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AQUAFISH COLLABORATIVE RESEARCH SUPPORT PROGRAM

AquaFish Collaborative Research Support Program Technical Reports: Investigations 2007-2009

The mission of the AquaFish Collaborative Research Support Program (CRSP) is to enrich livelihoods and promote health by cultivating international multidisciplinary partnerships that advance science, research, education, and outreach in aquatic resources. Bringing together resources from host country institutions and US universities, the AquaFish CRSP emphasizes sustainable solutions in aquaculture and fisheries for improving health, building wealth, conserving natural environments for future generations, and strengthening poorer countries' ability to self-govern.

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Cover photos:
Front cover center: Aquaculture ponds at a fish farm in Uganda. Photo by CRSP researcher Joe Molnar (Auburn University)

Front cover left: A Malian fisherman ready to go back out on Lake Sélingué. Photo by Nancy Gitonga.

Front cover right: A young Kenyan boy helps with the grinding step to produce pelleted feed for the fish raised at the Mwea Aquafish Farm. Photo by HC Lead PI Charles Ngugi.

Back cover: Giant freshwater prawns (*Macrobrachium rosenbergii*) are harvested at a farm in Vietnam in December 2007. Photo by Jim Bowman.

Bottom silhouette: Fishing boats parked at the Carriere landing beach, Lake Sélingué, Mali. Photo by Charles Ngugi.

Cover design by Tiffany Ruiz

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PRODUCTION SYSTEMS DESIGN AND BEST MANAGEMENT ALTERNATIVES

POLYCULTURE OF SAHAR (*TOR PUTITORA*) WITH MIXED-SEX NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

Production Systems Design and Best Management Alternatives/ Experiment/ 07BMA02UM

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ABSTRACT

Sahar (*Tor putitora*) were cultured with Nile tilapia (*Oreochromis niloticus*) to control tilapia recruitment in aquaculture ponds. Two experiments were conducted to assess the effects of the sahar to tilapia stocking ratio on the recruitment and growth of mixed-sex Nile tilapia. The first experiment was conducted on station to determine these effects, and the second experiment was conducted on farm to verify the results in working ponds. The on-station experiment was conducted in 100 m^2 earthen ponds at the Institute of Agriculture and Animal Science, Chitwan, Nepal, and the on-farm experiment was conducted in farmers' ponds at Kathar, Chitwan, Nepal. The on-station experiment had four treatments with three replicates each: tilapia monoculture (T1), 1:16 sahar to tilapia ratio (T2), 1:8 sahar to tilapia ratio (T3), and 1:4 sahar to tilapia ratio (T4). Tilapia were stocked at 2 fish m^{-2} (average size 11.3 g), and sahar were stocked at varying densities (15.2 g average size) in each pond. The ponds were fertilized weekly using diammonium phosphate (DAP) and urea at the rates of 0.1 g P m^{-2} d^{-1} and 0.4 g N m^{-2} d^{-1}, respectively. Tilapia were fed with a locally made pelleted feed (27 % crude protein), at the rate of 2 % body weight every other day after attaining a size of 100 g. Results showed significantly increased average harvest size (P<0.05) for treatment 2, when sahar were stocked with tilapia compared to the tilapia monoculture. The number of recruits significantly decreased (P<0.05) when sahar were stocked, and moreover, recruits were inversely proportional to stocking density of sahar. Thus, the results demonstrated that stocking of sahar controls tilapia recruitment in a mixed-sex Nile tilapia pond culture system and

could provide better tilapia growth and production. Stocking at a 1:16 sahar to tilapia ratio showed the best overall performance.

The on-farm experiment was composed of three treatments with three replicates each: tilapia monoculture (T1), 1:33 sahar to tilapia ratio (T2), and 1:16 sahar to tilapia ratio (T3). Ponds were fertilized every two weeks with DAP and urea at the same rate as the on-station experiment, but there was no feeding. On-farm results showed significantly higher tilapia growth with a 1:33 stocking ratio of sahar to tilapia compared to the tilapia monoculture. As with the on-station experiment, the number of recruits decreased with increasing stocking density of sahar. Lower sahar stocking provided higher growth and production of stocked tilapia, though there were fewer recruits at these levels. There might have some growth depression of tilapia by higher sahar stocking. Stocking sahar to Nile tilapia at 1:33 showed better overall performance than the monoculture but not the 1:16 treatment in terms of Nile tilapia growth production, growth of sahar and gross income.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is considered an ideal species for small or large-scale aquaculture (Pillay, 1999). Being a lower trophic level species, it represents the greatest potential for efficiency and is commonly cultured in semi-intensive systems (Welcome, 1996; Shrestha et al., 2000). It was introduced in Nepal in 1985 (Pantha, 1993), but it was confined to government farms until 2000 (Shrestha, 2004). Nile tilapia culture in Nepal is based on mixed-sex culture and is expanding to areas of the central Terai and among small-scale farmers. However, polyculture for controlled management of mixed-sex tilapia culture must be developed (Shrestha, 2004). Overproduction of recruits in mixed-sex culture causes stunted growth and produces undersized fish for the market (Pillay, 1999; Focken et al., 2000). This creates a significant economic pressure to control excessive tilapia recruitment in the culture system.

The sahar (*Tor putitora*) is an economically important indigenous fish species (Rai et al., 1997), and is in decline across much of its native range due to habitat loss and physical changes in Nepal's environment (Desai, 1994; Shrestha, 1997). This has led to efforts to conserve, manage and propagate the species (Shrestha, 1997). It is an omnivorous fish (Shrestha, 1997), but it also shows predatory habits (Acharya, 2004; Paudel, 2007; Yadav et al., 2007). Sahar predation is a simple mechanism that has been proposed to control tilapia recruitment in mixed-sex Nile tilapia culture. Combining sahar and Nile tilapia at a proper ratio could provide new recruits for continuous seeding and maintain a stable sahar population. An additional benefit would be conservation of the declining sahar population in Nepal.

The purposes of this study were to create a polyculture system of sahar with mixed-sex Nile tilapia fitted to the local conditions of Nepal, to assess the appropriate stocking density of sahar to control Nile tilapia recruitment, to grow and produce mixed-sex Nile tilapia in such a pond culture and to verify the best results of the on-station experiment in a working aquaculture pond.

METHODS AND MATERIALS

Two consecutive experiments were conducted in the subtropical climate of Nepal to quantify and optimize the potential benefits from polyculture of sahar and Nile-tilapia. The on-station experiment was conducted from 14 October 2007 to 10 June 2008 (240 d). Data from this trial were then used to inform parameters for the experiment at a working farm, which was carried out from 15 February to 19 July 2009 (151 d).

Experiment One: Pond trials at the Institute of Agriculture and Animal Science

The on-station experiment was conducted at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal in twelve 100 m^2 earthen ponds. There were four treatments with three replicates each: tilapia monoculture (control, T1); sahar with tilapia at a 1:16 ratio (T2); sahar with tilapia at a 1:8 ratio (T3); and sahar with tilapia at a 1:4 ratio (T4). The stocking density of tilapia was 2 fish/m^2 in each treatment, and sahar densities were varied according to the treatment. Ponds were randomly assigned a treatment, and tilapia (11.1-11.6 g) and sahar (14.5-16.3 g) were stocked in each. The ponds were fertilized weekly using diammonium phosphate (DAP) and urea at the rates of 0.1 g P m^{-2} d^{-1} and 0.4 g N m^{-2} d^{-1}, respectively. Tilapias were fed with a locally produced pellet feed (27% crude protein) at a rate of 2 % body weight every other day after they attained a size of 100 g.

Weekly and biweekly measurements of water quality parameters were conducted from 06:30 to 07:30 h starting on 14 October 2007. In situ water temperature at 0.2 m depth, dissolved oxygen (DO) at 0.2 m depth, pH, and Secchi disk depth were measured weekly using a Thermo Orion DO Meter (810A^+ Model), Microprocessor pH meter (WTW, pH 539 Model) and Secchi disk. Water samples were collected biweekly from each pond using a plastic column sampler (10 cm diameter, 1 m long) and analyzed for total alkalinity (as $CaCO_3$), total ammonium nitrogen (TAN), nitrite nitrogen (nitrite-N), nitrite-nitrate nitrogen (nitrite-nitrate-N), soluble reactive phosphorous (SRP), total phosphorous (TP), and Chlorophyll-*a* (APHA, 1985).

Periodically, fish were netted from the ponds to measure growth using an electronic balance. At least 15% of the fish were netted for sampling and weighed to determine growth. The total weight of sampled fish from each experimental pond was also recorded. All study ponds were harvested on 10 June 2008 by seining three times followed by a complete draining of the pond. At this point, the final total number and weight of each fish species in each pond was determined. Net fish yield (NFY) and apparent food conversion ratio (AFCR) were then calculated. Nile tilapia recruits were also collected, counted, and weighted to determine their growth and density in each pond during final harvest.

Experiment Two: Pond trials on a working aquaculture farm

Based on the results from experiment one, a verification trial was conducted in 12 earthen ponds (90-131 m^{-2}) at a working aquaculture farm in Kathar, Chitwan. The best performing treatment from experiment one and one additional treatment were tested against the tilapia monoculture. The three treatments were: tilapia monoculture control group (T1); sahar with tilapia at a 1:33 ratio (T2); and sahar with tilapia at a 1:16 ratio (T3). The stocking density of Nile tilapia was 2 fish/m^2 in all treatments, with sahar

stocking density varying according to treatment. Nile tilapia fingerlings (55.3-55.7 g) and sahar fingerlings (15.1-15.7 g) were stocked on 20-21 February 2009. Each treatment was replicated three times and ponds were randomly assigned a treatment. The water depth was maintained at about 1 m in each pond by filling with canal water to compensate water loss. Ponds were fertilized every two weeks with DAP and urea at the same rate as the on-station experiment.

In situ water temperature, dissolved oxygen, pH, Secchi disk depth and water depth were measured weekly at 07:00–09:00 h as previously described, while total alkalinity, total ammonium nitrogen, soluble reactive phosphorus, and Chlorophyll-*a* were analyzed monthly by collecting water samples from each pond using the plastic column sampler (APHA, 1985). Monthly growth of the Nile tilapia and sahar was determined by netting at least 20 % of the fish from each pond. All study ponds were harvested 19 July 2009 by seining three times followed by complete draining. The total numbers and weights of the Nile tilapia, sahar and tilapia recruits in each pond were then determined. NFY was calculated as kg $pond^{-1}$ $crop^{-1}$ and extrapolated by hectare and year (300-day period).

A partial budget analysis was conducted based on farm-gate prices for harvested fish and market prices for all costs in Nepal (Shang 1990). Farm gate prices of Nile tilapia and sahar were 140 and 250 NRs/kg, respectively (1US$ = 75 NRs), for the harvested sizes in this experiment. Prices for Nile tilapia and sahar fingerlings were 5 and 10 NRs/individual, respectively. Prices for DAP, urea and lime were 35, 18 and 15 NRs/kg, respectively. Daily wages for extra labors were 200 NRs/person. The calculation of working capital cost was based on an annual interest rate of 10%.

Data from both experiments were analyzed statistically by an analysis of variance (ANOVA) using the SPSS statistical software package (SPSS 14.0, SPSS Inc., Chicago). Differences were considered significant at the 95 % confidence level ($P<0.05$). All means are shown ± standard error (SE).

RESULTS

Experiment One: Pond trials at the Institute of Agriculture and Animal Science

The results showed that the survival rate of Nile tilapia ranged from 56.0 to 70.1%, without significant differences ($P>0.05$) among treatments (Table 1). Daily weight gain (DWG) and net fish yield (NFY) were significantly higher ($P<0.05$) in ponds with a 1:16 sahar to tilapia stocking ratio than they were in control ponds (Table 1; Figure 1). However, there were no significant differences in DWG or NFY within the different sahar densities. Moreover, DWG and NFY in the control group were not significantly different from the 1:8 and 1:4 sahar to tilapia treatments (Table 1). The survival rate of sahar ranged from 39.3% to 56.4%. DWG of sahar ranged from 0.28-0.39 g/d and was significantly different ($P<0.05$) among treatments (Table 1; Figure 2). However, the NFY did not differ significantly among treatments and ranged from 60-80 kg/ha.

The extrapolated NFY values were 1.2 ± 0.1, 2.2 ± 0.3, 1.4 ± 0.2 and 1.8 ± 0.2 t ha^{-1} yr^{-1} for control, 1:16, 1:8, and 1:4 sahar to Nile tilapia stocking ratios, respectively. The NFY for the treatment with a 1:16 sahar to tilapia stocking ratio was significantly higher ($P<0.05$) than the control; but it was not significantly different from the 1:8 and 1:4

treatments (Table 1). The apparent food conversion ratio ranged from 0.17-0.24 and was not significantly different among treatments (Table 1).

The number of recruits was significantly higher in control (324 ± 40) than polyculture treatments, ranging from 69-169 recruits (Table 2). There were no differences in the number of recruits among different sahar stocking ratios. However, the number of recruits decreased linearly with increasing stocking density of sahar (r^2 =0.54; P = 0.007, Figure 3) and the size of recruits increased with increased sahar to tilapia stocking ratio (Table2).

Mean values of water temperature, DO, pH, Secchi disk depth, total alkalinity, TAN, SRP, nitrite-N, nitrite-nitrate-N, TP, Chlorophyll-*a*, gross and net primary production were not significantly different among treatments (Table 3). Water temperature during the experimental period fluctuated but remained below 20^{o}C for 3.5 months and below 25^{o}C for almost 5 months, (Figure 4). At one point, DO decreased to a greater extent in the control ponds, reaching a minimum (0.2 mg/L) that was lower than the polyculture treatment minimum (0.7mg/L, Table 3). Secchi disk depth and total alkalinity increased at the beginning, peaked during the first part of the experiment, and then fluctuated depending upon the nutrient supply and fresh water supply during latter half of the experiment. Similarly, TAN, SRP, NO_2-N, NO_3-N, Chlorophyll-*a*, gross and net primary production and TP tended to fluctuate during the experimental period depending upon the nutrient supply.

Experiment Two: Pond trials on a working aquaculture farm

Survival rates of Nile tilapia ranged from 92.9% to 95.1% and were not significantly different among treatments (Table 4). Final mean weight and DWG of T2 was significantly higher *(P*<0.05) than tilapia monoculture (T1), but not significantly different from T3. The mean DWG of Nile tilapia ranged from 0.55-0.87 g/d (Table 4). Polyculture showed a trend of higher Nile tilapia growth rates (*P*<0.05) than monoculture (Figure 5). Survival of sahar ranged from 88.9 - 92.9%. The final mean weight and DWG of sahar in T2 was significantly higher (*P*<0.05) than T3 (Figure 6). However, the NFY of sahar was not significantly different between the two polyculture treatments. The extrapolated total yield of fish was 4.10 ± 0.86 t ha^{-1} yr^{-1}, 6.04 ± 0.52 t ha^{-1} yr^{-1}, and 5.40 ± 0.84 t ha^{-1} yr^{-1} in T1, T2, and T3, respectively (Table 4). The extrapolated total NFY in 1:33 sahar-tilapia polyculture was significantly higher (*P*<0.05) than tilapia monoculture, but was not different from the 1:16 treatment (Table 4).

The mean number of recruits in the control treatment (13.2 ± 4.0 recruits/m^2) was significantly higher than the 1:33 polyculture treatment (3.9 ± 0.3 recruits/m^2, Table 5). The number of recruits decreased with increasing stocking density of sahar. However, the mean total weight of recruits was not significantly different among treatments (Table 5).

The means and ranges of water quality parameters measured during the experimental period are presented in Table 6. Pond water temperature remained above 25^{o}C for most of the experimental period (Figure 7).

The budget analysis showed that polyculture with sahar and Nile tilapia produced a positive gross margin, with net returns between 3800 - 4400 NRs/100 m^2 pond compared to 2600 NRs/100 m^2 pond in the tilapia monoculture ponds (Table 7).

DISCUSSION

In the first experiment, growth rates of the Nile tilapia in polyculture with sahar at different stocking ratios were higher than those in the monoculture system. Nile tilapia gained 0.38-0.42 g/d when stocked with sahar on-station, which was lower than rates on the working aquaculture farm (0.55-0.87 g/d). This discrepancy could have been due to differences in water temperature between the two experiments; during the first experiment water temperatures remained below 20°C almost for 2.5 months while they were above 25°C for the majority of experiment two. The daily weight gains of Nile tilapia in both experiments were lower than the growth rate of Nile tilapia (1.15 g/d, Acharya, 2004) in polyculture with sahar at a 1:1 stocking ratio and conducted in a cement pond. This discrepancy is also likely explained by the duration of temperatures below 25°C (Green et al., 1997), feed type, and feed quantity. Sahar showed density-dependent growth with mean daily weight gains of 0.28-0.39 g/d in experiment one and 0.45-0.62 g/d in experiment two, indicating higher growth at lower stocking density of sahar. The results of Acharya (2004) are consistent with this density-dependence pattern, as they found a low sahar growth rate (0.32 g/d) in polyculture with Nile tilapia at a 1:1. The density dependence suggests that in order to grow well, this fish species requires a relatively large space to accommodate its riverine habits and provide an adequate forage base (tilapia recruits).

The extrapolated combined NFY of sahar and Nile tilapia was 4.37-4.93 t ha^{-1} yr^{-1} in experiment two, which was higher than in experiment one (1.4-2.2 t ha^{-1} yr^{-1}). However, both experiments showed lower yield than 6.0 t ha^{-1} yr^{-1}, obtained by Acharya (2004) in polyculture with sahar at 1:1 ratio. This research reaffirms previous findings that the addition of sahar effectively controls the number of tilapia recruits and increases NFY (Yadav et al., 2007). Furthermore, the mean weight of Nile tilapia recruits was significantly higher at higher sahar densities in both of the experiments, meaning that sahar selectively preyed upon relatively smaller recruits. The dorsal spines in large tilapia may have minimized predation in this size-class. The tilapia recruitment in earthen ponds was higher compared to other cement pond experiments (Mishra, 2002; Acharya, 2004; Yadav et al., 2007). This might have been due to the large quantity of local fish found in the earthen ponds, whose populations were also controlled by sahar (Table 2).

Most of the water quality parameters in both experiments, except water temperature in experiment one, were within acceptable ranges for fish culture (Boyd, 1990). There did not appear to be a relationship between water quality and the stocking ratio of sahar (Tables 3 and 6). Water temperature likely limited tilapia growth during most of experiment one, as it remained below 20°C for about three months (December to February). There was an occasional drop in early morning DO levels to below 0.2 mg/L in the tilapia monoculture pond, which was lower than the minima for the polyculture on-station treatments (0.8 mg/L). DO levels in the on-farm experiment remained fairly high compared to those in the on-station experiment. This might have been due to decreased

respiration from the lack of feed in the on-farm experiment. The fluctuations in DO concentration were due to variation in the rate of photosynthesis in different weather as well as the rate of oxygen consumption by the fish and other aquatic heterotrophs through respiration (Boyd, 1982). Nutrient-rich waters with high primary production would fuel high net respiration at night and generate large fluctuations in DO. Previous research supports this explanation, as increasing feed inputs fueled increasing primary production, Chlorophyll-*a* concentration, and fish growth (Diana et al., 1997).

Income in these experiments was estimated by a simple budget analysis. Fixed costs were not included in the analysis as the analysis was intended to only compare relative differences in efficiency between the treatments, and we assumed those to be similar for all treatments. The cost estimation was based on local market prices of fingerlings, fertilizers, lime, and labor wages. Results showed that net returns in the second experiment were higher in polyculture with sahar than tilapia monoculture. Among polyculture treatments, the highest returns were produced by a 1:33 sahar to Nile tilapia stocking ratio generating 4,400 NRs/100 m^2 pond (438,000 NRs/ha, Table 7).

The results of these experiments showed that the polyculture of mixed sex Nile tilapia with sahar has great potential for economic and ecological benefits. Sahar to tilapia stocking ratios between 1:33–1:16 appear to optimize tilapia growth. However, while higher stocking ratios of sahar controlled recruitment more strongly, tilapia growth was also affected. At high sahar densities, this might represent behavioral changes in tilapia to the perceived threat of sahar predation. Polyculture with sahar is the better economical choice, and more profitable in a semi-intensive polyculture system. While the cost of growing fish with fertilization and feeding in polyculture was relatively high, it would provide small scale farmers an opportunity to generate more income with higher valued fish. This system will also help to sustain sahar, whose populations are decreasing in the wild.

Figure 1. Mean weight of Nile tilapia in monoculture (T1), sahar-tilapia polyculture at a 1:16 ratio (T2), polyculture at a 1:8 ratio (T3), and polyculture at a 1:4 ratio (T4) during the experimental period in the on-station experiment.

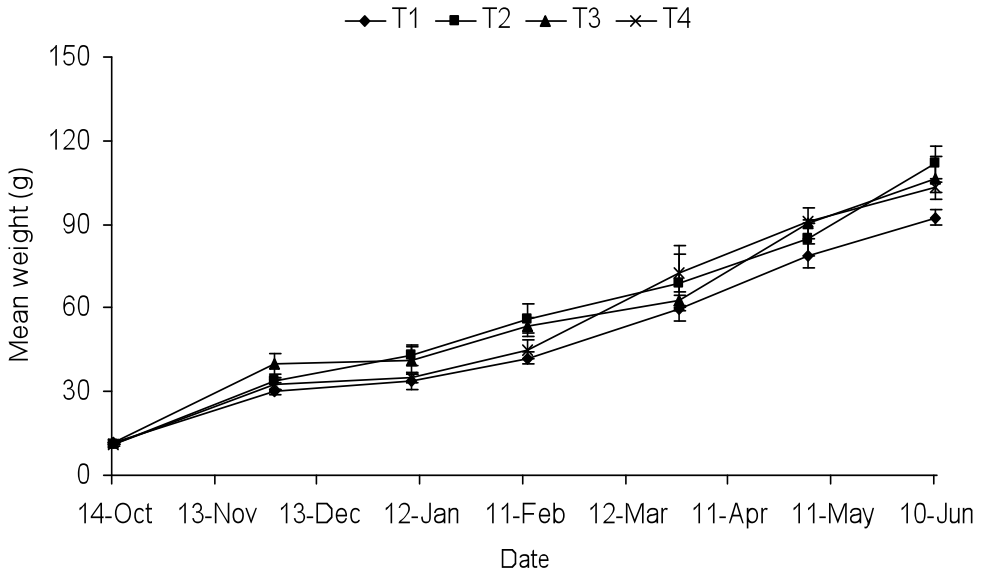

Figure 2. Mean weight of sahar in the three polyculture treatments in the on-station experiment.

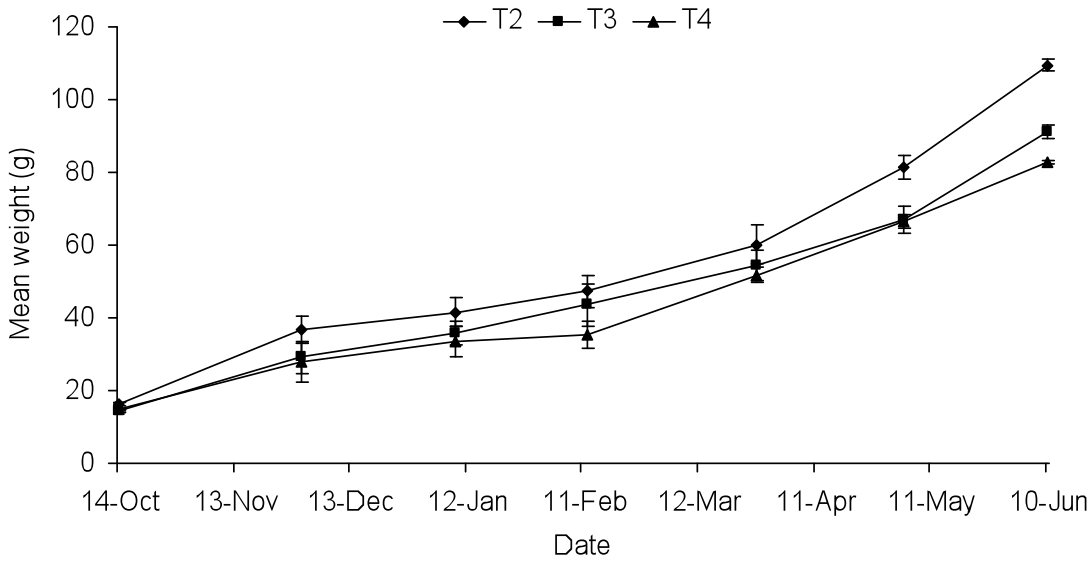

Figure 3. Relationship between sahar to tilapia stocking ratio and tilapia recruits (number m-2) during the experimental period in the on-station experiment.

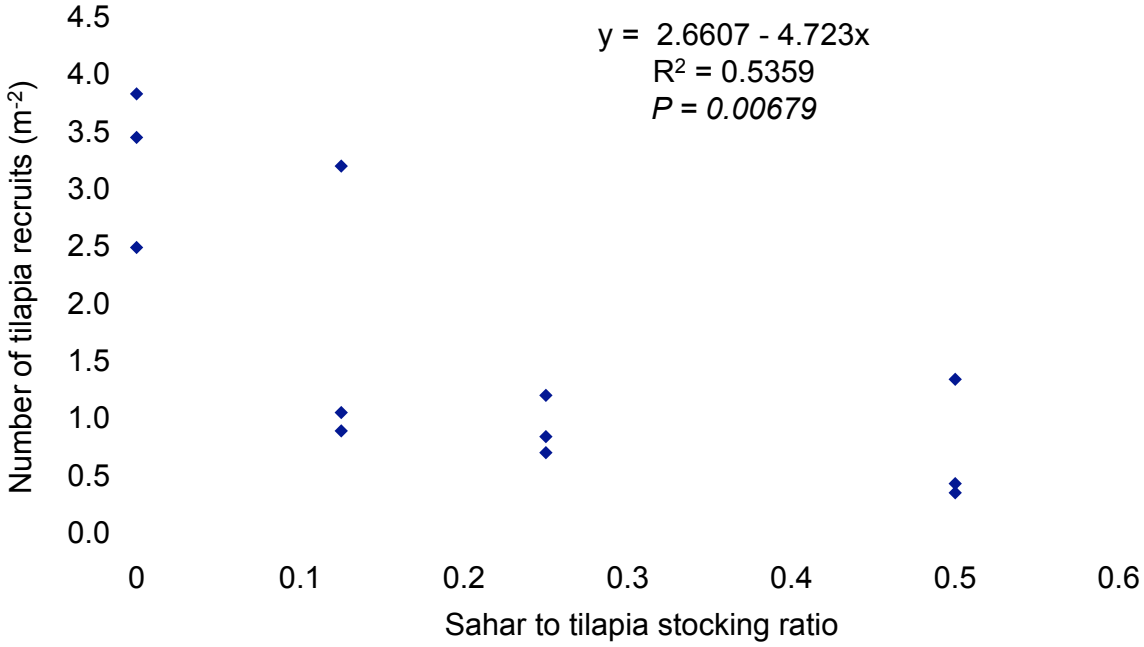

Figure 4. Weekly mean temperature (°C) of pond water at 6:30-7:30 AM for each treatment during the experimental period in the on-station experiment.

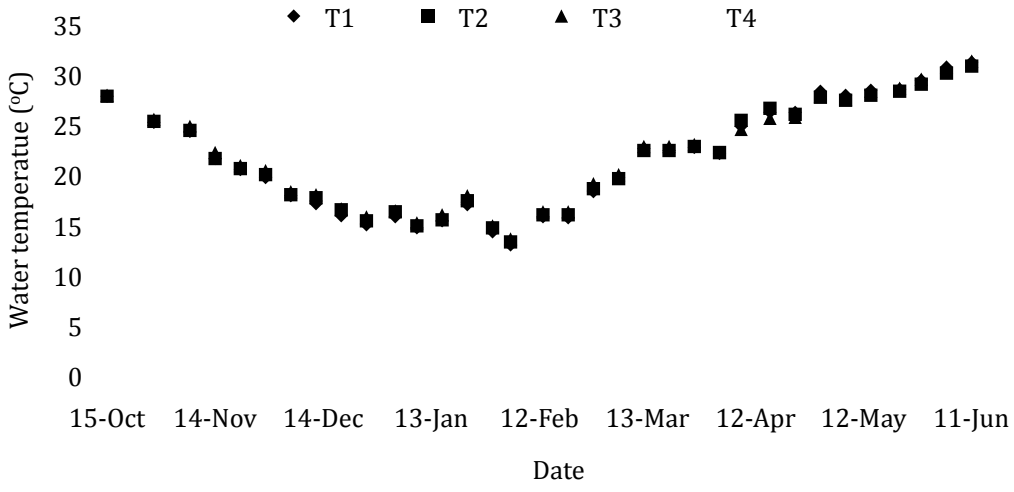

Figure 5. Monthly mean weight of Nile tilapia in the three treatments in the on-farm experiment.

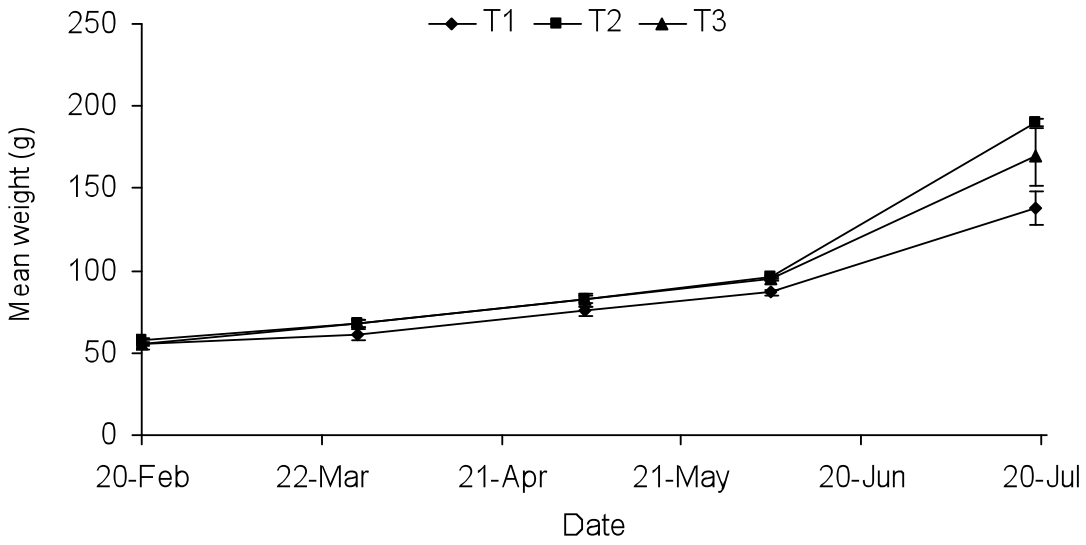

Figure 6. Monthly mean weight of sahar in the two sahar-tilapia polyculture treatments in the on-farm experiment.

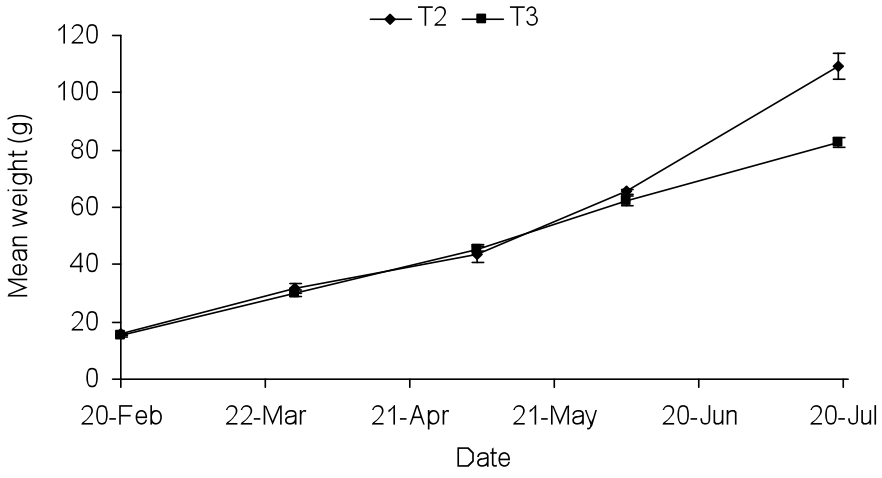

Figure 7. Weekly mean temperature (°C) of pond water at 7:00-8:00 AM the three treatments during the experimental period in the on-farm experiment.

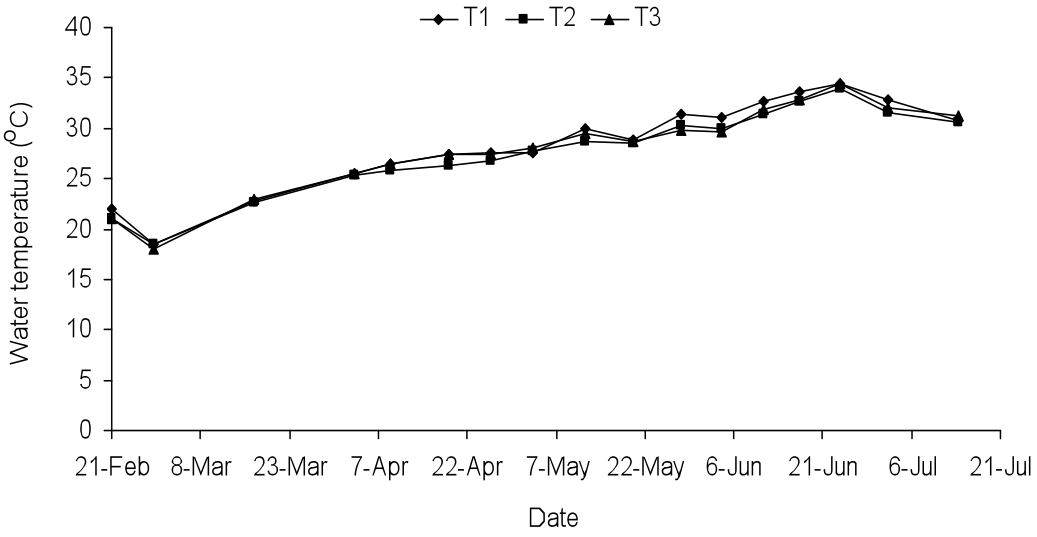

Table 1. Performance of Nile tilapia in monoculture (T1), sahar-tilapia polyculture at a 1:16 ratio (T2), sahar-tilapia polyculture at a 1:8 ratio (T3), and sahar-tilapia polyculture at a 1:4 ratio (T4) in the on-station experiment (Mean ± SE).

Parameters	Treatment			
	T1	T2	T3	T4
Nile tilapia				
Stocking				
Total weight (kg/pond)	2.3 ± 0.04	2.3 ± 0.0	2.3 ± 0.1	2.2 ± 0.1
Mean weight (g/fish)	11.6 ± 0.2	11.3 ± 0.1	11.5 ± 0.4	11.1 ± 0.4
Harvest				
Total weight (kg/pond)	10.4 ± 0.6^{a}	15.8 ± 2.0^{b}	11.9 ± 0.7^{ab}	13.1 ± 1.5^{ab}
Mean weight (g/fish)	92.4 ± 2.6^{a}	112.0 ± 6.0^{b}	106.6 ± 7.4^{ab}	103.1 ± 1.6^{ab}
Daily weight gain (g fish^{-1} d^{-1})	0.33 ± 0.0^{a}	0.42 ± 0.0^{b}	0.39 ± 0.0^{ab}	0.38 ± 0.0^{ab}
Survival (%)	56.0 ± 2.3	70.1 ± 5.0	56.8 ± 7.1	63.8 ± 8.5
NFY (t ha^{-1} yr^{-1})	1.2 ± 0.1^{a}	2.1 ± 0.3^{b}	1.5 ± 0.1^{ab}	1.7 ± 0.2^{ab}
Sahar				
Stocking				
Total weight (kg/pond)	-	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.7 ± 0.0^{c}
Mean weight (g/fish)	-	16.3 ± 0.3^{a}	14.5 ± 0.5^{b}	14.7 ± 0.1^{b}
Harvest				
Total weight (kg/pond)	-	0.8 ± 0.1^{a}	1.1 ± 0.1^{a}	1.5 ± 0.1^{b}
Mean weight (g/fish)	-	109.4 ± 1.5^{a}	91.1 ± 1.9^{b}	82.9 ± 0.4^{c}
Daily weight gain (g fish^{-1} d^{-1})	-	0.39 ± 0.0^{a}	0.32 ± 0.0^{b}	0.28 ± 0.0^{c}
Survival (%)	-	56.4 ± 6.7	49.3 ± 5.8	39.3 ± 3.5
NFY (t ha^{-1} yr^{-1})	-	0.10 ± 0.00	0.10 ± 0.00	0.14 ± 0.02
Total NFY (t ha^{-1} yr^{-1})	1.2 ± 0.1^{a}	2.2 ± 0.3^{b}	1.4 ± 0.2^{a}	1.8 ± 0.2^{ab}
Mean FCR	0.24	0.17	0.17	0.18

Table 2. Tilapia recruitment and local fish harvested from ponds (100 m^2) in the four treatments during the on-station experiment (Mean ± SE).

Parameter	Treatment			
	T1	T2	T3	T4
Mean number (count/pond)	324 ± 40[a]	169 ± 74[b]	89 ± 15[b]	69 ± 32[b]
Mean weight (g/fish)	3.3 ± 0.2[c]	6.2 ± 0.9[b]	7.9 ± 1.0[b]	10.6 ± 0.9[a]
Local fish (kg/pond)	4.7 ± 1.6	1.7 ± 0.7	2.8 ± 0.4	1.8 ± 0.9

Table 3. Mean (± SE and range) weekly and bi-weekly water quality parameters in each treatment during the on-station experiment.

Parameters	Treatment			
	T1	T2	T3	T4
Temperature (°C)	21.6 ± 0.5 (12.5-31.4)	21.7 ± 0.5 (12.6-31.8)	21.9 ± 0.5 (13.4-31.6)	21.5 ± 0.5 (12.6-30.9)
Dissolved oxygen (mg/L)	6.2 ± 0.4 (0.2-14.7)	6.3 ± 0.4 (0.8-18.4)	7.1 ± 0.4 (0.7-14.5)	7.3 ± 0.5 (0.7-17.7)
pH	8.4 (7.2-9.4)	8.1 (6.9-9.2)	8.4 (7.3-9.3)	8.6 (7.1-9.7)
Secchi disk depth (cm)	21.8 ± 0.8 (8-55)	27.4 ± 1.2 (8-56)	29.8 ± 1.5 (9-70)	22.5 ± 1.1 (8-60)
Pond water depth (cm)	52.8 ± 0.8 (40-70)	48.1 ± 0.9 (30-62)	49.5 ± 0.6 (34-63)	50.4 ± 0.8 (30-65)
Total alkalinity (mg/L $CaCO_3$)	110.9 ± 2.2 (78.7-152.1)	104.2 ± 2.3 (67.3-134.4)	104.6 ± 2.0 (73.7-145.2)	103.9 ± 1.4 (85.9-135.0)
Chlorophyll *a* (mg/m^3)	78.8 ± 8.8 (5.3-315.4)	58.1 ± 6.8 (2.7-240.6)	46.9 ± 5.4 (0.0-200.5)	78.4 ± 9.8 (2.7-291.4)
Gross primary productivity (g C m^{-2} 12 hr^{-1})	5.1 ± 0.3 (2.4-12.8)	5.4 ± 0.3 (1.8-11.8)	5.4 ± 0.3 (1.5-12.0)	5.3 ± 0.4 (1.6-13.0)
Net primary productivity (g C m^{-2} 12 hr^{-1})	2.6 ± 0.2 (1.3-6.8)	2.6 ± 0.2 (0.8-6.2)	2.7 ± 0.2 (0.6-6.2)	2.7 ± 0.2 (0.8-6.3)
Total ammonium nitrogen (mg/L)	0.10 ± 0.0 (0.01-0.3)	0.09 ± 0.0 (0.01-0.32)	0.10 ± 0.01 (0.01-0.35)	0.08 ± 0.0 (0.01-0.26)
Soluble reactive phosphorus (mg/L)	0.16 ± 0.01 (0.0-0.42)	0.12 ± 0.01 (0.0-0.37)	0.13 ± 0.01 (0.0-0.37)	0.13 ± 0.01 (0.0-0.37)
Nitrite nitrogen (mg/L)	0.10 ± 0.02 (0.0-0.57)	0.15 ± 0.03 (0.0-0.88)	0.14 ± 0.02 (0.0-0.59)	0.17 ± 0.02 (0.0-0.70)
Nitrate nitrogen (mg/L)	0.29 ± 0.05 (0.0-1.27)	0.30 ± 0.06 (0.01-1.49)	0.32 ± 0.05 (0.01-1.45)	0.30 ± 0.05 (0.01-1.29)
Total phosphorus (mg/L)	0.48 ± 0.04 (0.04-1.36)	0.39 ± 0.04 (0.04-1.05)	0.49 ± 0.04 (0.05-1.41)	0.41 ± 0.03 (0.02-0.97)

Table 4. Performance of Nile tilapia in the four treatments in the on-farm experiment (Mean ± SE).

Parameters	Treatment		
	T1	T2	T3
Nile tilapia			
Stocking			
Total weight (kg/pond)	11.42 ± 1.29	12.45 ± 0.83	12.36 ± 1.01
Mean weight (g/fish)	55.3 ± 3.1	57.4 ± 1.7	55.7 ± 1.4
Harvest			
Total weight (kg/pond)	26.20 ± 1.90[a]	40.57 ± 0.71[b]	35.32 ± 3.02[b]
Mean weight (g/fish)	138.0 ± 10.1[a]	190.0 ± 2.0[b]	169.3 ± 17.8[ab]
Daily weight gain (g $fish^{-1}$ d^{-1})	0.55 ± 0.08[a]	0.87 ± 0.01[b]	0.75 ± 0.11[ab]

Survival (%)	92.9 ± 1.7	94.98 ± 0.8	95.1 ± 2.6
NFY (t ha^{-1} yr^{-1})	2.91 ± 0.54[a]	4.82 ± 0.01[b]	4.22 ± 0.76[b]
Sahar			
Stocking			
Total weight (kg/pond)	-	0.11 ± 0.00[a]	0.21 ± 0.01[b]
Mean weight (g/fish)	-	15.7 ± 0.2	15.1 ± 0.5
Harvest			
Total weight (kg/pond)	-	0.71 ± 0.08	1.08 ± 0.24
Mean weight (g/fish)	-	109.5 ± 4.5[a]	82.5 ± 1.7[b]
Daily weight gain (g $fish^{-1}$ d^{-1})	-	0.62 ± 0.03[a]	0.45 ± 0.01[b]
Survival (%)	-	92.9 ± 4.1	88.9 ± 11.1
NFY (t ha^{-1} yr^{-1})	-	0.11 ± 0.01	0.15 ± 0.03
Total NFY (t ha^{-1} yr^{-1})	2.91 ± 0.54[a]	4.93 ± 0.01[b]	4.37 ± 0.74[ab]

Table 5. Nile tilapia recruitment in the three treatments in the on-farm experiment (Mean ± SE).

Parameter	Treatment		
	T1	T2	T3
Mean number (count/pond)	1371 ± 406[a]	899 ± 184[a]	434 ± 31[b]
Mean number (count/m)	13.2 ± 4.0[a]	8.0 ± 1.4[a]	3.9 ± 0.3[b]
Mean weight (g/fish)	4.4 ± 0.6[a]	6.6 ± 1.7[a]	13.1 ± 1.0[b]

Table 6. Weekly or monthly water quality parameters in the three treatments in the on-farm experiment (mean ± SE range).

Parameters	Treatment		
	T1	T2	T3
Temperature (°C)	28.4 ± 0.3 (18.5-34.5)	27.3 ± 0.2 (18.4-34.0)	28.0 ± 0.4 (18.0-34.8)
Dissolved oxygen (mg/L)	4.0 ± 0.6 (1.3-5.7)	4.9 ± 0.2 (0.75-8.4)	4.6 ± 1.1 (1.0-7.6)
pH	8.5 ± 0.0 (7.3-9.5)	8.5 ± 0.0 (7.0-9.8)	8.6 ± 0.0 (7.1-9.6)
Secchi disk depth (cm)	29.9 ± 2.3 (21.7-40.7)	24.7 ± 4.6 (19.3-31.0)	20.3 ± 1.2 (14.7-31.0)
Pond water depth (cm)	85 ± 2 (72-102)	82 ± 1 (70-92.5)	85 ± 3 (70-95)
Total alkalinity (mg/L $CaCO_3$)	101.9 ± 5.5 (95.9-107.1)	108.9 ± 1.5 (89.3-137.6)	115.1 ± 3.0 (100.0-141.4)
Chlorophyll - *a* (mg/m^3)	71.0 ± 10.7 (3.6-137.2)	47.7 ± 24.0 (10.0-124.3)	77.0 ± 6.3 (16.9-144.3)
Total ammonium nitrogen (mg/L)	0.095 ± 0.035 (0.015-0.167)	0.078 ± 0.010 (0.055-0.110)	0.096 ± 0.014 (0.068-0.114)
Soluble reactive phosphorus (mg/L)	0.048 ± 0.015 (0.001-0.096)	0.046 ± 0.019 (0.017-0.093)	0.057 ± 0.002 (0.010-0.093)

Table 7. Comparative economic analysis (Mean ± SE) of the three treatments in the on-farm experiment, based on a 100 m^2 pond and Nepalese currency (NRs).

Category	Price	T1	T2	T3
Gross Return				
Adult Nile tilapia	140.0	3,595.7 ± 304.2	5,049.2 ± 23.8	4,532.1 ± 571.7
Sahar	250.0	-	158.3 ± 15.1	236.9 ± 32.9
Nile tilapia recruits	0.50-2.00	685.3 ± 202.9	899.0 ± 184.0	867.3 ± 61.4
Total		4,281.0 ± 503.9[a]	6,106.6 ± 192.7[b]	5,636.4 ± 597.5[b]
Variable Cost				
Lime	15.00	75.0	75.0	75.0
Tilapia (fingerlings)	5.00	1,000.0	1,000.0	1,000.0
Sahar (fingerlings)	10.00	-	70.0	130.0
DAP	35.00	134.8	134.8	134.8
Urea	18.00	93.1	93.1	93.1
Labor	100.00	200.0	200.0	200.0
Cost of working capital	(10 %)	150.3	157.3	163.3
Total Variable Cost		1,653.2	1,730.2	1,796.2
Gross Margin (1,000 NRs/pond)		2.6 ± 0.5[a]	4.4 ± 0.2[b]	3.8 ± 0.6[ab]
Gross Margin (1,000 NRs/ha)		262.8 ± 50.4[a]	437.6 ± 19.3[b]	384.0 ± 59.8[ab]

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CO-SPONSORSHIP OF "SECOND INTERNATIONAL WORKSHOP ON THE CULTIVATION AND BIOTECHNOLOGY OF MARINE ALGAE: AN ALTERNATIVE FOR SUSTAINABLE DEVELOPMENT IN LATIN AMERICA AND THE CARIBBEAN"

Production System Design and Best Management Alternatives/ Activity/ 07BMA03UA

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ABSTRACT

At the 2009 World Aquaculture Society Meetings in Veracruz Mexico, we organized and co-chaired a workshop the - Second International Workshop on the Cultivation and Biotechnology of Marine Algae. As part of our support for this workshop, we provided transportation and registration funding for four participants in the workshop. The workshop itself included nine presentations and a panel discussion. As a follow-up to the workshop we collected the Presentations and converted to PDF's for posting on a conference website.

INTRODUCTION

Seaweeds and other algae are widely recognized as one of the most important sectors of the aquaculture industry. Many algae species are consumed directly by humans, especially in Asia, where they contribute significantly to general nutrition (Figure 1.). An even larger industry exists culturing seaweeds for processing with some of the constituents used as functional ingredients in all types of processed foods. Alginates, agar, and carageenan are the most common ingredients. Algae are critical components of developing sustainable integrated aquaculture systems, as important nutrient sources for rearing aquatic animals and to absorb their wastes (Figure 2 and 3). Multi-trophic aquaculture systems will address many of the complaints leveled at current aquaculture practices and generate an additional marketable crop (Figure 4). Algae also predominate in many of the second generation biofuel plans that endeavor to create sustainable sources of fuel that will also remove large quantities of carbon dioxide from the atmosphere. Currently, virtually all of the economically successful algae businesses are part of the aquaculture industry. The community interested in utilizing algae for biofuels has much to learn from aquaculture practitioners who have many years experience.

Our goal in sponsoring the workshop was to increase awareness and to begin sharing with a wider community the skills and experience from the aquaculture industry. We invited both established practitioners of algae production for human consumption, commercial producers of phytoplankton used in fish and shrimp hatcheries, researchers producing micro-algae for biofuels and students presenting their research results.

Conference highlights

The session included:

09:00 Mauricio Ondarza
ALGAE AS A SUSTAINABLE FEEDSTOCK ALTERNATIVE FOR BIOFUELS

09:20 Koen Vanhoutte, Victor Chepurnov, Luc Roef
A REVIVAL OF INDUSTRIAL APPLICATIONS OF MICRO-ALGAE IN AQUACULTURE

09:40 Kim Falinski, Charles Laidley, Michael Timmons, Leonard Lion
EFFECTS OF DIFFERENT AERATION CONDITIONS ON *Isochrysis galbana* (T-ISO) CCMP 1324 IN A BENCH-SCALE PHOTOBIOREACTOR

10:00 Simon Chung, Kao Chung Wang, Ping Hua Teng, T. M. Lee, Anne T. J. Chow
GENETIC MODIFICATION OF MICROALGAE FOR ANTI-MICROBIAL ACTIVITIES

11:10 Julieta Munoz, Ravi Fotedar
BASIC BIOLOGY OF THE AGAROPHYTE *Gracilaria cliftonii* FROM WESTERN AUSTRALIA

11:30 Abdollah Haghpanah, Lalik Sarikhani, Yousef Iri, Behrooz Gharavy
COMPARISON OF EXTRACTED ALGINIC ACID IN BROWN ALGAES; *Sargassum illicifolium*, *Cystoseira indica* AND *Nizimuddinia zanardini* IN THE OMAN SEA (CHABAHAR)

11:50 Raul Rincones, Daniel Robledo
THE INTRODUCTION AND CULTIVATION OF THE RED ALGA *Kappaphycus alvarezii* FOR THE PRODUCTION OF CARRAGEENAN IN THE CARIBBEAN AND THE WESTERN ATLANTIC: AN ALTERNATIVE LIVELIHOOD FOR COASTAL COMMUNITIES

12:10 Kevin Fitzsimmons, Anicia Hurtado, Michael Rimmer, Nelson Golez, Hasan Hasanuddin
Gracilaria AND *Euchuma* PRODUCTION IN TSUNAMI AFFECTED AREAS OF BANDA ACEH, INDONESIA

12:30 Rafael Martinez-Garcia, Stephen Nelson, Brendan Ambrose, Edward Glenn, Kevin Fitzsimmons

BIOMASS PRODUCTION, SEED YIELD, AND TISSUE OSMOLARITY OF *Salicornia bigelovii Torr.* (Chenopodiacae) IN RELATION TO IRRIGATION SALINITY

Workshop Posters

This poster was presented with partial travel support provided to graduate student Michael Mason. The research conducted by Michael was also partly supported by AquaFish CRSP funds.

