Pond Dynamics/Aquaculture Collaborative Research Support Program

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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 2000 to 31 July 2001.

The corresponding work plans for reports included appear in the CRSPs Ninth Work Plan and addenda. 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.

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POND SOIL CHARACTERISTICS AND DYNAMICS OF SOIL ORGANIC MATTER AND NUTRIENTS

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

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ABSTRACT

Soil cores were taken from ponds in Thailand, Honduras, Kenya, Peru, Brazil, and the Philippines. All cores could be delineated into F, S, M, T, and P horizons without difficulty. The major similarities among cores involved an increase in dry bulk density and a decrease in concentrations of total carbon, total nitrogen, and total sulfur with increasing depth into the sediment. There was wide variation in acidity, phosphorus, major cation and micronutrient concentrations, pH, texture, and color in samples from different sites, but differences in these variables with sediment depth did not follow clear trends. The S horizon, the main sediment layer that interacts with pond water quality, varied in thickness from 2 to 10 cm. Pond management should focus on neutralizing acidity and enhancing organic matter decomposition within the S horizon through liming and drying of pond bottoms between crops. Sediment accumulates in deeper parts of ponds over time, and soft sediment should be removed periodically.

Results of sediment analyses were used in formulating a pond soil classification system based on levels of primary and secondary sediment properties. The primary properties are pH, texture, sediment thickness, and organic matter status (mineral or organic in nature). The secondary properties are organic carbon, carbon:nitrogen ratio, acidity (exchangeable acidity and acidity from sulfide oxidation), carbonates, and sodium adsorption ratio. Two optional, tertiary properties, thickness of F horizon and oxidation status of the sediment surface, may be used in the classification system if desired. The classification system is described in this report.

INTRODUCTION

This project on pond dynamics was concerned with the composition of sediment profiles in ponds at PD/A CRSP host country sites and in other selected ponds in the host countries. During the Eighth and Ninth Work Plans, soil cores were obtained from ponds in Thailand, Honduras, Kenya, Peru, Brazil, and the Philippines. Data from all sampling sites other than those in Brazil and the Philippines have been presented and discussed in earlier progress reports (Boyd et al., 1998; 1999; 2000; 2001). Thus, only data from the sites in Brazil and the Philippines will be provided in this report. The data obtained on the composition of pond sediment were used to prepare a simple system for classifying pond soils, and the main objective of this report is to describe this system.

METHODS AND MATERIALS

Pond Soil Cores

The ponds in Brazil were located at the fish culture station of the Brazilian Environmental Institute, Pirassununga, São Paulo State, and on a private fish farm near Chapeco, Santa Catarina State. Ponds in the Philippines were at Central Luzon State University, Muñoz, Philippines. Three ponds were selected at each of the sites. Ponds had surface areas of 300 to 500 m^2, and maximum water depth was about 1 m. Ponds had been stocked for two to four months at the time of sampling, and tilapia was the primary culture species in all ponds. Ponds at Pirassununga, Brazil, and in the Philippines had a history of fertilization and feed application. The ponds at Chapeco, Brazil, were fertilized by manure from pig parlors constructed above them.

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 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), wet soil pH (direct, glass electrode), 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 phosphorus (perchloric acid oxidation), total sulfur (Leco Sulfur Analyzer), and 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. Particle size analyses were made by the pipette method. All methods followed details provided by Munsiri et al. (1995) and Thunjai and Boyd (2001).

Pond Soil Classification

The pond soil classification system was developed using data from the literature on pond soils (Boyd, 1995) and the results of the pond soil samples taken during the Eighth and Ninth Work Plans (Boyd et al., 1998; 1999; 2000; 2001). This effort represents the opinions of the investigators, and the system is likely to be revised in the future.

Table 1. Concentrations of moisture, dry bulk density, pH, and exchangeable acidity in soil cores from ponds at Pirassununga and Chapeco, Brazil. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Moisture (%)	Dry Bulk Density (g cm^{-3})	pH (s.u.)	Exchangeable Acidity (meq (100 g)$^{-1}$)
PIRASSUNUNGA				
0–2	518.85 ± 40.37	0.14 ± 0.02	4.85 ± 0.09	21.33 ± 1.87
2–4	376.52 ± 11.00	0.19 ± 0.01	4.90 ± 0.06	17.33 ± 2.82
4–6	307.26 ± 21.90	0.24 ± 0.04	4.70 ± 0.12	20.27 ± 1.62
6–8	240.65 ± 38.58	0.32 ± 0.08	4.77 ± 0.12	22.40 ± 0.80
8–10	252.14 ± 51.63	0.31 ± 0.09	4.73 ± 0.12	17.87 ± 2.82
10–12	240.08 ± 46.68	0.33 ± 0.09	4.77 ± 0.12	16.27 ± 2.71
12–14	210.96 ± 46.62	0.35 ± 0.10	4.88 ± 0.12	16.00 ± 5.00
14–16	208.29 ± 44.79	0.36 ± 0.09	4.92 ± 0.10	13.07 ± 2.37
16–18	184.33 ± 23.18	0.38 ± 0.06	5.28 ± 0.33	16.00 ± 6.67
18–20	148.90 ± 20.66	0.47 ± 0.12	5.10 ± 0.28	11.73 ± 0.71
20–22	118.47 ± 37.58	0.65 ± 0.37	5.07 ± 0.12	10.93 ± 0.96
22–24	103.55 ± 41.24	0.85 ± 0.67	5.02 ± 0.12	10.40 ± 1.22
24–26	90.56 ± 31.57	0.92 ± 0.63	5.08 ± 0.09	9.87 ± 1.87
26–28	66.29 ± 31.33	1.02 ± 0.45	5.05 ± 0.10	9.87 ± 1.62
28–30	56.82 ± 23.22	1.13 ± 0.40	5.07 ± 0.18	12.53 ± 3.53
30–32	38.99 ± 8.97	1.37 ± 0.36	5.03 ± 0.09	16.00 ± 6.84
32–34	43.02 ± 0.00	1.17 ± 0.00	5.30 ± 0.00	10.40 ± 0.00
34–36	37.33 ± 0.00	1.39 ± 0.00	5.10 ± 0.00	11.20 ± 0.00
CHAPECO				
0–2	339.11 ± 14.82	0.24 ± 0.02	5.88 ± 0.28	12.27 ± 2.81
2–4	248.31 ± 8.37	0.30 ± 0.02	6.03 ± 0.07	13.23 ± 3.79
4–6	199.78 ± 11.52	0.37 ± 0.04	5.92 ± 0.06	11.09 ± 3.21
6–8	176.37 ± 13.69	0.39 ± 0.03	5.87 ± 0.06	10.40 ± 2.12
8–10	137.02 ± 30.23	0.53 ± 0.13	5.90 ± 0.09	10.93 ± 1.85
10–12	123.58 ± 28.39	0.60 ± 0.15	5.85 ± 0.08	11.73 ± 1.22
12–14	128.56 ± 19.75	0.54 ± 0.09	5.82 ± 0.04	12.00 ± 2.88
14–16	118.60 ± 16.36	0.59 ± 0.10	5.90 ± 0.08	10.93 ± 1.22
16–18	101.55 ± 9.53	0.64 ± 0.07	5.85 ± 0.08	11.20 ± 1.39
18–20	92.87 ± 7.09	0.67 ± 0.05	5.75 ± 0.10	10.93 ± 1.85
20–22	86.93 ± 8.94	0.70 ± 0.06	5.75 ± 0.10	13.87 ± 3.23
22–24	85.17 ± 7.02	0.77 ± 0.03	5.70 ± 0.10	13.60 ± 3.67
24–26	83.00 ± 6.75	0.73 ± 0.08	5.63 ± 0.11	14.40 ± 2.77
26–28	85.29 ± 8.26	0.77 ± 0.05	5.58 ± 0.06	13.07 ± 2.01
28–30	82.45 ± 5.49	0.75 ± 0.05	5.68 ± 0.04	13.07 ± 1.22
30–32	80.77 ± 7.02	0.78 ± 0.03	5.60 ± 0.10	13.33 ± 0.44
32–34	79.32 ± 8.67	0.72 ± 0.05	5.77 ± 0.18	12.05 ± 4.17
34–36	78.63 ± 1.64	0.74 ± 0.04	5.60 ± 0.04	13.07 ± 3.23
36–38	76.04 ± 3.01	0.82 ± 0.09	5.53 ± 0.02	14.00 ± 3.96
38–40	64.96 ± 7.46	0.89 ± 0.07	5.58 ± 0.02	12.80 ± 3.39
40–42	58.07 ± 5.83	0.98 ± 0.11	6.00 ± 0.00	8.00 ± 0.00

RESULTS

The data for the analyses of soil cores from ponds in Brazil and the Philippines are summarized in Tables 1 to 14. Each entry in the tables represents the average concentration of a variable in three ponds, and the plus or minus (±) values following means represent standard deviations.

DISCUSSION

Pond Soil Cores

Chemical and physical analyses of soil core segments from Brazil and the Philippines did not reveal features or trends greatly different from those found during the Eighth and Ninth Work Plans for ponds at other sites (Boyd et al., 1998; 1999; 2000; 2001). In general, moisture content decreased and dry bulk density increased with increasing depth in the profile (Tables 1 and 2). The S, M, T, and P horizons could be easily delineated from dry bulk density, as has been the case in all ponds previously sampled. The pH and exchangeable acidity did not differ greatly among depths. The two sites in Brazil had acidic sediment, while sediment at the Philippine site was near neutral in reaction (Tables 1 and 2).

Soil color changed with depth from a lighter color near the surface to a darker color deeper within the profile at the Pirassununga site in Brazil, but soil color changed little with depth at the other two sites (Tables 3 and 4). Color is useful for determining if sediment is oxidized at the surface of the S horizon, but it is of little use in delineating horizons or for other aspects of pond soil classification.

Total carbon, total nitrogen, and total sulfur concentrations decreased with depth (Tables 5 and 6), as was observed in all other ponds of this study (Boyd et al., 1998; 1999; 2000; 2001). Concentrations of carbon in S and M horizons were about 2 to 3% in most samples. Total sulfur values were consistently below 0.1%. Phosphorus concentrations did not differ greatly with sediment depth (Tables 5 and 6).

There were no consistent trends of change in concentrations of major cations with respect to sediment depth (Tables 7 and 8).

Table 2. Concentrations of moisture, dry bulk density, pH, and exchangeable acidity in soil cores from ponds at Muñoz, Philippines. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Moisture (%)	Dry Bulk Density (g cm^{-3})	pH (s.u.)	Exchangeable Acidity (meq (100 g)$^{-1}$)
0–2	215.38 ± 3.82	0.37 ± 0.01	7.17 ± 0.22	5.60 ± 1.39
2–4	92.06 ± 8.06	0.68 ± 0.03	7.20 ± 0.20	6.67 ± 1.16
4–6	77.26 ± 4.81	0.76 ± 0.03	7.27 ± 0.18	7.20 ± 1.39
6–8	73.17 ± 2.49	0.77 ± 0.01	7.33 ± 0.15	7.20 ± 1.22
8–10	71.12 ± 2.04	0.83 ± 0.03	7.32 ± 0.13	5.60 ± 0.46
10–12	55.74 ± 7.16	0.90 ± 0.05	7.27 ± 0.09	6.40 ± 0.92
12–14	56.78 ± 2.86	0.94 ± 0.04	7.20 ± 0.10	6.93 ± 1.16
14–16	53.27 ± 1.27	0.94 ± 0.03	7.22 ± 0.14	7.47 ± 1.16
16–18	50.70 ± 1.22	0.95 ± 0.04	7.20 ± 0.13	6.67 ± 0.96
18–20	49.45 ± 1.95	0.96 ± 0.05	7.23 ± 0.07	5.87 ± 0.96
20–22	48.74 ± 1.68	1.02 ± 0.03	7.25 ± 0.03	5.87 ± 0.96
22–24	45.33 ± 2.31	1.00 ± 0.05	7.28 ± 0.04	5.33 ± 0.71

Table 3. Profile for color in soil cores from ponds at Pirassununga and Chapeco, Brazil. Color values are given as standard Munsell Color Chart Units.

Depth (cm)	Pirassununga			Chapeco		
	Pond A59	Pond D20	Pond A61	Pond 1	Pond 2	Pond 3
0–2	10YR 4/3	10YR 4/4	1G 2.5/10Y	10YR 3/6	10YR 3/3	10YR 3/3
2–4	10YR 4/2	1G 4/10GY	1G 2.5/10Y	10YR 3/3	10YR 3/4	10YR 3/3
4–6	10YR 3/2	1G 3/10GY	1G 3/10Y	10YR 3/3	10YR 3/3	10YR 3/2
6–8	10YR 3/2	1G 3/10GY	1G 3/10Y	10YR 2/2	10YR 3/4	10YR 3/2
8–10	10YR 4/1	1G 4/5G	1G 3/10Y	10YR 3/2	10YR 2/2	10YR 3/1
10–12	10YR 4/1	1G 4/5G	1G 3/10Y	10YR 3/2	10YR 3/2	10YR 3/2
12–14	10YR 4/1	1G 4/5G	1G 3/10Y	10YR 2/2	10YR 3/3	10YR 3/2
14–16	10YR 4/1	1G 3/10GY	1G 3/10Y	10YR 3/2	10YR 2/2	10YR 3/2
16–18	10YR 4/1	1G 3/10GY	1G 3/10Y	10YR 3/2	10YR 3/2	10YR 3/2
18–20	10YR 4/1	1G 3/10GY	1G 3/5GY	10YR 3/1	10YR 3/2	10YR 3/3
20–22	7.5YR 5/1	1G 2.5/5GY	1G 3/5GY	10YR 2/2	10YR 3/2	10YR 3/2
22–24	2.5YR 5/1	1G 4/10Y	1G 3/5GY	10YR 2/2	10YR 3/2	10YR 3/2
24–26	2.5Y 5/1	1G 5/10Y	1G 3/5GY	10YR 3/2	10YR 3/1	10YR 3/2
26–28	1G 5/N	7.5YR 4/4	1G 4/5GY	10YR 3/2	10YR 2/2	10YR 3/2
28–30	1G 4/N		1G 4/5GY	10YR 3/2	10YR 3/2	10YR 3/2
30–32	1G 4/5GY		1G 3/5GY	10YR 3/2	10YR 3/2	10YR 2/2
32–34			1G 3/10GY	10YR 3/2	10YR 3/2	10YR 3/2
34–36			1G 4/N		10YR 3/2	10YR 3/2
36–38					10YR 3/2	10YR 3/2
38–40					10YR 3/2	10YR 3/2
40–42					10YR 3/2	10YR 3/2

10YR 4/3—brown; 10YR 4/2—dark grayish brown; 10YR 4/1—dark gray; 7.5YR 5/1—gray; 2.5YR— reddish gray; 2.5Y 5/1—gray; 1G 5/N—gray; 1G 4/N—dark gray; 1G 4/5GY—dark greenish gray; 10YR 4/4—dark yellowish brown; 1G 4/10GY—dark greenish gray; 1G 3/10GY—dark greenish gray; 1G 4/5G—dark greenish gray; 1G 3/10GR—; 1G 2.5/5GY—greenish black; 1G 4/10Y—dark greenish gray; 1G 5/10Y—greenish gray; 7.5YR 4/4—brown; 1G 2.5/10Y—greenish black; 1G 3/10Y—dark greenish gray; 1G 3/5GY—dark greenish gray; 1G 4/5GY—dark greenish gray; 1G 3/10GY—dark greenish gray; 10YR 3/6— dark yellowish brown; 10YR 3/3—dark brown; 10YR 2/2—very dark brown; 10YR 3/2—very dark grayish brown; 10YR 3/1—very dark gray; 10YR 3/4—dark yellowish brown

However, concentrations of major cations were higher in samples from the Philippines site than in those from the two sites in Brazil. Concentrations of minor nutrients did not have consistent patterns of change with sediment depth (Tables 9 and 10), but concentrations were within the ranges of those found in other ponds sampled during this study (Boyd et al., 1998; 1999; 2000; 2001).

Soil particle size analysis revealed a high percentage of clay in sediment from all sites (Tables 11 and 12). All samples had clay or silty clay as texture names (Tables 13 and 14).

Pond Bottom Soil Classification

A systematic procedure for classifying terrestrial soil, known as soil taxonomy, is widely used by soil scientists (Soil Survey Staff, 1994). Soil taxonomy uses data on soil physical and chemical properties, but it also relies heavily on the appearance of the soil profile. Terrestrial soil profiles develop over many centuries and consist of several well-defined layers known as horizons. The presence or absence of specific horizons, the thickness of individual horizons, and the appearance and characteristics of soil in different horizons are major factors in soil taxonomy.

When aquaculture ponds are constructed, one or more of the upper horizons of the terrestrial soil is usually removed, and subsoil is exposed to water when ponds are filled. The main requirement is that soil for ponds consist of a mixture of particle sizes and contain enough fine, clay-size particles to resist seepage. Some references suggest that the soil should contain at least 20 to 30% clay, but soils with only 5 to 10% clay content can be used to construct ponds. However, as soon as a pond is constructed, the characteristics of the pond bottom begin to change. These changes result from erosion, sorting, and sedimentation of particles from within or from outside the pond and from the accumulation of organic matter. Pond soil classification should be based primarily on the sediment that accumulates in pond bottoms and is in direct contact with the pond water rather than upon the original soil from which the pond was constructed.

Table 4. Profile for color in soil cores from ponds at Muñoz, Philippines. Color values are given as standard Munsell Color Chart Units.

Depth (cm)	Pond 6B	Pond 6G	Pond 5F
0–2	2.5Y 4/2	2.5Y 4/2	2.5Y 4/1
2–4	2.5Y 4/2	2.5Y 4/2	2.5Y 4/1
4–6	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
6–8	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
8–10	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
10–12	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
12–14	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
14–16	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
16–18	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
18–20	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
20–22	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1
22–24	2.5Y 5/3	2.5Y 4/2	2.5Y 4/1

2.5Y 4/2—dark grayish brown; 2.5Y 5/3—light olive brown; 2.5Y 4/1—dark gray

Initial efforts at describing sediment cores revealed that several layers (horizons) were easily defined in ponds at Auburn

Table 5. Concentrations of total carbon, total nitrogen, total sulfur, acid-extractable phosphorus, and water-soluble phosphorus in soil cores from ponds at Pirassununga and Chapeco, Brazil. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	**Total Carbon** (%)	**Total Nitrogen** (%)	**Total Sulfur** (%)	**Acid–extractable Phosphorus** (ppm)	**Water–soluble Phosphorus** (ppm)
PIRASSUNUNGA					
0–2	3.51 ± 0.04	0.08 ± 0.01	0.04 ± 0.01	13.47 ± 0.58	0.05 ± 0.01
2–4	3.33 ± 0.05	0.08 ± 0.01	0.04 ± 0.02	14.27 ± 0.35	0.07 ± 0.01
4–6	3.13 ± 0.04	0.07 ± 0.01	0.05 ± 0.01	15.20 ± 0.46	0.06 ± 0.02
6–8	3.02 ± 0.05	0.06 ± 0.01	0.04 ± 0.01	14.80 ± 1.51	0.07 ± 0.03
8–10	2.89 ± 0.05	0.06 ± 0.01	0.04 ± 0.01	14.93 ± 0.96	0.07 ± 0.01
10–12	2.73 ± 0.06	0.06 ± 0.01	0.02 ± 0.02	15.87 ± 0.87	0.07 ± 0.01
12–14	2.28 ± 0.08	0.05 ± 0.01	0.02 ± 0.02	16.67 ± 1.09	0.08 ± 0.01
14–16	2.49 ± 0.08	0.05 ± 0.01	0.03 ± 0.02	17.07 ± 1.96	0.10 ± 0.02
16–18	2.15 ± 0.06	0.04 ± 0.01	0.02 ± 0.02	13.87 ± 1.96	0.09 ± 0.02
18–20	1.51 ± 0.04	0.03 ± 0.01	0.02 ± 0.02	17.60 ± 3.21	0.09 ± 0.04
20–22	1.35 ± 0.06	0.03 ± 0.01	0.01 ± 0.01	14.27 ± 2.87	0.09 ± 0.04
22–24	1.31 ± 0.06	0.03 ± 0.01	0.02 ± 0.02	16.93 ± 4.35	0.09 ± 0.04
24–26	1.34 ± 0.07	0.03 ± 0.01	0.01 ± 0.01	14.53 ± 2.47	0.06 ± 0.03
26–28	1.12 ± 0.06	0.03 ± 0.01	0.03 ± 0.02	13.20 ± 4.55	0.07 ± 0.04
28–30	1.21 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	13.07 ± 2.77	0.05 ± 0.02
30–32	1.19 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	12.67 ± 2.08	0.06 ± 0.02
32–34	1.38 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	12.80 ± 0.00	0.09 ± 0.00
34–36	1.11 ± 0.00	0.02 ± 0.00	0.07 ± 0.00	12.40 ± 0.00	0.10 ± 0.00
CHAPECO					
0–2	3.71 ± 0.32	0.09 ± 0.02	0.05 ± 0.02	14.93 ± 0.58	0.32 ± 0.04
2–4	3.46 ± 0.20	0.08 ± 0.01	0.06 ± 0.02	15.60 ± 0.83	0.32 ± 0.05
4–6	3.22 ± 0.15	0.07 ± 0.01	0.09 ± 0.01	13.87 ± 0.48	0.32 ± 0.05
6–8	3.07 ± 0.09	0.07 ± 0.01	0.06 ± 0.01	12.40 ± 2.84	0.27 ± 0.02
8–10	2.74 ± 0.30	0.06 ± 0.01	0.07 ± 0.01	15.60 ± 0.83	0.37 ± 0.05
10–12	2.73 ± 0.37	0.06 ± 0.01	0.07 ± 0.01	15.07 ± 1.50	0.33 ± 0.07
12–14	2.68 ± 0.30	0.06 ± 0.01	0.05 ± 0.01	15.33 ± 2.31	0.29 ± 0.04
14–16	2.42 ± 0.28	0.05 ± 0.01	0.05 ± 0.02	15.07 ± 0.87	0.30 ± 0.05
16–18	3.11 ± 0.67	0.06 ± 0.02	0.04 ± 0.01	15.60 ± 1.85	0.24 ± 0.04
18–20	3.00 ± 0.63	0.07 ± 0.01	0.05 ± 0.01	15.33 ± 0.35	0.30 ± 0.14
20–22	2.81 ± 0.66	0.06 ± 0.02	0.04 ± 0.01	14.13 ± 0.35	0.21 ± 0.03
22–24	2.90 ± 0.71	0.06 ± 0.02	0.03 ± 0.02	15.47 ± 1.27	0.23 ± 0.04
24–26	2.74 ± 0.60	0.06 ± 0.01	0.05 ± 0.01	17.60 ± 1.67	0.21 ± 0.02
26–28	2.79 ± 0.60	0.06 ± 0.01	0.04 ± 0.01	15.20 ± 1.40	0.21 ± 0.02
28–30	2.77 ± 0.64	0.06 ± 0.01	0.03 ± 0.01	14.00 ± 1.40	0.19 ± 0.02
30–32	2.69 ± 0.59	0.06 ± 0.02	0.03 ± 0.01	14.93 ± 0.35	0.17 ± 0.03
32–34	2.65 ± 0.64	0.05 ± 0.01	0.03 ± 0.02	15.60 ± 0.61	0.14 ± 0.01
34–36	2.74 ± 0.66	0.05 ± 0.01	0.04 ± 0.01	14.80 ± 1.74	0.14 ± 0.02
36–38	2.33 ± 0.59	0.04 ± 0.01	0.01 ± 0.00	14.60 ± 1.14	0.12 ± 0.01
38–40	2.26 ± 0.25	0.05 ± 0.01	0.04 ± 0.02	12.80 ± 0.33	0.14 ± 0.01
40–42	2.19 ± 0.30	0.05 ± 0.01	0.04 ± 0.02	18.40 ± 0.00	0.12 ± 0.01

Table 6. Concentrations of total carbon, total nitrogen, total sulfur, acid-extractable phosphorus, and water-soluble phosphorus in soil cores from ponds at Muñoz, Philippines. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	**Total Carbon** (%)	**Total Nitrogen** (%)	**Total Sulfur** (%)	**Acid–extractable Phosphorus** (ppm)	**Water–soluble Phosphorus** (ppm)
0–2	2.84 ± 0.36	0.09 ± 0.02	0.09 ± 0.01	15.20 ± 0.40	0.29 ± 0.20
2–4	2.27 ± 0.25	0.07 ± 0.01	0.07 ± 0.00	13.73 ± 2.22	0.24 ± 0.11
4–6	1.90 ± 0.11	0.06 ± 0.01	0.06 ± 0.01	13.07 ± 1.79	0.24 ± 0.07
6–8	1.66 ± 0.10	0.05 ± 0.00	0.04 ± 0.02	16.27 ± 3.53	0.27 ± 0.08
8–10	1.60 ± 0.05	0.05 ± 0.00	0.04 ± 0.01	15.20 ± 4.33	0.29 ± 0.09
10–12	1.31 ± 0.10	0.04 ± 0.00	0.03 ± 0.01	14.53 ± 2.28	0.22 ± 0.06
12–14	1.20 ± 0.11	0.04 ± 0.01	0.04 ± 0.02	14.13 ± 1.62	0.19 ± 0.03
14–16	1.24 ± 0.09	0.04 ± 0.00	0.05 ± 0.01	13.87 ± 1.92	0.21 ± 0.03
16–18	1.20 ± 0.12	0.05 ± 0.01	0.02 ± 0.02	14.13 ± 1.50	0.16 ± 0.03
18–20	1.12 ± 0.09	0.04 ± 0.00	0.04 ± 0.01	15.87 ± 1.33	0.15 ± 0.05
20–22	1.06 ± 0.06	0.03 ± 0.00	0.03 ± 0.01	17.33 ± 0.27	0.18 ± 0.05
22–24	0.94 ± 0.08	0.03 ± 0.00	0.02 ± 0.01	22.27 ± 6.67	0.18 ± 0.09

Table 7. Concentrations of calcium, magnesium, sodium, and potassium in soil cores from ponds at Pirassununga and Chapeco, Brazil. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Potassium (ppm)
PIRASSUNUNGA				
0–2	1,117 ± 262	253 ± 72	20 ± 3	64 ± 6
2–4	1,028 ± 208	251 ± 73	17 ± 3	58 ± 9
4–6	836 ± 152	182 ± 49	15 ± 2	45 ± 5
6–8	891 ± 138	211 ± 43	13 ± 2	49 ± 3
8–10	913 ± 191	217 ± 55	12 ± 1	49 ± 7
10–12	953 ± 202	227 ± 57	13 ± 1	45 ± 7
12–14	927 ± 261	217 ± 72	10 ± 1	41 ± 9
14–16	1,026 ± 293	236 ± 85	10 ± 2	42 ± 11
16–18	1,966 ± 731	303 ± 165	11 ± 1	37 ± 10
18–20	1,476 ± 335	251 ± 111	10 ± 2	33 ± 9
20–22	1,100 ± 321	229 ± 104	8 ± 1	33 ± 10
22–24	1,010 ± 255	219 ± 78	7 ± 1	32 ± 9
24–26	1,013 ± 362	272 ± 80	8 ± 2	32 ± 10
26–28	973 ± 377	226 ± 100	8 ± 1	28 ± 8
28–30	827 ± 241	320 ± 86	10 ± 1	28 ± 12
30–32	799 ± 232	253 ± 47	8 ± 1	27 ± 11
32–34	828 ± 0	441 ± 0	6 ± 0	14 ± 0
34–36	544 ± 0	204 ± 0	7 ± 0	21 ± 0
CHAPECO				
0–2	2,561 ± 198	802 ± 103	17 ± 1	127 ± 12
2–4	2,465 ± 184	808 ± 104	15 ± 2	109 ± 10
4–6	2,328 ± 289	769 ± 142	16 ± 2	98 ± 8
6–8	1,643 ± 539	519 ± 182	10 ± 2	68 ± 12
8–10	1,948 ± 204	599 ± 99	14 ± 2	90 ± 7
10–12	1,894 ± 232	586 ± 114	14 ± 1	95 ± 7
12–14	1,706 ± 126	523 ± 65	14 ± 1	93 ± 8
14–16	1,574 ± 23	494 ± 4	13 ± 2	93 ± 10
16–18	1,559 ± 89	509 ± 22	12 ± 1	93 ± 5
18–20	1,457 ± 46	486 ± 33	12 ± 1	95 ± 7
20–22	1,275 ± 22	413 ± 24	12 ± 1	96 ± 7
22–24	1,087 ± 55	346 ± 14	12 ± 2	97 ± 8
24–26	1,038 ± 96	326 ± 19	11 ± 1	98 ± 5
26–28	993 ± 78	298 ± 14	11 ± 1	99 ± 6
28–30	945 ± 41	269 ± 12	10 ± 1	95 ± 5
30–32	905 ± 34	254 ± 20	13 ± 1	91 ± 6
32–34	864 ± 43	225 ± 16	11 ± 1	86 ± 8
34–36	1,541 ± 740	424 ± 230	14 ± 3	109 ± 33
36–38	813 ± 101	204 ± 6	11 ± 2	80 ± 10
38–40	767 ± 116	190 ± 17	11 ± 4	77 ± 11
40–42	621 ± 0	158 ± 0	11 ± 0	58 ± 0

University. These layers were defined by Munsiri et al. (1995) as follows: F horizon, layer of flocculent material on the pond bottom consisting of recent sediment; S horizon, well-mixed sediment with high moisture content and low bulk density (< 0.3 g cm^{-3}); M horizon, mature, stable, un-mixed sediment with dry bulk density of 0.3 to 0.7 g cm^{-3}; T horizon, transition layer; and P horizon, original pond bottom with bulk density less than 1 g cm^{-3}. Chemical analyses revealed that a major characteristic of the soil profile, at least in ponds constructed on mineral soils, was a decrease in organic carbon with depth. Also, there tended to be greater accumulation of nitrogen and phosphorus in S and M horizons than in T and P horizons.

Data collected during the Eighth and Ninth Work Plans showed that a sediment layer develops in ponds within a period of only two or three years. Suspended solids settling in

Table 8. Concentrations of calcium, magnesium, sodium, and potassium in soil cores from ponds at Muñoz, Philippines. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Potassium (ppm)
0–2	4,435 ± 316	843 ± 69	174 ± 20	88 ± 8
2–4	4,140 ± 343	812 ± 67	158 ± 17	68 ± 5
4–6	4,060 ± 290	817 ± 68	156 ± 13	66 ± 1
6–8	4,173 ± 286	810 ± 71	162 ± 11	64 ± 3
8–10	4,052 ± 233	794 ± 81	164 ± 13	66 ± 3
10–12	4,052 ± 182	800 ± 87	164 ± 15	67 ± 6
12–14	3,886 ± 228	819 ± 88	166 ± 11	68 ± 7
14–16	3,955 ± 280	820 ± 94	159 ± 11	67 ± 6
16–18	3,822 ± 299	822 ± 98	158 ± 10	68 ± 7
18–20	3,850 ± 369	837 ± 103	155 ± 9	66 ± 7
20–22	3,654 ± 334	858 ± 118	155 ± 13	71 ± 6
22–24	3,691 ± 441	874 ± 131	153 ± 11	64 ± 8

ponds were derived from erosion on pond watersheds; erosion of pond bottoms and sides by wave action and water currents; and organic matter from uneaten fish feed, organic fertilizer, and dead plankton. The sediment contained layers (horizons), but horizons were not always as well-defined as those described for ponds at Auburn University (Munsiri et al., 1995). The F, S, M, T, and P horizons could be discerned visually, and dry bulk density tended to differ among ponds. Pond sediment tended to be a non-structured, fluid mixture of particles that increased in bulk density and decreased in organic matter with increasing depth. Although the horizons described by Munsiri et al. (1995) could be easily identified, the appearance of these horizons was similar among ponds, and the main visual difference in sediment among ponds was sediment thickness.

Soil taxonomy relies on both visual differences and measurable differences in chemical and physical characteristics among soil profiles. Unfortunately, visual differences among pond soil profiles were limited. Analyses of pond soils by Boyd et al. (1994) and pond soil cores from ponds at PD/A CRSP sites during the present study revealed large differences in chemical and physical characteristics among ponds. However, aquaculture was observed to be successful over a wide range of soil characteristics. Only a few soil properties are known to influence aquacultural production (Banerjea and Ghosh, 1967; Boyd, 1995), and pond soil classification should be based on these properties. Thus, we abandoned our initial plan to apply a modified system of soil taxonomy to aquaculture pond soil classification in favor of a simpler classification system.

We prepared a classification system based on the following soil properties: texture; sediment thickness; organic matter content; carbon:nitrogen (C/N) ratio; pH and acidity; amount of sulfur; amount of carbonate; and sodium content. The properties will be considered as either primary or secondary ones. The primary properties will be texture, sediment thickness, organic matter, and pH, and the other variables will be secondary properties. The condition of the flocculent layer (F horizon) and the surface of the sediment also can be included in the classification system as tertiary properties if desired.

Texture

Aquaculture ponds usually are located in mineral soils that contain less than 5% organic carbon. A few aquaculture ponds

Table 9. Concentrations of iron, manganese, zinc, and copper in soil cores from ponds at Pirassununga and Chapeco, Brazil. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)
PIRASSUNUNGA				
0–2	795 ± 11	253 ± 66	15 ± 1	18 ± 7
2–4	743 ± 71	251 ± 39	19 ± 2	19 ± 7
4–6	981 ± 69	182 ± 46	16 ± 1	20 ± 10
6–8	933 ± 58	211 ± 41	16 ± 1	23 ± 9
8–10	941 ± 28	217 ± 39	16 ± 1	26 ± 11
10–12	933 ± 31	227 ± 41	16 ± 1	24 ± 11
12–14	959 ± 41	217 ± 44	13 ± 2	22 ± 11
14–16	935 ± 54	236 ± 47	14 ± 3	23 ± 12
16–18	689 ± 193	303 ± 36	12 ± 2	21 ± 9
18–20	845 ± 138	251 ± 32	11 ± 2	18 ± 8
20–22	868 ± 138	229 ± 29	9 ± 3	18 ± 6
22–24	855 ± 115	219 ± 26	8 ± 3	16 ± 5
24–26	781 ± 143	272 ± 26	7 ± 3	14 ± 4
26–28	799 ± 202	226 ± 29	7 ± 3	12 ± 3
28–30	819 ± 61	320 ± 39	8 ± 3	20 ± 12
30–32	850 ± 69	253 ± 39	7 ± 2	18 ± 10
32–34	817 ± 0	441 ± 0	6 ± 0	7 ± 0
34–36	1,022 ± 0	204 ± 0	5 ± 0	11 ± 0
CHAPECO				
0–2	109 ± 49	802 ± 28	41 ± 3	12 ± 4
2–4	99 ± 31	808 ± 51	44 ± 4	13 ± 3
4–6	173 ± 86	769 ± 67	46 ± 2	17 ± 7
6–8	184 ± 29	519 ± 100	35 ± 11	16 ± 3
8–10	307 ± 56	599 ± 44	44 ± 5	26 ± 3
10–12	377 ± 102	586 ± 42	45 ± 6	27 ± 5
12–14	410 ± 95	523 ± 39	42 ± 5	30 ± 3
14–16	454 ± 36	494 ± 28	37 ± 4	31 ± 1
16–18	467 ± 85	509 ± 31	34 ± 1	29 ± 1
18–20	442 ± 15	486 ± 44	31 ± 2	32 ± 2
20–22	556 ± 71	413 ± 64	27 ± 3	36 ± 1
22–24	537 ± 49	346 ± 42	28 ± 6	38 ± 1
24–26	614 ± 52	326 ± 27	27 ± 4	39 ± 2
26–28	562 ± 72	298 ± 42	24 ± 2	40 ± 1
28–30	611 ± 20	269 ± 50	21 ± 5	40 ± 2
30–32	755 ± 81	254 ± 55	21 ± 6	40 ± 2
32–34	709 ± 54	225 ± 78	14 ± 3	39 ± 3
34–36	580 ± 277	424 ± 52	21 ± 10	29 ± 11
36–38	812 ± 23	204 ± 57	10 ± 1	40 ± 1
38–40	817 ± 72	190 ± 30	9 ± 1	37 ± 1
40–42	784 ± 0	158 ± 0	6 ± 0	44 ± 0

are built in organic soils, but such soils are not well suited for aquaculture. In mineral soils, texture is important because light texture (sandy or loamy) facilitates the exchange of dissolved oxygen and other substances between water and sediment. Also, when ponds are drained and their bottoms dried between crops, light-textured soils dry quicker and aerate better than heavier-textured soils. The system for assigning texture of terrestrial soils using a soil triangle provides 12 soil texture classes. Most of the soil texture classes given for terrestrial soils are suitable for pond aquaculture, so such a complex system is not needed. The following five soil texture classes should be enough for aquaculture pond sediment classification:

Clay	> 60% clay
Clayey	30 to 60% clay
Loamy	< 30% clay; < 60% sand
Sandy	< 10% clay; > 60% sand
Sand	> 90% sand

Table 10. Concentrations of iron, manganese, zinc, and copper in soil cores from ponds at Muñoz, Philippines. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)
0–2	44 ± 12	286 ± 103	3.5 ± 1.6	1.6 ± 0.5
2–4	46 ± 12	353 ± 100	4.3 ± 1.8	1.9 ± 0.9
4–6	39 ± 10	335 ± 77	4.5 ± 1.1	2.4 ± 0.9
6–8	36 ± 8	319 ± 62	5.2 ± 1.2	1.9 ± 0.5
8–10	34 ± 8	292 ± 45	5.3 ± 0.9	2.0 ± 0.4
10–12	35 ± 9	312 ± 34	5.3 ± 0.6	2.7 ± 0.1
12–14	41 ± 11	399 ± 67	5.6 ± 0.6	3.7 ± 0.5
14–16	40 ± 7	379 ± 56	5.7 ± 0.6	3.3 ± 0.9
16–18	63 ± 14	513 ± 169	5.6 ± 0.8	4.0 ± 0.7
18–20	106 ± 45	588 ± 219	5.2 ± 1.1	2.8 ± 0.0
20–22	126 ± 84	526 ± 156	5.3 ± 0.9	4.4 ± 0.4
22–24	213 ± 157	585 ± 113	5.1 ± 1.0	3.6 ± 0.4

Sediment Thickness

Sediment thickness is a critical variable in pond management because accumulation of soft sediment makes ponds shallow, encourages anaerobic conditions at the sediment-water interface, lengthens the fallow period for bottom dry-out, traps sinking feed pellets, and interferes with harvest (Boyd, 1995). Suggested categories for sediment thickness are as follows:

Thin	< 5 cm
Moderately thick	5 to 10 cm
Thick	11 to 25 cm
Very thick	> 25 cm

Pond bottoms quickly become covered with sediment, so the term sediment will be used instead of soil in the classification system.

Organic Matter

Organic matter is a minor component of mineral soils, but it is a major component of organic soils. Pond soils containing more than 15% organic carbon will be considered organic soils. Organic soils will be separated into three groups relative to organic carbon content as follows:

Low	15 to 25% organic carbon
Medium	26 to 40% organic carbon
High	> 40% organic carbon

Most aquaculture ponds are constructed in mineral soil of low organic matter content, but as ponds age, organic matter accumulates. Pond sediments can have too little organic matter for optimum growth of benthic organisms, or they may contain so much organic matter that anaerobic conditions frequently develop in the pond bottom (Boyd, 1995). Pond sediment usually contains between 0.5 and 5% organic carbon, and the optimum concentration is considered to be 1 to 3%. Thus, the following categories are suggested for organic carbon in sediment of ponds constructed on mineral soil:

Low	< 1% organic carbon
Moderate	1 to 3% organic carbon
High	> 3% organic carbon

Table 11. Profile for particle sizes in soil cores from ponds at Pirassununga and Chapeco, Brazil. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Pirassununga			Chapeco		
	Sand	*Silt*	*Clay*	*Sand*	*Silt*	*Clay*
0–2	11.25 ± 3.70	38.27 ± 3.99	50.48 ± 1.56	16.93 ± 4.02	42.49 ± 5.82	40.58 ± 2.01
2–4	14.94 ± 4.42	35.43 ± 5.88	49.63 ± 1.76	14.07 ± 3.47	44.56 ± 0.80	41.36 ± 3.09
4–6	20.82 ± 0.52	33.65 ± 6.58	45.53 ± 6.54	16.46 ± 3.73	45.52 ± 1.17	38.03 ± 2.56
6–8	20.71 ± 0.46	33.60 ± 5.55	45.69 ± 5.70	16.98 ± 2.74	41.63 ± 1.53	41.39 ± 2.10
8–10	19.74 ± 3.53	37.57 ± 8.02	42.69 ± 5.70	18.97 ± 4.59	35.84 ± 4.40	45.19 ± 2.93
10–12	18.22 ± 1.97	36.61 ± 6.74	45.17 ± 5.66	15.31 ± 4.33	42.42 ± 2.03	42.27 ± 2.33
12–14	21.56 ± 3.60	36.13 ± 3.65	42.31 ± 6.93	11.50 ± 3.53	45.58 ± 2.20	42.92 ± 1.96
14–16	22.79 ± 2.90	32.87 ± 5.79	44.34 ± 4.83	10.16 ± 2.60	46.48 ± 3.12	43.36 ± 0.78
16–18	23.47 ± 6.68	36.07 ± 9.09	40.45 ± 8.00	9.96 ± 3.64	44.48 ± 1.85	45.56 ± 1.84
18–20	27.95 ± 6.73	30.91 ± 8.10	41.14 ± 1.86	9.19 ± 2.39	44.07 ± 2.67	46.73 ± 0.43
20–22	37.70 ± 15.01	25.88 ± 11.05	36.41 ± 6.22	8.62 ± 1.84	45.32 ± 2.97	46.05 ± 1.17
22–24	39.10 ± 14.21	24.65 ± 10.73	36.25 ± 4.72	4.79 ± 1.19	46.91 ± 2.18	48.31 ± 2.25
24–26	41.87 ± 12.31	22.01 ± 8.53	36.12 ± 5.79	3.87 ± 0.92	45.55 ± 0.63	50.57 ± 1.22
26–28	46.75 ± 8.28	18.35 ± 5.40	34.89 ± 4.19	3.78 ± 0.79	47.50 ± 2.42	48.72 ± 3.00
28–30	41.15 ± 11.23	21.64 ± 8.02	37.21 ± 3.68	5.96 ± 2.45	47.86 ± 1.86	46.19 ± 4.25
30–32	47.99 ± 12.19	19.04 ± 7.53	32.97 ± 4.68	5.71 ± 1.11	45.30 ± 2.41	48.99 ± 1.30
32–34	59.79 ± 0.00	11.06 ± 0.00	29.16 ± 0.00	6.90 ± 1.48	42.96 ± 1.48	50.15 ± 1.12
34–36	41.31 ± 0.00	20.29 ± 0.00	38.40 ± 0.00	7.48 ± 3.71	42.25 ± 3.22	50.28 ± 0.49
36–38				7.86 ± 2.24	45.14 ± 3.65	47.00 ± 1.40
38–40				11.07 ± 1.34	45.11 ± 2.21	43.82 ± 0.87
40–42				12.55 ± 0.44	44.51 ± 1.05	42.94 ± 1.49

Table 12. Profile for particle sizes in soil cores from ponds at Muñoz, Philippines. Entries are the averages and standard errors of data from three ponds.

Depth (cm)	Sand	Silt	Clay
0–2	13.20 ± 3.86	41.48 ± 1.39	45.32 ± 4.14
2–4	11.46 ± 3.75	42.50 ± 2.85	46.04 ± 2.66
4–6	10.59 ± 3.59	41.67 ± 3.14	47.73 ± 3.80
6–8	8.89 ± 3.27	46.99 ± 2.17	44.12 ± 4.22
8–10	9.43 ± 2.97	42.49 ± 2.36	48.08 ± 3.10
10–12	9.13 ± 2.88	42.48 ± 2.33	48.39 ± 2.69
12–14	9.95 ± 2.46	41.59 ± 1.96	48.45 ± 2.52
14–16	9.42 ± 2.25	42.08 ± 1.79	48.51 ± 2.69
16–18	9.01 ± 1.94	41.11 ± 1.85	49.88 ± 2.87
18–20	11.41 ± 2.32	41.83 ± 3.24	46.76 ± 4.87
20–22	13.44 ± 4.69	37.82 ± 0.28	48.75 ± 4.42
22–24	16.33 ± 7.42	36.74 ± 0.38	46.93 ± 7.10

Table 13. Profile for soil texture in soil cores from ponds at Pirassununga and Chapeco, Brazil.

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

C/N Ratio

The C/N ratio can vary greatly in aquaculture pond soils, but in most ponds the values will fall between 8 and 15. Ponds with low C/N ratios tend to have highly decomposable organic matter, and anaerobic conditions at the sediment-water interface may be a common problem. At high C/N ratios, organic matter will decompose very slowly because of nitrogen deficiency. Three categories are suggested for C/N ratio:

Low	< 8
Medium	8 to 15
High	> 15

Usually, C/N ratios above 20 are found only in organic soils.

Acidity and pH

Acidity in sediment is derived from two major sources: 1) oxidation of sulfides and 2) exchangeable aluminum. Soils with a high sulfide content are called acid-sulfate soils, for when exposed to air, sulfide oxidizes to sulfuric acid. Soils with high sulfide content normally are found in coastal wetlands such as mangrove forests or in former coastal wetlands. Some sulfidic soils also may be organic soils. Aluminum hydroxides and oxides occur in many soils, and aluminum ions occur on the cation exchange sites of these soils. Exchangeable aluminum is

Table 14. Profile for soil texture in soil cores from ponds at Muñoz, Philippines.

Depth (cm)	Soil Texture
0–2	Silty Clay
2–4	Silty Clay
4–6	Silty Clay
6–8	Silty Clay
8–10	Silty Clay
10–12	Silty Clay
12–14	Silty Clay
14–16	Silty Clay
16–18	Silty Clay
18–20	Silty Clay
20–22	Clay
22–24	Clay

in equilibrium with aluminum ions in the pore water, and hydrolysis of aluminum ions releases hydrogen ions in the pore water. The greater the proportion of aluminum ions to basic ions (calcium, magnesium, sodium, and potassium) on cation exchange sites in sediment, the lower the pH. Although the pH is related to the ratio of acidic to basic ions, the acidity of soil is related to the total amount of exchangeable aluminum in soil. Two soils may have the same pH because they have equal ratios of aluminum ions to basic ions. However, if the cation exchange capacity (CEC) of these two soils differs, the soil with the highest CEC will have the greatest exchangeable acidity. The need for lime in a soil is indicated by pH, but the amount of liming material that must be applied to raise pH to the desired level is a function of exchangeable acidity. Exchangeable acidity is the most common type of acidity in soils, and even acid-sulfate soils have exchangeable acidity. The suggested categorization of soil based on pH contains seven classes:

Strongly acidic	< 4.0
Acidic	4.1 to 5.0
Moderately acidic	5.1 to 6.8
Neutral	6.9 to 7.1
Moderately basic	7.2 to 7.9
Basic	8.0 to 8.9
Strongly basic	> 9.0

However, three classes should be adequate for exchangeable acidity:

Low	< 10 meq (100 g)$^{-1}$
Medium	10 to 30 meq (100 g)$^{-1}$
High	> 30 meq (100 g)$^{-1}$

It is recommended that pond sediments be classified as sulfidic if they contain more than 0.5% total sulfur. Sulfidic sediment should be ranked according to sulfur content as follows:

Low	0.5 to 1.0% total sulfur
Medium	1.1 to 2.0% total sulfur
High	> 2.0% total sulfur

Carbonate and Sodium

A pH of 8 or above usually is found only in sediments containing free carbonates or high concentrations of sodium and potassium (alkali soils). Pond sediment can be categorized based on the presence or absence of free carbonates. Three levels of carbonate content should be recognized as follows:

Low	< 1% $CaCO_3$ equivalent
Medium	1 to 10% $CaCO_3$ equivalent
High	> 10% $CaCO_3$ equivalent

The pH of non-alkali soils containing carbonate usually will not exceed 8.

The sodium content of soil affects soil texture and pH. Soils with high concentrations of sodium relative to calcium and magnesium concentrations tend to be dispersed and to have a high pH. The sodium status of soil often is expressed as the sodium adsorption ratio (SAR):

$$SAR = \frac{Na}{[\frac{1}{2}(Ca - Mg)]^{0.5}}$$

where Na, Ca, and Mg are cation concentrations in meq kg^{-1}. The SAR can be used to divide soils into different classes with respect to sodium status. When SAR exceeds 12, soil pH usually will be above 8. Freshwater pond sediments usually will have an SAR below 10, while brackishwater pond sediments will have a higher SAR. There should be three levels of SAR:

Low	SAR < 10
Medium	SAR 10 to 30
High	SAR > 30

Flocculent Layer and Sediment Surface

The F horizon is more pronounced over some pond sediments than others. The surface of the S horizon may be either reduced or oxidized, and the thickness of the oxidized layer varies. The depth of the F horizon and the degree of oxidation of the upper part of the S horizon will change with time during the aquaculture crop, so these properties change more rapidly and more often than the other properties used in the classification system. Nevertheless, for some purpose it will be useful to include the thickness of the F horizon and the degree of oxidation of the S horizon in pond sediment classification. The following categories are suggested:

Thickness of F horizon - thin (< 1 cm); normal (1 to 3 cm); thick (> 3 cm)

Degree of oxidation of S horizon - thin (< 2 mm); normal (2 to 10 mm); thick (> 10 mm)

Depth of Samples for Classification

Pond sediment composition changes with depth, and in many sediment the levels of variables will change between horizons. This should not present a problem in pond soil classification because the classification system will be based on the characteristics of the S horizon. The reason for selecting the S horizon for classifying pond soils is that this layer is the first layer below the soil-water interface and is the only layer in direct contact with pond water. Exchanges of substances between pond water and bottom soil occur in this layer. There usually is no direct contact of pond water with the M, T, and P horizons below the S horizon.

A transparent core tube can be used to secure samples, and the S horizon can be identified and removed for analysis. The total sediment depth, thickness of the F horizon, and oxidation status of the sediment surface also can be obtained from inspection of the core before the S horizon is removed.

Use of Classification System

The proposed system allows a pond sediment to be classified using four primary and five secondary properties. There also is the option to use two tertiary properties if desired. Adoption of a standardized way to indicate the levels of these properties for a sample is essential, and for convenience, the indication of levels should result in a sediment name. A name consisting of words denoting levels of up to 11 properties would be very long. The name can be shortened if words are used only for the levels of the primary properties pH, organic matter, sediment thickness, and organic status (organic or mineral). A pond sediment could be described in words as an acidic, loamy, thin sediment. As most pond sediments are mineral in nature, it is not necessary to use the word mineral in the description. The word organic must be included in the designation of a sediment that contains more than 15% organic carbon, e.g., a moderately acidic, sandy, thick, organic sediment. The sediment name can be completed by providing, in parentheses and after the primary descriptor words, abbreviations denoting levels of secondary properties. The additional information should be abbreviated as follows: OC = organic carbon; CN = C/N ratio; EA = exchangeable acidity; CS = calcareous sediment; SS = sulfidic sediment; SR = sodium adsorption ratio. The degree of intensity—high, medium, or low—should be indicated with the subscript h, m, or l, respectively. Using the suggestions above, an example of a sediment name could be an acidic, loamy, thin sediment (EA_h; OC_l; CN_m; SR_l). Because the word organic is missing, this name implies that the sediment is of mineral nature even though the word mineral does not appear as a descriptor. Also, the absence of the abbreviations CS and SS reveals that the sediment is neither calcareous nor sulfidic.

Where it is desired to use the tertiary properties, we suggest the following abbreviations: F and S for F horizon and S horizon; subscripts o and r indicate oxidized and reduced conditions, respectively, at the surface of the S horizon; subscripts l, m, and h indicate thin, medium, and thick. These abbreviations should follow the parentheses containing the secondary property descriptors as follows: acidic, loamy, thin sediment (EA_h; OC_l; CN_m; SR_l) F_l S_r or acidic, loamy, thin sediment (EA_h; OC_l; CN_m; SR_l) F_l $S_{o,m}$. In the first case, F_l indicates a thin flocculent layer and S_r denotes that the sediment surface is reduced. In the second case, $S_{o,m}$ indicates that the sediment surface is oxidized to a normal depth.

An example of the use of soil chemical and physical data to classify and name a pond sediment is provided here. A sediment has the following composition: clay, 45%; pH, 6.2; sediment thickness, 24 cm; organic carbon, 2.1%; C/N ratio, 10; total sulfur, 0.11%; exchangeable acidity, 5 meq (100 g)$^{-1}$; free carbonate, 0%; SAR, 5.5; F horizon = 5 cm; S horizon reduced at surface. The name follows:

moderately acidic, clayey, thick sediment (EA_l; OC_m; CN_m; SR_l) F_h, S_r.

Note, CS (calcareous sediment) and SS (sulfidic sediment) do not appear as secondary properties because no free carbonate was present and sulfur was below 0.5%.

Consider another example for a sediment with the following composition: clay, 32%; pH, 3.5; sediment thickness, 8 cm; organic carbon, 17%; C/N ratio, 32; total sulfur, 2.1%; exchangeable acidity, 14 meq (100 g)$^{-1}$; free carbonate, 0%; SAR, 7; F horizon = 1 mm; sediment surface oxidized to 5 mm depth. The name follows:

strongly acidic, clayey, moderately thick, organic sediment (EA_m; OC_l; CN_h; SS_h; SR_l) F_l, $S_{o,m}$.

Note, CS does not appear because there was no free carbonate in the sediment.

We plan to present this pond classification system in a scientific journal. We hope the procedure described above, or a slight modification of it, will be adopted as a standard procedure in pond aquaculture.

ANTICIPATED BENEFITS

This research has demonstrated three important points about pond sediments:

1) Reactions between pond soil and pond water occur primarily in the upper 2 to 10 cm layer.
2) Low pH, high acidity, and elevated organic matter concentration in surface sediment appear to be the most common chemical problem with pond soils.
3) Accumulation of soft sediment in ponds is the most common physical problem with pond soils.

These observations suggest that soil management in ponds should focus on liming—to reduce acidity and increase pH—and drying of bottoms between crops. These two procedures can enhance the degradation of organic matter and improve soil quality. Also, soft sediment should periodically be removed from the deep areas of ponds. It is anticipated that these findings will greatly improve pond soil management procedures.

The research also provides a method of pond sediment classification. It is anticipated that this method, or a refinement of it, will become the standard procedure for describing aquaculture pond sediment. A uniform method of describing pond soils will be beneficial in describing soils from different areas. The information provided by the sediment name also will serve to indicate the type of bottom soil management needed.

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GROWTH PERFORMANCE AND ECONOMIC BENEFITS OF *OREOCHROMIS NILOTICUS*/*CLARIAS GARIEPINUS* POLYCULTURE FED ON THREE SUPPLEMENTARY FEEDS IN FERTILIZED TROPICAL PONDS

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

An experiment was conducted for 180 days at Sagana Fish Farm, Kenya, to evaluate the performance of two formulated pellet feeds and a locally available rice bran. A polyculture of *Oreochromis niloticus* and *Clarias gariepinus* in fertilized tropical ponds was used. Twelve 800-m^2 ponds were used, and each pond was limed at a rate of 20,000 kg ha^{-1} and stocked at a rate of 19,375 ha^{-1} with sex-reversed male *O. niloticus* and 625 ha^{-1} with *C. gariepinus*. The fish were fed daily at a rate of 2% body weight. Two formulated diets were compared with rice bran in three treatments that were replicated four times. The composition of the diets was as follows: Pig finisher pellet (PFP): crude protein 12.5%, lipids 10.9%, crude fiber 15.1%; Rice bran (RB): crude protein 6.5%, lipid 10%, crude fiber 37.9%; Test diet pellet (TDP): crude protein 12.5%, lipid 13.1%, crude fiber 14.1%. Diammonium phosphate (DAP) and urea were used at rates of 8 kg P ha^{-1} wk^{-1} and 20 kg N ha^{-1} wk^{-1}, respectively. After one month urea input was reduced from 2.7 to 2.2 kg $pond^{-1}$ to allow for the nitrogen contributions from the feed and to maintain the inputs at 35 kg N ha^{-1} wk^{-1} in the ponds. Water quality analyses showed no significant differences ($P > 0.05$) among treatments in the parameters measured. Exceptions were alkalinity, pH, and dissolved oxygen (DO), which were significantly ($P < 0.05$) different among treatments. The lowest dawn DO level (0.9 mg l^{-1}) was recorded in the PFP treatment, while the highest afternoon value (9.9 mg l^{-1}) was recorded in the RB treatment. The lowest pH value of 7.9 was recorded in PFP, while the highest value (8.3) was recorded in the RB treatment. The overall range of monthly mean total alkalinity was 98.0 to 118.8 mg $CaCO_3$ l^{-1}, and the lowest value was observed in the RB treatment. The phytoplankton community was dominated by green algae in the beginning of the culture period but later by the blue-greens towards the end of the experiment. The overall mean diversity index of phytoplankton was 0.7, and values were not significantly different ($P > 0.05$) among treatments. Gross primary production ranged from 0.1 to 11.5 g C m^{-2} d^{-1}. However, the values were also not significantly different ($P > 0.05$) among treatments. The RB treatment gave significantly ($P < 0.05$) lower values in fish growth rate and annualized net fish yield (0.69 g d^{-1} and 5,000 kg ha^{-1}, respectively) than both PFP (1.17 g d^{-1} and 9,298 kg ha^{-1}, respectively) and TDP (1.15 g d^{-1} and 8,828 kg ha^{-1}, respectively). The feed conversion ratio was highest in the RB treatment. There were no significant differences ($P > 0.05$) in survival rates and relative condition factors among the treatments. Profitability analysis by using partial and enterprise budgets revealed that locally available pig finisher pellets were the most profitable followed by rice bran at the local market price of US$1.17 kg^{-1} fish. At a higher price of US$1.56, PFP would still be the best choice, followed by TDP, while RB would be the least profitable. The net returns were positive for all the treatments. However, RB had the lowest break-even price and the least investment cost.

INTRODUCTION

Tilapia farming in tropical and subtropical developing countries is practiced at either extensive or semi-intensive levels. The extensive culture of fish is undertaken mostly by cash-poor farmers, while semi-intensive culture is practiced on a more commercial scale. The suitability of tilapia for culture revolves around its ability to tolerate a wide range of environmental conditions as well as utilize food from the lowest trophic level and the detrital food chain (Moriarty and Moriarty, 1973; Bowen, 1979, 1982). The fish also displays high plasticity in its food habits, thus accepting a wide range of materials that are available as food (Maitipe and De Silva, 1985).

The semi-intensive culture of tilapias is particularly ideal in developing countries because it provides a wide variety of options in management and capital investments. Management strategies in the lower levels of intensification involve the use of fertilizer to encourage natural productivity and to improve the levels of dissolved oxygen (DO). Fish yields from such techniques have been found to be higher than those from natural unfertilized systems (Hickling, 1962; Hepher, 1963; Green, 1992; and Diana et al., 1994a). Moreover, increases in fish yields above those attained by fertilization only require the use of feed-fertilizer combinations, which result in higher critical standing crop (CSC). This increment occurs either by allowing for an increase in fish size or a higher stocking rate (Hepher, 1978). The fertilizer-feed management technique boosts fish yields but also offers the possibility of reducing feed inputs (Teichert-Coddington and Rodriguez, 1995). Diana et al. (1994a) demonstrated that when fish were fed on half ration and full ration in fertilized ponds, the yields were similar in both treatments, indicating that the feed inputs could be reduced by as much as one-half.

A wide range of materials have been utilized as feed supplements in Nile tilapia farming, including single ingredients (Binh et al., 1996). The role of a single ingredient as a feed supplement in fish production is determined by the quality, quantity, and availability of its nutrients to fish. Cottonseed meal, for example, has a higher protein content and is a more efficient fish producer than rice bran. To achieve a higher production than that achieved with single ingredients, formulated feeds have been utilized in semi-intensive culture of *Oreochromis niloticus* (Diana et al., 1994b). Formulated diets produce significantly higher fish yields than single ingredients (Binh et al., 1996). Besides the improved quality, complete feeds provide some micronutrients that enhance fish production (Binh et al., 1996). However, formulated diets for fish in Kenya have not been adequately developed. Moreover, the few that are available are of questionable quality in addition to their high cost. Diets formulated for other domestic animals, however, are cheaper and readily available. Therefore, there is a need to evaluate the performance of these feeds as possible alternatives.

The performance of a diet in fish culture depends on the quantity and quality of the feed as well as its effects on water quality. Water quality is inversely related to the amount of feed inputs (Boyd, 1990). Un-ionized ammonia and dissolved oxygen (DO) are the two most critical water quality parameters that may depress fish growth, even though the supplemental feed may be of high nutritional quality. Ammonia toxicity is dependent on the pH of the water (Trussel, 1972). Therefore, diurnal pH variation may affect fish yields significantly. A complete evaluation of the performance of a supplemental feed should therefore include evaluation of the concomitant responses of the water quality variables.

This study was conducted to evaluate the performance of two formulated diets in fertilized ponds for polyculture of *O. niloticus* and *Clarias gariepinus*. Locally available pig finisher pellets (PFP) and specially prepared formulated test diet pellets (TDP) were compared with the widely used rice bran (RB). PFP were selected because they are less expensive than poultry pellets, and previous trials indicated that they were just as effective as poultry pellets. TDP were formulated to contain about 20% crude protein. The effects of these supplemental diets on water quality variables were also monitored.

METHODS AND MATERIALS

Twelve 800-m^2 ponds were stocked with male sex-reversed *O. niloticus* at a rate of 19,375 fish ha^{-1} and an average weight of 89.3 g. *C. gariepinus* was introduced at a stocking rate of 625 fish ha^{-1} and an average weight of 331.7 g as a predator in tilapia culture to control any breeding. The ponds were divided into three treatments with four replicates each. Ponds were randomly assigned the following diets: locally available PFP containing 14% protein, TDP formulated by using cottonseed cake and maize as the major components and containing 20% crude protein, and locally available RB with 6.5% protein.

The fish were fed at a rate of 2% body weight per day (BWD), equally divided between morning and afternoon feedings. Sampling was done fortnightly by seining to determine the average weight of the fish and adjust the feed accordingly. The experiment was run for 180 days. The TDP and PFP were manufactured by the same company. The ponds were fertilized with urea and diammonium phosphate at weekly doses of 20 kg ha^{-1} wk^{-1} nitrogen and 8 kg ha^{-1} wk^{-1} phosphorus.

Samples for pond water quality parameters were taken from three points in each pond by using a column sampler (Boyd and Tucker, 1992). The three samples were pooled into one. Water quality parameters analyzed include total nitrogen (TN), total ammonia nitrogen (TAN), total phosphorus (TP), total alkalinity (TA), dissolved reactive phosphorus (DRP), nitrate-nitrogen, nitrite-nitrogen, and total hardness (TH). All the parameters were analyzed as described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1980). Temperature and DO measurements were taken at 25-, 50-, and 75-cm depths, and the values were used in the calculations of phytoplankton primary productivity.

Partial and enterprise budgets were used to compare the relative profitability of the diets and their combinations. All costs were converted to monetary values, and the net returns to investments were determined. The analysis was based on the local market prices and expressed in US$ (US$1 = KSh 77).

Data from the experiment were subjected to single classification analysis of variance (ANOVA) using the Statgraphics program. Significant differences were judged at an alpha of 0.05.

RESULTS

The growth curves for *O. niloticus* for the entire culture period are illustrated in Figure 1. Separation of treatments became apparent during the first 20 days of culture. After 170 days of culture the curves for all the treatments had leveled off, indicating that the carrying capacity had been reached. The growth of fish fed PFP leveled off at an average weight of 364.5 g, while those fed TDP and RB leveled off at lower average weights of 335.2 g and 231.9 g, respectively.

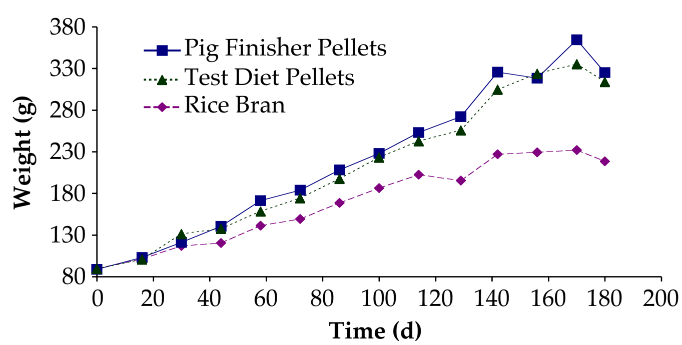

Figure 1. Growth of *O. niloticus* fed three supplemental diets during a 180-d experiment in earthen ponds at Sagana Fish Farm, Sagana, Kenya.

All three diets contained lower protein levels than expected. RB, which previously tested at 11% crude protein (CP) (Veverica et al., 1999), was analyzed and found to contain only 6.5% CP. The PFP was advertised as containing 14% CP but tested at only 12.5% CP. The TDP contained three types of oilseed meal, making up over 30% of the ingredients, but still tested at 12.5% CP.

The results of tilapia performance in various supplemental diets are summarized in Table 1. There were significant differences ($P < 0.05$) in growth rate of *O. niloticus* among treatments, with fish in PFP and TDP treatments having similar but higher growth rates (1.17 and 1.15 g d^{-1}, respectively) than those in the RB treatment (0.69 g d^{-1}). Survival rates varied among ponds but were not significantly ($P > 0.05$) different among treatments. The net yields and average weights showed trends similar to those for growth rates, with PFP and TDP having similar yields of 9,298 and 8,825 kg ha^{-1} yr^{-1}, respectively, and RB with a significantly ($P < 0.05$) lower value of 5,000 kg ha^{-1} yr^{-1}. Fish in PFP and TDP had statistically similar ($P > 0.05$) final average weights (325.1 and 313.9 g, respectively).

Although the harvest weight, growth rate, and annualized net fish yields were similar in the PFP and TDP treatments for *O. niloticus*, these variables differed significantly ($P < 0.05$) among the three treatments for *C. gariepinus* (Table 2). The PFP treatment was the best, while RB recorded the worst performance in these variables for the latter species. The mean individual growth rate of *C. gariepinus* was faster than that of *O. niloticus* in all the treatments. Overall production figures (for all fish—*O. niloticus* and *C. gariepinus*) are shown in Table 3.

Water quality parameters varied among and within treatments, but most of the parameters were not significantly different except for TA, DO, and pH. The TDP treatment had significantly ($P < 0.05$) higher TA values than PFP and RB, while the latter two had statistically similar values. Mean water temperature and pH ranged from 24.3 to 28.9°C and from 7.9 to 8.3, respectively. The pH values were significantly different ($P < 0.05$) among treatments. The dawn DO was lower than the afternoon value in all the treatments and differed significantly ($P < 0.05$) among treatments (Table 4). The highest pH values were recorded in the RB treatment, while the lowest values were recorded in the PFP treatment. Total hardness was slightly lower than total alkalinity in all treatments.

Minimum DO levels were significantly ($P < 0.05$) different among treatments, with the highest value (1.70 mg O_2 l^{-1}) recorded in the RB treatment. PFP had significantly ($P < 0.05$) the lowest value (0.92 mg O_2 l^{-1}), while the TDP value (1.26 mg O_2 l^{-1}) was intermediate. The value for TP was also high (1.21 mg l^{-1}) in the RB treatment but not significantly ($P > 0.05$) different from the other treatments.

Table 1. Stocking, harvest size, condition factor, fish growth, survival, gross fish yield, net fish yield, and net annualized production of *O. niloticus* with different feed treatments (mean ± standard error).

Parameter	**Treatment**		
	Rice Bran	*Pig Finisher Pellets*	*Test Diet Pellets*
Stocking Size (g)	89.5 ± 1.87 [a]	88.9 ± 2.53 [a]	89.6 ± 0.37 [a]
Harvest Size (g)	218.5 ± 8.27 [a]	325 ± 10.19 [b]	314 ± 16.5 [b]
Relative Condition Factor	0.9 ± 0.19 [a]	1.0 ± 0.15 [b]	0.9 ± 0.16 [a]
Growth Rate (g d^{-1})	0.9 ± 0.06 [a]	1.2 ± 0.13 [b]	1.2 ± 0.10 [b]
Survival (%)	90.8 ± 1.48 [a]	88.1 ± 2.94 [a]	90.1 ± 2.30 [a]
Gross Yield (kg ha^{-1})	4,100 ± 173 [a]	5,823 ± 350 [b]	5,746 ± 224 [b]
Net Yield (kg ha^{-1})	2,366 ± 148 [a]	4,101 ± 323 [b]	4,010 ± 225 [b]
Net Annualized Production (kg ha^{-1} yr^{-1})	4,731 ± 297 [a]	8,202 ± 647 [b]	8,020 ± 451 [b]

Note: Values followed by the same superscript in a row are not significantly different.

Table 2. Stocking, harvest size, condition factor, fish growth, survival, gross fish yield, net fish yield, and net annualized production of *C. gariepinus* in different feed supplements (mean ± standard error).

Parameter	**Treatment**		
	Rice Bran	*Pig Finisher Pellets*	*Test Diet Pellets*
Stocking Size (g)	333 ± 19.1 [a]	326 ± 19.9 [a]	336 ± 12.5 [a]
Harvest Size (g)	602 ± 47.1 [a]	1,148 ± 55 [b]	1,031 ± 75.0 [c]
Growth Rate (g d^{-1})	1.5 ± 0.27 [a]	4.6 ± 0.41 [b]	3.9 ± 0.42 [c]
Survival (%)	92.5 ± 3.6 [a]	104 ± 6.7 [a]	95.0 ± 9.85 [a]
Gross Fish Yield (kg ha^{-1})	349 ± 32.9 [a]	752 ± 93.0 [b]	612 ± 78.5 [c]
Net Fish Yield (kg ha^{-1})	138 ± 29.1 [a]	548 ± 104.8 [b]	402 ± 81.0 [c]
Net Annualized Production (kg ha^{-1} yr^{-1})	275 ± 58.0 [a]	1,097 ± 210 [b]	805 ± 162.0 [c]

Note: Values followed by the same letter in a row are not significantly different.

TN ranged among treatments between 4.9 and 5.6 mg l^{-1}, but the values were not significantly different. Mean gross primary productivity was also not significantly different ($P > 0.05$) among treatments and varied between 4.8 and 5.7 g C m^{-2} d^{-1}. However, the range between individual ponds was high, with minimum and maximum values of 0.1 and 11.6 g C m^{-2} d^{-1} recorded during the study. Similarly, chlorophyll *a* did not show significant differences among treatments, and the mean range was 133.6 to 176.8 mg m^{-3} (Table 4).

The results of partial and complete enterprise budgets shown in Table 5 indicate that PFP and fertilizer was the most profitable treatment for *O. niloticus* and *C. gariepinus* polyculture. It was followed by RB at a price of US$1.17 for fish under 300 g. However, when fish above 300 g were valued at US$1.56, RB was the least profitable. PFP gave significantly higher returns than RB and TDP but had a break-even price similar to that of RB. TDP had the highest break-even value among all the treatments. Feed and fertilizer together cost US$0.42, 0.23, and 0.51 kg^{-1} of fish produced for PFP, RB, and TDP, respectively.

DISCUSSION

The results of this study demonstrate that the use of formulated supplemental feeds is more efficient than single ingredient supplements, further illustrating the potential for expansion of semi-intensive management practice.

The CSC was reached in less than 20 days after commencement of the present experiment, when differences in growth rates of fish between the formulated diets and rice bran were observed. *O. niloticus* has been shown to reach the CSC during the first month of culture and at a size of 30 g (Diana et al., 1991). Diana et al. (1994b) observed differences in growth rates during the first month of culture of *O. niloticus* that was stocked at a size of 10.1 g. During an experiment that utilized staged feeding (Diana et al., 1996), fish growth differentiated soon after the first feeding was initiated. This differential growth rate occurred at a size of 50 g, indicating that the CSC had already been reached at that level of fertilization. In the present experiment, fish were stocked at average weights of 89.3 g, and separation occurred in fewer than 15 days of culture, suggesting that the CSC may have been exceeded at the time of stocking. This observation supports the evidence suggesting early achievement of CSC for *O. niloticus* (Diana et al., 1994a).

Supplemental feeding with formulated feeds resulted in much more rapid growth of *O. niloticus* than did supplemental feeding with a single ingredient. The two formulated feeds gave similar growth rates and yields that significantly differed from that of rice bran. Fish in the RB treatment recorded a

Table 3. Overall gross fish yield, net fish yield, net annualized production, and apparent feed conversion ratios for the *O. niloticus*/*C. gariepinus* polyculture with different feed treatments (mean ± standard error).

Parameter	Treatment		
	Rice Bran	*Pig Finisher Pellets*	*Test Diet Pellets*
Gross Fish Yield (kg ha^{-1})	4,448 ± 163.0 [a]	6,575 ± 325.0 [b]	6,359 ± 215.0 [b]
Net Fish Yield (kg ha^{-1})	2,503 ± 148.0 [a]	4,649 ± 309.0 [b]	4,412 ± 221.0 [b]
Net Annualized Production (kg ha^{-1} yr^{-1})	5,006 ± 295.0 [a]	9,298 ± 617.0 [b]	8,825 ± 443.0 [b]
Apparent Feed Conversion Ratio	5.1 ± 0.31 [a]	3.4 ± 0.24 [b]	3.5 ± 0.18 [b]

Note: Values followed by the same superscript in a row are not significantly different.

Table 4. Water quality parameters (mean ± standard error).

Water Quality Parameter	Treatment		
	Pig Finisher Pellets	*Rice Bran*	*Test Diet Pellets*
Dissolved Oxygen, dawn (mg l^{-1})	0.9 ± 0.05 [b]	1.7 ± 0.05 [a]	1.3 ± 0.05 [c]
Dissolved Oxygen, afternoon (mg l^{-1})	8.4 ± 0.17 [b]	9.9 ± 0.18 [a]	9.1 ± 0.18 [c]
Temperature (°C), dawn	24.6 ± 0.05 [a]	24.3 ± 0.05 [a]	24.4 ± 0.05 [a]
Temperature (°C), afternoon	28.1 ± 0.43 [a]	28.9 ± 0.44 [a]	28.2 ± 0.45 [a]
Alkalinity (mg $CaCO_3$ l^{-1})	108.5 ± 3.84 [b]	98.0 ± 3.84 [a]	118.8 ± 3.84 [b]
Total Hardness (mg $CaCO_3$ l^{-1})	88.7 ± 5.98 [a]	95.1 ± 7.72 [a]	107.8 ± 6.68 [a]
Primary Production (g C m^{-2})	4.82 ± 1.50 [a]	5.5 ± 1.95 [a]	5.7 ± 2.71 [a]
Total Ammonia Nitrogen (mg l^{-1})	0.9 ± 0.08 [a]	1.0 ± 0.10 [a]	0.9 ± 0.09 [a]
Total Phosphorus (mg l^{-1})	1.0 ± 0.10 [a]	1.2 ± 0.13 [a]	0.9 ± 0.12 [a]
Total Nitrogen (mg l^{-1})	5.6 ± 0.44 [a]	4.9 ± 0.57 [a]	5.2 ± 0.49 [a]
Total Suspended Solids (mg l^{-1})	255.9 ± 110.14 [a]	255 ± 85.57 [a]	278.1 ± 103.50 [a]
Chlorophyll *a* (mg m^{-3})	176.81 ± 6.81 [a]	133.60 ± 16.81 [a]	163.1 ± 16.81 [a]
pH	7.9 ± 0.06 [a]	8.3 ± 0.06 [b]	8.0 ± 0.06 [a]

Note: Values followed by the same letter in a row are not significantly different.

Table 5. Economic comparison among three experimental diets with interest rates at 24% per annum (US$1 = KSh 77).

Item	Unit	Treatment		
		Pig Finisher Pellets (PFP)	*Rice Bran (RB)*	*Test Diet Pellets (TDP)*
Gross Revenue	US$	7,685 [b]	5,199 [a]	7,433 [b]
Variable Cost	US$	5,761 [b]	3,880 [a]	6,293 [b]
Income above Variable Cost	US$	1,924 [b]	1,319 [a]	1,140 [a]
Fixed Cost	US$	431.6 [a]	431.6 [a]	431.6 [a]
Total Cost	US$	6,192.6 [b]	4,311.6 [a]	6,724.6 [b]
Net Return	US$	1,492.9 [b]	874.1 [a]	708.4 [a]
Break-Even Yields	kg	5,821 [b]	4,182 [a]	6,791 [b]
Break-Even Price (Variable Cost)	US$	0.88 [a]	0.87 [a]	0.99 [b]
Break-Even Price (Total Cost)	US$	0.94 [a]	0.97 [a]	1.06 [b]

Note: Values followed by the same letter in a row are not significantly different.

growth rate of 0.68 g d^{-1}, while fish in the PFP and TDP treatments recorded growth rates of 1.17 and 1.15 g d^{-1}, respectively. The values obtained in the present study for PFP and TDP are similar to the 1.17 g d^{-1} growth rate obtained with the same species based on fertilization alone, but are lower than the value of 3.10 g d^{-1} observed with feed and fertilizer in Thailand (Diana et al., 1996). Green (1992) obtained a value of 2.03 g d^{-1} in Honduras using feed and fertilizer. The differences in the growth rates between these studies may be attributable to temperature differences between regions and the use of manure (Green, 1992), which has been shown to produce higher fish yields than chemical fertilizers.

Rice bran (6.5% CP) in the present experiment recorded a net annualized fish yield of 5,000 kg, which is slightly lower than the 5,319 kg recorded for the same bran (11% CP) by Veverica et al. (1999). The way rice bran is processed greatly affects its quality. During periods of scarcity of rice bran, some traders mix rice bran with rice hulls, thus lowering its quality. It is therefore possible that the variability of the quality of rice bran contributed to the slightly lower yields observed in the present experiment.

While working on defatted loose and pelleted rice bran in Arkansas, Perschbacher and Lochmann (1995) recorded yields of 6,128 and 6,316 kg ha^{-1}, respectively, in fertilized ponds. Despite differences in the CP content of the two materials (13 and 18%, respectively), these yields were not significantly different. Although the protein contents of brans differed markedly within a country, fish yields showed only a slight difference. The more than one tonne increase in fish yields between Kenya and Arkansas could be attributed to the temperature differences under which these experiments were conducted and possible strain growth rate differences.

Diana and Lin (1998) obtained values close to 6,600 kg ha^{-1} yr^{-1} with chemical fertilization alone in Thailand, which are slightly higher than values reported for the rice bran and fertilizer combination in this study. These observations suggest that rice bran, which has a very high level of crude fiber, may be acting merely as a fertilizer rather than as a feed.

Single ingredients are of relatively inferior quality compared to formulated diets. The net annualized fish yields of 9,300 and 8,800 kg ha^{-1} for PFP and TDP, respectively, in the present experiment were much higher than that recorded for the RB treatment (5,000 kg ha^{-1}). The present values for the formulated diets fall within the range obtained in other studies. Diana et al. (1994b) obtained values between 8,400 and 11,600 kg ha^{-1} yr^{-1} in a fertilizer and supplemental feeding combination fed at 50% *ad libitum*. The values in the present experiment can be considered to be similar to the range above if the variability normally observed within treatments is taken into account.

Pond water in the RB treatment had lower alkalinity than in the formulated diet treatments. Long-term increases in alkalinity in ponds treated with organic matter has been associated with the release of carbon dioxide from the decomposing organic material (Knud-Hansen, 1998). The rice bran used in the present study had very high levels of crude fiber (37.9%) and therefore would be decomposed at a much slower rate than formulated feeds with low levels of crude fiber (14 to 15%). Rice bran residues were observed on the bottom mud after harvest and draining of the ponds. The rice bran residues contained some carbon, which would have been converted into carbon dioxide to boost the alkalinity through the carbonate equilibrium (Boyd, 1990). Furthermore, since the fish in the RB treatment grew at a slower rate than those fed the formulated diets, the input of organic matter was less in the RB treatment than in the formulated diets. This provides a plausible explanation for why pond water in the RB treatment had lower alkalinity than in the other treatments.

Primary production and other water quality parameters that affect fish performance were not significantly different among treatments in the present experiment. However, dawn DO levels were lowest in the PFP treatment. Despite these low levels, the treatment was the best performer, indicating that the low oxygen levels did not significantly affect fish growth. Therefore, the differences in fish growth can mainly be attributed to the quality of the diets offered. This observation also provides further evidence on the ability of *O. niloticus* to tolerate low DO levels. Formulated diets contribute more to the growth of fish than do single ingredients (Cao et al., 1998).

The PFP diet was formulated to contain 14% CP, while the TDP were formulated to contain 20% CP. Actual laboratory analysis indicated CP levels of 10 and 11% for PFP and TDP, respectively. The yields from these diets were similar despite their differences in cost.

Net returns were positive for all diets but varied among treatments. Pig finisher pellets were more profitable as a supplemental feed than rice bran and test diet pellets. Rice bran and pig finisher pellets had lower break-even prices than test diet pellets, while rice bran required the least operational cost. Although the test diet pellet resulted in similar yields to the pig finisher pellet, the break-even price for the former was higher. This difference occurred as a result of the high cost of fish diets compared to domestic animal diets. This study revealed that formulated diets produce higher fish yields than single ingredients and that locally available pig finisher is more cost-effective than specially prepared diets for *O. niloticus* and *C. gariepinus* polyculture.

ANTICIPATED BENEFITS

The development of cost-effective feeding and fertilizing strategies and the identification of economical feeds for use in aquaculture in Africa should increase the profitability of fish farming in the region and stimulate commercial aquacultural enterprises. Testing of pond management strategies that are locally and regionally practicable is required to identify those that are most cost-effective; such testing may require a number of sequential experiments using different feeds and fertilizers. This experiment and the previous Feeds and Fertilizers studies conducted at Sagana Fish Farm (8KR3 and 8KR3A) constitute two steps in such a testing program, and both have produced valuable results in the effort to develop cost-effective management strategies for Kenya and East Africa. Collaboration with local feed manufacturers and testing of already available feeds such as the pig finisher pellet can lead to viable partnerships with private enterprises and the development of cost-effective feeds for tilapia. The production of natural food organisms in feed-fertilizer management practices is often highly variable among sites. The need to evaluate this more intensive fish production practice and the results of experiments like this one under different environmental conditions may stimulate future intraregional collaboration.

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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 2A (9FFR2A)
Final Report

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ABSTRACT

Stable carbon and nitrogen isotopes were used to obtain estimates of the contribution of natural and supplemental feeds to the nutrition of *Oreochromis niloticus* in ponds (free-swimming or caged) receiving different inputs in Sagana, Kenya. Three dietary treatments were employed in the pond study: 1) the test diet; 2) a pig finisher diet; and 3) a rice bran diet. Feeding rates and fertilization regimes are detailed in the report for 9FFR2. For isotope analysis, samples of *Oreochromis* (free-swimming and caged) and plankton were taken from ponds in Sagana three times (January, March, and May) during the study. The carbon and nitrogen isotope ratios of the diets were analyzed once. Modest fish growth during the study on all dietary treatments (the fish acquired ≤ 50% of their final weight between January and May) limited the application of the stable isotope technique for determining the relative assimilation of plankton and the different diets. The patterns of change in the $\delta^{13}C$ and $\delta^{15}N$ of free-swimming and caged *Oreochromis* and plankton over time and their possible interpretation were described within and between treatments.

INTRODUCTION

Stable carbon and nitrogen isotope analyses are useful techniques to obtain quantitative estimates of the relative contributions of different food sources to the nutrition of aquatic animals in ponds (Schroeder, 1983; Anderson et al., 1987; Yoshioka et al., 1989; Lochmann and Phillips, 1996; Lochmann et al., 2001). Presumably, isotope ratios of the fish will resemble those of the food(s) they assimilate most. The carbon isotopes are used to delineate the directions of carbon flow throughout a food web, and the nitrogen isotopes can be used to indicate the trophic level of different organisms in a pond. The double tracer technique is usually more powerful than the use of a single isotope in determining patterns of nutrient flow in aquatic systems, particularly when organisms of multiple trophic levels are present. In this study stable carbon and nitrogen isotope ratios of diets and plankton were compared to those of *Oreochromis* (free-swimming in ponds or confined to cages in the same ponds). Although *Clarias* was included in the study as a predator, insufficient data were collected on this species to determine its nutrient sources during the study. The caged *Oreochromis* did not receive any prepared feed and were expected to consume mostly plankton, while the free-swimming fish in the same pond had access to both an artificial input (one of the three diets) and plankton. A comparison of the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively) between the two groups was expected to reveal the additional benefits, if any, of adding supplemental inputs (diets) to ponds containing plankton. We anticipated using the results to optimize nutrient utilization and minimize feed and fertilizer costs for *Oreochromis* production in Kenya and possibly other locations.

METHODS AND MATERIALS

Pond production data such as stocking rates, feeding rates, and fertilization regimes are detailed in the final report of Liti et al. (see "Growth performance and economic benefits of *Oreochromis niloticus*/*Clarias gariepinus* polyculture fed on three supplementary feeds in fertilized tropical ponds," 9FFR2; pp. 11–16 of this report) and will not be repeated here. The three diets tested in this study were a test diet, a pig finishing ration, and rice bran. Each diet was fed to fish in four ponds, for a total of twelve ponds, for approximately seven months. Because some of the experimental samples were lost in Sagana prior to analysis, this report includes data for only five months of the study (January through May). Dry matter and ash analysis of the diets was conducted at the University of Arkansas at Pine Bluff using standard methods (Association of Official Analytical Chemists, 1984); protein analysis was by the Kjeldahl method, and lipids were analyzed using a Folch extraction (Folch et al., 1957).

Plankton was the main natural component of the pond system assumed to contribute to the nutritional status of *Oreochromis* and *Clarias*. Therefore, samples of the plankton and the fish (*Oreochromis*, both free-swimming and caged) collected in January, March, and May were subjected to carbon and nitrogen isotope ratio analyses. There were not sufficient numbers of *Clarias* to sample more than once, so isotope data for *Clarias* were included for qualitative comparison only. Plankton was treated two different ways at the March sampling period only. Some of the plankton (called Plankton in the Results section) was collected by net and not treated further, like at the other sampling periods. The rest of the plankton collected by net was centrifuged to concentrate the phytoplankton. This phytoplankton concentrate was called Plankton 2 in the Results. The isotope values of both samples were analyzed. Diets, fish, and plankton were prepared for stable isotope analysis as described previously (Lochmann and Phillips, 1996). The analyses were conducted at the Stable Isotope Laboratory at the University of Arkansas, Fayetteville, Arkansas, using a micromass isotope ratio mass spectrometer.

RESULTS AND DISCUSSION

Proximate composition of the diets is shown in Table 1. The test diet and the pig finisher diet were similar to each other in proximate composition, and the rice bran was lower in protein and higher in ash than the other diets. Several batches of each diet were formulated during the study, and rice bran varied the most in proximate composition between batches.

The test diet and the pig finisher diet have similar $\delta^{13}C$ and $\delta^{15}N$ values (Tables 2 and 3). Rice bran had much more negative $\delta^{13}C$ values and positive $\delta^{15}N$ values than the other two diets (Tables 2 and 3). *Oreochromis* and plankton samples collected 5 January 2000 were used as the initial samples for isotope data. By January there were already significant differences in the $\delta^{13}C$ values of the free-swimming fish and plankton in ponds receiving rice bran (Table 2) and in the $\delta^{15}N$ values of the plankton in ponds receiving rice bran (Table 3). By contrast, there were no differences in $\delta^{15}N$ of free-swimming or caged tilapia in ponds receiving any of the diets in January. There were also no differences in $\delta^{13}C$ of caged tilapia in ponds receiving any of the diets in January (Table 2), probably reflecting their lower rate of weight gain and thus assimilation of different nutrients, as reflected by their isotope values.

By March the $\delta^{13}C$ values of the free-swimming and caged tilapia as well as the plankton were all significantly more positive in ponds receiving the test diet or the pig finisher diet compared to ponds receiving rice bran (Table 2). This trend continued in the fish and plankton samples taken in May (Table 2). The $\delta^{15}N$ values of free-swimming tilapia, by contrast, were distinctly different among all three diets (Table 3), while there were no significant differences in $\delta^{15}N$ of caged tilapia or plankton among diets in March (Table 3). This trend in the $\delta^{15}N$ values continued into May (Table 3).

Figure 1 shows the change in $\delta^{13}C$ values in ponds receiving each of the diets over time. The test diet (Figure 1a) was isotopically distinct from the plankton (both Plankton and Plankton 2) for the whole period. The values for plankton and both free-swimming (Pond) and caged fish stayed within a fairly narrow range throughout the study, and there was no large decrease in $\delta^{13}C$ of the fish, which would have indicated a high rate of assimilation of the test diet. However, free-swimming fish gained only about 50% of their final body weight during the period of observation. Generally, a weight increase of 250% is needed to determine whether a diet will be assimilated to a great degree (Anderson et al., 1987). The pattern of change in $\delta^{13}C$ over time was similar in ponds that received the pig finisher diet (Figure 1a). In this case the isotope values of the free-swimming tilapia were consistently more positive than those of the caged tilapia, indicating a higher rate of assimilation of the pig finisher compared to the

Table 1. Proximate composition (%) of diets used in a feeding trial with free-swimming and caged tilapia in ponds in Sagana, Kenya.[1]

Diet	Protein[2]	Lipid[3]	Dry Matter[4]	Ash[5]
Test	11.52 ± 0.00	6.33 ± 0.04	87.80 ± 0.00	8.10 ± 0.07
Pig Finisher	11.59 ± 0.49	4.18 ± 0.32	87.82 ± 0.00	9.04 ± 1.16
Rice Bran	8.10 ± 0.61	4.12 ± 0.18	90.59 ± 0.00	19.41 ± 0.08

1 Values are means of two replicates ± SD for the first batch of diets used in the experiment.
2 Protein in subsequent batches of the test diet was within 1% of the first batch. Protein ranged from 9.8 to 12.4% for subsequent batches of the pig finisher and 4.7 to 8.1% for subsequent batches of rice bran.
3 Lipid in subsequent batches of all diets was within 1% of the values for batch 1.
4 Dry matter in subsequent batches of all diets was within 2% of the values for batch 1.
5 Ash in subsequent batches of pig finisher was within 1% of the value for batch 1. Ash in subsequent batches of rice bran ranged from 15.5 to 21.1%.

Table 2. Stable carbon isotope ratios ($\delta^{13}C$ in ‰) of free-swimming tilapia in ponds, tilapia in cages in the same ponds, and plankton sampled in January, March, and May 2000 in Sagana, Kenya.[1] The $\delta^{13}C$ of the diets is indicated in parentheses.

Diet	Free-Swimming Tilapia	Caged Tilapia	Plankton
JANUARY			
Test (–16.9)	–18.8 ± 0.5 [a]	–19.4 ± 0.5	–20.5 ± 2.0 [a]
Pig Finisher (–16.2)	–18.9 ± 0.8 [a]	–19.6 ± 0.8	–21.2 ± 1.1 [a]
Rice Bran (–27.6)	–20.5 ± 0.4 [b]	–19.8 ± 0.2	–23.7 ± 1.2 [b]
MARCH			
Test (–16.9)	–18.5 ± 0.6 [a]	–18.8 ± 0.8 [a]	–18.1 ± 1.2 [a]
Pig Finisher (–16.2)	–18.3 ± 0.3 [a]	–18.9 ± 0.5 [a]	–18.9 ± 1.6 [a]
Rice Bran (–27.6)	–21.2 ± 2.3 [b]	–20.5 ± 0.4 [b]	–22.2 ± 1.0 [b]
MAY[2]			
Test (–16.9)	–18.0 ± 0.4 [a]	–17.7 ± 0.9 [a]	–18.5 ± 0.6 [a]
Pig Finisher (–16.2)	–18.0 ± 0.2 [a]	–18.7 ± 0.6 [a]	–18.2 ± 1.1 [a]
Rice Bran (–27.6)	–22.0 ± 1.0 [b]	–20.7 ± 0.4 [b]	–23.1 ± 0.5 [b]

1 Values are means of four replicates ± SD.
2 *Clarias* (stocked to control tilapia reproduction) samples were available for isotope analysis only in May. The mean $\delta^{13}C$ values for *Clarias* fed the test diet, pig finisher, and rice bran were –17.0 ± 0.5, –17.7 ± 0.8, and –0.8 ± 0.6‰, respectively. Fish fed the rice bran had significantly more negative $\delta^{13}C$ than those fed the test diet or pig finisher.
a,b Means for each month in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.

Table 3. Stable nitrogen isotope ratios ($\delta^{15}N$ in ‰) of free-swimming tilapia in ponds, tilapia in cages in the same ponds, and plankton sampled in January, March, and May 2000 in Sagana, Kenya.[1] The $\delta^{15}N$ of the diets is indicated in parentheses.

Diet	Free-Swimming Tilapia	Caged Tilapia	Plankton
JANUARY			
Test (3.8)	7.9 ± 0.5	8.1 ± 0.1	5.4 ± 0.7 [a]
Pig Finisher (3.9)	8.0 ± 0.5	8.2 ± 0.3	3.8 ± 1.0 [b]
Rice Bran (6.8)	8.2 ± 0.5	8.2 ± 0.6	5.3 ± 0.6 [a]
MARCH			
Test (3.8)	6.9 ± 0.5 [a]	7.9 ± 0.5	4.2 ± 0.4
Pig Finisher (3.9)	7.3 ± 0.8 [b]	7.6 ± 0.6	4.0 ± 0.7
Rice Bran (6.8)	8.2 ± 0.3 [c]	8.0 ± 0.4	4.2 ± 0.7
MAY[2]			
Test (3.8)	7.2 ± 0.4 [ab]	7.4 ± 1.2	2.9 ± 1.3
Pig Finisher (3.9)	6.9 ± 0.9 [a]	7.5 ± 0.5	3.4 ± 0.6
Rice Bran (6.8)	8.1 ± 0.3 [b]	7.9 ± 0.4	3.0 ± 4.5

1 Values are means of four replicates ± SD.
2 *Clarias* (stocked to control tilapia reproduction) samples were available for isotope analysis only.
a,b,c Means for each month in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.

caged tilapia. The $\delta^{13}C$ value of the plankton in the ponds receiving the pig finisher steadily increased (became more positive), possibly reflecting assimilation of the diet as well. The pattern of $\delta^{13}C$ values in ponds that received the rice bran was distinct from that of ponds that received the other two diets (Figure 1c). The rice bran was isotopically distinct from the plankton in this case also, but the diet value was much

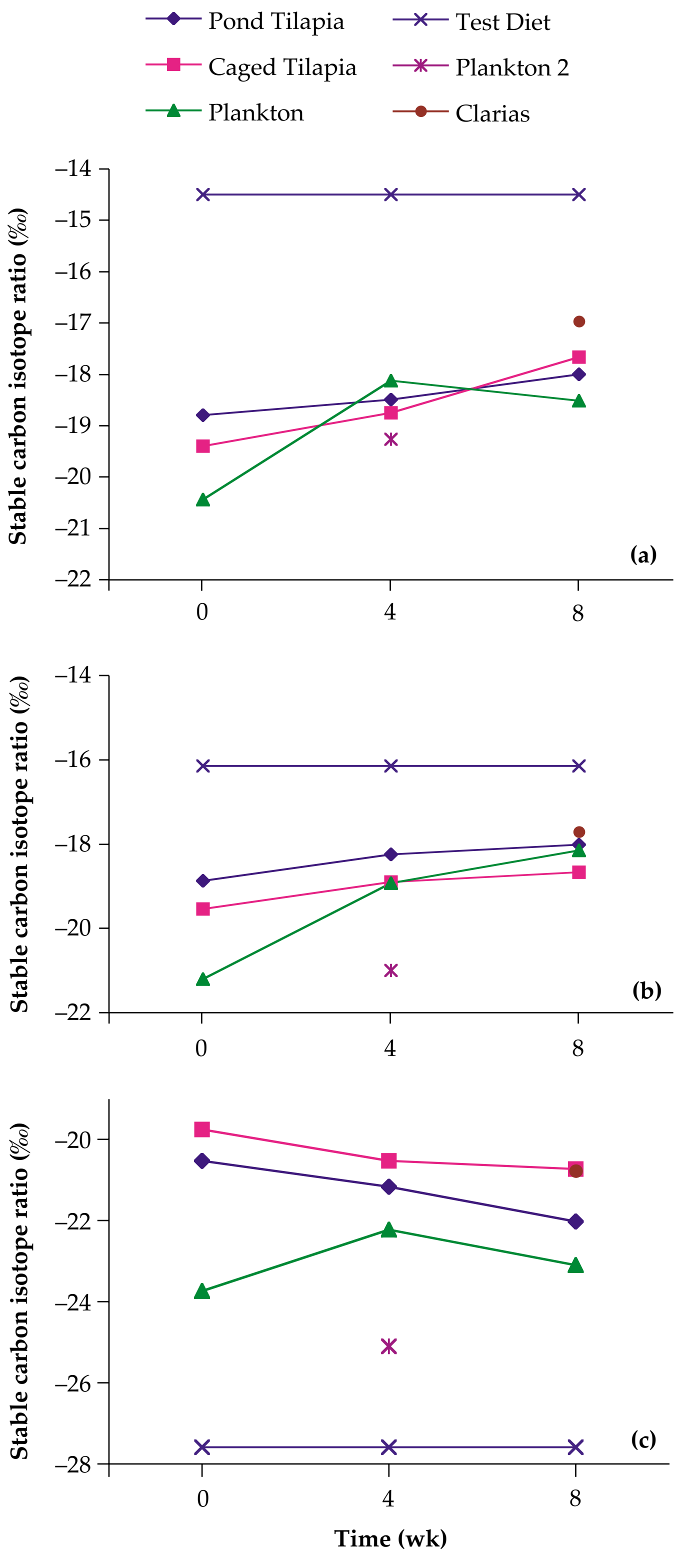

Figure 1. Mean $\delta^{13}C$ values over time in ponds that received (a) the test diet, (b) pig finisher, and (c) rice bran.

more negative than all other components in the pond, whereas the other two diets were much more positive than the other pond components (Figures 1a and b). The $\delta^{13}C$ of both free-swimming and caged tilapia gradually became more negative during the study. However, in this case the $\delta^{13}C$ of both the diet and the plankton were more negative than the fish (free-swimming and caged) initially. Therefore, it is not possible to determine the relative contributions of the plankton and the diet to the final $\delta^{13}C$. The $\delta^{13}C$ of the plankton in this treatment first increased then decreased during the study. The reasons for this pattern were not clear.

