Experiment at Sagana Fish Farm, Sagana, Kenya.

picked up occasionally during the warm season experiment but they never totaled more than 2 or 3 on any day. No C*larias* died on these occasions. In fact, the relationship between C*larias* yield and N input rate was linear; suggesting that they are less affected by high N.

Taking the first derivative of the regression equation gives the N input rate at which maximum GFY would be obtained: 21.0 kg N ha^{-1} $week^{-1}$ for the cool season experiment and 18.6 kg N ha^{-1} $week^{-1}$ for the warm season experiment. A highly significant relationship exists between GFY and average total N in ponds as well (Figure 4). Differences in initial fish size and density do not permit a valid comparison of growth and productivity as a function of season.

At draining of the cool season experiment, every pond had at least two large C*larias* (> 1 kg). These fish were probably left over from the previous experiment. Some ponds contained many more C*larias* than were stocked, and these extra fish may have entered with the tilapia fingerlings. It was easier to separate out C*larias* during stocking in the second experiment because fish were fewer in number and larger.

The weight of tilapia reproduction decreased with increasing N input (Table 2). Size of tilapia females, C*larias* size, or adverse water quality due to high N input rate could have been causes.

Water quality

During the warm season the ponds did have higher water temperatures and lower morning dissolved oxygen concentrations than they did during the cool season (Table 3). Chlorophyll *a* levels tended to be slightly higher during the warm season experiment than during the cool season experiment, so the warm season's lower fish production could not have been a result of low phytoplankton densities. Figures 5 and 6 illustrate the generally increasing trends in chlorophyll *a* levels that were observed during the experiment. In both seasons, chlorophyll *a* was significantly correlated to N input rates (P<0.01); the exponential model best described the relationship. In the first experiment (cool season), net tilapia yield was significantly correlated with both uncorrected chlorophyll *a* levels (r^2=60.6% using reciprocal × model) and chlorophyll *a* corrected for phaeophytin (r^2=54.4% using reciprocal × model). However, net tilapia yield was not significantly correlated with chlorophyll *a* levels (neither corrected for phaeophytin nor uncorrected) in the warm season experiment. Nitrogen input levels, gross primary production (GPP), and net primary production (NPP) for the cool- and warm-season experiments are shown in Table 4. NPP was not a good predictor of fish yields; nor was it related to N input rates. GPP was significantly correlated with net tilapia yield in the cool season experiment (r^2=81%, p=0.0001 using a log × model) and less well correlated in the warm season experiment (r^2=68% using multiplicative model). GPP was also a good indicator of N input rates in both experiments; for the cool season the relationship was quadratic, whereas for warm season the relationship was multiplicative, thereby indicating that GPP either did not increase much or actually decreased at the highest input rate of 30kg ha^{-1} wk^{-1}.

Observations for TH, TH, corrected and uncorrected chlorophyll *a*, soluble reactive phosphorus, pH, total N, ammonia N, nitrite N, and nitrate N for both the cool- and warm-season experiments are shown in Table 5. In both experiments, it can be concluded that P was not limiting in any way. Attempts to keep TA high were successful in the first experiment but not so in the second. Decreasing total hardness meant that there was less buffering capacity, which probably kept pH high in ponds receiving large amounts of sodium carbonate. Although TA averaged less than 60 mg l^{-1}, chlorophyll *a* levels were higher in the highest N input treatment.

Table 4. Nitrogen input rates and corresponding mean gross (GPP) and net (NPP) primary productivity observations during the cool and warm season trials of the PD/A CRSP's optimum nitrogen input experiment, conducted at Sagana Fish Farm (Sagana, Kenya) during 1998 and 1999.

Pond	N input rate (kg ha^{-1} wk^{-1})	Mean GPP (mg O_2 L^{-1})	Mean NPP (mg O_2 L^{-1})
Cool Season			
D07	0	5.46	0.38
D08	0	6.62	0.54
E06	0	7.13	0.67
D06	10	10.04	0.98
E04	10	12.88	0.78
E05	10	12.69	1.72
D05	20	11.55	1.80
E07	20	16.51	1.90
E08	20	18.81	2.91
D04	30	13.17	4.85
E03	30	16.12	0.92
E09	30	11.22	0.59
Warm Season			
D04	0	11.31	1.79
D08	0	6.68	0.44
E09	0	8.44	-0.07
D07	10	17.18	0.94
E03	10	13.37	0.04
E06	10	10.38	0.27
E04	20	13.19	0.89
E05	20	14.04	0.84
E07	20	17.45	2.34
D05	30	16.64	1.78
D06	30	14.64	0.90
E08	30	16.31	2.47

Soils

Because the soil was sampled to a depth of 5 cm at the end of the cool season experiment, comparison at beginning and end of the experiment is difficult. Although P input rates were high for all treatments (8 kg P $ha^{-1}wk^{-1}$) and the pond soils were supposedly saturated with P at the beginning of the experiment, soil P still tended to decrease over time and to decrease more as N inputs increased. In the warm season experiment, soil P at the end of the experiment was significantly negatively correlated to N input rate ($r = -0.69$; $p = 0.013$). There was however no relationship between N input rates and soil N or soil C.

Economic Analysis

The costs of inputs and revenues per hectare for these experiments are shown in Table 6. The highest marginal return (increase in revenues less increased costs) was obtained at the 20 kg N ha^{-1} wk^{-1} rate in both experiments. Maximum marginal returns were calculated at input rates of 19.9 and 16.0 kg N ha^{-1} wk^{-1} for the first and second season experiments, respectively, (Figure 7).

Carry-over Effect

Table 7 was assembled to examine the possible effects of previous treatments on the outcome of the second experiment. Ponds that received no N during the first experiment were the worst performers in the lower N input rates of the second experiment and the best performers in the high-N treatments of the second experiment. Ponds that received high N inputs were better performers (in terms of fish yields) in the no-N input treatments of the second experiment and the worst performers in the high-N input treatments. It therefore appears that overall production

Table 5. Mean (±SE) pond water quality observations during the cool- and warm-season experiments on optimum nitrogen fertilization at Sagana Fish Farm, Sagana, Kenya.

Weekly Nitrogen Application	Total Alkalinity (mg L^{-1} as $CaCO_3$)	Total Hardness (mg L^{-1} as $CaCO_3$)	Corrected Chlorophyl *a* (mg m^3)	Uncorrected Chlorophyll *a* (mg m^3)	Total Phosphorus (mg L^{-1})	Soluble Reactive Phosphorus (mg L^{-1})
Cool Season						
0 kg ha^{-1}	89.7 ±15.9	75.5 ±18.3	60.2 ±2.7	107.5 ±10.6	2.0 ±0.72	1.0 ±0.46
10 kg ha^{-1}	73.8 ±5.5	60.0 ±7.2	85.0 ±8.3	155.6 ±2.9	1.9 ±0.29	1.1 ±0.20
20 kg ha^{-1}	74.8 ±8.5	52.1 ±5.4	156.5 ±43.8	233.0 ±52.3	2.4 ±0.12	1.2 ±0.06
30 kg ha^{-1}	77.9 ±3.6	60.0 ±8.0	185.4 ±15.5	277.6 ±20.2	1.3 ±0.24	0.6 ±0.14
Best fit model			reciprocal-y	exponential		
correlation	NS	NS	-.86**	.866**	NS	NS
std error est.			0.00277	0.23		
Warm Season						
0 kg ha^{-1}	84.8 ±6.3	80.2 ±7.2	56.6 ±15.6	75.9 ±19.3	1.5 ±0.06	0.7 ±0.14
10 kg ha^{-1}	69.6 ±0.9	59.2 ±3.4	109.3 ±8.8	147.3 ±7.0	3.0 ±1.88	1.4 ±0.39
20 kg ha^{-1}	64.0 ±1.6	47.5 ±4.3	182.2 ±78.4	252.3 ±97.8	2.6 ±0.47	1.3 ±0.08
30 kg ha^{-1}	55.3 ±0.8	30.8 ±3.6	198.5 ±16.9	271.0 ±25.7	3.7 ±0.42	1.5 ±0.23
Best fit model	reciprocal	sq.root-y	exponential	exponential	reciprocal	exponential
correlation	.95**	-.94**	.77*	.80**	-.75**	-.65*
std error est.	0.0008	0.511	0.44	0.40	0.129	0.357

Weekly Nitrogen Application	pH	Total Nitrogen (mg L^{-1})	Ammonia-N (mg L^{-1})	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	Soda Ash Added to Ponds (kg ha^{-1})
Cool Season						
0 kg ha^{-1}	7.5 ±0.13	2.5 ±0.27	0.8 ±0.06	0.0 ±0.00	0.0 ±0.01	254 ±242
10 kg ha^{-1}	7.8 ±0.16	3.5 ±0.16	0.9 ±0.12	0.0 ±0.02	0.1 ±0.02	229 ±67
20 kg ha^{-1}	8.0 ±0.25	5.7 ±0.35	1.1 ±0.07	0.3 ±0.08	0.3 ±0.14	491 ±256
30 kg ha^{-1}	7.5 ±0.07	6.4 ±0.75	1.3 ±0.09	0.7 ±0.20	0.3 ±0.06	283 ±136
Best fit model		linear	linear	linear	sq.root-y	
correlation	NS	.96**	.82**	.83**	.79**	NS
std error est.		0.529	0.140	0.196	0.143	
Warm Season						
0 kg ha^{-1}	7.7 ±0.31	2.1 ±0.33	0.5 ±0.05	0.0 ±0.00	0.0 ±0.03	75 ±38
10 kg ha^{-1}	8.4 ±0.13	3.3 ±0.16	0.5 ±0.10	0.0 ±0.01	0.1 ±0.03	258 ±33
20 kg ha^{-1}	8.8 ±0.30	5.0 ±0.69	0.6 ±0.09	0.1 ±0.05	0.2 ±0.08	321 ±17
30 kg ha^{-1}	8.8 ±0.20	6.1 ±0.52	0.8 ±0.08	0.3 ±0.11	0.3 ±0.01	704 ±115
Best fit model	sq.root-x	sq.root-y	linear	sq.root-y	linear	sq. root-y
correlation	.77**	.92**	.67*	.90**	.77**	.89**
std error est.	0.388	0.179	0.144	0.101	0.119	1.05

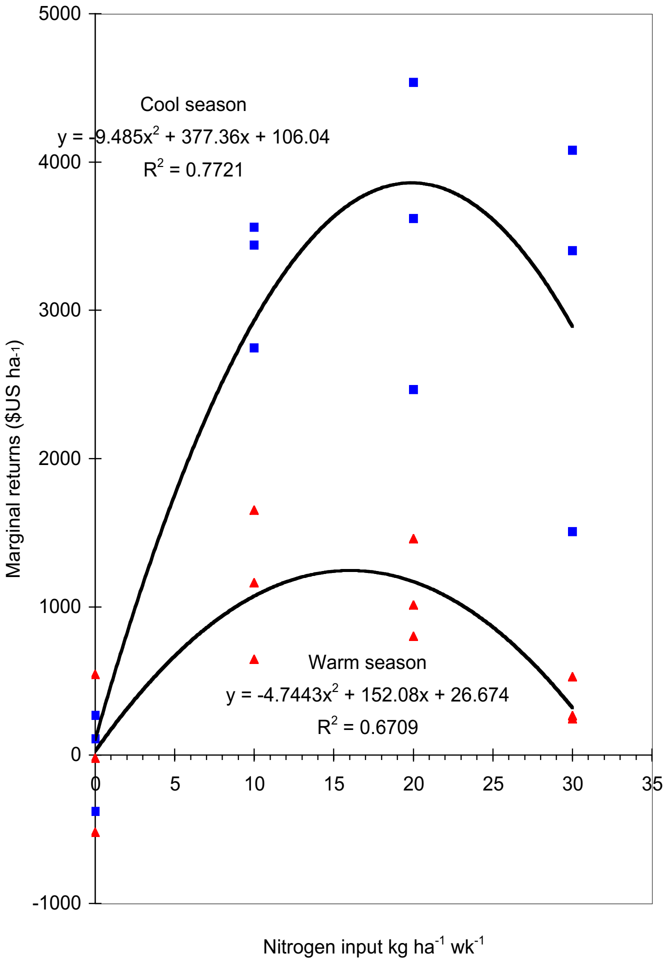

Figure 7. Marginal returns (increased revenues less increased costs) as a function of N input rates for the cool and warm season trials of the Global Experiment conducted at Sagana Fish Farm, Sagana, Kenya. Cost of capital was estimated at 12%.

over the two experiments was related to cumulative N input. Figure 8 shows this relationship. Combined net fish yields for the cool- and warm-season experiments were highly correlated to combined N inputs over the two experiments. Unfortunately, no pond received two consecutive highest N input rates. The calculated maximum net fish yield would occur at 1183.5 kg ha^{-1} N input over 45 weeks or 26.3 kg N ha^{-1} wk^{-1}. This relationship was the most highly correlated of any fish yield relationship examined.

DISCUSSION

Our observations on water quality parameters that usually correlate well with high tilapia production (chlorophyll *a*, total N) were very similar in both experiments. They definitely don't explain why fish production was so low in the warm-season experiment. Production was lower in the second experiment at the El Carao site as well but no explanation was given (Green et al. 1999). A lack of significant differences in fish yields in the second experiment at El Carao was attributed to carryover effects of the N from the preceding experiment. The results from Sagana also suggest some carryover effect or possibly the effect of differences in initial stocking size and density.

In addition to conducting the first round of the Global Experiment on optimum N, researchers at the Asian Institute of Technology (AIT) in Thailand conducted a complementary study in which they compared production from ponds that had been stocked with three different sizes of tilapia. They concluded that stocking with medium-sized fish (averaging 10 g in size) resulted in greater production than stocking with either small tilapia (averaging 4.6 g in size) or larger fingerlings (averaging 21 g in size). The fish used to stock ponds in the first round fo the Sagana experiment were in between the medium and large sizes used in the AIT study.

The results obtained at Sagana are more similar to those obtained at the El Carao site than to those from the AIT site. Increasing N input rates to 30 kg ha^{-1} $week^{-1}$ did not result in increased fish yields at either El Carao or Sagana. The high water temperatures that are typical at the AIT site (29 to 37°C) are a plausible explanation for a higher optimum N input rate there. Hatchery technicians who work with recirculating systems know that the capacity of a biofilter to process N is affected by temperature, with maximum N loading rates being higher at higher temperatures.

The two experiments conducted at Sagana took much longer than at the other two CRSP sites. The experiment was terminated after 91 days at AIT and after 121 and 107 days at El Carao. In contrast, the cool season experiment at Sagana lasted 147 days and the warm season experiment lasted 133 days.

These results and questions lead us to qualify our recommendations for farmers. A chemical fertilizer-only treatment will probably not form part of the extension recommendations to farmers in Kenya because of problems in controlling alkalinity. Organic matter inputs seem to stabilize the pond system and maintain total alkalinity

Table 6. Cost of inputs and revenue per hectare for the optimum nitrogen fertilization experiments (Global N Experiment) conducted at Sagana Fish Farm, Sagana, Kenya, during 1998 and 1999. Costs include initial P-saturation inputs of TSP applied to ponds for the cool-season experiment.

Nitrogen input in the 2nd experiment	Performance in the 2nd Experiment	Nitrogen Input in the 1st Experiment
0	Best pond: D04	30
	Worst pond: D08	0
10	Best pond: E03	30
	Worst pond: E06	0
20	Best pond: E05	10
	Worst pond: E07	20
30	Best pond: D06	10
	Worst pond: E08	20

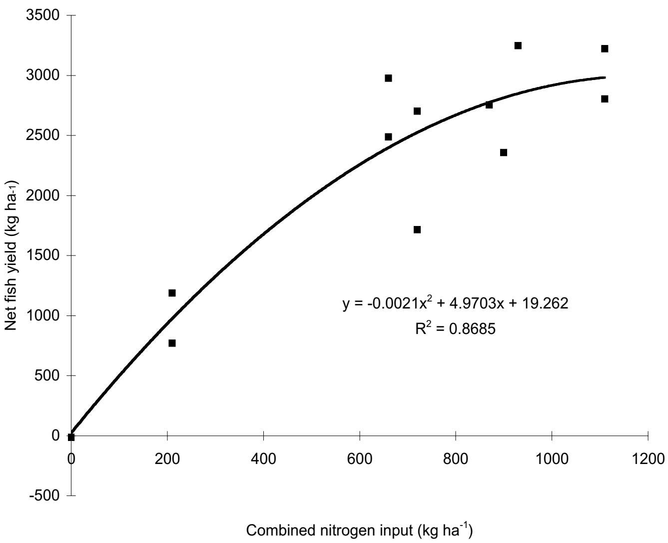

Figure 8. Combined net fish yield in relation to total nitrogen input for the cool and warm season trials of the Global Experiment conducted at Sagana Fish Farm, Sagana, Kenya.

(see carrying capacity test report) However, the optimum N input rate will be used in setting input recommendations and in estimating carrying capacity for certain input combinations. Farmers in cooler areas will be cautioned not to exceed 16 kg ha^{-1} wk^{-1} of chemical N.

Anticipated Benefits

The results from this experiment were valuable additions to the formulation of extension recommendations for tilapia culture in the East Africa region. Combining the discussions from the three PD/A CRSP sites that conducted the global experiment can lead to some additional questions as well: Fish size as a variable in carrying capacity estimates and temperature as the main reason for different optimum N input rates. The temperature question has been answered for the most part but the question of fish size versus carrying capacity merits further work, especially for systems using filter feeders and relying on natural production.

Acknowledgments

The work of Mr. James Karuri, lab technician, and Thomas Ndegwa, lab helper, was invaluable. Paul Wamwea Wabitah helped out in keeping data for the pond sampling. Our super seine crew demonstrated that twelve ponds can indeed be sampled in under two hours—a feat never thought possible at Sagana. Mr. John Kogi was a highly reliable pond manager and record-keeper.

Literature Cited

Boyd, C.E. 1976. Nitrogen fertilizer effects on production of tilapia in ponds fertilized with phosphorus and potassium. Aquaculture 7:385-390.

Boyd, C.E. 1990. Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station. Auburn University, Alabama. 482 pp.

Boyd, C.E., and J.W. Sowles. 1978. Nitrogen fertilization of ponds. Transactions of the American Fisheries Society 107:737-741.

Egna, H.S., J. Bowman, and M. McNamara. 1990. Title XII Collaborative Research Support Program Pond Dynamics/Aquaculture seventh annual administrative report 1989. Oregon State University, Corvallis, OR. 114 pp.

Egna, H.S., J. Bowman, and M. McNamara. 1991. Title XII Collaborative Research Support Program Pond Dynamics/Aquaculture eighth annual administrative report 1990. Oregon State University, Corvallis, OR. 166 pp.

Egna, H.S., J. Bowman, B. Goetze and N. Weidner. 1994. Title XII Collaborative Research Support Program Pond Dynamics/Aquaculture eleventh annual administrative report 1993. Oregon State University, Corvallis, OR. 178 pp.

Table 7. Performance of ponds in the second trial in relation to the N input level received in the first trial of the Global Experiment on optimum nitrogen fertilization.

N rate	DAP (KSh)	Urea (KSh)	TSP (KSh)	Lime (KSh)	Soda Ash KSh	Stockers (KSh)	Total Cost (KSh)	Total Cost ($US)	Tilapia Revenue (KSh)	*Clarias* Revenue (KSh)	Total Revenue (KSh)	Gross Profit (KSh)	Gross Profit ($US)
Cool													
0	-	-	39,625	10,000	2,796	124,440	176,861	2,948	121,800	24,960	146,760	(30,101)	(502)
10	24,000	2,776	15,625	10,000	2,521	124,440	179,362	2,989	312,240	32,400	344,640	165,278	2,755
20	24,000	12,688	15,625	10,000	5,408	124,440	192,161	3,203	354,360	30,000	384,360	192,199	3,203
30	24,000	22,601	15,625	10,000	2,521	124,440	199,186	3,320	301,200	59,400	360,600	161,414	2,690
Warm													
0	-	-	36,625	7,500	825	135,000	179,950	2,999	134,280	18,480	152,760	(27,190)	(453)
10	21,000	2,429	-	1,667	2,842	135,000	162,937	2,716	200,640	19,680	220,320	57,383	956
20	21,000	11,102	-	2,500	3,529	135,000	173,131	2,886	206,400	22,080	228,480	55,349	922
30	21,000	19,775	-	8,333	7,746	135,000	191,855	3,198	182,400	21,960	204,360	12,505	208

Prices	Per bag	Per kg (KSh)	Per kg ($US)
Diammonium phosphate (18-46-0) costs 1250 KSh/50kg bag	1250	25	0.42
Urea (46-0-0) cost 950 KSh/bag 50 kg	950	19	0.32
Triple superphosphate (0-46-0) cost 1250KSh/bag	1250	25	0.42
Lime: diatomite (Magmax) cost 200KSh/50 kg bag	200	4	0.07
Sodium carbonate (Na2CO3) cost 9KSh/kg+VAT=11KSh/kg	11	11	0.18
Fish priced at 120 KSh/kg (price for live fish), both for stocking and harvesting.	120	120	2.00

Fingerlings not attributed a value. We do not use fingerlings from S/R brooders.

Exchange rate is currently 70KSh/$ but was about 60 at the time of the experiment. Rate of 60 was used.

Prices of inputs have not increased, probably because we are finding cheaper suppliers all the time. For example, we can now get any of these fertilizers delivered to Sagana for equal to or less than the price in KSh.

Egna, H.S., J. Bowman, B. Goetze and N. Weidner. 1995. Title XII Collaborative Research Support Program Pond Dynamics/ Aquaculture twelfth annual administrative report 1994. Oregon State University, Corvallis, OR. 209 pp.

Egna, H.S., M. McNamara, and N. Weidner. 1992. Title XII Collaborative Research Support Program Pond Dynamics/Aquaculture ninth annual administrative report 1991. Oregon State University, Corvallis, OR. 172 pp.

Egna, H.S., M. McNamara, J. Bowman and N. Astin. 1993. Title XII Collaborative Research Support Program Pond Dynamics/ Aquaculture tenth annual administrative report 1992. Oregon State University, Corvallis, OR. 275 pp.

Green B.W., D.R. Teichert-Coddington, C.E. Boyd, N. Claros, and C. Cardona. 1999. Global experiment: Optimization of nitrogen fertilization rate in freshwater tilapia production ponds. Pages 27-37 *in* K. McElwee, D. Burke, M. Niles and H. Egna (Editors). Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon.

Hepher, B. 1962a. Primary production in fish ponds and its application to fertilization experiments. Limnology and Oceanography 7:131-135.

Hepher, B. 1962a. Ten years of research in fish pond fertilization in Israel. I. The effect of fertilization on fish yields. Bamidgeh 14:29-38.

Hickling, C.F. 1962. Fish culture. Faber and Faber, London. 295 pp.

Lin, C.K., Y. Yi, R.B. Shivappa, M.A.K. Chowdhury, and J.S. Diana.1999 Global experiment: Optimization of nitrogen fertilization rate in freshwater tilapia production ponds Pages 49-56 in K. McElwee, D. Burke, M. Niles, and H. Egna (Editors). Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon.

Mortimer, C.H. 1954. Fertilizers in fish ponds. Her Majesty's Stationery Office, London. Fish Publication No. 5. 155 pp.

Murad, A., and C.E. Boyd. 1987. Experiments on fertilization of sportfish ponds. Progressive Fish-Culturist 49:100-107.

PD/A CRSP Technical Committee. 1992. Handbook of analytical methods. Pond Dynamics/Aquaculture Collaborative Research Support Program. Program Management Office, Office of International Research and Development. Oregon State University, Corvallis, OR.

Statistical Graphics Corporation. 1997. Statgraphics Plus for Windows 3.0.

Swingle, H.S. 1947. Experiments on pond fertilization. Alabama Agricultural Experiment Station, Alabama Polytechnic Institute. Auburn, AL. Bulletin 264. 36 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Global Experiment: Optimization of Nitrogen Fertilization Rate in Freshwater Tilapia Production Ponds (Cool-Season Trial)

Eighth Work Plan, Feeds and Fertilizers Research 1 (8FFR1Ph)
Final Report

Christopher L. Brown
Marine Biology Program
Florida International University
North Miami, Florida, USA

Remedios B. Bolivar
Eddie Boy T. Jimenez
Freshwater Aquaculture Center
Central Luzon State University
Nueva Ecija, Philippines

James P. Szyper
Sea Grant College Program
University of Hawaii at Manoa
Hilo, Hawaii, USA

Abstract

An experiment was conducted, following a standardized experimental design, to determine optimum inputs of nitrogen to be used in pond cultivation of tilapia. Twelve 0.05-ha earthen research ponds were used at the Freshwater Aquaculture Center of the Central Luzon State University, Nueva Ecija, Philippines, from 14 December 1999 to 14 March 2000. A completely randomized design was employed, involving the use of three replicates per treatment of the following nitrogen fertilization rates: 0, 10, 20, and 30 kg N ha^{-1} wk^{-1} (termed Treatments 1, 2, 3, and 4, respectively). Productivity, water chemistry, and cost parameters were analyzed statistically. A trend in mean body weights favoring the ponds receiving supplemental nitrogen was detected, favoring higher mean body weights in Treatments 4, 2, 3, and 1 in that order, although the apparent difference was not statistically significant due in part to inherent variance in growth and also to the competition of unwanted tilapia recruits into the experimental ponds. Yields were improved in the fertilized ponds (Treatments 2, 3, and 4) relative to those receiving no nitrogen (Treatment 1), although only the presence or absence of fertilizer had a significant effect, and not the concentration that was applied. Some water chemistry differences were noted, specifically higher Secchi disk readings, alkalinity, and dissolved oxygen levels in ponds receiving less added nitrogen. The most cost-effective treatment was the addition of the lowest tested concentration of nitrogen (Treatment 2).

Introduction

Optimization of aquaculture production systems requires optimal use of inputs. Nitrogen, phosphorus, and carbon availability are important considerations in management of ponds for optimal fish production. PD/A CRSP research has addressed enhancement of primary productivity through inorganic and organic nutrient addition to ponds; however, previous findings on the optimum nitrogen, phosphorus, and carbon inputs required to improve fish yields at the PD/A CRSP sites appear inconsistent and demand clarification. Higher inputs have increased fish production at all PD/A CRSP sites, but optimum inputs of nitrogen, phosphorus, and carbon have not been defined.

Fertilization rates in PD/A CRSP experiments were higher than rates reported for earlier pond fertilization research. In an often-cited series of fertilization experiments conducted in Malaysia, Hickling (1962) never used more than 1.1 kg P and 1.1 kg N ha^{-1} wk^{-1}. In Israel, the standard fertilizer dose was 2.3 kg P and 6.5 kg N ha^{-1} wk^{-1} (Hepher, 1962a; 1962b). The highest rates of phosphorus and nitrogen used in most experiments at Auburn University were 1.26 and 3 kg ha^{-1} wk^{-1}, respectively (Swingle, 1947; Boyd, 1976; Boyd and Sowles, 1978; Murad and Boyd, 1987; Boyd, 1990). Rates in Europe seldom exceeded 1 kg ha^{-1} wk^{-1} for nitrogen and phosphorus (Mortimer, 1954). Rates used in Malaysia, USA, Israel, and Europe were adequate to give dense phytoplankton blooms and good fish production. Also, in all of the studies cited above, phosphorus was the most important limiting nutrient.

The objectives of this study were: 1) to determine optimal rates of nitrogen fertilizer to obtain tilapia yields with the greatest profit in ponds and 2) to develop a full-cost enterprise budget for the most profitable fertilization rate.

Methods and Materials

The experiment was conducted in twelve 0.05-ha earthen research ponds at the Freshwater Aquaculture Center of the Central Luzon State University, Nueva Ecija, Philippines, from 14 December 1999 to 14 March 2000.

Table 1. Growth performance of Nile tilapia in ponds fertilized with different rates of nitrogen in the 90-day culture period. Mean values with different superscript letters in the same row are significantly different ($P < 0.05$).

Performance	Treatment			
	1	2	3	4
Initial Biomass (kg pond^{-1})	50 ± 0[a]	50 ± 0[a]	50 ± 0[a]	50 ± 0[a]
Initial Mean Weight (g fish^{-1})	13.7 ± 0.9[a]	12.1 ± 0.3[a]	12.4 ± 1.2[a]	13.4 ± 0.3[a]
Final Mean Weight (g fish^{-1})	23.2 ± 5.9[a]	49.2 ± 11.4[b]	43.9 ± 11.2[b]	59.2 ± 1.4[b]
Net Fish Yield (kg pond^{-1})	6.9 ± 2.3[a]	82.1 ± 16.9[b]	72.8 ± 25.8[b]	83.6 ± 16.6[b]
Extrapolated Net Fish Yield (kg ha^{-1})	137 ± 45[a]	1,642 ± 338[b]	1,456 ± 516[b]	1,671 ± 332[b]
Gross Fish Yield (kg pond^{-1})	56.9 ± 2.2 [a]	132.1 ± 16.9[b]	122.8 ± 5.7[b]	133.6 ± 16.6[b]
Extrapolated Gross Fish Yield (kg ha^{-1})	1,138 ± 45[a]	2,642 ± 338[b]	2,456 ± 516[b]	2,671 ± 332[b]
Survival (%)	71 ± 16.5[a]	68 ± 16.6[a]	76.7 ± 23[a]	60.7 ± 8.6[a]

A completely randomized design with three replicates per treatment was used in this experiment. Four nitrogen fertilization rates served as treatments at 0, 10, 20, and 30 kg N ha^{-1} wk^{-1}, which correspond to Treatments 1, 2, 3, and 4, respectively. Urea and ammonium phosphate were the chemical fertilizer sources for nitrogen and phosphorus. Due to the unavailability of triple superphosphate, Treatment 1 did not receive any phosphorus fertilizer, but the rest of the treatments received 8 kg P ha^{-1} wk^{-1}.

Sex-reversed male Nile tilapia (*Oreochromis niloticus*) of the Genetically Improved Farmed Tilapia (GIFT) strain averaging 12.9 g were stocked at the rate of 1,000 kg ha^{-1}. This resulted in a stocking rate of about 78,000 fish ha^{-1}. The fish were sampled by seine net at biweekly intervals to measure growth. Approximately 10% of the initial stock was seined, counted, and weighed en masse from each pond during sampling. All fish were harvested by draining after 90 days of culture. Mean body weight of fish, fish yield (kg ha^{-1}), and survival rates were calculated.

Table 2. Mean (± SE) pond water quality parameters measured at the initial, midway, and final weeks in ponds fertilized with different rates of nitrogen fertilization. Mean values with different superscript letters in the same row are significantly different ($P < 0.05$).

Performance	Treatment			
	1	2	3	4
DO AT DAWN (MG L^{-1})				
Initial	1.68 ±0.05[a]	1.76 ±0.13[a]	1.70 ±0.08[a]	1.70 ±0.13[a]
Midway	5.27 ±0.07[a]	4.77 ±0.26[a]	4.40 ±0.35[a]	4.27 ±0.37[a]
Final	7.35 ±0.40[a]	4.76 ±0.74[b]	4.69 ±0.13[b]	4.27 ±0.26[b]
PH				
Initial	8.26 ±0.13[a]	7.96 ±0.46[a]	8.33 ±0.09[a]	8.67 ±0.19[a]
Midway	8.47 ±0.08[a]	8.56 ±0.11[a]	8.46 ±0.06[a]	8.62 ±0.14[a]
Final	8.32 ±0.08[b]	8.51 ±0.05[ab]	8.43 ±0.03[ab]	8.55 ±0.05[a]
TEMPERATURE (°C)				
Initial	24.47 ±0.15[ab]	24.60 ±0.06[a]	24.20 ±0.06[b]	24.33 ±0.12[ab]
Midway	20.23 ±0.15[a]	20.47 ±0.20[a]	20.33 ±0.24[a]	20.47 ±0.09[a]
Final	24.28 ±0.14[a]	24.47 ±0.11[a]	24.23 ±0.28[a]	24.42 ±0.15[a]
TOTAL AMMONIA NITROGEN (MG L^{-1})				
Initial	0.03 ±0.01[a]	0.04 ±0.02[a]	0.04 ±0.01[a]	0.05 ±0.01[a]
Midway	0.07 ±0.02[a]	0.08 ±0.01[a]	0.20 ±0.09[a]	0.24 ±0.06[a]
Final	0.13 ±0.03[b]	0.32 ±0.03[a]	0.35 ±0.03[a]	0.29 ±0.02[a]
TOTAL PHOSPHORUS (MG L^{-1})				
Initial	0.23 ±0.06[a]	0.32 ±0.04[a]	0.26 ±0.03[a]	0.32 ±0.06[a]
Midway	0.24 ±0.06[a]	0.23 ±0.03[a]	0.21 ±0.04[a]	0.24 ±0.06[a]
Final	0.39 ±0.03[a]	0.36 ±0.02[a]	0.34 ±0.02[a]	0.39 ±0.07[a]
TOTAL ALKALINITY (MG L^{-1})				
Initial	238 ±17.5[a]	214 ±7.5[a]	220 ±20.5[a]	198 ±3.5[a]
Midway	240 ±14.8[a]	199 ±9.2[b]	178 ±15.3[b]	167 ±1.8[b]
Final	151 ±20.0[a]	132 ±3.8[a]	132 ±10.4[a]	122 ±2.6[a]
PRIMARY PRODUCTIVITY (MG O_2 L^{-1} D^{-1})				
Initial	11.4 ±0.87[a]	14.2 ±2.24[a]	13.9 ±1.80[a]	16.6 ±2.4[a]
Midway	11.6 ±0.77[a]	21.5 ±0.38[a]	16.3 ±3.39[a]	18.9 ±2.34[a]
Final	8.0 ±0.17[b]	19.6 ±3.11[a]	20.9 ±2.29[a]	18.7 ±0.80[a]
SECCHI DISK DEPTH (CM)				
Initial	31.7 ±4.1[a]	29.0 ±5.7[a]	23.7 ±4.7[a]	28.3 ±3.5[a]
Midway	35.7 ±5.2[a]	19.0 ±4.0[b]	18.7 ±4.2[b]	18.7 ±1.2[b]
Final	33.7 ±2.4[a]	21.3 ±0.67[b]	16.0 ±1.5[b]	16.3 ±1.8[b]

Table 3. Partial budget analysis for Nile tilapia cultured in ponds fertilized with different rates of nitrogen fertilization (budget items in Philippine Pesos; US$1 = P45).

Item	Treatment			
	1	2	3	4
Income (Selling Fish)	1,138.00	4,623.50	4,298.00	4,676.00
Added Income (A)	----	3,485.50	3,160.00	3,538.00
Cost for Urea	0	189.00	280.98	372.96
Added Cost from Urea (B1)	----	189.00	280.98	372.96
Cost for Ammonium Phosphate	0	118.58	118.58	118.58
Added Cost from Ammonium Phosphate (B2)	----	118.58	118.58	118.58
Ratio of Added Income to Added Cost	----	11.3	7.9	7.2
Profit (A – B1 – B2)	----	3,177.92	2,760.44	3,046.46
US$	----	70.62	61.34	67.70

Dissolved oxygen (DO), pH, temperature, total ammonia nitrogen (TAN), total phosphorus (TP), total alkalinity, primary productivity, and Secchi disk depth (SDD) were determined during the second week, midway through the experiment, and two weeks before the harvest of fish. Analyses of water quality were done according to methodologies detailed in the PD/A CRSP Handbook of Analytical Methods (PD/A CRSP Technical Committee, 1996). DO, temperature, and pH measurements were taken on the three sampling dates at 0600 h, 1000 h, 1600 h, 1800 h, and 0600 h the following morning at 5-cm, 25-cm, 50-cm, and 75-cm depths in the water column. Water level in the pond was maintained at 1 m throughout the culture period by occasionally adding water to the ponds.

Data from the experiment were analyzed statistically by regression analysis and analysis of variance using SAS System for Windows v6.12 statistical software (SAS Institute, 1996). Partial budget analysis was conducted to determine which fertilization rate yielded the greatest profitability (Shang, 1990). A full-cost enterprise analysis was developed for the fertilization rate that yielded the greatest profitability.

RESULTS

Mean values with their standard errors for initial and final body weights, gross and net yields, and survival of Nile tilapia are shown in Table 1. Mean initial body weights did not differ significantly among treatments ($P > 0.05$). Mean final body weights were significantly different, with Treatment 1 having the lowest mean body weight of fish at harvest ($P < 0.05$) among treatments. The highest mean weight of fish at harvest was observed in Treatment 4 (59.2 ± 1.4 g), followed by Treatments 2 (49.2 ± 11.4 g) and 3 (43.9 ± 11.2 g). However, there was no significant difference in the final mean body weights of fish in these treatments. Similar trends were observed in the gross and net fish yields and in extrapolated values. Yields were significantly higher in the fertilized ponds relative to the unfertilized ponds, but treatment-dependent differences among the ponds given different concentrations of fertilization were otherwise absent. The mean growth of the Nile tilapia in the different treatments is shown in Table 1.

The percent survival of Nile tilapia was highest in Treatment 3 (77%), followed by Treatment 1 (71%), Treatment 2 (68%), and Treatment 4 (61%). Survival rates of fish in this study did not vary significantly among treatments ($P > 0.05$).

Water Quality

Mean values for water quality parameters are shown in Table 2. Except for temperature, mean values for the initial analysis of water quality parameters did not differ significantly among treatments ($P > 0.05$).

Analysis of water quality midway through the experiment indicated significant differences in total alkalinity and SDD. Total alkalinity was significantly highest ($P < 0.05$) in Treatment 1 (240 ± 14.8 mg l^{-1}), followed by Treatment 2 (199 ± 9.2 mg l^{-1}), Treatment 3 (178 ± 15.3 mg l^{-1}), and Treatment 4 (167 ± 1.8 mg l^{-1}). There were significant differences among Treatments 2, 3, and 4 with respect to total alkalinity ($P < 0.05$).

SDD was also significantly different among treatments ($P < 0.05$). Treatment 1 had the highest SDD (35.7 ± 5.2 cm) as compared to the other three treatments, which had similar SDD (19 cm). This roughly indicated the condition of the water in terms of phytoplankton growth. Treatment 1 had the poorest phytoplankton growth compared to the treatments that received nitrogen fertilization.

Analyses of final water quality parameters showed five parameters that were significantly different among treatments. These were DO concentration at dawn, pH, TAN, primary productivity, and SDD. DO concentration and SDD were

Table 4. A full-cost enterprise budget for Nile tilapia cultured in a 0.05-ha pond fertilized with 10 kg N ha^{-1} wk^{-1} (budget items in Philippine Pesos; US$1 = P45).

Item	Price (P kg^{-1})	Quantity (kg $pond^{-1}$)	Value (P $pond^{-1}$)
Gross Revenue (A)			
Harvested Tilapia	35.00	132.00	4,623.50
Cost (B)			
Fingerlings	35.00	50.00	1,750.00
Urea	7.30	12.60	91.98
Ammonium Phosphate	7.70	28.00	215.60
Net Returns (A – B)			2,565.92
Break-Even Price		15.58	

significantly higher in Treatment 1 than in the treatments with nitrogen fertilization. However, no significant differences were observed among Treatments 2, 3, and 4 ($P > 0.05$) in terms of the final DO concentration at dawn and SDD. For pH, TAN, and primary productivity, significantly lower mean values were recorded in Treatment 1, while Treatments 2, 3, and 4 did not differ significantly with respect to these parameters.

Tilapia Recruits

Due to unsuccessful hormonal sex reversal, tilapia recruits were observed in the ponds. The recruits were bulk-weighed at harvest, and the highest average weight was observed in Treatment 4 (13.6 ± 6.7 kg), while Treatments 2 and 3 had an average of 5.3 ± 2.04 kg and 5.1 ± 0.70 kg of recruits, respectively. Treatment 1 had only one pond with observed reproduction (1.4 kg). Although there was no significant difference in the weight of recruits among treatments ($P > 0.05$), the presence of recruits affected the final growth of Nile tilapia in Treatment 4 because of possible fish competition for food and space.

Budget Analysis

Partial budget analysis indicated that Treatment 2 (10 kg N ha^{-1} wk^{-1}) gave slightly more profit than the treatments with higher nitrogen fertilization (Table 3). A full-cost enterprise budget was developed for this nitrogen fertilization rate (Table 4). A net return of P2,566 (US$57) can be obtained in the culture of Nile tilapia in a 0.05-ha pond for 90 days.

Discussion

These results show the effects of adding exogenous nitrogen to tilapia culture ponds in the Philippines; the differences observed and the lack of differences among treatments may be considered equally instructive. Individual growth (mean body weight) was not significantly affected by fertilization, although a trend favoring the fertilized ponds was apparent. Additional replicates and the exclusion of competing recruits might show this effect to be biologically significant, but that remains to be seen. In general, tilapia production was enhanced by the addition of fertilizer relative to the lack of any fertilizer, but doubling or tripling the amount added had no effect on pond yields. Consequently we found that the addition of the minimal amount of fertilizer used in this study (10 kg ha^{-1} wk^{-1}) was most cost-effective.

Anticipated Benefits

Although this experiment was not designed by those who carried it out, it fits with the majority of the research being done by our team at Central Luzon State University. Specifically, the theme of moderate intensification of fish farming technology, with attention to reduction of operating costs, is consistent with our feed studies. This study illustrates the point that low-level fertilization improves yields, but that doubling or tripling the application of fertilizers is not cost-effective. We anticipate that this approach will be readily accepted by farmers in the Luzon area, who have already demonstrated a willingness to adopt low-cost aquaculture methods, as opposed to methods that increase intensification but which also drive up production costs.

Literature Cited

Boyd, C.E., 1976. Nitrogen fertilizer effects on production of Tilapia in ponds fertilized with phosphorus and potassium. Aquaculture, 7:385–390.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Alabama Agriculture Experiment Station, Auburn University, Alabama, 482 pp.

Boyd, C.E. and J.W. Sowles, 1978. Nitrogen fertilization of ponds. Trans. Amer. Fish. Soc., 107:737–741.

Hepher, B., 1962a. Primary production in fish ponds and its application to fertilization experiments. Limnol. Oceanogr., 7:131–135.

Hepher, B., 1962b. Ten years of research in fish ponds and its application to fertilization experiments. Bamidgeh, 14:29–38.

Hickling, C.F., 1962. Fish Culture. Faber and Faber, London, 295 pp.

Mortimer, C.F., 1954. Fertilization in Fish Ponds. Fish Pub., Her Majesty's Stationery Office, London, 155 pp.

Murad, A. and C.E. Boyd, 1987. Experiments on fertilization of sportfish ponds. Prog. Fish-Cult., 49:100–107.

PD/A CRSP Technical Committee, 1992. Handbook of Analytical Methods. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon.

SAS Institute, 1996. SAS System for Windows v6.12 Statistical Software. SAS Institute Inc., Cary, North Carolina.

Shang, Y.C., 1990. Aquaculture Economic Analysis: An Introduction. World Aquaculture Society, Baton Rouge, Louisiana, 211 pp.

Swingle, H.S., 1947. Experiments on Pond Fertilization. Alabama Agriculture Experiment Station Bull. 264, Auburn, Alabama, 36 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

FISH YIELDS AND ECONOMIC BENEFITS OF TILAPIA/*CLARIAS* POLYCULTURE IN FERTILIZED PONDS RECEIVING COMMERCIAL FEEDS OR PELLETED AGRICULTURAL BY-PRODUCTS

Ninth Work Plan, Feeds and Fertilizers Research 2 (9FFR2)
Progress Report

Karen Veverica
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

David Liti
Department of Zoology
Moi University
Eldoret, Kenya

Enos Were
Department of Fisheries
Moi University
Eldoret, Kenya

Jim Bowman
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

ABSTRACT

There is a clear need to develop feed/fertilizer combinations that are appropriate for fish farming in Kenya and other parts of Africa. The strategy of using high-quality nutritionally complete feeds to produce high fish yields, frequently employed in developed countries, is often impossible or inappropriate in countries where high-quality feed ingredients are in short supply or are very expensive. However, the use of lower-quality pelleted feeds formulated specifically for tilapia, combined with fertilization regimes to increase the availability of natural food organisms, may be an economically appropriate approach for intensification of tilapia culture in Africa. This experiment is the second in a series designed to compare fish performance and economic benefits under different fertilization/feeding regimes—using low-cost, locally available materials—in earthen ponds. Water quality and fish growth sampling data were collected throughout the experiment, but data have not yet been analyzed. This report includes preliminary observations regarding the experiment. The experiment was conducted in twelve 800-m^2 earthen research ponds at Sagana Fish Farm, Kenya, between November 1999 and May 2000. Four replicates of each of three combinations of feed and fertilizer were tested. The treatments were Rice Bran (RB), Pig Finisher Pellets (PFP), and Test Diet Pellets (TDP). The experiment was concluded when fish reached market size, which occurred after 180 days. Water quality parameters were not significantly different ($P > 0.05$) among the three treatments except for total alkalinity, for which PFP ponds had a significantly higher ($P < 0.05$) mean value. Phytoplankton communities exhibited a strong seasonal succession, being dominated by green algae in the beginning and by blue-greens later in the cycle. Gross primary productivity ranged from 0.1 to 11.9 g C m^{-2} d^{-1} for all treatments. It took almost two months to develop phytoplankton blooms in the ponds, and fish growth was relatively slow at first. Fish receiving RB grew much slower than in similar treatments in previous trials. This was probably due to the lower-than-normal protein content of the bran. Average fish yield was greatest in ponds receiving PFP, followed by TDP, and finally by RB. Less than 50% of the fish in the RB treatment attained market size (300 g), whereas over 80% of the fish from the other two treatments were over 300 g. *Clarias* in all treatments attained market size of 600 g. If price varies by fish size, using PFP for supplemental feeding would be the best choice. RB treatment had significantly lower fish growth rate, net fish yield, and annual production compared to PFP and TDP ($P < 0.05$). However, there were no significant differences in survival rate and relative condition factor among the treatments. Relative profitability analysis using partial and enterprise budgets revealed that the PFP treatment was the best, followed by the RB treatment. Net returns were positive for all treatment regimes. However, RB had the lowest break-even price and the least investment cost.

INTRODUCTION

Commercial fish culture in developed countries generally achieves greatest profits when high-quality, nutritionally complete feeds are used to produce high fish yields. This strategy is often impossible or inappropriate in countries where high-quality feedstuffs are limited. In Africa, nutritionally complete diets for tilapia are very expensive. However, poultry diets and some purchased inputs, such as brans, can be used to intensify fish production in ponds. Disadvantages are that commercial poultry rations are not nutritionally balanced for fish, containing more digestible energy per unit of protein than recommended for fish, and brans are nutritionally deficient and often unconsumed by the fish due to small particle size. Pelletizing reduces feed losses, especially when multiple ingredients are included in the formulation. There is a clear need to develop feed/ fertilizer combinations that are appropriate for fish farming in Kenya (Ngugi and Wangila, 1996). Lower-quality pelleted feeds formulated specifically for tilapia, combined with fertilization regimes to increase the availability of natural food organisms, may be an economically appropriate approach for intensification of tilapia culture in Africa.

This report describes the pond production aspects of an experiment conducted at Sagana Fish Farm, Kenya, to compare fish performance and economic benefits of Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) polyculture in fertilized ponds using three low-cost supplemental feeds. The supplemental feeds used were rice bran (RB), a commercially available pig finisher pellet (PFP), and a pelleted test diet (TDP) developed in collaboration with the same company that supplies the PFP.

Fertilization is often practiced to increase the abundance of natural food organisms in fish ponds receiving nutritionally deficient feeds. Assessment of the relative contribution of natural food organisms to fish growth in fed ponds would be a valuable contribution in the development of management practices involving nutritionally incomplete feeds. This assessment can be accomplished by analyzing the stable isotope ratios of nitrogen and carbon in the natural foods, feeds, and fish flesh; this is being undertaken by researchers at the University of Arkansas at Pine Bluff who will report separately on that work (see "Stable carbon and nitrogen isotope analysis of tilapia and *Clarias* fed commercial feeds or agricultural by-products," 9FFR2A; p. 31 of this report).

Methods and Materials

This experiment was originally planned to be conducted between July and November 1999, but its start was delayed by the late completion of the previous experiment ("Use of pond effluents for irrigation in an integrated crop/aquaculture system," 9ER1), which had taken longer than expected due to slower-than-expected fish growth. Ponds were finally prepared and stocked for the experiment on 5 November 1999.

Twelve 800-m^2 research ponds at Sagana Fish Farm, Sagana, Kenya, were used for the experiment. Prior to filling, all ponds were limed at a rate of 20,000 kg ha^{-1} and fertilized as part of the test treatments, as described below. Each pond was stocked with 1,550 sex-reversed male tilapia (19,375 ha^{-1}; 90 g average weight) and 50 *Clarias*. Fish were fed twice daily at 2% body weight per day. Twenty-five tilapia were stocked in a cage in each pond and were not fed. These fish were part of a companion study in which they will be analyzed for C and N isotopes for comparison to fish in the open pond, which were fed.

Three treatments were applied in quadruplicate as follows:

- Rice Bran (RB) plus inorganic fertilization by applying diammonium phosphate (DAP) and urea at rates of 4 kg P ha^{-1} wk^{-1} and 16 kg N ha^{-1} wk^{-1};
- Pig Finisher Pellets (PFP), available locally, plus the fertilization regime of Treatment RB; and
- Test Diet Pellets (TDP) formulated to contain about 20% crude protein plus the fertilization regime of Treatment RB.

Rice bran had been tested as a supplemental feed in earlier work and so was used as the basis for comparison in this study. Pig finisher pellets were selected because they were less expensive than poultry pellets and because previous work indicated they were just as good as long as fertilizer was added on a regular basis. The feed in Treatment TDP contained no fish meal and was developed in consultation with the local fish feed manufacturer.

Fish were sampled by seining every two weeks to determine growth rates and adjust feeding rations. Thirty fish of each species were measured and weighed individually to obtain standard deviations. Fish and food/feed samples were collected at stocking, every 1.5 to 2 months during the experiment, and at harvest for isotope analysis. Gut contents of caged and free-swimming fish were collected at least twice to back up results of the fish tissue analyses.

Dissolved oxygen, temperature, and pH were measured weekly in the morning and afternoon. Total alkalinity, chlorophyll *a*, Secchi disk visibility, and total ammonia nitrogen were measured every two weeks. Total nitrogen, mineral nitrogen, total phosphorus, and soluble reactive phosphorus were analyzed monthly. Samples for water chemistry were taken on Thursdays, fertilizing was done on Fridays, and dissolved oxygen and temperature readings were done on Mondays. All sampling and analysis was carried out according to standard CRSP sampling protocols.

Water lost through evaporation or seepage was replaced weekly. Otherwise no water was added to or allowed to flow through the ponds during the experiment.

The ponds were drained completely after 180 days, when the fish had reached market size. Tilapia were separated by sex, counted, and weighed. Tilapia reproduction was weighed and subsamples were counted. *Clarias* were counted and weighed.

Results

Data for the pond production aspects of the experiment were collected throughout the experiment but have not yet been fully analyzed. Following are some preliminary observations concerning the experiment.

Water quality parameters were not significantly different ($P > 0.05$) among the three treatments except for total alkalinity, for which ponds fed PFP had a higher ($P < 0.05$) mean value than the other two treatments. The lowest dawn oxygen level (0.92 mg l^{-1}) was observed in the PFP treatment, while the highest afternoon level (9.9 mg l^{-1}) was recorded in the RB treatment. The phytoplankton community was dominated mainly by green algae in the beginning and later by blue-greens, exhibiting a strong seasonal succession. Gross primary productivity ranged from 0.1 to 11.9 g C m^{-2} d^{-1} for all treatments.

Fish growth was relatively slow at first, and it took almost two months for the ponds to develop phytoplankton blooms. Fish growth in the TDP ponds fell behind that in the PFP ponds after the nitrogen input rate was reduced in order to stay under 35 kg ha^{-1} wk^{-1} total N input as feed and fertilizer combined. However, TDP fish growth almost caught up with the PFP growth by the end of the experiment. The fish receiving RB grew much slower than fish under similar

Table 1. Harvest data for fish in ponds fed rice bran (RB), pig finisher pellets (PFP), and test diet pellets (TDP) during a 180-day experiment at Sagana Fish Farm, Sagana, Kenya. Numbers followed by different letters in the same column are significantly different at $P \leq 0.05$ using LSD multiple range test.

Feed Type	Gross Fish Yield (kg ha^{-1})	Average Tilapia Weight (g)	Tilapia > 300 g (%)	Average *Clarias* Weight (g)
Rice Bran (RB)	4,288[a]	218[a]	41[a]	603[a]
Pig Finisher Pellets (PFP)	6,156[b]	315[b]	82[b]	1,031[b]
Test Diet Pellets (TDP)	6,329[b]	326[b]	82[b]	1,147[c]

Table 2. Enterprise budget summary for an *Oreochromis niloticus*/*Clarias gariepinus* polyculture system using three supplemental feeds in fertilized ponds at Sagana Fish Farm, Sagana, Kenya.

Treatment	Income above Variable Costs (US$)	Net Returns to Land, Labor, and Management (US$)	Break-Even Price (Variable Costs) (US$)	Break-Even Price (Total Cost) (US$)
Rice Bran (RB)	2,168	1,742	0.67	0.76
Pig Finisher Pellets (PFP)	2,767	2,341	0.73	0.80
Test Diet Pellets (TDP)	1,992	1,566	0.84	0.91

treatment regimes in previous trials. This was probably due to the bran's lower-than-normal protein content. Average fish yield was greatest in ponds receiving PFP, followed by TDP and finally by RB (Table 1). Less than 50% of the fish in the RB treatment attained market size (300 g), whereas over 80% of the fish from the other two treatments were over 300 g. *Clarias* in all treatments attained market size of 600 g (Table 1). If price varies by fish size, using PFP for supplemental feeding would be the best choice.

All three feeds contained much lower protein levels than expected. The RB that previously tested at 11% crude protein (CP) contained only 5% CP when re-analyzed. The PFP was advertised as containing 14% CP but tested at only 10%. The TDP contained three types of oilseed meal, making up over 30% of the ingredients, but still tested at only 11% CP.

The RB treatment had significantly lower fish growth rate, net fish yield, and annual production compared to PFP and TDP ($P < 0.05$). However, there were no significant differences in survival rate and relative condition factor among the treatments.

Relative profitability analysis using partial and enterprise budgets revealed that the PFP treatment was the best, followed by RB (Table 2). The net returns were positive for all the nutrient regimes. However, RB had the lowest break-even price and the least investment cost.

ANTICIPATED BENEFITS

The development of cost-effective feeds in Africa may increase the profitability of fish farming in the region and stimulate commercial aquacultural enterprises. Collaboration with a local feed manufacturer can lead to a viable partnership with private enterprise to develop the most cost-effective tilapia feed for ponds in Kenya and the region. The production of natural food organisms in feed/fertilizer management practices is often highly variable across sites; the need to evaluate this more-intensive fish production practice under different environmental conditions may stimulate future intra-regional collaboration.

LITERATURE CITED

Ngugi, C.C. and B.C.C. Wangila, 1996. Aquaculture in Kenya: Status and constraints. In: Fisheries for Sustainable Development. Technical Report No. 1, Department of Fisheries, Moi University, Eldoret, Kenya, pp. 33–42.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Stable Carbon and Nitrogen Isotope Analysis of Tilapia and *Clarias* Fed Commercial Feeds or Agricultural By-Products

Ninth Work Plan, Feeds and Fertilizers Research 2A (9FFR2A)
Abstract

Rebecca Lochmann
Department of Aquaculture and Fisheries
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

Abstract

Samples of feeds, fertilizers, fish, mud, and plankton from the final feeding study in Kenya were received in Pine Bluff, Arkansas, in late June 2000. These samples are being freeze-dried and ground. In addition, mud must be treated to remove carbonates. All prepared samples will be submitted to a laboratory for isotope analysis, which will begin in two to three months. Final results should be available by 31 December 2000. Since all samples were received only after the feeding trial was completed, there are no preliminary isotope data to report. Several sets of feeds and feedstuffs have been subjected to proximate analysis at University of Arkansas at Pine Bluff to verify results of analysis conducted in Kenya.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Timing of the Onset of Supplemental Feeding of Nile Tilapia (*Oreochromis niloticus*) in Ponds

Ninth Work Plan, Feeds and Fertilizers Research 4 (9FFR4)
Final Report

Christopher L. Brown
Marine Biology Laboratory
Florida International University
North Miami, Florida, USA

Remedios B. Bolivar
Eddie Boy T. Jimenez
Freshwater Aquaculture Center
Central Luzon State University
Nueva Ecija, Philippines

Abstract

An on-farm trial was conducted on seven farms in Nueva Ecija, Philippines, to investigate the effect of two onsets of feeding on the growth, yield, and survival of Nile tilapia. There were no significant differences in the performance data (final mean weight, daily weight gain, extrapolated gross fish yield, and survival rate) that were recorded in this study. The only statistically significant difference observed was in the total feed used in the trial. The 45-day onset in feeding produced more gross value of the crop (P205,617 ha^{-1}) compared with the 75-day delay (P197,063 ha^{-1}), but by delaying the start of feeding, the costs were reduced such that the net value of the crop was improved (P124,242 ha^{-1} in 75-day versus P106,026 ha^{-1} in 45-day delay).

Introduction

Tilapia farmers in the Philippines commonly provide supplemental feeds in their grow-out operation. However, the timing of the start of supplemental feeding is variable. Earlier studies under the PD/A CRSP showed that initiation of feeding of Nile tilapia after 80 days in the pond produced the same yield as initiation of feeding at 38 days. Later feeding also increased growth rates and yields to the target levels, but with some delay compared with earlier feeding (Diana et al., 1996). This means that fish do not need to be fed immediately after stocking, but can be supported by the natural food in the pond ecosystem.

This study was undertaken to demonstrate efficient supplemental feeding strategies for tilapia production in fertilized ponds. Specifically, the study aimed to evaluate growth, yield, and survival of tilapia with different periods of delay before feeding.

Methods and Materials

Seven farmers were enlisted for this trial. The farming practice of farmers ranged from extensive to semi-intensive. Two ponds from each farm were assigned each of the two treatments—supplemental feeding beginning at 45 and 75 days after fish stocking. The pond size ranged from 416 to 3,500 m^2 with water depth maintained at 1 m. The ponds were stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) of the Genetically Improved Farmed Tilapia (GIFT) strain. Fingerlings with mean weight of 0.11 g were used at a stocking rate of 4 fish m^{-2}. All ponds were fertilized weekly with urea and ammonium phosphate at a rate of 28 kg N ha^{-1} wk^{-1} and 5.6 kg P ha^{-1} wk^{-1}.

The fish were fed with prepared feeds consisting of 67% rice bran and 33% fish meal (crude protein [CP] = 28.6%) at 5% fish body weight per day (BWD) during the first 15 days of feeding and 4% BWD thereafter up to 30 days. For the remaining period prior to harvest, the fish were fed at the rate of 3% BWD. A sample of 50 fish was obtained in each pond every two weeks to measure average weights of the fish.

Water quality parameters were monitored monthly in all ponds. The water was analyzed for dissolved oxygen, pH, total alkalinity, total ammonia, and soluble reactive phosphorus. Analyses were done at the Freshwater Aquaculture Center Water Quality Laboratory according to standard methods (APHA, 1980; PD/A CRSP, 1992).

After 150 days, the ponds were harvested by seining and then complete draining. The total number of fish was counted and bulk-weighed. Final mean weight, daily weight gain, gross yield, and survival rates were calculated. The total amount of feed given in each treatment was also estimated at the end of the study. Data were analyzed statistically by paired T-tests.

Results and Discussion

Performance data (final mean weight, daily weight gain, extrapolated gross fish yield, and survival rate) were not significantly different between the two treatments (Table 1). The only statistically significant difference observed was in the

Table 1. On-farm growth performance of Nile tilapia at two periods of delay before feeding (45 and 75 days) in a 150-day experiment.

Performance	Treatment (Feeding Onset)	
	45 Days	*75 Days*
Final Mean Weight (g fish^{-1})	164.7	151.7
Mean Daily Weight Gain (g fish^{-1}d^{-1})	1.09	1.01
Extrapolated Gross Fish Yield (kg ha^{-1})	5,140	4,926
Survival (%)	85	87
Total Amount of Feed (kg ha^{-1})	8,299	6,068

total feed used in the trial. The extrapolated total feed used was 8,299 and 6,068 kg ha^{-1} for 45-day and 75-day delays in feeding, respectively. The growth patterns of tilapia in the two feeding schemes are shown in Figure 1.

The simple cost/benefit analysis of this farm trial, taking into consideration the gross sales and the cost of feed, indicated that the 45-day onset in feeding produced more gross value of the crop (P205,617 ha^{-1}) compared with the 75-day delay (P197,063 ha^{-1}), but by delaying the start of feeding, the feed cost was reduced such that the net value of the crop was improved (P124,242 ha^{-1} in 75-day versus P106,026 ha^{-1} in 45-day delay; US$1 = P45).

Table 2 presents the range of values of the water quality parameters measured at monthly intervals. Water quality parameters appeared to be within the acceptable ranges for tilapia culture and there were no treatment-dependent differences that were observed.

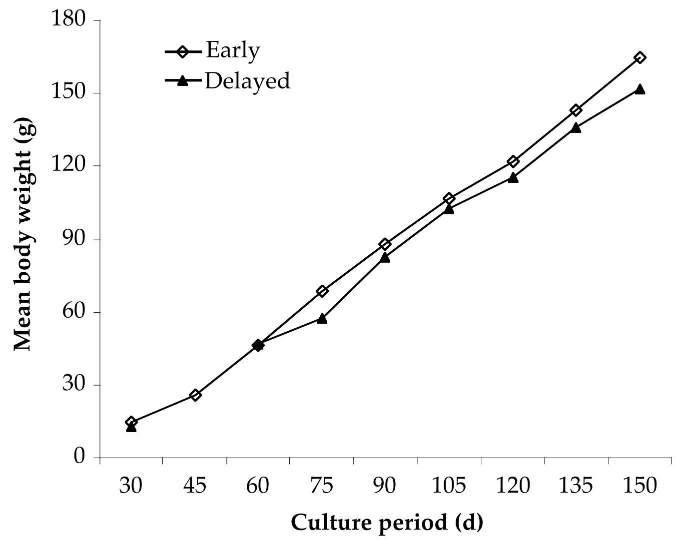

Figure 1. Mean body weight of Nile tilapia in the early (45 days) and delayed (75 days) feeding onset trials.

This study provides technical guidance to farmers on the efficient feeding practices that will optimize tilapia production. The on-farm trial indicated that a delay in the onset of feeding did not significantly reduce the production of tilapia but significantly reduced the cost of feed by about 37%. It is important, however, that proper pond fertilization be maintained to promote the production of natural foods in the pond.