Michael J. Mason, Joel Cuello
MICROALGAL PRODUCTION IN CLOSED SYSTEM BIOREACTORS BASED ON MIXING AND RESIDENCE TIME

The following poster was presented the second place student award by the AquaFish CRSP.

Socorro Jiménez-Valera, M. del Pilar Sánchez-Saavedra
EVALUATION OF GROWTH AND NUTRIENT REMOVAL OF MIXED PHYTOPLANKTON CULTURES

The other algae posters included:
Jeane Rimber Indy, Lenin Arias Rodriguez, Hajime Yasui
INDONESIAN SEAWEED BIODIVERSITY

Lúcia Helena Sipaúba Tavares, Rodrigo Ney Millan, Flávia de Almeida Berchielli
USE OF ALTERNATIVE MEDIUMS AND DIFFERENT TYPES OF RECIPIENTS IN THE LABORATORY CULTURE OF *Ankistrodesmus gracilis* (REISCH) KORSIKOV (CHLOROPHYTA)

Abdollah Haghpanah, Lalik Sarikhani, Yousef Iri, Behrooz Gharavy
CULTURE OF *Gracilaria corticata* IN THE EARTHEN PONDS OF BERIS SESSION (CHABAHAR)

Abdollah Haghpanah, Lalik Sarikhani, Yousef Iri, Behrooz Gharavy
CULTURE OF *Gracilaria corticata* IN THE EARTHEN POND AND SEA (CHABAHAR)

Antonio López, Norma Garcia, Anselmo Miranda, Nolberta Huerta, Antonio García
GROWTH AND BIOCHEMICAL COMPOSITION OF THE MICROALGAE *Thalassiosira pseduonana* AT 6 SALINTIES IN 3 GROWTH PHASES

Daniel Robledo, Eucario Gasca-Leyva, Roger Domínguez May
GROWTH MODEL FOR *Kappaphycus alvarezii* 'COTTONI'

Marcelo Shei, Marcelo Shei, Talia Bonfante, Oscar Barreto, Gastão Bastos
PRODUCTION AND COSTS OF THE MARINE DIATOM *Chaetoceros calcitrans* USING DIFFERENT BRANDS OF ARTIFICIAL SEAWATER

CONCLUSIONS

The workshop was very well attended and generated considerable discussion. The World Aquaculture Society has since followed-up our workshop with a similar session at the Asia-Pacific Aquaculture Meeting in Kuala-Lumpur, Malaysia in November 2009 (Figure 5). As we gather the final PDF's for the website and provide links and publicize the site we expect to garner a significant amount of web traffic. We also plan to build upon the linkages generated at the meeting. Raul Rincones from Venezuela has offered to assist with a workshop in Guyana. He has also been in correspondence with our Mexican colleagues. We hope to further expand these links with others we met at the Kuala Lumpur conference who have developed method to create high quality white paper from red algae.

Figure1: Raul

Figure 2. Algae Biofilters

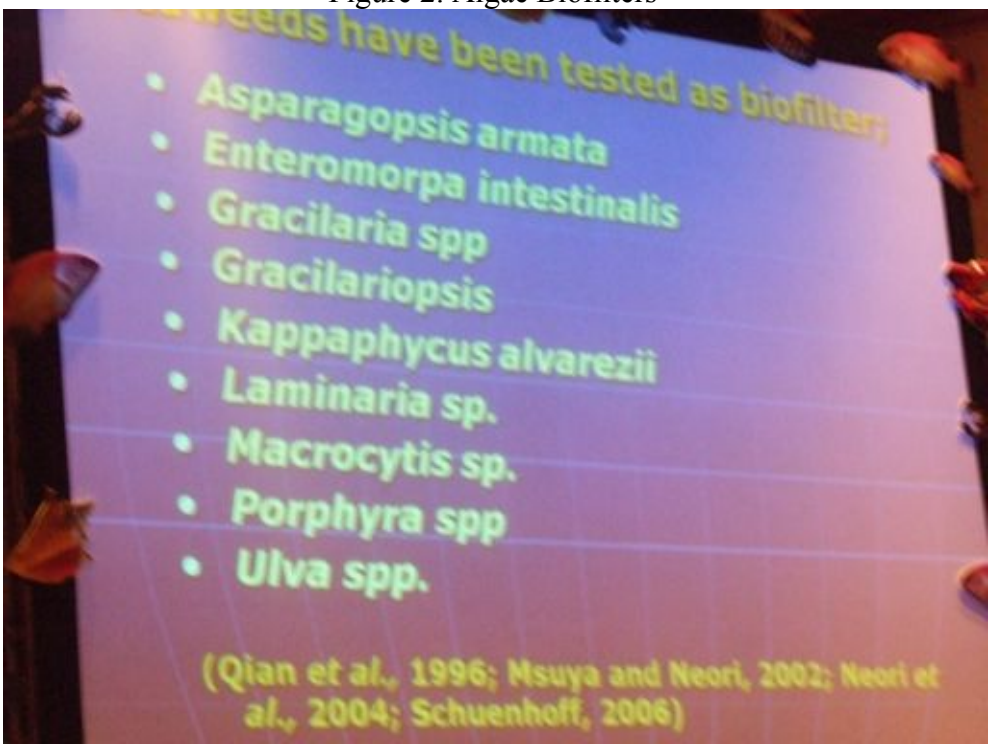


Figure 3. Larviculture algae

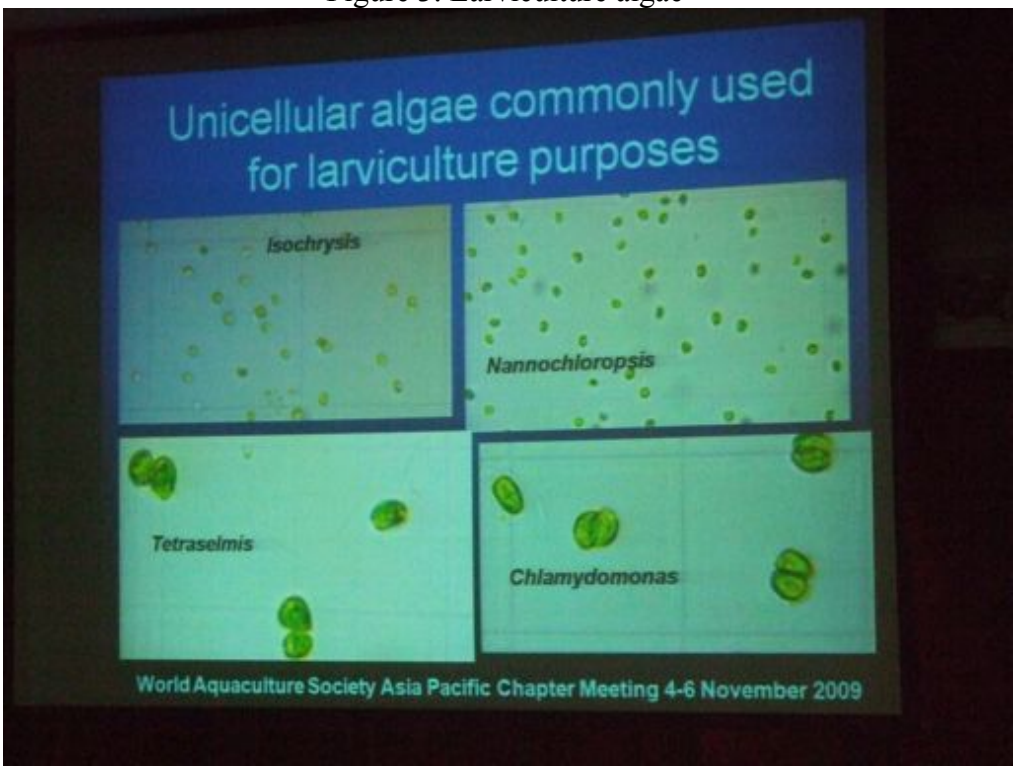


Figure 4. Seaweed Shrimp

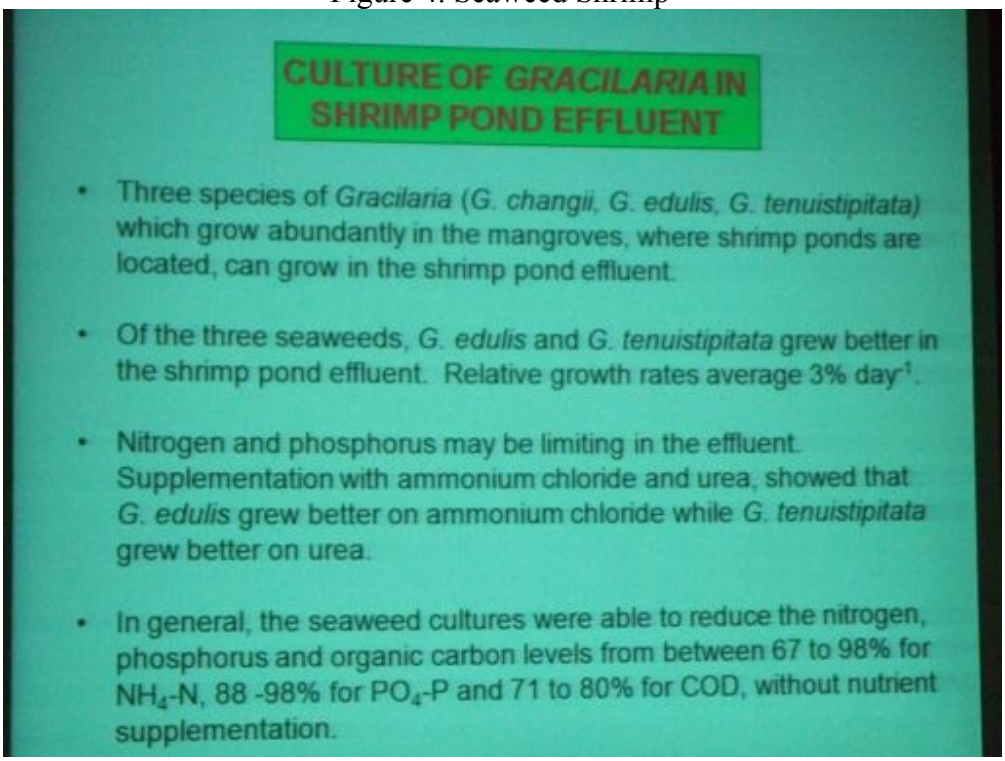
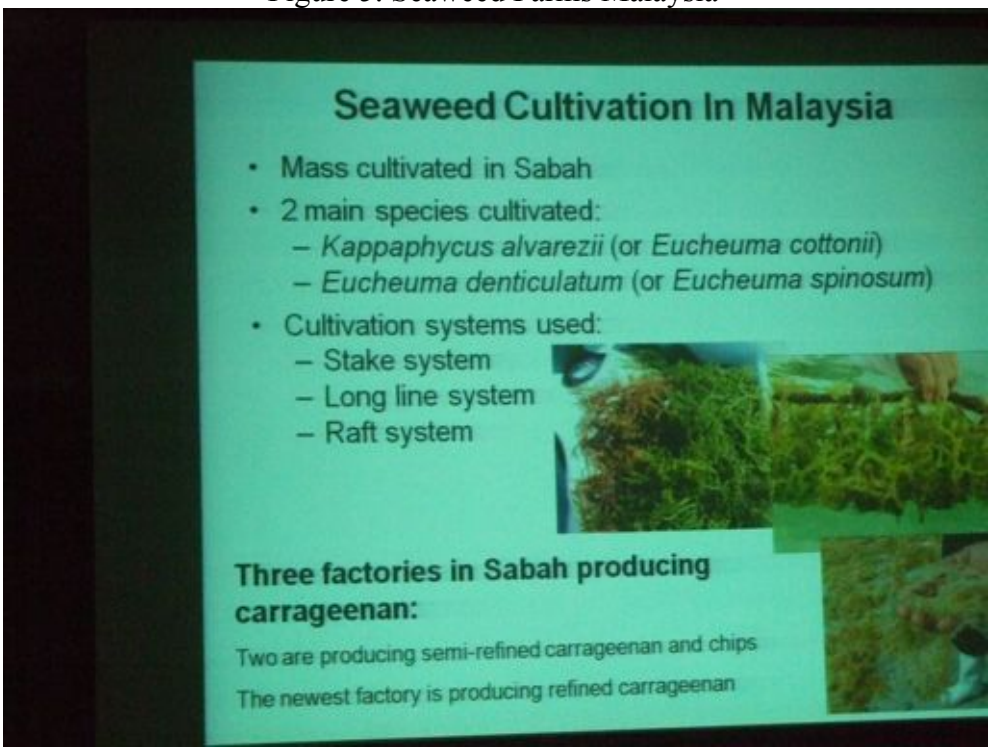


Figure 5. Seaweed Farms Malaysia



TRAINING IN BEST MANAGEMENT PRACTICES FOR THE PRODUCTION OF MOLLUSKS IN THE STATES OF NAYARIT AND SINALOA

Production System Design and Best Management Alternatives/ Activity/ 07BMA04UH

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ABSTRACT

Two intensive workshops were held at the UAS Marine Sciences Facility in Mazatlan, Mexico on November 21 and December 5, 2009. The purpose of the workshops was to increase capacity in the area of good practices for bivalve shellfish culture with an emphasis on the sanitation and health aspects. Much of the content was based on the findings of past and current CRSP investigations. Twenty-eight producers and technical assistance providers from Sinaloa and Nayarit were among the participants.

INTRODUCTION

Bivalve mollusk culture is a priority for aquaculture development throughout LAC. In the case of Mexico, state governments (e.g. North Baja California, South Baja California, Nayarit, Sinaloa, Sonora) and the federal government have prioritized shellfish culture for development for nearly ten years. The Autonomous University of Sinaloa and its numerous partners in aquaculture development have recently worked together in an integrated effort to accelerate development of the shellfish industry with long-term support from CRSP/USAID. Two intensive workshops were held on November 21 and December 5, 2009. Each half day represented a separate module.

The objectives of this training workshop were to:

- Present the results of research and development efforts by stakeholders from the Northern/Central Pacific Coast of Mexico;
- Increase technical capacity among farmers and technical assistance providers.
- Increase extension capacity and partnerships;

- Provide a net working opportunities; and
- Develop strategies for current and future collaborative efforts.

METHODS

The workshop was designed and planned as a collaborative effort between HC institutions (UAS, CESASIN, CIAD) and U.S. Universities (LSU and UHH). The workshop was held on November 21 and December 5, 2009. Originally this series of workshops had been intended to be held on four separate days, but given the many delays caused by the swine flu epidemic in Mexico which resulted in banning of public meetings for several months over the 2009 year, the workshops were condensed into 2 days with four separate modules.

Topics included:

- Five key methods for safe and healthy seafood to prevent food borne diseases (Dr. Omar Calvario, CIAD)
- Community Waste Management (Eladio Gaxiola, UAS)
- How to operate an "ecological latrine" (Eladio Gaxiola, UAS)
- Sanitary risks due to trash and human wastes in aquaculture areas and garbage from fisheries products and operations (Eladio Gaxiola,UAS)
- Oyster culture methods (Gilberto Soto, CESASIN)
- Presentation by community residents on the CRSP projects
- Determination of the carrying capacity of Boca de Camichin Estuary
- Native oyster spat (*Crassostrea corteziensis*) collection at Santa Maria Bay

RESULTS AND CONCLUSIONS

Fifteen stakeholders (12 men, 3 women) attended the first workshop and thirteen attended the second (10 men, 3 women). The workshop was also video-taped and DVD's with the video and PowerPoint presentations were delivered to the participants after conclusion of the workshop.

Benefits

There was a high level of satisfaction among the trainees and several have since put the acquired knowledge and skills to use. A total of 28 stakeholders representing the following institutions or cooperatives attended:

Ostricamichin (cooperative)
Concheros de los Campos (cooperative)
Pescadores de la Reforma (cooperative)
Ostricola Faro de Papillon (cooperative)
Ostioneros del Mar de Villa Juarez (cooperative)
Cultivos de Playa Colorado (cooperative)
Ayuntamiento de Santiago Ixcuintla (government)
UAS Mazatlan and Culican campuses (university)
CIAD (government)

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INTENSIVE TRAINING AND INTERNSHIP IN BIVALVE CULTURE AND SHELLFISH SANITATION

Production System Design & Best Management Alternatives/ Activity/ 07BMA05UH

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ABSTRACT

A seven day training workshop in hatchery methods and shellfish sanitation was held in Louisiana on June 8-15 and was hosted by the Louisiana State Sea Grant College Program. The training was highly effective in increasing capacity among the personnel of LSU, UHH, UAS and UCA for hatchery design, planning, management as well as for shellfish sanitation. Knowledge and skills acquired from this training have since benefited the CRSP efforts in the U.S., Mexico and Nicaragua. Four graduate students also benefited from the training.

INTRODUCTION

Bivalve mollusk culture is a priority for aquaculture development throughout LAC. In the case of Mexico, state governments (e.g. Nayarit, Sinaloa, Sonora) and the federal government have prioritized shellfish culture for development for nearly ten years. The Autonomous University of Sinaloa and its numerous partners in aquaculture development have recently worked together in an integrated effort to accelerate development of the shellfish industry with long-term support from CRSP/USAID. In the case of Latin America, there is wide spread recognition of the potential for shellfish aquaculture, but progress towards realizing its potential has been slow. The Central American University (UCA), has been promoting shellfish culture and management of the shellfish fisheries for over ten years with support from multiple donors, including CRSP. Throughout Latin America, molluscan culture has been most successful when wild spat collection is adequate to support industry development.

Further development into other areas and species has been limited by the lack of mollusk

hatcheries. In the case of the Pacific Coast of Mexico, the bivalve industry is divided between Nayarit, where spat collection of the native "Pleasure Oyster" *Crassostrea corteziensis* is abundant, and the northern States surrounding the Sea of Cortez, where the non-native species, *C. gigas* (Japanese Oyster) is predominant. The latter industry is largely supplied with eyed larvae or spat from U.S. hatcheries. In Nicaragua, UCA has been working since 1997 to research and development bivalve culture, particularly for cockle species. Throughout the LAC region, there is potential to develop other native species for culture and improve production methods for currently cultured species, but one major impediment is the lack of molluscan hatcheries in the region. The lack of hatcheries is due in part to the greater economic feasibility of shrimp production, but also in part due to lack of technical capacity.

The objectives of this training workshop were to:

- Build capacity among Mexican and Nicaraguan researchers and extension agents for hatchery methods;
- Increase inter-institutional sharing of knowledge and methods;
- Provide hands-on experience in hatchery and nursery production;
- Increase understanding of the U.S. model of shellfish sanitation; and
- Familiarize participants with other aspects of the U.S. Gulf Coast shellfish industry. The desired outcomes included:
- Sufficient capacity among UAS and UCA personnel to evaluate the feasibility of establishing small scale hatcheries at their home institutions
- That personnel would have sufficient technical capacity to design, build and operate small-scale hatcheries;
- To support on-going efforts to establish shellfish sanitation programs in Mexico and Nicaragua; and
- An increased degree of institutional collaboration between U.S. and HC institutions.

METHODS

A training workshop was held June 8-15 at the Louisiana State University (LSU) Sea Grant College Program Oyster Hatchery. Dr. John Supan is Director of the hatchery and led this effort with assistance from Dr. Maria Haws, who directs the UHH shellfish hatchery program. Participants met in New Orleans and first attended the Southeastern States Shellfish Sanitation Annual Meeting which included one day of presentations and one day of visits to surrounding industry sites. The latter included visits to Motavatit Seafood, which is one of only two companies in the U.S. which uses hydrostatic pressure to treat raw oysters. A second company was also visited which uses low temperature pasteurization for oysters. The group had a chance to see and sample various treated products and discuss value-added strategies. The group also saw oyster dredges and discussed harvesting and handling processes.

The LSU hatchery is located on Grand Isle in Louisiana. Six days of training were provided there on the topics of: microalgae culture; induced spawning; larval rearing; remote setting; water treatment; and related topics. Additionally, considerable discussion

was given to the topic of hazard preparedness in hatchery design and operation. The LSU hatchery had been destroyed by Hurricane Katrina in 2005, but due to special design and preparedness considerations, economic loss was minimized and recuperation was speeded up. This information is useful since both the Nicaraguan and Mexico coasts are vulnerable to storms and hurricanes.

Participants included: John Supan, Esther Young, Marc Stubbs (LSU), Maria Haws (UHH), Nelvia Hernandez and Abelardo Rojas (Nicaragua), Daren Garriques (Ecuador) and Olga Zamudio (Mexico). Young, Stubbs, Garriques and Rojas are graduate students.

RESULTS

There was a high level of satisfaction among the trainees and several have since put the acquired knowledge and skills to use. Olga Zamudio and Nelvia Hernandez have since begun to plan and design small-scale research hatcheries for their universities. The former hatchery at FACIMAR/UAS in Mexico is part of the CRSP workplan for 2010. UCA obtained funding for their hatchery efforts from other donors. Daren Garriques is now employed at the UHH oyster hatchery and is utilizing his increased skills in conducting his masters degree research. Maria Haws has since made modifications to the UHH hatchery, particularly for preparedness purposes, based on the methods used at the LSU hatchery.

CONCLUSION

The workshop was very beneficial in increasing capacity for hatchery design and development. Additionally it also increased the level of communication and cooperation between the four institutions participating in this work. Much of the future CRSP research and extension in Mexico and Nicaragua has since benefited from this training.

Benefits

Please see above.

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TOPIC AREA
SUSTAINABLE FEED TECHNOLOGY

ALTERNATIVE FEEDS FOR FRESHWATER AQUACULTURE SPECIES

Sustainable Feed Technology/Study/ 07SFT01UC

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INTRODUCTION

Aquaculture is growing rapidly in Vietnam and has the potential to do the same in Cambodia. Production of pangasiid catfish in the Mekong Delta of Vietnam alone exceeded 1 million metric tons in 2008. While some of the food provided to these fish, especially at the larger commercial farms, is pelleted feed from commercial feed mills, many small farmers still use "trash fish" from the Mekong in preparing feed by hand at the farm. In Cambodia, catfish culture is still at the small-farm stage and trash fish comprise the basic feed for the industry (which is considerably smaller in Cambodia than in Vietnam).

As aquaculture expands in Vietnam and Cambodia, the fish called snakehead is becoming popular to culture because of its high value in the market. There are actually two species currently being cultured, *Channa striata*, the snakehead murrel, and *Channa micropeltes*, the giant snakehead. While culture of these is permitted (and growing) in Vietnam, it is prohibited in Cambodia (except for some experimental work) due to its dependence on small fish in the diet. Catfish culture has available commercial pellet diets, so getting farmers to switch from small fish to pellets is a socioeconomic issue. On the other hand, pelleted diets do not yet exist for snakehead in Vietnam and Cambodia.

Piscivorous (fish-eating) fish like snakehead typically require high levels of protein in the diet, reflecting the high protein in their natural diet. The usual source of that protein in pellet diets is fish meal (FM), an international commodity made from species like anchovy, herring, menhaden, capelin and so on. Because of the high price of fish meal, and to reduce the fishing pressure on the aforementioned species, fish nutritionists and aquaculturists worldwide are trying to replace fish meal with plant proteins in diets for fish.

The objective of the study is the development of cost-effective alternative feed for carnivorous freshwater species to replace or reduce the dependence on low value/ trash fish. The results of this study are intended to provide information on alternative diets for snakehead, especially those diets that incorporate local plant materials, in order to build a long-term sustainable industry. Through an economic analysis of costs of the diets (based on costs of fish meal and plant proteins vs. trash fish) and the risks of the unavailability of trash fish in the future, study results allow decisions to be made on the development of feed mills for local production for the snakehead industry.

To meet the objective, the first study, qualitative and quantitative assessment of the regular freshwater trashfish diet for snakehead food, was done. Then, a series of formulated feed experiments were conducted at the wet laboratory and hapas at the College of Aquaculture and Fisheries (CAF) of Cantho University (CTU) to develop formulated feed for snakehead culture. The experiments conducted were:

(i) Weaning methods with formulated feeds for snakehead *(Channa striata)* larave

(ii) Replacement of fishmeal with soybean meal (SM) with or without phytase and taurine in diets for *Channa striata*, and *Channa micropeltes*

(iii) Utilization of rice bran in snakehead *Channa striata* feed

(iv) Replacement of fishmeal with soybean meal with additions of soluble fish attractant or alpha-galactosidase in diets for *Channa striata*

(v) Replacement of freshwater trash fish by formulated feed in snakehead (*Channa striata*) and (*Channa micropeltes*) fingerling diets

(vi) Taste analysis of snakehead fed by different feeds

RESULTS

2.1 Study 1: Qualitative and quantitative assessment of the regular freshwater trashfish diet for snakehead food

The Mekong River Basin hosts one of the most diverse freshwater faunas in the world. There are 1,200 recorded fish species and the number will increase as new species are discovered and classified. Diversity is based on a wide range of permanent and seasonal habitats, which are a result of the Mekong Basin's complex geological history. Most fish species depend on different habitats at different stages of their life and at different seasons of the year. During the flood season, most Mekong species take advantage of the floodplains for feeding, breeding and rearing their young (Sverdrup-Jensen, 2002).
In the Mekong Delta, the fisheries encompass a range of different gears and methods targeting different species groups. In a multi-species fisheries environment such as the Mekong system, it is useful to distinguish different species groups based on different life history strategies. Preliminary calculations suggest a 20 percent increase in fish demand in the Lower Mekong Basin over the next 10 years. Fishing is likely to increase due to population growth and ease of access and this may result in an increase in overall catches. On the other hand, the increase will be accompanied by a continued decrease in many important commercial species in the catches (Sverdrup-Jensen, 2002). However, the

freshwater fishes are used not only for human consumption but also for aquaculture, especially the juvenile fishes caught in flood seasons. Therefore, a study of the freshwater trash fishes was carried out in order to determine the species composition, size frequency and distribution of the low-value fishes used for aquaculture.

2.1.1 Methodology

The study has been carried out monthly during the flood season from August to October 2008. Trash fish samples were collected at snakehead culture farms in Chau Doc, Thoai Son and Chau Thanh district, An Giang province, the Mekong Delta, South of Vietnam. The fish samples were identified by the scientific names and the species composition, length frequency, seasonal occurrence and distribution were determined.

The common species: *Cirrhinus lobatus, Trichogaster microlepis, Anabas testudineus, Mystus mysticetus and Esomus metallicus* were analyzed for chemical composition (moisture, crude protein-CP, crude lipid-CL and crude ash-CF) according to AOAC (2000). Loss on drying was used to determine moisture content, protein (N x 6.25) was determined by Kjeldahl method, lipid was determined by Soxhlet method, and ash was determined by combustion in a muffle furnace.

2.1.2 Results

Species composition

The sampling surveys were carried out at three sampling sites from August to October 2008. Night samples of the trash fish were collected and analyzed. Results showed that 33 species of freshwater fish had been identified and the most common species were *Cirrhinus lobatus* (19.55%), *Trichogaster microlepis* (12.55%), *Anabas testudineus* (10.06%), *Trichogaster trichopterus* (8.24%), *Puntioplites proctozysron* (7.27%), *Mystus mysticetus* (5.63%), *Puntius orphoides* (5.59%), *Esomus metallicus* (4.75%), *Labiobarbus leptocheilus* (3.27%) and *Oreochromis niloticus* (2.95) (Figure 1; Table 1). In total, the species belong to 20 families, of which the families Cyprinidae (24%), Bagridae (10%), Cobitidae (10%) and Osphronemidae (10%) are most heavily represented (Fig 1).

Seasonal occurrence and length frequency distribution

The results showed seasonal occurrence of the most common species as follows:

+ In August: The most abundant species were *Cirrhinus lobatus* (50.4%); *Anabas testudineus* (11.1%); *Mystus mysticetus* (8.7%) and *Barbonymus gonionotus* (7.4%)

+ In September: The most abundant species were *Pangasius bocourti* (22.3%); *Puntius orphoides* (19.2%); *Helicophagus waandersii* (11%); *Ompok bimaculatus* (8.4%) and *Brachirus panoides* (7.9%)

+ In October: The most abundant species were *Puntioplites proctozysron* (47.5%); *Oreochromis niloticus* (25.3%) and *Labeo chrysophekadion* (10.3%).

The results also indicated that many commercial species were exploited at very small size or in the juvenile stage (Table 1) such as *Anabas testudineus*, Cá Rô Đồng (TL_{min}= 13mm); *Puntius orphoides,* Cá Đỏ Mang (TL_{min}= 17 mm). The length frequency analysis

showed that *Cirrhinus lobatus* was exploited mostly from 60 to75 mm in total length, while the other species were exploited with a larger range of total lengths.

Chemical composition
Chemical composition of the common species is shown in Table 2. Crude protein, crude lipid and crude ash of *Anabas testudineus* are the highest values reported, whereas those of *Trichogaster microlepis* are lowest for both protein and lipid.

To sum up, thirty-three species of freshwater fish have been used as "trash fish" or low-value fish for aquaculture in An Giang province, and the most abundant and common species is *Cirrhinus lobatus*. Chemical composition of some common species fluctuated from 14.3 - 16.5% protein, 1.97-8.39% lipid, and 2.48-4.67% ash. Many of those fishes are commercial species and some of them are target species for aquaculture in Vietnam, such as *Anabas testudineus*, *Pangasius bocourti, Oreochromis niloticus.* Therefore, those fish stocks should be assessed and the inland fishery should be managed properly, especially in flood season.

2.2 Study 2: Weaning methods using formulated feeds for snakehead *(Channa striata)* larvae

First feeding is one of the critical periods in fish larval rearing. Zooplankton such as *Brachionus, Moina* and *Daphnia* are frequently used as food resources in freshwater larviculture and for ornamental fish. They contain a broad spectrum of digestive enzymes such as proteinase, peptidase, amylase, lipase and even cellulase that can serve as exo-enzymes in the gut of the fish larvae (Lavens and Sorgeloos 1996). The quantity and quality of food given, including the types of food used in each of the developmental stages, can also be critical in larval rearing and most importantly can affect economic aspects. Larval rearing has been successful for freshwater and marine fish larvae using brine shrimp *Artemia* sp (Léger et al., 1986), for walking catfish *Clarias macroceplalus* using *Moina* (Fermin et al., 1991), and for European catfish *Silurus glanis* using *Tubifex* worms (Ronyai and Ruttkay, 1990). It was also reported that some catfish (*Clarias gariepinus* and *Heterobranchus longifilis*) can be reared exclusively on formulated diet (Appelbaum et al., 1988). However, it often resulted in lower growth and survival rate than live foods or trash fish. So the present study aims at comparing growth performance and survival rate of *Channa striata* larvae when weaning from live feed to formulated diets.

2.2.1 Methodology

After absorbing the yolk on day 3 after hatch, larvae were fed with *Moina*. The experimental treatments were initiated when the larvae were 10 days after hatch (dah). We tested three ages of larvae to begin weaning: 10, 17 and 24 dah. Larvae were fed ad libitum. For 10-dah treatments, the weaning procedure consisted of 10% or 20% of *Moina* biomass replaced daily by formulated diet until fish were feeding exclusively on formulated diet (i.e., the procedure took 10 days or 5 days, respectively, for the 10% and 20% treatments). Similarly, for the 17-dah and 24-dah treaments, trash fish biomass was replaced by formulated diet at a rate of 10% or 20% per day for each treatment. The control treatment was fed with *Moina* replaced by 20% per day trashfish within 5

consecutive days and then trashfish was fed to the end of the experiment (Table 3). Formulated feed contained 50% crude protein and 4.7 kcal.g^{-1}.

Larvae were stocked in 50-L tanks with stocking density of 5 fish/L. The fish were fed to satiation by hand twice daily. The remaining feed and faeces were siphoned out before feeding. Daily records were kept on mortality, food consumption and water quality, such as temperature, pH and dissolved oxygen. Larvae were weighed and measured at biweekly intervals. The water was maintained at 28±2^{o}C. At the beginning and the end of the experiment, samples of fish were sacrificed for proximate analysis.

At the end of the experiment, final body weight (FBW, mg) and wet weight gain (WWG, mg) were determined. Differences among treatments were determined by one-way ANOVA with means separated using Duncan's Multiple Range test at p = 0.05 using SPSS 13.0.

2.2.2 Results and Discussion

The growth of larvae in treatment 10day-20FF (3.21 g) differed significantly from other treatments (Table 4), but growth in other treatments was not significantly different. The survival rates of larvae in treatments 10day-10FF and 10day-20FF (15.23 and 2.33%) were significantly lower than other treatments and were significantly different from each other (Table 4). The total biomass gain was highest in the control treatment but not significantly different from the 17- and 24-dah at replacement treatments of 10% per day. Hence, *Channa striata* larvae can be fed with trash fish for 17 days and then be weaned to formulated diet. This could greatly reduce the amount of trash fish used and therefore protect freshwater fishes, which are usually fed to snakehead.

Lavens and Sorgeloos (1996) reported that many small fish larvae produce insufficient enzymes for digesting non-living diets. Enzymes present in their live prey carried out digestion (autolysis) in the fish larvae. Formulated diets often lead to poor growth and survival rates. Use of a commercial trout starter diet resulted in good survival rates with value of 12% in *Clarias gariepinus* (Fermin and Bolivar, 1991), 32% in *Heterobranchus longifilis* (Kerdchuen and Legendre, 1994) and 67.5% in *Pangasius bocourti* (Hung *et al.,* 1999).

In fishes, cannibalism is usually associated with heterogeneous size variation, lack of food, high density, lack of refuge area and light condition. Among these variables, size variation and unsuitable food are considered the primary causes of cannibalism. Cannibalism was reported in most larval rearing especially in artificial diet treatments. For example, the cannibalism in *Clarias gariepinus* larval rearing contributed more than natural mortality (Hecht and Appelbaum, 1987). In this study, *Channa striata* displayed a high cannibalism in dry diet treatments. Low survival rate may be related to snakehead behavior. Conversely, *Pangasius bocourti* larvae had a low cannibalism even in the artificial feeding treatment (Hung et al., 1999). In summary, weaning snakehead on formulated feed can begin at 17 dah with relacement of 10% per day.

2.3 Study 3: Replacement of fishmeal with soybean with or without phytase and taurine in diets for *Channa striata* and *Channa micropeltes*

Soybean meal is the most common fish meal-replacement source in aquafeeds, having the best amino acid profile among plant protein sources (NRC, 1993). Nevertheless, the use of soy protein in feeds presents a number of challenges associated with low methionine and cystine content, lower protein digestibility, indigestible oligosaccharides, low phosphorus availability, antinutritional factors and poor palatability (Hertrampf and Piedad-Pascual, 2000). Soybean meal contains phytic acid (NRC, 1993), which is the major compound for phosphorus storage (over 70%) in the plant seeds and can not be digested and absorbed by mono-gastric animals including fish (Jackson et al.,1996). Many fish nutritionists have tried to supplement phytase, myo-inositol-hexaphosphate phosphohydrolase enzyme, to liberate free phosphorus from phytic acid. Phytase is produced either by microorganisms or present in some plant ingredients. Therefore, phytase supplementation is advantageous when significant portions of plant protein meals such as soybean meal are used in fish feeds. Research on phytase supplementation was carried out with positive results in several species, including channel catfish (Jackson et al. 1996), striped bass (Hughes and Soares, 1998), Atlantic salmon (Sajjadi and Carter 2004), rainbow trout (Cheng and Hardy 2004), and *Labeo rohita* (Baruah et al. 2007). Taurine derives from methionine via cysteine and is not considered to be among the ten indispensable amino acids, nor is it incorporated into protein; however, it has several physiological roles and is relatively abundant in fish meal. The ability of fish to synthesize taurine is species dependent and possibly affected by the stage of development. The beneficial effects of dietary taurine supplementation have been demonstrated in many species. In sea bass fry, *Dicentrarchus labrax,* a taurine supplemented diet caused an increase in growth rate when fish meal and soybean meal were the primary sources of protein (Martinez et al., 2004). The main goal of this study is to find out the appropriate soybean meal level to replace fish meal with and without phytase or taurine supplementation in snakehead (C*hanna striata*) and giant snakehead (*Channa micropeltes*) diets. In addition, essential amino acids (EAA) were added to all the diets containing soybean, in order to eliminate any EAA deficiencies compared to the control diet.

2.3.1 Methodology

Experimental fish

Before starting the experiments, all the fish were reared in 2000-L round tanks and were fed with trash fish combined with pellet diets for 4 weeks. Replacement of trash fish by pellet feed was applied gradually at a rate 10% per day until 100% of trash fish was substituted by pellet feed.

Experimental design

In each of experiments 1 and 2, nine diet treatments were set up randomly into 27 experimental tanks (500-L composite tank) with three replicates for each treatment. Thirty *Channa striata* fingerlings (4.7g in initial weight) were assigned to each tank and were fed to satiety.

In experiment 3, five diet treatments were set up randomly into 15 experimental tanks (500-L composite tank) with three replicates for each treatment. *Channa micropeltes* fingerlings (4.70–4.82g in initial weight) were randomly distributed into the 15 tanks with 25 individuals per tank. At the beginning of each experiment, 20 fingerlings from the stock tank were sacrificed for assessment of their initial proximate body composition. In addition, at the end of the experiment, all fish from each replicate were collected for the final proximate body composition analysis.

During the trial, the fish were fed by hand three times a day at 8:00, 12:00 and 17:00 hours. The amount of feed consumed was adjusted on a daily basis and recorded. Total fish weight in each aquarium was determined every 4 weeks and dead fish were recorded and weighed for calculating feed conversion ratio (FCR). Water temperature, measured daily, ranged from 27.0–28.5^{o}C. pH and dissolved oxygen, measured weekly, varied from 7.0–7.2 and 5.0–7.6 ppm, respectively.

Experiment 1: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa striata *diets

Nine practical diets were formulated to replace 0%, 20%, 30%, 40% and 50% of fish meal by soybean meal without phytase supplemention (FM, SM 20%, SM 30%, SM 40% and SM 50%, respectively); and 20%, 30%, 40% and 50% with phytase addition (SM-P 20%, SM-P 30%, SM-P 40% and SM-P 50%, respectively) on a protein equivalent basis in the diet. In addition, the EAA's lysine, threonine and methionine were added to the SM diets to eliminate deficiencies caused by substitution of fish meal. All of the experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.5 kcal gross energy/g of diet. Composition of the experimental diets is shown in Table 5.

Experiment 2: Replacing fish meal by soybean meal with EAA and taurine additions in* Channa striata *diets

Nine practical diets were formulated to replace 0%, 20%, 30%, 40% and 50% of fish meal by soybean meal without taurine addition (FM, SM 20%, SM 30%, SM 40% and SM 50%, respectively); and 20%, 30%, 40% and 50% with taurine supplemention (SM-T 20%, SM-T 30%, SM-T 40% and SM-T 50%, respectively) on a protein equivalent basis in the diet. In addition, the EAA's lysine, threonine and methionine were added to the SM diets to eliminate deficiencies caused by substitution of fish meal. All of the experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.5 kcal gross energy/g of diet. Composition of the experimental diets is shown in Table 6.

Experiment 3: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa micropeltes *diets

Five basal diets were formulated to be isonitrogenous, 44% crude protein, and isocaloric, 4.5 Kcal / g diet in gross energy. The control diet (FM) was made with FM as the main protein source. In diets 2 - 5, SM was substituted for an isonitrogenous amount of FM control diet, to replace 20% (diet 2 - SM 20), 30% (diet 3 - SM 30), 40% (diet 4 - SM 40) and 50% (diet 5 - SM 50) of the FM crude protein (Table 7). In addition, the EAA's

lysine, threonine and methionine were added to the SM diets to eliminate deficiencies caused by substitution of fish meal. Also, phytase enzyme (Ronozyme, dry powder) was added in diets 2 to 5 at 0.02%/ kg feed.

The experimental diets were made in a laboratory pellet mill by blending all of the dry ingredients. The extruding temperature did not exceed 40^0C. After extruding, all diets were dried at 45°C within 48h and stored at 4°C prior to use.

Data calculation
At the end of each period, fish were weighed and counted to calculate survival rate (SR), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and economic conversion ratio (ECR)
SR (%) = (Numbers of fish at the end of experiment / numbers of initial fish) x 100
WG = Final body weight – Initial body weight
DWG = (Final body weight – Initial body weight) / Experiment time
FI = (Feed intake/no. fish)/ No. days
FCR = Feed intake / Weight gain
PER = (Final body weight – Initial body weight) / Protein intake
ECR = Feed cost x FCR

Chemical analysis
Feed was analyzed for chemical composition (moisture, crude protein-CP, crude lipid-CL, crude fiber-CF, nitrogen free extracts-NFE and gross energy) according to AOAC (2000). Loss on drying was used to determine moisture content; protein (N x 6.25) was determined by Kjeldahl method; lipid was determined by Soxhlet method; crude fiber was determined by acid and base hydrolysis; and gross energy was determined by bomb calorimetry. Carbohydrate-NFE equals 100-(CP+CL+CF). Moisture, crude protein, crude lipid, crude ash and nitrogen free extracts were determined in fish collected at the beginning and end of each experiment.

2.3.2 Results and Discussion

Experiment 1: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa striata *diets
The fish growth showed that the final weight, weight gain, daily weight gain of fish fed FM, SM 20%, SM 30%, SM-P 20%, SM-P 30% and SM-P 40% were not significantly different (Table 8). In contrast, the growth performance of fish fed SM 40%, SM 50% and SM-P 50% were significantly lower than those fed the control (FM) feed. There was no significant difference in survival rate among diets (Table 8).

FCR in fish fed the SM 50% was significantly higher than that of fish fed all the diets with less than 40% SM replacement; all the other diets were not significantly different from each other (Table 9). Similarly, PER in fish fed the SM 50% diet was significantly lower than that of fish fed FM, SM 20%, SM – P 20% and SM 30%; all the other diets were not significantly different from each other (Table 9).

From an economic point of view, it can be seen that replacing FM by SM up to 40% in protein content achieved economical benefit; however, the greatest gains were seen in the

SM – P 20% and SM – P 30% diets (Table 10). Thus, replacement of fish meal with SM, especially with phytase added, appears to be an economically benficial strategy.

Experiment 2: Replacing fish meal by soybean meal with EAA and taurine additions in* Channa striata *diets

In this experiment, the final weight and daily weight gain of fish fed FM, SM 20%, SM 30%, SM-T 20%, SM-T 30% and SM-T 40% were not significantly different; in contrast, the growth performance of SM 40%, SM 50% and SM-T 50% were significantly lower than control (FM) feed. There was no significant difference in survival rate of fish among diets (Table 11).

Feed intake (FI) data indicated significant differences only between fish fed the SM 50% diet and those fed the FM, SM 20%, and SM – T 20% diets: feed conversion ratio (FCR) data indicated significant differences between both of the SM 50% diets and all the diets up to 30% SM, with the exception that the SM – T 50% diet did not differ from the SM 30% diet; and protein efficiency ratio (PER) data indicated differences between the SM 50% diets and the FM diet, as well as between the SM – T 50% diet and the SM – T 20% diet (Table 12).

From an economic point of view, it can be seen that replacing fishmeal by soy meal up to 30% in protein content achieved economical benefit (Table 13). Cost for one kg fish weight gain was reduced by a maximum of 6.90 % in treatment SM-T 30% compared to the control treatment.

Experiment 3: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa micropeltes *diets

Fish in the FM treatment showed significantly higher final weight and daily weight gain (0.38 ± 0.05 g.day^{-1}) than those in the SM 50 treatment (0.28 ± 0.01 g.day^{-1}). Survival rate of fish among treatments was not significantly different (Table 14).
Significant differences in FCR and PER were seen only between the FM and SM 50 treatments (Table 15). Thus, soybean meal can replace up to 40% of fish meal in terms of protein with phytase supplementation in diets for *C. micropeltes*.

From an economic point of view, it can be seen that replacing FM by SM up to 40% in protein content achieved economical benefit (Table 16). Cost for one kg fish weight gain was reduced 9.59% in treatment SM 40 compared to control (FM) treatment. The substitution can be applied in practical terms because it did not affect growth rate and mortality of fish.

The study concluded that with phytase supplement of 0.02% in diet, FM could be replaced by SM up to 40% in terms of protein which not only achieved growth performances, feed utilizations and survival rates of giant snakehead juveniles, but also succeeded from economic point of view.

There was no significant difference in survival rate among treatments *($P>0.05$).* Daily weight gain of fish showed a downward trend when replacement fish meal with soybean meal level from FM to 50% SM without phytase or taurine supplemention. However, the growth was improved with phytase or taurine supplemention, namely, this growth

performance was good at SM-P 40% or SM-T 40% while it reached SM 30% without phytase or taurine additions. It is clear that soybean meal can replace up to 40% of fish meal diets for juvenile snakehead if phytase or taurine are added. However, taurine was disadvantagious in cost for one kg fish weight gain because feed conversion ratios in taurine diets were not optimal. Thus, taurine should not be used in snakehead diets. Many previous studies that used taurine in marine fish diets achieved positive results. In the results of Experiment 3 optimal results for both survival rate and economic efficiency were achieved with replacement up to 50%, but growth performance and efficiency ratio results indicated that the replacement level should only be up to 40%.

The present study demonstrated that SM with phytase supplements could replace dietary FM protein up to 40% without negative effects on growth performances, feed utilizations and survival of *Channa striata* and *Channa micropeltes* fingerlings. Many studies reported that the supplementation of phytase to P-inadequate diets has been shown to enhance growth performance. Soltan *et al*. (2008) studied the maximum replacement levels of fish meal (FM) by a plant protein mixture (cottonseed, sunflower, canola, sesame and linseed meals) in diets for Nile tilapia. They found that fishmeal can be replaced up to 45% with growth rate not differing significantly from that of fish fed control diet. The incorporation of plant protein mixture in diets did not significantly affect whole-body dry matter and crude protein of fish. From an economic view, it reduced feed costs/kg diet and feed costs/kg weight gain by 11.40 and 6.74%, respectively. An increase of weight gain has been reported in channel catfish fed phytase supplemented diets containing only plant protein or a combination of plant and animal protein sources (Jackson et al., 1996). The supplemental effect of phytase on growth performance in fish cannot simply be compared because it could be different depending on fish species and rearing conditions, and more specifically on dietary composition in each feeding study (Cao et al., 2007). Generally, growth improvements were observed in the studies that used diets entirely or almost entirely based on plant protein sources. However, growth performance responses to phytase supplementation were somewhat inconsistent (Cao et al., 2007).

The present study showed that fish meal protein in *Channa striata* and *Channa micropeltes* fingerlings diets can be replaced by soybean meal protein up to 40% with phytase supplements in which growth performances, feed utilizations and survival of two species are not affected.

2.4 Study 4: Utilization of rice bran in snakehead *Channa striata* feed

Introduction

Rice bran also is a rich source of protein (8.34-16.3%), oil, dietary fiber, and micro nutrients (Hien et al., 2006). Rice bran has been used in formulated feed for terrestrial animals and aquaculture species. Moreover, rice bran is an available and abundant crop by-product in Mekong Delta. In order to reduce the cost of feed for snakehead, diminish trash fish use and reduce the environmental impact, rice bran is thought to be the best ingredient. The goal of this study is to find out the appropriate level of rice bran in formulated feed that can achieve the optimum growth and cost-effectiveness. There has been some research on utilization of rice bran as feed of some species such as Nile

tilapia *Oreochromis niloticus* (Perschbacher and Lochmann, 1999; Liti et al., 2006), silver barb *Puntius gonuonotus* (Mohanta et al., 2006), *Streptocephalus proboscideus* (Ali and Dumont et al., 2002), sub - adult mud crab *Scylla paramamosain*. Hien et al., (2006) reported defatted rice bran could be used in diets for tilapia *Oreochromis niloticus* and striped catfish *Pangasius hypophthalmus* with increased growth rate and reduced feeding cost.

2.4.1 Methodology

Feed was formulated from main ingredients such as Kien Giang fish meal, defatted soybean meal, cassava meal, and dried rice-bran. Experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.7 Kcal gross energy/g diet. The ratio of protein fish meal and protein soybean meal level was 6:4. Diet 0% RB was considered as control treatment. The other treatments contained 10% , 20%, or 30% RB, as indicated in the composition of the experimental diets shown in Table17. The experiment consisted of four treatments with three replicates per treatment and 50 fish per replicate. Snakehead fingerlings (4.51-4.63g in initial weight) were assigned randomly to each 500-L composite tank. Water temperature, measured daily, ranged from 27.0–28.0^{o}C, pH and dissolved oxygen, measured weekly, were 7.6–7.7 and 6.68–6.76 ppm, respectively. The experiment lasted eight weeks.

Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the phytase experiments**.**

2.3.2 Results and Discussion

Daily weight gain of fish in treatment 10% rice bran (0.29±0.02 g.day^{-1}) was significantly higher than that of fish in the control treatment that had no rice bran in the diet; moreover, final weight and daily weight gain of fish in treatment 20% and 30% rice bran in diet was not significantly different from that of fish in the control treatment.and there was no significant difference among RB 10%, 20%, 30% treatments in daily weight gain (Table 18). Survival rates of fingerlings were high and ranged from 60 to 69.3% and there was no significant difference observed (Table 18). This study obviously indicates the possibility of using formulated diets for rearing snakehead in captive conditions. According to Trieu *et al.,* (2001), survival rates of snakehead fingerlings during 4 weeks in tank conditions ranged from 60.2 to 100%. The growth performance of fish in this experiment was better than that of snakehead fingerlings (initial weigh 5.22g) fed with 50% CP (1.19%.day^{-1}) which were studied by Trieu *et al.,* (2001).

Feed intake fluctuated between 227 and 293 mg fish^{-1}day^{-1} among treatments and showed significant idfferences, with the lowest FI in treatment RB 0% and the highest FI in RB 10% treatment; however, no significant differences were seen in FCR or PER (Table 19).

The improvement in growth performance of snakehead fingerling fed rice bran diets when compared to the result from 0% RB diets may have been caused by the presence of micronutrients in rice bran. Data shown in table 17 indicate the cassava meal content decreased along with the increase of rice bran in diets. Rice bran is abundant in trace minerals and vitamin B, especially vitamin B1 (thiamine) that are neccesary for growth.

Vitamin B1 plays a major role in carbohydrate metabolism (Jean *et al*, 2001). There was no significant difference in FI observed in RB 10% and RB 30% treatment. However, the growth response of snakehead in RB 10% treatment was better, inducing an FCR in this treament lower than the FCR in RB 30% and the lowest FCR in this experiment. That result may be caused by the highest fibre content in RB 30% diet (4.31%) whereas the cassava meal (which plays a role as a good binder for the diet) was absent, reducing the stability of the pellet and raising waste feed,alhough there was no significant difference in FCR and PER observed among treatments. The lower the FCR is in cultivation, the more efficient the feed utilization is. Ningrum (2005) found in Asian catfish (4.9 g/fish in initial weight) fed 55.6% rice bran in diet that FCR was low (1.3), and SGR and PER were high (4 % per day and 2.6, respectively)

From an economic point of view, it can be seen that replacing fishmeal and soybean meal by rice bran up to 30% in protein content achieved economical benefit (Table 20). Cost for one kg fish weight gain was reduced 6.88 % in treatment RB 30% compared to the control treatment.