Figure 2 shows the change in $\delta^{15}N$ values in ponds receiving each of the diets over time. The isotope values for the test diet and plankton overlapped and were both lower than the values for free-swimming and caged tilapia (Figure 2a). The $\delta^{15}N$ value of the free-swimming tilapia fluctuated more than that of the caged tilapia, indicating a more varied diet. The free-swimming tilapia appeared to be tracking the plankton and/or diet in the middle of the study, but then the final value became more positive, indicating a greater emphasis on the prepared diet at the end of the study, or possibly the assimilation of other more isotopically positive nutrient sources.

The $\delta^{15}N$ of the free-swimming and caged tilapia in ponds that received pig finisher were very similar throughout the study (Figure 2b). They became slightly lower by the end of the study. The diet and plankton values were not distinct at any point in the study, although both were much lower than those of the fish. It was not possible to determine the relative contributions of the plankton and pig finisher to the nutrition of the tilapia using $\delta^{15}N$ in this case. The $\delta^{15}N$ of the plankton and rice bran were distinct in ponds that received rice bran (Figure 2c). However, the $\delta^{15}N$ of the free-swimming and caged tilapia changed less in this treatment over time than in any of the others. The $\delta^{15}N$ of the plankton declined markedly during the study, but this decline was not reflected in the $\delta^{15}N$ values of the fish (free-swimming or caged). Again, the lower growth rate of fish fed rice bran compared to those fed the test diet or pig finisher limited the ability to discriminate between the food sources of the fish using stable isotopes.

The isotope technique is more effective in pinpointing nutritional inputs of an animal when the inputs are isotopically distinct from each other and from the animal itself (Anderson et al., 1987). The discriminating power of the isotope technique may be improved in future studies by fractionating the plankton into different categories consistently before isotope analysis, as fish gut content analysis in the Eighth Work Plan indicated that there were major differences in the relative use of zooplankton and phytoplankton for *Oreochromis* and *Clarias* in the different treatments (Lochmann and Perschbacher, 2000). The nitrogen isotope data clearly provide a distinct data set from the carbon isotope data. The point-in-time data for *Clarias* indicated that the dietary habits of all of the fishes had considerable overlap. However, in this study there were not sufficient data collected over time on *Clarias* to elucidate dietary habits in relation to trophic level of the fishes that were fed different diets.

ANTICIPATED BENEFITS

Production efficiency of *Oreochromis* and *Clarias* can be improved once the quantitative importance of different nutrients under defined experimental conditions is established

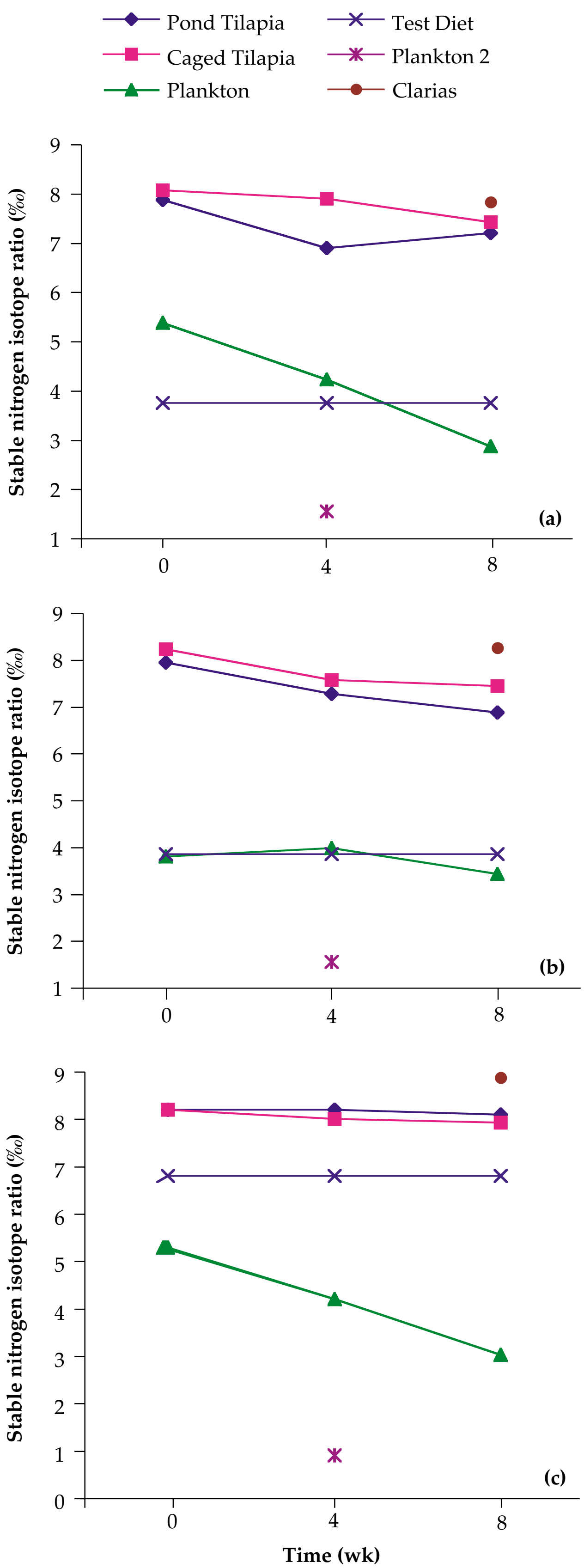

Figure 2. Mean $\delta^{15}N$ values over time in ponds that received (a) the test diet, (b) pig finisher, and (c) rice bran.

using the isotope technique in conjunction with comprehensive production data. Furthermore, the procedures used to define the importance of various components in this aquaculture production system may be modified and applied to other systems in other regions.

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REDUCTION OF FEED RATIONS BELOW SATIATION LEVELS IN TILAPIA POND PRODUCTION

Ninth Work Plan, Feeds and Fertilizers Research 3 (9FFR3)
Final Report

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ABSTRACT

The goal of this study was to evaluate feeding strategies that could be used to reduce tilapia grow-out costs. Growth, yield, and survival of tilapia fed daily were compared at 100 and 67% of experimentally determined satiation. Analysis of growth performance parameters demonstrated that the reduction of rations to 67% of satiation had no effect on growth or yield, suggesting that this approach may be useful to farmers wishing to reduce costs without compromising sales.

INTRODUCTION

The use of supplemental feed in addition to fertilization has improved tilapia yields economically. The availability of many types of commercial feeds has made it even more convenient for tilapia farmers to provide feed to their fish. But while feeding ensures rapid growth of fish, feed costs often demand 60 to 70% of the total production cost.

Feeding strategy can affect the profitability of a tilapia production operation. Good feeding procedures must be developed to minimize feed wastage and deterioration of water quality. In a tilapia feeding experiment in Thailand under the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP), results showed that feeding at 50% satiation ration, coupled with fertilization, gave comparable growth and yield to full satiation and provided better water quality (Diana et al., 1994).

The amount of feeds provided is usually determined as some percentage of the body weight of the culture animals. In culture conditions where some natural food is available, 3 to 5% of the total weight of a crop is a reasonable rule of thumb for providing pellet food to finfish (Avault, 1996; Nwanna and Bolarinwa, 2001). Another feeding practice is to provide all the food the fish will consume to ensure that all individuals obtain sufficient nutrition during the grow-out period. However, this latter practice can be costly and may create water quality problems.

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 fed daily at 100 and 67% of experimentally determined satiation. This experiment, conducted in cooperation with farmers in the central area of Luzon Island, also served to test the applicability of a reduced-feeding strategy under commercial tilapia aquaculture conditions in the Philippines.

METHODS AND MATERIALS

Nine tilapia farmers participated in this trial. At each participating farm site, two ponds were assigned one of each of the two treatments—feed ration at 100 and 67% of experimentally determined satiation levels. The determination of satiation requirements was made once a week on each farm by the project staff. The fish were given prepared feeds consisting of 67% rice bran and 33% fish meal (crude protein = 28.6%).

The pond size ranged from 0.1178 to 0.40 ha. The ponds were stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) of the Genetically Improved Farm Tilapia (GIFT) strain. This strain is genetically selected for rapid growth and presently distributed by the GIFT Foundation International, Incorporated. Fingerlings with mean weight of 0.05 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 and 5.6 kg P ha^{-1} wk^{-1}. Water depth was maintained at 1 m.

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 according to standard methods (PD/A CRSP, 1992).

A sample of 50 fish was obtained from each pond every month to measure average weights of the fish. After 120 days the ponds were harvested by seining and then complete draining. Total number of fish was counted and bulk-weighed. Final mean weight, daily weight gain, gross yields, and survival rates were calculated. The total amount of feed given in each treatment was also estimated at the end of the study. Growth performance was analyzed statistically by t-tests.

RESULTS

Growth Performance

Growth performance data are presented in Table 1. Initial stocking weight of the fish was similar in all farm sites. At harvest there were no significant differences observed in the final mean weights, daily weight gains, fish yields, extrapolated gross yields, and survival between the two satiation levels tested ($P > 0.05$).

Mean body weights measured at monthly intervals are shown in Figure 1. A higher mean final body weight of fish was observed in the 67% satiation than in the 100% satiation; however, the difference was not significantly different ($P > 0.05$). A better feed conversion ratio was obtained in the fish fed at reduced satiation level (2.38) than in those fed at full satiation (3.40).

The total feed given to the full satiation treatment was 9,396 kg ha^{-1} as opposed to 7,801 kg ha^{-1} in the reduced ration. This reduction in the amount of feed resulted in $399 savings in terms of feed costs (Table 2). The survival rate can be considered moderate, but there was also no evidence that this was treatment-related. Mortality could be due to handling stress at stocking and during regular sampling of the fish.

Table 1. Mean values ± standard deviation of on-farm growth performance of Nile tilapia at 100 and 67% satiation levels.

Performance	Satiation Level	
	100%	*67%*
Initial Mean Weight (g fish^{-1})	0.05	0.05
Final Mean Weight (g fish^{-1})	149 ± 45	154 ± 26
Mean Daily Weight Gain (g fish^{-1} d^{-1})	1.70 ± 1.0	1.76 ± 0.87
Gross Yield (kg pond^{-1})	202 ± 116	211 ± 104
Extrapolated Gross Fish Yield (kg ha^{-1})	3,135 ± 1,149	3,576 ± 1,258
Survival (%)	57 ± 22	65 ± 20
Feed Conversion Ratio	3.40 ± 1.60	2.38 ± 110

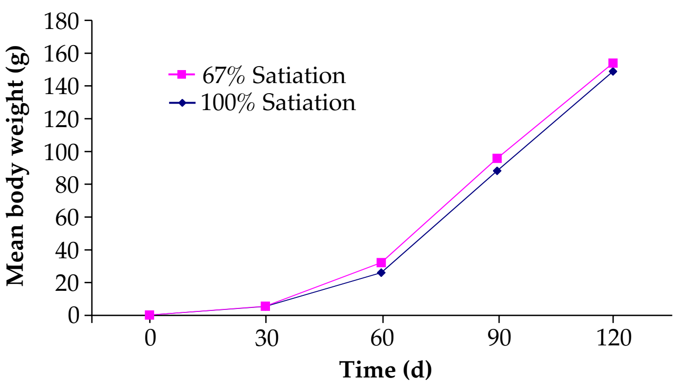

Figure 1. Mean body weight of Nile tilapia in the 100 and 67% satiation rations.

ANTICIPATED BENEFITS

This is the second experiment concluded under the current work plan in which a demonstrable reduction in operating costs is not accompanied by a reduction in fish yields. Farmers have adopted the methods demonstrated in the first part of our project (9FFR4, "Timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds") and will in all likelihood find the reduction of the ration to provide another viable cost-reduction option for them. The pattern that is developing is one of a number of methods being made available to minimize the cost of feeding tilapia grown in ponds in the Philippines. It seems likely that many conscientious farmers are reluctant to compromise their feeding schedules in the interest of growing fish rapidly and keeping them as healthy as possible. Our results indicate that reducing feeding rates, either by delaying the introduction of feeds (9FFR4) or by feeding less than the amount required for satiation (this investigation), does not cause any statistically detectable difference in pond yields or fish quality. We would predict that farmers will find this welcome and reassuring news. We anticipate that a significant number of tilapia farmers in Central Luzon will adopt and to some extent adapt this technique for their own applications, realizing some cost savings in the process.

ACKNOWLEDGMENTS

We wish to acknowledge the support of the farmer-collaborators who willingly made their pond facilities available for this study. This research was supported by the PD/A CRSP. The Philippine Phosphate Fertilizer Corporation is acknowledged for providing part of the chemical fertilizers used in this study.

Table 2. Cost and return of tilapia production per hectare using 100 and 67% satiation (in US$).

Item	Satiation Level	
	100%	*67%*
GROSS RETURN	3,919	4,470
COST		
Tilapia Fingerlings	420	420
Fertilizers	150	148
Feeds	2,349	1,950
NET RETURN	1,000	1,952

Note: Mean values for water quality parameters measured during the study were found within the acceptable ranges for tilapia culture (Table 3). No significant differences were found between the mean values of water quality parameters between the two treatments across the farms tested ($P > 0.05$).

Table 3. Mean values for water quality parameters measured monthly over a 120-day culture period in ponds fed at 100% (Pond A) and 67% (Pond B) satiation levels in the nine farm sites.

Farm	Pond	Secchi Disk Visibility (cm)	DO (mg l^{-1})	Temperature (°C)	pH	Alkalinity (mg l^{-1})	Ammonia (mg l^{-1})	Phosphorus (mg l^{-1})
1	A	17.8	6.2	25.6	8.5	167.2	0.21	0.55
	B	19.8	6.3	27.7	8.2	172.6	0.28	0.57
2	A	27.8	5.5	28.6	8.3	192.8	0.17	0.42
	B	24.8	10.4	29.3	8.7	179.4	0.16	0.34
3	A	27.4	11.9	28.9	9.0	87.4	0.19	0.20
	B	24.2	11.1	28.6	9.1	117.6	0.24	0.16
4	A	13.4	7.7	27.6	9.1	158.0	0.21	0.50
	B	17.8	6.2	27.7	8.8	137.0	0.22	0.38
5	A	45.2	7.7	28.6	8.6	193.8	0.22	0.42
	B	32.0	8.4	28.4	8.4	167.6	0.16	0.33
6	A	37.0	9.8	27.6	8.6	289.4	0.09	0.39
	B	39.8	10.3	27.6	8.6	256.2	0.08	0.32
7	A	28.4	10.3	29.9	8.7	99.2	0.12	0.32
	B	28.8	11.3	30.2	8.8	105.0	0.10	0.30
8	A	27.2	8.0	25.8	8.7	169.6	0.19	0.34
	B	40.4	6.4	26.8	8.6	166.4	0.15	0.36
9	A	24.6	10.8	30.4	8.6	178.2	0.16	0.43
	B	28.0	10.4	30.1	8.7	164.6	0.11	0.30

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EDUCATIONAL DEVELOPMENT ACTIVITIES IN SUPPORT OF TILAPIA AQUACULTURE IN THE PHILIPPINES

Ninth Work Plan, Feeds and Fertilizers Research 5 (9FFR5)
Final Report

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ABSTRACT

This host-country institutional capacity-building objective has been met and in fact exceeded, in part because of a small budget supplement that was made available late in the project. Three visitors from Central Luzon State University, Philippines, traveled to Florida International University as part of this objective, including two who received technical training. An additional graduate student at Central Luzon State University was supported in the course of his doctoral studies.

Physical improvements within the Freshwater Aquaculture Center were completed in the process of meeting the capacity-building objective; these improvements included the replacement of two obsolete computers, the renovation of teaching laboratories, and the construction of a set of poured-concrete fish-culture tanks on campus.

PROJECT SUMMARY

A modest capacity-building component of this project was added during the course of other pond-based and extension objectives. At the time, some commitments had been made to support the doctoral studies of Eddie Lopez. In keeping with the commitments that had been made earlier, some research support was made available to Mr. Lopez, who is in the concluding stages of his dissertation-writing.

Institutional ties between Central Luzon State University (CLSU), Philippines, and Florida International University (FIU), Florida, were formalized and strengthened with the execution of a Memorandum of Understanding, which recognized not only our common interest in the successful completion of the Pond Dynamics/Aquaculture CRSP project but also the common interests of our two institutions that extend well beyond the project.

Scientific exchanges between CLSU and FIU have occurred on several levels. Each project principal investigator has visited the corresponding institution and presented a guest lecture or seminar. CLSU graduate student Eddie Boy Jimenez visited FIU for a ten-day technical training period, in which he learned the basic elements of confocal microscopy, protein quantification by the Lowry method, and morphometric analysis of larval and juvenile fishes. CLSU faculty member Emmanuel Vera Cruz also visited FIU for technical training in the summer of 2001, for a one-week session covering the same material. The president of CLSU, Dr. Rodolfo Undan, visited FIU and met with institutional officials at the North Miami campus to discuss the continuation and expansion of the interactions between the two universities.

The simple exchange of students envisioned at the outset of work on this objective has grown into something significantly more ambitious. CLSU distinguished faculty member Emmanuel Vera Cruz is now an applicant for the doctoral program in Biological Sciences at FIU.

In addition to the exchange of students, faculty, principal investigators, and executives, this educational development component activity has provided resources for improvement of facilities at CLSU that have already begun having a positive effect on educational activities in aquaculture and fisheries. Fifteen $2 \times 1 \times 1$ m concrete tanks were constructed as an additional research facility for students and researchers. These will be used in future student and faculty research projects. The Fisheries Information and Learning Center (FILC) is being set up, which will maintain a modest collection of aquaculture and fisheries references. These are presently cataloged for easy retrieval using bibliographic system computer software. About 200 students can benefit from this once it becomes operational. Two additional computer units have been ordered and will be made available for students at the FILC.

ANTICIPATED BENEFITS

Human Capacity Development activities have resulted in a range of upgrades to key components of the educational mission of the Freshwater Aquaculture Center at CLSU. The availability of improved experimental facilities, teaching laboratories, and information technology (two computers) will significantly improve the quality of teaching and research at both the undergraduate and graduate levels. The most direct impact will be among the aquaculture students, but all students and faculty at the Freshwater Aquaculture Center are likely to benefit either directly or indirectly from these improvements. In addition, the research support for doctoral candidate Eddie Lopez has enabled him to reach the final stages of completion of his doctoral studies and dissertation-writing activities at CLSU.

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DEVELOPMENT OF TRAINING MODULES FOR AQUACULTURE EXTENSION WORKERS AND UNIVERSITY STUDENTS IN KENYA

Ninth Work Plan, Feeds and Fertilizers Research 6 (9FFR6)
Final Report

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ABSTRACT

A series of five highly successful short courses was conducted by the Kenya Project of the PD/A CRSP for Kenya Fisheries Department personnel during the period of the Ninth Work Plan. Activity leaders responsible for planning and carrying out these short courses felt constrained by a lack of training materials relevant to the aquaculture situation in Kenya. Although they were able to develop some materials and to borrow others for use in these courses, they did not have teaching modules specifically suited to Kenya on topics such as pond construction, composting, pond production of mixed-sex tilapia, fish nutrition, or production by species, all of which are key for the type of training currently needed in Kenya.

This activity was proposed to begin work on the development of such training modules. A faculty member from the Moi University Department of Fisheries spent eight weeks at Auburn University, Alabama, while beginning to develop training modules for use in future training sessions in Kenya. Three complete modules were developed, and work on nine others was begun. A digital camera and a new computer, to be used for continued work on module development back in Kenya, were provided. Over 1,800 slides and photographs suitable for use in training courses were digitized and saved to disk for further work in Kenya. While in the US, the participant was also able to attend and participate in the annual conference of the World Aquaculture Society (Aquaculture America 2001) and the Annual Meeting of the PD/A CRSP, as well as to visit commercial fish-farming operations in western Alabama. This activity was conducted between 16 January and 15 March 2001.

INTRODUCTION

Lack of technical training has been cited as a major reason for the low output of fish ponds in Kenya. This lack has been observed at all levels, from extension agents working in the field through university undergraduate and graduate students to upper-level fisheries officers in the Kenya Fisheries Department. It was this lack that led to the development of a training program undertaken by the Kenya Project under the PD/A CRSP Ninth Work Plan (9ADR3), which sought to improve training and to provide a cadre of trainers with extensive practical fish production experience (see "Aquaculture training for Kenyan fisheries officers and university students," 9ADR3; pp. 155–158 of this report). Under that activity Karen Veverica and Charles Ngugi conducted a series of five short courses, in which they trained officers of the Kenya Fisheries Department in pond construction, pond management, and commercial fish farming and developed training materials relevant to the needs and level of education of their trainees. However, Veverica and Ngugi often felt constrained by the lack of materials relevant to Kenya's situation. Although they were able to borrow some training modules from faculty at Auburn University (AU), they did not have teaching modules on topics such as pond construction, composting, pond production of mixed-sex tilapia, fish nutrition, and production by species, all of which are needed for training in Kenya.

It was therefore proposed that Ngugi spend six weeks at AU developing modules for training Fisheries Department extension officers and undergraduates in the Moi University (MU) Department of Fisheries. In addition, he would use the opportunity to further develop his computer skills and to work with AU faculty with experience in areas of module development, use of equipment and facilities, literature searches, and downloading extension files from the Internet.

The overall objective of this activity was to increase the capability of the Department of Fisheries at MU to contribute to sustainable utilization of aquatic natural resources through the development of aquaculture.

More specific objectives of the activity were to:

1) Develop educational materials such as extension bulletins to be used for training fisheries extension workers and university students;
2) Develop teaching modules for dissemination of research information to producers;
3) Learn how to use software and hardware needed for making teaching aids, e.g., digitizing slides and photos, using presentation programs; and
4) Search and retrieve information from the library and download and transfer extension bulletins for use in preparation of course modules.

METHODS AND MATERIALS

This activity provided funds and mentoring to allow one faculty member from the MU Department of Fisheries (Eldoret, Kenya) to travel to AU to work with experienced professionals there to learn the technical skills needed for the preparation of training modules for use in training courses in Kenya, both at MU and in the Kenya Fisheries Department.

Ngugi spent approximately eight weeks in the US between January and March 2001 working on this effort. At AU he was provided with office space at the main office of the Department of Fisheries and Allied Aquacultures, given tours of AUs extensive fish-culture facilities, and given access to library facilities on the main campus. He was supported and guided in his efforts at AU by Len Lovshin, Tom Popma, and Veverica, along with several other faculty and staff members there. Training and presentation modules previously developed at AU were used as models for his work. Jim Bowman of the Oregon State University (OSU) Department of Fisheries and Wildlife also provided guidance, as well as overall administration of the activity.

The activity also provided material support in the form of a state-of-the-art computer and printer, a digital camera, and start-up supplies (diskettes, CDs, transparencies, etc.) to begin the process of module preparation and for continued use in the development of training materials after Ngugi's return to Kenya.

RESULTS AND DISCUSSION

Ngugi departed Kenya on 16 January, arriving at AU the following day. During his first week there he met Lovshin and Popma and began planning the details of his work at AU. Veverica gave him a tour of AUs Fisheries Research Unit, and he made arrangements to audit the Fish Hatchery Management course given by Ron Phelps.

During the period of 22 to 27 January, Ngugi traveled with other members of the PD/A CRSP to Orlando, Florida, to attend the annual conference of the World Aquaculture Society (WAS) and the Annual Meeting of the PD/A CRSP. At the WAS meetings he was able to view posters, attend many presentations, meet with a number of professionals in the field, and view products and services displayed by the numerous exhibitors present. After the WAS conference he learned about other CRSP projects, got to know other CRSP participants, and developed a better understanding of the overall CRSP program by participating in the Annual Meeting.

Back at AU Ngugi rapidly settled into the task of learning the necessary computer skills and beginning to develop training modules for Kenya. The project provided a Dell™ Pentium® III 800 MHz computer with a color printer and Zip and CD-RW drives to help with the effort. Also provided were an Olympus digital camera and basic supplies for both the camera and the computer system. These items were purchased through OSU, delivered to Ngugi at AU, and taken back to Kenya (MU) to be available for continued module and training material development there.

Lovshin and Popma continued to work closely with Ngugi throughout his time at AU. They assisted him in getting started with the modules and allowed him to use or adapt their existing modules as necessary for the Kenyan situation. Veverica provided input as needed and in addition provided numerous opportunities for Ngugi to participate in and learn from aquacultural research activities taking place at the AU Fisheries Research Unit.

While at AU Ngugi learned to use the software programs needed for downloading materials from the web, for preparing presentations, and for making overhead transparencies to go with those presentations. As part of module development, he required a variety of photographs on various global aquaculture activities. Consequently, he was given access to photographic slides of fish and aquaculture by many individuals. He digitized 145 slides from Bowman and 235 slides from Popma. Veverica gave him over 155 photographs taken from Kenya, Cameroon, and Rwanda, and she also gave him 173 slides from these countries to digitize. Lovshin gave him nine CDs with over 800 slides to copy and use back in Kenya. Ngugi also copied slide presentations on fish hatchery management from Phelps. In all he digitized and saved over 1,800 slides from various sources, including downloading some from the Internet. Other sources of materials included Southern Regional Aquaculture Center (SRAC) fact sheets, the Fish Hatchery Management course given by Phelps, Carrying Capacity and Aquaculture Production by Popma, and Food and Agriculture Organization documents.

Through these efforts Ngugi was able to develop three complete training modules while in Auburn. They are:

- Introduction to Aquaculture—An Overview,
- Pond Construction—Site Selection, and
- Pond Management and Maintenance.

In addition to these complete training modules, Ngugi began work on a number of others, which will be adaptations of existing modules for the Kenyan situation. These are listed, along with the name of the original author of each:

- Fish Yield (Len Lovshin)
- Bait Minnows (Len Lovshin)
- Marine Shrimp (Len Lovshin)
- Golden Shiner (Len Lovshin)
- American Oyster (Len Lovshin)
- Atlantic Salmon (Len Lovshin)
- Channel Catfish (Len Lovshin)
- Crawfish (Matt Parker)
- Striped Bass (Mark Reddy)

He also received 18 other presentations from students and friends that he met in Orlando and at AU, including ones on culture of tilapia, carp, black bass, and trout.

During the first week at AU after the WAS and PD/A CRSP meetings, Ngugi was joined by Nancy Gitonga, Director of the

Kenya Fisheries Department and host country Principal Investigator (PI) for the Kenya Project; David Liti, a CRSP researcher from the MU Department of Zoology; and Jim Bowman, US Regional Coordinator for the Kenya Project. This group, along with Kenya Project researchers Veverica and Popma, spent a full day discussing Kenya Project progress and problems and outlining plans for proposals for the PD/A CRSP Tenth Work Plan. The group also took a full-day tour to catfish farms, a feed mill, and a fish processing plant in western Alabama.

Ngugi departed Auburn to return to Kenya on 13 March, arriving back in Eldoret on 15 March. He feels that his AU visit was very productive, informative, and educative. He gained immensely from the visit, attending numerous departmental seminars, workshops, and classes. It gave him the opportunity to meet and interact with world-renowned authors and publishers such as John Jensen (Head of the Department of Fisheries and Allied Aquacultures), Bart Green, Popma, Lovshin, and Claude Boyd, among others. Through this activity a new state-of-the-art computer, loaded with the most recent software, was acquired for use in training-course development back in Kenya; this computer will be very useful to the faculty and students of the MU Department of Fisheries.

ANTICIPATED BENEFITS

This activity has already led to the development of three new training modules to be used in training courses at several levels in Kenya. Additional modules are planned and are being worked on by Ngugi in Kenya. The activity has provided MU faculty, and in particular Ngugi, with improved computer operation and course preparation skills, which will lead to the preparation of better training materials and improved dissemination of aquaculture information to extension workers and university students. Ngugi will be able to pass on some of his new computer skills to colleagues at MU. In line with PD/A CRSP objectives, linkages between research and extension workers in Kenya are being strengthened. This activity has also provided Ngugi with additional international exposure, both at AU and at the WAS conference in Orlando.

Training modules will provide extension workers and fish farmers with better information on pond construction and management. This will lead to improved fish production and poverty alleviation for the rural communities. This will spin off small-scale commercial fish production operations that will improve farmers' incomes and serve to standardize the information given to farmers.

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MASCULINIZATION OF TILAPIA BY IMMERSION IN TRENBOLONE ACETATE: DETECTION OF TRENBOLONE ACETATE IN WATER AFTER TREATMENT

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

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ABSTRACT

In a previous experiment in which Nile tilapia fry were successfully masculinized, we investigated how the concentration of trenbolone acetate (TA) in the immersion water changed before and after treatment. The results from that experiment indicated that the concentration of TA before and after treatment of Nile tilapia fry was highly variable and below the expected levels. Therefore, we decided to corroborate those results by running two experiments in which fry were not present and by testing different water sources. These new experiments confirmed our previous findings, indicating that independently of the source of water, the concentration of TA is highly variable and below the expected levels.

INTRODUCTION

Previous experiments in our laboratory have demonstrated that short-term immersions in synthetic steroids such as 17α-methyldihydrotestosterone (MDHT) and trenbolone acetate (TA) are effective masculinizing treatments for Nile tilapia fry (Contreras et al., 1997, 1999, 2000; Gale et al., 1999). Recently, Bart et al. (2000) have found that ultrasound treatment enhances masculinization when short-term immersions are used. However, short-term immersions are far from being used on a commercial scale. One of the major criticisms to this method is that the concentration used is higher than the amount of 17α-methyltestosterone (MT) used in feeding trials. However, the immersion protocol presents advantages over feeding treatment for hormone control and disposal, as well as fewer risks of environmental contamination.

The use of TA in the cattle industry for growth enhancement has created expectations in the aquaculture industry. This steroid can be used for androgenic purposes and may have anabolic effects while fry are under masculinization treatment. It has been reported that TA administered in the food successfully masculinized channel catfish (Galvez et al., 1995) and blue tilapia (Galvez et al., 1996). However, Davis et al. (2000) found that the catfish treated with TA were not functional males but infertile organisms after three years of growth.

Research is needed to understand how much steroid is available to the fry during immersion treatment. Because of their hydrophobic properties, steroids may be very unstable in the water treatment, form precipitates, or bind to the walls of the containers. To address some of these questions, we performed experiments to determine the concentration of TA in dechlorinated water and distilled water at different times.

METHODS AND MATERIALS

Experiment 1: Steroid Solutions without Fry Immersed

Following an experiment reported on in Contreras et al. (2001) involving examination of TA solution following immersion of fry, a similar experiment with no fry immersion was planned. This experiment consisted of three 3.8-l glass jars containing 1 l of well water. To each replicate 500 µl of TA were added, mixed thoroughly, and maintained at 28 ± 1°C under constant aeration. Samples were collected at 0, 3, and 30 h at the surface, middle, and bottom of the jar (samples taken at 30 hours at middle depth were lost during processing).

Experiment 2: Short Trial in Borosilicate Tube

Ten ml of double-distilled water were placed in a borosilicate tube, to which 100 µl of TA were added and thoroughly mixed by vortexing. Samples of 1 ml were collected at time 0 (from the middle of the tube) and at 1 h (from the surface, middle, and bottom). Once all samples were collected, the water remaining in the tube was vortexed, and the tube was emptied and washed with 1 ml of ether to assay for TA attached to the tube walls.

TA Detection

All samples were stored frozen (–20°C) until processed for TA detection. 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-ml 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 min 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.

RESULTS

Experiment 1

Concentrations of TA in well water were variable at all times and at all sampling points (surface, middle, and bottom). At time 0, levels of TA in the surface water were lower than the expected value (mean = 300.7 µg l^{-1}, SD = 87.5). Initial values of hormone concentration range from 211.2 to 372.4 µg l^{-1}. Similar patterns were observed at 3 and 30 h after addition of the steroid. This trend was also observed in samples taken at the middle point and bottom of the jars (means = 279.5 and 271.8 µg l^{-1}, respectively). Concentration values of TA showed no consistent patterns (Figure 1).

Experiment 2

Concentration of TA at time 0 was 8,731.5 µg ml^{-1} (the expected value was 10,000 µg ml^{-1}). No significant changes were observed in the samples taken at the surface, middle, or bottom of the tube after one hour of mixing of the steroid with water (Figure 2). TA was detected in the ether used for rinsing the glass tube (1,887.7 µg l^{-1}). The estimated total amount of TA detected in the borosilicate tube after adding the concentration rinsed from the glass accounted for 92.4% of the total amount of TA added to the tube.

DISCUSSION

Our earlier findings (Contreras et al., 2001) indicate that the target dose for TA immersion is rarely achieved. The surprisingly low levels of TA found in our previous report have forced us to validate these results by conducting further experiments. However, we have found that the patterns are maintained independently of the source of water used (dechlorinated versus well water) or the location at which the samples are collected (surface, middle, or bottom of jars). Our first hypothesis focused on the precipitation of TA out of solution after mixing with water. However, the data from the experiments reported here indicate that samples from the bottom of the jar have similar patterns to those observed in the surface water. Another hypothesis for explaining the low levels of steroid in the water is that the steroid could be binding to the walls of the jars (which may be porous). The data obtained from a borosilicate tube (used for assays because of its low-binding properties) indicate that about 2% of the TA added binds to the glass. Therefore, it can be expected that binding to jars is higher. More research is needed to determine if the glass employed is trapping a significant amount of steroid, decreasing the efficacy of the immersion technique.

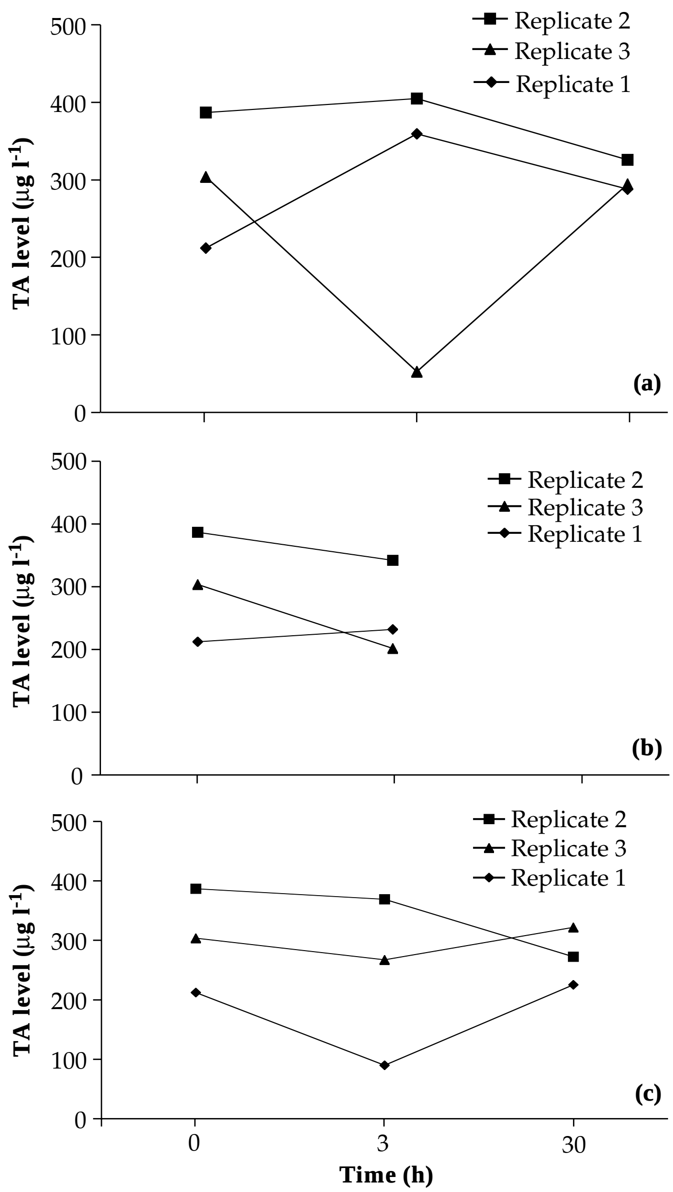

Figure 1. Trenbolone acetate (TA) levels in well water through time in experimental jars containing 500 µg l^{-1} of steroid with no fish added. Samples were taken at the surface (a), middle (b), or bottom (c) of the container.

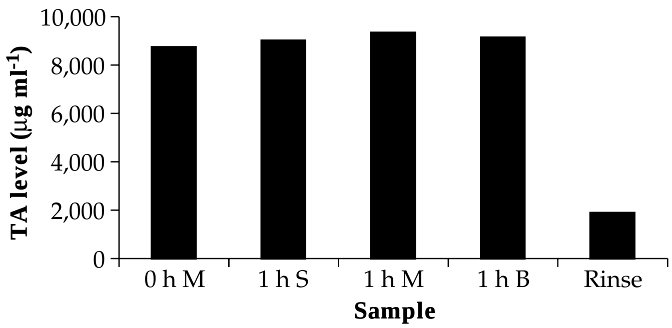

Figure 2. Trenbolone acetate (TA) levels in double-distilled water mixed in a borosilicate assay tube containing 1,000 µg ml^{-1} of steroid. Samples were taken from the middle (M) at 0 and 1 h, and from the surface (S) and bottom (B) at 1 h. Sample named “Rinse” was collected once the tube was emptied and rinsed with ether to determine if TA was binding to the glass.

ANTICIPATED BENEFITS

Masculinizing Nile tilapia fry by immersion can be a good alternative to feeding the fry with hormone-impregnated food. However, the amount of variability observed in the concentration of the steroid used during treatment indicates that this technique requires refinement to obtain more consistent results.

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Masculinization of Nile Tilapia Fry by Immersion in Trenbolone Acetate: Reuse of Hormone Solution and Effects of Temperature

Ninth Work Plan, Reproduction Control Research 5D (9RCR5D)
Final Report

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Abstract

Preliminary studies in our laboratory showed that short immersions in the synthetic androgen trenbolone acetate (TA) constitute a good option for masculinizing Nile tilapia fry produced by a single female. This technique offers the potential to replace MT feeding for 28 days and avoid steroid accumulation in pond sediments. We investigated the effects of TA treatment on fry collected from a tank containing batches produced in multiple spawnings. Our results suggest that masculinization involving short-term immersions in TA results in significantly more males in the treated groups (55.9 and 61.6%) than in the controls (44.5 and 38.9%). However, the percentage of males produced is far below that recommended for aquacultural purposes. We further investigated the potential enhancing effects of elevated temperatures in combination with TA treatment during immersion time and found no significant effects of temperature on the proportion of males obtained.

Introduction

The production of single-sex populations of tilapia (*Oreochromis* spp.) is an important tool for aquaculturists to avoid unwanted reproduction and to produce the sex with the larger growth potential (Macintosh and Little, 1995; Green et al., 1997). Aquaculturists usually administer hormones to fish through the diet, but this method often requires long-term administration of the steroid and poses the risk of contamination by the uneaten or unmetabolized hormone that eventually reaches the pond environment. In previous studies we have demonstrated that a substantial amount of the methyltestosterone administered with the food quickly appears in the tank water and remains in the sediments for at least four months (Contreras-Sánchez, 2001). Therefore, the development of techniques that can offer significant masculinization of fry involving short-term treatments and presenting little or no risk to the environment or hatchery workers may be advantageous.

Recent studies in our laboratory have shown that short-term immersions can result in significant masculinization of Nile tilapia fry (Gale et al., 1995, 1999; Contreras-Sánchez, 2001). These studies showed that immersion in androgen has the potential to be an alternative to dietary treatment with steroids for the masculinization of tilapia. This technique has the advantage of using the steroid solution under controlled systems allowing for reuse and safe disposal. However, little is known regarding the efficacy of this technique in large-scale systems. A variety of androgens—especially synthetic androgens—are effective masculinizing agents (Hunter and Donaldson, 1983). Recently, the synthetic steroid trenbolone acetate (Galvez et al., 1996) has been used to masculinize tilapia using hormone-treated food. Trenbolone acetate (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 (Contreras-Sánchez et al., 1997; Contreras-Sánchez, 2001). Such a treatment offers an alternative to the typical four weeks of feeding tilapia with MT. However, in order to be a viable alternative, short immersions in TA must be tested on fish.

Because recent studies have shown that elevated temperatures can induce masculinization to a certain degree, we decided to examine the potential enhancing effects that elevated temperatures may have when used in combination with short-term immersions in steroids. To investigate these potential effects, we carried out an experiment to compare masculinization rates between TA-immersed fry under normal temperatures (28°C), TA-immersed fry under elevated temperatures (32 and 36°C), and EtOH-immersed fry.

Methods and Materials

Multiple breeding families of Nile tilapia, *Oreochromis niloticus*, were placed in reproduction tanks (2 × 4 × 1 m) at a ratio of one male to three females per square meter. Fry release was monitored every day, starting on day 10. Once breeding occurred, fry were removed from the tank. Fry were selected by grading with a 3-mm mesh (Popma and Green, 1990), counted, and randomly assigned to experimental groups. All treatments were triplicated. The number of fry per replicate was determined by the amount of fry collected in a particular day. Each replicate was housed in a 10-l plastic container with dechlorinated tap water. The mean temperature at which fry were maintained was 29 ± 2°C. All containers were kept under constant aeration.

Experiment Ia: TA Immersions Using Multiple Broods

The objective of this experiment was to determine the masculinizing efficacy of TA in batches of fish produced by several females in fry production systems. Fry were immersed for three hours in 500 µg l^{-1} of TA or ethanol vehicle (both of which were mixed before addition of fry) at a density of 33 fish l^{-1}. All fish were immersed twice; one immersion was conducted at day 1 (day of fry harvest) and the other at day 3. Fry were collected after each immersion, containers were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. At the conclusion of the immersions, fry from all treatments were transferred to 0.5-m^3 hapas located in a grow-out cement pond.

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. Temperature and pH were monitored daily; dissolved oxygen was checked weekly.

Experiment Ib: Reuse of Steroid

The goal of this experiment was to determine the potential reuse of TA solutions after masculinizing tilapia fry. TA solutions were reused twice after the first immersion trial. Fry were immersed for three hours in 500 µg l^{-1} of TA or ethanol vehicle (both of which were mixed before addition of fry) at a density of 33 fish l^{-1}. All immersions were conducted as described in the previous experiment. Treatments were as follows:

1) Control (EtOH) first usage
2) Control (EtOH) first reuse
3) Control (EtOH) second reuse
4) TA first usage
5) TA first reuse
6) TA second reuse

Experiment II: Effect of Temperature

The goal of this experiment was to determine if elevated temperatures in combination with TA immersions could induce masculinization of Nile tilapia. Fry were collected and kept at either 24, 28, 32, or 36°C (± 1.5°C) from day 1 to day 4. Fry were immersed for three hours in 500 µg l^{-1} of TA or ethanol vehicle (both of which were mixed before addition of fry) at a density of 33 fish l^{-1}. All immersions were conducted as described in the previous experiment. Control groups were assigned to each temperature.

Growth Measurements and Sex Identification

Subsamples of fish (25 to 30) were measured to the closest 0.1 g after 65 days of growth. Sex ratios were determined by examination of gonads using squash (10 and 40X) preparations after Wright (Humason, 1972) staining.

Statistical Analysis

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™.

RESULTS

In Experiments Ia and Ib, TA immersions resulted in significant masculinization ($P < 0.05$); however, the efficacy of treatment was low. In Experiment Ia the control group had 44.5 ± 3.8% males while TA treatment resulted in 55.9 ± 4.1% males (Figure 1a). In Experiment Ib no significant differences were found between any of the control groups, and data were pooled (mean = 38.9 ± 4.3% males). TA treatment resulted in significantly more males than the control (mean = 61.6 ± 4.2%). Reuse of the hormone for the first and second time resulted in slightly more males (50 ± 4.8 and 51.7 ± 3.9%, respectively) than the control group; however, these results were not statistically different (Figure 1b).

In Experiment II we found no significant differences between control groups and any of the TA-treated groups. No significant effects on masculinization were observed when fry was kept at elevated temperatures (Figure 2). No significant differences in growth were found between any of the groups (weight = 1.39 ± 0.08 g; total length = 43.4 ± 0.7 mm).

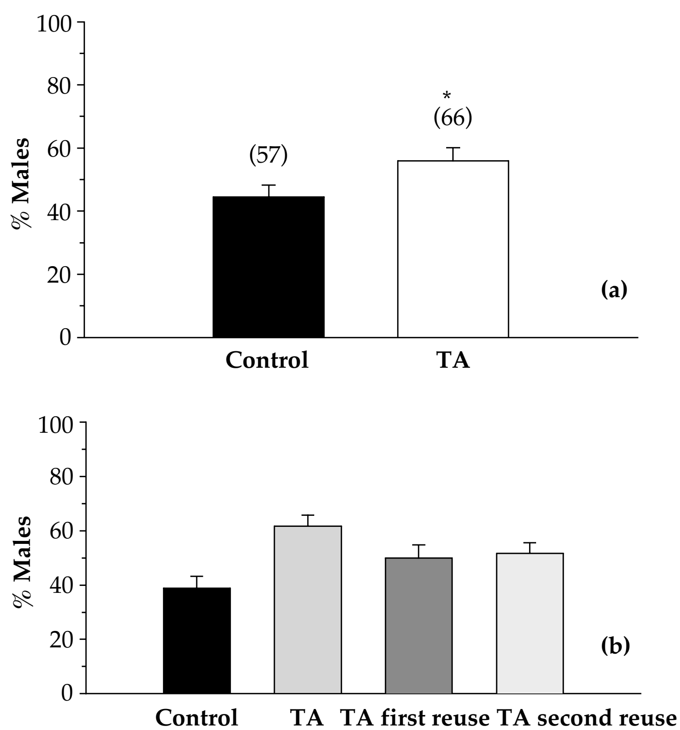

Figure 1. Effects of double immersions on masculinization of Nile tilapia fry. Graph shows mean percentage of males (± SE) obtained after immersions for 3 h in 500 µg l^{-1} of TA or EtOH vehicle (control; a). Effects of double immersions on fish immersed in TA and two cycles of reuse of the steroid solution (first and second reuse; b). The controls represent pooled data. Fish were immersed at days 1 and 3 after harvest. The numbers in parentheses indicate the numbers of fish sampled for each treatment. Asterisks indicate significant differences between control and treatment groups.

DISCUSSION

The results of this study indicate that short-term immersions of Nile tilapia fry (collected from spawning ponds) in masculinizing agents cause little or no masculinization of the fry. In a previous study Contreras-Sánchez (2001) reported that the period of gonadal sensitivity to exogenous steroids is limited to five or six days, with the gonad sensitivity to steroids resembling a normal distribution pattern. According to the cited paper, masculinization of tilapia fry kept at 28°C starts at 11 days post-fertilization (dpf), continues increasing through 12 dpf, and reaches a maximum at days 13 and 14. By 15 dpf, the response to steroid administration starts declining. The low masculinization rates found in our experiments may reflect the variability caused by multiple spawners, and it is possible that fry collected from spawning tanks have passed the labile period at which sex inversion can be accomplished. Several studies have been devoted to determining this valuable information in several species of salmonids (reviewed in Piferrer and Donaldson, 1993). However, most experiments have focused on attaining high numbers of individuals of one sex or the other using multiple-day immersion protocols, but few have approached the delimitation of the labile period by single immersion protocols.

The immersion technique poses disadvantages in terms of its feasibility for aquacultural purposes if more than 95% male populations are required. Early studies on immersion of tilapia fry in androgens reported 100% masculinization; however, these studies involved protocols that required one to five weeks of treatment (Varadaraj and Pandian, 1987; Torrans et al., 1988). Such a long-term protocol defeats the purpose of the immersion treatment (i.e., short-term usage of steroids, small amounts of hormone used, little manipulation of the fry).

It has been proposed that in Nile tilapia, female differentiation is inhibited by elevated temperatures during a sensitive period. Contreras-Sánchez (2001) suggested that this thermosensitive period may comprise the same days at which masculinization can be induced by the exposure of fry to synthetic steroids, and significant masculinization can be achieved by exposures to 34°C for as short as 3.5 d. In other studies tilapia fry were masculinized using long-term exposures (30 to 40 d) to elevated temperatures (Baroiller et al., 1995; D'Cotta et al., 1999; Wang and Tsai, 2000), but apparently such a long treatment may not be needed if conducted at the right time. Our results suggest that our treatments may have missed that window of sensitivity.

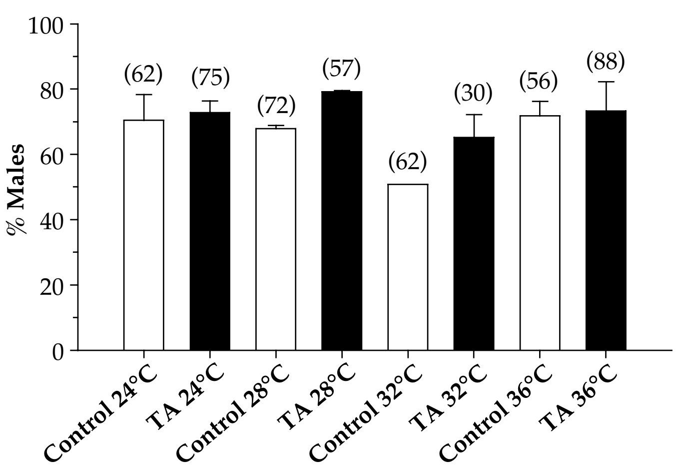

Figure 2. Effects of elevated temperatures and double immersions on masculinization of Nile tilapia fry. Graph shows mean percentage of males (± SE) obtained after immersions for 3 h in 500 µg l^{-1} of TA or EtOH vehicle (control). Fish were immersed at days 1 and 3 after harvest. The numbers in parentheses indicate the numbers of fish sampled for each treatment.

More research is needed to investigate if the immersion protocol can be improved by a combination of such factors and hormonal treatment. The development of this technology for masculinization of tilapia may enable farmers to masculinize tilapia with androgens while minimizing the risk of contamination of ponds with MT.

ANTICIPATED BENEFITS

The implementation of masculinizing trials using immersion will set up the base for the application of large-scale use of immersions under farm conditions. This will provide great opportunities for extending PD/A CRSP research and impacts to tilapia producers. In previous studies we have shown that short immersions in synthetic steroids can provide significant masculinization and that elevated temperatures may enhance the masculinizing effects of this treatment. However, our results indicate that more research is needed for large-scale trials.

The development of this technology for masculinization of tilapia fry may enable farmers to masculinize fish with androgens while minimizing the risk of contamination of ponds with MT. Furthermore, if steroid solutions can be reused for further masculinization trials, the benefits of the immersion technique may increase considerably.

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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)
Final Report

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ABSTRACT

Intraspecific breeding programs have been developed to exploit the sex inheritance mechanism in the tilapia *Oreochromis niloticus* to produce male populations. These programs are built on the premise that the mechanism of sex inheritance must conform closely to a monofactorial sex determination with a heterogametic male. Sex inheritance in tilapia, however, appears to be more complicated. The sex ratio of an individual spawn often does not conform to the expected 1:1 ratio. A better understanding of sex inheritance in tilapia and the identification of tilapia populations with a minimum variation in progeny sex ratios from individual spawns is needed for a successful intraspecific breeding program to produce male tilapia.

Nine families of *O. niloticus*, each from individual pair spawns, were selected based on the sex ratio in the family. Two families were highly skewed to male (100% male), three were near 50% male, and four were skewed to female. Fish within the same family were mated, and the sex ratios of the progeny were determined. In the two families that were all males, the males were mated to females from a family with a sex ratio near 1:1. Ten sets of progeny per family were sexed, with the exception of one family, from which only eight sets were available.

Sex ratios did not appear to be passed on from one generation to another in the fish used in our study. A realized heritability for sex ratio of –0.09 was calculated. No family with skewed sex ratios produced progeny from sibling matings with similarly skewed sex ratios. Family VIII, which had a 1:1 male:female ratio, had a range of 43 to 68% male in its sets of progeny. Family V, which was 22% male, gave 10 sets of progeny, of which five sets were > 70% male. Families II and VII, which were 100% male, when crossed with females from Family III gave sets of progeny ranging from 23 to 79% male. When the percentage of males in the female parent family (III, 40% male) was considered in matings with Families II and VII, 40 and 50% of the spawns, respectively, differed in sex ratios from the female parent family.

INTRODUCTION

Tilapia culture is one of the fastest-growing forms of finfish aquaculture in the world. It has broadened its base from being a subsistence-oriented technology to being a component of world commerce as a high-quality fillet product exported to Europe and the US. The commercial market requires a large fish suitable for providing fillets or being sold whole. Uncontrolled reproduction can result in less than 25% of the adults being greater than 250 g after a six-month culture period, with the majority of the population being progeny smaller than 10 g each, with few to no marketable fish.

Monoculture of males prevents reproduction while allowing the culture of the faster-growing sex. Male populations of tilapia can be created by hormone sex reversal, but there are concerns about consumer perceptions of eating hormone-treated fish. Inter- and intraspecific breeding programs can result in populations with highly skewed sex ratios, but these programs often give inconsistent results. Interspecific crosses have not proven to be practical due to difficulties in maintaining the parent species integrity. Intraspecific breeding programs have been developed to exploit the sex inheritance mechanism in the tilapia *Oreochromis niloticus*. Females are said to be homogametic (XX) and males heterogametic (XY). The presence of the Y chromosome establishes the sex as male. Selective breeding programs have been developed to produce YY males, where the progeny of such males mated with a normal female (XX) would be all male. For this approach the mechanism of sex inheritance must conform closely to a monofactorial sex determination with a heterogametic male. Sex inheritance in tilapia, however, appears to be more complicated. The sex ratio of an individual spawn often does not conform to the expected 1:1 ratio. This lack of conformity to a simple XX:XY sex inheritance mechanism complicates any intraspecific breeding program used to produce all-male progeny. The identification of tilapia populations with a minimum variation in progeny sex ratios from individual spawns would be a significant contribution to the development of an intraspecific breeding program. The following study addressed the question of the heritability of sex in *O. niloticus*.

METHODS AND MATERIALS

Broodstock

Nine families of *O. niloticus*, each from individual pair spawns, were selected based on the sex ratio in the family. Two families were highly skewed to male, three were near 50% male, and four were skewed to female. The total number of fish for each family, the strain, and the male:female ratio are provided in Table 1.

Fish were kept in 1 × 1 × 2 m hapas of 1.5-mm mesh in 20-m^2 concrete tanks. Four hapas were placed in each tank and suspended by a frame of treated 2 × 2 lumber. Water was kept at a 70-cm depth by an adjustable PVC pipe outlet. The water source was the station's reservoir of rainwater. Tank water was not fertilized. Daily maintenance of the tank water consisted of adjusting water flow in or out and removal of any algae build-up in the water or on the hapas. Dissolved oxygen and temperatures recorded from May to September 2000 averaged 9.8 mg l^{-1} and 28.7°C, respectively, and ranged from 1.1 to 15 mg l^{-1} and 19.9 to 33.7°C.

Fish of each family were sexed and separated equally into the four hapas at stocking, the exception being Family IX, for which only two hapas were needed. Once sorted, five males were placed in each female hapa. All fish were fed once a day with floating catfish feed to apparent satiation. Feed offered was adjusted daily as needed. In the case of all-male families, II and VII, ten females from Family III were used. Females from Family III were not transferred until after Family III had met its goal of 15 successful spawns.

Seed Collection

Adult females' mouths were checked for eggs every eight to ten days starting on 20 May 2000. Females with eggs, or fry, were placed individually in 19-l buckets and transferred to 75-l aquaria at the hatchery of the Fisheries Research Station, Auburn University, Alabama. Females were allowed to incubate the eggs and were then transferred to 19-l buckets for return to their respective families' tank. Females were not necessarily returned to the same hapa, but even numbers per hapa were maintained. In families that showed little spawning activity over two spawning cycles, the males were replaced by five different males from the same family. The last date females were examined for the spawns was 31 July 2000.

The hatchery was equipped with ninety 40-l aquaria on a recirculating system. Aeration was provided to each aquarium by a low-pressure blower through a single air stone. Average water temperature in the hatchery was 28°C. No feed was offered to females or the sampled spawns during the incubation and swim-up period.

Fry and Juvenile Management

Aquaria were checked for swim-up fry daily. Spawns were removed from aquaria upon swim-up. Each spawn was identified with a tag in a sealed vial. Those spawns that were estimated to contain over 100 fish were transferred via a 19-l bucket to either a 20-m^2 tank or a small suspended hapa. The 20-m^2 tank was used for grow-out. When one was not available, fry were kept in a 1.5-mm-mesh hapa, with a volume of 0.5 m^3, until a grow-out tank was ready. Fifteen small hapas were suspended per 20-m^2 tank. Fry were fed three times daily to apparent satiation, starting with a #0 floating trout feed. As fry grew larger, feed changed to #2, and finally #4 floating trout feed was fed twice a day. Each spawn was grown until fish were approximately 4 cm long. Upon the completion of the grow-out period, each spawn was harvested.

Harvest

Each tank was fully drained with a screen placed in the drain. Fish were then removed from the catch basin with a fine mesh net and placed in a 19-l bucket. Spawns were then transferred to the hatchery for processing. Each spawn was anesthetized in a solution of MS-222, counted, and 110 randomly selected fish preserved in a 10% formalin solution in a labeled jar. If fewer than 110 fish survived, then all were preserved. Preserved spawns were held a minimum of ten days before being sexed.

Sexing

Each spawn was rinsed in water for a minimum of 24 hours before dissecting. Sexing consisted of removing the gonads from each fish, placing each pair on a glass slide, staining the gonads with Fast Green, squashing the gonads with a second slide, and then determining sex by microscopic examination.

RESULTS

Families and Spawns

A total of 88 spawns were collected and sexed from the nine families of adults. Each family produced ten spawns for sexing except family VI, which produced eight spawns for the season. No family gave progeny sex ratios that closely conformed to that of the family. Family sex ratios and sex ratios of progeny

Table 1. Nine families of known sex ratios* selected for determining the heritability of sex and the number of siblings of each sex available for matings on 17 May 2000.

Family	Strain	Number of Males	Number of Females	Parent M:F Ratio*
I	Ivory Coast	59	61	45:55
II	Ivory Coast	111	0	100:0
III	Ivory Coast	34	42	40:60
IV	Ivory Coast	41	38	25:75
V	Egypt	57	38	22:78
VI	Ghana	13	72	19:81
VII	Egypt	94	0	100:0
VIII	Ghana	41	38	50:50
IX	Egypt	6	35	18:82

* Note that the parent male:female ratio is that of the family after sexual differentiation and not the number on hand at stocking.

Table 2. The percentage of males found and the percentage of males in each set of progeny for the nine parent families, where the percentage of males in the parent families is known.

Family	Males (%)										
	Parent	*Per Spawn*									
		1	2	3	4	5	6	7	8	9	10
I	45	23	24	26	48	50	51	52	54	58	61
II	100	23	40	42	44	48	48	51	52	53	56
III	40	25	40	43	44	47	54	56	56	59	59
IV	25	25	39	40	41	46	56	58	59	63	74
V	22	43	48	48	63	64	70	71	71	79	86
VI	19	19	38	53	56	57	62	63	68	--	--
VII	100	27	33	35	43	49	51	54	59	65	79
VIII	50	43	43	48	52	53	55	56	56	57	68
IX	18	31	34	41	43	53	59	66	69	76	80

Table 3. Percent of spawns that had a sex ratio different from the parent family sex ratio and from the mean sex ratio of its sibling pair spawns as determined by Chi square.

Family Number	Spawns Differing from Parent Sex Ratio (%)	Spawns Differing from Mean Sex Ratio of Sibling Family (%)
I	40	40
II	100	10
III	60	10
IV	90	20
V	100	40
VI	88	25
VII	100	50
VIII	10	10
IX	100	40

from matings within each family are given in Table 2, and the percentages of spawns that had a mean percentage of males different than that of the parent family are given in Table 3. When the original families were considered as to the skewness of their sex ratio and categorized as skewed to male, female, or neutral, the progeny varied in sex ratio but had category means of 47.6 ± 12.8, 55.6 ± 15.9, and 48.7 ± 11.4% male, respectively. No one family had a narrow distribution of sex ratios. The progeny from sibling matings within each family had a range of sex ratios from a low of 19% in Family VI to as high as 86% in Family V. The percentages of spawns in which the mean percentage of males was different than that of the sibling spawns within the same family are given in Table 3.

Values for inheritance were calculated using a linear regression between parents and offspring as described by Kirpichnikov (1981). Values for the regression were for all spawns percentage male (Y) compared to the parent percentage male (X), where h^2 is equal to the slope (b). Calculations revealed that h^2 was –0.09 with an R^2 value of 0.04.

Environmental Effects on Sex Ratios

High temperature did skew the sex ratio of spawns from Families VI and VIII but not Family V. The means are shown in Figure 1. Temperature data for each spawning and rearing cycle for fish cultured outdoors, along with the mean percent male for that period, are given in Table 4. There was no trend in percentage of males over this period related to ambient temperature.

DISCUSSION

The heritability of sex ratio is a fundamental question in tilapia breeding programs. Variability in sex ratios among individual spawns has been noted by a number of authors (Shelton et al., 1983; Mair et al., 1991; Al Hafedh, 1994; Tuan et al., 1999). Shelton et al. (1983) proposed that it might be possible to select for specific sex ratios. This would require that sex ratio be a heritable trait. Wohlfarth and Wedekind (1991) discuss the heritability of sex determination in tilapia using the data of Wedekind (1987), who found that selected males from spawns that were skewed to male gave progeny which were also skewed to male and that control males from families with normal ratios gave progeny with normal ratios. Al Hafedh (1994), using a strain of *O. niloticus* originally collected from Lake Manzala, Egypt, selected brooders from families with known sex ratios and mated various combinations of fish. Two pair spawns from males of a family skewed to males (75%), when crossed to females from that family, gave 68 and 75% male progeny. In three spawns from pairs in which the males from the male-skewed family were crossed with females from a family highly skewed to female (99.4%), the progeny were 27, 27, and 69% male. However, using males and females from more normal ratio families (males from 44 to 46% male families × females from 42 to 46% male families), seven spawns were obtained, of which five had sex ratios different than that of the parent family.

Sex ratios did not appear to be passed on from one generation to another in the fish used in our study. No family with skewed sex ratios produced progeny from sibling matings with similarly skewed sex ratios. Family VIII, which had a 1:1 male:female ratio, had a range of 43 to 68% male in its sets of progeny. Family V, which was 22% male, gave ten sets of progeny, of which five sets were > 70% male. Families II and VII, which were 100% male, when crossed with females from Family III gave sets of progeny ranging from 23 to 79% male. When the percentage of males in the female parent family (III, 40% male) was considered in matings with Family II and VII, 40% and 50% of the spawns, respectively, differed in sex ratios from the female parent family.

The low value of h^2 calculated from a linear regression between parents and offspring suggests little to no heritability of the trait sex ratio. This is contrary to studies concerning other populations where greater levels of heritability for sex ratio have been calculated. Lester et al. (1989), studying *O. niloticus* strains that may have been mixed with *O. mossambicus*, give a heritability value of 0.26 for half-sib families. Al Hafedh (1994), using a sire-dam model to determine sex ratio heritability, found 0.43 ± 0.05 in the first generation and 0.56 ± 0.10 in the second. The strains of *O. niloticus* used in our study have been inbred for a number of generations, experiencing several bottlenecks along the way. This may have been a factor

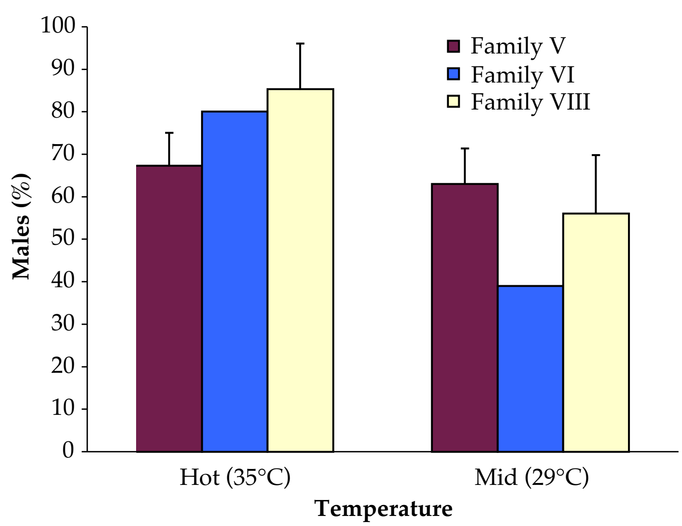

Figure 1. Percent males of fry from Families V, VI, and VIII held at a mid-range temperature (29°C) or at a hot temperature (35°C) during the period of gonadal differentiation.

Table 4. Temperature and percent male data for spawning and rearing periods.

Cycle	Spawning Period	Average Temperature (°C)	Rearing Period	Average Temperature (°C)	Mean Percent Male
A	5/1–6/3	26.90 ± 1.97	6/3–7/3	28.88 ± 2.34	51.17 ± 16.41
B	5/7–6/7	27.08 ± 1.55	6/7–7/7	29.23 ± 2.22	52.80 ± 10.48
C	5/14–6/14	27.93 ± 1.61	6/14–7/14	29.43 ± 1.08	55.71 ± 16.78
D	5/19–6/19	28.37 ± 2.38	6/19–7/19	29.59 ± 1.00	46.91 ± 10.14
E	5/26–6/26	28.72 ± 2.38	6/26–7/26	29.52 ± 0.87	44.00 ± 11.62
F	6/6–7/6	29.10 ± 2.32	7/6–8/6	29.48 ± 0.97	60.25 ± 17.02
G	6/10–7/10	29.53 ± 2.12	7/10–8/10	29.54 ± 1.00	50.33 ± 8.06
H	6/18–7/18	29.57 ± 0.98	7/18–8/18	29.42 ± 1.08	53.40 ± 22.70
I	6/25–7/25	29.52 ± 0.87	7/25–8/25	29.22 ± 1.13	52.25 ± 16.25
J	6/30–7/30	29.61 ± 0.92	7/30–8/30	29.07 ± 1.13	42.00 ± 20.07
K	7/3–7/31	29.45 ± 0.86	8/3–9/3	29.07 ± 1.19	38.00 ± 0.00

contributing to the low heritability. Low heritability for growth has been found in both the Ivory Coast and Ghana lines. Teichert-Coddington and Smitherman (1988) found a low realized heritability for fast growth in the Ivory Coast strain and attributed that to a small founder stock and subsequent generations of inbreeding. Hulata et al. (1986) found no response to selection for rapid growth using the Ghana strain of *O. niloticus*.

Water temperature during the time of gonadal differentiation did appear to affect sex ratios in two of the families tested. High water temperatures have been shown to skew sex ratios to male in other studies with *O. niloticus* and *O. aureus*. Baroiller et al. (1995) skewed the sex ratio of *O. niloticus* to more males by holding fry at 36°C during gonadal differentiation. Desprez and Mélard (1998) found similar results with *O. aureus*, where the sex ratio was altered to 97.8% male by holding non-hormone-treated fry at 34°C. Mair et al. (1990) also found that some matings of *O. niloticus* were more sensitive to the effects of temperature on sex ratio than others. This variation in response among individual fish to temperature is another factor to be considered in sex determination.

When all the matings from all families are considered as a whole, the percentage of males is normally distributed around a mean of 51.4 ± 14.2% (Figure 2).

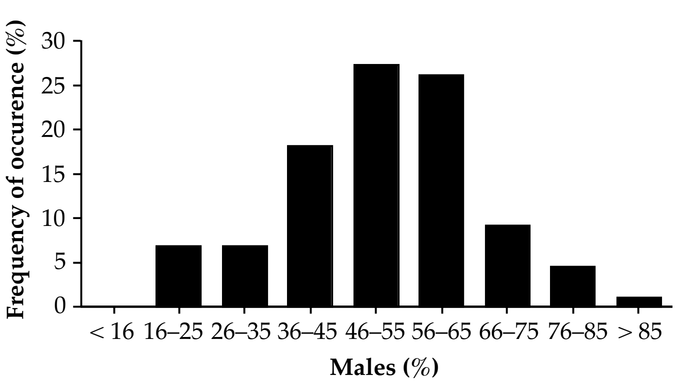

Figure 2. The frequency of sex ratios observed from the combined sets of progeny, where the parent sex ratios were skewed to male or female or not skewed.

This distribution pattern is similar to that seen in studies in which the sex ratios of individual spawns of normal fish are given (Shelton et al., 1983; Mair et al., 1991; Al Hafedh, 1994; Tuan et al., 1999; Warrington, 1999). This pattern suggests a polygenic mechanism of sex determination susceptible to environmental influences, where in any one mating a sex ratio that does not closely conform to 1:1 might be possible. None of the nine parent families when mated to siblings gave progeny sex ratios that closely matched that of the parent family. The diversity of sex ratios in such closed populations reflects a complex mechanism of sex determination making it difficult to produce a true-breeding line of fish for use in a YY breeding program.

Conclusions

The sex ratio produced by one set of parents was not a trait inherited and expressed by the progeny. The lack of consistency from one generation to another and among sibling matings to give a consistent sex ratio in their progeny provided evidence that a YY breeding program to produce male progeny will not be effective for the strains of *O. niloticus* evaluated.

Anticipated Benefits

The above study illustrates that sex determination in tilapia is a complicated mechanism of genetic expression not following a monofactoral mode of control. This degree of complication suggests that even a highly selective program for developing brooders for a YY breeding program will not be effective. This study illustrates the need for developing other methods of controlling tilapia reproduction.

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MONOSEX TILAPIA PRODUCTION THROUGH ANDROGENESIS

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

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ABSTRACT

Control of reproduction is vital to aquaculture and includes artificial propagation as well as management of unwanted recruitment. Developments in manipulation of the reproductive system provide options to enhance production. Nile tilapia, *Oreochromis niloticus,* spawning was managed by photoperiod and temperature manipulation. A controlled light cycle of 20L:4D and water temperature of 26 ± 2°C directed spawning to a predictable time frame. A developmental rate (t_0) relationship was described and applied to chromosome manipulation. Blond Nile tilapia are homozygous recessive for a color mutation that was used as a phenotypic marker in the development of protocol for androgenetic induction, while the color pigmentation for red Nile tilapia is dominant over the wild type color pattern. Androgenotes were produced by neutralizing the female genome of normal color Nile tilapia or that of red tilapia (600 J m^{-2} UV dose), eggs were activated with sperm from blond males or Ghana males, respectively, and then the eggs were diploidized with cold shock (11 ± 0.5°C for 60 min) applied at various times after incubation at 28 ± 0.2°C. Shock applied at 69 min post-activation produced greater numbers of androgenotes than shocks applied at 59 or 79 min post-activation; the shock application time of 69 min was used for induction with red tilapia stocks. Production of viable diploid androgenotes for crosses involving either red or blond and Ghana stocks was very low, and no progeny survived to maturity. Thus, neither verification of sex determination in androgenotes nor testing of monosex breeding was accomplished.

INTRODUCTION

Management of fish reproduction is important to aquaculture, as one of the major constraints to aquaculture development is the reliability and sustainability of seed organisms for culture. Reproductive management may involve production of seedstock through artificial propagation or, conversely, through development of limits to recruitment. Enhancement of spawning can be by habitat manipulation or through direct hormonal intervention, while reproductive control also can be direct or based on more complicated modifications (Shelton, 1989; Dunham, 1990). Within the context of tilapia farming, this limitation involves effective and practical control of unmanaged recruitment. Tilapia culture is one of the fastest growing forms of finfish aquaculture in the world. Production efficiency and product marketability in most economies depend on the capacity to control unwanted reproduction. Various approaches to monosexing fishes have been applied to culture. Techniques for managing recruitment have evolved from traditional means (predator control, hybridization, or hand-sexing) to the current practice of monosexing by steroid-induced sex reversal, which has been the industry standard during the past couple of decades. However, chemical treatment of food fish has become increasingly constrained, and since this tool could be withdrawn by governmental regulation, an alternative should be available. The use of chromosome manipulation is one of the more recently implemented practices (Shelton, 1989; Dunham, 1990).

Chromosome manipulation, which includes ploidy alteration or euploidy induction with single-parent genome contribution, can provide alternative control mechanisms. Ploidy manipulation includes the induction of triploids through polar body retention or tetraploidy through interference with first zygotic mitosis. Euploid alterations include gynogenetic (gynogenote) diploidization of the maternal genome through polar body retention (meiogynote) or mitotic interference (mitogynote). Gynogenesis has been investigated widely, but the development of techniques to induce androgenesis, i.e., progeny with only a paternal genome, is much less studied (Thorgaard and Allen, 1986). Diploidization of the paternal genome (androgenote) is accomplished by interference with first mitosis for eggs that have been genome-neutralized before activation. Androgenesis can provide a mechanism for the development of unique broodstock to use in producing all-male progeny without the use of sex-reversing steroids.

Within the contemporary atmosphere of increasing governmental regulation on the use of chemicals on food fish, continued dependency on steroid-induced monosexing places the culture of tilapias in a precarious position. The recently developed protocol to develop YY-male tilapia relies on estrogen treatment, and even though fish to be cultured are one generation removed from the treatment, the protocol still depends on progeny testing to identify target broodstock (Scott et al., 1989; Mair et al., 1997). In contrast, androgenesis offers the potential of direct induction for YY-male tilapia, without chemical treatment and ultimately without the need for progeny testing to identify the unique male broodstock. Although the genetics of sex determination are based on an incompletely characterized system (Wohlfarth and Wedekind, 1991; Mair, 1993), the perceived complications might be minimized by using select strains ("Monosex Tilapia Production through Androgenesis: Selection of Individuals for Sex Inheritance Characteristics for Use in Monosex Production," 9RCR6A; pp. 39–43 of this volume). A concurrent collaborative PD/A CRSP–funded study at Auburn University, Alabama, (R. Phelps, principal investigator) is examining strain variations in sex ratio inheritance. Androgenotes from the present study were to be progeny-tested (males and females) along with other strains as a component of examining the genetic basis of sex determination.

Assuming that sex determination is effected by control from a single pair of chromosomes, a monosex breeding program using YY-male broodstock might provide such a solution. Through androgenesis, YY-males can be induced directly, independent of steroids, and after verification, used without the need for subsequent progeny-test identification. Sex determination is characterized by a homogametic/heterogametic genotype; however, autosomal modifier genes may alter the theoretical 1:1 progeny sex ratio (Shelton et al., 1983; Wohlfarth and Wedekind, 1991; Mair, 1993). Therefore, deviations in the expected all-male progeny from YY-male breeding should be anticipated. Thus, this study proposed to investigate a protocol to produce androgenotes (progeny with only a paternal genome) and also to examine the basic mechanism of sex determination.

Androgenesis should result in offspring of equal sex ratio; females would be XX and males would be YY. Progeny testing will be required during experimental development to confirm that the males are fertile and that only male offspring (XY) will result when spawned with normal females (XX). Sex ratio of progeny from crosses of androgenote females with normal males should be 1:1, and presumptive YY-male androgenote crossed with normal females would be expected to produce only male progeny; these results would verify the assumptions of monochromosomal sex determination. The YY-males would then be a basis for developing a unique broodstock that would produce all-male progeny and add insight into the stability and fidelity of the sex-determining system in tilapia.

Tilapias do not respond well to hormonal induction for spawning control, but interruption of aquarium spawning so as to collect gametes might be sufficient to proceed with chromosome manipulation studies. Chromosome manipulation involves one or two basic treatments after obtaining fresh gametes (Thorgaard and Allen, 1986). For androgenesis the first treatment is the deactivation of the female genome. Ultraviolet (UV) irradiation is preferred for simplicity and safety but also because it dimerizes the DNA rather than fragmenting it. Egg activation with untreated spermatozoa then requires diploidization of the haploid zygote by some form of shock to interrupt the first mitotic division. Shock is most often physical, e.g., thermal (cold or hot) or pressure. Thermal treatment is usually preferred because of the ease of application and equipment simplicity. In order to prevent chromosome segregation, the shock must be timed to coincide with a cytological event, such as disruption of the spindle fibers during metaphase to prevent karyokinesis, or interference with the cell duplication during cytokinesis. Thus, shock type and intensity, duration, and time of application must be optimally combined into a protocol for maximum yield of diploid progeny. Further, because the rate of development is inversely temperature dependent, either the preshock incubation temperature must be standardized or the shock time must be calibrated to account for the temperature effect. Absolute shock time (minutes post-activation) can be transformed with reference to an index of development rate or mitotic interval (τ_0), also in minutes (Dettlaff and Dettlaff, 1961). Shock time (τ_s) can be related to tau (τ_s/τ_0) to report shock protocol in a dimensionless index, which is temperature compensated (Dettlaff, 1986). A tau curve for the Nile tilapia and gametic treatment with UV were described during an earlier segment of this study (Shelton, 1999), and one phase of the diploidization protocol was reported (Shelton, 2000). Both gametic treatments (UV and shock) are near lethal, increasing direct mortality, and further, the genomic diploidization will increase homozygosity, thereby reducing fitness. Thus, survival of viable androgenotes to maturity is expected to be quite low. This report describes the initial development of a protocol for the production of androgenetic progeny of Nile tilapia, *Oreochromis niloticus*.

METHODS AND MATERIALS

Broodstock

The University of Oklahoma stock of *O. niloticus* used in the earlier study segments was obtained from Auburn University, Alabama, in 1982, and it originated in Ivory Coast. The blond stock was derived from a population in Lake Manzala, Egypt (Scott et al., 1987; McAndrew et al., 1988) and was obtained from the University of Wales, Swansea, in 1987. Stock of the red mutant was also originally derived from Lake Manzala, Egypt, and a founder population for our work was obtained from Israel as about 50 juveniles in the fall of 1997. Broodstock development was delayed until 2000. The red color mutation is dominant to the wild pigmentation (McAndrew et al., 1988). During various studies the Ivory Coast population became hybridized to an unknown level with the blond stock. Since this is the phenotypic marker that was to be used in our study, a different stock was obtained for the final series of experiments. Nile tilapia from a Ghana source were obtained from Auburn University in 1998, but the fish in this founder stock were small. They were transferred to ponds for the summer to mature, then overwintered. Fish from this population were first used in experiments beginning in January 1999. Each stock (Ghana, blond, and Ghana × blond) was progeny-tested for color phenotype at developmental stages from hatching to swim-up; progeny testing of red stocks for color inheritance began in 2000.

Two basic developmental questions were initially addressed. One of these was the pigment pattern ontogeny in the various stocks—Ivory Coast, Ghana (G), and Egyptian (E)—and the two color mutations, blond (Bl) and red (R), used in the experiments that were developed from Egyptian stock (McAndrew et al., 1988). In the trials to induce androgenesis, one of the two gamete donors (male or female) was from one of the two color mutants. Pigment development was examined in the various stocks (IC, G, Bl, R) and their hybrids. The sequence of pigmentation provided a means of differentiating between normally fertilized offspring of IC or G or their hybrids with color mutants and larvae with only the genome of the color mutant stock (phenotypic marker). This latter would identify androgenote progeny as distinct from any offspring resulting from normal fertilization.

Spawning

Spawning was managed through regulation of photoperiod (20L:4D) and temperature (26 and 36°C), but this provided only limited control (Shelton, 1998, 1999). Although injection with gonadotropic materials is reported to lead to predictable ovulation (Gissis et al., 1988), we were not able to regularly duplicate this success. Therefore, behavior was observed so as to identify receptive females, and then stripping was accomplished in those instances when active courtship and spawning resulted; spawning was interrupted, and the eggs were collected from these females. Several females (four to six) were stocked with one male in four large aquaria (550 liters) or paired in 200-liter aquaria. Water was aerated and was circulated at the rate of one turnover per day, and temperature was maintained at 26 ± 2°C. The light cycle was controlled by adjusting a timer to turn on the overhead lighting at 0100 hours; the natural sunset regulated the end of the photoperiod for a total of about 20L:4D. Tilapia spawn about 8 to 10 h after the beginning of the light cycle (Myers and Hershberger, 1991). Ovulation and spawning readiness were judged by courtship behavior, coloration, and papilla erection (Rothbard and Pruginin, 1975; Rothbard, 1979). Females were stripped after initiation of spawning or as indicated by other characteristics that signal ovulation. Artificial propagation of tilapia includes gamete stripping, *in vitro* fertilization, and surrogate incubation; each component results in lower survival compared to normal spawning and *in vivo* incubation by the female (Rana, 1988).

Fertilization

For developmental rate experiments, eggs were collected in a clean container, milt was expressed over the eggs, and water was added directly to initiate activation (Rana, 1988). Fertilized eggs were placed in incubation chambers submerged in a water bath within 2 to 3 min, temperature was regulated closely, and 20 to 30 eggs were examined under magnification at intervals of 5 min to record cleavage rate. Developmental rate (τ_0) is defined by the duration of one mitotic cycle during early synchronous cleavage (Dettlaff and Dettlaff, 1961). Tau curves have been used to facilitate chromosome manipulation studies in various fishes (Shelton and Rothbard, 1993; Shelton et al., 1997). The tau curve of Nile tilapia was reported in an earlier progress report (Shelton, 1999); it was described from mean time intervals between the initiation of the first and third mitoses in 5 to 10% of the eggs over a range of temperatures (20 to 30°C). Subsequently, eggs were incubated at 28 ± 0.2°C in upwelling units of one liter capacity with sufficient flow to gently tumble the developing embryos. For progeny testing of pigment development, fertilized eggs from natural spawns as well as from *in vitro* fertilization were used; observations on the appearance and pattern of melanophores were recorded at prehatching, posthatching, and at swim-up stanzas for Ghana, blond, Ghana × blond, red, and both reciprocal crosses with Ghana. Fish occasionally spawned outside of periods of observation, but fertilized eggs were collected from the brooding females, moved to incubators, and pigmentation development observed.

Chromosome Treatment

Freshly ovulated eggs were stripped from normally pigmented Ghana females and allocated into four subsamples. Two to four hundred eggs (about 2 ml) were placed in each of four 10-cm petri dishes with enough 28°C water (10 ml) to provide slight buoyancy. Three subsamples were placed in a UV-crosslinker (FisherBiotech, FB-UVXL-1000) and exposed to 600 J m^{-2} UV; this dosage was determined in an earlier study to be sufficient to dimerize the female genome (Shelton, 1999). The UV-treated eggs were activated with freshly collected spermatozoa from a blond male, then maintained at 28°C on a rotating shaker table; the untreated eggs (control) were fertilized with milt from the same male. After 30 min all eggs were transferred from the petri dishes to screened, individual incubator chambers, which were submerged in a 28°C water bath. Treated eggs were subsequently plunged into water in a thermal bath at 11 ± 0.5°C for 60 min; one subsample was cold shocked at 59 min post-activation, the second at 69 min, and the third at 79 min. The first shock time coincided with the initial mitotic metaphase, the second just prior to cleavage furrow formation (cytokinesis), and the third immediately after cell division. After shock all eggs were returned to the water bath, and then they were transferred, including controls, into individual flow-through incubators. Development was monitored through hatching and swim-up. Beginning in 2000, red female broodstock were used as the egg donor and were fertilized (control) or activated (androgenesis) with sperm from Ghana males.

RESULTS AND DISCUSSION

Developmental Rate

Various experiments were conducted to measure developmental rate; 23 observations between 20 and 28°C established a tau curve relationship that permitted adjusting shock time to compensate for incubation temperature differences. For all experimental treatments, a replication as a control was included. Twenty-three tests of genome deactivation by UV-egg treatment at 400 to 600 J m^{-2} resulted in complete DNA dimerization. Spermatozoa from males of one of the color mutants was used to activate treated eggs. Twenty-three trials were used to optimize diploidization of the haploid male genome with a shock of 28°C for 60 min at 59 (1.8τ_0), 69 (2.1τ_0), and 79 (2.4τ_0) min post-activation at 28°C. The time of shock initiation was adjusted for incubation temperature using a developmental rate curve, or tau (τ_0) curve (Shelton, 1999). Gamete treatment with UV has been the mechanism used to remove the maternal genomic influence. Egg treatment for androgenesis is more difficult than treating spermatozoa in gynogenesis because of the difference in cell size and because of the complication of orientation with reference to nuclear position during exposure. A dose rate of about 600 J m^{-2} was determined to be effective to dimerize the egg DNA. After shock, eggs were hatched in flow-through incubators. The hatch rate of androgenotes was very low, and none of the androgenotes survived beyond juvenile stages. Thus, none reached maturity so that the expected sex ratio of 1:1 could be verified, nor could progeny testing of presumptive YY-males be accomplished so as to determine whether only male progeny would be produced.

Spawning and Progeny Testing

Photoperiod manipulation permitted spawning activity to be forced to mid-day. During experiments prior to ploidy studies, a total of 86 natural spawns and 87 strip spawns resulted in 44 and 16% hatch rate, respectively. Females ovulated and spawned between about 9 and 14 h (mean = 11.8 h) after the light-on cycle. Ovulation based on the light cycle was a reasonable means of predicting stripping time. Hormonal induction of ovulation would seem to be a logical extension, but cichlids have responded poorly to gonadotropic therapy (Rana, 1988). During the summer of 1998, while the Ghana broodstock were in ponds, progeny testing of blond stocks continued. Beginning in January 1999, the Ghana stock was incorporated in the spawning efforts. No spawning occurred among the Ghana stock until April, then progeny testing was initiated.

Five spawns were sufficient to establish the developmental pattern of pigment for this race. The appearance of melanophores in larval tilapia from normally pigmented broodstock is clearly differentiated from that for blond progeny; 26 crosses were made with red and Ghana broodstock (R female × G male, G female × R male , R female × R male), but only 16 resulted in progeny (Table 1). Again, the sequence of pigment development permitted identification of androgenotes from as early as prehatch embryos to swim-up; juvenile and adult pigment of red tilapia is entirely distinct from that of normal tilapia. The sequence of melanophore development in three areas is useful for identifying the parental source of progeny; these areas are the yolk, eye, and brain surface. Progeny of normally pigmented stocks (Ghana) developed melanophores first in the eyes and on the yolk surface; pigment cells could be seen 1 to 2 d prior to hatching. Eye color appeared about 12 h prior to hatch but was initially diffuse and then developed as a gradual blending of melanin and gold pigmentation. At hatching scattered chromatin-filled melanophores were present on the dorsal surface of the brain and laterally on the body, adjacent to the notochord and near the yolk sac, but subsequently, the melanophores progressed caudad and gradually increased in abundance. In contrast, progeny of blond stocks totally lacked pigmentation in these areas until post-hatching, and then coloration was limited to a gold cast in the eyes. Body color did not develop during the larval stages, and only non-pigment bearing melanophores could be seen on the yolk and brain surfaces. These appeared as shadowy cellular outlines that contained no melanin. Finally, hybrids between pigmented females (Ghana) and blond males developed pigmentation in the areas and in the sequence described for the Ghana stock. Thus, progeny that have dark coloration in the first several days post-hatching (until the mouth becomes functional) and possess genomic contribution from one or both pigmented parents, but not exclusively from the blond parent(s). The latter are non-pigmented throughout larval states. Pigment of the red color mutant is dominant in crosses with normally pigmented fish; development is first in the eyes, but pale and diffuse melanophores appear on the dorsal brain surface and lateral yolk. No permanent melanistic pigment develops subsequently; juveniles and adults are predominantly light colored. Androgenotes derived from blond male parents or red females can therefore be easily identified from pre-hatching through swim-up stages.

Chromosome Manipulation

Fertilization rate has been variable; in the six crosses involving red tilapia for androgenetic induction, some eggs from all crosses hatched (Table 2), but in the crosses with blond broodstock, 6 of 17 batches that were strip-spawned (artificially fertilized) from Ghana females had no development in controls, but in the 11 other strip-spawned groups that were artificially fertilized, the hatch rate in controls ranged from only 4.0 to 11.3% (mean 10.9%). Motility of spermatozoa was evaluated microscopically and was used only if more than 90% of cells were viable. Poor quality of ova is most often the primary cause of developmental failures. Initiation of egg resorption is common among tilapias, and although it does not prevent ovulation of the bad eggs, fertilization and hatching are poor (Peters, 1983). However, some physiological characteristics of tilapia gametes provide advantages in chromosome manipulation. Eggs retain high fertility for 3 to 6 h post-immersion in water (Myers et al., 1995), and sperm remain motile in water for several hours, in contrast to most fishes (Yeheskel and Avtalion, 1986).

Table 1. Pigment development during ontogeny of *Oreochromis niloticus* (G – Normal, Ghana; Bl – Blond, Egyptian; R – Red, Egyptian; functional lower jaw at swim-up) (+++ heavy melanophore pigment; ++ moderate; + light pigment; 0 no pigment; g gold pigment; melanophores gradually proliferate from initial scattered cells). Female is listed first in each of the progeny test crosses.

Stage at 28–30°C	Anatomical Feature	G × G	G × Bl	Bl × Bl	R × R	R × G	G × R
Pre-Hatch	Eye	++, g	0	0	+, g	+, g	+, g
	Head	0	0	0	0	0	0
	Yolk	++	++	0	0	0	0
Hatch	Eye	+++	+	0	++	++	++
	Head	++	+	*	+	+	+
	Yolk	+++	++	*	+	+	+
Swim-Up	Eye	+++	++	*	++	++	++
	Head	+++	++	*	+	+	+

* Empty cells but with no melanistic pigment.

Table 2. Summary* of induction trials to produce androgenetic Nile tilapia using UV-treated eggs (600 J m^{-2}) from Ghana strain females inseminated with sperm from blond (Egyptian strain) tilapia and diploidized by shock (11 ± 0.5°C for 60 min) at 1.8, 2.1, and 2.4τ_0.

Total Eggs	Control Hatch (N, %)	1.8τ_0 Hatch (N, %)	2.1τ_0 Hatch (N, %)	2.4τ_0 Hatch (N, %)	Survival to Maturity (N)
6,655	257, 16%	16, 6%**	103, 40%**	38, 14%**	0

* Data from 22 trials as reported in Shelton (2000).

** Note – Calculation error in published report (Shelton, 2000) for percent hatch, which is relative to the control; replicates were equal numbers of eggs subdivided into four groups (control and three treatments), and the original calculations used total egg number per batch as divisor rather than number hatched in the control.

Hatch rate of UV-treated and cold-shocked ova was significantly lower than that of controls, i.e., never more than a fraction of a percent. Relative to the controls, hatch rate ranged from about 3% of the control for the shock that started at 59 min after activation to about 22% of the control for the shock that started at 69 min in the Ghana × blond crosses and to about 7% in the red × Ghana crosses (Tables 2 and 3). Increased mortality resulting from the UV-dose was expected due to cell damage. The late-shock protocol for tetraploid induction that was successfully developed by Don and Avtalion (1988a, 1988b) and Don (1989) was used as the basis for induction of androgenesis. Cold shock has a wider time of application (60 min) compared to heat or pressure shock (2 to 5 min) and intuitively should provide a greater likelihood of some successful diploidization. Further, the shock application time used by the Israeli scientists is based on cytological correlations that relate well to time factors as described by tau adjustments (Saat, 1993; Shirak, 1996; Shirak et al., 1998). This is in sharp contrast to the empirical approach of Mair (1993), Hussain et al. (1993) and Myers et al. (1995), where shock time application had little relationship to cytological events.

Hatch in control groups for all six of the R × G crosses and in eleven trials (G × Bl) indicated reasonably good quality ova (Table 2). Induction of androgenetic progeny (UV-treated Ghana ova activated with sperm from blond males or UV-treated ova from red females and activated with sperm from Ghana males) was tested by cold-shocking at three post-activation times (59, 69, and 79 min at 36°C), which correspond to 0.75, 0.87, and 1.0T (T = time of first mitosis) or 1.8, 2.1, and 2.5τ_0, respectively. The former (0.75T) is the approximate time for first mitotic metaphase (Saat, 1993; Rubinshtein et al., 1997), while the second (0.87T) is just prior to first mitotic cytokinesis and the optimal induction time for late cold-shock in tilapia as determined by Shirak et al. (1998). The highest hatch rate of androgenotes (non-pigmented larvae) was at the 69-min post-activation shock time; the other two shock times had lower yields. Induction of androgenotes was attempted using eggs from red females and sperm from Ghana males, which were shocked only at 2.1τ_0 based on the earlier trials with G × Bl; induction success was generally lower at about 11% compared to about 40% (note, relative to control hatch) in the G × Bl crosses (Tables 2 and 3). Induction success for androgenotes to hatching does not ensure survival to later stages. From a total of 157 androgenotes that hatched from the G × Bl crosses, only 57 survived to swim-up, or yolk absorption (Table 2). Additional mortality during the juvenile stages further reduces the number that will be available for progeny testing to verify the production of YY males. None of the androgenotes of the G × Bl or R × G crosses survived to maturity, so no verification of sex determination in androgenotes was accomplished.

Optimization of shock protocol is affected by the asynchrony in cell division; at 28°C, the time from initiation of first cleavage to end of the last encompasses about 20 min for Nile tilapia (Shirak et al., 1998). Thus, the shock application and duration will affect only a small percentage of the cells at an optimal induction time. Further, even adjusting for the incubation temperature effect, optimization of treatment time for shock is complicated by apparent differences in effectiveness of various types of shock. Thermal shocks were used in the present study for practical reasons and because their effectiveness has been as good or better than pressure shock. Don and Avtalion (1988a) used cold shock for tetraploid induction based on comparative effectiveness for triploid induction (Don and Avtalion, 1988b). However, the timing for shock reported by Myers et al. (1995) at 27 min post-activation (28°C) markedly contradicts the optimal shock time of 92 min post-activation (26°C) reported by Don and Avtalion (1988a) and Don (1989), even when adjusted for the different incubation temperatures. We reported induction times in the context of developmental rates (τ_0) to adjust for temperature-induced differences. Further, significant differences in optimal shock initiation induction times for tilapia are apparently related to the type of shock (cold vs. heat or thermal vs. pressure). Palti et al. (1997) clearly demonstrated a difference in optimal shock time for gynogenetic induction with heat and pressure shock for rainbow trout, *Oncorhynchus mykiss*. Similarly, Hussain (1995) demonstrated differential optima between cold, heat, and pressure shock in Nile tilapia. Shirak et al. (1998) suggested that cold shock interferes with cell division, while the mechanism for heat shock has been considered to be disruption of the spindle fibers during karyokinesis (Mair, 1993).

Several factors contribute to the low yield and survival of androgenotes. Some of the effects are directly related to the severity of treatment (UV dose and temperature shock) as well as probable genetic influences. Ploidy manipulation depends on the exposure of gametes to near lethal conditions; consequently, elevated mortality is expected. Induced androgenesis includes several critical events: handling, UV exposure, and temperature shock. Eggs are more sensitive to handling than spermatozoa,

Table 3. Induction of androgenotes of Nile tilapia with UV-treated eggs from red tilapia (600 J m^{-2}) inseminated with sperm from Ghana strain tilapia and diploidized by cold shock (11 ± 0.5°C for 60 min) at 2.1τ_0.

Trial	Number of Eggs*	Number Hatched		Androgenote (Swim-Up)	Survival to Maturity
		Control	*Treatment*		
1	750	40	4	4	0
2	600	28	0	0	0
3	550	42	2	2	0
4	700	22	0	0	0
5	500	36	6	5***	0
6	450	16	0	0	0
Total	3,550	184 (10%)	12 (7%)**	11 (6%)**	0

* Divided between control and treatment; note androgenotes are normally pigmented.
** Relative to control.
*** One red-pigment individual (normally fertilized).

and the higher UV dose contributes to reduced viability; the treated eggs are subsequently subjected to a severe temperature. Because zygotes are diploidized by late shock, all loci are homozygous, which results in an abrupt increase in the pairing of detrimental or lethal genes. Thus, zygotes that survive the trauma of treatment have a reduced viability because of genomic influence.