Anticipated Benefits

A simple technique has been identified which reduces production costs by the equivalent of about $400 per hectare

Table 2. Range of values for water quality parameters measured monthly over a 150-day culture period in ponds fed 45 days (Pond A) and 75 days (Pond B) after stocking of tilapia.

Farm	Pond (A = 45 d) (B = 75 d)	Secchi Disk Visibility (cm)	DO (mg l^{-1})	Temperature (ºC)	pH	Alkalinity (mg l^{-1})	Ammonia (mg l^{-1})	Phosphorus (mg l^{-1})
1	A	7–23	4–10	28–31	7.8–8.9	36–95	0.08–0.17	0.18–0.80
	B	8–28	3–9	28–31	7.6–9.9	42–87	0.03–0.33	0.29–0.56
2	A	9–59	9–15	31–35	8.6–9.1	112–156	0.12–0.36	0.06–0.85
	B	14–60	11–16	31–38	8.4–9.6	92–220	0.10–0.30	0.06–0.62
3	A	15–30	11–12	30–31	8.4–10.3	49–81	0.02–0.26	0.08–0.13
	B	15–54	8–13	29–31	8.0–10.3	56–93	0.05–0.32	0.07–0.45
4	A	12–32	9–12	30–32	7.9–9.7	150–162	0.10–0.35	0.27–0.37
	B	10–22	9–11	29–31	8.0–10.4	115–140	0.03–0.37	0.03–0.30
5	A	9–65	6–16	29–32	8.0–9.9	127–175	0.05–0.25	0.14–0.68
	B	11–65	5–14	29–32	8.0–9.5	119–230	0.03–0.35	0.11–0.35
6	A	8–50	14–20	31–32	7.7–9.6	112–210	0.11–0.28	0.18–0.77
	B	12–46	14.6–20	31–34	7.7–9.5	86–207	0.05–0.24	0.09–0.63
7	A	8–40	4–15.4	29–33	8.4–9.7	239–302	0.06–0.22	0.04–0.51
	B	9–28	5–12	29–33	8.4–9.7	271–345	0.09–0.10	0.10–0.57

during a tilapia grow-out cycle without significantly reducing yields. That is a meaningful amount of money (P2,200) in the Philippines, especially considering the increased profit is the result of reduced effort on the part of the farmers. Following effective dissemination of these results (see "Workshop on the timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds," 9ADR6A and "Production of improved extension materials," 9ADR6B), it is expected that the technique of providing commercial feeds to tilapia later in the grow-out period will be broadly adopted, resulting in more cost-effective and profitable farming. It is also possible that the time gained by adding a month of very low-maintenance farming will facilitate other farm activities, such as vegetable gardening or other crop diversification, or educational and training activities.

ACKNOWLEDGMENTS

We wish to acknowledge the support of the farmer-cooperators who willingly made available their pond facilities for this study.

LITERATURE CITED

APHA (American Public Health Association), 1980. Standard Methods for the Examination of Water and Wastewater, Fifteenth Edition. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC, 1,134 pp.

Diana, J.S., C.K. Lin, and Y. Yi, 1996. Timing of supplemental feeding for tilapia production. J. World Aquacult. Soc., 27(4):410–419.

PD/A CRSP, 1992. Handbook of Analytical Methods. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Methods for the Contribution from the Male and Female Genome to Sex Inheritance

Eighth Work Plan, Reproduction Control Research 1C (8RCR1C)
Final Report

Ronald P. Phelps and Richard Lee Warrington
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Abstract

The variability in the factors affecting the sex ratios of Nile tilapia (*Oreochromis niloticus*) was studied using pair spawns from the Egypt, Ghana, and Ivory Coast strains. Sex ratios from 129 progeny groups were determined by microscopic inspection of the gonads. Of the 12,450 progeny sexed, 54.14% were males; this differed significantly from a 1:1 sex ratio ($P < 0.001$). Sex ratios from the 129 progeny groups produced a normal distribution ($P > 0.45$) but did not reflect a binomial distribution ($P < 0.01$) as would be expected from a simple monofactorial sex determination process. Therefore, other factors must account for the variation observed in sex ratios. Single spawn sex ratios ranged widely from 16 to 100% male. Chi-square tests revealed weak correlations between strain and progeny gender, individual parent and progeny gender, and male-female parent combinations and progeny gender. Sixty-five percent (11 of 17) of the repeat spawns by the same pairings produced sex ratios that extended beyond a range of 10%. The continuous range of sex ratios within the normal distribution suggests the presence of several minor sex-modifying factors in *O. niloticus* and raises questions as to whether sex inheritance in tilapia is predictable enough for a YY breeding program to be practical.

Introduction

The nature of sex determination in tilapia is not clear. Clemens and Inslee (1968) and Chen (1969) proposed that in *O. mossambicus*, the female was the homogametic sex (XX) and the male heterogametic (XY). However, in both studies the authors obtained some sex ratios that could not be explained by a simple Mendelian XX:XY sex determination. Jalabert et al. (1971) studied the sex determination of *O. niloticus* and *O. macrochirus* by evaluating the sex ratios of hybrid progeny and concluded that *O. niloticus* had a basic XX:XY sex determination but that the sex ratios from the back cross of the male hybrid to a female *O. niloticus* did not conform to the expected ratios. Both species and their hybrid progeny had an identical karyotype (2N = 44) with no evident sex chromosome. The authors proposed that autosomes may play a role in sex determination.

The widely noted variation and general unpredictability of tilapia sex ratios has made the selection of true-breeding fish difficult. Shelton et al. (1983) reported sex ratios from mass spawnings of *O. niloticus* that ranged from 31 to 83% male. Tuan et al. (1999) reported a range of 15 to 100% male among 95 families of *O. niloticus*. These examples of variation in sex ratio and the uncertainty concerning the exact process behind sexual determination in tilapia present a challenge to the production of all-male populations through a YY breeding program.

YY breeding programs have been based on the premise that sex determination in *O. niloticus* is a simple Mendelian inheritance process where the female *O. niloticus* is homogametic (XX), the male heterogametic (XY), and animals having a Y chromosome develop as males. There are several approaches to a YY breeding program. Scott et al. (1989), working with *O. niloticus*, obtained a YY male in a gynogenesis study in which the parent female was thought to be of an XY genotype. Another approach is through hormone-induced sex inversion followed by selective breeding, as outlined by Shelton et al. (1978). Yongquan et al. (1979) applied this approach with *O. mossambicus*, another species in which the female is thought to be homogametic for sex determination, and successfully produced all-male progeny using YY males. Mair et al. (1993) successfully applied this approach to *O. niloticus* to produce all-male populations. Androgenesis, in which viable X or Y sperm are used to fertilize an irradiated oocyte, has also been used to produce YY male tilapia (Avila and Gonzalez, 1995; Myers et al., 1995; Marengoni and Onoue, 1998).

Numerous authors have proposed theories on the mechanism of tilapia sex determination, as reviewed by Wohlfarth and Wedekind (1991). It appears, however, that none has described the mechanism specifically enough to account for all the variation observed. This study was conducted to further examine the variation in sex ratios and to elucidate parts of the sex-determining process involved while trying to develop a line of fish suitable for use in a YY breeding program.

Where an XX:XY pattern of inheritance is assumed, the male would produce either an X or Y sperm, and an X-containing egg would be fertilized. If no autosomal factors influence sex ratios, the fertilization of the egg with a Y sperm should result in male progeny. This would be particularly evident in a highly inbred population such as one produced by androgenesis.

Methods and Materials

It was the intent of this study to develop a clearer understanding of sex inheritance using androgenically produced males and females. However, it was not possible to produce a highly inbred population through androgenesis of adequate quantity for use in this study as initially proposed. Instead, three other inbred strains of tilapia were used to evaluate the contribution of the male and female genome to sex inheritance. The Ivory Coast strain of *O. niloticus* was introduced to Auburn University (AU) from Brazil in 1974 and was originally introduced into Brazil from the Ivory Coast in 1971. The Egypt strain was introduced from the Ismailia Canal near Cairo to AU in 1982. The Ghana strain was introduced to AU from Israel in 1982 but was originally collected in 1978 from Lake Volta near Accra, Ghana (Khater, 1985). These strains have been maintained at Auburn since their original introduction; no additional fish have been added to the respective populations from the outside.

Fish were allowed to spawn from June to October of 1997 and 1998 in outdoor 2-m^3 net hapas with a mesh size of 1.5 mm. Four net hapas per tank were suspended in 20-m^2 rectangular cement tanks at the Fisheries Research Unit, Alabama Experiment Station, Auburn University, Alabama. Each tank was maintained at an average water depth of 70 cm using water from a rain-fed reservoir with a mean alkalinity of 30 mg l^{-1}. Tanks were not limed or fertilized during the spawning period. Reference water temperature and dissolved oxygen readings were taken four times per week prior to daily feeding using a YSI Model 54ABP dissolved oxygen meter.

All broodfish were tagged for individual identification using a PIT tag (Destron Fearing Corporation). One male was placed in each net hapa. Females were added at a ratio of three to five females per male. Each strain was held and spawned separately. Females were inspected for eggs eight to ten days after stocking. Brooding females were collected with a fine mesh dip-net and transported individually to the Alabama Experiment Station hatchery. Each female was placed into a 95-l aquarium for the duration of the egg incubation. When the eggs hatched and the larvae reached the swim-up phase, the female was returned to another spawning hapa containing a different male. Swim-up fry were collected from the aquarium and placed in a 57-l aquarium, reared for approximately 30 d, and then transferred to outdoor tanks and ponds to grow to a sexable size. Water temperatures in all aquaria ranged from 29 to 35°C. Dissolved oxygen concentration was maintained near saturation through aeration using one to two air stones in each aquarium.

Determination of Sex

When the experimental fish had reached a length of at least 5 to 6 cm, a random sample was taken from each progeny group and immediately preserved in a 10% formalin solution. The sex of each fish was determined by surgical extraction of the gonads. The gonads were placed on a microscope slide and stained using Fast Green. A cover slide was laid over the stained gonads and pressed firmly so as to rupture the gonads. The gonadal squash was examined under a microscope. Each gonad was designated as ovary, testis, or ovotestis.

Data Analysis

Data on sex ratio of progeny were analyzed using SAS. S+ software was used to generate figures. Four Chi-square tests were performed relating chance of maleness of individual progeny members to strain, to individual sires, to individual dams, and to repeat pair spawns. A logistic regression analysis was used to distinguish differences in percent males among strains. A weighted least-squares regression analysis was used to compare average standard deviations between strains.

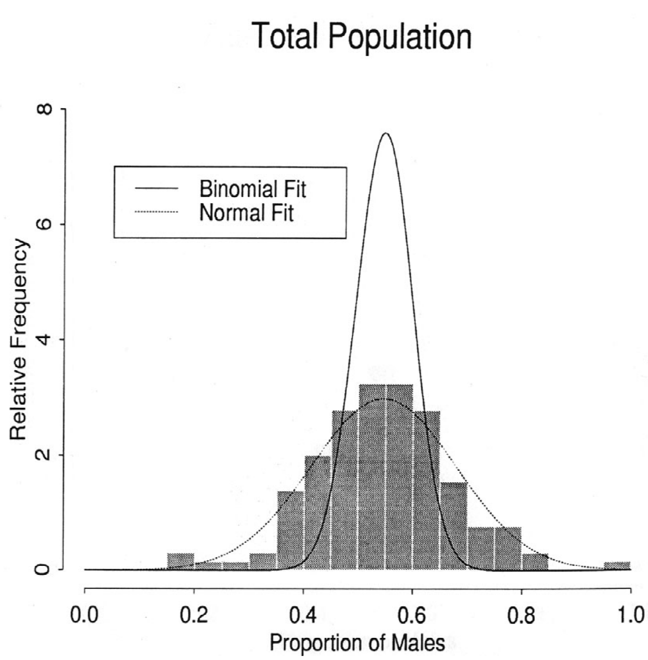

Figure 1. Proportion of males from the matings of the experimental population across strains (progeny groups = 129). Bars represent the relative frequencies of each proportion. The solid curve represents a binomial fit, and the dotted line represents a normal fit.

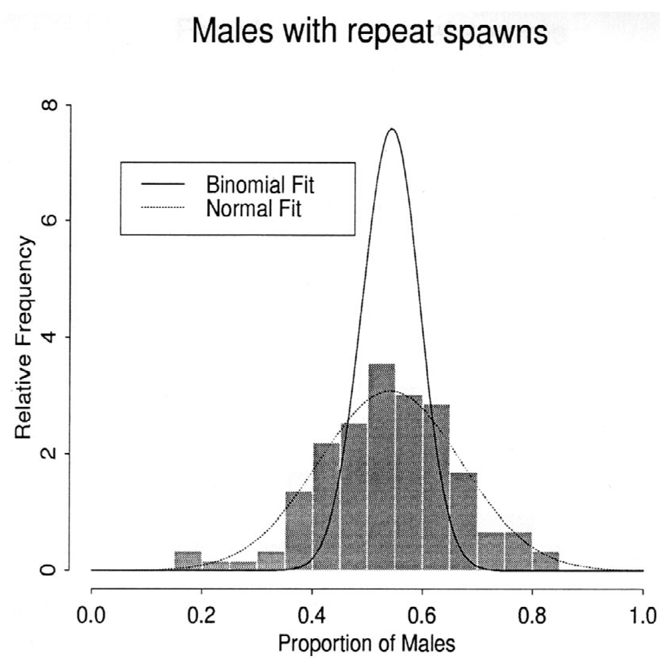

Figure 2. Proportion of males from the progeny groups of all male parents with repeat spawns (progeny groups = 119). Bars represent the relative frequencies of each proportion. The solid curve represents a binomial fit, and the dotted line represents a normal fit.

RESULTS

Sex Ratio of Total Population

Sex ratios were analyzed for 129 progeny groups from pair spawnings of three strains of normal (non-hormone-treated) *O. niloticus* broodstock. Fifty to one hundred randomly selected offspring were sexed from each progeny group, depending upon the number of individuals present at the time of harvest. Of the total number of progeny sexed (12,450), the mean percent males was 54.1%, with a standard deviation of 0.14 and a range of sex ratios from single spawns of 16 to 100% male. The mean percent males was significantly different ($P < 0.001$) from a 1:1 population sex ratio due to the slight excess of males. Sex ratios reflected a normal distribution ($P > 0.45$), but not a binomial distribution ($P < 0.01$), due to the overdispersion of sex ratios. The normal distribution possessed a skewness value of –0.07 and a range of 0.838. The normal curve was fitted using the sample mean and standard deviation. The binomial curve was fitted using the sample mean and a conservative sample size of 90 fish per progeny group due to the slight variation in progeny sample sizes (Figure 1). The summation of spawns (N = 119) from all male parents with repeat spawns yielded an average progeny of 54.1% male, with a standard deviation of 0.16 (Figure 2). The summation of spawns (N = 81) from all female parents with repeat spawns yielded an average progeny of 55.0% male with a standard deviation of 0.16 (Figure 3). The male and female summations also produced a normal distribution ($P > 0.42$) without producing a binomial distribution ($P < 0.01$), likewise a result of the overdispersion of sex ratios. All normal curves were again fitted using the respective sample mean and standard deviation, and binomial curves with the respective mean and a conservative sample size of 90 fish per progeny group.

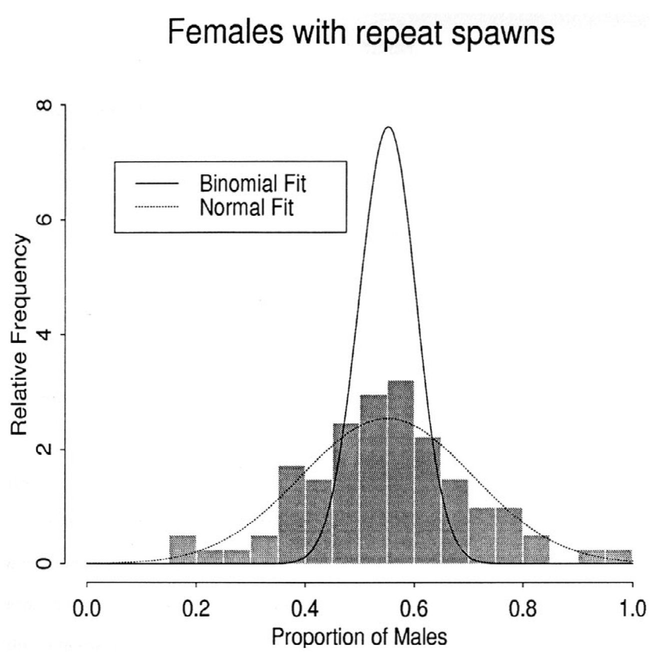

Figure 3. Proportion of males from the progeny groups of all female parents with repeat spawns (progeny groups = 81). Bars represent the relative frequencies of each proportion. The solid curve represents a binomial fit, and the dotted line represents a normal fit.

Single Male with Multiple Partners

Twelve Egypt males had multiple partners (Table 1). The mean percent male among the 3,716 progeny was 52.7%, with an average standard deviation of 0.09 and a range of 26 to 64% among pair spawns. Forty-two percent (5 of 12) of the males produced sex ratios that ranged less than 10% or less than two standard deviations. A statistically significant association ($P = 0.001$) existed between individual male parent and progeny sex. The Pearson correlation coefficient (–0.037), however, demonstrated that this association was very weak.

Seven Ghana males had multiple partners (Table 1). The mean percent male among the 2,559 progeny was 57.2%, with an average standard deviation of 0.11 and a range of 16 to 78% among pair spawns. Twenty-nine percent (2 of 7) of the Ghana males produced sex ratios that ranged less than 10%. An association ($P = 0.001$) also existed here between individual male parent and progeny sex. The correlation coefficient (0.102) again displayed the relative weakness of the association.

Table 1. Average percent male and average standard deviation of progenies from males with multiple partners.

Strain	Number of Spawns	Percent Male	Standard Deviation	Range (%)
EGYPT				
	5	48.9	0.083	39–61
	3	50.0	0.122	36–59
	3	54.2	0.090	48–63
	3	64.9	0.041	62–64
	3	55.7	0.050	51–61
	2	59.8	0.189	45–71
	2	48.5	0.219	33–64
	2	59.7	0.027	57–61
	2	38.5	0.169	26–50
	2	60.9	0.044	58–64
	2	48.3	0.031	46–49
	9	51.1	0.083	38–60
	9	51.1	0.083	38–60
GHANA				
	6	63.1	0.139	37–78
	5	43.2	0.163	16–57
	4	65.9	0.115	54–76
	3	59.9	0.081	51–67
	3	47.3	0.051	43–53
	3	58.4	0.037	56–62
	2	67.5	0.148	57–78
IVORY COAST				
	12	49.8	0.189	16–100
	7	56.4	0.182	31–79
	7	58.6	0.063	53–70
	6	47.5	0.080	39–55
	6	58.8	0.105	46–73
	4	54.9	0.108	44–68
	3	53.4	0.090	43–59
	2	46.2	0.124	37–55
	2	54.5	0.162	43–66
	2	57.3	0.060	53–61
	2	71.0	0.183	58–84
	2	48.5	0.007	48–49

Twelve Ivory Coast males had multiple partners (Table 1). The mean percent male among the 5,332 progeny was 54.1%, with an average standard deviation of 0.12 and a range of 16 to 100% among pair spawns. Eight percent (1 of 12) of the males produced sex ratios that ranged less than 10%. An association between the individual male parent and progeny sex did exist within this strain as well ($P = 0.001$) but again was shown to be weak by the correlation coefficient (–0.018).

Single Female with Multiple Partners

Ten Egypt females had multiple partners (Table 2). The mean percent male among the 2,632 progeny was 52.5%, with an average standard deviation of 0.11 and a range of 26 to 75% among pair spawns. Twenty percent (2 of 10) of the females produced sex ratios that ranged less than 10%. A Chi-square test showed an association ($P = 0.002$) between individual female parent and progeny sex. The association, as demonstrated by the correlation coefficient (0.029), was weak.

Six Ghana females had multiple partners (Table 2). The mean percent male among the 1,264 progeny was 58.5%, with an average standard deviation of 0.09 and a range of 16 to 78% among pair spawns. Thirty-three percent (2 of 6) of the females produced sex ratios that ranged less than 10%. The Chi-square test showed an association ($P = 0.005$) between individual female parent and progeny sex. The corresponding correlation coefficient (–0.017) demonstrated the weakness of the association.

Twelve Ivory Coast females had multiple partners (Table 2). The mean percent male among the 4,015 progeny was 54.5%, with an average standard deviation of 0.14 and a range of 16 to 100% among pair spawns. Eight percent (1 of 12) of the females produced sex ratios that ranged less than 10%. The Chi-square test showed an association ($P = 0.001$) between individual female parent and progeny sex. The weakness of this association was reflected in its small correlation coefficient (–0.054).

Table 2. Average percent male and average standard deviation of progenies from females with multiple partners.

Strain	Number of Spawns	Percent Male	Standard Deviation	Range (%)
EGYPT				
	4	58.6	0.190	38–75
	4	44.7	0.148	26–62
	3	53.3	0.058	47–60
	3	52.3	0.088	42–58
	3	54.2	0.064	49–61
	2	50.0	0.084	44–56
	2	61.0	0.042	58–64
	2	54.5	0.063	50–59
	2	55.5	0.106	48–63
	2	44.3	0.159	33–55
GHANA				
	3	53.9	0.332	16–78
	2	56.9	0.281	37–76
	2	66.5	0.035	64–69
	2	58.5	0.047	54–63
	2	51.5	0.078	45–57
	2	58.4	0.037	56–62
	2	67.2	0.107	59–74
IVORY COAST				
	5	48.5	0.210	16–73
	4	52.8	0.034	49–57
	4	57.3	0.071	50–67
	4	68.5	0.283	39–100
	4	58.0	0.133	39–70
	3	51.3	0.127	43–66
	3	46.0	0.080	38–54
	3	40.0	0.115	31–53
	3	39.3	0.158	21–49
	3	60.5	0.077	55–68
	3	74.7	0.111	62–83
	3	54.7	0.244	37–72

Repeat Spawns of Same Pair

Four pairs of Egypt individuals spawned with one another at least twice (Table 3). The mean percent male among the 855 progeny was 52.7%, with an average standard deviation of 0.11 and a range of 39 to 61%. None (0 of 4) of the Egypt pairs produced sex ratios that ranged less than 10%. The Chi-square test revealed no association between the combination of parents and progeny gender ($P = 0.44$).

Two pairs of Ghana individuals spawned with one another at least twice (Table 3). The mean percent male among the 370 progeny was 69.7%, with an average standard deviation of 0.06 and a range of 49 to 78%. Fifty percent (1 of 2) of the Ghana pairs produced sex ratios that ranged less than 10%. A Chi-square test showed no association between the combination of parents and progeny gender ($P = 0.16$).

Eleven pairs of Ivory Coast individuals spawned with one another at least twice (Table 3). The mean percent male among the 2,451 progeny was 55.5%, with an average standard deviation of 0.10 and a range of 16 to 79%. Forty-five percent

Table 3. Average percent male in progeny per spawn from pairs with repeat spawns.

Strain	Number of Spawns	Percent Male	Range (%)
EGYPT			
	3	50.1	39–61
	2	50.0	44–56
	2	53.5	47–60
	2	52.3	42–58
GHANA			
	2	66.5	64–69
	2	72.5	67–78
IVORY COAST			
	3	54.3	43–66
	3	57.0	50–67
	3	64.0	60–70
	2	54.5	43–66
	2	75.5	51–100
	2	28.7	16–41
	2	33.5	31–36
	2	70.5	62–79
	2	62.5	57–68
	2	55.0	55–55
	2	48.5	48–49

(5 of 11) of the Ivory Coast pairs produced sex ratios that ranged less than 10%. The Chi-square test did reveal an association between the combination of parents and progeny gender ($P = 0.001$), which may have been influenced by the larger sample size. The correlation coefficient (0.022) again proved the association to be extremely weak.

DISCUSSION

There was no clear indication that sex determination in *O. niloticus* is sex-linked. The mean percentage male of 54.10% from all spawns of males that produced repeat spawns closely approximates the population mean. The mean percentage male of 55.04% from all spawns of females that produced repeat spawns was slightly higher than either the repeat-spawning males' or the population mean. Male and female averages possessed an identical standard deviation of 0.16. The data on the population level appear quite similar, with no apparent differences due to the parents' sex.

Individual males with multiple partners and individual females with multiple partners were investigated to determine the influence of individual parents on sex ratios. Although each individual parent was found to have a statistically significant effect on progeny sex ratio ($P < 0.005$), the correlation was weak, and the overall average ratio of males produced by male parents with repeat spawns versus males produced by female parents with repeat spawns was similar within each strain. Wohlfarth and Wedekind (1991) concluded that for *O. niloticus* the sex ratio was determined by the male, and skewed sex ratios could be selected for based on selection of the male parent. Meriweather (1980) suggested that in *O. aureus* the female parent had a greater influence on sex ratio of the progeny than did the male. Mair et al. (1991) found no link between male parent, female parent, and progeny sex ratios from diallele-type crosses of five male and five female *O. niloticus*.

Considerable variation was observed among repeat spawns of the same pair. Some pairs produced sex ratios skewed to male in one spawn and to female in the next spawn. The variation from repeat spawns of the same pair is supported by Tuan et al. (1999), who reported that progenies derived from repeat spawns of the pair were significantly heterogeneous ($P < 0.01$). Several pairs in this study did, however, reproduce with consistent sex ratios. Thirty-five percent (6 of 17) of pairs with repeat spawns produced sex ratios that ranged less than 10%.

Wohlfarth and Wedekind (1991) cited Wedekind (1987), who found sex ratio to be a stable, heritable trait, with repeat spawns of the same pair giving almost identical sex ratios. Specific males and females within the current study also gave consistent skews over multiple partners, indicating that selection for sex ratio may be productive, as stated by Shelton et al. (1983). The limited number of repeat spawns by the same pair in the current study suggests that a portion of the brood population may breed relatively true for sex ratio. However, this characteristic was not observed in all pairs; it is possible that numerous spawns from the same pair would yield a normal distribution of sex ratios.

Sex ratios of *O. niloticus* do not always conform to a simple Mendelian pattern of inheritance. Of the 129 spawns in the current study, only 57% conformed to a 1:1 sex ratio. The frequency distribution of sex ratios within this study was normal; therefore, no multiple modes existed that might suggest sex ratio inheritance is based on major sex-determining genes. Hammerman and Avtalion's (1979) prediction of eight distinct sex ratios (0:1, 1:3, 3:5, 1:1, 9:7, 5:3, 3:1, 1:0) might also be expected to display multiple modes. The frequency distribution did not reflect a binomial distribution as would be predicted by a simple XX/XY determination process; therefore, other factors such as autosomal genes must be present to account for the overdispersion of sex ratios.

Shelton et al. (1983) found a range of 31% to 83% males, with 21% differing from a 1:1 sex ratio, among 71 progeny groups of the Ivory Coast strain of *O. niloticus*. Many generations later, 42% of 57 Ivory Coast pairs produced progeny that did not conform to a 1:1 sex ratio. Inbreeding results in a more homozygous gene pool (Tave, 1986), and a highly inbred line such as the Ivory Coast strain should show reduced genetic variability. Tave and Smitherman (1980) and Teichert-Coddington and Smitherman (1988) concluded that there was little genetic variability in this Ivory Coast strain involving selection for faster growth. In the current study there is no evidence of a reduction in sex ratio variability as a result of inbreeding.

The results suggest that it will not be possible to have a successful YY breeding program without first breeding to minimize variation in sex determination among normal fish. Variation in sex ratio occurs at all levels of selection, in general, yielding a normal distribution of sex ratios. The variation occurs at the level of individual male-female mating because repeat spawns of the same pair are not always consistent in the sex ratios produced. Sex determination appears to be a product of individual parent contribution, but how this effect occurs and to what extent each parent contributes could not be determined here. No current theory on sex inheritance is specific enough to explain the variation observed in this study.

ANTICIPATED BENEFITS

Monosex populations are an essential part of many tilapia production systems. These can be obtained by several methods, with direct inversion of sex through hormone administration the most common. Monosex populations of males can also be produced by genetic manipulation to obtain male brood fish of a YY genotype. This assumes that sex determination is based solely on the female being homogametic (XX) and the male being heterogametic male (XY) with no additional sex-modifying genes.

In this study it was evident that there are additional sex modifiers complicating a successful YY breeding program. There was no evidence that these modifiers were sex-linked or associated with a particular strain of fish. There was no evidence to justify a selection program for simple sex determination based on sex or strain of fish. However, the data suggest that it might be possible to build a line of fish acceptable for use in a YY breeding program based on single pair selections. Such an intensive selection will result in a highly inbred line of fish which may or may not have similar growth performance to other male tilapia.

LITERATURE CITED

Avila, M.C. and M.A.C. Gonzalez, 1995. Ensayo de androgenesis diploide en tilapia roja (*Oreochromis* sp.), Trewavas (1981), mediante manipulación cromosomal. Boletin Cientifico INPA, 3:66–78.

Chen, F.Y., 1969. Preliminary studies on the sex determining mechanism of *Tilapia mossambica* Peters and *T. hornorum* Trewavas. Verh. Int. Ver. Limnol., 17:719–724.

Clemens, H.P. and T. Inslee, 1968. The production of unisexual brood of *Tilapia mossambica* sex reversed with methyltestosterone. Trans. Amer. Fish. Soc., 97:18–21

Hammerman, I.S. and R.R. Avtalion, 1979. Sex determination in *Sarotherodon* (Tilapia). Part 2: The sex ratio as a tool for the determination of genotype—A model of autosomal and gonosomal influence. Theor. Appl. Genet., 55:177–187.

Jalabert, B., P. Kammacher, and P. Lessent, 1971. Déterminisme du sexe chez les hybrides entre *Tilapia macrochir* et *Tilapia nilotica*. Etude de la sex-ratio dans les recroisements des hybrides de première génération par les espèces parentes. Ann. Biol. Anim. Biochim. Biophys., 11(1):155–165.

Khater, A.A., 1985. Identification and comparison of three *Tilapia nilotica* strains for selected aquacultural traits. Ph.D. dissertation, Auburn University, Alabama, 67 pp.

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

Mair, G.C., J.B. Capili, J.A. Beardmore, and D.O.F. Skibinski, 1993. The YY-male technology for the production of monosex male tilapia, *Oreochromis niloticus* (L.). Proceedings of the AADCP International Workshop on Genetics in Aquaculture and Fisheries Management. University of Stirling, Scotland, pp. 93–95.

Marengoni, N.G. and Y. Onoue, 1998. Ultraviolet-induced androgenesis in Nile tilapia *Oreochromis niloticus* (L.), and hybrid Nile × blue tilapia, *O. aureus* (Steindachner). Aquacult. Res., 29:359–366.

Meriweather, F.H., 1980. Progeny sex ratios from pair spawning of *Tilapia aurea* (Steindachner). M.S. Thesis, Auburn University, Alabama, 61 pp.

Myers, J.M., D.J. Penman, Y. Basavaraju, S.F. Powell, P. Baoprasertkul, K.J. Rana, N. Bromage, and B.J. McAndrew, 1995. Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia (*Oreochromis niloticus* L.). Theor. Appl. Genet., 90(2):205–210.

Scott, A.G., D.J. Penman, J.A. Beardmore, and D. Skibinski, 1989. The "YY" supermale in *Oreochromis niloticus* (L.) and its potential in aquaculture. Aquaculture, 178(34):237–251.

Shelton, W.L., K.D. Hopkins, and G.L. Jensen, 1978. Use of hormones to produce monosex tilapia. In: R.O. Smitherman, W.L. Shelton, and J.L. Grover (Editors), Culture of Exotic Fishes Symposium Proceedings. Fish Culture Section, American Fisheries Society, Auburn, Alabama, pp. 10–33.

Shelton, W.L., F.H. Meriweather, K.J. Semmens, and E. Calhoun, 1983. Progeny sex ratios from intraspecific pair spawnings of *Tilapia aurea* and *Tilapia niloticus*. In: L. Fishelson and Z. Yaron (Compilers), International Symposium on Tilapia in Aquaculture, Nazareth, Israel. Tel Aviv University Press, Tel Aviv, pp. 270–280.

Tave, D., 1986. Genetics for Fish Hatchery Managers. AVI Publishing Company, Inc., Westport, Connecticut, 299 pp.

Tave, D. and R.O. Smitherman, 1980. Predicted responses to selection for early growth in *Tilapia nilotica*. Trans. Am. Fish. Soc., 109:439–445.

Teichert-Coddington, D.R. and R.O. Smitherman, 1988. Lack of response by *Tilapia nilotica* to mass selection for rapid early growth. Trans. Am. Fish. Soc., 117:297–300.

Tuan, P.A., G.C. Mair, D.C. Little, and J.A. Beardmore, 1999. Sex determination and the feasibility of genetically male tilapia production in the Thai-Chitralada strain of *Oreochromis niloticus*. Aquaculture, 173:257–269.

Wedekind, H., 1987. Untersuchungen zur Vererbung des Geschlechtes bei Afrikanischen Buntbarschen (*Oreochromis niloticus*). Diplomarbeit, Gottingen University, Gottingen, 110 pp.

Wohlfarth, G.W. and H. Wedekind, 1991. The heredity of sex determination in tilapias. Aquaculture, 92:143–156.

Yongquan, Y., Z. Zhonging, L. Kehong, W. Yusheng, X. Zhen, H. Erchun, and G. Zhihui, 1979. A preliminary study on physiogenetic control of sexuality in *Tilapia mossambica*. Acta Genet. Sin., 6(3):17–22.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Masculinization of Tilapia by Immersion in Trenbolone Acetate: Growth Performance of Trenbolone Acetate–Immersed Tilapia

Ninth Work Plan, Reproduction Control Research 5B (9RCR5B)
Final Report

Wilfrido M. Contreras-Sánchez and Martin S. Fitzpatrick
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon USA

Carl B. Schreck
Oregon Cooperative Fishery Research Unit
Biological Resources Division—US Geological Survey
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon USA

Abstract

Preliminary studies in our laboratory showed that the synthetic androgen trenbolone acetate (TA) is a good candidate for masculinizing Nile tilapia (*Oreochromis niloticus*) fry using short immersions. In this study we investigated the effects of TA treatment on the growth performance of Nile tilapia. We tested the potential anabolic effects of two treatments by growing treated and control fish for 81 and 114 days. Our results suggest that masculinizing treatments involving short-term immersions in TA and four-week feeding with 17α-methyltestosterone (MT) do not result in significant increases in fish growth. Despite significant masculinization (65 to 70% with TA and 100% with MT) in both treatments, we found no differences in final weight between treatments.

Introduction

Masculinization of tilapia continues to be an important tool for aquaculturists to prevent unwanted reproduction (which shunts energy away from growth towards gamete production) and to produce the sex with the larger growth potential (Green et al., 1997). Previous work in our laboratory has shown that short-term immersion in androgenic steroids can result in masculinization of Nile tilapia (*Oreochromis niloticus*) (Gale et al., 1995, 1999; Contreras-Sánchez et al., 1997). These studies showed that immersion in an androgen has the potential to be an alternative to dietary treatment with steroids for the masculinization of tilapia. A variety of androgens—especially synthetic androgens—are effective masculinizing agents (Hunter and Donaldson, 1983).

Tilapia have been effectively masculinized when fed trenbolone acetate (TA) (Galvez et al., 1996). TA has been widely used in the cattle industry for growth enhancement and is considered a potent androgenic and masculinizing agent (Galvez et al., 1996). We have previously shown that short-term immersion of tilapia fry in TA can result in significant masculinization. Such a treatment offers an alternative to the typical four-week feeding of tilapia with 17α-methyltestosterone (MT). However, in order to be a viable alternative, immersions in TA must be tested for its effects on fish performance. We decided to examine the potential androgenic effects of TA on Nile tilapia by analyzing fry and juvenile growth. To investigate these potential effects, we carried out an experiment to compare growth performance of fish under the following regimes: TA-immersed, EtOH-immersed, MT-treated food, and EtOH-treated food.

Methods and Materials

Breeding families of Nile tilapia were placed in 200-l aquaria (one male to three females). The temperature was maintained at 28 ± 1°C. Time of spawning was monitored every two hours. Spawning occurred between 1600 h and 1900 h. Once breeding occurred, the other fish were removed and the brooding female was left to incubate the progeny. At ten days post-fertilization (dpf; 280 CTU), fry were removed from the tank and randomly assigned to experimental groups. (Development of the fry was expressed in CTUs—mean water temperature in °C × the number of days since fertilization.) The fry used in the experiment came from an individual female. Each replicate was housed in a 3.8-l glass jar with dechlorinated tap water. The water in all treatments was maintained at 28 ± 1°C under constant aeration.

Experimental Design

Immersion treatments consisted of two immersions, one at 11 dpf (308 CTU) and one at 13 dpf (354 CTU). The TA treatment was immersed in water containing 500 µg l^{-1} (EtOH used as vehicle), and the control group was immersed in water containing vehicle only. Fry were collected after each immersion, jars were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. Feeding treatments consisted of MT-fed fish (60 mg kg^{-1} food) and control-fed fish

(EtOH-treated food). MT food was made by spraying crushed, flaked food with MT dissolved in EtOH; control food was made by spraying crushed, flaked food with EtOH. Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of ethanol (1 mg ml^{-1}) at 4 ± 1°C. This experiment was repeated with a different brood, and all treatments were triplicated each time.

To keep conditions similar for all treatments, fry to be used in the feeding treatments were counted and kept in jars until day 15 post-fertilization. On this day all fry from both immersion and feeding experiments were transferred to 50-l aquaria. Fry were fed the treatment diets for four weeks (from 15 to 43 dpf), commencing the day of the transfer to aquaria. Immersed fry received control food (no ethanol, no MT). Water temperature in the jars and aquaria was maintained at 28 ± 1°C. Temperature and pH were monitored daily, while ammonia, nitrites, and dissolved oxygen were checked weekly. Feeding rate was at 20% per calculated body weight for the first 23 days of treatment and then 10% per calculated body weight until the end of hormone treatment (day 28) (Popma and Green, 1990). Appropriate water quality was maintained using an activated charcoal filter (Whisper™ Filter I) and a 50% water exchange twice per week. At the conclusion of the 28 days of dietary treatment (on 40 dpf), fry from all treatments were transferred to the Oregon State University Warm Water Research Laboratory, Corvallis, Oregon, and reared in 75-l fiberglass tanks in a recirculating system. Fish from each replicate were placed into two grow-out tanks to allow faster growth. Water temperature in the grow-out system was maintained at 28 ± 1°C, and water quality parameters were also monitored.

Growth Measurements and Sex Identification

Subsamples of fish (15 to 20) were measured to the closest 0.1 g at 15 and 51 dpf (experiment 1), and all fish were measured at 81 dpf. Fish from experiment 2 were measured similarly at 15, 80, and 114 dpf. Sex ratios were determined by examination of gonads using squash (10 and 40X) preparations after Wright staining (Humason, 1972) at days 81 (experiment 1) and 114 (experiment 2) post-fertilization.

Statistical Analysis

Sex Identification

Data were pooled from replicate tanks because there was no evidence of tank effects within treatments (Chi-square test). Pairwise comparisons for sex ratio and mortality data were analyzed using Fisher's exact test with exact p-values (a more conservative test than the Chi-square test for small sample sizes) estimated in GraphPad Prism™.

Growth

The mean final weights of sampled fish were analyzed for differences between groups using one-way ANOVA, with density as a possible confounding variable. For all analyses,

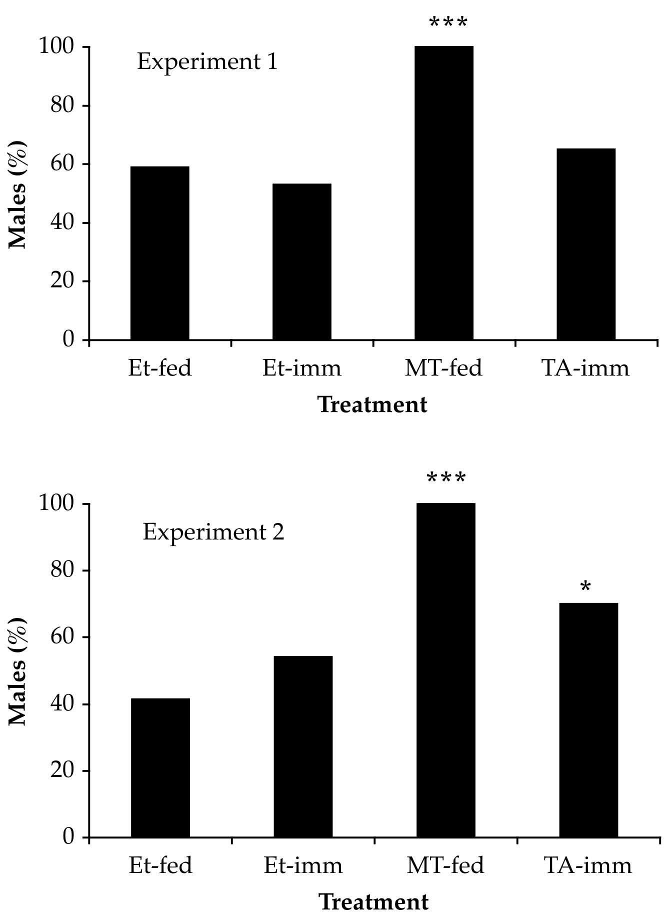

Figure 1. Masculinizing effects of MT-impregnated food (MT-fed) and TA immersion (TA-imm) on Nile tilapia fry. Control treatments were EtOH-impregnated food (ET-fed) and EtOH-immersion with regular food (ET-imm). Each treatment was triplicated. Significant differences between treatments and their respective control are denoted by asterisks (* $P < 0.05$; *** $P < 0.001$).

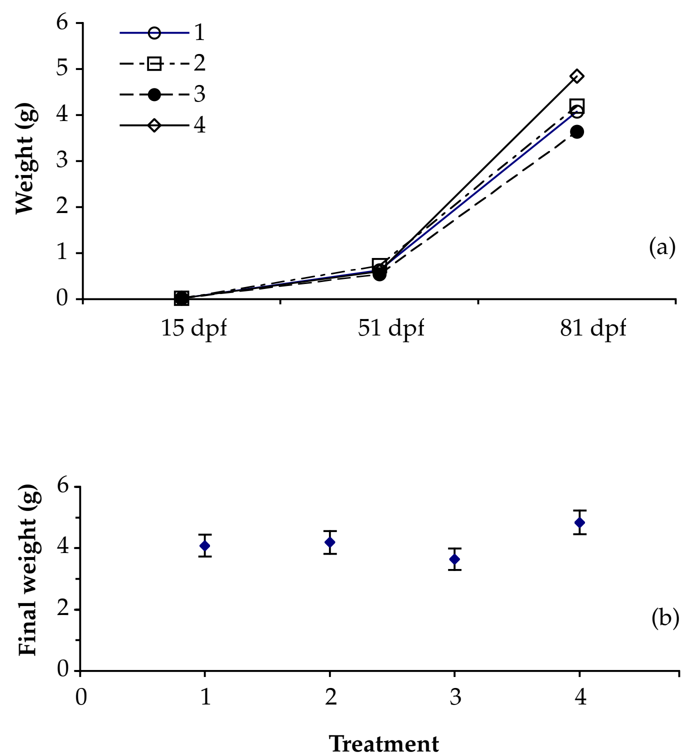

Figure 2. Growth in weight (a) and mean final weight (b) from experiment 1. Treatments were EtOH-impregnated food (1); EtOH-immersed with regular food (2); MT-impregnated food (3); and TA-immersed with regular food (4). Each experiment had three replicates.

differences were considered statistically significant when the p-value (P) was less than 0.05.

Results

In both experiments, TA immersions resulted in significant masculinization; however, the efficacy of treatment was significantly lower than that of the MT treatment, which resulted in 100% males, compared with 59% males in the control-fed group in experiment 1 and 42% males in the control-fed group in experiment 2 (Figure 1). In experiment 1, TA treatment resulted in 65% males, compared with 53% males in the immersed-controls; in experiment 2 the TA treatment had 70% males, compared with 54.1% males in the immersed-controls.

We found no significant differences in weight at either 51 or 81 dpf for experiment 1 (Figure 2a). Due to differential mortalities in the grow-out tanks, some replicates showed larger growth in individuals than other replicates (e.g., TA-immersion); however, these differences were accounted for once density was used in the statistical analysis as a possible confounding variable ($P = 0.17$). Final mean weights (± SE) at 81 dpf for each treatment were: EtOH-fed = 4.1 g ± 0.3; EtOH-immersed = 4.2 g ± 0.4; MT-fed = 3.6 g ± 0.4; and TA-immersed = 4.8 g ± 0.4 (Figure 2b). Similar results were observed in experiment 2, where mean weight values were not significant at any sampling time ($P = 0.29$; Figure 3a). Final mean weights (± SE) at 114 dpf for each treatment were: EtOH-fed = 11.3 g ± 0.9; EtOH-immersed = 13.2 g ± 1.1; MT-fed = 12.61 g ± 1.0; and TA-immersed = 10.9 g ± 0.9 (Figure 3b).

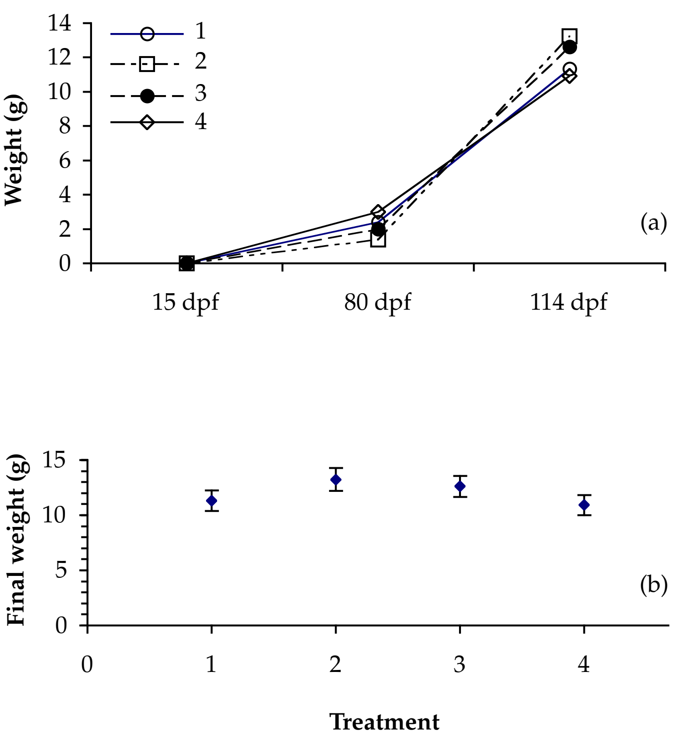

Figure 3. Growth in weight (a) and mean final weight (b) from experiment 2. Treatments were EtOH-impregnated food (1); EtOH-immersed with regular food (2); MT-impregnated food (3); and TA-immersed with regular food (4). Each experiment had three replicates.

Discussion

Several authors have argued that a masculinizing treatment using steroids causes a significant increase in fish growth. However, few papers have tried to determine if these synthetic steroids are in fact potent growth enhancers or if the masculinizing effects of the hormone cause the observed differences in growth, resulting in a male-biased population of fish. Our experiments show that immersion of Nile tilapia on days 11 and 13 post-fertilization for three hours each caused significant masculinization without affecting growth. Similarly, feeding MT-impregnated food for 28 days—the time required for efficient masculinization—did not enhance growth performance of the fish despite the production of 100% males in this treatment. Green and Teichert-Coddington (1994) showed similar results in experiments conducted in nursery ponds, finding no differences between MT-fed and control-fed fish sampled after 94 days of growth. Furthermore, Green and Teichert-Coddington (1994) found similar results after growing the fish for 165 days in fertilized earthen grow-out ponds.

Kuwaye et al. (1993) and Ron et al. (1995) have shown that oral administration of MT can significantly increase growth of the euryhaline tilapia (*Oreochromis mossambicus)* if administered for very long periods of time. These authors used hormone-treated food for 60 days (one month over the masculinizing period), 180 days, and 210 days (Kuwaye et al., 1993), and 168 days (Ron et al., 1995). It is important to mention that the fish used in these studies were grown for periods of time longer than the ones used in our experiments; however, if comparable sampling days are analyzed (90 to 120 days), then the fish used in the cited papers do not show significant differences in mean weight (not tested by the authors, estimated from graphs).

Our results may indicate that some of the differences between treatments can occur after 120 days of growth, perhaps when the females present in the control groups are actively producing eggs, spawning, and incubating fry, thereby shunting energy into reproduction rather than growth.

Anticipated Benefits

The use of anabolic steroids to produce 100% male populations of Nile tilapia showed no significant effects on mean final weight after 110 days of growth, despite the efficacy of both treatments on masculinizing fry. We have also shown in other experiments that the anabolic steroid 17α-methyltestosterone (MT) remains in the sediments of model ponds for up to three months after its use for masculinizing tilapia fry, representing a potential risk to hatchery workers and non-target species. Together, these results will be useful to those involved in aquaculture (e.g., farmers, researchers, and government workers) because they will discourage the unnecessary use of steroids for the purposes of enhancing growth.

Literature Cited

Contreras-Sánchez, W.M., M.S. Fitzpatrick, R.H. Milston, and C.B. Schreck, 1997. Masculinization of Nile tilapia (*Oreochromis niloticus*) by single immersion in 17α-methyltestosterone and trenbolone acetate. In: K. Fitzsimmons (Editor), Proceedings of the Fourth International Symposium on Tilapia Aquaculture. Orlando, Florida, pp. 783–790.

Gale, W.L., M.S. Fitzpatrick, and C.B. Schreck, 1995. Immersion of Nile tilapia (*Oreochromis niloticus*) in 17α-methyltestosterone and mestanolone for the production of all-male populations. In: F.W. Goetz and P. Thomas (Editors), Proceedings of the Fifth International Symposium on Reproductive Physiology of Fish. Fish Symposium 95, Austin, Texas, p. 117.

Gale, W.L., M.S. Fitzpatrick, M. Lucero, W.M. Contreras-Sánchez, and C.B. Schreck, 1999. Production of all-male populations of Nile tilapia (*Oreochromis niloticus*) by immersion in androgens. Aquaculture, 178:349–357.

Galvez, J.I., J.R. Morrison, and R.P. Phelps, 1996. Efficacy of trenbolone acetate in sex inversion of the blue tilapia *Oreochromis aureus*. J. World Aquacult. Soc., 27:483–486.

Green, B.W. and D.R. Teichert-Coddington, 1994. Growth of control and androgen-treated Nile tilapia, *Oreochromis niloticus* (L.), during treatment nursery and grow-out phases in tropical fish ponds. Aquacult. Fish. Manage., 25:613–621.

Green, B.W., K.L. Veverica, and M.S. Fitzpatrick, 1997. Fry and fingerling production. In: H.S. Egna and C.E. Boyd (Editors), Dynamics of Pond Aquaculture. CRC Press, New York, pp. 215–243.

Humason, G.L., 1972. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco, 641 pp.

Hunter, G.A. and E.M. Donaldson, 1983. Hormonal sex control and its application to fish culture. In: W.S. Hoar, D.J. Randall, and E.M. Donaldson (Editors), Fish Physiology. Volume 9B. Academic Press, New York, pp. 223–303.

Kuwaye, T.T., D.K. Okimoto, S.K. Shimoda, R.D. Howerton, H. Lin, P.K.T. Pang, and E.G. Grau, 1993. Effects of 17α-methyltestosterone on the growth of the euryhaline tilapia, *Oreochromis mossambicus*, in fresh water and in sea water. Aquaculture, 113:137–152.

Popma, T.J. and B.W. Green, 1990. Sex reversal of tilapia in earthen ponds. International Center for Aquaculture and Aquatic Environments Research and Development Series No. 35. Auburn University, Alabama, 15 pp.

Ron, B., S.K. Shimoda, G.K. Iwama, and E.G. Grau, 1995. Relationships among ration, salinity, 17α-methyltestosterone and growth in the euryhaline tilapia, *Oreochromis mossambicus*. Aquaculture, 135:185–193.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Masculinization of Tilapia by Immersion in Trenbolone Acetate: Detection of Trenbolone Acetate after Treatment

Ninth Work Plan, Reproduction Control Research 5C (9RCR5C)
Progress Report

Wilfrido M. Contreras-Sánchez and Martin S. Fitzpatrick
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Carl B. Schreck
Oregon Cooperative Fishery Research Unit
Biological Resources Division—U.S. Geological Survey
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Abstract

In previous experiments we have found that two 3-hour immersions in trenbolone acetate (TA) can successfully masculinize Nile tilapia fry. In this study we are investigating how the concentration of TA in the immersion water changes before and after treatment to determine the amount of hormone uptake and estimate the potential for reuse of the treatment water. Nile tilapia fry were subjected to two 3-hour immersions at 11 and 13 days post-fertilization (dpf) in water containing 500 µl of TA. Surprisingly, we have found that the concentration of TA before and after treatment is highly variable and below the expected levels. We are currently assessing whether TA comes out of solution and forms precipitates or binds to the jar glass.

Introduction

One of the major criticisms to masculinization by immersion in TA hormone solutions is that the concentration of hormone used is higher than the amount of 17α-methyltestosterone (MT) used in feeding trials. Although the TA immersion protocol presents advantages over MT feeding treatment for hormone control and disposal, as well as fewer risks of environmental contamination, it might not be welcomed by producers because of the potential increased costs in comparison to feeding treatment. In previous studies, we have shown that single and double immersions in the non-aromatizable synthetic androgens 17α-methyldihydrotestosterone (MDHT) and trenbolone acetate (TA) during early development are effective masculinizing treatments (Contreras-Sánchez et al., 1997, 1999, 2000; Gale, 1999). However, little is known about hormone uptake by the fry and the potential for reuse of treatment water in subsequent masculinization treatments.

Trenbolone acetate has been widely used in the cattle industry for growth enhancement and is considered a potent androgenic and masculinizing agent (Galvez et al., 1996). These factors make TA a very good candidate for fish masculinization and facilitate its acceptance among farmers and administrators involved in the regulatory process. In order to determine if the water used for the immersions contains TA in concentrations sufficient for reuse, we examined the fate of TA after three-hour immersions of Nile tilapia fry by analyzing water samples before and after immersions.

Methods and Materials

Breeding families of Nile tilapia (*Oreochromis niloticus*) were placed in 200-l aquaria (one male to three females). The temperature was maintained at 28 ± 1°C. Time of spawning was monitored every two hours. Spawning occurred between 1600 and 1900 h. Once breeding occurred, the other fish were removed and the brooding female was left to incubate the progeny. At 10 days post-fertilization (dpf; 280 CTU), fry were removed from the tank and randomly assigned to experimental groups. Development of the fry was expressed in CTUs (mean water temperature in °C × the number of days since fertilization). The fry used in the experiment came from an individual female. Each replicate was housed in a 3.8-l glass jar with dechlorinated tap water. The water in all treatments was maintained at 28 ± 1°C under constant aeration.

Experimental Design

Nile tilapia fry were immersed twice for three hours in either steroid (TA) or ethanol vehicle (EtOH), which were mixed before the addition of fry; one immersion took place at 11 dpf (308 CTU) and the other at 13 dpf (354 CTU). An additional treatment consisted of adding hormone solution to the water, but no fish were placed in it. Fry were collected after each immersion, jars were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of ethanol (1 mg ml^{-1}) at 4 ± 1°C.

Seven days after the final immersion, fry were transferred to Oregon State University's Warm Water Research Laboratory, Corvallis, Oregon, and reared in 75-l fiberglass tanks in a recirculating system. In all systems temperature and pH were monitored daily; ammonia, nitrites, and dissolved oxygen were checked weekly. Water temperature in the grow-out system was maintained at 28 ± 1°C. At 70 to 80 dpf, sex ratios were determined by microscopic examination (10 and 40X) of gonads using squash preparations in Wright's stain (Humason, 1972). The weights of sampled fish were recorded at this time.

TA Detection

Water samples were collected two times on day 0 (before and after treatment) and once on days 1, 2, 4, 7, and 14. From each sample 2.0 ml were extracted in 8 ml of diethyl ether. The organic phase of each sample was collected in a new tube after the aqueous phase was snap-frozen in liquid nitrogen. The extraction procedure was repeated and the ether extracts were pooled for each sample and dried down in a SpeedVac. Each dried extract was reconstituted in 1 ml of methanol. Aliquots of the reconstituted extracts were removed to 150 µl glass inserts for determination of TA concentration by High Performance Liquid Chromatography (HPLC). The HPLC methods followed the procedure outlined in Huang et al. (1983) and modified by Feist et al. (1990). The HPLC analysis was performed using a Waters System consisting of a 600 controller, 717 autosampler, 996 photodiode array detector, a Dell Dimension V400c computer, Millenium PDA software, and a reverse phase C18 column (flow rate 0.4 ml min^{-1}). We used an isocratic mobile phase of water: methanol:acetonitrile:isopropanol (62:28:5:5) followed by a linear gradient (3.3% min^{-1}) of water:methanol:butanol (35:45:20) for 30 minutes monitored at a wide variety of wavelengths but specifically analyzed at 254, 280, and 340 nm. This system allows for the separation of 19 steroid standards with detection limits of 3 ng for each steroid. Each sample was analyzed once.

Statistical Analysis

Sex ratio and mortality data were analyzed using Fisher's exact test with exact p-values (a more conservative test than the Chi-square test for small sample sizes) estimated in GraphPad Prism™. Intersex fish were counted as females for the purposes of analysis in order to be conservative. Concentrations of TA in water at the various sample times were not compared statistically because of the limited sample size (n = 3 per date) and because the goal of the study was descriptive (i.e., to detect presence/absence of steroid).

RESULTS

TA concentrations were highly variable at all times. At time 0, immediately after mixing the steroid stock solution, the expected concentration of hormone was 500 µg l^{-1}. Surprisingly, at this time (before addition of fry) levels of TA in the water were lower than the expected value (mean = 178.4 µg l^{-1}; SD = 134.1). Initial values of hormone concentration range from 27.2 to 386.7 µg l^{-1} (coefficient of variation (CV) = 75.2). Concentration values of TA after the fish were removed from the jars also varied, showing no consistent pattern (mean = 162.4 µg l^{-1}; SD = 134.3; CV = 82.7). In some cases TA values were higher after removing fish than before their introduction into the jars (Figures 1a and 1b). In jars containing TA but no fish, two of the three replicates had similar levels of TA through the first 24 hours; however, one replicate had very low values throughout most of the experiment, showing an increase in hormone concentration towards the last two sampling times (Figure 1c). Most of the jars with or without fish showed a depletion of TA after seven days, reaching non-detectable levels at day 14.

No significant masculinization was observed by TA immersions on 11 and 13 dpf (54% males compared with 49% males in the EtOH-immersed controls). We also observed substantial mortality in both TA and EtOH treatments (38 and 63%, respectively).

DISCUSSION

Our findings indicate that the target dose for TA immersion is rarely achieved, perhaps because TA precipitates out of

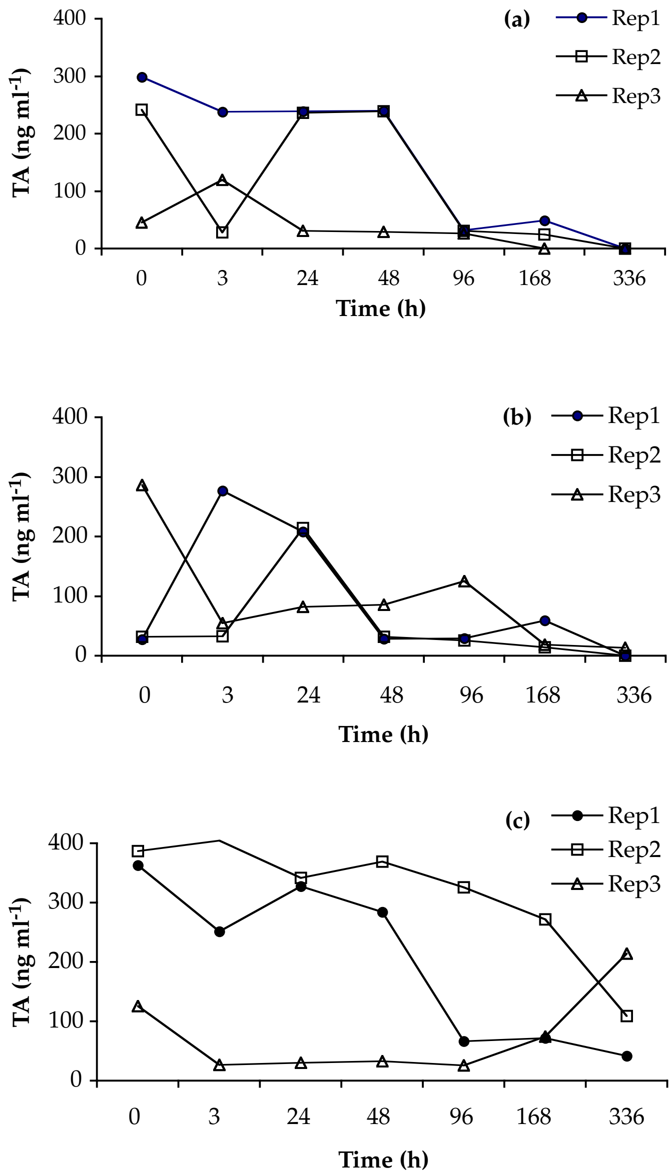

Figure 1. Trenbolone acetate (TA) levels in water through time in experimental jars containing either 33 fish l^{-1} that were immersed in TA for the first time on day 13 post-fertilization (a), or 33 fish l^{-1} immersed for the second time on day 13 post-fertilization (b), or jars containing hormone but no fish (c). Values in control jars with EtOH-vehicle were non-detectable.

solution after mixing with water. The surprisingly low levels of TA before the immersion of tilapia fry has forced us to validate these results by conducting further experiments. We have repeated the experiment using 500 µl l^{-1} of TA delivered using either ethanol or dimethyl sulphoxide (DMSO) as vehicle. Samples were obtained at 0 h (before fry immersion) and at 3 h (after fry immersion). We are currently processing these samples. In another experiment, we are comparing levels of TA and testosterone (T) as a way to determine if TA, which has lower solubility in water than T, precipitates out of solution at a higher rate than T.

The concentrations of TA found in the treatment water before the fish immersion may explain the lack of masculinization for this particular experiment. We suspect that a combination of factors influenced not only survival but treatment efficacy as well. These factors may include recent high levels of copper in our dechlorinated water, as well as the solution used to dechlorinate and eliminate copper (NovAqua®). We are presently analyzing these potential confounding factors by using well water in our treatments.