In summary, rice-bran could be well utilized by snakehead fingerlings with levels from 10% to 30% without any differences in growth performance and carcass composition. Hence, rice bran could be used in home-made formulated feed for snakehead fingerlings up to 30% to reduce feed cost.

2.5. Replacement of fishmeal with soybean with soluble fish attractant or alpha-galactosidase in diets for *Channa striata*

Soybean meal can replace up to 30% of fish meal in the diet of snakehead without addition of phytase or 40% of fish meal with the addition of phytase (Hien et al., 2009). To improve utilization efficiency of plant protein for snakehead, replacement of fish meal by soybean meal was conducted at higher levels of 50, 60 and 70% with soluble fish attractant or alpha-galactosidase added. Alpha-galactosidase is added to some animal feeds to improve utilization efficiency of plant protein. Feeding attractants such as betaine, squid viscera meal, and L-amino acids (L-alanine, L-glutamic acid, L-arginine) have been used to increase the palatability of plant protein diets for fish. Mackie and Mitchell (1985) summarized the results of various studies using dietary feeding attractants, and reported the positive effect of mixtures of dietary free amino acids as feeding stimulants in rainbow trout, *O. mykiss* (Adron and Mackie, 1978); European eel, *Anguilla anguilla* (Mackie and Mitchell, 1983); Japanese eel, *A. japonicus* (Takeda et al., 1983); sea bass, *Dicentrarchus labrax* (Mackie, 1982); and red seabream, *Chrysophyrys major* (glycine betaine plus L-amino acids; Goh and Tamura, 1980). Therefore, the present study investigated the effects of supplementing dietary α-galactosidase and a feeding attractant solution on the nutriient digestibility and growth performance in fingerling snakehead fed diets containing more than 40% SM.

2.5.1 Methodology

Diets were formulated to contain different levels of soybean meal from 50% to 70% for protein fish meal with α-galactosidase (Experiment 1) (Table 21) and feeding attractant solution (Experiment 2) supplementation (Table 22), compared to control treatment (fish meal-FM). All of the experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.5 kcal gross energy/g of diet. Rice

bran and cassava meal were also used as sources of plant protein in the diets. Methionine, lysine and phytase were all also added to the diets, based on results of our previous experiments.

In each of experiments 1 and 2, four diet treatments were set up randomly into 12 experimental tanks (500L composite tank) with three replicates per treatment. Thirty *Channa striata* fingerlings (2.24–3.79g in initial weight) were assigned to each tank and were fed to satiety. Water temperature, measured daily, ranged from 26.5-27.5^{o}C. pH and dissolved oxygen, measured weekly, varied 7.2-7.5 and 5.0–7.6 ppm respectively. Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the phytase experiments.

2.5.2 Results and Discussion

In experiment 1, there were no significant differences among the experimental treatments in final weight, daily weight gain, or survival rate (Table 23), as well as in FI (Table 25); However, there was a significant difference in FCR between the 70% SBM diet and all the other diets, as well as signficant differences in PER between the 60% and 70% SBM diets and the FM and 50% SBM diets (Table 25). We found in previous studies that SBM can replace up to 30% of FM in the snakehead diet without addition of phytase or 40% of FM with the addition of phytase (Hien et al., 2009). The results of this study showed that addition of α-galactosidase to snakehead diets could allow the replacement of FM with SBM to be as high as 70% (based on growth results), 60% (based on FCR results), or 50% (based on PER results. The decrease in PER with increased inclusion of plant protein was seen previously in Atlantic cod (*Gadus morhua L*.) (Hansen et al., 2007).

In experiment 2, there were again no significant differences among the treatments in final weight, daily weight gain, survival rate (Table 24) or FI (Table 26); howe ver, there were significant differences in FCR between the SBM 70% treatment and all the other diets, as well as in PER between the SBM 60% and 70% diets and the FM and SBM 50% diets (Table 26). Positive effects of feeding attractants was previously shown in *Epinephelus malabricus* by adding 1% of squid viscera meal for using a blend of rendered animal protein ingredients to replace fish meal in practical diets (Wang et al., 2008).

Considering economic efficiency, cost for one kg fish weight gain decreased 9.91% and 10.5% in 50% SBM diet compare to control diet in Experiments 1 and 2, respectively; however, the replacements over 50% were not economically efficienct because of increasing FCR (Table 27 and Table 28).

In summary, SBM can replace up to 60% of FM in the snakehead diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant. However, considering economic efficiency, SBM can only replace up to 50% of FM in the diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant.

2.6 Replacing freshwater trash fish by formulated feed in snakehead (*Channa striata*) and (*Channa micropeltes*) fingerling diets

Snakehead culture mainly relies on trash fish supplied and feeding cost is the biggest cost to the farmer, so many problems have been observed. The most important of these problems are poor quality of trash fish and variable nutritional composition because of inappropriate storage. Risk of disease introduction, environmental pollution and high feed conversion in snakehead rearing contributed more concerns. Moreover, the growing competition between human and aquaculture usage of low value fish (trash fish) led to increasing its price to the farmer (Rachmansyah et al., 2009). For those reasons, it is necessary to develop cost-effective and high-performing compounded feeds that would allow less reliance on trash fish and would have lower environmental impacts. It was recognized that snakehead previously fed on trash fish would not readily take a dry feed and thus development of an appropriate feed acceptable to the fish was an important aspect of our feed development work. This study, therefore, was designed to determine the percentage of trash fish that could be replaced by formulated feed for optimum growth and survival of snakehead. The cost of feed was also calculated.

2.6.1 Methodology

Based on the results of our previous experiments, we developed a feed formulation for snakehead with FM, SBM, rice bran and cassava meal, with addition of the EAA's methionine, threonine, and lysine and the the addition of phytase and fish solution feeding attractant (Table 29). We then set up the experimental replacement of trash/low-value fish diets with this 45%-protein formulated feed (FF) (Table 29) at levels of 0 (control), 25, 50 75, or 100%. The five treatments each had three replicates with 50 fish per replicate and were conducted in hapas. Before the startof the experiment, snakehead fingerlings had been acclimated to formulated feed for 30 days.

The fish (4.7g in initial average weight) were assigned randomly to each hapa. The experiment lasted eight weeks, during which fish were fed twice/day to satiation at 9:00 and 16:00 hours. Total fish weight in each hapa was determined every 4 weeks and dead fish were recorded and weighed for calculating feed conversion ratio (FCR). After feeding, the remaining feed was weighed daily. Water temperature, measured daily, ranged from 29.5-30.5^{o}C. pH and dissolved oxygen, measured weekly, varied from 5.2-5.5 and 6.0–7.4 ppm, respectively.

Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the phytase experiments**.**

2.6.2 Results

Experiment 1: Replacing freshwater trash fish by formulated feed in* Channa striata *fingerling diets

Survival rate of fish in the 100% FF treatment (complete replacement of trash fish by formulated feed) was 73.3%, significantly lower than all other treatments, which had very high survival rates, 95.3 to 92.7% (Table 30). There was a trend of reducing growth response with increased replacement by FF and fish fed diets consisting of 0% FF, 25% FF and 50 % FF exhibited significantly higher final weight and daily weight gain than did fish in the 75% FF and 100% FF treatments (Table 30).

FI and FCR were significantly reduced at each increasing level of FF replacement, but no significant differences were seen in PER (Table 31). It is likely that the significant reductions in FI and FCR were due to different moisture levels in the diets that the fish received, since trash fish is 72.7 % moisture, whereas formulated feed is 9.38% moisture.

According to the economic analysis, feed cost per kilogram weight gain decreased with increasing formulated feed in diets (Table 32), with a reduction up to 35.5% using 100% FF compared to the trash fish diet (0% FF). The aforementioned growth performance and survival rate of snakehead were not significantly different when 50% of trash fish was replaced by formulated feed. At this replacement proportion, the feed cost was reduced considerably by 22.1% compared to the diet containing 100% trash fish.

Experiment 2: Replacing freshwater trash fish by formulated feed in* Channa micropeltes *fingerlings diets

In this experiment, fish in the 0% FF treatment (i.e., fed only trash fish) had significantly higher survival than all of the other treatments (Table 33). Final weight and daily weight gain were significantly higher in the control, 25 FF and 50 FF treatments than were those in the 75 FF and 100 FF treatments.

In terms of feeding, FI differed significantly among all treatments, except that the 75 FF and 100 FF treatments did not differ significantly; FCR differed significantly among all treatments, except that the 50 FF and 75 FF treatments did not differ significantly; and PER in the 0 FF treatment was signifcantly higher than that of all other treatments, which did not differ significantly from each other (Table 34).

The economic analysis showed that cost per kg of fish weight gain was reduced the more the percentage of trash fish was replaced, except for the 25 FF treatment (Table 35).

Snakehead is a carnivorous species. In this study, although they were weaned from trash fish to formulated feed, live food was still their favorite feed. The diet containing no trash fish reduced attraction of fish to feed. Simply providing formulated feeds led to cannibalism in this species in a previous study (Qin et al., 1996). Utilization of commercial pellet feed nowadays is more popular, especially for carnivorous fish in order to reduce the dependence on trash fish, feeding cost and environmental impact. Several studies on replacing of trash fish by formulated feed in several species achieved better growth rate and more profit, such as tiger grouper, *Epinephelus fuscoguttatus* (Rachmansyah et al., 2009); Japanese sea bass, *Lateolabrax japonicus* and red drum, *Sciaenops ocellata* (Cremer et al., 2001); and sea bass, *Lates calcarifer* (Aquacop et al., 1989). Cremer et al. (2001) replaced trash fish with formulated diets in cage culture of red drum (*Sciaenops ocellata*) (172g/fish in initial weight) and Japanese sea bass *Lateolabrax japonicus*) (74g/fish) and concluded that fish consuming formulated diet (43% crude protein, 12% lipid) with 35% soybean meal showed better growth and less feeding cost than fish fed trash fish.

Replacing trash fish by formulated feed brought more benefits in feeding cost and less dependence in trash fish supply in Singapore (Aquacop et al., 1989). Sea bass (*Lates*

calcarifer) could use pellet feed (45% crude protein) in fingerling stages but required high levels of fishmeal quality. Grouper (*Epinephelus fuscoguttatus*), a carnivorous species which requires a high protein content (445-50%) in diet, fed a mixed diet of formulated feed and trash fish with the ratio 1:1, performed insignificantly differently from fish fed trash fish completely (Rachmansyah *et al.,* 2009).

To sum up, if only economic efficiency were considered, replacing fresh water trash fish by formulated feed in two species diets up to 100% is possible. If both growth performance and feed conversion ratio were considered, the replacement should stop at 50%. Thus, depending on the farmer's situation, they should choose the optimal solution for replacing fresh water trash fish by formulated feed in snakehead culture. The data provided in this study help to enable them to make that choice.

2.7 Taste analysis of snakehead fed by different feeds

Snakehead is a good quality aquaculture species which is highly prized by consumers. We have conducted several experiments to improve pellet feed for culture of this fish. One urgent question is whether improved feed affects snakehead product quality, so it was necessary to conduct an experiment for snakehead sensory analysis with fish fed by different feeds for experimental treatments.

2.7.1 Methodology

The experiment was carried out with 18 hapas (1m x 1m x 2m) in an experimental pond for two fish species (*Chana striata* and *Chana micropeltes*), which were transferred from An Giang province, Vietnam. Before starting the experiment, snakehead fingerlings had been acclimated to formulated feed for 30 days. The fish ranging from 4-5 $g.fish^{-1}$ were assigned randomly to each hapa, fifty fish per hapa in stocking density. Every treatment was triplicated and the experimental period was 16 weeks. During the trial duration, fish were fed twice/day to satiation at 9:00 and 16:00 hours.

The experiment was conducted with 3 treatments: freshwater trash fish and two formulated feeds containing 45% crude protein that had been developed from our previous studies. The first formulated feed was based on fish meal only and the other was based on a combination of fish meal and plant proteins (soy bean meal and rice bran) (Table 36).

At the end of the experiment, all fish were killed, filleted and washed, then they were steamed for 8 minutes. First, these fish were used to determine the difference in the quality of fish fillet between the control and experimental groups by a triangle test (2 controls and 1 sample) with three replacements per test.

There were three groups of control-samples for both *C. micropeltes* and *C. striata*: trashfish-fishmeal (TF-FM), trashfish-plant protein (soybean and rice bran) (TF-PP), fishmeal-plant protein (FM-PP).

If less than 8 out of 12 subjects detected the odd sample correctly, we determined that there was no significant difference and therefore no need to conduct a sensory test. A

pair test was run if there was any difference in any sensory attributes for texture or taste even if they were minor – called a 'descriptive pair test'. On the other hand, if 9 out of 12 people detected the odd sample correctly, there was a significant difference at $P<0.01$ or 8 out of 12 $P<0.05$. In this case, it was necessary to do a comprehensive pair test on appearance, texture and taste.

A pair test is hedonic and scored on an intensity scale (1-9 points) on appearance such as liking (1, least like – 5, o.k. – 9, like very much), whiteness (1, dark – 5,medium – 9, very white), and structural integrity (uniformity: 1, very irregular – 5, medium – 9, very uniform); taste, for example liking (1, least like – 5, o.k. – 9, like very much); snakehead-like taste (1, very little – 5, o.k. – 9, very much)' presence of objectionable taste (yes or no) and presence of objectionable odor (yes or no); texture, for instance, liking (1, least like – 5, o.k. – 9, like very much); firmness (1, very soft – 5, medium – 9, very firm); moistness (1, very dry – 5, medium – 9, very moist); chewiness (1, mushy – 5, medium – 9, very chewy); and flakiness(1, least or rubbery – 5, medium – 9, very flaky).

Statistical analysis
Mean values of results in different treatments were compared by paired sample t-test using SPSS 13.0 software. Treatment effects were considered with the significance level at $P< 0.05$.

2.7.2 Results

The result showed that there was no significant difference between paired samples in triangle tests (less than 8 out of 12 people detected the odd sample correctly) (Table 37). These samples were then subjected to "descriptive" pair tests, with the result that the quality of fish fillet samples did not significantly differ. In appearance, both *C.micropeltes* and *C.striata* were approximately 5 to 6 on the scale (Table 38 and Table 39), meaning that the fish fillet was passable or fairly likable for liking; medium or rather white for whiteness; medium or relatively uniform for structural integrity. In taste, the fish fillet was snakehead-like taste, and without the presence of objectionable taste and odor. In texture examination, for liking, the score was from 4 to 6, from not rather like – passable – fairly like. About firmness, the result was 3-5, soft – relatively soft – medium fish fillet. The fillet moistness was rather dry and medium (not dry and not moist). The fillet chewiness and flakiness was fairly mushy and relatively rubbery or medium (not mushy and not chewy; not rubbery and not flaky).

In summary, *C. micropeltes* and *C. striata* fillet quality was fairly liked and did not significantly differ between samples in triangle tests. The desciptive pair tests gave the same result as triangle tests and there was no significant difference between samples. So, these diets do not affect the quality of fish fillet for both *C. micropellets* and *C. striata.*

CONCLUSION

- Thirty-three species of freshwater fish were identified as being used as "trash fish" or low-value fish for snakehead culture and the most abundant and common species is *Cirrhinus lobatus*. Chemical composition of some common species fluctuated from 14.3 to 16.5, 1.97-8.39, 2.48-4.67 in crude protein, crude lipid and

crude ash, respectively. Most of those fishes are commercial species and some of them are target species for aquaculture in Vietnam, such as *Anabas testudineus* and *Trichogaster trichopteru*s. Therefore, those fish stocks should be assessed and the inland fishery should be managed properly, especially in flood season.

- Weaning onto formulated feed for snakehead larvae can begin by 17 days after hatch with relacement ratio of 10%.day^{-1}
- Up to 40% of fish meal in *Channa striata* and *Channa micropeltes* fingerling diets can be replaced by soybean meal with phytase supplements with no significant loss of growth performance, feed utilization or survival of the two species.
- Rice-bran could be well utilized by snakehead fingerlings with levels from 10% to 30% without any differences in growth performance and carcass composition. Hence, rice bran could be used in home-made formulated feed for snakehead fingerlings up to 30% to reduce feed cost.
- Soybean meal can replace up to 60% of fish meal in the diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant. However, considering economic efficiency, protein soybean meal only can replace up to 50% of protein fish meal in the diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant.
- Considering economic efficiency, replacing fresh water trash fish by formulated feed in two species diets up to 100% is possible. If both growth performance and feed efficiency ratio were of interest, the replacement should stop at 50%. Thus, depending on a farmer's situation, they should choose their own optimal solution for replacing fresh water trash fish by formulated feed in snakehead culture.
- *C. micropeltes* and *C. striata* fillet quality in a taste test was fairly liked and did not significantly differ between samples. In descriptive pair tests, there was no significant difference between samples. Thus, formulated feed (fish meal or plant protein) did not significantly affect the quality of fish fillet in both *C. micropeltes* and *C. striata* compared to a diet of trash fish.

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FEEDING REDUCTION STRATEGIES AND ALTERNATIVE FEEDS TO REDUCE PRODUCTION COSTS OF TILAPIA CULTURE

Sustainable Feed Technology/Experiment/07SFT02NC

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ABSTRACT

Feed constitutes 60-80% of total production costs of tilapia (*Oreochromis spp.*). Reductions in quantity of feed used for fish growout and in the cost of formulated feeds are two approaches to containing feed costs. We evaluated feed reduction strategies and replacement of fishmeal as potential options for improving production efficiency of Nile tilapia (O. niloticus). In a 120 day trial on commercial farms, fish subjected to 60-days delayed, followed by 30-days alternate day and then 30 days 67% subsatiation feeding showed lower growth rates and survivorship and overall harvest size then fish fed daily at full prescribed levels. Approximately 55% less feed was applied to animals on the combined reduced feeding regimen relative to those fed on a traditional full daily feeding schedule. In a separate study on commercial ponds, tilapia raised on 50% subsatiation ration levels had growth rates, survivorship, and final harvest size that did not differ significantly from fish fed a full daily ration over 120 days. Fish on the reduced ration also consumed 56% less feed and had 100% improved feed conversion relative to fish fed full ration. We then tested the utility of substituting less costly alternative protein sources (yeast extract, poultry meal, fermented deboned meat poultry byproduct) to fishmeal in tilapia diets over a 105 day tank trial. Fish fed the different diets exhibited similar specific growth rates, although animals fed diets containing yeast extract and fermented poultry byproduct had slightly lower body size. Using circulating insulin-like growth factor-I (IGF-I) as a potential proxy of growth, we found as with specific growth rates that IGF-I did not vary among fish on the different diets. In a fourth study fish were grown out in ponds for 120 days on an alternate day reduced feeding regimen using diets with and without 6% fishmeal that incorporated plant ingredients widely available in the

Philippines (cassava meal, copra meal, coconut oil, rice bran) and porkmeal to replace fishmeal. Fish showed similar performance on diets containing 0% and 6% fishmeal. A marginal budget analysis showed an 8% improved return on fish fed the cheaper diet lacking fishmeal. This along with the alternative day feeding strategy previously shown to be as effective as daily feeding protocols has the potential of reduce overall feed costs for growing marketable size tilapia by > 60%. Collectively, these series of studies show that reduced feeding strategies and substitution of diets containing fishmeal with cheaper and more sustainable sources of protein are effective options for reducing the costs without negatively impacting the production of tilapia.

INTRODUCTION

Feed is widely recognized as the most costly component of fish farming. A cost-farm budget analysis shows that feed constitutes 60- 80% of total production costs of tilapia (*Oreochromis niloticus*) for small-scale, rural farmers in the Philippines (ADB 2005). Because of this, any reductions of feed costs can effectively increase income for Philippine farmers. Reductions in both the amount of feed used for growout of marketable fish and in the cost of formulated feeds are two approaches to containing feed costs. Our previous studies show that 1) delaying the onset of supplemental feeding to either 45-days or 75-days in fertilized ponds reduces the amount of feed consumed without any negative impact on the production of marketable tilapia, 2) feeding at a sub-satiation level of 67% did not reduce measurable production of marketable fish relative to fish fed at 100% satiation level, and 3) feeding only on alternate days saved approximately half of feed cost without a significant reduction in growth, survival, or market yield of Nile tilapia in growout ponds (Brown et al. 2000, Bolivar et al. 2003, Bolivar et al. 2006). In this study we examined the utility of combined delayed feeding strategies of 60 days delayed and 30 day alternate day feeding with 67% satiation reduced feeding to evaluate if production costs of tilapia for farmers in the Philippines could be further reduced. An additional on-farm study evaluated whether further reductions in daily feeding to half the amount typically applied might produce additional cost savings for farmers.

The cost of commercial fish feeds are rising sharply as the market demand increases to supply growing aquaculture and the availability of fishmeal declines. About 40% of feed costs are attributable to fishmeal, which constitutes 15-20% of the feed formulation. Much of the fishmeal used for tilapia in the Philippines is imported, and costs are expected to rise in the future as global supplies become constrained by increasing demands from other aquaculture and declines in commercial bait fisheries. Because tilapia are omnivorous fish, which naturally feed on plankton, diatoms, small crustaceans, algae, higher plants and decomposing vegetable matter, they do not require fish in their diet and they are an ideal group of species to recycle food by-products into high quality food protein for humans (Brown 1983). Unlike carnivorous fishes, tilapia can digest high levels of carbohydrate in their diet (Anderson et al. 1984; National Research Council 1993), and they can effectively utilize human food by-products as feed ingredients, such

as rice bran, cocoa, various flowers, soya, nut oil, milling waste, brewer's wastage, poultry by-product meal and feather meal, cassava, and ipal-ipal leaf (Jackson et al. 1982). All of these lower-cost ingredients are readily available in the Philippines to completely replace or significantly reduce the inclusion of fishmeal in tilapia diets. Indeed, various animal protein meals (meat and bone meal, poultry by-product meal, feather meal, and blood meal) and plant proteins (soya, copra, cottonseed and others) have been shown to be either partially or completely replace fishmeal in tilapia diets (El-Sayed 1998; for reviews see Lim and Webster 2006 and El-Sayed 2006). We have determined that tilapia can be fed diets containing up to 33% sweet potato and lactic acid-stabilized poultry carcasses (60:40 co-extruded blend) without adverse effects on growth performance or consumer panel sensory indices (aroma, flavor and texture) (Middleton et al., 2000). However, the use of food by-products to produce least-cost fish feed is primarily constrained by their poor nutrient content, poor digestibility, or poor functional properties in manufacturing feed that can withstand the rigors of pond feeding (Li et al. 2006). Few studies have addressed the combinations of animal and plant protein types that might suffice in replacing or significantly reducing fishmeal in tilapia feed. Also, most investigations focus on the performance and nutritional characteristics of different protein sources rather than their ability to improve profit margins in tilapia production (see El-Sayed 2006). This study compares the utilization of protein sources that can replace fishmeal in tilapia diets, namely fermented mechanically deboned meat poultry byproduct, yeast extract protein, and poultry by-product meal. We also examined the use of pork by-product meal as a replacement for fishmeal in diets formulated with ingredients widely available in the Philippines on the grow out performance of Nile tilapia fed on alternate days in earthen ponds at Central Luzon University.

Based on our previous laboratory work (Vera Cruz et al. 2006), the abundance insulin-like growth factor-I (IGF-I), specifically hepatic mRNA levels, has the potential to be a rapid growth indicator in the Nile Tilapia (*Oreochromis niloticus* L.), We attempted to evaluate whether hepatic-derived IGF-I mRNA might correlate to growth under conditions in the field (ponds) and also to assess whether circulating hormone levels, thought to be derived primarily from liver, might also prove useful as a growth indicator (Picha et al. 2008). Evaluation of IGF-I as a growth biomarker was done in the context of the studies on feed reduction strategies and nutrition outlined above.

MATERIALS AND METHODS

Study 1 - Evaluation of combined 60 day delayed feeding, 30-day alternate day feeding, and 67% satiation feeding versus continuous satiation daily feeding on on-farm growout of Nile tilapia in earthen ponds

This study evaluated two (2) feeding program treatments on the growth performance of Nile tilapia. Treatment group 1 was subjected to 60 days of delayed feeding, 30 days alternate-day feeding and 30 days full feeding on a daily basis but at a sub-satiation level of 67%. Treatment group 2, the control group, were fed daily at 100% of recommended feed level based on fish biomass.

Sex-reversed Nile tilapia fingerlings (0.20 – 0.25 g body weight) of the Genetically Improved Farmed Tilapia (GIFT) strain were used in the study. The study utilized six separate tilapia farms in the Nueva Ecija region with areas ranging from 586 to 1,280 sq m. Each farmer-cooperator provided two ponds, one for each treatment group, for the growout study. Fingerlings were stocked at 4 fish m^{-2}. A day after stocking, fingerlings on the control regime (treatment group 2) were fed with commercial feeds following the recommended feeding guide of two feed suppliers. Fish were initially fed at a rate of 20% of the fish biomass that was lowered down to 2% of the fish biomass towards the end of the culture period. Ponds were fertilized with ammonium phosphate (16-20-0) and urea (46-0-0.) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water. Fish were subsampled every two weeks wherein one hundred (100) individual fish were captured by the cast net method and measured for average weight and length to assess the growth of stocks and for feed adjustment. Water quality parameters, such as dissolved oxygen, water temperature, total ammonia-nitrogen and water pH were monitored weekly starting at 0900 hours. After 120 days of culture, stocks were harvested for bulk weight of all the stocks, survival rate, and the extrapolated gross fish yield (total weight of fish at harvest (kg) / area of the pond (m^2)). Other variables, including total feed consumption, feed conversion ratio (FCR; feed consumed/body weight), specific growth rate (SGR; % daily body weight and length gain; $[(\ln W_f - \ln W_i)/(T_f - T_i) \times 100]$) were calculated. Differences among means between groups were analyzed using the paired Student's t-test.

Liver was collected from fish subsampled at monthly intervals and at the end of the experiment for analyses of IGF-I mRNA and its corollary to growth. Tissue was placed in RNA Later (Qiagen Inc., Valencia, California, USA) and stored at -20C until processing. Total liver RNA isolation and processing and hepatic IGF-I mRNA determinations were conducted according to previously described methods for Nile tilapia (Vera Cruz et al. 2006). Total RNA was isolated using Trizol® according to the manufacturer's protocol (Invitrogen™). High salt precipitation solution was used for glycogen removal in the samples. RNA was treated with DNA-free™ (Ambion®) to remove any possible genomic DNA contamination. RNA was quantified and purity was assessed by spectrophotometry (JASCO V-530 UV/VIS Spectrophotometer). On reverse transcription, first strand cDNA was synthesized in 20ul RT reactions with 1 ug total RNA template Omniscript® reverse transciptase, 10x RT buffer, 5uM dNTP, 10 uM oligo-dT primer (Promega®) and RNase inhibitor (RNasin®, Promega®). Normalization of samples was carried out against total RNA content and was done using absorbance measures at OD_{260} in JASCO V-530 UV/VIS Spectrophotometer. Samples were reverse transcribed by incubation at 37 °C for 60 min. IGF-I quantification using TaqMan® qRT-PCR was performed at the Southeast Asian Fisheries Development Center (SEAFDEC) at Tigbauan, Iloilo, Philippines on a Rotor Gene 6000 (Corbette Research), using the standard conditions at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. Gene-specific primers (Invitrogen) and FRET probes (Applied

Biosystems) for qRT-PCR were designed with the Primer Express® Program (Applied Biosystems). The forward primer was 5'-GTCTGTGGAGAGCGAGGCTTT-3', corresponding to bases 324 to 344 and the reverse primer was 5'-CACGTGACCGCCTTGCA-3', corresponding to bases 377 to 393. The sequence of the probe was 5'-TTTCAATAAACCAACAGGC-TATGGCCCC-3'. The selected reporter and quencher dyes for the probe were FAM and TAMRA, respectively. Reactions for each sample were done with each well containing 25 ul PCR mixture (10 ng cDNA template, 1X TaqMan® universal PCR master mix, 900 nM forward and reverse primers and 250 nM probe).

Study 2 – Evaluation of 50% daily feed ration levels versus full daily feed ration on on-farm growout of Nile tilapia in earthen ponds.

This study was composed of two (2) treatment groups and replicated six (6) times. Groups were as follows: I – 50% of recommended daily ration based on biomass, and II – a control group fed daily at 100% of recommended daily feed ration based on fish biomass (20% down to 3%). Sex-reversed Nile tilapia fingerlings (0.20 – 0.25 g body weight) of GIFT strain were used in the study. The study utilized six separate tilapia farms in the Nueva Ecija region with areas ranging from 400 – 1380 m^2. Each farmer-cooperator provided two ponds, one for each treatment group, for the growout study. Fingerlings were stocked at 4 fish m^{-2}. Fish were fed daily at either a 50% (Treatment I) or 100% (Treatment II) level based on fish biomass. Ponds were fertilized with ammonium phosphate (16-20-0) and urea (46-0-0.) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water.

Fish were subsampled and production parameters were measured and analyzed as described under Study 1. Liver was collected from fish subsampled monthly and at the end of the experiment for analyses of IGF-I mRNA and its corollary to growth as described above.

Study 3 – *Effects of Fermented Mechanically Deboned Meat Poultry by-Products, Poultry Meal and Nupro Yeast Extract as a potential protein source substitution for Fish Meal in tilapia feed formulations*

We evaluated other protein sources as a substitute for fishmeal in tilapia diets in tank trials using sex reversed male Nile tilapia (Aquasafra, Sarasota, FL). Fish were either fed a balanced tilapia diet (32% crude protein, 7% crude fat) containing 6% fishmeal (FM), or diets in which fishmeal was substituted with other protein sources; fermented mechanically deboned meat poultry byproduct (MDM), Nupro yeast extract (NUP) or poultry meal (PM). All four test protein meals had similar crude protein content (62-65% Crude Protein), but the MDM had a higher fat content, thus requiring less supplemental poultry fat to formulate experimental diets of similar nutrient composition (Table 3). All the feed was manufactured into pellets using a pellet press and crumbled into 2 mm crumbles.

Fish of similar size were raised in recirculating freshwater tanks (1000 L) at the Tidewater Research Station (Plymouth NC) for 105 days beginning at an average body weight of approximately 90 g (N = 2 tanks/group with 35 fish/tank). A separate group of smaller fish of identical age (56 g, N = 1 tank/group, 35 fish/tank) was also tested to ascertain whether growth responses might vary with body size. Unless otherwise indicated data presented represent that from the replicated groups containing larger fish, although the smaller fish responded virtually identically to the different diets as that of the larger fish. Fish were grown at 25ºC and water quality parameters were maintained well within the tolerance levels for tilapia. Fish were fed twice daily at 3% body weight per day throughout the experiment. Total feed applied to the tanks was adjusted based on body weight approximately every 3 weeks. All fish were weighed and measured for total length at 0, 34, 72 and 105 days of the experiment. Fish were sampled for plasma IGF-I analyses at the beginning (day 0) and end (day 105). Blood was taken via heparinized syringe from the caudal vein of anesthetized fish and plasma separated by centrifugation and frozen at -20C (Picha et al. 2008). At the end of the experiment a subsample of animals (3-4 fish/tank) were also frozen for proximate analyses.

For proximate analyses, fish were weighted, grinded, and dried under ventilation a 65ºC for 48 hours to determine dry matter content of each fish. Samples were subsequently further grinded to attain smaller particle sizes and analyzed for crude protein, crude fat, and energy content.
Protein efficiency ratio was calculated as the total amount of protein consumed divided by the total amount of body protein gained during the 105 day period.

Differences in mean body weight and total body length were analyzed by repeated measures ANOVA followed by the Fisher's LSD test for predetermined comparisons among groups. Specific growth rate (% body weight gain/day), feed conversion ratio, and body composition data were analyzed by a Student's t-test.

Study 4: Effect of pork meal replacement of fishmeal in diets formulated with locally-available ingredients in the Philippines on the growth of Nile tilapia cultured in earthen ponds using alternate-day feeding strategy

In this study we evaluated the utility of using pork meal, a source of animal protein substantially lower in cost than fishmeal and widely available in the Phillipines, as a replacement of fishmeal in diets of tilapia grown in ponds. Alternate day feeding was previously shown to reduce production costs of tilapia without significantly altering final yield as almost 50% less feed could be used to grow fish than that incorporating standard daily feeding practices. Hence, this study evaluated if replacement of fishmeal with pork meal is as effective in producing tilapia under an alternate-day feed reduction strategy, as those diets containing standard levels of fishmeal. We established a collaboration with Santeh Feed Corp. (Bulacan, Philippines), a major producer of fish feeds in the Philippines. Mr. Ning Pascaul of Santeh helped formulate the feeds using various locally

available ingredients in the Philippines.

This study was composed of two (2) treatment groups with four replicates per treatment. Groups were as follows: treatment I – formulated feeds with 6% fishmeal and treatment II – formulated feeds lacking fishmeal and containing pork meal. The constitution of the formulated
tilapia grower floating feeds is shown in Table 6. The grow-out phase of this study were done in eight 500 m^2 earthen ponds at the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Size 20 (weight range = 0.35-0.37 g) fingerlings of the GIFT strain were stocked in each pond at 4 fish m^{-2}. Fish were initially fed on alternate days with prestarter (36% crude protein) for 30 days and then starter feeds for 30 days. Following this 60-day period, animals were fed formulated grower feeds with and without fishmeal on alternate days until the end of the experiment. The feed ration was based on the average fish biomass amd ranging from 10% down to 3% body weight per day (Table 7). Fish sampling was done every two weeks using cast net method to monitor fish growth and for feed adjustment. Estimated survival were as follow: first month – 100%, second month – 95%, third month – 90% and fourth month – 85%. Ponds were fertilized weekly with ammonium phosphate (16-20-0) and urea (46-0-0.) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water. Water quality parameters (dissolved oxygen concentration, water temperature and Secchi disc visibility) were monitored weekly between 9 to 10 o'clock in the morning. Water depth was maintained at 1 m in each pond. The total numbers of fish were counted and bulk weighed at the end of the 120 days culture period. Final mean weight, daily weight gain, gross yield and survival rates were calculated. A simple cost and return analysis were computed to compare the cost benefits between the two treatments. Data were analyzed using paired t-Test.

RESULTS AND DISCUSSION

Study 1 - Evaluation of combined 60 day delayed feeding, 30-day alternate day feeding, and 67% satiation feeding versus continuous satiation daily feeding on on-farm growout of Nile Tilapia in earthen ponds

Figure 1 shows the body weight and total length of fish on the delayed/reduced feeding regime (treatment I) versus fish fed daily at full ration levels (treatment II). We found fish had lower body weights and lengths after 60-days of delayed feeding than control fish fed daily. During the third month of culture, fish on the delayed feeding were then fed on alternate days. These fish had an average body weight of 52.09 g compared with 84.77 g for fish fed daily. Body length did not change significantly among the two groups during this period. On the final month of culture, fish on the delayed/reduced feeding protocol were then fed daily at 67% satiation. During this period, fish showed a greater growth rate than fish fed daily, although full catch-up growth was not achieved. By the end of the experiment average body weight of treatment I fish on the delayed/reduced feeding protocol was 118.05 g, relative to 149.82 g for treatment II fish fed daily on a full ration throughout the study. Stocks were harvested wherein number of fish and total weight

were recorded for survival and gross fish production. Each farm was monitored during harvest ensuring the security of the data. Extrapolated gross yield, feed conversion and other production variables is presented in Table 1.

Following the 120-day culture period, the performance of the fish stock with regards to the mean final weight and length, daily gain in weight and length, specific growth rate, and feed conversion ratio were lower in fish on the delayed and reduced feeding protocol, albeit the effect was statistically insignificant relative to fish fed daily at full ration ($P > 0.05$; Table 1). Survival rate and extrapolated gross yield was significantly lower in fish on the delayed/reduced feeding protocol ($P < 0.05$; Table 1). It is likely the reduced gross yield of fish on the delayed/reduced feeding protocol, may have resulted in part to their lower survival rates (32%), although the fish on normal feeding also showed relatively low survivorship (< 50%). Although the reason for poorer survival rates is unclear, it may have been due in part to the presence of predators, as several adult catfishes and mudfishes were present in ponds or perhaps to extreme weather conditions where hot mornings and sudden afternoon rains prevailed during the culture period. These conditions may have led to lower survival rates of fish on the delayed/reduced feeding protocol, particularly early on when fish showed significant loss in condition factor (body weight/length^3) during the initial 60 days of delayed feeding. Results show that using combination of feed reduction strategies significantly reduced the quantity of feeds consumed by about 55%.

Study 2 – Evaluation of 50% daily feeding versus the typical 100% of daily feeding ration on on-farm growout of Nile tilapia in earthen ponds.

Figure 2 shows the body weight and total length of fish on the 50% reduced feeding ration (treatment I) versus fish fed daily at full ration levels (treatment II). Initial stocking size between the two groups was virtually identical (0.21 ± 0.03 g and 0.21 ± 0.02 g body weight). Following the 120-day culture period fish on the reduced feed ration showed had a lower final mean weight of 123.57 ± 15.71 g and mean daily weight gain of 1.03 ± 0.13 g ($P < 0.05$; Figure 2, Table 2) relative to fish on full daily ration that had a final weight of 148.95± 13.06 g and daily weight gain 1.24 ± 0.11 g ($P < 0.05$;). A 7% lower total length was also observed with fish on reduced ration. Despite the difference in final weight and length the specific growth rate did not differ between groups (reduced ration, 5.03 ± 0.18 %; full ration, 5.46 ± 0.13 %; $P > 0.05$; Table 2).

Survival rates were similar between fish on the reduced and full feeding rations (reduced ration, 62.5 ± 6.08 %; 63.1 ± 10.59 %; Table 2). The extrapolated gross yield of fish on the reduced ration (2754.9 ± 234.16 kg/ha) was moderately lower and did not differ from fish on the full ration (3440.9 ± 613.27 kg/ha; $P > 0.05$; Table 2). The total quantity of feeds consumed was 56% lower in fish on the reduced ration protocol relative to those provided a full ration. This dramatic reduction in feeds consumed was accompanied by a > 100% improvement in feed efficiency. Mean feed conversion ratio (FCR) was 1.0 ± 0.06 for fish grown on reduced ration while that for fish on the full ration was 2.1 ± 0.38

($P < 0.05$; Table 2).

Water quality parameters, including water temperature and dissolved oxygen concentration was monitored throughout the culture period (Figure 3). Temperature and DO concentration were within the normal range. However, frequent fluctuations especially on water temperature due to unstable weather condition was observed throughout the culture period.

We attempted to evaluate the utility of IGF-I mRNA as a biomarker of growth in pond reared fish from both *Study 1* and this study. However, after performing RNA isolation, DNA-free treatment, cDNA synthesis and IGF-I quantification, sample readings were negligible (data not shown). It is likely that the RNA was degraded as frequent power interruptions had exposed samples to temperatures higher than the recommened (-20 – -80ºC). It will be critical in future studies to rapidly process samples through the cDNA synthesis step, as DNA is considerably more stable than RNA.

Study 3 – *Effects of Fermented Mechanically Deboned Meat Poultry by-Products, Poultry Meal and Nupro Yeast Extract as a potential protein source substitution for Fish Meal in Nile tilapia feed formulations*

We evaluated protein sources as a substitute for fishmeal in tilapia diets in tank trials using sex reversed male Nile tilapia. Fish were either fed a balanced tilapia diet (32% crude protein, 7% crude fat) containing 6% fishmeal (FM), or diets in which fishmeal was substituted with other protein sources; fermented mechanically deboned meat poultry byproduct (MDM), Nupro yeast extract (NUP) or poultry meal (PM) (See Table 3)

The data presented are from the replicated groups containing larger fish, although the smaller fish responded virtually identically to the different diets as that of the larger fish. Fish fed the PM based diets achieved a similar final mean body weight as fish fed the FM-based diets (mean SEM; PM, 437 ± 1.26 g versus FM, 448 ± 1.31 g; Figure 4). Final mean weight was significantly greater in fish fed FM and PM than those fed MDM (411 ± 11.29 g) and NUP (422 1.43 g) diets. We observed that the feed pellets of the MDM feed had larger mean particle size than the FM diets (2000 microns versus 1200 microns), which may have compromised feed intake when the fish were small and thus significantly reduced specific growth rate during the 0-34 day time period. Body lengths did not differ among the groups. Although fish on the PM and FM diets had showed elevated growth rates, there was not a significant overall effect of the different diets on specific growth rates over the 105 day growout period (Figure 5) (mean ± SD; PM, 3.29 ± 0.007 % BW/day; FM, 3.39 ± 0.005 % BW/day; MDM, 3.03 ± 0.082 % BW/day; and NUP, 3.15 ± 0.037 % BW/day).

We evaluated whether changes in circulating IGF -I might parallel changes in growth rate

under controlled tank trials. Relative to the start of the study, we observed that plasma IGF-I increased significantly in fish sampled by the end of the experiment in all groups ($P > 0.05$; Figure 6). However, as with specific growth rate, there was no significant difference in systemic IGF-I among fish fed the different diets. Future studies should evaluate if measures of the IGF-I protein in blood might change under conditions where growth rates clearly differ.

Feed conversion ratios did not differ among the groups, except for fish on the MDM diet, where the feed conversion ratio was higher relative to animals on the FM diet ($P < 0.05$; Table 4). The effect of MDM inclusion on feed form may have resulted in more feed wastage, rather than compromising nutrient utilization or digestibility. Indeed, there was not a significant difference in protein efficiency ratio (a measure of dietary protein digestibility and utilization) among diets containing FM, MDM, or NUP. However, the protein efficiency ratio (weight gain over the period observed / total protein intake) was significantly lower in fish fed the PM diets than those fed the FM diet ($P < 0.05$; Table 4). There were no significant treatment effects on crude protein, crude fat (ether extract), and energy content of fish after 105 days of feeding the different diets (Table 5).

Collectively, these results suggest that alternate protein sources may serve as suitable substitutes of fishmeal in tilapia diets and could ultimately provide cost savings while reducing dependence on fishmeal derived from capture fisheries.

Study 4: Effect of pork meal replacement of fishmeal in diets formulated with locally-available ingredients in the Philippines on the growth of Nile tilapia cultured in earthen ponds using alternate-day feeding strategy

We conducted a study in collaboration with Santeh Feed Corporation (Philippines) to evaluate porkmeal, which is widely available in the Philippines, as a substitute for fishmeal on growout of tilapia in earthen ponds in the Philippines. Collaborations with industry insured that diet formulations incorporated locally-available ingredients including copra cake, cassava meal, local fish oils and coconut oil (Table 6). We also utilized an alternate day feeding scheme that was previously shown to work as effectively as daily feeding in producing marketable fish (Bolivar et al. 2006).

Figure 7 shows growth in body weight and length of fish raised in quadruplicate in ponds for 120 days using grower diets with and without 6% fishmeal. Changes in body weight and length were virtually identical among the groups fed isocaloric diets (Figure 7). Table 6 summarizes the production parameters of Nile tilapia grown on the different diets including weight and length gain, feed conversion, extrapolated yield per hectare and survival rate. Overall, results show that the different production parameters did not differ among fish fed the two diets.

Survival rate was high in fish fed the 0% (84.2%) and those provided the 6% fishmeal (89.3%) diet. Extrapolated yield and feed consumed per hectare was 3,080 kg/ha and

3,231.4 kg/ha, respectively, for fish on the 6% fishmeal formulated diet, and 3,062 kgs and 3,129.9 kgs per hectare, respectively, for fish on the 0% fishmeal diet. Feed conversion was slightly lower in fish fed 0% versus 6% fishmeal diets.

Water quality parameters including dissolved oxygen, water temperature and secchi disc visibility were similar among the two groups and fell within the range tolerable for tilapia growout. However, dissolved oxygen levels declined in ponds during the last month of growout for both groups of fish. This may have resulted in reduced feeding activity and overall growth of both groups of fish.

We conducted a simple cost and return analysis using current prices for all inputs and the value of marketable tilapia (Table 9). We found an approximate 8% higher net return for production of fish on the 0% fishmeal formulated diets (PhP 55,944.42) had than those grown on the 6% fishmeal diet (PhP 51,742.76).

CONCLUSION

It is estimated that 60-80% of total variable costs for growing tilapia is attributable to feeds. Through a series of four studies we found that alternative feeding strategies that reduce total feed consumption and replacement of fishmeal in tilapia diets have the potential to provide cost savings to tilapia farmers. The combined delayed and reduced feeding strategies that incorporate a sequential series of 60-day delayed, 30 days of alternate day, followed by 30 days of 67% subsatiation feeding produced fish with lower growth rates and approximately 40% lower extrapolated gross yield as compared with fish fed daily at full prescribed levels. Approximately 55% less feed was fed to the fish on the delayed/reduced feeding regimen relative to those fed on a traditional full daily feeding schedule. Based on these results, the combined feed reduction strategy showed reduced feed costs, but also resulted in significantly lower yields at harvest, suggesting this feeding strategy may not provide a signficant cost benefit relative to animals fed the typical continuous full daily feeding. Shortening the delayed feeding period, where most growth potential in weight and length was lost, to 30-45 days might prove useful in improving survival and the gross yield of fish subjected to combined reduced and delayed feeding strategies. It appears that combining both delayed and reduced feeding, at least based on the experimental paradigm tested here, is less effective than applying delaying feeding, 67% satiation, or alternate day feeding strategies alone.

In trials on six separate farms, we found that feeding at 50% subsatiation was effective in producing tilapia of similar gross yield as that of fish grown on full daily feeding. Fish on this reduced feeding protocol consumed almost 60% less feed, showed a 100% improvement in feed efficiency, and had no appreciable loss in specific growth rate or gross yield relative to animals fed a full ration level. Collectively, reducing feed rations by as much as 50% has the potential to produce substantial cost savings to farmers. A simple cost-return analysis shows a greater net return of approximately US$1300 for fish grown on half the ration relative to those grown on a typical full ration level.

In study 3, we found that fermented deboned meat poultry byproduct (MDM), Nupro

yeast extract (NUP) and poultry meal (PM) show strong promise as subtitutes for fishmeal in isocaloric balanced tilapia diets that incorporate 6% inclusion rate of fishmeal, a level typically used for commercial feeds in the Philippines. Performance of fish on the poultry meal diets was virtually identical to that of fish fed fismeal diets, while fish on the fermented poultry byproduct and yeast extract had similar specific growth rates. Even though the MDM and NUP resulted in slightly lower body weight and feed conversion (FCR) than the fishmeal (FM) diets, there were no significant differences in protein efficiency ratio (PER) among these three diets. Thus the quality of protein for tilapia in MDM and NUP is similar to FM. Evidently, the larger crumble size of the feeds containing the MDM and NUP may have comprimised feed intake and resulted in more feed wastage when the fish were small during the first 34 days of the trial. Because both the MDM and NUP had undergone minimal heat processing prior to pellet processing the feed, their functional properties as a pellet binder were much greater than the fishmeal and poultry meal products. This enhanced pellet binding characteristics of MDM and NUP is an advantage in manufacturing stable feed pellets provided pellet size is suitable for the size of fish consuming the feed. In contrast to the MDM and NUP, the PER of PM was determined to be significantly lower than fishmeal (1.95 vs 2.13, $p < 0.05$), even though dietary inclusion of these two protein meals resulted in statistically similar body weights and FCR.

Collectively, these results clearly indicate that the alternate protein sources evaluated in study 3 may serve as suitable substitutes of fishmeal in tilapia diets and could ultimately provide significant cost savings while reducing dependence on fishmeal derived from capture fisheries. Our results agree with the results of other researchers that evaluted the feeding value of fermented poultry byproducts, poultry meal and yeast protein in comparison to fishmeal for tilapia. Middleton et al. (2001) observed that fermented poultry byproduct can be included up to 13% in the diet of tilapia without adverse effects on growth performance and consumer sensory panel indicies (aroma, flavor, and texture) of tilapia filets. Likewise, Medri et al. (2000) observed that dietary inclusion up to 30% of distillery yeast (*Saccharomyces cerevisae*) can be fed to tilapia without adverse effects on growth performance. In contrast to the total replacement of dietary fishmeal with fermented poultry byproduct or yeast extract as done in our studies, Middleton et al (2001) and Medri et al. (2000) included at least 8% fishmeal in all of their experimental diets. Finally, Yildirim et al. (2009) found that poultry meal can replace 50% of the fishmeal in tilapia diets without adverse effects on growth, but 100% fishmeal replacement with poultry meal resulted in reduced growth rate and feed conversion.

We previously show that alternate day feeding resulted in significant cost-savings relative to daily feeding at full ration (Bolivar et al. 2006). Using this more cost-effective alternate day feeding strategy, we assessed whether elimination of fishmeal from diets and its replacement with a cheaper animal protein (porkmeal) might provide additional cost savings to tilapia production. The diets were produced by a local feeds company and incorporated locally available Philippine ingredients where possible. We

show that fish fed formulated feeds lacking fishmeal had similar daily weight gain, specific growth rate, and survivorship as fish fed fishmeal diets. Feed consumption, gross harvest yield and feed conversion were also similar among fish on the experimental diets. A cost-return analysis shows that incorporation of a diet lacking fishmeal produced an 8% or almost $100 in cost savings in feed for each hectare of tilapia farmed. This along with the alternative day feeding strategy has the potential of reduce overall feed costs for growing marketable size tilapia by 60% relative to the typical practice of applying fishmeal formulated diets on a daily basis. A future study directly comparing daily and alternative day feeding strategies with diets formulated with and without fishmeal througout the entire production cycle of tilapia is warranted to establish the actual cost savings farmers are likely to have.

Based on success in the laboratory, we attempted to field test the utility of using IGF-I mRNA as an indicator of tilapia growth. However, tissue samples from the pond studies evaluating feed reduction strategeis showed RNA degradation negating the capacity to reliably measure IGF-I mRNA. For future studies, tissues will be rapidly processed to produce cDNA, which is substantially more stable than RNA and less susceptbile to degradation that may arise from temperature fluctuations due to power outages. Samples will then be measured shortly thereafter for IGF-I gene expression. We did find that measurement of the protein in blood, that is circulating IGF-I, which we show is a strong indicator of growth in fish (Picha et al. 2008) may also be suitable as an indicator of growth in tilapia. Circulating IGF -I nor specific growth rate varied in animals subjected to alternative diets in which fishmeal was substituted for yeast and poultry based animal protein. Additional work should evaluate if circulating IGF-I correlates to growth status of fish that exhibit differential growth responses to nutritional or environmental parameters.

Collectively, these series of studies show that reduced feeding strategies and substitution of diets containing fishmeal with cheaper and more sustainable sources of protein are effective options for reducing the costs without negatively impacting the production of tilapia.