Finally, evidence is increasing to suggest that sex determination among tilapias is not exclusively controlled by sex chromosomes, i.e., monofactoral, and that Mendelian, and therefore sex, ratios cannot be uniformly expected (Lester et al., 1989; Wolfarth and Wedekind, 1991; Trompka and Avtalion, 1993; Müller-Belecke and Hörstgen-Schwark, 1995). Shelton et al. (1983) reported unexpected variations in sex ratios among progeny of pair-spawned Nile and blue tilapia; Tuan et al. (1999) repeated the pair-spawning for a different strain of Nile tilapia and verified the results of Shelton et al. (1983). Further, Phelps et al. (1999) compared progeny sex ratios from pair-spawns of the three races of Nile tilapia used in the present study and found departures from the expected 1:1.

Poor survival of androgenotes from each broodstock continued throughout the study; the low probability of producing mature YY-males and the increasing evidence of complications with reference to sex determination for tilapias convinced us to suspend the study. Funds for the second year of this study were reallocated in a subcontract to cover a partial stipend for an Auburn University graduate student and travel support for an overseas principal investigator to the annual meeting.

ANTICIPATED BENEFITS

Control of unwanted reproduction in tilapia culture has been one of the primary considerations affecting successful food production. Various approaches have been developed and utilized. Most recently efforts to develop systems that will allow breeding programs to produce monosex cultures have been sought. Androgenesis seemed to offer a mechanism that could directly produce YY-males for use as broodstock to breed all-male progeny. Development of the induction technology was successful, but for various reasons survival of androgenotes to maturity failed, as did the test of the theoretical basis for sex determination and monosex production from androgenote broodstock.

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

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

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ABSTRACT

Immersion protocols have been unsuccessful in consistently producing all-male tilapia at a high enough ratio for them to be commercially viable. This study explored ultrasound to improve on the results of previous immersion studies. Experiments were carried out to evaluate immersion with ultrasound as a sex-reversal procedure by 1) assessing duration of treatment (one vs. two hours) on the efficacy of hormone to sex-reverse female tilapia and 2) examining the efficacy of various androgens. Due to low survival in experiment 2, experiment 3 was conducted with fewer treatments that included only two hormones (trenbolone acetate, TBA and 17α-methyldihydrotestosterone, MDHT) at two concentrations (100 and 250 μg l^{-1}) and with ultrasound (cavitation level).

Two-hour treatments with ultrasound resulted in a significantly higher percentage of males (92%) than one-hour (73%) treatments. Ultrasound treatment resulted in significantly higher percentages of males (94%) compared to treatments without ultrasound (89%). Two of the three replicates of the TBA-250 μg l^{-1} treatment in the third experiment resulted in 100% males and also in the highest percentage of males (98%). Variability within and between treatments with ultrasound was significantly lower (91 to 98%) than treatments with no ultrasound (83 to 94%). While there was no concentration effect, treatment of fry in TBA-250 μg l^{-1} and ultrasound resulted in significantly higher percentages of males (98.5%) than treatment with MDHT and ultrasound (90.5%). This study thus demonstrated the potential of a short-term immersion protocol using ultrasound to more predictably produce all-male tilapia seed.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*), one of the most commonly cultured species, is a prolific breeder. Males usually grow faster than females, so propagation of all-male populations is desirable to control reproduction and increase production. Methods to create all-male populations have been developed using various kinds of androgens (Pandian and Sheela, 1995). A number of hormones have been found to be effective, although only 17α-methyltestosterone (MT) is used for commercial production.

Current methods to masculinize populations involve administration of androgen incorporated in feed. Using this technique, newly hatched larvae held in hapas or ponds are fed directly on the pond surface. This method contaminates the environment. An inevitable inefficiency of this method lies in the uneven distribution of hormone as feeding hierarchies develop and some fish get more food than others. Loss of hormone results from uneaten food as well as dissolution from feed in the water column. Although dissolved hormones have been shown to break down in the water column within a week, long-term persistence of hormones has been documented in pond sediments (Fitzpatrick and Schreck, 1999). As an alternative to feeding, immersion of fish in hormone-containing solution has been used to masculinize salmonids and tilapia (Piferrer and Donaldson, 1989; Feist et al., 1995; Contreras-Sánchez et al., 1999).

In a previous study, Fitzpatrick et al. (1998) achieved variable masculinization of ten-day-old tilapia fry (20 to 92%) by using 17α-methyldihydrotestoterone (MDHT), trenbolone acetate (TBA), and MT. Immersing fish in MT solution for 2 and 48 hours resulted in only 20 to 25% males. Gale et al. (1999) achieved better results (73 to 92% male) when immersion treatment was applied for three hours on both day 10 and day 13 after fertilization. This inconsistent inversion of sex may be, in part, due to insufficient and nonuniform uptake of hormone by tilapia larvae.

A novel method that uses cavitation level ultrasound at low frequency has been used to enhance transport of compounds in human as well as fish tissues (Mitragotri et al., 1995; Bart et al., 2001). While Bart et al. (2001) used a cavitation effect to enhance delivery of a target compound (calcein) across fish skin and gills, Mitragotri et al. (1995) used a modified (reduced voltage) ultrasonic bath to achieve the cavitation effect to enhance permeation of insulin-like protein across human skin tissue. Both methods resulted in enhanced uptake of dissolved materials.

In the present study, an immersion protocol combined with ultrasound exposure was expected to increase transport of hormones from water into fish. Increased transport was expected to result in a more consistent and higher rate of masculinization of tilapia fry. The objective was to assess the effect of cavitation level ultrasound on sex inversion using four types of hormones—MT, androstenedione (AN), MDHT, and TBA—at various concentrations and immersion durations.

METHODS AND MATERIALS

Three experiments were conducted to evaluate variation in hormone type, duration of exposure, and influence of cavitation level ultrasound. Three-day-old fertilized eggs were collected from the Chitralada strain of Nile tilapia from broodfish held at the Asian Institute of Technology (AIT) aquaculture research ponds in Thailand. Fertilized eggs (three days post-fertilization, dpf) from 11 females were pooled and incubated in hatchery trays as described by Little et al. (1993). The hatched larvae were collected on the second day of incubation. Three days after hatch, a high-protein (40% crude protein) diet consisting primarily of fishmeal was fed five times per day. Temperature throughout the experimental period was maintained at 27 to 29°C.

Experiment 1 was a factorial (2 × 2 × 2) randomized block design. Variables were two hormones (AN and MT), two concentrations (100 and 500 μg l^{-1}), and two treatment durations (1 and 2 h) with three replicates for each treatment. Experiment 2 used a similar design consisting of three hormones (MT, MDHT, and TBA) and three concentrations (50 μg l^{-1} for MT and 250 or 100 μg l^{-1} for MDHT and TBA). In experiment 3, variables included two hormones (MDHT and TBA) at two concentrations (100 and 250 μg l^{-1}) with or without ultrasound. A total of 150 larvae per treatment, consisting of 50 larvae per replicate, were subjected to ultrasound.

The immersion solutions were prepared by mixing hormones MDHT, TBA, AN, and MT with water containing 0.5 ml l^{-1} of ethanol vehicle, resulting in final concentrations of 50 (MT), 100 (AN and MT), 250 (MDHT and TBA), and 500 μg l^{-1} (AN and MT). Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri). Fifty 10-dpf fry were randomly picked for each replicate and immersed in a modified ultrasound bath (Bransonic 1210, Danbury, Connecticut) containing 500 ml of solution at the treatment concentration of hormone. With the exception of the first experiment, all treatments used two-hour exposure to hormones. Three levels of controls were used, in which the first did not receive ultrasound, the second did not receive hormones, and the third did not receive either ultrasound or hormones.

The ultrasound bath was modified by reducing the voltage. Voltage was divided by placing a series of 10 to 60 W/5W resistors connected to the transducer. An analog meter (Class 2.5, Mu 45) was used to read the voltage output that drove the transducer. Voltage ranging from 190 to 520 V was fixed in six arbitrary incremental steps (190, 230, 420, 440, 480, and 520 V). At step three, when the voltage was 420, cavitation effects (microbubble formation, growth, and collapse) were clearly visible with the aid of a hand-held magnifying glass. Larvae were subjected to each step during the preliminary trials, and 420 V was found to be the highest level that could be delivered without causing mortality. Mortality associated with 420 V of ultrasound exposure was less than 3%, similar to that of the control, and was therefore selected for the two following experiments.

Larvae (10 dpf) receiving ultrasound were held in a bath solution for two hours with continuous pulse at 47 (± 6%) kHz. Fry were removed after treatment and placed in an aquarium with continuous aeration until 13 dpf. On day 13 they were subjected to the same treatment as described above and allowed to recover in the aquarium three or seven days prior to stocking in hapas in a pond.

Mortality was recorded at each step of the process. Survival rates were also assessed between stocking and harvest. Sex ratio was determined after 144 days post-hatch by simple dissection and observation of maturing gonads. Observation *in situ* (40X) revealed the presence of eggs or sperm. Difference in sex ratios between six treatments was analyzed using analysis of variance and chi-square test (α ± 0.05; Zar, 1984).

RESULTS

Experiment 1: Duration of Treatment

The use of ultrasound during immersion was expected to reduce the time required to achieve masculinization. Of the two duration treatments (1 and 2 h) assessed using AN and MT at concentrations of 100 or 500 μg l^{-1}, the two-hour treatment was found to yield a significantly larger percentage of males (92%) than the one-hour treatment (73%) ($P < 0.05$; Figure 1).

The immersion treatments using MT resulted in a higher average percentage of males (90%) compared with AN treatment (75%) when ultrasound was applied. Controls were subjected to immersion but not treated with ultrasound. While controls had one replicate, treatments had two replicates due to limited number of fry and time to conduct the trials. Percentage of males from MT treatment was higher than AN treatment (75 and 65%, respectively) regardless of the duration of treatment. This suggested higher efficacy of MT for sex inversion compared to AN. Efficacy of MT was especially amplified when the ultrasound was applied. Greater variation between replicates treated with AN also indicated that AN is a less useful hormone for sex inversion in tilapia using this protocol.

Survival immediately after the first treatment (10 dpf) was lower (85%) compared to the control (99%). After the second treatment (13 dpf), survival was further reduced (79%) compared to the control (97%). Prior to stocking (7 d post-treatment), overall survival was 53% in the ultrasound-only treatment group and high in both hormone immersion-only and control groups (74 and 76%, respectively; Figure 2). Treatment durations of one or two hours did not affect survival. Survival after seven weeks of rearing was again slightly lower in the treatments than in the control (43 and 55%, respectively).

The difference in survival between treatment and control during the early part of the experiment could be due to acoustic stress caused by ultrasound. This study was conducted during the early part of July, when dissolved oxygen in the seed collection ponds was chronically low (2.2 to 3.1 mg l^{-1}

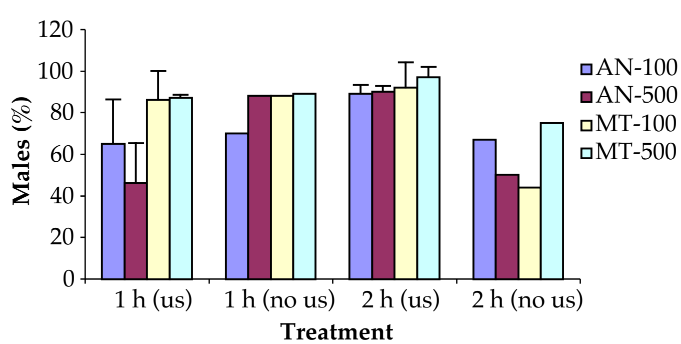

Figure 1. Percent males at harvest of Nile tilapia, immersed in AN and MT at 100 and 500 μg l^{-1} for one or two hour duration with or without ultrasound (us). The control was treated without ultrasound and without hormone but immersed in 5% ethanol solution.

due to overcast days), which may have affected the health of broodfish and subsequent health conditions of larvae. Another weakness of this study was the low number of replicates (two) per treatment, which made it difficult to carry out some statistical comparisons.

Experiment 2. Comparison of MT, MDHT, and TBA

The second experiment repeated the procedures of the first experiment, except it used three replicates per treatment and a lower MT dose (50 μg l^{-1}). It also tested MDHT and TBA as hormones at 100 and 250 μg l^{-1}.

Percent males resulting from this procedure was high in all treatments and significantly higher than in the control. The largest number of males was obtained from TBA-100 and TBA-250 treatments (97 and 96%, respectively). The lowest percentage of males resulted from immersion only in MDHT-250 (83%). No significant difference was found between fish treated with MT at 50 and 100 μg l^{-1}. While the effect of ultrasound was clearly evident for some hormone exposures, no difference in the effectiveness of ultrasound was observed among all three hormones and concentrations examined (Figure 3). The controls in this experiment were slightly male-biased (58%).

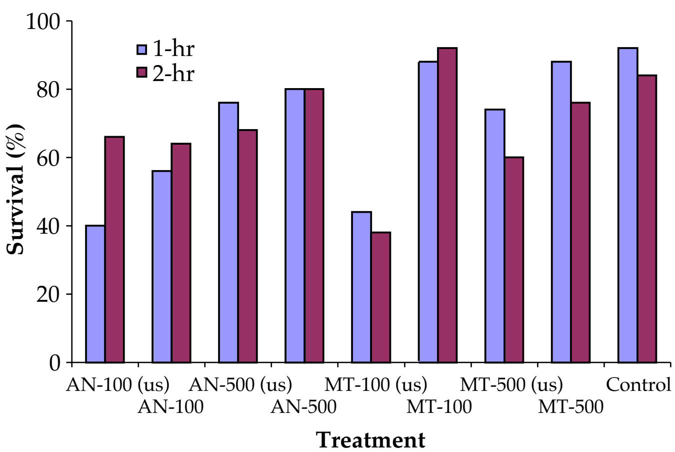

Figure 2. Percent survival at harvest of Nile tilapia immersed in AN and MT at 100 and 500 μg l^{-1} for one or two hour duration with or without ultrasound (us). The control was treated without ultrasound and without hormone but immersed in 5% ethanol solution.

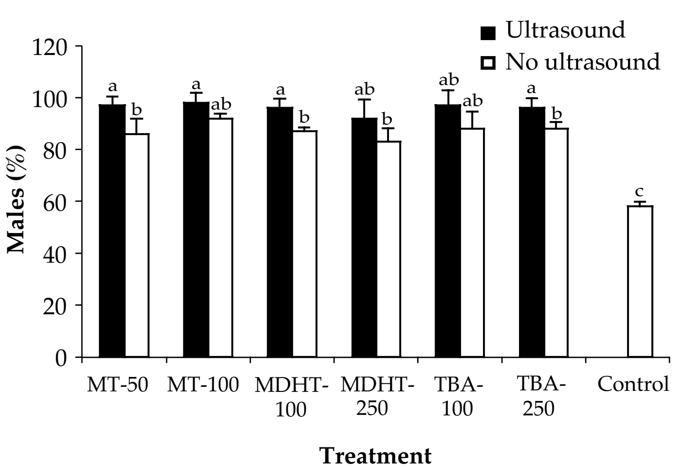

Figure 3. Percent males at harvest of Nile tilapia fry immersed in MT at 50 or 100 μg l^{-1} and MDHT or TBA at 100 or 250 μg l^{-1} with or without ultrasound (us). The control was treated without ultrasound and without hormone but immersed in 5% ethanol solution. Treatments with different letter superscripts indicate mean values that are significantly different ($P < 0.05$).

Although mortality measured immediately after both sets of ultrasound treatments was less than 4%, survival was severely affected by bacterial infection prior to stocking in the pond. All treatments were similarly affected and mean survival at harvest ranged from only 18 to 56% (Figure 4).

Experiment 3. Comparison of MDHT and TBA with and without Ultrasound

In order to verify the results from the second experiment, which had low survival at harvest in all treatments due to infection, a third experiment was carried out with only two hormones (MDHT and TBA), two concentrations (100 and 250 μg l^{-1}), and with or without ultrasound treatments.

Ultrasound resulted in significantly higher ($P < 0.05$) percentages of males (94%) compared to treatments without ultrasound (89%). The exception was MDHT-250, which had similar values between ultrasound treatments (Figure 5). Two of the three replicates of the TBA-250 treatment resulted in 100% males. Moreover, this entire treatment also resulted in the highest percentage of males (98%). Variation in percentage of males among fish not treated with ultrasound was significantly higher (83 to 94%) than variation among fish treated with ultrasound (91 to 98%). Within a treatment, fish not subjected to ultrasound were also slightly more variable in percentage of males (83 to 95%) than fish treated with ultrasound (89 to 100%). Although sex ratio was skewed toward males in the controls, those treated with ultrasound but no hormone also had a higher number of males ($P < 0.05$) than controls without hormone and ultrasound.

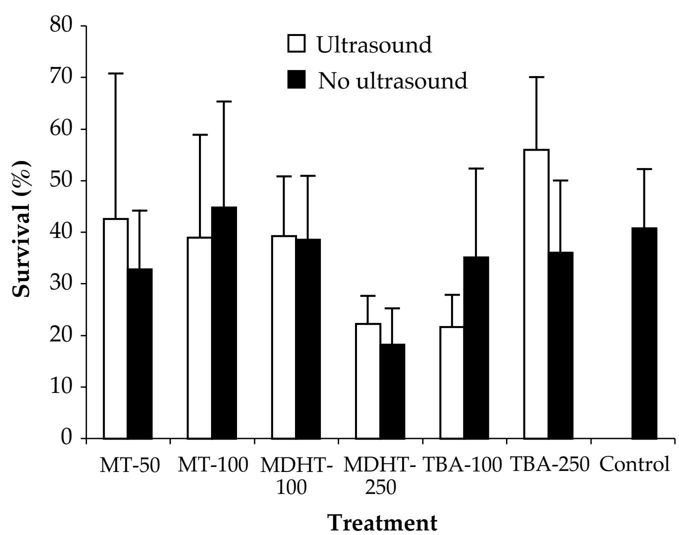

Figure 4. Percent survival at harvest of Nile tilapia fry immersed in MT at 50 or 100 μg l^{-1} and MDHT or TBA at 100 or 250 μg l^{-1} with or without ultrasound (us). The control was treated without ultrasound and without hormone but immersed in 5% ethanol solution.

Larvae treated in TBA resulted in a significantly higher percentage of males (94%) compared to MDHT (89%; $P < 0.05$). Furthermore, TBA treatment, when combined with ultrasound, resulted in a significantly greater number of males (98.5%) than MDHT with ultrasound (90.5%). There was no significant difference in the percentage of males resulting from fry in no-ultrasound treatments using the two hormones ($P > 0.05$). Higher concentrations of hormones (250 mg l^{-1}) did not result in a greater number of males for any treatment combination.

Survival to harvest for treated fish (90%) was not significantly different from controls (95% for fish with ultrasound and no hormones, 94% for fish with no ultrasound and no hormones; Figure 6). The survival of ultrasound-treated fry was 98.5% after the first and second treatments. Low mortality (< 2%) was observed during the treatment period, the post-treatment period, and at stocking (98.34%). This low mortality was not significantly different from control fish.

DISCUSSION

In a similar study Gale et al. (1999) used MT and MDHT immersion for 3 h at a rate of 500 and/or 100 µg l^{-1} and achieved an average of 92% males in the most effective treatment. Unfortunately, variability in the percentage of males between treatments ranged from 52 to 92%, with a mortality rate as high as 80% in one control. This enormous variation in the percentage of males between treatments and between similar experiments, although difficult to explain, could be partly because of nonuniform and differential diffusion of hormones. In comparison, our study using TBA and MDHT immersion for two hours at 100 and 250 µg l^{-1}, with continuous pulse ultrasound, had low mortality rates. The most effective treatment (TBA at 250 µg l^{-1}) resulted in 98% males, and in this treatment two replicates had 100% males. These results are comparable to those seen in larvae fed MT (Macintosh and Little, 1995). The number of males in any treatment never fell below 90% (90.5 to 98.4%) when ultrasound was used. Reduced variability in percentage of males between and within treatments may be due to a more uniform transport of hormones into fish larvae when ultrasound is applied. More focused studies are needed to identify the portal of entry and the kinetics of hormone movement across tissue that is facilitated by ultrasound.

TBA was clearly the most potent hormone, even when not exposed to ultrasound. At a higher concentration of TBA, immersed fry without ultrasound exposure resulted in more males (94%) than the MDHT treatment with ultrasound (91%). Despite lower concentrations (250 µg l^{-1}), more fish per batch (n = 50), and a higher density (1 fish $(5\ ml)^{-1}$) used in this study, the percentage of males was higher in all TBA treatments than in previous studies using other hormones (Varadaraj and Pandian, 1987; Leone and Ridha, 1993; Gale et al., 1999).

Although treatments were significantly different from the controls, controls were observed to have male-biased sex ratios (57%). The reason for such bias was not clear, and others (Gale et al., 1999) have reported a similar male-biased phenomenon. Similarly, controls that were exposed to ultrasound without hormones had a higher percentage of males (69%) and were significantly different ($P < 0.05$) from controls that did not receive hormones or ultrasound (57%). Ultrasound itself may have an effect on sex reversal, which needs further examination.

Immersion and ultrasound exposure did not significantly affect mortality in this experiment. Post-treatment evaluation immediately and three days after ultrasound exposure indicated no survival difference between treatments and controls. High survival was observed in all treatments (82 to 96%). The lowest survival (82%) was observed in treatment (TBA-250) without ultrasound, while the highest survival (96%) was observed in the treatment MDHT-100 with ultrasound. Controls had similar survival to the treatments.

ANTICIPATED BENEFITS

Three experiments were successfully conducted to assess the effect of ultrasound on production of all-male tilapia over six months. Each subsequent study benefited from lessons learned in the previous experiment. In all experiments the effect of ultrasound was clearly evident. The first experiment indicated that two-hour immersion combined with ultrasound produced consistently larger percentages of males than one-hour treatments. The second experiment illustrated that MT, MDHT, and

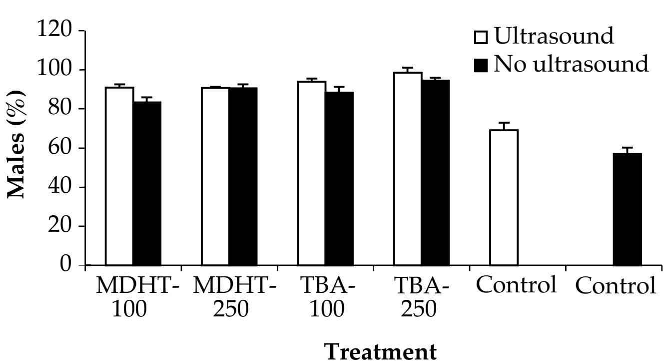

Figure 5. Percent males at harvest of Nile tilapia fry immersed in MDHT or TBA at 100 or 250 µg l^{-1} with or without ultrasound (us). The first control was treated with ultrasound and without hormone, and the second control was immersed in 5% ethanol solution without either ultrasound or hormone treatment. Treatments with different letter superscripts indicate mean values that are significantly different ($P < 0.05$).

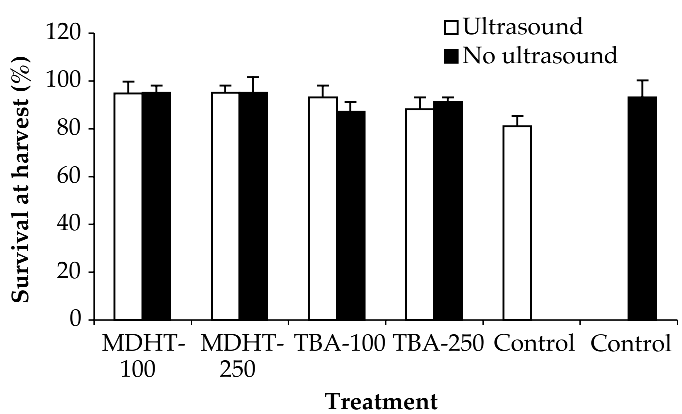

Figure 6. Percent survival at harvest of Nile tilapia fry immersed in MDHT or TBA at 100 or 250 µg l^{-1} with or without ultrasound (us). The first control was treated with ultrasound only, and the second control was immersed in 5% ethanol solution without either ultrasound or hormone treatment.

TBA are all potent inducers of masculinization in tilapia. However, the results were clouded by high mortality across all treatments, including the control. The third experiment was conducted to verify the observation of the second experiment with fewer variables. TBA at 250 µg l^{-1} was found to be the most effective hormone in consistently producing a large percentage (98 to 100%) of male tilapia. The results clearly indicate that this technique has the potential to replace the currently practiced technique of feeding testosterone for sex reversal of tilapia.

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LOTUS-FISH CULTURE IN PONDS: RECYCLING OF POND MUD NUTRIENTS

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

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ABSTRACT

An experiment was conducted in nine 200-m^2 fertilized earthen ponds at the Asian Institute of Technology, Thailand, from January to September 2000. This experiment was designed to assess the recovery of pond mud nutrient by lotus (*Nelumbo nucifera*), to assess pond mud characteristics after lotus-fish co-culture, and to compare fish growth with and without lotus integration. There were three treatments in triplicate: A) lotus-tilapia together; B) tilapia alone; and C) lotus alone. Seedlings (0.39 ± 0.09 kg) of Thai lotus variety were transplanted to ponds of treatments A and C at a density of 25 seedlings pond^{-1}, while sex-reversed all-male Nile tilapia (*Oreochromis niloticus*) fingerlings (8.6 to 10.3 g) were stocked at 2 fish m^{-2} in ponds of treatments A and B when the water depth had been increased to 50 cm due to increasing lotus height. Ponds stocked with tilapia (treatments A and B) were fertilized weekly with urea and triple superphosphate (TSP) at a rate of 28 kg nitrogen and 7 kg phosphorus ha^{-1} wk^{-1} after tilapia stocking. There was no fertilization in ponds of treatment C.

Lotus co-cultured with tilapia or cultured alone in ponds was able to effectively take up nutrients from old pond mud (about 300 kg N and 43 kg P ha^{-1} yr^{-1}) and resulted in the reduction of nutrients in mud by about 2.4 t N and 1 t P ha^{-1} yr^{-1}. There were no significant differences in lotus growth performance between treatments A and C, while Nile tilapia cultured alone grew significantly better than when co-cultured with lotus. The partial budget analysis indicates that lotus cultured alone generated the highest net return, and lotus contributed the largest portion of net income in lotus-tilapia co-culture. The present experiment has demonstrated the effectiveness of nutrient removal from old pond mud by lotus and the feasibility of rotation and co-culture of lotus and Nile tilapia technically and economically. Both systems can recycle nutrients effectively within ponds and are environmentally friendly culture systems.

INTRODUCTION

Regular fertilization and feeding in fish ponds result in nutrients being deposited in pond mud. One hectare of old pond mud was reported to have the equivalent of 1.85 tons of urea and 2.30 tons of triple superphosphate (TSP; Shrestha and Lin, 1997) or 2.8 tons of urea and 3.0 tons of TSP (Yang and Hu, 1989). Pond muds are a major sink for phosphorus, and adsorption capacity is related to mineral composition and clay content of pond muds (Shrestha and Lin, 1996). Release of adsorbed-P to the water column is minimal, and phytoplankton are not as effective in utilizing adsorbed-P as rooted crops. Roots extended in interstitial water of soil provide a better opportunity to extract P from soil (Denny, 1972; Boyd, 1982; Smart and Barko, 1985), and hence, nutrient-rich mud removed from fish ponds has been widely used to fertilize rooted land crops such as mulberry (Hu and Yang, 1984), forage crops (Yang and Hu, 1989), and maize (Christensen, 1989). However, removing pond mud is labor intensive and its practicability is questionable (Edwards et al., 1986; Little and Muir, 1987).

Alternatively, aquatic macrophytes may utilize reserve nutrients in muds by either rotation between two crops or co-culture with fish. Although in actual practice fish and aquatic macrophytes are rarely raised together in the same system, the co-culture and rotated culture of lotus (*Nelumbo nucifera*) and fish have been practiced in China for many years. Hoffmann (1934, cited by Edwards, 1987) reported that a farmer reared fish in the same pond as lotus in China but with only 50% of the usual number of fish because they grew more slowly than when raised alone. The rotation of fish and aquatic macrophytes may give farmers two crops to market rather than one and could sustain them if a loss occurred in one of the two ventures (Edwards, 1987).

Lotus is an aquatic emergent plant that grows as tall as 1.5 meters. Lotus is an important and popular cash crop in many Asian countries. Lotus has multiple uses, for example, stems as fresh vegetables; rhizomes as fresh vegetables, canned food, dessert, and starch; seeds as dessert and medicine; flowers as religious ornaments; and several parts as raw materials to produce cosmetics. It is commonly planted in fields or ponds with nutrient-rich mud and has a growing season of three to five months for the Chinese rhizome variety that does not flower or produce, and five to eight months for the Thai variety. It can extract nutrients from old pond mud efficiently.

Water levels of ponds can be increased as lotus grows. Fish can be stocked when water levels reach 30 cm and harvested four to five months after lotus is planted. Additionally, lotus shoots provide substrate for growth of epiphytic algae that are consumed by tilapia (Bowen, 1982; Lowe-McConnell, 1982; Shrestha and Knud-Hansen, 1994).

The purposes of this study were to:

1) Assess the pond mud nutrient recovery by lotus plants;
2) Assess pond mud characteristics after lotus-fish co-culture; and
3) Compare fish growth with and without lotus integration.

METHODS AND MATERIALS

The experiment was conducted using a randomized complete block design in nine 200-m^2 earthen ponds at the Asian Institute of Technology (AIT), Thailand, from January to September 2000. There were three treatments in triplicate: A) lotus-tilapia co-culture, B) tilapia alone, and C) lotus alone.

All ponds were used for intensive fish culture with commercial pelleted feed prior to this experiment. The ponds were dried for one month and filled with water to 10 cm deep one day prior to lotus transplanting. Seedlings of Thai lotus variety, purchased from a local farm, were transplanted to ponds of the lotus-tilapia and lotus alone treatments (A and C) at a density of 25 seedlings $pond^{-1}$ on 22 January 2000. The average length and weight of the transplanted lotus seedlings were 1 m and 0.39 ± 0.09 kg, respectively. After lotus seedlings were transplanted, water was added weekly to all ponds and water depth increased as the height of lotus increased. Sex-reversed all-male Nile tilapia (*Oreochromis niloticus*) fingerlings (8.6 to 10.3 g in size), obtained from AIT Hatchery, were stocked at 2 fish m^{-2} in the lotus-tilapia and tilapia-alone treatments (A and B) on 9 March 2000, when water depth reached 50 cm. Water depth was increased continuously up to 1 m with the growth of lotus, and it was maintained at 1 m throughout the rest of the experimental period by adding water weekly to replace evaporation and seepage losses. Ponds stocked with tilapia (treatments A and B) were fertilized weekly with urea and TSP at a rate of 28 kg nitrogen (N) and 7 kg phosphorus (P) ha^{-1} wk^{-1} after tilapia stocking. There was no fertilization in ponds of the lotus-alone treatment (treatment C).

During the experiment there was no fish sampling and no removal of dead lotus parts such as dead leaves from ponds. Matured lotus pods with seeds were harvested periodically and air-dried to separate seeds (with husk). On 14 September 2000, all ponds were drained. Tilapia were harvested after 189 days of culture, while different parts of lotus (flower, pod, leaf, stem, and root) were harvested separately (after 236 days of cultivation).

Integrated water samples were taken biweekly from the entire water column near the center of each pond at about 0900 h for analyses of pH, alkalinity, total ammonium nitrogen (TAN), nitrite-N, nitrate-N, total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), total phosphorus (TP), chlorophyll *a*, total suspended solids (TSS), and total volatile solids (TVS) (APHA et al., 1985; Egna et al., 1987). Water temperature and dissolved oxygen (DO) were also measured at the time of collecting water samples with a YSI model 54 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio).

The nutrient budgets for nitrogen and total phosphorus in ponds during the experimental period were calculated based on inputs from water, stocked tilapia fingerlings, transplanted lotus seedlings, fertilizers, and soil as well as on losses in harvested tilapia and lotus, discharge water, and mud. Mud samples were collected with 5-cm-diameter plastic tubes from the top 10 cm of pond bottom before lotus introduction and after fish and lotus harvest. Total nitrogen (TN) and TP in samples of mud and different parts of lotus and tilapia at the beginning and end of the experiment were analyzed using the methods described by Yoshida et al. (1976).

Data were analyzed statistically by analysis of variance and t-test (Steele and Torrie, 1980) using SPSS (version 7.0) statistical

Table 1. Growth performance of Nile tilapia cultured alone and integrated with lotus for 189 days in 200-m^2 ponds. Mean values with different superscript letters in the same row were significantly different ($P < 0.05$).

Parameters	Treatment A (*Lotus-Tilapia*)	Treatment B (*Tilapia Alone*)
STOCKING		
Density (fish m^{-2})	2	2
Total No. of Fish	400	400
Mean Weight (g $fish^{-1}$)	9.2 ± 0.4	9.6 ± 0.4
Total Weight (kg)	3.7 ± 0.2	3.9 ± 0.2
HARVEST		
Mean Weight (g $fish^{-1}$)	58.4 ± 3.1 ^{a}	117.5 ± 6.4 ^{b}
Total Weight (kg)	10.0 ± 1.3 ^{a}	38.5 ± 1.2 ^{b}
Survival Rate (%)	42.6 ± 3.7 ^{a}	82.3 ± 3.5 ^{b}
WEIGHT GAIN		
Mean Weight Gain (g $fish^{-1}$)	49.1 ± 3.5 ^{a}	107.8 ± 6.1 ^{b}
Daily Weight Gain (g $fish^{-1}$ d^{-1})	0.26 ± 0.02 ^{a}	0.57 ± 0.03 ^{b}
Total Weight Gain (kg)	6.3 ± 1.4 ^{a}	34.6 ± 1.2 ^{b}
Net Yield (kg ha^{-1} yr^{-1})	608.7 ± 134.8 ^{a}	3,344.6 ± 113.4 ^{b}
Gross Yield (kg ha^{-1} yr^{-1})	964.3 ± 123.3 ^{a}	3,717.0 ± 113.2 ^{b}

Table 2. Growth performance of lotus cultivated alone and integrated with Nile tilapia for 236 days in 200-m^2 ponds. Mean values with different superscript letters in the same row were significantly different ($P < 0.05$).

Parameters	Treatment A (*Lotus-Tilapia*)	Treatment C (*Lotus Alone*)
TRANSPLANTING		
Density (seedlings $pond^{-1}$)	25	25
Total Biomass (kg $pond^{-1}$)	9.90 ± 0.10	9.38 ± 0.48
HARVEST		
Leaf (kg $pond^{-1}$)	429.93 ± 37.07	450.19 ± 36.03
Pod (kg $pond^{-1}$)	11.50 ± 0.74	14.25 ± 1.61
Flower (kg $pond^{-1}$)	5.10 ± 2.86	7.16 ± 1.63
Root and Stem (kg $pond^{-1}$)	159.13 ± 14.92	177.33 ± 6.34
Total Biomass (kg $pond^{-1}$)	605.67 ± 32.89	648.92 ± 28.10
BIOMASS GAIN (kg $pond^{-1}$)	595.77 ± 32.87	639.55 ± 28.17
NET YIELD (t ha^{-1} yr^{-1})	46.07 ± 2.54	49.46 ± 2.18
GROSS YIELD (t ha^{-1} yr^{-1})	46.84 ± 2.54	50.18 ± 2.17

software package (SPSS Inc., Chicago, Illinois). Differences were considered significant at an alpha level of 0.05. All means were given with ± 1 standard error (SE).

A partial budget analysis was conducted to determine economic returns of lotus-tilapia integrated culture, tilapia alone, and lotus alone (Shang, 1990). The analysis was based on farm-gate prices in Thailand for harvested tilapia and lotus products (seeds and flowers) and on current local market prices for all other items expressed in US dollars (US$1 = 40 baht). Farm-gate price of Nile tilapia varied with size: \$0.250 kg^{-1} for size 50 to 100 g and \$0.375 kg^{-1} for size 100 to 200 g. Farm-gate prices of lotus seeds and flowers were \$0.75 kg^{-1} and \$0.125 $piece^{-1}$, respectively. Market prices of sex-reversed all-male Nile tilapia fingerlings (\$0.0125 $piece^{-1}$), lotus seedlings (\$0.125 $piece^{-1}$), urea (\$0.1875 kg^{-1}), and TSP (\$0.3125 kg^{-1}) were used. The calculation for cost of working capital was based on an annual interest rate of 8%.

RESULTS

All growth performance parameters showed that Nile tilapia grew significantly better in the tilapia-alone treatment (B) than in the lotus-tilapia treatment (A), indicating that lotus had significantly negative effects on tilapia growth when they were cultured together ($P < 0.05$, Table 1). Lower survival coupled with slower growth caused only a marginal gain of tilapia biomass in the lotus-tilapia treatment (A, Table 1). Although there were no nutrient inputs in ponds of the lotus-alone treatment (C), lotus growth performance was slightly higher in treatment C than in the lotus-tilapia treatment (A), but this was not significant ($P > 0.05$, Table 2). The addition of chemical fertilizers did not increase lotus biomass production in the present experiment.

The proximate compositions of Nile tilapia, lotus, and mud are summarized in Table 3. The nutrient budgets indicate that the dominant nutrient source was mud in all treatments (Table 4). At the end of the experiment, mud in all treatments still contained the most TN and TP, followed by lotus, while tilapia only contained a small fraction of nutrients from mud or fertilizers (Table 4). In treatments with lotus (A and C), there were no significant differences in nutrient contents between each output component ($P > 0.05$), which were significantly different in nutrient content from the treatment without lotus ($P < 0.05$, Table 4). The largest portion of both TN and TP that disappeared from ponds was not accounted for, and the unaccounted TN and TP contents were significantly higher in the lotus-tilapia treatment than in the tilapia-alone and lotus-alone treatments ($P < 0.05$, Table 4). There were no significant differences in amounts of nutrients recovered by lotus between the lotus-tilapia treatment and the lotus-alone treatment

Table 3. Moisture (%) and TN and TP composition (mg g^{-1}, dry matter basis) in Nile tilapia, lotus, and mud for each treatment.

Parameters	Beginning			End		
	A	*B*	*C*	*A*	*B*	*C*
TILAPIA						
Moisture	75.50	75.50	----	79.80	78.20	----
TN	100.15	100.15	----	102.00	95.97	----
TP	9.20	9.20	----	11.27	10.56	----
LOTUS SEEDLING						
Moisture	62.80	----	62.80	----	----	----
TN	5.65	----	5.65	----	----	----
TP	5.35	----	5.35	----	----	----
LOTUS LEAF						
Moisture	----	----	----	73.11	----	74.86
TN	----	----	----	31.16	----	30.94
TP	----	----	----	3.53	----	3.52
LOTUS FLOWER						
Moisture	----	----	----	77.63	----	75.98
TN	----	----	----	22.76	----	24.45
TP	----	----	----	3.54	----	3.72
LOTUS POD						
Moisture	----	----	----	74.24	----	74.22
TN	----	----	----	17.35	----	19.63
TP	----	----	----	2.41	----	2.89
LOTUS STEM AND ROOT						
Moisture	----	----	----	86.05	----	85.90
TN	----	----	----	14.96	----	13.06
TP	----	----	----	6.33	----	6.73
MUD						
Moisture	40.41	44.01	37.58	58.13	54.14	58.82
TN	2.18	1.79	2.12	0.34	0.94	0.35
TP	1.12	0.94	0.98	0.34	0.62	0.35

($P > 0.05$), while the amount of nutrients recovered by tilapia was significantly higher in the tilapia-alone treatment than in the lotus-tilapia treatment ($P < 0.05$, Table 5). The inclusion of lotus in ponds resulted in the significantly greater reduction of nutrient contents in mud compared to ponds without lotus ($P < 0.05$, Table 5). However, the application of chemical fertilizers in the lotus-tilapia treatment did not cause significantly greater amounts of nutrients to remain in pond mud than were present in the lotus-alone treatment, in which no chemical fertilizers were added ($P > 0.05$, Table 5). In the lotus-alone treatment (without adding fertilizers), lotus could recover about 301 kg N and 43 kg P ha^{-1} yr^{-1}, which resulted in the reduction of nutrients contained in mud by 2.38 t N and 0.91 t P ha^{-1} yr^{-1} (Table 5).

The mean and final values of water quality parameters indicated that DO concentrations at dawn were significantly higher in the tilapia-alone treatment than in the treatments with lotus throughout the experimental period ($P < 0.05$, Table 6, Figure 1). The pH fluctuated during the experimental period and was significantly lower in treatment A than in treatments B and C at the end of the experiment ($P < 0.05$). Mean pH values were not significantly different among treatments ($P > 0.05$). Water temperature ranged from 26.0 to 32.2°C over the experimental period, and the mean values were significantly lower in the treatments with lotus than in the tilapia-alone treatment ($P < 0.05$, Figure 1). Alkalinity in the treatments with tilapia decreased over time and was significantly lower than in the treatment without tilapia ($P < 0.05$, Figure 2). Concentrations of different nitrogen forms were significantly higher in the tilapia-alone treatment, intermediate in the lotus-tilapia treatment, and lower in the lotus-alone treatment ($P < 0.05$, Figure 2), while there were no significant differences in TP and SRP concentrations among treatments. Concentrations of chlorophyll *a* and solids (TSS and TVS) were also significantly higher in the tilapia-alone treatment, intermediate in the lotus-tilapia treatment, and lowest in the lotus-alone treatment ($P < 0.05$, Figure 3).

The partial budget analysis (Table 7) indicated that the lotus treatments produced positive net returns, while tilapia alone had a negative net return. The lotus-alone treatment produced the highest net return because there were no other inputs except lotus seedlings.

DISCUSSION

It is feasible to co-culture tilapia and lotus in the same ponds. Compared with Nile tilapia growth in most semi-intensive culture, Nile tilapia grew quite slowly even in the tilapia-alone treatments, which might be related to the decreasing alkalinity throughout the experimental period due to no liming in this experiment. The significantly lower growth and higher mortality of tilapia in the lotus-tilapia treatment might result from shading by lotus leaves, which could reduce phytoplankton production and cause low DO concentration in ponds. Dead lotus leaves were not removed from ponds, and the decomposition further worsened the water quality, especially DO. If the lotus density is optimized and dead lotus vegetation is well managed, such an integrated lotus-tilapia co-culture system may have potential in many Asian countries where lotus is commonly cultivated.

The production of lotus biomass in ponds without fertilization was not significantly different from that in ponds stocked with tilapia and fertilized. This indicates that the nutrient content in the old pond mud was sufficient or exceeded the amount required for lotus growth. Shrestha and Lin (1997) reported that nutrients diluted by 50% in old pond mud were sufficient to support the growth of cowpea (*Vigna unguiculata* L. Walp.), a terrestrial legume, and taro (*Colocasia esculenta* L. Schott), a semi-aquatic crop, in pot experiments. The extrapolated lotus

Table 4. Nitrogen and phosphorus budgets in different treatments over the 236-day experimental period. Mean values of nutrient outputs and gains with different superscript letters in the same row within each nutrient category were significantly different ($P < 0.05$).

Parameters (kg)	Total Nitrogen			Total Phosphorus		
	A	*B*	*C*	*A*	*B*	*C*
INPUTS						
Fertilizers	14.90 ± 0.00	14.90 ± 0.00	----	3.70 ± 0.00	3.70 ± 0.00	----
Lotus	0.02 ± 0.00	----	0.02 ± 0.00	0.02 ± 0.00	----	0.02 ± 0.00
Tilapia	0.09 ± 0.00	0.10 ± 0.00	----	0.01 ± 0.00	0.01 ± 0.00	----
Water	0.27 ± 0.01	0.35 ± 0.01	0.28 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Mud	36.06 ± 2.98	31.67 ± 1.59	35.04 ± 3.63	18.86 ± 1.49	17.18 ± 1.61	16.13 ± 1.76
Total	51.34 ± 2.99	47.02 ± 1.57	35.34 ± 3.63	22.63 ± 1.49	20.95 ± 1.61	16.19 ± 1.76
OUTPUTS						
Lotus	3.94 ± 0.54	----	3.92 ± 0.12	0.56 ± 0.05	----	0.58 ± 0.03
Tilapia	0.22 ± 0.03 [a]	0.79 ± 0.01 [b]	----	0.02 ± 0.00 [a]	0.09 ± 0.01 [b]	----
Water	0.51 ± 0.03 [a]	1.85 ± 0.19 [b]	0.46 ± 0.18 [a]	0.07 ± 0.02	0.06 ± 0.00	0.06 ± 0.01
Mud	5.68 ± 0.84 [a]	14.84 ± 1.77 [b]	4.27 ± 0.55 [a]	5.35 ± 0.52 [a]	9.68 ± 0.68 [b]	4.39 ± 1.14 [a]
Total	10.34 ± 1.33 [a]	17.48 ± 1.57 [b]	8.64 ± 0.75 [a]	6.00 ± 0.54 [a]	9.84 ± 0.68 [b]	5.03 ± 1.17 [a]
GAINS						
Lotus	3.92 ± 0.54	----	3.90 ± 0.12	0.54 ± 0.05	----	0.56 ± 0.03
Tilapia	0.13 ± 0.04 [a]	0.70 ± 0.01 [b]	----	0.02 ± 0.00 [a]	0.08 ± 0.01 [b]	----
Water	0.23 ± 0.02 [a]	1.50 ± 0.19 [b]	0.18 ± 0.18 [a]	0.03 ± 0.02	0.01 ± 0.00	0.02 ± 0.01
UNACCOUNTED	41.00 ± 3.05 [a]	29.54 ± 0.89 [b]	26.69 ± 2.90 [b]	16.63 ± 1.03 [a]	11.12 ± 1.12 [b]	11.16 ± 0.67 [b]

Table 5. The efficiency of nutrient removal from applied fertilizers and mud in different treatments over the 236-day experimental period. Mean values with different superscript letters in the same row were significantly different ($P < 0.05$).

Parameters	Treatments		
	A	*B*	*C*
NITROGEN			
Recovered by Lotus			
(kg pond^{-1})	3.92 ± 0.54	----	3.90 ± 0.12
(kg ha^{-1} yr^{-1})	303.05 ± 41.56	----	301.20 ± 9.49
(%)	7.78 ± 1.21 ^{a}	----	11.29 ± 0.83 ^{b}
Recovered by Tilapia			
(kg pond^{-1})	0.13 ± 0.03 ^{a}	0.70 ± 0.01 ^{b}	----
(kg ha^{-1} yr^{-1})	9.71 ± 2.69 ^{a}	54.02 ± 0.42 ^{b}	----
(%)	0.25 ± 0.07 ^{a}	1.50 ± 0.05 ^{b}	----
Total Recovered			
(kg pond^{-1})	4.04 ± 0.57 ^{a}	0.70 ± 0.01 ^{b}	3.90 ± 0.12 ^{a}
(kg ha^{-1} yr^{-1})	312.78 ± 44.20 ^{a}	54.02 ± 0.42 ^{b}	301.20 ± 9.49 ^{a}
(%)	8.03 ± 1.28 ^{b}	1.50 ± 0.5 ^{c}	11.29 ± 0.83 ^{a}
Reduction in Mud			
(kg pond^{-1})	30.38 ± 2.71 ^{a}	16.84 ± 0.94 ^{b}	30.77 ± 3.09 ^{a}
(t ha^{-1} yr^{-1})	2.35 ± 0.21 ^{a}	1.30 ± 0.07 ^{b}	2.38 ± 0.24 ^{a}
(%)	84.24 ± 2.06 ^{a}	53.37 ± 3.70 ^{b}	87.87 ± 0.32 ^{a}
PHOSPHORUS			
Recovered by Lotus			
(kg pond^{-1})	0.54 ± 0.05	----	0.56 ± 0.03
(kg ha^{-1} yr^{-1})	41.87 ± 3.87	----	43.44 ± 2.35
(%)	2.41 ± 0.21 ^{a}	----	3.53 ± 0.29 ^{b}
Recovered by Tilapia			
(kg pond^{-1})	0.02 ± 0.00 ^{a}	0.08 ± 0.01 ^{b}	----
(kg ha^{-1} yr^{-1})	1.17 ± 0.15 ^{a}	6.13 ± 0.62 ^{b}	----
(%)	0.07 ± 0.01 ^{a}	0.39 ± 0.06 ^{b}	----
Total Recovered			
(kg pond^{-1})	0.56 ± 0.05 ^{a}	0.08 ± 0.01 ^{b}	0.56 ± 0.03 ^{a}
(kg ha^{-1} yr^{-1})	43.04 ± 4.02 ^{a}	6.13 ± 0.62 ^{b}	43.44 ± 2.35 ^{a}
(%)	2.48 ± 0.22 ^{b}	0.39 ± 0.06 ^{c}	3.53 ± 0.29 ^{a}
Reduction in Mud			
(kg pond^{-1})	13.51 ± 1.02 ^{a}	7.50 ± 1.12 ^{b}	11.74 ± 0.69 ^{a}
(t ha^{-1} yr^{-1})	1.04 ± 0.08 ^{a}	0.58 ± 0.09 ^{b}	0.91 ± 0.05 ^{a}
(%)	71.70 ± 1.06 ^{a}	43.25 ± 3.33 ^{b}	73.56 ± 3.89 ^{a}

biomass gain was about 11 dry t ha^{-1} yr^{-1} in this experiment, which was similar to that (11 to 16 t ha^{-1} yr^{-1}) of lotus planted in an old pond (calculated from Mon, 2000) and that (10 to 12 t ha^{-1} yr^{-1}) of cowpea in pots but lower than that (35 to 46 t ha^{-1} yr^{-1}) of taro planted in pots filled with old pond mud (calculated from Shrestha and Lin, 1997).

One hectare of old pond mud has been reported to contain the equivalent of 1.85 t urea and 2.30 t TSP (Shrestha and Lin, 1997) or 2.8 t urea and 3.0 t TSP (Yang and Hu, 1989). The old mud of ponds used in this experiment contained higher nutrient concentrations, which were equivalent to 3.44 to 3.92 t urea and 4.11 to 4.81 t TSP ha^{-1}. After 236-day cultivation of lotus, pond mud nutrients decreased by about 1.53 t N ha^{-1} and 0.63 t P ha^{-1} (more than 80% of N and 70% of P contained in old pond mud), which are equivalent to 3.33 t urea and 3.22 t TSP. This reduction was much higher than that reported by Mon

Table 6. Final and mean values of water quality parameters measured in different treatments over the experimental period. Mean values with different superscript letters in the same row were significantly different among treatments ($P < 0.05$).

Parameters	Treatments		
	A	*B*	*C*
FINAL VALUES			
DO (mg l^{-1})	1.23 ± 0.12 ^{a}	4.27 ± 0.57 ^{b}	0.81 ± 0.40 ^{a}
Temperature (°C)	27.0 ± 0.2	27.7 ± 0.1	27.0 ± 0.6
pH	6.3 ± 0.1 ^{a}	6.7 ± 0.2 ^{b}	6.8 ± 0.1 ^{b}
Alkalinity (mg l^{-1} as $CaCO_3$)	23 ± 10.5 ^{a}	15 ± 1.8 ^{a}	125 ± 20.7 ^{b}
TKN (mg l^{-1})	2.72 ± 0.18 ^{a}	10.18 ± 1.14 ^{b}	2.51 ± 0.96 ^{a}
TAN (mg l^{-1})	0.59 ± 0.43 ^{a}	3.06 ± 0.12 ^{b}	0.05 ± 0.05 ^{a}
Nitrite-N + Nitrate-N (mg l^{-1})	0.09 ± 0.04 ^{a}	0.37 ± 0.08 ^{b}	0.00 ± 0.00 ^{a}
TP (mg l^{-1})	0.36 ± 0.11	0.34 ± 0.01	0.32 ± 0.04
SRP (mg l^{-1})	0.17 ± 0.12	0.04 ± 0.02	0.19 ± 0.06
Chlorophyll *a* (mg m^{-3})	21 ± 5.1 ^{a}	57 ± 8.9 ^{b}	19 ± 2.5 ^{a}
TSS (mg l^{-1})	20 ± 1.0 ^{a}	61 ± 6.9 ^{b}	10 ± 2.4 ^{a}
TVS (mg l^{-1})	10 ± 0.8 ^{a}	14 ± 1.4 ^{b}	7 ± 1.5 ^{a}
MEAN VALUES			
DO (mg l^{-1})	1.81 ± 0.16 ^{a}	4.35 ± 0.27 ^{b}	1.40 ± 0.26 ^{a}
Temperature (°C)	28.5 ± 0.1 ^{b}	29.8 ± 0.0 ^{a}	28.0 ± 0.2 ^{c}
pH	6.9 ± 0.0	7.0 ± 0.1	7.0 ± 0.1
Alkalinity (mg l^{-1} as $CaCO_3$)	61 ± 5.7 ^{a}	45 ± 1.9 ^{a}	98 ± 4.2 ^{b}
TKN (mg l^{-1})	3.22 ± 0.12 ^{a}	6.32 ± 0.46 ^{b}	1.96 ± 0.45 ^{a}
TAN (mg l^{-1})	2.14 ± 0.26 ^{b}	3.42 ± 0.22 ^{a}	0.23 ± 0.02 ^{c}
Nitrite-N + Nitrate-N (mg l^{-1})	0.55 ± 0.13 ^{a}	0.56 ± 0.05 ^{a}	0.12 ± 0.01 ^{b}
TP (mg l^{-1})	0.27 ± 0.03	0.18 ± 0.01	0.20 ± 0.03
SRP (mg l^{-1})	0.07 ± 0.04	0.01 ± 0.00	0.08 ± 0.03
Chlorophyll *a* (mg m^{-3})	22 ± 0.9 ^{b}	40 ± 2.2 ^{a}	14 ± 1.5 ^{c}
TSS (mg l^{-1})	22 ± 1.6 ^{b}	49 ± 1.5 ^{a}	14 ± 1.7 ^{c}
TVS (mg l^{-1})	9 ± 0.4 ^{a}	12 ± 0.5 ^{a}	7 ± 0.2 ^{b}

(2000; 1.16 t N ha^{-1} and 0.39 t P ha^{-1}), due probably to the shorter cultivation period (119 days) in that experiment. Lotus incorporated about 12.8% N and 4.4% P from pond mud in the present experiment. Lotus did take up N by 0.30 t ha^{-1} yr^{-1} and P by 0.04 t ha^{-1} yr^{-1}, which are rates similar to those taken up by lotus (0.30 to 0.44 t N ha^{-1} yr^{-1} and 0.04 to 0.05 t P ha^{-1} yr^{-1}) reported by Mon (2000), but lower than amounts taken up by cowpea and taro (0.68 t N ha^{-1} yr^{-1} and 0.06 t P ha^{-1} yr^{-1}, and 0.73 t N ha^{-1} yr^{-1} and 0.09 t P ha^{-1} yr^{-1}, respectively) in pot experiments (calculated from Shrestha and Lin, 1997).

In the aquatic macrophyte–fish co-culture system, the main problem we found was the low water quality for fish growth due to the shading effects of lotus. The shading effects of macrophytes may lead to reduced phytoplankton production, lower DO concentration, and increased concentration of free carbon dioxide in the water column with a concomitant fish kill (Edwards, 1980). In the present experiment, we believe the shading effects of lotus leaves caused lower phytoplankton standing crop and lower DO concentrations, resulting in the poor performance of Nile tilapia in the lotus-tilapia co-culture system. However, lotus might help maintain higher alkalinity and lower TAN levels, which might potentially benefit the co-cultured fish. Thus the lotus-tilapia co-culture system needs further testing.

The net return from selling lotus seeds and flowers was highest in the ponds without fertilization but with lotus alone; sale of lotus seeds and flowers contributed to the largest portion of net return from ponds with lotus and tilapia. If the experimental period were adjusted or extended for two months to cover the cool season for developing lotus rhizomes, the net return would be much higher because lotus rhizomes fetch a good price. In an experiment using the Chinese vegetable variety of lotus to recover nutrients from old pond mud, the extrapolated rhizome production from 6-m^2 compartments in a 200-m^2 pond for four-month cultivation of lotus reached 38 t ha^{-1} yr^{-1} and a value of about US$12,000 (Mon, 2000).

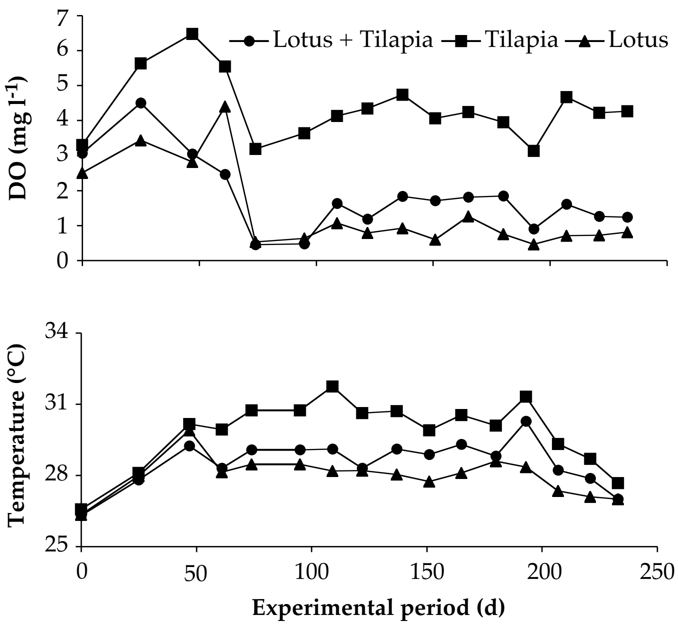

Figure 1. Mean DO and temperature (0900 h) in all treatments over the 160-day experimental period.

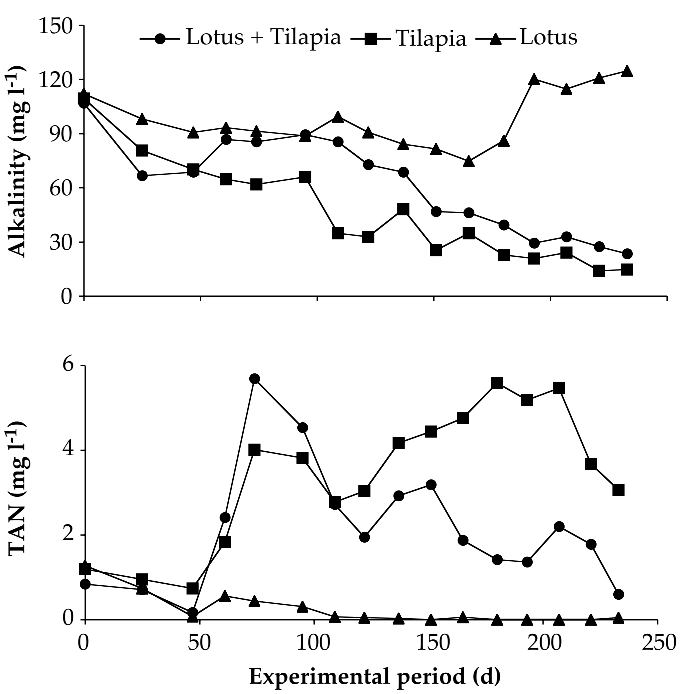

Figure 2. Mean total alkalinity and TAN (0900 h) in all treatments over the 160-day experimental period.

The practice of rotating fish with macrophytes is reported to have at least declined considerably in China due to greater profitability from raising fish year-round, while rotation of fish and agricultural crops was done much less frequently in Eastern Europe due to the wider use of fertilizers (Edwards, 1987). However, with the high net economic return from cultivating lotus in fish ponds shown by Mon (2000) and the present experiment, economic incentives may make the rotation or co-culture of lotus and fish attractive to farmers. For example, some farmers in China have changed their ponds from culturing fish to cultivating lotus in recent years because Chinese carp culture is less profitable than lotus cultivation due to oversupply of fish (Yang Yi, personal observation).

The present experiment has demonstrated the effectiveness of nutrient removal from old pond mud by lotus and the feasibility of rotation and co-culture of lotus and Nile tilapia. Both systems can recycle nutrients effectively within ponds and are environmentally friendly culture systems. Further research is needed to refine the lotus-tilapia co-culture system and make it profitable.

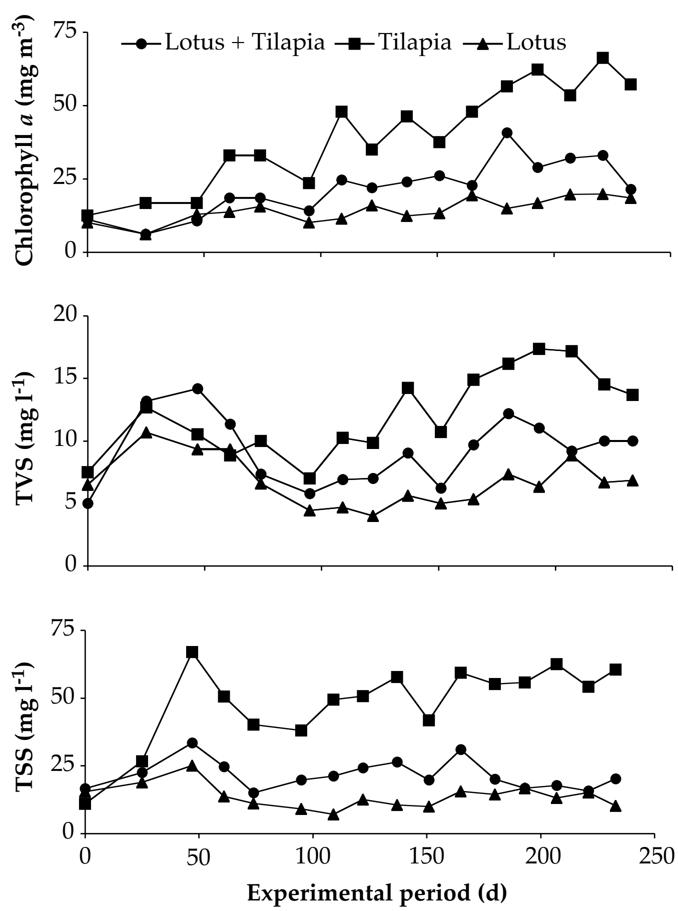

Figure 3. Mean chlorophyll *a*, TVS, and TSS (0900 h) in all treatments over the 160-day experimental period.

Table 7. Partial budget analysis for integrated lotus-tilapia co-culture (treatment A), tilapia alone (treatment B), and lotus alone (treatment C) in the 236-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								
Tilapia	kg	0.25–0.375	10.0	2.50	38.5	14.44	----	----
Lotus Seed	kg	0.75	5.11	3.83	----	----	5.33	4.00
Lotus Flower	piece	0.13	138	17.25	----	----	139	17.38
Total				23.58		14.44		21.37
VARIABLE COST								
Urea	kg	0.1875	32.4	6.08	32.4	6.08	----	----
TSP	kg	0.3125	18.9	5.91	18.9	5.91	----	----
Tilapia Fingerling	piece	0.0125	400	5.00	400	5.00	----	----
Lotus Seedling	piece	0.125	25	3.13	----	----	25	3.13
Cost of Working Capital	yr	8%	0.65	1.04	0.65	0.88	0.65	0.16
Total				21.15		17.86		3.29
NET RETURN				2.44		-3.42		18.09

ANTICIPATED BENEFITS

Results of the experiment will provide information on lotus-fish co-culture and rotation system to recycle pond mud nutrients that are otherwise wasted. The experiment generated information on bottom mud characteristics altered by rooted plants. It may benefit small-scale farmers in Asian countries for resource utilization where lotus is commonly grown as a cash crop.

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CULTURE OF MIXED-SEX NILE TILAPIA WITH PREDATORY SNAKEHEAD

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

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ABSTRACT

An experiment was conducted in eighteen 200-m^2 fertilized earthen ponds at the Asian Institute of Technology, Thailand, from March through October 2000. This experiment was designed to assess the efficiency of snakehead (*Channa striata*) in controlling recruitment of mixed-sex Nile tilapia (*Oreochromis niloticus*) in ponds and to assess growth and production characteristics of Nile tilapia in monoculture and polyculture with snakehead. There were six treatments: A) monoculture of sex-reversed all-male tilapia; B) monoculture of mixed-sex tilapia; C) polyculture of snakehead and mixed-sex tilapia at 1:80 ratio; D) polyculture of snakehead and mixed-sex tilapia at 1:40 ratio; E) polyculture of snakehead and mixed-sex tilapia at 1:20 ratio; F) polyculture of snakehead and mixed-sex tilapia at 1:10 ratio. Sex-reversed and mixed-sex Nile tilapia were stocked at 2 fish m^{-2} at sizes of 10.5 to 11.6 g and 7.2 to 8.1 g, respectively.

Results show that snakehead were able to completely control Nile tilapia recruitment at all tested predator:stocked-prey ratios, and the best predator:stocked-prey ratio was 1:80. The addition of snakehead into Nile tilapia ponds did not result in significantly greater tilapia growth, but it significantly lowered total net and gross yields of adult plus recruited tilapia. Snakehead growth was density-dependent, decreasing significantly with increasing stocking densities. While snakehead biomass gain was not significantly different at stocking densities from 0.025 to 0.1 fish m^{-2}, the gain was significantly lower at a stocking density of 0.2 fish m^{-2}. The present experiment demonstrates that snakehead are able to control Nile tilapia recruitment completely and provide an alternative technique for Nile tilapia culture.

INTRODUCTION

The aquaculture of species at lower trophic levels, such as tilapia, presents the greatest potential for efficiency (Welcomme, 1996). However, overpopulation of tilapia in confined ponds causes stunted growth due to shortage of natural food, particularly in semi-intensive culture. Various methods of population control have been applied (Mair and Little, 1991), such as culture in cages, culture with predators, intermittent harvesting, hybridization, induction of sterility, and production of super-male fish (YY-male). However, population control of tilapias by culture with predators has been practiced worldwide but not well studied. Various predatory fish species have been used with varying success in combination with different tilapia species depending on their availability. These species include snakehead (*Channa striata* or *Ophiocephalus striatus*) (Pongsuwana, 1956; Chimits, 1957; Tongsanga, 1962; Chen, 1976; Cruz and Shehadeh, 1980; Hopkins et al., 1982; Wee, 1982; Balasuriya, 1988); *Ophiocephalus obscuris* (de Graaf et al., 1996); *Micropterus salmoides* (Swingle, 1960; Meschkat, 1967; McGinty, 1985); *Lates niloticus* (Meschkat, 1967; Planquette, 1974; Lazard, 1980; Bedawi, 1985; El Gamal, 1992); *Hemichromis fasciatus* (Bardach et al., 1972; Lazard, 1980); *Cichla ocellaris* (Lovshin, 1977; McGinty, 1983; Verani et al., 1983); *Clarias* sp. (Meecham, 1975; Bard et al., 1976; Lazard, 1980; Janssen, 1985; de Graaf et al., 1996); *Cichlasoma managuense* (Dunseth and Bayne, 1978); *Elops hawaiiensis* (Fortes, 1980); and *Megalops cyprinoides* (Fortes, 1980). However, the difficulty in breeding or obtaining predators of the correct size often resulted in limited application of this population control method (Balarin and Hatton, 1979; Penman and McAndrew, 2000).

Snakehead have long been regarded as valuable food fish and widely cultured in the Far East (Wee, 1982). It was reported to be used in polyculture with tilapia to control tilapia population or with carps to keep out other extraneous pest fish in the pond system (Wee, 1982). Snakehead are highly predacious as they swallow their prey whole (Diana et al., 1985) and have been shown to effectively prey on live tilapia fry (Kaewpaitoon, 1992). A population including 5% (predator:stocked-prey ratio of 1:20) snakehead with tilapia has been demonstrated to control tilapia recruitment (Balasuriya, 1988). Negligible tilapia recruitment was generally found during harvest where snakehead existed in tilapia ponds.

The purposes of this study were to assess:

1) The efficiency of snakehead in controlling overpopulation of mixed-sex Nile tilapia (*Oreochromis niloticus*) in ponds and
2) The growth and production of Nile tilapia in monoculture and polyculture with snakehead.

METHODS AND MATERIALS

The experiment was conducted using a randomized complete block design in eighteen 200-m^2 earthen ponds at the Asian Institute of Technology (AIT), Thailand. There were six treatments with triplicates, one in each block:

A) monoculture of sex-reversed all-male tilapia;
B) monoculture of mixed-sex tilapia;
C) polyculture of snakehead and mixed-sex tilapia at 1:80 ratio;
D) polyculture of snakehead and mixed-sex tilapia at 1:40 ratio;
E) polyculture of snakehead and mixed-sex tilapia at 1:20 ratio;
F) polyculture of snakehead and mixed-sex tilapia at 1:10 ratio.

The Chitralada strain (Thai strain) of Nile tilapia was used in the present experiment. Nile tilapia fry were obtained from the AIT Hatchery, while snakehead fingerlings were purchased from a local market. Sex-reversed Nile tilapia (10.5 to 11.6 g size) and mixed-sex Nile tilapia (7.2 to 8.1 g size) were stocked at 2 fish m^{-2} in treatment A and treatments B through F, respectively, while snakehead (88.0 to 100.0 g size) were stocked at 0.025, 0.05, 0.1, and 0.2 fish m^{-2} in treatments C, D, E, and F, respectively, on 30 March 2000. During the experiment, approximately 10% of the initial Nile tilapia stock was seined, counted, and weighed en masse biweekly for each pond. All fish were harvested on 10 October 2000 after 194 days of culture. Daily weight gain (g $fish^{-1}$ d^{-1}), yield (kg $pond^{-1}$), and extrapolated yield (kg ha^{-1} yr^{-1}) were calculated.

All ponds were dried for one month prior to the experiment to eliminate wild fish. Each pond dike was enclosed with a fine mesh net fence about 1 m tall, supported by bamboo sticks, with the lower end of the net buried in the dike soil to prevent entry of wild fish and movement of stocked snakehead from one pond to another. All ponds were fertilized with urea and triple superphosphate (TSP) at a rate of 28 kg nitrogen (N) and 7 kg phosphorus (P) ha^{-1} wk^{-1}. Initial pond fertilization took place two weeks prior to fish stocking. Water depth in all ponds was maintained at 1 m throughout the experiment by adding water weekly to replace evaporation and seepage losses.

Integrated water samples were taken biweekly from the entire water column near the center of each pond at about 1000 h for analyses of pH, alkalinity, total ammonium nitrogen (TAN), nitrite-nitrogen, nitrate-nitrogen, total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), total phosphorus (TP), chlorophyll *a*, total suspended solids (TSS), and total volatile solids (TVS) (APHA et al., 1985; Egna et al., 1989). At the time of collecting water samples, Secchi disk visibility was measured using a Secchi disk, while temperature and dissolved oxygen (DO) were measured with a YSI model 54 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio,

Table 1. Growth performance (mean ± SE) of Nile tilapia in fertilized earthen ponds during 194-day culture. Rows of values with superscripts indicate variables with significant differences among treatments (ANOVA, $P < 0.05$). Values with similar letter superscripts are not significantly different.

Parameters	Treatments					
	A (Sex-reversed)	*B* (Mixed-sex)	*C* (1:80)	*D* (1:40)	*E* (1:20)	*F* (1:10)
STOCKING						
Density (fish m^{-2})	2	2	2	2	2	2
Total Number (fish $pond^{-1}$)	400	400	400	400	400	400
Mean Weight (g $fish^{-1}$)	9.0 ± 0.4	7.4 ± 0.1	7.7 ± 0.1	7.5 ± 0.3	7.6 ± 0.0	7.7 ± 0.3
Total Weight (kg $pond^{-1}$)	3.62 ± 0.17	2.96 ± 0.05	3.08 ± 0.04	3.01 ± 0.12	3.02 ± 0.01	3.07 ± 0.13
HARVEST						
Adult Tilapia						
Total Number (fish $pond^{-1}$)	331 ± 10	330 ± 12	322 ± 4	312 ± 8	323 ± 9	327 ± 10
Mean Weight (g $fish^{-1}$)	157.7 ± 17.5	149.8 ± 16.3	158.0 ± 10.0	152.9 ± 13.5	158.2 ± 15.3	155.3 ± 13.9
Total Weight (kg $pond^{-1}$)	51.90 ± 4.60	49.03 ± 3.89	50.88 ± 3.17	47.64 ± 4.18	50.94 ± 4.05	50.60 ± 3.92
Survival Rate (%)	82.90 (78.28–87.07)	82.61 (77.97–86.81)	80.52 (75.69–84.93)	77.99 (72.97–82.63)	80.93 (76.14–85.30)	81.88 (77.17–86.15)
Daily Weight Gain (g $fish^{-1}$ d^{-1})	0.77 ± 0.09	0.73 ± 0.08	0.77 ± 0.05	0.75 ± 0.07	0.78 ± 0.08	0.76 ± 0.07
Total Weight Gain (kg $pond^{-1}$)	48.28 ± 4.43	46.07 ± 3.93	47.80 ± 3.21	44.63 ± 4.12	47.92 ± 4.06	47.53 ± 3.87
Net Yield (t ha^{-1} yr^{-1})	4.54 ± 0.42	4.33 ± 0.37	4.50 ± 0.30	4.19 ± 0.39	4.51 ± 0.38	4.47 ± 0.36
Gross Yield (t ha^{-1} yr^{-1})	4.88 ± 0.43	4.61 ± 0.37	4.79 ± 0.30	4.48 ± 0.39	4.79 ± 0.38	4.76 ± 0.37
Recruited Tilapia						
Total Number (fish $pond^{-1}$)	----	951 ± 191	----	----	----	----
Mean Weight (g $fish^{-1}$)	----	9.9 ± 0.5	----	----	----	----
Total Weight (kg $pond^{-1}$)	----	9.32 ± 1.65	----	----	----	----
Net and Gross Yield (t ha^{-1} yr^{-1})	----	0.88 ± 0.15	----	----	----	----
Combined Adult and Recruited Fish						
Total Net Yield (t ha^{-1} yr^{-1})	4.54 ± 0.42 ^{a}	5.21 ± 0.22 ^{b}	4.50 ± 0.30 ^{a}	4.20 ± 0.39 ^{a}	4.51 ± 0.38 ^{a}	4.47 ± 0.36 ^{a}
Total Gross Yield (t ha^{-1} yr^{-1})	4.88 ± 0.43 ^{a}	5.49 ± 0.22 ^{b}	4.79 ± 0.30 ^{a}	4.48 ± 0.39 ^{a}	4.79 ± 0.38 ^{a}	4.76 ± 0.37 ^{a}

Table 2. Growth performance (mean ± SE) of snakehead in fertilized earthen ponds during 194-day polyculture with Nile tilapia. Rows of values with superscripts indicate variables with significant differences among treatments (ANOVA, $P < 0.05$). Values with similar letter superscripts are not significantly different.

Parameters	Treatments					
	A (Sex-reversed)	*B* (Mixed-sex)	*C* (1:80)	*D* (1:40)	*E* (1:20)	*F* (1:10)
STOCKING						
Density (fish m^{-2})	----	----	0.025	0.05	0.1	0.2
Total Number (fish $pond^{-1}$)	----	----	5	10	20	40
Mean Weight (g $fish^{-1}$)	----	----	94.5 ± 2.4	93.7 ± 3.5	95.8 ± 2.2	95.7 ± 2.2
Total Weight (kg $pond^{-1}$)	----	----	0.47 ± 0.01	0.94 ± 0.03	1.92 ± 0.04	3.83 ± 0.09
HARVEST						
Total Number (fish $pond^{-1}$)	----	----	4 ± 0	8 ± 0	19 ± 1	35 ± 2
Mean Weight (g $fish^{-1}$)	----	----	441.3 ± 18.1^{a}	292.0 ± 11.6^{b}	179.3 ± 12.0^{c}	123.3 ± 4.2^{d}
Total Weight (kg $pond^{-1}$)	----	----	1.91 ± 0.12^{a}	2.43 ± 0.04^{b}	3.33 ± 0.13^{c}	4.30 ± 0.08^{d}
Survival Rate (%)	----	----	90.75 (70.25–99.83)	83.64 (60.06–97.81)	95.47 (78.49–99.72)	88.23 (66.43–99.32)
Daily Weight Gain (g $fish^{-1}$ d^{-1})	----	----	1.79 ± 0.08^{a}	1.02 ± 0.04^{b}	0.43 ± 0.07^{c}	0.14 ± 0.02^{d}
Total Weight Gain (kg $pond^{-1}$)	----	----	1.43 ± 0.13^{a}	1.49 ± 0.04^{a}	1.42 ± 0.17^{a}	0.47 ± 0.15^{b}
Net Yield (t ha^{-1} yr^{-1})	----	----	0.14 ± 0.01^{a}	0.14 ± 0.00^{a}	0.13 ± 0.02^{a}	0.05 ± 0.01^{b}
Gross Yield (t ha^{-1} yr^{-1})	----	----	0.18 ± 0.01^{a}	0.23 ± 0.00^{b}	0.31 ± 0.01^{c}	0.41 ± 0.00^{d}

USA). Diel measurements for temperature, DO, and pH were conducted monthly in each pond at 0600, 1000, 1400, 1600, 1800, and 0600 h.

Data were analyzed statistically by analysis of variance and linear regression (Steele and Torrie, 1980) using SPSS (version 7.0) statistical software package (SPSS, Inc., Chicago, Illinois, USA). Differences were considered significant at an alpha level of 0.05. Statistical analyses for survival rates (%) were performed on the transformed data by arcsine transformation. Mean values of survival rates were given in the back-transformed scale followed by their confidence limits. All other means were given with ± 1 standard error (SE).

A partial budget analysis was conducted to determine economic returns of the different monoculture and polyculture systems tested (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 = 40 baht). Farm-gate prices of snakehead and Nile tilapia varied with size: snakehead at \$0.25 kg^{-1} for size 100 to 200 g, \$0.50 kg^{-1} for size 200 to 300 g, \$0.75 kg^{-1} for size 300 to 400 g, and \$1.00 kg^{-1} for size above 400 g; and Nile tilapia at \$0.125 kg^{-1} for size below 50 g and \$0.375 kg^{-1} for size 100 to 200 g. Market prices for fingerlings of snakehead (\$0.15 kg^{-1}), sex-reversed Nile tilapia (\$0.0125 $piece^{-1}$), and mixed-sex Nile tilapia (\$0.0042 $piece^{-1}$); urea (\$0.1875 kg^{-1}); and TSP (\$0.3125 kg^{-1}) were applied to the analysis. The calculation for cost of working capital was based on an annual interest rate of 8%.

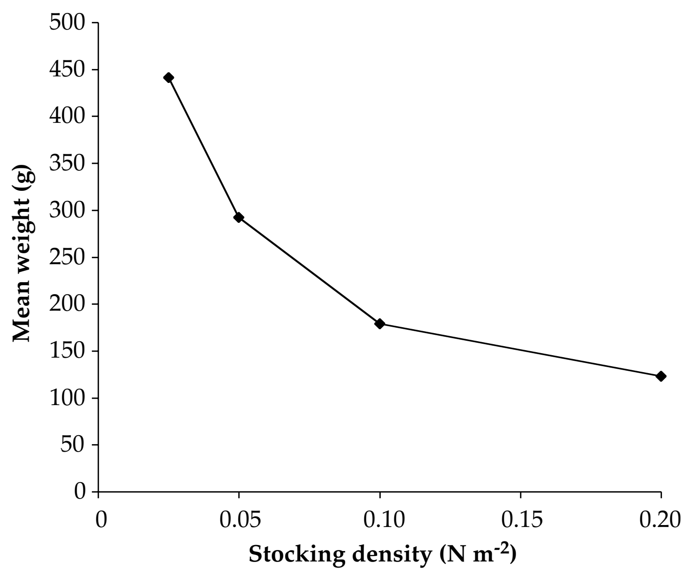

Figure 1. Comparison of final weight of snakehead (g) at different stocking densities (N m^{-2}) of snakehead in the snakehead and Nile tilapia polyculture after 194 days.

RESULTS

Growth performance parameters of adult Nile tilapia were not significantly different among all treatments ($P > 0.05$, Table 1). Tilapia offspring were found only in monoculture of mixed-sex tilapia. Both sex-reversal and predator techniques were able to control the recruitment of Nile tilapia completely. However, neither sex reversal nor polyculture resulted in significantly

weight; and r = –0.985, $P < 0.05$ for daily weight gain). Snakehead biomass gain (total weight gain and net yield) was not significantly different ($P > 0.05$) within stocking densities of 0.025 to 0.1 fish m^{-2} (treatments C, D, and E; Figure 1). At a stocking density of 0.2 fish m^{-2} (treatment F), snakehead biomass gain was significantly lower than in other densities ($P < 0.05$, Table 2, and Figure 2). The significantly reduced individual growth and biomass gain at the stocking density of 0.2 fish m^{-2} indicated that carrying capacity of snakehead was exceeded.

The additional net yield from snakehead did not cause a significantly higher combined net yield of adult tilapia and snakehead ($P > 0.05$), but it resulted in a significantly higher combined gross yield of adult tilapia and snakehead ($P < 0.05$, Table 3). When recruited tilapia were included, the combined net yield in the monoculture of mixed-sex tilapia (treatment B) was significantly higher than those in all other treatments ($P < 0.05$), while the combined gross yield in the monoculture of mixed-sex tilapia was similar to those in the polyculture with higher predator:stocked-prey ratios (treatments E and F, $P > 0.05$, Table 3). The results indicated that the predator: stocked-prey ratio of 1:80 (treatment C) was enough to completely control Nile tilapia recruitment.

Physical and chemical parameters of pond water were not significantly different among all treatments at all sampling times throughout the entire experimental period ($P > 0.05$). The mean values of water quality parameters were also not significantly different among all treatments ($P > 0.05$, Table 4).

The partial budget analysis (Table 5) indicated that all treatments in this experiment were profitable, and mixed-sex Nile tilapia culture (treatments B through F) produced significantly higher net return than sex-reversed Nile tilapia culture (treatment A). Snakehead and Nile tilapia polyculture at the

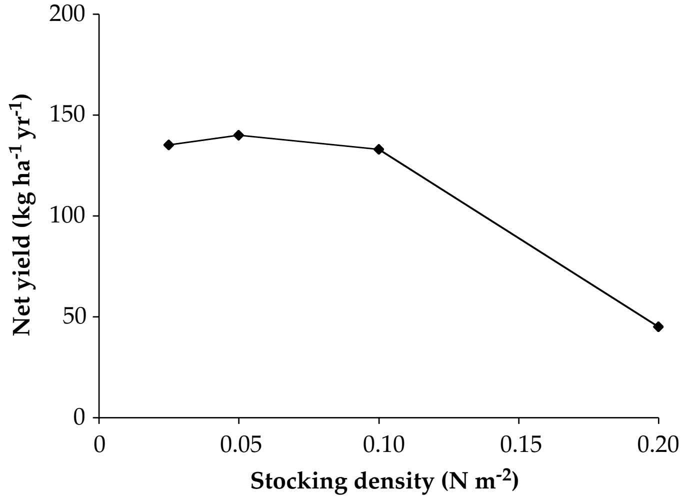

Figure 2. Comparison of net yield of snakehead (kg ha^{-1} yr^{-1}) at different stocking densities (N m^{-2}) of snakehead in the snakehead and Nile tilapia polyculture after 194 days.

Table 3. Combined yields (mean ± SE) of Nile tilapia and snakehead in fertilized earthen ponds during 194-day culture. Rows of values with superscripts indicate variables with significant differences among treatments (ANOVA, $P < 0.05$). Values with similar letter superscripts are not significantly different.

Parameters	Treatments					
	A (Sex-reversed)	*B* (Mixed-sex)	*C* (1:80)	*D* (1:40)	*E* (1:20)	*F* (1:10)
ADULT TILAPIA + SNAKEHEAD						
Net Yield (t ha^{-1} yr^{-1})	4.54 ± 0.42	4.33 ± 0.37	4.63 ± 0.30	4.39 ± 0.39	4.64 ± 0.39	4.52 ± 0.35
Gross Yield (t ha^{-1} yr^{-1})	4.88 ± 0.43 [abc]	4.61 ± 0.37 [a]	4.97 ± 0.29 [bc]	4.71 ± 0.40 [ab]	5.11 ± 0.38 [c]	5.16 ± 0.36 [c]
ADULT AND RECRUITED TILAPIA + SNAKEHEAD						
Net Yield (t ha^{-1} yr^{-1})	4.54 ± 0.42[a]	5.21 ± 0.22 [b]	4.63 ± 0.30 [a]	4.34 ± 0.39 [a]	4.64 ± 0.39 [a]	4.52 ± 0.35 [a]
Gross Yield (t ha^{-1} yr^{-1})	4.88 ± 0.43 [ab]	5.49 ± 0.22 [c]	4.97 ± 0.29 [ab]	4.71 ± 0.40 [a]	5.11 ± 0.38 [bc]	5.16 ± 0.36 [bc]

Table 4. Values of water quality parameters (mean ± SE) measured throughout the experiment.

Parameters	Treatments					
	A (Sex-reversed)	*B* (Mixed-sex)	*C* (1:80)	*D* (1:40)	*E* (1:20)	*F* (1:10)
DO at Dawn (mg l^{-1})	2.43 ± 0.39	2.25 ± 0.23	2.38 ± 0.19	2.05 ± 0.27	2.36 ± 0.17	2.03 ± 0.17
Temperature (°C)	27.9–37.4	27.4–36.7	27.8–37.3	27.8–36.9	27.8–36.6	27.8–35.9
pH	6.5–9.6	6.5–10.3	6.4–9.8	6.5–10.0	6.5–10.4	6.5–10.2
Alkalinity (mg l^{-1} as $CaCO_3$)	52 ± 6	54 ± 5	54 ± 9	59 ± 6	54 ± 4	58 ± 4
TKN (mg l^{-1})	6.62 ± 0.88	6.24 ± 0.73	6.48 ± 0.41	5.76 ± 0.26	6.42 ± 0.49	6.46 ± 0.34
TAN (mg l^{-1})	2.48 ± 0.57	2.36 ± 0.26	2.37 ± 0.28	2.04 ± 0.43	2.24 ± 0.27	2.18 ± 0.09
Nitrite-N (mg l^{-1})	0.19 ± 0.04	0.22 ± 0.01	0.20 ± 0.01	0.14 ± 0.02	0.21 ± 0.04	0.21 ± 0.02
Nitrate-N (mg l^{-1})	0.64 ± 0.17	0.63 ± 0.04	0.79 ± 0.13	0.49 ± 0.14	0.69 ± 0.12	0.76 ± 0.009
TP (mg l^{-1})	0.51 ± 0.24	0.51 ± 0.09	0.62 ± 0.08	0.62 ± 0.26	0.70 ± 0.13	0.66 ± 0.12
SRP (mg l^{-1})	0.29 ± 0.22	0.18 ± 0.08	0.35 ± 0.06	0.35 ± 0.24	0.38 ± 0.10	0.33 ± 0.10
Chlorophyll *a* (mg m^{-3})	35 ± 11	46 ± 8	31 ± 8	42 ± 13	44 ± 10	41 ± 12
Secchi Disk Visibility (cm)	24 ± 2	24 ± 3	25 ± 0	22 ± 1	24 ± 2	23 ± 3
TSS (mg l^{-1})	95 ± 15	109 ± 5	91 ± 6	107 ± 14	91 ± 11	117 ± 14
TVS (mg l^{-1})	20 ± 3	26 ± 2	20 ± 1	25 ± 4	22 ± 4	26 ± 4

lowest predator:stocked-prey ratio (1:80, treatment C) had the highest net return and ratio of added income to added cost, followed by the treatment at the ratio of 1:20 (treatment E).

DISCUSSION

This experiment showed that snakehead were able to completely control recruitment of Nile tilapia at a very low predator:stocked-prey ratio of 1:80, indicating high efficiency in recruitment control. A similar ratio (1:85) was used by Cruz and Shehadeh (1980) to control Nile tilapia recruitment successfully. *Lates niloticus* was reported to have a similar predation efficiency (1:84; Planquette, 1974), while other piscivorous species such as *Hemichromis fasciatus* (1:17 to 1:48; Bardach et al, 1972; Lazard, 1980), *Cichla ocellaris* (1:15; Lovshin, 1977), *Clarias lazera* (1:10; Bard et al., 1976), *Cichlasoma managuense* (1:4; Dunseth and Bayne, 1978), *Elops hawaiiensis* (1:20; Fortes, 1980), *Megalops cyprinoides* (1:10; Fortes, 1980), *Clarias gariepinus* (1:2.7; de Graaf et al., 1996), and *Ophiocephalus obscuris* (1:30; de Graaf et al., 1996) were less effective.

The present experiment clearly showed that the carrying capacity of snakehead was exceeded at the predator: stocked-prey ratio of 1:10, and poor growth occurred due mainly to the limited food items available. Snakehead is carnivorous and highly predacious on aquatic organisms such as insects; fish, including its own species; frogs; shrimps; and even small aquatic snakes (Wee, 1982). In another study snakehead had better growth at the same predator:stocked-prey ratio with smaller stocking size (0.3 g) and harvest size (108.5 g; Balasuriya, 1988). In comparison, stocking size was 95.7 g in the present experiment. The results of the present experiment suggest that the standing crop of snakehead at a stocking density of 2 Nile tilapia m^{-2} should be below 4.30 kg per 200 m^2, or 215 kg ha^{-1}, to achieve good growth.

In other studies, high yields of harvestable-size tilapia were reported, and the final size of harvested tilapia increased with effective predators (Swingle, 1960; Lovshin, 1977; Dunseth and Bayne, 1978; Edwards et al., 1994). However, there were no significant differences in final size and yield of harvested adult tilapia among treatments in this study, and total production combining adult and recruited tilapia was significantly reduced in all polyculture treatments as the recruits were eaten. This is consistent with the results using other piscivorous species reported by Maar et al. (1966), Lovshin (1977), Fortes (1980), McGinty (1983, 1985), and Edwards et al. (1994).

The growth of sex-reversed all-male Nile tilapia was only 5% faster than mixed-sex tilapia, and this difference was not statistically significant in the present experiment. In comparison, the sex-reversed all-male tilapia grew more than 10% faster than mixed-sex tilapia in other experiments (Pascual and Mair, 1997). Stunting with mixed-sex tilapia culture, caused by competition for food between recruits and stocked tilapia, was not observed in the present experiment. Green and Teichert-Coddington (1994) also did not find significant differences between sex-reversed and mixed-sex Nile tilapia growth in ponds. Dan and Little (2000) reported that growth difference between sex-reversed and mixed-sex Thai strain of Nile tilapia (new-season seed) was significant when cultured in ponds but not significant when cultured in cages. Clearly, there are system-specific differences that may affect the growth, production, and stunting of mixed-sex tilapia.

ANTICIPATED BENEFITS

The results of this study demonstrated that snakehead can control Nile tilapia recruitment completely at low predator: stocked-prey ratios. This provides an alternative technique for Nile tilapia culture system. Tilapia recruitment control by stocking predators can make Nile tilapia production expand

Table 5. Partial budget analysis (US$) for sex-reversed and mixed-sex Nile tilapia monoculture (treatments A and B) and snakehead and mixed-sex Nile tilapia polyculture (treatments C through F) in the 194-day experiment (based on 200-m^2 ponds).

Parameters	Treatments					
	A (Sex-reversed)	*B* (Mixed-sex)	*C* (1:80)	*D* (1:40)	*E* (1:20)	*F* (1:10)
GROSS REVENUE						
Adult Tilapia	19.46	18.39	19.08	17.87	19.10	18.98
Recruited Tilapia	----	1.16	----	----	----	----
Snakehead	----	----	1.91	1.82	1.67	1.08
Total	19.46	19.55	20.99	19.69	20.77	20.05
VARIABLE COST						
Tilapia Fingerlings	5.00	1.67	1.67	1.67	1.67	1.67
Snakehead Fingerlings	----	----	0.07	0.14	0.29	0.58
Urea	6.75	6.75	6.75	6.75	6.75	6.75
TSP	6.56	6.56	6.56	6.56	6.56	6.56
Cost of Working Capital	0.78	0.64	0.64	0.64	0.65	0.66
Total cost	19.09	15.62	15.69	15.76	15.92	16.22
NET RETURN	0.37	3.93	5.30	3.92	4.85	3.83
ADDED COST	3.33	----	0.07	0.14	0.29	0.57
ADDED RETURN	-0.09	----	1.44	0.14	1.22	0.50
ADDED INCOME/ADDED COST	-0.03	----	20.41	0.97	4.22	0.87

and increase, especially in rural areas where sex-reversed tilapia are not available. It will benefit culturists throughout Southeast Asia and other regions where tilapia are commonly cultured, snakehead are available, and there is no tilapia hatchery for sex-reversed all-male fry.

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DEVELOPMENT OF SUSTAINABLE POND AQUACULTURE PRACTICES FOR *COLOSSOMA MACROPOMUM* AND *PIARACTUS BRACHYPOMUS* IN THE PERUVIAN AMAZON

Ninth Work Plan, New Aquaculture Systems/New Species Research 3 and 6 (9NS3 and 6)
Final Report

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ABSTRACT

Studies on the sustainable aquaculture production of gamitana (*Colossoma macropomum*) and paco (*Piaractus brachypomus*) in the Peruvian Amazon were conducted at the Instituto de Investigaciones de la Amazonia Peruana (IIAP) Quistococha Aquaculture Station in Iquitos, Peru. Growth performance of paco stocked at 4,000, 6,000, and 8,000 fish ha^{-1} in an experiment was not significantly different among stocking densities. Fish were harvested after six and one-half months for the 4,000, 6,000, and 8,000 fish ha^{-1} study; mean weights of 418.2, 447.5, and 474.9 g, respectively, were attained. Specific growth rates (% d^{-1}) were 1.8, 1.8, and 1.7; feed conversion efficiencies were 72.9, 76.2, and 74.7; and condition factors were 2.7, 2.7, and 2.8 at the low to high densities, respectively. Survival for the paco experiment was > 80%. Paco fingerlings were fed a locally prepared diet (26.7% crude protein, 9.0% crude lipid). Water quality parameters (dissolved oxygen, temperature, total ammonia nitrogen, and pH) remained within acceptable levels for tropical aquaculture. The stocking density study suggests the economic feasibility of rearing paco in the Peruvian Amazon. The cost of production analysis in this and an earlier study indicated that gamitana production is economically more feasible than paco production because of its higher market value (US$3.00 vs. US$2.10 kg^{-1} fresh weight) and equal production costs (US$0.6 to US$0.9 kg^{-1} fresh weight). Production of gamitana and paco at densities of 2,500 fish ha^{-1} and higher will be more profitable than pineapple production, which is the highest market value agriculture cash crop produced in the Loreto region at the present time.

In another experiment, plasma concentrations of sex steroids—testosterone (T), 11-ketotestosterone (11-kT), estradiol-17β (E2) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP)—were measured by radioimmunoassay following ethyl-ether extraction to monitor and understand the dynamics of gonadal steroidogenesis during maturation of paco and gamitana. In paco, prior to hormonal treatments with luteinizing hormone-releasing hormone analog (LHRHa), the concentrations of 11-kT in males and E2 in females as well as the ones of their precursor T were significantly ($P < 0.01$) higher in fish maintained under normoxic conditions than in fish exposed to hypoxia. After ovulation and spermiation, the concentrations of T and 17,20βP significantly ($P < 0.05$) increased in both sexes in both experiments. However, the levels of plasma sex steroids reached under normoxic conditions were higher than the ones recorded under hypoxia, except the ones of 17,20βP in males. Additionally, the effect of oxygen concentration on human chorionic gonadotropin (hCG) was evaluated during final stages of induced maturation on blood steroid profiles in an attempt to correlate these data with gamete viability. From 8 to 11 November 2000, the second attempt at artificial spawning of paco and the first of gamitana were performed. Six pairs of paco were selected and transferred to indoor concrete tanks. Treatments involved injection of three pairs with LHRHa and three pairs with hCG (Sigma, St. Louis, Missouri) at 500 IU kg^{-1} (females) or 100 IU kg^{-1} (males). Fish were observed during the following 48 h and spawning attempted. Fish were weighed, tags identified, and blood samples taken prior to injection, at the time of ovulation, or 48 h after

injection. In the case of gamitana, four pairs were formed after preliminary selection (robustness or sperm presence). All fish were injected with LHRHa at the same dose as paco. Conditions in ponds and indoor tanks were monitored during spawning procedures. LHRHa proved to be the only sex hormone effective for inducing spawning in both males and females.

INTRODUCTION

The addition of a project in South America to the PD/A CRSP has provided considerable and unique opportunities to expand the CRSP network. In the Eighth Work Plan, a prime site was established at Iquitos, Peru, which is in the heart of the Peruvian Amazon (Loreto Region). The Loreto Region, with a population of 602,000, constitutes 27% of the country's total area. Approximately 46% of the region's population resides in the city of Iquitos. The main resource in the region is the integrated rain forest. The people in the region are primarily engaged in agriculture, cattle-raising, forestry, hunting, fishing, and tourist activities. Other economic activities of major importance to the region include the mining and drilling of nonrenewable resources such as oil, gold, and silica.

In the Peruvian Amazon, there are three important institutions working with aquaculture: Instituto de Investigaciones de la Amazonia Peruana (IIAP), Ministerio de Pesqueria (Peruvian Government), and Universidad Nacional de la Amazonia Peruana (UNAP). In the past ten years they have produced thousands of fry and have refined numerous aquaculture techniques. Gamitana (*Colossoma macropomum*) and paco (*Piaractus brachypomus*) are considered by local aquaculturists as the best fishes for commercialization in the tropical part of Peru. However, considerable potential exists to examine other species, as the Amazon Basin is home to over 2,000 freshwater species of fish.