ANTICIPATED BENEFITS

We have found that masculinizing Nile tilapia fry by immersion can be a good alternative to feeding the fry with hormone-impregnated food, posing fewer risks to hatchery workers and the environment. However, this technique requires refinement and more consistent results. The reusage of treatment water for consecutive sex inversion can be a significant improvement to this technique.

LITERATURE CITED

Contreras-Sánchez W.M., M.S. Fitzpatrick, R.H. Milston, and C.B. Schreck, 1997. Masculinization of Nile tilapia (*Oreochromis niloticus*) by single immersion in 17α-methyltestosterone and trenbolone acetate. In: K. Fitzsimmons (Editor), Proceedings of the Fourth International Symposium on Tilapia Aquaculture. Orlando, Florida, pp. 783–790.

Contreras-Sánchez, W.M., M.S. Fitzpatrick, R.H. Milston, and C.B. Schreck, 1999. Masculinization of Nile tilapia (*Oreochromis niloticus*) by immersion in synthetic androgens: Timing and efficacy. In: B.W. Green, H.C. Clifford, M. McNamara, and G. Montaño-Moctezuma (Editors), Proceedings of the Fifth Central American Symposium on Aquaculture. San Pedro Sula, Honduras, pp. 246–248.

Contreras-Sánchez, W.M., M.S. Fitzpatrick, R.H. Milston, and C.B. Schreck, 2000. Masculinization of Nile tilapia with steroids: Alternative treatments and environmental effects. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, and S.O. Stefansson (Editors), Proceedings of the Sixth International Symposium on Reproductive Physiology of Fish. John Grieg, A.S., Bergen, Norway, pp. 250–252.

Feist, G., C.B. Schreck, M.S. Fitzpatrick, and J.M. Redding, 1990. Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. Gen. Comp. Endocrinol., 80:299–313.

Gale, W.L., M.S. Fitzpatrick, M. Lucero, W.M. Contreras-Sánchez, and C.B. Schreck, 1999. Production of all-male populations of Nile tilapia (*Oreochromis niloticus*) by immersion in androgens. Aquaculture, 178:349–357.

Galvez, J.I., J.R. Morrison, and R.P. Phelps, 1996. Efficacy of trenbolone acetate in sex inversion of the blue tilapia *Oreochromis aureus*. J. World Aquacult. Soc., 27:483–486.

Huang, F.L., F.C. Ke, J.J. Hwang, and T.B. Lo, 1983. High-pressure liquid chromatography separation of a mixture of corticosteroids, androgens, and progestins. Arch. Biochem. Biophys., 225:512–517.

Humason, G.L., 1972. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco, 641 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Monosex Tilapia Production through Androgenesis: Selection of Individuals for Sex Inheritance Characteristics for Use in Monosex Production

Ninth Work Plan, Reproduction Control Research 6A (9RCR6A)
Abstract

Ronald P. Phelps
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Abstract

There is evidence that in domestic stocks of Nile tilapia (*Oreochromis niloticus*), sex ratios of individual pairs may vary from 1:1. Several authors have found wide variation in sex ratios from one single pair mating to another. The grand mean for the population might average 1:1, but individual pairs would produce sets of progeny where the male frequency may range from 5 to 100%. This variability in sex ratios is a challenge to the development of YY breeding programs, where sex determination is assumed to be controlled by the Y chromosome with no other factors involved. For YY production of males to be successful, variability in the percent males produced must be reduced, and parent lines that conform to a simple Mendelian inheritance must be established.

Nine families of Nile tilapia based on single pair matings have been selected, each with a sex ratio that was either highly skewed to male or female or that conformed closely to a 1:1 sex ratio. Matings within the families as well as across families are being conducted to determine the heritability of sex and the factors influencing it. Progeny from representative matings are being cultured in 45-l aquaria at temperatures of 27–28°C and 36°C during the period of gonadal differentiation to determine the effect of temperature on altering sex ratios and how that response may vary by family. A total of 80 within-family and 65 cross-family spawns have been obtained from the nine families. Additional pair spawns were made through 30 July 2000. The sets of progeny obtained to date are being grown to a sexable size to determine the frequency of each sex. Sex ratios from each set will be compared to those of other sibling matings and the frequency from the parent family.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Monosex Tilapia Production through Androgenesis

Ninth Work Plan, Reproduction Control Research 7 (9RCR7)
Abstract

William L. Shelton
University of Oklahoma
Norman, Oklahoma, USA

Abstract

A phenotypic marker in chromosome manipulation investigations is vital to interpreting induction results. During earlier studies on androgenesis in tilapias, males of the homozygous recessive color mutation (blond) in Nile tilapia (*Oreochromis niloticus*; Egyptian strain, Lake Manzala) were used as an induction control to verify that progeny carried only the paternal genome. Control crosses between blond males and normal colored females (Ghana strain) produced viable progeny, but survival of androgenotes (paternal, blond) was extremely low. Severity of the induction treatment and inbreeding of the blond mutant were considered possible factors. An alternative approach is being tested which involves another color mutation as the phenotypic marker. Red tilapia also originated from the same population (Egyptian strain, Lake Manzala), but the color mutation is a dominant trait. Thus, red females and Ghana males are being used since the relatively unselected paternal genome of the Ghana strain might be hardier. However, the inheritance of the color and the pigment development pattern must be verified through progeny testing. The color pattern of red × red and red × Ghana is now being examined. Broodstock of these phenotypes and in these combinations have been pair spawned during the latter part of this reporting period.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

THE APPLICATION OF ULTRASOUND TO PRODUCE ALL-MALE TILAPIA USING IMMERSION PROTOCOL

Ninth Work Plan, Reproduction Control Research 8 (9RCR8)
Abstract

Amrit Bart and A.R.S.B. Athauda
Aquaculture and Aquatic Resources Management
Asian Institute of Technology
Pathumthani, Thailand

James S. Diana
School of Natural Resources and Environment
The University of Michigan
Ann Arbor, Michigan, USA

ABSTRACT

This study was initiated in February of 2000 and is expected to be completed by December 2000. The experiment is designed to reveal the application of cavitation-level ultrasound to enhance delivery of androgens using immersion protocol. This experiment is in two parts: 1) a preliminary study to detect the effect of ultrasound on sex reversal using two androgens commonly available in Asia (17α-methyltestosterone and andostrenedione) and 2) an examination of the effects of two novel and more potent hormones using a protocol established by CRSP researchers. In the preliminary experiment the variables tested were androgens (17α-methyltestosterone and andostrenedione), duration of treatment (1 or 2 h), and hormone concentrations (100 or 500 $\mu g\ l^{-1}$). Fish were treated on days 10 and 13 post-hatch. This study has been completed, and the results clearly indicate an effect of ultrasound. Although the rate of sex reversal was less than 100% in all cases, significant differences were observed between those treated with and without ultrasound in two-hour treatments, irrespective of the hormone concentration used. The highest number of males (88 to 94%) was obtained from the two-hour ultrasound-treated group, while the lowest number of males (44 to 75%) was observed from the same group without application of ultrasound. The second experiment is underway to examine the hormones trenbolone acetate (TA) and 17α-methyldihydrotestosterone (MDHT) at different concentrations (250 or 500 $\mu g\ l^{-1}$) and duration of treatment (1 or 2 h). We expect a higher and more consistent rate of sex reversal between treatments by varying these parameters.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Lotus-Fish Culture in Ponds: Recycling of Pond Mud Nutrients

Ninth Work Plan, New Aquaculture Systems/New Species Research 1 (9NS1)
Abstract

C. Kwei Lin and Yang Yi
Aquaculture and Aquatic Resources Management Program
Asian Institute of Technology
Pathumthani, Thailand

James S. Diana
School of Natural Resources and Environment
The University of Michigan
Ann Arbor, USA

Abstract

This experiment started in February 2000 and will be terminated in September 2000. The purposes of the experiment were to: 1) assess the pond mud nutrient recovery by lotus plants (*Nelumbo nucifera*); 2) assess pond mud characteristics after lotus-fish culture; and 3) compare fish growth in ponds with and without lotus integration. There were three treatments: A) lotus-fish integrated culture; B) fish alone; and C) lotus alone. Lotus plants were transplanted at 20 plants per pond in treatments A and C. Sex-reversed all-male Nile tilapia (*Oreochromis niloticus*) fingerlings were stocked at 2 fish m^{-2} in ponds of treatments A and B. Treatment ponds stocked with tilapia (treatments A and B) were fertilized weekly with urea and triple superphosphate (TSP) at rates of 28 kg N and 7 kg P ha^{-1} wk^{-1}. No fertilizer was applied in treatment ponds with lotus alone (treatment C). Fish growth and survival will be assessed only at the end of the experiment due to sampling difficulties. Fish and lotus plants will be harvested by draining. Nutrient budgets will be determined for all ponds. Partial budgets will be estimated for cost of inputs and value of fish and lotus.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Culture of Mixed-Sex Nile Tilapia with Predatory Snakehead

Ninth Work Plan, New Aquaculture Systems/New Species Research 2 (9NS2)
Abstract

C. Kwei Lin and Yang Yi
Aquaculture and Aquatic Resources Management Program
Asian Institute of Technology
Pathumthani, Thailand

James S. Diana
School of Natural Resources and Environment
The University of Michigan
Ann Arbor, Michigan, USA

Abstract

An experiment was conducted in eighteen 200-m^2 earthen ponds at the Asian Institute of Technology, Thailand, during May through October 1999. The purposes of the experiment were to: 1) assess the efficiency of snakehead (*Channa striata*) in controlling overpopulation of mixed-sex Nile tilapia (*Oreochromis niloticus*) in ponds and 2) assess growth and production characteristics of Nile tilapia in monoculture and polyculture with snakehead. The six treatments were: A) monoculture of sex-reversed tilapia; B) monoculture of mixed-sex tilapia; C) polyculture of mixed-sex tilapia and snakehead at 10:1 ratio; D) polyculture of mixed-sex tilapia and snakehead at 20:1 ratio; E) polyculture of mixed-sex tilapia and snakehead at 40:1 ratio; and F) polyculture of mixed-sex tilapia and snakehead at 80:1 ratio. All ponds were fertilized weekly with urea and triple superphosphate (TSP) at rates of 28 kg N and 7 kg P ha^{-1} wk^{-1}. Sex-reversed all-male and mixed-sex Nile tilapia were stocked at 2 fish m^{-2} at sizes of 42.3 ± 1.0 g and 31.0 ± 0.5 g, respectively. Snakehead dug holes in the pond dikes and moved freely between ponds and the canal. The experiment showed that snakehead can control overpopulation of mixed-sex Nile tilapia. This experiment is currently being repeated, starting in March 2000 and terminating in September 2000.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Development of Sustainable Pond Aquaculture Practices for *Colossoma macropomum* in the Peruvian Amazon

Ninth Work Plan, New Aquaculture Systems/New Species Research 3 (9NS3)
Progress Report

Christopher C. Kohler
Fisheries and Illinois Aquaculture Center
Southern Illinois University at Carbondale
Carbondale, Illinois, USA

Susan T. Kohler
Economic and Regional Development Office
Southern Illinois University at Carbondale
Carbondale, Illinois, USA

Marcos J. De Jesus
Fisheries and Illinois Aquaculture Center
Southern Illinois University at Carbondale
Carbondale, Illinois, USA

Fernando Alcántara and Salvador Tello
Instituto de Investigaciones de la Amazonia Peruana
Iquitos, Peru

Printed as submitted

Abstract

Colossoma macropomum growth performance did not significantly differ in trials conducted in ponds at 2,500, 3,250, and 4,000 fish ha^{-1} in Iquitos, Peru. Fish initially weighing 3.4 g were fed a locally prepared diet (26.7% crude protein; 9.0% crude lipid) in rations ranging from 3 to 5% body weight per day. Fish were harvested after 168 days and had mean weights of 374.7, 307.7, and 287.0 g for the 2,500, 3,250, and 4,000 fish ha^{-1} stocking rates, respectively. Survival ranged from 67 to 96%, though all but two of nine ponds exceeded 80% survival. Feed conversion efficiency was 40.4, 43.4, and 61.3%, respectively, for the 2,500, 3,250, and 4,000 fish ha^{-1} treatments. Fish in two of the ponds were reared for an additional five months and attained a mean weight of 1 kg. Water quality parameters remained within acceptable ranges for tropical aquaculture. As with *Piaractus brachypomus* in a previous study, this study suggests the economic feasibility of rearing *Colossoma* in the Peruvian Amazon. Generally, the combined cost of fingerlings (US$0.14 each; corrected for 90% survival) and feed (US$1.02 kg^{-1} to produce 1 kg fresh fish) is under half the price (US$3.00 to $4.00 kg^{-1}) for which the fish are sold in the Iquitos market during flood periods.

Introduction

Native species aquaculture has been expanding in the Peruvian Amazon as research has played a major role in the positive evaluation of its potential. Alike *Piaractus brachypomus*, *Colossoma macropomum* is native to the Amazon basin, and shares many characteristics that also makes it suitable for aquaculture. Local production of this species is still in an extensive manner, but it possesses a high demand and attains a higher price at the market. This motivates local farmers to invest their time in the production of this valued fish. As research develops, more important information becomes available to producers, hence the improvement of native species aquaculture in the region. However, technology is still under-developed, but external aid has made it possible for the locals to become more aware and active with aquaculture practices of *Colossoma* and other native species.

Colossoma broodstock are many times collected from their habitat although they are also raised in captivity at research stations. These fish may be immediate descendants of wild broodstock or may even be a product of multiple generation breeding. Normally a large broodstock population is reared for wide selectivity and to increase the probability of successful reproduction. They are then selected upon external physical characteristics when ready to spawn.

No standardization exists for stocking densities for fry or fingerlings (Campos, 1993). Likewise, no uniform fish diets are available in the region (Cantelmo et al., 1986; Ferraz de Lima and Castagnolli, 1989). The purpose of this study is to determine suitable stocking densities for optimal and efficient production of *Colossoma macropomum* to market size (0.5 to 1.0 kg) using a prepared diet, manufactured from locally available ingredients. Replicated pond studies were carried out

Table 1. Ingredients and costs in U.S. dollars for feed[a] used in pond trials of Colossoma macropomum In Iquitos, Peru from 17 April to 20 October 1998.

Ingredient	Percent in Diet	Cost per Unit[b]
Fish Meal	19.9	1.00 kg-1
Soybean	19.9	0.72 kg-1
Wheat	19.9	0.26 kg-1
Rice	28.8	0.19 kg-1
Corn Meal	9.9	0.68 kg-1
Vitamin C	0.1	32.00 kg-1 [c]
Vitamin/Mineral Premix	1	----[c]
Fish Oil	0.5	1.60 kg-1

[a] Proximate analysis of diet by Rebecca Lochmann (9% lipid, 26.7% protein, 92.5% dry matter).
[b] Ingredient prices varied over the course of the study. Feed costs averaged U.S. \$0.67 kg^{-1}.
[c] Costs reflect price of vitamin C and vitamin/mineral premix combined.

at the Instituto de Investigaciones de la Amazonia Peruana (IIAP) research facilities at Iquitos.

Along with this density study, other experiments related to the performance of these fishes are currently in progress. A nutritional study performed by Rebecca Lochmann was set to determine an optimal broodstock diet at low cost, manufactured with locally available ingredients. Konrad Dabrowski is completing an experiment where he studies the fish's blood plasma steroid concentration during their annual breeding cycle. A study to compare the effects of different inducing hormones on *Colossoma* and/or *Piaractus* is also in progress. A second density study with *Piaractus* is presently under completion at the host country research facility. All together these studies will provide important information to develop an efficient protocol for the spawning and rearing of the Amazon fish species.

Table 2. Performance of Colossoma macropomum at three densities in pond trials conducted in Iquitos, Peru from 17 April to 20 October, 1998.

Date	Weight (g)	T.L. (cm)	FCE[a]	SGR[b]	K[c]
2,500 Fish ha^{-1}					
17-Apr	3.4	5.1	-	-	-
20-Jul	163.3	19.2	-	-	-
20-Oct	374.7	25.7	40.4	2.3	2.1
3,250 Fish ha^{-1}					
17-Apr	3.4	5.1	-	-	-
20-Jul	114.3	18.3	-	-	-
20-Oct	319.1	25.7	43.4	2.6	1.9
4,000 Fish ha^{-1}					
17-Apr	3.4	5.1	-	-	-
20-Jul	80	15.3	-	-	-
20-Oct	396.5	26.5	61.3	2.1	2.1

[a] Feed conversion efficiency (composite)
[b] Specific growth rate: $\ln(W_f) - \ln(W_o)/T \times 100$; where W_f and W_o = final and initial weights in grams, respectively, and T = time in days.
[c] Condition factor: $K = W/L^3$; where W = weight in grams, L = total length in cm.

METHODS AND MATERIALS

Nine ponds, ranging in size from 1,015 to 5,320 m^2, were stocked with *Colossoma macropomum* at three densities: three ponds at 2,500 fish ha^{-1}, three at 3,250 fish ha^{-1}, and three ponds at 4,000 fish ha^{-1}. The mean initial weight was 3.4 g. A locally manufactured feed using available ingredients was fed (Table 1). Fish were fed 5% body weight per day (bwd) for the first month and 3% bwd for the remainder of the trial. Rations were divided into three feedings. Fish were sampled (10% minimum population) by seining every two weeks to record lengths and weights. At harvest, biomass, feed conversion efficiency (FCE), specific growth rate (SGR), and condition factor (K) were calculated. The study commenced 17 April 1997 and continued until 20 October 1997. General water quality parameters (dissolved oxygen, temperature, total ammonia nitrogen, and pH) were measured daily or weekly, in the early morning. Harvest data were analyzed using the Statistical Analysis System (SAS Institute 1993) with an alpha of 0.05.

Materials and methods, as well as results for the nutrition and blood plasma studies will be presented respectively by Rebecca Lochmann and Konrad Dabrowski in their reports. The remaining density study and reproductive hormone experiment will be reported in the final workplan report.

RESULTS

No differences ($P > 0.05$) existed at harvest in *C. macropomum* weight (374.67 g at 2,500 fish ha^{-1}, 293.5 g at 3,250 fish ha^{-1}, and 377.33 g at 4,000 fish ha^{-1}; see figure 1), total length (26.03 cm at 2,500 fish ha^{-1}, 24.75 cm at 3,250 fish ha^{-1}, and 26.33 cm at 4,000 fish ha^{-1}), specific growth rate (2.32 at 2,500 fish ha^{-1}, 2.60 at 3,250 fish ha^{-1}, and 2.61 at 4,000 fish ha^{-1}),

Table 3. Early morning water quality levels[a] of ponds at used in work plan 9 at the IIAP CRI-Loreto research facility Iquitos, Peru 1998.

	Temperature	O^2	pH	TAN[b]
Mean	30.7	7.0	7.1	<1
High	37.0	13.0	9.1	<1
Low	24.0	1.7	3.4	<1

[a]/ Values in mg L^{-1} except temperature (°C) and pH
[b]/ TAN = total ammonia nitrogen

condition (2.1 at 2,500 fish ha^{-1}, 1.9 at 3,250 fish ha^{-1}, and 2.1 at 4,000 fish ha^{-1}), feed conversion efficiency (40.4 at 2,500 fish ha^{-1}, 43.4 at 3,250 fish ha^{-1}, and 61.3 at 4,000 fish ha^{-1}), or productivity (894.9 kg ha^{-1}, 715.3 kg ha^{-1}, and 1098.3 kg ha^{-1}; see table 2). Survival exceeded 80 %.

Water quality varied among ponds (Table 3). Mean maximum and minimum temperatures over the course of the study were 31 and 28.5 C, respectively. Minimum dissolved oxygen levels generally remained above 1.0 mg L^{-1}, and usually averaged in excess of 4.0 mg L^{-1}. Total ammonia nitrogen remained below 1.0 mg L^{-1}. Carbon dioxide was not recorded this study, but levels reached a high of 22 mg L^{-1} in one pond during the last trial. These waters can be classified as soft (hardness = 20 mg L^{-1}; alkalinity = 20 mg L^{-1}; conductivity = 96 ohms cm^{-2}) and slightly acidic (morning pH ranging from 3.4 to 9.1).

DISCUSSION

No significant differences were found in growout performance of *Colossoma macropomum* stocked in ponds at densities of 2,500, 3,250, and 4,000 fish ha^{-1}. The mean fish growth rate of 1.90g d^{-1} was lower than *Piaractus* in the previous study. Although, this can be attributed to certain ponds emerging with significant macrophyte infestations, which impeded the feeding process for an undetermined period of time. Some of the clearer ponds showed growth rates at about 3.0g d^{-1}, which is what is expected of this species according to studies by St. Paul 1986 and Gunther and Boza Abarca 1992.

Feed conversion was excellent throughout the trial. Exceptionally high values indicate the ability of *Colossoma* to filterfeed. In contrast to *Piaractus*, they are equipped with longer, finer gillrakers. In both species, filterfeeding is evident. Also important, is the fact that various seeds and fruit that grow around the banks of the ponds are ingested by the fish when these fall in the water. Fish that were fed for an additional five months (10 months total) reached about a kg in size (from 3.4 g). The prepared diet used in this study cost U.S. $1.02 to

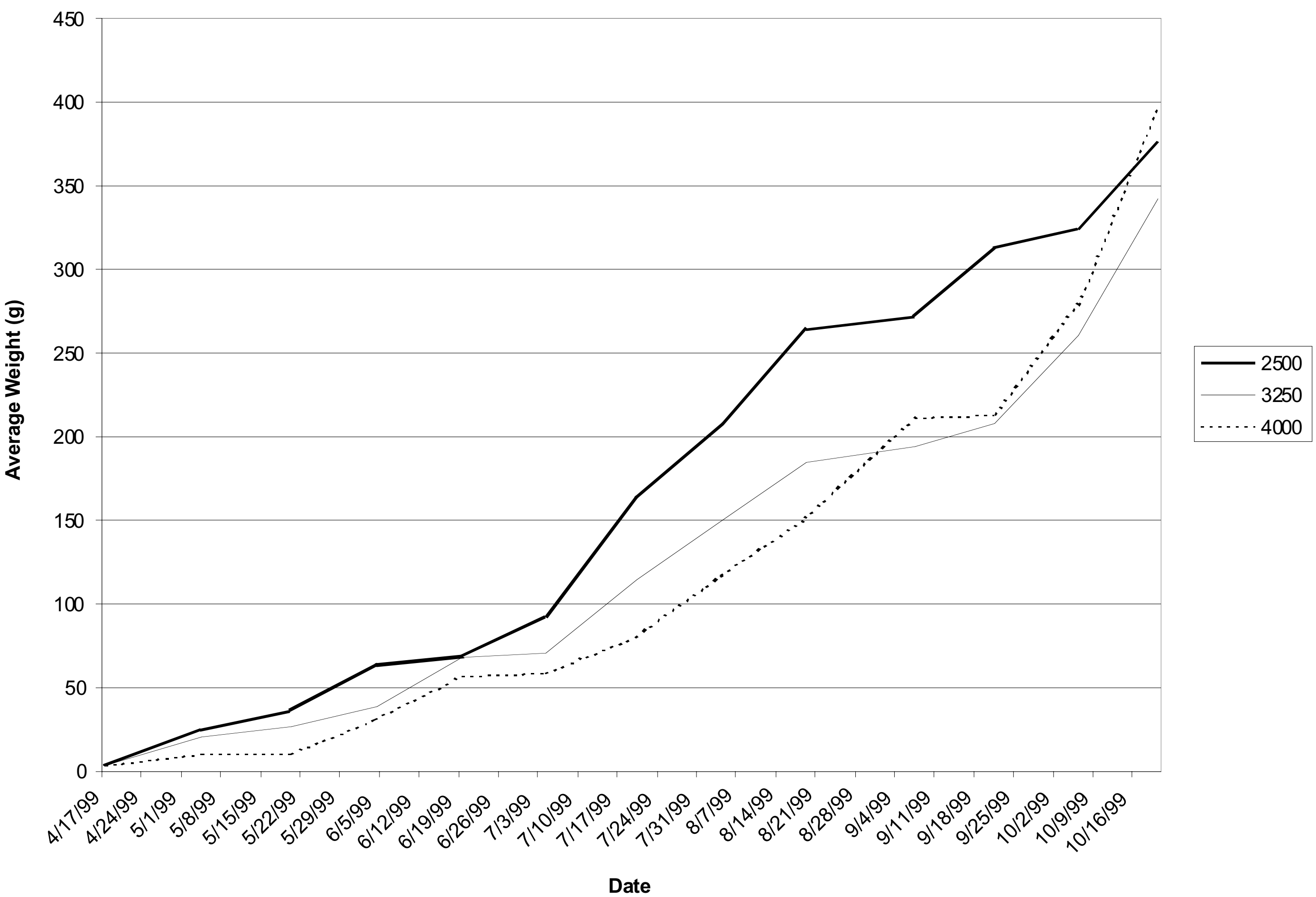

Figure 1. Average weight of *Colossoma macropomum* stocked at densities of 2,500, 3,250, and 4,000 fish ha^{-1} in Iquitos, Peru from 17 April through 20 October 1998.

produce 1 kg of whole fish. Fingerlings generally sell about U.S. $0.13 each. *Colossoma* will attain a market price of over U.S. $3.00 kg^{-1}.

Water quality remained well within the tolerances of *Colossoma* throughout the study. Truly, it must be recognized that this species has the ability to slow its metabolic rate during periods of stress. In fact, they can generate extended tubercles from their lower tip of the jaw that help them breath oxygen from the surface. So it would take days of low oxygen levels to adversely affect this species instead of hours, which is the case in many culture species.

This study revealed considerable potential for intensive aquaculture of *Colossoma* in the Peruvian Amazon. No significant differences were found between the three densities. Densities of 2,000 to 3,000 fish ha^{-1} are traditionally used in the region. According to results in this study, higher stocking densities may be possible. On 2 March 2000, triplicated ponds were stocked at 4,000, 6,000, and 8,000 *P. brachypomus* ha^{-1} at the same facility. This study is in its final stages, and harvest is coming up close. More comparisons will be available further on.

ANTICIPATED BENEFITS

Most farmers generally use organic fertilizers and periodically provide fruits, nuts and kitchen scraps. This research presents an economic prepared diet (26.7% protein and 9% lipids) used for the growout of *Colossoma*. Considering the excellent growth rates that occurred (3.4g to 1 kg in ten months), it appears that this diet meets or exceeds *Colossoma's* nutritional needs. More detailed nutritional studies performed by Rebecca Lochmann should recommend a well balanced diet for *Colossoma* and *Piaractus* that could be manufactured locally with locally available ingredients at a low cost. Results of the present and ongoing studies will be shared with local farmers via extension work.

Konrad Dabrowski will give us a perspective as to when these fish reach their peak during their annual reproductive cycle in order to better program spawning efforts. Along these measures, he will also investigate how to obtain best gamete quality from the broodstock. Compiling all this valuable information will allow us to efficiently and successfully culture this important fish in the Peruvian Amazon.

LITERATURE CITED

Campos, L.B., 1993. The culture of gamitana (*Colossoma macropomum*, Cuvier, 1818) in Latin America. M.S. thesis. Southern Illinois University, Carbondale, IL 148 pp.

Cantelmo, A., A. De Soura, and J. Senhorini, 1986. Dimencao da particula do alimento para alevinos de pacu, *Colossoma mitrei* e tambaqui, *Colossoma macropomum*. Proyecto Aquicultura (Editor), Sintese Dos Trabalhos Realizados Com Species Do Genero Colossoma, March 1982, Abril 1986., Pirassununga, Brasil, 28 pp.

Ferraz de Lima, J. and N. Castagnolli, 1989. Reproducao, larvicultura e genetica: cultivo de *Colossoma*. A. Hernandez (Editor), Primera Reunion Grupo de Trabajo Tecnico, Junio 1988. Pirassunuga, Brasil, pp. 315-322.

Gunther, J. and J. Boza Abarca, 1992. Growth performance of *Colossoma macropomum* (Cuvier) juveniles at different feed rations. Aquaculture and Fisheries Management 23:81-93.

Saint-Paul, U., 1986. Potential for aquaculture of South American freshwater fishes: a review. Aquaculture 54:205-240.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Practical Diet Development for Broodstock of *Colossoma macropomum* and *Piaractus brachypomus*

Ninth Work Plan, New Aquaculture Systems/New Species Research 3A (9NS3A)
Progress Report

Rebecca Lochmann
Department of Aquaculture and Fisheries
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

Abstract

Proximate analyses of broodstock and grow-out feeds for gamitana (*Colossoma macropomum*) and paco (*Piaractus brachypomus*) and their constituent feedstuffs were conducted. Literature values for specific nutrients known to affect fish reproduction were calculated from published sources for the broodstock diet. Broodstock eggs have not yet been obtained for proximate analysis. However, similar analyses conducted previously on eggs of similar species in Brazil have been described. Analytical information on the feedstuffs and diets currently being used in Iquitos, Peru, together with published information on the natural diets of colossomids and broodstock nutrition of other species were combined to formulate preliminary recommendations for the nutrition and feeding of gamitana and paco broodstock.

Introduction

Colossomid culture in Iquitos, Peru, may be limited currently by several factors, including the inability to obtain consistent spawning of captive broodstock. Inadequate nutrition of the broodstock may be contributing to this problem. However, little is known about broodstock nutrition in fish (De Silva and Anderson, 1995). Therefore, the objectives of this study were to identify nutritional factors that may be hindering reproductive success in captive colossomid broodstock and to formulate recommendations for improvement of broodstock diets.

Methods and Materials

Proximate analyses of feedstuffs and broodstock diets were conducted using standard methods (Association of Official Analytical Chemists, 1984). Protein was analyzed using the Kjeldahl method. Total lipid was analyzed using the Folch method (Folch et al., 1957). Other data used in this report were obtained from published sources. Small-scale feeding trials are being performed with paco to bracket their dietary requirements for vitamins C and E.

Results and Discussion

The analyzed protein content of the broodstock diet was approximately 32%. The calculated energy: protein (E:P) ratio (kcal of energy per gram of protein) of the current broodstock diet is about 8.7. This is lower than the range of values reported for good growth of colossomid species (10.7 to 13.9) (Castagnolli, 1991). Adult fish require more energy than juveniles simply for maintenance, and even more energy for production of gametes. The relative abundance of protein compared to energy in the diet may cause the fish to metabolize a large percentage of protein for basic maintenance requirements, possibly at the expense of gamete production. This imbalance is also not cost-effective, as protein is a more expensive energy source than lipid or carbohydrate. The E:P ratio of the current broodstock diet could be increased by replacing some of the wheat husks with lipid. Some of the broodstock fish are reportedly "fatty" (Fernando Alcántara, pers. comm.). If this is the case even on a relatively low-energy diet, there may be a lipid transport problem. This problem has multiple potential causes but might be relieved by including dietary lipid in the form of soybean lecithin (1%). Lecithin functions in lipid digestion and transport and improves performance in some fish (Hertrampf, 1992).

No supplemental lipid has been added to the broodstock diet, but it contains 7% lipid. Most of the lipid comes from fish meal (2.9%), wheat husks (1%), soybean meal (0.7%), and corn flour (0.7%). Lipids from fish meal supply n-3 fatty acids, while the other ingredients supply mainly n-6 fatty acids. Both families of fatty acids are necessary for reproduction. A ratio of 50/50 of n-3 to n-6 fatty acids has been suggested as optimal for most fish functions, including reproduction (Tacon, 1987). However, wild-spawned eggs of colossomids reportedly contain higher levels of n-3 fatty acids than eggs from captive broodstock (Araujo-Lima and Goulding, 1997). Therefore, it is still necessary to measure the fatty acid content of broodstock eggs to see if fatty acids are limiting spawning success of gamitana or paco in Peru. Until these data are available, 1% fish oil could be added to the broodstock diets.

There is little or no information on vitamin requirements of colossomid species. The natural diets of these fish are especially rich in vitamins C and E and carotenoids. All of these nutrients are known to affect reproduction in at least some fish species (De Silva and Anderson, 1995).

The broodstock diet is currently supplemented with 500 mg kg^{-1} of vitamin C. This supplement is critical, as the intrinsic vitamin C content of the feedstuffs is very low. Five hundred mg kg^{-1} of vitamin C meets or exceeds the known requirements of other fish species for weight gain and prevention of deficiency signs (National Research Council, 1993). The stability of vitamin C is poor under conditions of high heat and humidity, as found in Iquitos. Therefore, the form of vitamin C is critical—a stabilized form should be used. If this is not the case currently, a different form can be used and/or other antioxidants (e.g., ethoxyquin or equivalent) can be added to the diet to ensure stability.

The swine vitamin/mineral premix currently used in the broodstock diet supplies about 100 mg kg^{-1} of vitamin E, and the feedstuffs supply another 20 to 30 mg kg^{-1}. One hundred mg kg^{-1} meets or exceeds the vitamin E requirements of most fish species for weight gain and absence of deficiency signs (National Research Council, 1993). However, only alpha-tocopherol has high biological activity. The form of vitamin E in the premix is not specified. The form should be verified since the supplement supplies most of the dietary vitamin E. Also, a stabilized form of vitamin E should be used (e.g., alpha-tocopherol-acetate). Vitamin E is very prone to oxidation under conditions of high heat and humidity. In addition, vitamin E is quickly used up in the presence of unsaturated lipids (as from fish oil) because it is a powerful antioxidant. The amount of vitamin E should be increased proportionately if unsaturated lipids (especially those found in fish oil) are increased in the diet. Furthermore, there are studies showing that broodstock diets containing large amounts of vitamin E have positive effects when given to broodstock just prior to spawning (Kanazawa, 1988). In carp, vitamin E increases gonadosomatic index, facilitates vitellogenesis, and protects essential fatty acids in the oocytes. The specific amount of vitamin E needed to optimize these activities is not known, but vitamin E nutriture has not been investigated in gamitana or paco.

Of pigments reported to have beneficial effects in fish or other animals, only xanthophylls (about 17 mg kg^{-1} from the corn flour) are present in the broodstock diet. Other carotenoids such as beta-carotene are important for egg viability in some fish and are prevalent in the natural diets of colossomid species. Therefore, carotenoid supplementation of the broodstock diet may be beneficial for spawning success of gamitana or paco. Further research is necessary to identify inexpensive, available sources of carotenoids (e.g., fruits, vegetables, and/or flowers) that could be used in Iquitos.

The combination of fish meal (25%) and soybean meal (30%) should meet all of the essential amino acid requirements of the fish, as well as the available phosphorus requirement (National Research Council, 1993). However, reduction of the fish meal in the diet should be considered for environmental and/or economic reasons. Most studies indicate that there is little or no benefit to using fish meal in vegetarian or omnivorous colossomid species diets. The fish meal in the broodstock diet could be reduced to 10% (with a concomitant increase in soybean meal) without creating any amino acid deficiencies. If fish meal is reduced, available phosphorus may become limiting and a supplement should be considered (there is no phosphorus in the swine premix).

Large juvenile *Piaractus brachypomus* are now at the aquaculture research facility in Pine Bluff, Arkansas. Preliminary feeding trials to bracket the dietary vitamin C and vitamin E requirements of this species are underway.

ANTICIPATED BENEFITS

Improving the nutritional status of colossomid broodstock should increase spawning success and possibly the quality of resulting fry. These changes would enhance the economic viability of commercial colossomid farming in Peru.

LITERATURE CITED

Alcántara, F., personal communication, 1999.
Araujo-Lima, C. and M. Goulding, 1997. So Fruitful a Fish: Ecology, Conservation, and Aquaculture of the Amazon's Tambaqui. Columbia University Press, New York, 191 pp.
Association of Official Analytical Chemists, 1984. Official Methods of Analysis, Fourteenth Edition. Arlington, Virginia, 1,141 pp.
Castagnolli, N., 1991. Brazilian finfish, tambaqui, pacu, and matrinxã. In: R. Wilson (Editor), Handbook of Nutrient Requirements of Finfish. CRC Press, Boca Raton, pp. 31–34.
De Silva, S.S. and T.A. Anderson, 1995. Fish Nutrition in Aquaculture. Chapman and Hall, London, 319 pp.
Folch, J., M. Lees, and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem., 226:497–509.
Hertrampf, J.W., 1992. Feeding Aquatic Animals with Phospholipids II. Fishes. Publication #11. Lucas Meyer (GmbH and Co.), KG, Hamburg, 70 pp.
Kanazawa, A., 1988. Broodstock nutrition. In: T. Watanabe (Editor), Fish Nutrition and Mariculture. Kangawa International Fisheries Training Centre, Japan International Cooperation Agency, pp. 147–159.
National Research Council, 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC, 124 pp.
Tacon, A.G.J., 1987. The Nutrition and Feeding of Farmed Fish and Shrimp - A Training Manual. 1. The Essential Nutrients. GCP/RLA/075/ITA - FAO Field Document 2/E. Food and Agriculture Organization, Brasilia, Brazil, 117 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Semi-Intensive Culture of Tilapia in Brackishwater Ponds

Ninth Work Plan, New Aquaculture Systems/New Species Research 4 (9NS4)
Abstract

C. Kwei Lin and Yang Yi
Aquaculture and Aquatic Resources Management Program
Asian Institute of Technology
Pathumthani, Thailand

James S. Diana
School of Natural Resources and Environment
The University of Michigan
Ann Arbor, Michigan, USA

Abstract

This experiment was started in June and will be terminated in November 2000. The purposes of the experiment were to: 1) determine appropriate fertilization regimes in brackishwater ponds; 2) investigate nutritional value and digestibility of specific marine phytoplankton as food organisms to tilapia; and 3) exploit underutilized or abandoned shrimp ponds for tilapia production. Two fertilization regimes (28 kg N and 7 kg P ha^{-1} wk^{-1} and 28 kg N and 14 kg P ha^{-1} wk^{-1}) and three levels of salinity (10, 20, and 30‰) were tested by a 2×3 factorial design. Eighteen cement ponds (6 m^2) were filled with 15 cm of soil and then flooded with water of differing salinities by diluting hypersaline water to the appropriate concentrations. Thai red tilapia fingerlings were stocked at 2 fish m^{-2}. All ponds were fertilized weekly at the two rates using urea and triple superphosphate (TSP). Water levels will be maintained at 0.8 m depth, and salinity levels will be checked and adjusted weekly. Plankton composition will be assessed biweekly. Partial budgets will be calculated to estimate cost of inputs and value of fish crop.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Use of Pond Effluent for Irrigation in an Integrated Crop/Aquaculture System

Ninth Work Plan, Effluents and Pollution Research (9ER1)
Final Report

C. Wesley Wood
Department of Agronomy and Soils
Auburn University, Alabama, USA

M. Bernard Meso
Sagana Fish Farm
Sagana, Kenya

Karen L. Veverica
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Nancy Karanja
Department of Soil Science
University of Nairobi
Nairobi, Kenya

Abstract

When fish are recovered from ponds, the effluent is often drained, presenting both an environmental challenge and an agricultural opportunity. The effects of irrigation with pond effluent and its interaction with applied fertilizer were assessed in a field experiment using French bean (*Phaseolus vulgaris*) and kale (*Brassica oleracea*) over two growing seasons near Sagana, Kenya. Fresh and dry matter yields of the crops were recorded at harvest, and samples were collected for determination of tissue nutrient concentration. In the first season, French bean fresh pod yield differed significantly ($P = 0.05$) among treatments. Plots receiving canal water and fertilizer at recommended rates had the highest yield (9.1 t fresh pod ha^{-1}), while those receiving no fertilizer or irrigation had the lowest yield (1.3 t fresh pod ha^{-1}). In the second season, significant differences ($P = 0.05$) were observed among treatments in fresh bean pod and fresh kale leaf yields. The highest (4.4 t ha^{-1}) fresh pod yield was observed in pond-effluent-irrigated and fertilized plots, while the lowest (1.3 t ha^{-1}) was observed in non-irrigated, unfertilized plots. The highest fresh kale leaf yield (11.5 t ha^{-1}) was obtained using irrigation with canal water combined with fertilizer application, while the lowest (4.2 t ha^{-1}) was observed in non-irrigated, unfertilized plots. Low nutrient status in the pond water, together with inadequate water supplied to some crops due to emitter clogging, was responsible for low yields in treatments where pond water was substituted for canal water. Pond water from the Sagana Fish Farm supplied low amounts of nitrogen (N) and phosphorus (P) for crops, indicating that recommended rates of mineral fertilizers should be used when pond water is used for irrigation. In the second experiment, the effectiveness of two types of soil occurring at Sagana, Kenya—a vertisol (black clay soil) and a cambisol (red clay soil)—in retaining nutrients from pond effluent was investigated. A laboratory experiment was conducted with soil columns containing red or black clay soil. Pond effluent application rates of 31, 81, and 161 mm d^{-1} were tested on both soils. Both soils retained over 60% of total phosphorus from pond effluents, with red clay soil retaining 27% more phosphorus than black clay soil. At the high effluent loading rate, low % N removal was observed in both soils. Total nitrogen removal efficiency declined with time after 21 days at the high rate, and after that time no nitrogen removal was observed where red clay soil was used. Black clay soil was more enriched by nitrogen than red clay soil, while phosphorus enrichment was higher in red clay soil than in black clay soil. It appears that land application can remove substantial amounts of phosphorus and nitrogen from pond effluent.

Introduction

In Kenya there are approximately 46,000 fish ponds, producing about 1,100 Mg of fish annually (Government of Kenya, 1997). Fertilizers are applied to ponds to increase inorganic nutrient concentrations that favor phytoplankton growth, enhancing production of fish and crustaceans (Boyd, 1990). During harvesting, ponds are drained to levels where fish can be recovered via nets. A result of pond draining is effluent discharge (Sumari, 1982). Such effluents are often allowed to run into natural waterways. Effluents from fertilized ponds have relatively high nutrient concentrations and can be potential sources of pollution and eutrophication for receiving waters.

Pond effluents have been applied to crops as irrigation water (Prinsloo and Scoonbee, 1987; Al-Jaloud et al., 1993; Hussein and Al-Jaloud, 1995). Hussein and Al-Jaloud (1995) report wheat grain yields ranging from 770 to 5,010 kg ha^{-1} with well water and 2,140 to 5,790 kg ha^{-1} with aquaculture effluent. Improved water use efficiency (WUE) was also reported with aquaculture-effluent–irrigated crops having a WUE of 11 to 30 kg ha^{-1} mm^{-1}, whereas well water treatments had a WUE of 7 to 22 kg ha^{-1} mm^{-1}. Grain yield and WUE obtained with well water combined with 75 to 100% of the nitrogen requirement as fertilizer were comparable with treatments irrigated with aquacultural effluents combined with 25 to 50% of the nitrogen requirement. These results imply that the application of 150 to 225 kg N ha^{-1} for well water irrigation and 75 to 160 kg N ha^{-1} for aquaculture effluent irrigation containing 40 mg l^{-1} is sufficient for optimum grain yield and WUE. Similar results were obtained by Al-Jaloud et al. (1993).

When pond effluents are applied in arid and semiarid environments, greater crop returns may be obtained through more efficient application methods. In Kenya, where farm ponds can also serve as water reservoirs for irrigation, drip irrigation could be profitable. Drip irrigation is a technique whereby water and fertilizers can be placed directly over the root zone through use of emitters that are calibrated for low flow rates. Drip irrigation appears most promising when water and fertilizer application is split into several events over a cropping season.

Little work has been conducted in East Africa on the use of fish pond effluent as a source of irrigation water for high-value crops. A study was undertaken to determine the effects of irrigation with polyculture tilapia (*Tilapia aureus*) and African catfish (*Clarias gariepinus*) pond water on yield of French bean (*Phaseolus vulgaris*) and kale (*Brassica oleracea*). Specific objectives of the study were to:

1) Evaluate pond effluents as a source of irrigation water for French bean and kale;
2) Assess the ability of pond effluents to supply nitrogen and phosphorus to French bean and kale; and
3) Determine the effectiveness of two soil types from the Sagana, Kenya, area to retain nutrients from fish pond effluent.

METHODS AND MATERIALS

The project was conducted between October 1998 and September 1999 at the Department of Fisheries Fish Farm at Sagana in central Kenya. The farm lies at an elevation of 1,231 m above sea level. Rainfall at the farm ranges from 1,332 to 1,612 mm yr^{-1}, and daily average air temperatures range from 16.3 to 26.9°C. Water supply to the farm was from the Ragati River.

Separate studies were conducted in this project. The first study was conducted to investigate the potential benefits of applying pond effluents to crops and the effects of the effluents on the yield of French bean and kale. The second investigation examined the potential use of land to purify fish pond effluents.

Soils at the farm are "black cotton soils" (vertisols) of volcanic origin. Table 1 shows the pH, nutrient, and other chemical concentrations (Hue and Evans, 1986), bulk density, and hydraulic conductivity (Klute et al., 1986) of the vertisol (black clay soil) and cambisol (red clay soil) used in both the experiments at the start of the trials. Water analyses for both experiments were done using standard methods.

French Bean and Kale Field Experiment

The experiment was conducted during two growing seasons. The first season started in October 1998 and ended in February 1999. The second season started in June and ended in September 1999. For both runs of the experiment, one of the fish ponds on the Sagana Fish Farm was selected to supply effluent. The pond was fertilized with 8 kg P ha^{-1} as diammonium phosphate during a 17-wk period prior to stocking. The pond was then stocked with tilapia and African catfish. Subsequently, the pond received 20 kg N ha^{-1} wk^{-1} and 8 kg P ha^{-1} for the 17-wk grow-out periods for both runs of the experiment.

First Growing Season

Eighteen field plots measuring 10 × 6 m were prepared on land previously under star grass *(Digituara scalarum)*. Plots were hand-tilled and hand-harrowed sufficiently for planting French bean. In October 1998, plots were planted with French bean (var. Samantha) at a spacing of 0.6 × 0.1 m. Bean plants were sprayed with Antracol® and Ripcord® at a rate of 80 l ha^{-1} at 14-d intervals for pest and disease control.

The experimental design was an incomplete factorial arranged as a randomized complete block with six treatments replicated three times. Treatments consisted of:

- Non-irrigated, unfertilized (–I –F);
- Non-irrigated, fertilized (–I +F);
- Drip-irrigated with canal water, unfertilized (+I –F);
- Drip-irrigated with canal water, fertilized (+I +F);
- Drip-irrigated with fish pond effluent, unfertilized (+P –F); and
- Drip-irrigated with equal parts canal and pond water, unfertilized (+IP –F).

At planting, diammonium phosphate (DAP) (200 kg ha^{-1}) was applied to treatments receiving fertilizer. These treatments received an additional 200 kg ha^{-1} of calcium nitrate as top dressing after bean emergence. Plots receiving irrigation water were fitted with garden drip irrigation systems. A 10-l distribution bucket suspended on a post held water (canal or pond) to irrigate individual plots receiving irrigation treatments. Plots receiving water via drip irrigation were fitted with a F1 0.75-in filter (Lego Inc., Israel) that was intended to remove particulate matter. Drip-irrigated treatments received 0.33 mm water d^{-1} over a growing season of 74 days.

French bean harvest began 46 days after planting and continued for 28 days. Fresh and dry weight of bean pods and total biomass dry weight (dry pods, leaves, and stems) were recorded. Twenty-one days after transplanting, leaf samples were picked for nutrient analysis. After 76 days, the bean crop was uprooted and the above-ground biomass dried out on polythene sheets for biomass yield records.

Second Growing Season

Twenty-four plots measuring 5 × 6 m were prepared on the previous season's experimental site, with an additional twelve plots prepared on an adjacent uncultivated portion under star grass. The land was hand-tilled and hand-harrowed to the recommended tilth for French bean and kale.

The experiment consisted of twelve treatments arranged as a 2 (crops; French bean and kale) × 2 (fertilization; 0 and recommended rates) × 3 (drip irrigation; 0, canal water, and pond water) factorial laid in randomized complete block design with three replicates. Treatments were:

- No irrigation and no P or N for kale (K: –I, –F);
- No irrigation and no P or N for bean (B: –I, –F);
- No irrigation plus 78 kg N ha^{-1} in splits of 48 kg N ha^{-1} at

planting time and 30 kg N ha^{-1} four weeks after planting and 54 kg P ha^{-1} for kale (K: –I, +F);

- No irrigation plus 36 kg N and 40 kg P ha^{-1} for bean (B: –I, +F);
- Drip irrigation at 2.3 mm d^{-1} with canal water and no P or N for kale (K: +IC, –F);
- Drip irrigation at 2.3 mm d^{-1} with canal water and no P or N for bean (B: +IC, –F);
- Drip irrigation at 2.3 mm d $^{-1}$ with water from the canal and application of 78 kg N ha^{-1} in splits of 48 kg N ha^{-1} at planting time and 30 kg N ha^{-1} four weeks after planting and 54 kg P ha^{-1} for kale (K: +IC, +F);
- Drip irrigation at 2.3 mm d^{-1} with water from the canal and application of 36 kg N and 40 kg P ha^{-1} for bean (B: +IC, +F);
- Drip irrigation at 2.3 mm d^{-1} with pond effluent providing 6.3 kg N ha^{-1} and 2.6 kg P ha^{-1} for kale (K: +IP, –F);
- Drip irrigation at 2.3 mm d^{-1} with pond effluent providing 6.3 kg N ha^{-1} and 2.6 kg P ha^{-1} for bean (B: +IP, –F);
- Drip irrigation at 2.3 mm d^{-1} with pond effluent providing 6.3 kg N ha^{-1} and 2.6 kg P ha^{-1} with application of 78 kg N ha^{-1} in splits of 48 kg N ha^{-1} at planting time and 30 kg N ha^{-1} four weeks after planting and 54 kg P ha^{-1} for kale (K: +IP, +F); and
- Drip irrigation at 2.3 mm d^{-1} with pond effluent providing 6.3 kg N ha^{-1} and 2.6 kg P ha^{-1} with application of 36 kg N and 40 kg P ha^{-1} for bean (B: +IP, +F).

Kale seedlings were transplanted to the field on 9 June 1999 at a spacing of 0.9 × 0.3 m and watered for seven days using a watering can to facilitate establishment. The crop was sprayed with Dimethoate® and Antracol® every two weeks to protect it against insect and fungal attacks, respectively. French bean seeds were direct seeded on 12 June 1999 at a row spacing of 0.6 m and line spacing of 0.1 m. Other practices were done as in the first season.

Plots receiving irrigation water were fitted with garden drip irrigation systems. Water for drip irrigation was lifted to a 70-l distribution barrel in each irrigated plot using a pedal pump and applied daily at 1100 h. Plots under French bean receiving drip irrigation water were fitted with an Alkal 1.5-in filter (which was bigger and more efficient than F1 0.75-in used in the first season), while those under kale were fitted with F1 0.75-in filters used in the first season.

Kale harvesting began 22 days after transplanting and continued for 42 days by removal of the lowest three leaves per plant every four days. The weight of fresh kale leaves harvested was measured at the desired harvesting period from each of the plots receiving different treatments and summed over the harvest period. Samples for leaf nutrient analyses were collected from the second uppermost leaf of kale just before the final harvesting period. Harvest ceased 80 days after transplanting, and dry matter yield for kale was determined for all treatments as the sum of leaf dry matter over the course of the experiment.

Second-season French bean harvest began 52 days after planting and continued for 28 days. The weight of fresh French bean pods were measured at the desired harvesting period from each of the plots receiving different treatments. Samples for leaf nutrient analyses were collected for French bean from the third uppermost leaf during flowering. After 81 days the bean crop was uprooted and the above-ground biomass dried out on polythene sheets for biomass yield records. Total above-ground dry matter yield for French bean was determined from all the treatments at the end of the experiment.

Plant Tissue Analysis

For both growing seasons, plant tissue samples for nutrient analyses were oven-dried at 65°C for 24 hours, hand-crushed using a mortar and pestle, and kept in plastic cans for analysis in laboratories at the Department of Agronomy and Soils at Auburn University. Total nitrogen in plant tissue was determined by dry combustion with a LECO CHN-600 analyzer (LECO Corp., St. Joseph, Michigan) (Hue and Evans, 1986). Phosphorus, potassium, iron, manganese, and aluminum in plant tissue were measured by dry ashing, followed by dissolution in 1 M hydrochloric acid, followed by determination with a Jarrell-Ash inductively coupled argon plasma (ICAP) spectroscope (ICAP 9000, Thermo Jarrell Ash, Franklin, Massachusetts) (Hue and Evans, 1986).

Statistical Analysis

For both growing seasons, analyses of variance were performed to determine variation in French bean and kale (second season only) fresh and dry biomass and leaf nutrient concentrations owing to treatments.

Pond Effluent Nutrient Removal by Soil Experiment

The experiment, conducted in the laboratory at Sagana Farm, was designed with soil columns set up to filter and retain pollutants from fish pond effluents. Soil columns are commonly used in solute transport and nutrient leaching experiments. Columns simulating a soil profile allow easy access to through-flow water and hence their adoption in this study.

Two soil samples were obtained, one from an uncultivated field under star grass for the vertisol (black clay soil) and the other batch from a field previously cultivated with soybean (*Glycine max*) for the cambisol (red clay soil) by excavation to a depth of 45 cm using a soil auger. For the two types of soils, samples were taken from 0–15 cm, 15–30 cm, and 30–45 cm depths and maintained as individual samples. The soil samples were air-dried in the laboratory, crushed, and sieved through a 2-mm mesh screen. Dry bulk density, hydraulic conductivity, and initial concentration of total nitrogen and extractable phosphorus were determined from subsamples taken from each of the soil types as previously described.

In the same fields, undisturbed samples were obtained using the core ring method (Klute et al., 1986). Four sites were selected randomly on the experimental site. Mini pits were dug to a depth of 0.50 m and steps demarcated at 0–0.15 m, 0.15–0.3 m, and 0.3–0.5 m. Three cores were placed at random on each step and driven into the soil using a core driver. The cores were then dug using a sharp knife, wrapped in aluminum foil, and taken to the laboratory for analysis.

Three portions of pipe (10-cm diameter) were used to simulate a soil profile of three layers with depths of 0–15 cm, 15–30 cm, and 30–45 cm. Each portion of 15-cm length was filled with soil taken from a particular soil layer. Based on the determined bulk densities (Table 1), 1.56 kg of the red clay soil and 1.93 kg of the black cotton soil from the 30–45 cm soil layer were

Table 1. Initial soil chemical and physical characteristics at the onset of the experiment.

Soil Type	Depth	K_{sat}	Bulk Density	pH (water)	Total N	Total C	P	Fe	Al	Mn	K
	cm	*cm d⁻¹*	*kg m⁻³*	*1:1.5*	*(g kg⁻¹)*		*(mg kg⁻¹)*				
Black Clay	0–15	0.98	1,160	6.8	0.5	27.0	8.1	13.4	27.5	28.9	31.0
	15–30	0.99	1,260	7.3	0.4	20.8	6.2	11.4	25.2	11.3	9.6
	30–45	–	1,322	8.24	0.3	15.7	8.3	13.6	33.2	20.9	7.9
Red Clay	0–15	466	1,330	5.32	0.5	16.3	20.6	12.2	31.0	212.2	79.1
	15–30	339	1,373	5.01	0.4	12.1	7.3	10.7	26.7	110.4	57.9
	30–45	–	1,447	4.97	0.2	11.7	3.7	10.4	25.2	102.6	44.7

Table 2. Treatments in the soil column filters.

Treatment	Irrigation Intensity ($mm\ d^{-1}$)	Application Period (d)	Water per Land ($m^3\ m^{-2}$)	Pond:Land
1	0	0	0	0:0
2	31	32	1	1:1
3	81	62	5	5:1
4	161	62	10	10:1

Table 3. Average nutrient and total suspended solids (TSS) contents of canal and fish pond water.

Source	Season 1			Season 2		
	Total N	*Total P*	*TSS*	*Total N*	*Total P*	*TSS*
	($mg\ l^{-1}$)			($mg\ l^{-1}$)		
Canal	0.49	0.04	79.7	0.72	0.16	54
Pond	6.03	3.89	330.6	3.16	1.33	193

pushed down into the lowest portion. The second 15-cm portion of the pipe was fitted on the top side of the first portion already filled with soil from the 30–45 cm soil layer and fixed by duct tape; then 1.48 kg of the red clay soil and 1.84 kg of the black cotton soil from the 15–30 cm soil layer were packed into this second portion of the pipe. A third portion of the pipe, which was longer (22-cm depth) to hold pond effluent, was fitted on the top side of the system and fixed using the same procedure. Red clay soil (1.51 kg) and black cotton soil (1.63 kg) from 0–15 cm soil layer were packed into the portion to a depth of 15 cm and compacted by shaking so as to attain the bulk density of the field soils in the same horizon. The three portions fixed together formed an individual soil column filter, which was mounted on a collection pan. Pond water to be purified was then collected from the pond receiving 20 kg N $ha^{-1}\ wk^{-1}$, containing on average 5.18 mg l^{-1} N and 0.68 mg l^{-1} P, and passed through the soil column filters at varying depths of irrigation, which served as the treatments.

Four treatments were administered at the depths of water corresponding to varying loading rates of pond effluent to land as shown in Table 2. Three replicates of the soil column filters were arranged on the laboratory floor in a completely randomized design.

At the end of experiment, soil was retrieved from column filters at the three 15-cm depths, prepared, and analyzed for total nitrogen and extractable phosphorus. The through-flow water from soil columns was collected on Tuesdays and Fridays and stored at 4°C for chemical analysis. Using standard methods, pond effluents and through-flow water were analyzed for total nitrogen and phosphorus. The difference between the concentration of total nitrogen and phosphorus in pond effluents and the through-flow water obtained from the soil column gave the estimated nutrients retained from the pond effluents by the soil columns. Percent nutrient removal (% NR) from effluents was calculated as:

$$\%NR = (\text{conc. X} - \text{conc. F}) \times 100/\text{conc. X}$$

where

%NR = % nutrient removal,
conc. X = nutrient concentration in the pond effluent, and
conc. F = nutrient concentration in the filtrate.

Change in soil nutrient content after application of effluents was determined using the following equation:

$$dX = X_1 - X_2$$

where

dX = change in nutrient X content in the soil,
X_1 = concentration of nutrient X in the soil at 0 level of effluent application, and
X_2 = concentration of nutrient X in the soil at a given irrigation depth used to apply pond effluent.

Data on soil nutrient content from the columns were entered into a spreadsheet with the various treatments in rows and the nutrient levels in columns. Analysis of variance was performed using the general linear model of Statgraphics to compare the mean effects of water application rates on nutrient retention by the soil. The means were separated using the least significant difference procedure.

RESULTS AND DISCUSSION

French Bean and Kale Field Experiment

The two sources of irrigation water used in this study differed in nitrogen, phosphorus, and total suspended solids (TSS) concentrations (Table 3). Total nitrogen and phosphorus concentrations were higher in pond water than canal water. After filling the pond with canal water and subsequent fertilization, nitrogen and phosphorus concentrations in pond

Table 4. Nutrient concentration of French bean leaves in the first season.

Treatment	Total N	P	K	Mn	Fe	Al
		(g kg-1)			(mg kg-1)	
B: –I, –F	42.7	2.8	1.70	342	184	79
B: –I, +F	50.0	3.2	1.67	298	146	115
B: +IC, –F	44.4	3.0	1.89	302	166	144
B: +IC, +F	52.5	4.0	1.84	274	134	97
B: +IP and C (1:1), –F	43.3	2.6	1.65	296	152	125
B: +IP, –F	42.1	2.6	1.82	931	167	139
$LSD_{0.05}$	3.6	0.1	0.31	96	54	37
C.V.	9.5	16.78	10.40	15	23	26

water increased. Fish activities and their excreta increased the TSS in pond water.

Figures 1 and 2 show fresh, dry pod, and above-ground biomass yields for French bean in the first season. Significant differences were observed in fresh pod yield of bean among treatments ($P = 0.05$), with all treatments having higher fresh pod yield than the control. The control yielded 1.3 t fresh weight ha^{-1} (t fw ha^{-1}). When irrigation was combined with fertilization the highest yield of 9.1 t fw ha^{-1} was recorded. Irrigation with canal water alone supported 7.7 t fw ha^{-1} yield, with a gradual decline as fish pond water was substituted for canal water. Irrigation with fish pond and canal water at a ratio of 1:1 without fertilization and irrigation with fish pond water without fertilization provided 6.1 and 4.3 t fw ha^{-1}, respectively.

A 53% yield decline when pond water was substituted for fertilizer application was observed. This observation is in contrast to those of Prinsloo and Schoonbee (1987) using flood irrigation on tomatoes, Al-Jaloud et al. (1993) using flood irrigation on wheat, and Hussein and Al-Jaloud (1995) using flood and furrow irrigation on wheat, all of whom observed an increase in crop yield when pond water was used for irrigation instead of well water. Pond water supplied inadequate nitrogen and phosphorus to bean, owing to the low concentration of these nutrients (Table 3). Irrigation with pond water at 0.3 mm d^{-1} supplied only 1.6 kg N ha^{-1} and 1.03 kg P ha^{-1} to the root zone over the growing period. This input was equivalent to 4.2% and 2.4% of the recommended rates of nitrogen and phosphorus. The total nitrogen concentration in pond water (Table 3) was within the acceptable range for irrigation water but could not support yields similar to those obtained with fertilizers.

French bean foliar nutrient concentration in the first season is summarized in Table 4. Significant differences ($P = 0.05$) in leaf nitrogen and phosphorus concentrations among treatments were observed. Leaf nitrogen and phosphorus levels were higher in fertilized treatments, suggesting that availability of nitrogen and phosphorus to bean was higher in those treatments. These data help explain yield differences between treatments receiving fertilizers and those without fertilizer. Pond water containing 6 mg N l^{-1} and 4 mg P l^{-1} when applied at rates used in this study did not supply sufficient amounts of nitrogen and phosphorus to French bean to support optimum yields. It is apparent from this study that pond water cannot be a substitute for fertilizer application.

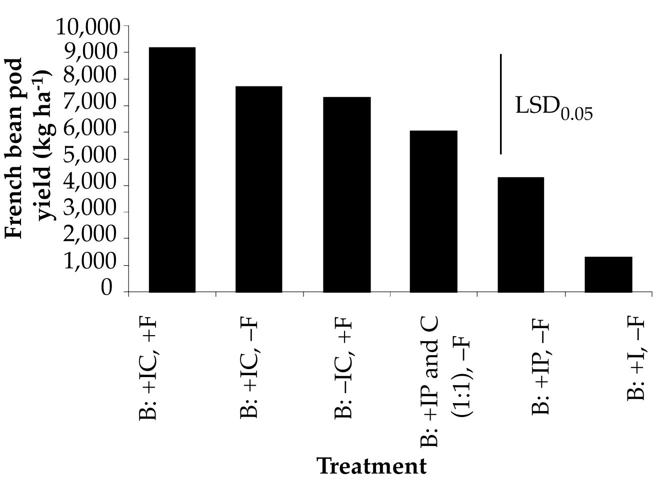

Figure 1. Fresh pod yield of French bean in the first season.