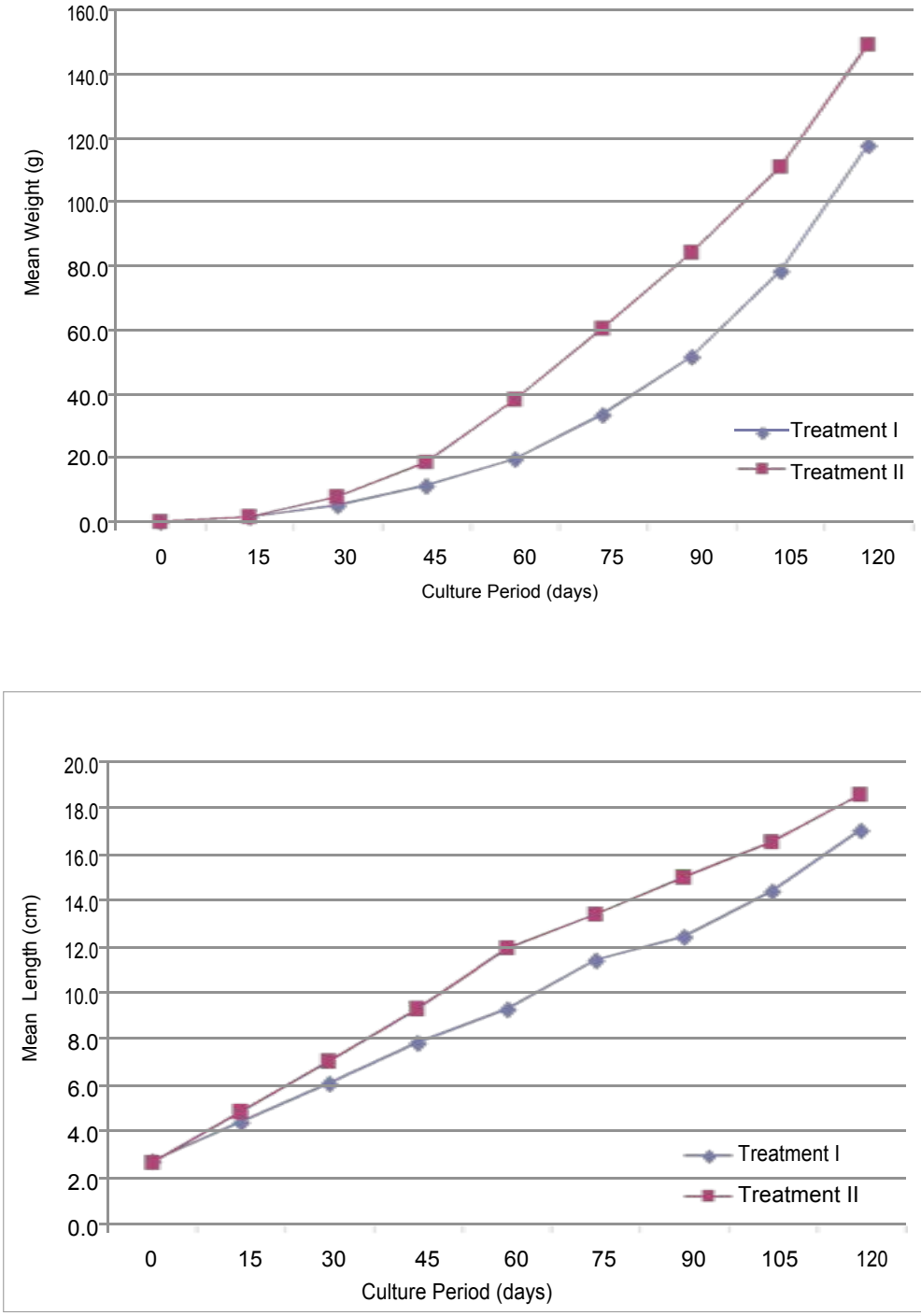


Figure 1. Mean body weight (top) and total length (bottom) of fish after a120 day culture period. Fish were grown under a combined delayed and reduced feeding protocol consisting of 60 days delayed feeding, 30 days alternate-day feeding, followed by 30 days full feeding on a daily basis but at a sub-satiation level of 67% (treatment I) versus those grown at 100% of recommended feed level based on fish biomass (treatment II).

Table 1. Production parameters (mean ± SEM) of fish grown under a combined delayed and reduced feeding protocol consisting of 60 days delayed feeding, 30 days alternate-day feeding, followed by 30 days full feeding on a daily basis but at a sub-satiation level of 67% (treatment I) versus those grown at 100% of recommended feed level based on fish biomass. Fish were grown on local farms in ponds for 120 days.

Parameter	Delayed/Reduced Feeding	Typical Daily Feeding
Mean Initial Weight (g)	0.35 ± 0.01^{a}	0.35 ± 0.01^{a}
Mean Final Weight (g)	118.05 ± 5.68^{a}	149.82 ± 13.53^{a}
Mean Daily Weight Gain (g)	0.98 ± 0.05^{a}	1.25 ± 0.11^{a}
Mean Specific Growth Rate (%/day)	4.86 ± 0.05^{a}	5.04 ± 0.07^{a}
Mean Initial Length (cm)	2.8 ± 0.05^{a}	2.7 ± 0.04^{a}
Mean Final Length (cm)	17.1 ± 0.44^{a}	18.6 ± 0.61^{a}
Mean Final Gain in Length (cm)	14.4 ± 0.42^{a}	15.9 ± 0.58^{a}
Mean Feed Conversion Ratio	2.4 ± 0.34^{a}	2.7 ± 0.45^{a}
Mean Survival Rate (%)	32.1 ± 9.59^{a}	47.9 ± 9.11^{b}
Mean Extrapolated Gross Yield (kg ha^{-1})	$1,283.1 \pm 264.95^{a}$	$2,674.6 \pm 670.39^{b}$
Mean Quantity of feeds (kg ha^{-1})	$2,590.0 \pm 269.11^{a}$	$5,711.3 \pm 399.07^{b}$

Treatment means within the same row with different superscript letters are significantly different ($P < 0.05$).

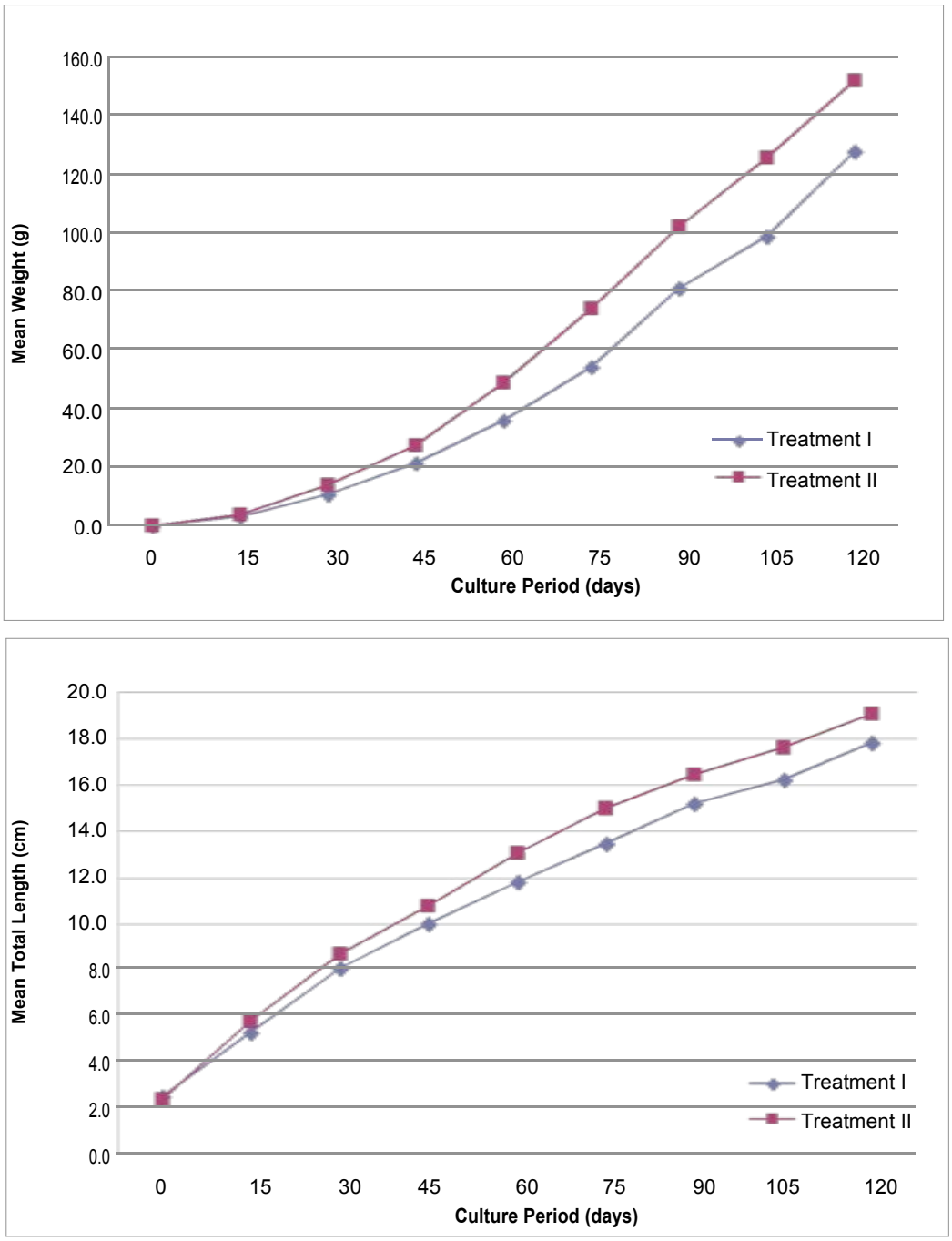
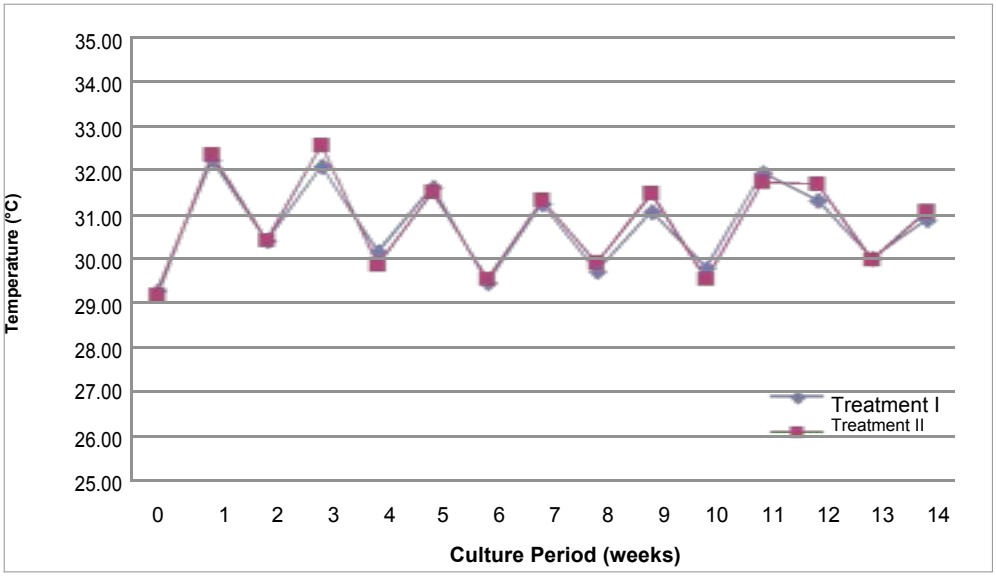


Figure 2. Mean body weight (top) and total length (bottom) of fish after a120 day culture period. Fish were grown at 50% reduced daily ration level (Treatment I) or at a full daily ration level (Treatment II).

Table 2. Production parameters (mean ± SEM) of fish grown on 50% reduced daily ration versus those grown on full daily ration. Fish were grown on local farms in ponds for 120 days.

Performance	50% Reduced Daily Feed Ration	Full Daily Feeding
Mean Initial Weight (g)	0.21 ± 0.03[a]	0.21 ± 0.02a
Mean Final Weight (g)	123.57 ± 15.71[a]	148.95 ± 13.06[b]
Mean Daily Weight Gain (g)	1.03 ± 0.13[a]	1.24 ± 0.11[b]
Mean Initial Length (cm)	2.5 ± 0.11[a]	2.4 ± 0.09[a]
Mean Final Length (cm)	17.6 ± 0.61[a]	18.9 ± 0.56[b]
Mean Final Gain in Length (cm)	15.2 ± 0.61[a]	16.5 ± 0.61[b]
Mean Specific Growth Rate (%/day)	5.03 ± 0.18[a]	5.46 ± 0.13[a]
Mean Survival Rate (%)	62.5 ± 6.08[a]	63.1 ± 10.59[a]
Mean Feed Conversion Ratio	1.0 ± 0.06[a]	2.1 ± 0.38[b]
Mean Extrapolated Gross Yield (kg ha^{-1})	2754.9 ± 234.16[a]	3440.9 ± 613.27[a]
Mean Quantity of feeds (kg ha^{-1})	2588.0 ± 121.75[a]	5928.7 ± 178.06[b]

Treatment means within the same row with different superscript letters are significantly different ($P < 0.05$).



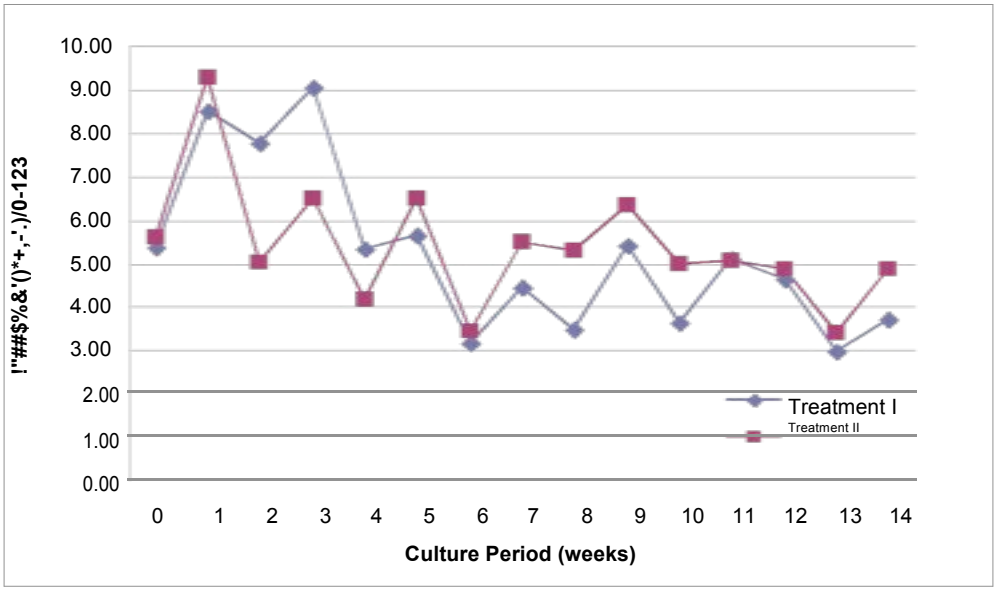


Figure 3. Mean water temperature (top) and dissolved oxygen concentration (bottom) in ponds during the 120 day culture period. Mean body weight (top) and total length (bottom) of fish after a120 day culture period. Fish were grown at 50% reduced daily ration level (Treatment I) or at a full daily ration level (Treatment II).

Table 3. Composition of caloric balanced test diets contained 6% fishmeal, and fishmeal replaced with 6% Nupro yeast extract, poultry meal, and fermented mechanically deboned meat poultry byproduct (MDM). Test diets were used in a 105-day growout of tilapia in tanks.

Ingredients	Fishmeal Diet	Nupro Diet	Poultry Meal Diet	Mechanically Deboned Meat
Soy Bean Meal	50.8	50.8	50.8	50.6
Corn	27.2	27.2	27.2	26.5
Wheat Midds	10	10	10	10
Fishmeal	6	--	--	--
Nupro	--	6	--	--
Poultry Meal	--	--	6	--
MDM	--	--	--	6
Poultry fat	4.43	4.43	4.43	3.94
Limestone	0.44	0.44	0.44	0.68
Dical P	0.33	0.33	0.33	1.29
Salt	0.22	0.22	0.22	0.27
Se Premix	0.15	0.15	0.15	0.15
Ascorbic Acid	0.13	0.13	0.13	0.13
TM Premix	0.10	0.10	0.10	0.10
Vit Premix	0.10	0.10	0.10	0.10
Choline CL 60	0.10	0.10	0.10	0.10
Lysine	0	0	0	0.14
TOTAL	**100**	**100**	**100**	**100**
Dry matter (%)	86.92	87.73	87.37	86.72
Crude Protein (%)	28.9	28.29	30.67	27.03
Acid Det. Fiber (%)	6.57	5.04	6.77	5.32
Crude Fat (%)	6.18	6.31	6.05	7.28
Calcium (%)	0.68	0.66	0.66	0.57
Phosphorus (%)	0.73	0.69	0.73	0.65
Sulfur (%)	0.27	0.31	0.28	0.26
Magnesium (%)	0.21	0.24	0.21	0.21
Sodium (%)	0.13	0.14	0.10	0.10
Potassium (%)	1.11	1.28	1.12	1.08
Cooper, ppm	14	18	14	14
Iron, ppm	276	271	236	250
Manganese, ppm	98	120	105	103
Zinc, ppm	116	142	121	116
Ash (%)	5.72	5.15	5.55	5.54
Neutral Det. Fiber (%)	8.72	9.33	9.89	9.52
Non-fiber CHO (%)	37.41	38.65	35.22	38.84
ME (kcal/kg)	3000	3000	3000	3000

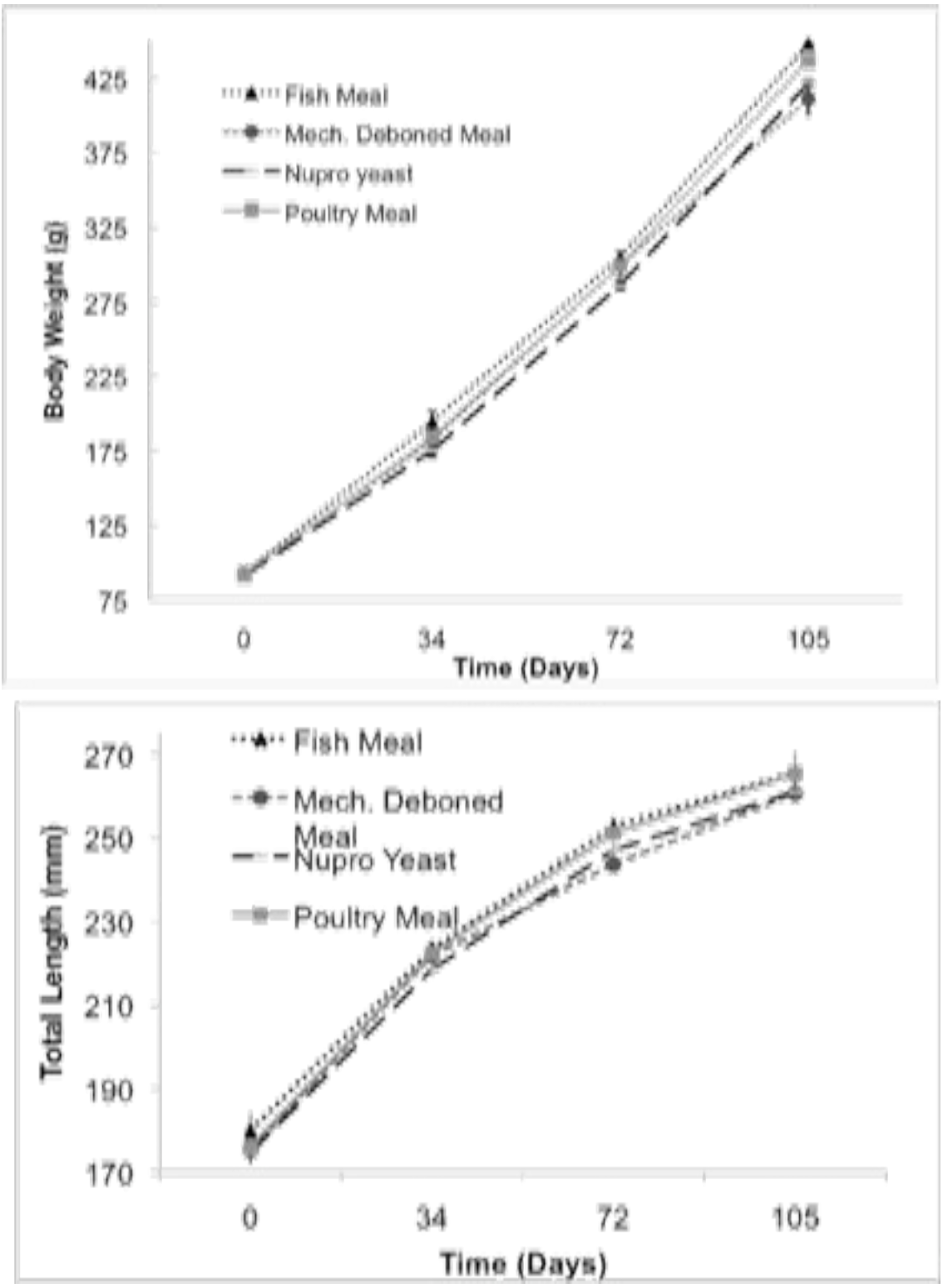


Figure 4. Body weight and total length (mean ± SD) of Nile tilapia following a 105-day growth study in tanks. Animals were fed diets containing 6% fishmeal or diets in which fishmeal was replaced with 6% Nupro yeast extract, poultry meal, and fermented mechanically deboned meat poultry byproduct. N = 2 tanks, 37 fish /tank. Fish on diets containing Nupro and deboned meat poultry byproduct had lower body weights than fish fed diets containing fishmeal or poultry meal ($P < 0.05$).

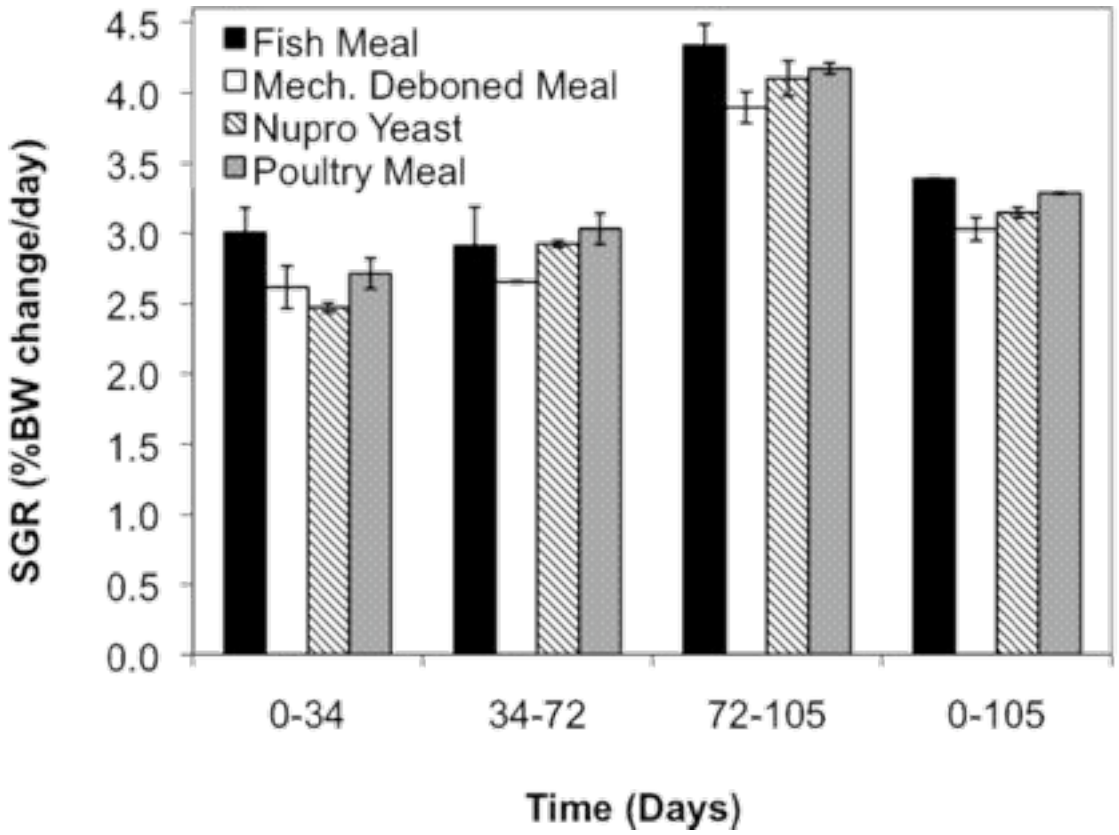


Figure 5. Specific growth rate (mean ± SD) of Nile tilapia over a 105-day growth study in tanks. Animals were fed diets containing 6% fishmeal or diets in which fishmeal was replaced with 6% Nupro yeast extract, poultry meal, and fermented mechanically deboned meat poultry byproduct. N = 2 tanks, 37 fish /tank.

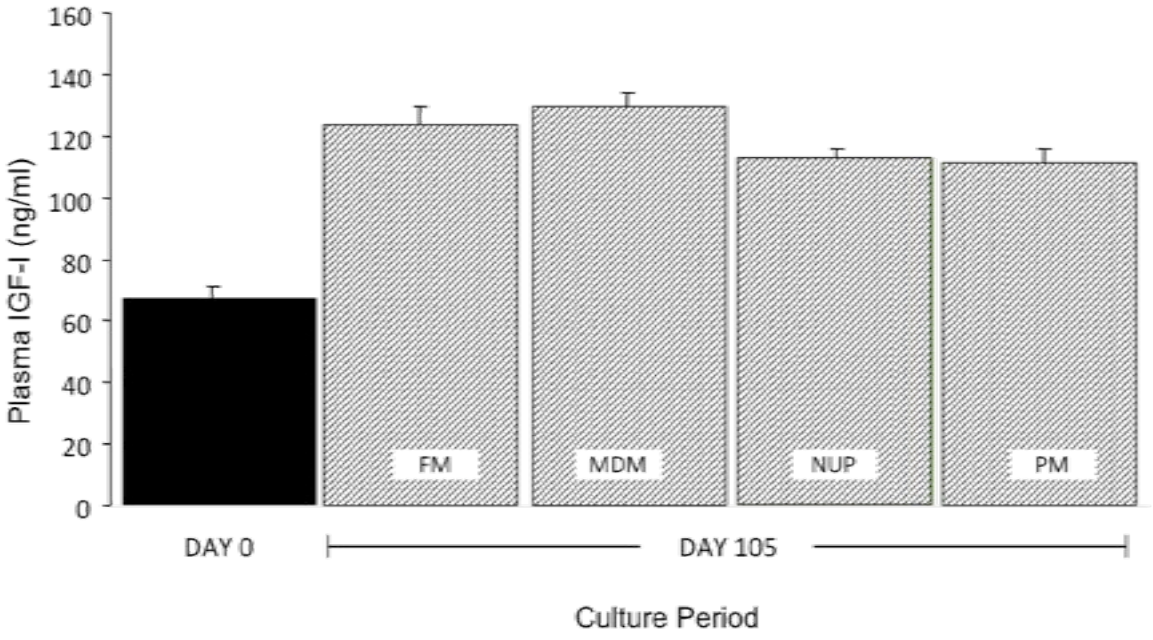


Figure 6. Plasma IGF-I levels (mean ± SEM, N = 8/group) in Nile tilapia over a 105-day growth study in tanks. Animals were fed diets containing 6% fishmeal (FM) or diets in which fishmeal was replaced with 6% Nupro yeast extract (NUP), poultry meal (PM), or fermented mechanically deboned meat poultry byproduct (MDM). Relative to levels at the start of the experiment, plasma IGF-I increased in all groups at day 105 ($P < 0.05$). There were no differences in plasma IGF-I in fish fed different diets.

Table 4. Feed Conversion Rate (FCR) and protein efficiency ratio (PER) of tilapia following 105 days growout on diets with 6% fishmeal or without fishmeal where fishmeal was replaced with 6% Nupro yeast extract (NUPRO), poultry meal (PM), and fermented mechanically deboned meat poultry byproduct (MDM). (mean ± standard deviation; N = 2 tanks/group, 37 fish/tank)

Treatments	FCR	PER
Fish Meal	1.62±0.008 B	2.13±0.004 A
NUPRO	1.75±0.07 AB	2.02±0.033 AB
MDM	1.82±0.03 A	2.04±0.078 AB
Poultry Meal	1.68±0.005 AB	1.95±0.006 B
Probability	*0.02**	*0.048**

FCR= total feed consumed by fish/weight gain by fish
PER= weight gain over the period observed / total protein intake
Values with different letters are significantly different ($P < 0.05$)

Table 5. Proximate analyses of tilapia carcasses following growout on diets containing 6% fishmeal or where fishmeal was replaced with 6% Nupro yeast extract, poultry meal, and fermented mechanically deboned meat poultry byproduct (MDM). Body weight, BW; and crude protein, CP; ether extract or crude fat (EE), and energy content (mean ± SEM; N = 6 animals/group).

Treatments	BW (g)	CP (%)	EE (%)	Energy (cal/g)
Fish Meal	412.91±91.40	23.14±1.49	9.95±1.40	5710±167
NUPRO	353.05±73.44	22.97±2.06	10.41±1.69	5803±160
MDM	393.61±63.73	23.09±0.99	10.07±1.58	5617±432
Poultry Meal	446.39±68.23	22.82±1.06	9.92±1.19	5697±196
Probability	*0.30*	*0.98*	*0.94*	*0.72*

Table 6. Composition of caloric balanced grower test diets with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal) that was used in the growout of tilapia for 120 days in earthen ponds at Central Luzon State University in the Philippines. Ingredients (inclusion rate in kg ton^{-1} of feed) represent those locally available in the Philippines.

RAW MATERIALS	Grower – 6% Fishmeal	Grower – 0% Fishmeal
Soybean Meal (HP) 45%	422.00	400.00
Corn Gluten	50.00	53.00
Hydrolyzed Animal Protein	30.00	30.00
Fishmeal Tuna 55%	60.00	0.00
Pork Meat Meal 55%	0.00	74.00
Copra Cake	73.00	76.00
Rice Bran	178.20	182.90
Cassava Meal	150.00	150.00
Fish Oil (Local)	5.50	5.00
Coconut Oil	5.00	5.00
Mono dicalcium phosphate	12.00	10.00
Salt	5.00	5.00
Mineral Premix	3.00	3.00
Vitamin Premix	6.30	6.10
TOTAL WEIGHT	**1000.00**	**1000.00**
DE Fish (kcal/kg)	2477.92	2484.50
Crude Protein (%)	30.99	31.07
Crude Fat (%)	6.21	6.23
Crude Fiber (%)	4.41	4.29
Starch (%)	17.36	17.40
Ash (%)	8.20	9.05
Ca (%)	0.96	0.91
Avail. Phosphorus (%)	0.67	0.66
Lysine (%)	1.51	1.50
Methionine (%)	0.54	0.50
Methionine + Cysteine (%)	0.97	0.96
Threonine (%)	1.06	1.07
Tryptophan (%)	0.33	0.42

Table 7. Feeding guide used for growing tilapia in ponds for 120 days using grower feed formulated with 31% crude protein (CP) with or without 6% fishmeal.

Culture Days	Estimated Survival (%)	Feeding Rate (%)	Type of Feed + Crude Protein (%)
0	100	10	Pre-starter (36% CP)
15	100	10	Pre-starter (36% CP)
30	95	7	Starter (34% CP)
45	95	7	Starter (34% CP)
60	90	6	Formulated Grower Feed (31% CP)
75	90	5	Formulated Grower Feed (31% CP)
90	85	4	Formulated Grower Feed (31% CP)
105	85	3	Formulated Grower Feed (31% CP)
120	-	-	-

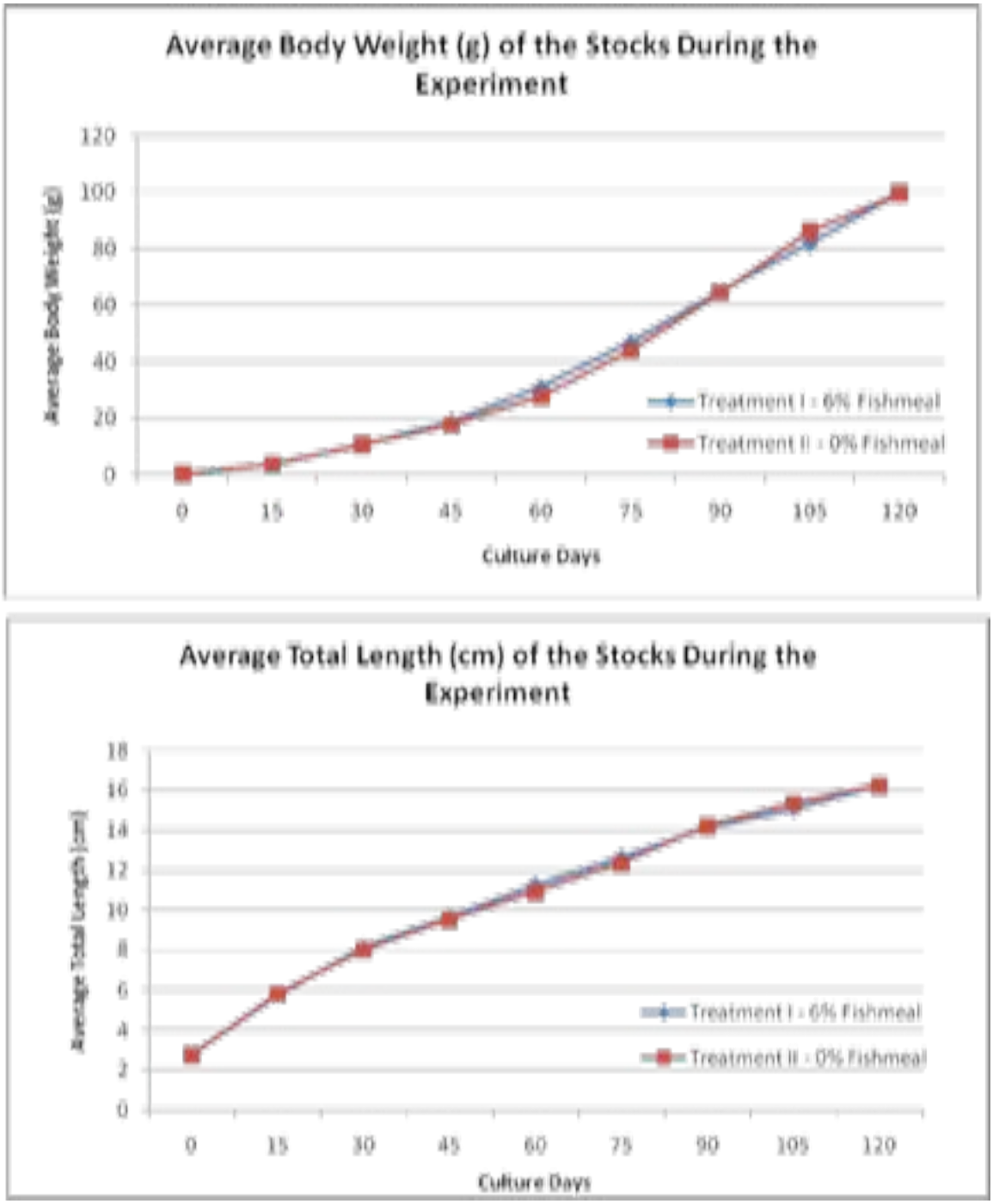


Figure 7. Mean body weight (top) and total length (bottom) of fish after a120 day culture period in earthen ponds. Fish were fed on alternate days with grower diets containing 6% fishmeal or 0% fishmeal (porkmeal substituted for fishmeal).

Table 8. Production parameters (mean ± standard deviation) of fish fed on alternate days with grower diets with 6% fishmeal or 0% fishmeal (porkmeal substituted for fishmeal). Fish were grown in ponds for 120 days.

Parameters	Treatment I (6% Fish Meal)	Treatment II (0% Fish Meal)
Initial Average Weight (g)	0.372 ± 0.049[a]	0.356 ± 0.028[a]
Final Average Weight (g)	99.531 ± 19.190[a]	99.746 ± 14.331[a]
Average Gain in Weight (g)	99.159 ± 19.175[a]	99.390 ± 14.355[a]
Average Daily Gain in Weight (g/day)	0.826 ± 0.160[a]	0.828 ± 0.120[a]
Specific Growth Rate (%)	4.652 ± 0.172[a]	4.693 ± 0.176[a]
Initial Average Total Length (cm)	2.8 ± 0.12[a]	2.8 ± 0.06[a]
Final Average Total length (cm)	16.261 ± 1.116[a]	16.241 ± 0.823[a]
Average Gain in Length (cm)	13.467 ± 1.107[a]	13.491 ± 0.880[a]
Average Daily Gain in Length (cm/day)	0.112 ± 0.009[a]	0.112 ± 0.007[a]
Survival Rate (%)	89.3 ± 5.3[a]	84.2 ± 3.1[a]
Extrapolated Feed Consumed per Hectare (kg/hectare)	3231.4 ± 711.5[a]	3129.9 ± 425.7[a]
Extrapolated Yield per Hectare (kg/hectare)	3080.0 ± 598.9[a]	3062.0 ± 520.8[a]
Feed Conversion Ratio	1.05 ± 0.05[a]	1.03 ± 0.05[b]

Treatment means within the same row with different superscript letters are significantly different ($P < 0.05$).

Table 9. Simple cost and return analysis per hectare of production of fish grown on diets with 6% fishmeal and 0% fishmeal (porkmeal substitution of fishmeal) over a 120-day culture period. Values are shown in Philippine pesos (~50 PhP = $1 USD)

Descriptions	Treatment I – 6% Fishmeal	Treatment II – 0% Fishmeal
Gross Return	PhP 169,400.00	PhP 168,410.00
Costs (PhP, Philippines peso):		
Fingerlings	17,200.00	17,200.00
Commercial Feeds	99,043.44	93,497.46
Fertilizers		
16-20-0	526.40	658.00
46-0-0	887.40	1,110.12
Total Cost:	117,657.24	112,465.58
NET RETURN	PhP 51,742.76	PhP 55,944.42

Assumptions:

Price of Fingerling: P 0.43 piece^{-1}
Price of Commercial Feeds:
Pre-starter: P35.00 kg^{-1}
Starter: P28.25 kg^{-1}
Formulated Feeds with 6% Fishmeal: P31.00 kg^{-1}

Formulated Feeds with 0% Fishmeal: P30.00 kg^{-1} Price of marketable Tilapia: P55.00 kg^{-1}
Price of Fertilizers:
16-20-0: P18.80 kg^{-1}
46-0-0: P17.40 kg^{-1}

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ALTERNATIVE FEEDING STRATEGIES TO IMPROVE MILKFISH PRODUCTION EFFICIENCY IN THE PHILIPPINES

Sustainable Feed Technology/ Experiment/ 07SFT03NC

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ABSTRACT

In the Philippines, cage culture of milkfish in marine environments is increasing. The practice uses high stocking densities, with significantly greater inputs of artificial feeds which more often than not, has led to excessive feeding and consequently excessive nutrient loading in receiving waters, exacerbating problems with pollution. These could have contributed to occurrence of periodic fish kills in areas of marine milkfish culture clusters. Sixty percent of milkfish farming expenses are attributable to feed costs. A series of experiments were conducted in an attempt to develop alternative feeding strategies that will reduce feed inputs without compromising growth and resulting production. In the first experiment, growth was compared in milkfish fed daily, on alternate days and on alternate 2-week or 4-week starvation and refeeding cycles in a tank environment provided with flow-through water system. Results show, that milkfish fed on alternate days do not grow as well as milkfish that are fed daily. Feed restriction for 2 weeks followed by 2 weeks of refeeding elicited a compensatory growth response such that average body weight (ABW) of fish was not significantly different from ABW of fish fed daily. Another experiment compared growth of fish given a ration equivalent to 10% of BW (usual practice) or 7.5% of BW. Results show no significant effect on growth or final ABW or biomass in fish fed daily or on 2-week cycles of feed restriction and refeeding. Thus, a lower feeding ration can be given to milkfish without compromising yield. Results of the tank experiments were verified in actual marine cage and brackishwater pond production systems using an initial feeding rate of 7.5% rather than 10% ABW. In the experiment in cages, survival rates between the control fish and the groups subjected to cycles of 2-week starvation followed by 2 weeks of normal feeding were comparable, except for one replicate of the starved-refed group where survival rate was very low (38.78%). After 3 cycles of starvation and refeeding, weight gain in the starved-refed groups were generally lower than in the control groups, which may suggest that small-sized fish cannot adapt very well to periodic starvation. In brackishwater ponds, growth, survival and total biomass at harvest was comparable between milkfish fed daily and those fed on alternate days. Hence, FCR was lower in the milkfish fed on alternate days compared to fish fed daily. On the other hand, survival was very low in fish subjected to 2 cycles of 2 weeks starvation followed by 4 weeks of normal feeding. These results suggest that as in tilapia, alternate day feeding can be adopted to milkfish culture in brackishwater ponds without

compromising production while at the same time lowering production cost and environmental impact. Overall, these studies provide two practical strategies, reduced ration size and alternate day feeding, to improve production efficiency of milkfish, the largest finfish aquaculture industry in the Philippines

INTRODUCTION

Aquaculture in the Philippines is a high food security priority particularly in the light of the country's rapidly growing populations and their continued dependence on fish protein. Milkfish has been cultured in the Philippines for almost a century (Bagarinao 1999). It is the largest finfish aquaculture industry in the Philippines with a harvest area of 280,000 ha and total production of 300,000 metric tons annually (BAS 2006). There is also a growing export market for milkfish at one million kilograms annually and a value of almost $3 million, with the U.S. topping imports among all countries. As part of the Philippine government's food security and poverty alleviation programs, expansion of milkfish culture is a high priority (Rosario 2006) both to wean fishers off capture fisheries by providing them with fish farming as an alternative or supplemental form of livelihood, and to increase income of farmers whose poverty levels are disproportionately high (Rivera et al. 2006). Milkfish production is increasing at 5% annually, with much of the production moving away from traditional culture in brackishwater (BW) ponds to fish cages in marine waters, where a 14% increase was seen in 2005 alone (BAS 2006). Cage culture of milkfish in marine environments is done at high densities and with significantly greater inputs of artificial feeds. The use of this practice, however, has led to wastage of artificial feeds and excessive nutrient loading in receiving waters (Sumagaysay-Chavoso et al. 2004), exacerbating problems with pollution and possibly periodic fish kills that have been observed in areas of marine milkfish culture clusters.

Sixty percent of milkfish farming expenses are attributable to feed costs (Rosario 2006, Rivera et al. 2006). Research on tilapia has demonstrated that feeding only on alternate days saves almost 50% on feed costs with little effect on growth, survival or market yield (Bolivar et al. 2006). Like the tilapia, milkfish is a low trophic species and plankton eater. On the other hand, previous research have also demonstrated that longer- term feed restrictions followed by a refeeding period can elicit a compensatory growth response in fish characterized by a 2-3 fold increase in specific growth and feed conversion (Skalski *et al*., 2005; Picha et al. 2008a, 2009; Turano et al. 2007; see Ali et al. 2003 for review). Cyclic feeding was also shown to reduce total phosphorous load in ponds by as much as 40% (Turano et al. 2008). Although general feed methods and nutritional requirements to raise marketable sized milkfish have been established, largely through research at SEAFDEC AQD (Juario *et al*., 1984; Alava and Lim, 1988; Sumagaysay 1995, 1998), studies have not assessed whether periodic episodes of feed restriction/refeeding can induce compensatory growth and improve production efficiency in this species.

The overall goal of this investigation is to develop feeding strategies as potential cost containment options for milkfish (*Chanos chanos*) production in both brackishwater (BW) and marine environments. We examined whether feeding schedules that incorporate feed restriction/refeeding increments might limit overall feed input without significantly impacting fish yield. We first conducted preliminary studies in tanks and used the data generated to test different feeding regimens in an intensive culture system in cages in marine waters, as well as in a semi-intensive culture system in brackishwater ponds. The

rationale for testing different feeding regimens in marine cage and in BW pond systems is because each may require different feed inputs due to natural productivity (BW ponds) or lack thereof (marine fish cages). Also, BW ponds represent 70% of total production and marine cage culture is on the rise. Thus, the sustainability of these two culture systems and farmer's profitability could be enhanced through methods that both reduce feed input and limit nutrient loading in receiving waters through excessive feed wastage.

Insulin-like growth factor-I (IGF-I) is the primary hormonal mediator of growth in fish. We and others have found that it may serve as a robust biomarker of growth rates in various fish species that include tilapia, hybrid striped bass, flounder and salmonids (Beckman et al. 2004; Vera Cruz et al. 2006; Luckenbach et al. 2007; Picha et al. 2008b). Its levels change rapidly prior to and in parallel with specific growth responses (Picha et al. 2006). Considering traditional methods of conducting lengthy and costly growth trials, a biomarker of growth such as IGF-I could prove valuable for researchers for the rapid assessment of the numerous, and often confounding number of environmental and nutritional parameters that regulate growth. We undertook studies under controlled growout environments to evaluate the utility of IGF-I mRNA expression as an instantaneous indicator of growth in milkfish.

OBJECTIVES

- Evaluate the efficacy of feed schedules that incorporate lower feed ration and different increments of feed restriction and refeeding on milkfish production characteristics and the compensatory ("catch-up") growth response in tanks.

- Determine effects of feed restriction on milkfish production characteristics in marine cages.

- Determine effects of different feeding regimens on full grow-out trials in brackishwater ponds

- Evaluate the potential for insulin-like growth factor-I (IGF-I) as a biomarker of growth status in milkfish.

MATERIALS AND METHODS

Study 1 - Evaluate the efficacy of feed schedules that incorporate lower feed ration and different increments of feed restriction and refeeding on milkfish production characteristics and the compensatory ("catch-up") growth response in tanks.

Experiments were initially conducted in tanks to test the effect of different feed schedules on milkfish growth and survival. Results from these experiments helped in defining the feeding regimens that might best work for milkfish and served as bases in the conduct of field production trials in BW ponds at the Dumangas Brackishwater Station and in fish cages in marine waters at the Igang Marine Station of SEAFDEC AQD, respectively.

The effect of different feeding regimes on growth and survival of milkfish in tanks was examined. The feeding regimes tested are as follows:

Treatment 1: control, daily feeding at 10% ABW
Treatment 2: feeding on alternate dates at 10%

Treatment 3: cycles of 2-week starvation followed by 2 weeks normal feeding Treatment 4: cycles of 4-week starvation followed by 4 weeks of normal feeding

Milkfish fingerlings produced at the SEAFDEC AQD fish hatchery and grown in brackishwater nursery ponds with an average body weight of 10-20 g were stocked in 1- ton fiberglass tanks at a density of 100 fish/ton. Fish were fed SEAFDEC AQD formulated feeds under the 4 different feeding regimens. The tanks were provided with flow-through seawater. There were 3 replicate tanks per treatment. All fish were sampled for body weight and standard length measurements every 2 weeks to monitor growth and adjust for feed ration. Samples of liver were also taken from 3 fish in each tank during each sampling schedule for IGF-I mRNA quantification.

Differences among treatment means at the end of the 8-week growth trial were analyzed by one-way analyses of variance (GraphPad Prism, LaJolla, CA. USA).

In another experiment, the effect of reducing feeding level from 10% to 7.5% of ABW on growth and survival of fish fed daily and those subjected to cycles of 2-week starvation followed by 2 weeks normal feeding was examined. The feeding regimes tested were as follows:

Treatment 1: control, daily feeding at 10% ABW

Treatment 2: cycles of 2-week starvation followed by 2 weeks normal feeding (10% ABW) Treatment 3: reduced feeding, daily feeding at 7.5% ABW
Treatment 4: cycles of 2-week starvation followed by 2 weeks of reduced feeding (7.5% ABW)

Milkfish fingerlings produced at the SEAFDEC AQD fish hatchery and grown in brackishwater nursery pond with an average body weight of 10- 20 g were stocked in 1- ton fiberglass tanks at a density of 100 fish/ton. Fish were fed SEAFDEC AQD formulated feeds under the 4 different feeding regimens. The tanks were provided with flow-through seawater. There were 3 replicate tanks per treatment. All fish were sampled for body weight and standard length measurements every 2 weeks to monitor growth and adjust for feed ration. Liver samples were taken from a subgroup of fish for measurement of hepatic IGF-I mRNA levels. Livers were processed and IGF-I mRNA measured by real time quantitative PCR according to our previously established protocols (Picha et al. 2007; Vera Cruz et al. 2006).

Differences among treatment means at the end of the 12-week growth trial were analyzed by one-way analyses of variance (GraphPad Prism, LaJolla, CA. USA).

Study 2 - Determine effects of feed restriction on milkfish production characteristics in marine cages.
Based on the results of the tank experiments (Figure 1 and 2) and preliminary experiments in a simulated cage culture system set up in a 50- ton tank (data not shown), an experiment

was conducted to compare milkfish production characteristics in an intensive culture system in fish cages in marine waters. Fish were subjected to two feeding regimes:

Treatment I: control, fed daily at 7.5% body weight

Treatment II: cycles of 2-week starvation followed by 2 weeks of daily feeding at 7.5% body weight.

Six units of fish cages measuring 5 x 5 x 3m were set up in Igang Marine Station of SEAFDEC AQD. Milkfish fingerlings (12-35 g ABW) produced at the SEAFDEC AQD fish hatchery and grown in a private milkfish nursery facility in Nueva Valencia, Guimaras were stocked at a density of 35 fish/m^3. Fingerlings were fed SEAFDEC AQD formulated feeds initially at 7.5% of ABW given in 4 rations daily. The amount of feed was reduced as the fish gained weight. This was shown to be as effective as the standard industry practice of feeding initially at 10% of body weight. Water quality parameters including dissolved oxygen level, chlorophyll a and phosphorous was monitored in relation to establish potential relationships with feed input and fish biomass. To monitor growth and adjust the feed ration, 25 fish in each cage were subsampled and measured for body weight and standard length every 2 weeks. At the termination of the experiment, actual head count and measurement of actual biomass harvested was done. Percent survival and feed conversion ratio (FCR) were determined.

Differences among treatment means in production parameters at the end of the 14-week growth trial were analyzed by paired t-Test (GraphPad Prism, LaJolla, CA. USA).

Study 3 – Determine effects of different feeding regimens on full grow-out trials in brackishwater ponds

Based on results of the tank experiments, an experiment on milkfish production in semi - intensive brackishwater ponds was conducted to compare the effects of alternate day feeding and 2-week restricted and 4-week refeeding cycles to that of fish fed daily. The pond production study was conducted at SEAFDEC AQD's Dumangas Brackishwater Station.

For this experiment, 3 treatment groups were used and each treatment had 2 replicates. The treatments are as follows:

T1: control, normal daily feeding at 7.5% of average body weight and gradually decreasing to 4% with increased fish biomass
T2: same feeding rate as control but given on alternate days only

T3: cycles of 2 weeks restricted feeding followed by 4 weeks normal feeding at a rate similar to controls

Six units of ponds (700 m^2) were used. Ponds preparation followed established procedures for eradication of unwanted species and enhancement of the growth of lablab, the natural food of milkfish in ponds. However, because of continuous heavy rains, good growth of lablab was not achieved. Milkfish fingerlings produced at the SEAFDEC AQD fish

hatchery and grown in brackishwater nursery ponds to an average body weight of 75 g were used for stocking. Fingerlings were stocked at a density of 350 fish/700m^3 and fed SEAFDEC AQD formulated feeds initially at 7.5% of ABW given in 4 rations daily. The amount of feed was reduced as the fish gained weight. Body weight measurements of 40 fish were taken every 2 weeks to monitor growth and adjust for feed ration. After the last sampling, total harvest was done where the total number of fish in each pond compartment was counted and total biomass was measured. Percent survival and feed conversion ratio (FCR) were determined.