A memorandum of understanding (MOU) is currently in place linking IIAP, UNAP, and Southern Illinois University at Carbondale (SIUC) (and collaborating US universities with SIUC under this umbrella) within the CRSP network. Between IIAP and UNAP there exist 49 earthen culture ponds ranging in size from 60 m^2 to nearly a hectare. Laboratory facilities also exist to monitor water quality variables of ponds and conduct pertinent research on sustainable aquaculture development of important fish species native to South America. Facilities have been significantly renovated at IIAP during the Ninth Work Plan.

Native species aquaculture has been expanding in the Peruvian Amazon as research has played a major role in the positive evaluation of its potential. Gamitana and paco are native to the Amazon basin and share many characteristics that also make them suitable for aquaculture. Local production of both species is still practiced in an extensive manner, but these species are in high demand and attain a higher price at the market. This motivates local farmers to invest their time in the production of these valued fish. As research develops, more important information becomes available to producers, hence the improvement of native species aquaculture in the region. Although technology is still underdeveloped, external aid has made it possible for the locals to become more aware of and active in aquaculture practices for gamitana, paco, and other native species.

Broodstock are usually collected from their wild habitat, although they are also raised in captivity at research stations. These fish may be immediate descendants of wild broodstock or may be a product of multiple-generation breeding. 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). This study determined suitable stocking densities to be as high as 8,000 fish ha^{-1} for optimal and efficient production of gamitana and paco to market size (0.5 to 1.0 kg) using a prepared diet manufactured from locally available ingredients. Replicated pond studies were carried out at IIAP, Iquitos.

METHODS AND MATERIALS

Methods and materials for research objectives are as follows:

Objective 1 (9NS3): Develop a Prepared Feed for Broodstock and Fingerlings

Additional information on nutrition of paco has been obtained via feeding experiments performed by Rebecca Lochmann at the University of Arkansas at Pine Bluff. Literature values for specific nutrients known to affect fish reproduction were calculated from published sources for the broodstock diet. Analytical information on the feedstuffs and diets currently being used in Iquitos, together with published information on the natural diets of paco and broodstock nutrition in other species, was combined to formulate preliminary recommendations for the nutrition and feeding of broodstock (see "Spawning and grow-out of *Colossoma macropomum* and/or *Piaractus brachypomus*," 9NS3A on pp. 85–88 of this report).

Objective 2 (9NS3): Determine Blood Plasma Steroid Concentrations in Relation to Gamete Quality

The plasma concentrations of steroids—testosterone (T), estradiol-17β (E2), 11-ketotestosterone (11-KT), and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP)—were measured by radioimmunoassay, similar to those used previously (Ottobre et al., 1989) following ethyl-ether extraction. The characteristics of these antisera have been reported previously (Dabrowski et al., 1995, for T; Butcher et al., 1974, for E2; Kime and Manning, 1982, for 11-KT; and Fostier and Jalabert, 1986, for 17,20βP).

Objective 3 (9NS3): Compare Hormones to Induce Spawning

In July 1999, paco and gamitana were collected from two separate broodstock ponds located at the IIAP field station in Iquitos, Peru. Twenty-five fish of both species were individually measured, weighed, and tagged. Blood was collected from the caudal vessel of unanesthetized fish using a heparinized syringe. Fish were then released into their respective ponds. Blood was centrifuged at 1,500 rpm for 15 min, and the plasma was stored at –20°C until assayed. During October and November 1999, mature paco (average weight 3,394 ± 575 g and 3,683 ± 606 g in males and females, respectively) were sampled. Spermiating males and robust females were selected and then transferred to an indoor facility. Ovarian maturity was assessed with a microscope using oocytes collected from the ovary with a catheter. Pairs of paco were moved into indoor concrete tanks (0.75 m^3). The male was separated from the female by a net in each tank. Six and four pairs were used in the first and second experiments, respectively. In the first experiment aeration was not provided in the broodfish tanks, and the concentration of oxygen decreased to 2.5 ± 0.3 mg l^{-1}, whereas intensive aeration in the broodstock tanks prior to the stocking resulted in an increased oxygen concentration of 7.5 mg l^{-1}. Both genders were injected with two doses of LHRHa (Conceptal®). The concentration of LHRHa was 0.0042 mg of equivalents of active hormone per ml. Males and females were injected with 1 ml kg^{-1} and 2.6 ml kg^{-1}, respectively. The priming dose (50 and 10% in males and females, respectively) was administered in the morning, whereas the resolving dose (50 and 90% in males and females, respectively) was injected at 2200 h. The presence of a few eggs at the bottom of the tank, as well as the "knocking" sound produced by the male, was used as a sign of female readiness (oviposition). Blood was collected from the caudal vessel of unanesthetized fish prior to the priming injection and after ovulation or spermiation using a heparinized syringe. Blood was centrifuged at 1,500 rpm for 15 min, and the plasma was stored at –20°C until assayed. From 27 to 30 March 2000, gamitana and paco broodstock were caught and transferred to indoor tanks for identification, blood sampling, and possible evaluation of gonad maturity. In total, 12 fish of each species were examined. In addition, we visited two private farmers in the vicinity of Iquitos and sampled gamitana (muscle) fed local diets, including fruits, for possible analysis of phytochemicals.

From 8 to 11 November 2000, the second attempt at artificial spawning of paco and the first of gamitana were performed. The weight of paco and gamitana sampled for blood steroid analysis reached 4,986 ± 511 g and 7,370 ± 538 g, respectively. Six pairs of paco were selected and transferred to indoor concrete tanks. Treatments involved injection of three pairs with LHRHa and three pairs with hCG (Sigma, St. Louis, Missouri), the former at dosages previously described and the latter at 500 IU kg^{-1} (females) or 100 IU kg^{-1} (males). Fish were observed during the following 48 h and spawning attempted. Fish were weighed, tags identified, and blood samples taken prior to injection, at the time of ovulation, or 48 h after injection. In the case of gamitana, four pairs were formed after preliminary selection (robustness or sperm presence). All fish were injected with LHRHa at the same dose as paco. Conditions in ponds and indoor tanks were monitored during spawning procedures for all studies.

Objective 4 (9NS3): Identify Proper Stocking Density for Paco and Gamitana for Pond Culture

Two earlier experiments (Kohler et al., 1999, 2001) tested paco and gamitana at several stocking densities. In this third experiment, nine ponds, ranging in size from 600 to 5,320 m^2, were stocked with paco at three densities: three at 4,000 fish ha^{-1}, three at 6,000 fish ha^{-1}, and three at 8,000 fish ha^{-1}. The mean initial weight was 21.3 g. The study was initiated 2 March 2000 and continued until 15 September 2000. General water quality parameters (dissolved oxygen (DO), temperature, total ammonia nitrogen (TAN), and pH) were measured daily or weekly in the early morning for all studies. Harvest data were analyzed using the Statistical Analysis System (SAS Institute, 1993) with an alpha of 0.05.

RESULTS AND DISCUSSION

The overall goal of the program was to provide food security for the region through the following objectives:

Objective 2 (9NS3): Determine Blood Plasma Steroid Concentrations in Relation to Gamete Quality

The assay characteristics are shown in Tables 1 and 2. Extraction blanks were below sensitivity of assay for examined hormones, and serial dilutions of plasma samples showed parallelism with the standard curve between 25 and 100 ml.

In July 1999, the weight of paco and gamitana sampled for blood steroid analysis reached 3,332 ± 73 g and 5,092 ± 144 g, respectively. At this time it was not possible to identify the sex based on external characteristics of the fish sampled. The average concentrations of plasma sex steroids were low (< 1 ng ml^{-1}) in both species (Table 3). High concentrations (1.2 to 3.9 ng ml^{-1}) of E2 were found in few paco (in 10 of 25 sampled), indicating that those fish were females and had started their gonad recrudescence. The concentrations of 11-kT (1 to 2.1 ng ml^{-1}) were high in 8 gamitana (out of 25 sampled). Those fish were suspected to be males and had started their gonad recrudescence.

Table 1. Radioimmunoassay characteristics of plasma sex steroid hormones—testosterone (T), 11-ketotestosterone (11-kT), estradiol-17β (E2), and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP)—in paco (*Piaractus brachypomus*).

Characteristics	T	E2	11-kT	17,20βP
Within-Assay CV (%) (n = 6)	1.7	2.0	2.5	2.0
Between-Assay CV (%) (n = 3)	9.2	3.9	6.1	5.9
Accuracy (Coefficient of Determination)	0.988	0.995	0.984	0.983
Sensitivity (pg ml^{-1})	2	1	2	1
Recovery of Extraction (%)	85.8	89.7	91.9	98.6

Table 2. Radioimmunoassay characteristics of plasma sex steroid hormones in gamitana (*Colossoma macropomum*). Abbreviations as in Table 1.

Characteristics	T	E2	11-kT	17,20βP
Within-Assay CV (%) (n = 6)	1.9	2.2	2.4	2.1
Between-Assay CV (%) (n = 3)	4.3	3.5	4.5	3.9
Accuracy (Coefficient of Determination)	0.978	0.983	0.948	0.968
Sensitivity (pg ml^{-1})	2	1	2	1
Recovery of Extraction (%)	89.9	89.2	90.5	93.5

Table 3. Plasma sex steroid hormones in a mixed-sex population of paco (*Piaractus brachypomus*) and gamitana (*Colossoma macropomum*) sampled in August 1999. Abbreviations as in Table 1.

Species	Plasma Sex Steroids (pg ml^{-1})			
	T	*E2*	*11-kT*	*17,20βP*
Paco	137 ± 13	106 ± 9	790 ± 108	37 ± 6
Gamitana	180 ± 28	991 ± 230	631 ± 58	15 ± 3

Table 4. Plasma sex steroid hormones of male and female paco (*Piaractus brachypomus*) under hypoxic and normoxic conditions before and after hormonal treatments. Abbreviations as in Table 1. Means within the same column grouped as males and females with different letters are significantly different ($P < 0.01$).

Conditions	Plasma Sex Steroids					
	Male			Female		
	T (ng ml^{-1})	11-kT (ng ml^{-1})	17,20βP (pg ml^{-1})	T (ng ml^{-1})	11-kT (ng ml^{-1})	17,20βP (pg ml^{-1})
HYPOXIA (N = 6)						
Before Treatment	0.53 ± 0.10 ^{a}	5.98 ± 1.61 ^{a}	30 ± 10 ^{a}	1.58 ± 0.19 ^{a}	3.92 ± 0.23 ^{a}	17 ± 7 ^{a}
After Treatment	5.82 ± 0.72 ^{b}	6.29 ± 0.52 ^{a}	116 ± 25 ^{b}	11.06 ± 1.80 ^{c}	3.65 ± 0.53 ^{a}	736 ± 253 ^{b}
NORMOXIA (N = 4)						
Before Treatment	6.76 ± 0.98 ^{b}	37.68 ± 5.48 ^{b}	22 ± 9 ^{a}	6.10 ± 1.15 ^{b}	7.46 ± 0.32 ^{b}	45 ± 24 ^{a}
After Treatment	11.11 ± 1.12 ^{c}	7.46 ± 1.73 ^{a}	106 ± 18 ^{b}	24.14 ± 3.49 ^{d}	4.58 ± 0.75 ^{a}	2,161 ± 865 ^{c}

In October and November 1999, the concentrations of plasma sex steroid hormones increased significantly in comparison to those reported in July. Moreover, before the hormonal injections, the concentrations of 11-kT in males and E2 in females, as well as the ones of their precursor (T), were significantly ($P < 0.01$) higher in fish maintained under normoxic conditions than the ones in hypoxia. In contrast, the plasma levels of 17,20βP were similar regardless of the oxygen concentrations (Table 4). The response patterns of plasma sex steroids to the hormonal treatments were similar in both genders. The concentrations of T and 17,20βP significantly ($P < 0.05$) increased in both experiments. However, the levels reached under normoxic conditions were higher than the ones recorded under hypoxia. The exception was the concentration of 17,20βP in males, which did not change significantly. The concentration of 11-kT in males and E2 in females after spermiation or ovulation, respectively, decreased significantly ($P < 0.01$) in fish maintained in normoxic conditions, whereas it remained similar in males in hypoxia (Table 4). In March 2000 the weight of paco and gamitana sampled for blood steroid analysis reached 3,347 ± 113 g and 5,645 ± 250 g, respectively.

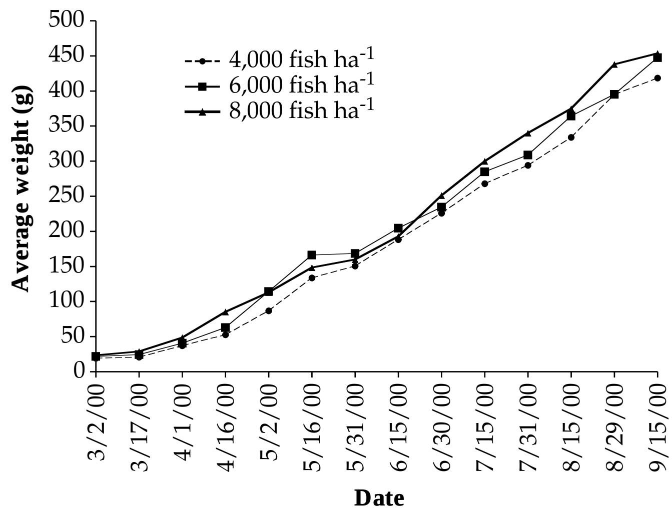

Figure 1. Average weight of *Piaractus brachypomus* stocked at densities of 4,000, 6,000, and 8,000 fish ha^{-1} in Iquitos, Peru, from 2 March through 15 September 2000.

Objective 3 (9NS3): Compare Hormones to Induce Spawning

Spawning induction of paco with LHRHa resulted in ovulation of three females, and a copious amount of sperm was obtained from males. Only one female, however, gave high quality of eggs (over 93% fertilization rate; embryonic-eyed stage). Neither males nor females were observed to mature following hCG injections. Gamitana were not induced to spawn following hormonal injections. Blood samples were collected from all examined fish, and steroid hormones were determined.

We do not recommend further use of hCG for gamitana and paco. Additionally, the effect of oxygen concentration on hCG was evaluated during final stages of induced maturation on blood steroid profiles, attempting to correlate these data with gamete viability.

Objective 4 (9NS3): Identify Proper Stocking Density for Paco and Gamitana for Pond Culture

It was possible to conduct research with both species (*Piaractus* and *Colossoma*) proposed originally in the Ninth Work Plan. No significant differences were present in the first study in grow-out performance of paco when stocked in ponds at densities of 3,000 and 4,000 fish ha^{-1} (Kohler et al., 1999). The mean fish growth rate of 3.0 g d^{-1} in this study is comparable to findings for gamitana (Saint-Paul, 1986; Gunther and Boza Abarca, 1992). Both characids grow slightly better under intensive culture conditions than tilapia (Peralta and Teichert-Coddington, 1989) and similar to *Clarias* (Hogendoorn et al., 1983; Verreth and Den Bieman, 1987). Feed conversion was excellent throughout the first study. The exceptionally high values during the early stages of the study reflect the ability of paco to filter-feed at the fingerling stage. These fish can also consume seeds and some plants found in the water. No differences ($P > 0.05$) existed in this experiment with paco at harvest in weight (418 g at 4,000 fish ha^{-1}, 447.5 g at 6,000 fish ha^{-1}, and 474.9 g at 8,000 fish ha^{-1}; Figure 1); total length (24.8 cm at 4,000 fish ha^{-1}, 26.1 cm at 6,000 fish ha^{-1}, and 25.7 cm at 8,000 fish ha^{-1}); specific growth rate (1.8 at 4,000 fish ha^{-1}, 1.8 at 6,000 fish ha^{-1}, and 1.7 at 8,000 fish ha^{-1}); condition (2.7 at 4,000 fish ha^{-1}, 2.7 at 6,000 fish ha^{-1}, and 2.8 at 8,000 fish ha^{-1}); or feed conversion efficiency (72.9% at 4,000 fish ha^{-1}, 76.2% at 6,000 fish ha^{-1}, and 74.7% at 8,000 fish ha^{-1}; Table 5). Survival exceeded 80%. No significant differences were found among any of the stocking densities at 3,000, 4,000, 6,000, and

Table 5. Performance of paco (*Piaractus brachypomus*) at three densities in pond trials conducted in Iquitos, Peru, from 2 March to 15 September 2000.

Date	4,000 fish ha^{-1} Weight (g)	TL (cm)	FCE (%)	SGR[a]	K[b]	6,000 fish ha^{-1} Weight (g)	TL (cm)	FCE (%)	SGR[a]	K[b]	8,000 fish ha^{-1} Weight (g)	TL (cm)	FCE (%)	SGR[a]	K[b]
02 March	19.0	8.5	--	--	--	21.7	8.5	--	--	--	28.3	9.8	--	--	--
17 March	20.9	9.1	--	--	--	24.3	9.3	--	--	--	33.8	10.6	--	--	--
01 April	37.0	10.9	--	--	--	40.4	11.3	--	--	--	47.5	12.1	--	--	--
16 April	52.7	12.8	--	--	--	62.9	13.5	--	--	--	85.0	14.3	--	--	--
02 May	86.7	14.7	--	--	--	114.0	15.7	--	--	--	133.0	16.3	--	--	--
16 May	133.7	17.0	--	--	--	166.0	17.5	--	--	--	170.3	17.8	--	--	--
31 May	150.7	17.8	--	--	--	168.3	17.7	--	--	--	178.0	18.7	--	--	--
15 June	188.0	19.6	--	--	--	204.3	19.8	--	--	--	192.7	19.4	--	--	--
30 June	225.7	20.2	--	--	--	234.3	20.7	--	--	--	261.7	21.0	--	--	--
15 July	268.0	21.4	--	--	--	284.7	22.0	--	--	--	320.3	23.0	--	--	--
31 July	294.0	22.2	--	--	--	308.7	23.7	--	--	--	350.3	24.7	--	--	--
15 August	333.8	22.9	--	--	--	364.0	24.4	--	--	--	403.0	25.4	--	--	--
29 August	394.7	24.7	--	--	--	395.0	24.9	--	--	--	445.3	25.9	--	--	--
15 September	418.2	24.8	72.9	1.8[c]	2.7[c]	447.5	26.1	76.2	1.8[c]	2.7[c]	474.9	26.5	74.7	1.7[c]	2.8[c]

(TL = Total length)
[a] Specific growth rate: SGR = ln (W_f) – ln (W_0)/T × 100; where W_f and W_0 = final and initial weights (g), respectively, and T = time (d).
[b] K = W/L^3; where W = weight (g), L = total length (cm).
[c] Composite

8,000 fish ha^{-1}. Accordingly, a density of at least 4,000 fish ha^{-1} can be recommended when supplemental feeding is provided. Densities of 2,000 to 3,000 fish ha^{-1} are traditionally used in the region.

Feed conversion for paco was excellent throughout this study. Exceptionally high values indicate its ability to feed on secondary natural sources in the ponds. In contrast to gamitana, they are not equipped with long, fine gillrakers; hence, filter-feeding is not as efficiently performed as in gamitana.

No significant differences were found in grow-out performance of gamitana stocked in ponds at densities of 2,500, 3,250, and 4,000 fish ha^{-1}. The mean fish growth rate of 1.90 g d^{-1} was lower than paco in the previous study. This can be attributed to certain ponds emerging with significant macrophyte infestations, which impeded the feeding process for an undetermined period of time.

Water quality varied among ponds in all density experiments (Table 6). Temperatures ranged from 25.0 to 30.0°C. Minimum DO levels were above 1.0 mg l^{-1} and averaged in excess of 3.0 mg l^{-1}. TAN remained below 1.0 mg l^{-1}. 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.

Table 6. Early morning water quality levels of ponds at the IIAP CRI-Loreto research facility in Iquitos, Peru, for *Piaractus brachypomus,* 2 March through 15 September 2000.

	Temperature (°C)	DO (mg l^{-1})	pH	TAN
Mean	28.0	3.4	7.1	< 1
High	30.0	8.5	9.1	< 1
Low	25.0	1.1	3.4	< 1

Water quality remained well within the tolerances of paco and gamitana throughout all the trials. Of course, it must be recognized that these fish can reduce their metabolic rate during periods of stress. Oxygen declines would need to remain low for days rather than hours to adversely affect them.

All the experiments revealed considerable potential for intensive aquaculture of gamitana and paco in the Peruvian Amazon. No significant differences were found between all the stocking densities.

Objective 5 (9NS3): Determine Cost of Production for Rearing in Ponds at Different Densities

In 2000 and 2001, IIAP produced 30,000 fingerlings of paco and 35,000 fingerlings of boquichico (*Prochilodus nigricans*) in a four- to six-month culture period. This production volume will be doubled in the future since the new hatchery construction has just been finished. Gamitana fingerlings were not produced since the broodstock were stressed from unusual physicochemical parameters in the holding pond.

The results of this study identified that both paco and gamitana are more profitable to produce per hectare at all stocking densities studied since they have a higher market value and equal production costs compared to other traditional agriculture crops (Tables 7 and 8). The production of gamitana and paco (assuming 90% survival) at densities higher than 2,500 fish ha^{-1} will be more profitable than pineapple production, which is the highest market value agriculture cash crop being produced in the Loreto region at the present time (Table 9).

Table 7. Enterprise budget for the production of gamitana at different stocking densities in earthen ponds in Iquitos, Peru.

	Costs per Unit	Stocking Rate (fish ha^{-1})				
		2,500	*3,250*	*4,000*	*6,000*	*8,000*
VARIABLE COSTS (US$)						
Fingerlings	0.13 each	325.0	422.5	520.0	780.0	1,040.0
Feed (kg fish)	0.31 kg^{-1}	775.0	1,007.5	1,240.0	1,860.0	2,480.0
Liming (500 kg ha^{-1})	214.3 ha^{-1}	214.3	214.3	214.3	214.3	214.3
Labor/Liming	25.0 ha^{-1}	25.0	25.0	25.0	25.0	25.0
Fertilizer (Chicken Manure)	125.0 ha^{-1}	125.0	125.0	125.0	125.0	125.0
Labor/Fertilizing	25.0 ha^{-1}	25.0	25.0	25.0	25.0	25.0
Maintenance	90.0 ha^{-1}	90.0	90.0	90.0	90.0	90.0
Total Variable ha^{-1}		1,579.3	1,909.3	2,239.3	3,119.3	3,999.3
FIXED COSTS (US$)						
Pond Construction Interest	313.0	313.0	313.0	313.0	313.0	313.0
Taxes	86.0	86.0	86.0	86.0	86.0	86.0
Pond Depreciation	120.0	120.0	120.0	120.0	120.0	120.0
Total Fixed ha^{-1}	519.0	519.0	519.0	519.0	519.0	519.0
TOTAL HA^{-1} (FIXED + VARIABLE)		2,098.3	2,428.3	2,758.3	3,638.3	4,518.3
SURVIVAL	90%	2,250	2,925	3,600	5,400	7,200
COST KG^{-1} HA^{-1} (COSTS/SURVIVAL, US$)		0.9	0.8	0.8	0.7	0.6
PRICE KG^{-1} (US$)		3.0	3.0	3.0	3.0	3.0
PROFIT POTENTIAL KG^{-1} (US$)		2.1	2.2	2.2	2.3	2.4
TOTAL PROFIT HA^{-1} (PROFIT × SURVIVAL, US$)		4,651.7	6,346.7	8,041.7	12,561.7	17,081.7

Table 8. Enterprise budget for the production of paco at different stocking densities in earthen ponds in Iquitos, Peru.

	Costs per Unit	Stocking Rate (fish ha^{-1})				
		2,500	*3,250*	*4,000*	*6,000*	*8,000*
VARIABLE COSTS (US$)						
Fingerlings	0.13 each	325.0	422.5	520.0	780.0	1,040.0
Feed (kg fish)	0.31 kg^{-1}	775.0	1,007.5	1,240.0	1,860.0	2,480.0
Liming (500 kg ha^{-1})	214.3 ha^{-1}	214.3	214.3	214.3	214.3	214.3
Labor/Liming	25.0 ha^{-1}	25.0	25.0	25.0	25.0	25.0
Fertilizer (Chicken Manure)	125.0 ha^{-1}	125.0	125.0	125.0	125.0	125.0
Labor/Fertilizing	25.0 ha^{-1}	25.0	25.0	25.0	25.0	25.0
Maintenance	90.0 ha^{-1}	90.0	90.0	90.0	90.0	90.0
Total Variable ha^{-1}		1,579.3	1,909.3	2,239.3	3,119.3	3,999.3
FIXED COSTS (US$)						
Pond Construction Interest	313.0	313.0	313.0	313.0	313.0	313.0
Taxes	86.0	86.0	86.0	86.0	86.0	86.0
Pond Depreciation	120.0	120.0	120.0	120.0	120.0	120.0
Total Fixed ha^{-1}	519.0	519.0	519.0	519.0	519.0	519.0
TOTAL HA^{-1} (FIXED + VARIABLE)		2,098.3	2,428.3	2,758.3	3,638.3	4,518.3
SURVIVAL	90%	2,250	2,925	3,600	5,400	7,200
COST KG^{-1} HA^{-1} (COSTS/SURVIVAL, US$)		0.9	0.8	0.8	0.7	0.6
PRICE KG^{-1} (US$)		2.1	2.1	2.1	2.1	2.1
PROFIT POTENTIAL KG^{-1} (US$)		1.2	1.3	1.3	1.4	1.5
TOTAL PROFIT HA^{-1} (PROFIT × SURVIVAL, US$)		2,626.7	3,714.2	4,801.7	7,701.7	10,601.7

Assumptions: 90% survival

Fixed costs are per year; assume a two-month "rest" before production begins again.

US$313 interest is for year 1 only; it drops to US$260, US$199, US$129, and US$48 for years 2 through 5, respectively.

US$3 kg^{-1} wholesale market price for gamitana and US$2.10 kg^{-1} for paco.

Fish that were fed for an additional five months (ten months total) reached about a kilogram in size (from 27.5 g). The prepared diet used in the study cost US$1.02 to produce 1.0 kg of whole fish, and fingerlings generally sell for about US$0.13 each. Food-size paco sell in the Iquitos market for US$2.10 kg^{-1} and gamitana for over US$3.00 kg^{-1}. Some success has been achieved in encouraging local consumers to purchase small farm-raised fish (250 g) over the large (> 5 kg) wild-caught ones, particularly during the dry-season months when these two species are scarce. Consumers are slowly changing their ways to a point where some are beginning to identify smaller fish as synonymous with better taste. Additionally, the family food preparer is discovering the advantage of serving a whole fish per plate to their family as opposed to serving only a portion of a large one. It is important to mention that the majority of the producers located along the Iquitos–Nauta road have identified the high economic value of their aquaculture crop to the point that they might utilize part of their traditional agriculture crops as supplemental or exclusive diets for gamitana or paco.

Objective 1 (9NS6): Conduct Outreach Activities to Regionalize CRSP Outcomes

The Chemistry Lab Will Be Upgraded, Equipped, and Supplied with Reagents to Become Functional for the Performance of All CRSP Water Quality Analyses

A new hatchery was recently finished. This hatchery will facilitate the production of fingerlings not only of paco and gamitana but also of *Pseudoplatystoma* and *Arapaima* for the experiments and studies of the Tenth Work Plan. Most of the equipment and reagents present at IIAP Quistococha Aquaculture Station were updated, including the following additions: microscope, stereoscope, spectrophotometer, oxygen and pH meters, minimum/maximum thermometers, weather equipment, and reagents to determine various water quality parameters.

A South American Network of Aquaculturists Will Be Initiated

An Amazon Aquaculture website was created at <ws1.coopfish.siu.edu/amazonia/index.html> to initiate a network specializing in Amazonian species for the purpose of bringing together Amazon aquaculture researchers from around the world for information exchange in their efforts to improve the culture and marketing of existing species and of expanding the species list even further. Several contacts have been established in Honduras as well as in our host country, Peru. All of these contacts were made in person.

Reinforce Extension Activities for Local Farmers

The extension activities conducted in the Eighth and Ninth Work Plans are still being conducted jointly with the Italian NGO Terra Nuova and Programa de la Seguridad Alimentaria (PROSEAL), serving the 260 local farmers along the road system between the cities of Iquitos and Nauta. Farmers will be provided guidance by Terra Nuova until the end of December 2001. PROSEAL will continue together with the CRSP through the Tenth Work Plan to administer this service to the local farmers. They now provide services to 71% of fish farmers, who account for almost 62% of total fish ponds in the region (Table 10). PROSEAL has been a direct beneficiary of the CRSP program in Peru. Results from research conducted at our host country facilities provided much of the information that PROSEAL extended to farmers. Thanks to leadership provided by our host country principal investigator (PI), Fernando Alcántara, as well as other IIAP and UNAP members, valuable information developed from our project has been transferred to the local area fish farmers. Efforts began in the Ninth Work Plan to create an extension working committee, which will allow us to formally integrate our extension activities for the Tenth Work Plan into the existing host country program. The committee will ensure proper cooperation among all participating entities and help avoid redundancy in the proposed work region. Alcántara, with PD/A CRSP support, will continue to serve as the lead aquaculture extensionist. The PROSEAL project is scheduled to terminate in December 2001. Accordingly, the continuity of this important effort will be reliant on PD/A CRSP support thereafter.

Technicians Will Be Provided with Training for Laboratory and Pond Practices Following CRSP Protocols

All IIAP aquaculture technicians have been trained for laboratory and pond practices through the workshops that have been offered to the fish producers by PROSEAL and sponsored by the PD/A CRSP and Terra Nuova.

A Workshop Will Be Planned

Alcántara (IIAP, host country PI) and Palmira Padilla (co-investigator) presented several weekend workshops for the regional farmers involved in local extension projects and for high school students. Padilla continues to offer general aquaculture courses to high school students in Iquitos. The purpose is to introduce the local population to this discipline and arouse interest in this form of food production. In addition to these workshops, several international presentations were given in numerous symposia and conferences.

Objective 2 (9NS6): Complete Spanish-Language Production Manual for Small-Scale Pond Aquaculture in the Peruvian Amazon

The production manual "Reproducción Inducida de Gamitana y Paco" was printed and distributed. It is currently being used as an extension manual by IIAP collaborators. The manual fully acknowledges the PD/A CRSP, as well as IIAP and SIUC. The manual includes the following sections: broodstock preparation, broodstock selection, hormonal treatment, ovulation and spawning, fecundity, incubation, larvae culture, and grow-out. Alcántara is planning to prepare a new edition to cover the new aspects that have been generated this year and that will be in subsequent years.

Table 9. Estimated production of other local agriculture crops in one hectare of land in the Loreto region, Peru.

Crop	Production Volume	Production Cost (US$)	Market Value (US$)	Total Value (US$)	Profit (US$)
Yucca	8,000 kg	160.0	2.00 $(50\ kg)^{-1}$	320.0	160.0
Palmito	8,000 stalks	280.0	0.07 $stalk^{-1}$	560.0	280.0
Aguaje	6,000 kg	342.6	5.71 $(50\ kg)^{-1}$	685.2	342.6
Plantain	400 bunches	228.0	1.14 $bunch^{-1}$	456.0	228.0
Pineapple	16,000 tons	1,232.0	0.14 kg^{-1}	2,240.0	1,008.0

Objective 3 (9NS6): Expand List of Locally Available Ingredients for Practical Diets Suitable for *Colossoma* and *Piaractus* Broodstock to Include Grow-out Diets

We have expanded the list of practical ingredients developed by Lochmann for broodstock diets to include grow-out diets. Alternative local grow-out sources have been identified. Numerous wild fruits and plant products for small-scale sustainable aquaculture production of gamitana and paco are currently being used by the local farmers as fish feed in and around Iquitos (Table 11).

The fruits and plants surveyed have the following seasonal availability: pijuayo (*Bactris gasipaes*), winter; guayaba (*Psidium guajaba*), winter; lady finger banana (*Musa paradisiaca*), all year round; papaya (*Carica papaya*), all year round; airambo (*Phytolaca rivinoides*), winter; mullaca (*Physalis angulata*) and camu camu (*Myrciaria dubia*), December to April (during flooding season); cetico (*Cecropia* sp.), winter; míspero (*Achras sapota*), winter; renaco (*Ficus* sp.), winter; yucca (*Manihot esculenta*), all year round, although during the dry season (from June through September) it is more abundant; mishquipanga (*Renealmia alpina*), winter; picho huayo (*Siparuna guianensis*), winter; cocona (*Solanum sessiliflorum*), all year round; cashew (*Anacardium occidentalis*), winter; caimito (*Chrysophyllum cainito*), all year round; anona (*Annona muricata*), winter.

ANTICIPATED BENEFITS

We were able to identify suitable stocking densities for paco and gamitana for profitable pond culture in the Peruvian Amazon. Most farmers generally use organic fertilizers and periodically provide fruits, nuts, and kitchen scraps. The experiment utilized an economic prepared diet (26.7% protein and 9% lipids) for grow-out. Considering the excellent growth rates that occurred (3.4 g to 1 kg in ten months), it appears that

Table 10. Local organizations and their extension responsibilities along the Iquitos–Nauta road as of January 2001 (Alcántara, pers. comm.).

Organization	Surface Area (ha)	Percentage of Total	Fish Farmers	Percentage of Total	Ponds	Percentage of Total
PROSEAL	44.00	35.40	260	70.65	308	61.97
CURMI (NGO)	8.99	7.23	41	11.14	42	8.45
CARITAS (NGO)	10.95	8.81	10	2.72	10	2.01
FONDEPES (GO)	1.80	1.44	6	1.63	6	1.20
Independent Farmers	52.32	42.09	49	13.32	84	16.90
Government Farms	6.25	5.03	2	0.54	47	9.45
Total	124.31	100.00	368	100.00	497	100.00

Table 11. Proximate analysis values (when known) of some fruits and other local plant products utilized to feed fish around Iquitos (food value per 100 g, modified from Morton, 1987).

Common Name	Scientific Name	Calories	Proteins (g)	Carbohydrates (g)	Lipids (g)	Fiber (g)	Ash (g)
Pijuayo	*Bactris gasipaes*	196	2.6	41.7	4.4	1.0	ND
Guayaba	*Psidium guajaba*	36–50	0.9–1.0	9.5–10.0	0.1–0.5	2.8–5.5	0.4–0.7
Lady Finger Banana	*Musa paradisiaca*	110.7–156.3	0.8–1.6	25.5–36.8	0.1–0.8	0.3–0.4	0.6–1.4
Papaya	*Carica papaya*	23.1–25.8	0.1–0.3	6.2–6.8	0.1–1.0	0.5–1.3	0.3–0.7
Airambo	*Phytolaca rivinoides*	ND	ND	ND	ND	ND	ND
Mullaca	*Physalis angulata*	ND	0.05	ND	0.16	4.9	1.0
Cetico	*Cecropia* sp.	ND	ND	ND	ND	ND	ND
Míspero	*Achras sapota*	ND	ND	ND	ND	ND	ND
Renaco	*Ficus* sp.	80	1.2–1.3	17.1–20.3	0.1–0.3	1.2–2.2	0.48–0.85
Yucca	*Manihot esculenta*	135	1.0	32.4	0.2	1.0	0.9
Mishquipanga	*Renealmia alpina*	ND	ND	ND	ND	ND	ND
Picho Huayo	*Siparuna guianensis*	ND	ND	ND	ND	ND	ND
Cocona	*Solanum sessiliflorum*	ND	0.6	5.7	ND	0.4	ND
Cashew	*Anacardium occidentalis*	ND	0.1–0.2	9.1–9.8	0.1–0.5	0.4–1.0	0.2–0.3
Caimito	*Chrysophyllum cainito*	67.2	72.0–2.33	14.7	ND	0.6–3.3	0.4–0.7
Anona	*Annona muricata*	53.1–61.3	1.0	14.6	1.0	0.8	0.60
OTHERS							
Rice Meal Powder	*Oryza sativa*	ND	6.2	36.0	5.2	28.9	15.7
Wheat Bran	*Triticum aestrivum*	ND	15.2	53.8	3.9	10.0	6.1

ND = No data available

this diet meets or exceeds nutritional needs of gamitana and paco. Results of the present study are being shared with local farmers via extension work, and future results will be shared similarly. We have also gained perspective as to when these fish reach their reproductive peak during their annual cycle in order to better program spawning efforts. We also determined some parameters that can be controlled to obtain improved gamete quality from the broodstock. Most importantly, the information gleaned from the Ninth Work Plan is being made available to local farmers through an extension program now fully in place.

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SPAWNING AND GROW-OUT OF *COLOSSOMA MACROPOMUM* AND/OR *PIARACTUS BRACHYPOMUS*

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

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ABSTRACT

Proximate analysis of broodstock and grow-out feeds for gamitana (*Colossoma macropomum)* and paco (*Piaractus brachypomus*) and their constituent feedstuffs was conducted. Literature values for specific nutrients known to affect fish reproduction were calculated from published sources for the broodstock diet. Fatty acid and amino acid profiles of broodstock eggs were obtained. Analytical information on the feedstuffs and diets currently being used in Iquitos, Peru, together with the egg data and published information on the natural diets of colossomids and broodstock nutrition in other species, was 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. Broodstock nutrition research in fish is rapidly expanding because reliable propagation of captive species is both difficult and vital to the success of most aquaculture industries. Key nutrients such as long-chain highly unsaturated fatty acids, amino acids, and antioxidant vitamins are implicated consistently in the spawning performance and production of quality offspring in many fish species (De Silva and Anderson, 1995). The amino acid profile of fish eggs has been used as an index of the appropriate amino acid balance for broodstock diets (Ketola, 1982), and the fatty acid profile may be used to determine the essential fatty acid composition of the diet (Tocher and Sargent, 1984). Chemical analyses of feedstuffs, prepared broodstock diets, and fish eggs were used in this study, along with published data, to identify nutritional factors that may be hindering reproductive success in captive colossomid brood-stock. In addition, preliminary recommendations were made for improvement of broodstock diets.

METHODS AND MATERIALS

Proximate analysis 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 content was analyzed using the Folch method (Folch et al., 1957). The amino acid profiles of fertilized and unfertilized colossomid eggs were determined with an amino acid analyzer. The fatty acid profiles of the eggs were determined with a gas chromatograph equipped with a flame ionization detector. Other data used in this report were obtained from published sources. Small-scale feeding trials were attempted with *Piaractus brachypomus* to bracket the dietary requirements for vitamins C and E. However, most of the fish died during prolonged power outages due to ice storms in Arkansas in December 2000 and January 2001.

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. Colossomid eggs are known to be lipid-rich, which appears to be an adaptation to support metabolism rather than growth of newly hatched larvae as they drift downstream for days without feeding (Araujo-Lima, 1994).

The relative abundance of protein compared to energy in the broodstock 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 nutrient-poor 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 is a phos-pholipid which functions in lipid digestion and transport and improves performance in some fish (Hertrampf, 1992). Phospholipid supplementation of broodstock diets also enhances egg quality in some fishes, such as red sea bream (Watanabe et al., 1991), and serves as a source of phosphorus. The present swine vitamin-mineral premix does not contain phosphorus, and fishmeal is the primary source of available phosphorus in the diet. Phospholipid content of the broodstock diet was not addressed in the present study, but will be measured in future studies with colossomid broodstock.

No supplemental lipid was added to the current broodstock diet, but it contains 7% lipid. Most of the lipid comes from fishmeal (2.9%), wheat husks (1%), soybean meal (0.7%), and corn flour (0.7%). The lipid content of the broodstock diet strongly influences the fatty acid profile of fish eggs (Watanabe et al., 1978, 1984). The lipid content and fatty acid profile of fertilized and unfertilized colossomid eggs from captive broodstock in Iquitos is shown in Table 1. The total lipid content of fertilized and unfertilized eggs ranged from 41.5 to 42.5%. This lipid level is considerably lower than the value (60%) reported for unfertilized colossomid eggs by Heming and Buddington (1988). The apparent lipid deficit in colossomid eggs in this study might have resulted directly from the low available energy content of the current broodstock diet. This problem can be resolved by supplementing lipid in the diet, as recommended earlier. The total lipid content of unfertilized eggs was significantly higher than that in fertilized eggs, probably signifying the use of lipid to fuel ontogenetic changes following fertilization (Table 1). There were no differences in the relative amounts of individual fatty acids between fertilized and unfertilized eggs.

Lipids from the fish meal supplied several of the long-chain (≥ 20 carbons) fatty acids known to be important for reproduction in fish: eicosapentaenoic (EPA, 20:5n-3), eicosatetraenoic (arachidonic, 20:4n-6), and docosahexaenic (DHA, 22:6n-3). The 20-carbon fatty acids are precursors of prostaglandins, which regulate ovulation in females (Goetz, 1983) and synchronization of reproductive behavior in males and females (Kobayashi et al., 1986a, 1986b). The DHA plays prominent roles in the development of the brain and other parts of the nervous system, especially the eye (Mourente and Tocher, 1998). Because the n-3 and n-6 fatty acids are antagonistic, a ratio of 1:1 of n-3 to n-6 fatty acids has been suggested as optimal for most fish functions, including reproduction (Tacon, 1987). Colossomid eggs in this study appeared to have about twice as much total n-3 fatty acids as n-6 fatty acids (Table 1). Eggs of wild colossomids were not available during this study, but comparison of fatty acid patterns from wild and cultured eggs should indicate whether or not the n-3:n-6 ratio of approximately 2:1 represents an imbalance in the eggs from cultured fish. A deficiency or imbalance of the n-3 and n-6, 20- to 22-carbon fatty acids in broodstock diets has been identified or implicated in impaired spawning or reduced gamete and larval quality or both in rainbow trout (Fremont et al., 1984), fathead minnows (Cole and Smith, 1987), goldfish (Wade et al., 1994), milkfish (Ako et al., 1994), and many marine fishes (Izquierdo et al., 2001). Because the main source of these fatty acids in the present colossomid broodstock diet is the lipid associated with the fish meal, any change in diet composition that results in a lower level of fish meal should be accompanied by an increase in supplementation of marine fish oil. There are very few other practical lipid sources that contain highly unsaturated fatty acids of both the n-3 and n-6 families.

The combination of fish meal (25%) and soybean meal (30%) meets the essential amino acid requirements of most warmwater fish for most functions (National Research Council, 1993). The amino acid profile of fertilized and unfertilized colossomid eggs is shown in Table 2. Leucine was the predominant indispensable amino acid in both fertilized and unfertilized eggs. Histidine was present in lower amounts than all other indispensable amino acids in both fertilized and unfertilized eggs. The high levels of leucine, glutamic acid, and alanine indicate that the broodstock diet is providing sufficient quantities of the amino acids needed to synthesize vitellogenin, the primary precursor of yolk proteins in teleosts. Although omnivorous and vegetarian fishes need little or no fish meal for optimal growth, *Oreochromis niloticus* broodstock fed diets with isonitrogenous amounts of either fish meal or legume meal had better ovarian growth and produced larger eggs when fed the fish meal diet (Cumaranatunga and Thabrew, 1990). The authors concluded that fish meal is richer in

Table 1. Total lipid (%) and fatty acid (% of total fatty acids) content of freeze-dried fertilized and unfertilized eggs from *Piaractus brachypomus* in Iquitos, Peru.[1, 2]

Lipid or Fatty Acid Name	Fatty Acid Formula[3]	Fertilized Eggs	Unfertilized Eggs
Total Lipid	NA	41.48 ± 0.72*	42.50 ± 1.36*
Myristic	14:0	2.77 ± 0.10	2.74 ± 0.13
Palmitic	16:0	27.45 ± 1.44	27.10 ± 1.33
Palmitoleic	16:1	4.03 ± 0.52	3.82 ± 0.57
Stearic	18:0	13.45 ± 1.53	13.24 ± 1.53
Oleic	18:1	31.08 ± 1.66	30.64 ± 2.05
Linoleic	18:2n-6	4.10 ± 0.16	4.12 ± 0.08
Eicosatrienoic	20:3n-3	1.03 ± 0.02	1.02 ± 0.03
Eicosatetraenoic	20:4n-6[4]	1.39 ± 0.09	1.37 ± 0.06
Eicosapentaenoic	20:5n-3[4]	1.10 ± 0.07	1.11 ± 0.05
Docosahexaenoic	22:6n-3[4]	8.68 ± 0.44	8.88 ± 0.37

1 Values are means of three replicates ± SD. Fatty acids present at less than 1% of the total are not shown.
2 Means in rows followed by an asterisk (*) are significantly different ($P < 0.10$) using a paired t-test.
3 Fatty acids are designated using the formula xy (n–z), where x = the number of carbons in the chain, y = the number of double bonds, and z = the carbon number where the first double bond from the methyl end (n) of the fatty acid is located.
4 The 20- and 22-carbon fatty acids with 4 or more double bonds are known to be important for reproduction in many fishes.

Table 2. Amino acid content (%) of freeze-dried fertilized and unfertilized eggs from *Piaractus brachypomus* in Iquitos, Peru.[1, 2]

Amino Acid[3,4]	Fertilized Eggs	Unfertilized Eggs
Phenylalanine[3]	2.55 ± 0.01	2.53 ± 0.03
Valine[3]	3.55 ± 0.01	3.48 ± 0.04
Threonine[3]	2.49 ± 0.07	2.53 ± 0.04
Isoleucine[3]	3.28 ± 0.02*	3.18 ± 0.03*
Methionine[3]	1.85 ± 0.05	1.86 ± 0.08
Histidine[3]	1.47 ± 0.01	1.48 ± 0.08
Arginine[3]	4.22 ± 0.08	4.23 ± 0.04
Leucine[3]	5.39 ± 0.03**	5.30 ± 0.05**
Lysine[3]	4.38 ± 0.01	4.32 ± 0.08
Aspartic Acid[4]	4.44 ± 0.03*	4.49 ± 0.05*
Serine[4]	2.70 ± 0.19	2.85 ± 0.07
Glutamic Acid[4]	7.08 ± 0.02	7.00 ± 0.09
Glycine[4]	1.87 ± 0.02	1.86 ± 0.03
Alanine[4]	6.50 ± 0.03*	6.37 ± 0.08*
Cystine[4]	0.64 ± 0.02	0.64 ± 0.04
Tyrosine[4]	1.98 ± 0.07	1.91 ± 0.09

1 Values are means of three replicates ± SD.
2 Means in rows followed by one or two asterisks (*) are significantly different at $P < 0.10$ and $P < 0.05$, respectively, using a paired t-test.
3 Indispensable amino acid. Tryptophan was the only indispensable amino acid excluded from analysis because it could not be analyzed simultaneously with the rest.
4 Dispensable amino acid.

vitellogenic proteins and n-3 fatty acids that enhance reproduction in many fishes. In this study isoleucine, leucine, and alanine were higher in fertilized eggs than in unfertilized eggs, and aspartic acid was higher in unfertilized eggs than in fertilized eggs (Table 2). These differences can be attributed to differences in the relative activities of energy generation and protein synthesis from amino acids in unfertilized and fertilized eggs.

There is little 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 vitamin C at a rate of 500 mg kg^{-1}. This supplement is critical, as the intrinsic vitamin C content of the feedstuffs is very low. A diet with 139 mg ascorbic acid kg^{-1} was optimal for growth of pacu (*P. mesopotamicus*) fingerlings (Martins, 1995). However, the requirement may be higher for larvae due to their more rapid growth rate. The quality of eggs and spermatozoa of rainbow trout was substantially higher when fish were fed diets containing vitamin C at 8 to 10 times the level required for optimal growth (Blom and Dabrowski, 1995). The stability of vitamin C is poor under conditions of high heat and humidity, as in Iquitos. Therefore, the form of C is critical; a stabilized form should be used. If a stable form is not currently used, a different form can be used 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 vitamin E kg^{-1} diet, and the feedstuffs supply another 20 to 30 mg. 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 marine fish oil) are increased in the diet. Furthermore, there are studies showing that broodstock diets containing large amounts of vitamin E given to broodstock just prior to spawning have positive effects (Kanazawa, 1988). In carp, vitamin E increases gonadosomatic index, facilitates vitellogenisis, 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 colossomids.

Of pigments reported to have beneficial effects in fish or other animals, only xanthophylls (from corn flour, with a xanthophyll content of 17 mg kg^{-1}) are present in the broodstock diet (2.2 mg kg^{-1}). 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 colossomids. Further research is necessary to identify inexpensive, available sources of carotenoids (such as fruits, vegetables, or flowers) that could be used in Iquitos.

Finally, most studies indicate that there is little or no benefit in adding fish meal to grow-out diets of vegetarian or omnivorous colossomid species. However, the nutritional requirements for growth are not always the same as for those for reproduction. If reduction of the fish meal in the current broodstock diet is considered for environmental or cost reasons, there are multiple nutritional factors to consider as well, as outlined in this report. The chemical composition of the colossomid eggs from the present study and other studies should serve as a guideline for formulation of broodstock diets in the future.

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.

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SEMI-INTENSIVE CULTURE OF RED TILAPIA IN BRACKISHWATER PONDS

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

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ABSTRACT

An experiment was conducted at the Asian Institute of Technology, Thailand, to investigate effects of fertilization rates and salinity levels on the growth of sex-reversed Thai red tilapia (*Oreochromis* sp.). The experiment was designed to test two fertilization rates (28 kg nitrogen and 7 kg phosphorus ha^{-1} wk^{-1}, N:P = 4:1; and 14 kg N and 7 kg P ha^{-1} wk^{-1}, N:P = 2:1) and three salinity levels (10, 20, and 30‰). An additional treatment using optimized fertilization rates (28 kg N and 7 kg P ha^{-1} wk^{-1}, N:P = 4:1) in freshwater ponds served as a control. Red tilapia fingerlings (20.2 to 23.7 g size) were stocked at 2.4 fish m^{-2} in 5-m^2 cement tanks with soil bottoms. They were cultured for 160 days.

Growth performance of red tilapia was better in brackish water than in fresh water. Growth of red tilapia in brackish water was inversely related to the salinity levels ($r = -0.63$, $P < 0.05$), decreasing significantly with increasing salinity. Best growth performance was achieved in the treatment with N:P ratio of 4:1 at 10‰ salinity. The highest net economic return was achieved in the treatment with N:P ratio of 2:1 at 10‰ salinity, and all treatments had positive returns.

Preliminary trials using a single species of marine phytoplankton showed that growth of red tilapia fed with *Chaetoceros* sp. and *Thalassiosira* sp. was significantly better than those fed with *Tetraselmis* sp. and *Chlorella* sp., and the former two resulted in a significantly higher protein utilization efficiency than the latter two. The prey ingestion rate of red tilapia for *Chaetoceros* sp. and *Thalassiosira* sp. was significantly higher than that for *Tetraselmis* sp. and *Chlorella* sp.

INTRODUCTION

Many tilapia species are euryhaline and can grow in saline water after proper acclimation (Suresh and Lin, 1992). Varieties of red tilapia have been successfully cultured in saline waters (Watanabe, 1991). However, most of those tilapia culture trials were conducted in intensive systems with pelleted feeds, requiring frequent water exchanges or cages. Compared to the voluminous literature available for semi-intensive culture of tilapia in freshwater ponds, literature on semi-intensive culture in saline ponds is almost nonexistent. The species composition, feeding, and nutritional value of phytoplankton for tilapia growth in freshwater are relatively well understood, notably in work by Moriarty and Moriarty (1973), Bowen (1982), Hepher and Pruginin (1982), and recently the Pond Dynamics/ Aquaculture Collaborative Research Support Program (PD/A CRSP) (Egna and Boyd, 1997). The PD/A CRSP project did conduct a brief experiment on Nile tilapia grow-out in fertilized brackishwater ponds in the Philippines in the early 1980s (Woessner et al., 1991). In that experiment fish production was extremely low, resulting from high mortality due to uncontrolled high salinity. We are assuming that fertilization rates for brackishwater ponds are similar to those for freshwater ponds. Common PD/A CRSP fertilization guidelines are 28 kg nitrogen (N) and 7 kg phosphorus (P) ha^{-1} wk^{-1}, giving a N:P ratio of 4:1 (Knud-Hansen et al., 1991).

During the last few years, there has been a strong desire to culture tilapia in brackishwater ponds in Southeast Asia, as well as in Central and South America (Green, 1997). The major reason for this need is that there are a large number of shrimp ponds available, resulting from either failure in shrimp farming or desires to diversify shrimp culture. Tilapias appear to be the most appropriate choice for such a culture system because there are few domesticated finfish species that feed on low-cost natural foods such as detritus and plankton. This interest in brackishwater culture is particularly strong in Thailand and Vietnam where shrimp culture is now commonly reduced to one crop per year, leaving the ponds empty for half a year. Tilapia culture is also attractive to shrimp farmers as a by-product to utilize abundant phytoplankton in either shrimp ponds or their effluents. Thai strain red tilapia (*Oreochromis* sp.) has been becoming more and more popular in Thailand, and there is a great potential to culture this species in brackishwater ponds.

The purposes of this study were to determine an appropriate fertilization regime in brackish water for culture of Thai red tilapia and to investigate the nutritional value and prey consumption of specific marine phytoplankton as food organisms for Thai red tilapia.

METHODS AND MATERIALS

This experiment was carried out at the Asian Institute of Technology (AIT), Thailand, from June through November 2000 to determine an appropriate fertilization regime and salinity level for culture of Thai red tilapia in brackish water. The experiments were conducted in a randomized complete block design with a 2 × 3 factorial arrangement to test effects of two fertilization regimes (28 kg N and 7 kg P ha^{-1} wk^{-1}, N:P = 4:1; and 14 kg N and 7 kg P ha^{-1} wk^{-1}, N:P = 2:1) and three salinity levels (10, 20, and 30‰) on growth of Thai red tilapia. There were six combinations (treatments) and an additional treatment using the PD/A CRSP standard fertilization regime (28 kg N and 7 kg P ha^{-1} wk^{-1}, N:P = 4:1) in fresh water (0‰) that served as a control. Three replicates were used per treatment. The experiment was conducted in twenty-one 5-m^2 (2 × 2.5 m) cement tanks filled with 10 cm of soil on the bottom. The tanks were grouped into three blocks, and treatments were allocated randomly to tanks in each block.

Thai strain red tilapia was used in the experiment. Sex-reversed all-male Thai red tilapia fingerlings (20.2 to 23.7 g size) were purchased from a local hatchery and acclimated to appropriate salinity levels in acclimation tanks by raising the salinity level 5‰ every two days until the target salinity was reached. The acclimated Thai red tilapia fingerlings were stocked at 2.4 fish m^{-2} in all experimental tanks on 8 June 2000. During the experiment 50% of the initial tilapia stock was seined, counted, and weighed en masse biweekly for each tank. All fish were harvested on 15 November 2000, after 160 days of culture. Daily weight gain (g $fish^{-1}$ d^{-1}), yield (kg $pond^{-1}$), and extrapolated yield (kg ha^{-1} yr^{-1}) were calculated.

All tanks were fertilized weekly with urea and triple superphosphate (TSP) to achieve the treatment dosage. Initial pond fertilization took place one week prior to fish stocking. Sodium bicarbonate was applied at 250 kg ha^{-1} in the third week. Salinity was regulated by trucking hypersaline water (150‰) to AIT and diluting to the appropriate concentrations. Salinity levels in all tanks were monitored weekly. Water depth in all tanks was maintained at 1 m throughout the experiment by adding water of appropriate salinity levels weekly to replace evaporation and seepage losses. All tanks were aerated for 24 hours daily using one airstone in each tank.

Water samples integrated from the entire water column were taken biweekly near the center of each tank at approximately 0900 h for analysis of pH, alkalinity, total ammonium nitrogen (TAN), nitrite-nitrogen, nitrate-nitrogen, total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), total phosphorus (TP), chlorophyll *a*, total suspended solids (TSS), total volatile solids (TVS), and plankton composition (Parsons et al., 1984; APHA, 1985; Egna et al., 1989). At the time of collecting water samples, Secchi disk visibility was measured using a Secchi disk, while temperature and dissolved oxygen (DO) were measured at the time of collecting water samples with a YSI model 54 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Diel measurements for temperature, DO and pH were conducted in each pond at 0600, 1000, 1400, 1600, 1800, and 0600 h once a month.

Preliminary trials were also carried out to investigate the nutritional value and prey consumption of specific marine phytoplankton species, *Tetraselmis* sp., *Chlorella* sp., *Thalassiosira* sp., and *Chaetoceros* sp., for Thai red tilapia. The first trial on nutritional value was conducted over 15 days in twelve glass jars of 15-l volume with three replicates for each species. Each jar was filled with 15‰ salinity water and continuously aerated using an airstone throughout the experimental period. Thai red tilapia (1.42 to 1.61 g size) were stocked at 0.4 fish l^{-1} and fed twice daily with the same quantity (dry matter, DM) of each phytoplankton species, which were cultured in the AIT laboratory and were approximately three to four days old. Red tilapia growth was determined by bulk-weight at the beginning and end of the culture period. Water in jars was changed

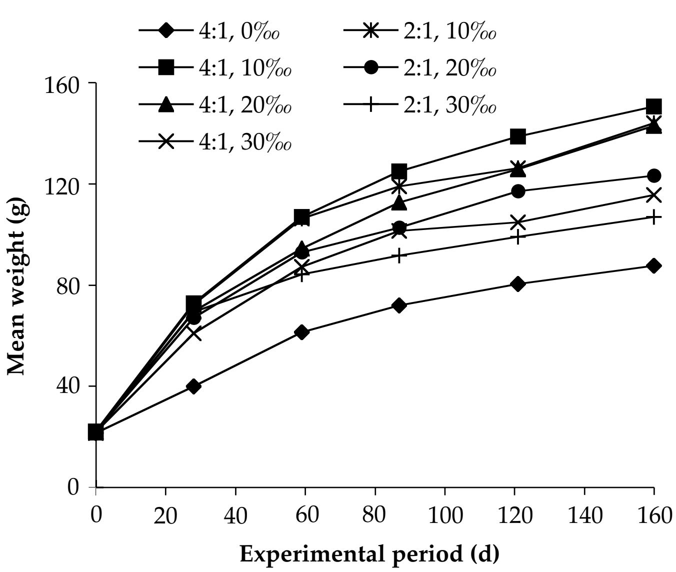

Figure 1. Mean weight of Thai red tilapia in all treatments over the 160-day experimental period.

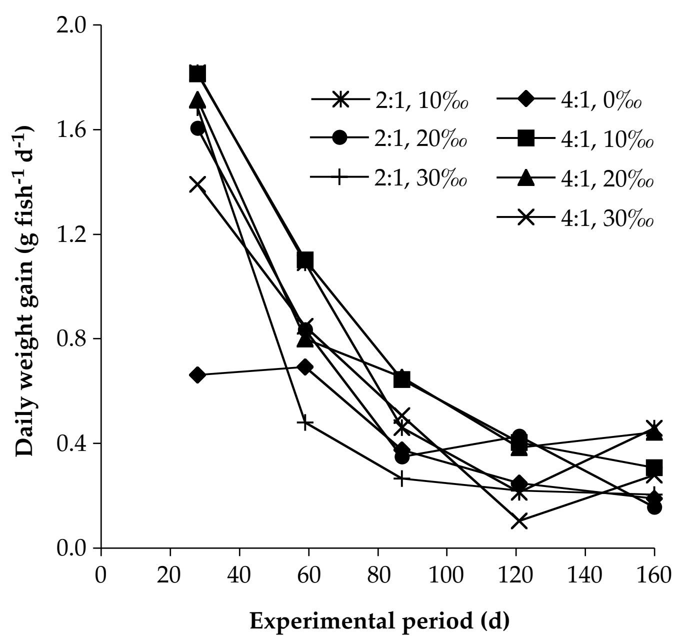

Figure 2. Daily weight gain of Thai red tilapia in all treatments over the 160-day experimental period.

by half each morning. Water temperature, DO, salinity, and pH were measured at 0800 h daily prior to changing water. Proximate analysis of red tilapia and the four phytoplankton species was done at the beginning and end of the culture period using methods described by Lowry et al. (1951), Yoshida et al. (1976), Buddington (1980), and Tecator (1983, 1987). The second trial on prey consumption was conducted twice, at the beginning and end of the first trial, in twelve glass jars of 4-l water volume with three replicates for each species for approximately two hours. Red tilapia were stocked at 1.5 fish l^{-1} with mean weights of 1.50 and 2.20 g for the prey consumption trials conducted at the beginning and end of the first trial, respectively. The same quantity (DM) of each phytoplankton species was put into each jar, and the dry weight of phytoplankton was determined immediately after the two-hour feeding period.

Data were analyzed statistically using analysis of variance and linear regression (Steel and Torrie, 1980) with SPSS (version 7.0) statistical software package (SPSS Inc., Chicago, USA). Differences were considered significant at an alpha of 0.05. Statistical analyses for survival rates (%) were performed on data after arcsine transformation. Mean values of survival rates in this text are listed in normal scale followed by their confidence limits. All other means are given with ± 1 standard error (SE).

A partial budget analysis was conducted to determine economic returns of red tilapia cultured at different salinity levels and different fertilization regimes (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 = 40 baht). Farm-gate price of red tilapia was fixed at \$0.50 kg^{-1}. Market prices of sex-reversed all-male red tilapia fingerlings (\$0.0125 $piece^{-1}$), electricity (\$0.05 kWh^{-1}), sodium bicarbonate (\$0.775 kg^{-1}), urea (\$0.1875 kg^{-1}), and TSP (\$0.3125 kg^{-1}) were applied to the analysis. The calculation for cost of working capital was based on an annual interest rate of 8%.

RESULTS

There was no mortality of red tilapia in any treatment. Red tilapia grew fast in the first two months, and then growth declined towards the end of the experiment period (Figures 1 and 2). Growth performance of red tilapia (Table 1) was inversely related to salinity levels ($r = -0.63$, $P < 0.05$), and size at harvest decreased with increased salinity (Figure 3). For a given salinity level, there was no significant difference in size at harvest for the two fertilization regimes ($P > 0.05$, Table 1 and Figure 3). All growth performance parameters were best in the treatment with high N:P ratio at 10‰ salinity and were significantly better than those in the treatment with low N:P ratio at 30‰ salinity and those in the freshwater treatment ($P < 0.05$) but were not significantly different from other

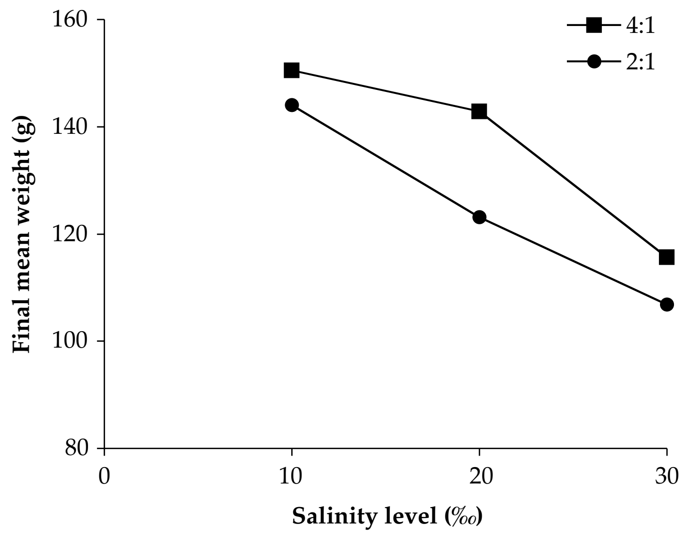

Figure 3. Comparison of final mean weight of Thai red tilapia cultured at different salinity levels under low and high fertilization rates after the 160-day experiment period.

Table 1. Performance values (mean ± SE) of Thai red tilapia in cement tanks at different fertilization regimes and salinity levels at stocking and harvest (160 d later). Data with superscripts showed significant differences among treatments, and treatments with the same superscript are not significantly different.

Parameters	Salinity and Fertilization Rate						
	N:P = 4:1				*N:P = 2:1*		
	0‰	10‰	20‰	30‰	10‰	20‰	30‰
STOCKING							
Density (fish m^{-2})	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Total Number (fish $tank^{-1}$)	12	12	12	12	12	12	12
Mean Weight (g $fish^{-1}$)	21.4 ± 0.3	22.0 ± 0.4	21.6 ± 0.3	21.9 ± 0.9	21.5 ± 0.6	22.0 ± 0.2	22.1 ± 1.0
Total Weight (kg $tank^{-1}$)	0.26 ± 0.00	0.26 ± 0.00	0.26 ± 0.00	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.00	0.26 ± 0.01
HARVEST							
Total Number (fish $tank^{-1}$)	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0
Mean Weight (g $fish^{-1}$)	87.5 ± 11.7 ^{a}	150.5 ± 9.2 ^{c}	142.8 ± 17.0 ^{bc}	115.6 ± 11.0 ^{abc}	144.0 ± 14.3 ^{bc}	123.1 ± 6.1 ^{abc}	106.8 ± 10.0 ^{ab}
Total Weight (kg $tank^{-1}$)	1.05 ± 0.14 ^{a}	1.81 ± 0.11 ^{c}	1.71 ± 0.20 ^{bc}	1.39 ± 0.13 ^{abc}	1.73 ± 0.17 ^{bc}	1.48 ± 0.07 ^{abc}	1.28 ± 0.12 ^{ab}
Survival Rate (%)	100	100	100	100	100	100	100
Daily Weight Gain (g $fish^{-1}$ d^{-1})	0.41 ± 0.07 ^{a}	0.80 ± 0.06 ^{c}	0.76 ± 0.11 ^{bc}	0.59 ± 0.06 ^{abc}	0.77 ± 0.09 ^{bc}	0.63 ± 0.04 ^{abc}	0.53 ± 0.07 ^{ab}
Total Weight Gain (kg $tank^{-1}$)	0.79 ± 0.14 ^{a}	1.54 ± 0.11 ^{c}	1.45 ± 0.21 ^{bc}	1.12 ± 0.12 ^{abc}	1.47 ± 0.17 ^{bc}	1.21 ± 0.07 ^{abc}	1.02 ± 0.13 ^{ab}
Net Yield (t ha^{-1} yr^{-1})	3.62 ± 0.64 ^{a}	7.04 ± 0.48 ^{c}	6.64 ± 0.95 ^{bc}	5.13 ± 0.56 ^{abc}	6.71 ± 0.76 ^{bc}	5.53 ± 0.33 ^{abc}	4.64 ± 0.58 ^{ab}
Gross Yield (t ha^{-1} yr^{-1})	4.79 ± 0.64 ^{a}	8.24 ± 0.50 ^{c}	7.82 ± 0.93 ^{bc}	6.33 ± 0.60 ^{abc}	7.88 ± 0.78 ^{bc}	6.74 ± 0.34 ^{abc}	5.85 ± 0.54 ^{ab}

Table 2. Mean (± SE) values of water quality parameters measured throughout the experiment. Data with superscripts showed significant differences among treatments, and treatments with the same superscript are not significantly different.

Parameters	Salinity and Fertilization Rate						
	N:P = 4:1				N:P = 2:1		
	0‰	10‰	20‰	30‰	10‰	20‰	30‰
DO at Dawn (mg l^{-1})	5.40 ± 0.26	5.74 ± 0.17	5.04 ± 0.19	4.77 ± 0.16	5.63 ± 0.20	5.07 ± 0.20	5.00 ± 0.17
Temperature (°C)	28.2–32.7	28.1–32.7	28.2–32.7	28.2–32.7	28.2–32.7	28.3–32.7	28.1–32.7
pH	7.1–11.1	6.4–10.2	6.3–10.0	6.1–9.6	5.7–11.0	6.7–10.2	6.4–9.8
Alkalinity (mg l^{-1} as $CaCO_3$)	46.5 ± 1.4 [a]	27.6 ± 2.9 [b]	29.7 ± 1.6 [b]	22.9 ± 1.2 [b]	29.3 ± 2.7 [b]	27.8 ± 2.9 [b]	27.8 ± 6.8 [b]
TKN (mg l^{-1})	1.90 ± 0.17 [ab]	2.91 ± 0.34 [c]	2.60 ± 0.32 [bc]	2.05 ± 0.25 [ab]	1.41 ± 0.18 [a]	1.75 ± 0.43 [a]	1.24 ± 0.08 [a]
TAN (mg l^{-1})	0.02 ± 0.00 [a]	0.05 ± 0.00 [a]	0.05 ± 0.00 [a]	0.09 ± 0.01 [b]	0.04 ± 0.01 [a]	0.04 ± 0.01 [a]	0.05 ± 0.02 [a]
Nitrite-N (mg l^{-1})	0.38 ± 0.03 [abc]	0.44 ± 0.01 [c]	0.42 ± 0.02 [bc]	0.39 ± 0.04 [abc]	0.35 ± 0.03 [ab]	0.31 ± 0.02 [a]	0.31 ± 0.04 [a]
Nitrate-N (mg l^{-1})	0.43 ± 0.06 [ab]	0.87 ± 0.03 [d]	0.85 ± 0.10 [d]	0.70 ± 0.08 [cd]	0.36 ± 0.04 [a]	0.63 ± 0.07 [bc]	0.56 ± 0.02 [bc]
TP (mg l^{-1})	0.83 ± 0.13	0.52 ± 0.07	0.56 ± 0.02	0.41 ± 0.07	0.47 ± 0.05	0.53 ± 0.09	0.61 ± 0.17
SRP (mg l^{-1})	0.56 ± 0.16	0.30 ± 0.07	0.35 ± 0.03	0.20 ± 0.06	0.26 ± 0.05	0.30 ± 0.08	0.44 ± 0.19
Chlorophyll *a* (mg m^{-3})	62 ± 15.0	53 ± 3.4	57 ± 7.5	72 ± 1.4	58 ± 3.7	68 ± 9.0	69 ± 7.1
TSS (mg l^{-1})	119 ± 8.6 [a]	132 ± 4.0 [a]	172 ± 6.9 [b]	232 ± 9.1 [c]	121 ± 6.4 [a]	163 ± 4.7 [b]	228 ± 15.6 [c]
TVS (mg l^{-1})	32 ± 3.4 [a]	38 ± 1.3 [a]	50 ± 4.0 [b]	71 ± 2.8 [c]	38 ± 3.0 [a]	51 ± 2.8 [b]	70 ± 4.4 [c]

Table 3. The most abundant phytoplankton genera (from high to low) in different treatments measured throughout the experiment.

Salinity and Fertilization Rate						
N:P=4:1				N:P=2:1		
0‰	10‰	20‰	30‰	10‰	20‰	30‰
Scenedesmus	*Entomoneis*	*Entomoneis*	*Chaetoceros*	*Entomoneis*	*Entomoneis*	*Chaetoceros*
Golenkinia	*Chaetoceros*	*Chaetoceros*	*Entomoneis*	*Chaetoceros*	*Chaetoceros*	*Entomoneis*
Euglena	*Coscinodiscus*	*Coscinodiscus*	*Coscinodiscus*	*Coscinodiscus*	*Chroomonas*	*Chroomonas*
Ankistrodesmus	*Navicula*	*Chroomonas*	*Oscillatoria*	*Chlorella*	*Coscinodiscus*	*Nitzschia*
Coelastrum	*Oscillatoria*	*Lyngbya*	*Lyngbya*	*Lyngbya*	*Lyngbya*	*Coscinodiscus*

Table 4. Partial budget analysis for red tilapia cultured in different salinity levels under different fertilization regimes (based on a 1-ha tank per year).

Parameters	Salinity and Fertilization Rate						
	N:P = 4:1				N:P = 2:1		
	0‰	10‰	20‰	30‰	10‰	20‰	30‰
GROSS REVENUE							
Red Tilapia (US$)	2,395	3,520	3,320	2,565	3,355	2,765	2,320
Total (US$)	2,395	3,520	3,320	2,565	3,355	2,765	2,320
VARIABLE COST							
Red Tilapia Fingerlings (US$)	300	300	300	300	300	300	300
Urea (US$)	594	594	594	594	297	297	297
TSP (US$)	578	578	578	578	578	578	578
Sodium Bicarbonate (US$)	194	194	194	194	194	194	194
Electricity (US$)	329	329	329	329	329	329	329
Cost of Working Capital (US$)	160	160	160	160	136	136	136
Total (US$)	2,154	2,154	2,154	2,154	1,834	1,834	1,834
NET RETURN (US$)	241	1,366	1,166	411	1,521	931	486

Table 5. Average proximate compositions of different phytoplankton species and red tilapia fed with these single phytoplankton species. Data with superscripts showed significant differences among treatments, and data with the same superscript are not significantly different.

Treatment	Moisture (%)	Crude Protein (%)	Lipid (%)	Ash (%)
PHYTOPLANKTON				
Tetraselmis sp.	91.25 ± 1.26 [a]	10.56 ± 0.85 [b]	7.86 ± 0.29 [a]	52.12 ± 2.05 [a]
Chlorella sp.	91.94 ± 1.54 [a]	12.14 ± 1.03 [b]	12.56 ± 1.22 [b]	52.36 ± 1.14 [a]
Thalassiosira sp.	91.13 ± 0.99 [a]	13.25 ± 0.82 [b]	8.63 ± 0.86 [a]	53.44 ± 1.05 [a]
Chaetoceros sp.	90.66 ± 1.47 [a]	8.53 ± 0.41 [a]	10.12 ± 0.92 [b]	51.32 ± 2.02 [a]
RED TILAPIA				
Tetraselmis sp.	83.02 ± 0.96 [a]	68.12 ± 0.39 [a]	17.60 ± 0.20 [a]	25.40 ± 0.42 [b]
Chlorella sp.	82.57 ± 0.29 [a]	67.14 ± 0.51 [a]	17.59 ± 0.09 [a]	25.94 ± 0.38 [b]
Thalassiosira sp.	82.68 ± 0.41 [a]	69.85 ± 0.87 [a]	17.64 ± 0.06 [a]	22.29 ± 0.19 [a]
Chaetoceros sp.	82.91 ± 1.57 [a]	69.89 ± 0.97 [a]	17.54 ± 0.16 [a]	22.93 ± 0.53 [a]

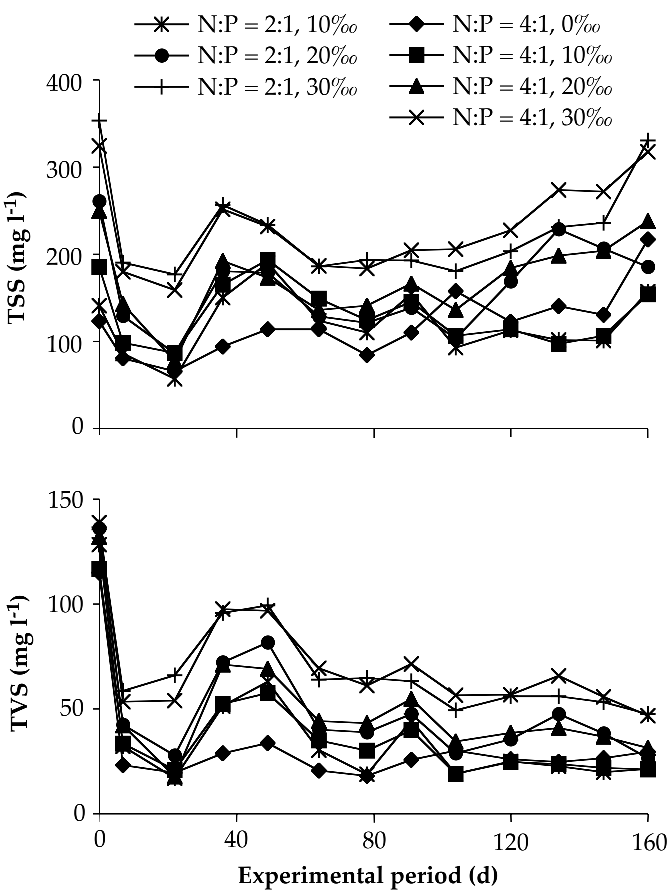

Figure 4. Fluctuations of TSS and TVS (0900 h) in all treatments over the 160-day experimental period.

brackishwater treatments ($P > 0.05$, Table 1). Growth performance in fresh water was lowest and significantly lower than that in the treatments with high N:P ratio at 10 and 20‰ salinity and with low N:P ratio at 10‰ salinity. Growth of red tilapia in brackish water was 22 to 72% faster than that in fresh water. There was no significant interaction between N:P ratio and salinity level on the growth of red tilapia ($P > 0.05$).

Mean values of water quality parameters measured throughout the experimental period are summarized in Table 2. Concentrations of DO at dawn were above 4 mg l^{-1} on average.

Table 6. Growth (mean ± SE) of Thai red tilapia fed with different phytoplankton species at initial and final (15 d later) times. Data with superscripts showed significant differences among treatments, and treatments with the same superscript are not significantly different.

Treatment	Initial Mean Weight (g fish^{-1})	Final Mean Weight (g fish^{-1})	Mean Weight Gain (g fish^{-1})
Tetraselmis sp.	1.42 ± 0.13 [a]	1.97 ± 0.05 [b]	0.54 ± 0.17 [b]
Chlorella sp.	1.47 ± 0.05 [a]	1.57 ± 0.06 [a]	0.11 ± 0.01 [a]
Thalassiosira sp.	1.42 ± 0.07 [a]	2.49 ± 0.06 [c]	1.07 ± 0.03 [c]
Chaetoceros sp.	1.61 ± 0.10 [a]	2.47 ± 0.12 [c]	0.87 ± 0.04 [c]

Water temperature and pH values ranged from 28.1 to 32.7°C and 5.7 to 11.1, respectively; both did not differ among treatments. Alkalinity was generally low in all treatments throughout the experimental period except for an increase after applying sodium bicarbonate in the third week. Alkalinity in the freshwater treatment was significantly higher than in the brackishwater treatments ($P < 0.05$), among which there were no significant differences. Concentrations of TKN, nitrite-N, and nitrate-N were higher in the treatments with high N:P ratio than in the treatments with low N:P ratio, and the highest values were obtained in the treatment with high N:P ratio at 10‰ salinity ($P < 0.05$). Concentrations of TAN were significantly higher in the treatment with high N:P ratio at 30‰ salinity than other treatments ($P < 0.05$), among which there were no significant differences ($P > 0.05$). However, concentrations of both TP and SRP were not significantly different in any treatment ($P > 0.05$). Chlorophyll *a* concentrations fluctuated throughout the experimental period without significant differences among treatments ($P > 0.05$). Nitrogen: phosphorus ratios did not result in significant differences in either TSS or TVS ($P > 0.05$); however, both increased significantly with increasing salinity levels ($P < 0.05$, Figure 4). The qualitative analysis of plankton composition showed that the most abundant phytoplankton genera were completely different between freshwater and brackishwater treatments (Table 3). In brackishwater treatments it seemed that phytoplankton composition was affected by salinity levels but not by fertilization rates, and the composition was similar in the 10 and 20‰ treatments. These two treatments were different from phytoplankton in the 30‰ treatment. In the 10 and 20‰ treatments, the most abundant phytoplankton genus was *Entomoneis*, followed by *Chaetoceros* and *Coscinodiscus*, while in the 30‰ treatment the most abundant genus was *Chaetoceros*, followed by *Entomoneis* and *Coscinodiscus* (Table 3).

The partial budget analysis for our data indicated that red tilapia culture in fertilized ponds was profitable (Table 4). The net return from freshwater ponds was lower than that from brackishwater ponds, while the net return decreased with increased salinity levels. The highest net return was achieved in the treatment with N:P ratio of 2:1 at 10‰ salinity, followed by the treatment with N:P ratio of 4:1 at 10‰ salinity, in which the best fish growth was achieved. At 10 and 30‰ salinity, the net return was higher in the ponds with N:P ratio of 2:1 than those with N:P ratio of 4:1, while at 20‰ salinity, the net return was higher in the ponds with N:P ratio of 4:1 than those with N:P ratio of 2:1.

The proximate analysis indicated that there was no significant difference in moisture and ash content of the four tested phytoplankton species ($P > 0.05$, Table 5). *Chaetoceros* sp. had the significantly lowest crude protein content, while *Chaetoceros* sp. and *Chlorella* sp. had significantly higher lipid content compared to other species ($P < 0.05$, Table 5). Red tilapia fed with different phytoplankton species did not show significant differences in moisture, crude protein, or lipid contents ($P > 0.05$), but ash content was significantly higher in red tilapia fed with *Tetraselmis* sp. and *Chlorella* sp. than in those fed with *Thalassiosira* sp. and *Chaetoceros* sp. ($P < 0.05$, Table 5). The best growth performance of red tilapia was achieved by feeding with *Thalassiosira* sp. and *Chaetoceros* sp., intermediate with *Tetraselmis* sp., and poorest with *Chlorella* sp. ($P < 0.05$, Table 6). *Chaetoceros* sp. had the lowest crude protein content but gave the highest protein utilization efficiency, which was not significantly different from that of *Thalassiosira* sp. but was significantly higher than that of *Tetraselmis* sp. and *Chlorella* sp. by more than two and ten times, respectively ($P < 0.05$, Table 7). The two-hour prey consumption trials showed that the prey ingestion rates of red tilapia for *Chaetoceros* sp. and *Thalassiosira* sp. were significantly higher than those for *Tetraselmis* sp. and *Chlorella* sp. ($P < 0.05$, Table 8).

DISCUSSION

Thai red tilapia grew faster in brackish water than in fresh water in this experiment, which is consistent with the results obtained by Hoa (1996). Similar results were also reported in monosex Florida red tilapia (Watanabe et al., 1988a, 1993), *O. mossambicus* (Canagaratnam, 1966; Juerss et al., 1984; Villegas, 1990), *O. mossambicus* × *O. hornorum* hybrid (Garcia and Sedjro, 1987, cited in Watanabe et al., 1993), mixed-sex Taiwanese red tilapia (*O. mossambicus* × *O. niloticus* hybrid) (Liao and Chang, 1983), and F_1 hybrid of *O. mossambicus* × *O. niloticus* (Villegas, 1990). The better growth performance in saline water might be attributed to higher osmoregulation energy costs in fresh water than in brackish water or seawater found in *O. mossambicus* × *O. hornorum* hybrid (Febry and Lutz,

Table 7. Protein utilization efficiency of red tilapia fed with different phytoplankton species in glass 15-l jars for 15 days. Data with superscripts showed significant differences among treatments, and data with the same superscript are not significantly different.

Treatment	Phytoplankton		Red Tilapia		Protein Utilization Efficiency (%)
	Fed Amount (g DM jar^{-1})	*Crude Protein* (g jar^{-1})	*Biomass Gain* (g jar^{-1})	*Crude Protein* (g jar^{-1})	
Tetraselmis sp.	26.06 ± 0.00	2.75 ± 0.01	2.60 ± 1.18	0.30 ± 0.14	10.91 ± 4.95 ^{b}
Chlorella sp.	26.06 ± 0.00	3.16 ± 0.02	0.63 ± 0.06	0.07 ± 0.01	2.33 ± 0.22 ^{a}
Thalassiosira sp.	26.06 ± 0.00	3.45 ± 0.01	6.39 ± 0.16	0.77 ± 0.02	22.40 ± 0.56 ^{c}
Chaetoceros sp.	26.06 ± 0.00	2.22 ± 0.01	5.21 ± 0.24	0.62 ± 0.03	27.99 ± 1.31 ^{c}

Table 8. Consumption of different phytoplankton species by red tilapia with mean weights of 1.50 g and 2.20 g in 4-l glass jars during the two-hour trial period.

Treatment	Initial Phytoplankton Concentration (mg DM jar^{-1})	Final Phytoplankton Concentration (mg DM jar^{-1})	Phytoplankton Consumed (mg DM jar^{-1})	Prey Ingestion Rate (mg DM g^{-1} fish h^{-1})
TILAPIA OF 1.50 G SIZE				
Tetraselmis sp.	273 ± 17.2 ^{a}	180 ± 20.0 ^{b}	93 ± 8.3 ^{a}	5 ± 0.5 ^{a}
Chlorella sp.	288 ± 18.2 ^{a}	177 ± 14.4 ^{b}	110 ± 19.3 ^{a}	6 ± 1.1 ^{a}
Thalassiosira sp.	269 ± 9.9 ^{a}	48 ± 5.0 ^{a}	221 ± 12.5 ^{b}	12 ± 0.7 ^{b}
Chaetoceros sp.	251 ± 10.3 ^{a}	51 ± 24.3 ^{a}	200 ± 18.4 ^{b}	11 ± 1.0 ^{b}
TILAPIA OF 2.20 G SIZE				
Tetraselmis sp.	306 ± 13.5 ^{a}	182 ± 5.0 ^{b}	124 ± 9.9 ^{a}	7 ± 0.5 ^{a}
Chlorella sp.	306 ± 9.9 ^{a}	185 ± 6.2 ^{b}	121 ± 15.3 ^{a}	7 ± 0.9 ^{a}
Thalassiosira sp.	318 ± 14.0 ^{a}	85 ± 5.8 ^{a}	233 ± 19.1 ^{b}	13 ± 1.1 ^{b}
Chaetoceros sp.	311 ± 21.1 ^{a}	76 ± 6.7 ^{a}	235 ± 27.7 ^{b}	13 ± 1.5 ^{b}

1987), suppressed territorial aggression in saline waters (Watanabe et al., 1988b), and inhibitory effects of aggressive behavior which varied among different salinities (Liao and Chang, 1983). In contrast, all-male Taiwanese red tilapia exhibited faster growth in fresh water than in salt water (Liao and Chang, 1983), and similar results were found in *O. niloticus* (Villegas, 1990).

Results of studies on effects of salinity on fish growth are controversial. Under saline water conditions, growth of Thai red tilapia decreased with increased salinity levels from 10 to 30‰ in both low and high fertilization rates in this experiment. The present experiment indicates that the growth of Thai red tilapia may reach a peak at the salinity levels of around 10‰, which is in accordance with the results (12‰) for red tilapia from the UK (Payne et al., 1988). These results support the common assumption that growth of euryhaline teleosts is increased at salinities near isosmotic since osmoregulation costs are minimal under these conditions. Febry and Lutz (1987) found that osmoregulation costs in *O. mossambicus* × *O. hornorum* hybrids were lowest in isosmotic seawater (12‰). However, growth at salinities near isoosmotic was found to be lower than that at higher salinities in Florida red tilapia (Watanabe et al., 1988a) and F_1 hybrid of *O. mossambicus* × *O. niloticus* (Villegas, 1990). Febry and Lutz (1987) and Watanabe et al. (1988b) proposed that non-osmoregulatory factors such as aggression might also influence the growth performance of tilapia cultured in saline water. Territorial aggression might account for one-third to one-half of the active metabolic rate in teleosts during intense contesting (Brett and Groves, 1979). Watanabe et al. (1988a) attributed the increased growth they found with increasing salinity for Florida red tilapia to increased food consumption and lowered food conversion ratio.

The fertilization regime with high N:P ratio (28 kg N and 7 kg P ha^{-1} wk^{-1}) resulted in good growth performance of Thai red tilapia at all tested salinity levels. The results suggested that the optimized fertilization regime in fresh water can be applied in brackish water. However, the simple partial budget analysis based on the market prices in Thailand indicated that net return from the fertilization regime with low N:P ratio (14 kg N and 7 kg P ha^{-1} wk^{-1}) was higher than that from high N:P ratio (28 kg N and 7 kg P ha^{-1} wk^{-1}). The fertilization regime in brackishwater ponds may be further fine-tuned based on the local conditions.

One surprising result of this experiment was that the growth of Thai red tilapia in fresh water (0.42 g d^{-1}) was far below the growth rate of sex-reversed normal *Oreochromis niloticus* (averaging 1 g d^{-1}; Diana, 1997), while growth in brackish water was also poor but approached the normal level. It is generally observed that tilapias exhibit poor growth in cement tanks. The same source of Thai red tilapia grew very well (1.17 g d^{-1}) at a density of 62.5 fish m^{-3} in cages suspended in a earthen ponds (Yi et al, 2002). However, it would be useful to compare the two tilapia strains for growth characteristics under different salinity conditions to better understand both economic and biological production.

ANTICIPATED BENEFITS

This experiment demonstrated that Thai red tilapia grows better in brackishwater ponds, especially at 10 to 20‰, than in freshwater ponds in semi-intensive fertilization culture systems. It also showed that Thai red tilapia culture was profitable in fertilized brackishwater ponds. Best growth performance was achieved in brackishwater ponds fertilized at 28 kg N and 7 kg P ha^{-1} wk^{-1} at 10‰, while the highest net return is achieved in brackishwater ponds fertilized at 14 kg N and 7 kg P ha^{-1} wk^{-1} at 10‰. These data provide farmers with low-risk alternatives to culture red tilapia in semi-intensive fertilization systems using underutilized or abandoned shrimp ponds in the coastal zones of Southeast Asia and of other regions. Culture of red tilapia in low salinity brackish water over time may help reclaim these pond areas to agriculture or to freshwater culture.

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SUPPLEMENTAL FEEDING FOR SEMI-INTENSIVE CULTURE OF RED TILAPIA IN BRACKISHWATER PONDS

Ninth Work Plan, New Aquaculture Systems/New Species Research 5 (9NS5)
Final Report

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ABSTRACT

An experiment was conducted at the Asian Institute of Technology, Thailand, to investigate effects of feeding regimes on growth of sex-reversed Thai red tilapia (*Oreochromis* sp.). There were five different supplemental feeding regimes: 0, 25, 50, 75, and 100% of satiation. Red tilapia fingerlings (33.2 to 33.4 g size) were stocked at 62.5 fish m^{-3} in fifteen 0.8-m^3 net cages suspended in a 200-m^2 earthen pond and cultured for 90 days. The pond was maintained at 10‰ salinity and fertilized weekly at rates of 4 kg N and 1 kg P ha^{-1} d^{-1}. Growth performance of red tilapia was significantly better in the feeding treatments than in the non-feeding treatment. Red tilapia growth and average feeding rate increased, but the Feed Conversion Ratio (FCR) and net economic return decreased with increasing percentages of satiation feeding levels from 25 to 100%. Considering low FCR, good growth and yield performance, high economic return, and potential for growing to greater size, 50% satiation feeding was the most efficient feeding rate.

INTRODUCTION

Many tilapia species are euryhaline and can grow in saline water after proper acclimation (Suresh and Lin, 1992). Varieties of red tilapia have been successfully cultured in saline waters (Watanabe, 1991). However, most of those tilapia culture trials were conducted in intensive systems with pelleted feeds, requiring frequent water exchanges or use of cages. Compared to the voluminous literature available for semi-intensive culture of tilapia in freshwater ponds, information on semi-intensive culture in saline ponds is almost nonexistent. On the other hand, there has been a strong desire to culture tilapia in brackishwater ponds in Southeast Asia as well as in Central and South America during the last few years (Green, 1997). There are a large number of shrimp ponds available in these regions, resulting from either failure in shrimp farming or desires to diversify shrimp culture. These ponds provide a good opportunity for aquaculture, and tilapia appears to be the most appropriate choice for such a culture system because there are few other domesticated finfish species that feed on low-cost natural foods, such as detritus and plankton. This interest in brackishwater culture is particularly strong in Thailand and Vietnam, where shrimp culture is now commonly reduced to one crop per year, leaving the ponds empty for half a year. Tilapia culture is also attractive to shrimp farmers to utilize abundant phytoplankton in shrimp ponds or in pond effluents.

The results from a recent PD/A CRSP experiment conducted in Thailand showed that Thai red tilapia (*Oreochromis* sp.) grew better at 10‰ than at other salinities in ponds fertilized using common CRSP fertilization guidelines (4 kg N and 1 kg P ha^{-1} d^{-1}; Yi et al., 2002). However, under this semi-intensive culture system with fertilizer as the sole nutrient input, growth of red tilapia slowed down after they reached 100 g, resulting in small size at harvest (about 150 g) after five months of culture. Market price in many countries is much higher for larger tilapia. To rear tilapia to a size greater than 500 g to fetch the higher market price, supplemental feeds are usually required. In freshwater ponds the most efficient culture system is to grow tilapia to 100 to 150 g with fertilizer alone, then begin supplemental feeding at 50% satiation feeding level (Diana et al., 1994, 1996). However, such information is not available for red tilapia culture in brackish water.

The purpose of this experiment was to determine the appropriate levels of supplemental feeding in fertilized ponds with 10‰ salinity for maximum growth of Thai red tilapia.

METHODS AND MATERIALS

This experiment was carried out at the Asian Institute of Technology (AIT), Thailand, from March through June 2001. The experiment was conducted using Thai red tilapia in a randomized complete block design in 15 cages (1 × 1 × 1.2 m) suspended in a 200-m^2 fertilized earthen pond at 10‰ salinity. Five treatments were used to test effects of different supplemental feeding regimes: 1) 0% (no feeding); 2) 25% satiation feeding; 3) 50% satiation feeding; 4) 75% satiation feeding; and 5) 100% satiation feeding. Satiation rations in treatments 2 through 5 were determined by estimating the total amount of floating pelleted feed (crude protein 30%, Charoen Pokphand

Co., Ltd., Bangkok, Thailand) consumed by red tilapia from 0800 to 0900 h and 1400 to 1500 h every Saturday. The tested percentages of satiation feeding were used to calculate the amount of feed to apply for respective treatments from Sunday through Friday.

Sex-reversed fingerlings of Thai red tilapia (33.2 to 33.4 g size) were purchased from a local farm and acclimated to 10‰ salinity in acclimation tanks by raising salinity level 5‰ every two days. The fingerlings were then stocked at 62.5 fish m^{-3} in all cages on 30 March 2001. During the experiment average weights of red tilapia were determined biweekly by bulk weighing 50% of the initial stock in each cage. Fish were randomly sampled by dip net. Red tilapia in every cage were harvested, counted, and bulk weighed on 28 June 2001 after 90 days of culture. Daily weight gain (g $fish^{-1}$ d^{-1}), yield (kg m^{-3}), and extrapolated yield (kg m^{-3} yr^{-1}) were calculated.

The pond was fertilized weekly with urea and triple superphosphate (TSP) at 4 kg N and 1 kg P ha^{-1} d^{-1}. Initial pond fertilization took place two weeks prior to stocking fish. Salinity was initially regulated by trucking hypersaline water (150‰) to AIT and diluting it to 10‰. Salinity was maintained at 10‰ and monitored weekly. Water depths in the pond and cages were maintained at 1.0 and 0.8 m, respectively, throughout the experiment by adding water at about 10‰ salinity weekly to replace evaporation and seepage losses. All cages were aerated for six hours daily from 0200 to 0800 h using one airstone in each cage.

Integrated water samples were collected from the entire water column near the two ends and the center of the pond and also from each cage biweekly at approximately 0900 h. Analyses were done for pH, alkalinity, total ammonium nitrogen (TAN), nitrite-nitrogen (nitrite-N), nitrate-nitrogen (nitrate-N), total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), total phosphorus (TP), chlorophyll *a*, total suspended solids (TSS), and total volatile solids (TVS) using standard methods (Parsons et al., 1984; APHA et al., 1985; Egna et al., 1987). At the time of collecting water samples, Secchi disk visibility was measured using a Secchi disk, while temperature and dissolved oxygen (DO) were measured with a YSI model 54 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Diel measurements for temperature, DO, and pH were conducted in each pond at 0600, 0900, 1600, 1800, 2200, and 0600 h once a month.

Data were analyzed statistically using analysis of variance, paired-sample t-test, and linear regression (Steele and Torrie, 1980) with SPSS (version 7.0) statistical software package (SPSS Inc., Chicago, Illinois, USA). Differences were considered significant at an alpha of 0.05. Statistical analyses for survival rates (%) were performed on data after arcsine transformation. Mean values of survival rates in this text are listed in normal scale followed by their confidence limits. All other means are given with ± 1 standard error (SE).

A partial budget analysis was conducted to determine economic returns of red tilapia in the different treatments (Shang, 1990). The analysis was based on farm-gate prices in Thailand for harvested red tilapia and current local market prices for all other items expressed in US dollars (US$1 = 40 baht). Farm-gate prices of red tilapia were $0.50 and $0.75 kg^{-1} for the sizes 100 to 200 g and 200 to 300 g, respectively. Market prices of sex-reversed red tilapia fingerlings ($0.0125 $piece^{-1}$), electricity ($0.05 kWh^{-1}), pelleted floating feed ($0.4875 kg^{-1}), urea ($0.1875 kg^{-1}), and TSP ($0.3125 kg^{-1}) were applied to the analysis. The calculation for cost of working capital was based on an annual interest rate of 8%.

RESULTS

Survival of red tilapia in cages ranged from 96.7 to 99.8% and did not differ significantly among treatments ($P > 0.05$). Significant difference in caged red tilapia growth between the non-feeding treatment (0% satiation feeding) and the feeding treatments (25 through 100% satiation feeding) was shown at the first fish sampling (15 d after stocking, $P < 0.05$), while

Table 1. Growth performance of all-male Nile tilapia fed at 0, 25, 50, 75, and 100% of satiation in 0.8-m^3 cages. Mean values with different superscript letters in the same row were significantly different among treatments ($P < 0.05$).