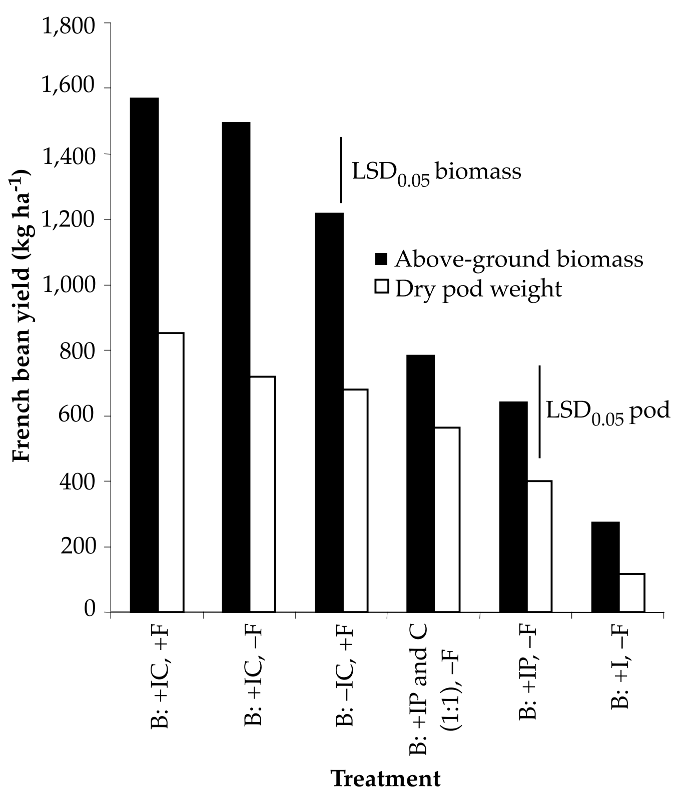

Figure 2. Dry pod weight and above-ground whole plant biomass (dry weight basis) of French bean in the first season.

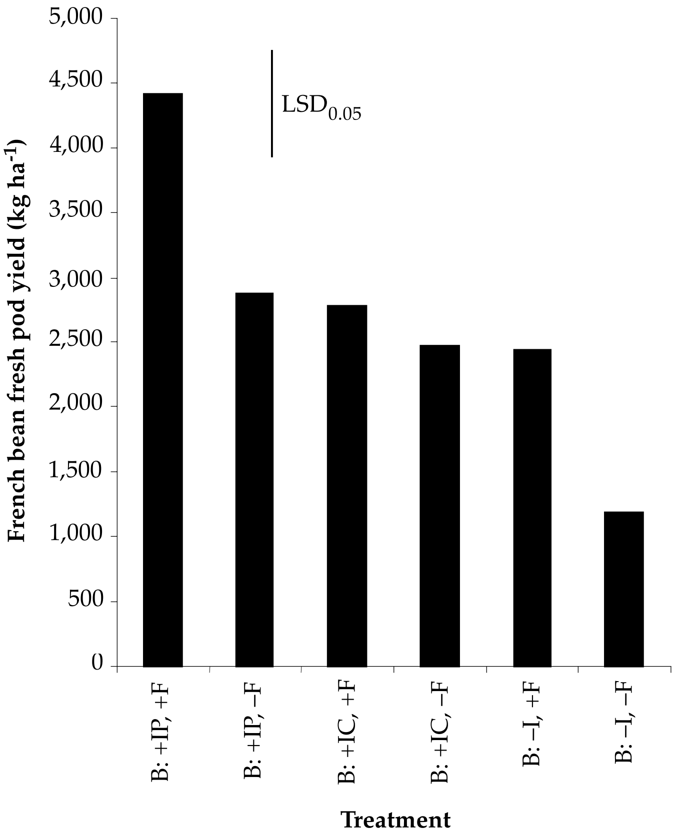

Figure 3. Fresh pod yield of French bean in the second season.

Beneficial effects of water application to crop yields are widely recognized (Hillel, 1980). A comparison made between the two water sources used in this experiment showed a 44% bean pod yield decline when pond water was substituted for canal water. The observed yield decline was due to insufficient water supplied to bean receiving irrigation from the pond. Pond water was poorly distributed along drip irrigation lines due to irregular clogging of emitters, resulting in patchy, inadequate water supply to bean plants. Clogging of emitters frequently occurred due to suspended particles and algae in pond water. Some of these particles may have escaped the filtration system and ended up in drip tapes. Particles of organic matter, minute spores, and separate cells of microorganisms probably infiltrated through filters and reached emitters. Under these conditions of reduced water velocity, oxidation-reduction potentials and favorable temperatures and aeration allowed microorganisms to proliferate, gradually plugging the outlets. When some emitters receiving water from the same bucket clogged, the remaining functioning emitters supplied excess water, resulting in excessive through-flow and leaching which took place directly under the drip tapes. As wetness increased below the emitters to the point of being waterlogged or flooded, reduced soil conditions likely occurred. Reducing conditions in a saturated or periodically inundated rhizosphere may have resulted in increased soluble manganese concentration in soil solution, promoting manganese toxicity to bean that was expressed as crinkled leaves (Bohn et al., 1985) and yield reduction. Generally, plants are affected at a foliar manganese concentration of about 300 to 500 mg kg^{-1}, and manganese concentration in mature bean leaves is typically 40 to 50 mg kg^{-1} (Tisdale et al., 1990). Thus, the concentration above 500 mg kg^{-1} observed in this study (Table 4) resulted in toxicity.

Table 5. Nutrient concentration of French bean leaves in the second season.

Treatment	Total N	P	K	Mn	Fe	Al
	(g kg-1)			(mg kg-1)		
B: –I, –F	45.9	2.4	1.52	62.5	671	551
B: –I, +F	55.3	3.6	2.05	55.5	614	473
B: +IC, –F	47.3	2.7	1.87	65.6	666	574
B: + IC, +F	48.4	3.0	1.72	81.7	894	806
B: + IP, –F	45.2	2.5	1.41	60.3	553	435
B: + IP, +F	50.2	2.9	1.99	85.1	769	664
$LSD_{0.05}$	4.7	0.9	0.37	30.2	549	586
C.V.	9.7	21.4	17.20	30	41	51

Figure 3 shows fresh pod yield obtained from French bean in the second season. There were statistically significant differences (P = 0.05) among treatments for fresh pod yields but not for dry pod weight and above-ground biomass (data not shown). Separation of means showed that fresh weight yield of bean pods from B: +IP, –F was significantly different from the yields of the control (B: –I, –F), B: +I, –F, and B: –I, +F treatments (Figure 3). The lowest yield, 1.2 t fw ha^{-1}, was obtained from the control. Irrigation with pond water combined with fertilization at the recommended rate resulted in the highest yield, 4.4 t fw ha^{-1}. Application of canal water combined with fertilization (B: +IC, +F) had a fresh pod yield that was not significantly different from application of pond water without fertilization (B: +IP, –F).

Contrary to observations made in the first season, no significant change in fresh pod weight was observed when pond water was substituted for canal water. This finding was due to the effects of improved distribution of pond water, the greater irrigation amount, or their interaction. An increase of irrigation amount from 0.33 mm d^{-1} in the first season to 2.3 mm d^{-1} assured sufficient water supply to the root zone. Consumptive use of water was thus satisfied and better yields were obtained.

Table 5 shows nutrient concentration of bean leaves collected in the second season. Analysis of variance showed a statistically significant difference in leaf nitrogen concentration (P = 0.05). No difference was observed for leaf phosphorus, potassium, magnesium, aluminum, iron, and manganese concentrations. Treatments receiving fertilizers without irrigation had the highest foliar nitrogen concentration. Low foliar nitrogen concentrations were observed in treatments irrigated with pond water without fertilizer application and canal water without fertilizer application, suggesting a reduced availability of nitrogen in irrigated plots or a dilution effect owing to greater biomass production.

TSS in pond water was 42% higher in the first season than in the second season (Table 3). In the second season, larger filters (Alkal filter 1.5-in Lego Inc., Israel) were fitted on the drip irrigation system, leading to improved filtration. Low concentrations of TSS in pond water coupled with improvement of the filtration system resulted in a better distribution of pond water along the drip line, reducing emitter clogging problems like over-irrigation, soil saturation, and insufficient water supply. Leaf manganese concentrations remained low and no toxicity occurred.

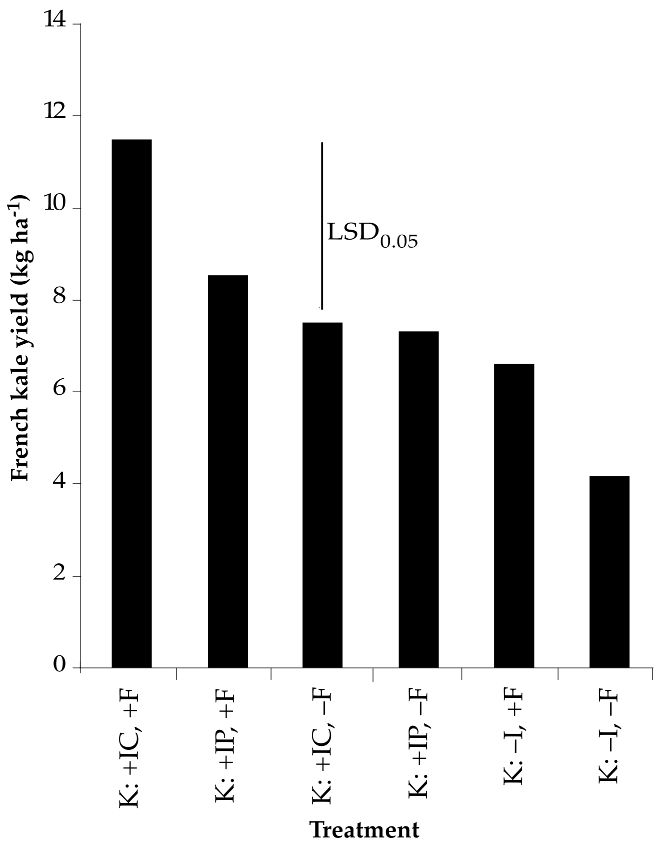

Figure 4. Fresh leaf yield of kale as affected by irrigation and fertilization.

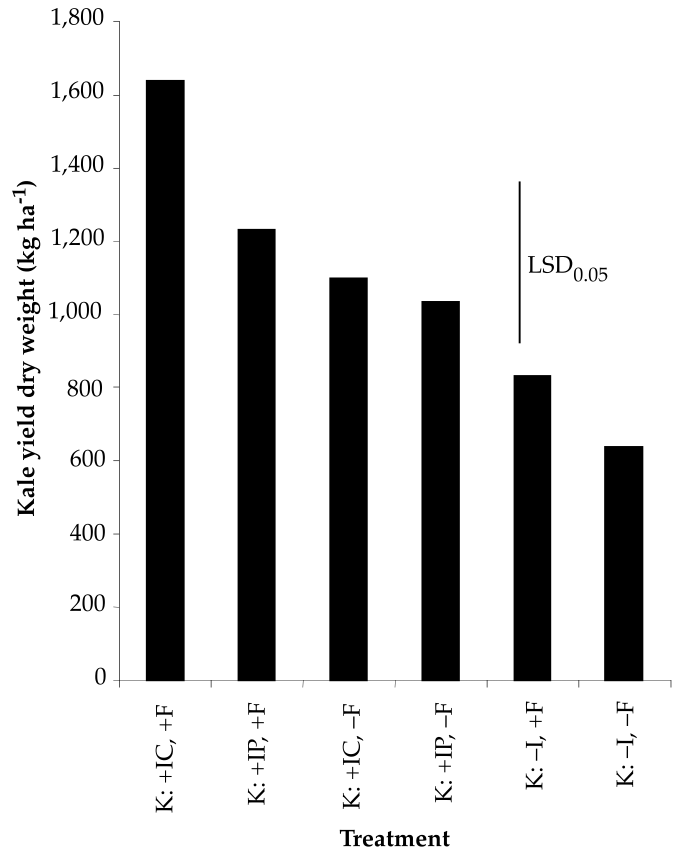

Figure 5. Leaf dry matter yield of kale as affected by irrigation and fertilization.

No other crops were grown in the neighborhood of the trial plots, and a higher pest incidence on the trial plots was witnessed. Therefore, lower yield in the second season bean compared to the first season could be due to the effects of pests and diseases that occurred due to late planting in the second season.

Fresh weight and dry weight yields of kale are shown in Figures 4 and 5. There were significant differences ($P = 0.05$) in kale fresh leaf yield among treatments. All treatments had higher fresh leaf weight and dry leaf weight than the non-irrigated, unfertilized control. When pond effluent was substituted for canal water, the net effect was a reduction in yield irrespective of nutrient application. A similar trend was observed for French bean in the first season. Yield reduction in kale owing to use of pond effluent observed in this study was related to moisture supply, probably due to poor water distribution and, hence, excess or insufficient water and inadequate nutrients reaching the kale crops. The F1 0.75-in filtration systems proved to be inadequate, releasing a low volume of water to the drip tapes and resulting in irregular clogging of the emitters and poor distribution of water along the drip irrigation lines. An imperfect and discontinuous drip irrigation system resulted in a continuous water stress as the moisture reservoir available to crops was extremely small. Discontinuously operated drip irrigation systems pose a disadvantage of moisture depletion and crop stress (Hillel, 1980).

Table 6. Leaf nutrient concentration of kale.

Treatment	Total N	P	K	Mn	Fe	Al
	g kg^{-1}			*mg kg^{-1}*		
K: –I, –F	56.1	3.9	2.34	34.1	358	303
K: –I, +F	57.6	3.8	1.54	46.9	310	186
K: +IC, –F	58.2	3.8	2.09	33.2	258	157
K: +IP, –F	57.6	5.6	1.83	43.7	550	330
K: +IC, –F	54.0	4.2	2.00	31.7	232	151
K: +IP, +F	54.7	5.2	1.99	52.4	389	172
LSD	8.8	1.3	0.50	22.5	507	390
C.V.	7.7	21.9	16.80	31.7	72	78

Table 6 shows foliar nutrient concentrations in kale leaves. Significant differences ($P = 0.05$) were observed in leaf phosphorus concentration. No significant differences were observed for leaf nitrogen, aluminum, iron, manganese, potassium, and magnesium concentrations. Irrigation with canal water with fertilizer application had the highest leaf phosphorus concentration, followed by pond water with fertilizer application. The lowest leaf phosphorus concentration was observed with zero fertilizer with or without irrigation, suggesting that the phosphorus taken up by kale was largely from applied fertilizers and not irrigation water. Similar to first season French bean results, it is apparent that pond water supplies inadequate nutrients to kale and that a larger filtration system is required to provide good quality pond effluent for drip irrigation.

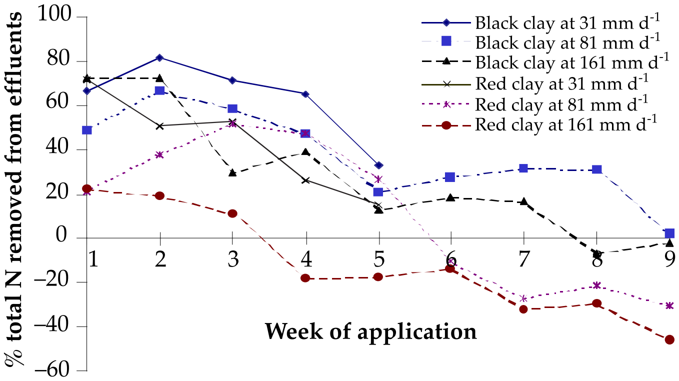

Figure 6. Nitrogen removal trends by soil in the columns at varying pond effluent application intensities.

Pond Effluent Nutrient Removal by Soil Experiment

Removal of total nitrogen from fish pond effluent via irrigation to soil columns at varying irrigation amounts is shown in Figure 6. In the first two weeks, 75% of applied nitrogen was removed from effluents in the 31 mm d^{-1} irrigation treatment by the black clay soil, followed by the 161 mm d^{-1} (72%) and 81 mm d^{-1} (60%) treatments. Within the same period, 60% of total nitrogen was removed from the effluent by red clay soil at the 31 mm d^{-1} effluent application intensity, 35% in the 81 mm d^{-1} effluent application intensity, and 20% in the 161 mm d^{-1} application intensity.

The highest percent nitrogen removal (70% for red clay soil and 84% for black clay soil) was observed with application rates of 31 mm d^{-1} during the first week, while the 161 mm d^{-1} effluent application rate resulted in 20% nitrogen removal in the same period for red clay soil. The intermediate 81 mm d^{-1} irrigation treatment resulted in 37% nitrogen removal from pond effluent added to red clay soil. High effluent loading resulted in less effective nitrogen removal. Black clay soil was 20%, 42%, and 72% more effective than red clay soil in nitrogen removal at 31, 81, and 161 mm d^{-1} effluent application intensities, respectively. Total nitrogen removal declined rapidly to less than 30% after the third week in the 81 mm d^{-1} and 161 mm d^{-1} application intensities.

Table 7 shows total nitrogen enrichment in soil after passing pond effluents through. Black clay soil retained 2.7%, 145%, and 155% more total nitrogen than red clay at the 31, 81, and 161 mm d^{-1} rates of effluent application, respectively. Low saturated hydraulic conductivity of black soil (Table 1) reduced the rate of downward movement of water, increasing contact time between nitrogen in effluent and the soil exchange surface, resulting in higher nitrogen adsorption. Water was probably transported through the small pores in the soil profile, enhancing adsorption of ammonia and nitrate. With alkaline pH (Table 1), black soil retained more total nitrogen than red clay soil; sorption of NO_3 was reported to increase with increased soil pH (Black and Waring, 1979). Sorption of NO_3 on positively charged surfaces delayed downward movement of NO_3. The location and rate of movement of the NO_3 front was determined primarily by water movement and adsorption in the two soils (Bohn et al., 1985). Transport of NO_3 was slow in black clay since displacement of the soil solution was less rapid. Due to expansion of the black soil upon wetting, volumetric water content was high (Russel, 1988), and assuming that the incoming water displaced the resident soil solution, the downward movement of the soil solution was less in black than red soil. For an equal quantity of effluent applied, dissolved forms of nitrogen were displaced farther in red clay soil and the soluble nitrogen front advanced faster than in black clay soil.

Table 7. Increase in total N content in the soil after pond effluent application

Application Intensity (mm d^{-1})	Red Clay	Black Clay	$LSD_{0.05}$	SE	C.V.
	Soil Total N (g m^{-2})				
31	1.1	1.13	0.48	0.16	40
81	0.51	1.25	0.35	0.12	52
161	0.44	1.12	0.38	0.13	44

For red clay soil, total nitrogen in pond effluent was equal to that in the through-flow water from the soil columns after 23 days and 40 days of operation in the 161 mm d^{-1} and 81 mm d^{-1} effluent application intensities, respectively, implying zero nitrogen removal. However, zero nitrogen removal was not observed in the 31 mm d^{-1} application intensity (Figure 6). In black clay soil, zero nitrogen removal was observed at 161 mm d^{-1} application intensity after 54 days; the other two application intensities did not attain zero removal. Any further application of the pond effluents after reaching zero nitrogen removal resulted in higher levels of total nitrogen in soil column through-flow than in pond effluent. This negative nitrogen budget implies nitrogen addition by the soil in the columns to the through-flow water, which can be attributed to the arrival of the NO_3 front at the bottom of the soil column. This observation agrees with those reported by Lance and Whistler (1972) and Pell and Nyberg (1989).

Figure 7 shows phosphorus removal trends from black and red clay soils. Over 60% of total phosphorus applied in effluent was removed at all application intensities. Concentration of phosphorus in through-flow from soil columns remained low from the second week throughout the experimental period. The efficiency of phosphorus removal was about 75% in black clays and 80% in red clays at all loading intensities. Phosphorus retained in the soil was mainly a product of application

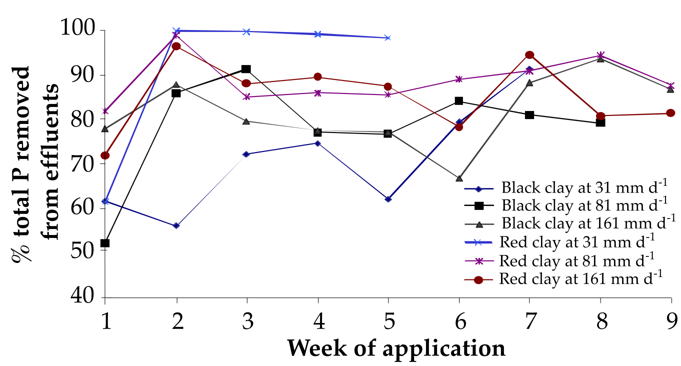

Figure 7. Phosphorus removal trends by soil in the columns at varying pond effluent application intensities.

Table 8. Increase in dilute-acid-extractable phosphorus content in the column soil after pond effluent application.

Application Intensity (mm d^{-1})	Red Clay	Black Clay	$LSD_{0.05}$	C.V.
	Soil Extractable P ($g\ m^{-2}$)			
31	1.03	0.99	0.16	14
81	1.5	1.03	0.83	46
161	1.34	1.02	0.24	23

rate and phosphorus concentration; similar observations were made by Chardon et al. (1997). The fact that very low levels of phosphorus were found in effluent from the soil columns suggests that most of the phosphorus was rapidly adsorbed to soil particles. No tendency for phosphorus saturation was observed with the loading rates used in this study, contrary to observations made by Pardue et al. (1994), who used higher concentration effluents.

Phosphorus sorption onto soil from pond effluents occurred where phosphorus was exchanged with other anions and with metal ligands in the soil or was changed from physical sorption to chemisorption, precipitated as insoluble phosphorus compounds, incorporated into hydroxy-aluminum or iron polymers, or diffused into the crystal lattice of soil minerals (Bohn et al., 1985). Data on column soil enrichment with dilute-acid-extractable phosphorus ($g\ m^{-2}$) pond effluent application are presented in Table 8. There was no significant difference ($P = 0.05$) in total phosphorus retention between the two soils as shown by an increase in dilute-acid-extractable phosphorus; however, red clay soil retained 4%, 45%, and 31% more phosphorus than black clay soil at the 31, 81, and 161 mm d^{-1} rates of effluent application, respectively.

CONCLUSIONS

Fish pond effluent applied as irrigation and as a supplemental source of nutrients is beneficial for French bean and kale growth and yield. However, pond effluent should not be used as the primary source of nitrogen and phosphorus for crops owing to its low concentration of these elements. Moreover, the small amount of nitrogen and phosphorus in pond water does not justify adjustment of recommended nitrogen and phosphorus rates for crops.

Nutrient-rich pond water supports growth of phytoplankton and other organisms in the pond. Phytoplankton and suspended solids present in pond water due to activities of fish increase turbidity of the water, causing clogging of distribution pipes, drip lines, and water emitters. The resulting poor water distribution leads to nonuniform crop stands and low yields. Improving filtration using Alkal 1.5-in in place of F1 0.75-in filters led to better French bean yield response to pond effluent application. Improved filtration via larger filters is therefore essential. Similar conclusions are apparent for growing kale under drip irrigation.

In the soil column study, removal of nitrogen from pond effluent was high in the first three weeks of application, but rapidly declined with time. Continuous application of pond effluent at rates of 81 and 161 mm d^{-1} for periods of 40 and 23 days, respectively, saturates the soil's ability to retain total nitrogen from pond effluent with total nitrogen concentrations ranging from 1.33 mg l^{-1} to 6.30 mg l^{-1}. As effluent leaches, soil enrichment with nitrogen is higher in black than in red clay soil due to a longer residence time of the effluent, allowing adsorption. Soil treatment resulted in an average 80% removal of total phosphorus from pond effluent for up to ten weeks. Pond effluent application at a rate of 81 mm d^{-1} in red clay soil resulted in the highest soil phosphorus enrichment, while an application at a rate of 31 mm d^{-1} in black soil had the least enrichment. Black and red clay soils in the Sagana, Kenya, area are able to retain substantial amounts of nitrogen and phosphorus from pond effluent and are most effective in removing phosphorus.

ANTICIPATED BENEFITS

When fish are recovered from ponds, the effluent is often drained, presenting both an environmental challenge and an agricultural opportunity. Application of chemical fertilizers in ponds and activities of fish increase the nutrient concentration of pond water. Application of pond water to crops during fish grow-out is feasible, but filters capable of removing particulates will be required if it is to be delivered through a drip irrigation system. Nutrient enrichment of pond water during aquaculture production is insufficient to meet crop nutrient demand, and fertilizer recommendations for crops should not be altered when pond water is used as an irrigation source. The soils in the Sagana, Kenya, region are capable of removing substantial quantities of nitrogen and phosphorus from pond effluent that otherwise would contribute to eutrophication of receiving water bodies.

LITERATURE CITED

Al-Jaloud, A.A., G. Hussain, A.A. Alsadon, A.Q. Siddiqui, and A. Al-Najada, 1993. Use of aquaculture effluent as a supplemental source of nitrogen fertilizer to a wheat crop. Arid Soil Res. Rehab., 7(3):233–241.

Black, A.S. and S.A. Waring, 1978. Adsorption of nitrate, chloride and sulfate by some highly weathered soils from southeast Queensland. Aust. J. Soil Res., 17:271–272.

Bohn, H., B.L. McNeal, and G.A. O'Connor, 1985. Soil Chemistry, Second Edition. John Wiley and Sons, New York, 341 pp.

Boyd, C.E., 1990. Water Quality in Warm Water Fish Ponds. Alabama Agricultural Experiment Station, Auburn University, Alabama, 482 pp.

Chardon, W.J, O. Oenema, P. del Castilho, R. Vreisema, J. Japenga, and D. Blaauw, 1997. Organic phosphorus in solutions and through-flows from soils treated with animal slurries. J. Environ. Qual., 26:372–378.

Government of Kenya, 1997. National Fisheries Statistics Bulletin. Department of Fisheries, Ministry of Natural Resources, Nairobi, Kenya.

Hillel, D., 1980. Applications of Soil Physics. John Wiley and Sons, New York, 301 pp.

Hue, N.V. and C.E. Evans, 1986. Procedures Used for Soil and Plant Analysis by the Auburn University Soil Testing Laboratory. Alabama Agricultural Experiment Station, Auburn University, Alabama, 31 pp.

Hussein, G. and A.A. Al-Jaloud, 1995. Effects of irrigation and nitrogen on water use efficiency of wheat in Saudi Arabia. Agric. Water Manage., 27(2):143–153.

Klute, A., G.S. Campbell, R.D. Jackson, M.M. Mortiland, and D.R. Nielson, 1986. Methods of Soil Analysis. Part 1. Physical and

Mineralogical Methods, Second Edition. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, 810 pp.

Lance, J.C. and F.D. Whistler, 1972. Nitrogen balance in soil columns intermittently flooded with secondary sewage effluent. J. Environ. Qual., 1:80–86.

Pardue, J.H., R.D. De Laune, W.H. Patrick, Jr., and J.B. Nyman, 1994. Treatment of alligator farm wastewater using land application. Aquacult. Eng., 13:129–146.

Pell, M. and F. Nyberg, 1989. Infiltration of wastewater in a newly started pilot sand-filter system: III. Transformation of nitrogen. J. Environ. Qual., 18:463–467.

Prinsloo, J.F. and H.J. Schoonbee, 1987. Investigations into feasibility of a duck-fish-vegetable integrated agriculture-aquaculture system for developing areas in South Africa. Water S.A., 13(2):109–118.

Russel, T., 1988. Soil Conditions and Plant Growth. Oxford University Press, London, 720 pp.

Sumari, O., 1982. Report on fish farm effluents in Finland. In: J.S. Alabaster (Editor), Report of the EIFAC Workshop on Fish-Farm Effluents, Silkeborg, Denmark, 26–28 May 1981. European Fisheries Advisory Commission (EIFAC) Tech. Paper 41. Food and Agriculture Organization, Rome, pp. 21–28.

Tisdale, S.L., W.L. Nelson, and J.D. Beaton, 1990. Soil Fertility and Fertilizers. MacMillan, Inc., New York, 480 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Fate of Methyltestosterone in the Pond Environment: Detection of MT in Pond Soil from a CRSP Site

Ninth Work Plan, Effluents and Pollution Research 2B (9ER2B)
Final Report

Wilfrido M. Contreras-Sánchez and Martin S. Fitzpatrick
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Carl B. Schreck
Oregon Cooperative Fishery Research Unit
Biological Resources Division—U.S. Geological Survey
Department of Fisheries and Wildlife
Oregon State University, Corvallis, Oregon, USA

Abstract

The following study examined the persistence of 17α-methyltestosterone (MT) in the environment after its use for masculinizing Nile tilapia in nursery ponds at two CRSP sites: Sagana Fish Farm, Kenya, and the Universidad Juárez Autónoma de Tabasco (UJAT), Mexico. Fry were treated with a masculinizing dose of MT (60 mg kg^{-1}) for four weeks beginning at the initiation of feeding. Concentrations of MT were determined by radioimmunoassay, which revealed that the levels of MT in the sediments from the Sagana Fish Farm had a mean value of 4,567 pg g^{-1}. The concentration of MT slightly decreased near the drain of the pond. Concentration of MT in water and sediments from UJAT showed background levels after first-time use of MT in the pond. These results suggest that accumulation of MT may take place after masculinization of a significant number of fish.

Introduction

Treatment of tilapia fry with 17α-methyltestosterone (MT)–impregnated food to produce all-male populations has become a common aquaculture practice. All-male populations are desirable because no energy is shunted toward reproduction and no competition for food with younger fish occurs (Green et al., 1997). Nevertheless, uneaten or unmetabolized food may leak significant amounts of MT into the pond environment, posing the risk of unintended MT exposure to hatchery workers as well as to aquatic and terrestrial organisms. We have investigated the fate of MT in semi-closed systems such as ponds. In a previous study, we reported that MT in the water peaks at approximately 3.6 ng ml^{-1} at 28 days after the onset of feeding with MT-impregnated food, decreasing to background levels by 35 days after the onset of feeding (i.e., within a week of ceasing treatment with MT-impregnated food). We have also shown that MT accumulated in sediments of model ponds, reaching 2 to 6 ng g^{-1} at 28 days after the onset of feeding with MT-impregnated food, and remained detectable in the soil between 2.8 and 2.9 ng g^{-1} after 84 days (eight weeks after ending treatment with MT-impregnated food), which demonstrated persistence of MT in soil for nearly three months after cessation of treatment (Contreras-Sánchez et al., 2000; Fitzpatrick et al., 2000). In this study we determined the concentration of MT in soil and water samples collected in nursery ponds from the Sagana Fish Farm (SFF), Kenya, and the Laboratory of Aquaculture at the Universidad Juárez Autónoma de Tabasco (UJAT), Mexico, after masculinizing treatment with MT (60 mg kg^{-1}) for four weeks. These two CRSP sites have different histories regarding MT usage; namely, MT has been used for several years at SFF while never used before at UJAT.

Methods and Materials

Sagana Fish Farm, Kenya

Soil samples were collected from three points (near pier, halfway between pier and drain, and near drain) in one of the sex-reversal ponds at the hatchery. Samples were then frozen and shipped to Oregon State University (OSU) for MT analysis.

Laboratory of Aquaculture at UJAT, Mexico

Nile tilapia (*Oreochromis niloticus)* fry were collected from a spawning tank. Fry were counted and randomly assigned to either MT-feeding or EtOH (vehicle)–feeding, and each treatment was triplicated. Replicates were housed in $1 \times 1 \times 0.75$ m hapas made of mosquito mesh, and hapas were placed in a 7×15 m earthen pond. The MT-treated replicates were located at one end of the pond and the control replicates at the other end.

MT-impregnated food was made by spraying crushed flaked food with MT dissolved in EtOH; control food was made by spraying crushed flaked food with EtOH. Fry were fed the MT (60 mg kg^{-1}) or control diet for four weeks (from 15 to 43 days post-fertilization [dpf]). Feeding rate was at 20% per calculated body weight for the first 23 days of treatment and then 10% per calculated body weight through day 28 of treatment (Popma and Green, 1990). After 28 days of dietary treatment (on 44 dpf), fry were fed with regular fish food. At 90 to 100 dpf, sex ratios were determined by microscopic examination (10 and 40X) of gonads using squash preparations in Wright's stain (Humason, 1972). The weights of sampled fish were recorded at this time.

To collect water and soil samples, seven sampling points were set along the pond as follows: points 1 and 7 were located under treatment hapas (1 = control treatment; 7 = MT treatment); all other sampling points were two meters apart from one another (points 2 to 6). Water samples (12 ml) were collected with pipettes into 15-ml polypropylene tubes and stored at –20°C until analysis for MT. Soil core samples were collected with long 1.25-cm-diameter PVC pipes, placed in whirl pak bags, had excess water poured off, and were stored at –20°C until shipment to OSU for analysis. Water and soil samples were taken at the onset (July 13) and end (August 9) of treatment.

Radioimmunoassay

For analysis of MT concentration, 1.0 ml of each water sample and 0.2 g of each soil sample were extracted in 8 ml of diethyl ether. The organic phase of each sample was collected in a new tube after the aqueous phase was snap-frozen in liquid nitrogen. The extraction procedure was repeated and the ether extracts were pooled for each sample and dried down in a SpeedVac. Each dried extract was reconstituted in 1 ml of phosphate-buffered saline containing gelatin. Aliquots of the reconstituted extracts were removed to 12 × 75 mm tubes for determination of MT concentration by radioimmunoassay (RIA). The RIA methods followed the procedure outlined in Fitzpatrick et al. (1986; 1987). Antisera specific to MT were purchased from UCB-Bioproducts SA, and ^{3}H-MT (Amersham) was generously donated by Dr. Gordon Grau of the Hawaii Institute of Marine Biology. Standards of known concentration of MT were made in EtOH and used in each assay to generate a standard curve. The assay was validated by demonstration of parallelism between serial dilutions of several samples and the standard curve, and by demonstration of low cross-reactivity with testosterone and 11-ketotestosterone. Extraction efficiency for MT for the RIA was checked by adding a known amount of ^{3}H-MT to water and soil (n = 5 for each) and then extracting the samples as described above. Once each of these tubes was reconstituted in 1 ml of phosphate-buffered saline containing gelatin, 0.5 ml was removed from each and the amount of radioactivity counted by scintillation spectroscopy (extraction efficiencies were 73.3% for water and 71.4% for soil). Concentrations of MT in water, soil, and interface at the various sample times were not compared statistically because of the limited sample size (n = 1 per date) and because the goal of the study was descriptive (presence/absence).

RESULTS

Sagana Fish Farm, Kenya

Soil samples obtained from Kenya showed MT concentrations between 3,900 and 4,800 pg g^{-1} (Figure 1), having the lowest concentration near the drain and similar values near the pier and the middle point. Unfortunately, we had no samples from ponds that have not been used for sex inversion to determine background levels.

Laboratory of Aquaculture at UJAT, Mexico

MT concentrations in soil at the beginning of the experiment ranged between 400 and 800 pg g^{-1} with a mean value of 482 pg g^{-1}. After 28 days of feeding we detected slightly higher values of MT, ranging between 320 and 900 pg g^{-1} with a mean of 691 pg g^{-1} (Figure 2). We observed no pattern related to the location of the treatments (i.e., sampling locations near MT-fed hapas did not show higher levels of MT). Detected values of MT in the water were low at both the onset and end of the experiment (Figure 3). Values ranged between 4 and 10 pg ml^{-1} of water (mean = 6.8 pg ml^{-1}) at the beginning and between 3.7 and 7 pg ml^{-1} (mean = 4.4 pg ml^{-1}) at the end of the experiment. The pond used for sex inversion at UJAT was never used before for treatments with MT.

DISCUSSION

Despite the widespread use of methyltestosterone for masculinizing tilapia in aquaculture facilities, little is known about the fate of this potent synthetic steroid in the pond environment. Few studies have been dedicated to detect MT or its metabolites in body tissues of the sex-inversed fish (Cravedi et al., 1989; Goudie et al. 1986a; 1986b; Curtis et al., 1991);

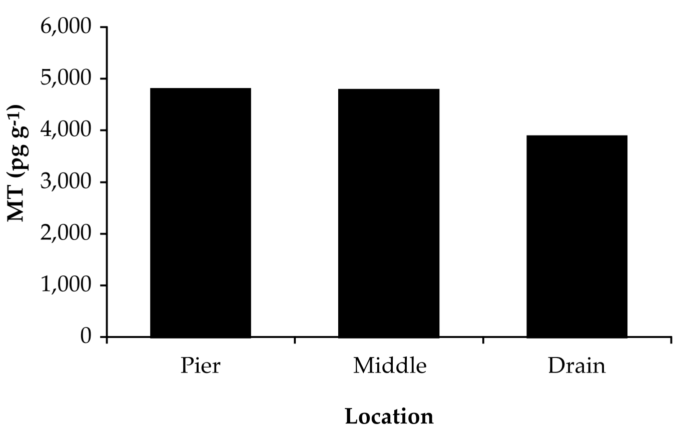

Figure 1. Concentration of methyltestosterone in sediments from a sex-reversal pond in Sagana Fish Farm, Kenya.

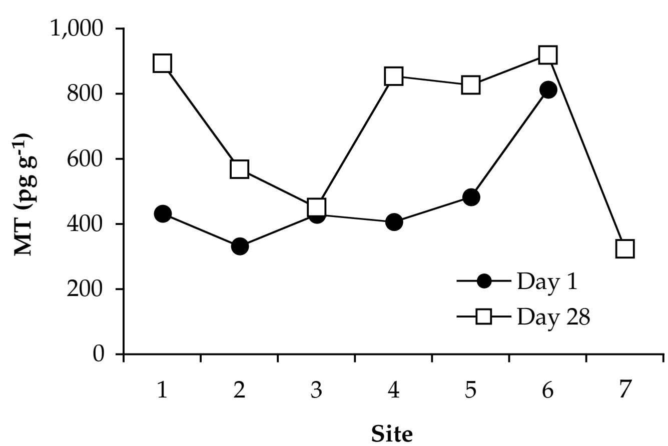

Figure 2. Concentration of methyltestosterone in sediments from UJAT, Mexico; sampling sites are distributed between control hapas (1) and MT-treated hapas (7).

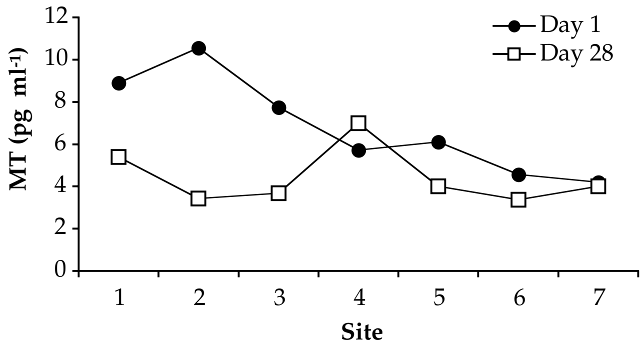

Figure 3. Concentration of methyltestosterone in water from UJAT, Mexico; sampling sites are distributed between control hapas (1) and MT-treated hapas (7).

however, little has been done to detect if MT or its metabolites can dissociate from the impregnated food and accumulate in the pond environment. Recent studies in our laboratory have shown that MT can be detected in the water during MT treatment and that it eventually accumulates and remains in the sediments of model ponds for up to eight weeks (Fitzpatrick et al., 1999; 2000).

We found that MT levels in sediments from SFF were higher at all three sampling points than any background levels that we have detected in previous studies (e.g., 200 to 400 pg g^{-1} at Oregon State University (Fitzpatrick et al., 1999); 700 to 900 pg g^{-1} at Auburn University (Phelps et al., 1999); and 400 to 800 pg g^{-1} at UJAT), suggesting that the usage history of MT at SFF ponds might have allowed an accumulation of MT in the sediments. Since we were not able to analyze soil samples from SFF ponds without a history of MT treatment, we cannot rule out the possibility that these high levels resulted from background levels of compounds that cross-react with the antibody used to measure concentrations. Nevertheless, these high levels from SFF soil samples point to the need for further monitoring. The values obtained from water and sediments from UJAT are low and show no trend regarding location of the treated hapas in the pond. These results suggest that MT levels are still near background values. Another possibility to explain these results is that the number of fish treated with MT is very low (n = 1500) and, considering the dimensions of the pond, the amount of MT used is too low to cause detectable accumulation.

In an effort to determine the fate of steroids used in experiments involving live fish and recirculating systems, Budworth and Senger (1993) reported that testosterone injected into rainbow trout (*Oncorhynchus mykiss*) leaked out of the fish body and eventually reached other fish present in the system. These authors highlighted the need to avoid exposure of untargeted organisms when steroids are administered in aquatic systems. Recent studies have reported that exposure of untargeted organisms to MT can result in biased sex ratios. Gomelsky et al. (1994) found significant masculinization of common carps (*Cyprinus carpio*) exposed to water used in MT-impregnated feeding trials. They also reported that the masculinizing effects of MT were stronger in recirculating systems than in tanks with flow-through water. These observations suggest that MT (and/or its metabolites) can persist in the water at concentrations capable of causing sex inversion. Incidental sex reversal in tilapias has been reported recently (Abucay and Mair, 1997; Abucay et al., 1997). These authors indicated that in aquaria and concrete tanks, sex ratios are significantly biased when non-target fish are housed in the same tank where groups of fish are fed with MT.

The potential risks posed by unintentional exposure to MT in aquacultural facilities are not confined to the androgenic effects of the steroid (e.g., masculinization of genital tract, gonads, or secondary sexual characteristics). In laboratory studies, it has been demonstrated that long-term exposure to MT can act as a weak hepatocarcinogen in male and female mice (Taylor et al., 1984). It has also been reported that long-term exposure to MT eliminated male sexual behavior and suppressed serum testosterone levels in male rats (Clark et al., 1997). Furthermore, it has been demonstrated that overexposure of fish to MT can result in paradoxical feminization due to a hypothesized conversion of MT into estrogen by the enzymatic action of aromatase (Piferrer and Donaldson, 1991; Piferrer et al., 1993; Eding et al., 1999; Rinchard, et al., 1999). Unfortunately, very little is known about the potential effects of residual MT in ponds where large amounts of fish are masculinized. In our laboratory, we found that reusing tanks with sediments from trials involving feeding with MT-impregnated food resulted in the appearance of few intersex fish; however, we did not address the possibility of other effects. These findings suggest that caution should be used when using steroids for aquacultural purposes and that more research is needed to understand the fate of exogenous hormones in the pond environment as well as the potential effects on the health of non-target organisms, including farm workers.

ANTICIPATED BENEFITS

We detected the anabolic steroid 17α-methyltestosterone (MT) in the sediments of sex-inversion ponds from the Sagana Fish Farm while background levels were found at UJAT, Mexico. Although we have no evidence that the detected levels at SFF represent a health or environmental risk, these results suggest that caution should be exercised because of the risk of unintended MT exposure of pond workers, fish, and other organisms.

LITERATURE CITED

Abucay J.S. and G.C. Mair, 1997. Hormonal sex reversal of tilapias: Implications of hormone treatment application in closed water systems. Aquacult. Res., 28:841–845.

Abucay J.S., G.C. Mair, D.O.F. Skibinski, and J.A. Beardmore, 1997. The occurrence of incidental sex reversal in *Oreochromis niloticus* L. In: K. Fitzsimmons (Editor), Proceedings from the Fourth International Symposium on Tilapia in Aquaculture. Natural Resource, Agriculture, and Engineering Service (NRAES), Cooperative Extension, Ithaca, New York, pp. 729–738.

Budworth, P.R. and P.L. Senger, 1993. Fish to fish testosterone transfer in a recirculating system. Prog. Fish-Cult., 55:250–254.

Clark, A.S., E.V. Harrold, and A.S. Fast, 1997. Anabolic-androgenic steroid effects on the sexual behavior of intact male rats. Horm. Behav., 31:35–46.

Contreras-Sánchez, W.M., M.S. Fitzpatrick, R.H. Milston, and C.B. Schreck, 2000. Masculinization of Nile tilapia with steroids: Alternative treatments and environmental effects. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, and S.O. Stefansson (Editors), Proceedings of the Sixth International Symposium on Reproductive Physiology of Fish. John Grieg, A.S., Bergen, Norway, pp. 250–252.

Cravedi, J.P., G. Delous, and D. Rao, 1989. Disposition and elimination routes of 17α-methyltestosterone in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci., 46:159–165.

Curtis, L.R., F.T. Diren, M.D. Hurley, W.K. Seim, and R.A. Tubb, 1991. Disposition and elimination of 17α-methyltestosterone in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 99:193–201.

Eding, E.H., A. Bouwmans, and J. Komen, 1999. Effects of methyltestosterone and 11-keto-androstenedione on sex differentiation in African catfish, *Clarias gariepinus*. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, and S.O. Stefansson (Editors). Proceedings of the Sixth International Symposium on Reproductive Physiology of Fish. John Grieg, A.S. Bergen, Norway, p. 258.

Fitzpatrick, M.S., G. Van Der Kraak, and C.B. Schreck, 1986. Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. Gen. Comp. Endocrinol., 62:437–451.

Fitzpatrick, M.S., J.M. Redding, F.D. Ratti, and C.B. Schreck, 1987. Plasma testosterone concentration predicts the ovulatory response of coho salmon (*Oncorhynchus kisutch*) to gonadotropin-releasing hormone analog. Can. J. Fish. Aqua. Sci., 44:1351–1357.

Fitzpatrick, M.S., W.M. Contreras-Sánchez, R.H. Milston, R. Hornick, G.W. Feist, and C.B. Schreck, 1999. Detection of MT in aquarium after treatment with MT food. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 81–84.

Fitzpatrick, M.S., W.M. Contreras-Sánchez, and C.B. Schreck, 2000. Methyltestosterone persists in the environment after use for masculinizing Nile tilapia. In: B. Hart and B. Brown (Co-Chairs), Book of Abstracts, Aquaculture America 2000. World Aquaculture Society, New Orleans, Louisiana, p. 112.

Gomelsky, B., N.B. Cherfas, Y. Peretz, N. Ben-Dom, and G. Hulata, 1994. Hormonal sex inversion in the common carp (*Cyprinus carpio* L.). Aquaculture, 126:265–270.

Goudie, C.A., W.L. Shelton, and N.C. Parker, 1986a. Tissue distribution and elimination of radiolabelled methyltestosterone fed to sexually undifferentiated blue tilapia. Aquaculture, 58:215–226.

Goudie, C.A., W.L. Shelton, and N.C. Parker, 1986b. Tissue distribution and elimination of radiolabelled methyltestosterone fed to adult blue tilapia. Aquaculture, 58:227–240.

Green, B.W., K.L. Veverica, and M.S. Fitzpatrick, 1997. Fry and fingerling production. In: H. Egna and C. Boyd (Editors), Dynamics of Pond Aquaculture. CRC Press, Boca Raton, Florida, pp. 215–243.

Humason, G.L., 1972. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco, 641 pp.

Phelps, R.P., M.S. Fitzpatrick, W.M. Contreras-Sánchez, R.L. Warrington, and J.T. Arndt, 1999. Detection of MT in pond water after treatment with MT food. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 57–59.

Piferrer, F. and E.M. Donaldson, 1991. Dosage-dependent differences in the effect of aromatizable and nonaromatizable androgens on the resulting phenotype of coho salmon (*Oncorhynchus kisutch*). Fish Physiol. Biochem., 9:145–150.

Piferrer, F., I.J. Baker, and E.M. Donaldson, 1993. Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex differentiation in genotypic female chinook salmon (*Oncorhynchus tshawytscha*). Gen. Comp. Endocrinol., 91:59–65.

Popma, T.J. and B.W. Green, 1990. Sex Reversal of Tilapia in Earthen Ponds. Research and Development Series No. 35. International Center for Aquaculture and Aquatic Environments, Auburn University, Alabama, 15 pp.

Rinchard, J., K. Dabrowski, M.A. García-Abiado, and J. Ottobre, 1999. Uptake and depletion of plasma 17α-methyltestosterone during induction of masculinization in muskellunge, *Esox masquinongy*: Effect on plasma steroids and sex reversal. Steroids, 64:518–525.

Taylor, W., S. Snowball, and M. Lesna, 1984. The effects of long-term administration of methyltestosterone on the development of liver lesions in BALB/c mice. J. Pathol., 143:211–218.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Fate of Methyltestosterone in the Pond Environment: Impact of MT-Contaminated Soil on Tilapia Sex Differentiation

Ninth Work Plan, Effluents and Pollution Research 2C (9ER2C)
Final Report

Wilfrido M. Contreras-Sánchez and Martin S. Fitzpatrick
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Carl B. Schreck
Oregon Cooperative Fishery Research Unit
Biological Resources Division—U.S. Geological Survey
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Abstract

The following study examined the effect of environmentally persistent 17α-methyltestosterone (MT) on sex differentiation of Nile tilapia (*Oreochromis niloticus*). Three different broods of fry were treated one after the other with a masculinizing dose of MT (60 mg kg^{-1}) for four weeks beginning at the initiation of feeding in model ponds which consisted of 60-l tanks that contained 5 kg of soil. Four weeks after the last MT treatment, two different broods of tilapia fry were fed control feed while being maintained in the tanks that had contained the MT-treatment groups. Water and soil samples were taken before the onset of treatment and on the last day of treatment during each treatment cycle. Concentrations of MT were determined by radio-immunoassay, which showed that the levels of MT in the water were elevated between about 200 and 1,250 pg ml^{-1} during the three cycles of MT and then returned to background levels during the remaining cycles of control diet feeding. Analysis of water samples taken shortly after adding the MT-impregnated food to the tanks revealed that MT leaks into the water within a minute of treatment. The levels of MT in the soil were elevated to about 2,000 pg g^{-1} after one feeding cycle and remained elevated between 1,400 and 3,300 pg g^{-1} through three months after the conclusion of the last MT feeding cycle, including the time during which the control-fed fry were raised in these tanks. The sex ratios of the groups fed control food while being maintained in the tanks that had contained the MT-treatment groups were not different from control fish; however, several individuals in the former groups had intersexual gonads, suggesting some impact on development.

Introduction

Treatment of tilapia fry with methyltestosterone (MT)-impregnated food for producing all-male populations is a common practice throughout the world. All-male populations offer the production advantage of enhanced growth potential (Green et al., 1997). However, significant "leakage" of MT into the pond environment may occur from uneaten or unmetabolized food. This leakage poses a risk of unintended exposure of hatchery workers as well as fish or other non-target aquatic organisms. Furthermore, in some countries, pond sediments are dredged and used to "prepare soil" for crop production, thereby spreading the risk of MT exposure to terrestrial organisms.

Previously, we conducted studies on the fate of MT in small 3.7-l model ponds that indicated that MT leaked into the water during the feeding treatment period but decreased to background levels once the treatment ended. However, MT was found in the soil of these model ponds at the end of the treatment period and persisted in the soil for at least three weeks after treatment (through the end of the study). Thus, MT persists in the soil for a considerable time after treatment of tilapia fry, suggesting the possibility that if the sediments were subsequently disturbed by tilapia during nest building or searching for food, then MT might be resuspended into the water, which could cause further exposure of fry.

The following study was undertaken to determine if MT contamination of the soil could affect sex differentiation of subsequent broods of fry that were placed in the same aquaria.

Methods and Materials

Breeding families of Nile tilapia (*Oreochromis niloticus*) were placed in 200-l aquaria (one male to three females) where water temperature was maintained at 28 ± 1°C. Once breeding occurred, the other fish were removed and the brooding female was left to incubate the progeny. The female was forced to release the fry from her mouth at 280 Celsius Temperature Units (CTU) or ten days post-fertilization (dpf). Fry were removed from the tank and randomly assigned to model ponds (60-l aquaria) at a stocking rate of 175 fry $tank^{-1}$ (1 fry per 7.9 cm^2). This value corresponds to 1/3 of the recommended stocking rate (by area) for masculinization of 3,000 fry m^{-2} (Popma and Green, 1990). However, the volume used in our model ponds was limited by the tank height, conferring a stocking rate by volume of 3.5 fish l^{-1} (0.5 fish l^{-1} more than the recommended stocking rate). Model ponds were set up two days before the expected time of fry release. Each aquarium contained 5,000 g (approximately 3 cm) of packed soil, which was obtained from a meadowed hill near the Principal Investigator's house located north of Corvallis, Oregon. Each model pond contained 50 l of dechlorinated tap water.

To determine the effect of MT contamination in sediments on sex differentiation of tilapia, three separate broods of fry were treated with MT-impregnated food in sequence (with 32 and 11 days, respectively, elapsing between the end of one treatment and the beginning of the subsequent treatment). Following MT treatment, two separate broods of fry were fed a control diet in sequence in the MT-treatment tanks (11 and 36 days, respectively, between the end of one 4-wk feeding and the beginning of the next). At the same time as all these treatment groups, different groups of fish from the same brood were fed a control diet. All experiment treatments were replicated. MT-impregnated food was made by spraying crushed flaked food with MT dissolved in EtOH; control food was made by spraying crushed flaked food with EtOH. Fry were initially fed with Hatchfry Encapsulon™ (Argent Chemical Laboratories) until 15 dpf. Delaying treatment allowed the fry to reach the initial size proposed by Popma and Green (1990) for treatment. Fry were fed MT (60 mg kg^{-1}) or control diet for four weeks (from 15 to 43 dpf). Water temperature in the model ponds was maintained at 28 to 30°C, except for the first week of the study when water temperatures were 24 to 25°C. Temperature was monitored daily; pH, ammonia, nitrites, and dissolved oxygen were checked weekly. The feeding rate was at 20% per calculated body weight for the first 23 days of treatment and then 10% per calculated body weight through day 28 of treatment (Popma and Green, 1990). Water lost by evaporation from the model ponds was restored twice weekly.

After 28 days of dietary treatment (on 44 dpf), fry were transferred to the Oregon State University Warm Water Research Laboratory, Corvallis, Oregon, and reared in 75-l fiberglass tanks in a recirculating system. Water temperature in the grow-out system was maintained at 28 ± 1°C, and water quality parameters described above were also checked. At 80 to 90 dpf, sex ratios were determined by microscopic examination (10 and 40X) of gonads using squash preparations in Wright's stain (Humason, 1972). The weights of sampled fish were recorded at this time.

Water samples (12 ml) were collected with pipettes into 15-ml polypropylene tubes and stored at –20°C until analysis for MT. Soil core samples were collected with 0.5-cm-diameter plastic pipes, placed in whirl pak bags, excess water poured off, and stored at –20°C until analysis. In tanks with gravel and no substrate, a film of fine sediments was formed. This material was collected with a pipette and stored at –20°C until analysis. Fine sediments were precipitated by centrifugation, and then a 1-ml subsample was dried in an oven at 50°C. For analysis of MT concentration, 1.0 ml of each water sample, 0.2 g of each soil sample, and 0.2 g of each fine sediment sample were extracted in 8 ml of diethyl ether. The organic phase of each sample was collected in a new tube after the aqueous phase was snap-frozen in liquid nitrogen. The extraction procedure was repeated, and the ether extracts were pooled for each sample and dried down in a SpeedVac. Each dried extract was reconstituted in 1 ml of phosphate-buffered saline containing gelatin. Aliquots of the reconstituted extracts were removed to 12 × 75 mm tubes for determination of MT concentration by radioimmunoassay (RIA). The RIA methods followed the procedure outlined in Fitzpatrick et al. (1986; 1987). Antisera specific to MT were purchased from UCB-Bioproducts SA, and ^{3}H-MT (Amersham) was generously donated by Dr. Gordon Grau of the Hawaii Institute of Marine Biology. Standards of known concentration of MT were made in EtOH and used in each assay to generate a standard curve. The assay was validated by demonstration of parallelism between serial dilutions of several samples and the standard curve and by demonstration of low cross-reactivity with testosterone and 11-ketotestosterone. Furthermore, soil samples were subjected to analysis by HPLC after extraction (as above), filtering, and reconstitution in MeOH to search for possible metabolites of MT. Extraction efficiency for MT for the RIA was checked by adding a known amount of ^{3}H-MT to water and soil, (n = 5 for each), and then extracting the samples as described above. Once each of these tubes was reconstituted in 1 ml of phosphate-buffered saline containing gelatin, 0.5 ml was removed from each and the amount of radioactivity counted by scintillation spectroscopy (extraction efficiencies were 73.3% for water and 71.4% for soil).

Sex ratios were analyzed using Fisher's exact test with exact p-values (a more conservative test than the chi-square test for small sample sizes) estimated in GraphPad Prism™. Intersex fish were counted as females for the purposes of analysis in order to be conservative. Concentrations of MT in water and soil at the various sample times were not compared statistically because of the limited sample size (n = 1 or 2 per date) and because the goal of the study was descriptive (presence/absence). The mean final weights of sampled fish were analyzed for differences between groups using a one-way ANOVA including mortality as a possible confounding variable. For all analyses, differences were considered statistically significant when the p-value (*P*) was less than 0.05.

RESULTS

Each cycle of MT treatment of tilapia fry resulted in significant masculinization; however, the efficacy of treatment differed between cycles of MT treatment (Figure 1). In the first cycle,

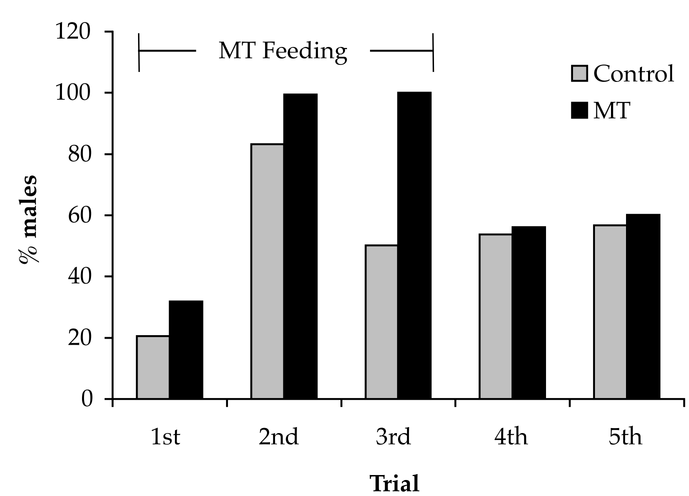

Figure 1. Masculinizing effects of MT in model ponds under consecutive feeding trials (first through third) and residual effects of MT in control-fed fish (fourth through fifth trials). Each treatment was run in duplicates.

the MT-treatment groups had 31.8% males compared with 20.5% males in the control groups; in the second cycle, the MT-treatment groups had 99.5% males compared with 83.2% males in the controls; and in the third cycle, the MT-treatment groups had 100% males compared with 50.2% males in the controls.

In the two subsequent cycles of MT treatment of tilapia fry all fed a control diet, no difference in sex ratios was found between groups in tanks that had been exposed to MT treatment compared to groups held in the control tanks (Figure 1).

The use of MT-impregnated food resulted in rapid leakage of MT into the water and subsequent deposition into the sediments. Within one minute of adding MT-impregnated food to the water, the levels of MT in the water jumped to nearly 100 pg ml^{-1}; within 15 minutes of feeding, levels of MT in the water were around 160 pg ml^{-1} (Figure 2). Mean levels of MT in the water were elevated for the first 60 days of the experiment, ranging between about 234 and 1,179 pg ml^{-1} (Figure 3), with levels dropping to background and remaining near background levels through the remaining days of the experiment. Mean levels of MT in the soil were elevated to about 15,976 pg g^{-1} after one feeding cycle; levels decreased to 2,332 after 58 days of treatment and remained elevated thereafter between 1,265 and 3,193 pg g^{-1} through three months after the conclusion of the last MT feeding cycle (Figure 4), including the time during which the control-fed fry were raised in these tanks. Four months after cessation of MT administration, an estimated 6.3 g of MT were still present in the soil.

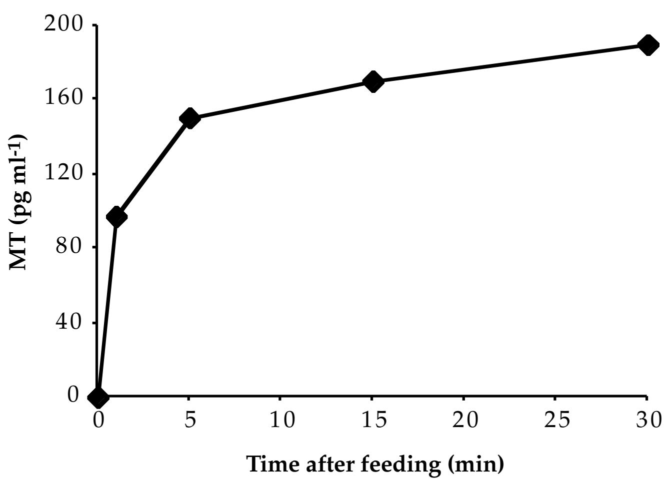

Figure 2. Detection of MT in water from an MT-fed model pond immediately after feeding.

Discussion

These experiments confirm previous studies (Fitzpatrick et al., 1999; Contreras-Sánchez et al., 2000) that demonstrated that considerable amounts of MT can be found in the pond environment during and after dietary treatment with MT. MT levels in water peaked during the three cycles of dietary treatment and returned to background levels thereafter. Soil MT reached high levels after the first cycle of MT treatment and remained elevated months after the conclusion of MT treatment.

A significant level of masculinization was obtained from dietary treatments with MT tanks; however, the level of masculinization achieved after the first cycle of treatment was below what has been reported by other researchers (Green et al., 1997). This confirms our earlier results (Fitzpatrick et al., 2000), which also had suboptimal masculinization (in these studies soil was not reused, so it is comparable to the first cycle of treatment). We had previously ruled out the possibility of improper diet preparation as an explanation for the lack of masculinization achieved in the first cycle. Once one cycle of MT treatment was complete, nearly complete masculinization was achieved in subsequent cycles of MT treatment. Therefore, some "conditioning" of the soil with MT may be necessary to optimize masculinization.