Differences among treatment means in production parameters at the end of the 12-week growth trial were analyzed by one-way analyses of variance (GraphPad Prism, LaJolla, CA. USA).

RESULTS

An experiment was conducted to assess the effects of different feeding regimes on growth of milkfish in tanks provided with flow-through water. Figure 1 shows the changes in average body weight and daily growth rates of milkfish under the different feeding regimes. Expectedly, negative growth is observed during periods of starvation. On the other hand, milkfish exhibited increased growth upon refeeding after periods of prolonged starvation. These results show that milkfish fed a ration equivalent to 10% of ABW on alternate days did not grow as well as milkfish fed the same ration daily ($P < 0.05$). On the other hand, ABW of milkfish subjected to 2-week alternate starvation and refeeding cycle was comparable to the control group that was fed daily, suggesting that compensatory growth mechanisms were at work ($P > 0.05$). Milkfish subjected to a 4-week starvation and refeeding cycle attained final ABW that was significantly lower than the control.

Attempts were made to quantify IGF-I expression in livers of milkfish subjected to different feeding regimes. IGF-I expression was normalized against the expression of the housekeeping gene, GAPDH. Ideally, the expression of housekeeping genes does not vary between tissues, developmental stage or physiological state and hence can be used to control for possible differences in sample loading or as normalization to the gene of interest. We found that changes in IGF-I expression observed in the current experiment did not show clear trends, possibly because the expression of GAPDH, which was used to normalize IGF-I expression was variable (data not shown). Other studies also show that housekeeping genes may vary in a tissue-specific manner and with the physiological state of the animal. We are currently exploring the use of other housekeeping genes and methods for normalization of IGF-I mRNA expression.

Based on anecdotal observations we conducted a second, supplemental experiment not initially proposed for this investigation to assess the effects of lower feed ration on milkfish growth. We compareed growth of milkfish fed the typical ration of 10% average body weight daily with a lower ration level of 7.5% of body weight. Results show that growth of milkfish measured as body weight and growth rate was not affected by reduction of ration to 7.5% of the body weight, regardless of whether fish were fed daily or on 2-week feed restriction/refeeding cycles ($P > 0.05$: Figure 2).

Another preliminary experiment was conducted to assess the effects of different feeding

regimes on growth of milkfish in a simulated marine cage environment. Results generally reflect the result of the tank experiment. Milkfish fed on alternate days did not grow as well as milkfish fed daily (data not shown). Milkfish subjected to a 2-week alternate starvation and refeeding cycle did not exhibit compensatory growth of comparable magnitude as was observed in tanks. This lack of a robust compensatory growth response may have been due to the presence algae growing on the nets and of plankton in the water that the fish can feed on. This other natural sources of food may have reduced the level of catabolism during the starvation period, such that animals did not undergo as significant a compensatory growth response when realimentated to daily feeding as was seen in tanks (see Picha et al. 2006). Prolonging the starvation period to 3 weeks also did not enhance the compensatory growth response.

Nevertheless, we proceeded to test the alternative feeding strategies that were effective in the tank trials on milkfish production in intensive marine cage culture. The effect of 2-week starvation and refeeding cycles on milkfish production in an intensive culture system in marine cages was compared to the standard continuous daily feeding strategy at 7.5% body weight. Six units of 5x5x3m cages were stocked with milkfish fingerling at a stocking density of 35 fish/m^3. Fish in 3 cages were fed daily while fish in the other 3 cages were subjected to 2-week starvation and refeeding cycles. The stocks (50 fish from each cage) were sampled every two weeks to monitor growth and to adjust for feed ration. Figure 3 shows changes in body weight (BW) and total length (TL) of control fish fed daily and size-matched groups that were subjected to starvation and refeeding cycles. Production parameters after 3 starvation and refeeding cycles are summarized in Table 1. Except for replicate 3 of the starved-refed group, survival rates between the 2 groups are comparable. After 3 cycles of starvation and refeeding, weight gain in replicate 2 of the starved-refed group (initial ABW: 12.2g) was only 72.8% compared to the control group with similar initial body weight (ABW: 12.4g) which exhibited 155% weight gain, which may suggest that small sized fish cannot adapt very well to periodic starvation. Overall, we found that the growth, survival, and feed efficiency of fish in the starved/fed group was lower than that of fish fed daily. The total biomass harvested was significantly lower in the starved/fed group relative to fish continuously fed on a daily basis.

We also evaluated the effect different feeding regimes on milkfish production in a semi - intensive culture system in brackishwater ponds. Six pond compartments with an area of 700 m^2 were stocked with milkfish fingerlings at a stocking density of 0.5 fish/m^2. The following feeding regimens were tested: daily feeding (control), feeding on alternate days and 2 cycles of 2-week starvation followed by 4 weeks of normal feeding. There were two pond replicate per feeding regime tested. Stocks (40 fish from each pond compartment) were sampled every two weeks to monitor growth as well as for adjusting feed rations. Data after 2 starvation and refeeding cycles are summarized in Figure 4 and Table 2. Results of the pond experiment show that growth of milkfish fed daily was better (DGR: 3.12% and 3.64%) than milkfish fed on alternate days (DGR: 2.97% and 2.68%) or fish subjected to 2 cycles of 2-week starvation followed by 4 weeks of normal feeding (DGR: 2.79% and 2.75%). However, the differences were not statistically significant. Survival rate of milkfish fed on alternate days (81.43% and 94.57%) was comparable to milkfish fed daily (63.43% and 90.29%), but were significantly lower in fish subjected to 2 cycles of 2-week starvation followed by 4 weeks of normal feeding (24.29% and 46.57%; $P < 0.05$). Total biomass at harvest was similar for milkfish in

ponds that were fed daily (68.6 kg and 100.8 kg) versus those fed on alternate days (88.57 kg and 96.5 kg). Because the number of surviving fish was very low, total biomass at harvest and FCR in fish subjected to 2 cycles of 2-week starvation followed by 4 weeks of normal feeding ranged from only 23.05 kg to 47.75 kg and 4.73 to 9.62 for the 2 replicates, respectively. FCR of milkfish fed daily ranged from 2.89 to 4.5 while that of milkfish fed on alternate days was between 1.68 and 1.90, respectively. Although the FCR was over 50% lower in fish on the alternate day versus those on daily feeding, there was no overall statistical difference. In addition to having similar biomass at harvest and lower FCR, fish on alternate day feeding also consumed almost 50% less than fish on daily feeding ($P < 0.05$).

DISCUSSION

The results of the series of experiments on feeding strategies and milkfish production show fish respond differently depending on the feeding regime and the culture system in which they are raised. In initial studies in tanks, growth of milkfish subjected to cycles of 2-week starvation followed by 2 weeks of normal feeding was comparable to fish fed daily. Moreover, reductions of feeding rate from 10% to 7.5% of total biomass also resulted in comparable growth regardless of whether animals were fed daily or on 2-week restricted-refeeding cycles that elicit compensatory growth responses. These results have important implications in terms of reduction of costs for the culture of milkfish as well as the negative environmental impact of milkfish farming. Feed costs make up about 50-60% of the total cost for milkfish production. Excessive feeding, especially in intensive culture systems has likewise led to feed wastage and high nutrient loading in the culture environment and pollution resulting in periodic episodes of fish kills. Our results suggest a 25% reduction in feed ration, at least during the intial period of fingerling growout, in addition to reducing the feed amount by half through the use of prolonged periods of feed restriction, can significantly reduce feed costs in tank cultured milkfish.

In the experiment in marine cages, survival rates between the control fish and the groups subjected to cycles of 2-week starvation followed by 2 weeks of normal feeding were comparable, except for replicate 3 of the starved-refed group where survival rate was very low (38.78%). After 3 cycles of starvation and refeeding, weight gain in the starved-refed groups were generally lower than in the control groups. Notably, weight gain in replicate 2 of the starved-refed group (initial ABW: 12.2 g) was only 72.8% compared to the control group with similar initial body weight (ABW: 12.4 g) which exhibited 155% weight gain, which may suggest that small sized fish may not adapt as well to periodic starvation. In addition to lower final weight and weight gain, the total biomass harvested was significantly lower with fish on the restricted-refeeding cycles. This was likely exacerbated by the poorer survival rate of one of the replicates on the cyclic feeding regimen. Although fish on the cyclic feeding consumed approximately 50% less feed, the lower growth rate and biomass harvested suggests that two week-cycles of feed restriction followed by refeeding may not be a suitable cost containment strategy for producing milkfish in intensive cage culture in marine environments. Considering fish responded to the cyclic feeding regimen differently in tanks versus cage culture (see Figures 1 and 2 versus Figure 3), it is possible that alternate day feeding may prove useful in the cage environment, despite it not having been as effective in tank culture. This should be tested as it may significantly improve FCR relative to daily feeding, with a more limited impact on growth and harvest biomass relative to fish restricted and refed for prolonged periods.

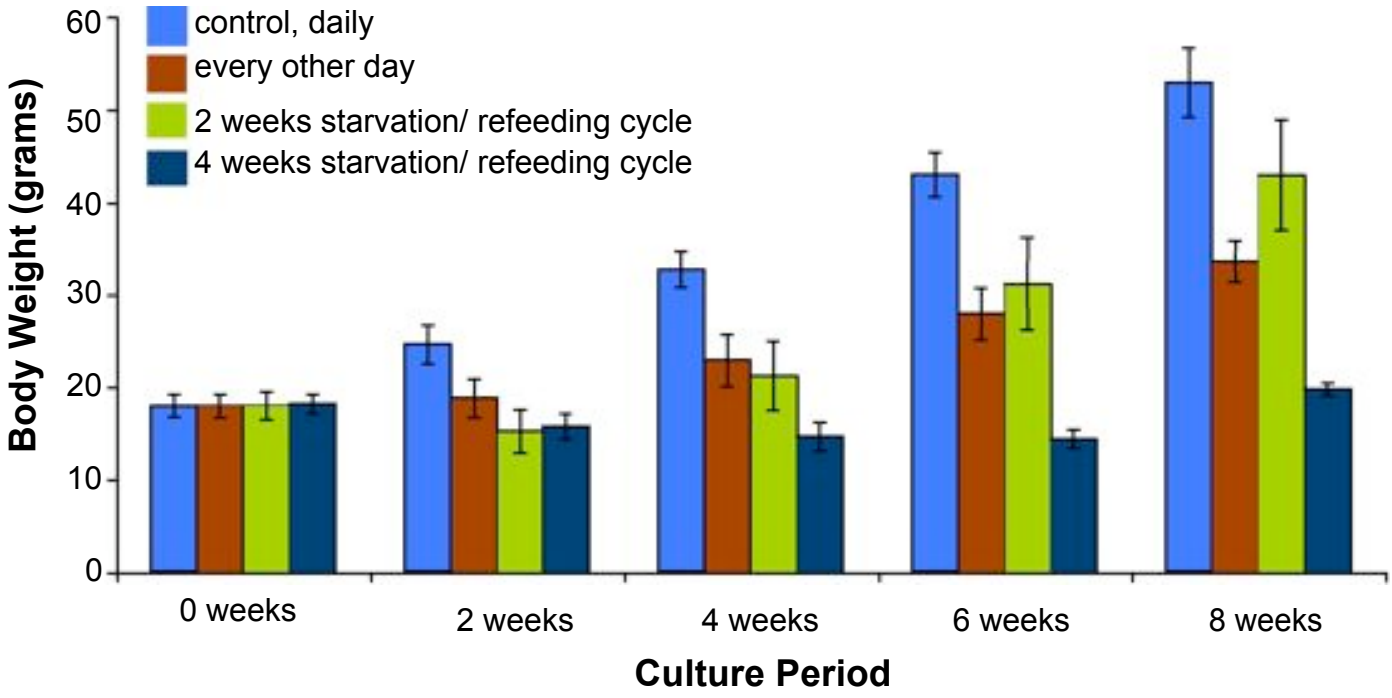
Industry FCR values in fish raised in marine cages normally range from 2.9 or higher. We found that fish fed daily at a ration level of 7.5% ABW had substantially lower FCR values of 2.29 on average (Table 1). This lower FCR may have resulted from the use of the lower ration level relative to the standard of 10% ABW/day used by industry. Additionally, in related work at SEAFDEC, feeds have been reformulated to better target growout of milkfish in marine environments. Hence, the reformulation of feeds along with lower ration level may have contributed to the generally lower FCR we observed in fish fed daily.

Results of the pond experiment show that growth of milkfish fed daily was a slightly better than milkfish fed on alternate days and lowest in those subjected to 2 cycles of 2-week starvation followed by 4 weeks of normal feeding. The differences were not statistically significant however. Similarly, survival rate of milkfish fed on alternate days was comparable to milkfish fed daily, but very low in fish subjected to 2 cycles of 2-week starvation followed by 4 weeks of normal feeding ($P < 0.05$). This was surprising since no mass mortalities were observed during the duration of the experiment. Average total biomass harvested was similar in fish on the alternate day (mean ± SD; 92.5 ± 5.6) versus those on the standard daily (89.7 ± 29.8) feeding regimen. Fish on the alternate day feeding consumed almost 50% less feed and showed improved feed efficiency (mean FCR of 1.74) relative to fish on the daily feeding schedule (mean FCR = 3.70). By contrast fish on the 2-week restricted and 4-week refeeding protocol had an average FCR value of 7.18. Collectively, these results suggest alternate day feeding may prove useful in reducing feed costs by almost 50% in milkfish culture in brackishwater ponds without compromising production, while simultaneously lowering nutrient loading in the environment. These results are similar to that which has been established with feed reduction strategies that employ alternate day feeding of tilapia in semi-intensive culture in ponds (Bolivar et al. 2006).

It should be noted that in a semi-intensive culture system for milkfish, feeding is supposed to be supplemental and is normally given once the natural food in the pond is exhausted. However, good growth of natural food was not achieved in the course of the experiment because of long spells of heavy rains, thus feeding was done from the start of the experiment until it was terminated. Because of this and limitations in the number of ponds available (2 per treatment) for this study an additional trial should be conducted to confirm the utility of alternate day feeding with production of milkfish in ponds, particularly under conditions where fish are fed only when natural foods (lablab) are exhausted. Nevertheless, the studies point to the strong possibility that reduced feeding may prove highly useful to enhancing production efficiency of milkfish. This is particularly noteworthy since some farmers in Region 6 in the Philippines (a major milkfish producing region comprising the provinces of Iloilo, Capiz, Aklan, Antique and Negros Occidental) who traditionally relied solely on natural food production for milkfish culture are seeing smaller harvests because natural food production has become increasingly difficult to maintain. With the increased reliance on supplemental feeding in the brackishwater culture of milkfish in the Philippines, the alternate day feeding protocol tested here may prove an ideal practical method to reduce costs for small-scale farmers while limiting environmental impacts of their activities.

Collectively, the studies conducted here indicate that a 25% reduction in feed ration levels during a portion of the production cycle can reduce costs of milkfish culture. Moreover, 2-

week cycles feed restriction followed by refeeding and alternate day feeding are effective in providing additional cost savings for production of milkfish in tanks and in the semi-intensive culture of milkfish in brackishwater ponds, respectively.



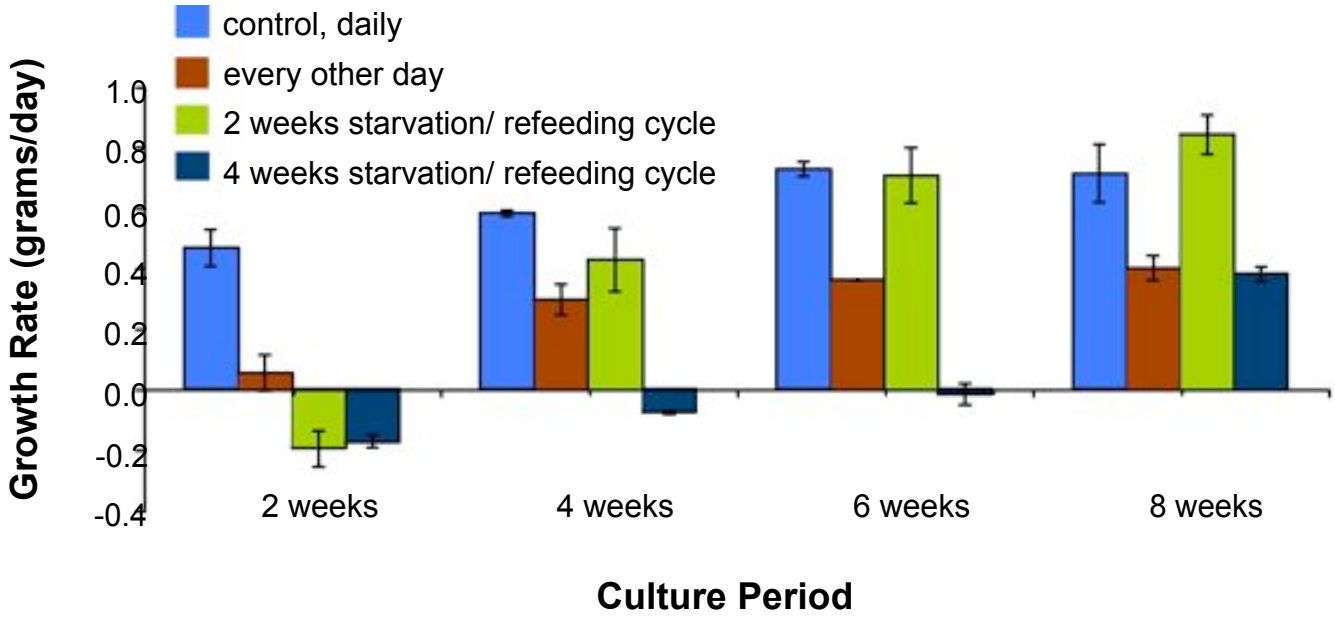


Figure 1. Changes in body weight (grams; top graph) and daily growth rate (grams/day; bottom graph) of milkfish in tanks under different feeding regimes: daily feeding at 10% of total biomass, feeding at 10% of total biomass but on alternate days, alternate 2-week starvation and refeeding cycles, and alternate 4-week starvation and refeeding cycles. During the refeeding period ration level was 10% of biomass daily. Data are mean ± SEM of three replicate tanks per group. There was no significant difference in body weight and growth rate for fish fed daily and those fed on 2-week restricted/refeeding cycles.

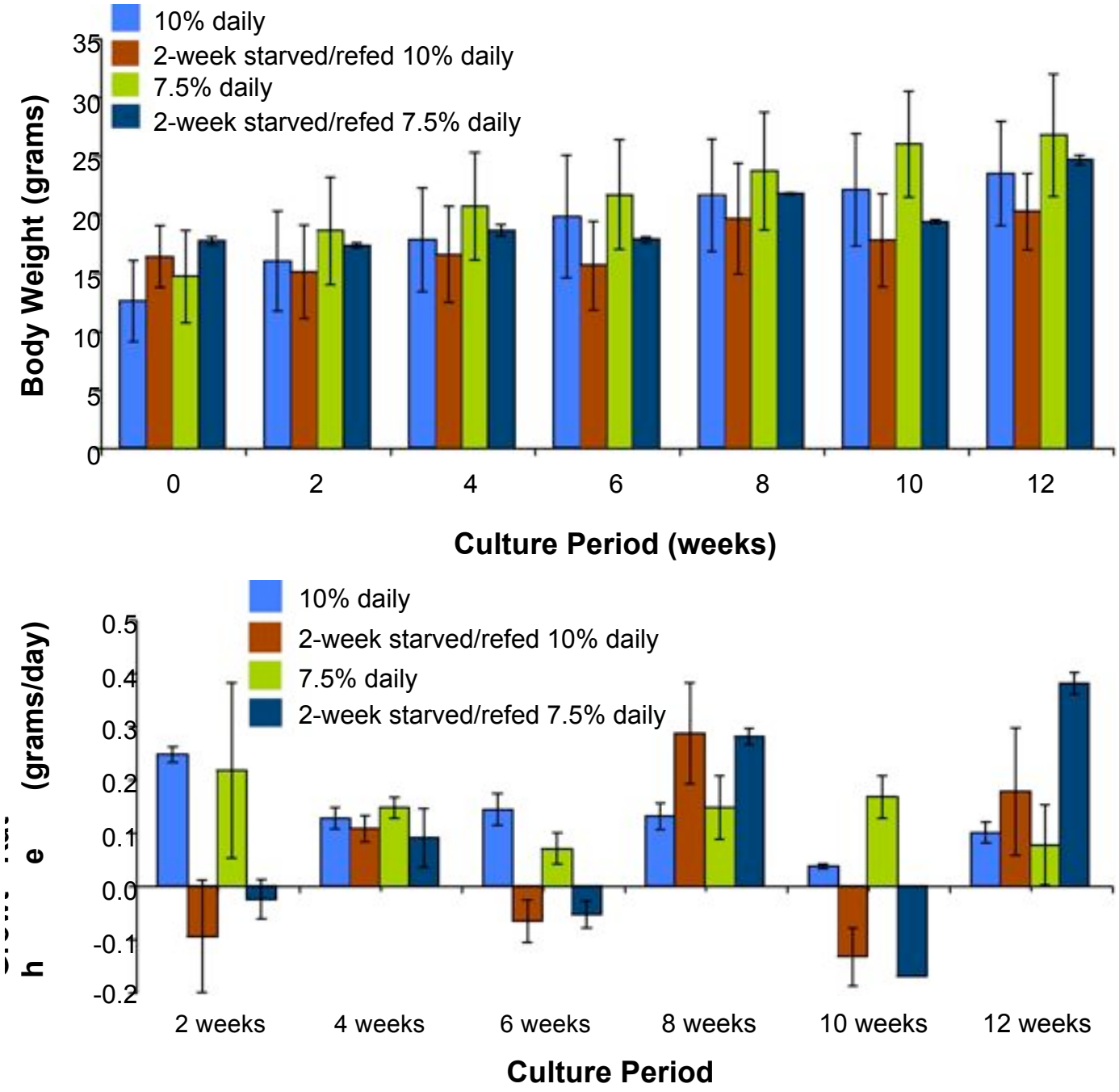
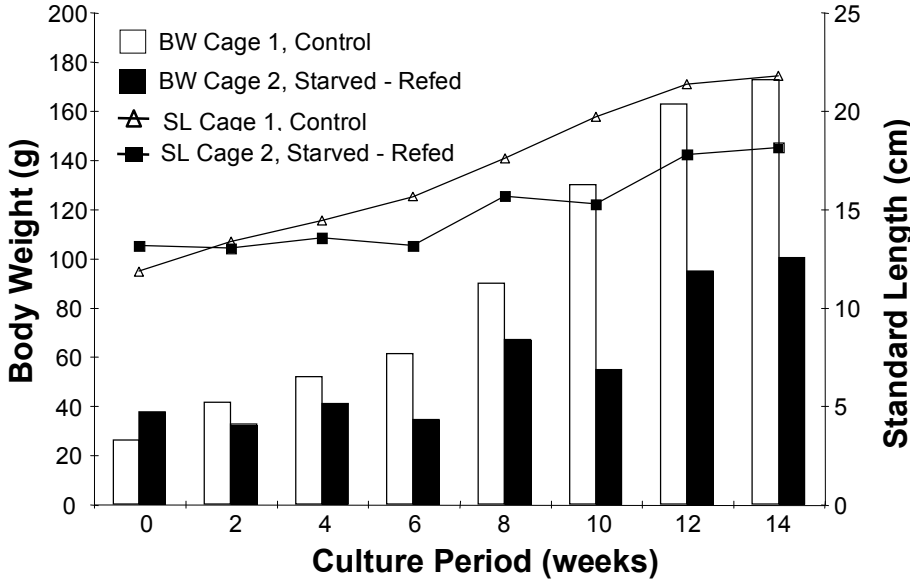
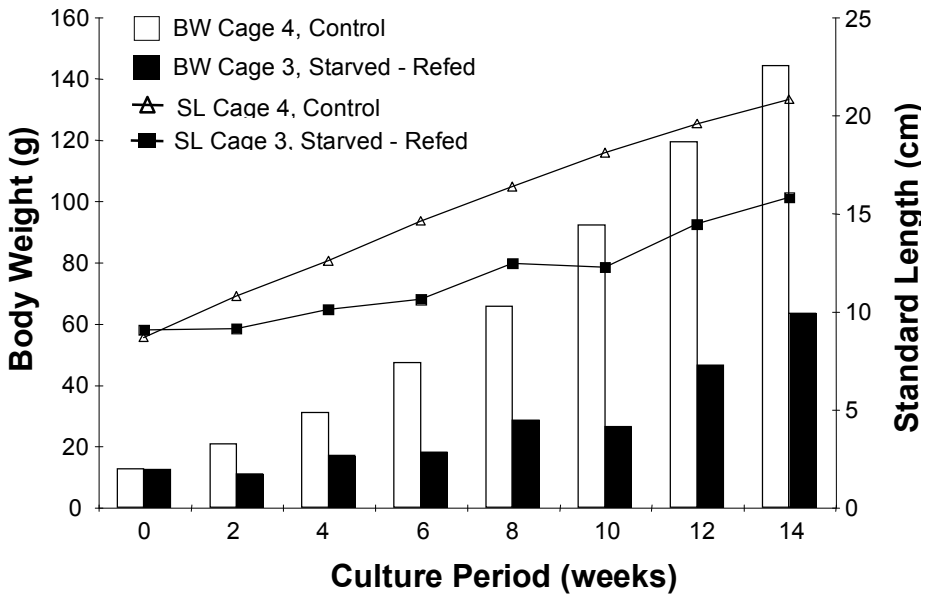


Figure 2. Changes in body weight (grams; top graph) and daily growth rate (grams/day; bottom graph) of milkfish in tanks under different feeding regimes: daily feeding at 10% of total biomass, alternate 2-week starvation and refeeding daily at 10% of total biomass, daily feeding at 7.5% of total biomass, and alternate 2-week starvation and refeeding daily at 7.5% of total biomass. During the refeeding period ration level was 10% of biomass daily. Data are mean ± SEM of three replicate tanks per group. There were no differences in body weight among groups following 12-weeks of growout.





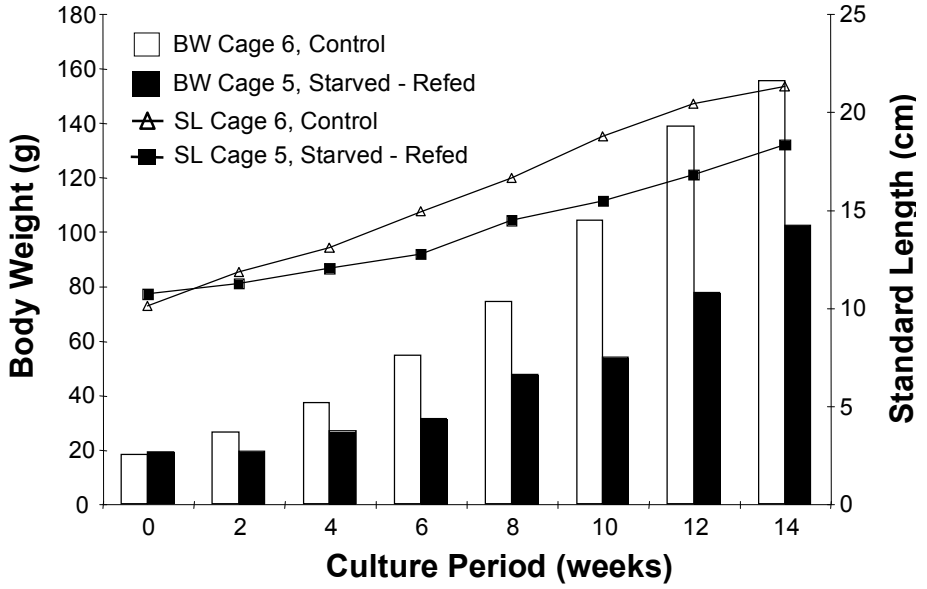


Figure 3. Changes in body weight (grams) and standard length (SL) of milkfish in an intensive culture system in marine cages fed either daily at 7.5% of the total biomass (control) or subjected to alternate 2-week starvation and refeeding cycles (7.5% biomass). The different figures represent size-matched replicates. Data are shown as means of 50 fish sampled from each cage during each sampling schedule. Error bars represent standard errors of the means. Fish on the cyclic feeding schedule grew significantly less than those on the daily feeding schedule ($P < 0.05$).

Table 1. Production indicators for the intensive culture of milkfish in marine cages under different feeding regimes. Each cage was stocked with milkfish at a density of 35 fish /m^3. There were three replicate cages for each treatment.

Parameter	**Treatment A** (Fed daily, control)	**Treatment B** (2-week starvation and refeeding cycles)
Initial ABW (g)	25.95 ± 0.93	37.35 ± 1.25
	12.4 ± 0.93	12.2 ± 1.25
	18.3 ± 1.14	19.05 ± 0.90
Group Mean ± SD	*18.9 ± 6.8 (a)*	*22.9 ± 13.0 (a)*
Final ABW (g)	220.0 ± 8.38	144.0 ± 5.27
	167.4 ± 2.27	85.0 ± 4.76
	168.0 ± 0.25	192.5 ± 0.24
Group Mean ± SD	*166.3 ± 24.2 (a)*	*117.6 ± 51.2 (a)*
% Weight Gain	194.05	106.65
	155.0	72.8
	149.7	173.42
Group Mean ± SD	*185.1 ± 30.2 (a)*	*140.5 ± 53.84 (a)*
Survival Rate (%)	98.28	91.43
	72.38	69.33
	103.0	38.78
Group Mean ± SD	*91.2 ± 16.5 (a)*	*66.5 ± 26.4 (a)*
Actual Biomass Harvested (kg)	567.6	345.1
	318.0	154.7
	567.6	196.0
Group Mean ± SD	*484.4 ± 144.1 (a)*	*231.9 ± 100.2 (b)*
Total Feed Consumed (kg)	1268.68	727.48
	898.5	368.68
	1035.30	652.30
Group Mean ± SD	*1067.5 ± 187.2 (a)*	*582.8 ± 189.2 (b)*
FCR	2.24	2.10
	2.82	2.38
	1.82	3.33
Group Mean ± SD	*2.29 ± 0.5 (a)*	*2.60 ± 0.64 (a)*

Different letters shown in parentheses among rows reflect significant differences between groups ($P < 0.05$).

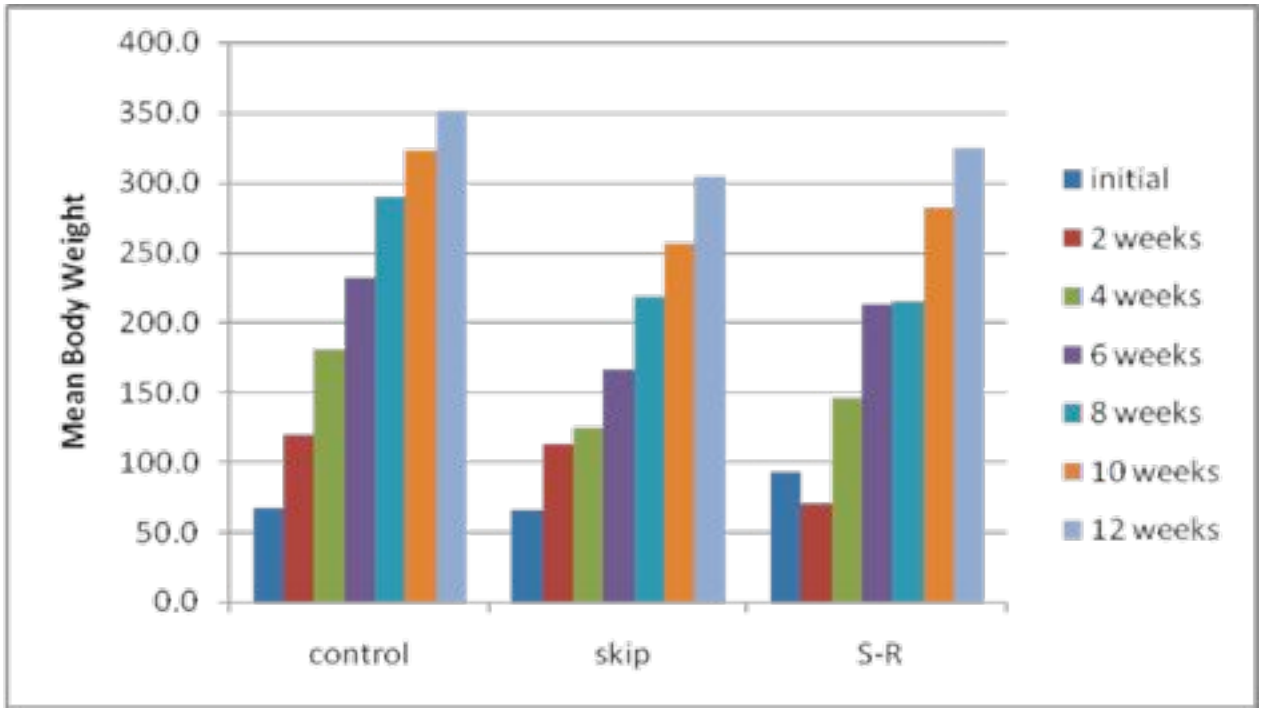


Figure 4. Changes in body weight (grams) of milkfish in a semi -intensive culture system in brackishwater ponds fed either daily at 7.5% of the total biomass (control) or on alternate days (7.5% of biomass) or subjected to alternate 2-week starvation and refeeding cycles (7.5% of biomass daily). Data are shown as means from two replicate ponds.

Table 2. Production indicators for the semi-intensive culture of milkfish in brackishwater ponds under different feeding regimes. Animals were fed either daily at 7.5% of the total biomass (control) or on alternate days (7.5% of biomass) or subjected to alternate 2-week starvation and refeeding cycles (7.5% of biomass daily). Each 700 m^2 pond was stocked with 350 pcs of milkfish. There were 2 replicate ponds for each treatment. Initial and final body weights were determined from 40 fish sampled from each pond.

Parameter	**Treatment A** (Fed daily)	**Treatment B** (Fed alternate days)	**Treatment C** (2 weeks starvation, 4 weeks refeeding
Initial ABW (g)	71.0 ± 4.46	71.91 ± 4.5	75.6 ± 3.64
	63.07 ± 3.75	61.27 ± 5.78	108.61 ± 6.31
Group Mean ± SD	*67.0 ± 5.6 (a)*	*66.6 ± 7.5 (a)*	*92.1 ± 23.3 (a)*
Final ABW (g)	332.74 ± 12.51	321.51 ± 7.92	309.94 ± 9.47
	369.30 ± 13.01	286.42 ± 8.54	339.51 ± 11.18
Group Mean ± SD	*351.0 ± 25.9 (a)*	*304.0 ± 24.8 (a)*	*324.7 ± 20.9 (a)*
% Weight Gain	368.65	347.10	309.97
	485.54	367.47	212.60
Group Mean ± SD	*427.1 ± 82.7 (a)*	*357.3 ± 14.4 (a)*	*261.3 ± 68.9 (a)*
DGR (g/day)	3.12	2.97	2.79
	3.64	2.68	2.75
Group Mean ± SD	*3.38 ± 0.37 (a)*	*2.83 ± 0.21 (a)*	*2.77 ± 0.03 (a)*
Survival Rate (%)	63.43	81.43	24.29
	90.29	94.57	46.57
Group Mean ± SD	*76.9 ± 19.0 (a)*	*88.0 ± 9.3 (a)*	*35.4 ± 15.8 (b)*
Actual Biomass	68.60	88.57	23.05
Harvested (kg)	110.80	96.50	47.75
Group Mean ± SD	*89.7 ± 29.8 (a)*	*92.5 ± 5.6 (a)*	*35.4 ± 17.5 (a)*
Total Food	308.98	159.32	221.76
Consumed (kg)	320.46	162.31	226.10
Group Mean ± SD	*314.7 ± 29.8 (a)*	*160.8 ± 2.1 (b)*	*223.9 ± 3.1 (c)*
FCR	4.50	1.80	9.62
	2.89	1.68	4.73
Group Mean ± SD	*3.70 ± 1.14 (a)*	*1.74 ± 0.08 (a)*	*7.18 ± 3.46 (a)*

Different letters shown in parentheses among rows reflect significant differences between groups ($P < 0.05$).

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UTILIZATION OF LOCAL FEED INGREDIENTS FOR TILAPIA AND PACU PRODUCTION

Sustainable Feed Technology/ Activity/ 07SFT04UA

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ABSTRACT

The focus of this investigation was an effort to work with fish farmers and a feed mill operator to develop a list of potential fish feed ingredients available on local markets in Guyana and to design a practical diet based on these ingredients. A primary focus was to reduce or eliminate the use of expensive imported ingredients including fishmeal and soybean oil meal. The design of a practical diet incorporates nutritional balance, pellet stability and homogeneity, and economic feasibility. To accomplish this, we collected samples of local ingredients, submitted the samples for proximate analyses, and compared the results to published reference ingredient analyses. We then used feed formulation software to develop a diet that met the criteria for protein content, fat level, protein to energy ratio, caloric value and price that would meet the nutritional demands for tilapia and pacu on a commercial basis.

INTRODUCTION

Objectives:

a. To discuss semi-intensive culture of Tilapia and Pacu, with a focus on nutritional requirements
b. To identify and examine local ingredients suitable for use in formulation of Tilapia and Pacu Feed
c. To examine simple feed formulation techniques using identified ingredients

Significance:

Tilapia and pacu aquaculture has been practiced for starting in the 1980's in Guyana. In recent years the growers have organized into a farmer's cooperative, the National Aquaculture Association of Guyana. With the assistance of the Ministry of Agriculture and the USAID/GTIS Program, the farmers have begun to develop local markets and were able to participate in the 7th International Symposium on Tilapia in Aquaculture. The Mon Repos Aquaculture station has worked with the farmers to provide technical assistance and training.

In Guyana, aquaculture is now starting to emerge as an industry. Maharaja Mills, an existing feed and oil manufacturer has been willing to engage in fish feed manufacture. However, they require assistance in suitable ingredient identification, feed formulation, manufacture of low cost feed, production with existing machinery, and training in aquatic feed production techniques. Development of economic diets utilizing locally available ingredients was a central focus point. The pacu and tilapia producers have several advantages over regional producers that they feel will allow them to compete effectively in local markets as well as in North American markets. There is an abundance of high quality water with ideal temperatures, a stable government and economic system, a native English-speaking workforce and a local population that has already proven the acceptance of tilapia and other fish as a staple in the diet.

Sustainability has been identified as a priority for the aquaculture producers. Utilization of local feed ingredients, minimizing the use of fish meal, and increasing the efficiency of the feed that is input to the system are immediate targets.

The fish farmer's group (NAAG) had several dozen of their management and staff attend the workshops. They reported that the training and instruction would allow them to operate in a more efficient manner and improve both profitability and environmental sustainability. Specifically, they wanted to receive advice and guidance on locally available ingredients that could be substituted into practical feeds and how to process them into usable pellets utilizing existing feedmill infrastructure. It was important that this should be done in a cooperative manner with both the farmers, who have concerns of cost and quality and the feedmill operators who have concerns over expenses and operational characteristics. Of course both groups were also interested in aspects of shelf life of the feed, % of fines in the products and other details. Another area of interest was operational characteristics of feeding and especially the Best Management Practices associated with feeding practices. There was considerable confusion regarding the certification programs from the Aquaculture Certification Council, Global GAP, NaturLand and World Wildlife Fund. The farmers report that they would like to incorporate BMP's both for their own farm improvement, but also as a step towards certification of their product as being more sustainable and capable of receiving a higher price on international markets. However, working through the plethora of competing standards is overwhelming at this stage. We determined to focus on the common aspects of all the BMP's and teach the basics that will allow the farmers to address certification processes in the near future as the programs develop and offer this option to the farmers.

RESULTS

a. *To discuss semi-intensive culture of Tilapia and Pacu, with a focus on nutritional requirements*

A series of workshops were held in August 2008 at the Mon Repos Aquaculture station and the Maharaja Mill. The workshops brought together farmers, feedmill operators and staff, government researchers and potential investors. We also met with several government leaders including the Ministers of Agriculture and Business Development and even a brief introduction to the Prime Minister. During the initial visit we also

visited several farms, the National Agricultural Research Station, and the University of Guyana Experiment Station.

In July 2009 a second series of workshops were conducted at the Maharaja Mill and the Mon Repos station. In these workshops we reviewed the results of the ingredient analyses and provided copies of the Least Cost Feed Formulation software that we were using to evaluate the diet formulations. We also worked with the farmers and feedmill operators to input additional parameters into the database utilized by the software. During the second series of workshops we also reviewed the basics of Best Management Practices and the current state of competing certification groups. In September 2009 we met with our host country PI, Pamila Ramotar, at the World Aquaculture meetings in Mexico to review progress and analyze data from the feeding trials based on the initial research in this investigation.

July 2008 Workshop Details
Locations: Mon Repos Aquaculture Station and Maharaja Oil Mill
(with contributions from:)

i. Mon Repos Aquaculture Station (facilities, equipment, personnel)
ii. Maharaja Oil Mill (facilities, equipment, personnel)
iii. NAAG (transportation, accommodation)
iv. USAID/GTIS: Training materials and curriculum preparation

Participants; Tilapia and Pacu farmers, Feed producers, Fish Processors, Government Representatives, Kevin Fitzsimmons, University of Arizona (Presenter)

The presentations included an overview of the tilapia industry covering producing countries, major production systems and processing and marketing. Then we covered more detailed sections with breaks and lunches included.

1. Aquaculture Theory: pond design, water quality, Tilapia biology, feeding, fingerling production, grow-out, health and disease etc.)
2. Practical Aquaculture: Plankton examination, male and female Tilapia ID, Tilapia dissection, transportation, acclimatization, pond fertilization etc.
3. Feed Ingredient ID: examining local feed materials, and determining suitable ones for feed ingredients
4. Feed Formulation Theory: calculation of protein and other nutrient content, balancing of ingredients, etc.
5. Practical Feed Formulations: Preparation and mixing of ingredients, mixing, pelletizing and drying of feed.

An associated opportunity included in the project was an investment opportunity for a large-scale project to be funded by Goldman-Sachs Sustainable Investment Fund. Several members of the Fund invited Fitzsimmons to meet with them during a vacation stop in London. Fitzsimmons reviewed the opportunity for sustainable integrated tilapia production with tropical irrigated agriculture in Guyana and other locations. One member of the Goldman Sachs team met with Fitzsimmons on his August 2008 mission and participated in the workshops, government agency meetings and the farm tours.

Goldman Sachs closed the Fund during the financial crises, but a spin off operation, Integrated Agriculture Ventures is still pursuing a potential investment in Guyana.

b. *To identify and examine local ingredients suitable for use in formulation of Tilapia and Pacu Feed*

Ingredients were collected during the visit to Guyana from a number of sources including the Maharaja Mill, a fish meal plant, poultry farm/feed operation, and various farms. The samples were brought back to the US and submitted to lab for proximate analyses. The results were compared to published values found in the peer reviewed literature to check accuracy. The Guyana ingredients were in general accord with published values, but in some cases were close to higher or lower limits, which needed to be accounted for in diet formulations. This information was shared with farmers some of whom had decided to develop their own on-farm formulations. One of the farmers had purchased a low cost hammer mill to grind grains and fish carcasses and a simple pellet mill. During the July 2009 visit one day was spent working at this farm to train the staff on the proper selection, processing and blending of the ingredients to formulate an on-farm diet. We also trained the staff on the operational aspects of the hammer mill and pelletizer. Several operating "tricks" were shared with the staff, which protected the equipment and improved the pellet stability.

Proximate composition of local Guyana ingredients and/or reference ingredients

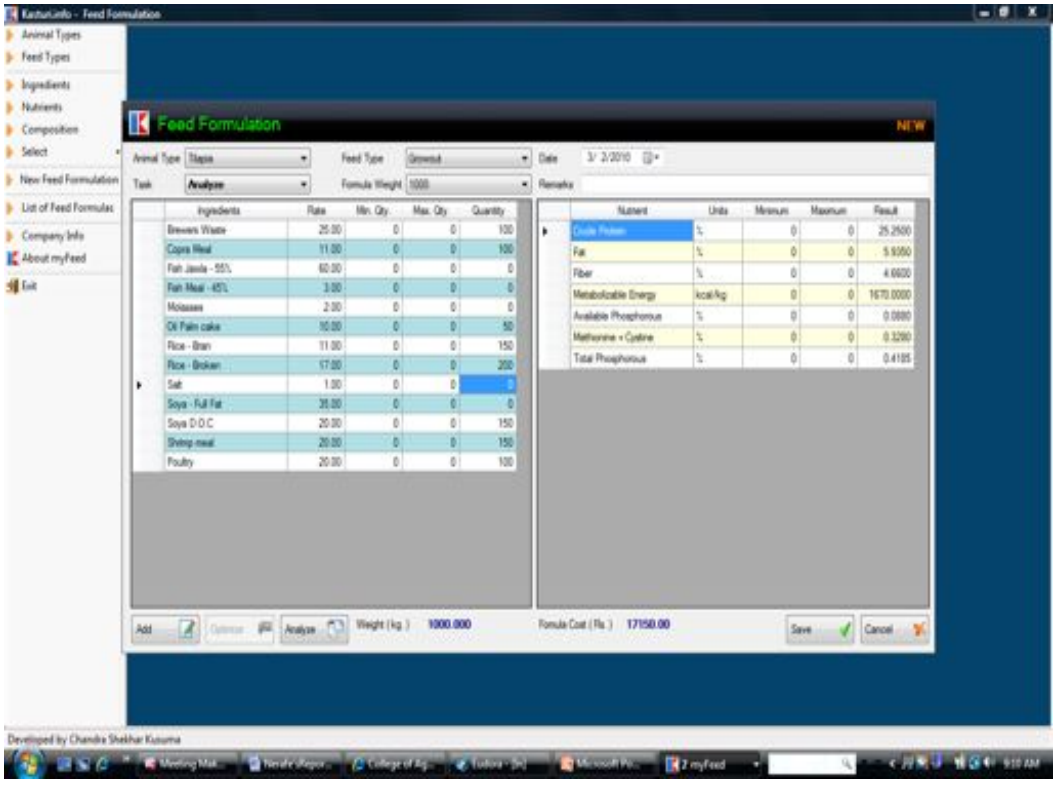
Feedstuff	Dry Matter	Crude Protein	Crude Fat	Crude Fiber	Ash	NFE	Ca	P	Reference
Banana meal (fruit, dehydrated)	87.00	3.90	2.70	4.70	4.60	71.04	0.11	0.15	6
Brewer's spent grains, dried	92.00	18.50	4.30	18.30	4.30	46.17	0.3	0.5	6
Cassava flour	95.56	12.45	7.06	6.53	5.21	67.49	0.03	0.61	2
Cassava leaf meal	86.50	30.40	7.60	9.80	6.30	32.40	-	-	4
Cassava meal, unpeeled	93.00	2.40	0.80	3.60	2.70	83.82	0.12	0.10	3
Copra meal cake	92.40	19.20	12.20	11.50	5.40	47.00	-	-	4
Copra Meal cake GUYANA	**92**	**25.1**	**7.0**		**5.5**	**Carbs 54%**			
Copra meal, expeller process	89.72	19.32	4.51	11.88	6.75	46.20	0.08	0.60	1
Corn, yellow, USA	87.00	8.30	3.80	2.40	1.20	71.30	-	-	4
Corn meal, white	86.51	7.31	0.45	0.74	0.51	76.59	0.01	0.06	1
Corn meal, yellow	88.19	8.37	2.10	1.00	0.73	73.82	0.02	0.12	1
Corn bran, coarse, white	85.89	10.64	7.41	5.51	4.77	57.57	0.04	1.03	1
Corn bran, fine, white	85.20	10.57	7.26	5.07	3.93	57.76	0.04	0.80	1

Fish Meal GUYANA	**92**	**52**	**13.5**		**26.2**	**Carbs <1%**			
Molasses, cane	79.25	1.86	2.50	0.02	5.90	68.88	0.45	0.14	6
Oil palm cake, GUYANA	**88**	**<1**	**73.4**		**15**	**Carb <1 %**			
Palm Kernal Meal, Malaysia	93	15-19	5-8	13-20	3-12	40.88	0.11	0.64	1
Palm nut whole	93	7	42	11	16.19	39.70	0.09	0.46	1
Rice broken GUYANA	**87**	**6.6**	**2.7**		**0.5**	**Carbs 77%**			
Rice bran, GUYANA	**90**	**12.9**	**1.15.2**		**6**	**Carbs 56%**			
Rice middlings, cono	85.29	9.69	2.66	2.97	3.33	66.73	0.04	0.55	1
Rice bran, D1	88.00	11.80	11.30	8.80	9.70	46.20	0.8	1.7	6
Sorghum, grain	87.92	9.29	2.54	2.46	1.59	72.09	0.05	0.33	1
Soybean meal, Brazil	88.00	42.30	5.60	4.50	5.30	30.30	-	-	4
Soybean meal, GUYANA	**88**	**47**	**2.2**		**6.3**	**Carb 33%**			
Wheat bran, soft	88.65	11.32	2.12	7.53	3.90	63.78	0.19	0.98	2
Wheat flour	88.63	16.27	1.76	1.36	1.01	79.70	0.04	0.24	2
Wheat bran, hard	89.14	15.44	3.11	10.07	4.90	55.61	0.12	0.95	1
Wheat pollard, soft	88.32	12.95	4.51	7.38	5.98	58.22	0.12	0.73	1
Fry feed, GUYANA	**91**	**36.5**	**10.8**		**10.8**	**Carbs 33%**			
Fingerling Feed GUYANA	**91**	**39.1**	**13.35**		**13.7**	**Carbs 25%**			
Growout Feed GUYANA	**90.6**	**32.3**	**6.7**		**9.6**	**Carbs 42%**			

Source:
1 - Gerpacio and Castillo (1979)
2 - SEAFDEC Central Analytical Laboratory (unpublished data)
3 - Philippine Society of Animal Nutritionists (1990)
4 - Yamazaki, Lopez and Kaku (1988)
5 - Manufacturer's/supplier's product brochure
6 - PCARRD (1984)

c. *To examine simple feed formulation techniques using identified ingredients*

Several feed formulation software packages were evaluated.



The software selected has proven to be simple but covers all pertinent aspects for the project. Copies were provided to our host country Principal Investigator, the Maharaja Mill manager/owner, and to several of the farmers who have expressed interest in developing their own on-farm diets. A number of diet simulations were developed and evaluated for nutritional values. A couple of the most promising were shared with the Mill owner to test with the local ingredients to determine if the equipment would process the mixture into workable pellets. A couple formulations were discarded as the mixture just did not have sufficient binding characteristics and would not bind into a pellet.

CONCLUSIONS

The project successfully identified a suite of potential ingredients that are locally produced and available in Guyana. The series of workshops and farm visits also served to encourage and assist the farmers and potential farmers to advance their plans and/or operating fish farms. Governmental officials in the Fisheries Department and the Ministry of Agriculture have professed additional support for aquaculture development and have included aquaculture as a preferred sector within the national sustainable development plan. The ingredients identified were then utilized in the follow-up investigation with positive results.