Performance Measures	Treatments				
	0%	*25%*	*50%*	*75%*	*100%*
STOCKING					
Total Weight (kg $cage^{-1}$)	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0
Mean Weight (g $fish^{-1}$)	33.3 ± 0.0	33.3 ± 0.1	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0
HARVEST					
Total Weight (kg $cage^{-1}$)	6.9 ± 0.1^a	9.8 ± 0.1^b	10.8 ± 0.4^c	11.8 ± 0.1^d	12.4 ± 0.3^d
Mean Weight (g $fish^{-1}$)	138.4 ± 2.2^a	203.2 ± 2.5^b	221.5 ± 5.0^c	241.9 ± 5.1^d	253.9 ± 2.8^d
Mean Weight Gain (g $fish^{-1}$)	105.1 ± 2.2^a	169.9 ± 2.5^b	188.2 ± 5.0^c	208.6 ± 5.1^d	220.6 ± 2.8^d
Daily Weight Gain (g $fish^{-1}$ d^{-1})	1.17 ± 0.10^a	1.92 ± 0.14^b	2.11 ± 0.18^c	2.33 ± 0.15^d	2.47 ± 0.19^e
Net Yield (kg m^{-3} $crop^{-1}$)	6.5 ± 0.1^a	10.2 ± 0.1^b	11.4 ± 0.5^c	12.7 ± 0.2^d	13.4 ± 0.3^d
(kg m^{-3} yr^{-1})	26.4 ± 0.4^a	41.3 ± 0.3^b	46.3 ± 2.2^c	51.6 ± 0.6^d	54.2 ± 1.4^d
Gross Yield (kg m^{-3} $crop^{-1}$)	8.6 ± 0.1^a	12.3 ± 0.1^b	13.5 ± 0.5^c	14.8 ± 0.2^d	54.2 ± 1.4^d
(kg m^{-3} yr^{-1})	34.8 ± 0.4^a	49.8 ± 0.3^b	54.7 ± 2.2^c	60.0 ± 0.6^d	62.6 ± 1.4^d
FCR	----	0.67 ± 0.01^a	0.93 ± 0.04^b	1.15 ± 0.01^c	1.28 ± 0.03^d
Survival (%)	99.8	96.7	98.3	99.3	98.3
	(93.8–100.0)	(93.0–99.1)	(82.0–100.0)	(82.1–100.0)	(82.0–100.0)

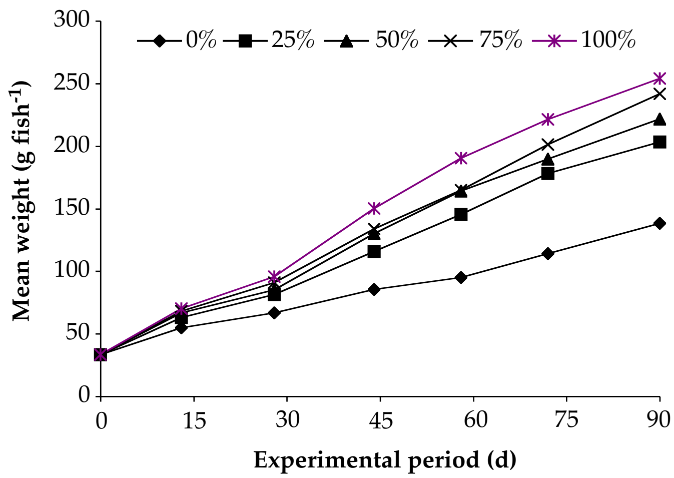

Figure 1. Growth of Thai red tilapia fed at 0, 25, 50, 75, and 100% satiation feeding levels over the 90-day experimental period.

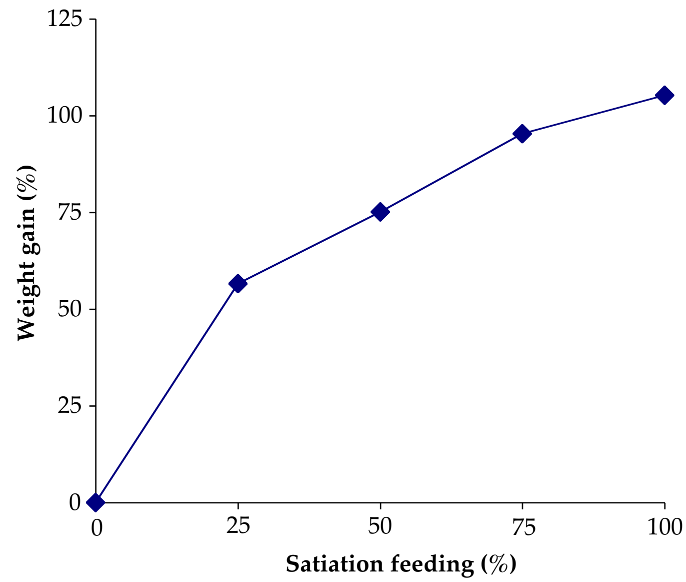

Figure 3. Percentages of additional total weight gains for each feeding treatment compared to the total weight gain of non-feeding treatment.

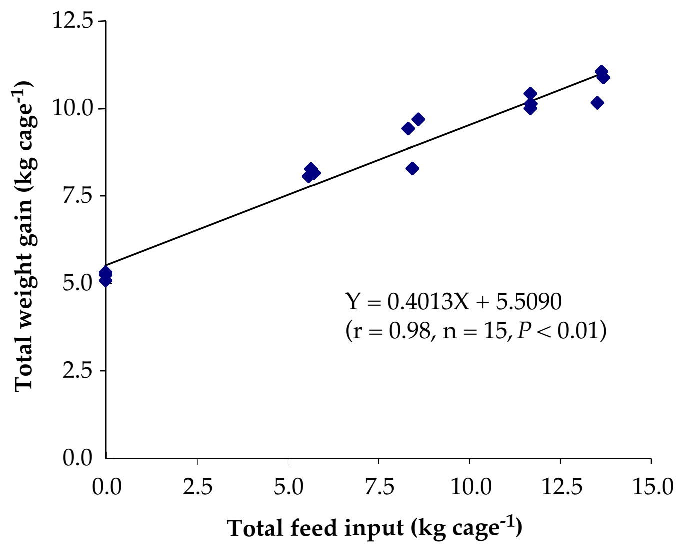

Figure 2. Relationship between total feed input to cages and total weight gain of caged red tilapia.

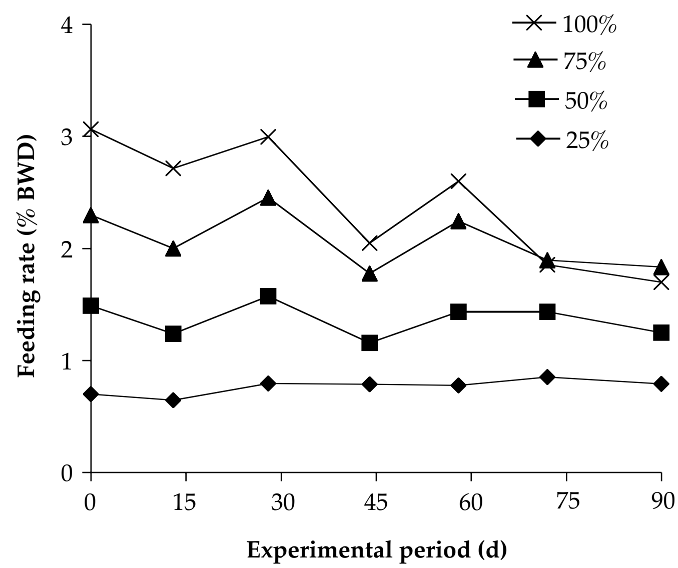

Figure 4. Mean feeding rates (% BWD) for red tilapia at 25, 50, 75, and 100% satiation feeding levels over the 90-day experimental period.

significant differences among feeding treatments were detected starting from the second fish sampling (30 d after stocking, $P < 0.05$, Figure 1). Growth and yield of caged red tilapia increased significantly with increased feeding rates from 0 to 75% satiation ($P < 0.05$), while there were no significant differences between the 75 and 100% satiation feeding treatments ($P > 0.05$). Mean daily weight gains were significantly different among treatments, with the highest value in the 100% satiation feeding treatment ($P < 0.05$, Table 1).

The feed conversion ratio was significantly better in treatments with lower percentages of satiation feeding ($P < 0.05$, Table 1). Total weight gain of caged red tilapia was linearly and positively correlated ($r = 0.98$, $n = 15$, $P < 0.01$) with total feed input to cages (Figure 2). Compared to weight gain in the non-feeding treatment, addition of feed at 25, 50, 75, and 100% satiation feeding resulted in 57, 75, 95, and 105% greater weight gain, respectively (Figure 3). By subtracting weight gain in the non-feeding treatment from that in the feeding treatments, natural food appears to contribute to 64, 57, 51, and 49% of the energy for weight gain in treatments with 25, 50, 75, and 100% satiation feeding levels, respectively. Feeding rates varied from 0.64 to 3.06% body weight per day (BWD) in the feeding treatments and increased with increasing percentage of satiation feeding levels (Figure 4). The average feeding rates were 0.76, 1.37, 2.07, and 2.42% BWD in the feeding treatments with 25, 50, 75, and 100% satiation feeding. Feeding rates also appeared to decline over time in the highest feeding treatments.

All water quality parameters except Secchi disk visibility were measured in cages and open water, and no significant differences were found among cages or between cages and open water ($P > 0.05$). The mean values of water quality parameters measured in cages and ponds at each sampling are summarized in Table 2. Mean temperature ranged from 29.9 to 32.2°C. Values of pH at the end of the experiment were significantly higher that those at the beginning ($P < 0.05$). Concentrations of

Table 2. Mean (± SE) values of water quality parameters measured in cages and open water at 0900 h (except DO, which was measured at dawn) at various points during the experimental period. Parameters (excluding Secchi disk visibility) marked with an asterisk had significant differences among the time periods.

Parameters	Day 6	Day 19	Day 34	Day 47	Day 60	Day 74	Day 88
*DO at Dawn (mg l^{-1})	4.61 ± 0.00	----	2.33 ± 0.02	----	1.68 ± 0.01	----	0.30 ± 0.00
Temperature (°C)	31.8 ± 0.0	32.2 ± 0.0	30.0 ± 0.0	32.9 ± 0.0	31.0 ± 0.0	29.9 ± 0.0	30.3 ± 0.0
*pH	7.9 ± 0.0	6.5 ± 0.0	6.2 ± 0.0	6.7 ± 0.0	6.5 ± 0.0	5.2 ± 0.0	5.9 ± 0.0
*Alkalinity (mg l^{-1} as $CaCO_3$)	78 ± 1	42 ± 1	42 ± 1	37 ± 0	15 ± 1	10 ± 0	13 ± 1
*TKN (mg l^{-1})	2.61 ± 0.08	3.29 ± 0.20	6.11 ± 0.13	5.79 ± 0.17	2.41 ± 0.18	3.13 ± 0.08	5.70 ± 0.24
*TAN (mg l^{-1})	0.56 ± 0.03	2.30 ± 0.02	4.59 ± 0.04	3.62 ± 0.15	0.89 ± 0.09	2.21 ± 0.02	2.84 ± 0.03
*Nitrite-N (mg l^{-1})	0.05 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.80 ± 0.00	0.28 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
*Nitrate-N (mg l^{-1})	0.23 ± 0.00	0.19 ± 0.00	0.06 ± 0.00	0.58 ± 0.02	0.09 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
*TP (mg l^{-1})	0.16 ± 0.00	0.10 ± 0.00	0.43 ± 0.00	0.17 ± 0.00	0.22 ± 0.00	0.91 ± 0.01	0.44 ± 0.01
*SRP (mg l^{-1})	0.01 ± 0.00	0.03 ± 0.00	0.14 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.43 ± 0.01	0.07 ± 0.00
*Chlorophyll *a* (mg m^{-3})	61 ± 3	14 ± 1	33 ± 1	39 ± 4	49 ± 1	52 ± 4	100 ± 3
*TSS (mg l^{-1})	76 ± 2	79 ± 1	54 ± 1	55 ± 1	96 ± 4	61 ± 1	69 ± 1
TVS (mg l^{-1})	29 ± 2	48 ± 1	19 ± 1	24 ± 1	48 ± 3	27 ± 1	33 ± 1
Secchi Disk Visibility (cm)	55	100	64	64	62	61	41

Table 3. Partial budget analysis (in US$) for red tilapia in each experimental treatment.

Parameters	Feeding Treatments				
	0%	*25%*	*50%*	*75%*	*100%*
GROSS REVENUE					
Red Tilapia	3.45	7.35	8.10	8.85	9.30
Total	3.45	7.35	8.10	8.85	9.30
VARIABLE COST					
Red Tilapia Fingerlings	0.63	0.63	0.63	0.63	0.63
Urea	0.21	0.21	0.21	0.21	0.21
TSP	0.21	0.21	0.21	0.21	0.21
Pelleted Feed	0.00	2.75	4.12	5.69	6.64
Electricity	1.35	1.35	1.35	1.35	1.35
Cost of Working Capital	0.19	0.41	0.52	0.65	0.72
Total	2.59	5.56	7.04	8.74	9.76
NET RETURN	0.86	1.79	1.06	0.11	–0.46

DO at dawn and alkalinity decreased significantly over time ($P < 0.05$). Concentrations of nutrient parameters fluctuated throughout the experimental period, with TKN, TAN, TP, and SRP increasing significantly but nitrite-N and nitrate-N decreasing significantly through the experiment ($P < 0.05$). Concentrations of chlorophyll *a* and TSS were significantly higher at the end than at the beginning of the experiment ($P < 0.05$), while TVS concentrations were not significantly different ($P > 0.05$).

The partial budget analysis for our data indicated that red tilapia culture with supplemental feeding at 25 to 75% satiation feeding levels was profitable (Table 3). Net return in the feeding treatments decreased with increased feeding levels, and the 100% satiation feeding resulted in a negative net return. The net return in the non-feeding treatment was lower than that in both the 25 and 50% satiation feedings but greater than that in the 75 and 100% satiation feedings.

DISCUSSION

Physical, chemical, and biological parameters did not differ among cages or between cages and open water in the present experiment, indicating that all red tilapia had a similar culture environment and equal access to natural foods. Compared to non-feeding cages, the addition of supplemental feed resulted in significantly higher yields and growth rates. The growth differential between non-fed and fed red tilapia started during the first two weeks of culture, similar to results for Nile tilapia (*Oreochromis niloticus*) cultured in freshwater ponds (Diana et al., 1994), while the growth differential among different levels of food input began after the first two weeks of culture and continued to differentiate throughout the experiment. Moav et al. (1977) reported insignificantly different production between fish with and without supplemental feeding in fertilized freshwater polyculture ponds, which was likely due to lower densities of stocked fish (Diana et al., 1994).

Hepher (1978) developed the concept of critical standing crop (CSC), which is the biomass of fish in any aquaculture system that results in growth reductions for each individual. In the present experiment, treatments differed significantly in growth of red tilapia from the first biweekly fish sampling, and growth continued linearly until harvest, indicating that CSC had to occur at a size less than 50 g. The results support findings in Nile tilapia grown in freshwater ponds (Diana et al., 1994).

The good growth (1.17 g d^{-1}) of red tilapia at high density in non-feeding cages indicates that natural foods were abundant and important to growth in this experiment. This growth rate was comparable to or higher than that in other studies (Green, 1992; Diana et al., 1994, 1996). The good growth of non-fed red tilapia also indicate the abundant natural foods in the experimental pond in the present experiment, which was further implied by declining alkalinity throughout the experiment due to carbon utilization for photosynthesis (Knud-Hansen et al., 1991). Tilapias under culture conditions prefer artificial to natural foods (Schroeder, 1978), and thus, the contribution of natural foods to red tilapia may decrease with increasing availability of artificial feed, giving higher FCR in the treatments with higher percentages of satiation feeding. In other words, supplemental feed was more effective for incremental fish growth at lower percentages of satiation feeding.

Combined fertilizer and feed application resulted in efficient use of nutrients (Green, 1992; Diana et al., 1994, 1996). Applying feed at a lower percentage of satiation makes tilapia more efficient at utilizing natural food. In a previous study, Diana et al. (1994) determined 50 to 75% satiation feeding to be the most efficient feeding rates for Nile tilapia culture in freshwater ponds. The partial budget analysis in the present experiment showed that 25% satiation feeding was most profitable, followed by 50% satiation feeding. However, growth started to slow at the end of the culture period in the 25% satiation feeding (Figure 1). It appears that a 25% ration was not sufficient to support further growth to a size (> 500 g) that would fetch higher market prices. Considering low FCR, good growth and yield performance, high economic return, and potential for growing to greater size, 50% satiation feeding is the most efficient feeding rate in the present experiment.

ANTICIPATED BENEFITS

This experiment showed that Thai red tilapia culture is profitable in cages without feeding or with 25 to 75% satiation feeding in fertilized brackishwater ponds. It also demonstrated that 50% satiation feeding is the most efficient rate. These data provide farmers with a cost-efficient alternative to culture red tilapia in semi-intensive fertilization systems supplemented with pelleted feed using underutilized or abandoned shrimp ponds in coastal zones. Culture of red tilapia in low-salinity brackish water over time may help reclaim these pond areas for agriculture or freshwater aquaculture.

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FATE OF METHYLTESTOSTERONE IN THE POND ENVIRONMENT: USE OF MT IN EARTHEN PONDS WITH NO RECORD OF HORMONE USAGE

Ninth Work Plan, Effluents and Pollution Research 2D (9ER2D)
Final Report

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ABSTRACT

The following study examined the persistence of 17α-methyltestosterone (MT) in the environment after its use for masculinizing Nile tilapia in nursery ponds located in the Universidad Juárez Autónoma de Tabasco, Mexico. Fry harvested from spawning ponds were treated with a masculinizing dose of MT (60 mg kg^{-1}) for four weeks. Concentrations of MT were determined by radioimmunoassay. MT was not detectable in the water at any time. In the sediments, MT was not detectable during the first 10 days of treatment. Afterwards MT was detectable in all sampling points (mean = 146.7 pg g^{-1}; SE = 21.3). MT values varied from not detectable to 368.9 pg g^{-1}. Masculinizing efficiency was low in the first trial (87.4% males) but increased significantly afterwards, reaching 92.6% males in the second trial and 98.7% in the third trial.

Another outcome of this investigation is a manual on tilapia masculinization using synthetic steroids. This manual is intended to reach fry producers, extension agents and technicians; it contains a general description of the biology of the tilapias, traditional culture practices, masculinization methods, and a detailed section on safe handling of steroids.

INTRODUCTION

In tilapia culture the production of all-male populations through treatment of fry with 17α-methyltestosterone (MT)–impregnated food has become the most popular procedure. All-male populations have greater growth potential because no energy is shunted toward reproduction and no competition with younger fish occurs (Green et al., 1997). Contreras-Sánchez (2001) demonstrated that significant "leakage" of MT to water and sediments occurs in small, closed systems, probably 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 to anabolic steroids if MT persists in the environment after treatment of tilapia fry.

Some researchers have warned about unintended effects of steroid administration, such as fish-to-fish transfer of steroids (Budworth and Senger, 1993), biased sex ratios in untargeted organisms (Abucay et al., 1997), and paradoxical feminization (Rinchard et al., 1999; Eding et al., 1999). If MT is being added to the food in amounts that efficiently masculinize fish despite steroid loss to the environment, then determining the fate of MT in semi-closed systems such as ponds will yield important information on both safety and efficacy of MT use for masculinization. To determine if MT is detectable within the pond environment, the following study was undertaken.

METHODS AND MATERIALS

Laboratory of Aquaculture at UJAT

Nile tilapia, *Oreochromis niloticus*, fry were collected daily from a spawning tank. Fry were selected by grading with a 3-mm mesh (Popma and Green, 1990), counted, and randomly assigned to either MT-feeding or ethanol (EtOH; vehicle)–feeding treatments. Fry 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 experimental units were located at one end of the pond and the control units at the other end. Three masculinization trials were established at different dates throughout the experiment. A total of seven hapas were assigned to each treatment: three for trial 1 (density = 3,000 fry $hapa^{-1}$), two for trial 2 (density = 2,000 fry $hapa^{-1}$), and two for trial 3 (density = 5,000 fry $hapa^{-1}$). The number of fry per treatment was established based on fry availability.

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 MT (60 mg kg^{-1}) or control diet for four weeks. Feeding rate was at 20% per calculated body weight for the first 23 days of treatment and then 10% per calculated body weight through 28 days of treatment (Popma and Green, 1990). After 28 days of dietary treatment, fry were moved to a grow-out pond and 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, three sampling points were set along the pond as follows: points 1 and 3 were located under treatment hapas (1 = control treatment; 3 = MT treatment); the other sampling point was located in the middle of the pond. 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, excess water poured off, and stored at –20°C until shipment to Oregon State University (OSU) for analysis. All water and soil samples were collected early in the mornings, before initiation of feeding. Samples were taken weekly starting five days before the onset of the experiment (23 February).

Radioimmunoassay

For analysis of MT concentration, 1.0 ml of each water sample and 1.0 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 Animal Pharm Services, 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 64.3% for water and 61.6% for soil). Concentrations of MT in water and soil 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

Detection of MT in the Pond Environment

MT was not detectable in water at any sampling point throughout the entire experiment. MT concentrations in soil at day 10 of treatment were below the detectable limit (Figure 1). At day 17, MT was detectable at all sampling points; values ranged between 113.0 pg g^{-1} at the sampling point located under the MT treatment hapas to 186.2 pg g^{-1} at the sampling point underneath the EtOH-fed cages. After this sampling date, values of MT varied significantly in both ends of the pond (under the MT and the control hapas), while at the middle of the pond MT concentrations remained nearly constant. 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). The pond used for sex inversion at UJAT had not been used for six months for treatments with MT.

Masculinizing Efficiency of MT

The efficacy of MT for masculinizing Nile tilapia fry was low during the first trial (87.4% males); however, these values increased thereafter and remained elevated throughout trials 2 (92.6% males) and 3 (98.7%). These results were significantly higher than those of the control groups maintained in the masculinizing pond (40.5, 36.4, and 53.3% males, respectively) and the control groups raised in a grow-out pond that received no MT treatment (46.2, 35.0, and 52.5% males, respectively). No significant differences were found between control groups.

Manual on Tilapia Masculinization and Safe Handling of Steroids

A manual on tilapia masculinization in Spanish was developed that contains a general description of the biology of the tilapias, traditional cultural practices, masculinization methods, and a detailed section on safe handling of steroids. This manual is intended to reach farms that are currently producing fry (some of which are already using masculinizing steroids), extension agents who provide training to farmers, technicians, and high school and undergraduate students in fields related to aquaculture. We consider the section on safe handling of steroids a very important part of the manual since most farmers are not aware of the potential detrimental effects of mishandling steroids. The manual will be published at UJAT and will be available through the Internet via UJAT and CRSP websites.

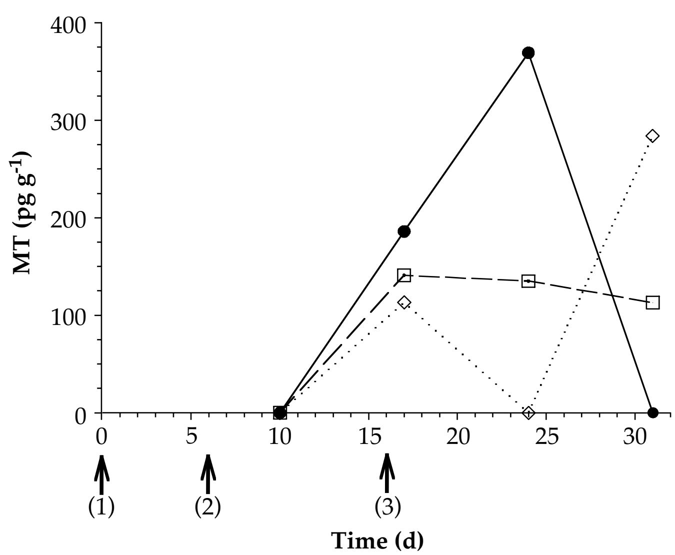

Figure 1. Concentration of 17α-methyltestosterone (MT) (pg g^{-1}) in sediments from an earthen pond used for tilapia fry masculinization. Arrows indicate initiation of feeding trials. Graph depicts MT concentration under MT-fed hapas (filled circles), at the middle of the pond (open diamonds), and under EtOH-fed hapas (open squares).

DISCUSSION

Recent studies in our laboratory have shown that MT can be detected in the water during MT treatment and eventually accumulates and remains in the sediments of model ponds for up to eight weeks (Fitzpatrick et al., 1999; Contreras-Sánchez, 2001). In the current study, we determined that during the masculinization of 23,000 tilapia fry, MT levels in sediments were lower than during a previous experiment conducted last year in the same facility (undetectable to 368.9 vs. 400 to 800 pg g^{-1}; Contreras-Sánchez et al., 2001). In this experiment, as well as in the previous one, MT values showed no trend with location of the treated hapas in the pond.

MT was not detectable in sediments for the first ten days of treatment, suggesting a lack of background levels. This is the first time we have been able to detect no background in our sediment samples. These results may be explained because we are using a new antibody and the limit for detectable values for the RIA was established at 10 pg $tube^{-1}$. Levels of MT in the pond water were below detectable limits, suggesting that the MT that leaks out to the environment may be precipitating to the sediments.

The large amount of variability detected in this experiment shows a similar pattern to that detected in previous studies (Fitzpatrick et al., 1999; Contreras-Sánchez, 2001). These patterns may be related to active bacterial degradation of the steroid (discussed in Contreras-Sánchez, 2001) or to a patchy distribution of the steroid in the pond due to dominant winds or uneaten food deposition or both.

The need to avoid exposure of untargeted organisms when steroids are administered in aquatic systems should be of great importance. Recent studies have reported that exposure of untargeted organisms to MT can result in biased sex ratios. Significant masculinization of common carps (*Cyprinus carpio*) exposed to water used in MT-impregnated feeding trials was reported by Gomelsky et al. (1994). Their findings suggested that MT (or its metabolites) can persist in the water at concentrations capable of causing sex inversion. Abucay and Mair (1997) and Abucay et al. (1997) reported incidental sex inversion in tilapias kept in aquaria and concrete tanks. These authors reported that sex ratios were significantly biased when nontarget fish were housed in the same tank where groups of fish are fed with MT.

Paradoxical feminization has been identified as a potential problem during steroid treatment. This process is thought to be caused by the enzymatic aromatization of testosterone to estradiol, and it has been documented that potent synthetic androgens (such as MT) are aromatizable (LaMorte et al., 1994). However, the mechanism of paradoxical feminization by MT has not been elucidated.

The problems with contamination of water and sediments are not only related to the immediate contact of the animal with the contaminated media; many effects are related to bioaccumulation and the transfer of the contaminants and their metabolites through the food web (Kime, 1998). Therefore, it is important to evaluate if the use of MT in aquacultural facilities requires preventive measurements such as filtration or biodegradation of the hormone and its metabolites in water and sediments.

CONCLUSIONS

Masculinization of tilapia fry using MT-impregnated food results in accumulation of small amounts of the steroid. Despite large variability in the data, it can be concluded that MT does not remain in the water but deposits in the sediments. More research is needed to determine if the concentration of MT increases when large numbers of fish are masculinized. There is no evidence in the literature that indicates if the detected levels of the steroid measured in the UJAT pond represent a health or environmental risk. However, we suggest caution when masculinizing tilapia fry because of the risk of unintended MT exposure of workers and other organisms. It is important to take measures now, either by demonstrating that the hormone and its metabolites are not a health hazard for humans or the environment or by removing these compounds from the farm effluents.

ANTICIPATED BENEFITS

We detected the anabolic steroid 17α-methyltestosterone (MT) in the sediments of sex-inversion ponds from UJAT, Mexico. The new antibody used in our latest RIA runs appears to produce no background. This antibody will allow more accurate detection of low levels of MT.

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EFFECTS OF WATER RECYCLING ON WATER QUALITY AND BOTTOM SOILS IN SHRIMP PONDS

Ninth Work Plan, Effluents and Pollution Research 4 (9ER4)
Final Report

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ABSTRACT

This study evaluated changes in chemical characteristics of production pond water, soils, and shrimp yields in response to water recycling through an oxidation pond. Nine 0.1-ha ponds were stocked with *Litopenaeus vannamei* post-larvae. Three ponds were stocked with a high density of shrimp (50 m^{-2}), three were stocked with a low density of shrimp (25 m^{-2}), and three others were stocked with a high density of shrimp while pond water was recycled through an adjacent pond of equal volume not stocked with shrimp. The density of shrimp in low-density and high-density-with-recycling treatments was equal when based on the total water area of production and recycling ponds. Mean shrimp yields for low-density (LD), high-density (HD), and high-density-recycling ponds (HDR) were 1,706 kg ha^{-1}, 4,648 kg ha^{-1}, and 4,534 kg ha^{-1}, respectively. There was no significant difference ($P > 0.05$) in yields between HD and HDR treatments or between LD and HDR treatments when based on total water surface area. Mean harvest weights of individual shrimp ranged from 22 to 25 g and were not different ($P > 0.05$) among treatments.

Recycling water from HDR ponds through an oxidation pond resulted in significant reductions in the mean mass weight of total nitrogen (TN) and total ammonia nitrogen (TAN) compared with HD ponds because HDR ponds used twice the water volume. The sum of the mean mass weight (kg) for water quality variables found in HDR ponds and oxidation ponds was significantly greater than the mean mass weight in HD ponds, except for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and TAN. No differences were noted for water quality in HDR and LD ponds. No differences were observed among treatments for soil pH; concentrations of carbon, sulfur, and nitrogen; soil respiration; and phosphorus absorption capacity. We concluded that recycling water from a production pond through an oxidation pond of equal volume had minimum to no effect on water quality and shrimp yields. The major operational disadvantages of recycling water were that pond space was put into nonproductive use as oxidation ponds, and 3.3 times more energy was used for aeration and water circulation. It would be better to stock two ponds at half the rate instead of doubling the volume of water per pond by recycling through an oxidation pond.

INTRODUCTION

Shrimp aquaculture pond water can become eutrophic when excessive amounts of fertilizer and feed are used to produce shrimp (Boyd, 1985). Feeds and fertilizers increase nitrogen and phosphorus concentrations in pond waters and stimulate the growth of phytoplankton, the base of the pond natural food web (Boyd, 1995). Green et al. (1997) estimated that feed accounted for 47 and 55% of added nitrogen and phosphorus, respectively, in semi-intensive shrimp ponds when using a 30% protein diet, and harvested shrimp accounted for 37% of applied nitrogen and 20% of applied phosphorus. Briggs and Funge-Smith (1994) estimated that 95% of the nitrogen and 71% of the phosphorus applied to intensive shrimp ponds was in the form of feed and fertilizers, but harvested shrimp accounted only for 24% of the nitrogen and 13% of the phosphorus. Thus, a portion of the nutrients in the feed consumed by shrimp is converted to shrimp flesh, but a greater proportion is wasted in the water column. Uneaten feed, dead phytoplankton, and other organic wastes are mineralized by microbial action to inorganic nutrients such as ammonia, nitrite, nitrate, phosphate, and carbon dioxide, which stimulate algal growth in the pond, sometimes leading to dense blooms (Boyd, 1985). It was estimated that the nutrients originating from the production of 1 kg of live weight of the culture species in ponds can lead to the production of 2 to 3 kg dry weight of phytoplankton (Boyd, 1985).

When pond management is intensified and stocking and feeding rates are increased beyond the capacity of the pond to assimilate nutrients, water quality deteriorates (Cole and Boyd, 1986). Water quality deterioration is first manifested by dissolved oxygen concentrations that become too low to support growth and life. In ponds without aeration, feeding rates should not be greater than 25 to 30 kg ha^{-1} d^{-1} because of the high probability of dissolved oxygen depletion at greater feeding rates (Tucker et al., 1979). Mechanical aeration can be used to sustain higher feeding rates, up to 100 to 120 kg ha^{-1} d^{-1} and prevent extremely low dissolved oxygen concentrations (Boyd, 1998). Wyban et al. (1988) obtained shrimp production (*L. vannamei*) of 2,852 kg ha^{-1} using 3.7 kW ha^{-1} in ponds stocked at 25 post-larvae m^{-2}. In ponds stocked at 45 post-larvae m^{-2}, Sandifer et al. (1991) obtained 7,500 kg ha^{-1} using 7.5 kW ha^{-1} of aeration and 17% water exchange. McIntosh et al. (1999) reported production of *L. vannamei* as high as 13,500 kg ha^{-1}. Ponds are stocked up to 160 post-larvae m^{-2}, aerated 24 h d^{-1} with 20 HP aerators ha^{-1} to sustain heterotrophic bacteria that decompose organic matter in suspension and metabolize nutrient wastes. However, production cannot be increased without limit, even if enough aeration is applied to prevent dissolved oxygen depletion at higher feeding rates because other water quality variables, such as ammonia, may impose limits on production (Boyd and Tucker, 1998).

A conventional management practice to resolve degraded water quality in shrimp production ponds is to exchange water

(Stern and Lettelier, 1992). During water exchange, pond water with high nutrient and algal concentrations is discharged from the pond and ideally replaced by water with lower nutrient and algal concentrations and greater dissolved oxygen concentration (Chien, 1992). Water exchange as a pond management tool must be used judiciously because of the potential negative impact effluents may have on receiving waters (Pruder, 1992). A study of semi-intensive shrimp farms in Honduras revealed that the increase in total nitrogen and total phosphorus between the intake and discharge was 0.24 and 0.04 mg l^{-1}, respectively (Teichert-Coddington, 1995). According to Dierberg and Kiattisimkul (1996), concentrations of total suspended solids, total nitrogen, total phosphorus, and biochemical oxygen demand in effluents from intensive shrimp ponds (stocked at 50 post-larvae m^{-2}) in Thailand are 461, 0.15, 0.53, and 28.9 mg l^{-1}, respectively.

An alternative to water exchange may be to recycle production pond water in an oxidation pond. In this scheme, production pond water is exchanged with oxidation pond water instead of estuarine water. Among the reasons for application of recycling systems are to minimize the spread of diseases (Boyd and Tucker, 1998); to conserve high-quality source waters (Treece, 2000); to improve growth performance because of greater control over water quality; and to decrease pond effluents. Usually a portion of the farm ponds is set aside as oxidation ponds to treat production pond effluents. In the oxidation ponds, organic matter is decomposed and nutrients are fixed.

A variety of shrimp pond water recycling schemes have been proposed or used (Fast and Menasveta, 1998). All rely on photosynthesis and biological and microbial decomposition to process organic matter and nutrients in oxidation ponds. The Choroen Pakphand Group (CP) operated a recirculation pond system for shrimp production at their Research and Development Center, Maeklong area, Thailand (Anonymous, 1996). Effluents from ten 0.5-ha shrimp culture ponds were discharged into four 0.55-ha treatment ponds and then circulated back into the production ponds. Treatments ponds sometimes included seaweeds or mollusks to help remove nutrients.

In Indonesia, shrimp water recycling was developed because of poor source water quality. A typical Indonesian recycling system consisted of 50% shrimp culture and 50% water reclamation (Anonymous, 1996). Shrimp pond effluents first entered a sedimentation pond, then fish/bivalve ponds, and lastly an aeration pond, before returning to the shrimp grow-out ponds.

Another proposed but untested system consists of a small intensive shrimp culture pond nested within a much larger extensive culture pond (Menasveta and Jarayabhand, 1998). Water would circulate through the intensive pond using low-energy water movers (Rogers and Fast, 1988) and discharge back into the extensive pond where suspended solids would settle and nutrients would stimulate primary and secondary productivity for a secondary aquaculture crop.

Little research has been done to evaluate the effectiveness in reducing the nutrient load either by proposed systems or those in use. Major disadvantages of recycling water are that pond space is put into non-productive use as treatment ponds and energy is used to circulate water. Instead of setting aside some ponds for treating production pond water, it may be more efficient simply to reduce the stocking densities of shrimp in all ponds. In that way all ponds are used for both production and oxidation purposes.

Bottom soils have been considered a major factor influencing water quality and aquatic animal production in ponds (Hajek and Boyd, 1994). Much of the recycling of organic matter into inorganic nutrients occurs at the pond bottom (Boyd, 1992). Excessive nutrient input may result in organic matter build-up on pond bottoms and high concentrations of partially oxidized or unoxidized components of nitrogen (NH_3, NO_2) and sulfur (H_2S), which may become stressful or toxic to shrimp. Organic input in a recycling system takes place in the unit where the culture organisms are offered feed. Water containing dissolved nutrients and suspended solids is circulated to the oxidation pond where they theoretically are mineralized or fixed. It is assumed, therefore, that water recycling would lessen the nutrient load on soils of the production pond. Many nutrients present in the soil, such as phosphorus, nitrogen, carbon, and sulfur, come from the organic matter inputs, so concentrations of these nutrients should be altered by lowering organic matter accumulation.

The objectives of this study were to evaluate effects on water quality, pond soils, and shrimp yields of recycling production pond water with a non-producing pond and to compare water recycling with the alternative of reducing stocking densities.

METHODS AND MATERIALS

Site Description

This experiment was conducted at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center (CPMC), Gulf Shores, Alabama. Ponds were lined with high-density polyethylene, and pond bottoms were covered with a 25-cm layer of natural soil. Ponds measured 1,075 m^2, and averaged 1 m deep. Ponds were filled with water pumped from the Intercostal Waterway, which connects Bon Secour Bay and Wolf Bay. Pond water salinity varied within a range of 11 to 16‰ during the experimental period.

Treatments

The experimental design consisted of three treatments with three replications each. Ponds were assigned randomly to treatments, and stocked with *Litopenaeus vannamei* as follows: 1) High-density stocking rate (50 post-larvae m^{-2}) and no water recycling (HD); 2) High density (50 post-larvae m^{-2}) with water recycling (HDR); and, 3) Low density (25 post-larvae m^{-2}) and no water recycling (LD).

Pond Preparation

Ponds were filled one week before stocking. After filling, the ponds were fertilized with 2 l of 38-8-0 liquid fertilizer three days before shrimp were stocked. A 1:16 mixture of motor oil and diesel fuel was applied evenly over the water surface in all ponds 24 h before stocking to eliminate or reduce populations of air-breathing insects. Ponds were stocked with specific pathogen-free *L. vannamei* post-larvae (PL 10-11) purchased from Shrimp Improvement Inc., Miami, Florida. Shrimp were stocked on 17 May and harvested 21 weeks later on 29 September 1999.

Water was not exchanged in any pond, but was added only to replace evaporation. Each replicate in the HDR treatment comprised two adjacent ponds, where one pond was the culture pond and the other was the oxidation pond. Water movement between the two ponds was accomplished by placing one 0.5-HP submersible pump at the deep end of the culture pond (50 cm from the bottom) and another 0.5-HP submersible pump at the shallow end (20 cm from the bottom) of the oxidation pond. Pumps were operated simultaneously 20 h d^{-1} (7 d wk^{-1}) to give a 7-d residence time in the oxidation pond.

All ponds, including oxidation ponds, were equipped with oxygen sensing and aerator activation systems (McGraw et al., in press), and one 1.5-kW propeller-aspirator type aerator. The automatic aerator activation system was programmed to maintain a dissolved oxygen concentration of 3.5 mg l^{-1} in the pond water. Shrimp were fed twice a day 7 d wk^{-1} with a 35%-protein pelleted feed (Burris Feed Mill, Franklinton, Louisiana). Daily feeding rate for all treatments was 5 kg ha^{-1} during the first week, 9 kg ha^{-1} during the second and third weeks, and 12 kg ha^{-1} during the fourth week. A nondestructive sample of 50 shrimp was taken weekly by cast net from each pond beginning the fifth week to monitor growth and to adjust the feeding rates. The initial daily feeding rate of 5% of body weight decreased to a final rate of 2.5% as shrimp increased in weight. The daily feed allowances were calculated using an assumed survival of 70% for the entire culture period. Equal quantities of feed were applied to all ponds in the same treatment. Trays were used to evaluate and verify feed consumption. The maximum daily feeding rate did not surpass 86 kg ha^{-1} for LD and 140 kg ha^{-1} for HD and HDR.

Water Quality

Chemical analyses of water were performed weekly. Samples of water in all ponds were taken with an 80-cm water column sampler (Boyd and Tucker, 1992) between 0630 and 0800 h. Subsamples of water were collected from three locations in the deep section of each pond and combined for analysis in the laboratory. Soluble reactive phosphorus (SRP), total phosphorus (TP), total nitrogen (TN), nitrite (NO_2-N), nitrate (NO_3-N), total ammonia nitrogen (TAN), and total suspended solids (TSS) were determined weekly. At least twice a month, 5-d biochemical oxygen demand (BOD_5) and chlorophyll *a* analyses were performed.

TP and TN were determined by persulfate digestion method (Clesceri et al., 1998). SRP was determined by persulfate oxidation method (Clesceri et al., 1998). TAN was determined by phenate method (Clesceri et al., 1998). NO_2-N was determined by the diazotization procedure (Boyd and Tucker, 1992). NO_3-N was analyzed by the Szechrome NAS reagent (diphenylamine sulfonic acid chromogene) method according to Gross and Boyd (1998). TSS was measured according to protocol presented by Boyd and Tucker (1992).

The BOD_5 method consists of placing the sample at 1:1 dilution in full, airtight 300-ml BOD bottles and incubating in the dark at 20°C for five days (Clesceri et al., 1998). Distilled water adjusted to the same salinity as the sample was used for dilution. Dissolved Oxygen (DO) was measured with a YSI BOD bottle probe and a YSI oxygen meter (Model 54 A). Frequent readings were taken in order to ensure that DO concentrations were not lower than 2 mg l^{-1}. Samples were reaerated after each reading. BOD nutrient buffer pillows (Hach Company, Loveland, Colorado), were used in dilution water for samples and blanks. Chlorophyll *a* was determined by acetone-methanol extraction according to pigment extraction from Pechar (1987).

Soil Analysis

Pond soil samples were collected before stocking and harvesting by taking a series of subsamples in an S-shaped pattern along the pond bottom from the deep to the shallow end. Samples collected before stocking were taken two days after filling of the ponds started, and samples collected before harvesting were taken a week before draining the ponds. Cores were taken to a depth of 10 cm, and cut into three segments: 0 to 2.5 cm, 2.5 to 5 cm, and 5 to 10 cm, according to methods described in Masuda and Boyd (1994). Soil core segments were dried in a forced-draft laboratory oven at 60°C. Soil samples were pulverized with a mechanical soil crusher (Custom Laboratory Equipment, Inc., Orange City, Florida) prior to analysis.

Soil was analyzed for pH in a 1:1 water-soil slurry (Boyd and Tucker, 1992). Total carbon analyses were made with a LECO Carbon Determinator Induction Furnace Analyzer EC12 (Leco Corporation, St. Joseph, Michigan). Total nitrogen was measured with a Leco Carbon-Hydrogen-Nitrogen Analyzer CHN 600 (Leco Corporation, St. Joseph, Michigan). Total sulfur was determined by incinerating the soil samples in a LECO Induction Furnace HP10 (Leco Corporation, St. Joseph, Michigan) and titrating the liberated sulfur with standard KIO_3 using a LECO Sulfur Titrator (Leco Corporation, St. Joseph, Michigan). Aerobic soil respiration was determined for the top 2.5 cm of each soil core according to Boyd (1995). Phosphorus absorption capacity (PAC) was determined according to Boyd and Munsiri (1996).

Data Analysis

Results of water quality determinations were averaged over time for each pond. Treatment means were averages of the replicate ponds in each treatment. Data were analyzed with a one-way analysis of variance using SAS StatView software, v. 5.0 (SAS Institute Inc., Cary, North Carolina). Significant differences among treatment means were determined by Fishers Protected Least Significant Difference (PLSD) test at a probability level of $P < 0.05$.

For the soil data analysis, the difference between the concentrations found just before harvesting and before stocking was determined for each soil variable. Treatment means were the averages of the differences (as specified above) of the replicate ponds in each treatment. A split plot design was used to analyze pH, sulfur, nitrogen, and carbon data ($P < 0.05$). Computer programming by SAS v. 6.12 (SAS Institute Inc., Cary, North Carolina) was used for data analyses. The whole plot was the pond and the split plot was the depth of soil within each pond. Soil respiration and phosphorus absorption capacity means were analyzed by one-way analysis of variance using SigmaStat v. 2.03 (SPSS, Chicago, Illinois) with a confidence level of $P < 0.05$.

RESULTS

Shrimp Production

Because of aerator failure, high mortality of shrimp occurred in one pond each of the LD and HDR treatments.

Table 1. Mean gross yields, average individual shrimp weight, food conversion ratio, survival, and kilowatt-hours (used for aeration in the production cycle) (± standard errors) for three different shrimp culture systems, low density (LD), high density (HD), and high density with recycling (HDR). The R (treatment column) represents the oxidation pond only of the HDR treatment. Numbers followed by different letters differ significantly within each column ($P < 0.05$).

Treatment	Gross Yield (kg ha^{-1})	Final Weight (g individual^{-1})	FCR	Survival (%)	Kilowatt-hours (kWh ha^{-1})
LD	1,706 ± 660.9 [a]	22.4 ± 2.95 [a]	5.6 ± 2.13 [a]	26.9 ± 7.26 [a]	8,984.2 ± 318.6 [a]
HD	4,648 ± 534.4 [b]	25.2 ± 0.73 [a]	3.4 ± 0.35 [a]	33.6 ± 2.93 [a]	13,123.7 ± 633.5 [b]
HDR	4,534 ± 629.8 [b]	24.0 ± 1.03 [a]	3.0 ± 0.04 [a]	34.9 ± 6.30 [a]	12,593.5 ± 1,020.9 [b]
R	--	--	--	--	3,623.3 ± 254.9 *

* R ponds were not included in the statistic model.
-- Does not apply.

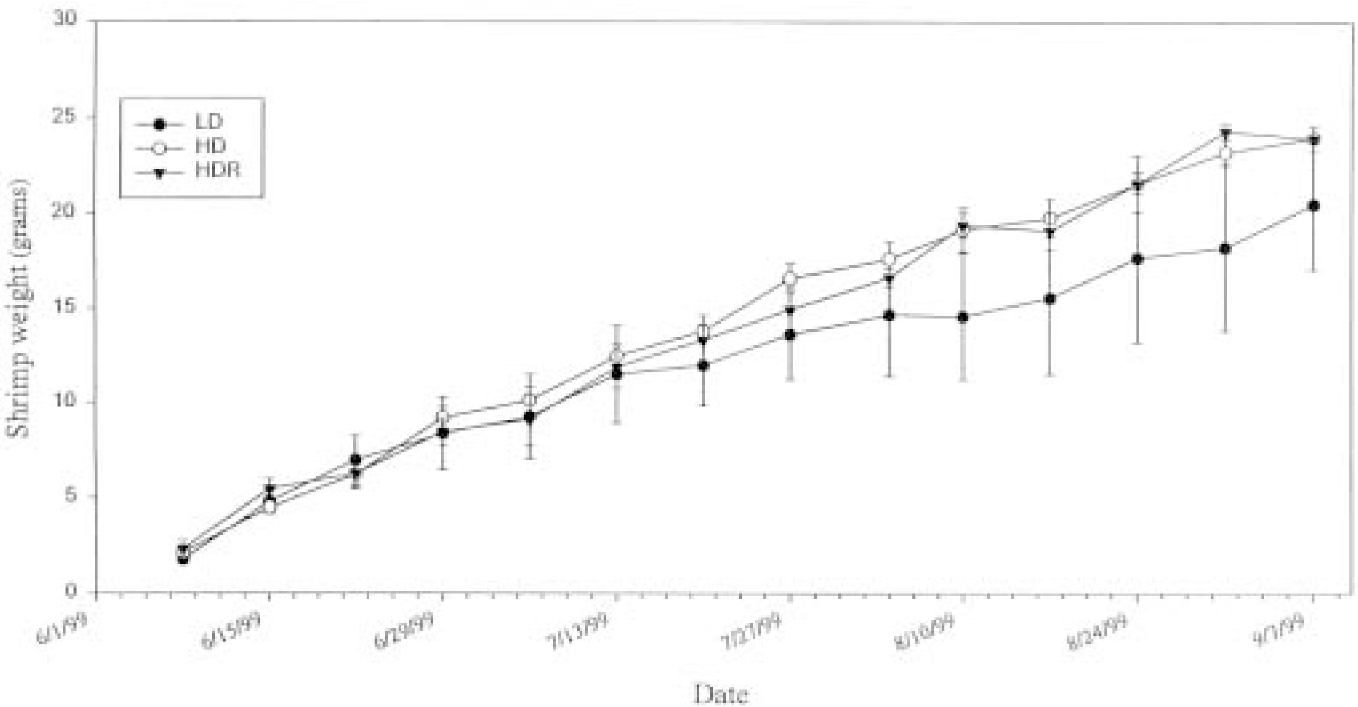

Figure 1. Shrimp growth under three different culture systems (means of three replicates in each treatment ± SE): low density (LD), high density (HD), high density with water recycling (HDR).

Table 2. Water quality variables (means of three replicates ± standard errors) for three different systems of shrimp culture: low density (LD), high density (HD), high density with recycling (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. Relevant comparisons were run 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 l^{-1})
LD	91.3 ± 4.32	0.28 ± 0.00	0.92 ± 0.04	4.95 ± 0.20	0.93 ± 0.15	0.06 ± 0.01	0.02 ± 0.01	18.53 ± 0.09	181.73 ± 24.09
HD	98.8 ± 3.06	0.18 ± 0.03	0.88 ± 0.07	5.18 ± 0.06	1.76 ± 0.17	0.11 ± 0.04	0.04 ± 0.00	20.37 ± 0.86	209.27 ± 11.60
HDR	93.1 ± 5.34	0.34 ± 0.01	0.98 ± 0.08	4.73 ± 0.07	0.74 ± 0.05	0.11 ± 0.09	0.02 ± 0.00	18.51 ± 1.02	178.10 ± 4.22
R	82.5 ± 1.10	0.51 ± 0.13	0.96 ± 0.15	3.84 ± 0.11	0.43 ± 0.08	0.07 ± 0.06	0.02 ± 0.00	14.12 ± 0.60	97.32 ± 30.22
HDR vs. HD	NS	S	NS	S	S	NS	NS	NS	NS
HDR vs. LD	NS	NS	NS	NS	NS	NS	NS	NS	NS
HD vs. LD	NS	S	NS	NS	S	NS	NS	NS	NS
HDR vs. R	S	NS	NS	S	NS	NS	NS	S	S

Data from these ponds were excluded from statistical analyses.

Mean shrimp gross yields for LD, HD, and HDR were 1,706, 4,648, and 4,534 kg ha^{-1}, respectively (Table 1). There was no significant difference between HD and HDR treatment yields. Based on a total water surface area of 2,150 m^{-2}, the HDR yield was 2,267 kg ha^{-1}, not significantly different from the LD yield. Mean harvest weights of individual shrimp ranged from 22 to 25 g and did not differ significantly among treatments (Table 1, Figure 1). Feed conversion ratios for LD, HD, and HDR treatments did not differ significantly and were 5.61, 3.40, and 3.02, respectively. Mean survival did not differ among treatments and was 27, 33.6, and 35% for LD, HD, and HDR treatments, respectively (Table 1).

Significantly less electricity (kWh) was required to maintain a minimum pond DO concentration (3.5 mg l^{-1}) in LD treatment ponds compared to HD and HDR treatment ponds (Table 1). However, there was no significant difference between HD and the culture unit of HDR treatments with respect to energy consumption for aeration.

Water Quality

No significant differences were observed among HD, HDR, and LD treatment means for TSS, TP, NO_3-N, NO_2-N, BOD_5, and chlorophyll *a* concentrations (Table 2, Figures 2 through 10). TN and TAN concentrations were greater in the HD treatment compared to the HDR treatment. The mean mass weight (Table 3) for TSS, SRP, TP, TN, and BOD_5 in HDR and R (HDR+R) were significantly higher than in the HD ponds. Mean mass weight for TAN, NO_3-N, NO_2-N, and chlorophyll *a* were not significantly different between HDR+R and HD. When comparing concentrations in the two units of the recycling system—HDR (culture ponds) and R (recycling ponds)—TSS, TN, BOD_5, and chlorophyll *a* were significantly higher in HDR.

Soils

No differences were found for soil pH, carbon, and nitrogen (Tables 4, 5, and 6) among treatments, among sample strata among treatments, or among sample depths within treatments. Sulfur concentration was significantly greater at the soil surface (0–2.5 cm and 5 cm) than at the deepest layer, but no significant differences were found among treatments (Table 7). No differences were found among treatments for net change of soil respiration and phosphorus absorption capacity in pond sediments (Tables 8 and 9).

DISCUSSION

Shrimp Production

In general, the shrimp yields in this experiment were as high as the yields found in other studies with similar culture conditions in the same research center; Hornsby (1997) obtained yields of 5,300 kg ha^{-1} in ponds stocked at 66 post-larvae m^{-2}, and McGraw et al. (in press) obtained yields of 2,970 to 3,975 kg ha^{-1} in ponds stocked at 33 post-larvae m^{-2}. In other research centers in the US, similar results have been found; Wyban et al. (1988) obtained 2,852 kg ha^{-1} from ponds stocked at 25 post-larvae m^{-2}. Higher yields (7,500 kg ha^{-1}) have been obtained by Sandifer et al. (1991), but using 7.5 kW ha^{-1} and 17% water exchange.

The stocking density of shrimp in the low-density treatment was equal to the stocking density of shrimp in the high-density-with-recycling treatment based on the total water area of production and recycling ponds. The mean HDR yield based

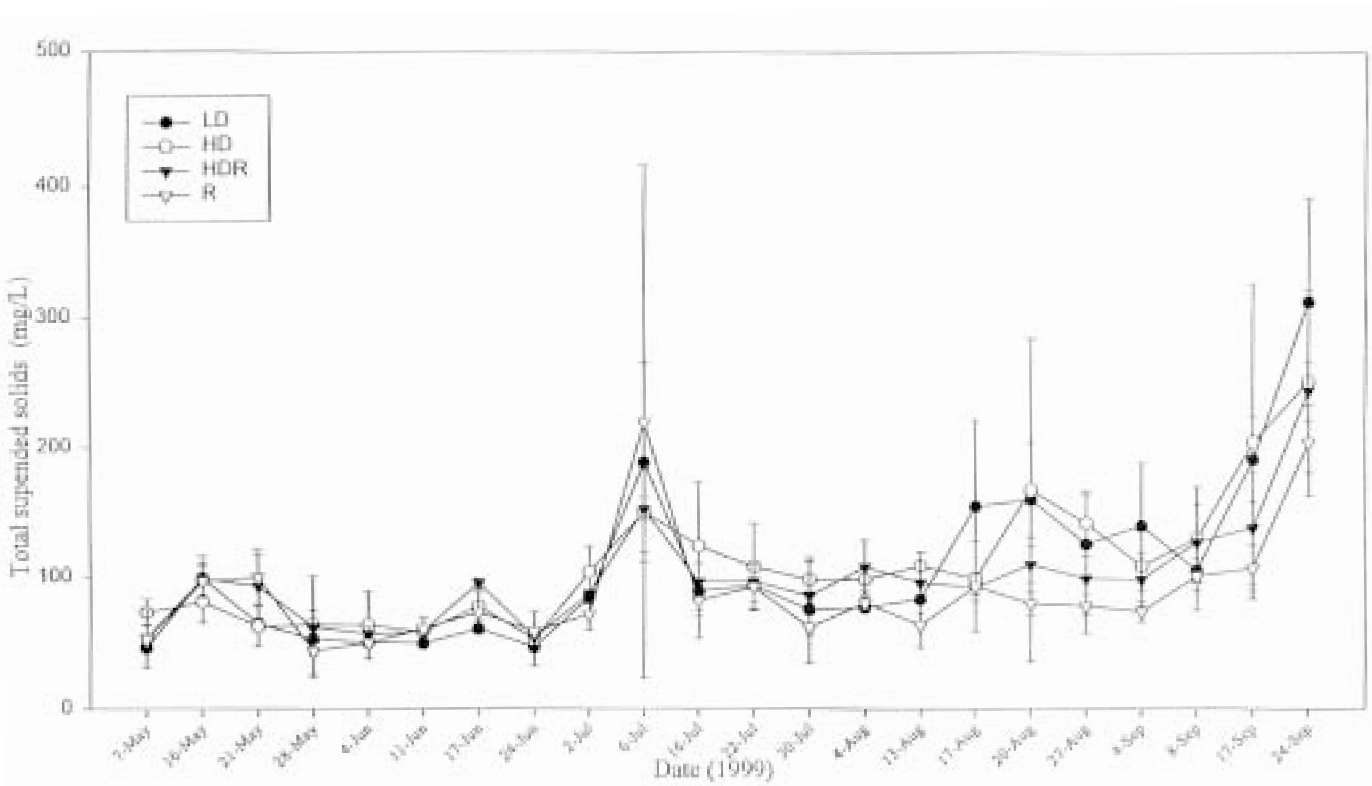

Figure 2. Mean total suspended solids (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

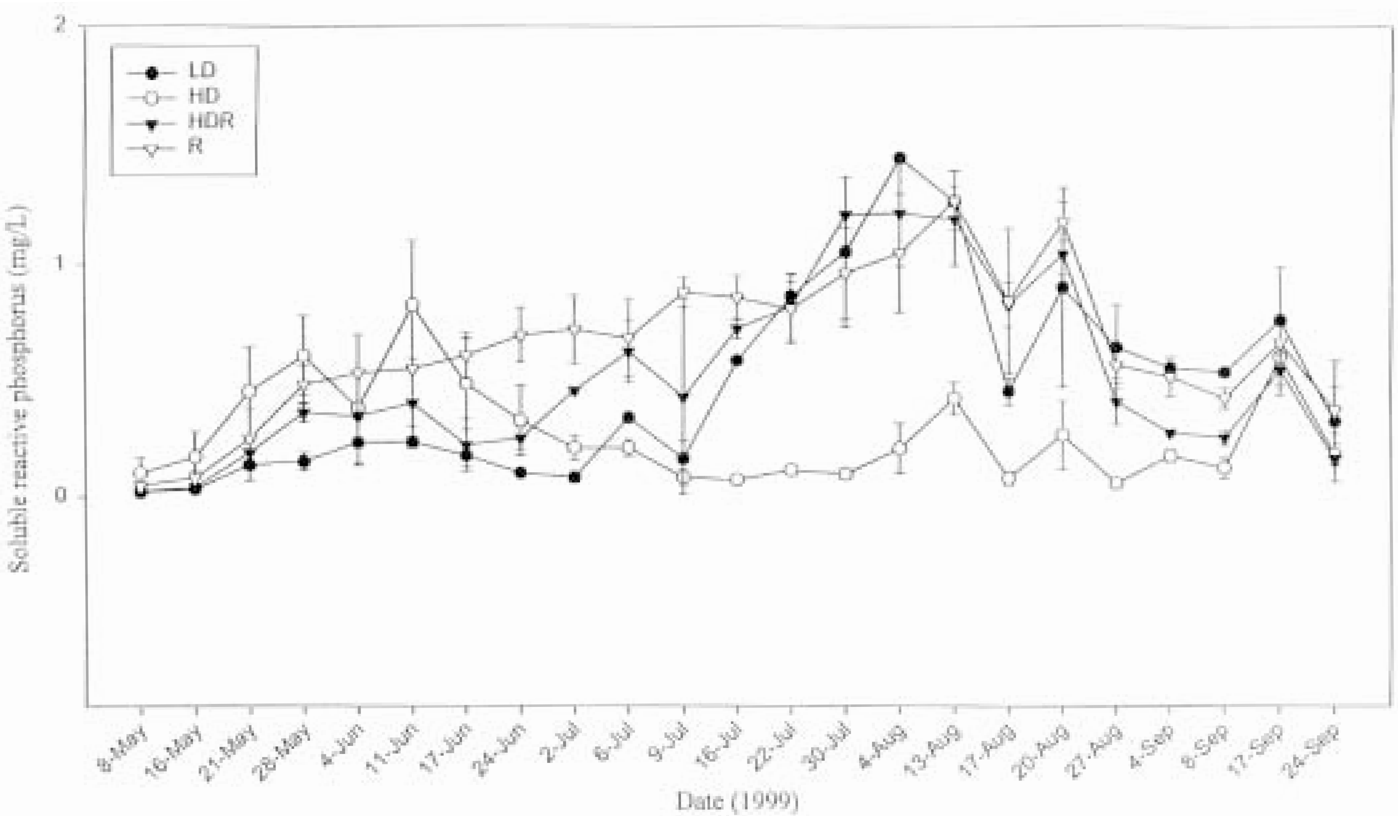

Figure 3. Mean soluble reactive phosphorus (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

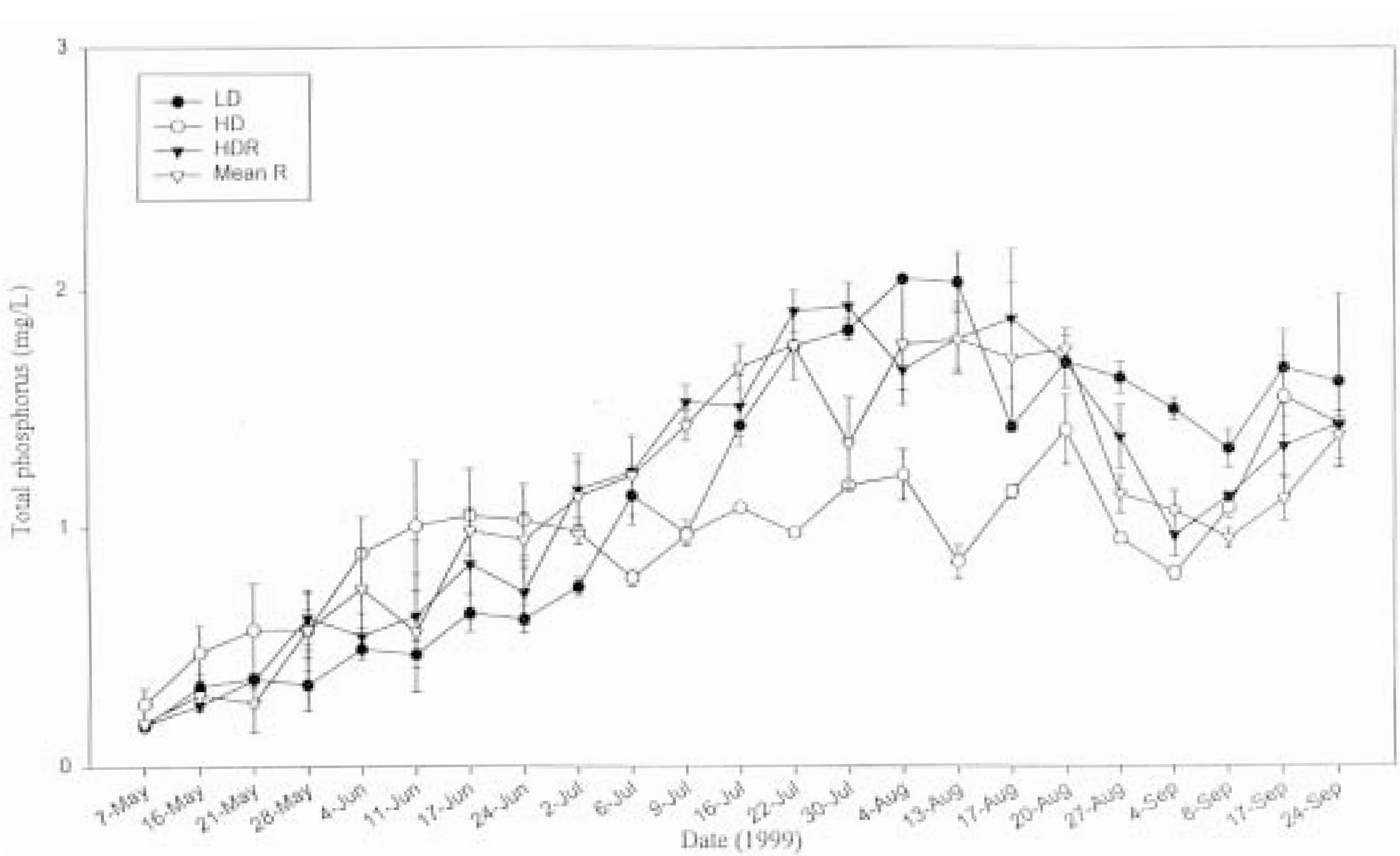

Figure 4. Mean total phosphorus (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

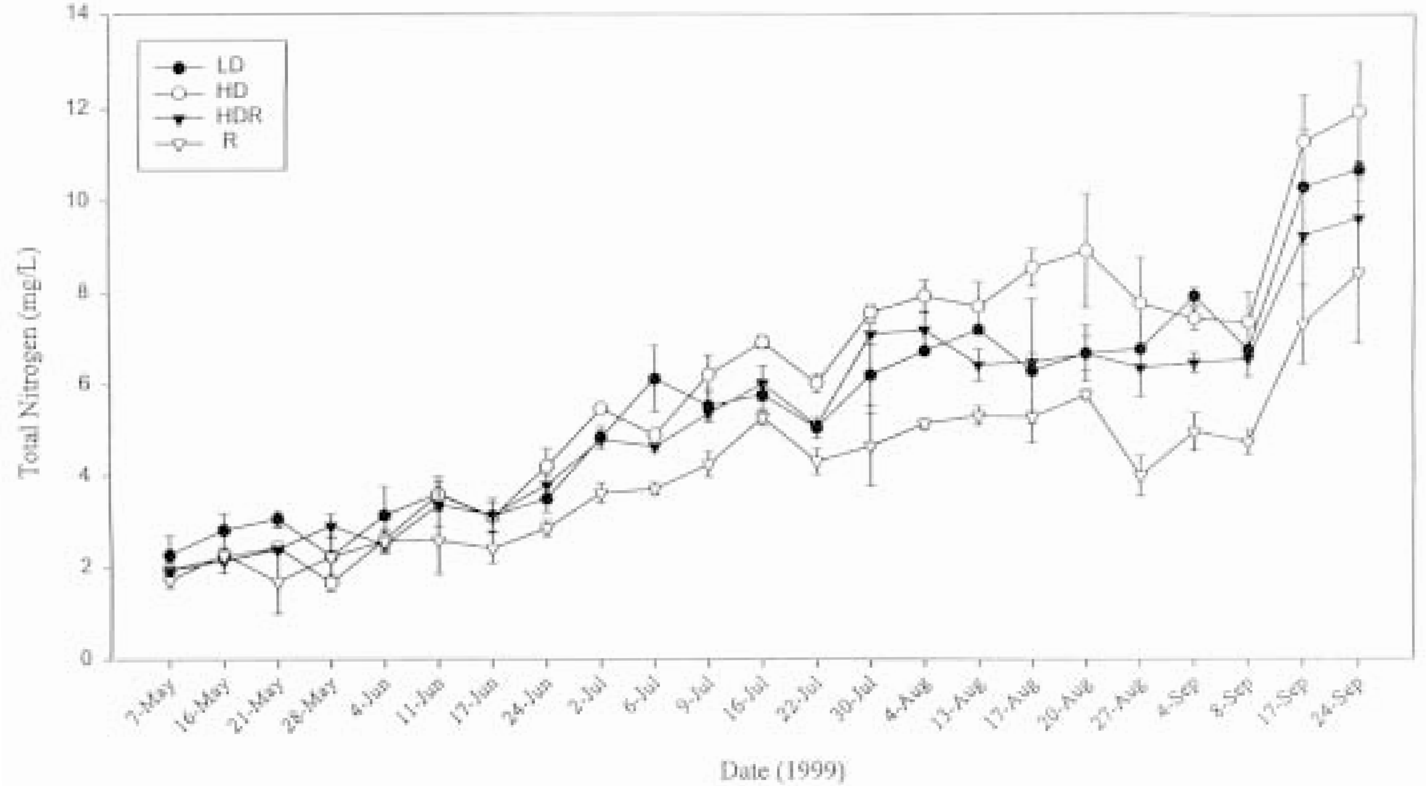

Figure 5. Mean total nitrogen (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling.

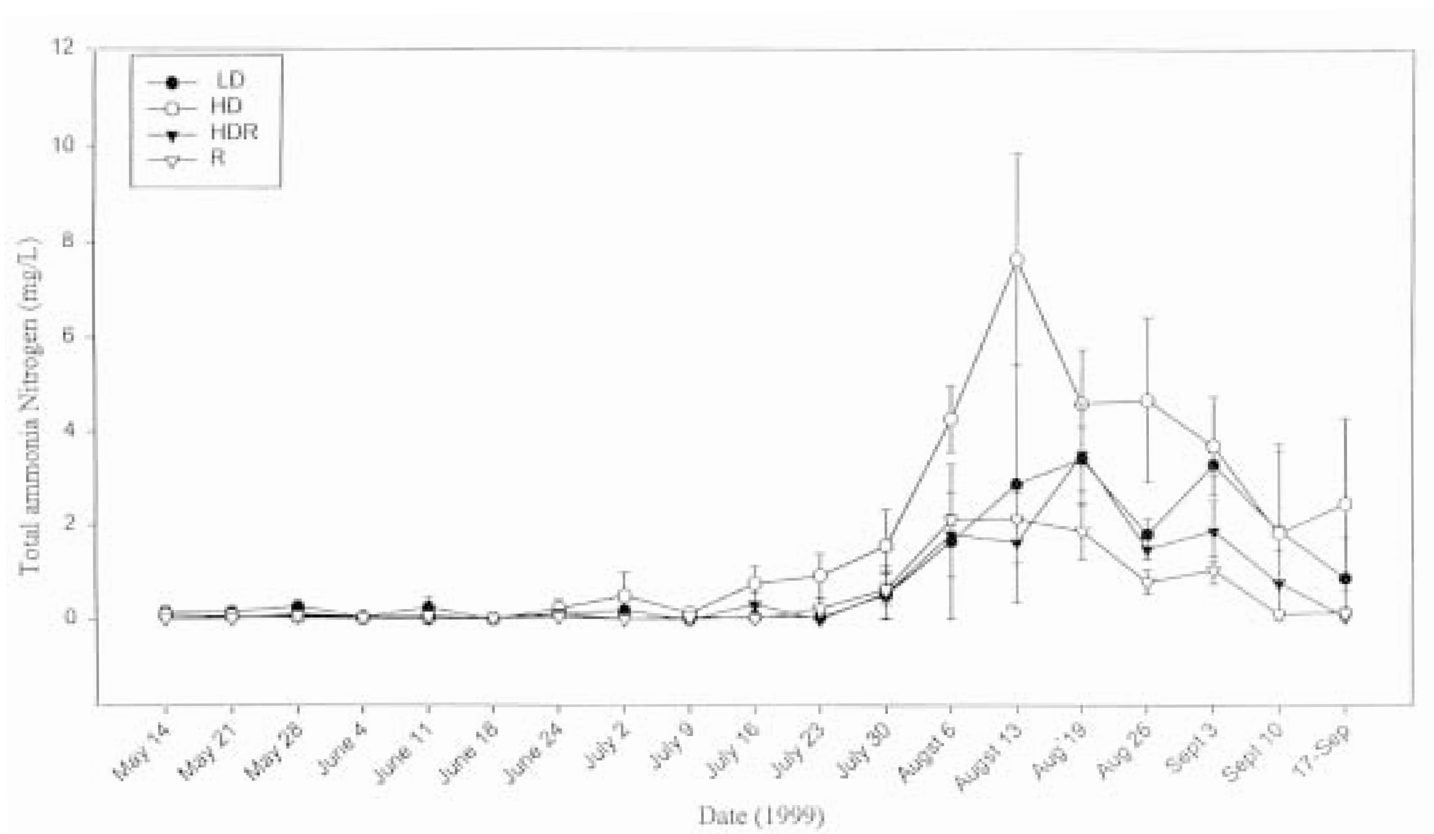

Figure 6. Mean total ammonia nitrogen (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

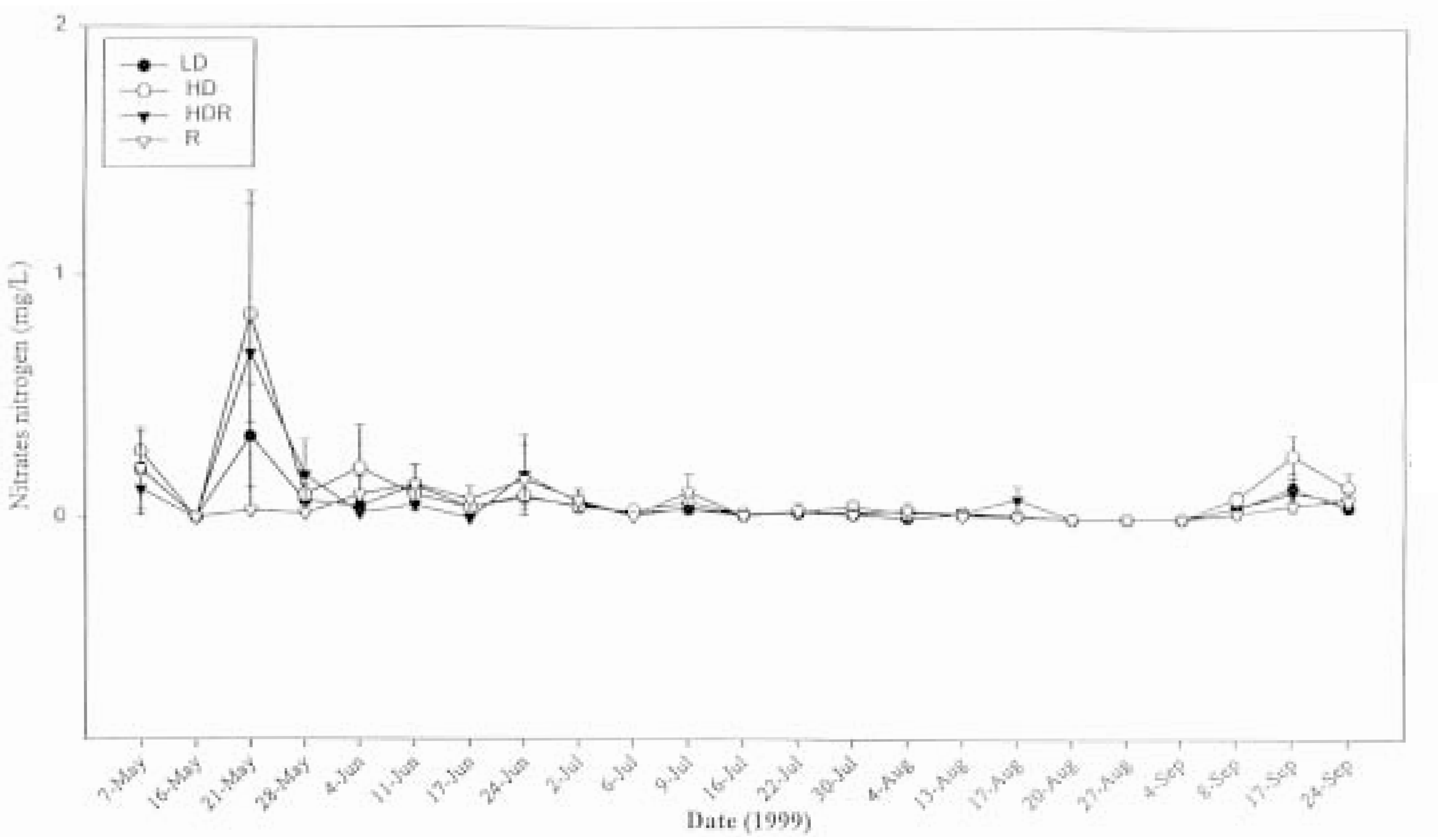

Figure 7. Mean nitrate-nitrogen (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

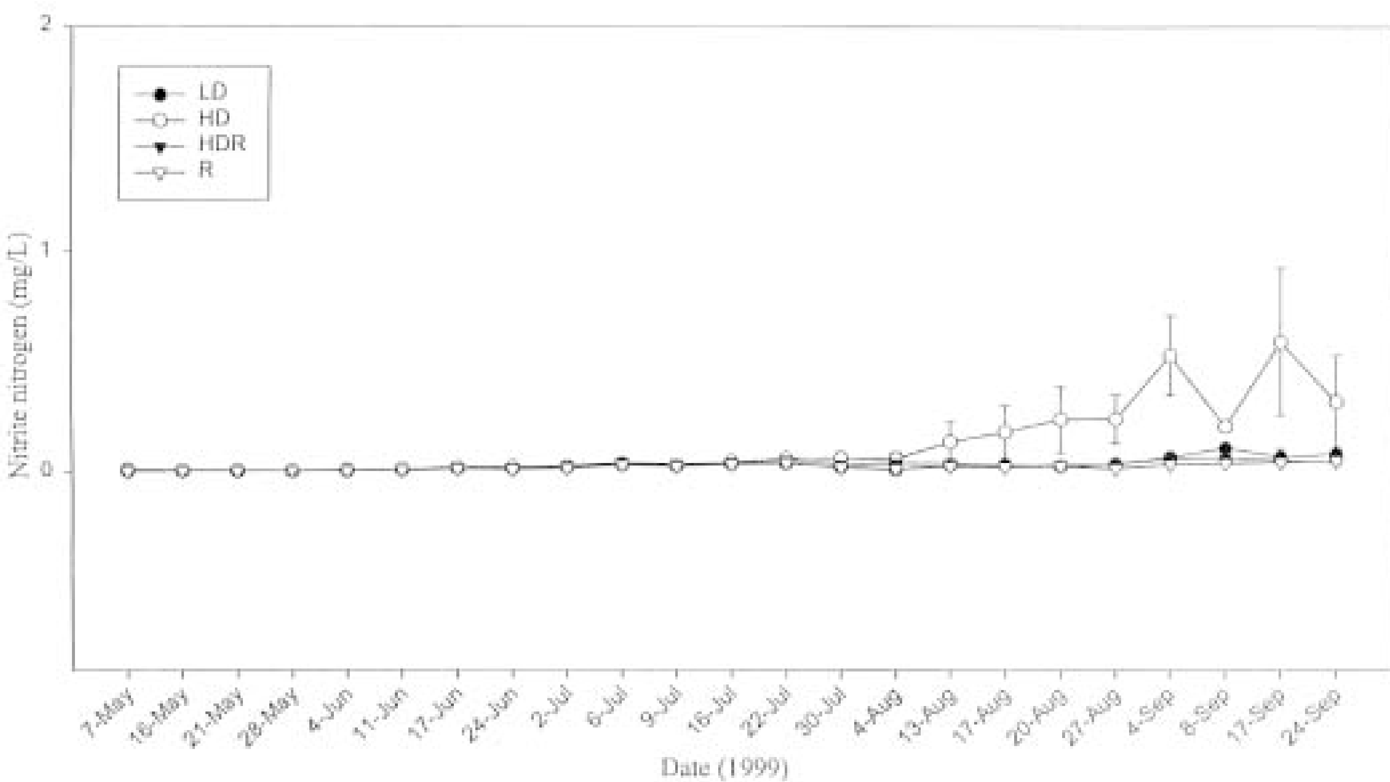

Figure 8. Mean nitrite-nitrogen (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

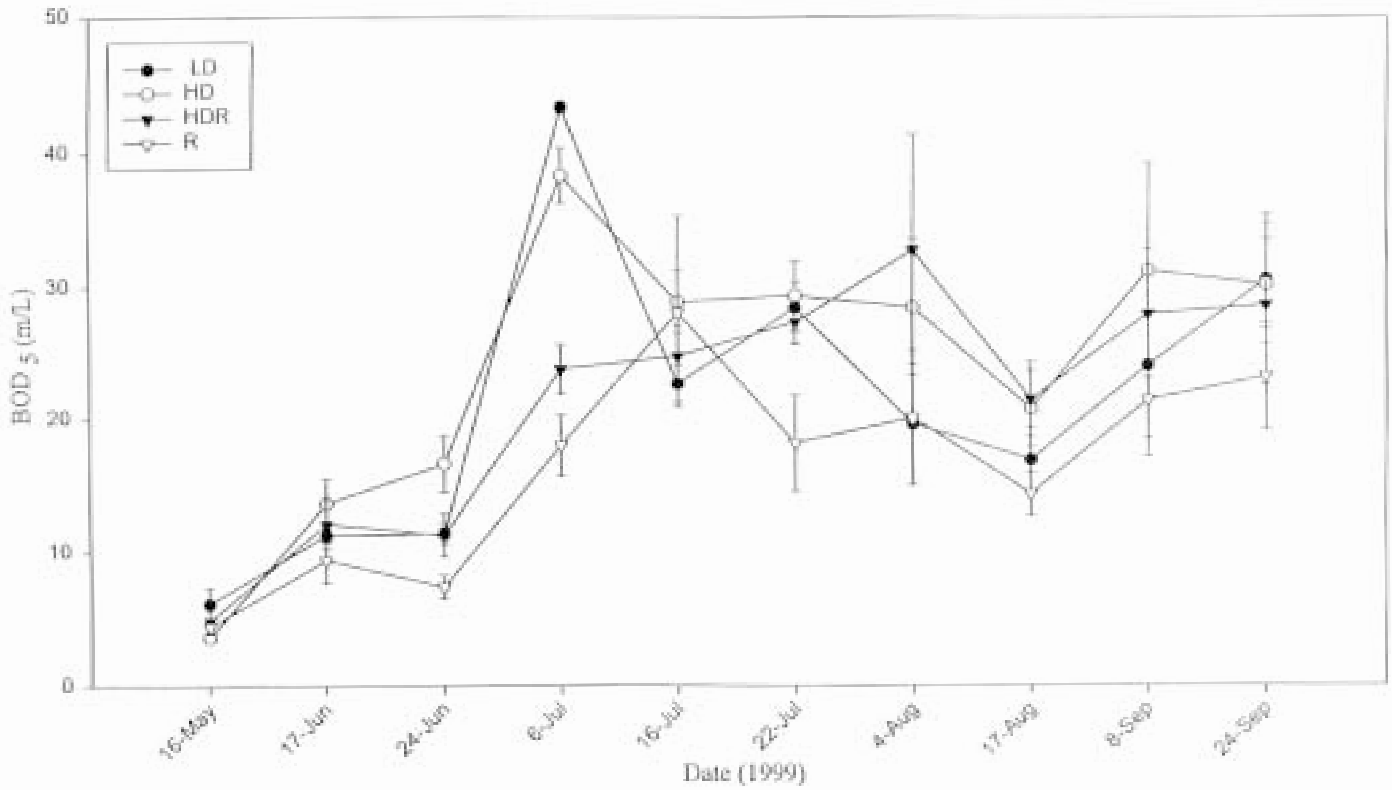

Figure 9. Mean biochemical oxygen demand (± SE) concentration in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

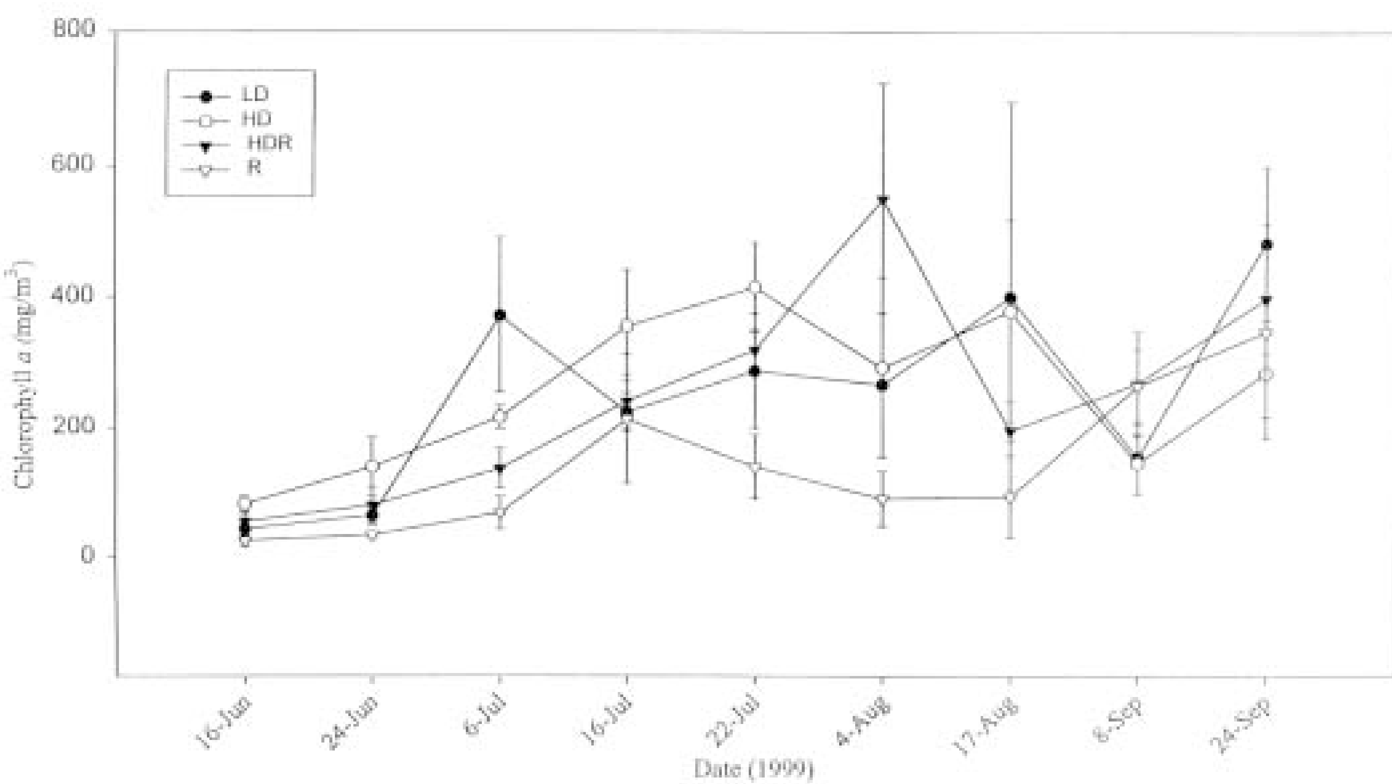

Figure 10. Mean chlorophyll *a* (± SE) concentrations in pond water during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

on the total area was 2,267 kg ha^{-1} and not significantly different from the mean LD yield of 1,706 kg ha^{-1}. These data suggest that yields cannot necessarily be increased merely by recycling production pond water through an oxidation pond. Instead of increasing the production intensity of one pond and recycling water through another non-production pond, it may be more efficient to stock both production and recycling ponds at a lower rate. In so doing, both ponds would be used for both production and oxidation, and no energy would be expended pumping water from pond to pond.

Survival was about half of what was expected. This is not attributed to management practices or water quality conditions, but to an overestimation of the post-larvae population reported by the supplier hatchery, since lower population counts were determined at the research site. However, survival calculations and feeding management practices were based upon the hatchery counts. Consequently, feed conversion ratios were high in all treatments because the daily feed allowances were calculated using an assumed survival of 70% for the whole culture period. Feed conversion ratios in the LD

Table 3. Mean mass weight (means of three replicates ± standard deviation) determined from water quality variables concentrations found in treatment ponds of 880 m^3 of water volume used to evaluate high density (HD) and high density with recycling (HDR) treatments. Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. Relevant comparisons were run among treatments; significance (S) and no significance (NS) differences are indicated within each column ($P < 0.05$).

Treatment	**TSS** (kg)	**SRP** (kg)	**TP** (kg)	**TN** (kg)	**TAN** (kg)	**NO_3-N** (kg)	**NO_2-N** (kg)	**BOD_5** (kg)	**Chlorophyll *a*** (g)
HD	86.9 ± 2.69	0.16 ± 0.03	0.77 ± 0.06	4.56 ± 0.05	1.55 ± 0.15	0.10 ± 0.03	0.03 ± 0.00	17.93 ± 0.76	184.16 ± 10.21
HDR	81.9 ± 4.70	0.30 ± 0.01	0.86 ± 0.07	4.16 ± 0.06	0.65 ± 0.04	0.10 ± 0.08	0.02 ± 0.00	16.29 ± 0.90	156.73 ± 3.71
R	72.6 ± 0.97	0.45 ± 0.11	0.84 ± 0.13	3.38 ± 0.10	0.38 ± 0.07	0.06 ± 0.05	0.04 ± 0.00	12.42 ± 0.53	85.64 ± 26.60
HDR + R	154.5 ± 4.02	0.75 ± 0.08	1.70 ± 0.16	7.54 ± 0.13	1.03 ± 0.04	0.16 ± 0.11	0.06 ± 0.00	28.71 ± 1.28	242.37 ± 22.52
HDR vs. R	S	NS	NS	S	NS	NS	NS	S	S
(HDR + R) vs. HD	S	S	S	S	NS	NS	NS	S	NS

Table 4. Averages (from three replicates ± standard errors) of hydrogen ion concentrations expressed as pH found in soil samples collected before stocking and after harvest at different depths (cm) in each treatment: low density (LD), high density (HD), and high density with recycling (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. No significant differences were found within treatments or within layers of a treatment.

Treatment	**pH**					
	2.5 cm		*5.0 cm*		*10.0 cm*	
	Before Stocking	After Harvest	Before Stocking	After Harvest	Before Stocking	After Harvest
LD	5.86 ± 0.20	6.45 ± 0.05	5.2 ± 0.40	5.9 ± 0.00	5.72 ± 0.35	6.18 ± 0.25
HD	5.96 ± 0.06	6.42 ± 0.13	5.6 ± 0.10	5.6 ± 0.27	5.76 ± 0.27	6.13 ± 0.03
HDR	5.98 ± 0.25	6.32 ± 0.15	5.4 ± 0.50	6.1 ± 0.25	5.40 ± 0.30	6.10 ± 0.30
R*	5.51 ± 0.37	5.60 ± 0.13	5.3 ± 0.50	5.6 ± 0.66	5.51 ± 0.45	5.70 ± 0.36

* R ponds were not included in the statistical model.

Table 5. Averages (of three replicates ± standard errors) of carbon percentages found in soil samples collected before stocking (BS) and after harvest (AH) at different depths (cm) in each treatment: low density (LD), high density (HD), and high density with recycling (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. No significant differences were found within treatments or within layers on a treatment.

Treatments	**Carbon Percentages**								
	2.5 cm			*5.0 cm*			*10.0 cm*		
	BS	AH	Difference	BS	AH	Difference	BS	AH	Difference
LD	1.55 ± 0.15	1.11 ± 0.00	–0.44 ± 0.15	1.06 ± 0.17	0.85 ± 0.06	–0.21 ± 0.12	0.78 ± 0.04	0.71 ± 0.03	–0.07 ± 0.01
HD	1.65 ± 0.12	1.61 ± 0.27	–0.04 ± 0.23	1.00 ± 0.07	1.09 ± 0.32	0.09 ± 0.35	0.66 ± 0.05	0.58 ± 0.07	–0.08 ± 0.04
HDR	1.42 ± 0.04	1.05 ± 0.08	–0.37 ± 0.11	0.95 ± 0.04	0.83 ± 0.10	–0.12 ± 0.06	0.63 ± 0.08	0.66 ± 0.10	0.03 ± 0.02
R*	1.55 ± 0.41	1.66 ± 0.46	0.11 ± 0.11	0.82 ± 0.15	1.08 ± 0.46	0.26 ± 0.32	0.60 ± 0.12	0.49 ± 0.08	–0.11 ± 0.03

* R ponds were not included in the statistical model.

treatment were especially poor because the calculated survival was particularly poor.

The energy consumption in LD treatment ponds to maintain DO at 3.5 mg l^{-1} was lower than in HD and HDR treatments (Table 1) because feed inputs were lower (Figure 11). The HDR and HD treatments were managed similarly, but recycling HDR production pond water system did not cause a reduction in the amount of energy needed to maintain the minimum DO concentration above 3.5 mg l^{-1}. The HDR treatment required

Table 6. Averages (of three replicates ± standard errors) of total nitrogen percentages found in soil samples collected before stocking (BS) and after harvest (AH) at different depths (cm) in each treatment: low density (LD), high density (HD), and high density with recycling (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. No significant differences were found within treatments or within layers on a treatment.

Treatments	**Total Nitrogen Percentages**								
	2.5 cm			*5.0 cm*			*10.0 cm*		
	BS	AH	Difference	BS	AH	Difference	BS	AH	Difference
LD	0.14 ± 0.10	0.09 ± 0.01	–0.05 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	–0.03 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	–0.02 ± 0.01
HD	0.16 ± 0.01	0.16 ± 0.04	–0.00 ± 0.04	0.08 ± 0.01	0.09 ± 0.04	0.01 ± 0.04	0.04 ± 0.00	0.02 ± 0.01	–0.02 ± 0.01
HDR	0.14 ± 0.01	0.09 ± 0.01	–0.05 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	–0.02 ± 0.02	0.04 ± 0.01	0.03 ± 0.00	–0.01 ± 0.01
R*	0.14 ± 0.03	0.16 ± 0.07	0.02 ± 0.05	0.05 ± 0.01	0.08 ± 0.04	0.03 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	–0.00 ± 0.01

* R ponds were not included in the statistical model.

Table 7. Averages (of three replicates ± standard errors) of sulfur percentages found in soil samples collected before stocking (BS) and after harvest (AH) at different depths (cm) in each treatment: low density (LD), high density (HD), and high density with recycling (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. Numbers followed by different letters differ significantly within mean column or mean row ($P < 0.05$).

Treatments	**Sulfur Percentages**									
	2.5 cm			*5.0 cm*			*10.0 cm*			*Treatment Mean*
	BS	AH	Difference	BS	AH	Difference	BS	AH	Difference	
LD	0.13 ± 0.01	0.21 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.14 ± 0.02	0.07 ± 0.03	0.05 ± 0.00	0.06 ± 0.01	0.01 ± 0.01	0.053 ± 0.390 [a]
HD	0.12 ± 0.01	0.22 ± 0.03	0.10 ± 0.03	0.07 ± 0.01	0.18 ± 0.03	0.11 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	0.073 ± 0.060 [a]
HDR	0.11 ± 0.03	0.22 ± 0.03	0.11 ± 0.01	0.06 ± 0.00	0.13 ± 0.01	0.07 ± 0.03	0.06 ± 0.01	0.05 ± 0.02	–0.01 ± 0.02	0.058 ± 0.053 [a]
Depth Means			0.10 ± 0.04 [a]			0.08 ± 0.04 [a]			0.01 ± 0.02 [b]	
R*	0.09 ± 0.02	0.27 ± 0.03	0.18 ± 0.03	0.27 ± 0.06	0.17 ± 0.06	0.11 ± 0.06	0.10 ± 0.07	0.06 ± 0.03	–0.04 ± 0.09	

* R ponds were not included in the statistical model.

Table 8. Average (of three replicates ± standard errors) of soil respiration rates found in soil samples collected before stocking and after harvest at the first 2.5 cm of depth in each treatment: low density (LD), high density (HD), and high density with recycling (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. Numbers within the difference column followed by different letters differ significantly ($P < 0.05$).

Treatments	**Soil Respiration Rates (CO_2)** (mg cm^{-2} h^{-1})		
	Before Stocking	*After Harvest*	*Difference*
LD	5.44 ± 0.43	5.83 ± 1.11	0.39 ± 1.54 [a]
HD	5.03 ± 0.56	7.35 ± 0.54	2.32 ± 1.10 [a]
HDR	3.66 ± 0.38	6.74 ± 0.31	3.08 ± 0.08 [a]
R	5.25 ± 0.16	6.26 ± 0.32	1.01 ± 0.97 *

* R ponds were not included in the statistical model.

Table 9. Average (of three replicates ± standard errors) of phosphorus absorption capacity found in soil samples collected before stocking and after harvest at the first 2.5 cm of depth in each treatment: low density (LD), high density (HD), and high density with recirculation (HDR). Data reported for the HDR treatment represent only the culture pond. The R (treatment column) represents the oxidation pond only of the HDR treatment. Numbers in the difference column with different letters differ significantly ($P < 0.05$).

Treatments	**Phosphorus Absorption** (mg l^{-1})		
	Before Stocking	*After Harvest*	*Difference*
LD	2.02 ± 0.21	0.61 ± 0.05	1.40 ± 0.26 [a]
HD	2.25 ± 0.40	0.65 ± 0.35	1.60 ± 0.33 [a]
HDR	1.65 ± 0.08	0.20 ± 0.20	1.45 ± 0.16 [a]
R	1.93 ± 0.40	1.09 ± 0.51	0.85 ± 0.21 *

* R ponds were not included in the statistical model.