The subsequent groups of fish fed a control diet in the MT-treatment tanks did not show any statistically significant effect on sex ratios; however, instances of intersex fish were observed in each of the subsequent cycles of control diet feeding. No intersex characteristics were observed in any fish from the control diet tanks. This suggests that the level of MT contamination in the soil was not sufficient to alter sex ratios;

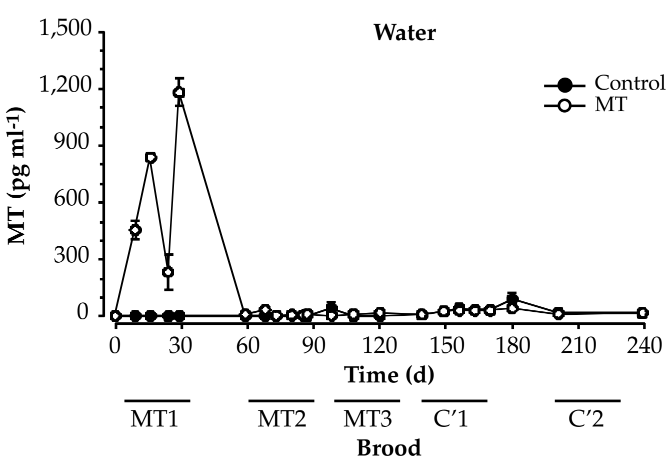

Figure 3. Mean values of MT (± SD) in water from control and MT-fed model ponds. Samples were taken at initiation and ending of each feeding trial.

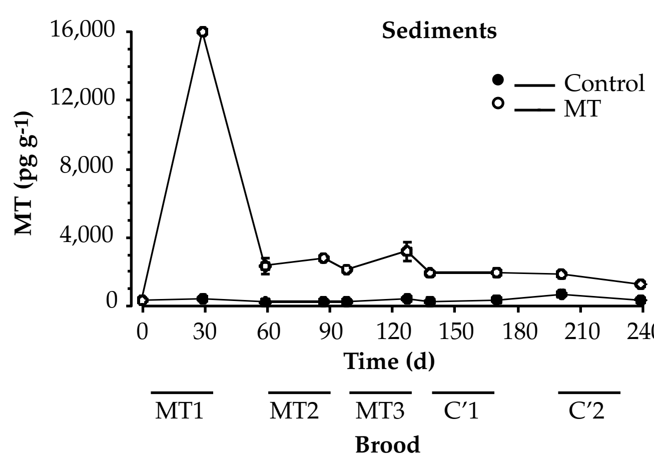

Figure 4. Mean values of MT (± SD) in sediments from control and MT-fed model ponds. Samples were taken at initiation and ending of each feeding trial.

nevertheless, some biological effects may have occurred. We have confirmed previous results (Fitzpatrick et al., 1999) showing that treatment of tilapia with MT results in leakage of this anabolic steroid into the environment. We have extended our previous work by showing that three cycles of MT feeding leads to contamination of the soil but does not alter the sex ratios of fish subsequently fed control diet.

Anticipated Benefits

The anabolic steroid 17α-methyltestosterone (MT) remains in the sediments of model ponds for up to three months after its use for masculinizing tilapia fry. The contaminated sediment is not associated with significant alterations in sex ratios of fry fed a control diet; nevertheless, the instances of intersexual individuals in these groups suggest that MT contamination may have a biological effect. These results will be useful in persuading those involved in MT use (e.g., farmers, researchers, and government workers) to exercise caution because of the risk of unintended MT exposure of pond workers, fish, and other organisms.

Literature Cited

Contreras-Sánchez, W.M., M.S. Fitzpatrick, R.H. Milston, and C.B. Schreck, 2000. Masculinization of Nile tilapia: Alternate treatments and environmental effects. In: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, and S.O. Stefansson (Editors), Proceedings of the Sixth International Symposium on the Reproductive Physiology of Fish. Institute of Marine Research and University of Bergen, Bergen, Norway, pp. 250–252.

Fitzpatrick, M.S., W.M. Contreras-Sánchez, and C.B. Schreck, 2000. Fate of methyltestosterone in the pond environment: Detection of MT in soil after treatment with MT food. In: K. McElwee, D. Burke, M. Niles, X. Cummings, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 109–112.

Fitzpatrick, M.S., G. Van Der Kraak, and C.B. Schreck, 1986. Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. Gen. Compar. Endocrin., 62:437–451.

Fitzpatrick, M.S., J.M. Redding, F.D. Ratti, and C.B. Schreck, 1987. Plasma testosterone concentration predicts the ovulatory response of coho salmon (*Oncorhynchus kisutch*) to gonadotropin-releasing hormone analog. Can. J. Fish. Aquat. Sci., 44:1351–1357.

Fitzpatrick, M.S., W.M. Contreras-Sánchez, R.H. Milston, R. Hornick, G.W. Feist, and C.B. Schreck, 1999. Detection of MT in aquarium water after treatment with MT food. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 81–84.

Green, B.W., K.L. Veverica, and M.S. Fitzpatrick, 1997. Fry and fingerling production. In: H. Egna and C. Boyd (Editors), Dynamics of Pond Aquaculture. CRC Press, Boca Raton, Florida, pp. 215–243.

Humason G.L., 1972. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco, 641 pp.

Popma, T.J. and B.W. Green, 1990. Sex Reversal of Tilapia in Earthen Ponds. International Center for Aquaculture and Aquatic Environments Research and Development Series No. 35, Auburn University, Alabama, 15 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Integrated Recycle System for Catfish and Tilapia Culture

Ninth Work Plan, Effluents and Pollution Research 3 (9ER3)
Final Report

Yang Yi and C. Kwei Lin
Aquaculture and Aquatic Resources Management
Agricultural & Aquatic Systems and Engineering Program
Asian Institute of Technology
Pathumthani, Thailand

James S. Diana
School of Natural Resources and Environment
The University of Michigan
Ann Arbor, Michigan, USA

Abstract

An experiment was conducted to test using effluents from intensive hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) ponds as nutrient inputs for Nile tilapia (*Oreochromis niloticus*) culture, which would reduce effluent effects from catfish culture, gain extra fish production at low cost, and possibly make aquaculture more profitable to farmers. Three treatments were done with three replicates each in seven 200-m^2 earthen ponds at the Asian Institute of Technology (AIT), Thailand, for 87 days. The treatments were A) catfish alone (control); B) catfish and tilapia without artificial water circulation; and C) catfish and tilapia with artificial water circulation. The pond used for control treatments was partitioned by 1.0-cm mesh plastic net into three equal compartments with 67 m^2 each for the replicates. The six other ponds were partitioned into two compartments: 1/3 for catfish and 2/3 for tilapia. Sex-reversed all-male Nile tilapia were stocked at 2 fish m^{-2}, and catfish at 25 fish m^{-2}. Catfish were fed twice daily with commercial pelleted feed at rates of 3 to 10% body weight per day. During the first month, tilapia compartments were fertilized weekly using urea and triple superphosphate (TSP) at rates of 28 kg N and 7 kg P ha^{-1} wk^{-1}. In the artificial water circulation treatment, the water in the catfish compartment was continuously circulated by a submersed pump to the tilapia compartment at a rate of one exchange per week, starting the second month.

There were no significant differences in growth performance of catfish among all treatments. Mean catfish weight at harvest ranged from 237.8 to 249.0 g, giving extrapolated net yields of more than 200 t ha^{-1} yr^{-1}. Final mean tilapia weight was 114.9 g in treatment B and 115.0 g in treatment C without significant differences. Although there was no significant difference in survival rates between treatments B (92%) and C (70%), high mortality of tilapia was observed in two replicates of treatment C due to heavy waste loading in the tilapia compartment by artificial water circulation. Extrapolated net tilapia yields (7.2 ± 1.3 t ha^{-1} yr^{-1} in treatment B and 4.9 ± 0.3 t ha^{-1} yr^{-1} in treatment C) obtained by using catfish wastes in this study were comparable to those achieved in organically and inorganically fertilized tilapia ponds. Nutrient budgets showed that total nitrogen and total phosphorus contents in pond effluents in treatments B and C were significantly lower than those in treatment A. Nile tilapia recovered 3.30 and 2.12% of total nitrogen and 1.29 and 0.84% of total phosphorus from feed wastes and fertilizer inputs in treatments B and C, respectively. Concentrations of total Kjeldahl nitrogen (TKN), total phosphorus (TP), and soluble reactive phosphorus (SRP) were also significantly lower in treatments B and C than in treatment A. This experiment indicates that Nile tilapia can effectively recover nutrients contained in wastewater of intensive catfish culture and suggests that natural water circulation between catfish and tilapia compartments can reduce nutrient contents in pond effluents and is cost-effective.

Introduction

Hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) has been one of the most popularly cultured freshwater fish in Southeast Asia. The present annual production in Thailand is estimated to be 50,000 tons. As an air breather, catfish can be grown at extremely high density (100 fish m^{-2}) with standing crops in pond culture reaching as high as 100 t ha^{-1} (Areerat, 1987). The fish are mainly cultured intensively and fed with trashfish, chicken offal, or pelleted feed, which generally cause poor water quality and heavy phytoplankton blooms throughout most of the grow-out period. To maintain tolerable water quality for fish growth, pond water is exchanged at later stages of the culture cycle (120 to 150 days). The effluents containing concentrated phytoplankton and nutrients are unsuitable to irrigate rice fields because unbalanced N:P ratios (high nitrogen content) can cause fruiting failure in the rice. Wastewater disposal from catfish ponds has become a serious problem, especially in Northeast Thailand where surface waters are in short supply. Farmers often discharge wastewater to adjacent rice fields, which are damaged by this input. To fully utilize the effluents, unproductive wetlands could be excavated for Nile tilapia (*Oreochromis niloticus*) culture. Such diversification and integration are regarded as important practices to enhance aquaculture sustainability (Adler et al., 1996; Pillay, 1996).

The wastes from catfish cultured in cages have been shown to be effective for producing phytoplankton to support Nile tilapia culture in the same pond (Lin et al., 1990; Lin and Diana, 1995). Similarly, tilapia reared in cages, feeding on phytoplankton in intensive channel catfish ponds, were shown

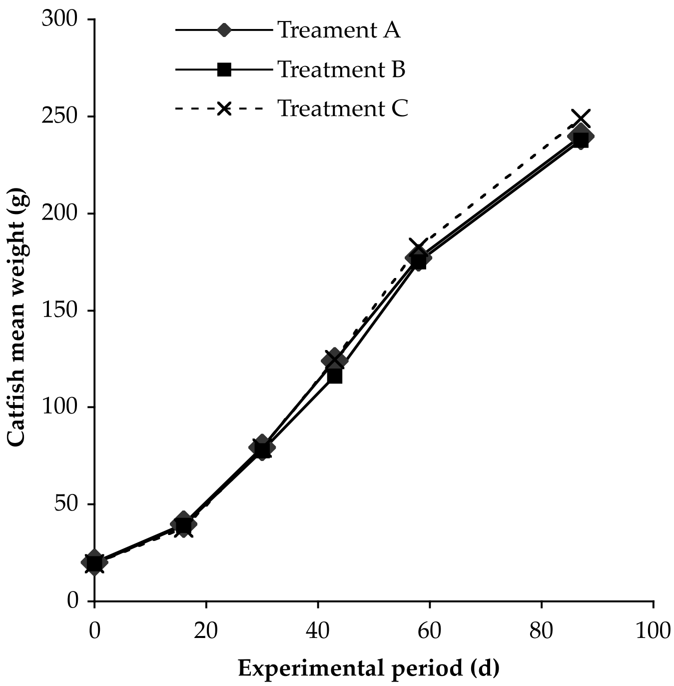

Figure 1. Growth of hybrid catfish in treatments A, B, and C over the 87-day experimental period.

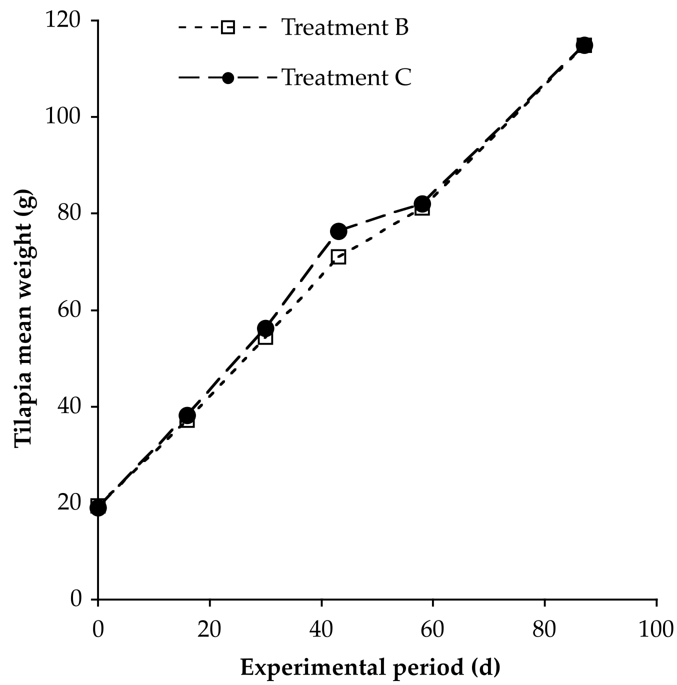

Figure 2. Growth of Nile tilapia in treatments B and C over the 87-day experimental period.

Table 1. Growth performance of hybrid catfish and Nile tilapia in integrated culture for 87 days.

Parameter	Catfish Treatment			Tilapia Treatment	
	A	*B*	*C*	*B*	*C*
STOCKING					
Density (fish m^{-2})	25	25	25	2	2
Total No. of Fish	1,675	1,675	1,675	266	266
Mean Weight (g fish^{-1})	20.0 ± 0.1	19.2 ± 0.2	19.3 ± 0.3	19.5 ± 0.1	19.1 ± 0.1
Total Weight (kg)	33.5 ± 0.2	32.2 ± 0.3	32.3 ± 0.5	5.2 ± 0.0	5.1 ± 0.0
HARVEST					
Mean Weight (g fish^{-1})	239.7 ± 3.8	237.8 ± 1.9	249.0 ± 7.9	114.9 ± 14.1	115.0 ± 13.5
Total Weight (kg)	383.3 ± 6.6	373.3 ± 5.8	394.3 ± 14.5	28.1 ± 4.0	20.7 ± 1.0
FCR (Feed Conservation Ratio)	1.25 ± 0.00[a]	1.31 ± 0.01[b]	1.26 ± 0.02[a]	----	----
SURVIVAL RATE (%)					
Mean	96.3	93.8	94.5	92.8	71.5
Range	79.6–99.4	88.6–97.4	91.6–96.8	65.4–99.3	21.2–99.9
WEIGHT GAIN					
Mean Weight Gain (g fish^{-1})	219.7 ± 3.7	218.6 ± 2.0	229.8 ± 8.0	95.4 ± 14.1	95.9 ± 13.4
Daily Weight Gain (g fish^{-1} d^{-1})	2.53 ± 0.04	2.51 ± 0.02	2.64 ± 0.09	1.10 ± 0.16	1.10 ± 0.15
Total Weight Gain (kg)	349.8 ± 6.8	341.1 ± 6.1	362.0 ± 14.6	23.0 ± 4.0	15.7 ± 1.0
NET YIELD (kg m^{-2} crop^{-1})	5.2 ± 0.1	5.1 ± 0.1	5.4 ± 0.2	0.2 ± 0.0	0.1 ± 0.0
NET YIELD (t ha^{-1} yr^{-1})	219.1 ± 4.3	213.6 ± 3.8	226.7 ± 9.1	7.2 ± 1.3	4.9 ± 0.3
GROSS YIELD (kg m^{-2} crop^{-1})	5.7 ± 0.1	5.6 ± 0.1	5.9 ± 0.2	0.2 ± 0.0	0.2 ± 0.0
GROSS YIELD (t ha^{-1} yr^{-1})	240.0 ± 4.2	233.8 ± 3.6	246.9 ± 9.1	8.9 ± 1.3	6.5 ± 0.3

* Mean values with different superscript letters in the same row within the same compartment were significantly different ($P < 0.05$).

to improve pond water quality as well as produce an extra crop (Perschbacher, 1995).

Therefore, the purposes of this study were to:

1) Use effluents from intensive catfish ponds as nutrients for tilapia culture ponds and thus reduce effluent effects from catfish culture and
2) Gain extra fish production at low cost, making aquaculture more profitable to farmers.

METHODS AND MATERIALS

The experiments were conducted in a completely randomized design in seven 200-m^2 ponds at the Asian Institute of Technology, Thailand. There were three treatments with triplicates each: A) catfish alone (control); B) catfish and tilapia without artificial water circulation; and C) catfish and tilapia with artificial water circulation. One randomly selected pond used for the control was partitioned by 1.0-cm mesh plastic net into three equal compartments of 67 m^2 each. The six remaining ponds were partitioned into two compartments: 1/3 of pond area (67 m^2) for catfish and 2/3 (133 m^2) for tilapia. The ponds were assigned randomly to treatments B and C.

Sex-reversed male Nile tilapia (19.0 to 19.7 g in size) were stocked at 2 fish m^{-2} in tilapia compartments of treatments B and C, and hybrid catfish (18.7 to 20.2 g in size) at 25 fish m^{-2} in treatment A and catfish compartments of treatments B and C on 3 August 1999. Catfish were fed twice daily with small-, medium-, and large-size commercial pelleted feed (crude protein 30%, Charoen Pokphand Co., Ltd., Bangkok, Thailand) at 0830 and 1530 h. Feeding rates of 10% body weight per day (BWD) for fish smaller than 20 g, 8% BWD for 20 to 50 g, 5% BWD for 50 to 100 g, and 3% BWD for fish larger than 100 g were applied six days per week. During the first month, tilapia compartments were fertilized weekly with urea and triple superphosphate (TSP) at rates of 28 kg N and 7 kg P ha^{-1} wk^{-1}. No fertilizers were applied during the rest of the experimental period. No feed was given to tilapia, which depended solely on natural foods. In the artificial water circulation treatment, water in the catfish compartment was continuously circulated at a rate of one exchange per week to the tilapia compartment by a submersed pump, starting the second month. Water depth in all ponds was maintained at 1 m throughout the experiment by adding water weekly to replace evaporation and seepage losses.

Water quality analysis was conducted biweekly by taking integrated column water samples at 0900 h from walkways extending to the center of compartments. Pond water samples were analyzed for pH, total Kjeldahl nitrogen (TKN), total ammonium nitrogen (TAN), nitrite, nitrate-nitrite, soluble reactive phosphorus (SRP), total phosphorus (TP), total alkalinity, chlorophyll *a*, total suspended solids (TSS), and total volatile solids (TVS) using standard methods (APHA, 1985; Egna et al., 1987). Secchi disk visibility, dissolved oxygen (DO), and temperature were measured before taking water samples, the latter two with a YSI model 54 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Diel measurements for DO, pH, and temperature were conducted monthly for each compartment at three different depths (25 cm below water surface, middle, and 25 cm above pond bottom) at 0600, 0900, 1400, 1600, 1800, and 0600 h. Un-ionized ammonia-nitrogen (UIA) was calculated by a conversion table for given pH and temperature (Boyd, 1990).

The nutrient budgets for nitrogen and phosphorus in ponds were calculated based on inputs from water, stocked fish, fertilizers, and pelleted feed and losses in harvested fish, discharged water, and sediment. Sediment samples were collected with 5-cm-diameter plastic tubes from the top 5 cm of each compartment before initial pond filling and after fish harvest. Total nitrogen and total phosphorus in sediment samples, monthly pelleted feed samples, and fish samples at stocking and harvest were analyzed using the methods described by Yoshida et al. (1976).

During the experiment, 40 Nile tilapia or 100 hybrid catfish were sampled by seining and group-weighed biweekly for each compartment. All fish were harvested on 29 October 1999 after 87 days of culture. Daily weight gain (g $fish^{-1}$ d^{-1}), yield (kg m^{-2} $crop^{-1}$), and extrapolated yield (t ha^{-1} yr^{-1}) were calculated.

Data were analyzed statistically by one-way analysis of variance (Steele and Torrie, 1980) using SPSS (version 7.0) statistical software package (SPSS Inc., Chicago, USA). Differences were considered significant at an alpha level of

Table 2. Moisture, total nitrogen, and total phosphorus composition (%, dry matter basis) of hybrid catfish, Nile tilapia, feed, and sediment.

Variable (%)	At Stocking			At Harvest		
	A	*B*	*C*	*A*	*B*	*C*
CATFISH						
Moisture	72.04	72.04	72.04	71.82	71.58	71.23
TN	11.06	11.06	11.06	9.26	9.10	8.68
TP	1.09	1.09	1.09	2.38	2.31	2.28
TILAPIA						
Moisture	----	74.40	74.40	----	78.42	79.03
TN	----	9.40	9.40	----	10.33	10.12
TP	----	0.67	0.67	----	0.81	0.80
FEED 1						
Moisture	4.50	4.50	4.50	----	----	----
TN	5.47	5.47	5.47	----	----	----
TP	1.04	1.04	1.04	----	----	----
FEED 2						
Moisture	2.07	2.07	2.07	----	----	----
TN	5.43	5.43	5.43	----	----	----
TP	0.91	0.91	0.91	----	----	----
FEED 3						
Moisture	6.66	6.66	6.66	----	----	----
TN	4.59	4.59	4.59	----	----	----
TP	1.37	1.37	1.37	----	----	----
SEDIMENT IN CATFISH COMPARTMENT						
Moisture	59.84	53.21	51.28	62.75	61.25	44.21
TN	0.23	0.24	0.20	0.29	0.32	0.25
TP	0.01	0.01	0.01	0.03	0.03	0.03
SEDIMENT IN TILAPIA COMPARTMENT						
Moisture	----	59.13	54.11	----	62.95	63.28
TN	----	0.22	0.23	----	0.25	0.29
TP	----	0.01	0.01	----	0.04	0.03

0.05. Statistical analyses for survival rates (%) were performed on arcsine transformed data. Survival rates in the text were recalculated and are expressed as actual mean and confidence limits. All other means were given with ± 1 standard error (SE).

A partial budget analysis was conducted to determine the economic feasibility of the integrated catfish-tilapia recycle system (Shang, 1990). The analysis was based on farm-gate prices in Thailand for harvested fish and current local market prices for all other items expressed in US dollars (US$1 = 38 baht). Farm-gate prices of catfish and tilapia were $0.58 and $0.39 kg^{-1}, respectively. Market prices of catfish ($0.017 each) and tilapia ($0.013) fingerlings, urea ($0.20 kg^{-1}), TSP ($0.33 kg^{-1}), small- ($0.51 kg^{-1}), medium- ($0.51 kg^{-1}), and large-size ($0.50 kg^{-1}) pelleted feed were applied to the analysis. Market price of electricity was $0.053 kWh^{-1}, and price of the 1.0-cm mesh plastic net was $0.264 m^{-1}. The calculation for cost of working capital was based on an annual interest rate of 10%.

RESULTS

Both hybrid catfish and Nile tilapia grew steadily in all treatments over the 87-day culture cycle (Figures 1 and 2). There were no significant differences in growth performance of both hybrid catfish and Nile tilapia among all treatments ($P > 0.05$, Table 1). At harvest, hybrid catfish reached 240 ± 3.8, 238 ± 1.9, and 249 ± 7.9 g with daily weight gains of 2.53 ± 0.04, 2.51 ± 0.02, and 2.64 ± 0.09 g $fish^{-1}$ d^{-1} in treatments A, B, and C, respectively. Extrapolated net yields of hybrid catfish were 219.1 ± 4.3, 213.6 ± 3.8, and 226.7 ± 9.1 t ha^{-1} yr^{-1} in treatments A, B, and C, respectively. Survival rates ranged from 93.8 to 96.3%, and there were no significant differences among all treatments ($P > 0.05$). However, the best feed conversion ratios (FCRs) were achieved in treatments A (1.25 ± 0.00) and C (1.26 ± 0.02), between which there was no significant difference ($P > 0.05$), while FCRs in both treatments A and C were significantly higher ($P < 0.05$) than in treatment B (1.31 ± 0.01). Final mean weights of Nile tilapia were 114.9 ± 14.1 g and 115.0 ± 13.5 g in treatments B and C, respectively. Although there were no significant differences in survival rates between treatments B (92%) and C (70%) ($P > 0.05$), high mortality of Nile tilapia was observed in two replicates of treatment C. Extrapolated net yields of Nile tilapia were 7.2 ± 1.3 and 4.9 ± 0.3 t ha^{-1} yr^{-1} in treatments B and C, respectively. The results indicated that neither natural nor artificial water circulation between catfish and tilapia compartments improved the growth of hybrid

Table 3. Nitrogen budgets in different treatments in integrated culture for 87 days.

Parameter (kg)	**Treatment**		
	A	*B*	*C*
INPUTS			
Feed	21.339 ± 0.152	21.388 ± 0.485	21.765 ± 0.614
Fertilizers	----	2.208 ± 0.000	2.208 ± 0.000
Catfish	1.035 ± 0.005	0.997 ± 0.010	0.998 ± 0.016
Tilapia	----	0.125 ± 0.001	0.122 ± 0.001
Water in Catfish Compartment	0.211 ± 0.000	0.187 ± 0.008	0.225 ± 0.008
Water in Tilapia Compartment	----	0.441 ± 0.036	0.446 ± 0.016
Sediment in Catfish Compartment	4.204 ± 0.202	4.935 ± 0.386	4.621 ± 0.072
Sediment in Tilapia Compartment	----	7.413 ± 0.193	9.404 ± 2.014
Total	26.789 ± 0.293	37.695 ± 0.691	39.789 ± 2.492
OUTPUTS			
Catfish	9.768 ± 0.578	9.648 ± 0.492	9.835 ± 0.240
Tilapia	----	0.629 ± 0.102	0.444 ± 0.046
Water in Catfish Compartment	2.440 ± 0.057[a]	0.753 ± 0.108[b]	0.776 ± 0.034[b]
Water in Tilapia Compartment	----	1.243 ± 0.216	1.592 ± 0.067
Sediment in Catfish Compartment	6.885 ± 0.256	6.804 ± 0.401	6.103 ± 0.436
Sediment in Tilapia Compartment	----	9.572 ± 0.291	11.916 ± 1.676
Total	19.094 ± 0.320[a]	28.649 ± 0.505[b]	30.666 ± 1.872[b]
GAIN			
Catfish	8.734 ± 0.582	8.651 ± 0.495	8.837 ± 0.254
Tilapia	----	0.504 ± 0.102	0.322 ± 0.046
LOSS			
Water in Catfish Compartment	2.229 ± 0.057[a]	0.565 ± 0.116[b]	0.551 ± 0.038[b]
Water in Tilapia Compartment	----	0.802 ± 0.186	1.146 ± 0.078
Subtotal in Water	2.229 ± 0.057[a]	1.367 ± 0.302[b]	1.698 ± 0.115[ab]
Sediment in Catfish Compartment	2.682 ± 0.389	1.868 ± 0.142	1.482 ± 0.370
Sediment in Tilapia Compartment	----	2.159 ± 0.321	2.512 ± 0.775
Subtotal in Sediment	2.682 ± 0.389	4.028 ± 0.287	3.994 ± 0.496
UNACCOUNTED	7.695 ± 0.391	9.046 ± 0.220	9.123 ± 1.058

* Mean values with different superscript letters in the same row were significantly different ($P < 0.05$).

catfish; however, artificial water circulation caused high mortality of Nile tilapia in two replicates. Based on the pond partition ratio for catfish and tilapia culture (2:1) and the stocking ratio of catfish to tilapia (6.3:1) in the experiment, a 1-ha pond could produce 78 tons of catfish and 6 tons of tilapia per year.

Proximate compositions of inputs, sediments, and fish indicate that the dominant nutrient input was the pelleted feed in all treatments (Table 2). Total nitrogen and total phosphorus contents in effluents in catfish compartments were significantly higher in treatment A than in treatments B and C ($P < 0.05$, Tables 3 and 4). However, artificial water circulation (treatment C) did not significantly increase total nitrogen and total phosphorus contents in tilapia compartments compared with natural water circulation (treatment B) ($P > 0.05$). There were no significant differences in sediments of each treatment for total nitrogen and phosphorus contents of catfish or tilapia compartments ($P > 0.05$). Losses of total nitrogen and total phosphorus in effluents from catfish compartments in treatment A were significantly higher than in treatments B and C ($P < 0.05$), and even significantly higher than the total effluents from both catfish and tilapia compartments in treatment B ($P < 0.05$; Tables 3 and 4). Additional fertilizer inputs in tilapia compartments of both treatments B and C did not result in higher nutrient outputs in effluents or nutrient deposited in sediments.

Hybrid catfish incorporated 40.87, 40.48, and 40.62% of total nitrogen and 50.01, 47.62, and 49.38% of total phosphorus from feed input in treatments A, B, and C, respectively (Table 5). Wastes derived from feed accounted for 59.13, 59.52, and 59.38% of total nitrogen and 49.99, 52.38, and 50.62% of total phosphorus from feed input in treatments A, B, and C, respectively (Table 5). No significant differences in incorporated and wasted nutrients were found among treatments ($P > 0.05$). These wastes fertilized the entire pond at loading rates of 7.32 ± 0.44 and 7.43 ± 0.29 kg N ha^{-1} d^{-1}, and 1.49 ± 0.102 and 1.46 ± 0.01 kg P ha^{-1} d^{-1} in treatments B and C, respectively. Nile tilapia recovered 3.30 and 2.12% of total nitrogen and 1.29 and 0.84% of total phosphorus from feed wastes and fertilizer inputs in treatments B and C, respectively (Table 5). The percentages of nutrient losses in effluent water in treatment A were significantly higher than those in treatments B and C ($P < 0.05$). Total nitrogen losses in effluent water were significantly lower in treatment B than in treatment C while total

Table 4. Phosphorus budgets in different treatments in integrated culture for 87 days.

Parameter (kg)	**Treatment**		
	A	*B*	*C*
INPUTS			
Feed	4.938 ± 0.088	4.941 ± 0.125	5.033 ± 0.139
Fertilizers	----	0.553 ± 0.000	0.553 ± 0.000
Catfish	0.102 ± 0.001	0.098 ± 0.001	0.098 ± 0.002
Tilapia	----	0.009 ± 0.000	0.009 ± 0.000
Water in Catfish Compartment	0.005 ± 0.000	0.004 ± 0.000	0.005 ± 0.000
Water in Tilapia Compartment	----	0.008 ± 0.000	0.010 ± 0.001
Sediment in Catfish Compartment	0.205 ± 0.035	0.277 ± 0.049	0.283 ± 0.051
Sediment in Tilapia Compartment	----	0.296 ± 0.030	0.488 ± 0.140
Total	5.249 ± 0.118	6.186 ± 0.185	6.478 ± 0.315
OUTPUTS			
Catfish	2.574 ± 0.116	2.447 ± 0.083	2.589 ± 0.158
Tilapia	----	0.050 ± 0.009	0.035 ± 0.002
Water in Catfish Compartment	0.151 ± 0.004[a]	0.057 ± 0.015[b]	0.034 ± 0.006[b]
Water in Tilapia Compartment	----	0.028 ± 0.003	0.029 ± 0.004
Sediment in Catfish Compartment	0.833 ± 0.047	0.721 ± 0.146	0.903 ± 0.158
Sediment in Tilapia Compartment	----	1.305 ± 0.267	1.390 ± 0.295
Total	3.557 ± 0.098	4.608 ± 0.390	4.979 ± 0.541
GAIN			
Catfish	2.472 ± 0.116	2.349 ± 0.083	2.490 ± 0.158
Tilapia	----	0.041 ± 0.009	0.026 ± 0.002
LOSS			
Water in Catfish Compartment	0.146 ± 0.004[a]	0.053 ± 0.015[b]	0.029 ± 0.006[b]
Water in Tilapia Compartment	----	0.020 ± 0.003	0.019 ± 0.005
Subtotal in Water	0.146 ± 0.004[a]	0.073 ± 0.018[b]	0.048 ± 0.010[b]
Sediment in Catfish Compartment	0.628 ± 0.075	0.443 ± 0.105	0.620 ± 0.107
Sediment in Tilapia Compartment	----	1.009 ± 0.260	0.902 ± 0.182
Subtotal in Sediment	0.628 ± 0.075	1.453 ± 0.348	1.522 ± 0.247
UNACCOUNTED	1.693 ± 0.019[a]	1.579 ± 0.270[b]	1.500 ± 0.256[b]

* Mean values with different superscript letters in the same row were significantly different ($P < 0.05$).

phosphorus losses in effluent water in treatment B were significantly higher than those in treatment C ($P < 0.05$). No significant differences in nutrient losses in sediment were found among all treatments ($P > 0.05$).

Water temperature and pH throughout the experimental period in all compartments ranged from 27.6 to 31.5°C and 6.6 to 7.4, respectively. At the end of the experiment, pH values in catfish compartments were significantly higher in treatment

Table 5. Distribution (mean and range in percentages) of total nitrogen and total phosphorus in different treatments in the integrated recycle culture for 87 days.

Parameter (%)	Treatment		
	A	*B*	*C*
TOTAL NITROGEN			
Catfish Compartment			
Feed Input	100.00	100.00	100.00
Gain in Catfish	40.87 (30.58–51.58)	40.48 (29.43–52.05)	40.62 (36.23–45.06)
Wastes	59.13 (48.42–69.42)	59.52 (47.95–70.57)	59.38 (54.94–63.74)
Total	100.00	100.00	100.00
Wastes and Fertilizers	100.00	100.00	100.00
Gain in Tilapia	----	3.30 (1.27–6.23)	2.12 (0.84–3.96)
Effluent Water	17.70 (15.81–19.67)[a]	9.90 (2.66–18.20)[b]	11.23 (7.01–16.30)[c]
Loss in Sediment	21.15 (10.29–34.39)	26.91 (22.89–31.13)	26.61 (11.27–45.23)
Unaccounted	61.15 (47.90–73.61)	60.70 (53.64–67.55)	60.04 (37.51–80.54)
Total	100.00	100.00	100.00
TOTAL PHOSPHORUS			
Catfish Compartment			
Feed Input	100.00	100.00	100.00
Gain in Catfish	50.01 (42.81–57.22)	47.62 (37.29–58.06)	49.38 (41.53–57.23)
Wastes	49.99 (42.78–57.19)	52.38 (41.94–62.71)	50.62 (42.77–58.47)
Total	100.00	100.00	100.00
Wastes and Fertilizers	100.00	100.00	100.00
Gain in Tilapia	----	1.29 (0.38–2.64)	0.84 (0.57–1.15)
Effluent Water	5.97 (4.82–7.13)[a]	2.29 (0.80–4.40)[b]	1.54 (0.45–3.16)[b]
Loss in Sediment	25.27 (15.97–35.90)	45.61 (11.36–82.23)	49.24 (17.29–81.55)
Unaccounted	68.76 (59.47–77.35)[a]	50.81 (13.23–87.91)[b]	48.38 (15.88–81.58)[b]
Total	100.00	100.00	100.00

* Mean values with different superscript letters in the same row were significantly different ($P < 0.05$).

Table 6. Mean values of water quality parameters measured at the end of the experiment.

Parameter	Catfish Treatment			Tilapia Treatment	
	A	*B*	*C*	*B*	*C*
DO at Dawn (mg l^{-1})	0.20 ±0.00	0.30 ±0.00	0.23 ±0.03	0.27 ±0.03	0.17 ±0.03
Temperature (°C)	27.8–28.3	27.9–28.1	28.0–28.2	27.6–27.8	27.7–28.2
pH	7.1–7.2[a]	6.9[c]	7.0–7.1[b]	7.1–7.2	7.0–7.1
Alkalinity (mg l^{-1})	513 ±7[a]	108 ±7[c]	155 ±14[b]	109 ±12	135 ±9
TKN (mg l^{-1})	36.34 ±0.88[a]	11.16 ±1.58[b]	11.52 ±0.53[b]	9.32 ±1.62	11.75 ±0.47
TAN (mg l^{-1})	3.09 ±0.09	3.43 ±0.07	3.52 ±0.17	3.76 ±0.32[x]	4.13 ±0.06[y]
UIA (mg l^{-1})	0.05 ±0.01[a]	0.03 ±0.00[b]	0.05 ±0.00[a]	0.04 ±0.00	0.04 ±0.01
Nitrite-N (mg l^{-1})	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00[x]	0.02 ±0.00[y]
Nitrate-N (mg l^{-1})	0.07 ±0.04	0.07 ±0.04	0.05 ±0.01	0.02 ±0.02[x]	0.20 ±0.04[y]
TP (mg l^{-1})	2.25 ±0.06[a]	0.85 ±0.23[b]	0.51 ±0.08[b]	0.42 ±0.05	0.43 ±0.06
SRP (mg l-1)	0.93 ±0.03[a]	0.01 ±0.01[b]	0.00 ±0.00[b]	0.01 ±0.01	0.00 ±0.00
Chlorophyll *a* (mg m^{-3})	44 ±1	72 ±22	132 ±57	90 ±29	131 ±48
TSS (mg l^{-1})	622 ±49	505 ±197	190 ±47	136 ±27	145 ±22
TVS (mg l^{-1})	101 ±5	62 ±24	42 ±6	30 ±5	42 ±11

* Mean values with different superscript letters (a, b, and c, and x and y) in the same row were significantly different among catfish compartments and between tilapia compartments of the treatments, respectively ($P < 0.05$).

A than in treatments B and C, and there were also significant differences in final pH values between the latter two ($P < 0.05$, Table 6). Measured DO concentrations at dawn decreased steadily from initial levels of 1.63–3.93 mg l^{-1} to 0.17–0.30 mg l^{-1} over the 87-d culture period in all compartments, and no significant differences in final DO concentrations were found among all treatments ($P > 0.05$, Figure 3 and Table 6). Concentrations of total alkalinity, TKN, TP, and SRP in catfish compartments were significantly higher in treatment A than in treatments B and C ($P < 0.05$, Table 6). In tilapia compartments, final concentrations of inorganic nitrogen forms (TAN, nitrite-N, and nitrate-N) were significantly lower in treatment B than those in treatment C ($P < 0.05$) while there were no significant differences for other water quality parameters between the two treatments ($P > 0.05$, Table 6). Un-ionized ammonia-nitrogen concentrations fluctuated throughout the experimental period but increased toward the end of the experiment (Figure 3). There were no significant differences in final concentrations of un-ionized ammonia-nitrogen in catfish compartments between treatments A and C ($P > 0.05$), and both were significantly higher than those in treatment B ($P < 0.05$, Table 6). The phytoplankton standing crop as expressed in chlorophyll *a* concentration steadily increased over the first two months and decreased slightly at the end in both catfish and tilapia compartments of treatments B and C (Figure 3). In treatment A, however, it increased sharply and reached the peak at the end of the second month, then decreased dramatically to a level even below those in all other compartments (Figure 3). At the end of the experiment, no significant differences in chlorophyll *a* concentrations were found among all compartments ($P > 0.05$, Table 6).

The partial budget analysis (Table 7) indicated that all of the treatments in the present experiment produced a negative net return, and treatment B had the least negative net return. The high electricity cost made treatment C produce a similar negative net return to treatment A.

DISCUSSION

Water circulation did not improve growth of catfish compared to the control in the present experiment. One of the most unique features of catfish is their air-breathing ability, which enables them to live at extremely high population density and gives great yields in various culture systems (Lin and Diana, 1995). Extrapolated gross catfish yield in this experiment ranged from 233.8 to 246.9 t ha^{-1} yr^{-1}, which was higher than yields achieved in previous experiments (Lin, 1990; Ye, 1991; Lin and Diana, 1995; Sethteethunyahan, 1998) and also higher than those obtained in the traditional pond culture in Thailand (Panayotou et al., 1982; Tonguthai et al., 1993). Although the phytoplankton standing crop (chlorophyll *a* concentration) was significantly higher in the controls than in the water circulation treatments during most of the experimental period, there were no significant differences in growth and yield among treatments. This supported the finding by Pearl (1995) that a phytoplankton-based food chain is relatively unimportant in pond culture that relies on artificial feed to promote rapid fish growth.

Artificial water circulation caused mass mortality of Nile tilapia in two replicates. The reason might be the heavy loading of wastes from the catfish compartment to the tilapia compartment, causing significantly higher concentrations of TAN than that in natural water circulation. The sharp in-

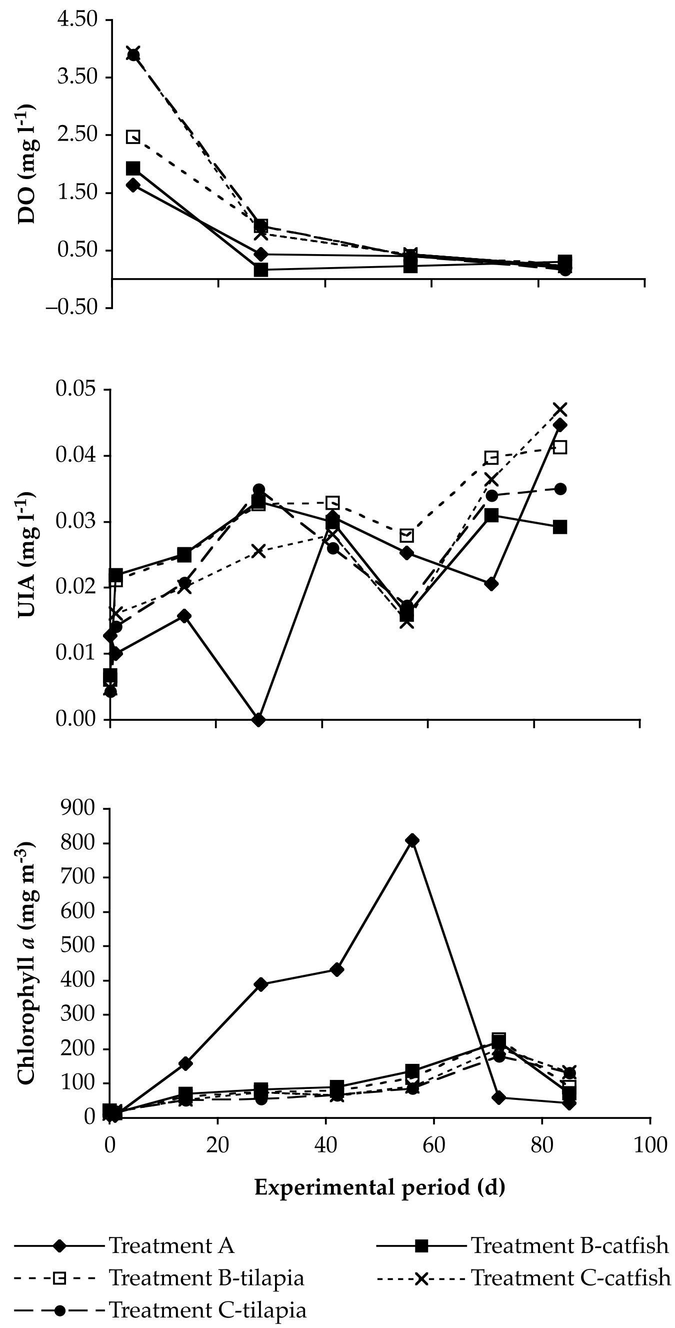

Figure 3. Fluctuations in DO (at dawn), UIA (at 0900 h), and chlorophyll *a* (at 0900 h) in catfish compartments of treatments A, B, and C and in tilapia compartments of treatments B and C over the 87-day experimental period.

creases in TAN concentration, decreases in dissolved oxygen, and possible prolonged exposure to poor water quality were probably the main causes of increased mortality in circulated ponds. The extrapolated tilapia yields in the water circulation treatments were similar to those achieved in conventional integrated fish-livestock systems (AIT, 1986), systems optimally fertilized with chicken manure (Diana et al., 1988) or chemical fertilizers (Diana et al., 1991), and fish-fish integrated culture systems (Lin, 1990; Ye, 1991; Lin and Diana, 1995; Yi et al., 1996; Yi, 1997). However, the fertilization rates by wastes from intensive culture were much higher in the present experiment than those reported by the above authors, due to the high catfish to tilapia ratio (6.3:1). The high loading of catfish wastes caused depletion of water quality after the first month of culture and thus did not result in higher yields of Nile tilapia compared to cultures with lower loading rates. The much higher concentrations of TKN, TAN, and TP also suggested that loading rates of catfish wastes were excessive in the present experiment. Lowering the catfish to tilapia ratio may result in better growth performance of Nile tilapia.

Nutrients released from the catfish compartment were about 59% of TN and 50% of TP in the present experiment. These were less than values reported by Boyd (1985) in channel catfish culture, or by Ye (1991) and Sethteethunyahan (1998) in catfish-tilapia integrated culture (62 to 73% of TN and 55 to 70% of TP). The released phosphorus percentages in the present experiment were also much less than those (79 to 84%) calculated by Beveridge (1984) for intensive cage culture of trout.

Nile tilapia in this integrated culture system recovered nutrients by utilizing natural foods derived mainly from catfish wastes. The nutrient recovery percentages (2.12 to 3.30% of TN and 0.84 to 1.29% of TP) in the present experiment were lower than those (4 to 13% of TN and 5 to 17% of TP) reported by Ye (1991) and Lin and Diana (1995). The main reason for the low nutrient recovery was a much higher catfish to tilapia ratio (6.3:1) in the present experiment, compared to the ratios (2:1 to 5:1) reported by them. Another reason was that the percentages in this experiment included fertilizers applied to promote tilapia growth during the first month when catfish waste loading was low. The percentages in the present experiment were higher than those (0.68 to 1.94% of TN and 0.86 to 2.48% of TP) reported by Sethteethunyahan (1998) due to the higher catfish to tilapia ratio (9:1) in that experiment.

In the present experiment, negative net returns in all treatments occurred mainly because of the expensive commercial pelleted feed. In terms of farmers' practices, however, the most common diets for catfish culture are combinations of chicken offal, trash fish, and pelleted feed, from which farmers generate marginal profits. For one cycle of catfish culture (87 days in the present experiment), tilapia did not reach marketable size and thus were sold at lower prices. The ideal culture period for the integrated recycle system is six months to produce two crops of catfish and one crop of tilapia. If feed cost was lowered by 20% through partially replacing expensive pelleted feed with low-cost diets and the culture period was extended to six months for two crops of catfish and one crop of tilapia, then the catfish monoculture treatment (treatment A) and natural water circulation treatment (treatment B) would be profitable (about $17 per 200-$m^2$ pond) while the artificial water circulation treatment (treatment C) would still lose money due to the high cost of electricity.

This integrated recycle system is the first example of an integrated pen-cum-pond culture system, which is based on the same principle of an integrated cage-cum-pond culture system developed and practiced by Lin (1990), Lin and Diana (1995), Yi et al. (1996), and Yi (1997). Compared with the cage-cum-pond culture system, the advantages of the pen-cum-pond culture system are simplicity, convenience, and low cost. However, the biggest disadvantage is that wastes derived from intensive culture may not circulate well to the semi-intensive culture system due to possible restriction of water exchange through netting materials.

The present experiment demonstrated that Nile tilapia can be cultured in such an integrated pen-cum-pond system to

Table 7. Partial budget analysis for hybrid catfish monoculture (treatment A) and integrated catfish-tilapia recycle system (treatments B and C) in the 87-day experiment (based on 200-m^2 ponds).

Item	Unit	Price (US$)	Treatment A		Treatment B		Treatment C	
			Quantity	*Value (US$)*	*Quantity*	*Value (US$)*	*Quantity*	*Value (US$)*
GROSS REVENUE								
Catfish	kg	0.580	1,144.0	663.52	373.3	216.51	394.3	228.69
Tilapia	kg	0.390	0.0	0.00	28.1	10.96	20.7	8.07
Total				663.52		227.47		236.76
VARIABLE COST								
Small-Size Feed	kg	0.510	167.5	85.43	55.3	28.20	55.2	28.15
Medium-Size Feed	kg	0.510	497.3	253.62	162.0	82.62	165.0	84.15
Large-Size Feed	kg	0.500	665.4	332.70	230.8	115.40	235.9	117.95
Urea	kg	0.200	0.0	0.00	4.8	0.96	4.8	0.96
TSP	kg	0.330	0.0	0.00	2.8	0.92	2.8	0.92
Catfish Fingerlings	piece	0.017	5,000	85.00	1,675	28.48	1,675	28.48
Tilapia Fingerlings	piece	0.013	0	0.00	266	3.46	266	3.46
Electricity (Pump)	kWh	0.053	0	0.00	0	0.00	1,212	64.24
Net	m	0.264	0	0	10	2.64	10	2.64
Cost of Working Capital	year	10%	0.25	18.92	0.25	6.57	0.25	8.27
Total				775.67		269.25		339.22
NET RETURN				–112.15		–41.78		–102.46

recycle nutrients in wastes which might be otherwise released into the environment and indicated that artificial water circulation may cause mass mortality of Nile tilapia due to heavy loading of wastes. The catfish to tilapia ratio should probably be lowered to allow Nile tilapia to reuse more nutrients derived from catfish wastes and thus enhance the nutrient utilization efficiency.

ANTICIPATED BENEFITS

This is the first trial to develop an integrated pen-cum-pond culture system. This integrated system can recycle wastes from an intensive culture system into a semi-intensive culture system, thereby reducing the nutrient input for fertilization and minimizing the environmental impacts of pond effluents. This experiment provides a new way—integrated pen-cum-pond system—for the integration of intensive and semi-intensive culture systems, which can be adapted by small-scale farmers and is especially suitable for low capital investment. A portion of ponds can be used to culture high-valued species for more income and to efficiently utilize costly commercial or local feed through recycling feeding wastes. Identification of the optimal catfish to tilapia ratio would maximize the profits and minimize the environmental impact of pond effluents.

ACKNOWLEDGMENTS

The authors wish to acknowledge the Asian Institute of Technology, Thailand, for providing the research, field, and laboratory facilities. Mr. Chumpol S., Mr. Manoj Y., and Mr. Supat P. are greatly appreciated for their field and laboratory assistance.

LITERATURE CITED

Adler, P.R., F. Takeda, D.M. Glenn, and S.T. Summerfelt, 1996. Enhancing aquaculture sustainability through utilizing byproducts. World Aquaculture, 27(2):24–26.

AIT (Asian Institute of Technology), 1986. Buffalo/Fish and Duck/Fish Integrated Farming Systems for Small-Scale Farmers at the Family Level. AIT Research Report No. 198, Asian Institute of Technology, Bangkok, Thailand, 138 pp.

APHA (American Public Health Association), 1985. Standard Methods for the Examination of Water and Wastewater, Sixteenth Edition. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C., 1,268 pp.

Areerat, S., 1987. *Clarias* culture in Thailand. Aquaculture, 63:355–362.

Beveridge, M.C.M., 1984. Cage and Pen Fish Farming: Carrying Capacity Models and Environmental Impacts. FAO Fisheries Technical Paper No. 255. Food and Agriculture Organization of the United Nations, Rome, Italy, 131 pp.

Boyd, C.E., 1985. Chemical budgets of channel catfish ponds. Trans. Am. Fish. Soc., 114:291–298.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Auburn University, Auburn, Alabama, 482 pp.

Diana, J.S., P.J. Schneeberger, and C.K. Lin, 1988. Relationship between primary production and yield of tilapia in ponds. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 1–6.

Diana, J.S., C.K. Lin, and P.J. Schneeberger, 1991. Relationship among nutrient inputs, water nutrient concentrations, primary production and yield of *Oreochromis niloticus* in ponds. Aquaculture, 92:323–341.

Egna, H.S., N. Brown, and M. Leslie (Editors), 1989. Pond Dynamics/Aquaculture Collaborative Research Data Reports, Volume 1: General Reference: Site Descriptions, Material and Methods for the Global Experiment. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, 84 pp.

Lin, C.K., 1990. Integrated culture of walking catfish (*Clarias macrocephalus*) and tilapia (*Oreochromis niloticus*). In: R. Hirano and I. Hanyu (Editors), The Second Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines, pp. 209–212.

Lin, C.K., and J.S. Diana, 1995. Co-culture of catfish (*Clarias macrocephalus* × *C. gariepinus*) and tilapia (*Oreochromis niloticus*) in ponds. Aquat. Living Resour., 8:449–454.

Lin, C.K., K. Jaiyen, and V. Muthuwan, 1990. Integration of intensive and semi-intensive aquaculture: Concept and example. Thai Fisheries Gazette, 43:425–430.

Panayotou, T., S. Wattanutchariya, S. Isvilanonda, and R. Tokrisna, 1982. The Economics of Catfish Farming in Central Thailand. ICLARM Technical Report 4, Kasetsart University Research and Development Institute, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines, 60 pp.

Pearl, H.W., 1995. Ecology of blue-green algae in aquaculture ponds. J. World Aquacult. Soc., 26:109–131.

Perschbacher, P.W., 1995. Algal management in intensive channel catfish production trials. World Aquacult., 26:65–68.

Pillay, T.V.R., 1996. The challenges of sustainable aquaculture. World Aquacult., 27:7–9.

Sethteethunyahan, S., 1998. Recycling wastewater from intensive hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) culture for semi-intensive Nile tilapia (*Oreochromis niloticus*) culture in cement tanks. M.S. Thesis, Asian Institute of Technology, Bangkok, Thailand, 75 pp.

Shang, Y.C., 1990. Aquaculture Economics Analysis: An Introduction. World Aquaculture Society, Baton Rouge, Louisiana, 211 pp.

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

Tonguthai, K., S. Chinabut, C. Limsuwan, T. Somsiri, P. Chanratchalkool, S. Kanchanakhan, and I.H. Macrae, 1993. Handbook of Hybrid Catfish: Husbandry and Health. Aquatic Animal Health Research Institute, Bangkok, Thailand, 37 pp.

Ye, Q.F., 1991. Nutrient budget and water quality in integrated walking catfish-tilapia culture. M.S. Thesis, Asian Institute of Technology, Bangkok, Thailand, 88 pp.

Yi, Y., 1997. An integrated rotation culture system for fattening large Nile tilapia (*Oreochromis niloticus*) in cages and nursing small Nile tilapia in open ponds. Ph.D. Dissertation, Asian Institute of Technology, Bangkok, Thailand, 169 pp.

Yi, Y., C.K. Lin, and J.S. Diana, 1996. Effects of stocking densities on growth of caged adult Nile tilapia (*Oreochromis niloticus*) and on yield of small Nile tilapia in open water in earthen ponds. Aquaculture, 146:205–215.

Yoshida, S., D.A Forno, J.H. Cock, and K.A. Gomez, 1976. Laboratory Manual for Physiological Studies in Rice, Third Edition. International Rice Research Institute, Manila, Philippines, 61 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Effects of Water Recirculation on Water Quality and Bottom Soil in Aquaculture Ponds

Ninth Work Plan, Effluents and Pollution Research 4 (9ER4)
Progress Report

Oscar Zelaya, Claude E. Boyd, David R. Teichert-Coddington, and Bartholomew W. Green
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Abstract

There is considerable interest in reducing negative environmental impacts of shrimp and fish farming. One of the most promising methods for reducing the environmental effects of pond aquaculture is to use water-recirculating systems to minimize effluents. However, few studies have been performed to evaluate the effect of recirculation upon soil and water quality in ponds. This study evaluates changes in physical and chemical characteristics of pond water and soils in response to varying density of production and in the presence or absence of water recirculation. Ponds were stocked with *Litopenaeus vannamei* and arranged in three treatments: 1) high-density stocking (50 post-larvae m^{-2}) (HDR) with water recirculation into another pond of equal volume without shrimp (R); 2) high-density stocking (50 post-larvae m^{-2}) without recirculation (HD); and 3) low-density stocking (25 post-larvae m^{-2}) without water recirculation (LD). Water quality variables determined weekly included soluble reactive phosphorus, total phosphorus, total nitrogen, nitrites, nitrates, and total suspended solids. Every two weeks determinations were done for 5-d biochemical oxygen demand and chlorophyll *a*. Soil variables determined at the beginning and end of the study included total nitrogen, soil respiration, pH, carbon, and sulfur. Analysis of variance techniques were used to determine if significant differences existed among treatments with respect to soil and water quality variables.

Introduction

Eutrophication of surrounding coastal areas can result from nutrients discharged in shrimp pond effluents. This is especially true for semi-intensive and intensive culture systems with rates of feeding and fertilization such that water exchange rates require frequent discharge of pond effluents.

Shrimp pond effluents are often high in suspended and dissolved organic matter (Boyd, 1992). The high biological oxygen demand of the pond effluents can cause oxygen depletion in receiving waters—especially in estuaries already receiving organic wastes from nearby urban and agricultural areas. In addition to nutrients and organic matter discharged from shrimp culture ponds, sediments removed from pond bottoms are often discharged into receiving waters (Boyd, 1990). These sediments can increase turbidity in receiving waters.

Large amounts of uneaten feed, feces, and metabolic wastes accumulate in pond waters and pond soils. These wastes are degraded through microbial processes to produce ammonia, nitrite, nitrate, and phosphate. These nutrients stimulate algal growth and may lead to dense blooms in the pond. In addition, some of these degradation products are toxic to shrimp at high concentrations. Collapse of algal populations can also cause shrimp stress and mortality through oxygen depletion. A conventional solution to this problem has been increased water exchange. Excess metabolites and algae are thus removed from the pond and replaced with better quality water. The water exchange, however, may have detrimental effects in the receiving waters. Water recirculation with digestion lagoons has been proposed as an alternative to high rates of water exchange and flushing. The purpose is to provide a system to degrade organic matter and fix nutrients before effluent discharge to receiving waters or to improve water quality in production ponds. The efficiency of this system has not been evaluated. This study evaluates the changes in physical and chemical characteristics of pond water and soils that occur in response to water recirculation with digestion ponds.

Methods and Materials

The experiment was conducted in ponds at the Alabama Department of Conservation, Claude Peteet Mariculture Center (CPMC) at Gulf Shores, Alabama. Ponds were 0.1 ha in area and averaged 1 m in depth. Ponds were lined with high-density polyethylene to prevent seepage and were filled with 25 cm of soil. Pond water was pumped from the intercoastal waterway.

The experimental design consisted of three treatments, each replicated three times:

1) High-density stocking (50 post-larvae m^{-2}) with water recirculation (HDR);
2) High-density stocking (50 post-larvae m^{-2}) without water recirculation (HD); and
3) Low-density stocking (25 post-larvae^{-2}) without water recirculation (LD).

Each recirculation replicate was stocked with shrimp, and water was recirculated with an adjacent pond of equal size and volume that was not stocked with shrimp (R). The recirculation rate was one pond volume per week.

Ponds without water circulation were filled, and water was added only to replace evaporation. In recirculation ponds, water was pumped from culture pond to treatment pond and then pumped back to the culture pond. Water retention time in treatment ponds was one week, and culture and treatment ponds had equal volume.

The production cycle was 21 weeks (May 17 to September 29, 1999). All ponds had automatic aeration systems with a capacity of 24 HP ha^{-1} using propeller aspirator aerators. The feeding rate administered to all ponds was adjusted according to shrimp growth but was not higher than 150 kg ha^{-1} at the peak. Shrimp were fed using a 35% protein pelleted feed purchased from Burris Feed Mill, Slidell, Louisiana. The feeding rates were increased according to a standard feeding table as shrimp biomass increased. Feeding trays were used to prevent overfeeding.

Water analyses were done weekly. Samples of water representing the surface 80-cm stratum were removed with a water column sampler. Analysis included soluble reactive phosphorus (SRP) by the ascorbic acid method according to APHA et al. (1975), total phosphorus (TP) and total nitrogen (TN) by digestion and persulfate oxidation (Eaton et al., 1995), total ammonia nitrogen (TAN) by the indophenol method (Boyd and Tucker, 1992), nitrites (NO_2-N) by formation of colored azo compounds (Boyd and Tucker, 1992), nitrates (NO_3-N) by NAS reagent method (Gross and Boyd, 1998), and total suspended solids (TSS). At least twice a month fresh samples were collected for 5-d biochemical oxygen demand (BOD_5) (Eaton et al., 1995) and chlorophyll *a* analysis.

Soil samples were collected before stocking the ponds and just before harvesting. Soil samples represented three consecutive levels: the first 2.5 cm, the second 2.5 cm, and the last 5 cm. Samples were dried immediately after collection. Parameters for the soil analyses included: pH measured in a 1:1 water-soil slurry (Boyd, 1992); carbon measured with a LECO EC12 induction furnace analyzer; and total nitrogen and total sulfur determined by incinerating the soil samples in a LECO induction furnace HP10 and titrating the liberated sulfur with standard KIO_3 using a LECO sulfur titrator. Soil respiration analyses were run for the samples taken from the first 2.5-cm layer according to the method described by Boyd (1995).

Analysis of variance techniques were used to determine if there were significant differences among treatments in water quality variables.

RESULTS

Mean shrimp yields for LD, HD, and HDR were 1,706 kg ha^{-1}, 4,648 kg ha^{-1}, and 3,687 kg ha^{-1} (1,843 kg ha^{-1} total water area), respectively. There was no significant difference ($P < 0.05$) in yields between HD and HDR or between LD and HDR treatments based on total water area. Mean harvest weights of shrimp ranged from 22 to 25 g and were not significantly different between treatments. Significant reductions in concentrations of TN, TAN, and NO_2-N were found in HDR ponds compared with HD ponds (Table 1). When concentrations of variables in HDR and treatment (R) ponds were summed (HDR + R), the mean total for TAN was still significantly less than in HD ponds. However, mean totals for all other variables, except NO_3-N and NO_2-N, were significantly greater than in HD ponds. No differences were noted in water quality between HDR and LD ponds. No significant treatment differences were discovered for soil carbon, nitrogen, or phosphorus. In conclusion, recirculation of water from a production pond through a digestion lagoon of equal volume had no effect on shrimp yields. Nitrogen concentrations in the production pond were reduced by dilution, but only TAN was effectively reduced by digestion in the recirculation system.

Soil samples are currently being analyzed and submitted to statistical analysis.

ANTICIPATED BENEFITS

The findings will allow a discussion of the feasibility of using water recirculation to minimize the discharge of pond effluents and the environmental implications of aquaculture with or without recirculation in Honduras.

This research has contributed to a better understanding of pond dynamics. It has also provided an environment for a Honduran graduate student to learn research techniques, sampling methods, water and soil analysis methods, and analytical protocols that are very useful to fill the need for research and improvement of sustainable aquaculture in Honduras.

Table 1. Water quality means and relevant comparison among treatments, Significance (S) and No Significance (NS) differences are indicated within each column ($P < 0.05$).

Treatment	TSS (mg l^{-1})	SRP (mg l^{-1})	TP (mg l^{-1})	TN (mg l^{-1})	TAN (mg l^{-1})	NO_3-N (mg l^{-1})	NO_2-N (mg l^{-1})	BOD_5 (mg l^{-1})	Chlorophyll *a* (mg m^{-3})
LD	91.3	0.28	0.92	4.95	0.928	0.06	0.02	18.53	181.73
HD	98.8	0.18	0.88	5.18	1.76	0.11	0.04	20.37	209.27
HDR	93.7	0.43	1.04	4.72	0.65	0.08	0.02	18.46	188.84
R	78.9	0.51	0.92	3.70	0.498	0.05	0.02	14.04	88.05
HDR + R	172.6	0.93	1.96	8.42	1.51	0.13	0.04	32.50	276.9
COMPARISON									
HDR vs HD	NS	S	NS	S	S	NS	S	NS	NS
HDR vs LD	NS	NS	NS	NS	NS	NS	NS	NS	NS
HDR vs R	S	NS	NS	S	NS	NS	NS	S	S
(HDR + R) vs HD	S	S	S	S	S	NS	NS	S	S

LITERATURE CITED

APHA (American Public Health Association), 1975. Standard Methods for Examination of Water and Wastewater, Fourteenth Edition. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC, 1,193 pp.