LOCAL INGREDIENTS SUBSTITUTING FOR FISHMEAL IN TILAPIA AND PACU DIETS IN GUYANA

Sustainable Feed Technology/Activity/ 07SFT05UA

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ABSTRACT

Following the findings of the preliminary study examining the potential availability and quality of local ingredients for aquaculture diets, we conducted a series of feeding trials to determine if the locally formulated feeds would be significantly different from the commercially available feed using imported ingredients. Tilapia feeding trials determined that an equal mix (50-50%) of poultry by-product meal and shrimp meal (heads and peels) that are locally available in Guyana could be substituted for the imported fish meal currently used in the locally formulated and manufactured diet currently available to tilapia farmers. The investigation determined this to be the situation with both fingerlings (20g average) and growout (100g average) fish. Tilapia fed a mix of 25-75% or 75-25% poultry and shrimp replacement of fish meal had significantly less growth and a higher Feed Conversion Ratio,

INTRODUCTION

Objectives:

a. To test experimental diets on Tilapia and Pacu reared in cages and ponds.
b. Trial one will replace 20%, 50% and 80% of fish meal with locally derived poultry by-product meal (Null hypothesis – growth rates and Feed Conversion Ratios will not be significantly different)
c. Trial two will replace 20%, 50% and 80% of fish meal with locally derived shrimp by-product meal (Null hypothesis – growth rates and Feed Conversion Ratios will not be significantly different)
d. Trial three will incorporate both poultry by-product and shrimp meal in a combination suggested by the results of the earlier trial

Aquaculture, for marine and brackish water species as well as for freshwater species, is expanding rapidly throughout the world. Some of the driving force for this expansion is simply the need for additional food resources. However, in other cases the demand is caused by the recognition of fish oils and other products as healthy substitutes

for other traditional products. New potential aquatic species are being studied and cultured each year creating a need for specialized formula feeds and feed ingredients.

Fishes have no particular requirement for specific raw materials instead they require essential nutrients such as amino acids, fatty acids, minerals, vitamins and pigments. This means that a variety of raw materials used in combination with others could potentially provide all the necessary nutrients and energy required to support good growth performance.

Farming of fishes requires that the diets supplied are nutritionally complete. Consequently, the choice of which raw materials to include in commercial feeds is critically important if fish are to obtain the correct balance of nutrients to sustain good growth, good health and good flesh quality.

Production of farmed tilapia is among the fastest expanding food sectors in the world. The Nile tilapia is the most cultured freshwater species among the farmed tilapias. However, the sustenance and expansion of production is limited by the high cost of fish feeds, which comprise over 60% of the production costs. Protein usually is the most expensive nutrient and its level and quality determine the cost of fish feeds (Tacon 1993).

Most fish feeds contain a proportion of animal protein, which is considered superior to other sources in terms of palatability and nutrient availability (Tacon 1993; Hardy and Tacon 2002). The majority of fish feeds contain fish meal as the main sources of dietary animal protein. However, due to worldwide decline in fishery products, fish meals are increasingly becoming scarce and expensive. Consequently, nutritionists and feed manufacturers have been searching for alternative dietary animal protein from non-conventional sources to replace the conventional sources and reduce feed costs (El-Sayed 1998; Abdelghany 2003; Liti et al 2005).

Tilapia (*Oreochromis niloticus*) and pacu (*Colossoma macropomum*) aquaculture has been developing in recent years in Guyana. In 2006 the growers organized into a farmer's cooperative, the National Aquaculture Association of Guyana. With the assistance of the Ministry of Agriculture and the USAID/GTIS Programme, the farmers have begun to develop local markets and would like to develop an export trade. The Mon Repos Aquaculture station has worked with the farmers to provide technical assistance and training (Geer 2004, 2006).

Feed costs represent 60-75% of the variable production costs in Guyana. The currently available, locally produced, commercial feed has ingredient costs of approximately $0.36 / kg ($360 /mt) and contains almost 25% fish meal. Fish meal prices are rising and the sustainability of fishmeal harvest is questionable. Use of alternatives ingredients, especially locally available by-product meals would be preferable for economic as well as environmental reasons.

One of our goals is to work collaboratively with the commercial feed mill and the fish farmers to develop economically attractive feeds that contribute to sustainability, which

has been identified as a priority for the aquaculture producers. Utilization of local feed ingredients, minimizing the use of fish meal, and increasing the efficiency of the feed that is input to the system are immediate targets.

Reducing costs, using local ingredients, and reducing fish meal usage are all laudable goals, but will be meaningless if the feed quality or efficiency are reduced. Fish effluents leaving a cage or being discharged from a pond will be increased is food is not converted to fish biomass. Our goal is to improve the diets nutritional efficiency, by being more nutrient dense, that is matching the nutritional need with the dietary ingredients (Lim and Webster 2006). Poultry by-product meal has been shown to be an effective protein replacement for up to 50% of fish meal in tilapia diets (Viola and Zohar 1984) and Williams et al. (1998) demonstrated that shrimp meal could also be used as a suitable ingredient to replace fishmeal.

METHODS AND RESULTS:

Objectives:

a. To test experimental diets on Tilapia reared in cages and ponds.

Due to several flooding events, the feeding trials were eventually completed in tanks. The tanks were arrayed with three replicates of each experimental diet and the tanks were fitted with separate recirculation systems. Stocks of pacu were not available during the experimental period. Cage and ponds trials are still planned and should be completed in the future but after the required deadline for submission of this report. Trials with pacu are also expected to be completed in the future, but again after the submission of this report.

b. Trial one replaced 20%, 50% and 80% of fish meal with locally derived poultry by-product meal (Null hypothesis – growth rates and Feed Conversion Ratios were not be significantly different)

This experimental diet was developed for tilapia fingerlings. The experiment was conducted in twelve 1m³ tanks. Each tank had 15 fingerlings with average weights between 16-23 grams per tank. There were 30 fingerlings in one 2m³ tank for the control. All the tanks had 1 liter of media 1/3 crushed snail shell and 2/3 gravel. The water in the tanks was exchanged using a slow drip system.

The results demonstrated that there were no significant differences in growth rates between the diets nor were FCR's significantly different between the control or the experimental diets.

c. Trial two replaced 20%, 50% and 80% of fish meal with locally derived shrimp by-product meal (Null hypothesis – growth rates and Feed Conversion Ratios were not be significantly different)

This experimental diet was developed for early growout stage tilapia. The experiment was conducted in twelve 1m³ tanks. Each tank had 10 mature fish with average weights between 62-126 grams per tank. There were 20 mature fish in one 2m³ tank for the control. All the tanks had 1 liter of media 1/3 crushed snail shell and 2/3 gravel. The water in the tanks was exchanged using a slow drip system.

The results demonstrated that there were no significant differences in growth rates between the diets nor were FCR's significantly different between the control or the experimental diets.

d. Trial three incorporated both poultry by-product and shrimp meal in a combination suggested by the results of the earlier trials

The study was conducted at the Satyadeow Sawh Aquaculture Station, Mon Repos, East Coast Demerara, Guyana from 28th July – 21st Sept 2009. As no pacu were available for this feed trial, it was repeated for fingerlings and growout size fish. The diet formulations are described below in table format.

Fingerlings trial - Tank Preparation
The experiment was conducted in twelve 1m³ tanks. Each tank had 15 fingerlings with average weights between 16-23 grams per tank. There were 30 fingerlings in one 2m³ tank for the control. All the tanks had 1 liter of media 1/3 crushed snail shell and 2/3 gravel. The water in the tanks was exchanged using a slow drip system.

Treatments The study had three treatments i) 25% shrimp meal and 75 % poultry meal, ii) 50% shrimp meal and 50 % poultry meal, iii) 75% shrimp meal and 25 % poultry meal. There was one control with 50% fish meal.

Grow-out trial - Tank Preparation for growout
The experiment was conducted in twelve 1m³ tanks. Each tank had 10 mature fish with average weights between 62-126 grams per tank. There were 20 mature fish in one 2m³ tank for the control. All the tanks had 1 liter of media 1/3 crushed snail shell and 2/3 gravel. The water in the tanks was exchanged using a slow drip system.

Treatments The study had three treatments i) 25% shrimp meal and 75% poultry meal, ii) 50% shrimp meal and 50% poultry meal, iii) 75% shrimp meal and 25 % poultry meal. There was one control with 27.7% fish meal

The diets were randomly allocated to groups of all male *Oreochromis spp.* fingerlings and mature fishes. They were fed all the three diets in four treatments for the fingerling trial and the same was done for the grow-out trials.

Fish were acclimatized for one week prior to the start of each experiment. The feed was sifted and the fish were fed manually, three times per day for the fingerling trial and two times per day for the grow-out trial. They were fed 5% and 3% of body weight per day for the fingerling and grow-out trials. All the fishes in the fingerling and grow-out tanks were weighed and measured on a weekly basis in the inception of the experiment to

monitor growth and adjust feeding rates. However, it was conducted fortnightly from the fifth week of the experiment.

Final weights were determined at the end of nine weeks by weighing and measuring each fish. Data was analyzed with Single Factor ANOVA.

	F/ling Fish Feed					Protein		
% Incl	Ingredient	Amount	Cost	Total	Protein	Content		shrimp/pou
	Regular	g	$	$	%	%		
0	Soy Meal	0	0	0	44	0		
50	Fish meal	10227	0	1,636	61.49	31		
0	Shrmp meal	0	0	0	39.1	0		
0	Pltry Meal	0	0	0	48	0		
11.2	Rice bran	2290	0	50	12.9	1		
16.6	Broken	3395	0	209	8.7	1		
22.2	Cocofat	4540	0	160	23	5		
100		20452		**$2,056**		**39**		
	Sample #1							
0	Soy Meal	0	0	0	44	0		
0	Fish meal	0	0	0	61.49	0		25/75
12.5	Shrmp meal	2556	0	256	39.1	5		
37.5	Pltry Meal	7670	0	862	48	18		
11.2	Rice bran	2290	0	50	12.9	1		
16.6	Broken	3395	0	209	8.7	1		
22.2	Cocofat	4540	0	160	23	5		
100		20451		**$1,537**		**31**		
	Sample #2							
0	Soy Meal	0	0	0	44	0		
0	Fish meal	0	0	0	61.49	0		50/50
25	Shrmp meal	5113	0	511	39.1	10		
25	Pltry Meal	5113	0	575	48	12		
11.1	Rice bran	2290	0	50	12.9	1		
16.6	Broken	3395	0	209	8.7	1		
22.2	Cocofat	4540	0	160	23	5		
99.9		20451		**$1,506**		**30**		
	Sample #3							
0	Soy Meal	0	0	0	44	0		75/25
0	Fish meal	0	0	0	61.49	0		
37.5	Shrmp meal	7670	0	767	39.1	15		
12.5	Pltry Meal	2556	0	287	48	6		
11.1	Rice bran	2290	0	50	12.9	1		
16.6	Broken	3395	0	209	8.7	1		
22.2	Cocofat	4540	0	160	23	5		
99.9		20451		**$1,474**		**29**		

				Growout Fish Feed					Protein
			% Incl	Ingredient	Amount	Cost	Total	Protein	Content
				Regular	g	$	$	%	%
			27.7	Fish meal	5665	0	906	61.49	17
			22.2	Soy Meal	4540	0	520	44	10
			22.2	Cocofat	4540	0	160	23	5
			16.6	Broken	3395	0	209	8.7	1
			11.2	Rice bran	2270	0	50	12.9	1
			0	Shrmp meal	0	0	0	39.1	0
			0	Pltry Meal	0	0	0	48	0
			99.9		20410		**$1,846**		**35**
				Sample #1					
	25/75		22.2	Soy Meal	4540	0	520	44	10
			0	Fish meal	0	0	0	61.49	0
Tanks 17,18,19,20			6.95	Shrmp meal	1421	0	142	39.1	3
			20.75	Pltry Meal	4244	0	477	48	10
			11.2	Rice bran	2270	0	50	12.9	1
			16.6	Broken	3395	0	209	8.7	1
			22.2	Cocofat	4540	0	160	23	5
			99.9		20410		**$1,559**		**30**
				Sample #2					
	50/50		22.2	Soy Meal	4540	0	520	44	10
			0	Fish meal	0	0	0	61.49	0
			13.85	Shrmp meal	2832	0	283	39.1	5
Tanks 21,22,23, 24			13.85	Pltry Meal	2832	0	318	48	7
			11.2	Rice bran	2270	0	50	12.9	1
			16.6	Broken	3395	0	209	8.7	1
			22.2	Cocofat	4540	0	160	23	5
			99.9		20409		**$1,541**		**30**
				Sample #3					
	75/25		22.2	Soy Meal	4540	0	520	44	10
			0	Fish meal	0	0	0	61.49	0
			20.75	Shrmp meal	4244	0	424	39.1	8
			6.95	Pltry Meal	1421	0	160	48	3
			11.2	Rice bran	2270	0	50	12.9	1
Tanks 13,14,15,16			16.6	Broken	3395	0	209	8.7	1
			22.3	Cocofat	4540	0	160	23	5
			100		20410		**$1,524**		**29**

Note that the fingerling diets without fishmeal are about $500 per ton less expensive while the growout diets with fishmeal are about $300 per ton less expensive. The experimental diets also are reduced in protein level as the shrimp and poultry are both lower in protein 39% and 48% respectively. Further we should note the high cost of the diets compared to typical world prices for similar diets which would be less than half the price in Guyana.

Both trials had a high level of survival. The few mortalities were directly related to interruptions in water flow to the affected tanks.

Figure 1. Growth of fingerlings fed experimental and control diets.

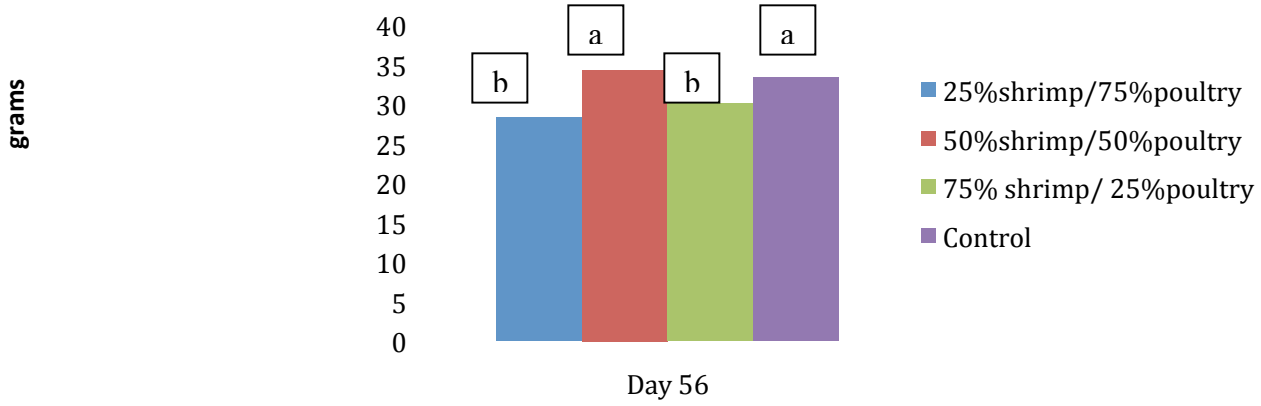
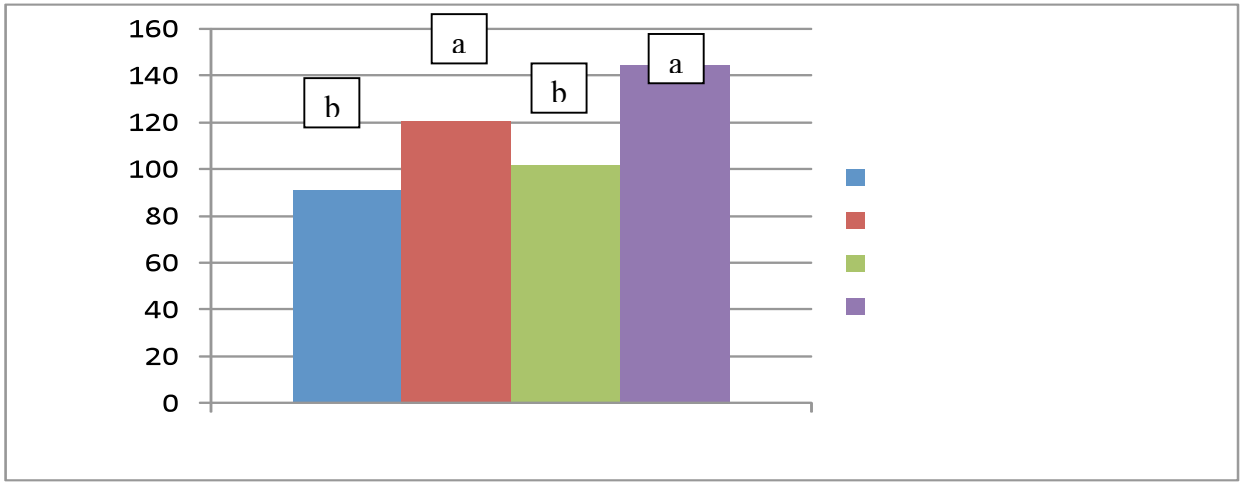


Figure 2. Growth of fish in growout phase fed the experimental and control diets.



CONCLUSIONS

The fingerling diet with a 50/50 mix of poultry and shrimp meal replacing fish meal in the diets was found to be similar to the control diet with no significant difference, while the 25/75 and 75/25 diets were found to provide significantly less growth for the tilapia fingerlings after nine weeks (one week acclimation and eight week trial). The Feed Conversion Ratios demonstrated the same pattern with control and 50-50% mix diets having FCR's that were not different and the 25-75 and 75-25% mixes having FCRs that were significantly higher, but not different from each other.

The larger growout size fish fed a 50/50 % mix of poultry and shrimp meal were also found to add weight at an equivalent rate to the control diet with fish meal. The diets with 25/75% and 75/25% mixes of poultry and shrimp were found to be significantly

less than the control and not significantly different from each other. The Feed Conversion Ratios demonstrated the same pattern with control and 50-50% mix diets having FCR's that were not different and the 25-75 and 75-25% mixes having FCRs that were significantly higher, but not different from each other.

We theorize that the 50-50% mix of poultry and shrimp meal may have an amino acid profile that better approximates that of the fish meal than either of the 25-75 or 75-25% mixtures.

ECONOMIC ANALYSIS

As the fingerling diet with the 50-50% mix was approximately $500 per ton less expensive and the growout diet was $300 less, the experimental diets would appear to be very cost effective and should be able to provide a significant savings for farmers.

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DEVELOPMENT OF LOCALLY AVAILABLE FEED RESOURCE BASE IN TANZANIA

Sustainable Feed Technology/Study/ 07SFT06PU

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ABSTRACT

Soybean meal has been recommended as a substitute for fishmeal in fish diets because of its high protein content and relatively low price. However, small-scale fish farmers in sub-Saharan Africa have not adopted it because soybean meal is pricey and supply is limited. Thus, there is a need to look for cheap alternative protein sources from locally available feed resources. This study was carried out to evaluate the effects of substituting soybean meal with either *Moringa oleifera* leaf meal (MOLM) or *Leucaena leucocephala* leaf meal (LLLM) in feed and the effects on pond water quality and growth performance of tilapia. The study also assessed whether the replacement of soybean meal with leaf meals increases profitability in tilapia farming. Nine diets were formulated and all of them contained 40% protein (soybean meal, MOLM, LLLM and mixtures of the three),

58% energy source (maize bran) and 2% mineral mix. Diet 1, diet 2 and diet 3 contained, respectively, LLLM, MOLM and soybean meal as sole protein sources. In diet 4, diet 5 and diet 6 LLLM replaced soybean at the levels of 25, 50% and 75%, respectively. In diet 7, diet 8 and diet 9, soybean meal was replaced with MOLM at the levels of 25, 50% and 75%, respectively. The fish were fed daily on the respective diets at a rate of 10% of body weight for 90 days. Body weights and length of the fish were measured at the start of the experiment and then at day 30, 60 and 90. Pond water temperature, dissolved oxygen (DO) and pH were measured at weekly intervals for the whole experimental period. Water temperature ranged from 27.7 to 28.5^{0}C, DO was between 8.7 and 11.3 mg/l and pH ranged between 7.68 and 8.18. The growth of fish was significantly ($P \leq 0.001$) influenced by the diets. Fish on diet 3 showed the highest average growth rate (GR) (0.76 ± 0.07 g/d), lowest average feed convention ratio (FCR) (2.7) and highest mean body weight (72.06 ± 1.25 g) and length (11.83 ± 1.90 cm) at 90 days. These were followed by the fish on diet 7 (GR = 0.57 ± 0.06 g/d, FCR = 2.8, mean body weight at 90 days = 47.43 ± 1.17 g and length = 11.25 ± 1.7 cm). Fish on diet 1 had the lowest GR (0.37 ± 0.03 g/d), mean body weight (37.79 ± 1.13 g) and length (9.60 ± 1.4 cm). Fish on diet 2 had the next lowest values (GR = 0.38 ± 0.03 g/d, body weight = 41.92 ± 1.21 g and length = 10.30 ± 1.4 cm).The economic analysis indicated that the diets which contained MOLM and LLLM as sole sources of protein resulted in more profits while the diet which had soybean meal resulted into a loss. The profits decreased as the proportion of soybean meal increased in the diets because of the costs. The use of MOLM and LLLM in place of soybean can result in profitability in tilapia farming, although the yield of Nile tilapia would be low. MOLM is found to be relatively better as a protein source in tilapia diets than LLLM.

INTRODUCTION

In Tanzania, fish farming provides vital animal protein to human population residing in areas, which are located far away from the major fishery resources. It is also an important enterprise to the economic wellbeing of households in rural areas. The most cultured species is the Nile tilapia (*Oreochromis niloticus*). The aquaculture industry is dominated by freshwater fish farming in which small-scale farmers practice both extensive and semi-intensive fish farming. Fish ponds of an average size of 10m x 15m (150 m^2) are the predominant production system (URT, 1997). These ponds are usually integrated with other agricultural activities such as gardening, livestock and poultry production on small pieces of land. The ponds are commonly fertilized with the droppings of domesticated animals or tender leaves as compost manure.

The fish farmers use naturally available feeds to feed the cultured fish. The most frequently used feed are rice and maize bran, kitchen leftovers, and garden remains. These are of low quality and fish reared on these feeds are unable to meet their maintenance and production requirements, especially for protein. This prolongs the time to reach the market weight and consequently leads to production of poor quality fish and low profitability of fish farming.

For several decades, fishmeal and soybean meal have been used as the main sources of protein in fish feeds (El-Sayed 1999). However, supplies are limited due to competition

from humans and livestock. Also, the continuous rise in prices of fish meal and soybean meal make them too expensive and affordable by small-scale fish farmers in developing countries. In order to enhance aquaculture production, improve food security and reduce the level of poverty in rural areas, a search for cheap and locally available feedstuffs is required. There is a need to identify alternative cheap sources of protein from locally available feed resources and to select protein sources that do not conflict with human food security interests (El-Sayed 1999; El-Saidy and Gaber, 2002), as is the case with fish meal and soybean. Leguminous tree leaves and their pods seem to be appropriate alternative protein sources to fishmeal and soybean meal (Fernandes et al., 1999; El-Saidy and Gaber, 2003; Richter et al., 2003; Kaushik et al., 2004). *Moringa oleifera* and *Leucaena leucocephala* are the most useful trees as feed supplements to animals. The leaves of *Leucaena leucocephala* are highly nutritious with excellent palatability, digestibility and balanced chemical composition of protein, minerals and amino acids (Hughes, 1998). Protein concentration in *Leucaena leucocephala* is usually high i.e. 23.5-31.5% (Kimbi, 1997; Ndemanisho et al.,1998; El-hassan et al., 2000; Kimoro, 2003). *Moringa oleifera* is a multipurpose tree of significant importance with several industrial and medicinal uses. All parts of the plant are used in various ways; young leaves, mature leaves, pods and seeds (Becker and Makkar, 2000).The leaves of *Moringa oleifera* are rich in protein and have been used as feed for animals such as goats (Aregheore, 2002; Sarwatt et al., 2002; Manh et al., 2005), for fish (Richter et al., 2003) and for cattle (Sarwatt et al., 2004). According to the Gidamis et al. (2003) the leaves of *Moringa oleifera* contain high concentrations of crude protein, essential vitamins, calcium, iron and proteins.

However, there is limited information on the feeding value of *Moringa oleifera* and *Leucaena leucocephala* leaf meals for use as protein supplements in tilapia feeding. Hence, there is a need to find out the extent to which these leguminous tree leaves can replace fishmeal or soybean in small scale fish production. In this study it was hypothesized that the replacement of soybean meal with *Leucaena leucocephala* leaf meal and *Moringa oleifera* leaf meal as protein sources in fish diets would lower feed costs and hence increase the profitability of fish farming enterprises. This study was, therefore, carried out with the major objective of evaluating the feeding value of *Leucaena leucocephala* leaf meal and *Moringa oleifera* leaf meal as protein supplements in tilapia diets.

The objectives of the study were;

1. To evaluate the feeding value of *Moringa oleifera* and *Leucaena leucocephala* leaf meals in terms of their chemical composition.
2. To evaluate the effect of feeding *Moringa oleifera* and *Leucaena leucocephala* on growth performance, feed conversion ratio and survival rate of cultured tilapia.
3. To examine the effects of *Moringa oleifera* and *Leucaena leucocephala* leaf meals on the quality of pond water.
4. To assess the economic profitability of using *Moringa oleifera* and *Leucaena leucocephala* as feed supplements for tilapia.

MATERIALS AND METHODS

Location of study area

The study was carried out at Kingolwira Fishery Centre, Morogoro, Tanzania. Morogoro lies at latitude 600 20' south and longitude 370 39' East. Morogoro is located at an altitude of about 525 m above sea level. Morogoro region has bimodal rainfall pattern, with short rains starting in November and ending in December and long rains starting in March and ending in May, with an average rainfall of 800 mm per annum. The relative humidity at the location of study is about 81%, while the monthly mean minimum and maximum temperatures are 14.8^{o}C and 32.4^{o}C, respectively.

Preparation of feed materials

Moringa oleifera and *Leucaena leucocephala* leaves were harvested in Morogoro District in September 2008.These leaves were dried in a shaded area to avoid nutrient degradation by direct sunlight. The dried leaves were chemically analyzed and used as plant protein sources in feed compounding. Soybean, maize bran and mineral mix were also purchased in Morogoro municipality. Soybean was boiled for 30 minutes, dehulled, then sun dried for three days. These feed ingredients were chemically analyzed to determine their chemical composition. *Moringa oleifera*, *Leucaena leucocephala* leaves, soybean and maize bran were crushed to enable uniform mixture during feed compounding process.

The chemical composition (dry matter (DM), ether extract (EE), crude fibre (CF), crude protein (CP) and ash) of *Moringa oleifera*, *Leucaena leucocephala* leaves, soybean and maize bran were determined using the proximate analysis scheme (AOAC, 1990). Mineral contents (Ca, Mg, P and K) were determined by using an Atomic absorption spectrophotometer. Extractable condensed tannins were measured by using the method of Makkar (2000). The concentrations of mimosine in diets were determined by using the procedure described by Matsumoto and Sherman (1951) with the modification of Okot (1998).

Feed formulation

Nine diets were formulated. Diet 1, diet 2 and diet 3 contained *Leucaena leucocephala* leaf meal (LLLM), *Moringa oleifera* leaf meal (MOLM) and soybean, respectively, as sole source of protein. In diet 4, diet 5 and 6 soybean was substituted with LLLM at the levels of 25, 50 and 75% while in diet 7, diet 8 and diet 9 MOLM replaced soybean at the levels of 25, 50 and 75%, respectively. All diets contained 40% protein source, 58% energy source and 2%. The proteins sources were soybean, LLLM and MOLM while maize bran was used as energy source.

Experimental procedure

Two concrete tanks were allocated for each diet and 40 fingerlings were stocked in each tank at a rate of 2 fingerlings/m^2. The fingerlings were of mixed sex and had mean (± se) weight of 3.1 ± 0.24 g. A total of 18 round concrete tanks with surface area of 7.06m^2 and depth of 1m were used in the study. The fish were fed twice a day at 0900 h and 1600 h for a period of 90 days. The amount of feed provided was 10% of the body weights, and was adjusted upwards according to monthly body weights. The data were collected in the months of November, December 2008 and January 2009. Body weights of 40 fingerlings

stocked in each tank were measured by using an electronic balance at the start of the experiment and then at day 30, 60 and 90 of the experiment and growth rates were computed. Fork length and body width of 10 fish randomly selected from each tank were measured using a measuring board and vernier caliper, respectively, at day 30, 60 and 90 of the experiment. Death was recorded as it occurred and survival rate was computed. In addition, temperature was determined using a digital thermometer, dissolved oxygen (DO) concentration was determined using a digital DO meter (Jennway 2001) and pH was determined using a digital pH meter (Portmass 911), at weekly intervals during the experiment.

Data analysis

The data collected were analyzed using General Linear Model procedure of Statistical Analysis System (SAS, 1998). The diets were used as fixed effect and the initial body weights were used as the covariates. The dependent variables were body weight, growth rate, dissolved oxygen, pH, and temperature. The chi-square test was used to analyze the data on mortality rate. In addition, gross margin (GM) analysis was used to estimate the profit margin. Gross margin (GM) =Total revenue (TR) – Total variable costs (TVC). The main input costs used in the calculation were prices of fingerlings, prices of feeds, and labour costs. The revenue was obtained from the sales of fish.

RESULTS

Chemical composition of feed ingredients

The analysis for chemical composition of feed ingredients indicated that average DM ranged from 96.52 to 97.13%. The CP content was highest in soybean meal (52.72 %) and lowest in maize bran (11.23%).The CF was highest in LLLM (17.23%) and lowest in soybean (5.85%).The ash content was highest in LLLM (10.63%) and lowest in soybean (4.3%). The Ca content ranged from 0.23% in LLLM to 0.61% in maize bran. The content of Mg was highest in soybean meal (0.32 %) and lowest in LLLM (0.10%).The K content was highest in maize bran (0.13%) and lowest in MOLM (0.05%).The P content was highest in soybean (0.55%) and lowest in LLLM (0.21%).

The analysis for anti-nutritive factors showed that LLLM had higher levels of mimosine (28.50 g/kg DM), condensed tannin (18.50 g/kg DM) and lignin (36.5 g/kg DM) compared to MOLM, MB and soybean. *Moringa oleifera* did not contain mimosine and condensed tannin, but contained lignin (22.05 g/kg DM). Water temperature in the tanks ranged from 27.7 to 28.5 0C, DO was between 8.7 and 11.3 mg/l and pH ranged between 7.68 and 8.18.

Growth Performance

The diets had significant ($P < 0.05$) influence on weight gain of fish. The fish on the control diet 3 had the highest weight gain (68.25 ± 2.48 g) compared to the fish on the other diets. However, fish on *Moringa oleifera* based diets (diets 2, 7, 8 and 9) showed slightly higher weight gain (32.70 ± 2.39 - 51.52 ± 2.37g) compared to the fish on *Leucaena leucocephala* based diets (diets 1, 4, 5 and 6). The diets significantly influenced ($P < 0.05$) the growth rate of fish. The fish on the control diet (diet 3) had the highest growth rate (0.76 ± 0.07 g/d) compared to those on the other diets. Fish on

Moringa oleifera based diets (2, 7, 8 and 9) had slightly higher growth rate (0.38 ± 0.03 - 0.57 ± 0.06 g/d) compared to those on *Leucaena leucocephala* based diets (1, 4, 5 and 6) (0.37 ± 0.03 – 0.46 ± 0.05 g/d).

Body length also differed significantly ($p< 0.05$) among the fish fed different diets. The highest body value for body length was observed on fish fed diet 1 (11.83 ±1.9 cm), this was followed by those on diet 7 (11.25 ±1.7 cm) and fish on diet 1 had the lowest body length (9.60 ± 1.4 cm). Fish on diet 3 had the highest body width (4.28 ± 0.04 cm) while those on diet 7 (4.15 ± 0.04 cm) had the second highest body width and those on diet 1 had the lowest (3.60 ± 0.03 cm). Feed conversion ratios of the fish under different diets ranged from 2.67 ± 0.05 (diet 3) to 2.91±0.02 (diet 1).

The results for the chi-square test indicated that fish mortality in the ponds was not significantly ($P \geq 0.05$) influenced by the diets. However, fish under *Moringa oleifera* based diets showed slightly higher survival rate than those under *Leucaena leucocephala* based diets.

The economic analysis for the different diets showed that the production cost for fish fed diets which contained soybean meal at levels more than 25% was higher compared to the revenue obtained after selling the fish. The diets which contained leaf meals as sole sources of protein had high returns, despite the fact that the fish on these diets had lower body size compared to the diets which contained soybean meal protein supplements. Diet 2 which had 100% MOLM as sole source of protein supplement resulted in the highest profit (TSHs 158,303.14≈ US$ 121.8), followed by diet 1 (125,402.38 ≈ US$ 96.5) which had 100% LLLM as sole source of protein supplement. Diets 9 (25% MOLM) and 6 (25%LLLM) ranked third and fourth in terms of profitability. Generally, the gross margin decreased as the level of soybean meal increased in the diets, indicating that soybean meal is very expensive and that feeding fish with MOLM and LLLM as sole sources of protein alone can increase the profitability of fish farming.

CONCLUSIONS

Based on the findings obtained from the present study, the following conclusions were made: 1) The relatively higher weight gain, growth rate and survival rate observed on fish fed MOLM based diets, indicate that MOLM can be used as protein source in tilapia diets to replace soybean meal; 2) Fish fed MOLM based diets performed relatively better than those fed LLLM based diets, indicating that MOLM is better than LLLM as protein source in tilapia diets; and 3) The results of the present study show that producing fish using diets with higher levels of leaf meals is more economical than using diets with higher levels of Soya bean meal.

ANTICIPATED BENEFITS

The study provided fish farmers some knowledge of *Moringa oleifera* and *Leucaena leucocephala* as possible protein sources for fish feed. Farmers acquiring this knowledge can prepare home-made fish feed using locally available feed resources. As more farmers use *Moringa oleifera* and *Leucaena leucocephala* in home-made fish diets, the cost of fish production will decline and profitability of small-scale fish farming in rural areas

will improve. It is expected that profitability can increase by more than 100%, which would help increase household income and food security. In addition, as the technology is adopted nation-wide, *Moringa oleifera* and *Leucaena leucocephala* could be utilized on a commercial scale to produce commercial fish diets at lower cost. This will have a positive impact on fish nutrition, aquaculture productivity, and profitability of fish farming in Tanzania.

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TOPIC AREA
INDIGENOUS SEPCIES DEVELOPMENT

DEVELOPMENT OF SNOOK (*CENTROPOMUS* SPP) SEED PRODUCTION TECHNOLOGY FOR APPLICATION IN AQUACULTURE AND RESTOCKING OF OVER-FISHED POPULATIONS

Indigenous Species Development/Experiment/ 07IND01UA

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INTRODUCTION

Most fisheries stocks in southeastern Mexico have been reduced or depleted due to an increase in the demand for fish products and in the number of fishermen, natural changes on stocks, and to anthropogenic alterations of aquatic habitats. Aquaculture is a viable

option to capture fisheries, proving alternatives for local employment and income generation. The development of aquaculture techniques for native species not only would help reduce fishing pressures on wild stocks, but it also would provide a source of fingerlings for the development and implementation of plans to restore depleted stocks. Snooks are the most valuable fishery species in southeastern Mexico. Common snook (*Centropomus undecimalis*) and fat snook (*C. parallelus*) support the regional snooks fisheries in the Mexican Atlantic coast. The natural range of common snook extends from North Carolina to Brazil (Muller et al., 2001), making the status of wild snook populations of international concern in the Americas due to local interest in each country for recreational, commercial fisheries and the possibility of fishes are migrating at different life stages in these habitats.

Knowledge of the reproductive biology of snooks is limited. Most information available concerns the common snook. Snooks are considered to be protandric hermaphrodites (Taylor et al., 2000). Spawning has been successfully induced by hormone treatment in wild-caught common snook broodstock (Neidig et al., 2000) and in captive fat snook broodstock (Alvarez-Lajonchere et al., 2002). Common snook broodstock has been difficult to maintain in captivity and thus the few available hatchery-spawning programs have mostly relied on wild-caught fish. Wild-caught broodstock are either immediately processed upon capture to obtain gametes for in vitro fertilization, or they are brought to the hatchery where they are promptly injected with hormones to induce spawning (Anonymous, 2001). Alvarez-Lajonchere and colleagues (2002) developed spawning techniques for fat snook that are based on chronic and acute hormone treatment methods. Although fat snook larvae have been successfully reared and weaned in other places (Alvarez-Lajonchere et al., 2002; Alves et al., 2006).

Snook larvae are small (approx.1.5 mm) and require small prey organisms for their survival and growth. The rearing of larval fish is the most critical stage in the production cycle for many species, and the primary obstacle is that of an adequate food supply (Léger et al., 1986; Abi-Ayad and Kestemont, 1994). A diet which is ready and available and of high nutritional quality, and that is accepted and digested by larval fish must be used (Kim et al.., 1996). Feeding protocols using microalgae, rotifers and Artemia nauplii are currently used for many species. At first feeding, adequate live food density and particle size are also essential for larval fish survival during this critical stage (García-Ortega and Lazo, 2004). Little information is currently available on the dietary requirements of captive common snook larvae. However, fat snook larvae have been successfully reared on a combination of rotifers and Artemia nauplii (Alvarez-Lajonchere et al., 2002), or solely on Artemia nauplii (Alves et al., 2006).

Seed production of fat snook at an experimental scale in Brazil and of the related species, Barramundi (Lates calcarifer) at a commercial scale has been successful achieved (Alvarez-Lajonchere et al., 2002). The laboratory of aquaculture at UJAT also has conducted pilot tests that demonstrate the feasibility of rearing common snook juveniles on artificial diets (Silvercup™ trout pellets; J.M. Vidal et al., unpublished data).

Spawning and larval rearing technologies and methods available for snooks need to be validated on site at the target locations to confirm their general applicability. Specific methods used elsewhere (or for other species) need to be tested on site to determine the precise conditions that result in larvae of good quality. Information regarding the timing, hormone type and dose and water quality conditions is particularly needed. There is the possibility of differences between species in their "manageability."

Implementation of aquaculture activities in Mexico and Central America strongly depends on the availability of fingerlings (seed); which until now has primarily focused on exotic tilapias. Recently, some fishermen in the region successfully raised snooks in freshwater earthen-ponds utilizing wild-caught seed. This practice was subsequently banned by the Secretariat of Natural Resources (SEMARNAT) and by state agencies because it was rapidly spreading, raising concerns about the potential impact on wild stocks recruitment. Despite its short life, this experience justified the development of snook seed production technologies because it showed (1) an interest in and a demand for snook seed for the purpose of aquaculture, (2) the feasibility of growing snook juveniles in freshwater ponds; and (3) a commitment by state and federal management agencies to manage the snook fisheries and aquaculture industry on the basis of ecologically sound principles.

Recent institutional efforts have been organized for snook information exchange between researchers, students, government and NGO´s technicians, farmers and, economical and recreational fishermen. In spring 2004, TTU and UJAT organized a successful international workshop on the biology and culture of snooks. Presenters included leading world experts on these topics. Since then, we have generated new information on the use of artificial diets for juvenile common snook (Vidal, 2009), status of common snook in Texas (Pope et al., in press), and freshwater habitat for juvenile common snook (Huber , 2007); and important information on the larval rearing of snooks has been developed by others (e.g., Alves et al., 2006). As part of this project we organized the Second International Workshop on Snook Biology and Culture for the purpose of collecting and widely disseminating the latest information on snooks.

METHODS AND MATERIALS

Objective 1: To develop techniques for the production of good quality snook eggs

General Experimental Procedure. All experiments were conducted in the recently built snook research facility in the coastal county of Centla, in the fishermen community of Jalapita, Tabasco. This rural facility was established by precedent collaboration projects (CRSP-UJAT-Cooperativa Pesquera San Ramón).

Common and fat snook wild broodstock were collected in coastal areas near to the facility during spawning season using 3 or 7 inch gill nets according with fish size. Collected fishes were transported to the facility and maintained in a 25 m^3 holding tank and fed with live food (local sardines, *Clupeidae*). Thirty six fishes were selected for each experiment (12 females and 24 males). All fat snook experiments were conducted using

recently caught wild broodstock and common snook were conducted with animals kept in captivity for 1-2 years in our facilities.

Before manipulation for gamete sampling, all fish were anesthetized using MS-222 (Finquel ™). Female maturity was confirmed using the gonad biopsy protocol suggested by Alvarez-Lajonchère & Hernandez Molejón (2001). Egg diameter and location of the oocyte´s germinal vesicle were determined; whereas male maturity was consider by abundant milt presence when abdominal pressure was applied. Ripe fish were transferred to holding tanks.

One female and two males were used in each experimental unit. Total length and weight were recorded from each fish. Treatments consisted of the application of either an injection or a pellet containing different concentrations (µg/female) of GnRHa (Argent Labs, USA). Pellet elaboration and application followed the methodology proposed by Alvarez-Lajonchère & Hernandez Molejón (2001) using a mixture of cholesterol/cellulose (95:5) and cocoa butter used for agglutination. After treatment, all fish were placed in 2,000 L tanks (for fat snook) or 9,000 L tanks (for common snook) connected to a recirculation system. Small tanks had a 20 L egg collector while large tanks had an 80 L egg collector. Egg collectors were equipped with a 400 um mesh bag for capturing the eggs. Sand-filtered sea water was supplied to the system and the water quality was maintained by daily 50% water exchange. Temperature, pH and dissolved Oxygen were measured daily (Hanna Instruments & YSI Corp). A second biopsy was performed to females post-spawning in order to determine the continuous action of GnRHa.

Treatment effectiveness was determined by presence or absence of spawnings and egg quality, subsequent implantation was performed and oocyte diameter was recorded. Eggs were collected and buoyant eggs selected for incubation in a 80 L cylindroconical tank. Number of eggs produced was estimated taking three 50 mL samples from the egg collector and eggs counted using a Petri dish to obtain total estimation. Fertilization was recorded after 30 minutes by blastomer observation of 100 eggs using a dissecting microscope (Álvarez-Lajonchere & Hernández-Molejón, 2001). Fertilized egg diameter was obtained from 100 viable embryos using an ocular micrometer attached to a dissecting microscope.

For hatching estimation, viable eggs were incubated in a 300 L tank using sea water similar to the one used in spawning tanks (similar temperature, oxygen level and salinity). After 48 h of incubation, three 50 mL samples were taken from each incubation tank for larval counting under the microscope. Larval survival was determined by the same counting method and total length was recorded under a dissecting microscope at first feeding time.

In all cases, the experimental designed used was a randomized complete block design using one blocking criteria. Due to a lack of enough ripe female availability a pseudo-replication (by date) was used to complete all treatments. Statistical analysis for treatments in the response variables: egg number, egg diameter and larvae total length

was performed using a one-factor ANCOVA. Replication trial (pseudo-replicates) was used as a blocked factor. Results from fertilization rates, hatching, and survival to first feeding were compared among treatments by Chi square test using contingency tables (CT). All statistical analyses were conducted using STATGRAPHICS™ 5.1 and graphical description by Sigma Plot V. 11.0™. We consider statistical differences when the probability level was less than 0.05.

Experiment 1a: Fat Snook spawning induction by GnRHa-saline injections. Except for hormone delivery, most procedures and analyses were conducted as described previously. GnRHa vials were dissolved in 0.9 % saline solution just before injections. A second gonadal biopsy was performed to females that did not spawned in order to determine GnRHa effects.
Treatments: Females were injected with saline solution (vehicle, no GnRHa), 75 µg GnRHa/kg, or 150 µg GnRHa/kg. All males were injected with 50 µg GnRHa/kg.
This experiment was conducted two times (1a-i).

Experiment 1b: Common snook spawning induction by GnRHa-saline injections. This experiment was not conducted due to a lack of broodstock. Despite several attempts, no wild fish (broodstock quality) were caught during 2009. Twenty-eight fish that were kept in captivity for three years died during the flooding episode of 2008, since electricity and communications were lost for two weeks. During this time, our emergency plant failed and the lack of power affected water quality.

Experiment 2a: Fat snook spawning induction by pelleted-GnRHa implantation. Females were implanted with pelleted vehicle (no GnRHa), 100 µg GnRHa, or 200 µg GnRHa/fish. All males were implanted with 100 µg GnRHa pellets.

Experiment 2b: Common snook spawning induction by pelleted-GnRHa implantation (Captive broodstock). Due to the size of the organisms, experimental tanks used for spawning induction were 4 m in diameter. Each tank had an 80 L egg collector. All other procedures and analyses were as described previously. When spawnings were not observed, a second gonadal biopsy was performed to females in order to determine the GnRHa effects.

Treatments: Females were implanted with pelleted vehicle (no GnRHa), 100 µg GnRHa pellet/fish, or 200 µg GnRHa/fish. All males were implanted with 100 µg GnRHa/male pellets.

Experiment 2b-i: Common snook spawning induction by pelleted-GnRHa implantation (Wild broodstock). Recently caught organisms were used according to availability. All procedures and analyses were as described previously.

Objective 2. To develop techniques for the production of snook seed
Experiment 3. Evaluation of Initial stocking rates for fat snook larvae. Because of the lack of spawnings with common snook and better results with fat snook in experiments 1 and 2, fat snook larvae were used for density trials at UJAT. Additional

efforts were conducted in collaboration with researchers from Universidad Nacional Autonoma de Mexico (Sisal, Yucatan-Unit) to obtain larvae from their common snook broodstock, resulting in two spawnings; but, in both cases, not enough larvae were obtained in order to run our density trials.

Two batches of fat snook larvae were obtained at our UJAT-CRSP-Cooperativa San Ramon- facility by GnRHa pellet induction (200 ug/fish dosage; experiment 2a). Embryos were transported to the Tropical Aquaculture Laboratory in Villahermosa, Tabasco and incubated for hatching. Incubation was performed in 100-L cylindroconical incubation tanks using sea water (at 24 ºC and 35 ppt of salinity). The number of yolk-sac larvae was estimated according to survival at hatching (87% fertilization & 80% hatching rate). Yolk-sac larvae were transferred from the incubator to fifteen culture tanks at desired treatment densities (densities were estimated by volumetric method). Initial stocking densities for snook larvae were 20, 40, 60, 80, and 100 larvae/L. Each treatment was conducted in triplicate. Each experimental unit was supplied with filtered sea water (35 ppt) and aeration and the room temperature was maintained at 24°C. Two trials were performed as follows:

Experiment 3a. Five liter culture tank. Desired densities were stocked in tanks containing a total volume of 5 L. The general feeding schedule was as follows: rotifers *Brachionus plicatilis* during days 2-14 post-hatch. Prior to presentation to larvae, rotifers were intensively fed with *Nannochloris* spp. and enriched with Selco (INVE) for 4 h. Random samples of approximately 10 larvae per tank (30 per density) were taken every five days. Fish were anaesthetized with MS-222 and fixed with 4% formaldehyde. Larvae were measured from a digitized image (Sigma Scan Pro V. 4.0) captured with an optical microscope Zeiss (8X). Due to the size of the larvae, weight was not recorded.

Experiment 3b. Ten liter culture tank. All procedures were similar to those described in the preceding experiment, only the tank volume used was different (10 L) in order to avoid excessive larval manipulation.

Growth data was compared using a nested ANOVA (treatment; tanks nested into treatments) followed by Tukey's HSD.

Objective 3. To conduct an international workshop on snook biology and culture. **T**he Second International Workshop on Snook Biology and Culture was organized at UJAT on the basis of our successful experience with the first (2004) workshop. The meeting consisted of four days of workshops and three days of conferences (International Snook Symposium). The meeting was widely advertised by using listservs, newsletters, and web site outlets that targeted both fisheries and aquaculture audiences as well as by personal contacts.

RESULTS

Objective 1: To develop techniques for the production of good quality snook eggs

Experiment 1a: Fat snook spawning induction by GnRHa-saline injections (wild broodstock). Initial statistical differences in oocyte diameter were observed among treatments (ANCOVA, $p<0.001$). The effect of treatment assignment was statistically significant ($p<0.001$). The blocked factor (date of pseudo-replication) had a significant effect in oocyte diameter ($p<0.001$), and the covariate (female weight) also had a significant effect ($p<0.001$). The random allocation of females resulted in differences in oocyte diameter, been larger in the 150 µg/kg treatment (359.82 ± 76.62 µm), while oocytes from the 75 µg treatment showed the lowest size (337.40 ± 76.31 µm; Fig 1); therefore, initial size of oocytes was included as covariate in the post-injection analysis. After injections, no spawning activity was observed in any of the treatments. Therefore, oocyte diameter was measured as response of the treatments. Post-injection ovarian biopsy from no-spawned females showed an increase in oocyte diameter when GnRHa was present (ANCOVA, $p<0.001$). The effect of hormone was statistically significant ($p<0.001$). The blocked factor (date of pseudo-replication) had a slight significant effect in oocyte diameter ($p=0.04$) and the covariates, initial oocyte diameter and female weight had no significant effects ($p= 0.48$ and 0.70, respectively). Oocyte diameter was larger in the 150 µg/kg dosage (392.87 ± 88.80 µm), while no changes were observed in control females (325.00 ± 68.73 µm; Fig. 2).

Experiment 1a-i: Fat snook spawning induction by GnRHa-saline injections (wild broodstock). All replicates were initiated at the same time; therefore, no pseudo-replication was needed. Initial statistical differences in oocyte diameter were observed among treatments (ANCOVA, $p<0.001$). The effect of treatment assignment was statistically significant ($p<0.001$). The covariate (female weight) also had a significant effect ($p<0.001$). The random allocation of females in treatments resulted in differences in oocyte diameter, sampled oocytes in the 75 µg GnRHa/kg treatment were the largest (402.12 ± 70.98 µm), while oocytes from control treatment showed the lowest size (380.12 ± 61.09 µm; Fig. 3). High mortality of adult fish was observed in all treatments.

Post-injection ovarian biopsy of no-spawned females, indicate an increase in oocyte diameter when GnRHa was present (ANCOVA, $p< 0.001$). Oocyte diameter was larger in the 150 µg/kg dosage (481.25 ± 120.03 µm), while no significant changes where observed in control females (399.37 ± 51.47 µ µm) when compared with initial oocyte diameter. The covariates, initial oocyte diameter and female weight had no significant effects ($p= 0.33$ and 0.09, respectively).

Experiment 2a: Fat snook spawning induction by pelleted-GnRHa implantation (wild broodstock). Initial statistical differences in oocyte diameter were observed among treatments (ANCOVA, $p<0.001$). The effect of treatment assignment was statistically significant ($p<0.001$). The blocked factor (date of pseudo-replication) had a significant effect in oocyte diameter ($p<0.001$), and the covariate (female weight) also had a significant effect ($p<0.001$). The random allocation of females resulted in differences in oocyte diameter. In all cases the nucleus position was the same, initial diameter (µm) was 371.25 (± 63.31) for the control group, 383.25 (± 68.41) for 100 µg GnRHa/fish, and 388.62 (± 57.09) for females in treatment with 200 µg GnRHa/fish. Results on the second

biopsy (post-spawning) indicated that new oocyte batches are ready for consecutive spawning events when GnRHa was used.