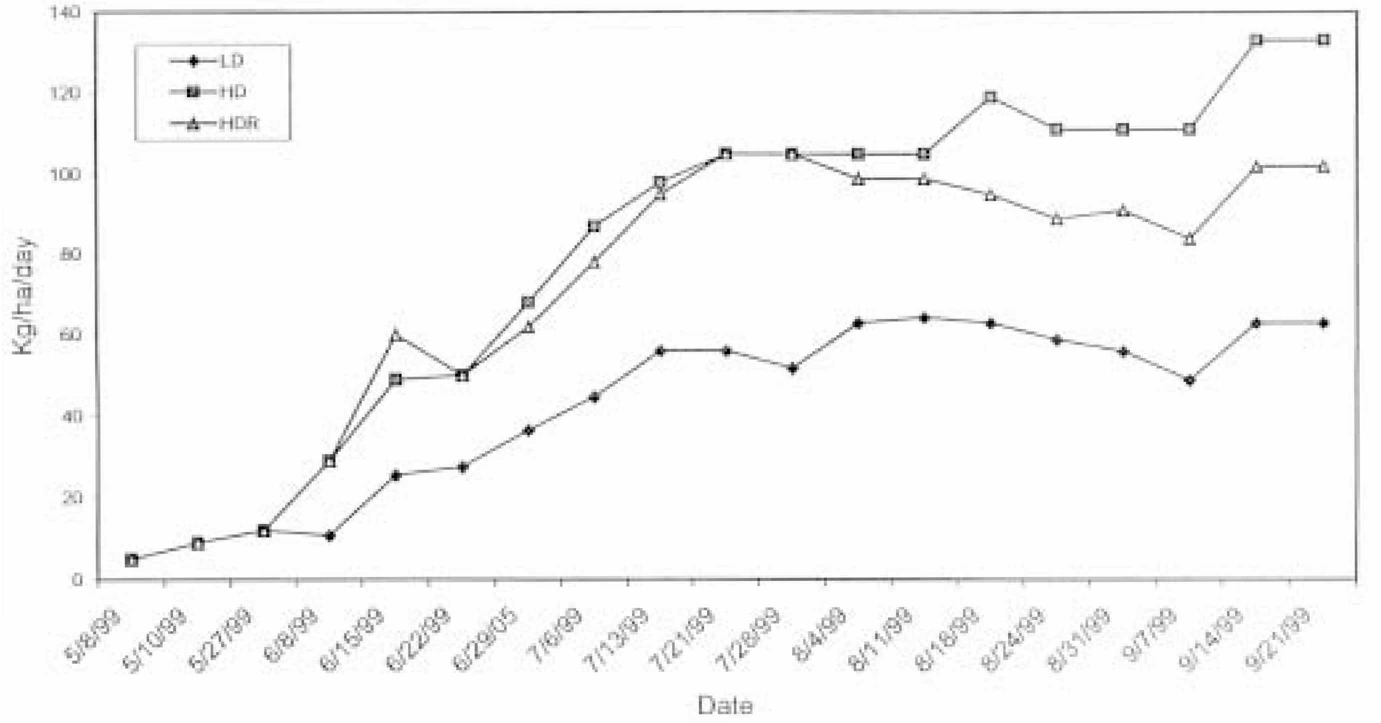

Figure 11. Feeding rates during the experimental period to test the effect of shrimp culture at low density (LD), high density (HD), and high density with water recycling (HDR).

27% more energy than the HD treatment to aerate ponds, if the energy to aerate the oxidation pond is added to that required to aerate the production pond (Table 1, p. 110).

Recycling incurred an additional cost for moving water between the two ponds. Two recycling pumps were operated simultaneously 20 h d^{-1}, consuming 0.5 kW each for a daily total of 186 kWh ha^{-1} or 27,342 kWh ha^{-1} for the whole cycle.

The total energy consumption of the HDR treatment, considering the additional energy in aerating the oxidation pond and the energy used to move the water, was 3.3 times the energy used in the HD treatment.

Water Quality

Effects of water recycling and the alternative of reducing stocking densities on water quality were evaluated by comparing the HDR and LD treatments, but no significant differences for water quality were observed between the two treatments. These results are expected because the total water volume per stocked shrimp was similar in both treatments. Results also suggest that no improvement in water quality was obtained by implementing a recycling system.

The results of comparison between LD and HD treatments were not expected. The HD treatment ponds were expected to have higher concentrations of nutrients and organic matter, but only TAN was significantly higher. Moreover, concentrations of SRP were actually higher in the LD than HD treatment, and all other variables were not significantly different between the two treatments (Table 2, p. 110).

Higher SRP concentrations were expected from HD treatment ponds compared to LD treatment ponds because of the greater quantity of feed used in the HD treatment. However, the SRP concentrations in the HD treatments were not only significantly lower than in the LD treatment but also lower than in HDR. This was also noticeable in the tendency through the experiment cycle (Figure 3, p. 112), where HD showed the lowest concentrations. The low concentrations of SRP in the HD treatment were not related to the presence of a greater amount of algae since no significant differences among treatments were found in chlorophyll *a* concentrations (Table 2, p. 110; Figure 10). It has to be considered that overfeeding took place and LD was more overfed than the other treatments. Phosphorus absorption capacity analyses were done to verify if the SRP concentrations were related to the soil phosphorus absorption; however, treatments did not differ significantly (Table 9).

To evaluate effects of recycling water to a non-culture pond, the comparison of HD and HDR was considered. Since the HDR treatment was using twice the water volume of the HD, it was expected that just by dilution the concentrations in the HDR treatments would be about half of the concentrations in the HD treatment. However, only TN and TAN concentrations were greater in the HD treatment compared to the HDR treatment. No water quality variable improvement was truly found that could be attributed to water recycling. When the mean mass weight determined from the water quality variable concentrations of culture and recycling pond were summed (HDR+R), they were significantly greater than the HD treatments or no significance differences were found (Table 3).

Soils

Differences between percentages found in initial and final samples did not differ ($P > 0.05$) within layers of the same treatment or when compared to other treatment layers for pH, carbon and nitrogen. Much of the nitrogen in soils is associated with organic matter (Alexander, 1977), so it is common that the pattern in soil nitrogen concentration in pond soils is similar to that of carbon, only in lower concentrations (Ghosh and Mohanty, 1981). Related to carbon results, the differences in soil respiration found between initial and final samples were not significantly different among any of the treatments. This suggests that water recycling did not lessen the nutrient load on soils of the culture unit of the recycling system.

Sulfur was significantly higher at the soil surface (0–2.5 cm and 5 cm) than at the deepest layer, but no significant differences because of treatment were found (Table 7). Sulfur is also contained in organic matter, and when it decomposes, sulfur is released as sulfide, which in the presence of oxygen is oxidized to sulfate (Boyd, 1990). Greater percentages of sulfur in the first two layers could have been generated by reduction of sulfate to sulfide followed by precipitation of sulfide as ferrous sulfide (Boyd, 1990).

CONCLUSIONS

Findings of this study indicate that recycling water through an oxidation pond did not cause improvement in any production variable, in water quality, or in pond soil conditions.

The major operational disadvantages of recycling water were that pond space was put into non-productive use as oxidation ponds and 3.3 times more energy was used for aeration and water circulation.

Recycling water through a non-production pond should not be used to intensify yields in the production pond. Rather, the shrimp should be stocked in all ponds at a lower density.

ANTICIPATED BENEFITS

The findings allowed a discussion of the feasibility of using water recycling to minimize the discharge of ponds effluents and the environmental implications of aquaculture with or without recycling.

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 soils analysis methods, and analytical protocol that are very useful to fill the need of research and improvement of sustainable aquaculture in Honduras.

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ON-FARM TRIALS: EVALUATION OF ALTERNATIVE AQUACULTURE TECHNOLOGIES BY LOCAL FARMERS IN KENYA

Ninth Work Plan, Appropriate Technology Research 1 (9ATR1)
Progress Report

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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. Such trials also allow project personnel to work with and train the fisheries extension officers who are involved in the trials at the various locations, thus complementing the training they receive through "regular" training activities.

Thirty farmers were selected to participate in on-farm trials in four districts of Central Province and one district of Eastern Province, Kenya, in 1999 and 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, to agree on how the trials would be conducted, and to 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 tested included high, medium, and low management levels. Ponds were sampled for fish growth at four- to six-week intervals, and farmers kept records of input type and weight, input costs, pond water additions, fish mortality, and fish sampling data. A post-trial workshop was held in March 2001 to summarize and evaluate the results of the trials. As a result of their participation in these trials, farmers learned that improved management can indeed lead to increased production, something that they were not convinced of prior to the trials. The average increase in fish harvested during these trials was 330% (3.5 t ha^{-1}, as compared with an estimate of just over 1 t ha^{-1} prior to the trials). Almost two-thirds of the ponds gave net revenues exceeding KSh 250,000 ha^{-1} yr^{-1}; the average was KSh 310,832 ha^{-1} yr^{-1}. Farmers also concluded that increasing the size of their ponds would further contribute to increases in production.

Phase two of the trials—in the western region of Kenya—began with a visit to the six districts' headquarters in December 2000. In May 2001, a pre-trial farmers workshop was held at the Bungoma Farmers Training Center to discuss and select management options suitable to the farmers. Ponds for the western region trials were stocked in May and June, and the first sampling visits were conducted in August. The trials are ongoing as of this report. As in the Central and Eastern Provinces, a post-trial workshop will be held to evaluate the results of these trials.

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 typically combined to result in 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, thus complementing the training they receive through the Kenya Project's training activity ("Aquaculture training for fisheries officers in Kenya," 9ADR3).

The specific objectives of this activity are to:

1) Collaborate with local fish farmers to test technologies developed through PD/A CRSP research at Sagana Fish Farm and elsewhere;
2) Demonstrate improved management techniques to extension officers and farmers; and
3) Teach simple methods for evaluating costs and benefits to farmers and extension agents.

This progress report mainly addresses on-farm trials conducted in the Central and Eastern Provinces during the period of 1999 to 2001. It also introduces the second phase of the trials, now being conducted in the western region of Kenya. A final report on the entire activity will be compiled when the western region trials are completed later this year.

METHODS AND MATERIALS

Preparatory contacts with farmers in Central Province and organizing activities were begun well before the beginning of this reporting year, but the pre-trial workshop and the beginning of the trials themselves were delayed. However, 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 per week.

For each district it was decided to select two focal points that, if possible, would be in areas having different climates or soil types. Each focal point had an extension agent assigned to it. 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. A total of 20 fisheries officers and extension agents were also involved in the Central and Eastern on-farm trials.

Pre-Trial Workshop

A workshop to discuss pond management options and to make stocking and management plans for each farmer's ponds was conducted from 14 to 17 December 1999. Farmers, extension agents, CRSP personnel, and some of the students involved in thesis work at Sagana participated. Farmers elected to try 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 treatments proposed in the Ninth Work Plan, which were:

1) Monosex tilapia (*Oreochromis niloticus*) + catfish (*Clarias gariepinus*), with bran + inorganic fertilizer, based on most recent recommendations from Sagana Fish Farm;
2) Same as Treatment 1 except with mixed-sex tilapia;
3) Monosex tilapia and catfish, with weekly additions of manure or organics at 500 kg total solids (TS) ha^{-1} wk^{-1}; and
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.

Trials

Ponds were stocked beginning 15 January 2000, using 10-g sex-reversed male or mixed-sex *O. niloticus*, depending on the treatment selected, and 5-g *C. gariepinus*. Stocking densities were 2 fish m^{-2} for *O. niloticus* and 0.2 fish m^{-2} for *C. gariepinus*. All fingerlings came from Sagana Fish Farm. 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.

Farmers were visited monthly by their extension agents and either monthly or every other month by their area fisheries officers accompanied by the extension agents. Sampling of ponds for fish growth was attempted with a four- to six-week frequency. Water chemistry parameters were not measured due to lack of personnel, high transport and per 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.

Visits were made by Judy Amadiva (Social Development Officer at Sagana Fish Farm), Enos Mac'Were (post-graduate student at Moi University), and Charles Ngugi to all farmers on three to five occasions during the trials. The fisheries officers often joined in the visits, especially in Kiambu and Embu, because they could get a ride with the Sagana staff. Nyeri fisheries officers had trouble in joining the visits because it was considerably out of the way for Sagana staff to pass by the Nyeri office on the way to and from the farmers' ponds. Although the CRSP vehicle was put at the disposal of the Kirinyaga officer when requested, requests were not made after the ponds were stocked. Extension agents were present for all of the visits at all of the sites except for those in Kirinyaga.

During the trials it became clear that the farmers who intended to purchase bran and fertilizer were having a difficult time because prices had soared. For example, a 40-kg bag of wheat bran that previously sold for KSh 200 to 400 was selling for KSh 800 in Embu. Farmers did not think that feeding bran was a good decision under this price regime. With an anticipated feed conversion ratio of 3 to 5 and a fish selling price of KSh 100 kg^{-1}, paying KSh 20 kg^{-1} wheat bran would not be advisable. So farmers were advised to switch to cassava leaves. Some of the farmers still purchased some chemical fertilizer, but others switched to manure as fertilizer. Therefore, instead of classifying the management levels of "cash vs. no-cash inputs," they were classified as high, medium, and low management. High management was defined as daily feeding of a high-value feedstuff like formulated animal feeds and slaughterhouse wastes. Medium-level management was characterized as consistent feeding (about 5 days a week), even if it was only leaves, and fertilizing every week or two. Ponds that received intermittent feeding and fertilization, with mostly manures and some leaves, were considered to have received low-level management. The management levels were assigned before harvest data were assembled, based on interviews and observations by Mac'Were and Amadiva.

Table 1. Responses obtained during the post-trial workshop to Question A: Were you satisfied with the extension services offered?

District	Yes	No	Not Sure	Total Farmers
Kiambu	4	0	1	5
Nyeri	5	0	0	5
Murang'a	3	0	2	5
Kirinyaga	1	2	1	4
Kiangwachi*	2	0	0	2
Embu	3	0	0	3
Total	**18**	**2**	**4**	**24**

* Two rice farmers who received advice from Sagana

Post-Trial Workshop

Farmers who had harvested their ponds were invited to the final meeting held on 5 and 6 April 2001. Some of those who had not yet harvested attended as well, bringing the total number of farmers present to 27. Extension agents and fisheries officers also attended. Most of the harvest information had been sent to Sagana earlier, but remaining questions were cleared up and all harvest data verified in interviews with farmers and extensionists conducted by Ngugi and Mac'Were. Geraldine Matolla and Amadiva interviewed farmers upon their arrival at the meeting using the questions in Tables 1 through 8 as a guide. Questions D and E (Tables 4 and 5) of the

Table 2. Responses obtained during the post-trial workshop to Question B: How can extension services be improved?

Suggested Improvements	Number of Responses	Percent of Farmers
Provide transport for extension agents	8	33
Better training of extension agents (more practical)	3	13
Extension agents need to make more regular visits to farmers	2	8
Extension agents should help the farmers with record keeping	1	4
Extension agents should try to encourage more people to farm fish	1	4
Farmers should be given printed technical information bulletins	1	4
Government should assist with distribution of fingerlings and inputs	1	4
Provide harvesting gear for extension agents	1	4
Total	**17**	

Table 3. Responses obtained during the post-trial workshop to Question C: What new management techniques did you learn?

New Techniques	Number of Responses	Percent of Farmers
Various options on feed types	13	59
Various options on fertilizer types (includes compost cribs)	10	45
Record keeping	7	32
Proper pond construction	5	23
Application rates for feeds and fertilizers	4	18
Adjusting stocking density to management level and desired fish size	3	14
Methods of predator control	3	14
Culturing a new species (catfish)	2	9
Integration of fish and livestock/poultry	2	9
Correct water management (static water)	1	5
Total	**50**	

interview were asked after the plenary session in which results were discussed. Answers were classed and tabulated by Veverica and Amadiva. Beatrice Nyandat and Susan Imende interviewed the extensionists.

After the first evening of the workshop, harvest data were compiled and sorted according to best yields (kg ha^{-1} yr^{-1}) and highest net revenue (KSh ha^{-1} yr^{-1}). The results were annualized to accommodate the different lengths of the production cycle practiced by the farmers. The results were presented to the farmers, and those who obtained the highest productions and the highest net revenues were invited to tell the group how they did it. Net revenues per pond were presented, and farmers noticed that the larger ponds had the highest net revenues. A table organized in order of highest to lowest net revenues per hectare was then presented and discussed.

Table 4. Responses obtained during the post-trial workshop to Question D: How did your results in the trial compare with your previous production results?

Response	Number of Responses
New Farmer	3
No Records/Can't Remember	3
Better	15
The Same	0
Worse	0
Total	**21**

Western and Rift Valley Province Trials

Trials in the western region began with a visit to the six districts' headquarters in December 2000. On 18 to 20 April 2001 we held a pre-trial farmers workshop at the Bungoma Farmers Training Center to discuss and select management options suitable to the farmers. Stocking dates, stocking densities, management levels, record keeping, and other issues were also agreed on at the meeting. In May and June 2001 Ngugi and Amadiva traveled to the districts and assisted with stocking on-farm trial ponds. Stocking information for the western region trials is summarized in Table 9. There are six fisheries officers and ten extension agents involved in this set of trials. Stocking densities used are the same as those used for the Central and Eastern trials. However, many of the farmers in the western trial are using a relatively high-quality feed consisting of fish meal, blood, and flour. This is expensive and

Table 5. Responses obtained during the post-trial workshop to Question E: What part of the harvest did you sell and what did you do with the revenues?

Response	Number of Responses	Percent of Farmers
Purchase more inputs for the pond	10	42
Used in general household/Institution budget	10	42
Paid school fees	5	21
Paid for pond renovation	2	8
Bought inputs for other farm enterprises	1	4
Paid medical/Purchased medicines for family	1	4
Total	**29**	

Table 6. Responses obtained during the post-trial workshop to Question F: Will you change your management? How?

Response	Number of Responses	Percent of Farmers
Will feed and fertilize at more regular intervals	15	68
Will use more inputs available on the farm	8	36
Will switch to fingerling production and sale	5	23
Will use some chemical fertilizer	4	18
Will construct more and better ponds	3	14
Will increase pond size	3	14
Will keep better records	2	9
Will renovate old ponds	2	9
Will sell some fish as well as consume at home	2	9
Will try harder to prevent predation	2	9
Will purchase "stockers" for quick grow-out	1	5
Will stop flowing water through pond	1	5
Will stop relying on extension service	1	5
Will switch to catfish culture	1	5
Will try carp culture	1	5
Total	**51**	

Table 7. Responses obtained during the post-trial workshop to Question G, Part 1: How many other people knew about these trials and about your participation in them?

District	Other Farmers	Family Members	School Kids, Parents, Teachers	Total	Number of Farmers Who Want to Begin	Number of Farmers Who Culture Fish	Number of Ponds Constructed
Kiambu	9	10	599	618	6	1	1
Nyeri	52	10	0	62	18	8	8
Murang'a	40	0	0	40	23	16	15
Kirinyaga	43	2	59	102	0	6	3
Kiangwachi*	10	0	0	10	10	0	0
Embu	48	0	60	108	5	0	1
Total	**202**	**22**	**718**	**942**	**62**	**31**	**28**

* Two rice farmers who received advice from Sagana

Table 8. Responses obtained during the post-trial workshop to Question G, Part 2: How many other people knew what farmers were doing with these trials—according to the Fisheries Officers?

District	Number Who Knew
Kiambu	> 400
Nyeri	> 500
Murang'a	> 300
Kirinyaga	0*
Embu	800–1,000
Total	**2,000–2,500**

* One agent privately said 300

farmers often stop feeding their fish if they do not have the money to purchase feed. Many were surprised to hear that other things on their farm could be used as feeds, and several of them decided to test farm by-products and compare results with those obtained using-high quality feed. As in Central and Eastern Provinces, a post-trial workshop will be held in December 2001 to evaluate the results of these trials.

RESULTS

Of the total 52 ponds stocked, 16 did not report any harvest data (Table 10). Some farmers combined data from more than one pond, so their total surface area was used to calculate yields and revenues, and management intensity was judged based on all the ponds. One group that combined mixed-sex and monosex pond data could not be included in the comparison of these two stocking strategies. The farmers, fisheries officers, and extension agents from the district of Nyeri are to be congratulated for complete reporting.

Some natural phenomena prevented harvest data collection. Some ponds dried up during the drought, and another farmer had his pond wash out after heavy rainfall. One farmer reported a total loss due to predation by otters. Thieves stole the fish from the prison ponds in Kiambu. Prison pond sampling data were used to calculate their pond productivity, but these ponds were not included in the economic analysis. Many of those reporting harvest had not yet drained their ponds, but they thought they had harvested the majority of the fish.

Table 9. Numbers of farmers participating and numbers of ponds stocked in districts and provinces of the western region for on-farm trials begun in 2001.

District	Province	Farmers	Ponds
Bungoma	Western	3	6
Busia	Western	4	6
Kakamega	Western	3	6
Trans Nzoia	Western	3	4
Vihiga	Western	2	4
Uasin Gishu	Rift Valley	3	4
Total		**18**	**30**

Table 10. Summary of ponds stocked and reported harvests for the Central and Eastern on-farm trials prior to final meeting.

District	Ponds Stocked	Ponds with Reported Harvest
Embu	7	5
Murang'a	11	9
Nyeri	9	9
Kirinyaga	14	6
Kiambu	11	7
Total	**52**	**36**

Table 11 presents the harvesting data for all the ponds. More than 50% of the ponds reported gross annualized production of over 3 t ha^{-1} yr^{-1} (Figure 1). The overall average was 3,475 kg ha^{-1} yr^{-1}. Previous records indicate about 1 t ha^{-1} yr^{-1} as the average production for those ponds that actually had recordable harvests. Interviews with farmers indicated that they may have produced even less than 1 t ha^{-1} yr^{-1}. Most of the ponds in the Central and Eastern trials are located at rather high elevations except for those in Kirinyaga. For example, the Nyeri ponds are all at about 1,800 m elevation, and several of them are higher up Mt. Kenya than the government trout farm at Kiganjo. In on-farm trials conducted in Rwanda, gross annualized yields of 1,600 kg ha^{-1} yr^{-1} (with inputs of chemical fertilizer and cut grasses) were reported from the 1,800-m elevation zone, whereas the average for Nyeri was 2,474 kg ha^{-1} yr^{-1} (Veverica and Rurangwa, 1997).

Table 11. Data from ponds belonging to farmers who attended the final meeting for the Central and Eastern on-farm trials.

Farmer Name	Pond Code	Area (m^2)	Months	GFY[1] (kg)	GFY ha^{-1} (kg ha^{-1})	GAFY[2] (kg $ha^{-1}yr^{-1}$)	Species Stocked[3]	Mgmt Level	Harvest Value (KSh)	Expenditure (KSh)	Net Rev. (KSh)	NAR[4] (KSh $ha^{-1}yr^{-1}$)	Rev. Sold (KSh)	Remarks
Justin I. Mwangi	MRA11	50	9	20	4,000	5,333	C	High	2,800		2,800	746,667	2,600	10 kg est. remain. Farmer insists got total > 100 kg!
Nicasco Ndambiri	KIR10	261	9	77	2,950	3,934	Mixed, C	High	7,700	3,600	4,100	209,451	6,200	
Nicasco Ndambiri	KIR11	54	9	46.7	8,648	11,531	Mono, C	High	5,150	900	4,250	1,049,383	5,150	
Gikuuri S. Grp	EBU1-3	378	9	41.95	1,110	1,480	T + C all	Low	6,035	2,560	3,475	122,575	6,035	Data from all 3 ponds combined.
Henry Kamau	KIR6	504	11	134.8	2,675	2,918	Mono, Mixed, C	Low	13,480	1,760	11,720	253,680	12,480	\
A. Chuma	KIR13	170	9	0	0	0	Mixed, C	Low	0	1,250	–1,250	(98,039)	0	No fish harvested.
A. Chuma	KIR12	220	9	30.43	1,383	1,844	Mixed, C	Low	3,043	1,250	1,793	108,667	3,043	
Joseph Thuo	MRA3	80	8	10	1,250	1,875	Mixed, C	Low	1,000		1,000	187,500	1,000	Otter predation, pond too shallow.
Kihara Gathuri	KBU7	180					Mixed, C	Low						Not harvested yet.
Romano Koigi	KIR5	100					Mixed, C	Low						
Francis Ndonga	NYI3	150	10	24	1,600	1,920	Mono, C	Low	2,400		2,400	192,000	2,400	
Kiritu Gitahi	KBU6	150	8	24	1,600	2,400	Mono, C	Low	4,800	403	4,397	439,700	1,950	Avg. 250 g tilapia @ 50/=, some fish escaped.
Kuria Mukiri	KBU8	80	8	26	3,250	4,875	Mono, C	Low	2,600	2,440	160	30,000	1,000	Still some fish left.
Charles Njeru	EBU4	168					Mono, C	Low		320				
Charles Njeru	EBU5	112					Mono, C	Low		320				So far only 1.5 kg for home use, rest not harvested.
Romano Koigi	KIR4	70					Mono, C	Low						
Isaac Ndirangu	MRA1	130	9	8	615	821	C	Med	800		800	82,051	800	Most fish went away with flood water.
Peter Wangaruro	KBU5	90	11	35	3,889	4,242	C	Med	3,500	1,290	2,210	267,879	2,500	Inputs–wastes, blood, pig finisher.
Thomas Nguo	EBU7	102	9	1.5	147		Mixed	Med		472				Home consumption, not all harvested yet.
D.M. Kabia	NYI6	144	8	14	972	1,458	Mixed, C	Med	2,295	684	1,611	167,813	1,945	
John Ng'ang'a	MRA7	162	10	25.0	1,543	1,852	Mixed, C	Med	3,125	424	2,701	200,074	3,125	Mostly leaves besides manure + fert.
S. Mwangi	MRA6	100	10	20.2	2,020	2,424	Mixed, C	Med	5,920	984	4,936	592,320	5,920	Has re-stocked with fingerlings from Sagana.
Francis Waigwa	NYI2	119	10	28.5	2,395	2,874	Mixed, C	Med	2,850		2,850	287,395	2,850	
Sagana W. Grp. 105	NYI5	108	10	28	2,593	3,111	Mixed, C	Med	2,800	349	2,451	272,333	2,100	
M. Ndung'u	MRA5	100	10	30	3,000	3,600	Mixed, C	Med	3,000		3,000	360,000		Not sure when harvested.
Francis Ndonga	NYI4	108	10	32.4	3,000	3,600	Mixed, C	Med	4,830		4,830	536,667	4,830	
James Ngotho	MRA10	100	9	29	2,900	3,867	Mixed, C	Med	2,900	985	1,915	255,333	2,400	
Mishak Kiragu	MRA8&9	280	9	97.8	3,493	4,657	Mixed, C	Med	11,610		11,610	552,857	11,610	Both ponds combined.
Prisons Dept.	KBU2	94	9	40	4,255	5,674	Mixed, C	Med		535				Stolen fish est. at 40 kg based on samples.
D.M. Kabia	NYI7	120	8	12.2	1,017	1,525	Mono, C	Med	3,100	1,086	2,014	251,750	1,500	
Francis Waigwa	NYI1	104	10	16.5	1,587	1,904	Mono, C	Med			0		1,650	Water crisis a serious problem; lost many fish.
Peter Wangaruro	KBU4	100	11	20	2,000	2,182	Mono, C	Med	2,000	250	1,750	190,909	1,000	Catfish ate all tilapia.
J. Kibugu	NYI9	302	8	48.62	1,610	2,415	Mono, C	Med	5,750	345	5,405	268,460	4,770	
Thomas Nguo	EBU6	105	9	26.5	2,524	3,365	Mono, C	Med	2,650	472	2,178	276,571	2,650	
R.M. Wachira	NYI8	117	8	27	2,308	3,462	Mono, C	Med	2,940	2,895	45	5,769	640	
Simon Gachieki	KIR1	190	9	51.15	2,692	3,589	Mono, C	Med	5,115	300	4,815	337,895	5,115	
Prisons Dpt.	KBU3	101	9	58.5	5,792	7,723	Mono, C	Med		535			2,290	Stolen fish est. at 40 kg based on samples.
Limuru G. Centre	KBU1	128	9	84	6,563	8,750	Mono, C	Med	10,080	1,762	8,318	866,458	10,080	Inputs–manure, wheat bran, DAP.

[1] Gross Fish Yield
[2] Gross Annualized Fish Yield
[3] C – *Clarias*, Mono – monosex tilapia, Mixed – mixed-sex tilapia
[4] Net Annualized Revenues

During the plenary session of the final meeting, it was concluded that management intensity explained most of the high net revenues. When farmers saw the net revenues reported in terms of KSh ha^{-1} yr^{-1}, they realized that they really need to expand their total pond surface area if they want to make money. However, it was cautioned that, whereas lower-level management such as use of manures and leaves works well for small ponds, it becomes increasingly difficult to use only farm by-products as total pond area increases.

Almost two-thirds of the ponds resulted in net revenues exceeding KSh 250,000 ha^{-1} yr^{-1}; the average was KSh 310,832 ha^{-1} yr^{-1} (Figure 2). A university graduate who works for the government earns an annual salary of about KSh 240,000. However, we did not include the expense of fingerlings used to stock the ponds, which would bring the overall average down to KSh 242,000 ha^{-1} yr^{-1}, nor did we include the value of fingerlings harvested when calculating revenues.

Although the average gross annualized yield was higher in ponds stocked with monosex tilapia + *Clarias* than in ponds stocked with mixed-sex tilapia + *Clarias* (Table 12), statistical analysis of variance was not done because the assumption of equal variance by treatment was violated. The high-intensity management option resulted in much greater net yields than the medium- or low-intensity management options, but there were only three ponds that were classed as having high management intensity: one was monosex tilapia + *Clarias*, one was mixed-sex tilapia + *Clarias*, and one was *Clarias* only. It appears that increasing management intensity is the first priority for fish farmers, and changing the species mix or switching to monosex should be considered only after that is done. This corroborates what farmers have been told for the past few years: If there is no food in the pond, it does not matter what kind of fish you have; nothing will grow. If we eliminate the three high-intensity management ponds and examine the medium- and low-intensity ponds, it appears that farmers are slightly better off if they increase management intensity from low to medium rather than switch from low-intensity mixed-sex to low-intensity monosex. In terms of net revenues, mixed-sex medium management gave greater net revenues than did monosex medium management. However, farmers did not report any expenditures for most ponds stocked with mixed-sex fingerlings, whereas all ponds stocked with monosex had expenditures reported.

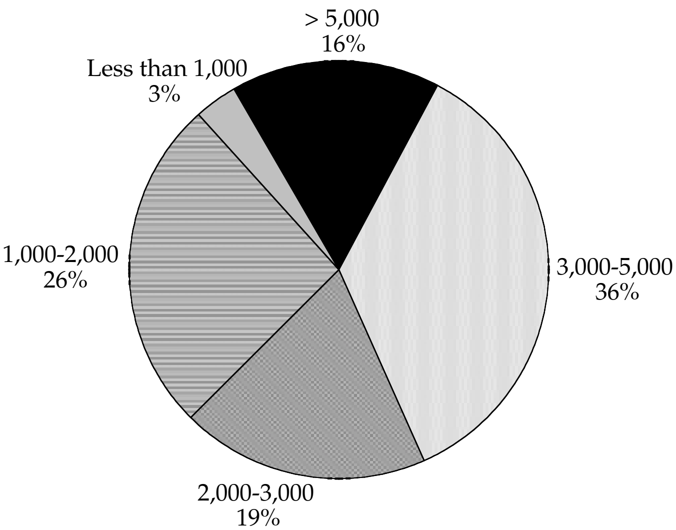

Figure 1. Percent of on-farm trial ponds that harvested each production category, expressed as gross annualized production (kg ha^{-1} yr^{-1}).

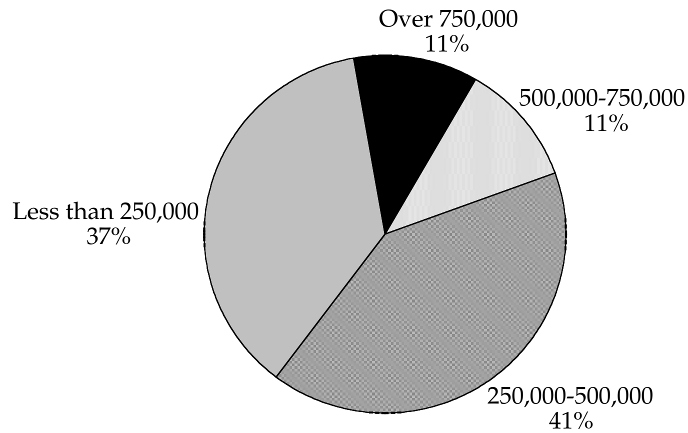

Figure 2. Percent of on-farm trial ponds in each net revenue category, expressed as net annualized revenues (KSh ha^{-1} yr^{-1}).

Farmers Questionnaire

Farmers said they were satisfied with the extension services offered by the Fisheries Department during these trials; the few exceptions came from Kirinyaga district (Table 1). The few people who were not sure about the quality of the services cited infrequency of visits in the case of Kirinyaga, lack of gear in Murang'a, and the apparent lack of technical expertise of extension agents in Kiambu. Suggestions to improve the extension services point to the problem of transport for extension agents (Table 2). The agents themselves have cited this problem repeatedly, and transportation assistance provided by the on-farm trials was short-lived. The need for training was also apparent by the time the trials began, and training of extensionists is an intended benefit of on-farm trials. This problem has been remedied to some extent with the extension agent training program initiated in November 1999, but only a small percent of the extension agents have been trained.

The numerous options for feeding fish and fertilizing a pond were overwhelmingly cited as the new things the farmers learned during these trials (Table 3). This is not surprising since data collected by Molnar et al. (1999) also suggested that farmers needed more information about how to manage their ponds. The organizers of the trials were aware of this and took the opportunity to inform farmers. It appears that the water management question was adequately addressed in our field days, but controlling water flow-through may not have been a problem in 1999 and 2000, as Kenya had experienced one of its worst droughts in recent history.

Many of the farmers interviewed said either they could not remember previous production results or they never got anything from their ponds previously (Table 4). Of the individuals who actually had previous harvests that they reported, harvest from these trials ranged from 1.7 to 10 times greater as compared to harvests from previous years. The

Table 12. Average gross annualized fish yield (GAFY; kg ha^{-1} yr^{-1}) and net annualized revenues (NAR; KSh ha^{-1} yr^{-1}) grouped by management intensity and by species stocked. Some farmers combined data, which did not allow some comparisons to be made for their ponds. Several ponds at low management intensity were not yet harvested.

Management Option	GAFY (kg ha^{-1} yr^{-1})	NAR (KSh ha^{-1} yr^{-1})	Number of Ponds Reporting
INTENSITY			
High	6,933	668,500	3
Medium	3,481	320,696	21
Low	2,164	154,510	8
STOCKING			
Tilapia Monosex + *Clarias*	4,280	355,354	13
Tilapia Mixed-sex + *Clarias*	2,912	279,413	14
Clarias Only	3,465	365,532	3
COMBINED INTENSITY AND STOCKING			
Medium Monosex + *Clarias*	3,879	313,973	9
Low Monosex + *Clarias*	3,065	220,567	3
Medium Mixed-sex + *Clarias*	3,312	358,310	10
Low Mixed-sex + *Clarias*	1,623	114,876	5

average was a 330% increase in harvest. This corroborates the estimated increase in production based on previously estimated average annual production of 1 t ha^{-1} yr^{-1}. The overall average for these trials was 3.5 t ha^{-1} yr^{-1}.

More than one-third of the farmers used their revenues for further pond management or renovation (Table 5). However, it is difficult to say how many of those who used the revenues in their general family operating budget also spent some of it on the ponds. One of the problems associated with increasing management intensity is that farmers have trouble saving some of the revenues to re-invest in their ponds. It appears that this was not the case with most of the farmers in this trial. It also seems that farmers were able to use some of the revenues to directly aid in family expenditures. This is the reason the ponds were there in the first place.

The way the farmers will change their management is interesting (Table 6). It appears that the increase in production during these trials resulted from better management, e.g., more frequent feeding and fertilizing, due to the fact that visitors were coming to see how the farmer and pond were doing. At the beginning of the trials, the farmers were not necessarily of the opinion that better management would result in better and more profitable production. However, after seeing what the high and medium management levels produced, they were convinced. It remains to be seen if they were convinced sufficiently to maintain the higher management levels in coming years or not. The other responses farmers gave demonstrate that the pond is considered a viable source of revenue. For example, several farmers reported a demand for fingerlings in their area, and they hope to supply this demand. Others will increase pond size and number (Table 6).

When asked how many other people knew that they were involved in the trials and what was going on, many farmers neglected to mention family members and were very conservative about numbers (Table 7). Perhaps they thought our next question would be to name the people who knew. The next questionnaire will ask specifically how many people from each category knew about the trials. According to the farmers, almost 1,000 people knew about the trials. According to extensionists, there were well over two times that number (Table 8). It therefore looks as if there was at least a 10:1 multiplier effect. More people may be in contact with the farmers later as well. The farmers reported a total of 62 people who they know want to begin fish farming, another 31 who have already begun, and 28 new ponds constructed so far (Table 7). The farmers themselves may become the advisors to these beginners because they see the potential for fingerling sales.

One of the notable things to come out of these trials was the farmers' eagerness to pay their own way for training sessions. Several of them asked if they could attend training at Sagana if they paid for their own lodging and meals. Production of tilapia and *Clarias* fingerlings, pond harvest techniques, and gear were most frequently requested subjects for further training.

Extension Agents Questionnaire

A separate set of questions was asked of the extension agents. They responded in much the same way as the farmers. They cited lack of transport, lack of gear, and lack of technical training as constraints to their job. The trials afforded them a broadening of experience. Those who attended training sessions before or during the on-farm trials felt more confident in their job. Their responses to the question asking what new things they learned were very much the same as the farmers' responses.

CONCLUSIONS AND RECOMMENDATIONS

Pond management, including water flow control and feeding and fertilization options, has been one of the biggest problems in aquaculture extension in Africa. These trials have helped farmers and extensionists to gain a better understanding of pond management. It is hoped that the Kenya Fisheries Department under the Ministry of Agriculture and Rural Development will continue to train its extension agents in pond management and, once agents are trained, provide them with sufficient transport opportunities to make frequent visits to farmers. In areas where fish culture will be practiced in a few small ponds per family, extension efforts should be concentrated for some years to bring farmers' understanding up to speed but then can be cut back to fewer visits by more highly trained extension specialists. Field days and annual meetings can be used to pass on information after the farmers have graduated from the intensive extension effort.

The small seines assigned to the extension offices have been of immense value. Most farmers in the Central and Eastern Provinces make partial harvests of their ponds over a period of several months, and the seines facilitate this. While partial harvesting is good from the biological production point of view, it can cause problems in tilapia ponds because of tilapia's prolific nature. Some of the problems are listed below:

- Large fish are removed first, thereby leaving the slowest growers as broodstock, and skewing the sex ratio towards female.
- A large biomass of fingerlings accumulates, and growth can virtually cease. The farmer delays draining the pond because the fish are not yet large enough but does not understand that the pond has reached carrying capacity.
- Predators such as *Clarias* can reach large sizes if they evade capture, and they will target larger tilapias, not the fingerlings.
- If draining does occur, large fingerlings are often selected for re-stocking, but farmers do not distinguish between large fingerlings and stunted females.

The extension service and farmers should be made aware of the problems associated with partial harvest. The best recommendation would be to drain and dry the pond after a maximum of 12 months and to restock with fingerlings produced by well-selected broodstock. Partial harvests should not be forbidden by the extension service because farmers will do them any way. The PD/A CRSP in Kenya has not yet done any research on partial harvesting options.

There were some problems with farmers thinking the fish belonged to the Sagana Station because the fingerlings were given free of charge. Some farmers were reticent to report harvests or sales for this reason. The free fingerlings also attracted farmers who probably should not have been in the trials. The fingerlings for western Kenya will not be given free of charge, but the project will make a considerable investment in getting the fingerlings to the farmers and in helping farmers meet the costs. Another problem, isolated to one district, seemed to be the "ownership" of the data from the ponds.

The fisheries officers in most districts were extremely helpful and recognized the contribution required of the Kenya Fisheries Department. They helped in sampling and compiling harvest data. The farmers did as well. This study financed almost all of the transport required for the trials, which allowed the officers and extensionists to make the visits. For the next set of trials, to be conducted in western Kenya, we recommend that the fisheries officers report the harvest data in separate reports for their districts and that these reports be cited as sources.

ANTICIPATED BENEFITS

This is the first set of trials in which stocking strategies and management options on farms in Central and Eastern Provinces have been systematically examined. Although the climate is cooler in Central Province compared to many parts of western Kenya, it was relatively easy to obtain a three-fold increase in production. Farmers evaluated and compared alternative technologies and made informed decisions about increasing fish production in their own ponds. Record keeping was greatly enhanced, and its value in future decision-making was appreciated. These trials immediately increased interest in fish farming, and many of the farmers who participated in the trials will be seen as models for new beginners. The trials also led investigators to management questions of interest to fish farmers such as possible partial harvest strategies and potential *Clarias* growth rates at higher elevations.

ACKNOWLEDGMENTS

Several individuals made outstanding contributions towards conducting and completing the Central and Eastern trials and towards getting the Western trials started. These include J. Amadiva and E. Mac'Were, who gathered data from farmers and offered advice; many fisheries officers and the extensionists working with them, who gathered and recorded sampling data; E. Mac'Were, who entered the sampling and harvest data on a standardized spreadsheet (he also included his notes on management level and types of inputs used; these were used to verify harvest data reported by the farmers and to attribute a management level to each pond record); and Charles Ngugi and E. Mac'Were, who re-verified harvest data at the final meeting.

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LINKAGES OF AQUACULTURE WITHIN WATERSHEDS AND CONCURRENT DESIGN OF HILLSIDE PONDS

Ninth Work Plan, Appropriate Technology Research 2 (9ATR2)
Final Report

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ABSTRACT

The hillsides in Latin America cover about one million square kilometers and provide livelihood for some 200 million people. Nearly one-half of this population is classified as "poor." There is a possibility of introducing tilapia production to the hillside regions in Latin America for improving nutrition of farm families and local communities and for providing a means of additional income. The objective of this paper is to present a levee pond design model for NGO personnel to use as they assist local producers. The model resides on the Excel® platform. The model is based on a monthly volume balance. The model enables iterative computation of the inflow needed to balance seepage and net evaporation. One can also determine the pump-in and pump-out rates needed to reach a target volume change rate per month. The model also includes an empirical spillway design.

INTRODUCTION

The hillsides in Latin America cover about one million square km and provide livelihood for some 200 million people (Verma et al., 2000). Nearly one-half of this population is classified as "poor." The typically hilly landscape is heterogeneous, and populated segments consist largely of small plots. Farming on hillsides has resulted in progressive deterioration of natural resources due to a combination of overgrazing, poor tillage and farming practices, deforestation, and poor water management.

There is a possibility of introducing tilapia production to the hillside regions in Latin America for improving nutrition of farm families and local communities and for providing a means of additional income. NGO organizations have effectively stimulated the production of tilapia by residents in a few localities of Honduras where water is plentiful. In most regions where water is not as plentiful, design tools are necessary for properly evaluating pond designs in order to make optimal use of the available water. The objective of this paper is to present a levee pond design model for NGO personnel to use as they assist local producers.

METHODS AND MATERIALS

The levee pond model is an Excel® spreadsheet that computes a volume balance on a levee pond. The model is organized into the following pages: Directions and Overview, Table of Contents, Input, Pond Model, Results, and Principal Spillway. The design is based on answers to 16 key questions on the Input page. Each question has guidance in the form of a comment that becomes visible when clicked upon. The model is designed to assist competent NGO personnel in helping small- to medium-scale producers. Input questions are shown in Figure 1. Each of the six regions selectable on the Input page has monthly precipitation and evaporation data built into the model. The Input page provides all inputs necessary to evaluate the volume balance, shown in Figure 2, on a monthly basis. The model allows one to iteratively compute the pumped inflow required to offset seepage and net evaporation. Knowing that value, one can then determine the inflow and outflow rates to change the levee pond volume a set number of times per month, based on management targets.

A spillway design is also provided based on an empirical spillway design approach. The riser diameter is based on the circumference acting as a weir. The circumference required for the flow through the levee to pass over a weir with a 5 mm head is computed.

RESULTS

After completing the initial inputs, one proceeds to the Results page, exemplified in Figure 3. Maximum, average, and minimum pond volume changes are computed based on net inflow and net outflow.

The recommended procedure for using the levee pond model is to first set the output pump rate to zero. One may then determine the inflow pump rate necessary to balance seepage, rainfall, and evaporation in a given climatic region, based on monthly net outflow as shown on the Pondmodel page. Monthly rainfall and evaporation are used in the monthly balances. A soil seepage field is included in the model, and it should be determined by soils analyses or seepage tests. Volume balances on net input should be near zero to have a sustainable pond. Next, one may determine the pump-out and pump-in rates needed to meet the volume change target. This process begins by entering a trial pump-out rate. To do this, enter the initial pump-in rate determined above, plus the trial pump-out rate for the new trial pump-in value. The volume balance based on net output should be near the volume change target. Maximum, average, and minimum volume ratios are reported based on monthly ratio computations. The principal spillway design is included. There is no watershed supply; therefore, an emergency spillway was not included.

Volume changes based on net inflow should approach the volume change target based on the level of management anticipated. After achieving the initial water balance, one adjusts both the pump-in and pump-out rates to achieve the desired volume change targets. The pump-in rate exceeds the pump-out rate by the pump-in rate found with the initial volume balance, which preserves the initial volume balance. Adjust these inputs until the desired volume changes are achieved based on net inflow. One may then proceed to the Principal Spillway page for a pipe-riser spillway design. The equivalent sharp-crested weir length is given for those who may prefer to use other spillway styles.

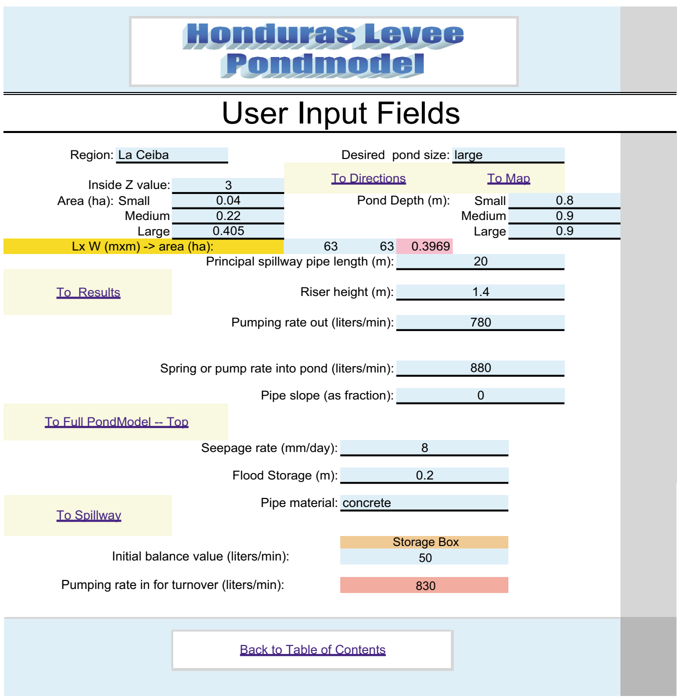

Figure 1. Input page for the levee pond model.

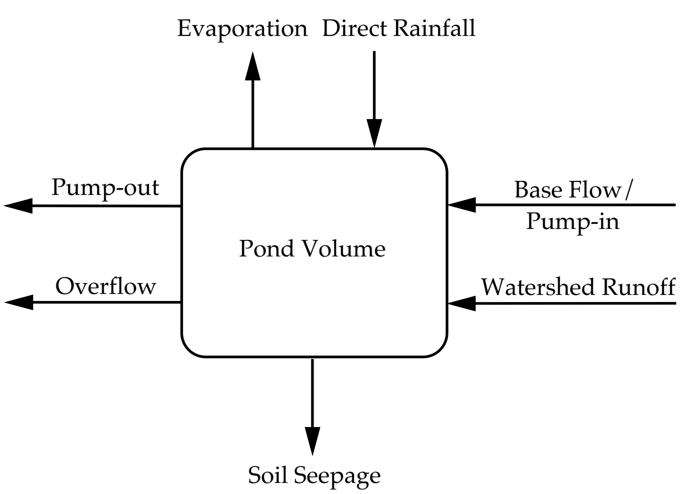

Figure 2. Volume balance for the levee pond model. Worst case overflow is zero.

DISCUSSION

Suppose a levee pond, with 0.4 ha surface and 0.9 m deep, is to be constructed in the La Ceiba region of Honduras. The spillway pipe is made of concrete and is 20 m long on a flat slope with a 1.4 m riser. The soil has a seepage rate of 8 mm d^{-1}. Pond managers have set a volume change rate of 10 per month.

On the Input page, choose the large pond size and select the La Ceiba region. Enter the 20 m spillway pipe length, concrete material, 1.4 m riser height, zero pipe slope, and 8 mm d^{-1} seepage rate. Initially, enter a pump rate out of the pond as zero. Enter a trial pump-in rate of 20 l min^{-1}. On the Results page, one will find the minimum volume change per month based on net outflow is negative. This implies that the pond will lose volume and not be sustainable. Increase the pump-in rate to 30 l min^{-1}. This brings the minimum monthly volume change per month to zero. One can manage the 30 l min^{-1} flow rate throughout the remainder of the year so as not to overflow the pond. The distribution of rainfall and evaporation throughout the year cause the variation. In the case of small levees, the design is usually complete after specifying a standard spillway of 101 mm diameter pipe and riser.

Slightly larger, more intensively managed ponds typically specify a volume change target per month. To do this, place the initial input rate of 30 l min^{-1} in the Storage Box on the Input page. Next, propose a pump-in rate of 500 l min^{-1}. To maintain the pond, the pump-in rate is 500 + 30 = 530 l min^{-1}. Go to the Results page and find that the net volume change based on inflow is 7.1 volume changes per month, below the target of 10. Therefore the pump-in is increased to, say, 780 l min^{-1}, and the pump-out rate is changed to 780 + 30 = 810 l min^{-1}. This raises

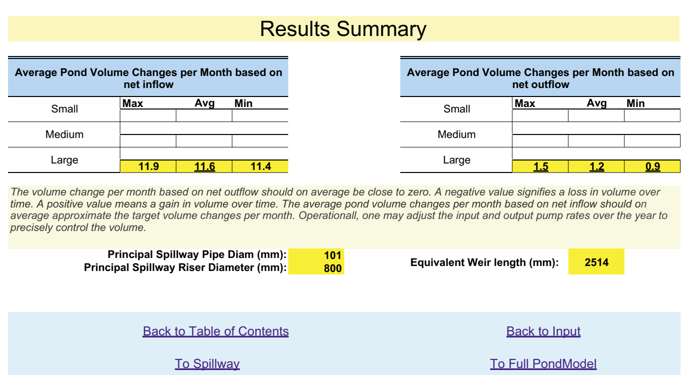

Figure 3. Results summary of the levee pond model.

the volume change based on net inflow to a value in excess of 10. Pump-in and pump-out rates are decreased to 730 and 760 l min^{-1}, respectively, yielding an average volume change rate of near 10. This outflow requires a 101 mm diameter pipe with a 0.7 m diameter riser. The equivalent weir length of 2,171 mm is useful to those desiring a principal spillway style other than the pipe riser. Other structures such as a drop-inlet or box-inlet should contain an effective weir length of the reported equivalent weir length.

If springs or stream flow are not adequate for the desired pond size and management, one may wish to consider a watershed pond or a hillside pond for water harvesting. Water harvesting is dependent on diverting runoff from a watershed collection zone to the pond. The design of the watershed pond or hillside pond is very site-specific. Hillside pond developers are strongly encouraged to seek the help of competent pond designers.

Experience suggests that valleys with available springs are the best levee pond candidates. Valleys frequently have soils of adequate clay for sealing purposes. Elevations above 1,000 m become problematic for finding springs. In Latin America there seems to be a correlation between both coffee and rice production and water availability. Areas with nearby hardwood forests tend to bode well for water availability.

CONCLUSIONS

The model is currently in verification and testing.

ANTICIPATED BENEFITS

The levee pond model is a useable tool for pond designers and managers. The feasibility of locations previously determined to possess desirable market potential and other promise of supporting tilapia production may be determined based on soil and terrain properties and water availability.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Mr. Steve Andrews in coding the Excel® spreadsheet containing the levee pond model.

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DEVELOPMENT OF CENTRAL AMERICAN MARKETS FOR TILAPIA PRODUCED IN THE REGION

Ninth Work Plan, Marketing and Economic Analysis Research 3 (9MEAR3)
Final Report

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ABSTRACT

Domestic markets for farm-raised tilapia could reduce market risk by providing alternative market outlets. Surveys of restaurants, supermarkets, and fish markets were conducted in Honduras and in Nicaragua between 1999 and 2000. Tilapia was well known in both countries. Wild-caught tilapia was sold by a majority of fish market vendors in both countries. Market penetration of tilapia products was greater in Honduras than in Nicaragua. In Honduras 40% of supermarkets and 30% of restaurants sold tilapia compared to 21% of restaurants and 26% of supermarkets that sold tilapia in Nicaragua. Both restaurants and supermarkets in both countries indicated that tilapia sales had increased over the previous year. Half of respondents who were not selling tilapia indicated that they were likely to begin selling tilapia the next year. In Honduras the primary reasons for not selling tilapia were availability problems, lack of demand, and freshness. In Nicaragua, however, the fear on the part of consumers that freshwater fish may be from Lake Managua and may be contaminated was a major constraint to tilapia sales. Marketing strategies in Honduras could focus on sales of whole-dressed tilapia to larger chain supermarkets with specialized fish sections and to international and middle-high income clients. In-store demonstrations, samples, and point-of-purchase information should be used to increase demand. Restaurant sales in both countries could likely be enhanced through catch-of-the-day promotions in upscale restaurants that feature product information supplied by tilapia growers. Fish markets in both countries and supermarkets in Nicaragua do not appear to be viable market outlets because wholesale tilapia prices appear to be too low for farm-raised products.

INTRODUCTION

The tilapia industry has grown rapidly in recent years in Central America. The United States is the primary export market for Central American tilapia, yet tilapia production companies have had to develop their own marketing companies in the US or work through a very limited number of brokers who are willing to make the effort required to develop markets for a new species like tilapia. The low number of brokers who are willing to handle tilapia increases the market risk involved in tilapia businesses. The development of domestic markets for tilapia would provide stability to Central American tilapia markets. Domestic markets could provide an outlet for smaller tilapia, enhance profits, and reduce risks.

METHODS AND MATERIALS

Six surveys were conducted between 1999 and 2000 in Honduras and Nicaragua, three in each country. Supermarkets, fish markets, and restaurants were surveyed in both countries. Data were collected to characterize each market outlet in terms of the type of business, types of clientele, and types of fish and seafood sold. Questions specific to tilapia sales included volumes, prices, suppliers, product forms, supply problems, and market channels used. Preferences for tilapia were measured with response scales and compared with responses for other types of fish and seafood.

Honduras

The three surveys were conducted in the two main urban population centers in Honduras (Tegucigalpa and San Pedro Sula) and in selected small rural towns. The following small rural towns were included in the survey: Catacamas, Siguatepeque, Santa Barbara, Comayaguela, Lago de Yojoa, Choluteca, Puerto Cortes, Juticalpa, Comayagua, La Paz, Santa Maria de Real, and Campamento La Lima. Response rates were very high (95 to 100%). This was likely due to the novelty of market surveys in Honduras.

Restaurant Survey
Fast-food establishments, bars, cafes, and Chinese restaurants were excluded from the restaurant survey; only full-service restaurants were represented. The direct personal interviews of randomly selected restaurants resulted in 72 completed questionnaires.

Supermarket Survey
Direct personal interviews were based on a census of the supermarkets published in telephone listings and resulted in 54 completed questionnaires. Supermarkets represented in the survey excluded convenience (Start Mart) stores.

Fish Market Survey
Vendors with a stand in the market were interviewed. Itinerant vendors selling fish outside the market area were excluded from the survey.

Nicaragua

Interviews for all three surveys were conducted throughout the populated region of Nicaragua in August and September 2000. The Atlantic Coast was not considered due to its low population density and a dense rain forest with difficult access.

Interviews were conducted in Managua, Los Pueblos, Masaya, Granada, Boaco, Jinotega, Matagalpa, Estelí, Chinandega, León, and Rivas. Response rates were very high (95 to 100%). This was likely due to the novelty of market surveys in Nicaragua.

Restaurant Survey
The sampling universe consisted of full-service restaurants registered with the Nicaraguan Institute of Tourism and those listed in the telephone directory. Fast-food eating establishments, roast-chicken specialty stores, catering shops, and pizza stores were excluded from the sampling universe for the survey. In all, 118 restaurant owners were interviewed in Nicaragua.

Supermarket Survey
A complete census of supermarkets was conducted in the major urban and rural population centers. Managers of all the supermarkets published in the telephone listings were interviewed, resulting in 35 completed questionnaires. Supermarkets represented in the survey excluded convenience (Start Mart) stores.

Fish Market Survey
A complete census of open-air markets was conducted in the major urban and rural population centers. Only fish market vendors with a stand in the open-air market areas were interviewed. Overall, 108 fish vendors were interviewed in Nicaragua.

RESULTS

Honduras

Restaurant Survey
Complete summaries of the Honduran restaurant survey data are available in Monestime et al. (2001). Approximately 90% of all the respondents surveyed in Honduras reported being familiar with tilapia. However, only 28% of restaurants also reported selling tilapia. Sixty-seven percent of the respondents in the north reported having sold more tilapia over the past year. More than half (54%) of the respondents who were not selling tilapia were willing to try it within the next year.

The most commonly purchased forms of tilapia throughout the country were fresh whole-dressed tilapia (60%), followed by fresh fillets (26%), live (9%), and frozen whole-dressed tilapia (5%). Restaurants reported off-flavor problems. Half also reported problems with freshness, and the other half reported problems with the size of the fish.

Respondents reported problems with insufficient quantity (100%), lack of availability of tilapia at certain times of the year (50%), availability of preferred sizes (50%), and unreliable quality of tilapia products (50%).

The highest rating overall from respondents who sold tilapia was for "tilapia is a high quality fish" (9.50). This rating was followed in decreasing order of agreement by "tilapia is easy to prepare" (9.44), "tilapia has a nice fresh flavor" (9.39), "tilapia is always readily available" (8.50), "tilapia supply is of reliable quality" (8.39), and "the patrons of my restaurant would like the variety that adding tilapia would provide" (7.78). The attribute that received the lowest rating was the "price of tilapia is too high relative to my patrons' desire to buy it" (4.11). A similar series of statements was presented to managers of restaurants who reported not selling fish or seafood. The highest rating was for the statement "the patrons of my restaurant would like the variety that adding fish or seafood to the menu would provide" (7.50).

Supermarket Survey
Complete summaries of the Honduran supermarket survey data can be found in Fúnez et al. (2001b). Overall, 41% of the supermarkets sold tilapia, 24% used to sell tilapia, and 35% had never sold tilapia. Larger stores, especially those with specialized seafood sections, tended to be those that sold tilapia. Forty-four percent of supermarkets that sold tilapia and reported a specialized fish section had a weekly sales volume from US$36,000 to US$67,500. Among the stores that sold tilapia, the most frequently mentioned type of clientele was international. This was followed by high-income mestizos, then middle-income mestizos. Of the stores that never sold tilapia, the most frequently mentioned clientele were low-income mestizo and black clients.

More of the supermarkets (34%) that had either stopped selling or had never sold tilapia mentioned lack of demand as the principal reason. This was followed by storage problems (22%), seasonal availability problems (16%), freshness, lack of space, and had not heard of it (9%) as reasons for not selling tilapia. Ninety-five percent of the respondents indicated that they did not have problems with the quality of tilapia. Only 5% said that they experienced problems with the quality of tilapia that they purchased. Of those that mentioned quality problems, freshness and off-flavor were the problems most frequently cited. Forty-one percent of the respondents indicated that they were selling more tilapia in the current year compared to one and two years ago. Overall, only 18% indicated that they were selling less tilapia compared to two years ago.

The top-selling tilapia product form was fresh whole-dressed tilapia (68%), followed by fresh tilapia fillets (24%) and frozen whole-dressed fish (8%). However, tilapia volumes sold daily were low. Thirty-six percent of the supermarket respondents indicated that they sold from 0 to 10 lb d^{-1}, and another 32% said that they sold from 20 to 30 lb d^{-1}.

The most frequently mentioned price (47% of responses) for fresh whole-dressed tilapia was \$0.83 lb^{-1}, but prices as high as \$2.35 lb^{-1} were reported. Fresh fillet prices were mostly in the range of \$1.06 to \$2.70 lb^{-1}. Prices of frozen whole-dressed tilapia averaged \$1.50 lb^{-1}. Wholesale prices of fresh whole-dressed tilapia products averaged \$0.83 lb^{-1} and ranged from \$0.33 to more than \$1.33 lb^{-1}. Fresh tilapia fillet wholesale prices averaged \$1.20 lb^{-1} and ranged from \$0.73 to \$2.13 lb^{-1}, but the majority of respondents (80%) reported prices higher than \$1.33 lb^{-1}. Wholesale prices of frozen whole-dressed tilapia products averaged \$0.87 lb^{-1} and ranged from \$0.73 to \$1.00 lb^{-1}.

More than three-quarters (77%) of respondents indicated that their supply of tilapia had been consistent. Another 23% indicated that it had not been. The majority of the supermarkets that purchased from fish farmers indicated that their tilapia supplies were consistent. The most commonly cited supply problem was that tilapia was unavailable at certain times of the year. This response was followed by high price.

Quality received the highest rating along with ease of preparation. The only three statements with which respondents disagreed were "tastes like earth," "price is too high," and "size too small." In other words, respondents indicated that off-flavor was not a problem and that tilapia price and sizes were acceptable.

Supermarkets that reported a specialized fish section or that were likely to add such a section rated tilapia higher on all attributes compared to other supermarkets. The only supermarkets that responded that tilapia tasted like earth were those that used to sell tilapia and were unlikely to add a specialized fish section. Half of the supermarket managers responded that they were likely to begin to sell tilapia the next year. Most supermarkets (80%) that were very likely to begin to sell tilapia the next year indicated that they were very likely to add a specialized seafood section. Moreover, all supermarkets that responded that they were very unlikely to begin to sell tilapia the next year said that they were very unlikely to add a specialized fish section.

Fish Market Survey

Complete results of the Honduran fish market survey can be found in Fúnez et al. (2001a). Overall, 70% of the respondents sold tilapia, 17% used to sell it, and 14% never sold tilapia. Equal percentages of respondents indicated that they were selling more and less tilapia compared to two years ago, but more respondents indicated that sales had remained constant from 1999 to 2000. Over half of the respondents (65%) indicated that they were very or somewhat likely to begin to sell tilapia the next year.

The top-selling tilapia form was fresh whole-dressed (74%) followed by frozen whole-dressed tilapia and fish fillets (4%). The most frequently mentioned price (93% of respondents) for fresh whole-dressed tilapia was $0.68 lb^{-1}, but prices as high as $0.87 lb^{-1} were reported. Wholesale prices of fresh whole-dressed tilapia averaged $0.51 lb^{-1} and ranged from $0.20 to more than $0.60 lb^{-1}. Fresh tilapia fillets and frozen whole-dressed tilapia wholesale prices averaged $0.58 lb^{-1} and ranged from $0.20 lb^{-1} to $0.80 lb^{-1}.

More than half (56%) of respondents indicated problems with tilapia supply. Approximately 24% of tilapia supplies were purchased from Nicaragua. The most frequently mentioned problem (69%) was seasonal availability. Half of the vendors mentioned insufficient quantity. A smaller number mentioned unreliable quality, availability of product form, and earthy flavor.

Nicaragua

Restaurant Survey

Complete results of the Nicaraguan restaurant survey can be found in Neira and Engle (2001). Only 21% of the restaurants sold tilapia. Sixteen percent used to sell tilapia, and 8% did not sell fish or seafood of any kind. Overall, 64% of the respondents were very likely and 21% were somewhat likely to begin to sell tilapia the next year if they had a consistent tilapia supply and could get a farm-raised tilapia product.

Restaurants that sold tilapia tended to be local restaurants selling primarily Nicaraguan food, steaks, Chinese food, seafood, and a variety of cuisine. Restaurants that sold tilapia tended to have more seating capacity than restaurants that used to sell or never sold tilapia. Tilapia sales were most often indicated to have remained stable. Forty percent of the respondents indicated that they were selling more tilapia compared to one year ago, but only 4% said they were selling less.

The most popular tilapia product form reported by the respondents was fresh tilapia fillets (53%), followed by fresh whole-dressed tilapia (27%) and frozen tilapia fillets (20%). Volumes of tilapia sold were generally low. The most frequently mentioned size of fresh tilapia fillets (75%) was between 0.21 and 0.60 lb per fillet. More than half of the respondents (56%) prepared breaded tilapia to serve in the restaurants. This was followed by grilled (48%), ceviche (32%), fried (28%), garlic (24%), boiled (20%), and soup (16%).

The most frequently mentioned reason for either not selling or having stopped selling tilapia (31% of respondents) was off-flavor (tastes like earth). Off-flavor was followed in importance by lack of awareness. Twenty-one percent of respondents overall had not heard of tilapia. This reason was followed by mention of the contamination of Lake Managua and lack of supply. Other reasons mentioned included lack of demand, selling only marine fish, negative consumer attitudes, poor consistency after frying, bony, patrons do not like to eat tilapia, size, gas flavor, price too high, and selling only fillets.

More than two-thirds of the respondents (68%) indicated that their supply of tilapia has been consistent. The most commonly mentioned problems with the supply of tilapia were the availability of preferred sizes (62%) and insufficient quantity (38%).

The highest rating overall was for ease of preparation. This was followed in descending order by positive ratings on the following attributes: adding variety to the menu, high-quality fish, nice fresh flavor, little fishy odor, reliable supply, availability, consumers like to eat, and tilapia is similar to guapote. Low ratings for price being too high and the size being too small indicated that respondents viewed both the size and the price of tilapia as appropriate. Respondents, however, also agreed that marine fish is better than tilapia. Some respondents indicated that tilapia tasted like earth or had an off-flavor.

The most important fish attribute mentioned by restaurant managers was quality. Supply and size were mentioned as the second and third most important characteristics that influenced restaurants that sold or used to sell tilapia. For restaurants that never sold tilapia, price was the second most important characteristic, while size was second most important for restaurants that did not sell fish. Of those restaurants that never sold tilapia and never sold fish, availability was the third most important characteristic.

Supermarket Survey

Complete results of the Nicaraguan supermarket survey can be found in Engle and Neira (2001b). Overall, 26% of the supermarkets sold tilapia. Another 29% sold fish but never sold tilapia. Supermarket respondents indicated that sales of tilapia in 2000 were higher than those in the previous year.

In the south-central region, the larger stores (401 to 2,000 m^2) were those that tended to sell tilapia. This was not the case in the Northwest region. Those that sold tilapia in the Northwest region tended to be the smaller supermarkets.

The primary reasons given for not selling tilapia were that it tasted like earth and that there was no supply. Other reasons given included contamination of Lake Managua, hadn't heard of it, negative consumer attitudes, and storage problems.

Fresh fillets were indicated to be the preferred tilapia product form by 60% of the respondents. This was followed by frozen fillets. Supermarkets sold more fresh fillets than any other product form. On a weekly basis, supermarkets sold an average of 396 lb wk^{-1}. This quantity was followed by frozen fillets with an average weekly sales volume of 134 lb wk^{-1}. The weekly volume sold of fresh whole-dressed fish averaged 30 lb wk^{-1}.

The average size of fresh fillets sold was 0.60 lb. The average size of frozen fillets was 0.37 lb, and the average size of fresh whole-dressed fish was 2.5 lb. There was only one respondent that sold fresh whole-dressed tilapia. Wholesale prices of fresh tilapia fillets averaged \$1.34 lb^{-1}. Wholesale prices of frozen fillets averaged \$1.43 lb^{-1}, while wholesale prices of fresh whole-dressed tilapia averaged \$0.64 lb^{-1}. Retail prices of fresh fillets averaged \$1.97 lb^{-1}, and retail prices of frozen fillets averaged \$1.94 lb^{-1}.

Overall, the majority of respondents indicated that their supply of tilapia was not consistent. The most frequently mentioned supply problem was an insufficient quantity of tilapia. This was followed by the lack of availability of tilapia at certain times of the year and by competition with Honduran exporters.

Quality was the most important characteristic that influenced the choice of fish products for supermarkets that sold tilapia, used to sell tilapia, never sold tilapia, or did not sell fish of any kind. Those who used to sell tilapia rated quality much higher than the other supermarkets. The second most important characteristic was price for all categories of sales with the exception of those that never sold tilapia. For this group of supermarkets, availability was the second most important characteristic. Availability was the third most important characteristic for most of the categories of supermarkets. For supermarkets that used to sell tilapia and that sold tilapia, the third most important characteristic was consumer preferences.

Overall, supermarkets rated tilapia highest on ease of preparation, nice fresh flavor, patrons like variety, high quality fish, and little fishy odor. However, respondents gave a high rating to the statement that "marine fish is better." Low ratings on the statements that "price was too high" and the "fish were too small" indicated that respondents found the prices and sizes of fish acceptable. Supermarkets that never sold tilapia rated it lower on reliable supply and on the attribute "consumers like to eat." Those who did not sell fish also rated tilapia low on reliable supply.

A very high percentage of respondents indicated that they were very likely to begin to sell tilapia the next year. This was true both for supermarkets that used to sell tilapia and those that never sold tilapia.

Fish Market Survey

Complete results of the Nicaraguan fish market survey can be found in Engle and Neira (2001a). Overall, 65% of the fish market vendors sold tilapia. Another 22% sold fish and seafood but never sold tilapia, while another 13% used to sell tilapia and stopped doing so. The majority of fish market vendors indicated that they were selling less tilapia than they sold in the previous year.

Tilapia has been sold by fish market vendors for 10 years on average. One respondent indicated that tilapia had been sold for 42 years in Nicaragua. Tilapia appeared to be sold equally frequently by vendors who had been in business a long time and by those who had been in business only a few years.

Vendors with larger stand areas tended to be those who sold tilapia, particularly in the south-central region. The vast majority of the clientele groups of fish market vendors were low-income clients. There appeared to be a slightly higher percentage of low-income clients of vendors who sold tilapia. More of those who never sold tilapia indicated that they had middle-income clients.

Fish market vendors sold an average of 57 lb of fish and seafood per day. Vendors who sold tilapia tended to have lower daily sales volumes of fish and seafood. Respondents indicated that the most preferred product form of tilapia was a fresh fillet. Vendors who sold tilapia sold 184 lb wk^{-1} of fresh whole-dressed tilapia and 90 lb wk^{-1} of fresh fillets. The average size of tilapia sold was 1 lb for fresh whole-dressed tilapia and 0.32 lb for fresh fillets. However, the majority of respondents indicated that they sold fresh whole-dressed tilapia in the range of 0.61 to 1.00 lb. For fresh fillets the most common size was 0.10 to 0.20 lb.

Wholesale prices averaged \$0.41 lb^{-1} for fresh whole-dressed tilapia. These prices ranged from \$0.15 lb^{-1} to \$1.30 lb^{-1}. Fresh fillet wholesale prices averaged \$0.91 lb^{-1} and ranged from \$0.46 lb^{-1} to \$1.30 lb^{-1}. Retail prices averaged \$0.56 lb^{-1} for fresh whole-dressed tilapia. Retail prices ranged from \$0.15 to \$1.30 lb^{-1}. Fresh fillet retail prices averaged \$1.20 lb^{-1} and ranged from \$0.71 to \$1.70 lb^{-1}.

A majority of respondents indicated that their supply of tilapia was not consistent. The most frequently mentioned problem with tilapia was an insufficient quantity. An additional 20% of responses indicated that lack of availability at certain times of the year was a problem. Other problems mentioned included off-flavor, too expensive, lack of availability of certain product forms, unreliable quality of the product, inconveniently sized purchase lots, and fish being too small.

All vendors, with the exception of those who never sold tilapia, indicated that quality was the most important characteristic that influenced the choice of fish products. Size of fish and price were the next two most important characteristics across all types of vendors. For those vendors who never sold tilapia, supply and odor were the most important characteristics. Price was the second most important characteristic (after quality) for those vendors who used to sell tilapia.

Respondents rated tilapia highest on attributes such as can prepare many dishes with tilapia, easy to prepare, nice fresh flavor, tilapia is a good fish, consumers like to eat tilapia, and supply is reliable. However, respondents thought that marine fish tasted better. Those who never sold tilapia rated it much lower on reliable supply, consumers like to eat, price, size, flavor, and odor.

The primary reason open-air fish market vendors stopped selling or never sold tilapia was the contamination of Lake Managua. This was followed by lack of supply, price being too high, negative consumer attitudes, and lack of demand. Among those who never sold tilapia, contamination of the lake and price being too high were the most frequently mentioned reasons.

In spite of the frequent comments about supply problems, over half (53%) of the respondents said that they were very likely to begin selling tilapia the next year. Higher percentages of those who used to sell tilapia were very likely to begin selling the next year.

DISCUSSION

During the course of the survey, it was observed that restaurants that used to sell or never sold tilapia were hesitant to admit that they were aware of tilapia. This was due to concerns that they would be accused of selling contaminated fish from Lake Managua. Some of these respondents indicated a lack of awareness of tilapia. This was particularly true of some restaurants that indicated that they did not sell tilapia. There were cases in which the managers denied selling tilapia, but the chef admitted that they cooked tilapia and showed the product to the interviewers. In addition some suppliers showed names of restaurants that had denied selling tilapia on their buyers' list.

CONCLUSIONS

Tilapia was a well-known product in Honduras in restaurants, supermarkets, and fish markets. More than 40% of surveyed supermarkets, 30% of restaurants, and 70% of open-air markets reported selling tilapia. Moreover, tilapia sales were reported to have increased in restaurants, supermarkets, and open-air fish markets in recent years. Of those that were not selling tilapia, half of the restaurants, supermarkets, and fish market vendors indicated that they were likely to begin selling tilapia in the coming year.

Availability problems (including lack of availability, seasonal availability, and inconsistency of supply) were mentioned as the primary reasons for not selling tilapia by all market segments. Restaurants, supermarkets, and fish market vendors all mentioned the lack of demand and freshness as problems. Restaurants had additional problems related to off-flavor and small fish, whereas fish market vendors complained of high wholesale prices. Preference ratings were high on most attributes of tilapia, but tilapia was rated unfavorably on reliable quality and ready availability by restaurants.

San Pedro Sula had higher percentages of market outlets interested in tilapia, but Tegucigalpa represents the largest market in Honduras. Attitudes towards tilapia were also favorable in Tegucigalpa, and long-term marketing efforts will need to be focused on this larger market. Small, rural towns not only had lower populations, but there was less interest in and lower perceptions of tilapia in all market segments in the small towns.

Maintaining a reliable supply and high quality of tilapia products will be essential factors in the development of domestic markets for tilapia in Honduras. Farmers will need to work with prospective clients to provide samples, recipes, and background information on farm-raised tilapia. Marketing strategies for farm-raised tilapia could focus on offering catch-of-the-day promotions in restaurants to generate increased demand. Effective forms of preparation such as grilled tilapia and garlic tilapia may be attractive to international and high-income clientele groups.

Larger, chain supermarkets with specialized fish sections and middle-high income mestizo and international clients appeared to have the greatest potential to increase tilapia sales in Honduras, particularly in the short term. Longer-term strategies should focus on supermarkets that cater to middle-income clientele groups. Lack of demand could be addressed in supermarkets through in-store demonstrations, samples, and point-of-purchase consumer information. Freshness should be emphasized. Given the low daily volumes of tilapia purchased, these same in-store promotions should be developed to increase sales for stores that currently sell tilapia.

The negative ratings of tilapia by some respondents may be related to the poor quality of the wild-caught product also circulating on the market. It will be important for tilapia growers to differentiate their product from wild-caught tilapia and to promote quality-control programs on tilapia farms and in processing plants. The results of the surveys suggest that if tilapia farmers can combine adequate marketing strategies with availability of high-quality tilapia, it may be possible to further develop the domestic market for tilapia in Honduras.

It is unlikely that farm-raised tilapia can be sold profitably to open-air fish markets. Production costs that have been estimated for tilapia in Honduras appeared to be greater than current wholesale prices paid by fish market vendors for tilapia.

Tilapia were also well known in Nicaragua. Tilapia have been sold for more than 10 years in the open-air markets and were sold by 65% of the fish market vendors. However, open-air fish market vendors indicated that they were selling less tilapia than before. Tilapia was considered as the fourth most important finfish species sold in restaurants. Only 26% of the supermarkets sold tilapia in Nicaragua, but those that sold it indicated that their sales in 2000 were higher than they had been in 1999. The vendors who did not sell tilapia indicated that the consistency and quantity of supply and odor problems were the most important reasons they did not sell tilapia.

Underlying all other reasons for not selling tilapia in Nicaragua was the fear of selling contaminated fish from Lake Managua. Restaurants were reluctant to admit selling tilapia due to off-flavor and fear of confusion with wild-caught tilapia viewed as contaminated. Consumers perceived tilapia as a freshwater fish caught in a polluted lake and were unaware of the advantages of a high-quality farm-raised fish. These fears extended to other preferred freshwater fish like guapote.

Nevertheless, half of restaurant, supermarket, and open-air fish market vendors indicated that they were very likely to begin to sell tilapia the next year if they had a consistent supply and if they could get a farm-raised tilapia product differentiated from wild-caught tilapia.

The reluctance to admit selling freshwater fish led to an emphasis on selling fillets instead of whole-dressed products in supermarkets and even in fish markets. This is in direct contrast to sales in Honduran supermarkets and fish markets. Given overall cost structures in Nicaragua, the preference for fillets in supermarkets and fish markets may prevent tilapia farmers from using these outlets as profitable market outlets. A few tilapia farms could potentially target a few upscale supermarkets. Moreover, fish and seafood overall comprised a low percentage of total supermarket sales (0.5 to 5%) in Nicaragua. In contrast to those in Honduras, few of the stores had specialized seafood counters. The combination of low fish sales and the emphasis on fillet products for freshwater fish result in low potential to develop supermarket sales for tilapia. Retail supermarket prices for fillets are not likely to be sufficiently high to cover the production costs of tilapia fillets. Similarly, for open-air fish markets the wholesale prices of wild-caught tilapia appeared to be too low to constitute an economically viable market outlet for farm-raised tilapia.

It may be possible to develop markets for tilapia in upscale Nicaraguan restaurants. Tilapia marketing strategies might be developed to compete with whole-dressed guapote and red snapper and with drum and red snapper fillets.

However, for a farm-raised tilapia industry to develop a domestic market for tilapia in Nicaragua, the issue of consumer fears of contamination must be addressed. Broad-based consumer education, third-party certification, and labeling programs may be needed to assist consumers to differentiate between farm-raised and wild-caught tilapia. Tilapia farms and processors in Nicaragua will need to guarantee and ensure the flavor, quality, and safety of their product and promote these attributes. A consistent supply of high-quality tilapia positioned as an upscale, export-quality product will be essential.

ANTICIPATED BENEFITS

Results of these surveys will be of value to tilapia growers as they seek alternative market outlets for their products.

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ECONOMIC AND SOCIAL RETURNS TO TECHNOLOGY AND INVESTMENT IN THAILAND

Ninth Work Plan, Marketing and Economic Analysis Research 4 (9MEAR4)
Final Report

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ABSTRACT

Thailand has a long and rich history of aquaculture production. The Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) has been involved in fish production research in Thailand since 1983. The economic impact of new technologies will depend upon farm-level benefits of the new technologies. However, if the new technologies require resources at a level not readily available to farmers, then the overall adoption rates and economic impacts will be lower. This study compares three aquaculture production technologies in Thailand with a fertilization technology developed by the PD/A CRSP to evaluate the farm-level economic impacts. Enterprise budgets were developed for each production technology. Price and cost data used in the analysis were pre-1998 data. These were used to formulate a whole-farm mathematical programming model. Farm resource levels specified in the model were based on survey data of small-scale fish farmers in northeastern Thailand. The production technologies evaluated included an extensive polyculture system that included tilapia, a more intensive polyculture of herbivorous fish including tilapia, monoculture of sex-reversed tilapia, and production of hybrid catfish. The enterprise budget analysis indicated that profits were highest for the tilapia monoculture system, second highest for the hybrid catfish production system, third highest for the more intensive polyculture system, and lowest for the extensive polyculture system. However, total annual costs were highest for hybrid catfish production, followed in descending order by tilapia monoculture and more intensive polyculture, and were lowest for the extensive polyculture system. The majority of the costs of catfish production were for the feed, whereas feed, fry, and fertilizer costs were the most important cost categories for tilapia monoculture production. Urea and triple superphosphate (TSP) costs were most important for the more intensive polyculture system, whereas manure was the greatest cost for extensive polyculture production. Results of the whole-farm mathematical programming analysis showed that if adequate resources exist on the farm, the optimal production mix is to stock all ponds in tilapia monoculture in order to maximize profits. However, when the model was constrained to the level of resources typically available on farms in northeastern Thailand, four of five ponds would be stocked in the extensive polyculture system with only one pond stocked in tilapia monoculture. Parametric analyses indicated that operating capital was the key limiting factor and constraint. Net returns increased dramatically as operating capital levels increased and the mix of production technologies moved more towards the more profitable tilapia monoculture production. The technologies were not labor intensive and the availability of labor did not change the mix of production activities, even at very low levels of labor. Yield of monosex tilapia production was also a key factor. The analysis indicated that a yield of 4,000 kg ha^{-1} constituted a threshold below which the production mix excluded tilapia monoculture and substituted the more intensive polyculture system. The price analysis showed that, if tilapia prices fell to Baht 27 kg^{-1}, the production mix would switch to the more intensive polyculture from tilapia monoculture. Feed and fertilizer prices, even at four times the level identified from the survey, did not affect the choice of production technology. Hybrid catfish production would only enter the mix if extremely high levels of operating capital were available and if the price of catfish were at least Baht 29 kg^{-1}.

INTRODUCTION

Farmers have raised aquaculture crops in northeastern Thailand for a number of years. The Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) has conducted aquaculture research in Thailand since 1983. Much of the effort of the PD/A CRSP research program in Thailand has been on tilapia production technologies, with a specific emphasis on fertilization types and strategies. Studies have evaluated both organic and inorganic fertilizers including chicken manure (Knud-Hansen et al., 1993), urea (Knud-Hansen and Pautong, 1993), and triple superphosphate (TSP).

Organic pond fertilization with chicken manure requires more dissolved oxygen and was shown to produce a significantly higher fish yield when compared to inorganic fertilization alone (Diana et al., 1990). However, inorganic pond fertilization has a lower oxygen demand but adds high nutrient content (Yamada, 1986). PD/A CRSP researchers developed a fertilization strategy that has been referred to as high-input green water (HIGW) technology. HIGW production uses relatively intensive applications of urea and TSP. Shrestha et al. (1997) showed that HIGW technology using sex-reversed tilapia has produced yields of over 5,000 kg ha^{-1} yr^{-1}.

Engle and Skladany (1992) evaluated PD/A CRSP technologies utilizing chicken manure. Results showed that net returns increased by 85 and 98% for chicken manure collected for use on fish ponds and for chicken manure applied directly from integrated systems, respectively. Returns on average investment on ponds and equipment increased by 60 to 70%, while returns on average total investment (including land) increased by 5% by adopting CRSP technologies of chicken manure fertilization regimes.

The predominant type of fish culture system in northeastern Thailand can be classified as semi-intensive herbivorous fish culture (Phromthong and Demaine, undated). Within this general category, three different levels of intensification can be identified: extensive polyculture, a more intensive polyculture, and monoculture of sex-reversed tilapia. The extensive polyculture practiced in northeastern Thailand is used to culture fish primarily for household consumption. Small fingerlings (1 to 2 cm) of tilapia and other herbivorous species are stocked. Fish are fed only occasionally with agricultural by-products, and the ponds are fertilized on a limited basis with livestock manure. Production levels range from 375 to 500 kg ha^{-1} yr^{-1}.

The more intensive polyculture production system is used to produce fish for household consumption with some surplus for sale. Farmers using this type of production system release a larger size of fingerling (6 to 8 cm) or nurse small fry to fingerlings in net cages before releasing fingerlings into the grow-out pond. Inorganic fertilizers and manures are used to fertilize the pond, and some supplementary feed from agricultural by-products is fed. Production levels typically range from 1,562 to 2,188 kg ha^{-1} yr^{-1}.

Monoculture production of sex-reversed tilapia is destined primarily for sale. Combined with the PD/A CRSP fertilization strategies of intensive fertilization and management, yields can exceed 5,000 kg ha^{-1} yr^{-1}.

Hybrid catfish production can produce approximately 10 tons $farm^{-1}$ yr^{-1}. Most hybrid catfish is sold to provincial markets in the region. A farmer can produce as much as three crops a year of catfish.

METHODS

Enterprise budgets were developed for the four aquaculture production technologies (extensive polyculture, more intensive polyculture, hybrid catfish culture, and high-input monoculture of sex-reversed tilapia) based on standard techniques (Kay and Edwards, 1994). Production, cost, and price information were taken from Phromthong and Demaine (undated), Edwards et al. (1986), Engle and Skladany (1992), and Shrestha et al. (1997). Data used were pre-1998 data.

The enterprise budgets formed the basis for constructing a whole-farm mathematical programming model for a representative fish farm in northeastern Thailand. The objective of the model was to maximize annual net returns subject to a series of constraints. Each production technology constituted a potential production activity. An additional activity modeled is a fish sales submodel. Fish of different sizes sold in different types of markets will sell for different prices. The fish sales submodel accounts for these marketing differences. Other activities modeled include purchases of feed (for nursing fry and for grow-out), purchases of fertilizers (urea, TSP, and lime), and application of manure.

Constraints included the availability of land and labor. Appropriate balance constraints were included as needed. The availability of sex-reversed and catfish fingerlings were modeled as constraints in the model. Financial constraints included the availability of both operating and investment capital.

Each submodel was tested and validated independently and then in conjunction with the other submodels. The model was run with unconstrained levels of right-hand side variables. Once the consistency tests were completed, the right-hand side variables were adjusted to represent typical farms as defined by means encountered in the survey data reported by Phromthong and Demaine (undated).

The effect of varying levels of operating capital on the optimal mix of production activities was modeled by parameterizing the levels of operating capital in the model. Similar parameterizations were conducted for varying levels of investment capital, land, and labor.

Fish yield is frequently a key determinant of fish farm profitability. The effect of yield level of tilapia in the high-input monoculture of sex-reversed tilapia was evaluated by testing model results at ± 25, 50, and 75% of the mean yields reported in the literature. The availability of sex-reversed tilapia fry, a previously constraining factor in monosex tilapia production in Thailand, was evaluated by varying the right-hand side values in increments of 25,000 fry.

Fish prices, prices of the different fertilizers, and feed prices were subjected to sensitivity tests to evaluate the effect on optimal production mix of varying levels. Input prices were increased by as much as four times the average price, while fish prices were decreased incrementally to price levels that represented half of the average price.

RESULTS

The extensive polyculture production system utilized a very low level of inputs (Table 1). The primary input was animal manure in addition to some feed fed in the grow-out phase of production. In addition to these inputs, there was some amount of pond maintenance. In all, total annual variable costs were Baht (Bt) 1,550 per year. Of these, manure represented the largest resource utilized and represented the greatest cost. In these budgets, these costs represent opportunity costs of resource utilization because most of these inputs were obtained from the farm itself. An average of 437.5 kg of fish were produced per year. Given the relatively small size of fish, the market price, if sold locally, would be Bt 30 kg^{-1}. In reality most of these fish are consumed by the household. The estimated revenue represents an opportunity cost of the consumption of these fish and allows for a comparison of resource utilization with other, market-oriented fish production activities. The fish production then was estimated to have a value of Bt 13,125. After accounting for total annual costs, the net returns to operator's labor, management, land, and risk were Bt 12,335 ha^{-1}.

The more intensive polyculture system utilized higher levels of inputs and produced higher yields of fish (Table 2). In addition to higher levels of feed for both the nursery and grow-out phases of production and much higher levels of fertilizer, additional marketing costs were associated with marketing fish

Table 1. Annual costs and returns of extensive polyculture system utilized on small-scale fish farms in northeastern Thailand (1 ha pond).

Budget Items	**Quantity** (kg)	**Price** (Bt kg^{-1})	**Value** (Bt)	**Percent of Total Variable Cost** (%)
GROSS RETURNS	437.5	30.00	13,125	
VARIABLE COSTS				
Fry	0		0	0
Fingerlings	0		0	0
Fertilizer				
Urea	0		0	0
TSP	0		0	0
Lime	0		0	0
Manure	960.0	0.75	720	46
Feed				
Nursery	0	0	0	0
Grow-out	25.4	9.44	240	16
Labor				
Harvesting (Bt)	0		0	0
Pond Maintenance (Bt)	0		550	36
Marketing			0	0
TOTAL ANNUAL VARIABLE COSTS			1,550	
TOTAL ANNUAL COSTS			1,550	
NET RETURNS TO OPERATOR'S LABOR, MANAGEMENT, LAND, AND RISK (Bt ha^{-1})			12,335	

Table 2. Annual costs and returns of a more intensive polyculture system utilized on small-scale fish farms in northeastern Thailand (1 ha pond).

Budget Items	**Quantity** (kg)	**Price** (Bt kg^{-1})	**Value** (Bt)	**Percent of Total Variable Cost** (%)
GROSS RETURNS	1,875.0	40.00	75,000	
VARIABLE COSTS				
Fry	0		0	
Fingerlings	0		0	
Fertilizer				
Urea	1,332.0	5.00	6,660	30
TSP	872.0	8.00	6,976	31
Lime	766.0	1.00	766	3
Manure	1,920.0	0.75	1,440	6
Feed				
Nursery	297.6	11.80	3,512	16
Grow-out	25.4	9.44	240	1
Labor				
Harvesting (Bt)	0			0
Pond Maintenance (Bt)			2,550	11
Marketing	469.0	.08	38	0
TOTAL ANNUAL VARIABLE COSTS			22,181	
TOTAL ANNUAL COSTS			22,181	
NET RETURNS TO OPERATOR'S LABOR, MANAGEMENT, LAND, AND RISK (Bt ha^{-1})			52,819	

in nearby villages. Urea and TSP represented the greatest levels of inputs used, constituting 30 and 31%, respectively, of total variable costs. Total annual costs of production were Bt 22,181. The value of fish produced in this technology was Bt 75,000 yr^{-1}, generating annual net returns to operator's labor, management, land, and risk of Bt 52,819 ha^{-1}.

Hybrid catfish production required the highest level and usage of inputs (Table 3). Catfish ponds were fed intensively, and feed constituted 92% of all total variable costs of production. High numbers of fingerlings were stocked into the pond. Fertilizer application rates were moderate, but there were additional costs associated with harvesting the high weight of fish produced in the system. Total annual costs of production were Bt 2,172,612. However, given the 82,794 kg ha^{-1} of catfish produced, the gross returns were Bt 2,235,438, even at the relatively low price of Bt 27 kg^{-1} for catfish. Thus, hybrid catfish appears to be profitable, and it generated net returns to operator's labor, management, land, and risk of Bt 62,826 ha^{-1}.

Monoculture of sex-reversed tilapia with high-input inorganic fertilization strategies utilized more moderate levels of input resources when compared with that of hybrid catfish (Table 4). Fry stocked into nursery phases were less than the number of catfish fingerlings stocked. However, fertilizer rates and costs were higher, especially the manure application rates. Feed levels were lower than those for hybrid catfish production, but higher than those for the more intensive polyculture production. Total annual variable costs were Bt 61,032 yr^{-1}. With the 6,022 kg ha^{-1} of tilapia produced, gross revenues were Bt 240,880 yr^{-1}, generating net returns to operator's labor, management, land, and risk of Bt 179,848 ha^{-1}.

Thus, the enterprise budget analysis indicated that profits were highest for the tilapia monoculture system, second highest for the hybrid catfish production system, third highest for the more intensive polyculture system, and lowest for the extensive polyculture system. However, total annual costs were highest for hybrid catfish production, followed in descending order by tilapia monoculture and the more intensive polyculture, and were lowest for the extensive polyculture system. The majority of the costs of catfish production were for the feed, whereas feed, fry, and fertilizer costs were the most important cost categories for tilapia monoculture production. Urea and TSP costs were most important for the more intensive polyculture system, whereas manure was the greatest cost for extensive polyculture production.

Table 5 presents results of the whole-farm mathematical programming analysis when resources are not constrained. In this case the optimal production mix is to stock all ponds in tilapia monoculture in order to maximize profits. Net returns in this scenario are Bt 540,988 yr^{-1}.

However, in reality, farms do not have access to unlimited resource levels. When the model was constrained to those levels of resources identified by survey data of farms in northeastern Thailand, the solution changed (Table 6). Only one pond was put into tilapia monoculture with high-input inorganic fertilization methods, and the remaining four were stocked in the extensive polyculture production system. Net returns decreased to Bt 245,810 yr^{-1}. The key constraint to production in this scenario was the level of operating capital. In other words, the farm did not have adequate operating capital to put more than one pond into the more profitable tilapia monoculture system and substituted the extensive polyculture system that required only very low levels of operating capital.

Varying the amounts of operating capital available resulted in the selection of different mixes of optimal production tech-

Table 3. Annual costs and returns of hybrid catfish production for small-scale fish farms in northeastern Thailand (1 ha pond).

Budget Items	**Quantity** (kg)	**Price** (Bt kg^{-1})	**Value** (Bt)	**Percent of Total Variable Cost** (%)
GROSS RETURNS	82,794	27.000	2,235,438	
VARIABLE COSTS				
Fry	0			
Fingerlings	1,324,700	0.100	132,470	6
Fertilizer				
Urea	859	5.000	4,295	0
TSP	562	8.000	4,496	0
Lime	494	1.000	494	0
Manure	828	0.750	621	0
Feed				
Nursery	0	11.800	0	0
Grow-out	211,061	9.440	1,992,416	92
Labor				
Harvesting (Bt)	82,794	0.270	22,354	1
Pond Maintenance (Bt)			2,550	0
Marketing	82,794	0.156	12,916	1
TOTAL ANNUAL VARIABLE COSTS			2,172,612	
TOTAL ANNUAL COSTS			2,172,612	
NET RETURNS TO OPERATOR'S LABOR, MANAGEMENT, LAND, AND RISK (Bt ha^{-1})			62,826	

Table 4. Annual costs and returns of the high-input inorganic fertilization tilapia production system for small-scale fish farms in northeastern Thailand (1 ha pond).

Budget Items	Quantity (kg)	Price (Bt kg^{-1})	Value (Bt)	Percent of Total Variable Cost (%)
GROSS RETURNS	6,022	40.00	240,880	
VARIABLE COSTS				
Fry	25,000	0.50	12,500	20
Fingerlings	0		0	0
Fertilizer				
Urea	1,719	5.00	8,595	14
TSP	1,125	8.00	9,000	15
Lime	988	1.00	988	2
Manure	12,431	0.75	9,323.25	15
Feed				
Nursery	297.6	11.80	3,511.68	6
Grow-out	1,476.5	9.44	13,938.16	23
Labor				
Harvesting (Bt)	0		0	0
Pond Maintenance (Bt)			2,550	4
Marketing	6,022	0.104	626	1
TOTAL ANNUAL VARIABLE COSTS			61,032	
TOTAL ANNUAL COSTS			61,032	
NET RETURNS TO OPERATOR'S LABOR, MANAGEMENT, LAND, AND RISK (Bt ha^{-1})			179,848	

Table 5. Mathematical programming results when resources are not constrained.

Item	Value
Net Returns (Bt yr^{-1})	540,988
Number of Ponds Used (n)	5
Production System Selected	High-Input Inorganic Fertilization
Monosex Tilapia Fry Purchased (n)	116,822
Urea Purchased (kg)	8,033
TSP Purchased (kg)	5,257
Lime Purchased (kg)	4,617
Animal Manure Used (kg)	58,005
Tilapia Sold (kg)	28,140
Fry Feed Purchased (kg)	1,391
Grow-out Feed Purchased (kg)	6,900
Grow-out Labor Used (h)	234
Harvesting Labor Used (h)	56
Pond Maintenance Cost (Bt)	11,916
Investment Capital (Bt)	30,000

Table 6. Results of mathematical programming analysis of model with resources limited to mean values as identified in Phromthong and Demaine (undated).

Item	Value
RESOURCE AVAILABILITY LIMITS	
Land (ha)	5
Labor (h)	7,488
Operating Capital (Bt)	75,000
Investment Capital (Bt)	30,000
MODEL SOLUTION	
Net Returns (Bt yr^{-1})	245,810
Ponds Used (n)	
High-Input Inorganic Fertilization	1
Extensive Polyculture	4
Tilapia Sales (kg)	
Locally	1,672
District	7,102
Key Constraint: Operating Capital	

nologies (Figure 1). Decreasing operating capital to Bt 25,000 resulted in stocking all five ponds in the extensive polyculture system. Increasing available operating capital above the Bt 75,000 mean value resulted in additional ponds stocked with tilapia monoculture. At the highest rates analyzed (Bt 250,000), four ponds were stocked in tilapia monoculture and only one stocked in the extensive polyculture system. Net returns also increased with increasing levels of operating capital available (Figure 2).

Varying the level of investment capital available to the farmer between Bt 14,000 and Bt 50,000 did not affect the choice of optimal production mix. However, at investment capital levels of Bt 13,000, the production mix switched to only three ponds in extensive polyculture while still keeping one pond in tilapia monoculture. As investment capital levels were lowered even farther, additional ponds were taken out of production. At investment capital levels of Bt 5,000 to 8,000, only one pond was put into production and that one was stocked in tilapia monoculture. These results indicate that it was more profitable to use the limited investment capital resources to produce the more profitable tilapia monoculture than to have more ponds in extensive polyculture. In this parameterization analysis, operating capital was held constant at Bt 75,000, so there was adequate operating capital to manage the one pond in tilapia monoculture throughout this analysis.