APHA, 1995. Standard Methods for Examination of Water and Wastewater, Nineteenth Edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC, 1,268 pp.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama, 482 pp.

Boyd, C.E., 1992. Shrimp pond bottom soil and sediment management. In: J. Wyban (Editor), Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, Louisiana, pp. 166–181.

Boyd, C.E., 1995. Bottom Soils, Sediment, and Pond Aquaculture. Chapman and Hall, New York, 348 pp.

Boyd, C.E. and C.S. Tucker, 1992. Water Quality and Pond Soil Analyses for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama, 183 pp.

Gross, A. and C.E. Boyd, 1998. A digestion procedure for the simultaneous determination of total nitrogen and total phosphorus in pond water. J. World Aquacult. Soc., 3:300–303.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

On-Farm Trials: Evaluation of Alternative Aquaculture Technologies by Local Farmers in Kenya

Ninth Work Plan, Appropriate Technology Research 1 (9ATR1)
Progress Report

Karen L. Veverica
Department of Fisheries and Allied Aquacultures
Auburn University
Auburn, Alabama, USA

Charles Ngugi
Department of Fisheries
Moi University
Eldoret, Kenya

James R. Bowman
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Abstract

Research conducted by the PD/A CRSP at Sagana Fish Farm has begun to identify alternative management practices and technologies that may be suitable in the region, but it should not be assumed that results obtained under controlled experimental conditions at Sagana are directly transferable to farms in the area. On-farm testing is therefore a logical step in transferring research-based technologies to the farm. On-farm testing of various alternatives allows farmers to assess their costs and benefits under local conditions as well as to receive instruction and training in basic pond management skills. Conducting such trials also allows project personnel to work with and train the fisheries extension officers who are involved in the trials at the various locations, which complements the training they receive through "regular" training activities.

Thirty farmers were selected to participate in on-farm trials in five districts of Central Province, Kenya, in 1999–2000. A pre-trial workshop including farmers, extension agents, Kenyan and US CRSP personnel, and students working on research projects at Sagana was held in December 1999 to discuss and select management schemes for testing, agree on how the trials would be conducted, and plan for proper record keeping during the trial period. Fifty-two ponds were stocked with monosex male tilapia (*Oreochromis niloticus*), mixed-sex tilapia, and/or catfish (*Clarias gariepinus*) between January and March 2000. Stocking densities were 2 fish m^{-2} for tilapia, 0.2 fish m^{-2} for catfish stocked with tilapia, and 1 fish m^{-2} for catfish stocked alone. Management schemes being tested include a "no cash expenditure" type of management, which relied on inputs such as manures and leaves found on farms, and a "purchased feed/fertilizer" management scheme, which featured chemical fertilizer and a feed such as bran or maize germ. Ponds are sampled for fish growth at four- to six-week intervals, and farmers keep records of input type and weight, input costs, pond water additions, fish mortality, and fish weight and length. The trials are underway, but harvests were not expected to begin until at least September 2000. A post-trial workshop will be held to summarize and evaluate the results of the trials. A similar set of trials is planned for western Kenya.

Introduction

Fish farmers throughout Kenya, as well as the extension agents who serve them, have suffered from a lack of information about good pond management practices and technology alternatives that may be available to them. Some of the major consequences of this are that many current farmers do not achieve good fish production in their ponds, other farmers become "inactive," and potential farmers avoid going into fish culture because its profitability has not been demonstrated to them. These and other factors have combined to result in typically low productivity from Kenyan fish ponds.

Research conducted by the PD/A CRSP at Sagana Fish Farm has begun to identify alternative management practices and technologies that may be suitable in the region, but it should not be assumed that results obtained under controlled experimental conditions at Sagana are directly transferable to farms in the area. On-farm testing is therefore a logical step in transferring research-based technologies to the farm. On-farm testing of various alternatives allows farmers to assess their costs and benefits under local conditions as well as to receive instruction and training in basic pond management skills. Conducting such trials also provides opportunities for project personnel to work with and train the fisheries extension officers who are involved in the trials at the various locations, which complements the training they receive through the Kenya Project's training activity ("Aquaculture Training for Fisheries Officers in Kenya").

The specific objectives of this activity are:

1) To collaborate with local fish farmers to test technologies developed through PD/A CRSP research at Sagana Fish Farm and elsewhere;
2) To demonstrate improved management techniques to extension officers and farmers; and
3) To teach simple methods for evaluating costs and benefits to farmers and extension agents.

METHODS AND MATERIALS

Preparatory contacts with farmers in Central Province and organizing activities were begun well before August 1999, but the pre-trial workshop and the beginning of the trials themselves were delayed. Still, contacts with potential participants were maintained, and pond visits and surveys were made during the month of November 1999. Each fisheries officer was asked to interview farmers wishing to participate in the trials and select ponds based on the following criteria:

1) The owners are interested in participating in the trials.
2) Pond surface areas are 100 m^2 minimum and 1,000 m^2 maximum.
3) The ponds are drainable.
4) The average water depth of each pond is 80 ± 10 cm.
5) The pond is not prone to flooding.
6) Seepage from the pond is less than 10 cm wk^{-1}.

For each district it was decided to select two focal points that, if possible, would be in areas having different climates or soil types. By December 1999, 30 farmers with 52 ponds had been selected to participate although some farmers needed to renovate some of their ponds prior to beginning the trials. This number is considerably higher than was originally planned. The numbers of farmers, fisheries officers, and extension agents involved in the Central Province trials are shown in Table 1.

Table 1. Numbers of farmers, fisheries officers, and extension agents involved in CRSP-sponsored on-farm trials in Central Province, Kenya, 1999–2000.

District	Number of Farmers	Number of Officers	Number of Extension Agents
Embu	3	1	2
Muranga	8	1	4
Nyeri	7	1	3
Kirinyaga	6	1	3
Kiambu	6	1	3

A workshop to discuss pond management options and make stocking and management plans for each farmer's ponds was conducted 14–17 December 1999. Farmers, extension agents, CRSP personnel, and some of the students involved in thesis work at Sagana participated. Farmers elected to practice either a "no cash expenditure" type of management, which relied on inputs such as manures and leaves found on their farm, or a "purchased feed/fertilizer" management scheme, which featured chemical fertilizer and a feed such as bran or maize germ. These options were based on the alternatives proposed in our work plan, which were:

- Treatment 1) Monosex tilapia (*Oreochromis niloticus*) + catfish (*Clarias gariepinus*), with bran + inorganic fertilizer, based on most recent recommendations from Sagana Fish Farm;
- Treatment 2) Same as Treatment 1 except with mixed-sex tilapia;
- Treatment 3) Monosex tilapia and catfish, with weekly additions of manure/organics at 500 kg TS ha^{-1} wk^{-1}; and
- Treatment 4) Same as Treatment 3, except with mixed-sex tilapia.

Many farmers had more than one pond and elected to try monosex tilapia in one pond and mixed-sex tilapia in another. Most farmers who stocked tilapia also stocked a small number of *Clarias* (about 10%). A few farmers opted for all *Clarias* (stocked at 1 m^{-2}) because they had access to meat scraps and manures.

Pond management and record-keeping techniques were also discussed at the pre-trial workshop. Considerable flexibility was allowed with respect to the management schemes that farmers chose to test, provided they agreed to keep good records of their efforts.

Ponds were stocked beginning 15 January 2000 using 10-g sex-reversed male or mixed-sex *O. niloticus*, depending on the treatment selected, and *C. gariepinus* of 5 g. Stocking densities were 2 fish m^{-2} for *O. niloticus* and 0.2 fish m^{-2} for *C. gariepinus*. All fingerlings came from Sagana Station. Some farmers could not finish renovations in time, and stocking of their ponds had to be delayed until February or March. More than 12,500 tilapia fingerlings and 1,600 *Clarias* fingerlings were stocked, as shown in Table 2.

Farmers are being visited monthly by their extension agent and either monthly or every other month by the area fisheries officer accompanied by the extension agent. Sampling of ponds for fish growth is attempted on a four- to six-week frequency. Water chemistry parameters are not being measured due to lack of personnel, high transport and per

Table 2. Numbers of ponds, monosex tilapia, mixed-sex tilapia, and *Clarias* stocked in ponds in districts of Central Province, Kenya, for on-farm trials sponsored by the PD/A CRSP, 1999–2000.

District	Number of Ponds	Number of Tilapia—Monosex	Number of Tilapia—Mixed-Sex	Number of *Clarias*
Embu	7	424	1,250	166
Muranga	11	600	1,848	494
Nyeri	9	662	1,876	255
Kirinyaga	14	1,316	2,706	452
Kiambu	11	1,332	602	276
Total	52	4,334	8,282	1,643

diem costs for fisheries officers, and the lack of electrical power at Sagana. Most travel money was used to pay for fisheries officers and extension agents to visit the farmers. Farmers record the following information when applicable:

- Input type and weight – as added;
- Pond water additions – weekly;
- Mortality – when observed;
- Expenses – weekly; and
- Fish weight and length – at each sampling.

An evaluation workshop will be conducted after the trials to present the results to farmers and discuss probable causes and economic consequences of the differences observed. Farmers' evaluations of the tested management schemes, including both pond productivity and economic aspects of production, will be solicited.

Fisheries officers and Moi University Department of Fisheries personnel are currently selecting farmers and ponds for similar trials in the Western Region (Western and Nyanza Provinces).

Results

Pond operation is proceeding normally, with frequent visits to ponds by Fisheries Department extension personnel and occasional supporting visits by Sagana personnel (CRSP and/or Fisheries Department) and sometimes by advanced students working at Sagana.

To date no harvests of trial ponds have occurred. The trials are expected to last up to 12 months, depending on when fish reach a size considered marketable by the farmer. Ponds in warmer areas may be ready for harvest as early as September 2000, but most will probably not be ready until November or December.

One of the management problems that stands out continues to be improper water management. Still believing that it is a good management practice, farmers continue to flow water through their ponds. They may be required to stop this practice, however, as Kenya is in the midst of one of the worst droughts it has experienced in recent decades.

Discussion

There is a severe lack of basic fish farming extension equipment in Kenya, and the lack of training of its personnel is immediately evident, almost overwhelming the good intentions of extension personnel. This activity will help by making available some equipment such as seines, weighing balances, and record sheets, as well as by providing at-the-pond training for some Fisheries Department staff and farmers. Unfortunately, the training provided by this activity touches only a small segment of the fish farming extension service; it does illustrate, however, what can be done and how to do it.

Anticipated Benefits

Farmers are evaluating and comparing alternative technologies by testing them in their own ponds; they will subsequently be able to adopt those technologies that are most appropriate under their specific conditions. Farmers and extension officers are receiving on-the-job training and gaining skills in basic pond management practices. Farmers and extension agents are also learning to keep good records on the operation of their fish ponds so that they can base their evaluations on documented facts. Adoption of some of the technologies tested should result in higher fish production and increased revenues from fish sales for participating farmers although these kinds of impacts are not likely to be seen immediately. Neighboring farmers may also adopt the new technologies or apply the improved management practices they have observed at the trial sites.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Linkage of Aquaculture within Watersheds and Concurrent Design of Hillside Ponds

Ninth Work Plan, Appropriate Technology Research 2 (9ATR2)
Abstract

Brahm Verma and E. William Tollner
Department of Biological and Agricultural Engineering
University of Georgia
Athens, Georgia, USA

Thomas Popma
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Daniel Meyer
Department of Biology
Escuela Agrícola Panamericana El Zamorano
Zamorano, Honduras

Joseph J. Molnar
Department of Agricultural Economics and Rural Sociology
Auburn University, Alabama, USA

Abstract

Hillsides in Latin America cover about one million square kilometers and provide livelihood for some 200 million people. Farming on the hillside has resulted in progressive deterioration of natural resources due to a combination of overgrazing, poor farming practices, deforestation, and poor water management. The introduction of tilapia production could improve the nutrition of farm families and local communities and provide a means of additional earning for improving economic status. However, improper pond designs and construction and maintenance methods can result in failed attempts to introduce tilapia. An important design aspect for the successful introduction of tilapia in Honduras and the adjoining regions is to have all stakeholders identify needs that include technical requirements as well as social and environmental issues important in the design of ponds and the production of tilapia.

The fundamental method of pond design is based on the principles of concurrent engineering design methodology. Our stakeholder list included Honduran farmers, extension agents, government agencies, nongovernmental organizations, builders, and design engineers. To insure that pond design meets the identified needs, specific measurable requirements for each need were listed and quantitative targets set.

The hillside pond was defined as a pond that is built on land slopes ranging from 2 to 15%. Two types of hillside ponds were considered in this study: 1) a watershed pond in which water availability is entirely dependent on rainfall in the watershed catchment area; and 2) a spring-fed pond in which water supply is entirely dependent on springs. Critical analysis of water balance considering water source, availability, distribution over time, and losses is important in the design of ponds. Thus, water balance models are being constructed for both pond types. For the watershed pond both runoff and evapotranspiration were modeled whereas for spring-fed ponds only the evapotranspiration and rate of water exchange were used for modeling.

Climate plays an important role in estimating water balance. Based on monthly average temperature and monthly rainfall, we selected six geographic locations distributed across Honduras. Selection was also based on number of years of available records for the candidate locations and on the results of in-depth analysis of rainfall to estimate water availability for watershed ponds. These locations are Comayagua, Choluteca, Santa Rosa, Catacamas, La Ceiba, and Sico. Rainfall data from these locations were used to estimate 90% probability distribution.

Thus, conditions for pond design are as follows:

a) Two pond types: watershed and spring-fed ponds.
b) Three pond sizes: small = 0.05 ha, medium = 0.05 to 0.5 ha, and large = 0.5 ha.
   The three sizes of pond were selected based on current farm sizes in Honduras and on meeting the needs at the following three levels: tilapia production for meeting needs of the farm family only, the farm family plus the immediate neighbors, and the farm family and the local market on a consistent basis.
c) Three slopes: low = 2 to 5%, medium = 5 to 10%, and high = 10 to 15%.
d) Three ground covers: forest, pasture, and mix of forest and pasture.
e) Selection of regions in Honduras based on adequate rainfall, appropriate slopes, and soil with greater than 20% clay content (to seal the pond).

To address diverse design needs of various communities, we have decided to identify modules in the design of ponds and develop concepts that will likely meet a range of anticipated conditions in Honduras. This approach will enable users to receive design information for a customized pond based on their own constraints and needs.

At this time we have selected nine conditions to provide the design for. They include a combination of three sizes of pond (small, medium, and large) and three levels of land slope (low, medium, and high), giving the nine alternatives. The following structural features will be included in the design recommendations: shape of pond, dimensions of pond, outlet pipe, spillway, diversion ditch, pond sealing, drainage outlet, construction methods, materials, cost, labor requirements, and maintenance.

In summary, we are using a design approach that concurrently considers the needs of all individuals and entities that can impact the construction, operation, and maintenance of a pond. Market considerations relevant to the pond design are also being considered. Furthermore, we are developing models for estimating the water balance to make a more informed decision when selecting pond size and type. Although many specifics of pond dimensions and design features have been reported earlier, this approach provides a means for the user to interactively input his/her needs and select a design for the conditions unique to his/her environment and constraints. Finally, concurrently considering needs of all "customers" in the design and selection of construction methods provides a powerful method to have users educated and invested in the design. This approach presents an increased possibility of introducing acceptable pond design and tilapia production as economic enterprises in Honduras and Central America.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Development of Central American Markets for Tilapia Produced in the Region: Potential Markets for Farm-Raised Tilapia in Honduras

Ninth Work Plan, Marketing and Economic Analysis Research 3 (9MEAR3)
Progress Report

Carole Engle, Ivano Neira, and Diego Valderrama
Department of Aquaculture and Fisheries
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

Abstract

Three surveys were conducted in Honduras from September through December 1999. A random sample of restaurants and a census of supermarkets and fish markets were conducted in the major urban areas and in selected small rural towns. Descriptive analyses of the restaurant and supermarket surveys are attached; the descriptive analysis of the fish market vendor survey is nearly complete. Quantitative analyses of the survey data have been initiated. Similar surveys are scheduled to be conducted in Nicaragua from August through October 2000. Descriptive and quantitative analyses will also be conducted with the Nicaraguan survey data.

Introduction

Tilapia culture was initiated in Honduras in the late 1970s (Teichert-Coddington and Green, 1997). In the early years, tilapia production was primarily a small-scale, family operation that was managed either extensively or semi-intensively as a supplemental agricultural activity. Sarmiento and Lanza Nuñez (1995) found a total of 113.6 ha of small-scale, family-level fish ponds (2,738 ponds) in every department (province) of Honduras.

Export oriented production of tilapia began in 1990 and has grown rapidly since 1991 and 1992 (Teichert-Coddington and Green, 1997). In 1997, there were 15 tilapia farms with a total water surface of 185.3 ha that produced for export and domestic markets. These farms produce tilapia exclusively and are owned by individuals, local investors, and international investors (Green and Engle, in press). Exports of tilapia to the US from Honduras have grown consistently since 1992.

The rapid growth in tilapia production is expected to generate a supply that could be available domestically in Honduras. The development of a strong domestic market for tilapia in Honduras could diversify market opportunities for tilapia growers and serve to stabilize this young aquaculture industry from the external shocks common in export-oriented markets. Furthermore, the development of a domestic market could enhance the income generating potential of small-scale tilapia production.

A limited amount of work has been done on markets for finfish in Central America. The few studies that have been carried out focused on the catch from commercial fisheries in Panama (Matton, 1981) and Costa Rica (Scheid and Sutinen, 1979). Head et al. (1994) developed market guidelines for saltwater-cultured Florida red tilapia in Puerto Rico. Several studies conducted in the US have examined the potential to develop markets for tilapia (Crawford et al., 1978; Nelson et al., 1983; Galbreath and Barnes, 1981). More recently, Swanson (1995) described US market requirements for tilapia. Engle (1997) interviewed intermediate seafood buyers in the US to determine the potential to increase sales of fresh and frozen tilapia fillets in the US. However, virtually no work has been done on the potential to develop domestic markets in Central America for tilapia. Engle (1997) describes the domestic markets that have emerged in Colombia for Colombian and Ecuadorian produced tilapia.

Methods

Direct personal interviews were conducted in Honduras in 1999 based on a census of supermarkets and fish market vendors and a random sample of restaurants in Tegucigalpa and San Pedro Sula (the two main urban population centers in Honduras) and in selected small rural towns. Small rural towns were selected along the primary route from north to south through the country to collect data along a possible gradient of preferences between the Pacific and Atlantic coasts. Honduras is the only Central American country with good access between the two coasts where this might be possible. Additional towns that were large enough to be included on maps and located to the east and west of the Tegucigalpa-San Pedro Sula highway were included. In all, the following small rural towns were included in the survey: Catascamas, Siguatepeque, Santa Barbara, Comaguela, Lago de Yojoa, Choluteca, Puerto Cortes, Juticalpa, Comayagua, La Paz, Santa Maria del Real, and Campamento La Lima.

Fast-food eating establishments, bars, cafes, and Chinese restaurants were excluded from the restaurant survey; only full-service restaurants were represented. Supermarkets represented in the survey excluded convenience stores. In the fish markets, only those vendors with a market stand within the market were interviewed. If there were itinerant vendors selling fish outside the market area they were excluded from the survey.

The survey instruments were designed to obtain descriptive information about the restaurants, supermarkets, and fish market vendors. This information included the other types of fish and seafood sold, prices, most frequently sold fish products, and supplier information. Awareness and availability of tilapia were addressed through questions related to the managers' familiarity with tilapia as well as questions related to the supply of tilapia. Information on restaurant and supermarket manager and fish market vendor attitudes towards attributes such as flavor, odor, supply, quality, ease of preparation, size, and price was elicited by asking respondents to assign a value from 1 to 10 in response to statements concerning each attribute. A score of 1 represented complete disagreement with the sentence, and a score of 10 represented complete agreement.

Characteristics related to the clientele and to the restaurant, supermarket, and fish markets interviewed were necessary to interpret responses to the survey. Questions were asked about the size of the stores and restaurants, the type of ownership, location, years in business, and the type of food sold or served.

The response rate was very high. This is likely due to the novelty of marketing surveys in Honduras. People were surprised to be asked to participate but were extremely cooperative. The response rate was 99% for the restaurant survey, 100% for the supermarket survey, and 100% for the fish market survey. In all, 73 restaurant, 54 supermarket, and 66 fish market questionnaires were completed.

Restaurant and supermarket managers and fish market vendors participated in the study. There were 86 potential questions on the restaurant questionnaire, 56 potential questions on the supermarket questionnaire, and 40 potential questions on the fish market vendor questionnaire. The restaurant interviews lasted the longest. Frequently, the managers provided samples of various fish and seafood preparations and wanted to discuss a variety of issues in addition to the survey. These interviews often lasted from 15 minutes up to one hour. The supermarket interviews lasted approximately 12 minutes while the fish market vendor interviews were the shortest at approximately 7 minutes.

RESULTS

Restaurant Survey

Of the 73 completed questionnaires, 64% of the respondents were located in the Pacific coast, or central-south, region of Honduras while 36% were located in the north, reflecting the

Table 1. Number and percentage of restaurants that sell and do not sell fish and tilapia by region, restaurant survey, Honduras, 1999.

Category	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Do Not Sell Fish	1	2	1	4	2	3
Sell Fish, But Not Tilapia	35	74	18	69	53	73
Sell Tilapia	11	23	7	27	18	25
Total	47	64	26	36	73	101

Table 2. Number and percentage of restaurants surveyed by percentage of total sales from fish and seafood, restaurant survey, Honduras, 1999.

Region	Seafood Sales						
	> 40%		*20–40%*		*0–19%*		*Total*
	No.	%	No.	%	No.	%	No.
CENTRAL-SOUTH							
Sell Tilapia	5	45	3	27	3	27	11
Don't Sell Tilapia	15	44	13	38	6	18	34
Total	20	44	16	36	9	20	45
NORTH							
Sell Tilapia	4	57	2	29	1	14	7
Don't Sell Tilapia	9	50	8	44	1	6	18
Total	13	52	10	40	2	8	25
TOTAL	33	47	27	39	13	19	70

Table 3. Most popular species of fish and seafood on menu by region, restaurant survey, Honduras, 1999.

Species	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Shrimp	37	28	20	27	57	27
Conch	17	13	10	13	27	13
Other Fish	11	8	15	20	26	12
Corvina	23	17	2	3	25	12
Other Shellfish	8	6	6	8	14	7
Robalo	7	5	6	8	13	6
Lobster	7	5	3	4	10	5
Red Tilapia	5	4	5	7	10	5
Crab	9	7	1	1	10	5
Red Pargo	6	4	3	4	9	4
Black Tilapia	4	3	4	5	8	4
Total	134	100	75	100	209	100

Table 4. Peak demand season for fish and seafood by region, restaurant survey, Honduras, 1999.

Season	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Mar.–Apr. (Easter)	23	44	10	40	33	43
None	19	37	8	32	27	35
Sept.–Dec. (Christmas)	8	15	7	28	15	19
Jan.–Feb.	1	2	0	0	1	1
May–Aug.	0	0	0	0	0	0
Don't Know	1	2	0	0	1	1
Total	52	100	25	100	77	100

Table 5. Current sales volumes of fish and seafood compared to 1998 and to 1997 by region, restaurant survey, Honduras, 1999.

Change in Sales	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
ONE YEAR AGO						
Same	21	46	5	20	26	37
More	14	30	5	20	19	27
Less	7	15	11	44	18	25
New Restaurant	3	6	3	12	6	8
Don't Know/ No Answer	1	2	1	4	2	3
Total	45	100	25	100	71	100
TWO YEARS AGO						
Same	17	37	4	16	21	30
More	9	20	12	48	21	30
Less	15	33	4	16	19	27
New Restaurant	3	6	3	12	6	8
Don't Know/ No Answer	2	4	2	8	4	5
Total	46	100	25	100	71	100

greater population density in the central-south part of the country (Table 1). Only 3% of the restaurants that responded to the survey did not sell fish at all. Seventy-three percent sold fish but not tilapia, and 25% of the restaurants sold tilapia. There was little difference in the percentages of those selling different types of fish from those selling tilapia between the two regions in the country.

Forty-seven percent of the restaurants surveyed had fish and seafood sales that composed greater than 40% of their total sales; 39% of the restaurants had fish and seafood sales that composed 20–40% of their total sales; and 19% had fish and seafood sales that composed 0–19% of their total sales (Table 2). The north region had a higher percentage (52%) of restaurants that had greater than 40% of their total sales from fish and seafood compared to the central-south (40%). The central-south region also had a higher percentage (20%) of restaurants that had only 0–19% of their total sales from fish and seafood compared to the north region (8%). There was only one restaurant in each region that did not include some type of fish or seafood on the menu. While nearly all restaurants surveyed included some type of fish and seafood, restaurants in the north relied more heavily on fish and seafood sales than did restaurants in the central-south part of the country. This corresponds with various observations made during the survey that people in the north consume fish and seafood more frequently than do people in the central-south region of Honduras.

Shrimp was indicated most often as one of the most popular types of fish or seafood on restaurant menus (Table 3). Shrimp was followed in descending order by conch, "other fish," corvina, "other shellfish," robalo, lobster, red tilapia, crab, red pargo, and black tilapia. "Other fish" refers to a frequent menu item that can be any of a number of different species of finfish depending on the daily catch and what the suppliers have available fresh that day. "Other fish" often is corvina, robalo, pargo, or other marine species. Regionally, corvina was identified as the second most popular type of fish and seafood after shrimp in the central-south region, while "other fish" was the second most popular item mentioned in the north region. Crab was identified as the fifth most popular in the central-south region but the least mentioned in the north region.

Table 6. Top fish and seafood dishes and appetizers in terms of sales, restaurant survey, Honduras, 1999.

Dish	Preferred Dish	
	No.	*%*
Breaded Shrimp	33	47
Fried Fish	30	43
Garlic Shrimp	24	34
Shrimp Cocktail	15	21
Soup	29	41
Breaded Fish	13	19
Fish *Ceviche*	11	16
Grilled Fish	8	11
Garlic Fish	8	11

The peak demand period was indicated to be the Easter season during March and April by 43% of the respondents (Table 4). Interestingly, another 35% responded that there was no one peak demand period, and 19% said that the period from September through December, or around Christmas, was the peak demand period. Similar percentages of respondents in the central-south and north regions agreed that March through April was the peak demand period, but more respondents in the north (28%) indicated that September through December was the peak demand period than did respondents in the central-south region (15%).

Thirty-seven percent of respondents said that they were selling the same amount of fish in 1999 and 1998 (Table 5). Twenty-seven percent said they were selling more, and 25% said they were selling less in 1999 compared to 1998. There were apparent regional differences in the response to this question. In the north, 44% of respondents said that they were selling less compared to only 15% who said they were selling less in the central-south region. Thirty percent of respondents in the central-south region said that they were selling more compared to only 20% in the north. In the central-south region, 46% said they were selling the same amount as one year ago, but only 20% of respondents in the north made the same assessment.

There were fewer restaurants with similar sales in 1999 and 1997 (Table 5). Higher percentages of respondents were recorded for both the categories of selling more and for selling less in 1999. Fewer respondents in both regions indicated that their sales were the same in 1999 and 1997 than the percentages indicating similar sales in 1999 and 1998. Higher percentages of central-south respondents were recorded for both categories of selling more and selling less in 1999 as compared to 1997. However, there were more respondents in the north indicating lower sales in 1999 as compared to 1997 than did when comparing 1999 to 1998 sales. Fewer respondents in the north said they were selling more in 1999 compared to 1997 than did when comparing 1999 to 1998 sales. These results seem to indicate a good deal of fluctuation in fish and seafood sales between 1997 and 1999. Some of the fluctuation may be due to the effects of hurricane Mitch on both the capture fisheries and the Honduran economy.

Breaded shrimp was mentioned most often as the top fish and seafood dish or appetizer in terms of sales (Table 6). It was followed in descending order of importance by fried fish, garlic shrimp, shrimp cocktail, soup (mariner's and conch), breaded fish, fish *ceviche*, grilled fish, and garlic fish.

Fried fish was the dish mentioned most often as having the fastest sales growth in the last year (Table 7). It was followed by breaded shrimp, conch soup, garlic shrimp, shrimp cocktail, breaded fish, fish *ceviche*, mariner's soup, and grilled shrimp.

Table 7. Fish and seafood dishes with fastest sales growth in last year, restaurant survey, Honduras, 1999.

Dish	Sales Growth	
	No.	*%*
Fried Fish	22	34
Breaded Shrimp	21	33
Soup	19	30
Garlic Shrimp	13	21
Shrimp Cocktail	10	16
Breaded Fish	9	14
Fish *Ceviche*	8	13
Grilled Shrimp	5	9
Shellfish *Ceviche*	4	6
Grilled Fish	4	6

Wholesalers were mentioned as the major suppliers of fish and seafood by the highest percentage (74%) of respondents in both the central-south and north regions (Table 8). Some restaurants (20%) also purchased fish and seafood directly from fishermen or fish farmers. One notable regional difference was that only restaurants in the central-south region indicated that they purchased from fish farmers.

Fifty-nine percent of respondents indicated that their wholesalers were from the central-south region while another 28% reported that their wholesalers were from the north.

Over half (59%) of the respondents had two or fewer different suppliers. An additional 27% had from three to five different suppliers, and only 13% had more than seven different suppliers.

Eighty percent of respondents did not transport their own fish. Twenty percent did haul their own fish. A few restaurants (8.5%) delivered fish to other restaurants.

Overall, 46% of respondents indicated that they were likely to begin adding tilapia to their menu in the next year (Table 9). Thirty-eight percent said that they would be unlikely to add tilapia to their menu in the next year, and 15% did not know. There was a marked regional difference in response to this question. Over half of the respondents in the central-south region expressed the likelihood of adding tilapia and only 26% were unlikely to add tilapia. However, in the north, only one-third (33%) were likely to add tilapia, but 61% were unlikely to do so.

Sixty-seven percent of respondents indicated that they could get tilapia supplied on a consistent basis (Table 10). However, respondents in the central-south region were more likely (72%) to indicate that tilapia supplies were consistent than respondents in the north (57%).

Across all respondents, the most often cited tilapia supply problem was seasonality of supply or unavailability at certain times of the year (Table 11). Insufficient quantities of tilapia, lack of availability of preferred sizes, and other, unspecified, problems of supply were mentioned. However, only respondents in the north mentioned problems of insufficient quantity.

Half (50%) of the respondents had been selling tilapia for more than five years, 22% for more than two but less than five years.

Table 8. Type of fish and seafood suppliers by region, restaurant survey, Honduras, 1999.

Type of Supplier	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Wholesalers	35	73	19	76	54	74
Fishermen	4	8	3	12	7	10
Fish Farmers	7	15	0	0	7	10
Processor/Producers	0	0	1	4	1	1
Importers	0	0	1	4	1	1
Other	2	4	1	4	3	4
Total	48	100	25	100	73	100

Table 9. Likelihood of restaurants beginning to sell tilapia by region, restaurant survey, Honduras, 1999.

Likelihood of Selling Tilapia	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Likely	18	53	6	33	24	46
Unlikely	9	26	11	61	20	38
No Answer	7	21	1	6	8	15
Total	34	100	18	100	52	100

Table 10. Availability of consistent supply of tilapia by region, restaurant survey, Honduras, 1999.

Consistent Supply of Tilapia	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Yes	8	72	4	57	12	67
No	3	28	3	43	6	33
Total	11	100	7	100	18	100

Table 11. Problems indicated with the supply of tilapia by region, restaurant survey, Honduras, 1999.

Supply Problems	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Unavailable at Certain Time of Year	3	60	2	29	5	42
Insufficient Quantity	0	0	3	43	3	25
Availability of Preferred Sizes	1	20	1	14	2	17
Other	1	20	1	14	2	17
Total	5	100	7	100	12	100

Table 12. Years restaurant has been selling tilapia, restaurant survey, Honduras, 1999.

Years Selling Tilapia	Restaurant	
	No.	*%*
> 5 years	9	50
2–5 years	4	22
1–2 years	2	11
0.5–1 year	1	6
< 0.5 year	2	11

Table 13. Current sales volume of tilapia compared to 1998 and to 1997 by region, restaurant survey, Honduras, 1999.

Change in 1999 Sales	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
1998						
More Tilapia	6	54	5	71	11	61
Less Tilapia	2	18	2	29	4	22
Same	2	18	0	0	2	11
No Answer	1	9	0	0	1	6
Total	11	100	7	100	18	100
1997						
More Tilapia	5	46	2	29	7	39
Less Tilapia	2	18	3	43	5	28
Same	2	18	1	14	3	17
No Answer	2	18	1	14	3	17
Total	11	100	7	100	18	100

Another 28% had been selling for less than two years (Table 12).

Sixty-one percent of respondents indicated that they sold more tilapia in 1999 compared to 1998 (Table 13). However, only 39% said that they sold more in 1999 than in 1997. In the north region, 71% said they sold more in 1999 than in 1998, but only 29% said that 1999 sales were higher than 1997 sales. In the central-south region, 54% said that they sold more in 1999 than 1998, and 46% said that they sold more in 1999 than 1997. These results indicate that 1998 sales were lower than normal, but sales in 1999 had recovered and tended to be somewhat higher than in 1997.

Table 14. Product forms purchased of tilapia, restaurant survey, Honduras, 1999.

Product Forms	Restaurant	
	No.	*%*
Fresh Whole-dressed	14	78
Fresh Fillets	6	33
Live	2	11
Frozen Whole-dressed	1	6
Total	23	100

Table 15. Forms of preparation of tilapia by region, restaurant survey, Honduras, 1999.

Form of Preparation	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Fried	11	41	7	100	18	100
Breaded	5	19	1	14	6	33
Boneless	1	4	3	43	4	22
Garlic	2	7	2	29	4	22
Grilled	1	4	2	29	3	17
Onion	3	11	0	0	3	17
Other	4	15	3	43	7	39

Seventy-eight percent of respondents said that they sold fresh whole-dressed tilapia (Table 14). An additional 33% sold fresh fillets; 11% sold tilapia live; and 6% sold frozen whole-dressed tilapia. The majority (57%) of those selling tilapia sold less than 50 lb wk^{-1}. Sixty-seven percent of respondents sold 1-lb fresh whole-dressed tilapia, while 33% sold 2-lb fresh whole-dressed tilapia. All respondents who sold fillets indicated that they sold 1-lb fillets.

Only 22% of respondents transported their own tilapia. Seventy-eight percent had the tilapia delivered to their restaurant.

All respondents fried tilapia to serve in their restaurants (Table 15). In addition, 33% breaded tilapia. This was a more common form of preparation in the central-south region (19%) than in the north (14%). However, 43% of respondents in the north also served tilapia as a boneless product compared to only 4% in the central-south region. An additional 17 to 22% of respondents served tilapia grilled or in a garlic sauce. Respondents in the central-south region also used an onion sauce to prepare tilapia.

All respondents served tilapia as a main dish. An additional 11% also served it as an appetizer.

Table 16. Quality problems of tilapia products by region, restaurant survey, Honduras, 1999.

Quality Problems	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Yes	9	82	7	100	16	89
No	2	18	0	0	2	11
Total	11	100	7	100	18	100

Table 17. Use of promotion for tilapia by region, restaurant survey, Honduras, 1999.

Use of Promotion	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Yes	3	27	4	57	7	39
No	8	73	3	43	11	61
Total	11	100	7	100	18	100

Table 18. Mean ratings of various attributes of tilapia by region, restaurant survey, Honduras, 1999.

Attribute	Sold Tilapia			Did Not Sell Tilapia		
	Central-South	*North*	*Total*	*Central-South*	*North*	*Total*
Reliable Quality	8.18	8.71	8.39	2.71	3.56	3.00
Available	8.00	9.29	8.50	3.06	4.44	3.53
Consumers Like to Eat	7.45	8.14	7.72	4.14	3.17	3.81
High Quality Fish	9.64	9.29	9.50	5.03	3.83	4.62
Little Fishy Odor	7.09	8.14	7.50	4.26	3.94	4.15
Taste Like Earth	3.36	3.14	3.28	3.37	4.11	3.62
Nice Fresh Flavor	9.45	9.29	9.39	5.09	4.06	4.74
Easy to Prepare	9.55	9.29	9.44	7.46	5.67	6.85
Price is Too High	4.45	3.57	4.11	2.89	1.11	2.28
Patrons Like Variety	7.18	8.57	7.72	5.00	5.11	5.04
Size Too Small	5.09	5.43	5.22	4.54	4.56	4.55

Table 19. Most important services of restaurants, restaurant survey, Honduras, 1999.

Degree of Importance	Meal Service									
	Breakfast		*Lunch*		*Dinner*		*Carry-out*		*Banquet*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Very Important	9	12	60	82	58	80	54	74	63	86
Somewhat Important	3	4	11	15	11	15	12	16	5	7
Less Important	5	7	1	1	4	6	4	6	3	4
Service Not Available	56	77	1	1	0	0	3	4	2	3
Total	73	100	73	100	73	100	73	100	73	100

Table 20. Number and percentage of supermarkets that sell and don't sell fish and tilapia by region, supermarket survey, Honduras, 1999.

Category	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Do Not Sell Tilapia	15	40	4	25	19	35
Sell Tilapia	12	32	10	62	22	41
Used to Sell Tilapia	11	29	2	12	13	24
Total	38	100	16	100	54	100

Eighty-nine percent of respondents indicated that they did not have any problems with the quality of tilapia products (Table 16). None of the respondents in the north indicated any quality problems while 18% of respondents in the central-south region indicated problems with the quality of tilapia products. Problems mentioned, while few, included off-flavor, freshness concerns, and fish being too small.

Only 39% of respondents reported using some form of promotion of tilapia products (Table 17). Promotion of tilapia products was more common in the north than in the central-south region. Types of promotion used included in-store signs, radio, discounted specials, news circular, TV, newspaper, and in-store samples. In-store signs and radio announcements were the most common.

Table 21. Number and percentage of stores with reasons for why supermarkets either do not sell or stopped selling tilapia by region, supermarket survey, 1999.

Reasons For Not Selling Tilapia	Region of Country								Total	
	Central-South				*North*					
	Did Not Sell		Used To Sell		Did Not Sell		Used To Sell			
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
There Is No Demand	4	27	3	27	2	50	2	100	11	34
Storage Problems	7	47	0	0	0	0	1	50	8	25
Unavailable at Certain Times of Year	2	13	2	18	0	0	1	50	5	16
Not Fresh	0	0	3	27	0	0	0	0	3	9
Don't Know/No Answer	1	7	1	9	1	25	0	0	3	9
Other	5	33	3	27	3	75	1	50	12	38

Table 22. Likelihood of supermarkets beginning to sell tilapia in the next year by region, supermarket survey, Honduras, 1999.

Likelihood of Selling Tilapia	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Very Likely	6	23	4	67	10	31
Somewhat Likely	4	15	2	33	6	19
Somewhat Unlikely	0	0	0	0	0	0
Very Unlikely	8	31	0	0	8	25
No Answer	8	31	0	0	8	25
Total	26	100	6	100	32	100

Respondents were asked to rank tilapia on a series of attributes on a scale of 1 to 10, in which 1 meant strong disagreement and 10 indicated complete agreement (Table 18). Restaurants that sold tilapia rated it highly on all attributes, except they disagreed that the price of tilapia was too high. Respondents of restaurants that had tilapia on the menu were neutral about the size of the fish supplied.

Restaurants that did not include tilapia on the menu rated tilapia low on reliable quality, availability, and preferences of consumers. Ratings on flavor, odor, and quality attributes were more neutral. Price was not considered to be too high, and tilapia was rated highly on ease of preparation. Responses to the size statement were neutral. Responses to the statement that patrons would like the additional variety that tilapia would add were nearly neutral (5.04 mean rating).

These results indicate clear differences in attitudes towards tilapia. Restaurants that serve tilapia rate it highly on all attributes; restaurants that do not have tilapia on the menu rate it much lower. The lowest ratings by restaurants that did not offer tilapia were for reliability of quality and availability. Still, ratings on the reliability of tilapia quality were neutral, particularly in the central-south region. Flavor and odor rankings were also nearly neutral. These results suggest that if tilapia farmers can guarantee supplies and availability of high-quality tilapia and combine this with samples and recipes, they could take advantage of the importance of variety to consumers by suggesting tilapia dishes as daily specials. This may be the best means of introducing tilapia to both restaurant mangers and their clientele as long as supplies can be guaranteed and reliable.

The most important services of the restaurant respondents were banquets (86%), lunch (82%), dinner (80%), and carry-out (74%) (Table 19).

Supermarket Survey

Of the 54 completed supermarket questionnaires, 70% were from the central-south region and 30% from the north (Table 20). This reflects the greater population levels in the central-south part of the country. All of the supermarkets sold some type of fish product. Overall, 41% sold tilapia, 24% used to sell tilapia but did not currently sell tilapia, and 35% had never sold tilapia. A much higher percentage of respondents in the north region were selling tilapia as compared to only 32% of the respondents in the central-south region. The central-south region had a higher percentage of stores (29%) that used to sell tilapia as compared to only 12% in the north.

In the north, the lack of demand was mentioned as a reason for either not selling or ceasing to sell tilapia (Table 21). However, in the central-south region, storage problems were cited most frequently as the reason for not having ever sold tilapia. This was followed by the citing of a lack of availability during certain times of the year. Supermarkets in the central-south region that used to sell tilapia indicated that it was not fresh and that it was not available at certain times of the year. None of the stores indicated that they had not heard of tilapia, that the wholesale price was too high, that fish were too small, that consumers had negative attitudes towards tilapia, or that tilapia tasted like earth.

Overall, 50% of supermarket managers responded that they were either somewhat or very likely to begin to sell tilapia in the next year (Table 22). There were an additional 25% who did not know if they might and 25% who were very unlikely to begin to sell tilapia. There was a marked regional difference in response to this question. All of the respondents in the north region said that they were likely to sell tilapia in the next year, but only 38% of central-south respondents indicated that they were likely to do so. Thirty-one percent said that they were very unlikely to do so.

The majority (60%) of the supermarket respondents began to sell tilapia in the last three years (1997–1999) (Table 23). Another 23% of supermarket managers responded that they began to sell tilapia in the last 6 years (1993–1996) while only 17% began selling tilapia prior to 1992. There was little difference between responses from the central-south and north region.

Twenty-six percent of respondents indicated that they sold more tilapia in 1999 compared to each of 1998 and 1997 (Table 24). Only 17% said they were selling less tilapia in 1999 as compared to each of 1998 and 1997.

Table 23. Years supermarket has been selling tilapia, supermarket survey, Honduras, 1999.

Category	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Do Not Sell Fish	1	2	1	4	2	3
Sell Fish, But Not Tilapia	35	74	18	69	53	73
Sell Tilapia	11	23	7	27	18	25
Total	47	64	26	36	73	101

Table 24. Current sales volumes of tilapia compared to 1998 and to 1997 by region, supermarket survey, Honduras, 1999.

Change in 1999 Sales Volume	1998		1997	
	No.	*%*	*No.*	*%*
More Tilapia	9	26	9	26
Less Tilapia	6	17	6	17
Same	9	26	4	11
No Answer	11	31	16	46
Total	35	100	35	100

Over half (54%) of respondents purchased tilapia from wholesalers (Table 25). Another 17% purchased tilapia from fish farms. There were no regional differences in type of suppliers of tilapia. The majority (57%) of the supermarkets that sold tilapia purchased from only one to two different suppliers. None had more than four suppliers.

Table 26 lists preference ratings for supermarkets that purchased tilapia for a variety of attributes. On a scale of 1–10, a score less than 5 indicates disagreement; 5 is a neutral score, and a score above 5 indicates agreement with the statement. Respondents rated tilapia favorably on supply, availability, patrons' preferences, odor, flavor, ease of preparation, price, variety, and size. Of these, quality received the highest ratings along with ease of preparation. In general, responses of those who purchased tilapia from the central-south were lower, although still positive, than responses from supermarkets with suppliers from the north.

The majority (71%) of respondents did not transport their own tilapia. The remaining 29% did not answer this question.

Less than half (49%) indicated that their supply of tilapia had been consistent (Table 27). Another 23% indicated it had not been, and 28% did not answer. Responses were similar for supermarkets with suppliers in the north and central-south regions. However, 75% of the respondents who obtained fish from Nicaragua said that their supply was consistent.

Table 25. Type of tilapia suppliers by region, supermarket survey, Honduras, 1999.

Type of Supplier	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Wholesalers	12	52	7	58	19	54
Fishermen	0	0	1	8	1	3
Fish Farmers	4	17	2	17	6	17
Processor/Producers	1	4	1	8	2	6
No Answer	8	35	2	17	10	29
Total	25	100	13	100	38	100

Table 26. Mean ratings of various attributes of tilapia by region, supermarket survey, Honduras, 1999.

Attribute	Sold Tilapia			Did Not Sell Tilapia			Used to Sell Tilapia			Total
	Central-South	*North*	*Total*	*Central-South*	*North*	*Total*	*Central-South*	*North*	*Total*	
Reliable Quality	6.83	7.70	7.23	4.93	4.50	5.96	5.18	7.00	5.46	5.96
Available	7.67	8.80	8.18	4.20	2.00	5.69	3.73	7.50	4.31	5.69
Consumers Like to Eat	6.67	9.00	7.73	5.47	7.75	5.95	7.09	5.50	6.85	6.89
High Quality Fish	8.33	9.00	8.64	7.87	4.75	7.21	8.00	7.50	7.92	7.96
Little Fishy Odor	7.50	9.10	8.23	4.60	1.75	4.00	5.91	5.50	5.85	6.17
Taste Like Earth	4.33	3.00	3.73	4.00	4.50	4.11	4.45	9.00	5.15	4.20
Nice Fresh Flavor	7.42	9.20	8.23	6.07	4.75	5.79	6.91	7.50	7.00	7.07
Easy to Prepare	7.75	8.60	8.14	8.27	2.25	7.00	8.45	7.50	8.31	7.78
Price is Too High	3.83	4.90	4.32	2.53	1.50	2.32	2.82	3.50	2.92	3.28
Patrons Like Variety	3.75	8.30	5.82	4.40	6.25	4.79	7.18	7.00	7.15	5.78
Size Too Small	4.75	3.30	4.09	2.67	n.a.	2.11	3.18	10.00	4.23	3.43

Table 27. Availability of consistent supply of tilapia by region, supermarket survey, Honduras, 1999.

Consistent Supply of Tilapia	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Yes	8	35	9	75	17	49
No	7	30	1	8	8	23
No Answer	8	35	2	17	10	28
Total	23	100	12	100	35	100

Table 28. Problems indicated with the supply of tilapia by region, supermarket survey, Honduras, 1999.

Supply Problems	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Unavailable at Certain Times of the Year	6	26	1	8	7	20
Insufficient Quantity	3	13	0	0	3	9
Availability of Preferred Product Forms	1	4	0	0	1	3
Unreliable Quality	1	4	0	0	1	3
Fish Tastes Like Earth	1	4	0	0	1	3
Other	2	9	0	0	2	6
No Answer	17	74	11	92	28	80

Table 29. Product forms purchased of tilapia, supermarket survey, Honduras, 1999.

Product Forms	Central-South		North		Total	
	No.	*%*	*No.*	*%*	*No.*	*%*
Fresh Whole-dressed	13	56	7	58	20	57
Fresh Fillets	2	9	5	42	7	20
Frozen Whole-dressed	3	13	0	0	3	9
No Answer	8	35	2	17	10	29
Total	26	100	14	100	40	100

The most commonly cited supply problem was that tilapia was unavailable at certain times of the year (Table 28). This was followed by insufficient quantity and a few responses related to unavailability of certain product forms, unreliable quantity, and off-flavor fish. There were more respondents with suppliers from the north who indicated insufficient quantity as the major supply problem.

The top selling tilapia product was fresh whole-dressed fish (57%) followed by fresh fillets (20%) (Table 29). There was little difference between respondents in the north and central-south regions on fresh whole-dressed fish; however, 13% of supermarkets in the central-south region that sold tilapia sold them as frozen whole-dressed products and even fewer (9%) sold fresh fillets. By comparison, 42% of the supermarkets in the north that sold tilapia handled fresh fillets, and none sold frozen whole-dressed fish.

Tilapia volumes sold daily were low (Table 30). Most of the supermarket respondents indicated that they sold from 0 to 10 lb d^{-1} of fresh whole-dressed tilapia. An additional 30% sold from 20 to 30 lb d^{-1}. Sales of fresh fillets were of similar magnitude.

Table 30. Pounds per day sold by product form, supermarket survey, Honduras, 1999.

Product Form	Amount Sold (lb d^{-1})							
	0–10		*20–30*		*40–50*		*> 70*	
	No.	%	No.	%	No.	%	No.	%
Fresh Whole Dressed	9	45	6	30	2	10	3	15
Fresh Fillets	3	43	2	29	1	14	1	14
Frozen Whole Dressed	1	33	0	0	1	33	1	33

Table 31. Retail price of tilapia by product form, supermarket survey, Honduras, 1999.

Product Form	Retail Price (Lempiras lb^{-1})							
	10–15		*16–20*		*21–25*		*30–40*	
	No.	%	No.	%	No.	%	No.	%
Fresh Whole Dressed	13	65	4	25	2	10	1	5
Fresh Fillets	1	14	1	14	1	17	4	57
Frozen Whole Dressed	2	67	1	33	0	0	0	0

The most frequent prices for fresh whole-dressed tilapia were in the range of 10 to 15 Lempiras lb^{-1} (Table 31). Fewer respondents sold fresh whole-dressed tilapia at higher prices. Fresh fillet prices were mostly in the range of 30 to 40 Lempiras lb^{-1}.

Wholesale prices of fresh whole-dressed tilapia were in the range of 5 to 10 Lempiras for 68% of the supermarket respondents (Table 32). Thirty-two percent of supermarkets purchased fresh whole-dressed tilapia at higher prices.

Only 11% of respondents indicated that they experienced problems with the quality of tilapia that they purchased (Table 33). Sixty percent said that they did not have problems with the quality of the tilapia. Of those that mentioned quality problems, off-flavor and freshness were the problems most frequently cited (Table 34).

Only 34% of the supermarket respondents used any form of promotion (Table 35). However, 42% of the respondents in the north did promote tilapia as compared to only 30% in the central-south region. In-store signs, discounted specials, and newspaper advertisements were the most common forms of promotion (Table 36). Some supermarkets also used radio advertising to promote tilapia sales.

Most supermarkets (54%) reported that suppliers of fish and seafood were wholesalers (Table 37). A few respondents purchased directly from fishermen or from a processor.

Half of the supermarket respondents had been in business less than 5 years (Table 38). An additional 12% had been in business for more than 20 years. The recent economic expansion in Honduras may have resulted in this evident growth in the supermarket sector.

Half of the supermarkets responding to the survey had weekly sales volumes of less than 540,000 Lempiras (Table 39). An

Table 32. Wholesale price of tilapia by product form, supermarket survey, Honduras, 1999.

Product Form	Wholesale Price (Lempiras lb^{-1})					
	5–10		*11–15*		*> 20*	
	No.	%	No.	%	No.	%
Fresh Whole Dressed	13	68	4	21	2	11
Fresh Fillets	1	33	1	33	1	33
Frozen Whole Dressed	2	67	1	33	0	0

Table 33. Quality of tilapia products by region, supermarket survey, Honduras, 1999.

Quality Problems	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Yes	3	13	1	8	4	11
No	12	52	9	75	21	60
No Answer	8	35	2	17	10	29
Total	23	100	12	100	35	100

Table 34. Quality problems of tilapia products by region, supermarket survey, Honduras, 1999.

Quality Problems	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Off-flavor	0	0	3	75	3	75
Freshness	1	100	3	75	3	75
Don't Know/No Answer	0	0	1	25	1	25
Other	0	0	1	25	1	25

Table 35. Use of promotion for tilapia by region, supermarket survey, Honduras, 1999.

Use of Promotion	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Yes	7	30	5	42	12	34
No	8	35	4	33	12	34
No Answer	8	35	3	25	11	31
Total	23	100	12	100	35	100

Table 36. Means of promotion used, supermarket survey, Honduras, 1999.

Use of Promotion	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
In-store Signs	5	71	5	100	10	83
Discounted Specials	6	86	4	80	10	83
Newspaper	4	57	3	60	7	58
Radio	2	29	1	20	3	25
Other	2	29	0	0	2	17
Total	19		13		32	

Table 37. Type of fish and seafood suppliers by region, supermarket survey, Honduras, 1999.

Type of Supplier	Region of Country				Total	
	Central-South		*North*			
	No.	%	No.	%	No.	%
Wholesalers	20	53	9	56	29	54
Fishermen	1	3	6	38	7	13
Processor/Producers	1	3	0	0	1	2
No Answer	17	45	1	6	18	33
Total	39		16		54	

additional 32% of respondents had weekly sales from 540,000 to 1,012,500 Lempiras, and the remaining 17% had weekly sales greater than 1,012,500 Lempiras.

Middle-income mestizos were the clientele group mentioned most frequently (82% of the time) as patrons of the responding supermarket managers (Table 40). Equal proportions by both high- and low-income mestizo groups were next most frequently mentioned. Twenty-four percent of the clientele was characterized as international.

The top fish and seafood product in terms of sales in the supermarkets was shrimp, mentioned by 52% of respondents (Table 41). This was followed by conch (35%), jaiba (33%), robalo (26%), "red" fish (22%), "other fish" (20%), corvina (18%), "fish fillets" (17%), red tilapia (17%), red pargo (17%), "other shellfish" (15%), crab (9%), and tilapia fillets (13%). There were some regional differences in the listing. More respondents in the north rated conch, "red" fish, red tilapia, crab, and tilapia fillets as top products than in the central-south region. However, in the central-south region, corvina and red pargo were more

Table 38. Years in business, supermarket survey, Honduras, 1999.

Years in Business	**Central-South**						**North**						**Total**	
	Sold Tilapia		*Did Not Sell Tilapia*		*Used to Sell Tilapia*		*Sold Tilapia*		*Did Not Sell Tilapia*		*Used to Sell Tilapia*			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0–5	7	58	8	53	6	54	6	60	0	0	0	0	27	50
6–10	2	17	2	13	2	18	1	10	2	50	0	0	9	17
11–15	2	17	1	7	1	9	1	10	2	50	0	0	7	13
16–20	0	0	0	0	1	9	2	20	0	0	1	50	4	7
20–25	1	8	1	7	0	0	0	0	0	0	1	50	3	6
30–40	0	0	2	13	1	9	0	0	0	0	0	0	3	6
No Answer	0	0	1	7	0	0	0	0	0	0	0	0	1	2
Total	12	100	15	100	11	100	10	100	4	100	2	100	54	100

Table 39. Weekly sales volume, supermarket survey, Honduras, 1999.

Sales Volume	**Region**												**Total**	
	Central-South						*North*							
	Sold Tilapia		Did Not Sell Tilapia		Used to Sell Tilapia		Sold Tilapia		Did Not Sell Tilapia		Used to Sell Tilapia			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
< Lps 540,000	5	42	11	73	3	27	3	30	3	75	2	100	27	50
Lps 540,000–1,012,500	4	33	2	13	4	36	6	60	1	25	0	0	17	32
Lps 1,012,501–1,336,500	2	17	0	0	1	9	0	0	0	0	0	0	3	6
Lps 1,336,501–2,011,500	1	8	0	0	0	0	0	0	0	0	0	0	1	2
Lps 2,011,501–2,700,000	0	0	1	7	0	0	0	0	0	0	0	0	1	2
> Lps 2,700,000	0	0	1	7	3	27	0	0	0	0	0	0	4	7

Table 40. Clientele groups of supermarkets that did and did not sell tilapia by region, supermarket survey, Honduras, 1999.

Clientele Group	**Region**								**Total**	
	Central-South				*North*					
	Sold Tilapia		Sold Fish, Not Tilapia		Sold Tilapia		Sold Fish, Not Tilapia			
	No.	%	No.	%	No.	%	No.	%	No.	%
High-Income Mestizo	6	50	3	20	8	80	2	50	24	44
International	7	58	0	0	2	20	0	0	13	24
Middle-Income Mestizo	9	75	13	87	8	80	3	75	44	82
Low-Income Mestizo	4	33	11	73	1	10	2	50	22	41
Middle-Income Black	0	0	1	7	1	10	0	0	4	7
Low-Income Black	4	33	11	73	1	10	2	50	3	6

Table 41. Top fish and seafood products in terms of sales by region, supermarket survey, Honduras, 1999.

Species	Region of Country				Total	
	Central-South		North			
	No.	%	No.	%	No.	%
Shrimp	15	40	13	81	28	52
Conch	8	21	11	69	19	35
Jaiba	12	32	6	38	18	33
Red Fish	5	13	7	44	12	22
Other Fish	6	16	5	31	11	20
Corvina	9	24	1	6	10	18
Fish Fillets	6	16	3	19	9	17
Other Shellfish	5	13	3	19	8	15
Robalo	9	24	5	31	14	26
Red Tilapia	5	13	4	25	9	17
Crab	0	0	5	31	5	9
Red Pargo	8	21	1	6	9	17
Tilapia Fillets	3	8	4	25	7	13
No Answer	17	45	1	6	18	33

Table 42. Seafood items with the fastest sales growth by region, supermarket survey, Honduras, 1999.

Species	Region of Country				Total	
	Central-South		North			
	No.	%	No.	%	No.	%
Shrimp	11	29	10	62	21	39
Conch	6	16	9	56	15	28
Red Fish	4	10	4	25	8	15
Jaiba	3	8	1	6	4	7
Other Fish	3	8	4	25	7	13
Corvina	4	10	1	6	5	9
Fish Fillets	4	10	2	12	6	11
Other Shellfish	1	3	2	12	3	6
Robalo	6	16	2	12	8	15
Red Tilapia	2	5	2	12	4	7
Crab						
Red Pargo	8	21	1	6	9	17
Tilapia Fillets	4	10	4	25	8	15
No Answer	17	45	2	12	19	35

frequently mentioned as top products. The seafood items reported to have the fastest sales growth were often identified as those with the highest sales volumes (Table 42).

Literature Cited

Crawford, K.W., D.R. Dunseth, C.R. Engle, M.L. Hopkins, E.W. McCoy, and R.O. Smitherman, 1978. Marketing tilapia and Chinese carps. In: R.O. Smitherman, W.L. Shelton, and J.H. Grover (Editors), Culture of Exotic Fishes Symposium Proceedings. Fish Culture Section, American Fisheries Society, Auburn, Alabama, pp. 240–257.

Engle, C.R., 1997. Economics of tilapia (*Oreochromis spp.*) aquaculture. In: B. Costa-Pierce and J. Rakocy (Editors), Tilapia Aquaculture in the Americas, Volume One. World Aquaculture Society, Baton Rouge, Louisiana, pp. 229-243.

Engle, C.R., 1997. Marketing of the tilapias. In: B. Costa-Pierce and J. Rakocy (Editors), Tilapia Aquaculture in the Americas, Volume One. World Aquaculture Society, Baton Rouge, Louisiana, pp. 248–258.

Galbreath, P. and T.A. Barnes, 1981. Consumer preference for color and size of tilapia sold in supermarkets. Proceedings Catfish Farmers of America, Research Workshop, 3:47–48.

Green, B.W. and C.R. Engle, in press. Commercial tilapia aquaculture in Honduras. In: B. Costa-Pierce and J. Rakocy (Editors), Tilapia Aquaculture in the Americas, Volume 2. World Aquaculture Society, Baton Rouge, Louisiana.

Head, W.D., A. Zerbi, and W.D. Watanabe, 1994. Preliminary observations on the marketability of saltwater-cultured Florida red tilapia in Puerto Rico. J. World Aquacult. Soc. 25(3):432–446.

Matton, E., 1981. Mercadeo de los recursos piscicolas de los grandes lagos de Panama. Proyecto TCP/PAN/2303. Organización de las Naciones Unidas para la Agricultural y la Alimentacion, Roma, Italia.

Nelson, R.G., L.L. Behrends, P.F. Galbreath, and T.A. Barnes, 1983. Estimating relative sales potential of tilapia in supermarkets. Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies, 37:314-326.

Sarmiento, M.T., W. Lanza Nuñez, 1995. Situacion actual del cultivo de tilapia en Honduras. In: N. Galvez, J. Gunther, A. Porras, H. Perez Athanasiadis, and W. Zurburg (Editors), Actas del Primer Simposio Centroamericano sobre Cultivo de Tilapia, San Jose, Costa Rica, 15–17 November 1995, pp. 16–21.

Scheid, A.C. and J.G. Sutinen, 1979. The structure and performance of wholesale marketing of finfish in Costa Rica. ICMRD Working paper No. 4, University of Rhode Island, Kingston, Rhode Island.

Swanson, W., 1995. Marketing farm-raised tilapia. Mimeo. D.A. Larson Company, Minneapolis, Minnesota.

Teichert-Coddington, D.R. and B.W. Green, 1997. Experimental and commercial culture of tilapia in Honduras. In: B.A. Costa-Pierce and J.E. Rakocy (Editors), Tilapia Aquaculture in the Americas, Volume One. World Aquaculture Society, Baton Rouge, Louisiana, pp. 142–162.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Economic and Social Returns to Technology and Investment in Thailand

Ninth Work Plan, Marketing and Economic Analysis Research 4 (9MEAR4)
Progress Report

Siddhartha Dasgupta and Carole R. Engle
Department of Aquaculture and Fisheries
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

Abstract

The first phase of this study was to conduct an analysis of the economic trade-offs associated with PD/A CRSP-developed technologies in Thailand using secondary data. This paper outlines conditions under which a small-scale tilapia producer in Thailand chooses among four PD/A CRSP–developed technologies: low-intensity inorganic fertilization (inorganic technology); organic fertilization with collected chicken manure (organic technology); organic fertilization in layer-fish integrated ponds (integrated technology); and high-input green water (HIGW) technology using intensive inorganic fertilization treatments. A mixed-integer programming (MIP) model of annual operations of a small-scale Thai tilapia farm was developed and used to attribute technologies to the production ponds based on maximization of net income. Eleven scenarios were developed that were based on the advantages of each of the four technologies. Results of this first-phase analysis will provide important insights into the key relationships to be explored in the survey data collection phase. The following progress report presents the results of the analysis conducted in Phase I and a draft of the survey instrument to be used in data collection in the second phase. (A draft of this survey may be obtained from the PD/A CRSP—Eds.)