Spawning was observed around 30 hour post-implantation only in treatments when GnRHa was present, whereas no spawning activity was observed when in the control group. The number of females that spawned per treatment were: 0/4 for the control group, 3/4 for 100 µg GnRHa/fish and 4/4 for 200 µg GnRHa/fish.

Average total number of eggs by treatment was 28,919 eggs (± 3897.3) for the treatment with 100 µg and 40,370 eggs (± 4478.7) for the treatment with 200 µg. Fertilization rate was higher when the lower dosage was used (CT, $p<0.001$; Fig 4a). While the opposite was observed for hatching rate; resulting higher when 200 µg were used (CT, $p<0.001$; Fig 4a).

No statistical differences were observed in diameter of spawned eggs (ANCOVA, $p = 0.6$). Egg diameter in the treatment with 100 ug/fish was 607.17 (± 68.0 µm) and 605.98 (± 51.60) for the treatment with 200 ug/fish. Statistical differences were found for total length of first feeding larvae; larvae from the treatment with 100 ug were higher (1.80 ± 0.25 mm) than those from the treatment with 200 ug (1.60 ± 0.12 mm; KW, $p= 0.0$) (Fig. 5).

Similar water quality (temperature, dissolved oxygen and salinity) was observed in all treatments during the entire experiment (Table 1).

Experiment 2b: Common snook spawning induction by pelleted-GnRHa implantation (Captive broodstock). Due to a lack of mature females, only one female per treatment was used. Statistical differences were observed for the initial oocyte diameter among treatments (ANOVA, $p< 0.001$; Fig 6). The biggest oocytes obtained from the ovarian biopsy were observed for females implanted with the 200 µg GnRHa pellet (328.27 ± 71.86 µm), whereas small oocytes were obtained from females implanted with 100 µg GnRHa pellets (201.80 ± 94.12 µm).

One spawning event was observed from the 200 µg GnRHa/pellet treated female; however, no fertile eggs were obtained. Egg diameter obtained from this spawn was 696.42 ± 16.45 µm. Post-spawning ovarian biopsy showed no significant changes in oocyte diameter for this spawned female (369.89 ± 141.29 µm).

Experiment 2b-i: Common snook spawning induction by pelleted-GnRHa implantation (Wild broodstock). Because of no female availability from several catching efforts (1 female and 19 males), the only female captured was implanted with a 200 µg GnRHa/pellet.

Initial oocyte diameter was 376.86 ± 77.47 µm and no spawning activity was observed. Second ovarian biopsy indicated that oocyte growth achieved 412.13 (± 69.47 µm; Fig. 7).

Objective 2. To develop techniques for the production of snook seed

Experiment 3. Evaluation of Initial stocking rates for fat snook larvae.

Experiment 3a. Five liter culture tank. Initial average larval length was 1.66 ± 0.09 mm. No significant differences among treatments (caused by random allocation in tanks) were found the first day of experimentation ($p > 0.05$). Yolk sac was absorbed at day three post-hatching. After this time, all tanks received their feeding regimes (*Brachionus plicatilis)* three times a day. Sampling for growth evaluation was conducted as planned on day five, resulting in significant differences between treatments ($p < 0.001$). The largest larvae were found in the treatment with a density of 60 larvae/L (2.10 ± 0.10 mm). The smallest larvae were measured in the treatment with 100 larvae/L (1.9 ± 0.10 mm; Fig. 8). Larvae in all treatments died at day 8 post-hatching. After revision of stomachal content, no rotifers were found. Water quality was maintained in optimal conditions in all treatments (temperature = 22.7, DO = 6.2 and salinity = 33.0; Table 2).

Experiment 3b. Ten liter culture tank. Initial average larval length was 1.64 ± 0.10 mm. No significant differences among treatments (caused by random allocation in tanks) were found the first day of experimentation ($p > 0.05$). Yolk sac was absorbed at day three post-hatching. No significant differences between treatments ($p > 0.05$) were found after 5 days of experimentation. The largest larvae were found in the treatment with a density of 80 and 100 larvae/L (2.00 ± 0.01 mm). The smallest larvae were measured in the treatment with 40 larvae/L (1.85 ± 0.15 mm; Fig. 8). Larvae in all treatments died at day 8 post-hatching. After revision of stomachal content, no rotifers were found. Water quality was maintained in optimal conditions (temperature 23.1, DO 6.4 and salinity 32.9; Table 3).

Objective 3. To conduct an international workshop on snook biology and culture. The 7-day workshop was divided in two sections. The first section consisted of four days of workshops contained the following topics: Snook age determination, Recirculation in aquaculture, Larval Culture and grow-out of Fat snook juveniles and Histological determination of gonadal development on Teleost fish; with emphasis on common snook. The second section consisted of a symposium on snook biology and aquaculture that lasted three days; the symposium was divided in three main topics: Biology and ecology, captive breeding and snook culture. All activities were conducted at División Académica de Ciencias Biológicas, UJAT.

An abstract for each workshop is presented below:

Snook age determination, July 8 2009, Villahermosa, Tabasco. Dr. Allyse Ferrara (Nicholls State University). Thirty participants assisted. The workshop initiated with a presentation of the theory behind age determination in fish and the importance of determining the fish age based on otoliths development. The instructor explained about the use of some other bony structure, such as: radio, spines, etc. Several techniques for measuring age were described with emphasis on snook species. After the theory, a practical session was held using different fish species (cichlids, catfish and snook). In the

practical activity the participants performed otolith extraction, cleaning, mounting and ring counting for age determination.

Recirculation in aquaculture. July 9, 2009, Villahermosa, Tabasco. Dr. Quenton Fontenot (Nicholls State University). Thirty-one participants assisted. The workshop initiated with aspects related to the importance of the use of recirculation systems in aquaculture. In this workshop there was given a review on aspects of water re-use and exchange and its importance on fish growth. All these aspects included; tanks size as determining factor for fish volume production, as well as, the cautious selection of high quality inputs to achieve the best production without having negative impacts on the system. Physiological aspects were included in the workshop; fish classification according to osmoregulatory challenges in fresh water and marine water fish and the effect of salinity on plasma osmolarity. Other aspects of fish included were: exact and constant amount of feed and enough oxygen supply in order to achieve better growth rates, feed selection (based on protein percentage, lipid content and floatability). Another important point presented was feed fish intake, correct feed supply according to fish biomass and those aspects will vary with each species, culture system and environmental conditions. The final topic addressed the need of controlling physicochemical water quality parameters (ammonia, nitrates and nitrites) which may have a direct effect on production rates.

Larval Culture and grow-out of fat snook juveniles (*Centropomus parallelus*). July 10, 2009, Villahermosa, Tabasco. Dr. Vinicius Ronzani Cerqueira (Universidad Federal de Santa Catarina, Brazil). Thirty-five participants assisted. The present workshop was divided into 6 parts. 1. Brazilian Aquaculture, 2. Snook biology: distribution, habitat, reproduction and growth, 3. Reproduction in captivity: broodstock, spawning induction, eggs hatching, eggs development and embryo development, 4. Larvae culture: embryo development, culture systems, intensive systems, environment management, feeding, growth and survival, 5. Pre- grow-out; and 6. Grow-out. All aspects presented represent experiences on snook aquaculture in Brazil; particularly on fat snook which have high importance in commercial and scientific areas in addition with the achievements and experiences in Tabasco and Gulf of Mexico.

Histological determination of gonadal development on Teleost fish, with emphasis on common snook. July 10, 2009. Villahermosa, Tabasco. Dr. Harry Grier (University of South Florida). Thirty-two participants assisted 32 participants. This workshop was divided in two parts. The first part was directed to review and compare the different stages of development and snook oocytes maturation. A new proposal for reviewing the ovarian tissues was presented, providing more accuracy for determining the exact degree of development in snook eggs. In the second part Dr. Grier explained how to differentiate physiologically and anatomically males from females when the reproductive season is close, making emphasis to the shape of the belly, inflammation of the genital papilla, etc. He also explained how to perform ovary biopsies and be able to determine the maturation level of the oocytes.

Second International Symposium on the Biology and Culture of Snooks.

Villahermosa, Tabasco, Mexico. 13 – 15, July 2009. Fifty seven persons attended the symposium composed of twenty-seven talks. Fifteen talks were on biology and ecology of snooks, three on captive breeding and nine on snook aquaculture (program in appendix 1 and abstracts in appendix 2). Participant countries were: Mexico (five states), United States (three states), Brazil (one state) and Guatemala (one state). The audience was composed of 20 females and 37 males; fifteen were students, forty were researchers, one was an extension agent and one was a sport fisherman (list of participants in appendix 3). The entire afternoon of the last day of the symposium was dedicated to build the international network of snook researchers. Officials and delegates were named and objectives delineated.

DISCUSSION

In this experimental study we were able of obtaining larvae from the fat snook (*C. parallelus*) in Tabasco, Mexico, while no larvae of the common snook (*C. undecimalis*) were produced, despite achieving oocyte maturation. Implantation of GnRH-a at doses of 100 and 200 µg/fish were effective inducing both maturation and spawning, while injections of the same hormone only reached maturation with no spawning activity. Egg quality was considered good since 85-99% of fertilized eggs and 89-97% hatching were reached. Álvarez-Lajonchere et al. (2002) reported 90% fertilization rates for the same species in Brazil. Our results also indicate that implants significantly induced egg maturation, similar to results reported by Cerqueira y Canarín (2008). These authors also reached spawning of *C. parallelus* using 30 and 50 µg/kg of GnRH-a when wild broodstock was induced. Ferraz *et al.* (2002), obtained spawnings with the same species after 35-42 hours of induction using injections of 50 µg/kg of LHRH-a. However, this group found that females in the control group also released eggs. They concluded that injections and implants were effective inducing fat snooks to spawn finding no significant differences in the number of eggs produced, fertilization and hatching rates. In our study, we were able of identifying mature females from recently caught wild fish. Spawning was attained after a week of induction. Despite differences in egg diameter caused by the time of capture, the use of hormone successfully resulted in final maturation and release. The size of the eggs was also directly influenced by the size of the female, consisting in larger females producing larger eggs. This has been confirmed by several authors including Bagenal (1969). Duncan *et al*. (2003), concluded that LHRH-a induces final maturation in *Sphoeroides annulatus*, using both injections or implants; however, spawning was not reached by the majority of the females treated. The advantage of using implants consists in less manipulation of the fish, reducing stress considerably.

Maturity of the eggs in *C. parallelus*, measured as egg diameter was very significant in our study. All females induced responded to GnRH-a treatments and several released the eggs. In some cases not al lthe eggs were fertilized. With respect to this, Berlinsky *et al.* (1996), indicated that GnRH-a implants produce viable eggs in repeated spawnings of *Paralichtys lethostigma*. The fact that the implant slowly reduces the hormone is considered the main factor for spawning induction. Similar results were reported by Lee *et al*. (1986), reaching maturity in males and females of the milkfish *Chanos chanos.* Khay (1980), succesfully stimulated vitelogenesis in the goldfish *Carassius auratus* and the aruan *Ophiocephalus striatus* using hCG implants. In our study we obtained positive

results with the lowest dose used (100 µg/fish); however, no differences were found when the largest dose was used (200 µg/fish).

Regarding results obtained with injections, we were able of increasing the size of the eggs without been released, despite application of repeated injections. Other studies with *C. parallelus* have reported successful spawning using this technique with recently caught fish (Reis y Cerqueira, 2003). It is possible that the fish that they used from the wild were in an advanced stage of maturation and potentially close to ovulation or even during spawning time. It is important to mention that *C. parallelus* is a multiple-spawner species; therefore the fish could be caught even in between spawning events been at the maximum stage of maturity. Cerqueira *et al.* (2005) reported that injections of hCG in dosages of 1,100 IU were sufficient to get ovulated females. Álvarez-Lajonchere and Hernández-Molejón (2001) recommended that injections must be performed in partial dosages to allow the entire maturation of the oocyte. In our study, we used single dosages; therefore, it is possible that final maturation was not reached. It is possible that the injections we applied to the snooks were not at the right time. Fitzpatrick *et al.* (1987), determined that GnRH-a is efficient accelerating final maturation of oocytes in the coho salmon, *Oncorhynchus kisutch*, however, best results are obtained when enough fish are used so that females are selected at the most favorable time. Álvarez-Lajonchere and Hernández-Molejón (2001) recommended application of injection in snooks when oocyte diameter reached between 400 y 500 µ, unfortunately our fish where always significantly below that diameter. Sullivan *et al*. (2003) suggested induction at the time when the oocytes completed growth, since most fish initiate ovarian regression when placed in captivity.

Other researchers have mentioned that failure in fish induced spawning may be caused by ovarian atresia caused by captivity. Zohar and Mylonas (2001) emphasized this particularly at the end of egg maturation. On the other hand, males can also limit the success of an induction experiment, since they may not fertilize the eggs (Diana, 1951). Captivity may also alter hormonal cycles in males resulting in a lack of circulating LH Zohar and Mylonas (2001). Males of *Sparus aurata,* may present spawnings behavior, lacking mature spermatozoids (Zohar, 1989). Females may release the eggs, but never get fertilized.

All stressful conditions present during captivity can definitely alter final maturation of oocytes and affect spawning. Contreras-Sánchez et al., (1998) reported that reproduction patterns were significantly altered by stress present during farming conditions of adult rainbow trout (*Oncorhynchus mykiss*). The impact in reproduction was also related to the time at which stress was applied to the fish.

The quality of the eggs has been related to the nutritional condition of the broodstock. Tucker (1994), emphasized that even seasonal changes may affect female condition and the quality of the spawning events. This author pointed out that in *Epinephelus striatus* the size of the egg varies between fish maintained in captivity and fish from the wild. In our study, fish induced came from wild captures and we assume that nutrition was not a

factor affecting the results. More information is needed regarding comparisons between wild and captive broodstock in this species.

The size of the larvae obtained in our experiments was very similar to that reported by Álvarez-Lajonchere *et al*. (2002). Unfortunately, larvae obtained from fat snooks died on day eight of experimentation. Our explanation is that the size of the rotifers used was larger than the size of the mouth opening. Cerqueira and Tsuzuki (2009) also reported that the majority of the mortality presented in some experiments with *C. parallelus* is present during the first week of life, been higher around the third or fourth day after disappearance of the yolk sac. Cerqueira *et al*. (1995) suggested *Brachionus plicatilis* and *Bachionus rotundiformis* as food for *C. parallelus* starting on day three post-hatching, and posteriorly changing to artemia nauplii. We used *B. plicatilis*, but apparently the size of the rotifers was too large. Regarding to this, Verreth (1994) pointed out the need for optimal conditions Turing larval rearing as well as appropriate size of the food; particularly for marine species with a very small larval size. Planas and Planas y Cunha (1999) highly recommended the use of *Brachionus plicatilis* and *B. rotundiformes*) when feeding marine fish; these species were very efficient when feeding larvae of *Sparus aurata* (Polo *et al*., 1992). Tucker (1998) suggested *B. plicatilis*, with emphasis in rotifers between 100 and 240 μm in size. Our results could be affected if the rotifers used where adults surpassing the size needed for the larvae obtained.

More research is needed in order to obtain a good protocol for first feeding of snook. One option could be the use of mesocosms systems when feeding the larvae, making sure that feeds with different sizes are available for the fish (Prieto *et al*., 2006; Hunter, 1981). Addition of essential fatty acids (HUFA) may also be needed for improving these feeds (Koven et al., 1992). Silva (1999) mentioned that other microalgae such as *Isochrysis galbana*, can significantly improve larval rearing

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Table 1. Water quality measured as average temperature, Dissolved Oxygen and Salinity during experiment 2a.

Treatment (μg GnRHa/fish)	**Temperature (°C)**	**DO (mg/L)**	**Salinity (S)**
0	26.0 ± 0.68	6.5 ± 0.16	32.2 ± 0.43
100	26.5 ± 0.25	6.5 ± 0.10	35.0 ± 0.0
200	26.8 ± 0.59	6.5 ± 0.27	35.2 ± 0.43

Table 2. Water quality measured as average temperature, Dissolved Oxygen and Salinity during experiment 3a.

Treatment (Larvae/L)	**Temperature (°C)**	**DO (mg/L)**	**Salinity (S)**
20	22.69 ± 6.13	6.13 ±1.65	33.67 ± 1.25
40	22.60 ± 0.38	6.22 ± 1.79	33.50 ± 1.24
60	22.67 ± 0.46	6.11 ±1.54	33.14 ± 1.24
80	22.62 ± 0.60	6.21 ± 1.71	33.62 ± 1.09
100	22.70 ± 0.42	6.21 ± 1.76	31.29 ± 4.23

Table 3. Water quality measured as average temperature, Dissolved Oxygen and Salinity during experiment 3b.

Treatment (Larvae/L)	**Temperature (°C)**	**DO (mg/L)**	**Salinity (S)**
20	22. 93 ± 0.33	6.42 ±1.89	32.76 ± 0.62
40	22.95 ± 0.42	6.53 ± 1.90	32.52 ± 0.87
60	23.65 ± 2.66	6.62 ±1.63	33.10 ± 0.94
80	22.88 ± 0.28	6.41 ± 1.80	32.90 ± 0.89
100	22.88 ± 0.49	6.13 ± 1.60	33.00 ± 1.10

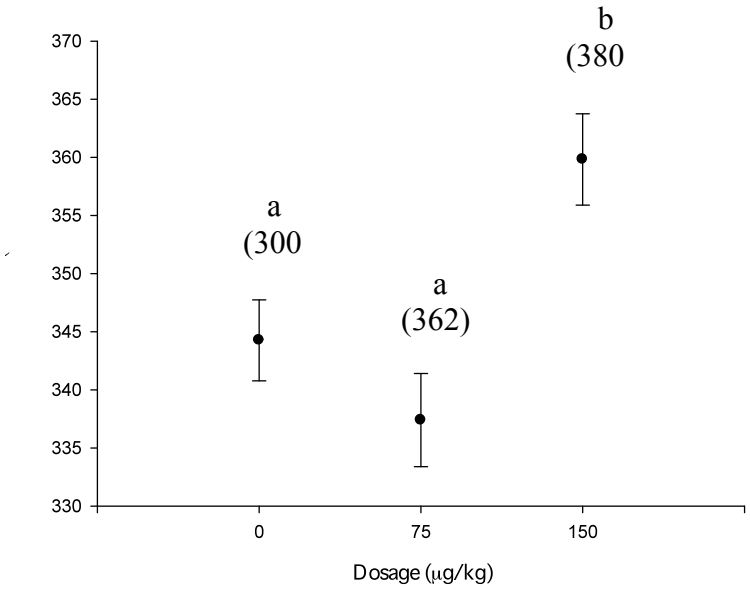

Figure 1. Oocyte diameter of *C. parallelus* before GnRH-a injection. Numbers in parenthesis indicate the number of oocytes measured. Different letters indicate significant statistical differences.

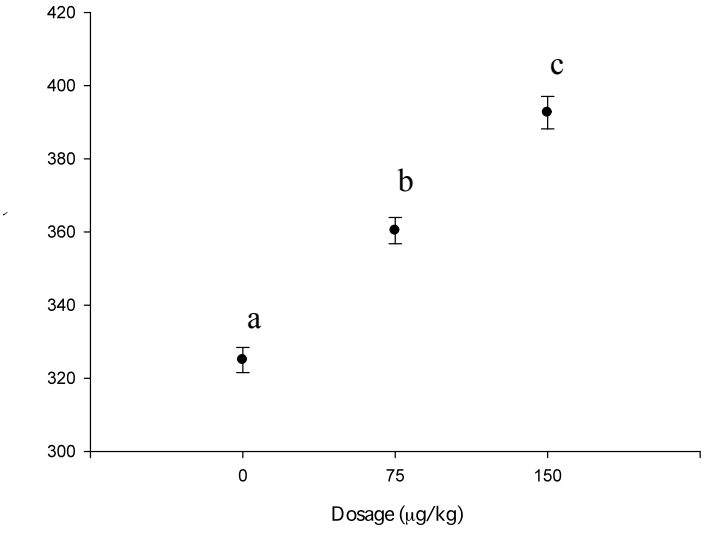

Figure 2. Oocyte diameter of *C. parallelus* after GnRH-a injection. In all treatments n = 400 eggs. Different letters indicate significant statistical differences.

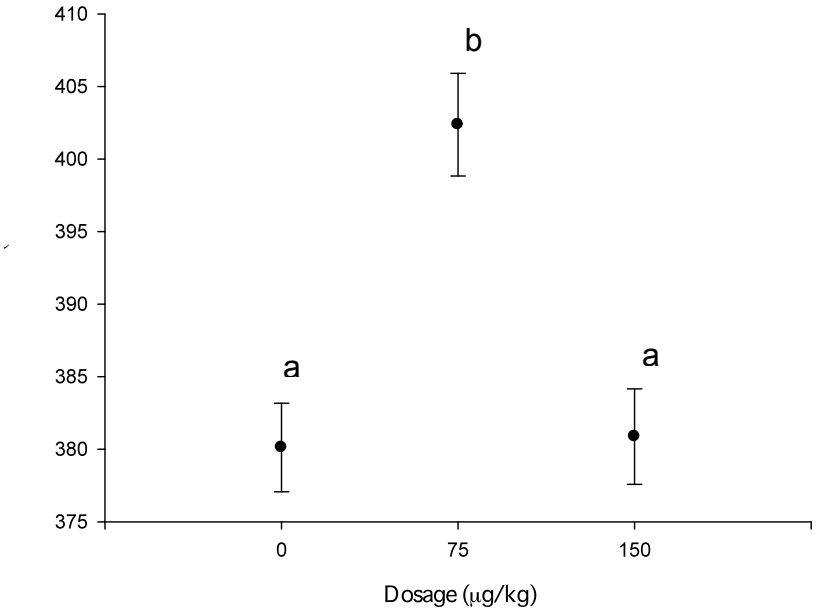

Figure 3. Oocyte diameter of *C. parallelus* before GnRH-a injections application. In all treatments n= 400. Different letters indicate significant statistical differences.

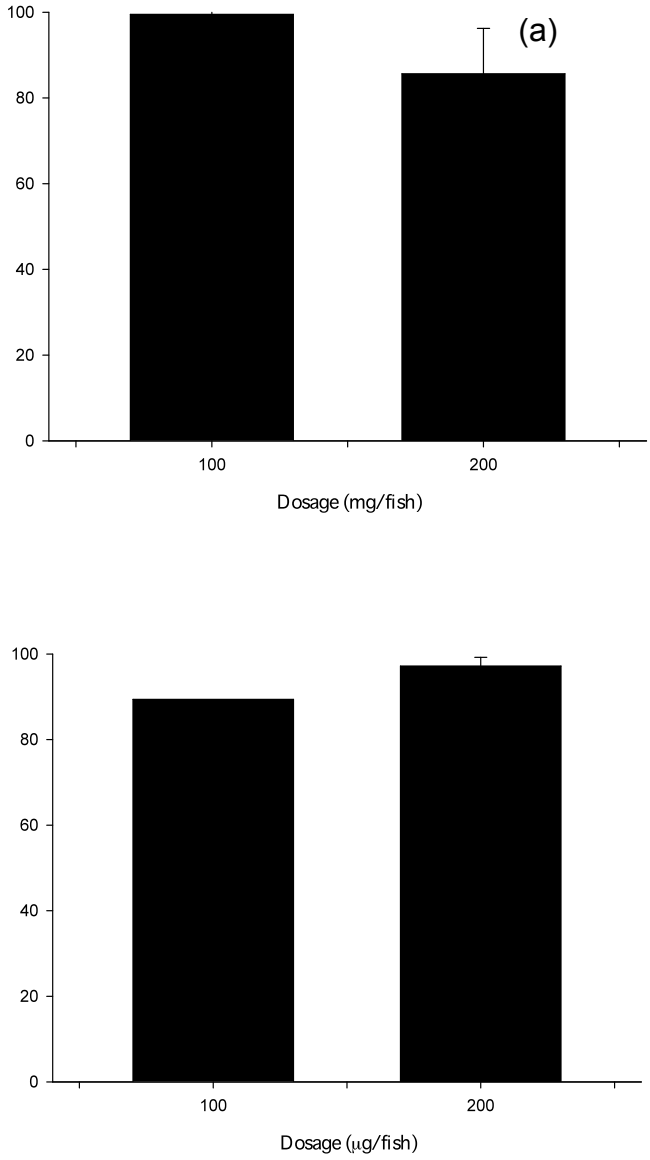

Figure 4. Fertilization (a) and Hatching (b) rates for *C. parallelus* viable eggs obtained from females induced by implantation of pelleted GnRHa. Asterisks indicate statistically significant differences.

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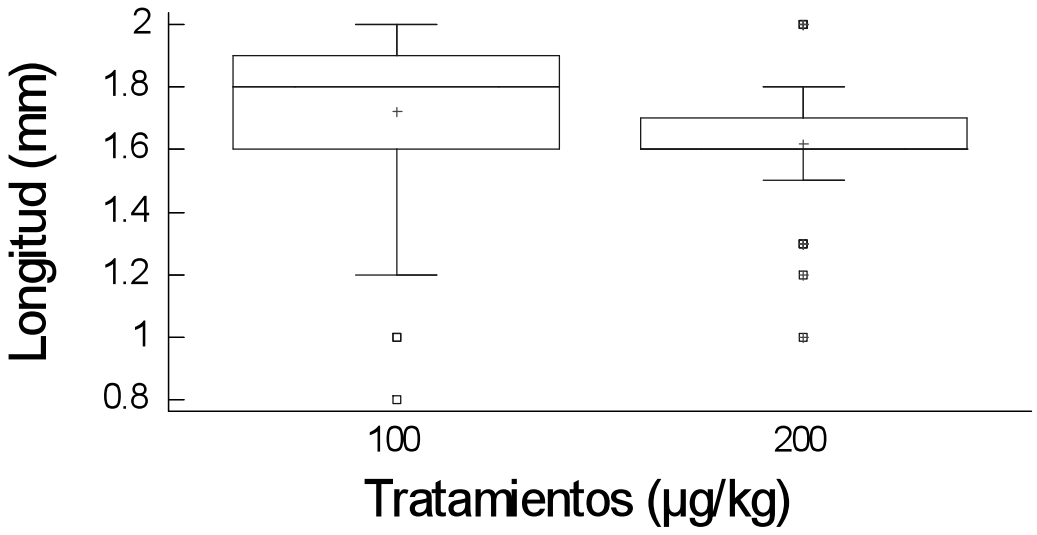

Figure 5. Initial size for larvae of *C. parallelus* obtained from females induced by implantation of pelleted GnRHa.

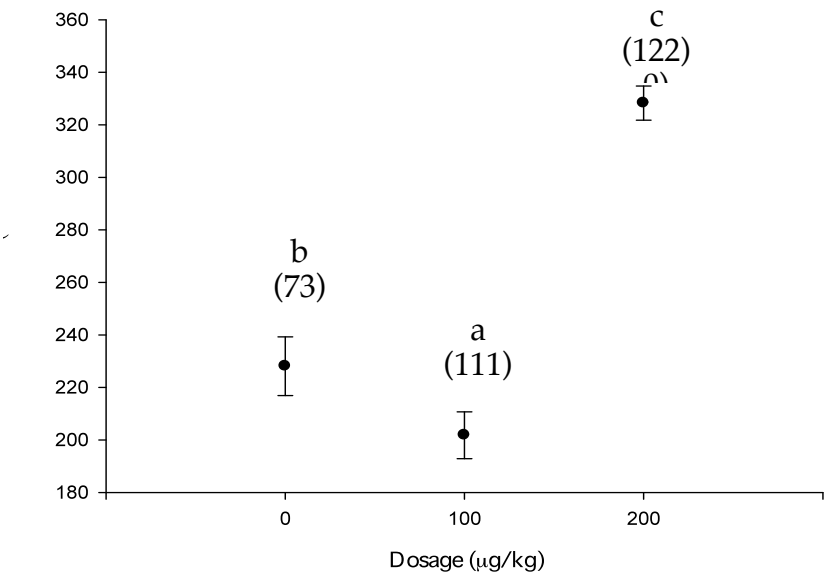

Figure 6. Oocyte diameter of *C. undecimalis* before GnRH-a implant application. Numbers in parenthesis indicate the number oocytes reviewed. Different letters indicate significant statistical differences.

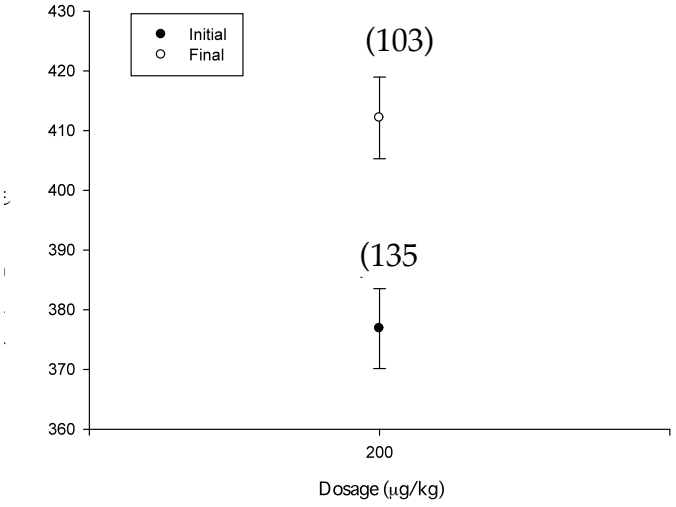

Figure 7. Oocyte diameter of *C. undecimalis* before and after GnRH-a implants application. Number in parenthesis correspond to the number oocytes reviewed. Both values are from the only female sampled at different times (before and after treatment).

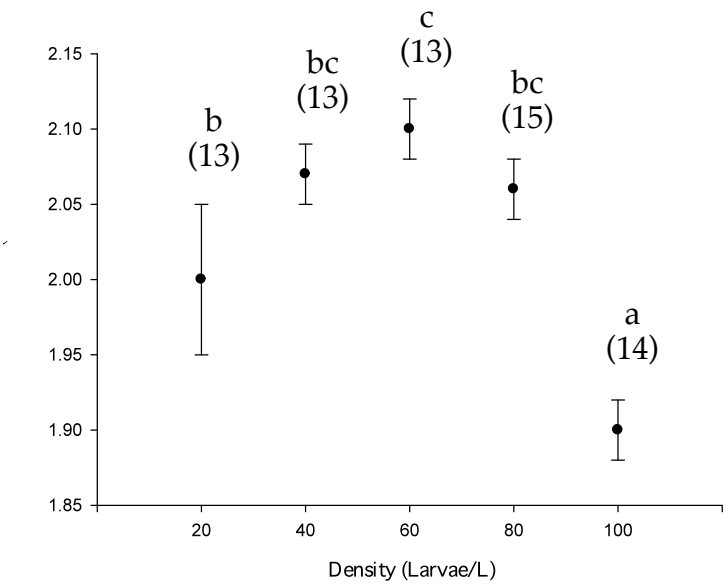

Figure 8. Average larval length of *C. parallelus* after five days of experimentation in experiment 3a. Numbers in parenthesis indicate the number larvae reviewed. Different letterS indicate statistical significant differences.

APPENDIX
1


UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO
División Académica de Ciencias Biológicas
Laboratorio de Acuicultura Tropical
Segundo simposio Internacional sobre Biología y Cultivo de Robalos
Villahermosa, Tabasco, México del 13 al 15 de Julio del 2009
Resúmenes/Abstracts

Second International Symposium on the Biology and Culture of Snooks
Villahermosa, Tabasco, Mexico
13 – 15, July 2009
Villahermosa, Tabasco, México

División Académica de Ciencias Biológicas
Universidad Juárez Autónoma de Tabasco

Room: Alexandr I. Oparin

PROGRAM

ORGANIZING COMITEE

UJAT
Wilfrido Miguel Contreras Sánchez
Ulises Hernández Vidal
Arlette Hernández Franyutti
Carlos Alfonso Álvarez González
Lenin Arias Rodríguez
Salomón Paramo Delgadillo
Gabriel Márquez Couturier
Alejandro Macdonal Vera

TTU
Reynaldo Patiño

CONFERENCES
July 13

Registration 8-9 AM

Inauguration Ceremony 9:00-9:30

Biology and Ecology

Time	Authors	Title
10:00-10:40	Carole McIvor, Adam Brame, and Justin Krebs.	**Habitat ecology of young of the year Common Snook (*Centropomus undecimalis*) in two Gulf of Mexico estuaries, Florida, USA.**
10:40-11:20	Philip W. Stevens, David A. Blewett, and Gregg R. Poulakis	**A conceptual model of juvenile Snook habitat use and application to a variety of estuarine systems**.
11:20-12:00	Caleb G. Huber, Kevin L. Pope, and Reynaldo Patiño	**Habitat preferences of juvenile Common Snook in the Lower Rio Grande/Bravo del Norte**.
12:00-12:30	**BREAK**	
12:30-13:10	Martha A. Perera-García, Manuel Mendoza-Carranza, Wilfrido Contreras-Sánchez, Eunice Pérez-Sánchez, Allyse Ferrara, Maricela Huerta-Ortiz, and Salomón Páramo-Delgadillo	**Comparison of age and growth of Common Snook (*Centropomus undecimalis*) in two different tropical systems (coast and river), in Tabasco, México**.
13:10-13:50	David A. Blewett, Philip W. Stevens, Ronald G. Taylor, Thomas R. Champeau, and Brent L. Winner.	**Use of rivers and an open estuary by common snook, *Centropomus undecimalis*, and comments on factors influencing cyclical seasonal movements.**
13:50-14:30	Ronald G. Taylor, David A. Blewett, Alexis A. Trotter, Phillip W. Stevens, and Robert Muller.	**Aperiodic, novel migrations and use of riverine habitats by common snook: evidence for skip spawning**.
14:30-17:00	**LUNCH**	
17:00-17:40	Martha A. Perera-García, Manuel Mendoza-Carranza,	**Reproductive biology and status of populations of the common snook**

	Wilfrido Contreras-Sánchez, Eunice Pérez-Sánchez, Maricela Huerta-Ortiz, and Salomón Páramo-Delgadillo	**(*Centropomus undecimalis*), in Tabasco, México**.
17:40-18:20	Rosa María Lorán-Núñez, Fco. Rolando Martínez-Isunza and Manuel Garduño-Dionate	**Total mortality of the Mexican snook (*Centropomus poeyi)* in Laguna de Alvarado, Veracruz, México (2005)**.
18:20-19:00	Richard J. Kline and G. Joan Holt	**The difficulties with defining the spawning season for snook in Texas**.

19:00-19:40	Manuel Mendoza-Carranza, Martha A. Perera-Garcia, Salomon Paramo-Delgadillo and Eunice Perez-Sanchez	**Variations in Common snook (*Centropomus undecimalis*) catch per unit effort and climatic variation in southeastern Mexico coasts.**
Reception		

CONFERENCES
July 14

Biology and Ecology

Time	Authors	Title
9:00-9:40	Ma. Guadalupe Gómez Ortiz, Rodolfo Arteaga Peña, Juan Balderas Télles, Guillermo Acosta Barbosa, and Ariel López Salazar	**Reproduction of the common snook (*Centropomus undecimalis*) in the Panuco River, Veracruz.**
9:40-10:20	Rosa María Lorán-Núñez, Fco. Rolando Martínez-Isunza, Manuel Garduño-Dionate and Víctor Martín Zarate-Noble	**Reproduction of the mexican snook (*Centropomus poeyi*) and common snook (*C. undecimalis*) in Laguna de Alvarado, Veracruz (2005-2008)**.
10:20-11:00	Manuel Garduño Dionate, Rosa María Lorán Nuñez, Francisco Rolando Martínez Isunza and Erik Márquez García.	**Fecundity of the common snook (*Centropomus undecimalis*), in the coast of Ciudad del Carmen, Campeche**.
11:00-11:30	**BREAK**	
11:30-12:10	Vequi Caballero Chávez	**Size for fish maturation and capture**

		composition of common snook in Southern Campeche.
12:10-12:50	Francisco Rolando Martinez-Isunza, Rosa María Lorán-Nuñez, Manuel Garduño-Dionate, Vequi Caballero-Chavez, María Guadalupe Gómez-Ortiz, Victor M. Zarate-Noble, Martha Alicia Perera-García, Salomón Paramo-Delgadillo y Carlos Alfonso Alvarez-González	**Proposal for the regulation of snook capture in the Gulf of Mexico**.
Captive breeding		
12:50-13:30	Adolfo Sanchez Zamora	**Status of the Common Snook reproduction in captivity at UMDI, UNAM, Sisal.**
13:30-14:10	Matthew Resley, Kevan Main, and John Stubblefield	**An overview of Common Snook broodstock maturation and spawning research**.
14:10-14:50	María de Jesús Contreras-García, Wilfrido M. Contreras-Sánchez, Ulises Hernández-Vidal, Alejandro Mcdonal-Vera	**Induction of reproduction of Fat Snook in captivity using LHRHa implants and injections**.
14:50-17:00	**LUNCH**	
Culture		
17:00-17:40	Ulises Hernández-Vidal, Wilfrido M. Contreras-Sánchez, Reynaldo Patiño, Juan M. Vidal-López, Ana Y. Torres-Marín, Carlos A. Alvarez-González y Arlette A. Hernández-Franyutti	**Maintenance in captivity of the Common Snook (*Centropomus undecimalis*), Fat Snook (*Centropomus parallellus*) and Mexican Snook (*Centropomus poeyi*)**.
17:40-18:20	Sergio Escárcega-Rodríguez	**Fist evidence of aclimation to captivity for Pacific black snook (*Centropomus nigrescens*)**
18:20-19:00	G. Joan Holt and Rick Kline	**Culturing Texas Snook - What have we learned so far**.

CONFERENCES
July 15

Time	Authors	Title
9:00-9:40	Leonardo Ibarra-Castro	**Culture of the Asian Snook (Lates calcalifer) in Australia**.
9:40-10:20	Eduardo A. Zarza-Meza	**Grow-out of *Centropomus undecimalis* (Bloch, 1792) and *Centropomus parallelus* (Poey, 1860) in fresh water in Veracruz, Mexico.**
10:20-11:00	Vinicius Ronzani-Cerqueira	**Spawning and larviculture of the fat snook (*Centropomus parallelus*) in Brazil**.
11:00-11:30	**BREAK**	
11:30-12:10	Kevan Main, Carlos Yanes-Roca, and Nicole Rhody	**Status and challenges in larval rearing and fingerling aquaculture of Common Snook in Florida**.
12:10-12:50	Juan Manuel Vidal-López, Wilfrido M. Contreras-Sánchez, Carlos A. Álvarez-González, Arlette Hernando-Franyutti, Ulises Hernández-Vidal	**Feminization of Common snook (*Centropomus undecimalis*) juveniles using Estradiol in the diet.**
12:50-13:30	Carlos A. Alvarez-González*, Natalia Perales-García, Bartolo Concha-Frías, Adolofo Sanchez-Zamora, Gabriela Gaxiola, Luis D. Jiménez-Martínez, Leticia Arena-Ortiz, Talhia Martínez-Bruguete, Arlette A. Hernández-Franyutti, Juan M. Vidal-López, Lenin Arias-Rodriguez, Gabriel Marquez-Couturier, Dariel Tovar-Ramírez, Enric Gisbert, Francisco J. Moyano, Francisco J. Alarcón, Pedro H. Toledo-Agüero, Alfonso E. Silva-Arancibia	**Status of digestive biology studies of the common snook (*Centropomus undecimalis*) in Tabasco, Mexico**.
13:30-14:10	**CLOSING REMARKS**	

APPENDIX 2

HABITAT ECOLOGY OF YOUNG OF THE YEAR COMMON SNOOK (*Centropomus undecimalis*) IN TWO GULF OF MEXICO ESTUARIES, FLORIDA, USA

Carole McIvor*, Adam Brame, and Justin Krebs, U.S.

Geological Survey, Florida Integrated Science Center
, St Petersburg, FL, 33715, USA.

Characterization and protection of nursery habitats of juvenile common snook is an important issue for proper management of the species. We conducted three different studies to examine habitat use and ecology of juvenile snook in two different regions of Florida. In each of the studies we used small haul seines to collect snook from permanently subtidal and shallow intertidal areas. We recorded water quality and habitat characteristics at each site to allow inferences about snook nursery areas. The first study was a 3-yr survey of habitat associations and density estimates of young of the year (YOY) common snook in four types of mangrove-lined, estuarine water bodies in Tampa Bay. Four hundred and thirteen snook were collected from 833 samples during this project. YOY snook were most abundant from August through January at salinities of 5-15 psu. Average densities were highest in natural tidal creeks followed closely by well-flushed, man-made, mosquito-control ditches. YOY were also relatively abundant in estuarine ponds that were well-connected to adjacent tidal channels. Man-made stormwater-control ditches consistently had only low densities of these fish, and apparently provided poor habitat for YOY snook. Such ditches are often scoured with high-velocity flows following heavy rain events. A second more-focused study specifically investigated small-scale habitat associations of YOY snook within a single tidal-creek drainage (Frog Creek in lower Tampa Bay), during the 2006 fall recruitment. Here Adam Brame collected YOY snook along the shorelines of a creek and four connected ponds, with the intent of better defining optimal nursery habitat. He collected 436 YOY snook from 144 samples and followed the growth of the cohort from a modal size class of 20 mm SL in September through 60 mm SL in February when sampling was terminated. Densities peaked in November and again in February indicating a prolonged recruitment. YOY snook were captured most frequently at 5-10 psu. Fish in ponds were slightly smaller than snook in adjacent tidal creeks. On average, the quiescent waters of the two upstream ponds contained four times as many YOY as any of the other three microhabitat types (downstream ponds, downstream creek, upstream creek). We hypothesize that YOY snook entering Frog Creek recruit preferentially to (or else exhibit higher survival in) low-salinity ponds in the upstream-most portion of the tidal section of Frog Creek. While working on a separate project in southwest Florida, we serendipitously discovered a previously unknown snook nursery area in Tarpon Bay, a large (48 km shoreline) upstream embayment along Shark River, the primary drainage for the Greater Everglades Ecosystem. Although there has long been a thriving recreational fishery for adult snook in the remote rivers and bays of SW Florida and in Florida Bay at the southern tip of the state, the nursery areas supporting this adult population were unknown. Our discovery paved the way for the third study whose objectives were to describe both the demographics and habitat use of juvenile snook in Tarpon Bay. We

collected 66 YOY and year-1 snook along shorelines in the NE-E part of Tarpon Bay between January 2006 and April 2007, most at salinities of 1-5 psu. However, subsequent directed random sampling from February 2008 through April 2009 (89 hauls over 6 dates) yielded no juvenile snook. Electrofishing in the uppermost low-salinity portion of the bay in November 2008 yielded 1 YOY and 6 year-1 fish. Why common snook demonstrated such wide recruitment variability in three consecutive years in this newly-identified, apparently suitable habitat is presently unclear. In summary, we found that YOY snook are predictably associated with shallow shorelines, underwater structure (prop roots, snags), relatively quiescent waters of low velocity, and low to moderate salinity. In west-central and SW Florida, such habitats are usually up-river away from the mouth of the estuary or tidal creek. Unless modified, these shorelines are generally mangrove-lined although in the lowest salinity habitats, there may be a mixture of mangroves with sawgrass (*Cladium jamaicense*) or cattails (*Typha* spp.). Some altered waterways (well-flushed mosquito-control ditches) may offer adequate nursery habitat whereas others (residential stormwater-control ditches) do not.

A CONCEPTUAL MODEL OF JUVENILE COMMON SNOOK *CENTROPOMUS UNDECIMALIS* HABITAT USE AND APPLICATION TO A VARIETY OF ESTUARINE SYSTEMS

Philip W. Stevens*, David A. Blewett, and Gregg R. Poulakis

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Habitats used by juvenile fishes can dramatically differ among estuaries. Thus, it is important to apply life-history models in a variety of estuarine settings to fully define a species' suite of juvenile habitats. To determine the locations of juvenile common snook *Centropomus undecimalis* habitat and to describe changing habitat affinities through ontogeny, datasets collected by the Florida Fish and Wildlife Conservation Commission, Fisheries-Independent Monitoring program were analyzed for Tampa Bay, Charlotte Harbor, the Caloosahatchee River estuary, Estero Bay, and the Indian River Lagoon.

Suitable habitat near larval sources (i.e., ocean passes) had higher juvenile densities than similar habitat farther into the estuary. In estuaries where rivers occurred in close proximity to passes, densities of small juveniles (15-150 mm SL) in riverine habitats were high, particularily in backwater wetland habitat. In the Calooshatchee River estuary, juvenile snook densities were 7 times greater in backwater embayments, tributaries, and oxbows than along the river's main stem. Not surprisingly, species known to be important prey for juvenile snook (e.g., mosquitofish *Gambusia holbrooki*, sailfin molly *Poecilia latipinna*, rainwater killifish *Lucania parva*) were an order of magnitude more abundant in the riverine backwaters.

In Charlotte Harbor, juvenile snook appear to make less use of riverine habitats and more exclusive use of coastal wetlands. The long distance of rivers from ocean passes (>30 km) may be unfavorable for dispersal of juvenile snook into upper Charlotte Harbor; less than 3% of small juvenile common snook captured during stratified-random sampling were found within riverine habitats compared to 67% in the Indian River Lagoon, and 97% in Tampa Bay. Moreover, the primary juvenile snook habitats in Charlotte Harbor appear to be located in coastal wetland ponds, creeks, and island networks, which are more widely available as potential habitat than in the other estuaries sampled in Florida, where wetlands have been impounded or have been lost to development.

As juvenile snook reach 100–150 mm SL, marked changes in their tolerance to high temperature and low dissolved oxygen occur, and juvenile snook are no longer abundant at the initial nurseries described above. Larger juveniles (151–350 mm SL) were found downstream or bayward of the initial nurseries. These data suggest that small juveniles occupy coastal-wetland ponds, creek networks, and riverine backwaters and subsequently inhabit the entrances to these areas as large juveniles before dispersing more broadly throughout the estuary. The general pattern of snook movement toward open water with size could be complicated at the most remote and isolated locations, which are highly dependent on water level in establishing connectivity to adjacent habitats. Anthropogenic changes in freshwater and tidal delivery to these wetland systems could effectively disconnect juvenile

snook habitat from the estuary. Continuing to refine life-history models to reflect habitat use in individual estuaries will aid in resource management at a local level, enabling managers to target specific areas for protection, land acquisition, and restoration.

HABITAT PREFERENCES OF JUVENILE COMMON SNOOK IN THE LOWER RIO GRANDE/BRAVO DEL NORTE

Caleb G. Huber, Kevin L. Pope, and Reynaldo Patiño*

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The common snook, *Centropomus undecimalis*, is a euryhaline species that can tolerate a wide range of salinities. This species also displays protandric hermaphroditism, in which all individuals first develop as males and then change sex to females as they reach a certain size range. Common snook were once abundant off the Texas coast and supported commercial and recreational fisheries. However, its populations are now characterized by low abundance and erratic recruitment that may be caused by habitat degradation and historical overfishing. Although the lower stretch of the Rio Grande is believed to provide nursery habitat for common snook in Texas, little is known about the specific biology and habitat needs of juvenile snook along the Texas coastline. Knowledge of the general biology of common snook is a prerequisite for the development of management strategies designed to increase the numbers of wild snook. The primary objective of this study was to describe the habitat preferences of juvenile common snook in the lower portion of the Rio Grande, Texas. Fish were collected during January-March 2006 from the lower 51.5 km of the river using multiple gears; a trawl net was used to sample the river channel, and a castnet and boat electrofishing were used to sample the river bank. Measurements of water quality (temperature, dissolved oxygen, conductivity, etc.) and other habitat traits (bank slope, presence of vegetation or woody debris, flow, etc.) were recorded at each sampling site.

A total of 211 common snook were captured. Fish size-frequency distribution and otolith analyses revealed that most common snook collected were age-1 or age-2 fish of up to 303 mm SL. Histological analysis of the gonads indicated that these fish were juvenile males (a single fish of 360 mm SL was caught that appeared to be of a larger size class). A single, incidental electroshock of the river channel indicated that adult male and female common snook (up to 595 mm SL) are also present in the river. All common snook were captured in freshwater habitat (above river kilometer 12.9.) Because juvenile (age 1 and 2) common snook are able to withstand saline waters, their absence in the estuarine portion of the river suggests that they are choosing riverine habitat based on traits other than water salinity. Multivariate analyses revealed that the distribution of juvenile common snook within the freshwater portion of the river was not random but weakly associated with turbidity, temperature, conductivity, pH, and perhaps substrate type. This observation suggested that habitat preferences of juvenile common snook in the freshwater portion of Rio Grande are dictated by a complex interaction of

multiple environmental variables or by factors not measured and not included in the present analysis such as available forage or predation pressure. It is concluded that nursery habitat for common snook is available only in the freshwater portion of the Rio Grande.

COMPARACIÓN DE EDAD Y CRECIMIENTO DEL ROBALO BLANCO (*Centropomus undecimalis*), EN DOS DIFERENTES SISTEMAS TROPICALES, TABASCO, MEXICO

Martha A. Perera-García*, Manuel Mendoza-Carranza, Wilfrido Contreras-Sánchez, Eunice Pérez-Sánchez, Allyse Ferrara, Maricela Huerta-Ortiz, Salomón Páramo-Delgadillo.

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El robalo blanco, *Centropomus undecimalis,* constituye una de las principales actividades económicas de las comunidades que se encuentran a lo largo del litoral y aguas interiores del Golfo de México. La captura de robalo, es considerada una pesquería artesanal y se ha estudiado de manera aislada; existen trabajos del área de la biología, pero son escasos aquellos que dimensionan la dinámica poblacional. El objetivo de este estudio fue comparar la estructura de edad y crecimiento del robalo blanco *C. undecimalis* de la zona costera y zona ribereña.

Mensualmente, desde julio de 2006 a marzo de 2008, se obtuvieron organismos de la zona costera (Barra el Bosque, Barra San Pedro) y de la zona ribereña (Tres Brazos, San Pedro, Balancán). De cada ejemplar, se tomó la longitud furcal (cm), peso eviscerado (g), se determinó el sexo y se extrajeron los otolitos *sagitta.* Los otolitos fueron seccionados (0.5 mm) con una cortadora Buehler Isomet, los cortes fueron montados con resina sintética, se contaron las bandas de crecimiento con un microscopio. Se realizó el análisis de incremento marginal para validar la formación de bandas de crecimiento. Se estimo la relación longitud-peso, y la relación longitud-radio del otolito. Los parámetros de la ecuación de Von Bertalanffy en longitud fueron estimados por métodos lineales y el método no lineal de Levenberg-Marquardt's.

Se analizó 557 otolitos seccionados. Las edades estimadas fueron de 2 a 17 años. La relación longitud-peso por sexos fue significativa (R^2=0.9). No se encontró diferencias significativas (X^2, P>0.05), en la formación de incremento marginal entre las áreas geográficas. Existe relación significativa entre la longitud furcal (LF) y radio del otolito (RO) de la zona costera y zona ribereña (P<0.01).