Figure 3 shows the results of the analysis parameterizing the level of land available to the farmer while holding all other values constant. With increasing amounts of land, additional ponds were put into extensive polyculture because, with the mean value of operating capital available, there was insufficient operating capital to add more tilapia monoculture ponds.

The technologies modeled in this analysis were not labor intensive. The parameterization analyses of land availability did not change the mix of production activities, even at very low levels of labor.

Yield of tilapia in the high-input monoculture system was a determining factor in its selection in the profit-maximizing mix of production activities (Figure 4). The analysis indicated that a yield of 4,000 kg ha^{-1} constituted a threshold below which the production mix excluded tilapia monoculture and substituted the more intensive polyculture system. The 4,000 kg ha^{-1} yr^{-1} production level represented a reduction of more than 25% from the average yield reported from survey data. This threshold also represented a sharp decrease in net returns from this production activity.

Reductions in the availability of sex-reversed tilapia fry did not have a dramatic impact until the low level of 25,000 fry per farm. At this level some hectareage began to switch to the more intensive polyculture system from the high-input tilapia monoculture production system (Table 7).

The price analysis showed that, if all tilapia prices were the same for local, village, and provincial markets, tilapia monoculture would still be the profit-maximizing option. However, if tilapia prices fell to Bt 27 kg^{-1}, the production mix would switch to the more intensive polyculture from tilapia monoculture. Feed and fertilizer prices, even at four times the level identified from the survey, did not affect the choice of production technology.

Hybrid catfish production never entered the profit-maximizing mix of production activities in this analysis. Catfish production is resource-intensive but not as profitable as tilapia monocul-

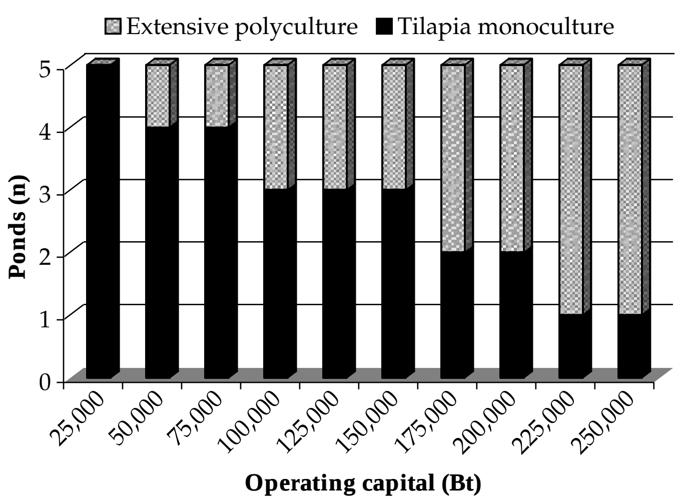

Figure 1. Optimal mix of tilapia production technologies at varying levels of operating capital availability.

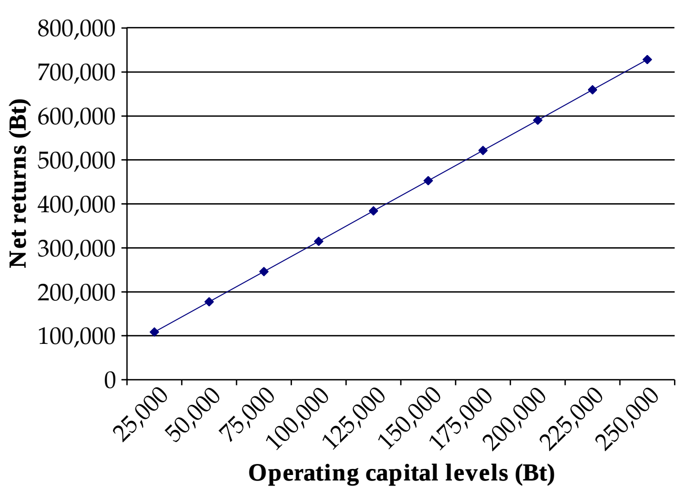

Figure 2. Net returns at different levels of operating capital availability.

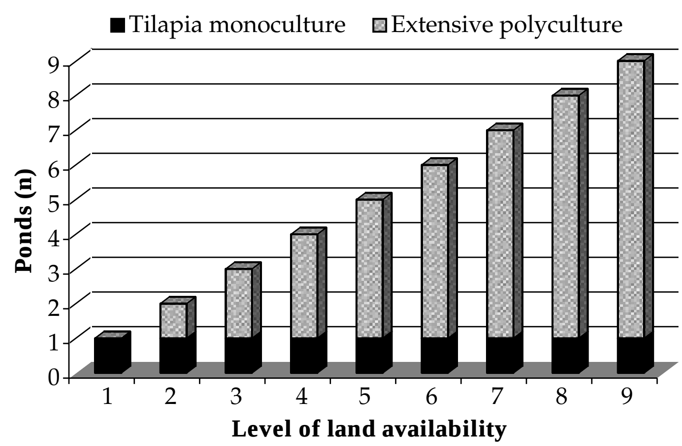

Figure 3. Optimal mix of tilapia production technologies at varying levels of land availability.

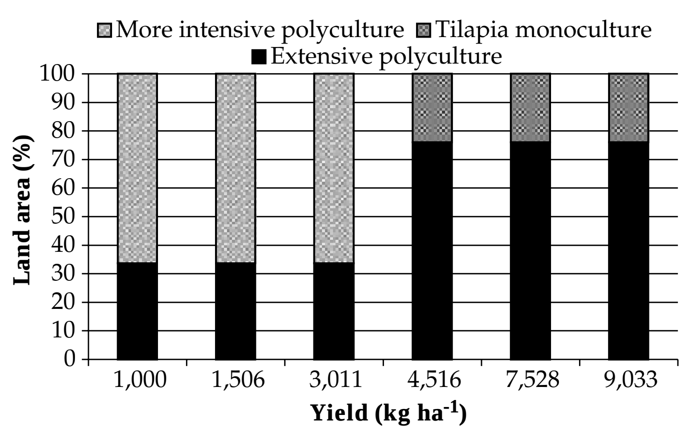

Figure 4. Optimal mix of tilapia production technologies with varying yields of monosex tilapia.

Table 7. Optimal mix of tilapia production technologies with varying levels of availability of sex-reversed tilapia.

Level of Availability of Sex-Reversed Tilapia Fry	**Net Returns** (Bt ha^{-1} yr^{-1})	**Production Technology** (ha of ponds)		
		Extensive Polyculture	*More Intensive Polyculture*	*High-Input Inorganic Fertilization*
25,000	236,039	3.5	0.5	1.0
50,000	245,810	3.8	0	1.2
75,000	245,810	3.8	0	1.2
100,000	245,810	3.8	0	1.2
125,000	822,390	3.8	0	1.2

ture. It may be that market demand for more diverse types of fish would limit the quantities of tilapia sold such catfish would begin to enter the mix of activities. In additional simulations, hybrid catfish production would enter the mix only if extremely high levels of operating capital were available and if the price of catfish were at least Bt 29 kg^{-1}.

DISCUSSION

The PD/A CRSP technologies developed to improve fertilization techniques and to produce sex-reversed tilapia fry result in strong economic incentives for adoption of this technology. Nevertheless, this analysis is based upon pre-1998 data in Thailand. Additional analysis is needed to evaluate the impacts of the 1998 economic change on cost and price relationships.

CONCLUSIONS

The combination of more intensive fertilization practices with monoculture of sex-reversed tilapia resulted in the most profitable production technology of those evaluated. Thus, the impact of the PD/A CRSP technologies is likely to be quite high. Nevertheless, this technology is strongly constrained by the availability of operating capital. In the absence of adequate levels of operating capital, the farmers revert to the very extensive type of traditional types of aquaculture. At the mean levels of operating capital from the survey data, the farm was restricted to putting only one pond into the more profitable tilapia monoculture system. Thus, financial conditions in the country will likely restrict the adoption, implementation, and impact of this technology.

ANTICIPATED BENEFITS

The economic impact of new technologies will depend upon farm-level benefits of the new technologies. Yet the resource requirements of new technologies, farm availability of resources, farm economic structure, and other considerations may prevent adoption of new technologies. The principal benefit of this project was to shed light on the economic incentives and trade-offs among the traditional aquaculture production systems, CRSP-developed technologies, and a very intensive aquaculture pond enterprise. This will provide guidance for the direction of CRSP research in terms of the types of inputs and management systems that are likely to have the highest rates of adoption among farmers.

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RAPID ECONOMIC EVALUATION TOOLS

Ninth Work Plan, Marketing and Economic Analysis Research 5 (9MEAR5)
Final Report

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ABSTRACT

The main objective of the project was to provide a user-friendly rapid economic evaluation tool that allows research and extension personnel to quantify possible outcomes of new tilapia production techniques and assess potential economic risk consequences of these techniques. We developed such a tool, having produced a first version for release. The quantitative tool allows the examination of not only mean response of tilapia production systems as reflected in economic budgets but also the risk associated with them. We present results from using the evaluation tool in the text. These results are based on available literature, a case study from an independent producer in Honduras, and results from the identification of three basic tilapia production technologies in Honduras. These results are not meant to be a diagnostic of the risk situation facing Honduras producers, rather to illustrate the potential uses of the evaluation tool by end users.

RISK: CONCEPTS AND DEFINITIONS

Knight (1921) defined risk as the state of the decision-making process where the probability of occurrence of an event is known. Accordingly, Reilly (1979) defines risk as "the uncertainty of future outcomes, or alternatively the probability of obtaining an adverse economic result." In this report we will concentrate on the second half of Reilly's definition. Traditionally, economists have divided risk into three components: market, social, and production. These components interact in different degrees and change in importance in the decision-making process.

Market risk is associated with the producer's lack of knowledge of future prices and quantities of input and outputs, uncertainty about timely availability of inputs, and inability to forecast changes in consumers' tastes and preferences. Social risk is associated with the producer's lack of knowledge of the availability of labor and the social interactions and conflicts that may affect his or her production. Production risk is associated with the producer's lack of knowledge of the components of the production process and the inherently stochastic nature of agriculture. It is important to point out that as producers learn about production systems, subjective evaluations about risk decrease in importance and objective risk increases.

THE @RISK PROGRAM

We modeled risk in tilapia production systems by using the @Risk add-in for the Excel® program. @Risk generates random numbers from selected distributions and calculates outcomes based on the chosen values. For example, instead of inputting a deterministic value for mortality, 10%, we can input this as a triangular distribution using values for least likely, 5%; most likely, 10%; and maximum, 15%.

The @Risk program allows correlations and dependencies between variables, which is an innovative and useful aspect of @Risk. This implies particular relationships between variables or distributions. For example, we can avoid having results that are not common sense such as having higher stocking density and higher average size or having lower dissolved oxygen and higher yield. The user of the tool inputs his or her own estimation of the degree of association between variables by inputting a correlation coefficient that varies between –1 and 1.

THE RAPID EVALUATION SPREADSHEET

Standard economic use of Excel® is to generate a spreadsheet that calculates budgets. This implies the estimation of fixed and variable costs, net returns, and break-even points. The evaluation tool using Excel® with @Risk requires that users enter subjective estimates of how much a decision variable affects production or other variable(s). The evaluation tool also requires users to enter values for the distributions of key variables. The current version of the evaluation tool uses a triangular distribution, which requires minimum, maximum, and most likely values for prices and quantities in the production system. In addition the user can enter values for distribution and correlations.

VALIDATION OF THE EVALUATION TOOL

We illustrate here and in Hatch and Falck (2001) the evaluation tool using three different methods: 1) replication of published material from the literature (Hatch and Falck, 2001), 2) a case study of a Honduran producer, and 3) evaluation of three tilapia production systems. It is important to note that the version of the evaluation tool that addressed the first two methods was simplified to improve user accessibility and use. It is also important to point out that results presented below

Table 1. Baseline parameters for tilapia stocking density of 4 fish m^{-2}.

Parameters	Unit	Value	Variation
Feed Consumption	%	2	
Harvest Weight	lb	0.48	0.45, 0.50, 0.55
Days to Harvest	d	180	
Mortality	%	11.3	8, 12, 14
Tilapia Price	Lps lb^{-1}	15.17	14.5, 15.0, 16.0
Fingerling Price	Lps each	0.38	0.25, 0.40, 0.50
Feed Price	Lps lb^{-1}	2.98	2.85, 3.00, 3.10
Interest Rate	%	14	10, 14, 18

Table 2. Baseline parameters for tilapia stocking density of 8 fish m^{-2}.

Parameters	Unit	Value	Variation
Feed Consumption	%	2.5	
Harvest Weight	lb	0.42	0.36, 0.42, 0.48
Days to Harvest	d	180	
Mortality	%	12.8	9, 14, 16
Tilapia Price	Lps lb^{-1}	15.17	14.5, 15.0, 16.0
Fingerling Price	Lps each	0.38	0.25, 0.40, 0.50
Feed Price	Lps lb^{-1}	2.98	2.85, 3.00, 3.10
Interest Rate	%	14	10, 14, 18

are not diagnostic of risk production practices in Honduras or elsewhere. Results presented are meant to illustrate the potential uses of the evaluation tool.

Method 2: Case Study of a Honduran Producer

We used data collected during the 2000 workshop "La Cria Exitosa de la Tilapia" held at the Escuela Agrícola Panamericana (Zamorano). In this workshop we identified a small independent producer from Olancho, who has 5,000 m^2 of total pond surface, with 800 m^2 being cultivated at the time of the meeting. The producer sold whole fish and cultured only tilapia. The temperatures of his pond were 25 to 28°C at the surface and 23°C at the bottom. Seventy percent of his production is red tilapia and 30% is *T. nilotica*. For the purposes of this exercise, we allowed only a change in population density from 4 to 8 fish m^{-2}.

The baseline parameters are presented in Tables 1 and 2 for tilapia densities corresponding to 4 and 8 fish m^{-2}, respectively. Figure 1 presents the distribution of net returns from a density of 4 fish m^{-2}. Because of the very low density, the probability of having a loss is almost certain. Mean net returns were –5,795 lempiras per lot. In contrast, Figure 2 presents results from the distribution of net returns from increasing density to 8 fish m^{-2}. Mean net returns increased to –327 lempiras per lot; however, there is a 43% probability that the producer would obtain a positive net return.

Because one of the secondary objectives was to diffuse the technology as much as possible, the decision was made to simplify the complexity of the evaluation tool as used in methods 1 and 2. In effect the revised tool would be easier and more flexible to use.

Method 3: Case Study of the Application of the Rapid Evaluation Tool

Popma (pers. comm., 2001) identified three production practices for small-scale tilapia production. The three included production practices are:

MF-1 = Manure + Low Quality Feed–Low
MF-2 = Manure + Low Quality Feed–High
INT-1 = Tilapia-Broiler Integrator

Note: The following descriptions are based on the assumption of 100-m^2 ponds. Extrapolation above or below this size (50 to 300 m^2) is generally valid for harvest and nutrient inputs, but labor requirements will likely be distorted because larger ponds generally require proportionally less labor than smaller ponds. After the pond is full, the only water added is just sufficient to compensate for losses from evaporation and seepage—no water exchange.

Unless otherwise indicated, the duration of most production cycles is six months but is affected by climate (possibly requiring only three to four months at temperatures near 30°C and up to eight months at temperatures near 23°C).

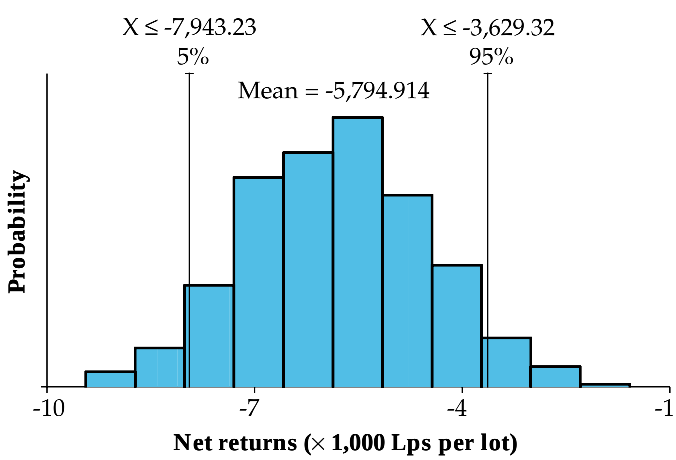

Figure 1. Distribution of net returns from a Honduras case study for tilapia density of 4 fish m^{-2}.

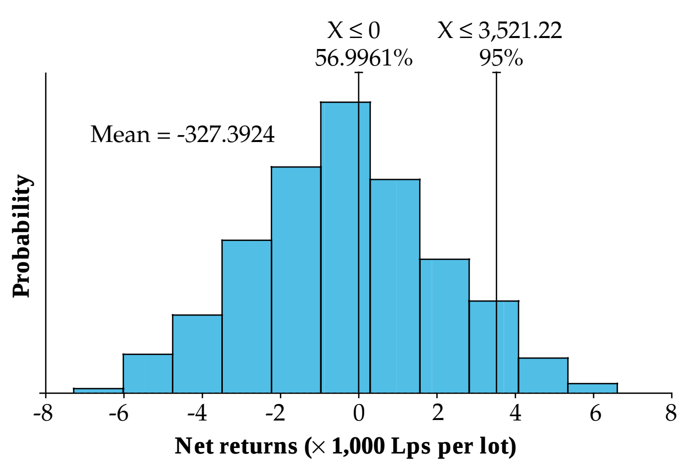

Figure 2. Distribution of net returns from a Honduras case study for tilapia density of 8 fish m^{-2}.

Manure + Low Quality Feed–Low, Management Code: MF-1
Nutrient inputs for this management practice are generally available on-farm, including mostly animal manures and low-quality supplemental feeds such as rice bran, spoiled corn, and household leftovers. The quantities added are less than optimum, usually because they are of limited availability. Average labor requirements are about two hours per week. Approximately 15 kg of fish will be harvested per cycle from a 100-m^2 pond.

Tilapia fingerlings (about 5 g each) are stocked at 2 m^{-2} (200 fingerlings in a 100-m^2 pond). Predator fish may be added (usually 10 to 20) to prevent overpopulation and stunting; the use of a predator will increase the final weight of the harvested fish, but the total weight of fish at harvest is about the same whether or not a predator is added. The nutrients (mostly manure but also some agricultural by-products, if available) are added at a rate of 0.5 to 1.0 kg about two to three times per week.

After four to eight months, and depending on whether a predator fish was added, the producer may decide to harvest. If fingerlings are difficult to obtain for the next production cycle, the best time to harvest is about one month after reproduction begins. At this time new fingerlings will be available for restocking as long as the producer has a place to keep them until the pond refills. Harvest will be about 15 kg. It is very common for most fish (possibly 80% of total weight) to be < 15 cm in length, but a higher percentage will be larger if predator fish are present.

Manure + Low Quality Feed–High, Management Code: MF-2
Nutrient inputs for this management practice are generally available on-farm, including mostly animal manures and low-quality supplemental feeds such as rice bran, spoiled corn, and household leftovers. However, in contrast with the previous management practice, the amount of nutrients added is near optimum for this class of nutrients. Average labor requirements are about 4 h wk^{-1}. Approximately 23 kg of fish will be harvested per cycle from a 100-m^2 pond.

As in the previous management scheme, tilapia fingerlings (about 5 g each) are stocked at 2 m^{-2} (200 fingerlings in a 100-m^2 pond). Predator fish may be added (usually 10 to 20) to prevent overpopulation and stunting; the use of a predator will increase the final weight of the harvested fish, but the total weight of fish at harvest is about the same whether or not a predator is added. The nutrients (a mixture of manure and some agricultural by-products, if available) are added at a rate of about 1.5 kg two to three times per week.

After four to eight months, and depending on whether a predator fish was added, the fish are harvested. If fingerlings are difficult to obtain for the next production cycle, the best time to harvest is about one month after reproduction begins. At this time new fingerlings will be available for restocking as long as the producer has a place to keep them until the pond refills. Harvest will be about 23 kg. It is very common for most fish (possibly 70% of total weight) to be < 15 cm length, but a higher percentage will be larger if predator fish are present.

Tilapia-Broiler Integration, Management Code: INT-1
The primary nutrient input for this management practice is fresh manure from chickens that are housed in a coop above the pond and fed a chicken concentrate. Fresh chicken droppings are a concentrated nutrient supply, making this management scheme more productive than the previous two non-integrated schemes. Three 7-week batches of broilers are produced during a six-month fish production cycle. When broilers are small (consuming less feed), some manure and agricultural by-products are added to maintain good nutrient loading into the pond. The integrated system has higher costs, greater labor requirements, and higher potential profitability than the previous two non-integrated systems. It is a more risky management practice because of the higher capital requirements and may have greater marketing problems. Average labor requirements are about 5 to 6 h wk^{-1}. Approximately 30 to 40 kg of fish and a total of 190 to 200 kg of broilers (from the three batches) will be harvested per six-month fish production cycle from a 100-m^2 pond. Note that no cost has been assessed for construction of the broiler coop.

As in the previous management schemes, tilapia fingerlings (about 5 g each) are stocked at 2 m^{-2} (200 fingerlings in a 100-m^2 pond). Predator fish may be added (usually 10 to 20) to prevent overpopulation and stunting; the use of a predator will increase the final weight of the harvested fish, but the total weight of fish at harvest is about the same whether or not a predator is added.

When broiler chicks are able to tolerate the cooler overnight temperatures (two to four weeks old), about 40 to 50 chicks are placed in the coop (0.1 m^2 of coop per chick) and fed a commercial broiler diet to satiation. About 2.2 to 3.0 kg of feed will be needed to produce a 1.5-kg broiler in seven weeks.

The primary nutrients for the fish are the droppings from the broilers. During the first week or two of each broiler cycle, additional nutrients should be added to the pond (about 1 kg of manure and agricultural by-products three days per week). After a couple weeks of the broiler cycle, no additional nutrients are added to the pond.

After six months, and depending on whether a predator fish was added, the fish are harvested. If fingerlings are difficult to obtain for the next production cycle, the best time to harvest is about one month after reproduction begins. At this time new fingerlings will be available for restocking as long as the producer has a place to keep them until the pond refills. Fish harvest will be about 30 to 40 kg. These fish are in a very productive environment and growing rapidly; about 60 to 70% of the total fish weight at harvest will be individuals > 15 cm length.

Evaluation of Production Systems
The labels for production practices (MF-1, MF-2, and INT-1) identify the individual evaluation tools.

The assumptions are that the producer already owns the land, has been or is willing to invest about 20 person-days for hand construction of a 100-m^2 pond, and considers his fixed costs to be zero. His primary concerns are total cash investment required and the expected return on his labor. In this tool the type and quantity of input and output line items are fixed, based on previous experiences. A default value has been assigned for the per unit value of each line item. The user of the tool can use these default values or can insert a more accurate value in the “actual unit price” column. Results, given for a 100-m^2 pond, include total variable cost, total net return to labor, and daily rate of return to labor (assuming an 8-hour work day).

Figure 3 presents the distribution of returns above variable cost and labor (RAVCL) for production system MF-1. Mean RAVCL are 23.09 lempiras per lot produced. A producer has a 30.8% probability of having negative RAVCL and a 5% probability of having RAVCL higher than 88 lempiras per lot produced. Figure 4 presents the distribution of RAVCL for production system MF-2. Mean RAVCL are 74.77 lempiras per lot produced. A producer has a 10% probability of obtaining negative RAVCL and a 5% probability of having RAVCL higher than 157 lempiras per lot produced. Figure 5 presents results from the INT-1 production system. In this production system, mean RAVCL per lot produced are 637 lempiras. There is a 27% probability of having negative RAVCL and a 5% probability of having RAVCL above 2,071 lempiras per lot produced.

In rural economies it is important to present the returns to labor of the activities undertaken. In the specific case of Honduras, the 2001 minimum wage salary in rural areas stands at 1,200 lempiras per month. This is equivalent to 8.75 lempiras per day or 1.09 lempiras per hour. This salary is adjusted to incorporate current Honduran labor laws, which demand the payment of fourteen months of salary per year. Of course, in rural areas minimum salary laws are not well enforced, and thus in some areas rural labor is not paid even the minimum salary.

Figure 6 presents the distribution of returns to labor (RTL) for production system MF-1. In this production system, RTL have a mean value of 0.74 lempiras per hour. There is a 30% chance that RTL will be negative. There is also a 5% chance that RTL will be higher than 2.87 lempiras per hour. From the standpoint of a small-scale farmer there is a very good chance that he or she will not earn as much as working as a second-hand laborer for other persons. A small-scale farmer working as a second-hand laborer may earn around 1.09 lempiras per hour. In the case of the MF-2 production system, the mean RTL are not very different from the previous example (Figure 7). Mean RTL are 0.79 lempiras per hour. However, the probability of having negative RTL decreases to 10%, whereas the probability of having a return higher than 1.68 lempiras per hour is 5%. The MF-2 production system is composed of manure as well as low-quality feeds added in amounts near optimum. The inclusion of high amounts of feed serves to decrease the variability of production at the lower scale of results; however, it is not enough to increase overall mean production.

In contrast, Figure 8 presents results for RTL for the broiler-tilapia integration. Mean RTL for this production system are 4.61 lempiras per hour. The probability of having negative RTL

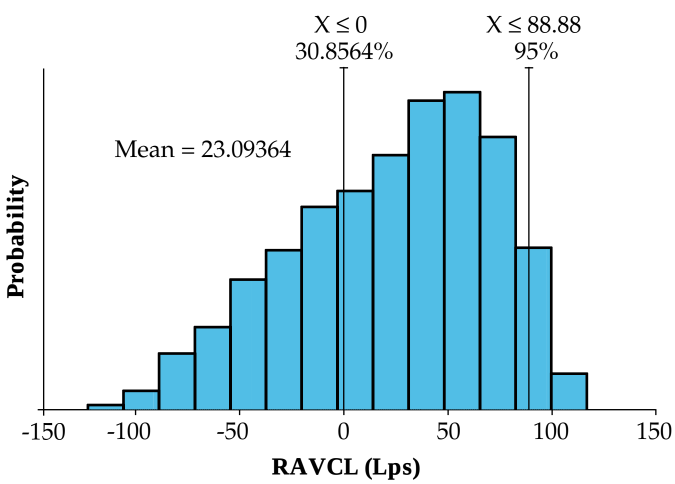

Figure 3. Distribution of returns above variable cost and labor (RAVCL) for the MF-1 production system.

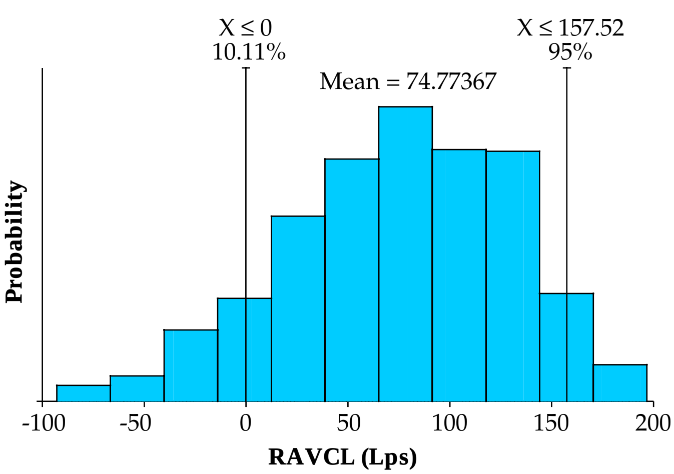

Figure 4. Distribution of RAVCL for the MF-2 production system.

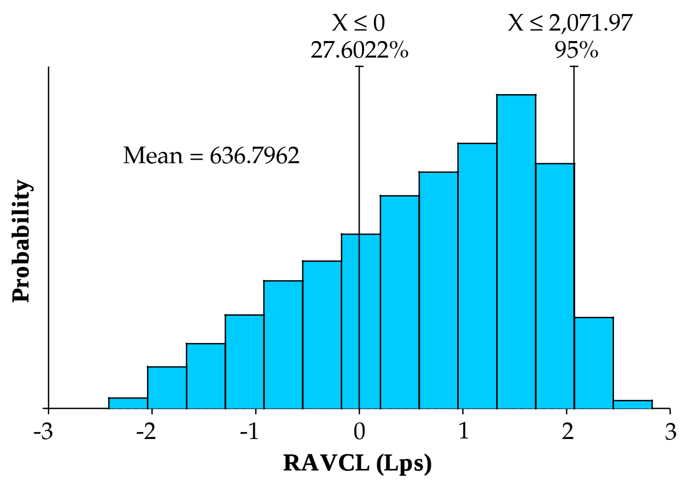

Figure 5. Distribution of RAVCL for the INT-1 production system.

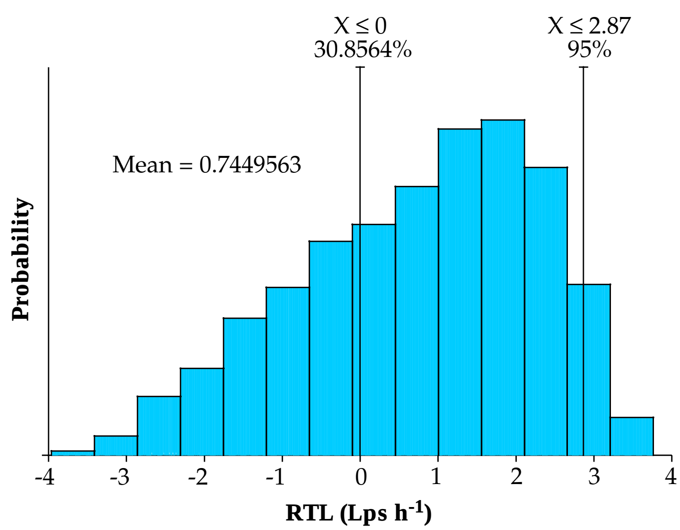

Figure 6. Distribution of returns to labor (RTL) for the MF-1 production system.

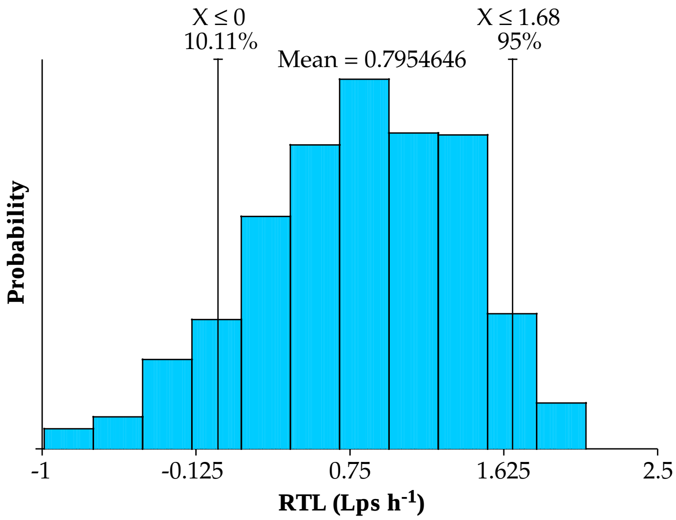

Figure 7. Distribution of RTL for the MF-2 production system.

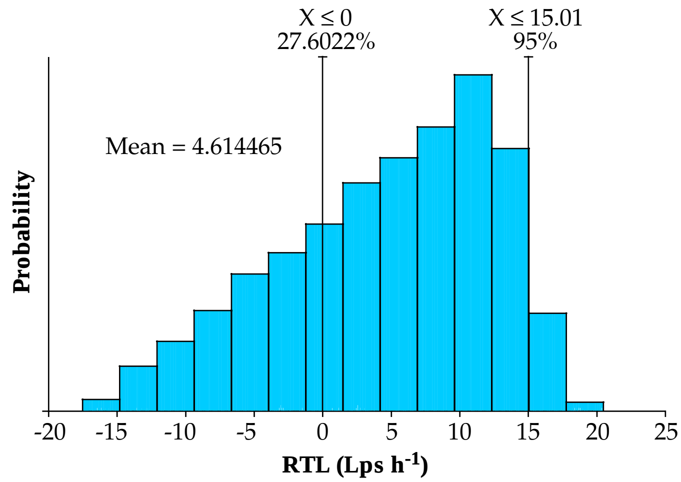

Figure 8. Distribution of RTL for the INT-1 production system.

is 27%, and there is a 5% probability of having RTL greater than 15 lempiras per hour. As expected, mean returns from the broiler-tilapia integration yield a significantly higher return to labor; however, these results do not seem to indicate that risk is higher, except perhaps for production system MF-2.

It is important to point out that the current version of the tool does not take into consideration fixed costs and that the baseline values for the price and quantity variables may not hold when departing from the baseline size of 100 m^2 as indicated in the description of the technology.

The current version of the tool provides a very simple and flexible way to examine production risk in small-scale tilapia production systems. By inputting data on the minimum, most-likely, and maximum values for prices and quantities, the user is able to model risk through a systematic procedure of simulations.

ANTICIPATED BENEFITS

We anticipate that there will be strong interest in the availability of a user-friendly rapid economic evaluation tool that allows research and extension personnel to quantify possible outcomes of new tilapia production techniques and assess potential economic risk consequences of these techniques. The quantitative tool allows the examination of not only mean response of tilapia production systems as reflected in economic budgets but also the risk associated with them. This tool will have benefits for instruction to provide a more realistic picture of the feasibility of any selected production system in that it estimates the negative possibilities as well as the "average or typical." These negative potential outcomes are very useful for decision makers to gain a better understanding of the "economic risk" they may face associated with alternative production decisions. Results presented here are intended to be illustrative of the Honduran growing conditions and would need to be revised for other locations.

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AQUACULTURE TRAINING FOR KENYAN FISHERIES OFFICERS AND UNIVERSITY STUDENTS

Ninth Work Plan, Adoption/Diffusion Research 3 (9ADR3)
Progress Report

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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 Kenya Project in Kenya seeks to improve training and to provide a cadre of trainers who have extensive practical fish-production experience.

This year the Kenya Project continued scholarship support for two M.S. students, one at Moi University's Chepkoilel Campus, Eldoret, Kenya, and the other at Auburn University, 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.

The series of short courses for personnel of the Kenya Fisheries Department (FD), begun in 1999 and 2000, was concluded this year with the fifth and final course planned under this activity. In this series of courses, more than 80 FD staff received two weeks of training in pond construction methods and pond management techniques, and an additional 26 persons (24 Fisheries Officers and 2 outside-funded participants) received three weeks of advanced training in pond construction, pond management, and business planning. Additional farmer field days for approximately 50 farmers are also planned for later in 2001.

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 Kenya 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 was expanded to include farmer training as well.

The objectives of this investigation are to:

1) Increase the pond management skills of fisheries personnel currently involved in aquaculture extension activities in Kenya.
2) 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 continuing to sponsor two M.S. students with full scholarships, conducting the final session in our series of short courses in pond construction and management for Kenya

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.

TRAINING OF UNIVERSITY STUDENTS

Two M.S. students have received full scholarship support from the CRSP Kenya Project for the past two years. Robinson Mugo began receiving CRSP support for his graduate program in the Department of Fisheries at Moi University (MU, Eldoret, Kenya) in October 1999. Mugo has finished his course work, field work, and data analysis and has submitted the first draft of his thesis, entitled "Effects of maize bran, rice bran and wheat bran on the growth of Nile tilapia," to his committee. He is currently working on revisions suggested by his committee and expects to submit the thesis for examination by the end of September 2001. Bethuel Omolo, selected by the Kenya FD in 1999 to receive training for the Department's new Research/ Extension Liaison position, began an M.S. program in the Department of Fisheries and Allied Aquacultures at Auburn University, Alabama, in January 2000. Omolo's studies have focused on extension methods and programming and general aquaculture. He also expects to complete and defend his thesis, entitled "Feed conversion efficiency as a function of fish size in channel catfish," by the end of 2001.

This year Patricia Mwau finished her program and was awarded the degree of Master of Science from the University of Nairobi (Department of Zoology) for her work, entitled "Nutrient dynamics, with special reference to nitrogen and phosphorus in tilapia (*Oreochromis niloticus*)/catfish (*Clarias gariepinus*) polyculture ponds at Sagana Fish Farm, central Kenya." Mwau conducted her thesis research at Sagana with on-site supervision by the resident CRSP research coordinator, Karen Veverica.

Earlier this year we received word that Paul Bilal Izaru, a graduate student supported by the Kenya Project, passed away. We have been unable to get official confirmation of this but continue our efforts to do so.

Daniel Oenga Nyanchiri, supported through October 1999, has submitted a draft thesis to Moi University, but some revisions are still needed. He is still employed by the Kenya Marine Fisheries Research Institute (KMFRI), so the going is slow with regard to completion of his thesis.

Last year we reported that Enos Mac'Were and Robert Olendi, both from MU, had begun M.S. research at Sagana Fish Farm. Mac'Were received CRSP stipend assistance and did his thesis research on commercial tilapia and *Clarias* production systems. He has completed his thesis, entitled "Comparison of tilapia and *Clarias* polyculture yields and economic benefits resulting from a locally available animal feed (pig finisher pellet), agricultural by-product (rice bran), and a pelleted test diet in fertilized ponds," and is expected to defend his thesis in September or October of 2001. Mac'Were's work was supervised by Charles Ngugi of MU and Veverica. 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 last year and will submit a draft by the end of August 2001. He was already employed as chief technician of the MU Department of Fisheries before entering graduate school and has returned to that position.

University undergraduates have continued to come to Sagana to do "attachments," in which they learn the practical aspects of fish farm management 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.

TRAINING FOR FISHERIES DEPARTMENT PERSONNEL

A series of two-week short courses in pond construction and pond management, begun during the previous reporting year (1999 to 2000), was completed this year. Each course was designed to accommodate 20 participants. These courses were typically a collaborative effort between the Sagana CRSP and FD group and the faculty of the Department of Fisheries of MU. Some courses were held at MUs Chepkoilel Campus in Eldoret (home of the MU Department of Fisheries), whereas others were held at Sagana. Primary MU participants included Ngugi, professor in the Department of Fisheries, Mucai Muchiri, Head of the MU Department of Fisheries, and David Liti, of the MU Department of Zoology. The full MU report on this five-session sequence of short courses is not included in this report because of space constraints but is available upon request to the Program Management Office. That report also includes, as attachments, course evaluations provided by the Training Assessor of the Kenya FD.

The final course in the series, held from 13 November through 3 December 2000, was aimed at senior Fisheries Officers in the Kenya FD (graduate student–level) and was expanded to cover a three-week period rather than the two-week duration of previous courses. As part of a farm planning and management exercise, trainees were given a three-week project assignment on enterprise budget development. This course will become the model for other university aquaculture classes. Twenty places in the course were reserved for Fisheries Officers, but as a result of the high interest in this course and requests to allow other trainees to attend the course, an additional five places were made available for outside-funded trainees (i.e., those under sponsorship from other projects or private entities). One private business and one government department took all the open places. The names, positions, home provinces, and sex of the participants in this course are shown in Table 1.

In addition to the skills gained by the trainees themselves, a further effect of this 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 Ngugi of the MU Department of Fisheries. Mac'Were, a graduate student from MU who has assisted with these programs, now has five training programs of experience. Liti (MU Department of Zoology) and Geraldine Matolla (MU Department of Fisheries) have also obtained considerable experience in these programs. Moi University now has eight ponds for use in student research projects and future training programs. The team-teaching technique that combines CRSP researcher Veverica, MU 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 FD. Professors at MU

Table 1. List of participants in the three-week course on Pond Construction and Commercial Fish Farm Management held for Fisheries Officers of the Kenya Fisheries Department at Sagana Fish Farm, Sagana, Kenya, from 13 November to 3 December 2000.

	Name	Designation	Institution	Province	Sex
1	Agwata Ototo	Lab Tech	KMFRI		M
2	Anne K. Mokoro	Fisheries Officer (FO)	Nyamira	Nyanza	F
3	Bernerd M. Fulanda	FO	Sagana	Central	M
4	George O. Owiti	FO	Sagana	Central	M
5	Isaac W. Wamalwa	FO	Bungoma	Western	M
6	James Mahaja	FO	Meru North	Eastern	M
7	Joab O. Okoto	FO	Teso	Western	M
8	John Oluoch Otiego	FO	Vihiga	Western	M
9	Joseph Kasuti	FO	Trans Nzoia	Rift Valley	M
10	Leornard W. Kundu	FO	Uasin Gishu	Rift Valley	M
11	Mahongah Wala Joseph	FO	Kiganjo	Central	M
12	Mbugua H. Mwangi	FO	Nairobi	Nairobi	M
13	Michael D. Omondi	FO	Kericho	Rift Valley	M
14	Michael M. Mumbi	FO	Kakamega	Western	M
15	Michael Mutuku Silla	FO	Gucha	Nyanza	M
16	Moses Munialo Simiyu	FO	Lugari	Western	M
17	Norman O. Munala	FO	Bomet	Rift Valley	M
18	Othniel Mwabili Mwanjala	FO	Kiambu	Central	M
19	Patricia Nduku Mwau	Graduate Student	Sagana	Central	F
20	Paul Kiprono Maritim	FO	Nandi	Rift Valley	M
21	Peterson Njeru Njue	FO	Sagana	Central	M
22	Philip Ruto	FO	Kisii	Nyanza	M
23	Rodrick Kundu Wakukha	FO	Turkana	Rift Valley	M
24	Simon Wahome Warui	FO	Nyahururu	Central	M
25	Stephen Gichunge	FO	Meru South	Eastern	M
26	Thomas M. Magara	Manager (Fisheries)	Taita D. Centre	Coast	M

Group Summary	Females	2	7.70%
	Males	24	92.30%

and officials of the Kenya FD and KMFRI have all independently reached the conclusion that Sagana is the best place to hold training of this sort.

This year the CRSP continued its support of James Karuri in a three-year diploma program in Applied Biology at the Murang'a College of Technology. He began his first year in January 1999 and will finish by the end of 2001.

FARMER TRAINING

Farmer education days planned for this reporting year have been unavoidably delayed but are expected to be held in September 2001. However, a considerable training effect for farmers and the Fisheries Officers and extension agents who work with them was realized as a result of the on-farm trials that are underway in Central Province. That activity is reported on separately (see "On-farm trials: Evaluation of alternative aquaculture technologies by local farmers in Kenya," 9ATR1; pp. 121–129 of this report).

ANTICIPATED BENEFITS

The Pond Construction and Management Training program has been included in the MU 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 untrained 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 September 2000. So far, none of the staff who received a certificate has 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. Senior fisheries officers who participated in the final course from November to December 2000 received more intensive training than previous groups, including experience in enterprise budget development and farm operation planning. 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, farm income, amounts of fish available to communities and markets, and employment opportunities.

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ESTABLISHMENT OF COMPANION SITES IN THE AFRICA REGION

Ninth Work Plan, Adoption/Diffusion Research 4 (9ADR4)
Final Report

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ABSTRACT

The establishment of companion sites was proposed as a way of expanding CRSP efforts in each region by assisting with needed research at sites other than CRSP host country sites and verifying the results of CRSP research at its host country sites. For the Ninth Work Plan the Kenya Project set out to identify and establish at least one companion site in the Africa region and to design and implement investigations at that site in support of the goals and needs of both the PD/A CRSP and the companion site. Discussions in 1999 between CRSP Kenya Project personnel and ICLARM-Malawi (Zomba, Malawi) and Bunda College of Agriculture (near Lilongwe) led to an agreement to collaborate. With oversight from Daniel Jamu, Director of ICLARM-Malawi, two studies were conducted between May 2000 and January 2001, one at the National Aquaculture Center near Zomba, and the second at Bunda College, Lilongwe. Reports on these two studies are included in this volume (see 9ADR4A, "Effect of stocking size and nutrient inputs on productivity of *Oreochromis shiranus* in ponds" and 9ADR4B, "Studies on potential use of salinity to increase growth of tilapia in aquaculture in Malawi"). An additional spin-off study conducted by a Bunda College student, "*Tilapia rendalli* fry production under a *Tilapia rendalli*/*Oreochromis shiranus* polyculture: The role of competition and predation," may be requested from the Program Management Office.

INTRODUCTION

The establishment of one or more companion sites in the Africa region was proposed as a way of expanding the regional effort 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 host country site. The objectives specifically listed for this effort in the Ninth Work Plan were to:

1) Identify and establish one or more companion sites for the Africa region (Year 1) and
2) 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 ICLARM-Malawi, at the National Aquaculture Center (Zomba), and with Bunda College of Agriculture, near Lilongwe (Veverica and Jamu, 2000). Agreement to collaborate on two experiments was reached in early 2000, and the research was conducted between May 2000 and January 2001. A preliminary (progress) report on this effort was made in 2000 (Jamu et al., 2001), and this is the final report for the activity. The two reports that follow are the results of work done in Malawi with support from the PD/A CRSP. They are:

- Effects of stocking size and nutrient inputs on productivity of *Oreochromis shiranus* in ponds (9ADR4A), by K. Chaula, D. Jamu, J. Bowman, and K. Veverica
- Studies on potential use of salinity to increase growth of tilapia in aquaculture in Malawi (9ADR4B), by J.S. Likongwe

An additional study, "*Tilapia rendalli* fry production under a *Tilapia rendalli* / *Oreochromis shiranus* polyculture: The role of competition and predation," was completed by an undergraduate student at Bunda College, L.S. Chimwala. The report can be requested from the Program Management Office.

ANTICIPATED BENEFITS

Fish farmers in Malawi and the 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 firsthand knowledge of the culture characteristics of several aquaculture species important in Malawi as well as from learning good research methods through their work with Jamu and Likongwe. The growth characteristics of species cultured in Malawi might be compared with CRSP findings from host country sites. 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.

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Effect of Stocking Size and Nutrient Inputs on Productivity of *Oreochromis shiranus* in Ponds

Ninth Work Plan, Adoption/Diffusion Research 4A (9ADR4A)
Final Report

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Abstract

A study to investigate the effects of three different stocking sizes (5, 10, 20 g) and two isonitrogenous input regimes (maize bran × urea and napier grass × urea) on the production of *Oreochromis shiranus* was conducted between June and November 2000 at the Malawi National Aquaculture Center. Six treatments (three stocking sizes × two input regimes), each in triplicate, were used in the study. Inputs were applied to ponds stocked with fish at the three stocking sizes such that each input regime supplied 20 kg N ha^{-1} wk^{-1}. Fish were stocked at 2 fish m^{-2} and sampling (mean weight of 100 fish) was conducted biweekly. Water quality parameters (dissolved oxygen, pH, electrical conductivity, and Secchi disk visibility) were measured weekly, and total ammonia nitrogen and chlorophyll *a* were measured biweekly. The experiment was conducted over a period of 150 days.

The two isonitrogenous input regimes did not significantly affect fish net yield and growth rate. There were significant differences ($P < 0.05$) in fish growth rate and net yield between treatments. The highest fish growth rates and production (net yield) were achieved in ponds when fish were stocked at 5 g and either input regime was used, while ponds stocked with 20-g fingerlings and supplied with either napier grass × urea or maize bran × urea had the lowest net mean yield. There were significant differences ($P < 0.05$) in gross margins between treatments, with treatments where fish were stocked at 5 g and napier grass × urea were applied giving higher gross margins than the rest of the treatments. Mean fish survival rate was not significantly different between treatments. Results from this study suggest that stocking *Oreochromis shiranus* at 5 g results in higher fish production and gross margins compared to stocking larger fish. The results further show that under conditions where inorganic fertilization is used, substituting napier grass for maize bran increases profitability without affecting overall fish yield.

Introduction

Extensive tilapia culture using the indigenous *Oreochromis shiranus* is widely practiced by Malawian small-scale farmers, with initial stocking sizes and inputs varying widely in these systems. The major contributing factor to the stocking of fingerlings of mixed sizes is the lack of information on the optimal fingerling size for increased resource use efficiency and fish production. Various studies have demonstrated that stock manipulation procedures—e.g., intermittent stocking and stocking different sizes of fish—can significantly affect fish growth rate and net fish yield (McGinty, 1985; Knud-Hansen and Lin, 1996). Kaunda (1996) reported significant differences in net fish yield between different recruit-removal ratios. Stock manipulation during the production cycle is complicated and may be difficult to implement under small-scale conditions; therefore, simple stocking procedures that optimize fish yield are required. Simulation model results as well as practical experience show that high initial biomass and stocking rates reduce final yield in tilapia ponds (Lanhai, 1997). Jamu (1998), using a simulation model, also showed that final fish biomass and individual fish weights were very sensitive to changes in initial fish weights. Determination of the effects of initial fish size and inputs on fish yield is essential if stocking size and input strategies that optimize fish yields and profitability under small-scale fish farming conditions in Malawi are to be developed.

Table 1. Description of treatments used in the study.

Treatment	Description
1	• 5-g fingerlings at stocking • Maize bran applied at 3% of mean body weight per day (BWD) • Urea applied at a rate such that maize bran × urea constituted a total pond nitrogen input of 20 kg ha^{-1} wk^{-1}
2	• 10-g fingerlings at stocking • Maize bran applied at 3% BWD • Urea applied at a rate such that maize bran × urea constituted a total pond nitrogen input of 20 kg ha^{-1} wk^{-1}
3	• 20-g fingerlings • Maize bran applied at 3% BWD • Urea applied at a rate such that maize bran × urea constituted a total pond nitrogen input of 20 kg ha^{-1} wk^{-1}
4	• 5-g fingerlings • Napier grass applied at 350 kg ha^{-1} wk^{-1} (8.5 kg N ha^{-1} wk^{-1}) • Urea applied at 25 kg ha^{-1} wk^{-1} (11.5 kg N ha^{-1} wk^{-1})
5	• 10-g fingerlings • Napier grass applied at 350 kg ha^{-1} wk^{-1} (8.5 kg N ha^{-1} wk^{-1}) • Urea applied at 25 kg ha^{-1} wk^{-1} (11.5 kg N ha^{-1} wk^{-1})
6	• 20-g fingerlings • Napier grass applied at 350 kg ha^{-1} wk^{-1} (8.5 kg N ha^{-1} wk^{-1}) • Urea applied at 25 kg ha^{-1} wk^{-1} (11.5 kg N ha^{-1} wk^{-1})

The objectives of this study were to:

1) Determine the effects of different fish stocking sizes on *O. shiranus* productivity;
2) Evaluate the effect of two different isonitrogenous input regimes on *O. shiranus* productivity and profitability; and
3) Recommend, based on objective 2 above, stocking strategies that optimize fish productivity and input regimes on fish yield.

METHODS AND MATERIALS

Eighteen randomly selected 200-m^2 ponds located at the Malawi National Aquaculture Center in Zomba district were used for this study. Three fingerling sizes (5, 10, and 20 g) and two isonitrogenous input regimes (urea × maize bran and urea × napier [*Pennisetum purpureum*] grass) served as treatments in this study (Table 1). The total Kjeldahl nitrogen contents of the input materials used in these treatments are shown in Table 2. Each treatment was replicated three times. Fish were stocked at a rate of 2 fish m^{-2}. The experiment ran for 150 days (22 June to 24 November 2000).

Water was added weekly to replace losses due to evaporation and seepage. Water temperature was taken daily, while dissolved oxygen (DO), pH, electrical conductivity, and Secchi disk visibility were measured weekly. Total ammonia nitrogen and chlorophyll *a* were analyzed every fortnight using standard methods (APHA, 1989).

Ponds were harvested by seining followed by complete draining to remove any fish that remained in the pond sediments. Data were analyzed using Statistical Analysis Package for Scientists (SAS Institute, Inc., 1988). Specific fish growth rate (g d^{-1}) and extrapolated gross and net yield (kg ha^{-1} yr^{-1}) were calculated for each replicate pond. Gross margins (Upton, 1987) were calculated using input cost estimates for fingerlings, maize bran, urea fertilizer, and gross revenue from fish sales. Treatment means (± 1 standard deviation) were considered to be significantly different at an alpha level of 0.05. Means that were significantly different were separated using the Duncan's Multiple Range Test (Montgomery, 1997). Gross margins were calculated using the following formula (Upton, 1987):

$$GM = TR - (F_c + M_c + FG_c)$$

where

GM = gross margin (Malawian Kwacha ha^{-1} yr^{-1});
TR = total revenue (MK ha^{-1} yr^{-1}) from fish sales;
F_c = cost of urea fertilizer (MK ha^{-1} yr^{-1});
M_c = cost of maize bran (MK ha^{-1} yr^{-1}); and
FG_c = cost of fingerlings (MK ha^{-1} yr^{-1}).

The cost of labor required for napier grass application was not included in the analysis because farmers use their own and family labor for this operation.

RESULTS

Water Quality

There were no significant differences in water quality parameters between treatments ($P > 0.05$), and all water quality parameters except water temperature were within acceptable limits for *O. shiranus* (Wolfharth and Hulata, 1983). Pond water temperatures gradually increased over time from 16°C in June, July, and early August to 29°C for the remainder of the experimental period (Figure 1). The mean water temperature

Table 2. Total Kjeldahl nitrogen contents of the inputs used in this study.

Inputs	Nitrogen Content (% Dry Weight)
Maize Bran	2.22
Napier Grass (*Pennisetum purpureum)*	2.24
Urea	46.0

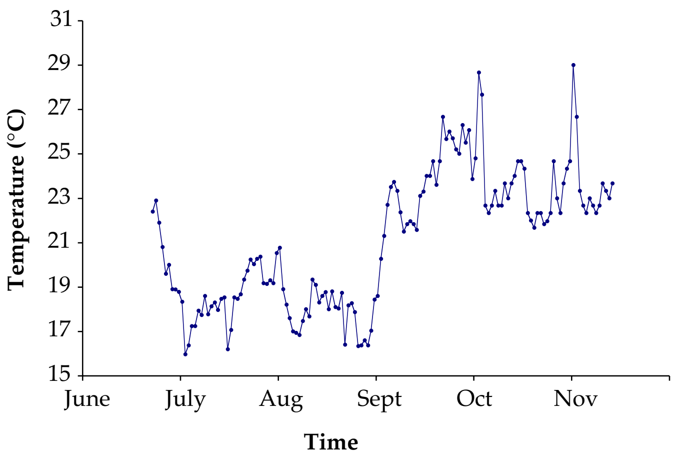

Figure 1. Mean monthly temperatures from 22 June to 24 November 2000.

during the experimental period was 22°C. Total ammonia nitrogen (TAN) concentrations were low and ranged from 0.01 to 1.12 mg l^{-1}. Conductivity increased over time, ranging from 29 to 83 mmho cm^{-1}, becoming highest towards the end of the experiment. Values of pH ranged from 5.3 to 8.1, increasing toward the end of the experiment. Early morning DO concentrations ranged from 3.1 to 9.6 mg l^{-1}. Chlorophyll *a* concentrations ranged from 10.4 to 42.6 µg l^{-1}. Secchi disk visibility decreased gradually with time, ranging from 17 to 39 cm.

Effect of Different Stocking Sizes on Production of *Oreochromis shiranus* in Ponds

Performance indicators for *O. shiranus* stocked at three different sizes and managed under two input regimes are presented in Table 3. There were significant differences in specific growth rates due to fingerling size at stocking ($P < 0.05$). Fish growth rate was highest in ponds stocked with 5-g fingerlings. There were significant differences in both gross and net yields due to fingerling size at stocking ($P < 0.05$). The highest net yield was obtained in ponds stocked with 5-g fingerlings, whereas those ponds stocked with 20-g fingerlings had the lowest net yield (Table 3).

Effect of Two Different Isonitrogenous Input Regimes on Production and Profitability of *Oreochromis shiranus*

Fish growth under the two input regimes is presented in Figure 2. There were no significant differences ($P > 0.05$) in specific fish growth rates and net fish yield between the two input regimes (Table 3). Fish growth was exponential up to the end of the experiment (Figure 2), suggesting that the pond carrying capacity was not reached in any of the treatments.

However, there were significant differences between the treatments in gross margins due to isonitrogenous input regimes ($P < 0.05$). Gross margin (MK ha^{-1} yr^{-1}) analysis showed that napier grass × urea gave higher returns than maize × urea at all stocking sizes. The highest gross margin was obtained in the treatment where fingerlings were stocked at 5 g and a combination of napier grass and urea was used (Table 3).

DISCUSSION

The mean weights of the fish in all treatments did not reach the desired size (> 60 g). This could be attributed mainly to low water temperatures that were experienced between the months of June and August. Specific growth rate was highest in ponds stocked with 5-g fingerlings, followed by those stocked with 10-g fingerlings and finally in ponds stocked with 20-g

Table 3. Performance indicators of *O. shiranus* in response to three different fish stocking sizes and two isonitrogenous input regimes. Treatments are as defined in Table 1 and the data are means of three replicates. Note: The price for fish was MK60.00 kg^{-1} (US\$0.83 kg^{-1}). Cost of labor was not included in determining gross margins. Means with similar superscript in the same row are not significantly different ($P > 0.05$).

Performance Measures	Treatments					
	1	2	3	4	5	6
STOCKING						
Total Weight (kg $pond^{-1}$)	2.1 ± 1.5	4.1 ± 1.2	7.9 ± 1.9	2.1 ± 1.4	3.9 ± 1.2	8.3 ± 0.8
Mean Weight (g $fish^{-1}$)	5.2 ± 0.9	10.3 ± 0.6	20.7 ± 1.8	5.1 ± 0.7	9.9 ± 1.2	20.1 ± 1.9
HARVESTING						
Total Weight (kg $pond^{-1}$)	14.6 ± 0.7	15.1 ± 0.6	15.9 ± 1.5	13.4 ± 0.5	14.2 ± 0.9	15.7 ± 1.1
Mean Weight (g $fish^{-1}$)	36.4 ± 1.5	37.8 ± 0.9	41.2 ± 0.6	34.8 ± 1.4	36.1 ± 1.6	40.1 ± 0.8
Total Weight Gain (kg $pond^{-1}$)	12.6 ± 1.5 ^{a}	11.1 ± 1.1 ^{a}	7.9 ± 1.2 ^{b}	11.4 ± 0.8 ^{a}	10.2 ± 2.6 ^{a}	7.7 ± 1.1 ^{b}
Mean Weight Gain (g $fish^{-1}$)	31.6 ± 1.6	27.8 ± 0.9	21.1 ± 0.6	29.8 ± 1.4	26 ± 1.6	20 ± 0.8
Specific Growth Rate (g $fish^{-1}$ d^{-1})	2.0 ± 0.9 ^{a}	1.3 ± 1.3 ^{b}	0.7 ± 0.6 ^{c}	1.9 ± 0.2 ^{a}	1.3 ± 0.9 ^{b}	0.7 ± 0.3 ^{c}
Net Yield (kg ha^{-1} yr^{-1})	1,508 ± 82 ^{a}	1,336 ± 66 ^{b}	965 ± 158 ^{c}	1,372 ± 56 ^{a}	1,238 ± 108 ^{b}	932 ± 128
Survival (%)	90 ± 0.8 ^{a}	88 ± 2.8 ^{a}	90 ± 5.2 ^{a}	89 ± 0.5 ^{a}	87 ± 5.8 ^{a}	88 ± 3.1 ^{a}
Gross Yield (kg ha^{-1} yr^{-1})	1,748 ± 82 ^{b}	1,816 ± 66 ^{a}	1,911 ± 182 ^{a}	1,613 ± 56 ^{b}	1,704 ± 103 ^{a}	1,892 ± 128 ^{a}
TOTAL COST (MK ha^{-1} yr^{-1})	59,712.44	63,284.82	78,545.12	53,492.11	57,492.00	63,492.00
GROSS MARGIN (MK ha^{-1} yr^{-1})	45,244 ± 3,549 $^{a, b}$	43,408 ± 3,641 ^{b}	38,384 ± 2,766 ^{c}	48,441 ± 2,553.2 ^{a}	47,677 ± 3,704 ^{a}	52,987 ± 1,907 ^{a}

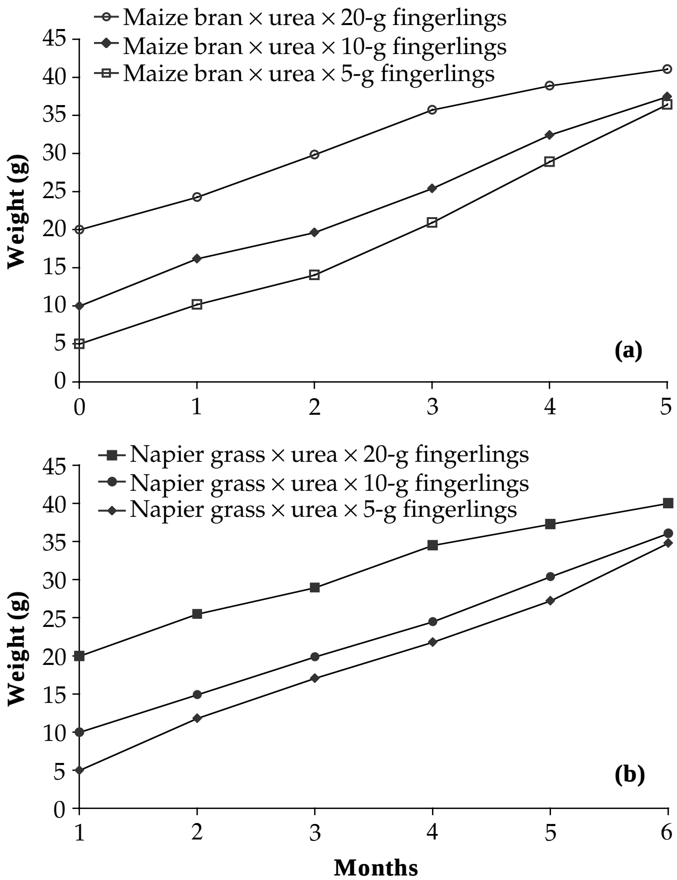

Figure 2. Effects of different stocking weights on growth of *O. shiranus* in ponds receiving two isonitrogenous (20 kg N ha^{-1} yr^{-1}) input regimes [maize bran × urea (a) and napier grass × urea (b)]. Fish were sampled from 22 June to 24 November 2000.

fingerlings. The specific growth rates for the 20-g fingerlings were similar to those obtained by Chikafumbwa (1996). Early breeding in tilapias has been reported to be related to the slowing of somatic growth (Lowe-McConnell, 1982). In this study, breeding was observed one month after stocking in ponds where 20-g fingerlings were stocked, and this could explain the low growth rates of fish in this treatment compared to treatments where fish were stocked at 5 and 10 g, respectively. High mean net yield of *O. shiranus* in treatments where 5-g fingerlings were used could be attributed to low initial biomass at stocking coupled with high growth rates that resulted in higher final net weight gain per unit time than in ponds stocked with 10-g and 20-g fingerlings, respectively. However, overall net yields were similar to those reported in other studies for similar systems (e.g., Chikafumbwa, 1996; Brummett, 2000).

There were no significant differences in fish growth rate and net yield between the two input regimes used in the study. Since the napier grass was applied as an organic fertilizer and maize bran was applied as a feed, it was expected that the maize bran × urea input regime would result in higher growth rates and fish production. Chikafumbwa et al. (1993), using delta carbon (δC) analysis, showed that *O. shiranus* derived most of its diet from detritus or phytoplankton. It is therefore likely that most of the maize bran (which small-scale farmers apply as a feed) contributed to the detrital food web, which was eventually targeted by the fish as food. Gross margin comparison favored the use of napier grass × urea as opposed to maize bran × urea. The cost of maize bran significantly contributed to high costs of growing *O. shiranus* in treatments where it was used. Although the labor required to cut and apply napier grass to ponds was not included in the gross margin analysis, available data (Chikafumbwa, 1996) show that 33 to 60 person-hours are required to cut and apply napier grass at a rate of 100 kg ha^{-1} d^{-1}. Because fish growth rate and net fish production and yield were similar between the two input regimes and gross margins were higher when napier grass and urea inputs were used, a combination of napier grass and urea inputs and an initial stocking size of 5 g could result in better fish production and profitability in low-input *O. shiranus* production systems where labor for cutting grass is cheap and readily available.

ANTICIPATED BENEFITS

The study was designed to evaluate the production and profitability of *O. shiranus* in experimental ponds where three different fingerling stocking sizes and two isonitrogenous pond input regimes were used, and it was designed to recommend a fingerling stocking size and input regime that result in better production and profitability. Results indicate that stocking fish at 5 g and using a combination of napier grass and urea as pond inputs gives the highest fish production and profitability. This management strategy will allow farmers to optimize fish production and profits while utilizing on-farm resources that would otherwise not be utilized on the farm. This study will also provide extensionists with information on stocking strategies and profitability of fertilized ponds in Malawi.

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STUDIES ON POTENTIAL USE OF SALINITY TO INCREASE GROWTH OF TILAPIA IN AQUACULTURE IN MALAWI

Ninth Work Plan, Adoption/Diffusion Research 4B (9ADR4B)
Final Report

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ABSTRACT

In a series of studies conducted in Malawi to determine the effects of different salinity concentrations on survival, growth, feed conversion, reproduction, and whole-body composition of five taxonomic groups of tilapia—*Oreochromis shiranus chilwae* (Lake Chilwa strain), *O. shiranus chilwae* (Bunda College strain), *O. karongae*, *O. shiranus shiranus*, and *Tilapia rendalli*—it was observed that the first three species grew faster in 10‰ salinity and would be recommended as potential candidates for brackishwater aquaculture in Malawi. *T. rendalli* and *O. shiranus shiranus* grew faster in fresh water (0‰ salinity) and are unsuitable for brackishwater aquaculture. With the exception *of O. shiranus chilwae* (Lake Chilwa strain) and *O. shiranus chilwae* (Bunda College strain), all species had lost carcass protein at the end of the study, suggesting that they used tissue protein as an additional energy source for osmoregulation and homeostasis. Salinity tolerance varied ontogenetically in almost all the above taxonomic groups, with younger individuals tolerating salinity longer than larger individuals. This study has also shown that the range of *T. rendalli* and *O. shiranus shiranus* would effectively be limited by salinity. The interactive effect of salinity and water temperature was not investigated in this study since all experiments were conducted at room temperature and ambient photoperiod. Temperature, however, has an influence on salinity tolerance, and in that light, we strongly recommend further investigations on the combined influence of the two abiotic factors (salinity and temperature) since they fluctuate together in nature, and their fluctuations may positively or negatively influence growth and reproductive performance of the above cichlids.

INTRODUCTION

Use of natural resources, including those of seemingly marginal value, is an important human activity designed to increase food production and income. In agriculture, soils may be too saline to support profitable crop husbandry, yet such soils may be used alternatively for productive aquaculture if a salinity-tolerant fish species is used. The cichlids of the genera *Tilapia* and *Oreochromis*, known to have evolved from the marine environment, are euryhaline, as they have the genes for salinity tolerance and can adapt, grow, and even breed in seawater. Morgan and Iwama (1991) provided a comprehensive classification of fishes based on differences in their metabolic rates in seawater and fresh water, but a similar classification is totally unknown for the cultured tilapias in Malawi. Reports and reviews of tilapia salinity tolerance have been published by several researchers (Fryer and Iles, 1972; Whitfield and Blaber, 1976; Chervinski, 1982; Payne and Collinson, 1983; Wohlfarth and Hulata, 1983; Watanabe et al., 1985, 1993; Zale and Gregory, 1989; Likongwe at al., 1996) among others. With the exception of *Tilapia rendalli*, none of the species reported by the above authors is one of the tilapia species currently cultured in Malawi. The present study was conducted to investigate how salinity tolerance in tilapias may be used to culture these fish in marginal areas of Malawi where the soils may be too saline for productive crop husbandry.

METHODS AND MATERIALS

The study was conducted in a wet laboratory at Bunda College of Agriculture in central Malawi. Five taxonomic groups of tilapia were used: *Oreochromis shiranus shiranus*, *O. shiranus chilwae* (from the fish ponds at Bunda College and Lake Chilwa), *O. karongae*, and *T. rendalli*. Approximately 900 fingerlings of *O. shiranus chilwae* were collected from Lake Chilwa and transported to Bunda College on 15 January 2001 in plastic bags under oxygen. On arrival at Bunda College, the fish were introduced into a fiberglass tank containing fresh water (salinity range 0.10 to 0.20‰) in the hatchery. The fish were maintained and acclimated in this tank for a week. The rest of the taxonomic groups above were collected from the fish ponds at Bunda College.

Four salinity concentrations—0, 10, 20, and 30‰—were originally proposed as treatments in the experiments, but the failure of all five taxonomic groups to adapt to 30‰ forced us to drop that treatment. The growth experiments, each lasting approximately seven to eight weeks, were designed to study the effects of salinity on growth, feed utilization, reproduction, and whole-body composition of the fish. The other five short-duration supporting experiments, conducted over variable periods of time depending on species and fish size, were designed to study the effect of salinity stress on survival of the fish after their direct transfer into 30‰ salinity.

All experiments were conducted in plastic tanks in a wet laboratory supplied by well water. In the various experiments that were conducted, nine 100-l, twelve 30-l, twelve 200-l, and twelve 50-l plastic tanks were used. Each tank was adequately aerated using a blower, and fish were cultured at room temperature under ambient photoperiod. The fish were fed twice a day on a pelleted diet containing 30% crude protein.

The fish were fed at 5% body weight per day (BWD). Fishmeal and soybeans were the main sources of dietary protein, while wheat offal and rice bran were the main carbohydrate energy sources in the formulated diet.

During the experimental periods the fish were checked every day for mortalities. Salinity, temperature, and pH were also checked daily. Dissolved oxygen (DO), ammonia, conductivity, and turbidity were checked three times a week (Monday, Wednesday, and Friday). Fish were sampled every two weeks to monitor changes in weight. All species ranged in average weight from 5.5 to 10.0 g. In each experiment only one statistically uniform size of fish was used.

The effect of salinity on whole-body composition of fish (dry matter, crude protein, fat, and ash) was determined by taking initial fish samples before starting the experiments. Final fish samples were taken at the end of the experiments to determine salinity effects on whole-body composition. There was an overlap in the experimental periods for the five experiments, but each had different stocking and sampling dates. In the next series of five supporting experiments, selected individuals of each species were introduced into 30‰ salinity water to determine their response to salinity stress. Specific growth rate (SGR), expressed as percent body weight per day, was calculated from:

$$SGR = 100 (\ln Wf - \ln Wi)/t$$

where

Wf = final mean weight,
Wi = initial mean weight, and
t = experimental time in days.

Feed conversion efficiency (FCE) was expressed as the ratio of growth (weight gain) to total feed consumed (dry weight). The mean concentration of salinity across treatments ranged from 0.10 to 20.6‰. DO ranged from 7.89 to 9.21 mg l^{-1}, water temperature ranged from 22.9 to 27.0°C, and pH ranged from 6.18 to 7.99. Ammonia concentration ranged from 0.70 to 2.60 mg l^{-1}, conductivity ranged from 0.292 to 38.40 mS cm^{-1}, and turbidity ranged from 2.0 to 48.0 mg l^{-1}.

Experiment 1: Growth and Whole-body Composition of *Oreochromis shiranus chilwae* (Lake Chilwa Strain) Cultured in Four Salinity Concentrations

After a week of acclimation in a fiberglass tank, juveniles of *O. shiranus chilwae* (Lake Chilwa strain) were transferred into twelve 50-l rectangular plastic tanks. Four salinity levels (0, 10, 20, and 30‰) were assigned to these tanks in triplicate. Each tank was filled with well water and was stocked with ten fingerlings (average weight = 8.7 to 9.6 g) at 1.58 fish cm l^{-1}. Fish were acclimated to their respective experimental salinity concentrations by adding salt at 2.5‰ d^{-1}. Fish were fed twice a day on a diet formulated from fish meal, soybean meal, wheat offal, and rice bran and containing 30% protein. Fish were fed at about 5% BWD. Fish mortality, salinity, and temperature were checked daily, while DO, ammonia, pH, conductivity, and turbidity were monitored three times weekly. Fish started dying within two weeks in the highest (30‰) salinity treatment. Between 20 January and 17 February 2001 (28 d), all fish in 30‰ salinity died, and that treatment was withdrawn from the study. We then continued with three treatments (0, 10, and 20‰ salinity). The effect of salinity on reproduction was evaluated by determining gonadosomal indices (GSI) of the experimental fish.

Experiment 2: Response of *O. shiranus chilwae* (Lake Chilwa Strain) to Salinity Stress

This experiment was conducted in two parts. In the first part of the experiment, smaller fish (average weight = 2.36 g) were subjected to a sudden transfer into an equivalent of 86% seawater (30‰ salinity). In the second part, larger individuals of the same species (average weight = 15.22 g) were also transferred to the same environment (30‰ salinity). The objective of this experiment was to determine differences in the response of *O. shiranus chilwae* to acute stress following direct transfer to 30‰ salinity. Thirty-seven fingerlings (average weight = 2.36 g) were collected from a fiberglass tank in the hatchery where they were maintained. Ten of these individuals were transferred into a 30-l circular tank half-filled with well water containing 30‰ salinity. The experiment was started at 1030 h and was timed. The water and fish in the tank were adequately aerated with a blower while the experiment was in progress. Cessation of opercular movement and the fish's failure to respond to physical touch or gentle prodding were used as the criteria for death. The opercular and jaw movements in the fish were therefore closely monitored. Dead individuals were instantly removed from the tank. The experiment was conducted under ambient photoperiod. The second part of this experiment started at 1045 h when ten individuals of the above species (average weight = 15.22 g) were transferred into another 30-l tank half-filled with well water containing 30‰ salinity.

Experiment 3: Growth and Whole-body Composition of *O. shiranus chilwae* (Bunda College Strain) Cultured under Laboratory Tank Conditions in Three Salinity Concentrations.

The cichlid *O. shiranus chilwae* (Bunda College strain) was collected locally from a breeding pond at Bunda College of Agriculture. After a week of acclimation to their respective experimental salinity concentrations in tanks, the fish were stocked in nine 100-l plastic tanks in a wet laboratory. Three salinity levels (0, 10, and 20‰) were assigned to the tanks in triplicate. The highest salinity concentration (30‰) was not included in the experiment due to a shortage of experimental tanks. Fish were acclimated to their respective salinity concentrations by adding salt at 2.5‰ d^{-1} as in the first experiment. Each tank was stocked with ten fingerlings (average weight = 5.42 to 5.60 g) at 0.66 fish cm l^{-1}. Fish were fed a formulated diet containing 30% protein, two times a day at 5% body weight. Fish mortality, salinity, pH, and temperature were checked every day, while DO, ammonia, conductivity, and turbidity were monitored three times weekly.

Experiment 4: Response of *O. shiranus chilwae* (Bunda College Strain) to Salinity Stress

At 1535 h, ten juveniles of *O. shiranus chilwae* (Bunda College strain) (average weight = 6.00 g) were placed in a 50-l plastic tank half-filled with well water of 30‰ salinity. The experiment was timed and the tank was aerated. The pH and the DO concentration of the water were both normal (refer to range above). In the second part of this experiment, we used slightly larger fish (average weight = 10.0 g). At 1515 h, ten of these individuals were again placed in a 50-l plastic tank containing well water at 30‰ salinity, and the experiment was again timed.

Experiment 5: Growth and Whole-body Composition of *Tilapia rendalli* Reared under Laboratory Tank Conditions at Two Salinity Concentrations

Tilapia rendalli fingerlings (average weight = 6.9 to 9.0 g) were collected from a breeding pond at Bunda College and introduced into nine 200-l circular plastic tanks filled with well water. Each tank was stocked with 14 fingerlings at 0.53 fish cm l^{-1}. Initially, three treatments (0, 10, and 20‰ salinity) were assigned in triplicate (the highest salinity concentration (30‰) proposed earlier in this study was not included). Fish succumbed to 20‰ very early (within two to three weeks), and this treatment had to be withdrawn from the study. The treatment of 20‰ salinity may not have been necessary since *T. rendalli* has been reported to tolerate a maximum salinity level of 19‰. We therefore continued the study using 0 and 10‰ salinity as treatments. The general maintenance of the experimental animals (in relation to water quality, feeding, and sampling) was the same as that outlined above for other experiments.

Experiment 6: Response of *T. rendalli* to Salinity Stress

In this experiment only juveniles of one size were used. It was not possible to get another size for this study. Ten juveniles of *T. rendalli* (average weight = 11.4 g) were introduced into a 50-l plastic tank containing well water at 30‰ and pH 7.46. The tank was adequately aerated. The experiment started at 1510 h and was also timed.

Experiment 7: Growth and Whole-body Composition of *O. karongae* Cultured under Laboratory Tank Conditions in Two Salinity Concentrations

Fingerlings of *O. karongae* (average weight = 6.9 to 9.0 g) were collected from a breeding pond at Bunda College and introduced into nine 200-l circular plastic tanks filled with well water. For this experiment each tank was stocked with 14 fingerlings at 0.53 fish cm l^{-1}. Initially, three treatments (0, 10, and 20‰ salinity) were assigned, but lack of adequate tanks forced us to investigate two salinity levels, 0 and 10‰. Salinity was increased in the experimental tanks by adding salt at 2.5‰ d^{-1}. The general maintenance of the experimental animals (in relation to water quality, feeding, and sampling) was carried out as outlined in the above experiments.

Experiment 8: Response of *O. karongae* to Salinity Stress

The method used was similar to that used for the other species above. The tank was stocked using *O. karongae* (average weight = 6.89 g). The experiment was started at 1540 h and was timed. The experiment was carried out using one size range of fish, as larger sizes were not available for the experiment.

Experiment 9: Growth and Whole-body Composition of *O. shiranus shiranus* Cultured under Laboratory Tank Conditions in Three Salinity Concentrations

Juvenile *O. shiranus shiranus* (average weight = 7.03 to 7.09 g) were collected from a nursery pond at Bunda College of Agriculture and placed in a concrete tank for five days acclimation. Twelve 30-l plastic tanks were cleaned and set up in the wet laboratory. Three treatments (0, 10, and 20‰ salinity) were assigned to the plastic tanks in triplicate. Each tank contained aerated well water. The fish were collected from the concrete tank and transferred into the experimental tanks at a stocking density of 8 fish $tank^{-1}$ (1.88 fish cm l^{-1}). Salinity was increased in the experimental tanks by adding salt at 2.5‰ d^{-1}. The fish were fed on 30% protein diet, twice a day at 5% body weight. Fish were sampled every two weeks to monitor changes in weight. Fish mortality, salinity, pH, and temperature were monitored every day, while DO, ammonia, conductivity, and turbidity were checked three times a week.

Experiment 10: Response of *O. shiranus shiranus* to Salinity Stress

At 1428 h, ten juveniles (average weight = 6.52 g) and ten others (average weight = 11.0 g) were simultaneously introduced into two separate 30-l plastic tanks containing water at 30‰ salinity.

RESULTS

Experiment 1: Growth and Whole-body Composition of *O. shiranus chilwae* (Lake Chilwa Strain)

<u>Survival</u>
Survival was highest in both the 0 and 10‰ salinity treatments; it was lowest (53.33%) in the highest-salinity treatment (20‰).

<u>Growth</u>
Growth of *O. shiranus chilwae* (Lake Chilwa strain) differed significantly ($P < 0.05$) among treatments (Table 1). Final mean weight was highest in 10‰ salinity water, where the fish gained weight by 15.10%. Growth was negative in fresh water (0‰), where fish lost about 4.20% of their initial weight. There was no significant difference ($P > 0.05$) in the final mean weights of fish in 10 and 20‰. Similarly, there was no significant difference in the final mean weights of fish in 0 and 20‰ salinity. In 20‰ water, fish gained weight by 7.91% (Table 1).

<u>Whole-body Composition</u>
Dry matter increased in proportion to an increase in salinity. In fresh water, where fish lost weight, there was a 13.80% decrease in whole-body protein (Table 2). In 10‰ salinity, where growth was highest, there was a 21.8% decrease in whole-body fat. Whole-body ash decreased in all treatments, with a maximum drop of 20.40% from the initial value in the 20‰ treatment.

Table 1. Growth of *O. shiranus chilwae* (Lake Chilwa strain) cultured under laboratory tank conditions in three salinity concentrations.

Salinity (‰)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	8.34 ± 1.63 [a]	7.99 ± 2.78 [b]	---	96.67
10	8.41 ± 1.52 [a]	9.68 ± 1.85 [a]	0.254	96.67
20	8.34 ± 1.42 [a]	9.00 ± 1.78 [ab]	0.140	53.33

[a, b] Means in a column followed by the same letter are not significantly ($P > 0.05$) different (Duncan's multiple range test).

Reproduction
In all treatment groups, the gonads (ovaries and testes) were too small to give accurate GSI values. In addition, visual examination of the gonads did now show any differences in gonadal development among treatments.

Experiment 2: Response of *O. shiranus chilwae* (Lake Chilwa Strain) to Salinity Stress

Survival of Younger Fish
On being introduced into the tank, almost all the fish floated. At 1245 h all fish stopped breathing and were pronounced dead. It took 2 h 15 min for them to succumb to the salinity challenge.

Survival of Older Fish
On being introduced into 30‰ salinity, the fish started swimming vigorously, and they did not float. At 1228 h (103 min after introduction), the fish were still alive. At 1232 h they died after exactly 1 h 47 min.

Experiment 3: Growth and Whole-body Composition of *O. shiranus chilwae* (Bunda College Strain)

Survival
Survival was inversely proportional to salinity. Survival was highest (93.33%) in fresh water, followed by 90% in 10‰, and finally 66.67% in 20‰ water (Table 3).

Growth
Growth did not differ significantly ($P > 0.05$) across treatments (Table 3). Specific growth rate was highest (0.81%) in 10‰ and lowest (0.71%) in 0‰ water.

Whole-body Composition
Whole-body dry matter increased in proportion to an increase in salinity. Final values for all treatments were higher than the initial values. The increase ranged from 6.25% (in 0‰) to 6.97% (in 20‰). Whole-body protein also increased in proportion to an increase in salinity. The increase ranged from 11.90% (in 0‰) to 22.89% (in 20‰). All treatments showed far lower final whole-body fat levels than the initial values. Whole-body fat and experimental salinity were inversely proportional. Whole-body fat decreased most (by 12.73%) in 20‰ water (Table 4).

Table 2. Effect of salinity on whole-body composition of *O. shiranus chilwae* (Lake Chilwa strain).

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	91.20	63.47	19.18	18.35
0‰	85.80	54.70	17.00	15.80
	(–5.4)	(–8.70)	(–2.18)	(–2.55)
10‰	89.20	63.47	15.00	15.80
	(–2.0)	(0)	(–4.18)	(–2.55)
20‰	93.40	63.47	27.07	14.60
	(2.20)	(0)	(7.89)	(–3.75)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

Experiment 4: Response of *O. shiranus chilwae* (Bunda College Strain) to Salinity Stress

Survival of Younger Fish
On direct transfer to the 30‰ salinity, fish maintained their normal swimming postures. At 1600 h (25 min later) the fish still responded to touch by swimming away from the point of stimulus. A little while later one fish floated on the surface but resumed swimming. At 1620 h (45 min after introduction) five individuals died. At 1631 h (56 min after introduction) only one individual was swimming in the tank while showing clear signs of salinity stress. At 1642 h (67 minutes after introduction) the last surviving fish was still able to use its fins for locomotion. The last fish died at 1650 h, after exactly 1 h 15 min.

Survival of Older Fish
On their direct transfer to this medium, the fish swam in their normal positions and did not float on the surface as was the case with the younger individuals. At 1600 h none of the fish floated on the surface. They were able to respond to touch. At 1607 h one fish was seen migrating to the bottom of the tank. At 1609 h (54 min after introduction) one fish died, and it was removed from the water. By 1620 h (65 min after introduction) all fish died. When they were withdrawn from the water, only two of them made some reflex movements, but they had already stopped breathing. This group died after exactly 1 h 5 min, showing a slight difference of 10 min sooner than the younger individuals.

Table 3. Growth of *O. shiranus chilwae* (Bunda College strain) cultured under laboratory tank conditions in three salinity concentrations.

Salinity (‰)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	5.596 ± 0.447 [a]	8.833 ± 1.297 [a]	0.71 [a]	93.33
10	5.479 ± 0.800 [a]	8.639 ± 1.482 [a]	0.81 [a]	90.00
20	5.418 ± 0.685 [a]	8.285 ± 1.779 [a]	0.76 [a]	66.67

[a] Means in a column followed by the same letter are not significantly ($P > 0.05$) different (Duncan's multiple range test).

Table 4. Effect of salinity on whole-body composition of *O. shiranus chilwae* (Bunda College strain) reared under laboratory tank conditions in three salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	83.20	50.09	24.47	21.20
0‰	88.40	55.80	16.00	15.50
	(5.20)	(5.71)	(–8.47)	(–5.70)
10‰	88.60	61.28	16.00	16.40
	(5.40)	(11.19)	(–8.47)	(–4.80)
20‰	89.00	62.37	15.00	18.50
	(5.80)	(11.47)	(–9.47)	(–2.70)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

Experiment 5: Growth and Whole-body Composition of *T. rendalli*

Survival
Survival was higher (97.60%) in fresh water than in 10‰ salinity (80.90%) (Table 5).

Growth
Growth differed significantly ($P < 0.05$) between the two treatment groups in 0 and 10‰ salinity. Growth was significantly ($P < 0.05$) higher (by 26.3%) in fresh water (0‰) than in 10‰ water (Table 5).

Whole-body Composition
There was a decrease in tissue dry matter, crude protein, and ash in both treatments at the end of the experiment. In fresh water (0‰), fish had a lower fat content than those raised in 10‰ salinity. The initial fat content was very low in the fingerlings at the time of stocking, but values increased significantly ($P < 0.05$) by 354.15 to 395.10%. Ash was lower in 0‰, whereas growth was higher than in 10‰ (Table 6).

Experiment 6: Response of *T. rendalli* to Salinity Stress

Survival of Fish (One Size Group)
In this trial, fish of only one size (11.40 g) were used. On direct transfer to the tank, all fish floated, while some of them made jerking movements. At 1530 h (20 min after introduction) most of the fish failed to maintain their normal posture. At 1545 h (35 min after introduction) two fish died. At 1548 h (38 min after introduction) two more fish succumbed to the salt concentration. The remaining fish continued making jerking movements. At 1558 h (48 min after introduction) three more fish died. The remaining fish stopped breathing but continued showing reflex action in their muscles. At 1600 h the remaining fish died. This group took 50 min to succumb to 30‰ salinity.

Experiment 7: Growth and Whole-body Composition of *O. karongae*

Survival
Survival was much lower in this species than in the other taxonomic groups. Survival was about 71% in each of the two treatments (Table 7).

Growth
Growth differed significantly ($P < 0.05$) between the two treatment groups in 0‰ and 10‰ salinity. Growth was significantly ($P < 0.05$) higher (by 26.3%) in 10‰ salinity than in fresh water (0‰) (Table 7).

Whole-body Composition
Salinity changed the dry matter values of *O. karongae*. There was a slight increase in dry matter in fresh water, while in 10‰ salinity there was a reduction in dry matter. Whole-body protein decreased in both treatments, with more protein lost in 10‰ water. Fish lost about 7.9% of their carcass protein in 10‰ water compared with 3.1% loss of protein in 0‰ water. Carcass fat increased significantly by 88.07 and 96.88% in 0 and 10‰ salinity, respectively. There was an inverse relationship between carcass ash and salinity. As salinity increased, ash levels decreased. Ash decreased by 39.15% in 0‰ and 44.60% in 10‰ (Table 8).

Experiment 8: Response of *O. karongae* to Salinity Stress

Survival (One Size Group)
On being introduced into the tank, the fish swam in all directions initially, and 8 min later (1548 h) most of them were

Table 5. Growth of *T. rendalli* cultured under laboratory tank conditions in two salinity concentrations.

Salinity (‰)	Initial Weight (g)	Final Weight (g)	Specific Growth Rate (%)	Survival (%)
0	8.12 [a]	11.05 [a]	0.56	97.6
10	8.02 [a]	8.60 [b]	0.127	80.9

[a, b] Means in a column followed by the same letter are not significantly ($P > 0.05$) different (Duncan's multiple range test).

Table 6. Effect of salinity on whole-body composition of *T. rendalli* reared under laboratory tank condition in two salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	91.00	56.90	5.30	29.40
0‰	88.10	52.52	24.07	13.13
	(–2.90)	(–4.38)	(18.77)	(–16.27)
10‰	87.00	50.34	26.24	15.27
	(–4.0)	(–6.56)	(20.94)	(–14.13)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

Table 7. Growth of *O. karongae* cultured under laboratory tank conditions in two salinity concentrations.

Salinity (‰)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	5.58 ± 1.02	6.58 ± 1.09	0.308 [a]	71.42
10	5.27 ± 1.09	6.86 ± 1.19	0.508 [b]	71.42

[a, b] Means in a column followed by the same letter are not significantly ($P > 0.05$) different (Duncan's multiple range test).

Table 8. Changes in the whole-body composition of *O. karongae* cultured under laboratory tank conditions in two salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	90.00	61.28	10.90	21.20
0‰	90.20	59.38	20.50	12.84
	(0.20)	(–1.90)	(9.60)	(–8.30)
10‰	89.04	56.44	21.46	11.74
	(–0.40)	(–4.84)	(10.56)	(–9.46)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

swimming near the bottom of the tank. They moved to the surface at about 1610 h (30 min after introduction). At 1625 h (45 min after introduction) two fish died, settled at the bottom, and were instantly removed. At 1629 h (49 min after introduction) most fish started swimming horizontally with their heads oriented slightly upwards. At 1700 h (80 min after introduction) five more fish died. At 1705 h, another fish died. At 1710 h (90 min after introduction) one more fish died. The remaining fish died by 1722 h (1 h 42 min after introduction).

Experiment 9: Growth and Whole-body Composition of *O. shiranus shiranus*

Survival

Survival ranged from 83.33% in both 0 and 10‰ treatment groups to 87.50% in 20‰ salinity (Table 9).

Growth

Growth was significantly ($P < 0.05$) influenced by salinity. Specific growth rate was highest ($P < 0.05$) in fresh water (0‰ salinity), followed by growth rate in 10‰, but differences in weight in the two treatments did not differ significantly ($P > 0.05$). Specific growth rate was lowest ($P < 0.05$) in the highest salinity (20‰) treatment where growth was significantly different ($P < 0.05$) from the growth rate in fresh water (0‰) (Table 9).

Whole-body Composition

There was a decrease in dry matter in all treatment groups at the end of the experiment. Similarly, there was a decrease in both carcass protein and ash, but there was a slight increase in carcass fat in the 0 and 10‰ salinity treatments (Table 10).

Experiment 10: Response of *O. shiranus shiranus* to Salinity Stress

Survival of Younger Fish

On transfer into the tank, juvenile *O. shiranus shiranus* swam in different directions, but they did not float. One individual swam up to the surface and started gasping for air. At 1508 h (40 min after introduction) the fish had not yet started dying. At 1517 h (49 min after introduction) six fish died and settled at the bottom. A minute later another fish also died. The remaining fish died by 1730 h (after 182 min).

Survival of Older Fish

The first fish died after 82 min from the time they were introduced into 30‰ salinity water. The last fish died after 135 min.

Table 9. Growth of *O. shiranus shiranus* cultured under laboratory tank conditions in three salinity concentrations.

Salinity (‰)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	7.03 ± 0.48 [a]	8.92 ± 1.58 [a]	0.444 [a]	83.33
10	7.07 ± 0.52 [a]	8.14 ± 1.46 [ab]	0.272 [ab]	83.33
20	7.09 ± 0.69 [a]	7.46 ± 1.37 [b]	0.094 [b]	87.50

[a, b] Means in a column followed by the same letter are not significantly ($P > 0.05$) different (Duncan's multiple range test).

DISCUSSION

Experiment 1: Growth and Whole-body Composition of *O. shiranus chilwae* (Lake Chilwa Strain)

O. shiranus chilwae (Lake Chilwa strain) required adequate time to convincingly demonstrate the effects of salinity on growth. Long-term exposure to fresh water (0‰ salinity) did not seem to result in their positive adaptation to that environment. Growth of fish in 10 and 20‰ salinity did not differ significantly ($P > 0.05$). From this study it appeared that 10‰ salinity concentration is isotonic to the blood of this species. Negative growth in fresh water (0‰) may be a reflection of the fish's earlier adaptation to the brackishwater environment of Lake Chilwa. The lowest whole-body fat, in 10‰, where growth was highest, may suggest that body fat was efficiently burned to provide fuel energy for growth, and this may have helped to spare body protein, which did not change at the end of this experiment. The effect of salinity on reproduction was not conclusive in all the experiments conducted in this study. Two possible reasons for this may be cited: either the experimental period was too short, or the sizes of the experimental animals were still very small. Visual observation of the reproductive products clearly showed that the eggs were still very small. It was difficult to distinguish ovarian size differences due to treatment effect. The most important observation in this experiment was that *O. shiranus chilwae* (Lake Chilwa strain) should be recommended as one of the most ideal candidate species for stocking brackishwater ponds that may be developed in saline soils. Raising this fish in fresh water would be analogous to subjecting it to a reverse acclimatory process, from saline water to fresh water.

Experiment 2: Response of *O. shiranus chilwae* (Lake Chilwa Strain) to Salinity Stress

The results of this study suggested that if the fingerlings of *O. shiranus chilwae* (weight = 2.0 to 16.0 g) are introduced into 86% seawater, they will most likely die within a maximum period of 3 h at the experimental temperature recorded in this experiment. Since the water in the experimental tanks was adequately aerated, anoxic conditions could not be implicated in fish mortality. The pH of the water was about 7.5 and was considered normal for cichlids. Floating of the younger fish on direct transfer into 30‰ salinity could not be considered as an early indicator of mortality, but simply the failure of the fish to

Table 10. Changes in whole-body composition of *Oreochromis shiranus shiranus* cultured under laboratory tank conditions in three salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	89.80	61.28	14.40	21.30
0‰	66.68	49.18	16.44	16.40
	(–23.22)	(–12.10)	(2.04)	(–4.90)
10‰	87.60	55.23	15.92	16.00
	(–2.20)	(–6.05)	(1.52)	(–5.30)
20‰	88.60	52.39	14.08	16.91
	(–1.20)	(–8.89)	(–0.32)	(–4.39)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

swim in their normal position in a dense saline environment (30‰). Earlier studies have shown that premature transfer of red tilapia juveniles to seawater can impair their survival (Watanabe et al., 1990). In the present study, salinity tolerance also varied ontogenetically in *O. shiranus chilwae* (Lake Chilwa strain) with larger individuals (average weight = 15.22 g) dying much earlier than smaller individuals (average weight = 2.36 g) following direct transfer to the same 30‰ salinity concentration. This disparity in salinity tolerance between the two sizes may be due to a larger surface:volume ratio in the smaller fish. It is possible that the younger individuals took comparatively small quantities of ions through their normal respiration. Their higher metabolic rates may have helped them to process the ingested salt faster than in larger fish.

In larger fish (average weight = 15.22 g), the individuals may have taken in large amounts of water through respiration and in the process flooded their systems with comparatively large amounts of salt, which needed to be cleared fast through the chloride cell intervention in the brachial epithelium. This may have demanded a lot of energy for osmoregulation at the expense of energy requirements for maintenance and homeostasis. This study therefore suggests that it would take longer to intentionally eradicate smaller fish of *O. shiranus chilwae* by taking advantage of their osmotic dysfunction. Watanabe et al. (1985) studied the effect of ontogeny on salinity tolerance in three taxonomic groups of tilapia. They used $MST\text{-}_{96}$ and $ST\text{-}_{50}$, in which $MST\text{-}_{96}$ was defined as mean survival time over a 96-h period following direct transfer from fresh water to full seawater. $ST\text{-}_{50}$ was defined as the time at which survival fell to 50% following direct transfer from fresh water to full seawater. Based on these indicators, Watanabe et al. (1985) were able to detect distinct age-specific differences in salinity tolerance in juveniles of *Oreochromis aureus*, *O. niloticus*, and an *O. mossambicus* × *O. niloticus* hybrid. In the present study, we used $ST\text{-}_0$, a modification of $ST\text{-}_{50}$, with $ST\text{-}_0$ being defined as the time at which survival fell to 0% as was the case in the present study.

Experiment 3: Growth and Whole-body Composition of *O. shiranus chilwae* (Bunda College Strain)

Growth of *O. shiranus chilwae* (Bunda College strain) was not significantly influenced in proportion to salinity, and growth differences were not significant ($P > 0.05$). In general, whole-body fat was depleted in all treatments at the end of the experiment, suggesting that this species was able to utilize body fat as a source of energy and to spare body protein. In this study body protein increased in all treatments, suggesting that it was not used to supply energy for maintenance even in the highest salinity treatment. The highest loss in fat was observed in the highest salinity treatment (20‰), where extra energy demanded by fish in this treatment may have been met by burning the available fat to release the required energy for maintenance. This species showed a lower salinity tolerance than *O. shiranus chilwae* collected directly from Lake Chilwa. The study suggested that *O. shiranus chilwae* (Bunda College strain) could be another potential candidate for stocking any available brackishwater pond constructed on a piece of saline land.

Experiment 4: Response of *O. shiranus chilwae* (Bunda College Strain) to Salinity Stress

In a salinity challenge test in 30‰ salinity, larger fish died a little earlier (1 h 5 min) than smaller fish (1 h 15 min). This observation is similar to the other experiments.