Introduction

Factors Affecting the Choice of Production Technology in Small-Scale Tilapia Farms in Thailand

The Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) has been involved in fish production research in Thailand since 1983 (Engle and Skladany, 1992). Much of this effort has been focused on improving traditional tilapia culture technologies and developing new technologies that are accessible to Thai farmers. The goal of this paper is to identify conditions that influence a producer's choice of alternative tilapia technologies.

PD/A CRSP research on tilapia production in Thailand included topics such as

1) Relationships between primary production and yield of tilapia in ponds (Diana et al., 1988);
2) The role of chicken manure in the production of Nile tilapia (*Oreochromis niloticus*) (Knud-Hansen et al., 1993); and
3) The role of urea in pond fertilization (Knud-Hansen and Pautong, 1993).

As a result of these and other studies, several alternative tilapia culture technologies were developed. Examples of such technologies include low-intensity inorganic pond fertilization, organic pond fertilization with chicken manure, and high-input green water (HIGW) technology using relatively intensive applications of urea and triple superphosphate (TSP). Inorganic pond fertilization has the advantages of high nutrient content and low oxygen demand (Yamada, 1986). Organic pond fertilization with chicken manure requires more dissolved oxygen and has the advantage of producing significantly higher fish yields when compared to inorganic fertilization alone (Diana et al., 1990). The HIGW technology, using sex-reversed tilapia, has produced yields of over 5,000 kg ha^{-1} yr^{-1}, more than a tenfold increase over yields from traditional farms using ruminant manure and other organic farm refuse (Shrestha et al., 1997). In the face of such variety, producer choice of technology will depend on several factors that include fish yield, input prices, input availability, and financial considerations. Results of this paper will be useful in assisting Thai tilapia producers to choose the optimal technology under various production, market, and financial conditions.

Methods and Materials

Secondary data from four tilapia pond production technologies were available for this study:

1) Tilapia polyculture (TP) with mrigal (*Cirrhina mrigala*), puntius (*Puntius gonionotus*), silver carp (*Hypophthalmichthys molitrix*), and common carp (*Cyprinus carpio*) using low-intensity inorganic fertilization only;
2) TP using organic fertilization by applying collected (or purchased) chicken manure;
3) TP in layer-fish integrated ponds with chicken coops (with fresh manure and spilled feed dropping directly into ponds); and
4) Tilapia monoculture using HIGW technology and sex-reversed tilapia fingerlings.

The above production technologies are denoted hereafter as 1) inorganic, 2) organic, 3) integrated, and 4) HIGW, respectively.

Engle and Skladany's (1992) study of the economic benefits of using chicken manure in Thai tilapia polyculture provided annual production and price data for the inorganic, organic, and integrated technologies. Information about HIGW technology is available in Shrestha et al. (1997). Table 1 provides a summary of annual fish yield, inputs used, and cost associated with the four technologies. Prices are 1990 annual input and output prices in Thai Baht (1 Baht = US$0.04) from Engle and Skladany (1992). Table 1 shows that tilapia polyculture with the inorganic technology has the lowest yields, followed by tilapia monoculture with HIGW technology. However, the HIGW technology requires much lower fish stocking density when compared with the remaining technologies. Table 1 also shows that the integrated technology provides farmers with three sources of income: fish, eggs, and hens. The costs and benefits of having egg-laying hens in the integrated technology were calculated from Engle and Skladany's (1992) report.

The above data were used to develop a mixed-integer mathematical programming model (MIP) of annual operations of a Thai fish farm, assuming a producer has access to the above four technologies and also has the option of taking ponds out of production. The objective was to maximize net annual returns subject to the following constraints:

1) A pond balance constraint, which ensures that the total number of ponds stocked with fish (or out of production) is equal to the number of ponds in the farm;
2) Fingerling balance constraints, which account for the total number of fingerlings of each fish species stocked in all ponds;
3) Urea and TSP balance constraints, which account for the total use of inorganic fertilizers in all ponds; and
4) Financial constraints.

The financial constraints can be subdivided into loan balance constraints and a loan repayment constraint. The loan balance constraints evaluate the total amount of operating loans and investment loans that a producer must have in order to finance variable input purchases and new capital (e.g., chicken coops over ponds as are used in the integrated technology approach). The loan repayment constraint ensures that the total annual revenue is sufficient to pay back the operating loan (with interest), make annual payments on the investment loan, and cover the annual capital depreciation. We assume that net returns are calculated as returns above fixed costs, and the only capital payments included in the MIP model are associated with construction of chicken coops on ponds using the integrated technology.

Engle and Skladany's (1992) report indicated that a 1-ha pond using the integrated technology has approximately 1,100 hens housed in three bamboo coops (3 m × 18 m × 1.5 m) that cost 21,000 Baht (5-yr service life). Other capital expenditures associated with layer-fish integrated ponds are:

1) Coop roofs (3-yr service life), which require 4,500 Baht;
2) Plastic netting (1-yr service life), which requires 120 Baht; and
3) 45 feed trays (3-yr service life), 45 water trays (3-yr service life), 105 baskets (2-yr service life), and electric wiring and bulbs (10-yr service life), all of which require an additional 6,125 Baht investment.

The total capital investment in chicken coops accumulates to 31,745 Baht per 1-ha pond.

Table 1. Annual yield received and variable inputs applied in a 1-ha pond with respect to four alternative tilapia production technologies: ponds using low-intensity inorganic fertilizers (inorganic); ponds using collected (or purchased) chicken manure (organic); ponds integrated with layers (integrated); and high-input green water ponds (HIGW). All prices are in 1990 Thai Baht (1 Baht = US$0.04).

Output	**Units**	**Inorganic**		**Organic**		**Integrated**		**HIGW**	
		Quantity	*Price* (Baht unit^{-1})	*Quantity*	*Price* (Baht unit^{-1})	*Quantity*	*Price* (Baht unit^{-1})	*Quantity*	*Price* (Baht unit^{-1})
YIELD									
Total Fish Yield[a]	kg	4,646	12	6,034	12	6,034	12	5,049	12
Hens	head					1,100	40		
Eggs[a]	head					277,200	1.45		
VARIABLE INPUTS									
Fingerlings									
Tilapia	head	90,520	0.10	90,520	0.1	90,520	0.1	25,000	0.1
Mrigal	head	129,310	0.05	129,310	0.05	129,310	0.05		
Puntius	head	12,930	0.05	12,930	0.05	12,930	0.05		
Silver Carp	head	8,620	0.15	8,620	0.15	8,620	0.15		
Common Carp	head	17,241	0.15	17,241	0.15	17,241	0.15		
Lime	kg	43	6	43	6	43	6	1,250	6
Urea	kg	43	7					1,593	7
TSP	kg	143	12					910	12
Collected Manure	kg			9,600	0.25				
Chicken Feed	kg					Cost[b]: 213,175 Baht (1,100 hens)$^{-1}$			
Chicken Vaccines	vial					10	100		
Electricity for Chickens	months					12	100		
Diesel	l	43	12	43	12	43	12	43	12
Rent for Plastic Net	d	6	100	6	100	6	100	6	100

Source: Engle and Skladany (1992) and Shrestha et al. (1997).

[a] These are expected values.

[b] Chicken feed for the first year consisted of: 1) 1.09 kg chicken^{-1} of commercial feed I (7.16 Baht kg^{-1}) for the first six weeks; 2) 0.82 kg chicken^{-1} of commercial feed II (10.16 Baht kg^{-1}), 0.41 kg chicken^{-1} of corn grain (3.5 Baht kg^{-1}), and 1.36 kg chicken^{-1} of fine rice bran (4.3 Baht kg^{-1}) for the next six weeks; and 3) 7.64 kg chicken^{-1} of commercial feed III (10.16 Baht kg^{-1}), 15.27 kg chicken^{-1} of medium rice bran (2.4 Baht kg^{-1}), 5.09 kg chicken^{-1} of fine rice bran (4.3 Baht kg^{-1}), 8.91 kg chicken^{-1} of broken rice (3.5 Baht kg^{-1}), and 0.26 kg chicken^{-1} of minerals (12 Baht kg^{-1}) for the remaining 40 weeks.

The objective function is expressed as the difference of total annual payments (i.e., the sum of variable costs, annual interest payment on the operating loan, and the annual payment on the investment loan) from the total annual revenue. Variable inputs (outlined in Table 1) include fingerlings, chicken manure, urea, TSP, lime, diesel, electricity, chicken feed, and chicken vaccines. Annual revenue per pond is calculated for each technology by using the output quantity and price data contained in Table 1. Since ponds devoted to the integrated technology include hens that produce eggs for two years, a terminal condition is included in the objective function, which accounts for the current value of future returns over variable costs of selling eggs and hens during the following year.

The MIP model was developed for a farm with two 1-ha ponds. Such farm sizes are not atypical of small-scale Thai aquaculture; Shrestha et al. (1997) reported an average farm size of 3.4 ha among the farms participating in trials of the HIGW technology. Decision variables for the MIP model include the number of ponds (integer variables) that are devoted to one of the four possible technologies or are taken out of production. Other decision variables include the total quantity of fingerlings, urea, and TSP purchased during the year, and the amount of operating and investment loans taken by the producer. We also assumed a fixed annual interest rate of 12% (Engle and Skladany, 1992).

RESULTS

Annual operating costs (per 1-ha pond) under each technology are evaluated from Table 1 as follows (operating costs of ponds with no liming are included in parentheses): 1) 23,435 Baht (23,177 Baht) for inorganic technology; 2) 23,818 Baht (23,560 Baht) for organic technology; 3) 245,593 Baht (245,335 Baht) for integrated technology; and 4) 33,183 Baht (25,683 Baht) for HIGW technology. Clearly, integrated ponds are the most expensive to operate. Ponds using the inorganic technology are the least expensive to operate, followed by ponds using the organic technology. Gross income per pond from each technology can also be evaluated from Table 1 (in ascending order): 55,352 Baht (inorganic technology); 60,588 Baht (HIGW technology); 72,408 Baht (organic technology); and 474,348 Baht (integrated technology).

Table 2 reports the results of solving the MIP model for 11 different scenarios. Consistent with the goals of this paper, we designed the scenarios to determine input availability conditions, input price conditions, and financial conditions that would induce producers to select different technologies based on maximizing their expected net annual income.

In the first scenario all decision variables are unbounded, i.e., there is an infinite availability of fingerlings, chemicals, and loan funds. Results of the MIP model show that a producer should use layer-fish integration in both ponds for a net annual return of 673,583 Baht. Since Table 1 indicates that the integrated technology has the highest income potential, this result is not surprising. Scenarios 2, 3, and 4 impose upper bounds on the operating loan and investment loans. If the maximum operating loan is 246,000 Baht (scenario 2), given that the annual variable costs per pond with integrated technology is 245,593 Baht, then the optimal solution is to use layer-fish integration in one pond and leave the other pond empty. Although a 246,000 Baht operating loan is sufficient to put both ponds in production using the inorganic, organic, or HIGW technology, that loan amount is insufficient for having two production ponds if one pond uses the integrated technology. Hence, scenario 2 results are indicative of the income superiority of the integrated technology over the other three technologies. Scenarios 3 and 4 establish an upper bound for an investment loan (although an unlimited operating loan is available). In scenario 3, the investment loan is restricted between 63,490 Baht and 31,745 Baht. Since 63,490 Baht is required for capital investments in two ponds using the

Table 2. Annual net income, operating loan, and investment loan of a Thai tilapia farm with two 1-ha ponds.

	Scenario	Solution	Net Income (Baht)	Operating Loan (Baht)	Investment Loan (Baht)
1	No bound on any decision variable.	2 ponds: integrated	673,582	491,184	63,490
2	Operating loan bounded above by 246,000 Baht.	1 pond: integrated, 1 pond: empty	336,791	245,592	31,745
3	Investment loan restricted between 63,490 Baht and 31,745 Baht.	1 pond: integrated, 1 pond: organic	383,953	269,409	31,745
4	Investment loan restricted to be less than 31,745 Baht.	2 ponds: organic	94,923	47,634	0
5	Operating loan bounded above by 245,000 Baht.	2 ponds: organic	94,923	47,634	0
6	Operating loan bounded above by 245,000 Baht. Manure price increases to 1.39 Baht kg^{-1}.	2 ponds: HIGW	71,250	66,367	0
7	Operating loan bounded above by 245,000 Baht. All fingerling prices increased by 63%.	2 ponds: HIGW	67,911	69,517	0
8	Operating loan bounded above by 245,000 Baht. Upper bound on tilapia fingerling availability: between 181,040 and 115,520.	1 pond: organic, 1 pond: HIGW	82,787	57,001	0
9	Operating loan bounded above by 245,000 Baht. Upper bound on tilapia fingerling availability: between 90,520 and 115,520.	2 ponds: HIGW	71,250	66,367	0
10	Operating loan bounded above by 47,000 Baht.	2 ponds: inorganic	61,823	46,868	0
11	Operating loan bounded above by 47,000 Baht, no liming of ponds.	1 pond: inorganic, 1 pond: organic	78,620	46,735	0

integrated technology (a 31,745 Baht capital investment is required for one integrated pond), scenario 3 allows only one pond to be managed under the integrated technology. The second pond is managed using the organic technology. If the maximum investment loan falls below 31,745 Baht (scenario 4), insufficient funds are available to invest in chicken coops on either of the two ponds. Hence, in scenario 4 both ponds are managed under the organic technology.

Scenario 5 is related to scenario 2; the maximum available operating loan is restricted to 245,000 Baht, which is insufficient for a pond to be stocked using the integrated technology. The MIP model chooses both ponds to be managed under the organic technology. Hence, scenarios 4 and 5 indicate that if operating and investment loans are restricted such that insufficient funds are available for the integrated technology, *ceteris paribus*, a producer should manage both ponds using the organic technology, provided sufficient funds are available to implement the inorganic, organic, and HIGW technologies.

Scenarios 6 and 7 were designed to evaluate conditions additional to scenario 5, under which the producer should opt for the HIGW technology instead of the organic technology. Since chicken manure is purchased for ponds using organic technology, sufficiently high manure prices might make inorganic or HIGW technologies more profitable. In scenario 6, the results indicate that manure prices must increase from 0.25 Baht kg^{-1} (Engle and Skladany, 1992) to 1.39 Baht kg^{-1} in order for the HIGW technology to generate a greater expected profit than the organic technology. Similarly, scenario 7 was designed with the notion that since fingerling expenditure is lowest in HIGW technology, producers may select the HIGW over the organic technology if fingerling prices increase. Results of the MIP model indicate that for the organic to HIGW technological shift to occur, all fingerling prices must increase by at least 63%.

Scenarios 8 and 9 were intended to study the specific conditions associated with restricting the availability of fingerlings in order for producers to select the HIGW technology over the organic technology. Similar to scenario 7, these conditions were based on relatively low stocking requirements of the HIGW technology: 25,000 tilapia fingerlings ha^{-1} compared to 90,520 tilapia fingerlings ha^{-1} in the other three technologies. Scenario 8 is identical to scenzario 5, except an upper bound is imposed on the availability of tilapia fingerlings. The MIP model's solution is unchanged from scenario 5, provided sufficient tilapia fingerlings are available for stocking both ponds using the organic technology. If the tilapia fingerling availability drops between 181,040 and 115,520 fingerlings (scenario 8), one pond is managed using the organic technology and the other pond using the HIGW technology. In scenario 9, the tilapia fingerling availability is further reduced between 90,520 and 115,520 fingerlings. Under these conditions, there are insufficient fingerlings for stocking two ponds, such that at least one pond could be managed by the organic technology. Instead, the MIP model chooses both ponds to be managed using the HIGW technology. Similarly, if the availability of mrigal, puntius, silver carp and common carp fingerlings were sufficiently reduced, the results show that only the HIGW technology should be implemented.

The inorganic technology was not optimal in any of the above scenarios. As indicated above, the inorganic technology has the advantage of requiring the lowest annual operating investment of all four technologies; however, this technology suffers from producing the lowest expected annual revenue. Hence, whenever production and financial resources were sufficient, the MIP model rejected the inorganic technology in favor of the other three technologies. Scenarios 10 and 11 investigate conditions under which the inorganic technology might enter the solution. In scenario 10, the upper bound of the operating loan is reduced to 47,000 Baht, making it impossible for a farmer to keep both ponds in production unless one pond uses a non-inorganic technology. Only under such stringent conditions does the MIP model select the inorganic technology as an optimal solution. The low-income attribute of the inorganic technology is also highlighted in a 61,824 Baht net return in scenario 10, the lowest net return of all 11 scenarios. Scenario 11, an extension of scenario 10, requires no liming of ponds. Since liming is typically a function of soil characteristics, the liming regimen reported in Engle and Skladany (1992) and Shrestha et al. (1997) need not be representative of all fish farms in Thailand. If lime is not applied, *ceteris paribus*, the operating costs under organic and inorganic technologies decrease sufficiently for one pond to be managed using the organic technology and the other pond to be managed using the inorganic technology.

DISCUSSION AND CONCLUSIONS

This paper outlines conditions under which a small-scale tilapia producer in Thailand chooses among four PD/A CRSP–developed technologies: low-intensity inorganic fertilization (inorganic technology); organic fertilization with collected chicken manure (organic technology); organic fertilization in layer-fish integrated ponds (integrated technology); and high-input green water (HIGW) technology using intensive inorganic fertilization treatments. A mixed-integer programming (MIP) model of annual operations of a small-scale Thai tilapia farm was developed and used to attribute technologies to the production ponds based on maximization of net income. Eleven scenarios were developed that were based on the advantages of each of the four technologies. For example, the low-intensity inorganic fertilization technology has the advantage of having the lowest operating costs. The organic fertilization technology using collected chicken manure has the advantages of simultaneously having the second-lowest operating costs and the second-highest revenues. The HIGW technology has the advantage of requiring the lowest stocking density of all four technologies. The layer-fish integrated ponds technology has the advantage of generating the highest revenues of all four technologies.

Of these technologies, ponds with layer-fish integration provide the greatest net returns. However, implementing such technology necessitates relatively heavy operating and investment loans. When farmers are restricted by the amount that they can borrow, the results of the MIP model indicate organic fertilization with collected chicken manure to be their optimal choice.

Scenarios 6, 7, 8, and 9 in Table 2 outline conditions under which a farmer might shift from using the organic technology to HIGW technology. Scenarios 6 and 7 show that producers would be better off using the organic technology unless there is a relatively massive increase in chicken manure price or fingerling price. Scenarios 9 and 10 report that producers might opt for HIGW technology if there is an insufficient supply of fingerlings to implement the high stocking demands of the organic technology.

Scenarios 10 and 11 illustrate conditions under which the low-intensity inorganic fertilization technology might be chosen as optimal. Given that this technology has the lowest operating costs, the MIP model chooses this technology only when the maximum operating loan is insufficient to select another technology. Use of this technology always results in the lowest net returns.

Hence, the conclusions of this paper are that tilapia producers using PD/A CRSP–developed technologies would be better off by restricting their choice to organic fertilization of ponds using chicken manure. They should choose the high-input green water technology only when there are severe restrictions to the availability of fingerlings.

Anticipated Benefits

This study provides insights into the economic trade-offs associated with four pond production technologies developed by the PD/A CRSP in Thailand. These economic relationships will be further explored in the second phase of this project.

Literature Cited

Diana, J.S., C.K. Lin, T. Bhukaswan, V. Sirsuwanatach, and B.J. Buurma, 1990. Pond Dynamics Collaborative Research Data Reports, Volume 2, Number 2, Thailand: Cycle II of the Global Experiment. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, 54 pp.

Diana, J.S., P.J. Schneeberger, and C.K. Lin, 1988. Relationships between primary production and yield of tilapia in ponds. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J.L. Maclean (Editors), ICLARM Conference Proceedings 15. Department of Fisheries, Bangkok, Thailand, and the International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 1–6.

Engle, C.R. and M. Skladany, 1992. The Economic Benefit of Chicken Manure Utilization in Fish Production in Thailand. CRSP Research Report 92-45. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, 8 pp.

Knud-Hansen, C.F., T.R. Batterson, and C.D. McNabb, 1993. The role of chicken manure in the production of Nile tilapia, *Oreochromis niloticus*. Aquacult. Fish. Manage., 24:483–493.

Knud-Hansen, C.F. and A.K. Pautong, 1993. On the role of urea in pond fertilization. Aquacult. Fish. Manage., 114:273–283.

Shrestha, S.M., P. Phromthong, D. Turongruang, J. Pant, C.K. Lin, and H. Demaine, 1997. High input inorganic fertilization on-farm trials in northeast Thailand. Working Paper, New Series No. T-5. Asian Institute of Technology, Bangkok, Thailand, pp. 1–23.

Yamada, R, 1986. Pond production systems: Fertilization practices in warmwater fish ponds. In: J.E. Lannan, R.O. Smitherman, and G. Tchobanoglous (Editors), Principles and Practices of Pond Aquaculture. Oregon State University Press, Corvallis, Oregon, pp. 97–110.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Rapid Economic Evaluation Tools

Ninth Work Plan, Marketing and Economic Analysis Research 5 (9MEAR5)
Progress Report

Upton Hatch and Jose Falck
Department of Agricultural Economics and Rural Sociology
Auburn University, Alabama, USA

Abstract

A demonstration of the first prototype of a rapid decision economic evaluation tool is presented. We performed an economic evaluation of two tilapia production technologies in Honduras as described by CRSP researchers: 1) chemical fertilization (CF) and 2) fertilization followed by supplemental feed (FSF). The demonstration's initial results agree with the Honduran results in that the FSF treatment has a higher net income than the CF treatment. In addition, results from our prototype program indicate that the FSF treatment is associated with a lower risk of losing money in the short run.

Introduction

Tilapia researchers could use a rapid decision economic evaluation tool to help examine the economic consequences of both experimental results and the design of experiments. We are in the process of developing such a tool and have produced a very preliminary first prototype of the software. The software will allow one to examine not only mean response of tilapia production systems as reflected in economic budgets but also the risk associated with them. A demonstration of some of the economic results, based on published work comparing two tilapia production technologies in Honduras, is shown.

Methods and Materials

Relevant literature on tilapia research activities performed in Honduras has been identified. Published research results have assisted in identifying key production, industry, and economic variables and in establishing a baseline production state to initiate the computer simulations. Interviews and consultations with tilapia experts with experience in Central America (T. Popma, B. Green, D. Teichert-Coddington, and T. Hanson), and particularly Honduras, have assisted in the identification of key component variables and the development of the decision tools.

We are currently developing the decision tool through the use of the software programs Microsoft Excel and @RISK to analyze risk consequences of the application of research results. In addition, we are investigating other software options such as SIMTOOLS. It is desirable that we use software that is readily available, affordable, and easy to use. We foresee a need for two distinct computer tools. The first computer tool will help researchers evaluate possible research outcomes *ex ante* an experiment, which may help in the experimental design phase. With the second tool, the researcher will be able to evaluate the economic consequences of *ex post* results of different experimental treatments.

We present results of the first prototype version of the software. It is important to point out that many improvements are anticipated, especially after in-country field validation. Results presented here will show some of the potential uses a rapid decision economic evaluation tool may provide. The degree to which the new tool will be integrated with POND© simulation software is now also under consideration.

For the purposes of illustrating initial results of the prototype, we used information contained in Green et al. (1994), in which two tilapia production technologies are compared across small- to medium-scale farms. The two technologies are chemical

Table 1. Mean, maximum, and minimum values of select financial variables of two tilapia technology production systems in Honduras based on Green et al. (1994). ($US1 = 5.40 Lps. [Lempiras], 1991)

Financial Variable	Technologies					
	Fertilizer + Feed			*Chemical Fertilization*		
	Min.	Mean	Max.	Min.	Mean	Max.
Variable Cost (Lps.)	2,713	2,773	2,836	2,303	2,469	2,601
Income above Variable Costs (Lps.)	220	1,141	2036	–307	323	1,017
Net Income (Lps.)	–473	447	1,342	–1,000	–371	324

Table 2. Mean, standard deviation, and probability of short-run losses of two tilapia production systems in Honduras based on Green et al. (1994). ($US1 = 5.40 Lps. [Lempiras], 1991)

Treatment/ Financial Variable	Mean	Standard Deviation	Probability of Obtaining Negative Value of Financial Variable (%)
FERTILIZER + FEED			
Income above Variable Costs (Lps.)	1,141	370	0
Net Income (Lps.)	447	370	10
CHEMICAL FERTILIZATION			
Income above Variable Costs (Lps.)	323	260	10
Net Income (Lps.)	–371	260	90

fertilization (CF) and fertilization followed by supplemental feed (FSF).

RESULTS

Results of the first run of the software prototype are presented in Tables 1 and 2. Table 1 presents the range of variation of income above variable costs and net income for the CF and FSF tilapia production systems presented in Green et al. (1994). Our initial results qualitatively agree with the original report in that the FSF treatment yields higher income above variable costs than the CF treatment. Quantitative differences in our results are a consequence of assumptions used in the software prototype. As we continue developing the prototype we expect these results to converge.

Table 2 presents the mean, standard deviation, and the probability of incurring losses from the examined technologies. This is an additional benefit of this methodology, as it allows us to examine not only mean response but also the risk of the production systems. In the initial results presented here, the FSF treatment not only has a higher mean response but also reduces the probability of losing money in the short run.

Refinements to the decision tool may change these results. For example, the incentive for supplemental feeding may be mitigated by availability or quality of feed. These results are presented only to demonstrate the technique's capabilities. In addition, this technique could be adapted for aquaculture under different conditions in other locations.

ANTICIPATED BENEFITS

The decision tool will be beneficial to aquaculture researchers and extension agents in judging economic incentives to adopt PD/A CRSP technologies. Of particular importance will be an appreciation for the additional risk that new technologies often encompass. This technique will not only provide economic estimates of the most likely outcomes for new users of PD/A CRSP technologies but also the likelihood and size of negative outcomes. Use by researchers *ex ante*, as they are developing treatments for investigation, has the potential of refining the new technology to address the source of economic risk. Also, new techniques could be categorized by extension agents as low-, medium-, or high-risk to let the potential new user appreciate possible future economic outcomes.

LITERATURE CITED

Green, B.W., D.R. Teichert-Coddington, and T.R. Hanson, 1994. Development of Semi-Intensive Aquacultural Technologies in Honduras. Research and Development Series, Number 39. International Center for Aquatic Environments, Auburn University, Auburn, Alabama, 48 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Sources of Technical Assistance for Fish Farmers in the Peruvian Amazon

Eighth Work Plan, Adoption/Diffusion Research 1-2 (8ADR1-2)
Final Report

Joseph J. Molnar
Department of Agricultural Economics and Rural Sociology
International Center for Aquaculture and Aquatic Environments
Auburn University, Alabama, USA

Fernando Alcántara Bocanegra and Salvador Tello
Instituto de Investigaciones de la Amazonia Peruana
Iquitos, Peru

Abstract

Data were collected from a sample of 146 practicing fish farmers in the Napo, Tamishiyacu, and Tahuayo river systems areas north and south of Iquitos, Peru, as well as in the Iquitos-Nauta Road area directly south of the city. Fish farmers were identified in selected communities who were provided technical assistance in aquaculture by CARE/Peru and several other nongovernmental organizations. The data suggest few differences in extension experience and perceptions by species cultured, but there is a notable pattern of differences across three measures of farm size. Larger operators tended to have more contact with extension and were slightly more likely to want extension contact in the future. There was little difference by farm size regarding contact with university technicians working in aquaculture or contact with government fish stations. Nearly all farmers wanted extension contact in the future.

Introduction

The reciprocal relationship between fisheries and aquaculture in the Peruvian Amazon is enhanced by the well-established patterns of fish consumption and marketing in the region. Fish are a central part of the *ribereños* diet; many species are accepted, and fish sales seem to be readily accomplished locally or at market centers. The purpose of this analysis is to examine the perceptions concerning the amount and kind of technical assistance available to fish farmers in the Peruvian Amazon as a function of the type of fish raised and the land holdings of the farmer.

In 1992 CARE/Peru began an effort to increase food security and raise incomes by working with families in nine villages along the Napo River, a tributary of the Amazon about 20 kilometers north of Iquitos, Peru. In 1995 a parallel effort was begun in six villages along the Tamishiyacu River and in another six villages along the Tahuayo River about 30 kilometers south of Iquitos. At each of the 21 villages, an initial pond was established for training and demonstration purposes. Subsequently farmers dug approximately 250 ponds— between 10 and 30 per village. Aquaculture is part of a broader strategy of community development, health education, and food security improvement.

The CARE/Peru project also provided fingerlings, nets, small loans for pond construction costs, and continuing technical support for aquaculture. One aquaculture technician works with previous extension contact villages while five others provide technical assistance to the Tamishiyacu and Tahuayo river regions. When cultured fingerlings were available, ponds were stocked with gamitana (*Colossoma macropomum*), the focal species of PD/A CRSP research in Peru. When cultured fingerlings are not available, farmers use wild-caught fry and juveniles or delay restocking until they can obtain seed stock. Each CARE/Peru pond is operated by a single family, primarily for food security purposes. As many of these ponds were built only in the last year, few had yet to harvest fish.

The Spanish nongovernmental organization (NGO) Agencia Española de Cooperación Internacional (AECI) supports the services of a technician who advises approximately 75 pond operators located primarily along the as-yet-incomplete Iquitos-Nauta Road. In 1998 approximately 15 ponds had achieved at least one harvest. The remaining pond operators were currently growing out their first crop of fish. The aquaculture technician is one of about five staff members working in various aspects of agricultural and community development in the Iquitos area. Farmers are provided technical assistance in pond construction and instruction in production management. Farmers can readily obtain fingerlings from river fishers. Terra Nuova, an Italian NGO supported by European Union funds, is extending the work initiated by AECI in this area.

Caritas is a Catholic assistance agency that targets poor communities not presently served by government programs or other NGOs. The Iquitos office is one of four in the Loreto Department. It has seven technicians—including one specialist in aquaculture—who spend two months in target communities followed by one week in Iquitos. With the exception of some lowland area communities where flooding makes fish culture impossible, most Caritas communities have families with functioning fish ponds. These villages are mostly located on rivers west of Iquitos and are farther from Iquitos than the communities served by other organizations in this discussion.

The regional headquarters of the Peruvian Ministry of Fisheries has ten offices in the Loreto Department. Each has a staff of between two and ten persons. Eleven aquaculture specialists, including those at the Iquitos headquarters, provide technical assistance and training programs throughout the region. Recently, efforts have been directed toward protein-deficient communities near the Ecuador frontier, largely composed of indigenous tribal people. These populations are in great need of assistance.

Fondo Nacional de Desarrollo Pesquero—Acción Promo-tora para el Desarrollo de la Acuicultura Unidad de Producción Acuícola Aguas Calientes (FONDEPES) is a Peruvian fisheries development agency. It operates a 10-ha fingerling production station near Nuevo Horizonte, 35 km southwest of Iquitos. The agency projects a shortfall in the stock of wild fingerlings and therefore plans to engage in production of grown fish for the market. Plans are to produce fingerlings for a variety of species, but this agency's strategy features boquichico (*Prochilodus nigricans*). This species requires a less intensive level of cultivation and is mainly suitable for home consumption and local markets. This species was the single most frequently reported kind of fish grown.

Each of these organizations is presently or potentially a partner with the PD/A CRSP. New technology for increasing the yield of current breeding techniques and expanding the period during which breeding is possible will provide widespread benefits for aquaculture producers in the selva (Kohler et al., 1999).

METHODS AND MATERIALS

Sample and Data Collection

Fish farmers were identified in selected communities who were provided technical assistance in aquaculture by CARE/Peru and several other NGOs in the Napo, Tamishiyacu, and Tahuayo River systems, which combine to form the Amazon, as well as in the Iquitos-Research Station Contact area south of Iquitos. Structured interviews were conducted with a sample of 146 fish farmers having accomplished at least one harvest in the past two years (Casley, 1988; Townsley, 1996).

The survey instrument was adapted from previous research conducted by Molnar et al. (1996) in five PD/A CRSP countries—Honduras, Thailand, the Philippines, Rwanda, and Kenya. The Peru survey, however, reflects the unique conditions and context of Amazonian fish culture, the diversity of species, and the singular relationship of aquaculture to the river fishery in the region. Ponds were identified in communities on three river systems north and south of Iquitos as well as the Research Station Contact area south of Iquitos. Data collection took place in early 1999 and was conducted by graduate students from the Department of Fisheries at Universidad Nacional de la Amazonia Peruana.

Analysis

The analysis tabulates the responses to five survey questions about technical assistance by type of fish raised and three measures of land holding. From this information, a number of patterns in farmer experiences and expectations for fish culture technical assistance are identified.

RESULTS

Type of Species Cultured

Table 1 shows contacts and experiences with extension by type of species cultured. The species are shown in order of the frequency in which they were grown by fish farmers in the sample. Boquichico was the most frequently cultured fish in the Peruvian Amazon, grown by 75% of the farmers. There were not many notable differences in perceptions of extension across the groups growing various species. Eighty-seven percent had contact with some type of extension in the previous two years. Overall, 4% reported contacts with university personnel working in aquaculture, with 16% of the Bujurqui farmers reporting such contacts.

Twelve percent of the sample had contact with personnel at government stations or technicians associated with these installations. Twenty-eight percent of the 25 Bujurqui growers in the sample had such contacts, a much higher proportion than any of the other species. Across all types of growers, nearly all (97%) wanted extension contact in the future.

Gamitana, the target species for PD/A CRSP research in Peru, was the second most frequently cultured fish. Technical

Table 1. Contacts and experiences with extension by type of species cultured, fish farmers in the Peruvian Amazon, 1999.

Survey Item	Yes Response by Species Cultured (%)									
	Boquichico (N = 106)	*Gamitana (N = 90)*	*Paco (N = 85)*	*Sábalo (N = 69)*	*Yaraqui (N = 44)*	*Bujurqui (N = 25)*	*Lisa (N = 15)*	*Oscar (N = 55)*	*Tucanare (N = 58)*	*All Species (N = 141)*
Have you had contact with any type of extensionist?	88	86	86	81	80	72	87	86	88	87
Have you had contact with any university technicians working in aquaculture?	5	6	5	7	9	16	7	6	2	4
Have you had contact with government station or technician?	13	12	13	16	18	28	13	18	12	12
Do you want extension contact in the future?	98	96	97	97	100	100	100	96	97	97

Table 2. Contacts and experiences with extension by pond characteristics, fish farmers in the Peruvian Amazon, 1999.

Survey Item	How Many Pieces of Land in Your Farm? (%)			How Much Land Owned? (%)			Compared to Other Farmers, How Much Land Do You Have? (%)			All (%)
	1–2 Parcels (N = 101)	*3–9 Parcels (N = 22)*	*10 + Parcels (N = 4)*	*< 1 Hectare (N = 6)*	*1–10 Hectares (N = 49)*	*> 10 Hectares (N = 73)*	*Less (N = 24)*	*Same (N = 89)*	*More (N = 19)*	*(N = 132)*
Have you had contact with any type of extensionist?	86	77	100	83	90	89	89	86	79	88
Have you had contact with any university technicians working in aquaculture?	4	4	50	0	4	4	4	2	16	5
Have you had contact with government station or technician?	8	27	50	17	12	12	17	10	16	14
Do you want extension contact in the future?	95	100	100	100	94	96	96	94	100	96

assistance perceptions for the 90 farmers who cultured this species differed little from the growers of other types of fish. Alcántara (1994) presents a detailed analysis of fish landings at Iquitos ports and the kinds of species that are brought to market.

Farm Size

Table 2 shows technical assistance perceptions tabulated by three measures of farm size. Seventy-seven percent of the sample owned one or two parcels of land. Holders of multiple parcels were primarily pond groups organized for constructing and maintaining fish ponds. While only half the sample owned more than 10 ha of land, 67% of the respondents felt that they owned the same amount of land as their neighbors.

Those owning three to nine parcels of land reported less contact with extension, in contrast to those holding 1 to 10 ha of land. A lower proportion of respondents who felt that they owned less land than their neighbors reported the most contact with extension. Farmers with more than ten parcels of land had more contacts with university technicians than farmers with fewer parcels of land. Few differences in contact were noted across land holding by area, but farmers who felt they owned more land than their neighbors had more contact with university technicians compared with those who felt they had the same or less land.

Contact with government stations tended to increase markedly with the number of parcels owned, but there was little difference by landholding. Those who felt they owned more land reported about the same level of contact with government stations or technicians. Respondents uniformly wanted more contact with extension in the future though smaller-scale farmers were slightly less interested when measured by parcel ownership and self-perception. Those who felt they owned more land than their neighbors also were more likely to want extension contact in the future.

Conclusions

The communication process linking experimental pond to farm practice involves several layers of translation and transmission. It is clear that the PD/A CRSP must work with the local network of institutions that provide technical assistance and services to rural producers if its research findings and insights into production practices are to provide widespread benefits to rural producers. In the Peruvian Amazon, a broad array of institutions and NGOs produce fingerlings and provide technical assistance to fish farmers. The enduring impacts of PD/A CRSP activities will be multiplied through the network of NGO partners and government agencies that utilize PD/A CRSP understandings and technologies in their ongoing programs of outreach and information assistance to villages and rural people throughout the selva.

Anticipated Benefits

The direct benefits of this study primarily accrue to CARE/Peru and other agencies endeavoring to promote and support fish culture among the rural poor in villages along the Amazon and its tributaries. Technicians and others working with farmers now have systematic information about the target population of rural producers and about their practices and needs. This study has provided some insight into the high level of receptivity to technical assistance in aquaculture in the PD/A CRSP target population.

Acknowledgments

We thank CARE/Peru Director Juan Guerrero for his organization's help in obtaining the interview data. Without this assistance, our work would have been much narrower in scope.

Literature Cited

Alcántara, F., 1994. Diagnóstico de los recursos hidrobiológicos de la Amazonía. Report. Tratado de Cooperación. Amazonica, Lima, Peru, 120 pp.

Casley, D.J. and K. Krishna, 1988. The Collection, Analysis, and Use of Monitoring and Evaluation Data. Johns Hopkins University Press, Baltimore, 174 pp.

Kohler, C., S. Kohler, M.J. DeJesus, F. Alcántara Bocanegra, E. Rios Isern, and G. Llosa Talavera, 1999. Development of sustainable pond aquaculture practices for *Piaractus brachypomus* in the Peruvian Amazon. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 99–102.

Molnar, J.J., T.R. Hanson, and L.L. Lovshin, 1996. Social, Institutional, and Economic Impacts of Aquacultural Research: The Pond Dynamics/Aquaculture CRSP in Rwanda, Honduras, the Philippines, and Thailand. Research and Development Series No. 40. International Center for Aquaculture and Aquatic Environments, Auburn University, Alabama, 63 pp.

Townsley, P., 1996. Rapid Rural Appraisal, Participatory Rural Appraisal, and Aquaculture. FAO Fisheries Technical Paper 358. Food and Agriculture Organization, Rome, 109 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

IDENTIFYING GOALS AND PRIORITIES OF FISH FARMERS IN THE PERUVIAN AMAZON

Eighth Work Plan, Adoption/Diffusion Research 1-3 (8ADR1-3)
Final Report

Joseph J. Molnar
Department of Agricultural Economics and Rural Sociology
International Center for Aquaculture and Aquatic Environments
Auburn University, Alabama, USA

Fernando Alcántara Bocanegra and Salvador Tello
Instituto de Investigaciones de la Amazonia Peruana
Iquitos, Peru

ABSTRACT

The Peruvian Amazon is in an advantageous situation for fish culture. Survey data from 146 practicing fish farmers show that they culture a variety of species, but regardless of the kind of fish they grow, farmers view fish culture in a positive light. While gamitana (*Colossoma macropomum*) is not the only Amazon fish to deserve special attention, it is the first species about which enough is known to both manage wild stocks and develop aquaculture. PD/A CRSP research at the Quistococha Station near Iquitos, Peru, focuses on this species. Most respondents grew a number of different species, planned to build more ponds, were content with growing fish, and felt the pond was the best use of the land it occupies. In addition, most felt that the pond was worth the work put into it. One of the most problematic aspects of owning a fish pond is the loss of inventory due to human or animal predation. The data show 58% of respondents indicating problems with people stealing fish; 75% of the tucanare (*Cichla ocellaris*) growers had this problem.

INTRODUCTION

The Peruvian Amazon surrounding the regional city of Iquitos has been subjected to large-scale commercial exploitation for the last two centuries (Barnham and Coomes, 1996). As Nauta, Tamishiyacu, and then Iquitos grew during the late 1800s, they became centers of urban consumption and international export. Petroleum-based tires have effectively ended the rubber trade in the northeastern Peruvian Amazon (Villarejo, 1988; Coomes, 1992a; 1992b).

The Amazon river fishery plays a fundamental role in the livelihoods and survival of rural populations in this region (Chibnik, 1994; McDaniel, 1997). Fishing is by far the most important source of animal protein in the Amazon Basin and the main generator of cash for people living along the river. Araujo-Lima and Goulding (1997) argue that fishing is the most promising means for increasing animal protein in the Amazon Basin with a minimum of environmental degradation. Aquaculture plays a unique and dynamic role in the forest- and river-based farming system of the Peruvian Amazon or the selva (Pinedo-Vasquez et al., 1992; Tomich et al., 1995).

The number of fish species in the Amazon hydrographic basin has been estimated at 2,000. Only about three-quarters of these have been described scientifically. These represent approximately 10% of the planet's ichthyofauna (Penn, 1998). Araujo-Lima and Goulding (1997) maintain that while gamitana (*Colossoma macropomum*) is not the only Amazon fish to deserve special attention, it is the first species about which enough is known to both manage wild stocks and develop aquaculture. Amazon's fishing potential, previously regarded as inexhaustible, was overestimated. Though most waters in the Iquitos area are brown, black- and clear-water rivers are poor in fish. It is foreseeable that sufficient fish supplies for the rapidly growing Amazonian population cannot be guaranteed for long. Management of fish for subsistence use or local consumption is done by the inhabitants of the areas around the lakes, whose interests in preservation conflict with those of the professional fishermen. The river people supplement fishing with subsistence agriculture activities, jute plantations, and extraction of wood and other products. Now aquaculture is widely perceived as a farm-based activity that complements traditional sources of food and livelihood.

Aquaculture in the Selva

There is no fish-breeding tradition in Amazonia. The aboriginal populations kept fish, manatees, and turtles in large corrals for periodic consumption, but no techniques for reproduction in captivity were developed. However, the efforts of government agencies, nongovernmental organizations (NGOs), missionaries, and others have led to a certain level of indigenous knowledge and interest in aquaculture.

There is a unique relationship between aquaculture and fisheries in many parts of the Amazon region (Hall, 1997). The abundance of large, rapidly growing fish species supports an extensive capture fishery in the Amazon, its tributaries, and a large number of oxbow lakes. The fishery, however, is cyclic, as fishing is more difficult during the high water period of December through March. At this time, fish prices for some species are as much as twice the low-water-period price. This cyclical deficit in the supply of fish coupled with a widespread perception that river and lake fish stocks have declined and will continue to do so are the primary motivations for fish culture in the selva. Commercial-scale fishers using large-scale fishing gear have depleted fish stocks in many oxbow lakes, further encouraging pond-based fish production.

Abundant supplies of warm water, generally available pond inputs, and easily obtainable grow-out stock are some of the favorable conditions for fish culture in the Amazon River system. Fingerlings can be obtained through individual effort in rivers or oxbow lakes or can be purchased from fisherman.

The reciprocal relationship between fisheries and aquaculture in the Peruvian Amazon is further enhanced by the well-established patterns of fish marketing present in the region. Alcántara's (1994) study of fish landings in Iquitos documents the diversity of fish in the markets and the centrality of boquichico (*Prochilodus nigricans*) as the most heavily harvested species. Gamitana had a steady, albeit slightly declining, level of reported fish landings over the extended period of data that were available. Fish are a central part of the *ribereños*' diet, many species are accepted for consumption, and fish sales seem to be readily accomplished locally or at market centers.

In 1992, CARE/Peru began an effort to increase food security and raise incomes by targeting families in nine villages along the Napo River. Aquaculture is part of a broader strategy of community development, health education, and food security improvement. CARE/Peru also provides fingerlings, nets, small loans for pond construction costs, and continuing technical support for aquaculture. One aquaculture technician works with Napo river villages while five others provide technical assistance to the Tamishiyacu and Tahuayo river regions. When cultured fingerlings are available, ponds are stocked with gamitana. When cultured fingerlings are not available, farmers use wild-caught fry and juveniles or delay restocking until they can obtain seed stock. Most CARE/Peru ponds are operated by a single family, primarily for food security purposes. Many of these ponds have been built and are now only beginning to harvest fish. New technology for increasing the yield of current breeding techniques and expanding the period during which breeding is possible will provide clear and widespread benefits for aquaculture producers in the selva (Kohler et al., 1999).

METHODS AND MATERIALS

Sample and Data Collection

Fish farmers were identified in selected communities in the Napo, Tamishiyacu, and Tahuayo River systems, which combine to form the Amazon, as well as in the Iquitos-Nauta Road area south of Iquitos. Structured interviews were conducted with a sample of 146 fish farmers having accomplished at least one harvest in the previous two years (Casley and Krishna, 1988; Townsley, 1996). The sample was drawn from available subjects in selected communities that were provided technical assistance in aquaculture by CARE/Peru and several other NGOs.

The survey instrument was adapted from previous research conducted by Molnar et al. (1996) in five PD/A CRSP countries—Honduras, Thailand, the Philippines, Rwanda, and Kenya. The Peru survey, however, reflects the unique conditions and context of Amazonian fish culture, the diversity of species, and the singular relationship of aquaculture to the river fishery in the region. Ponds were identified in communities on three river systems north and south of Iquitos, as well as the Nauta Road area south of Iquitos. Data collection took place in early 1999 and was conducted by graduate students from the Department of Fisheries at Universidad Nacional de la Amazonia Peruana.

Analysis

The analysis portrays patterns of survey responses by type of species cultured. The responses are tabulated within the subset of those producers who said they grew a particular species. From this information, central patterns of comparison and difference in experience and expectation for fish production can be discerned.

RESULTS

In the survey, farmers were asked a series of questions about their experience with fish culture, its relation to other farm activities, and the overall role the activity played in their farming system. A selected subset of these questions is summarized in terms of the species that the producers reported growing in their ponds.

We asked farmers whether they grew each of a series of nine species. Figure 1 charts the frequency distribution of the number of species grown. Only 8% grew only one species of fish, 24% grew two species, and more than 50% grew between two and five species.

Other analysis shows that the most frequently grown combination of species was tucanare (*Cichla ocellaris*) and bujurqui (*Cichlasoma amazonarum*), undertaken by about 13% of the sample. About 5% grew yaraqui (*Prochilodus amazonensis*) alone, 3% raised a lisa (*Leporinus* sp.)-tucanare-yaraqui combination, and 3% grew all the species but gamitana. No other combination of species accounted for more than 3% of the respondents. There seems to be some specialization or focus of growers on yaraqui as a culture species.

Table 1 describes the goals and priorities fish farmers in the Peruvian Amazon have for their aquaculture activities. The data for each respondent are tabulated under each species the

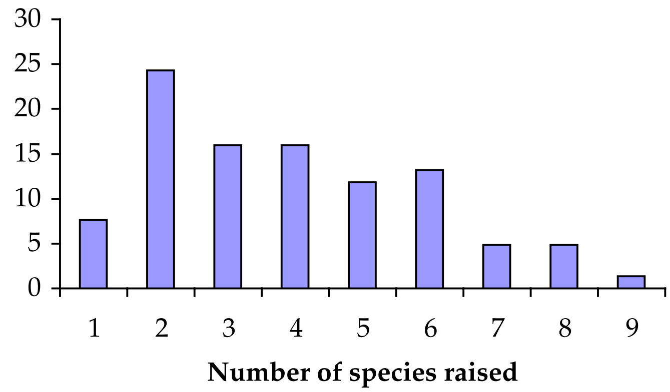

Figure 1. Number of species raised, Peruvian Amazon fish farmers, 1999.

Table 1. Goals and priorities for fish ponds by type of species cultured, fish farmers in the Peruvian Amazon, 1999.

Survey Item	Yes Responses by Species Cultured (%)									
	Boquichico (N = 109)	*Gamitana (N = 92)*	*Paco (N = 87)*	*Sábalo (N = 71)*	*Yaraqui (N = 60)*	*Bujurqui (N = 55)*	*Lisa (N = 45)*	*Oscar (N = 28)*	*Tucanare (N = 16)*	*All Species (N = 143)*
Do you have difficulty caring for your other crops because of your ponds?	1	2	2	1	2	0	2	0	0	1
Is it more difficult to take care of your family if you have ponds?	1	2	2	4	0	2	0	0	0	2
Have you had difficulty with other work because of your ponds?	3	3	3	4	3	4	7	4	0	2
Are there times during the year when the pond is too much work?	4	5	5	4	2	6	0	7	6	4
Have you had trouble getting enough water?	10	16	13	11	7	11	7	4	6	12
Have you had problems with people stealing your fish?	57	61	58	69	58	58	58	57	75	58
Is it easier to buy things for your family because of your ponds?	62	63	62	66	63	78	82	75	81	61
Have you had problems with birds or other animals?	70	62	60	66	73	64	62	71	75	64
Have you had trouble getting fingerlings?	62	61	63	62	65	56	44	43	25	65
Do you plan to build more ponds?	86	79	78	90	82	89	89	93	88	80
Is the pond more profitable compared to other activities?	89	90	90	88	86	86	91	88	100	87
Are you content with growing fish?	95	94	93	96	97	93	98	89	88	96
Is the pond the best use of the land it occupies?	96	98	98	96	93	98	98	93	94	97
Is the pond worth the work you put into it?	99	98	99	99	100	96	100	100	100	99

respondent reported growing. The columns (or species cultured) are also ordered in terms of the frequency that study respondents reported growing each type of fish.

Boquichico (*Prochilodus nigricans*) is the most frequently grown fish in the sample. A total of 109 of the 143 respondents reported growing this fish, about 76% of the total. It is easy to grow, fingerlings are readily obtained from the rivers, and it is popular as an ordinary low-priced food item among residents of the region.

Two-thirds of the sample raised gamitana in their ponds. This fish is the target of PD/A CRSP research in Peru. A fruit-eating species, it is a well-liked, high-value fish that is popular as a restaurant item.

Sixty-one percent grew paco (*Piaractus brachypomus*). About half the sample culture cultured sábalo (*Brycon* sp.), and 42% grew yaraqui. Almost 39% had bujurqui, 32% grew lisa, 20% grew oscar or acarahuazú (*Astronotus ocellatus*); at 11% tucanare was the least frequently cultured fish.

Table 1 shows the percent of respondents that said yes or agreed with each survey item. Few respondents reported difficulty taking care of other crops because of their ponds. No differences are notable across species. Similarly, few found it more difficult to take care of their family if they had ponds. Two percent reported difficulty with other work because of their ponds, and 4% said that there were times during the year when the pond was too much work. Nonetheless, 61% said that it was easier to buy things for their family because of the ponds, with over 80% of the lisa and tucanare farmers noting this advantage.

About 12% of respondents reported that they had trouble getting enough water to maintain their ponds, but gamitana farmers experienced this difficulty somewhat more often. One of the most problematic aspects of owning a fish pond is the loss of inventory due to human or animal predation. The data show 58% indicating problems with people stealing fish; this was highest among the tucanare farmers, of whom 75% had this problem. Similarly, 64% experienced problems with birds or other animals. Despite the extensive number of people engaged in at least casual fishing activity in this region of the Amazon, 65% of the sample said they had trouble getting fingerlings though only 25% of the tucanare growers noted this problem.

Eighty percent of respondents said that they planned to build more ponds, apparently basing their optimism on the premise shared by 87% that the pond was more profitable compared to other activities that might use the same land. Similarly, 96% indicated that they were content with the activity of growing fish, 97% felt that the pond was the best use of the land it occupied, and 99% believed that the pond was worth the work put into it. These findings show the central location of fish culture in the farming system of the Peruvian selva.

CONCLUSIONS

The Peruvian Amazon is in an advantageous situation for fish culture. The data show that farmers encounter few barriers to building ponds, obtaining fingerlings, feeding their fish, or marketing the product. Fruits and other forest-based fish foods are widely available to support extensive production systems. The natural cycle of the Amazonian river systems ensures a market period of relatively high prices for farm-reared fish. The research agenda is appropriately focused on enhancing the availability of hatchery-reared fry of a popular, high-value species. Nonetheless, additional attention is needed on identifying and communicating technical insights that will reduce production risk and enhance the benefits of aquaculture for the many small- and medium-scale farms in the selva.

Anticipated Benefits

The data presented here provide empirical specification of the needs and preferences of the actual intended beneficiaries of PD/A CRSP activities in Peru. As such, they provide a baseline or template for interpreting the cumulative impact of PD/A CRSP and NGO partner activities, as well as a starting point for identifying new directions and emphases that will help realize the promise of aquaculture for farmers and their families in developing countries. The data suggest that farmers are practicing diverse forms of polyculture that often feature the gamitana species, which is the focus of PD/A CRSP research in the region.

Acknowledgments

We thank the several PD/A CRSP principal investigators, host country counterparts, and NGO colleagues who facilitated our work and advised us on the many problems and issues. In particular we are grateful to CARE/Peru's Juan Guerrero for his organization's help in obtaining the interview data. Without this assistance, our work would have been much narrower in scope.

Literature Cited

Alcántara, F., 1994. Diagnóstico de los recursos hidrobiológicos de la Amazonía. Report. Tratado de Cooperacion. Amazonica, Lima, Peru, 120 pp.

Araujo-Lima, C. and M. Goulding, 1997. So Fruitful a Fish: Ecology, Conservation, and Aquaculture of the Amazon's Tambaqui. Columbia University Press, New York, 191 pp.

Barnham, B.L. and O.T. Coomes, 1996. Prosperity's Promise: The Amazonian Rubber Boom and Distorted Economic Development. Westview Press, Boulder, 179 pp.

Casley, D.J. and K. Krishna, 1988. The Collection, Analysis, and Use of Monitoring and Evaluation Data. Johns Hopkins University Press, Baltimore, 174 pp.

Chibnik, M., 1994. Risky Rivers: The Economics and Politics of Floodplain Farming in the Amazon. University of Arizona Press, Tucson, 267 pp.

Coomes, O.T., 1992a. Making a living in the Amazon rain forest: Peasants, land, and economy in the Tahuayo river basin of northeastern Peru. Ph.D. thesis. The University of Wisconsin, Madison, Wisconsin, 450 pp.

Coomes, O.T., 1992b. Blackwater rivers, adaptation, and environmental heterogeneity in Amazonia. Am. Anthropol., 94:698–701.

Hall, A., 1997. Sustaining Amazonia: Grassroots Action for Productive Conservation. Manchester University Press, Manchester, UK, 269 pp.

Kohler, C., S. Kohler, M.J. DeJesus, F. Alcántara Bocanegra, E. Rios Isern, and G. Llosa Talavera, 1999. Development of sustainable pond aquaculture practices for *Piaractus brachypomus* in the Peruvian Amazon. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 99–102.

McDaniel, J., 1997. Communal fisheries management in the Peruvian Amazon. Human Organization, 56:147–152.

Molnar, J.J., T.R. Hanson, and L.L. Lovshin, 1996. Social, Institutional, and Economic Impacts of Aquacultural Research: The Pond Dynamics/Aquaculture CRSP in Rwanda, Honduras, the Philippines, and Thailand. Research and Development Series No. 40. International Center for Aquaculture and Aquatic Environments, Auburn University, Alabama, 63 pp.

Penn, J., 1998. A brief view of market-oriented extraction in the Peruvian Amazon. Internet. URL: <www.rainforestconservation.org/articles/market_view.html>.

Pinedo-Vasquez, M., D. Zarin, and P. Jipp, 1992. Economic returns from forest conversion in the Peruvian Amazon. Ecol. Econ., 6:163–173.

Tomich, T.P., P. Kilby, and B.F. Johnston, 1995. Transforming Agrarian Economies: Opportunities Seized, Opportunities Missed. Cornell University Press, Ithaca, New York, 474 pp.

Townsley, P., 1996. Rapid Rural Appraisal, Participatory Rural Appraisal, and Aquaculture. FAO Fisheries Technical Paper 358. Food and Agriculture Organization, Rome, 109 pp.

Villarejo, A., 1988. Así es la Selva. CETA Press, Iquitos, Peru, 330 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

AQUACULTURE TRAINING FOR KENYAN FISHERIES OFFICERS AND UNIVERSITY STUDENTS

Ninth Work Plan, Adoption and Diffusion Research 3 (9ADR3)
Progress Report

Karen L. Veverica
Resident Research Coordinator, Sagana Fish Farm
Auburn University

Mucai Muchiri
Head, Department of Fisheries
Moi University

Charles C. Ngugi
Professor, Department of Fisheries
Moi University

James R. Bowman
US Regional Coordinator
Oregon State University

Judith Amadiva
Social Development Officer
Sagana Fish Farm

Printed as Submitted

ABSTRACT

Lack of technical training has been cited as a major reason for the low output of fish ponds in Kenya. The lack was observed at all levels, from the lowest level extension agent through university levels. The training program undertaken by the Africa Project in Kenya seeks to improve training and to provide a cadre of trainers who have extensive practical fish production experience.

This year the Africa Project has begun scholarship support for two MS students, one at Moi University's Chepkoilel Campus, Eldoret, Kenya, and the other at Auburn University, Auburn, Alabama. Small stipends for student research conducted at Sagana Fish Farm have allowed undergraduate as well as graduate-level university students to remain longer to complete projects and gain valuable field experience. A small research projects program for station staff has allowed them to further their professional development and carry out their own research, which can have a positive impact on station management.

The program of farmer education days developed during the first half of 1999 (Veverica et al, 2000) was followed this year by a series of four short courses for personnel of the Kenya Fisheries Department (FD). In this series of courses, more than 80 FD staff received two weeks of training in pond construction methods and pond management techniques. Additional farmer field days for approximately 50 farmers are also planned for later in 2000.

INTRODUCTION

Lack of technical training has been cited as a major reason for the low output of fish ponds in Kenya. The lack has been observed at all levels, from the lowest-level extension agent through university levels. The Africa Project's training program in Kenya seeks to improve training and to provide a cadre of trainers who have extensive practical fish production experience. This activity was originally planned to include training only for university students and Fisheries Officers at all levels, but has been expanded to include farmer training as well.

Objectives

1. To increase the pond management skills of fisheries personnel currently involved in aquaculture extension activities in Kenya.
2. To enhance the research and extension capabilities of Kenyan university students likely to be employed in the aquaculture sector.

Activities undertaken during the current reporting year include beginning full-scholarship sponsorship for two M.S. students, conducting a series of short courses in pond construction and management for Fisheries Department (FD) extensionists, and continuing to support undergraduate and graduate students conducting research at Sagana Fish Farm by providing guidance and mentorship by the US Research Coordinator stationed at Sagana as well as stipends for some students. The US Research Coordinator also continued to provide some guidance to Fisheries Officers assigned to Sagana who wished to develop their professional skills by conducting small research projects at the station.

TRAINING OF UNIVERSITY STUDENTS

Two MS students are now receiving full scholarship support from the CRSP Africa Project. Mr. Robinson Mugo began receiving CRSP support for his graduate program in the Department of Fisheries at Moi University (Eldoret, Kenya) in October, 1999. Mr. Mugo just finished his course work and will begin his research at the end of year 2000. He will be working on a project combining aquaculture and genetic diversity/ systematics questions. Mr. Bethuel Omolo, selected last year by the Kenya Fisheries Department to receive training towards taking up the Department's new Research/Extension Liaison position, was accepted into an M.S. program in the Department of Fisheries and Allied Aquacultures at Auburn University, Alabama, and began his program there in January, 2000. Mr. Omolo's studies will focus on extension methods and programming and general aquaculture.

Of the three University of Nairobi MS students reported on last year (Gichuri, Izaru, and Mwau), Wilson Gichuri was awarded his degree during this reporting year. His thesis was entitled "Relative contribution of rice bran and inorganic fertilisers in semi-intensive tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) polyculture in Kenya." Paul Izaru and Patricia Mwau finally were given a date for defense a year after submitting their theses. Ms. Mwau successfully defended and is in the process of making corrections. Mr. Izaru must resubmit. All of these students conducted their thesis research at Sagana with supervision by the resident CRSP research coordinator, Ms. Karen Veverica.

Mr. Oenga is still writing and the going is slow given that he has been employed by KMFRI. He may run one more experiment at Auburn University in the Spring of 2001.

New MS students at Sagana this year include Enos MacWere and Robert Olendi, both from Moi University. MacWere received CRSP stipend assistance and did his thesis research on commercial tilapia and *Clarias* production systems. He has completed his second thesis draft and will probably submit it in November. Olendi, who also received stipend support from the CRSP, did his research on the effects of suspended silt on primary production and fish growth. He finished his thesis research in May and will probably submit a draft by the end of the year. He was already employed as chief technician of the Moi University Department of Fisheries before entering graduate school, and will return to that job.

Two graduate courses in aquaculture were given at Sagana Fish Farm. One was for Moi University graduate students, given in February 2000 by Dr. David Liti. The second course was given for hydrobiology students of the University of Nairobi Department of Zoology; it was organized by Dr. Liti and included short talks given by a variety of Sagana station staff and students.

University undergraduates continue to come to Sagana to do "attachments," in which they learn the practical aspects of station work and often take on a special subject for an "Attachment Report." Those who can stay longer often also conduct "Senior Project" research, for which a report is due at the end of their senior year. Student stipends from the CRSP have made it possible for some students to remain at Sagana for the whole break between their junior and senior years, thus allowing them to complete a senior project. Three undergraduates from Moi University and one from Kenyatta University finished their junior/senior year attachment in September 1999 and returned to Sagana for December break. They graduated with bachelor's degrees in May 2000. Two other recently graduated Moi University students (Arthur Tuda and Charles Achuodo) worked at Sagana under the CRSP for one to four months and left to take jobs that they obtained as a result of their Sagana experience. They had already finished a senior project prior to arriving at Sagana. Names and senior project subjects for students are presented in Table 1. Although students handed in their reports to the station head, the CRSP

Table 1. University level training supported by the PD/A CRSP Africa Project in 1999 -2000. All undergraduate students were finished by May 2000.