Experiment 5: Growth and Whole-body Composition of *T. rendalli*

In this study, *T. rendalli* was adversely affected by salinity, suggesting that the species would not be considered as a potential candidate species for productive brackishwater aquaculture in Malawi. Although the species is reported to be isosmotic at 10‰ (Whitfield and Blaber, 1976), its failure to grow faster at this salinity concentration than at 0‰ salinity may have been caused by other factors (excluding temperature) that limit growth. Maximal salinity tolerance is also reported to be achieved by this species when the temperature range is 20 to 29°C. In the present study, the temperatures ranged from 22.90 to 27.0°C, which was within the range conducive to normal physiological reactions in *T. rendalli*. Rapid growth in 0‰ may have indicated the ability of this species to efficiently utilize body protein as a source of energy. The observation that *T. rendalli* succumbed to 20‰ salinity much earlier in this study agrees with earlier reports (Whitfield and Blaber, 1976) that the maximum salinity that *T. rendalli* tolerates is 19‰. There was a decrease in carcass protein and an increase in carcass fat at the end of the study, suggesting that *T. rendalli* derived most of its energy from protein rather than fat. This is different from *O. shiranus chilwae* (Bunda College strain), in which fat was depleted at the end of the study rather than protein, suggesting that it was fat that provided energy to *O. shiranus chilwae* for maintenance and growth. This experiment showed that salinity levels in the range of 20 to 30‰ would effectively limit the range of *T. rendalli* in the natural waters of countries in the Southern African Development Community (SADC) region. The experiment has further shown that *T. rendalli* is the least salt-tolerant among the five taxonomic groups studied and may not be recommended for brackishwater aquaculture at a given temperature range.

Experiment 6: Response of *T. rendalli* to Salinity Stress

Death of *T. rendalli* on direct transfer to 30‰ salinity occurred after only 50 min. This is in agreement with the important observation that *T. rendalli* cannot be recommended in Malawi to stock brackishwater ponds. A comparison between salinity tolerance of *T. rendalli* and that of *O. shiranus chilwae* would only be meaningful here if individuals of both species of the same size were subjected to the same salinity. In this study the latter species succumbed to 30‰ salinity after 60 min, which may not be considered to be much different from the 50 min that elapsed before *T. rendalli* died.

Experiment 7: Growth and Whole-body Composition of *O. karongae*

In this experiment growth of *O. karongae* was significantly ($P < 0.05$) higher in 10‰ salinity water than in fresh water (0‰), suggesting that 10‰ may be isotonic to the blood of *O. karongae*. The results also seem to indicate that *O. karongae* quickly converted the diet into carcass fat since there was a significant accumulation (gain) of carcass fat in this species at the end of the experiment. A decrease in whole-body protein in the 10‰ treatment also suggests that the fish utilized whole-body protein as a source of energy for somatic growth, while body fat was little used for the same purpose.

Experiment 8: Response of *O. karongae* to Salinity Stress

Salinity tolerance of *O. karongae* at two distinct sizes was inconclusive. We were unable to get fish of 15 to 20 g average weight to compare with small size fish. In a practical situation, one would not expect *O. karongae* of the size used in this experiment to survive in 30‰ beyond two hours.

Experiment 9: Growth and Whole-body Composition of *O. shiranus shiranus*

O. shiranus shiranus grew best in fresh water, where they increased carcass fat most. This is another species that may have mainly used carcass protein as a source of energy. Considering a slight increase in the fat content at the end of the study, it may be suggested that the fish also used some carcass fat as an additional source of energy for osmoregulation.

Experiment 10: Response of *O. shiranus shiranus* to Salinity Stress

This experiment suggests that *O. shiranus shiranus* grow faster in fresh water than in a saline environment. Some of these salinity preferences may have been influenced by temperature preferences. All experiments were conducted at room temperature within a single rainy season. It has become clear that most of the fish studied cannot tolerate 30‰ salinity for a period of more than two hours at the room temperature that was recorded. The exact levels of tolerance would have to be redefined at each temperature within a species temperature tolerance range.

ANTICIPATED BENEFITS

In landlocked Malawi, we have only freshwater aquaculture at both small-scale and semi-commercial levels with a weak base of knowledge about the environmental requirements of and best management practices for many of our cultured species. The predominant species that are cultured are the ones used in the present study. This study has contributed to our knowledge base about these species, showing that at least three of them—*O. shiranus chilwae* (both Bunda College and Lake Chilwa strains) and *O. karongae*—are potential candidates for brackishwater aquaculture in Malawi. Use of these species for productive brackishwater aquaculture would be beneficial as Malawi's marginal lands would contribute significantly to: 1) increased food production and security; 2) improved human health; 3) improved income generation; and 4) increased total national fish production from aquaculture. Both *T. rendalli* and *O. shiranus shiranus* demonstrated the best growth in fresh water, and these two species would not be recommended for brackishwater aquaculture. In this study a water body on a piece of land where the soils are saline is assumed to become saline (brackish). The species that was found to be least salt-tolerant is *T. rendalli*, so the range of this species would effectively be limited by salinity in the natural waters of countries in the SADC.

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REGIONAL OUTREACH IN AFRICA

Ninth Work Plan, Adoption/Diffusion Research 5 (9ADR5)
Final Report

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ABSTRACT

The goal of the Kenya Project's regional outreach activity has been to promote contact and communication among aquaculture research and extension personnel and organizations throughout the region. This was originally intended to be achieved mainly through participation at regional meetings and conferences, not only by presenting papers but also through participation in planning and organizing the meetings. It was hoped that such participation would 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 regional linkages among research and extension programs in the region. Several CRSP participants attended the Annual Conference of the World Aquaculture Society and the Annual Meeting of the PD/A CRSP in Orlando, Florida, in January 2001. This was followed by visits to research facilities at Auburn University and commercial operations in West Alabama.

INTRODUCTION

The intent of this activity has been to promote contact and communication among aquaculture research and extension personnel and organizations throughout the region. This was 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 could be shared, linkages with other African researchers could be established and future collaboration encouraged, and CRSP partners would be able to learn about research and extension efforts in other parts of the region. Regional meetings originally targeted for attendance included annual meetings of the Southern African Development Community (SADC) Inland Fisheries Sector Technical Coordination Unit and of the Fisheries Society of Africa (FISA), but other conference opportunities, including some outside of Africa were also attended when deemed appropriate.

The objectives specifically listed for this effort in the Ninth Work Plan were to:

1) Promote the dissemination of information emanating from PD/A CRSP research results;
2) Learn about fish culture practices and research priorities and activities in Kenya and neighboring countries in Africa; and
3) Encourage the establishment of regional linkages between research and extension programs in Africa. This activity continues from a similar activity (8KR5, "Regional outreach in Africa") in the Eighth Work Plan.

OUTREACH ACTIVITIES CONDUCTED

Opportunities for travel to and participation in meetings within the region were limited in the current reporting period, so we took the opportunity to fund travel for David Liti (Moi University Department of Zoology and a very active CRSP collaborator at Sagana Fish Farm) to attend Aquaculture 2001, the annual conference and exposition of the World Aquaculture Society (WAS) in Orlando, Florida; to participate in the Annual Meeting of the PD/A CRSP (also in Orlando); and to visit aquaculture programs and facilities at Auburn University (AU) and in other parts of Alabama in early 2001. Although their travel was funded through other CRSP activities, Liti was joined by Nancy Gitonga, Director of Fisheries for the Kenya Fisheries Department, and Charles Ngugi, of the Moi University Department of Fisheries, for much of this travel. At the WAS meetings in Orlando, they were able to hear presentations on a variety of topics by aquaculture researchers from around the world, to meet with and interact with many of those researchers, and to see the kinds of supplies and equipment available for aquaculture displayed by the many industry exhibitors present. Liti presented a poster based on CRSP research at Sagana Fish Farm (9FFR2) entitled "Growth performance and economic benefits of *Oreochromis niloticus* and *Clarias gariepinus* polyculture in fertilized tropical ponds" (co-authored by Veverica and Moi University graduate student Enos Mac'Were). By participating in the CRSP Annual Meeting, Liti, Gitonga, and Ngugi were able to meet CRSP researchers from other countries and to get a better feeling for how the CRSP functions and what some of the other CRSP projects are about.

Following the meetings in Orlando, the Kenyan researchers were able to spend almost a week in Alabama, where they met with faculty members of the AU Department of Fisheries and Allied Aquacultures, observed research activities and farm operations at the AU Fisheries Research Unit, and visited commercial aquaculture operations in West Alabama, including two catfish farms, a fish processing plant, and a fish feed mill. During the week they also met with Kenya Project participants Veverica, Tom Popma, and Bowman for brainstorming sessions regarding past and future CRSP activities in Kenya.

ANTICIPATED BENEFITS

Participants in this activity, through presentations given and contacts made, have furthered the dissemination of information stemming from PD/A CRSP research. Through their contacts with researchers and extension personnel in Kenya, in the region, and overseas, they have learned about fish culture practices and research priorities, and they have gained a better understanding of research and extension needs. Application of the knowledge and experience gained from this activity will result in improved research-extension linkages in Kenya and the region. 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.

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PRODUCTION OF IMPROVED EXTENSION MATERIALS

Ninth Work Plan, Adoption/Diffusion Research 6B (9ADR6B)
Final Report

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ABSTRACT

Experimental results accumulated over two years of study indicate that a number of methods are available by which farmers can minimize the cost of feeding tilapia grown in ponds in the Philippines, with no adverse effects. Farmers have been clearly impressed with these results and the graphic demonstration of the potential to expand profit margins without adversely affecting fish health or uniformity. The distribution of extension brochures will allow the spread of this information more widely than is currently occurring (that is, by word of mouth, workshops, and newsletters). The inclusion of data and the contact information for project personnel will ensure the diffusion of useful technical details to tilapia farmers in and potentially beyond the Central Luzon region.

SUMMARY

A new extension brochure has been prepared for distribution to tilapia farmers in and beyond the Luzon Island fish farming area of the Republic of the Philippines.

The practical aspects of our research objectives, realized over the two years of work on the Ninth Work Plan Philippines Project, generated information of use to farmers in the Central Luzon area of the Philippines. Specifically, our results suggest a series of measures that can be used by farmers to reduce production costs without compromising yields, consequently improving profitability. These include reducing costs in the initial phase of grow-out (Brown and Bolivar, 2001), the use of sub-satiation feed levels (Brown et al., 2002), and the cost benefit of using only light application of fertilizers (Brown et al., 2001).

Each of these lines of work has been included in the presently reported extension effort. The results of the studies have already been accepted by farmers near Central Luzon State University (CLSU), both through word of mouth and by way of a highly successful series of workshops and meetings. Our new extension brochure has been prepared, which will allow broader dissemination and consequently enhanced impact of these results. An illustrated brochure has been designed and drafted, and it will be made available prior to the end of our contract (presently operating under a no-cost extension).

ANTICIPATED BENEFITS

Area farmers are turning increasingly to CLSU for technical guidance; a recent Farmer's Day Event at the CLSU Freshwater Aquaculture Center had a much greater than expected turnout, with 60 farmers, 50 students, and about 30 faculty and staff from the Bureau of Fisheries and Aquaculture Research, CLSU, and other agencies in attendance. It appears likely that the brochures produced in the course of meeting this objective will reach hundreds of farmers, and the receptiveness of area farmers to new ideas generated at CLSU suggests that a significant portion of these farmers will implement what they learn from the brochures.

Note: The brochures have been sent to CLSU for revision, approval, and distribution to area farmers. They will be made available to the PD/A CRSP Management Entity at the end of our contract period, as discussed previously.

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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)
Final Report

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ABSTRACT

The project focused on identifying and developing methods to create an enabling environment for sustainable development of aquaculture in Honduras. Honduras has a large network of nongovernmental organizations (NGOs) operating at the village level, an exceptional educational institution in Zamorano with commitment to extend training and knowledge in aquaculture, and an established in-country sustainable-development electronic network operated by Red de Desarrollo Sostenible–Honduras (RDS-HN). We developed the concept of training the trainers (NGOs working with farmers at the village level) by bringing together Zamorano and RDS-HN and developing a Web-based Information Delivery System for Tilapia (WIDeST). In this approach WIDeST captures the already-developed electronic information technology network and capacity of RDS-HN while providing easy-to-use information developed by Zamorano. Furthermore, it provides a way to connect local NGOs, farmers, and decision-makers so they can exchange information and make informed decisions. The WIDeST provides information on tilapia production and related topics, natural resources of Honduras, contact information for NGOs, and chat-room facilities for conducting virtual forums and discussions. The email facility enables the user to ask questions and get answers from an expert. Since its inauguration session in March 2001, the website has had more than 6,800 hits, and more than 300 individuals have formally registered to receive information. The participants at training and workshop sessions have found this to be an easy and useful approach, and they have provided strong encouragement for adding new information. The number of individuals already reached, as evidenced from the numbers of visits to the website, strongly indicates that this may be a way to build the capacity of local institutions to develop an environment that enables farmers to adopt aquaculture as an alternative on their farms.

INTRODUCTION

The concept of an enabling environment has been identified as a key prerequisite for sustainable aquacultural development (Shehadeh and Pedini, 1997). Experiences in natural resource management initiatives for the hillside regions of Latin America (CIAT, 1997) suggest that creating partnerships among stakeholders involved in managing or using natural resources is part of the process of fostering an enabling environment. Another aspect is to adopt an integrated decision-making framework for use in such environments (Nath et al., 1999).

Implementing small- and medium-scale aquaculture on a widespread and sustained basis is a long-term process (Harrison, 1991; Molnar et al., 1991). Consumer demands and dietary preferences are not obstacles to fish culture in Honduras, nor are sales problems necessarily a reason for abandoning ponds. Tilapia is widely accepted as a consumer item. In a 1996 survey, a majority of Honduran farmers noted "my understanding" as the major obstacle to obtaining larger harvests from their ponds (Molnar et al., 1996). Thus, there exists an opportunity for sustainable development of small- and medium-scale aquaculture in Honduras.

The "Red Nacional de Acuicultura" (National Aquaculture Network) created by the Food and Agriculture Organization (FAO) in 1992 was an effort to integrate international institutions and the private and public sectors of Honduras. Among the participants were Dirección General de Pesca y Acuicultura (DIGEPESCA), Universidad Nacional Autónoma de Honduras (UNAH), Escuela Agrícola Panamericana El Zamorano, Escuela Nacional de Agricultura (ENA), Agricultural School John F. Kennedy, Peace Corp Honduras, Federación de Productores y Exportadores (FPX), Asociación de Acuicultores de Honduras (ANDAH), and Instituto Nacional de Agricultura (INA). In place for about a year, FAO organized the network with the intention of eventually withdrawing in favor of Honduran management. Unfortunately, leadership problems caused most organizations to suspend participation, and FAO moved on.

We realized the need for a systematic method for enabling communication to reawaken the dialogue. In this context the University of Georgia and its collaborators from Zamorano and Auburn University identified that a successful approach for developing is one in which the following three already existing elements in Honduras are brought together for effective communication and organized decision-making.

1) Host Country Nongovernmental Organizations (NGOs). Currently numerous NGOs in Honduras are extending advice to small- and medium-scale farmers and are keenly interested in providing information on aquaculture systems. However, they need information and educational materials.
2) Escuela Agrícola Panamericana El Zamorano is a well-established academic institution with outstanding programs in agricultural sciences. In addition to educating students, Zamorano has active programs for and a commitment to extending knowledge to local farmers.
3) The Red de Desarrollo Sostenible–Honduras (RDS-HN). RDS-HN was created with an initial grant from the United Nations Development Programme (UNDP) 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. The RDS-HN was among the first to establish a network and now provides Internet services to over 700 customers and hosts numerous websites in such areas as forest and natural resource systems. Similar SDNs have been created in other Latin American countries, e.g., Costa Rica, Dominican Republic, Nicaragua, Panamá, Guatemala, Haiti, México, Guyana, Bolivia, and Colombia. Together these SDNs can constitute a formidable information network to facilitate contact and exchange among farmers, government organizations, NGOs, and private entrepreneurs.

Thus, we believe there is a unique opportunity for capacity building and institutional strengthening for aquaculture in Central and South America by providing an enduring method that integrates NGOs, Zamorano, and RDS-HN into a team. In this team Zamorano leads in providing current knowledge on aquacultural systems; RDS-HN leads in making the knowledge accessible to the users via electronic information technology; and the NGOs use the knowledge to educate and advise small- and medium-scale farmers on aquacultural systems. In other words, the task in this project was to identify and implement those methods that will provide information to small- and medium-scale fish farms to ensure that they can be sustained as productive enterprises in Honduras.

The objective of this project was to create an enabling environment for developing linkages among organizations and to build institutional capacity for providing information expeditiously to small- and medium-scale farmers for sustainable development of aquaculture. This report presents a new approach with the use of electronic information technology for developing a Web-based Information Delivery System for Tilapia (WIDeST). The complementary training sessions provided information to NGOs and farmers about aquacultural systems and introduced them to the use of WIDeST. This approach diminishes the dependence of small- and medium-scale farmers on technical assistance from outside sources. It will enable host country NGOs and private firms to provide services, and it will enable Zamorano to provide technical assistance locally. This approach could fortify the partnerships between Zamorano, RDS-HN, and host country NGOs and their ability to sustain aquaculture development in Honduras.

METHODS AND MATERIALS

Sample and Data Collection

The first meeting of the collaborative investigators took place in Honduras at the inception of the project to discuss the objectives and timetable of activities. The meeting was devoted to our understanding of local capacity and familiarizing ourselves with the host country activities. In Zamorano we toured facilities, met key faculty and administrators, and assessed outreach capacity of the institution. We visited 12 national and international NGOs, extension agents, governmental officials, and policy-makers to evaluate their interest and capacity in aquaculture. We visited Comayagua research station in El Carao, a site of earlier work supported by the PD/A CRSP, to evaluate the possibility of utilizing these facilities for training. And we visited small, medium, and large farms to understand their farming decisions and their perceived limitations for adopting aquaculture.

Based on the observations of the first meeting, a working session was set up with RDS-HN to discuss in detail the capacity of electronic information technology in Honduras and the role it could play to meet the project objectives. As a result of these discussions it was concluded that a method should be developed to make information available to local NGOs and extension agents and that they in turn train small- and medium-scale farmers in aquacultural systems. It was also concluded that the method should have features to receive questions and comments from farmers, NGOs, and others to identify stakeholder needs and provide timely responses.

Developing Web-based System

The method selected was to develop a WIDeST. The WIDeST entailed developing a partnership between RDS-HN and Zamorano for reaching out to NGOs, extension agents, and farmers via the website, focus groups, training meetings, and printed documents to increase awareness of the website as a source of information. Additional features to be included were capacity to conduct electronic meetings through a chat facility, a whiteboard for posting questions and observations for public viewing, and links to other significant websites with pertinent information on aquaculture as well as on resources in Honduras.

To receive input from stakeholders, a workshop was arranged in Zamorano with 87 participants who were directors and coordinators of NGOs, farmers, educators, representatives of government agencies, and decision-makers with interest in tilapia. An overview of the concept of WIDeST was presented, and the participants provided inputs identifying the content and needs for making this method successful. Also, a questionnaire assessed the interest and judgment of stakeholders on the web-based approach in general and WIDeST in particular. The response was highly supportive.

RDS-HN, in collaboration with Zamorano and project investigators, was engaged in developing the WIDeST. A formal announcement of the website and exposure to the decision-makers was also planned. The target time was set for the first quarter of the 2001, followed by a formal training session on the use of the WIDeST towards the end of the project in July 2001. It was anticipated that during this project, the website will become useful but much improvement will be needed in having more complete information about tilapia, pond design, methods of assessing availability of water and other resources, and ease of use. Also, a lack of time and resources may hamper our ability to make available in Spanish some critical materials currently available only in English.

RESULTS AND DISCUSSION

The beginning project meeting with all co–principal investigators in Honduras led to the following observations:

- There is a large network of NGOs in Honduras operating at the village level.
- These NGOs do not have good communication among themselves and linking them could increase the effectiveness of their work.
- Many NGOs are interested in adding technical assistance capabilities in tilapia culture.
- NGOs and governmental policy-makers are interested in water, water harvesting, and hillside stabilization, which directly impact aquaculture development.
- The Comayagua research station in El Carao can be an appropriate site for training NGOs technicians and extension personnel.
- The current limited capacity of fingerling production is an impediment to aquaculture development.
- Women and children play key roles in farm families and are key to aquaculture development.
- Home consumption and local markets are primary outlets for small-scale aquaculture.
- There is a need for a manual with simple instructions for pond siting, design, and construction for local use.
- RDS-HN, with its electronic information technology network, can be an important NGO in developing communication among various extension agents and decision-makers.
- Innovative methods for delivering information are needed that are developed through significant input from the stakeholders and that permit informed decision-making at the local level.

These observations and the follow-up discussions with RDS-HN helped us reach the conclusion that using the electronic information technology capacity of RDS-HN and the excellent educational capacity of Zamorano in partnership can be an effective way to develop aquaculture in Honduras. This approach will also enable local NGOs to develop aquaculture and institutional capacity of the host country.

A website has been developed and is hosted by RDS-HN. It can be accessed at <acuacultura-ca.org.hn>. The following welcome statement conveys the overall purpose of the website, the collaborators, and the source of support.

> *Acuacultura CA is the result of an important collaboration among several universities and the Sustainable Development Network Honduras. Our purpose in establishing this interactive website was to provide a versatile linkage point to assist NGOs and individuals to attain success in small-scale fish culture projects utilizing low-cost inputs.*
>
> *The materials presented in the website are from diverse sources. They have been selected with the objective of providing information comprehensible to persons with some training in the agricultural and natural sciences, possibly beginning fish culturists.*
>
> *In addition, the website offers the possibility to establish a fluid communication between persons with an interest in learning about fish culture and experts in the different fields of aquaculture. The universities collaborating on this work are: the University of Georgia and Auburn University, both of the USA, and Zamorano in Honduras. The principal source of financing for this website comes from the Pond Dynamics/Aquaculture Collaborative Research Support Program of USAID.*

The website is organized to provide information on tilapia in the following 11 categories. These categories may change as more is learned about the needs of the farmers and decision-makers. Currently more than 100 documents are available. Also an Excel-based pond design model developed in another activity of this project provides users the ability to estimate the watershed size, the available water based on local rainfall estimates, and the design of pond for their local conditions.

1) Ponds   Includes information on methods for assessing watershed and water availability, pond design, and management of ponds for fish culture.
2) Biology of Tilapia   Includes introductory materials on history, fish biology, and reproductive biology.
3) Fingerling Production   Includes information on fingerling production, sex reversal, transportation, and other related subjects.
4) Grow-out of Tilapia   Includes information on all aspects of tilapia production.
5) Pathology and Disease   Includes information on fish diseases and control methods.
6) Water Quality and Aquaculture   Has four subcategories in aquaculture, fertilization, fish culture, and polyculture.
7) Product Quality   Includes fish quality, processing, and controls.
8) Production Systems and Costs   Includes information on economics of fish production systems.
9) Tilapia and Development   Includes subcategories in host country policy and agreements, relevant projects and current research, and a description of WIDeST.
10) News and Events   Provides a place to make users aware of interesting information and useful upcoming activities.
11) Fish Gallery   Provides a location to present attractive specimens of fish for visual recognition and satisfaction.

The website provides a chat room to conduct meetings and exchange information. The project leaders used the chat room on several occasions to discuss the content of the website when participants were in two locations in Athens, Georgia, two locations in Auburn, Alabama, in Zamorano, and at the RDS-HN Tegucigalpa location in Honduras. The real-time conversations provided an excellent means to interact. This facility will be useful for holding stakeholder meetings and discussions with experts and decision-makers. It will not only provide information on tilapia but will also permit users to communicate those needs that hamper the development of aquaculture.

The WIDeST was formally inaugurated on 3 March 2001 on the Zamorano campus. The participants included representatives of government agencies and NGOs, farmers, extension agents, and educators from Zamorano. The activity included an explanation of the WIDeST and a description and demonstration of the website, a hands-on exercise for the participants, and a chat session in which participants asked questions and an expert responded in real-time. The participants quickly learned the use of the website as well as the use of chat-room facilities. The participants, without exception, provided great encouragement to move faster in this direction and include materials that will be useful to commercial farmers. However, this project is focused on small- and medium-scale farmers, and this need can be met only with a more focused support perhaps in a separate but complementary project.

Since its inauguration, the website has had more than 6,800 hits, more than 300 individuals have registered to access documents available to registered guests only, nearly 25 emails have been sent to the webmaster, and nearly 30 emails with questions have been sent to experts. This is a reasonable response at this time with little publicity and general awareness of the website. The final meeting with stakeholders in August to be held in conjunction with the Sixth Central American Symposium on Aquaculture in Tegucigalpa will provide an opportunity to describe the project and the web-based system. The scheduled hands-on exercises and the exposure to the conference participants will add to our understanding and assessing the value of a web-based approach for institutionalizing aquaculture in Honduras as well as in other developing countries.

CONCLUSIONS

An enabling environment for sustainable aquacultural development will require a partnership between Escuela Agrícola Panamericana El Zamorano and the national and international NGOs in Honduras. In this partnership Zamorano will lead in providing scientific and technical information through training workshops and literature for the electronic information technology network for NGOs, decision-makers, and farmers. The NGOs will then work directly with small- and medium-scale farmers to develop aquaculture on their farms. This "training the trainer" concept is being institutionalized through a web-based information exchange system. In this system, information from the farmers is also shared with other farmers and users. Furthermore, this system will enable the identification of needs for and impediments to the sustainable development of aquaculture in Honduras and perhaps in developing countries throughout Central and South America.

In this project, the WIDeST was developed with a host country NGO, RDS-HN, and is accessible at <acuacultura-ca.org.hn>. This website has already received much attention. Furthermore, the participants at the training workshops and inauguration events provided enthusiastic encouragement. Since developing countries lack conventional communication and transportation infrastructures, the electronic communication network is a powerful way to bridge the gap. Providing information through the web to local NGOs, extension agents, and decision-makers helps them make informed decisions. Access to information and an ability to make informed decisions are fundamental to building the capacity of local institutions. The work in this project is beginning a new approach that appears very feasible. Additional work and longer experience with this approach are needed before its impact can be fully measured.

ANTICIPATED BENEFITS

The partnership between Zamorano and RDS-HN is expected to enable host country NGOs to increase their capacity to train farmers in aquaculture development. The web-based system will increase communication among NGOs, decision-makers, farmers, Zamorano, and other researchers. This will increase our capacity to provide useful information to farmers. Also, needs for developing the enabling environment for developing aquaculture in Honduras will increase. Finally, this work could serve as a model for other Central and South American countries to utilize their in-country Sustainable Development Networks (SDNs), which were established with an initial grant from the UNDP.

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PRODUCTION STRATEGIES CHARACTERIZING SMALL- AND MEDIUM-SCALE TILAPIA FARMS

Ninth Work Plan, Adoption/Diffusion Research 8 (9ADR8)
Final Report

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ABSTRACT

This report examines samples of farms from Honduras departments that have and do not have tilapia ponds as part of their farming systems. Data were obtained through personal interviews with 128 farmers, including 64 tilapia producers, in five departments: Olancho, Intibuca, El Paraíso, Francisco Morazán, and Santa Bárbara.

To obtain information about farms without tilapia, farmers were selected at random from within the same community as the identified tilapia producers. Interviews were conducted in communities where the small-scale farmers with production of tilapia were located. The data are intended to constitute a representative sample of the population of the Honduran small-scale aquaculture farmers in these departments. The analysis presents basic comparisons of landholding, farm, and personal characteristics of tilapia producers with the mirror sample of the farmers without tilapia. The analysis profiles basic differences between the two categories of farms, the operators, and their households. Younger farmers were more likely to become involved with tilapia farming. Those farmers more dedicated to their work inside their farm from which they obtain all their income, and whose principal occupation is farming, were more inclined to adopt farming of tilapia. Farmers who use their land more intensively and who dedicate themselves more to the farming of basic grains were more likely to adopt the farming of tilapia. Since Honduran small-scale farmers tend to be a depressed segment economically, they tend to satisfy first their subsistence necessities by maximizing the use of their resources. The financing for both tilapia growers and nongrowers tends to be a limiting factor because more than 80% of the population work without financing, a clear barrier to farm investments. Tilapia growers participated more in development projects.

INTRODUCTION

Rural people in Honduras constitute almost 61% of the total population (Barham and Childress, 1992; Stonich, 1993). They have little access to basic development goods—food, shelter, potable water, sanitation systems, education, communications, roads, and markets (Rosero, 1997). Eighty percent of all rural people live in poverty. Sixty-six percent of farmers who produce basic grains, the country's staple food, have access to only eight percent of all cultivable land. This 66% operate, on average, slightly more than one hectare of land to secure a year's supply of basic grains to feed a family with approximately six children and to produce a surplus for the nation. Given the high levels of poverty in Honduras, it will be particularly important to attend to the problems that small- and medium-scale farmers have in realizing the cash potential of their tilapia crop (Green et al., 1992, 2000).

The farming of tilapia was introduced in Honduras during the 1970s. The production of tilapia at that period was primarily small-scale (Teichert-Coddington and Green, 1997; Arias et al., 1998). Originally it was groups of families that were the primary participants in tilapia farming in an extensive or semi-intensive form. It was done as a supplementary activity inside their processes of agricultural production. In 1995 it was estimated that a total of 113.6 ha belonging to small-scale family farmers were operational in Honduras. However, the exact number of small-scale producers is not known (Sarmiento and Nuñez, 1995). Export-oriented production of tilapia began in 1990 and has had rapid growth in Honduras since then (Teichert-Coddington and Green, 1997). In 1997 there were 15 commercial farms of tilapia with a total water surface of 185.3 ha, which were producing for export and national markets. The Honduran export of tilapia to the United States has grown consistently since 1992 (Ponce, 1986; Cerezo, 1993).

To illustrate the problems farmers face in rural Honduras, Centro Internacional de Agricultura Tropical (CIAT) researchers' account of Yoro, in Central Honduras, is helpful. The principal commercial distribution channel is the intermediary or coyote. Such persons generally do not live in the community but instead travel from San Pedro Sula, Morazán, El Progreso, El Negrito, Comayagua, Siguatepeque, or El Salvador. Sometimes the intermediary provides equipment services at high prices and finance at high interest rates. Although there are ponds and aquaculture activities in Yoro, this activity is not described in the CIAT account. The most important marketing problems facing rural producers center on price, followed by the availability of opportunities to sell their product on a regular basis (Abbot, 1993; Molnar et al., 1996). Rural producers in Honduras face particular difficulties due to setbacks from periodic hurricanes (UNDP, 1999), difficult terrain, poor road systems, and fragmentation in the rural sector (Arriaga, 1986; Engle, 1997).

Although tilapia can be a source of steady income, the enterprise is not likely to generate rapid or large profits. Producers holding exaggerated expectations tend to define normal results as disappointment or failure. Thus, some of the negative sentiment about tilapia in Honduras stems from unrealistic views of the rate of adoption and impacts of tilapia production (Molnar and Lovshin, 1995). Small- and medium-scale farmers may more profitably rely on strategies such as pond bank sales, partial harvesting for local delivery to restaurants or markets, or other niche arrangements that reflect situational opportunities.

At present there are in existence organizations and institutions that are working to support the development of the production of tilapia on a small scale in different zones of the country. The support is financed by international agencies and is oriented towards small-scale farmers. The support for medium-scale farmers also exists. These organizations have tried to improve the livelihoods of small-scale farmers by implementing and promoting tilapia enterprises in their farm systems, as well as providing a means to improve the diet of their family members.

In Honduras, many efforts to promote the farming of tilapia have been developed by various development agencies. In addition to the PD/A CRSP, the Christian Commission for Development, the Program of Rural Reconstruction, Proyecto Guayape, and El Instituto Nacional de Información Profesional are prompting the small-scale production of tilapia as an alternative system of production to improve the diet in the families of small-scale farmers. However, up to now there has been no detailed characterization of those small-scale farmers willing to adopt the farming of tilapia inside their production systems. In this sense, the efforts of promotion may have been less effective by not having good information about the target category of producers. The present work focuses on the investigation of socioeconomic conditions of small-scale farmers in Honduras as its initial step in the development of a more effective program of extension and production.

The objective of this study is to compare socioeconomic characteristics of small-scale farmers, with and without a tilapia production system, as one means for understanding the adoption of tilapia farming. The purpose of the research is to identify the social and economic conditions that distinguish small-scale farmers who have incorporated the production of tilapia inside their system of production from those who have not.

METHODS AND MATERIALS

Given the lack of a national census of the producers of tilapia in Honduras, a partial census managed by specific developmental programs was used as a sampling frame for this study. The frame is incomplete and biased toward households and communities participating in NGO projects but is nonetheless representative of the total population. The projects that were considered were the following: Program of Rural Reconstruction, National Institute of Professional Development, Christian Commission for Development, Watershed Management Unit of the El Cajon Dam, and the Experimental Station of El Carao at Comayagua. Through these developmental agencies a list of tilapia producers was obtained. No other current list of producers, tilapia or otherwise, is available.

Data were obtained through personal interviews with 128 farmers, including 64 tilapia producers, in five departments: Olancho (Sta. María del Real and Juticalpa), Intibuca (Yamaranquila), El Paraíso (Danlí and El Paraíso), Francisco Morazán (Lizapa and Galeras), and Santa Bárbara (Las Vegas).

To obtain information about farms without tilapia, farmers were selected at random from within the same community as the identified tilapia producers. Interviews were conducted in communities where the small-scale farmers with production of tilapia were located (Casley and Kumar, 1988). The data are intended to constitute a representative sample of the population of the Honduran small-scale aquaculture farmers in these departments. The analysis presents basic comparisons of landholding, farm, and personal characteristics of tilapia producers with the mirror sample of the farmers without tilapia.

RESULTS

Table 1 compares the characteristics of farm households with and without tilapia, presenting Chi-square statistics to suggest which differences are worth considering as significant. In some instances, an ANOVA F-test is reported in the text when an interval-level variable is considered.

Age

Age was considered a factor that could have had influential effect on the adoption of the culturing of tilapia. In the case of farmers with tilapia, the average age was 39.5 years, significantly younger that the 43.6 years mean age for those without tilapia ($F = 3.6$, $P < 0.057$). Although older farmers may have more capital, nutrition, and food security, younger tilapia farmers may be more amenable to new enterprises and perhaps more motivated to seek alternate uses of farm resources.

Gender

Although 60% of the sample was male, there was no difference in the rate at which men and women participated in tilapia culture. About half the respondents of each gender in the sample were tilapia growers.

Marital Status

Marital status was related to whether or not farmers grew tilapia ($\chi^2 = 9.8$, $P < 0.01$). The married farmers were more likely to grow tilapia. It can be said that those legally married families present more stable socioeconomic conditions, which made it easier for them to adopt a technology that would affect their system of production. This is why projects of development should focus their work on promoting the production of tilapia to those families with family stability.

Income

Tilapia farmers reported higher average annual incomes than non-tilapia growers (18,918 Lempiras vs. 17,811 Lempiras).

Table 1. Association between tilapia growing and selected personal, family, and household characteristics, Honduran farmers, 2001.

Characteristic	Number	Tilapia	No Tilapia
		%	
GENDER	(χ^2 = 0.533, P < 0.465, df = 1)		
Male	80	53	48
Female	48	46	54
MARITAL STATUS	(χ^2 = 9.8, P < 0.01, df = 4)		
Single	7	71	29
Married	94	48	52
Common-law Union	21	67	33
Widow	4	0	100
Single Mother	2	0	100
PRINCIPAL INCOME SOURCE	(χ^2 = 23.7, P < 0.001, df = 3)		
Agriculture	84	60	40
Food Shop	10	10	90
Day Laborer	16	6	94
Others	18	67	33
HOLD LAND TITLE	(χ^2 = 0.533, P < 0.465, df = 1)		
Titled	77	53	48
No Title	54	46	54
USE OF LAND	(χ^2 = 11.1, P < 0.01, df = 2)		
Annual Crops	94	47	53
Perennial Crops	24	42	58
Combination	10	100	0
PRINCIPAL OCCUPATION	(χ^2 = 3.0, P < 0.25, df = 2)		
Farmer	30	63	37
Housewife	41	49	51
Farmer/Day Laborer	57	44	56
SOURCE OF FARM LABOR	(χ^2 = 1.0, P < 1.0, df = 1)		
Family Only	106	50	50
Family and Hired	22	50	50
SOURCE OF FARM FINANCE	(χ^2 = 1.0, P < 1.0, df = 1)		
ONG	4	50	50
Bank	8	62	38
Cooperative	7	43	57
Self	106	49	51
Other	3	66	34
PRINCIPAL ENTERPRISE	(χ^2 = 5.5, P < 0.1, df = 2)		
Grains	88	49	51
Coffee	16	75	25
Others	24	38	63
PARTICIPATION IN PROJECTS	(χ^2 = 28.3, P < 0.001, df = 1)		
Yes	81	68	32
No	47	19	81

However, this difference was not statistically significant (F = 0.127, P < 0.7).

Principal Source of Income

Farmers were asked about their principal source of income: agriculture, day labor, and small food store business. Apart from this, other sources of income such as the sale of items sent from abroad, among others, were considered. The Chi-square test showed significantly more farmers with tilapia obtain their income principally from agriculture (χ^2 = 23.7, P < 0.001). Those without tilapia have more income coming from working as day laborers, and others depend on income from profits made from a small food store or tavern.

Agriculture is commonly the main source of income for farmers in Central and South America; they depend on it for their subsistence. A study carried out in Guatemala about small-scale farmers showed that the principal source of income is agriculture (Castillo et al., 1992). Meanwhile, the production of tilapia should be promoted to those whose principal income is from agriculture. In this study farmers with tilapia have less land, which is why they have more intensive management of their farm. At the same time, they are more productive and prefer to work their lands instead of going out looking for work, as is more often the case of farmers without tilapia.

Size of Farm

The amount of land available showed significant difference between tilapia-growing and non-tilapia-growing farms; consequently, the size of the farm could be a factor of influence in the adoption of the farming of tilapia. Farmers without tilapia had more land in comparison to those with tilapia; on average farmers with tilapia had 2.45 ha, and those without tilapia had an average of almost 3.5 ha (F = 4.8, P < 0.03). Those with tilapia had smaller farms. Apparently, the intensity of the use of the land is greater as the size of the farm diminishes. In this case, it is true that those farmers with small farms have a tendency to diversify their operations. Similarly, Castillo et al. (1992) found that Guatemalan farmers with less than 2 ha of land were more likely to farm tilapia.

Type of Crop

Tilapia farmers differed depending on the type of crop they planted. The majority of farmers with tilapia showed a combination of annual and perennial crops. This could be because they have less availability of land than the traditional farmer does, and they utilize it better for greater productivity for their survival. This type of combination observed in farms is one in which farmers plant corn and beans and have part of their land in coffee as a perennial crop.

Use of Land

The association between tilapia-growing and the use of the land was not significant (χ^2 = 11.1, P < 0.1). Nonetheless, tilapia farming development programs should focus on the farmers who have a combination of annual and perennial crops, since they are the majority of those who have adopted the system of tilapia. The previous result signifies that those farmers with a more stable and diversified system of production would be more willing to adopt new technology.

Principal Occupation

Dependence on farming as a principal economic activity is important in determining the likelihood of adoption of tilapia farming because farmers with more or less continued presence on their operation may have more time and inclination to attend to a farm pond. This variable was significantly related to tilapia growing ($\chi^2 = 11.1$, $P < 0.1$). Farmers with shops or labor employment were less likely to be involved in tilapia culture.

Principal Farm Enterprise

The nature of the main farm enterprise was not associated with tilapia growing ($\chi^2 = 5.5$, $P < 0.1$). Although more respondents who grew tilapia reported coffee as the principal activity of their farm, the differences were not significant. Perhaps not detected in these data, some link may exist between the conditions that are favorable for coffee production and those for fish culture; soil quality and rainfall sufficiency in particular may also be more conducive to fish culture. Generally coffee areas have at least somewhat fertile soils and sufficient rainfall to replenish ponds and the watersheds that supply ponds.

Project Participation

Development agencies such as missionaries, NGOs, and units of the Honduran government often conduct training programs and provide technical assistance intended to improve and diversify the livelihoods of rural producers. Often these activities feature fish culture among the array of alternatives that are supported. The data suggest that these efforts bear greatly on whether small-scale farmers become involved in the promoted technology. There was a strong association ($\chi^2 = 28.3$, $P < 0.001$) between culture of tilapia and participation in NGO projects.

Organized programs of technical and organizational assistance may influence the adoption of tilapia farming. For one, such endeavors may provide technical assistance for fish culture. Others may provide low interest loans or access to pond construction services that might not otherwise be available in a locale. Such projects are often a central audience for PD/A CRSP research results and technical assistance efforts. The training programs they organize and the regular contacts that NGO technicians have with fish farmers are important conduits of information about fish farming. PD/A CRSP scientist participation in technician and farmer training are central mechanisms for propagating research results.

DISCUSSION

Development agencies should take into consideration the findings of this study in their programs of extension and research, in the promotion of tilapia, and in planning for rural people. One central insight of the study is the need to consider the role of financing to small-scale farmers. Small loans can help producers acquire technology and infrastructure that can make a long-term contribution toward developing their well-being. The results show that the majority of farmers work with their own finances, and this is a limitation for them.

Honduras is a diverse country, but the several different sites chosen for investigation augment the durability or robustness of the findings. Nonetheless, it remains necessary to investigate the possibilities for tilapia production in other zones of the country in relation to the socioeconomic characteristics of small-scale farmers and the production strategies appropriate to their conditions.

ANTICIPATED BENEFITS

The results of this study provide additional guidance to the technology development and outreach efforts of the PD/A CRSP research program in Honduras. There is growing recognition of the need to focus and target outreach efforts that encourage farmers to undertake tilapia culture. Understanding how tilapia farmers differ from the general population of producers in terms of their personal and household characteristics, coupled with knowledge about appropriate soils and water-holding capacity, can lead to a more effective presentation and use of PD/A CRSP research results. Enhanced understanding of production barriers, distribution difficulties, and disincentives to participation in tilapia culture are important ingredients in efforts to assist farmers in selecting tilapia as a farm activity and in increasing their production. The project must understand and anticipate those factors that give farmers reservations about the benefits they will receive from new or reactivated fish ponds.

Fish culture has a future in Honduras. The government can help best by doing a few things well and otherwise staying out of the way where it does not have the resources or commitment to act effectively. The donor community can share information with each other and the farmers, paying particular attention to the small-scale sector. Our project can help the nongovernmental community to train its technicians and to develop its capabilities for the day when PD/A CRSP will not be in Honduras. Tilapia is something Hondurans will eat and something an identifiable segment of operators is capable of growing. The market will work if a broad array of public institutions, NGOs, and large-scale farms are enlisted to improve the distribution of information about opportunities to grow and sell tilapia in Honduras.

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TECHNICAL ASSISTANCE FOR FINGERLING PRODUCTION SERVING SMALL- AND MEDIUM-SCALE TILAPIA PRODUCERS AND TRAINING AND TECHNICAL ASSISTANCE FOR HONDURAS INSTITUTIONS WORKING WITH SMALL- AND MEDIUM-SCALE TILAPIA PRODUCERS

Ninth Work Plan, Adoption/Diffusion Research 9 and 10 (9ADR9 and 10)
Final Report

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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. The Zamorano principal investigator and his technician in this project confirmed that the Comayagua research station El Carao was not a reliable supplier of fingerlings for area producers. Private fingerling producers are few and generally geared to supply large-scale commercial operations. The overriding objective of activity 9ADR9 was to provide technical assistance and training to current and potential fingerling suppliers to small- and medium-scale tilapia producers in Honduras.

A 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.

In November 1999 we consulted with 13 representatives of national and international, government and nongovernmental organizations (NGOs). From these consultations a strategy and timetable were developed for implementing technical assistance and training for fingerling suppliers and technicians working with NGOs currently or potentially involved in small- and medium-scale fish culture development. At least 33 small- and medium-scale tilapia producers (each with 150 to 12,000 m^2 of water surface) and 26 restaurants were subsequently interviewed to assess the production and marketing demands for tilapia in Honduras. With the collaboration of a local NGO, representatives of NGOs with actual or potential interest in aquaculture development were invited to a one-day seminar to describe opportunities and constraints for family-scale fish culture in Honduras. The Zamorano team continues to identify and provide technical assistance to regional fingerling producers and organizations involved in aquaculture extension. During the life of this activity, three technical workshops were provided for actual and prospective fingerling producers and extensionists. More than 30 publications on fingerling production and pond management practices have been incorporated in a web-based information system developed by a local NGO, primarily in response to the needs of local NGOs.

INTRODUCTION

A critical issue in the development of tilapia farming in Honduras is fingerling supply. Molnar and Lovshin (1995) found that fingerlings were hard to obtain for many farmers, but fingerling supply was not a reason for withdrawing from fish farming. For many farmers in remote areas, fingerling transport may be difficult, costly, and hard to organize. These conditions underscore the importance of increasing the number of private fingerling suppliers, enhancing autonomous fingerling production among small-scale producers in remote locales, and stabilizing the public and nongovernmental sectors as broodstock suppliers. Given the historically uneven performance of the public sector, it is vital that private sources of seed stock become the foundation for the industry. The objective of this activity was to collaboratively assist in providing the technical information required to develop and strengthen small- and medium-scale producers of tilapia fingerlings.

A 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 technical expertise is often insufficient to provide critical information required for productive pond management. An analysis of previous fish culture development projects involving family-scale fish culture in Central America, where aquaculture is not well established, emphasized that such development projects require many years of effort before fish farming becomes a stable agricultural activity (Castillo et al., 1992; Lovshin et al., 2000). Interruptions in the development process have often led to disillusionment and a generalized rejection of fish culture by participants. Given the relatively short horizon of our project, we concluded that we must work collaboratively with local organizations with long-term vision and commitment to development. The second objective of this activity was 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.

METHODS AND MATERIALS

Planning

In November 1999 the principal investigators (PIs) from Zamorano, the University of Georgia, and Auburn University visited 13 directors and representatives from 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 for fingerling suppliers and NGO extensionists.

Implementation

In early 2000, 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 market demands for tilapia in Honduras. With the collaboration of a local NGO, the Zamorano PI invited representatives of NGOs with actual or potential interest in aquaculture development to a one-day seminar to describe opportunities and constraints for family-scale fish culture in Honduras. Based on this information exchange, these organizations could better decide about the appropriateness of fish farming in their development program.

In September 2000 a two-day fingerling production technical workshop was presented by Zamorano and Auburn PIs for approximately 20 actual and prospective fingerling producers. The workshop included an analysis of conditions and fingerling demands, formal presentations on production techniques, and roundtable discussions.

In March 2001 a one-day workshop for approximately 15 tilapia producers and NGO representatives was held at Zamorano to discuss potential production techniques and to develop an interactive mechanism by which the economic impact of pond management practices could be assessed. The biological production characteristics of a given management can reliably be extrapolated from results obtained in regions with similar climates, but profitability is highly variable due to variation in the value or cost of individual inputs and outputs. Spreadsheets were therefore developed for each potential management practice, in which production inputs and outputs were fixed but the per unit value or cost of each line item could be entered by the user. Economic conclusions were expressed as Return to Labor above Variable Costs and Return to Labor per Unit of Labor Expended.

Institutionalizing Access to Technical Information

In conjunction with another activity in this project and in collaboration with a local NGO, Red de Desarrollo Sostenible–Honduras (RDS-HN), more than 30 documents related to tilapia fingerling production and grow-out techniques for small- and medium-scale operations were posted on a Spanish-English website <www.aquacultura-ca.org.hn>. The documents include theses, manuscripts published by the International Center for Aquaculture and Aquatic Environments (ICAAE) at Auburn University, Southern Regional Aquaculture Center (SRAC) publications, and original documents prepared by project PIs.

RESULTS AND DISCUSSION

During the 1999 planning activity in Honduras that was attended by all project PIs, 13 officials from relevant organizations and entities were consulted, including the following:

Angel Carcamo	Global Village
Raquel Isaula	Director, RDS-HN
Raul Zalaya	World Neighbors
Mike Giles	CARE, Honduras
Carlos Zelaya	Director of the local office, Food and Agricultural Organization (FAO)
Carlo Elvir	Coordinator of Local Watershed Management, FAO
Marco Lopez	Former Minister of Agriculture, Honduras
Marco Polo Micheletti	Ministry of Agriculture (MOA), Honduras
Adalberto Sorto	Director General, National Development Program
Marc de Lamotte	National Director of Human Resource, CARE
Cesar E. Duron	Human Resource Manager, Honduras
Arthuro Galo	Coordinator of Development Projects, MOA
Dennis Sharma	USAID, Honduras

All officials who were visited expressed a commitment and willingness to collaborate in the development of small- and medium-scale fish culture in Honduras, but an overriding concern was the budgetary commitment for a long-term effort. The need for a team approach was obvious.

With the collaboration of one of the local NGOs visited (RDS-HN), the Zamorano PI invited more than 50 representatives of NGOs with actual or potential interest in aquaculture development to a one-day seminar to describe opportunities and constraints for family-scale fish culture in Honduras. Based on the informal discussions during and following this meeting, about 20 NGOs expressed their determination to incorporate aquacultural development in their program, provided their outreach personnel could receive adequate training.

A two-day fingerling production technical workshop was organized at Zamorano for approximately 20 actual and prospective fingerling producers. The workshop included an analysis of conditions and fingerling demands, formal presentations on fingerling and grow-out production techniques, and a roundtable discussion with participants. The technical competence of the participants was highly variable. A single workshop appeared to be sufficient for some participants, but follow-up sessions, including additional hands-on field experience, seemed appropriate for the majority of the participants.

In conjunction with another activity in this project and in collaboration with RDS-HN, more than 30 documents related to tilapia fingerling production (Popma and Green, 1990) and grow-out techniques (Bocek, 1989; Green et al., 1994; Popma and Lovshin, 1996; Meyer and Triminio, 2001) for small- and medium-scale operations were posted on a Spanish-English website <www.aquacultura-ca.org.hn>. The majority of the documents were posted from February through August 2001. The number of "hits" on the relevant documents as of 9 August 2001 was 398. The affiliation and objectives of the users and their perception of the usefulness of the information are as yet undetermined.

CONCLUSIONS

Personnel of the ICAAE and the PIs in this project have dedicated more than 110 person-years of effort to development projects in 97 countries. One lesson learned from these experiences was that the development of family-scale fish farming in regions where aquaculture is not a traditional farm activity has a higher failure rate than large-scale commercial aquaculture enterprises and cannot be accomplished in a few short years. Constraints faced by prospective farmers relate to infrastructure (roads, electricity, etc.), availability of production inputs, access to markets, economic status of both producers and potential consumers, business management skills, and timely access to technical information. The cost of pond construction (cash or family labor) requires that ponds be utilized intensively. To accomplish this, ponds may have multiple functions (fish farming, irrigation, integrated animal husbandry, etc.) or nutrient inputs can be increased. Feeds are used on most commercial farms but are physically or economically unavailable for most small, family-scale farms with little land. Fish feeds, when available and generally profitable, add a degree of economic risk often inappropriate for resource-limited producers with little business management skill. For farmers with little land, on-farm available nutrients (manure, agricultural by-products, etc.) are sufficient for only small ponds. The opportunity for cash income is a strong motivator for prospective small-scale producers, but transport of the fish harvest to market is often difficult, and potential consumers often lack the cash to purchase the product. In spite of these constraints, a fish-farming development program can lead to the most productive use of their small plots of land, provided the development effort is committed, competent, and sustained.

Constraints faced by a group promoting the development of family-scale aquaculture in a region where it is nontraditional are many. Low fish production from small, relatively isolated ponds makes an extension program very expensive in terms of cost per ton of fish produced. The transition of fish farming from a nontraditional to a traditional farm activity is slow during early development because cash-poor and overworked producers are reluctant to invest scarce resources in questionable enterprises. The cost-benefit ratio of the outreach effort during early development may prematurely break the will of sponsoring groups, resulting in a development failure and disillusioned producers who reject any subsequent fish-farming effort.

The assessment of the appropriateness of fish culture and the training needs of a development group require an understanding of the socioeconomic characteristics of the community and the cost and availability of potential aquaculture inputs. The selection of appropriate culture species and management practices are critical. Contrary to operations utilizing nutritionally complete feeds, the species selected for most small, family-scale farms must be able to effectively utilize natural food organisms to supplement the nutritional deficiencies of agricultural by-products and on-farm available nutrients. Transportation and economic constraints also impose the eventual need for regional independence of fingerling supplies. This implies the selection of a species and production techniques appropriate for many local producers.

Information is often limiting on the biologically and economically most appropriate production strategies. Experimenting with resource-limited farmers in the target region is a dangerous development strategy because it often places them at unacceptable risk, and a failed experimental management practice often leads to a generalized rejection of fish farming. Expected production results can often be accurately extrapolated from experiences in other regions with similar climate and conditions, but economic results may be highly variable because of regional differences in input costs and the market value of the fish. Pond management practices should not be promoted until adequate information is available on the biological and economic benefits. Successful fish-farming development projects require a team effort. Local NGOs often have long-term vision and mid-level extensionists with generalized agricultural and development skills but little specialization in fish culture. Specialized training and case-specific research is often provided by another group. Training must be cost-effective, dynamic, and responsive to developing questions.

This CRSP project does not have the long-term horizon required to undertake fish-farming development in regions where there is little previous tradition. Our objective was to assist in "training the trainers," those individuals with direct contact with prospective producers and with long-term commitment to the community. The mechanisms for accomplishing this included using case-studies to assess conditions and to research incompletely understood management practices, offering short-courses, and providing information to incorporate into a dynamic and interactive web-based network.

ANTICIPATED BENEFITS

The described anticipated benefits of these two activities were improved quality and quantity of fingerlings for the private sector, including small- and medium-scale producers, and an increased understanding by NGO technicians and independent producers of the benefits and constraints of low-input tilapia farming. A high percentage of outreach personnel working in rural development often have an understanding of the overall needs of their target constituents and a broad, general knowledge of many potential agricultural enterprises. However, they often have a poor understanding of fish farming, leading to unrealistic expectations or offhand rejection of the potential benefits of this activity. In regions where fish farming is nontraditional, the benefits of training the trainers can vary from a simple rejection of an outreach effort where inappropriate to a slow transition of fish farming into a profitable and widely accepted farm activity. A historic analysis of such development efforts worldwide reveals mostly failures, especially where efforts were not sustained for several years.

However, the high risk of failure is balanced by the understanding that the target group is faced with the greatest constraints and is in greatest need of improving their nutritional, health, and economic status.

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A MANUAL OF FERTILIZATION AND SUPPLEMENTAL FEEDING STRATEGIES FOR SMALL-SCALE NILE TILAPIA CULTURE IN PONDS

Ninth Work Plan, Adoption/Diffusion Research 11 (9ADR11)
Final Report

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ABSTRACT

This study was conducted at the Asian Institute of Technology from January to June 2001 to summarize the PD/A CRSP work on Nile tilapia (*Oreochromis niloticus*) pond culture in Thailand and thus to develop a manual of fertilization and supplemental feeding strategies for small-scale Nile tilapia culture in ponds. The manual consists of eight sections. Section 1 gives a brief introduction to PD/A CRSP work and Nile tilapia culture; section 2 introduces pond preparation; section 3 examines different sources of pond inputs and quality; sections 4 and 5 focus on fertilization and supplemental feeding strategies, respectively; section 6 presents methods to control overpopulation of Nile tilapia; section 7 introduces pond management; and section 8 gives a simple economic analysis to select the suitable strategy. The aims of the manual are to provide simple guidelines of fertilization, supplemental feeding, and pond management for small-scale Nile tilapia pond culture and to provide simple extension and training materials to extension workers, trainers, and well-educated farmers. We expect that small-scale fish farmers in Asian countries, especially in Southeast and South Asia, will benefit from this manual to produce Nile tilapia through effectively using organic and inorganic fertilizers and feeds to increase fish production, achieve higher economic returns, and reduce environmental impacts.

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Establishment of New Collaboration in Bangladesh

Ninth Work Plan, Regional Analysis: Human-Environment Interactions 1 (9RA1)
Final Report

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Abstract

This activity was conducted from January to June 2001. Through this activity, a new link between the PD/A CRSP and a Bangladesh institution has been established. The potential PD/A CRSP collaborators in Bangladesh were identified, including an academic institution, Bangladesh Agricultural University, and three nongovernmental organizations (NGOs), namely Bangladesh Rural Advancement Committee (BRAC), Caritas, and PROSHIKA. The needs in aquaculture research in Bangladesh were also identified with a priority to optimize the fertilization regimes in pond culture. This report describes the potential site, current status of aquaculture development in Bangladesh, and the potential role of the PD/A CRSP. The establishment of collaboration with academic institutions and NGOs in Bangladesh will provide great opportunities for extending research and impacts of the PD/A CRSP to Bangladesh and South Asia, which is a potential site of the project in the future. Bangladesh researchers, NGO and government extension staff, and fish farmers will benefit from the experiences, research results, and approaches of the PD/A CRSP through the collaboration.

Introduction

The PD/A CRSP has conducted research/outreach activities in Southeast Asia for nearly two decades. However, the project has not been expanded to South Asia where a large part of the population is poor, especially in countries like Bangladesh.

Bangladesh is one of the most densely populated countries in the world. Additionally, three-quarters of it is covered by flat floodplains rich in water resources and supporting enormous amounts of freshwater fish. Fisheries and aquaculture in particular are vital to Bangladesh's national economy in terms of nutrition, income, employment generation, and foreign exchange earning (Alam et al., 1996). Fish is the traditional source of animal protein for Bangladesh people, and currently approximately 80% of the animal protein supply for residents is provided by fish. Population growth is rapidly overwhelming the productive potential of the Bangladesh fishery (O'Riordan, 1992). Since the 1960s, per capita availability of fish has dropped from 12 kg to only 7 kg, due to destruction of natural habitat. Moreover, among lower income groups, per capita consumption of fish is only 4.4 kg. For the poorest of the poor, fish is simply unaffordable (O'Riordan, 1992). Thus, aquaculture plays a more important role to meet the nutritional needs of poor Bangladesh people. Indian major carps are the dominant cultured species, but tilapia is playing an increasing role in solving problems of malnutrition, health, and alleviating poverty.

The purposes of the activities, conducted by researchers of the PD/A CRSP Thailand Project at the Asian Institute of Technology, were to:

1) Establish a new link to a Bangladesh institution and
2) Identify a potential PD/A CRSP site in Bangladesh.

Activities

The following activities were conducted from January to June 2001:

1) Primary identification of a Bangladesh institution as potential collaborator with the PD/A CRSP from January to March 2001, through:
   a) Following up initial contacts of Dr. H.S. Egna (Director of the PD/A CRSP) with Bangladesh institutions during her trip in July 2000;
   b) Consulting with Prof. J.H. Grover (ICLARM project leader in Bangladesh) and AIT colleagues who have conducted outreach activities in Bangladesh.
2) Contact was made with the primarily identified Bangladesh institution, Bangladesh Agricultural University (BAU), and Prof. Dr. Md. Abdul Wahab, Faculty of Fisheries, BAU, from February to June 2001. In March 2001, a researcher of the PD/A CRSP Thailand Project visited BAU, as well as fish ponds in villages (Kashimpcer and Muktugacha) in Mymensingh District. He also discussed potential collaboration between the PD/A CRSP and BAU with Dr. Wahab and visited Prof. Grover to discuss the research needs in Bangladesh.

3) A variety of NGOs in Bangladesh were contacted, including BRAC, Cooperative for Assistance and Relief Everywhere (CARE), Caritas, and PROSHIKA, from February to June 2001 for potential collaboration. Through emails we contacted Mr. Md. Mokarrom Hossain, Senior Regional Manager (Fisheries) of BRAC Rural Development Program; Mr. Greg Chapman, Coordinator of CARE Rice-Fish Project; Ms. Anwara Begum Sheely, Director of Caritas Fisheries Program; Dr. Thomas Costa, Development Director of Caritas; and Mr. Md. Abdur Rahman, Senior Coordinator of PROSHIKA Fisheries Program. A researcher of the PD/A CRSP Thailand Project visited Ms. Sheely at the head office of Caritas Fisheries Program at Dhaka in March 2001.
4) A junior staff member from BAU, Mr. Muhammad Mustafizur Rahman, was invited to visit AIT and several fish farms in Thailand in June 2001. He was briefed about the history and current activities of the PD/A CRSP, trained for analyses of water quality parameters using PD/A CRSP standard methods, and also trained for data collection, management, and analysis by researchers of the PD/A CRSP Thailand Project at AIT.

OUTPUTS, OBSERVATION, AND DISCUSSION

Identified Potential Collaborator: Bangladesh Agricultural University

Bangladesh Agricultural University is the largest institution in the country providing teaching, research, and extension support in agriculture. The Faculty of Fisheries is the youngest faculty and has some 40 academic staff. It remains the sole source of fisheries and aquaculture graduates in Bangladesh and is thus the principal trainer of fisheries and aquaculture extension staff. The Faculty of Fisheries maintains a good range of laboratories and field facilities.

On behalf of Bangladesh Agricultural University Research System (BAURES), Prof. Abidur Reza (Director of BAURES) has expressed interest and willingness to develop collaborative research activities between the PD/A CRSP and the Faculty of Fisheries of BAU. He has also ensured that BAURES and the relevant departments and laboratories will provide all necessary support for future collaborative research and training activities. Dr. Wahab has been nominated by BAURES as the principal investigator for collaborative activities with the CRSP.

Wahab started his teaching career as a lecturer in limnology at BAU in 1979. He obtained his Ph.D. in aquaculture from Stirling University, UK, in 1986. He studied nutrient enrichment in a flow-through land-based aquaculture system and its effects on benthic animals of the ponds. Since then, his major research interest has been in water quality and nutrient dynamics in various pond aquaculture systems. He has carried out extensive research on species combinations of both major Indian and exotic carps towards development of suitable polyculture technologies for small-scale aquaculture in rural Bangladesh. Wahab has been successful in receiving research grants from a number of national and international bodies, which include the International Foundation for Science (IFS), Department for International Development, UK (DFID), Norwegian Agency for Development Cooperation (NORAD), Danish International Development Agency (DANIDA), European Commissions International Cooperation Programme (EU-INCO), and USAID. The excellent performance of Wahab and his team will make the future collaborative activities successful.

Description of the Potential Site

The Fisheries Faculty Field Laboratory of BAU has been identified as a potential experimental site for a future PD/A CRSP project. BAU is about 100 km north of Dhaka and 4 km south of Mymensingh town, Mymensingh District, and occupies 486 ha by the side of Old Brahmaputra River.

<u>Description of Area/Region</u>
Climate: Köppen classification Af : Humid tropical group (A), tropical rainforest type (f). Bangladesh has a subtropical monsoon climate characterized by wide seasonal variations in rainfall, moderately warm temperatures, and high humidity. Regional climate differences in this flat country are minor. Three distinct seasons are observed: a cool, dry winter from October to March; a hot, humid summer from March to June; and a cool, rainy monsoon season from June to October. In general, the maximum summer temperature is about 36°C. The hottest month is April and the coldest month is January, when the average temperature is 11°C. Average daily humidity ranges from a March low of about 57% to a July high of about 86%. Heavy rainfall is characteristic of Bangladesh. The annual rainfall in Mymensingh is about 3,000 mm, 80% of which occurs during the monsoon season.

Topography: Broad deltaic plain. Chittagong Hills in southeast, low hills in northeast, and modest-elevation highlands in north and northwest.

<u>Description of BAU</u>
The Fisheries Faculty Field Laboratory of BAU consists of a hatchery, 72 earthen ponds, and a large earthen water storage pond 4 ha in size. The hatchery can reproduce Indian major carps, common carp, and Chinese carps. The size of earthen ponds varies from 75 to 800 m^2 (9 ponds are 75 m^2, 17 ponds 100 m^2, 16 ponds 400 m^2, 12 ponds 500 m^2, 12 ponds 800 m^2). The pond bottom soil is loam. The water supply for the ponds is groundwater from an adjacent deep tube-well. The Fisheries Faculty has a well-equipped Water Quality Laboratory.

Current Status of Aquaculture Development in Bangladesh and Potential Roles of the PD/A CRSP

Aquaculture is commonly practiced using polyculture of four to seven species of Indian and Chinese carps mainly in manured ponds (Wahab et al., 1991, 1999). The major pond inputs are cow dung and chicken manure, while the chemical fertilizers are either expensive, unavailable, or competitively used for agriculture. In spite of extensive research that has been conducted on fertilization in carp polyculture ponds in many parts of the world, such information is rather sparse in Bangladesh (Haq et al., 1993). In Bangladesh, most fish ponds are rain-fed and have multiple uses such as washing clothes, household, and kitchen items; serving as crop irrigation and drinking water for livestock; and even being used for bathing. During the dry season, the pond water level decreases and ponds may even dry up. The multiple use of fish ponds is a constraint for high nutrient inputs and high production. Thus, Bangladesh scientists estimate that the upper level of fish production in such ponds is 4,000 kg ha^{-1} yr^{-1}. However, current fish production is quite low in Bangladesh, averaging 2,800 kg ha^{-1} yr^{-1} (DOF, 1999). In rural aquaculture ponds, fish production is often lower than 1,500 kg ha^{-1} yr^{-1}. Compared with manure, chemical fertilizers may help to enhance fish production and maintain better water quality at the same time. The two decades of worldwide experiences by the PD/A CRSP can contribute significantly to aquaculture development in Bangladesh through future research, outreach, and training activities, especially with tilapia becoming more popular there.

Bangladesh has a variety of aquaculture and fisheries projects that have been funded by international aid. Many NGOs such as PROSHIKA, BRAC, CARE, and Caritas have been making significant progress in promoting aquaculture development in Bangladesh. However, NGOs are working more or less independently through their own extension networks, and researchers in academic institutions are seldom involved in such extension work. NGOs have been working with farmers to increase fish production, but different NGOs often recommend different fertilization regimes to farmers, and these regimes do not all seem to increase yields. Fertilization regimes do vary with different local conditions such as soil and source water. However, in some cases, the same farmers receive very different recommendations on fertilization regimes from different extension partners. Both over- and under-fertilization may cause adverse effects on fish production, water quality, pond effluents, and economic returns. Liming ponds is an important management practice in aquaculture depending on the soil conditions. Almost every pond is limed before fish stocking in Bangladesh. It has been questioned whether liming is needed in many ponds, but no such research has been conducted.

There is a critical need for good research to support the development efforts in Bangladesh, and there is very little real substance in the institutions and development programs to support this needed research (Grover, pers. comm.). Future potential PD/A CRSP activities may fill this gap and bring NGOs, academic institutions, and even government agencies together to optimize pond fertilization and management strategies for farmers in order to maximize fish production, maintain good water quality, reduce environmental degradation, and maximize economic returns. Among the contacted NGOs, BRAC, Caritas, and PROSHIKA have expressed interest in the optimization of fertilization regimes through collaborative research with the PD/A CRSP and BAU. CARE is focusing on rice-fish integrated culture and cage culture but is willing to explore other areas of possible future collaboration with the PD/A CRSP. A research proposal entitled “On-station and on-farm trials of different fertilization regimes used in Bangladesh” was developed for the Tenth Work Plan of the PD/A CRSP to begin with this collaboration. The purposes of the proposed research were to evaluate the different fertilization regimes currently used for aquaculture in Bangladesh; to compare effects of different fertilization regimes on fish production, water quality, pond effluents, and economic returns; and to recommend the best fertilization regimes to small-scale rural farmers from the joint efforts of the PD/A CRSP Thailand Project, BAU, BRAC, Caritas, and PROSHIKA.

Anticipated Benefits

The establishment of collaboration with academic institutions and NGOs in Bangladesh will provide a great opportunity for extending research and impacts of the PD/A CRSP to Bangladesh and South Asia, which is a potential site of this project in the future. Bangladesh researchers, NGO and government extension staff, and fish farmers will benefit from the experiences, research results, and approaches of the PD/A CRSP through the collaboration.

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.

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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

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Printed as Submitted

ABSTRACT

We report on the development of a new software tool for the analysis of fish population size distributions, focusing initially 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) continued development of models of 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. Software development has focused on developing entity descriptions and resulting implementations for describing populations and population dynamics in the model. Further, interoperability with datasets used by other catfish production software, (Fish98) was developed. The resulting Catfish Inventory Management (CIM) software allows users to represent populations of catfish in any number of ponds, and simulate the population dynamics and associated variability in response to differential growth rates and harvest practices of these ponds.

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. We give a description of the model below and report on its basic structure and functioning. 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 where found that describe differential growth with respect to fish size within a fish population. Since no acceptable models where 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

$$\frac{\partial}{\partial t}\mathrm{P}(w) = input - output$$

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 $d(w_1,w_2)$ that describes the fraction of fish at mass w_1 that grows to mass w_2. Now *input* and *output* can be described as:

$$input_{w_0} = \int_0^\infty \mathrm{P}(w)\,\delta(w,w_0)\,dw, \text{ and}$$

$$output_{w_0} = \int_0^\infty \mathrm{P}(w_0)\,\delta(w,w_0)\,dw,$$

where w_0 is a constant. Furthermore,

$$output_{w_0} = \mathrm{P}(w_0)$$

because $\mathrm{P}(w_0)$ is constant with respect to the variable of integration and the integral is equal to 1. So we have:

$$\frac{\partial}{\partial t}\mathrm{P}(w) = \int_0^\infty \mathrm{P}(w)\,\delta(w,w_0)\,dw - \mathrm{P}(w_0).$$

This system can easily be discretized by first defineing n weight intervals, w_i, i=0..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

$$\lim_{n\to\infty}\sum_{j=0} P_{j,k}\delta(w_j,w_i)=\int_0^\infty \mathrm{P}(w)\,\delta(w,w_0)\,dw.$$

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, Δ, 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 d 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 d by using the probability density function for the normal distribution;

$$\delta(w_1,w_2)=\left[\frac{1}{2}\frac{\sqrt{2}}{\sqrt{\pi\sigma}}\right]e^{\left(-\frac{1}{2}\frac{(w_2-\mu)^2}{\sigma^2}\right)}.$$

Database Development

The existing POND software uses proprietary database code. Relationships between entities in the database are "hard coded" into the software and cannot be changed without recompiling the software. While this implementation produces fast and efficient database functionality, the implementation also slows the development process when changes to database structure are required. Also, the POND database uses specially formatted text files to store database contents. This also hinders interoperability between other database software.

One of the design goals for the Catfish Inventory Management (CIM) software was to provide compatibility with Fish98 database files and provide mechanisms for interoperability with other Windows Software. To achieve this goal it was decided that the CIM tool should used the Microsoft DAO database technology. Choosing DAO simultaneously provides interoperability with existing Microsoft tools like Excel and Access and compatibility with Fishy98 files (Dbase 2 format).

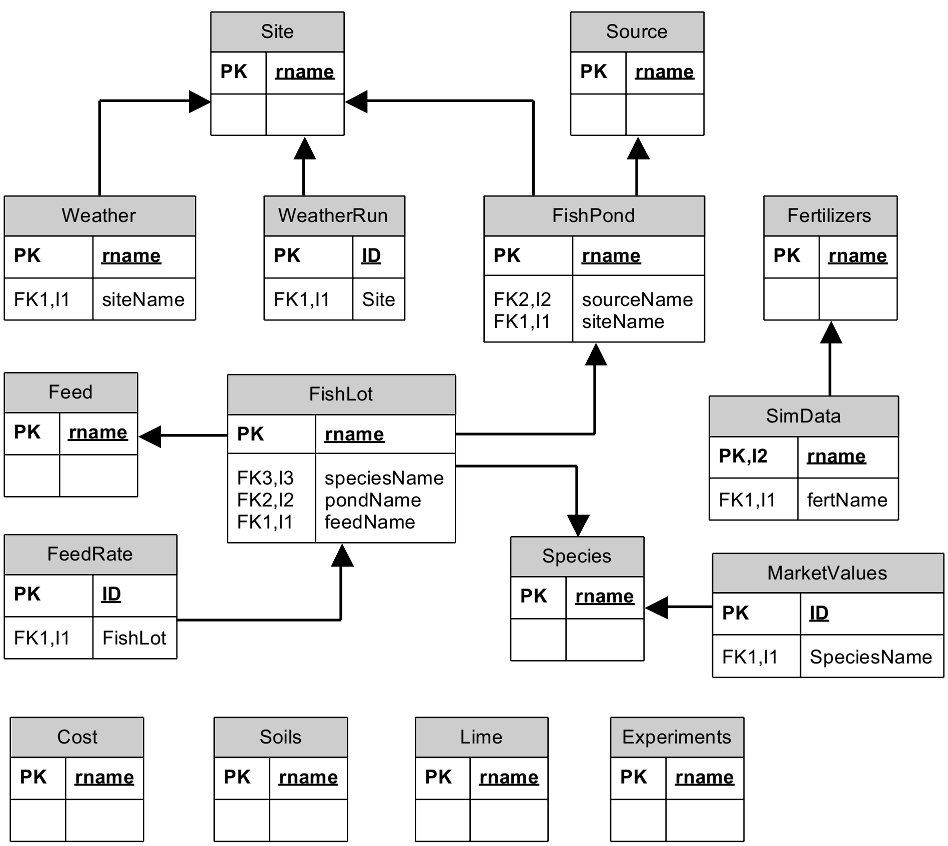

Figure 1: New POND Database schema

Three significant drawbacks where associated with choosing DAO. First the POND software had to be restructured to use DAO instead of the existing database code. Second, the POND database structure had to be implemented in an external database accessible via DAO. Finally the POND database had to be modified to address the different model structure used by the CIM software.

Development of the CIM database proceeded in three phases. First, the POND database was re-implemented in Microsoft Access. The DB structure of the new POND DB is shown in figure 1 (in abbreviated form; table names, relations, and keys only). While the DB structure is not extraordinarily complex, implementation required a considerable amount of time. The new POND DB contains over 300 fields in 16 tables. Table 1 shows what metadata information was included in the database. Much of this information had to be extracted directly from the POND source code.

Table 1: Pond Database Metadata

TableName	Textual name of the data table
VariableName	Name of variable used in source code
DisplayTitle	Name used when displaying variable with interface or user manual
Units	Physical units of the variable (if applicable)
Type	Data type of the variable
Min	Minimum value of the variable (if applicable)
Max	Maximum value of the variable (if applicable)
Default	Default value of the variable (if applicable)
Description	Textual description of the variable's purpose or meaning

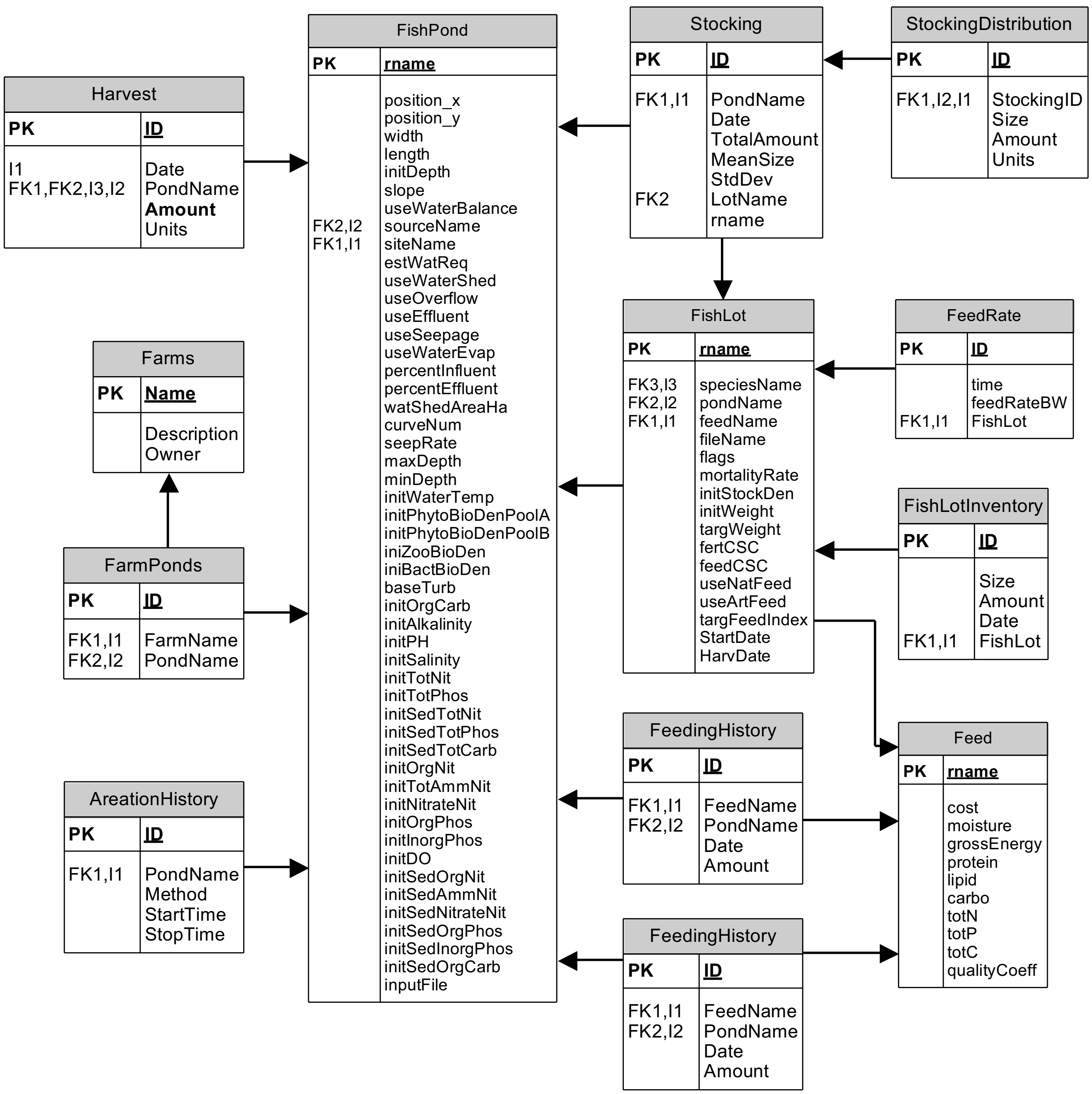

Figure 2 : Revised POND database schema

The second phase of CIM database development involved adding additional tables account for the different model structure used by CIM. POIND makes the assumption that all the fish within a single FishLot are approximately the same and can be modeled as a single entity. The CIM tool does not make this assumption. Instead, the entire distribution is modeled in a piecewise fashion. Figure 2 shows the DB structure with the new tables highlighted (note: some tables shown in fig. 1 are omitted for brevity).

The third phase of database development involved integration of the revised POND database structure into the existing POND software. Figure 3 is a Booch class diagram depicting the POND DB classes before and after modification. The modifications where chosen to minimize the impact on existing POND database structure.

CONCLUSIONS

The CIM tool allows users to effective model dynamic changes in size class distributions in fish ponds by modeling the changes in these distributions in response to fish growth and management. By using basic growth models and databases in common with the POND software tool, it can be readily adapted to new species and situations. We will be making CIM available on the web as it becomes ready for release at http://biosys.bre.orst.edu.

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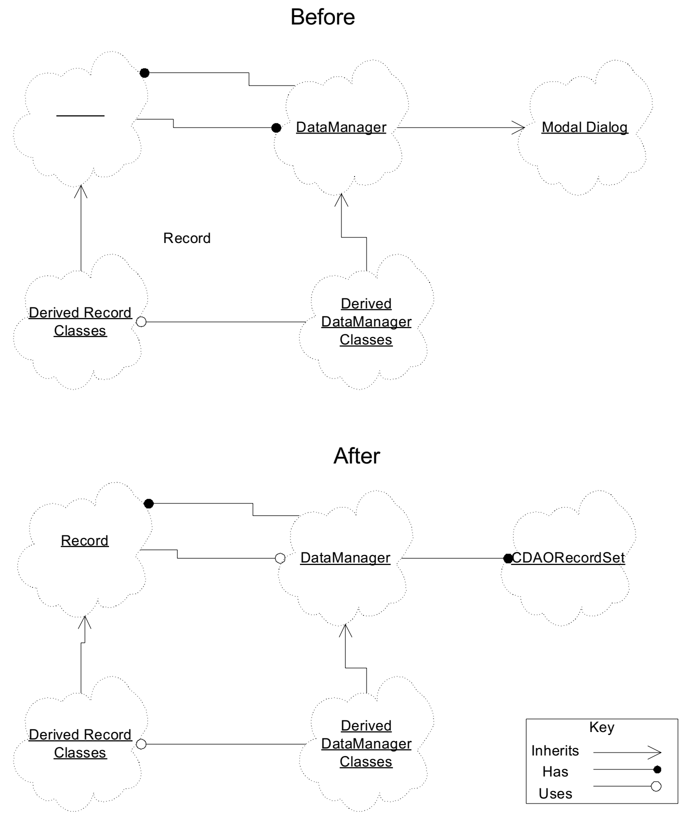

Figure 3. Booch Diagram of key objects in CIM

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ENHANCING THE POND DECISION SUPPORT SYSTEM FOR ECONOMICS, EDUCATION, AND EXTENSION

Ninth Work Plan, Decision Support Systems Research 3 (9DSSR3)
Progress Report

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ABSTRACT

This study deals with development of decision support tools for warmwater pond aquaculture. Efforts are directed at refining the POND software. Refinements to POND discussed here include development of tutorials and user interaction enhancements to facilitate the use of POND in educational and extension environments, and the development of a shrimp growth and development model incorporated into the POND framework. A POND tutorial has been developed, addressing problems relating to fertilization and liming calculations and to simulation setup. A new set of tutorial databases have been developed to facility the use of POND in these environments. In the interest of familiarizing the user with POND, discussions of how to create new ponds, fishlots, and other database objects have been expanded. The development of a marine shrimp model in POND is complete. It was developed using the BIOE bioenergetic model built into POND, and calibrated using the results from twenty six datasets related to shrimp production in the CRSP database.

INTRODUCTION

Decision support systems for pond aquaculture can aid in the analysis and understanding of the dynamics of pond production. 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 cost and returns from a particular facility to determine short- and long-term profitability. The growth and waters 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.

Recently, under the PD/A CRSP program, POND has been extended in several significant ways. Feedback from POND users indicated that refinements to POND where needed in the areas of 1) improved flexibility in enterprise budget capabilities; 2) provision of more extensive tools for helping users take advantage of the analytical tools available in POND, and 3) extension of POND's bioenergetic model to include analysis of shrimp production. These capabilities have been added to POND. The improvements in the enterprise budgeting capabilities are largely focused on providing more flexibility in scheduling periodic and non-periodic costs, and improvements in the user interface designed to increase ease of use. Overall usability improvements and a detailed characterization of the shrimp production models are described below.

POND USABILITY IMPROVEMENT

The POND user's manual has been revised and updated to reflect the major changes which were implemented in POND version 4.0. The most important change in the software is the addition of an application 'wizard', which guides the user through a step-by-step process to accomplish a number of basic tasks, such as creating new fish ponds and fish lots, calculating the fertilization and liming requirements for a pond, and setting up and running a simulation. The updated manual includes a chapter which documents the eight wizard-mediated tasks.

Other changes included the addition of installation instructions for Windows 95/98 operating systems, as the POND 3.0 manual was written for a Windows 3.x environment. The interface for the weather database was significantly revamped, and minor changes in other databases were also reflected in the documentation revision.

The POND manual was converted to a file library for use in AuthorIT, a help authoring program which can create Microsoft Word documents, Windows Help files, and HTML files from the same source files. The manual is thus being made available in any of these formats, facilitating flexible use on individual machines or via the Internet.

The existing POND tutorial has been reworked in accordance with the updated software. In some cases, particularly in problems relating to fertilization and liming calculations and to simulation setup, the basic tasks are now accomplished using a completely different method than in version 3.0. A new set of tutorial databases has been developed to support these new methods, as the default databases distributed with POND no longer include the necessary objects for the tutorial. In the interest of familiarizing the user with POND, discussions of how to create new ponds, fishlots, and other database objects have been expanded and incorporated into the tutorial and help systems.

The POND tutorial has been added to the same AuthorIT library which contains the user's manual files, so that information may be shared between the two documents. This enables us to distribute the tutorial as a Microsoft Word document, Windows Help file, or as HTML pages.

DEVELOPMENT OF A GROWTH AND FEEDING MODEL FOR MARINE SHRIMP

Methods

A total of 26 datasets from the CRSP Database (http://biosys.bre.orst.edu/crspDB/) were utilized for model development (Table 1). These datasets consisted of raw data from individual experimental treatments for the production of Penaeus vannamei (Pacific white shrimp) and P. monodon (tiger shrimp) in ponds. These ponds received various water exchange rates, various application rates of inorganic and organic fertilizers during pre-conditioning and/or culture periods, and some use of supplemental feeds. Additional datasets of raw, experimental data for laboratory and intensive tank culture of P. vannamei were obtained from the Oceanic Institute (Leonard Obaldo, personal comm.).

The bioenergetic model for finfish and shrimp (BIOE model) available in the POND software was utilized for the development and application of growth and feeding models for shrimp production. The BIOE model is a comprehensive, unified modeling approach, with respect to the multiple environmental and food-resource variables impacting shrimp performance (Nath, 1996). POND provides functionality for calibrating model parameters using shrimp production datasets, as well as functionality for the application of these calibrated models to the simulation of shrimp production. This methodology has been fully explained by Nath (1996).

Prior to regression procedures using the BIOE model in POND, datasets were analyzed using the double-log specific growth

Table 1. Shrimp production datasets (26 total) in the PD/A CRSP Central Database used to calibrate a shrimp growth model for POND. Given study and treatment codes can be used at the Database web site for additional information on experimental protocols. Given here are culture period, use of fertilizers and feeds, and shrimp species and stocking density.

Study	Treatment	Per. (days)	Inorg. Fert.	Org. Fert.	Supp. Feed	Species	Shrimp/m2
A_01_01	A	102	false	true	false	P. vannamei	5
A_01_02	A	140	false	true	false	P. vannamei	5
A_02_01	A	93	false	false	false	P. vannamei	4
	B		false	false	false	P. vannamei	4
A_02_02	A	153	true	true	true	P. vannamei	4
	B		true	true	true	P. vannamei	4
A_03_01	A	73	false	false	false	P. vannamei	4
A_03_02	A	132	false	false	true	P. vannamei	4
G_02_01	H	164	true	true	true	P. monodon	4
	I		true	true	true	P. monodon	4
	K		true	true	true	P. monodon	4
G_02_02	H	196	true	true	true	P. monodon	4
	I		true	true	true	P. monodon	4
	K		true	true	true	P. monodon	4
G_03_01	A	129	true	true	true	P. monodon	4
G_03_02	A	71	true	true	true	P. monodon	4
J_08_HR3A	A	138	false	false	true	P. vannamei	16
	B		false	false	true	P. vannamei	16
J_08_HR3B	A	119	false	false	true	P. vannamei	16
	B		false	false	true	P. vannamei	16
J_08_HR4	A	242	false	false	true	P. vannamei	16
	B		false	false	true	P. vannamei	16
	C		false	false	true	P. vannamei	16
J_INT_1B	A	87	false	false	true	P. vannamei	30
	B		false	false	true	P. vannamei	30
	C		false	false	true	P. vannamei	30

rate model (DSGR model) (Hepher, 1988; Ernst 2000). The DSGR model is:

$$SGR = SGR_c\, W^{-SGRe}$$

or $$GR = SGR_c\, W^{(1.0 - SGRe)}$$

by integration

$$W_t = [W_o^{\,SGRe} + (SGR_c\, SGR_e\, t)]^{(1.0 / SGRe)}$$

where

SGR = specific growth rate (1/day)
GR = growth rate (g/day)
SGR_c = coefficient (intercept of log transform regression)
SGR_e = exponent (slope of log transform regression)
W = shrimp body weight (g) (subscripts 0 and t denote time zero and current time)
t = time (days)

SGR_c is a function of (1) water temperature, using a second-order polynomial scalar function (Miao and Tu, 1995), (2) additional water quality scalars, and (3) food availability (Ernst, 2000). Model parameters can be determined by log-transform linear regression. Geometric-mean fish weight (W_{gm}, g) and SGR values are calculated for each growth interval of the sample fish weights (W_o and W_t), and their natural logs are used:

where

$$W_{gm} = e^{\{ [\log_e(Wo) + \log_e(Wt)] / 2.0 \}}$$

$$SGR = [(\log_e(W_t) - \log_e(W_o)] / t \text{ and}$$

$$\log_e(SGR) = \log_e(SGR_c) - [SGR_e \log_e(W_{gm})]$$

RESULTS AND DISCUSSION

Calibration (regression) procedures for the BIOE model in POND can variably consider a number of environmental and food-resource variables. All significant driving variables of shrimp performance must be included in model regressions in order to achieve meaningful results. Drivers of shrimp growth include (1) body weight, (2) daily feed intake (natural food resources and prepared feeds), (3) water temperature, and (4) any limiting water quality (e.g., dissolved oxygen and un-ionized ammonia). In addition, assessment of calibration results for the BIOE model is enhanced by excluding independent variables that are not significant drivers of shrimp performance. Therefore, the DSGR model was used to first check datasets for expected behavior and to identify constraints to shrimp growth, prior to BIOE calibration procedures.

Under excellent culture conditions of water quality and food availability, P. monodon are reported to achieve a weight of 30 g in about 100 days (Rosales, 1995). Under commercial culture in SE Thailand for average conditions, P. monodon are reported to grow from postlarval stages (PL 15-20) to 22 g in 124 days at an FCR of 2.0 (Briggs and Funge-Smith, 1994). Recommended daily feeding rates for shrimp (% body weight per day) range from 20 to 30 %/day for postlarval stages to 3 to 4 %/day for shrimp weights above 20.0g, with initial feeding crude protein contents ranging as high as 40% (Lovell, 1988).

Montoya et al. (1999) reported that Pacific white shrimp (Litopenaeus vannamei) reached 25 g in about 6 months, showing exponential and linear growth stanzas but no inflection point in growth rate or asymptotic stanza for the culture conditions used. Tian et al. (1993) reported growth from 1.0 g (53 days from hatching) to 16 – 18 g (winter, < 26 C) and to 20-21 g (summer, > 26 C) in 140 days. The asymptotic weight of P. vannamei is reported by Tian et al. to be about 80 g under non-limiting conditions. Under production conditions, Tian et al. report approximate asymptotic weights of 50 g at 5 shrimp/m2, reducing further to 30 g at higher densities (50 – 150 shrimp/m2). Some additional production benchmarks reported by Tian et al. include shrimp weights at 15 to 20 g for a growth period of 5 to 6 months (Hawaii), 23 g at 5.8 months (Texas), and 20 g at 5.3 months (South Carolina).

Laboratory culture of P. vannamei at the Oceanic Institute (Leonard Obaldo, personal comm.; control treatment) was assumed to represent non-limiting conditions of water quality and feed availability (Figure 1). In this study, shrimp were fed three times daily to satiation over the entire culture period. Water quality parameters were measured once a week and mean values were: water temperature 26 C, dissolved oxygen 6.3 mg/L, pH 7.6, unionized ammonia 0.002 mg/L, and salinity 33ppt. Growth data showed an expected good fit to the DSGR model.

Given this background information, and regression results of the DSGR model for each of the 26 CRSP datasets, it was determined that a number of the CRSP datasets showed that shrimp growth was limited by food availability and not by

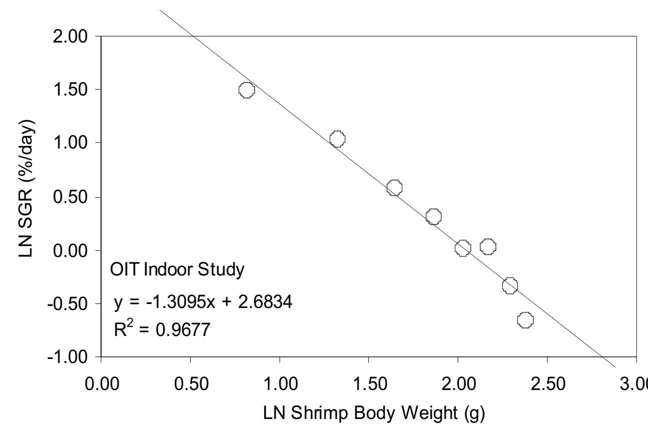

Figure 1. Regression of the double-log specific growth rate model for shrimp growth data from the laboratory culture of P. vannamei at the Oceanic Institute (Leonard Obaldo, personal comm.; control treatment).

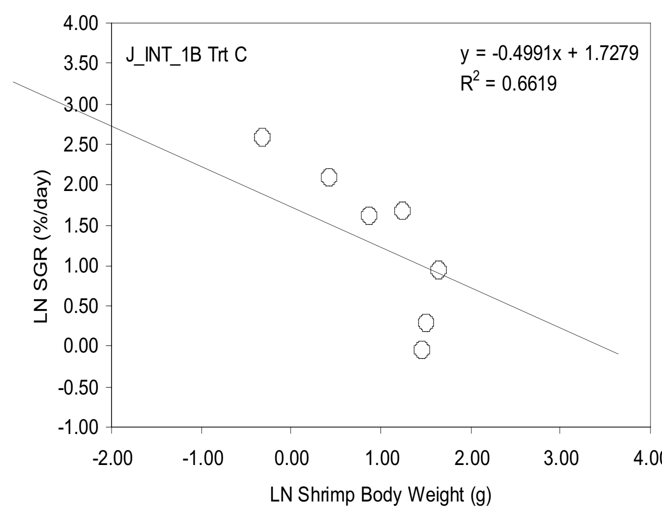

Figure 2. Regression of the double-log specific growth rate model for shrimp growth data from CRSP study J_INT_1B treatment C, illustrating a food resource limitation towards the end of the culture period.

water quality or maximum shrimp size. In all of the CRSP studies, shrimp body weights were less than half of reported maximum (maturation) asymptotic weights. Therefore, the catabolic term in the bioenergetic model (as opposed to the anabolic term) was determined to be a negligible factor with respect to observed asymptotic growth patterns. In addition, reported water quality for the CRSP studies did not indicate that water quality (e.g., dissolved oxygen and unionized ammonia) was a significant constraint to growth. To illustrate these trends with a specific example, the data and regression results shown in Figure 2 indicate a food resource constraint to shrimp growth and resulting poor fit of the DSGR model when declining natural food resources are not considered. In contrast, Figure 3 shows relatively non-constrained shrimp growth and a good fit of the DSGR model.

Natural food resources for shrimp in fertilized ponds are comprised of a number of benthic organisms and substrates, and as a result are difficult to quantify through purely mechanistic modeling. POND is able to consider various natural food resources, including phytoplankton, zooplankton, and bacteria. This food resource model is mainly intended for application to omnivorous finfish (e.g., tilapia and carp). For applications of the BIOE model to shrimp, a much simplified, empirically based approach to natural productivity was used. This method utilizes critical standing crop (CSC, kg shrimp/ha) and carrying capacity (CC, kg shrimp/ha) biomass density parameters with respect to food availability (Hepher, 1988). At shrimp densities less than CSC, the availability of natural food resources exceeds maximum consumption rates and does not limit growth. As shrimp density increases above CSC due to growth, natural food resources are utilized beyond their sustainable yield and depleted, causing a decline in natural productivity. When shrimp density achieves FBDcc, natural food resources are depleted to a level at which net growth is no longer supported and natural productivity is reduced to zero. Use of natural productivity without supplemental feeding yields sigmoidal fish growth curves, as natural food resources are initially unlimiting, then overwhelmed, and finally exhausted.

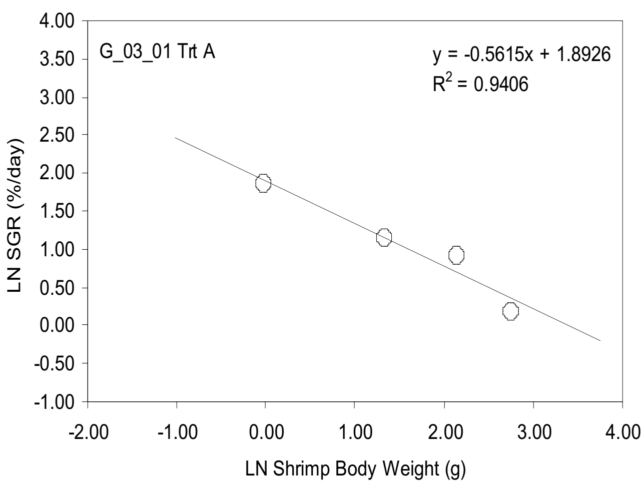

Figure 3. Regression of the double-log specific growth rate model for shrimp growth data from CRSP study G_03_01 treatment A, illustrating the availability of sufficient food resources throughout the culture period.

In conclusion, the BIOE and CSC/CC models available in POND were successfully calibrated for applications to marine shrimp culture. Results are available in the component databases of POND, available at the POND web site.

CONCLUSIONS

The POND decision support tool continues to evolve. Recent efforts, documented in this report, have focused primarily on increasing the useability of the software through incremental improvements in the user interface, enhanced online and offline help support, and better tutorial support. Further, the BIOE model has been adapted for use in simulating marine shrimp production, allowing POND to be used to incorporate marine shrimp production schemes into it facility analysis. With these additions, POND has become a mature, robust pond facility decision support tool. It is available for download from the POND website at http://biosys.bre.orst.edu/pond/pond.htm.

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