Name	University / Department	Project	Date started
Undergraduates			
Paul Wamwea Wabitah	Kenyatta Univ. Zoology	Attachment. Senior project in comparison of tanks and hapas for sex reversal of tilapia	May 1998-May 2000
David Mirera	Moi University Fisheries	Attachment. Senior project on primary productivity indicators	May 1999-May 2000
William Nyaga	Moi University Fisheries	Attachment. Senior project on treatments to enhance survival of goldfish larvae.	May 1999-May 2000
Cosmas Munga	Moi University Fisheries	Attachment. Senior project on *Clarias* larvae feeding strategies.	May 1999-May 2000
Graduate students (M.Sc.)			
Daniel Oenga Nyanchiri	Moi University Fisheries	Largemouth bass introductions and fingerling production	July 1998; continuing
Robert Olendi	Moi University Zoology	Effects of suspended silt on primary production and fish growth	October 1999; research finished May 2000
Robinson Mugo	Moi University Fisheries	studies on Kenyan killifishes	just beginning research
Enos MacWere	Moi University Fisheries	commercial tilapia and Clarias production systems	October 1999; research finished May 2000

Table 2. Summary of Fisheries Department (FD) staff and non-FD participants who completed two-week training programs in pond construction and management. A total of four programs were held between November 1999 and August 2000. Trainees came from all provinces except Northeast.

Designation	Male	Female	Total	Remarks
Kenya Fisheries Department (FD)				
Fishery Officer	2	1	3	Four additional FOs sat in on the course
Assistant Fishery Officer	4	1	5	Three did not complete the pond management section
Fisheries Assistant	52	9	61	
Fish Scout	9	1	10	
Subordinate Staff		1	1	
Total FD staff	**67**	**13**	**80**	
Non-FD participants				
KMFRI staff*	3		3	One Research Officer, two technicians
private farmers	14		14	Includes 13 from African Bulldozers
university students	6	1	7	Three were undergraduates; four were graduate students
Total others	**23**	**1**	**24**	
OVERALL TOTAL	**90**	**14**	**104**	

Dates:
Group A: 28 Nov.-3 Dec. 1999 at Moi University and 22-25 Feb. 2000 at Sagana.
Group B: 14-25 Feb. 2000 at Sagana.
Group C: 8-22 May 2000 at Moi University.
Group D: 13-26 Aug. 2000 at Moi University.

* Kenya Marine Fisheries Research Institute

was never provided a copy. Copies have been requested from Moi University.

Three additional undergraduate students were accepted for attachment by Dr. Liti for the year 2000.

TRAINING FOR FISHERIES DEPARTMENT PERSONNEL

A series of two-week short courses in pond construction and pond management was begun this year. Each course is designed to accommodate twenty participants. These courses are typically a collaborative effort between the Sagana CRSP/FD group and the faculty of the Department of Fisheries of Moi University. Some courses are held at Moi University's (MU) Chepkoilel Campus, in Eldoret (home of the MU Department of Fisheries), whereas others are held at Sagana. Moi University participants include Dr. Mucai Muchiri, Head of the MU Department of Fisheries, and Dr. Charles Ngugi, Professor in the Department of Fisheries.

The first course focused on pond construction and was held at the Chepkoilel Campus from 29 November through 4 December, 1999. Fifteen extension workers, four private pond contractors and one farmer participated. As part of their training, participants constructed two small ponds at the Moi University Department of Fisheries site near Eldoret. In February (2000) this group received an additional four days of training at Sagana, where they joined the second session to study pond management.

The second two-week course was held at Sagana from 14-25 February, 2000. This group included one Fisheries Officer, 19 Fisheries Assistants and Fisheries Scouts, and three workers from the crew of a private contractor. Oversight for this training session was provided by Judith Amadiva, Social Development Officer assigned to Sagana by the Kenya Fisheries Department . Both the first and second sessions (Eldoret and Sagana) were co-taught by Karen Veverica and Charles Ngugi of Moi University. Mr Enos MacWere, a graduate student working with the CRSP, provided assistance.

A third course, involving 20 FD officers and another three private contractor crew members was held at the Chepkoilel Campus of MU from 14–27 May, 2000. This course also focused on pond construction and management. Dr. Charles Ngugi had the major responsibility for teaching this course.

The fourth and last training course of this type held under the CRSP was held from 13-26 August at Chepkoilel campus. Ms. Geraldine Matolla helped teach the course. KMFRI requested three spots in this training program. Their officers began constructing two new ponds themselves immediately following the training. A total of 104 individuals received hands-on training in these short-courses during this reporting year (Table 2).

Because the FD saw the training program as a way to advance the grade of their staff, and the PD/A CRSP considered the training to be a tool to aid in dissemination of correct information to farmers, the selection of trainees met with some disagreement. The CRSP wanted people who actually work with farmers. The FD wanted to train Fisheries Assistants, whether or not they were involved actively in aquaculture extension. We have been able to resolve the differences by allowing the FD to choose the people to be trained but with the stipulation that they assure us that those trained will be reassigned to positions where they can use their training.

A further effect of the training has been the creation of a cadre of individuals experienced in the teaching of pond construction and pond management. This team is led by Dr. Charles Ngugi of the Moi University Department of Fisheries. Enos MacWere, a graduate student from Moi University who has assisted with these programs, now has four training programs of experience. Dr. David Liti (MU Department of Zoology) and Geraldine Matolla (MU Department of Fisheries) have also obtained considerable experience in these programs. Moi

University now has six ponds for use in student research projects and/or future training programs. The team teaching technique that combines CRSP researcher K. Veverica, Moi University professors, and private pond contractors (African Bulldozers) has resulted in a very strong field-work oriented training program—the first of its kind for the Fisheries Department. Professors at Moi University and officials of the Kenya Fisheries Department and KMFRI have all independently reached the conclusion that Sagana is the best place to hold training of this sort.

Judith Amadiva, the Social Development Officer at Sagana and a valuable assistant to the training programs when they were held at Sagana, has developed a follow-up questionnaire to document the effects of the training on the job performance of the graduates. Trainees will be asked what changes they have made in their recommendations to farmers and some visits will be made to ponds newly constructed following the training program.

One more training program is planned for the ninth Work Plan: Fishery Officer training to be held in November 2000. The FD recently requested that the program be extended to three weeks instead of two weeks. It will be team taught by Moi University and CRSP researchers Veverica and Bowman, at a graduate student level. The course will form the model for other university aquaculture classes. Only 20 places for Fishery Officers will be available. There has been high interest in this course and we have been requested to allow other trainees to attend the course under sponsorship from other projects or private entities. Therefore an additional five places are being added for outside-funded trainees. One private business and one government department have taken all the open places.

During the first part of the reporting year the CRSP resident research coordinator continued to provide guidance for Fisheries Officers assigned to Sagana Fish Farm in conducting small research projects, giving advice on experimental design and some material assistance. These projects further the professional development of the officers and provide background information and experience for writing proposals for more detailed research. Some of the projects, such as those looking at *Clarias* fingerling production techniques, have immediate impact on station management. Two such projects were undertaken during the year, as shown in Table 3. However, no report has come from ANY of the small projects conducted by any Fishery Officer. This effort had to be discontinued in 2000, following the reduction of CRSP support for the US Research Coordinator to 0.25 FTE.

By the end of this reporting period, almost all of the Fisheries Officers at Sagana had been transferred. One obtained a scholarship to Belgium; some were transferred for disciplinary reasons. The newly transferred FO's who replace them have little practical training in aquaculture but they had demonstrated good initiative and high interest. However, this means that further training is even more important.

The CRSP continues its support of Mr. James Karuri in a three-year diploma program in Applied Biology at the Muranga College of Technology. He began his first year in January 1999, and will finish by the end of 2001.

FARMER TRAINING

Last year we reported on a program of farmer education days that was developed in response to requests received from the farmers themselves. Under that program a series of five farmer education days involving 107 farmers and 40 extension workers was held during the first half of 1999. All districts in Central Province were covered and one district each from Eastern and Rift Valley Provinces was included. Only informal farmer training was conducted this year, through individual contacts when farmers visited Sagana or during farm visits. However, a considerable training effect is expected for farmers and the Fisheries Officers and extension agents who work with them as a result of the on-farm trials that are under way in Central Province. That activity is reported on separately (see 9ATR1, "On-Farm Trials: Evaluation of Alternative Aquaculture Technologies by Local Farmers in Kenya"). Field days for 100 additional farmers are planned as part of *this* activity, but these are scheduled to begin after August 2000.

Last year we reported that the water flow question appears to be the single most important issue in improving fish growth and production. This issue continues to be one of the most important to address. However, the biggest impediment to transferring water management understanding to farmers is, in fact, the extension service. Extensionists continue to advise what they were taught via hearsay (that flowing water through ponds is the preferred management approach), and they can only advise on subsistence level management. If subsistence level management is all the extension agents know, then aquaculture will not progress beyond the subsistence level.

ANTICIPATED BENEFITS

The Pond Construction and Management Training program has been included in the Moi University training curriculum and has been accepted as an official course by the FD. This means that those who have completed the program are eligible for promotion or they have an advantage over their un-trained colleagues when it comes time for downsizing of staff. This is why the FD elected to send mostly Fishery Assistants to the training. The FD is phasing out its use of Fish Scouts, so both the FD and the CRSP were hesitant to include many of these in the training. In fact, hundreds of fish scouts were retrenched in

Table 3. Fisheries Officers assigned to Sagana that have worked on their own research projects during this reporting year, with advice and materials provided by the PD/A CRSP.

Name	Project title	Status
Francis Mwonjoria	Cage culture of tilapias: trials using low volume/high density cages based on work done in China.	Began Nov. 1999; Officer lost interest as of Feb 2000. Coordinator examined data with him and made suggestions on reporting.
Raphael Mbaluka	*Clarias* fingerling production technologies: simultaneous fry growout with tilapia fry	Second experiment to began Aug. 1999. Worked up data and discussed with CRSP coordinator.

September 2000. So far, none of the staff who received a certificate have been retrenched.

This activity is providing university students, FD personnel (including those involved in extension efforts), and farmers with knowledge about proper pond construction and skills for improved fish handling and pond management. Short training courses are improving technical confidence and morale among Fisheries personnel involved in extension work. Linkages between research and extension activities in Kenya are being strengthened. Support and hands-on guidance of undergraduate and graduate aquaculture students will strengthen their degree programs and help promote productive and sustainable aquaculture growth in Kenya and in the region by providing a cadre of trained staff for commercial aquaculture. Ultimately, better pond management by farmers will lead to increased fish production, increased farm income, increased amounts of fish available to communities and markets, and increased employment opportunities.

REFERENCES

Veverica, K.L., B. Omolo, J. Amadiva, and J.R. Bowman. 2000. Aquaculture training for Kenyan fisheries officers and university students. In: K. McElwee, M. Niles, X. Cummings, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 167-170.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Establishment of Companion Sites in the Africa Region

Ninth Work Plan, Adoption/Diffusion Research 4 (9ADR4)
Progress Report

Daniel Jamu
ICLARM-Malawi
National Aquaculture Center
Zomba, Malawi

James R. Bowman
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Karen L. Veverica
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Abstract

The establishment of one or more companion sites in the Africa Region was proposed as a way of expanding regional efforts of the CRSP by assisting with the conduct of needed research at other sites in the region and of verifying the results of CRSP research at its site in Kenya. The objectives specifically listed for this effort in the Ninth Work Plan are 1) to identify and establish one or more companion sites for the Africa Region (year 1) and 2) to define and implement investigations at the companion site in support of PD/A CRSP and companion site goals (year 2). During the first year of the Ninth Work Plan (December 1998 to November 1999), CRSP Kenya Project personnel continued discussions with possible collaborators in Malawi, leading to a proposal to collaborate with the International Center for Living Aquatic Resources Management (ICLARM) at the National Aquaculture Center, Zomba, and with Bunda College, near Lilongwe. During year 2 an agreement was reached whereby two studies supported by the CRSP Kenya Project will be conducted in Malawi under the oversight of Dr. Daniel Jamu. One will be conducted at the National Aquaculture Center, and the second will be conducted at Bunda College. Work on the Zomba study began in May 2000.

Introduction

The establishment of one or more companion sites in the Africa Region was proposed as a way of expanding regional efforts of the CRSP by assisting with the conduct of needed research at other sites in the region and of verifying the results of CRSP research at its site in Kenya. The objectives specifically listed for this effort in the Ninth Work Plan are:

1) To identify and establish one or more companion sites for the Africa Region (year 1); and
2) To define and implement investigations at the companion site in support of PD/A CRSP and companion site goals (year 2).

During the first year of the Ninth Work Plan (December 1998 to November 1999), CRSP Kenya Project personnel continued discussions with possible collaborators in Malawi, leading to a proposal to collaborate with the International Center for Living Aquatic Resources Management (ICLARM-Malawi) at the National Aquaculture Center, Zomba, and with Bunda College, near Lilongwe. (Veverica and Jamu, 2000). This report outlines progress made during the current reporting year, 1 August 1999 through 31 July 2000.

Methods and Materials

Discussions among Daniel Jamu of ICLARM-Malawi, Karen Veverica and Jim Bowman of the CRSP Kenya Project, Jeremy Likongwe of Bunda College, and other Kenya Project personnel led to the identification of two studies suitable for support from the CRSP Kenya Project early in the second year of the Ninth Work Plan (December 1999 to February 2000). Following this, in May 2000, a collaborative agreement between Oregon State University (OSU), representing the Kenya Project, and ICLARM-Malawi was signed. This agreement cleared the way for the transfer of funds and the beginning of work in Malawi. Under the agreement, one study will be conducted at each of the two collaborating Malawi institutions—ICLARM-Malawi and Bunda College of Agriculture—with supervision by Jamu and Likongwe, respectively. Aquaculture students from Bunda College will be heavily involved in both studies, with much of the CRSP funding going to their support. In-country oversight of both studies is being provided by Jamu.

Work on a study entitled "Effect of Stocking Size and Nutrient Inputs on Productivity of *Oreochromis shiranus* in Ponds" began at the National Aquaculture Center in late May. The objectives of this study are:

1) To determine the effects of different fish sizes on *O. shiranus* productivity;
2) To evaluate the effect of two different isonitrogenous input regimes on *O. shiranus* productivity and profitability; and
3) To recommend, based on objective 2, stocking strategies that optimize fish productivity.

Eighteen ponds were stocked at a density of 2 fish m^{-2} with juvenile *O. shiranus shiranus* of three different sizes (5 g, 10 g, and 20 g). Two isonitrogenous input regimes (20 kg N ha^{-1} wk^{-1}) are being tested; these consist of: a) maize bran (3% of mean body weight per day) plus urea fertilizer and b) napier grass (50 kg dry matter ha^{-1} d^{-1}) plus urea fertilizer. Organic inputs (bran and napier grass) are applied daily whereas urea is applied weekly. All treatments are being done in triplicate. Fish (batch weight of 100 fish in the pond), total ammonia nitrogen, and chlorophyll *a* are measured biweekly; pH and electrical conductivity are measured weekly; and DO and water temperature are measured daily. The sampling is being implemented by a final-year undergraduate student from Bunda College of Agriculture in collaboration with students from the Malawi Fisheries College who are attached to the National Aquaculture Center. This work is directly supervised by Jamu.

At Bunda College, a study entitled "Use of Salinity to Increase Growth of Tilapia in Aquaculture" will be conducted under the supervision of Likongwe, Head of the Aquaculture and Fisheries Management Department. This work is of significance in Malawi because some areas currently considered to be marginal for fish production due to the presence of saline waters may have the potential for productive fish culture using species such as *O. shiranus shiranus*, *O. shiranus chilwae* (from pond and lake), *O. karongae,* and *Tilapia rendalli* if the relationships between fish growth and water salinity are better understood.

The objectives of this study are:

1) To determine the effect on survival of direct transfer into waters of different salinities for four taxonomic groups of tilapia: *O. shiranus shiranus*, *O. shiranus chilwae* (from pond and lake), *O. karongae*, and *T. rendalli*;
2) To determine the effects of different salinity concentrations on growth, feed conversion, and reproduction of tilapia; and
3) To identify salinity preferences of the above species at different sizes.

There will be five main experiments, each lasting 56 days, to examine survival, growth, and feed conversion of one of the species/subspecies cultured in four salinity concentrations (0, 10, 20, and 30 ppt). Five supporting experiments will examine the tolerance of these same species/subspecies to direct transfer into saline water, the duration of each depending on the ability of the fish to tolerate the test salinities. Each experiment will be conducted indoors using 12 glass or fiberglass tanks. Each tank will be stocked with 16 fingerlings of uniform size (10 g). Tanks will be aerated using a blower, and fish will be fed on pelleted diets containing 35% crude protein. Sampling of fish to monitor growth in weight and length will be done every two weeks. Water quality variables (temperature, salinity, dissolved oxygen, un-ionized ammonia, hardness, and pH) will be monitored daily or weekly, depending on the variable. All species (except *O. shiranus chilwae*) will be collected from Bunda College.

RESULTS

The stocking size experiment being conducted at the National Aquaculture Center was begun in late May and is expected to last approximately six months. Data are being collected as planned but will not be analyzed until after conclusion of the experiment. Fish weights from samples taken during the first two months of the experiment indicate that fish growth has been hindered by low water temperatures (18°C), which have persisted into the month of August.

The beginning of work on the salinity experiment to be conducted at Bunda College has been delayed. It is expected that this study will begin in August 2000.

ANTICIPATED BENEFITS

Fish farmers in Malawi and the surrounding region will benefit from information gained through this research because researchers will be able to provide better guidance with respect to appropriate stocking densities and to the use of saline waters for fish production. Aquaculture students from Bunda College who are involved in the research will benefit by gaining first-hand knowledge of the culture characteristics of several aquaculture species important in Malawi as well as by learning research methods through their work with Jamu and Likongwe. The growth characteristics of species cultured in Malawi might be compared with CRSP findings from other sites. The CRSP Central Database will be broadened through the inclusion of data from additional sites in Africa. Companion site researchers will benefit from data collected during the course of experiments, and improved fish farming methods resulting from the experiments will be available for adoption by fish farmers in the area around the companion site. Ultimately, fish farmers in new areas will experience increased fish yields, and greater amounts of fish will be available for consumption in communities and markets in those areas.

LITERATURE CITED

Veverica, K.L. and D. Jamu, 2000. Establishment of companion sites in the Africa region. In: K. McElwee, D. Burke, M. Niles, X. Cummings, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 171–172.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Regional Outreach in Africa

Ninth Work Plan, Adoption/Diffusion Research 5 (9ADR5)
Progress Report

Karen L. Veverica
Department of Fisheries and Allied Aquacultures
Auburn University
Auburn, Alabama, USA

Bethuel Omolo
Sagana Fish Farm
Sagana, Kenya

James R. Bowman
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

Abstract

The intent of the Kenya Project's regional outreach activity is to promote contact and communication among aquaculture research and extension personnel and organizations throughout the region. This is to be achieved mainly through participation at regional meetings, not only by presenting papers but also through participation in planning and organizing the meetings. It is hoped that such participation will help promote the dissemination of information emanating from PD/A CRSP research, help conference participants learn about fish culture practices and research priorities and activities in Kenya and in neighboring countries, and encourage the establishment of linkages among research and extension programs in the region. During the current reporting year Sagana Fish Farm and the CRSP hosted a meeting of the Aquaculture Committee of the Lake Victoria Environmental Management Programme. The Kenya Project sponsored travel to several workshops and conferences, including a two-week study tour on agro-aquaculture in Malawi for then–Sagana Head of Station B. Omolo; attendance at the LV2000 conference in Jinja, Uganda, for K. Veverica, J. Ngatuni (current Head of Station at Sagana), K. Kahareri, R. Mbaluka, and E. Were; and attendance at the IIFET 2000 conference "Microbehavior and Macroresults" by M. Muchiri, Head of the Department of Fisheries at Moi University, Eldoret, Kenya, and a collaborator on several CRSP Kenya Project activities. Graduate student B. Meso presented information on the first season of the study "Use of Pond Effluents for Irrigation in an Integrated Crop/Aquaculture System" at the 25th Conference and Silver Jubilee of the Soil Science Society of East Africa, in Kampala, Uganda, and was awarded the prize for academic excellence and diversity in soil science at the university post-graduate level for his presentation. As it has for several years, the CRSP supported the participation of the Kenya Fisheries Department and Sagana Fish Farm in the Agriculture Society of Kenya's annual "Nairobi Show." Although the Fisheries Society of Africa (FISA) did not hold any conferences this year, Veverica continues to maintain contact with officers of that organization, which is headquartered in Nairobi.

Introduction

The intent of this activity is to promote contact and communication among aquaculture research and extension personnel and organizations throughout the region. This is to be achieved mainly through participation at regional meetings, not only by presenting papers but also through participation in planning and organizing the meetings and in helping to develop and implement plans to increase participation in them. Through this effort, research results from current and previous CRSP activities can be shared, other African researchers can be met and encouraged, CRSP workers can learn about research and extension efforts in other parts of the region, and linkages for future collaboration can be established. Examples of regional meetings that might be included are annual meetings of the Southern Africa Development Community (SADC) Inland Fisheries Sector Technical Coordination Unit and the Fisheries Society of Africa (FISA), but other meeting opportunities will also be taken advantage of.

The objectives specifically listed for this effort in our work plan were:

1) To promote the dissemination of information emanating from PD/A CRSP research results;
2) To learn about fish culture practices and research priorities and activities in Kenya and neighboring countries in Africa; and
3) To encourage the establishment of regional linkages between research and extension programs in Africa.

This activity continues from a similar activity in the Eighth Work Plan.

This Year's Activities

In mid-1999, P.O.J. Bwathondi of the Lake Victoria Environmental Management Programme (LVEMP) asked to hold a meeting of the Aquaculture Committee of the LVEMP at Sagana so they could benefit from CRSP experiences and enhance our collaboration. K. Veverica and B. Omolo responded by organizing and leading a workshop at Sagana from 17–21 December 1999. Participants came from Tanzania, Uganda, and Kenya. LVEMP provided travel costs for some participants while the CRSP helped with organization and leadership of the workshop as well as provision of training materials, including the CRSPs *Dynamics of Pond Aquaculture* (Egna and Boyd, 1997) and *Pond Fertilization* (Knud-Hansen, 1998) books. Copies of some of the more relevant CRSP *Research Reports* and *Notices of Publication* and Sagana *Fact Sheets* (extension bulletins) were also given to interested participants. Veverica has interacted with LVEMP personnel for some time, and it is expected that this collaboration will continue. The LVEMP-CRSP linkage was greatly strengthened by this seminar. It was concluded that Sagana Fish Farm, in conjunction with the CRSP, would train the lab technicians working in the three LVEMP countries in water quality analyses.

Omolo attended a two-week study tour on agro-aquaculture in Malawi in November 1999 where he gave a presentation on *Aquaculture Development in Kenya*. This study tour was sponsored by the Technical Centre for Agricultural and Rural Co-operation (CTA) and organized by D. Jamu of ICLARM-Malawi. Jamu now collaborates with the CRSP Kenya Project as Principal Investigator for studies conducted in Malawi under our Companion Site activity (9ADR4).

LV2000, a conference on the Lake Victoria Basin originally scheduled to be held in Jinja, Uganda, 23–26 November 1999, was actually held from 14–19 May 2000. Veverica traveled with J. Ngatuni (Head of Station, Sagana Fish Farm), K. Kahareri, R. Mbaluka, and E. Were to attend the conference and to visit two Ugandan fish stations and a number of rural ponds. Veverica made a presentation addressing the business/economics aspects of aquaculture production in Central Kenya entitled "Commercial Tilapia Production Recommendations and Enterprise Budgets of East Africa in the Absence of Formulated Feeds." Although Lake Victoria fisheries management issues were the major theme of the conference, Veverica's presentation was very well received by conference attendees, who said that the business development perspective is just what is needed for aquaculture to develop further in the Lake Victoria basin. During this visit, an NGO working in wetlands development asked for help in training in fish farming. A training plan was developed and the organization is seeking funds to have Sagana station staff train 140 farmers.

M. Muchiri, Head of the Department of Fisheries at Moi University, Eldoret, Kenya, and a collaborator on several CRSP activities in Kenya, attended the IIFET 2000 conference "Microbehavior and Macroresults," held at Oregon State University in Corvallis, Oregon, 10–14 July 2000. Muchiri gave a presentation entitled *Break-Even Price And Investment Costs Under Different Loan Schemes For Small-Scale Fish Farmers In Kenya* at one of the several aquaculture sessions of the conference. Muchiri was able to meet and confer with fisheries and aquaculture professionals from several other African countries while at the conference. He stayed over in Corvallis for a few days following the conference, and was able to meet with several members of the Department of Fisheries and Wildlife and of the Office of International Research and Development to explore possibilities for linkages and for future exchanges of faculty and/or students between the universities. Prior to his departure from Corvallis, Muchiri and J. Bowman visited East Lake, in Central Oregon, to learn about a population dynamics study that was begun there this year. Following his time in Oregon, Muchiri traveled to Auburn University, Alabama, to learn about the aquaculture and fisheries programs there. There he again explored possibilities for future inter-institutional collaboration and faculty and student exchanges, leading to the drafting of an MOU between Moi University and Auburn University.

Graduate student B. Meso presented information on the first season of the study "Use of Pond Effluents for Irrigation in an Integrated Crop/Aquaculture System" (9ER1) at the 25th Conference and Silver Jubilee of the Soil Science Society of East Africa, held in Kampala, 6–10 September. He was awarded the prize for academic excellence and diversity in soil science at the university post-graduate level for his presentation.

The Agriculture Society of Kenya held its annual Nairobi Show from 27 September to 2 October 1999. The show is similar to a US county fair, with 230 stands and attendance of up to 100,000 visitors per day. Sagana Fish Farm and the Fisheries Department contributed to the stand for the Ministry of Natural Resources. Veverica and Omolo arranged for a pet store ornamental fish dealer to loan a 10-foot aquarium to display aquaculture species, including tilapia, *Clarias*, and carp. Sagana also donated fish for the Ministry of Agriculture's display. *Clarias* fillets were donated by Sagana to be served in the fish preparation demonstration. The Ministry of Agriculture won second place, and the Ministry of Natural Resources took third place for government stands. Sagana's Social Development Officer J. Amadiva worked at the stand and answered questions for three days, followed by S. Njau, who covered another three days.

Although the Fisheries Society of Africa (FISA) has not sponsored any conferences during this period, Veverica has maintained contact with officers of that organization, which is headquartered in Nairobi.

ANTICIPATED BENEFITS

Contact with researchers and extension personnel in Kenya and in other countries in the region will result in a better understanding of research needs and enhanced research-extension linkages. Extension services in Kenya and other African countries will benefit by being more closely linked with research institutions, and African researchers will have an enhanced understanding of research needs. Ultimately, fish producers throughout the region will benefit because these linkages will enable extension services not only to more easily convey farmers' needs to researchers but also to extend new research results back to the farmers.

LITERATURE CITED

Egna, H.S. and C.E. Boyd (Editors), 1997. Dynamics of Pond Aquaculture. CRC Press, Boca Raton, Louisiana, 437 pp.

Knud-Hansen, C.F., 1998. Pond Fertilization: Ecological Approach and Practical Applications. PD/A CRSP, Oregon State University, Corvallis, Oregon, USA, 125 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

WORKSHOP ON THE TIMING OF THE ONSET OF SUPPLEMENTAL FEEDING OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) IN PONDS

Ninth Work Plan, Adoption/Diffusion Research 6A (9ADR6A)
Final Report

Christopher L. Brown
Marine Biology Laboratory
Florida International University
North Miami, Florida, USA

Remedios B. Bolivar
Eddie Boy T. Jimenez
Freshwater Aquaculture Center
Central Luzon State University
Nueva Ecija, Philippines

ABSTRACT

Ninth Work Plan Feeds and Fertilizers research (9FFR4) has determined that delaying the onset of feeding in tilapia grow-out ponds reduces farmer investment while maintaining production levels. The application of these research results is encouraged, and a workshop was held at the Freshwater Aquaculture Center, Central Luzon State University, Nueva Ecija, Philippines, to extend the results to area farmers. Eight farmers participated in the workshop, which included both presentation of results and group discussion of impacts. Farmers who attended stated that they would immediately adopt the delayed feeding strategy. Since the workshop, farmers who heard of delayed feeding through word-of-mouth have already adopted the practice.

SUMMARY

Following a successful on-farm trial conducted in seven farms in Nueva Ecija, Philippines ("Timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds," 9FFR4), a workshop was held to disseminate the results to area farmers. Six of the seven participating farmers, and two additional farmers who have since volunteered to participate in the next round of trials, attended the meeting held at the Freshwater Aquaculture Center, Central Luzon State University (CLSU), Nueva Ecija, Philippines, on 6 December 1999. Other attendees included CLSU aquaculture students, faculty, and administrators.

Lunch was served to the guests and introductions were made, followed by a succinct presentation of farm trial results. These results included conclusive evidence that the postponement of initial feeding did not significantly compromise the performance of tilapia in terms of size, yield, uniformity, or crop value. The performance of sex-reversed Nile tilapia (*Oreochromis niloticus*) of the Genetically Improved Farmed Tilapia (GIFT) strain was equally impressive in all experimental conditions, and farmers were in agreement that those with the lowest operating cost were clearly the preferable feeding strategies. There was considerable discussion about the practical utility of these results.

ANTICIPATED BENEFITS

Farmers attending this workshop expressed their gratitude for being allowed to participate in a project that they felt could help them directly. The delay in the initial provision of a locally milled feed consisting of rice bran and fish meal until the fish reached 75 days of age did not significantly reduce growth, survival, or other production parameters although grow-out costs were favorably impacted by this treatment. Consequently, many of the participating farmers indicated at the workshop that they planned to adopt this feeding strategy. The consensus among the farmers was that waiting for additional results would serve no particular purpose, as the results suggested convincingly enough that there is no point in beginning to pay for feed early in the grow-out when pond productivity obviously can support growth adequately; the profitability of this approach seemed intuitive to the farmers. Word-of-mouth has since spread, and other area farmers have been using this approach to reduce their operating costs. We believe it is unusual that results are converted into profitable techniques in the commercial sector this rapidly.

In general, we felt that this was a good and productive workshop. In critiquing it, we felt that it would have been beneficial to try to reach a broader audience.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Production of Improved Extension Materials

Ninth Work Plan, Adoption/Diffusion Research 6B (9ADR6B)
Abstract

Christopher L. Brown
Marine Biology Program
Florida International University
North Miami, Florida, USA

Remedios B. Bolivar
Eddie Boy T. Jimenez
Freshwater Aquaculture Center
Central Luzon State University
Nueva Ecija, Philippines

James P. Szyper
Sea Grant College Program
University of Hawaii at Manoa
Hilo, Hawaii, USA

Abstract

In our first year of work on the Ninth Work Plan Philippines Project, we have generated meaningful results in two areas: the reduction of feeds used in the initial phase of grow-out ("Timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds," 9FFR4) and the cost-benefit of using only light application of fertilizers ("Global Experiment: Optimization of nitrogen fertilization rate in freshwater tilapia production ponds," 8FFR1Ph). Both of these lines of work lend themselves to extension effort. The results of the studies have been accepted by farmers near Central Luzon State University, suggesting that broader dissemination will have broader impact. We anticipate that a broad base of the fish farming industry in the Philippines will adopt methods shown convincingly to have the potential to improve their profits while reducing the amount of labor required. Thus far, we have presented results of the first of these two series of feeds experiments in a workshop ("Workshop on the timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds," 9ADR6A), at an annual CRSP meeting, and at an international meeting and have drafted a manuscript. We are presently working on conceptual design for extension materials for dissemination and have collected similar extension materials (technical bulletins, fact sheets, etc.) from the US to use as models.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

DECISION SUPPORT FOR POLICY DEVELOPMENT: PLANNING CONFERENCES FOR COLLABORATING RESEARCHERS, PUBLIC AGENCIES, AND NONGOVERNMENTAL ORGANIZATIONS WORKING IN AQUACULTURE

Ninth Work Plan, Adoption/Diffusion Research 7 (9ADR7)
Abstract

Brahm Verma and E. William Tollner
Department of Biological and Agricultural Engineering
University of Georgia
Athens, Georgia, USA

Daniel Meyer
Department of Biology
Escuela Agrícola Panamericana El Zamorano
Zamorano, Honduras

Tom Popma
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Joseph J. Molnar
Department of Agricultural Economics and Rural Sociology
Auburn University, Alabama, USA

Freddy Arias
Department of Agribusiness
Escuela Agrícola Panamericana El Zamorano
Zamorano, Honduras

ABSTRACT

This work relates to fostering linkages among national and regional organizations to pursue capacity building and institutional strengthening for aquaculture. All Co-Principal Investigators met in Honduras for one week in October/November 1999. The meeting was devoted to understanding the local conditions that will impact the effectiveness of envisioned linkages for development. The investigators:

1) Toured facilities of Zamorano University and met key university faculty and administrators;
2) Met 12 national and international nongovernmental organizations (NGOs), as well as extension agents, government officials, and policy makers;
3) Visited several small, medium, and large tilapia farms;
4) Visited sites of earlier work developed with PD/A CRSP support; and
5) Developed plans for identifying individuals and groups who should be included in training workshops on tilapia production, pond design, and decision-making approaches in which tilapia is an alternative for economic development.

Some of the observations made during this meeting were:

- There is a large network of NGOs operating at the village level that need to be linked.
- Many NGOs are currently offering assistance to small farms and are interested in adding technical assistance capabilities in tilapia culture.
- NGOs and government policy makers are interested in water, water harvesting, and hillside stabilization.
- The Comayagua research station in El Carao can be an appropriate site for providing training to NGO technicians and extension personnel.
- There is a need to expand the number and geographical distribution of fingerling producers and to improve fingerling quality in Honduras.
- Women and children often bear primary responsibilities for managing small-scale aquaculture ponds.
- Home consumption, pond-bank sales, and local markets are important outlets and can be the primary marketing opportunities for small- and medium-scale producers.
- Innovative methods of delivering information on tilapia culture and markets as a source of economic development should be considered. The project should provide for stakeholders to give input for developing these methods.
- There is a need for a method and a manual describing pond siting (that includes source of water and quality of watershed), construction, and management of ponds.

Based on these observations, much work is in progress. A one-day meeting with select stakeholders has been organized to present objectives of this research and receive inputs. This will contribute to the development of innovative methods for

delivering information and training of individuals and groups that can affect introduction of tilapia culture as an alternative in sustainable economic development. This meeting is scheduled at Zamorano University and a follow-up questionnaire is planned to identify NGOs and policy makers for a three-day training session on tilapia biology and culture, pond design, and water harvesting. The three-day workshop is scheduled for September 2000. The group will be introduced to an envisioned *W*eb-based *I*nformation *De*livery *S*ystem for *T*ilapia (WIDeST). The work on development of (WIDeST) is in progress. WIDeST has three important ingredients, namely:

1) Sources of data and information for successful production and marketing of tilapia;
2) The knowledge of decision-making methodology for sustainable economic development; and
3) The knowledge and experience of developing electronic information networks and Web-based online information exchange.

Its goal is to develop a system of information delivery by using the Web-based technology for making available the knowledge on tilapia production and management to farmers, NGOs, policy makers, businesses, consumers, and other stakeholders. WIDeST will contribute to successful introduction of tilapia production and marketing as an alternative in the economic development of Honduras and other parts of Latin America. A key cooperator in this work is the Red de Desarrollo Sostenible-Honduras (RDS-HN). RDS-HN was created with the initial grant from the United Nations Development Programme in response to the 1992 Earth Summit, which mandated assistance to “developing” countries for establishing in-country Sustainable Development Networks (SDNs). These networks were envisioned to provide infrastructural support for rapid communication through electronic information technology. RDS-HN is a very successful organization, and in partnership with Zamorano we planned the three components for the envisioned WIDeST:

1) A website accessible via the Internet;
2) A newsletter published and distributed periodically giving updates; and
3) Presentations and training through meetings and conferences.

Work on the website has begun, and materials for presentations and training are planned for upcoming events in September, November/December, and March/April. The newsletter is not planned at this time due to lack of firm funding. We feel that WIDeST will be a strong contribution from the PD/A CRSP that will initiate an innovative method to foster linkages and communication among all stakeholders. This will be an outstanding legacy of the PD/A CRSP. Of course, considerable development beyond the duration of this project will be needed to complete WIDeST and to increase its effectiveness in sustainable economic development and decision making. This level of development is beyond the scope of this project.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Production Strategies Characterizing Small- and Medium-Scale Tilapia Farms: Approaches, Barriers, and Needs

Ninth Work Plan, Adoption/Diffusion Research 8 (9ADR8)
Abstract

Joseph J. Molnar
Department of Agricultural Economics and Rural Sociology
Auburn University, Alabama, USA

Freddy Arias
Department of Agribusiness
Escuela Agrícola Panamericana El Zamorano
Zamorano, Honduras

Tom Popma
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Abstract

This study portrays Honduran tilapia producer perceptions of production processes, limitations, constraints, and possibilities through on-farm interviews with a selected sample of growers. Wholesalers, distributors, and urban restaurant buyers typically rely on connections to large-scale producers who can provide a regular supply of uniform product. Small- and medium-scale farmers rely largely on a diverse set of local strategies for realizing cash from their tilapia crops. One significant commercial distribution channel for small-scale and limited-resource farmers often is the intermediary or "coyote." Such persons generally do not live in the community but instead travel from community to community buying and selling farm products. Rural producers in Honduras face particular difficulties due to the difficult terrain, poor road system, and fragmentation in the rural sector. Students from the Escuela Agrícola Panamericana El Zamorano are currently conducting interviews with approximately 20 tilapia farmers at each of five regionally representative fingerling supplier sites throughout Honduras. As many women producers as possible will be interviewed so that the study results can identify their special problems and needs. An interview instrument was collaboratively developed by the researchers, who are extending the instrument used in a previous study, adapting it to focus on experiences and perceptions of the distribution process. At least 20 interviews have been completed. Previous research showed that almost half the Honduran farmers report that middlemen purchase some or all of their fish. A higher proportion of farmers sold tilapia to restaurants in Honduras than in the other PD/A country samples. Honduran farmers were the most confident about being able to sell their tilapia at some price, even if it was not what they originally asked. The most common distribution method for farmers is pond-bank sales to neighbors and to others coming to the ponds at harvest. Word-of-mouth knowledge about prospective harvests or the willingness to partial-harvest for immediate sale was a primary means for marketing tilapia for most small- and medium-scale farmers. Data collection continues from a new sample of tilapia producers that will provide longitudinal data on production practices and distribution strategies to verify and extend the previous research findings.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

TECHNICAL ASSISTANCE FOR FINGERLING PRODUCTION SERVING SMALL- AND MEDIUM-SCALE TILAPIA PRODUCERS

Ninth Work Plan, Adoption/Diffusion Research 9 (9ADR9)
Abstract

Thomas Popma
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Dan Meyer
Department of Biology
Escuela Agrícola Panamericana El Zamorano
Zamorano, Honduras

ABSTRACT

A central issue for aquacultural development in Honduras is fingerling supply. Previous PD/A CRSP research reported that farmers in remote places found that fingerlings were difficult to obtain but did not consider this sufficient reason for withdrawing from fish farming. Prior researchers reported that the Comayagua research station, El Carao, is not a reliable supplier of fingerlings for area producers. This observation was recently confirmed by the Zamorano Principal Investigator (PI) and technician in this project. Private fingerling producers are few and generally geared to supply large-scale commercial operations. The overriding objective of this Adoption/Diffusion activity is to provide technical assistance and training to current and potential fingerling suppliers to small- and medium-scale tilapia producers in Honduras. During the project team visit to Zamorano in November 1999, a strategy and timetable were developed for implementing technical assistance and training of fingerling suppliers. Since then, at least 33 small- and medium-scale tilapia producers (each with 150 to 12,000 m^2 of water surface) and 26 restaurants were interviewed by the Zamorano PI and technical team to assess the production and marketing demands for tilapia in Honduras. The Zamorano team continues to identify and provide technical assistance to regional fingerling producers. During September 2000, a fingerling production technical workshop will be provided by Zamorano and Auburn PIs for actual and prospective fingerling producers.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Training and Technical Assistance for Honduras Institutions Working with Small- and Medium-Scale Tilapia Producers

Ninth Work Plan, Adoption/Diffusion Research 10 (9ADR10)
Abstract

Thomas Popma
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Dan Meyer
Department of Biology
Escuela Agrícola Panamericana El Zamorano
Zamorano, Honduras

Abstract

The Peace Corps program of technical support to fish farmers was possibly the most focused on-farm assistance to small-scale fish farmers in Honduras, but this program ended in 1995. The national extension program in aquaculture has a presence in many regions, but the effort is fragmented and underfunded. A number of nongovernmental organizations (NGOs) have been active in rural development, including several active fish farming projects, but expertise in this activity is often insufficient to provide critical technical information required for productive pond management. The objective of this PD/A CRSP activity is to identify the NGOs and agencies interested in incorporating small-scale fish farming in their development programs and then to provide technical assistance and training to their field staff. The training is a collaborative effort between Auburn University and the Escuela Agrícola Panamericana El Zamorano ("Zamorano").

In November 1999, the Principal Investigators from Zamorano, the University of Georgia, and Auburn University visited directors and representatives from eleven educational and national and international governmental, nongovernmental, and private agencies involved in tilapia culture in Honduras. During this visit, a strategy and timetable were developed for implementing technical assistance and training of NGO technicians.

As a result of the Ninth Work Plan activity entitled "Decision support for policy development—Planning conferences for collaborating researchers, public agencies, and nongovernmental organizations working in aquaculture," (9ADR7), NGOs involved in rural development and with interest in evaluating tilapia culture as a component in the programs were identified. Meyer and F. Arias of Zamorano will offer a one-day workshop in August 2000 on the technical and economic aspects of tilapia culture, with emphasis on its potential value in rural and community development programs. The resulting clearer understanding of the benefits and constraints of tilapia culture will help NGOs make more knowledgeable decisions about the appropriateness of tilapia culture in their overall rural development program. In September 2000, technical staff of interested NGOs will attend a three-day workshop on technical aspects of fingerling production and grow-out of tilapia.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

Decision Support Systems for Fish Population Management and Scheduling in Commercial Pond Aquaculture Operations

Ninth Work Plan, Decision Support Systems Research 2 (9DSSR2)
Progress Report

John Bolte and Charles Hillyer
Department of Bioresource Engineering
Oregon State University
Corvallis, Oregon, USA

Abstract

This study is focused on developing software tools for the analysis of fish population size distributions, focusing initially on commercial catfish operations in the southeastern United States but generalizable to other types of operations and locations. Progress has been made in two primary areas: 1) modeling size distributions and their dynamics through time related to biological and management factors and 2) software development for the decision tool deliverable from this study. The current status of both is described in this progress report.

Introduction

A study was initiated to develop decision support software to assist commercial aquaculture producers in managing fish population dynamics and variability in stock size and weight resulting from biological and management factors. The objectives of this study are:

1) To modify previously developed fish growth models so as to enable simulation of population growth of multiple fish lots in pond environments;
2) To develop methods for estimating fish biomass in ponds that are stocked and harvested at various time intervals in continuous production systems;
3) To implement software support for inventory management of fish stocks in operational farms; and
4) To provide training to farmers on the use of decision support software for routine pond management.

Progress has been made in the first three of these objectives as described below. This report documents model development for size distribution analysis and software development for decision support. Presentation of the growth models has been described previously (Nath, 1996).

Approach and Results

Models for Size Distribution Analysis

Explicitly describing the distribution of fish sizes within a pond population is one of the required features for the population management model. Few models exist which offer a mathematical description of size classes within a population. None were found that describe differential growth with respect to fish size within a fish population. Since no acceptable models were found, a new model of population size distribution has been developed. The model is based on the concept of mass balances. Let $P(w)$ describe the fraction of the population that has mass w. Then

$$\frac{\partial}{\partial t} P(w) = input - output$$

where *input* is the fraction of the population that grows to size w and *output* is the fraction of the population that grows out of size w. We can define *input* and *output* by first defining a function $\delta(w_1,w_2)$ that describes the fraction of fish at mass w_1 that grows to mass w_2. Now *input* and *output* can be described as:

$$input_{w_0} = \int_0^{\infty} P(w)\, \delta(w,w_0)\, dw, \text{ and}$$
$$output_{w_0} = \int_0^{\infty} P(w_0)\, \delta(w,w_0)\, dw,$$

where w_0 is a constant. Furthermore,

$$output_{w_0} = P(w_0)$$

because $P(w_0)$ is constant with respect to the variable of integration and the integral is equal to 1. So we have:

$$\frac{\partial}{\partial t} P(w) = \int_0^{\infty} P(w)\, \delta(w,w_0)\, dw - P(w_0).$$

This system can easily be discretized by first defining n weight intervals, w_i, $i = 0 \ldots n$ and letting $P_{i,k}$ be the fraction of the population at weight w_i at time interval k. Then:

$$P_{i,k} = \left(\sum_{j=0}^{n} P_{j,k}\, \delta(w_j,w_i) \right) - P_{i,k}.$$

It is shown via the fundamental theorem of calculus that:

$$\lim_{n \to \infty} \sum_{j=0} P_{j,k} \delta(w_j,w_i) = \int_0^{\infty} P(w)\, \delta(w,w_0)\, dw.$$

Let P^k denote the columns of $P_{i,k}$ so that P^k is a vector of fractions for each size class at time interval k. If we define a matrix, D, such that:

$$\Delta_{j,i} = \delta(w_j,w_i),$$

then

$$P^{(k+1)} = \Delta P^k.$$

This allows computation of P^k for all desired k assuming that Δ does not change significantly with time. What still remains, however, is a definition for δ.

δ represents the amount of growth of a fish at a certain weight. The bioenergetics model (BE) provides this information; however, we cannot use it directly. The BE model (as it is used in POND©) can be represented in a simplified form as:

$$\frac{\partial}{\partial t} W(t) = H(t) w^n - k(t) w^m .$$

The first term represents anabolism and the second catabolism. This equation represents the growth of a fish at a certain weight, based on anabolic and catabolic balances. To use the bioenergetics model as δ, we must first make an assumption about the distribution of growth within the population. If we assume that growth for a given size class is normally distributed with variance s and mean given by:

$$\mu = w_1 + \mathrm{g}(w),$$

where g(w) is the growth predicted by the BE model, then we can define δ by using the probability density function for the normal distribution:

$$\delta(w_1, w_2) = \left[\frac{1}{2} \frac{\sqrt{2}}{\sqrt{\pi\sigma}} \right] e^{\left(-\frac{1}{2} \frac{(w_2 - \mu)^2}{\sigma^2} \right)} .$$

Figure 1 shows some sample calculations of P at various time intervals. These calculations were made assuming a fairly large (and constant) $\sigma = 0.4$. Notice that the distribution "flattens out" as time increases. This would be characteristic of an underfed population where a significant portion of the population was not reaching its full growth potential.

The drawbacks of the model are twofold. First, it requires a large amount of computational resources. Computing the difference matrix used in Figure 1 took five minutes using Mathcad on a 400Mhz Pentium II computer. This time could be reduced to under one minute via some optimization techniques. Long computation times will reduce the level of interactivity that will be possible with the software. Also, given that the range of size classes will vary over several orders of magnitude (a few grams to a few kilograms) the difference matrix must be large enough to provide reasonable accuracy. A difference matrix for 5,000 weight classes would require approximately 4 MB of RAM. The second problem is with the assumption that growth is normally distributed. Suitable values for the variance parameter, σ, must be determined using field data. The variance essentially represents the effects of competition for resources. These factors are likely to change as the population changes. Thus, fixed values of σ may not provide acceptable accuracy.

The model does have several advantages. First, it is fairly straightforward. This will simplify statistical analysis and software development. Second, it is practical. The existing POND© software contains growth calculations that are essentially the growth function, g(w), needed for the model. Third, the model can be fast in certain situations. If the difference matrix can be held constant, the only computation required is a vector-matrix multiplication of each time step. Finally, since weight classes are modeled explicitly (via the P^k vector), the user will be able to edit the distribution of size classes without modifying the difference matrix. Since harvest operations basically involve editing the distribution vector, the difference matrix will not need to be recomputed. Thus, changing harvest dates will not incur significant computational overhead, and the desired level of interactivity can still be maintained.

Software Design and Development

A requirements and specifications document has been developed in collaboration with Steve Killian. The document describes the features and functionality that will be provided by the inventory management software. The complete document is available online at <biosys.bre.orst.edu/hillyer/catfish_req.htm>.

It was decided that a high degree of interactivity and visual feedback was needed to best provide decision support for scheduling harvest operations and assessing economic outcomes. A slider-based interface coupled with rapid update of the interface will allow the user to experiment with scheduling harvest and stocking operations. This will allow the user to easily determine what is both optimal and practical for his

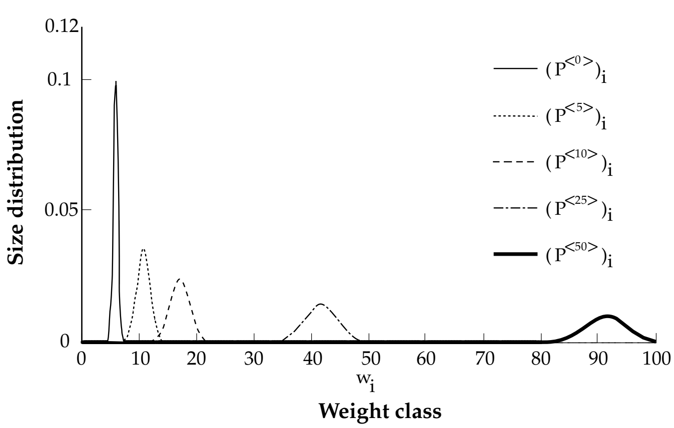

Figure 1. Size distributions at five time intervals. The size distribution is the fraction of the population within a given weight class. Weight classes are defined by the mass of a fish in the population.

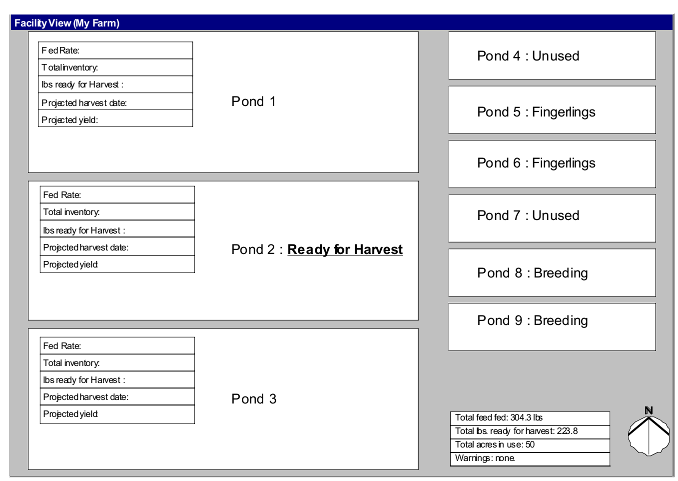

Figure 2. Facility-level view showing a schematic of the entire farm, as provided by modeling software.

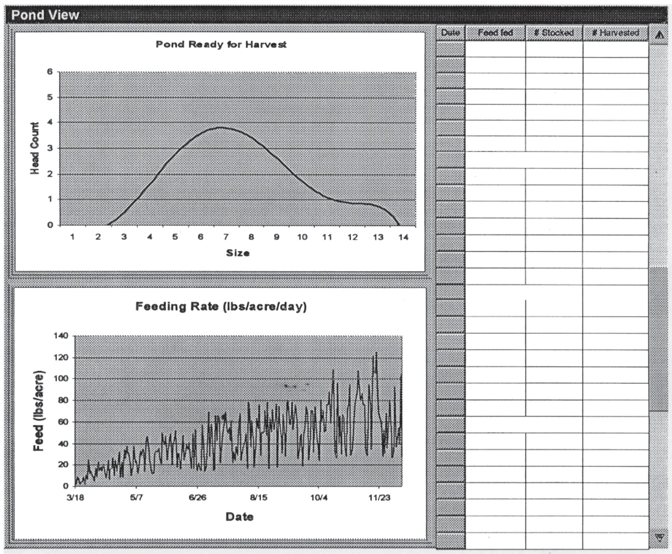

Figure 3. Pond-level view showing fish size distribution, feed rate history, and data spreadsheet, as provided by modeling software.

or her operation. Also, by integrating POND©s economic functionality, users will be able to base their decisions on financial criteria as well as yield.

The software will provide two principle views, a facility-level view and a pond-level view. Examples of these are shown in Figures 2 and 3. The facility view shows a schematic of the entire farm, focusing on the production ponds. The view will show summary data for each pond and will color ponds so that the user can quickly and easily view a pond's readiness for harvest. Other pond statistics can also be displayed (e.g., dissolved oxygen warnings) using color as the key indicator. Users will also be able to click on a pond and view it in greater detail using the pond view.

The pond view (Figure 3) is divided into three sub-areas. First, the estimated pond inventory (upper left) is displayed as a distribution of fish sizes. Second, a plot of the feed rate history (lower left) is shown. Finally, an editable spreadsheet display of the feeding, stocking, and harvest data is shown on the right. When changes are made in the spreadsheet, the pond inventory and feed history will be automatically updated to reflect the changes.

CONCLUSIONS

We are continuing to develop the models presented here and are implementing them in software. Current challenges include integrating the sophisticated bioenergetic growth models contained in the POND© software into this decision tool. Further outreach to the grower community will be developed during the remaining portion of the study, in cooperation with University of Arkansas at Pine Bluff extension staff.

ANTICIPATED BENEFITS

The proposed study is expected to result in the following benefits:

1) Models for projecting fish weight distributions over time and estimating harvest events;
2) Improved capabilities for managing multiple fish lots and their distributions in ponds;
3) Support for inventory management of ponds and lots in existing facilities;
4) Improved support for economic analysis of commercial pond operations; and
5) Farmers trained to apply decision support software for real-time management.

LITERATURE CITED

Nath, S.S., 1996. Development of a decision support system for pond aquaculture. Ph.D. dissertation, Oregon State University, Corvallis, Oregon, 273 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

ENHANCING THE POND© DECISION SUPPORT SYSTEM FOR ECONOMICS, EDUCATION, AND EXTENSION

Ninth Work Plan, Decision Support Systems Research 3 (9DSSR3)
Progress Report

John Bolte
Department of Bioresource Engineering
Oregon State University
Corvallis, Oregon, USA

ABSTRACT

This study deals with development of decision support tools for warmwater pond aquaculture. Efforts are directed at refining the POND© software and developing a new farm-level decision support tool. Refinements to POND© focus on general issues related to program maintenance, enhancements to the enterprise budgeting tool within POND©, and a task-oriented interface for assisting users in accomplishing specific activities within the tool related to educational and extension application. A brief summary of AquaFarm©, a new aquaculture decision support tool developed in part with PD/A CRSP support, is presented.

INTRODUCTION

Successful pond planning and management requires the integration of a host of information related to pond dynamics and water quality interactions, fish growth and development of diverse culture organisms, and economic analyses. Decision support software provides a vehicle for collecting, organizing, and analyzing this information in a consistent manner. Decision support tools can capture current state-of-the-art knowledge of system dynamics, processes, and interactions, and organize these in manner that allows users a convenient ability to further their understanding of the system of interest.

The PD/A CRSP has been involved with the development of decision support software for aquaculture systems for a number of years, resulting first in the PONDCLASS software (Lannan, 1993) and then the POND© software (Bolte et al., 2000). POND© provides the ability to simulate pond dynamics and fish growth for warmwater pond aquaculture facilities and to compute enterprise budgets relating various costs and returns from a particular facility to determine short- and long-term profitability. The growth and water quality models embedded in POND© have been widely validated using data from both PD/A CRSP sites and other warmwater aquaculture sites. Previous efforts at developing POND© have focused primarily on developing the underlying model used by POND© for decision support, and these efforts have been largely successful. Current efforts are focused more on improving the usability of POND© for addressing specific, frequent tasks and increasing its enterprise budget capabilities.

The POND© software has had limits in the types of systems it could be applied to. For example, to maintain a relatively simple learning curve, POND© focused exclusively on semi-intensive, warmwater systems. More recently, as more-intensive systems have become an important part of commercial aquaculture, the need for more-sophisticated models and decision support tools, addressing more-sophisticated systems, has become apparent. To address this need, a new tool, developed partially with PD/A CRSP support, has been created.

APPROACH AND RESULTS

The underlying approach for the development of the POND© software has been described elsewhere in detail (Nath, 1996); here we provide only a brief summary of this approach. POND© was built using an object-oriented design paradigm. Object-based systems represent the target system by providing representations for the underlying components of the real systems and by defining behaviors describing the interactions of these components. For POND©, fish ponds and fish lots provide the key representations of objects in the production facility. Additional objects representing "experts" managing the facility were defined. These experts included:

1) An aquatic chemist, with the ability to perform a wide range of water chemistry calculations;
2) An aquatic biologist, with the ability to perform functions related to fish growth and algal dynamics;
3) A weather manager, with the ability to estimate weather conditions for specific sites;
4) An aquacultural engineer, with the ability to perform heat and water balance calculations, among others; and
5) An economist, capable of performing enterprise budget analyses and managing costs of various facility operations.

By collecting instances of these objects together in a simulation framework, it becomes possible to simulate facility dynamics in a way that closely mimics the dynamics of real aquaculture facilities. In POND© these include fish performance, water temperature, water quality dynamics, and primary and secondary productivity. Models of water quality dynamics are organized hierarchically into two levels, allowing users to perform different kinds of analyses based on data availability and output resolution requirements. 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, but the model optionally generates supplementary feeding schedules. 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.

An important aspect of POND© is the ability to incorporate the results from the pond dynamics simulations, with additional information, into an enterprise budget. These 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.

Current efforts for POND© development are focusing on enhancing the economic capabilities of ponds. The first of these is the support of partial budgeting. The second is the inclusion of time-based costs. These can include periodic costs (i.e., costs that recur at specific periodic intervals) and costs that are scheduled to occur only at specific times and with specific durations. The inclusion of such costs has allowed POND© to be used to more directly simulate the medium- to long-term dynamics of production facilities, as well as to facilitate the examination of the economics of facility production during specific production windows.

Additional efforts are focusing on creating task-oriented user interfaces. We started this recently with the inclusion of "wizards" for automating key simulation tasks. We are now extending this metaphor for the development of design and analysis tasks. For example, wizards are being developed to size ponds to meet specific production targets under particular climatic and production regimes, provide optimized feeding schedules to hit particular production targets, and develop nutrient budgets for specific production strategies. We will be continuing to develop these wizards over the next year in collaboration with our cooperators.

In addition to these activities related to the POND© software, we are releasing this year an additional decision support tool, AquaFarm©. AquaFarm© provides simulation of physical, chemical, and biological unit processes, facilities, and management systems for a broad class of aquaculture systems, including semi-intensive and intensive systems. It incorporates and extends many of the components of the POND© model, including economic analyses but further provides more detailed unit process descriptions and more robust analytical capabilities. It allows simulation of broodfish maturation, egg production, and grow-out of finfish or crustaceans in cage, single pass, serial reuse, recirculation, or solar-algae pond systems. AquaFarm© allows the iterative refinement of design specifications for a production facility by simulating production dynamics with feedback when production goals are not satisfied, allowing users to rapidly fine-tune facility design and management to meet specific criteria. A more complete description of AquaFarm© is given by Ernst et al. (2000).

CONCLUSIONS

The design, development, and implementation of the POND© software have provided useful lessons in several areas. As the user base for POND© has grown, we have had the opportunity to solicit feedback on how well POND© is addressing user needs. The first lesson is that a diverse group of users has used POND© to address a diverse group of needs. Although we originally anticipated a research-focused audience, our largest group of users has been commercial aquaculture facility managers. The primary focus of this group has been economic analyses, with the utility of the biological models contained within POND© of secondary importance. An additional audience has been educators using POND© in the classroom as a tool for examining pond dynamics, where the biological models play a more important role. Each of these groups has a different set of interests and a different user interface requirement, and a "one size fits all" approach will not be optimally successful at addressing their needs.

A primary feedback from POND© users involved the ease of use of the software. Although we have spent considerable effort in developing a modern user interface for POND©, because of the underlying complexity of the models POND© employs and our desire to fully expose these models, the user interface proved to be burdensome for many users. While exposure of the underlying models is helpful to those focused on understanding the detailed biological dynamics of these systems, it is less helpful or irrelevant to those focused primarily on economic analyses of facility operations. We are addressing this issue in upcoming releases of POND© in two ways. First, the focus of POND©, from a user interface perspective, is more on decision support and less on models of the underlying biological system. While the underlying models continue to be an essential tool for supporting decision making, they are less apparent to the user, operating in the background but playing a secondary role to higher-level decision-making processes. Second, we have introduced a series of wizards into POND©. These wizards are software tools that walk users through specific frequently used tasks, hiding much of the complexity of these tasks and providing immediate help to users in accomplishing their goals. We anticipate continued development of these wizards to address specific needs of different user groups and to improve ease of use of the program.

The target audience for POND© continues to evolve. The bulk of our requests come from commercial producers looking for tools to improve their ability to design and manage their facilities. A second audience for which we have seen numerous requests is small commercial interests, often new to aquaculture, who are looking for tools to help them explore the financial feasibility of launching an aquaculture venture. Both these groups tend to focus primarily on economic analyses but require some basic understanding and consideration of the biological and chemical processes underlying facility operation. A final audience outside the research community is aquaculture educators. Their requirement is for readily accessible tools that students can use in class to enhance their understanding of the biological processes controlling aquaculture ponds, as well as to complete specific design tasks related to facility management.

ANTICIPATED BENEFITS

This study is expected to result in the following benefits:

- Enhanced ability to perform sophisticated economic analyses of production facilities;
- Development of feed optimization strategies for pond management;
- Improved student understanding of pond dynamics and production management principles;
- Enhanced capability to utilize POND© in applied pond management and facility planning; and
- Enhanced decision support tools for facility design and consulting.

LITERATURE CITED

Bolte, J.P., S.S. Nath, and D.E. Ernst, 2000. Development of decision support tools for aquaculture: The POND© experience. Aquacult. Eng., 23:103–109.

Ernst, D.E., J.P. Bolte, and S.S. Nath, 2000. AquaFarm©: Simulation and decision support for aquaculture facility design and management planning. Aquacult. Eng., 23:121–179.

Lannan, J.E., 1993. User's Guide to PONDCLASS: Guidelines for Fertilizing Aquaculture Ponds. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, 60 pp.

Nath, S.S., 1996. Development of a Decision Support System for Pond Aquaculture. Ph.D thesis, Department of Bioresource Engineering, Oregon State University, Corvallis, Oregon, 273 pp.

Cite as: [Author(s), 2001. Title.] In: A. Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), Eighteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]