Los parámetros de la ecuación de von Bertalanffy en longitud fueron para la zona costera: Barra Bosque $Lf=109.21(1-e^{-0.21(t+0.57)})$, Barra San Pedro $Lf=94.56(1-e^{-0.27(t+0.48)})$, y para la zona ribereña: San Pedro, Balancán $Lf=97.15(1-e^{-0.17(t+1.32)})$ y Tres Brazos $Lf=83.77(1-e^{-0.26(t+0.49)})$, es importante mencionar que los resultados para Tres Brazos no se deben considerar sólidos debido a que la muestra total no fue significativa durante el periodo de estudio.

La prueba T^2 Hotelling mostró diferencias significativas entre los parámetros de crecimiento de machos y hembras (P<0.01). El análisis de la suma de cuadros residuales (RSS) indicaron que las curvas de crecimiento entre las poblaciones fueron estadísticamente diferentes (F=74.08, P=0.05). En general los valores obtenidos son característicos de un crecimiento relativamente lento, especies longevas, lo que hace la necesidad de estudiar y proteger esta especie de la sobreexplotación pesquera.

USE OF RIVERS AND AN OPEN ESTUARY BY COMMON SNOOK *Centropomus undecimalis* AND COMMENTS ON FACTORS INFLUENCING CYCLICAL SEASONAL MOVEMENTS

David A. Blewett*, Philip W. Stevens, Ronald G. Taylor, Thomas R. Champeau, and Brent L. Winner.

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dave.blewett@myfwc.com

Common snook were collected monthly in the Charlotte Harbor estuary from 1997 to 2007 using a 183-m haul seine (2,244 stratified-random samples) to determine their distribution, abundance, size, habitat use, and diet. Common snook were abundant in the estuary (~3.5 fish/100 m shoreline) and ranged in size from 100 to 1,140 mm TL. They were found in a variety of habitats; however, their abundance was significantly greater at sites with mangrove shoreline vegetation, seagrass bottom vegetation, and a high overall abundance of potential prey. Most large catches of common snook (>20) were made during summer in higher saline waters within the sounds and along the barrier islands. They fed on a wide variety of prey within the estuary; at least 37 different prey taxa were identified, including 19 taxa that had not been previously reported. Fishes made up 71% of the prey by number and three prey taxa made up almost 50% of the diet numerically – *Lagodon rhomboides*, *Anchoa* spp., and *Farfantepenaeus duorarum*. An ontogenetic shift in the prey preference of common snook was identified at around 550 mm SL. A significant, positive relationship between predator size and prey size was observed, and the size selection of their prey contributed to some seasonal differences in their consumption of *L. rhomboides*. Seasonal electrofishing surveys targeting common snook were conducted from 2004 to 2006 in the three major rivers leading to the Charlotte Harbor estuary. Common snook collected in the rivers ranged in size from 100 to 1,085 mm TL. They were abundant (~2.8 fish/100 m shoreline) and widespread in all three rivers, with catch rates even greater than those of a dominant freshwater predator, the largemouth bass. Common snook fed on a wide variety of prey in the rivers; at least 30 different prey taxa were recorded. The most numerous and frequently collected taxa were *Gambusia holbrooki*, *Hoplosternum littorale*, and *Procambarus* spp.

Sampling the rivers of southwest Florida provided an opportunity to examine a long held but untested theory – that common snook move from open estuarine and coastal marine habitats into rivers during colder months to find warmer or more stable water temperatures (the overwintering paradigm). Seasonal abundance of common snook in the tidal freshwater portions of the rivers was high in spring and summer, doubled in fall, and then decreased slightly in winter. In the open estuary, their abundance was significantly lower in winter compared to spring, summer, and fall. No specific size group alone appeared to be involved in these seasonal changes, as length frequencies did not differ between seasons. Although these results provide evidence that a portion of the population moves between the rivers and the open estuary, the reasons for these movements require further study. The strong presence of common snook in the rivers during summer and their peak abundance in fall as opposed to winter were unexpected and suggests that their

use of freshwater and marine environments is more complex than previously considered. Our current research is exploring the effects of river flow in relationship to the abundance of common snook, which may prove to be an important factor regarding their seasonal movements to rivers.

APERIODIC, NOVEL MIGRATIONS AND USE OF RIVERINE HABITATS BY COMMON SNOOK: EVIDENCE FOR SKIP SPAWNING

Ronald G. Taylor*, David A. Blewett, Alexis A. Trotter, Phillip W. Stevens, and Robert Muller.

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Common snook support a valuable recreational fishery that contributes significantly to Florida's economy and have been the subject of numerous diverse studies that defined population level aspects of their life history, reproductive biology, and fishery dynamics. High levels of exploitation have resulted in numerous stock assessments with allied regulations; however the stocks remain in inexplicable over-fished conditions. Current assessment models contain assumptions based on historical reproductive schedules measured at the population level during the early 90's while integration of newly discovered variation of spawning dynamics may reveal that the stocks are more or less robust than predicted and that the current level of regulation is not appropriate. We conducted an acoustical telemetry study of the movements and migrations of individual common snook in the tidal reaches of the Caloosahatchee River, a major tributary of Charlotte Harbor, the largest estuary of southwest Florida. We implanted acoustic transmitters into adult common snook and monitored their movements along a 50 km stretch of the Caloosahatchee River. Locations and movements of individual snook were detected and recorded with an array of permanently deployed acoustic receivers along the lower 50-tidal km of the river. We determined that 40% (6/15), 44% (4/9), and 20 % (1/5) of the tagged snook remained inside the monitored portion of the river during 2005, 2006, and 2007, respectively. Overall, 65% of the tagged snook completed annual migrations outside our study area during the three year investigation, presumably to spawn. The mean total length (TL) of the 'residents' was significantly smaller than the mean TL of the 'migrants'. The onset, duration, and destination into the high salinity locations of the estuary of these annual migrations range within the findings of previous reproductive studies, however individual dynamics vary. The mean date of departure from and return to the array for the migrants was mid-June and early September, respectively. The mean number of days the migrants was absent from the array was 73 d (range 40 – 106 d). Fishery dynamics for the study group were calculated for year 1 (2005-2006) as follows: migrants = 9 (60%), residents = 6 (40%), exploitation (μ) = 20 or 27%, annual survival (S) = 9/15 or 60%, total instantaneous mortality (Z) = 0.51, total instantaneous fishing mortality = 0.25 or 0.34, and non-reporting = ~ 25%. For year 2 (2006- 2007) the parameters were: migrants 5 (56%), residents 4 (44%), μ = 11 or 33%, S = 5/9 or 56%, Z = 0.58, F = 0.15 or 0.43, and non-reporting = 66%. This high resolution information on the behavior and fate of individual snook should be incorporated into future stock assessments because this detailed data indicates differential individual contribution to total stock biomass which may affect the predicted condition of stocks.

BIOLOGIA REPRODUCTIVA Y POBLACIONAL DEL ROBALO BLANCO, *C. undecimalis*, EN DOS AMBIENTES TROPICALES, TABASCO, MÉXICO

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En Tabasco, la abundancia de *C. undecimalis* es escasa durante cierta época del año debido a los efectos de la sobreexplotación, principalmente cuando esta especie realiza migraciones durante el periodo reproductivo hacia las desembocaduras de los ríos, lo cual representa un peligro para las poblaciones. El objetivo de este estudio fue estudiar la biología reproductiva y poblacional de *C. undecimalis* capturados en la zona costera y ribereña de Tabasco.

Mensualmente, desde julio de 2006 a marzo de 2008, se obtuvieron organismos de la zona costera (Barra el Bosque, Barra San Pedro) y de la zona ribereña (Tres Brazos, San Pedro, Balancán). De cada ejemplar, se tomó la longitud furcal (cm), peso eviscerado (g), se determinó el sexo y el grado de madurez gonádica macroscópicamente. Se extrajeron los otolitos *sagitta,* los otolitos fueron seccionados (0.5 mm), los cortes fueron montados con resina sintética, se contaron las bandas de crecimiento. Se estableció la estructura poblacional, la relación longitud-peso, proporción sexual, el índice gonadosomático (IGS) y la talla y edad promedio de madurez sexual.

Se analizaron 790 organismos, el intervalo de la longitud furcal se muestra en la Figura 1. Se detectó diferencias significativas entre las longitudes medias de machos y hembras en ambas zonas (K.W., $p<0.05$). La proporción total de sexos, difirieron significativamente (X^2; $p<0.05$). La relación longitud-peso en la zona costera para ambos sexos fue $Pev=0.0059(LF)^{3.07}$ y para zona ribereña de $Pev=0.0086(LF)^{2.98}$, no se detectó diferencias significativas en la relación talla-peso para machos y hembras (Ancova, $P>0.5$). En la zona costera, el promedio mensual del IGS de machos mostró una tendencia similar al de las hembras, el periodo máximo de reproducción fue de abril a septiembre, se observó una relación con la época de lluvias y la temperatura. La L_{50} fue a los 64cm en los machos y de 80cm en las hembras. En relación a la edad de primera madurez sexual, los resultados fueron para los machos=5.8 años y hembras=8 años. El entendimiento de la biología reproductiva del robalo blanco, en tiempo como geográficamente, es fundamental para su manejo y determinar como la presión de pesca afecta la proporción de sexos a largo plazo.

Figura 2. Frecuencia relativa por tallas para machos y hembras de *C. undecimalis*, en (a) zona costera y (b) zona ribereña, Tabasco, México.

MORTALIDAD TOTAL DE ROBALO PRIETO (*Centropomus poeyi*) EN LA LAGUNA DE ALVARADO, VER., MÉXICO (2005-2008)

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Los robalos son especies que tienen importancia comercial tanto en la Laguna de Alvarado como en otros estados de la república Mexicana, y Veracruz se caracterizaba por tener la mayor captura dentro en el Golfo de México, pero a partir del año 2000 disminuyeron sus capturas, en cambio en Tabasco y Campeche aumentaron. Esto despertó la inquietud de las autoridades y pescadores para llevar a cabo estudios que se encaminaran a la regulación de la pesca, considerando uno de los lugares con capturas importantes de estas especies, inicialmente el objetivo principal de estimar la Captura por Unidad de Esfuerzo (CPUE) como un índice de abundancia de robalo prieto (*Centropomus poeyi*), pero posteriormente se cambio al estudio biológico pesquero y se incluyó el robalo blanco (*C. undecimalis*), y entre los objetivos se contempló la estimación de la mortalidad.

El estudio se inició en el año de 2005. Las zonas de muestreo fueron principalmente en la boca-barra de la Laguna de Alvarado, y de las capturas provenientes de los diferentes ríos que confluyen en dicha laguna y de lagunas cercanas que conforman el sistema lagunar de Alvarado. Se tomaron datos de tallas, pesos, sexo y madurez sexual de cada ejemplar, además se registró información relacionada con el esfuerzo pesquero, y se continuaron registrando los mismos datos para las dos especies hasta el 2008.
Para estimar la mortalidad total se requieren los parámetros de crecimiento, y para ello se procedió a estimar primeramente los grupos de edad por el método de Cassie (1954). A partir de los grupos de edad, se obtuvieron los parámetros de crecimiento: L_∞ se calculó con el método de Ford-Walford (1946), el valor de K se determinó con el valor de *b* resultante de la regresión de las tallas promedio de los grupos de edad (L_t y L_{t+1}) con la fórmula k = -1*lnb, y t_o con el gráfico de von Bertalanffy. Una vez obtenidos estos parámetros, se estimó la mortalidad total (Z) con la ecuación de Beverton y Holt (1956), $Z = k*((L_\infty - x)/(x-L'))$.

Obteniendo como resultados cinco grupos de edad, L_∞ igual a 116.9 cm, para *k* 0.342 y para t_o de 0.8955. Las estimaciones de mortalidad total (Z) para el año 2005 fue de 0.99, en el 2006 de 1.11, en el 2007 de 1.18 y en el año 2008 1.64.

Los valores de mortalidad total, indican que en el año 2005 este recurso estaba siendo explotado medianamente, pero en los años siguientes ha estado sometido a una explotación intensiva.

Palabras clave: Parámetros de crecimiento. Mortalidad. Explotación.

THE DIFFICULTIES WITH DEFINING THE SPAWNING SEASON FOR SNOOK IN TEXAS

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Prior to 1940, Common Snook, *Centropomus undecimalis*, were relatively abundant on the Texas coast and were commercially fished. In recent times, the Texas snook population has been small and highly variable. This depletion in snook can be attributed to various factors, namely over-fishing, reduced freshwater inflow and periodic freezes. More information regarding spawning season and size of the spawning population is needed to adequately manage this species along the Texas coast and to condition captive broodstock with appropriate photoperiod temperature regimes. Currently the snook population appears to be increasing in south Texas and juveniles are commonly captured in brackish canals and the Rio Grande. However, to date only one female has been captured in late stage maturation out of 100 adult snook sampled in the spring summer and fall. Thus the spawning season for common snook in south Texas is uncertain. In the bays and inlets sampled, the sex ratio for Texas snook appears skewed towards males, even at larger sizes. The size at female sex is similar to that reported for the East coast of Florida ranging from 650 to 900 mm TL. An analysis of trends in gonadal development seen in two years of sampling will be discussed as well as ongoing research to locate snook spawning aggregations in south Texas.

VARIATIONS IN COMMON SNOOK (*Centropomus undecimalis*) CPUE AND CLIMATIC VARIATION IN SOUTHEASTERN MEXICO COASTS.

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In this research are presented evidences of the relationship among common snook (*Centropomus undecimalis*) CPUE variations and environmental conditions (sea surface temperature and pluvial precipitation) in the coastal zone of Tabasco, Mexico from September 1999 to December 2007. Pluvial precipitation data were obtained from the Comisión Nacional del Agua and SST data from satellite images provided by The International Research Institute for Climate and Society. Monthly average data were standardized (Std. Score = (raw score -mean)/Std. deviation) and smoothed with moving average, multiple and simple correlation were tested. Results show that in the first quart of the analyzed period (May 2000 to December 2007) the peaks of Effort and CPUE precede the highest values of PP and SST (June to August each year). While in 2002 the highest values of effort (fishermen behavior) were observed from October to December (152 vessels/month), this is probably related to a change in annual climatic conditions. Along 2002 were registered anomalous peaks of PP during June (250mm), September (425mm), and November (300mm).

This change in rain pattern is coincident with 2002 El Niño event. After it the effort and CPUE repeated the behavior previously described, although CPUE was low (average 13.7 kg/vessel). We concluded that effort (fishermen behavior) is influenced principally the previous experiences about prevailing climatic conditions based principally on pluvial precipitation (Fig 1). Nevertheless, CPUE reflects success of the recruitment process of common snook into fishing area in response to the change in climatic conditions.

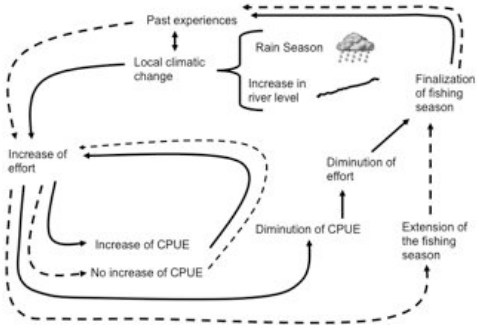

Fig. 1. Fishermen behavior in two climatic conditions.

ASPECTOS REPRODUCTIVOS DE ROBALO BLANCO (*Centropomus undecimalis*) EN EL RÍO PANUCO, VERACRUZ

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En la población del robalo existe en la actualidad una fuerte presión pesquera y un decremento tanto en su captura como en el hábitat disponible para su desarrollo, debido a que es una especie que sostiene dos pesquerías importantes, la que se desarrolla en las lagunas y ríos y la que se desarrolla en la costa y altamar. A pesar de que existe la veda de reproducción, en esta zona se considera desfasada la fecha propuestas en esa normatividad, incrementándose la captura en fechas cuando la especie se encuentra más vulnerable al agruparse en cardúmenes con fines reproductivos. Aunado a ello es un recurso que alcanza su madurez sexual en tallas mayores de los 50cm y la captura se realiza con artes de pesca poco selectivos además de un sistema de pesca inadecuado y el incremento de pescadores libres.

Se realizaron muestreos mensuales de junio a septiembre de 2008, con robalos capturados en el Río Pánuco, con red de arrastre y red trasmallo de luz de malla 6 pulgadas, registrando los datos morfométricos, sexo y madurez sexual. Se midieron un total de 332 robalos blancos, los cuales se distribuyeron entre las tallas 520 y 1,350mm de longitud total, con peso total variable de 2,650 a 12,750grs; la talla promedio en las hembras fue de 849mm y en machos de 798mm. La proporción hembra-macho fue de 1:1; el periodo reproductivo se observó desde junio, apareciendo la fase 5 de desove con el 21%, el 53% en julio, y para agosto y septiembre se presenta el máximo valor promedio del 68% (fig.1). La hembra sexualmente madura mas pequeña fue de 710mm de longitud total y la talla de madurez al 50% (Lc50) en hembras maduras fue de 870mm, mientras que el macho sexualmente maduro mas pequeño fue de 690mm, y el Lc50 fue de 800mm. De acuerdo a la curva de selectividad la L50% obtenida con redes de luz de malla de 6 pulgadas utilizada para la captura fue de 790mm en el total de la muestra.

Con los resultados obtenidos se puede observar que los machos son ligeramente mas pequeños que las hembras y que su madurez sexual la alcanzan a tallas menores. Se considera como fecha tentativa de veda adecuada para esta zona entre agosto y septiembre y una luz de malla en las redes optima para su captura de 6 pulgadas.

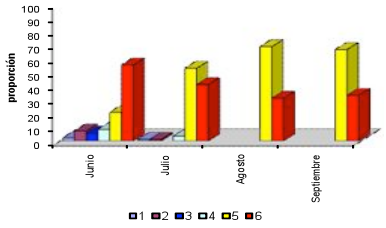

Figura 1. Proporción de las fases de madurez gonádica de robalo blanco *Centropomus undecimalis*, en el Río Panuco, Veracruz. 2008.

REPRODUCCIÓN DE ROBALO PRIETO (*Centropomus poeyi*) Y ROBALO BLANCO (*C. undecimalis*) EN LA LAGUNA DE ALVARADO VER., (PERIODO 2005- 2008)

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Debido a la importancia comercial de estas especies en la Laguna de Alvarado, a la disminución de la captura y la solicitud de los pescadores para cambiar el periodo de veda establecido oficialmente (del primero de julio al quince de agosto) se planteo este estudio cuyo propósito fue conocer la época de reproducción, con el fin de ratificar o rectificar dicho periodo.

Para ello se hicieron muestreos biológicos de cuatro a diez días por mes, en los años 2005, 2006, 2007 y 2008 en la boca-barra de la Laguna de Alvarado, y en las capturas provenientes de los diferentes ríos que confluyen en la Laguna y de lagunas cercanas que conforman el sistema lagunar de Alvarado, los datos registrados de cada ejemplar, incluyeron longitud total, sexo, madurez sexual, y peso, también se colectó información acerca de las características ambientales. La información obtenida se proceso mes por mes, se obtuvo el periodo de reproducción, la talla L_{C50} de madurez sexual, el crecimiento en relación a su peso, crecimiento individual entre otros.

En el año 2005 se midieron 223 robalos prieto y 163 robalos blancos; en el año 2006 fueron 210 robalos prietos y 885 robalos blancos y en el año 2007, fueron 1261 robalos blancos y 493 robalos prietos; y en el año 2008 se muestrearon 199 robalos prietos y 715 robalos blancos.
La reproducción para robalo prieto en el año 2005 se desarrollo en los meses de junio, julio y agosto siendo en julio en pico máximo, y se observó que durante estos meses se intensificó la época de lluvias; en el 2006 el periodo de reproducción fue de julio a septiembre, con un pico máximo en julio, también durante estos meses se intensifica las lluvias; en el año 2007 el periodo de reproducción fue de agosto a septiembre con pico en agosto (en este año las lluvias iniciaron a finales de julio), y en el año de 2008 la reproducción fue de junio a septiembre con un pico máximo en julio.
Con respecto al robalo blanco: El periodo de reproducción en el año 2005, fue de junio a agosto con un pico máximo en julio; en el 2006 fue de julio a septiembre con pico máximo en julio; en el año 2007 fue de junio a septiembre con un máximo en julio; en el año 2008 fue de junio a agosto con un máximo en agosto.

Se concluyó que las dos especies tienen un comportamiento reproductivo similar y que aparentemente las lluvias influyen en la reproducción.

Palabras clave: *Centropomus poeyi*. *C. undecimalis*. Veda. Características ambientales

FECUNDIDAD DE ROBALO BLANCO, *Centropomus undecimalis* EN LA COSTA DE CIUDAD DEL CARMEN, CAMPECHE

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Resumen
La población de robalo blanco *Centropomus undecimalis*, constituye una de las pesquerías ribereñas de mayor tradición e importancia económica en los estados de Campeche, Tabasco, Veracruz y Tamaulipas. Los estudios de fecundidad de los recurso pesqueros de escama son básicos dentro de las investigaciones biológico-pesqueras, que integrados a los estimados de la dinámica de las poblaciones, permiten las evaluaciones del tamaño de la población, caracterización de unidades de población, estimación del potencial reproductivo, entre otros. Se realizaron muestreos mensuales de la captura comercial ribereña de robalo blanco, en la localidad pesquera de Ciudad del Carmen, Campeche. Durante el período de reproducción de la especie de mayo a julio de 1987, se colectaron 33 gónadas maduras, estadio IV (Nikolsky, 1963) así como los datos de longitud total y peso total de las hembras. El sistema de captura utilizado fue la red de enmalle de 500 m de largo, 50 mallas de caída y tamaño de luz de malla de 6 pulgadas. La estimación de la fecundidad, se realizo a través del método propuesto por Vasconcelos-Pérez *et al.* (1976). La fecundidad promedio de robalo blanco se estimó en 3,260850 huevos, considerada alta entre las especies de escama. Asimismo se estableció las relaciones de fecundidad-longitud y fecundidad-peso de acuerdo a Bagenal *et al*. (1978a) con los siguientes resultados, respectivamente.

$F = 0.0109\ L^{4.2693}$

$F = -\ 1{,}917585.78 + 841.289\ P$

El análisis estadístico, de ANOVA indicó que existen diferencias significativas ($p < 0.05$) entre las fecundidades por talla y peso, calculadas.

TALLA DE PRIMERA MADUREZ Y COMPOSICIÓN DE LAS CAPTURAS DEL ROBALO BLANCO EN SUR DE CAMPECHE

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El robalo blanco (*Centropomus undecimalis*) es una de las especies de mayor importancia comercial en la región suroeste de Campeche. Se captura con redes de enmalle y soporta dos pesquerías. La que se realiza en lagunas y ríos y la de la franja costera. Las capturas más abundantes son durante la época de reproducción y cuando se inicia el reclutamiento de juveniles a las zonas de pesca, que es cuando más se capturan organismos que no han llegado a su primera reproducción. Para contribuir con información a la elaboración de los planes de manejo y la NOM (Norma Oficial Mexicana), el presente trabajo tuvo como objetivo ampliar el conocimiento sobre la relación que existe entre la talla de primera madurez y la composición de la captura comercial de la especie en el sur de Campeche.

Se analizó la composición de la longitud furcal (LF) de las capturas, la talla de primera madurez y la del 50% de organismos maduros, de robalo blanco de los años 1997, 2002, 2003, 2007 y 2009. Se hizo la comparación de la información por mes y por año, tanto entre la composición de las capturas como con las tallas de madurez y el 50% de madurez sexual. Por otra parte se hizo un análisis la relación que existe entre la composición de las capturas con la talla de primera madurez. Se tomó como talla de primera madurez cuando en la información que se obtuvo se encontró al menos una hembra o macho totalmente maduro. El 50% de organismos se obtuvo haciendo la frecuencia de LF acumulada por intervalos de clase, tanto de machos como de hembras.

Del análisis se obtuvo que la composición de la captura es muy similar en todos los años, sin embargo la talla de primera madurez y el 50 % de organismos maduros, ha disminuido de 86 cm. LF en 1997 a 76-78 cm. LF en 2007.
Aún y cuando se han encontrado organismos maduros entre 53 y 60 cm. de LF, si es importante mencionar que no se han encontrado tallas inferiores a los 50 cm. ovados.

En la figura1 se observa la estimación de $L_{m50\%}$ de robalo en 2007.

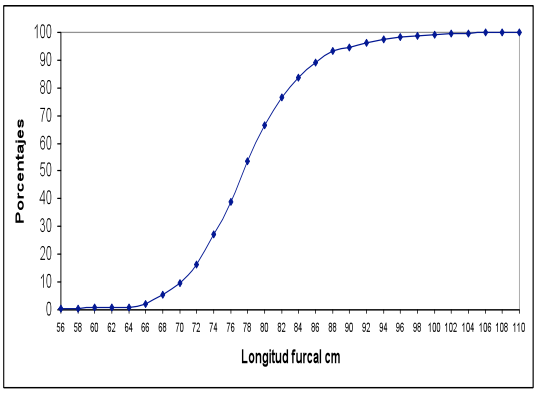

Fig. 1 Talla $L_{M50\%}$ de robalo blanco para sur de Campeche 2007.

PROPUESTA PARA LA REGULACIÓN DE LA CAPTURA DE ROBALO EN EL GOLFO DE MEXICO

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De acuerdo con las estadísticas oficiales (Anuarios Estadísticos de Pesca), en el Golfo de México, el recurso pesquero de robalo históricamente ha tenido importancia comercial, en Campeche, Tabasco y Veracruz. En los dos primeros estados la producción ha aumentado en 2001 y 2002, en cambio en Veracruz ha disminuido a partir del año 2000. No existen medidas de regulación pesquera generales aplicables al Golfo de México. A pesar de que existe una veda diferencial en tiempo en Veracruz y Tamaulipas, además, de manera local en Campeche se manejan, avisos de veda temporal implementadas por la Subdelegación Federal de Pesca (durante cinco días antes y cinco después de luna llena en los meses de junio a agosto). Por ello se consideró importante, elaborar un anteproyecto de Norma pesquera para regular la captura de los robalos en los Estados del Golfo de México.

Se revisaron todos los trabajos realizados en los diferentes estados y la normatividad aplicable y compatible, por lo que se hizo un documento base entre los investigadores de la Dirección General de Investigación Pesquera en el Atlántico, que se sometió a discusión en cuatro reuniones, con los investigadores de diferentes instituciones que han trabajado con la pesquería del robalo blanco (*Centropomus undecimalis*) y robalo prieto (*C. poeyi*). Este documento fue analizado y discutido en especial las recomendaciones técnicas, se incorporaron nuevas y se afinaron las que ya estaban, para quedar consensuadas en un documento final el cual quedo sustentado con la bibliografía correspondiente.

Las recomendaciones resultantes fueron encaminadas básicamente a las tallas mínimas de captura y a establecer un período de veda para las capturas de ambas especies. Del robalo blanco, se estimó una talla mínima de captura para las hembras de 85 cm de longitud total y en los machos de 80 cm; respecto del robalo prieto, se estimó una talla mínima de captura para las hembras de 82 cm de longitud total y en los machos de 78.

El periodo de veda propuesto fue, del 1 de julio al 15 de agosto de cada año, para proteger a los organismos durante el pico reproductivo. Se plantea una talla mínima de captura la de 85 cm con el fin de proteger a ambas especies y a los dos sexos en estado maduro. Con mismo enfoque se plantearon, las especificaciones de los artes de pesca, que serian redes de enmalle o agalleras, con luz de malla mínima de 152 mm (6 pulgadas) o mayores.

SITUACIÓN ACTUAL DE LA REPRODUCCIÓN DEL ROBALO BLANCO EN CAUTIVERIO, EN LA UMDI, SISAL, YUCATAN

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A la fecha, Una las especies de peces trabajadas en la UMDI sobre aspectos reproductivos es: el robalo blanco, *Centropomus undecimalis*; con esta especie se inicio el Programa; Cultivo de Peces Marinos; y se mantiene el objetivo original, de adecuar y/o desarrollar la tecnología existente para su cultivo con énfasis en la reproducción controlada.

La UMDI, es de reciente creación en Yucatán pero el Proyecto; Cultivo del robalo blanco, tuvo su origen años atrás en Cd del Carmen Campeche, donde se hicieron los primero ensayos sobre reproducción y se inició el banco de reproductores. Ahí fue donde se obtuvieron los primeros resultados positivos en maduración, desove en cautiverio y cría de larvas; de igual manera surgieron muchas interrogantes que consideramos como problemas prioritarios; por ejemplo, que factores determinan la maduración del robalo blanco en cautiverio?; cual es el mejor esquema para el cultivo larvario?. Las hembras de robalo blanco muy rara vez alcanzan la maduración final espontáneamente en cautiverio, pero a partir de cierto grado de maduración, se puede inducir con hormonas sintéticas; así, al hablar de maduración, nos referimos a, cuando los ovocitos alcanzan como mínimo 350 µ de diámetro.

Respecto al primer problema, surgieron algunas hipótesis respecto a los factores principales que inician la maduración, algunas de las cuales hemos intentado probar: el fotoperíodo fue uno de las variables a considerar, pero no hubo éxito; También, se utilizo la testosterona como agente promotor de la espermiación y el éxito fue parcial; la calidad de la dieta y la salinidad han sido otras variable incluidas; en cuanto a las larvas; únicamente se han ensayado los protocolos sugeridos por algunos investigadores, para la cría larvaria y la sobrevivencia ha sido baja; además se han hecho algunos ensayos sobre su fisiología.

En el 2008 se hizo un ensayo donde se aplicaron shocks de baja salinidad a 4 lotes de reproductores durante un mes; además se incluyo una dieta semihumeda, con pescado, calamar y Breed-M de INVE. Encontramos que, en agosto, el lote donde se aplico el cambio de salinidad en abril, maduraron 4 de 7 hembras y los dos machos, y en el control (alta salinidad constante) maduraron 2 de 7 hembras y los dos machos; en el resto de los tratamientos, no se encontraron hembras maduras y el 70% de los machos eran espermiantes, aunque con poco esperma. Actualmente se corre un experimento con el mejor tratamiento anterior, con el fin de conocer la repetibilidad de los resultados anteriores, esperando un número importante de hembras maduras y machos espermiantes. Es notorio el hecho que las hembras solamente han madurado de julio a septiembre.

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AN OVERVIEW OF COMMON SNOOK (*Centropomus undecimalis*) BROODSTOCK MATURATION AND SPAWNING RESEARCH IN FLORIDA

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Common snook (*Centropomus undecimalis*) are a very popular and important sportfish along the coast of Florida. Population declines in Florida, Texas and Mexico have generated interest in developing reliable aquaculture techniques to produce snook for stock enhancement and commercial production. Early attempts to spawn common snook in captivity were unsuccessful and efforts shifted to obtaining larvae from strip spawned wild fish or to capturing and inducing spawning in mature fish with hormones following capture. These approaches were not effective for large-scale production due to variability in catch of mature female snook and environmental influences on spawning success (e.g., presence of red tide). To more effectively obtain fry for stock enhancement, research at Mote Aquaculture Research Park shifted in 2005 to maturation and spawning of captive snook. Maturation methods include photo-thermal manipulation and hormone induced final maturation. Broodstock populations consist of wild caught fish, with an average of 14 fish per tank (7♂and 7♀). The broodstock systems (45,000 L volume) are 6.1 m diameter by 1.83 m deep tanks including biofilter, solids filter, UV sterilizers and heater-chiller unit. Snook were fed a regime consisting of a fresh frozen diet (50% shrimp, 25% squid, and 25% herring). After observing a possible nutrition problem, we tried many different feeding approaches in 2007 to increase and insure adequate levels of vitamins and later arachidonic acid (ARA). We determined that the best approach to delivering vitamins and ARA was a protein capsule containing the vitamin premix, which was placed in the fish or squid. In 2005, our captive snook populations where shifted into a shortened winter cycle, which included a 2 month winter (24°C; 12 hr light), one month spring (26°C; 13 hr light), followed by a prolonged summer (30°C; 14-15 hr light). Both photoperiod and lunar cycles are believed to be important in snook maturation. Solar 1000 lighting units allow us to control both daylight and lunar cycling. Previous research indicated that snook do not reach final maturation under photo-thermal control; although, maturation to a vitellogenic/post-vitellogenic stage was possible, which was observed in our fish. Using a dose of 50 µg/kg of gonadotropin releasing hormone analog (GnRHa) for mature females, we achieved the first captive spawns with common snook in May 2006. Fish were implanted three additional times in 2006, each roughly a month apart with varying spawning and larval success. In 2007, we successfully spawned snook out of season, nearly 2 months before the natural spawning timeframe. We had four successful spawning events and fish were then cycled back to winter in September. Besides spawning out of phase, the other major differences in 2007 were modifications in diet, allowing at least 6 weeks between sampling and hormone implantation, allowing fish to recover from sampling stress and implanting immature females and all males at 25 µg/kg GnRHa. In 2008, we added protein pellet enrichments to the feed and had 4-5 successful spawning events in our 3 tanks. Also in 2008 and in 2009, the feeding regimes were set up to look at ARA diet enrichments and determining the importance of ARA for egg quality and early larval survival. At this point in our study, there is a positive

correlation with the level of ARA in the eggs and the fish being fed the ARA supplement. More data is needed to determine egg/larval success based on ARA enrichment.

INDUCCIÓN DE LA REPRODUCCIÓN EN *Centropomus parallelus* BAJO CONDICIONES DE CAUTIVERIO EMPLEANDO INYECCIONES E IMPLANTES DE GnRH-a

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El robalo blanco (*Centropomus undecimalis*) y el chucumite (*Centropomus parallelus*), son especies de peces ampliamente distribuidas en la costa Atlántica de América, desde el sur de Estados Unidos hasta el sur de Brasil. En México son comunes en los Estados costeros del Golfo, particularmente en Tamaulipas, Veracruz y Tabasco en los cuales poseen un alto valor comercial. En el presente estudio se realizaron dos experimentos para la inducción de la reproducción *Centropomus parallelus* empleando inyecciones e implantes de GnRH-a para evaluar la efectividad de la hormona y determinar la calidad de huevos y larvas obtenidos a partir de esta hormona. En el primer experimento se utilizó la técnica de inyección (probando dosis de 75 y 150 µg/kg de pez), en el cual solo se evaluó la efectividad de la hormona y el diámetro de los huevos antes y después de la inyección puesto que no hubo desoves. De este modo se determinó que la dosis de 150 µg/kg fue la mejor al presentar el mayor diámetro de huevos antes y después de la inducción (Fig. 1). En el segundo experimento se empleó la técnica de implantes hormonales, determinándose la efectividad de la hormona, así como la calidad de huevos y larvas puesto que en las dosis probadas (100 y 200 µg/pez) se presentaron desoves; ambas dosis fueron mejores con respecto al control para el diámetro inicial de los huevos y huevos desovados (Fig. 2). Sin embargo, con la dosis más alta se obtuvo mejores resultados con huevos de hembras canuladas días después del desove. Se obtuvo un porcentaje de fertilización de 100 % con dosis de 100 y 200 µg. Para el caso de las larvas ambas dosis fueron similares en la talla inicial.

Esta investigación fue financiada por The F&A Collaborative Research Support Program a través del proyecto: Development of snook (*Centropomus spp*) seed production technology for application in aquaculture and restocking of over-fished populations.

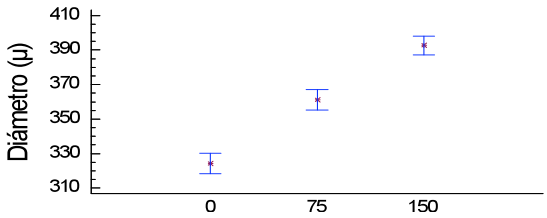

Fig. 1. Experimento 1. Diámetro final de huevos de *C. parallelus.*

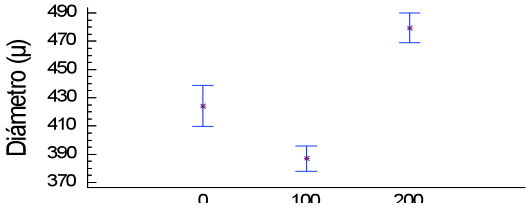

Fig. 2. Experimento 2. Diámetro final de huevos de *C. parallelus.*

PRIMERAS EVIDENCIAS EN LA ACLIMATACIÓN AL CAUTIVERIO DEL ROBALO PRIETO DEL PACÍFICO ORIENTAL, *Centropomus nigrescens* (GÜNTHER, 1864), EN LA COSTA DE MICHOACÁN

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Palabras Clave: Cautiverio, robalo prieto, estanques, costa de Michoacán.

El objetivo del trabajo fue documentar las primeras evidencias en México sobre la aclimatación al cautiverio del robalo prieto, *Centropomus nigrescens*, como una aportación tendente a generar las bases para su cultivo y como una fase inicial de un proyecto de reproducción y crianza de la especie en mesocosmos. El diseño consistió en la integración de un primer lote de juveniles de la especie a partir de la recolecta de crías en el delta del río Balsas, en la zona limítrofe de los estados de Michoacán y Guerrero, México, así como de dos fases de crecimiento. Una primera en agua dulce y una fase final en agua salobre a 22 $^{o}/_{oo}$, obteniendo la información de respaldo sobre la ubicación de los sitios de recolecta, calidad del agua, condiciones en el transporte, confinamiento en estanquería rústica, uso de anestésicos para la identificación de la especie, alimentación, crecimiento y sobrevivencia. A partir de la recolecta de 198 crías de 5-7 cm de L.T. en el brazo izquierdo del río Balsas y de las fases señaladas, en un período comprendido de noviembre de 2004 a junio de 2008 (43 meses), se integró un lote de 29 juveniles en una fase de final de confinamiento en estanquería rustica en Boca de Apiza, Mich. Los resultados alcanzados muestran a *C. nigrescens* como una especie con atributos para su cultivo, entre los que se destacan: facilidad para la recolecta de crías en el río Balsas, a 11 Km de la desembocadura, adaptabilidad para su manejo y crecimiento en estanques de agua dulce a valores de: 27.5° C, 5.38 mg/litro de oxígeno disuelto, 0.2 $^{o}/_{oo}$ de salinidad, pH de 8.0, 95 mg/litro de alcalinidad total, 153 mg/litro de dureza total y 0.051 mg/litro de amonio no ionizado; factibilidad en el uso de benzocaína a 60 mg/litro para la sedación total y el manejo de los organismos, adaptabilidad y resistencia al manejo con redes, factibilidad para la captura y aclimatación de ejemplares adultos en la zona de la desembocadura del río, rápida adaptación a cambios de salinidad (de 26 a 0.2 y de 0.2 a 22 $^{o}/_{oo}$ en dos horas), resistencia al manejo, y factibilidad para su transportación a tasas de 15 kg de biomasa/m^3 por espacio de 5 horas a una temperatura de 26° C. Será recomendable continuar con el proceso de maduración en agua salobre, valorar las condiciones de su protandrismo y maduración e integrar un lote de reproductores que permita avanzar a la fase de reproducción y crianza.

CULTURING TEXAS SNOOK - WHAT WE HAVE LEARNED SO FAR

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Research at the Fisheries and Mariculture Lab of the University of Texas Marine Science institute is based on spawning large marine fishes in captivity in recirculating tank systems using photoperiod and temperature conditioning to simulate natural seasonal changes (Fig. 1) that will induce natural spawning. Long term goals are to develop and publish reliable production technologies to farm new species of marine fish in recirculating systems. We have spawned red drum *Sciaenops ocellatus* for many years and cobia *Ranchycentron canadum* since 2001. We have carried out numerous studies to determine optimum conditions for larvae and juveniles. Recently we have applied the same techniques to the rearing of southern flounder *Paralichthys lethostigma* and hope they will be successfully applied to common snook *Centropomus undecimalis*. During the summer and fall of 2007 and 2008, more than 50 common snook (38-93 cm) were caught in the Laguna Madre and transported to Port Aransas. Snook were put into a 45,000 L recirculating seawater raceway (2.4m w x 13.7m L x 1m d) with a heat pump for temperature control and a cover with internal lights for photoperiod control. The temperature and photoperiod was cycled from 28^0 C and 14 hr light for summer to 21^0 C and 10 hr light for winter. A lunar cycle was also programmed to mimic natural moon phases. The goal was to induce natural spawns in the summer of 2008. No viable eggs were collected; a small number of unfertilized eggs were released on the full moon in August and again four days later. In early October the largest snook were checked for gonadal condition. Surprisingly, the majority of the large fish (80–94 cm) were males including the largest. Out of 10 fish checked, only 2 were identified as females (both 83.8 cm). Small, early stage eggs (200 um) were collected from one of the females and milt was collected from the males which could be activated and observed under a microscope. We had expected at least 50 % of these large snook would be males. Very little is known about the common snook in Texas including where and when they spawn and the sex ratio of large fish. New large snook were collected from the Brownsville Ship Channel in late October 2008 and transported back to Port Aransas and started on another seasonal photo-thermal cycle to induce spawning in the summer of 2009. Spawning by August is anticipated and if not the fish will be checked and eggs measured; if the size is $\geq$ 300 um they will be implanted with GnRHa pellets to try to induce spawning. We have raised larvae from eggs obtained from Kevan Main and are confident of production once good quality eggs have been produced.

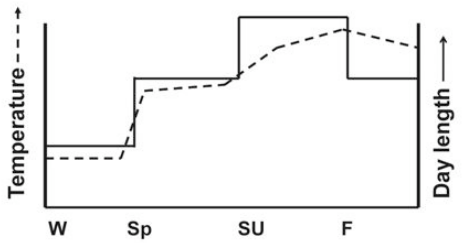

Figure 1. Photothermal cycle used at UTMSI to condition marine fish to spawn in the l

CULTURE OF THE ASIAN SNOOK (*Lates calcarifer,* Bloch) IN AUSTRALIA

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Asian snook (*Lates calcarifer* Bloch), also known as barramundi, is cultured in the Asian Pacific region, mainly in Thailand, Taiwan, Malaysia, Indonesia, as well as Australia. It belongs to the new family Latidae, which is closely related to "robalos" of the family Centropomidae in America. Barramundi is an important species for aquaculture in Southeast Asia and Australia were is one of the most suitable finfish for brackish water farming in earthen ponds and in floating net cages.

At Darwin Aquaculture Centre, Northern Territory, Australia, the breeders were maintained in two 20-m^3 fibreglass tank with recirculation system, at 28-32 ppt and 28-30^oC, with control of temperature and photoperiod. They were fed to satiation, three times a week with 60% of mullet, 34% squid and 6% of pellet INVE Breed-M 25mm, added with pre-mixture of vitamins. Two females and two males were induced to spawn with injections of LHRHa, 50 mg kg^{-1} to females and 25 mg kg^{-1} to males. The eggs were collected between 9-10 hr after the spawn and this were treated with ozone to a concentration between 0.4 to 0.5 mg L^{-1} during 2 minutes and incubated at 2000 eggs L^{-1} in 1-m^3 cylindroconical tanks with open flow until the larvae were transferred to culture larval tank on their second day after hatching (dah). The larval culture was carried out over 28 days in two 6-m^3 fibreglass tanks, with a recirculation system. Larvae were stocked at densities of 86.3 and 88.7 larvae L^{-1} in green water (*Nannochloropsis oculata* and *Isochrysis sp* (T-ISO)).

Recently fertilized egg diameter was 0.807 ± 0.17 mm and the oil droplet diameter 0.250 ± 0.06 mm, with 90 % of fecundity. A total of 3.5 x10^6 eggs obtained. The hatching period started 13 hr after the spawn. Hatching was 90 %, and between 23 to 25 hr later they were transferred to the larval rearing tanks. The average total length during the larval rearing is represented in Fig 1.

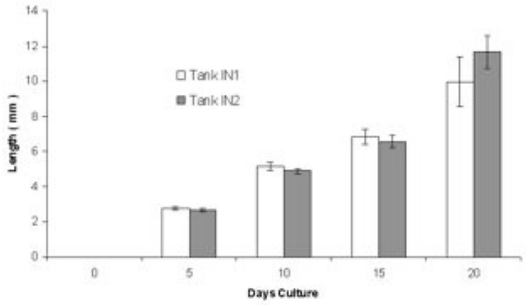

Fig. 1 Growth in total length of barramundi larvae ± standard deviations in two 6,000-L larval tanks in one rearing trial at Darwin Aquaculture Centre.

In the present work larval survival at day 25 was 60.8% for tank IN1 and 51.8% for tank IN2, and at the end of the larval rearing period (28th dah), total average survival was 69.5%. After 53 days of nursery a total of 497,866 juveniles survived (51.7%), with an average weight of 20 g, and a food conversion factor of 0.656. Usually the juvenile are grown to marketable size in ponds between 0.8 and 1.3 ha, and 2 m deep. After 14 months of pond culture fish are harvested with yields between 30 and 39 t ha^{-1} and average

individual weights from 2.5 to 3.0 kg, with a market value of 8 AUS dollar per kg of whole product.

ENGORDA DE ROBALO *Centropomus undecimalis* (Bloch, 1792) Y DEL CHUCUMITE *Centropomus parallelus* (Poey, 1860) EN AGUA DULCE EN EL ESTADO DE VERACRUZ, MEXICO.

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El robalo (*Centropomus undecimalis* Bloch, 1792) y el Chucumite (*Centropomus parallelus* Poey, 1860), son dos especies de importancia económica en el Golfo de México y dada esta importancia se llevaron a cabo 2 estudios para evaluar el crecimiento de estas 2 especies en estanques rústicos y de concreto. En los estanques rústicos se sembraron juveniles en diferentes proporciones: 1:1, 4:1 y 3:2. Previamente se sembraron reproductores de Tilapia, *O. niloticus,* en una proporción de 2 hembras por cada macho, para la producción de alimento. Se utilizaron 1,500 crías para los estanques rústicos (500 en cada uno) y 200 en el de concreto. Mensualmente durante 14 meses se pesaron y midieron. En ambos experimentos se estimo la curva de crecimiento para peso y longitud a partir de un modelo logarítmico $y = aL^b$, los resultados mostraron un crecimiento isométrico para ambas especies, en los estanques rústicos fue de 3.01 para el robalo y de 2.96 para el chucumite y en el de concreto 3.02 y 3.13 respectivamente. La tasa absoluta de crecimiento para longitud en robalo y chucumite en los estanques rústicos fue de 26.43 ± 0.135 cm y 12.0 ± 0.105 cm mientras que la tasa absoluta en peso fue de 265.3 ± 0.623 g y de 55.1 ± 0.191 g respectivamente, con crecimiento diario de 0.062 cm para el robalo y de 0.028 cm para el chucumite. En el estanque de concreto el crecimiento fue de 17.3 ± 0.74 cm y 14.9 ± 2.6 cm, respectivamente, con un crecimiento diario de 0.047 y 0.041 cm, mientras que la tasa absoluta en peso fue de 183.6 ± 2.02 g y 118.1 ± 1.38 g, con ganancia diaria de 0.50 y 0.32 g para el robalo y el chucumite respectivamente.

La velocidad de crecimiento en los estanques rústicos en talla fue semejante en estas especies 0.133 y 0.091 e igualmente en peso 0.347 y 0.279 para el robalo y el chucumite respectivamente, con una alimentación a base de especies forrajeras. En el estanque de concreto fue similar el crecimiento en longitud 0.063 y 0.085, sin embargo el crecimiento en peso en el chucumite mostro una mayor velocidad en relación a su especie con respecto el robalo 0.273 y 0.180, bajo un sistema de alimentación *ad libitum.*

Los datos obtenidos demuestran la factibilidad de que ambas especies puedan desarrollarse en cautiverio en estanques rústicos y con agua dulce de forma conjunta con la tilapia como alimento.

SPAWNING AND LARVICULTURE OF THE FAT SNOOK (*CENTROPOMUS PARALLELUS*) AND THE COMMON SNOOK (*C. UNDECIMALIS*) IN BRAZIL

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The fat snook, *Centropomus parallelus* Poey, 1860 and the common snook, *C. undecimalis* (Bloch, 1792) are distributed along the coast of Brazil, from Amapá to Rio Grande do Sul State, but they are more commonly found on the northern coast in tropical waters.

Presently the dominant coastal aquaculture activity in Brazil is shrimp culture. However, renewed interest in snooks is been stimulated largely by a need for the existing marine shrimp farming to diversify. Despite the studies carried out in the recent years, there is still no commercial production of these valuable fish.

In this work we describe the present status of snook reproduction and larviculture in Brazil, focusing on the results obtained at the experimental fish hatchery of the Universidade Federal de Santa Catarina.

Induced spawning of wild fat snook were first obtained in 1991 with a single injection of hCG. However, when broodstock were conditioned in maturation rooms and induced to spawn, there was a substantial increase in eggs quality. Although cultured females have ovaries containing oocytes at the tertiary yolk stage during the spawning season, they do not ovulate and spawn naturally. Different dosages of LHRH-a with saline injection and cholesterol implant were also tested to induce final maturation. As the fat snook exhibits group-synchronous oocyte development, females could be induced to spawn once a month resulting in up to four consecutive spawnings.

Experimental production of fingerlings has been successfully conducted from eggs. Results of larval culture have been highly variable at the

beginning, survival rates were frequently around 1% until the juvenile stage. With the improvement of the spawning induction technique and better larviculture practices, survival rates increased to 10-20%, using 5,000-L tanks, with initial stocking densities of approximately 40 eggs/L. Using tanks up to 15.000 L capacity, batches of 50.000 juveniles have been produced. Several experiments have been conducted to evaluate the effect of some factors on larval growth and survival: prey quality, photoperiod, light intensity, dietary fatty acids, prey density, weaning age, stocking density, etc.

Spawnings of the common snook were recently obtained by means of hormonal induction with LHRHa of wild fish captured during the natural reproduction season. First common snook juveniles were obtained with the same larviculture techniques employed with the fat snook. Adult females maintained in laboratory were not able to develop ovaries to attain vitelogenesis. Research is been conducted

in order to improve sexual maturation in captivity.

The fat snook proved relatively ease to rear through the larval stages. The common snook is a more promising species, due to a higher growth rate. However, to develop its maturation and routine spawning in laboratory to produce large amount of healthy eggs and larvae is still a challenge.

STATUS AND CHALLENGES IN LARVAL REARING AND FINGERLING AQUACULTURE OF COMMON SNOOK (*Centropomus undecimalis*) IN FLORIDA

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For more than twenty years, researchers have been investigating methods to produce common snook (*Centropomus undecimalis)* for stock enhancement by capturing mature fish and inducing spawning in tanks within a few days following collection, by strip spawning wild fish in the field, and more recently by maturing and spawning snook broodstock in captivity. Early research revealed that newly collected mature snook could be induced to spawn using hormone induction during the normal reproductive season, but this technique often resulted in small numbers of poor quality eggs and the broodstock often died following induced spawning. Another approach has been to strip spawn snook collected from natural spawning aggregations, which allowed us to obtain large numbers of fertilized eggs and to track the egg quality progression over the spawning season. Variability in field spawning success led us to develop maturation and spawning systems at Mote Aquaculture Research Park (MAP) and from 2006 through 2009 we matured and spawned snook in the photo-thermal controlled, recirculating systems at MAP. Common snook spawning primarily occurs in southwest Florida from May through September, but egg quality varies greatly within this time frame. A review of larval rearing data from several years revealed the highest survival rates from spawns collected just past the new and full moon cycle. We strip spawned wild snook from April through September 2003. Egg samples were collected 1 day before and up to 5 days after new and full moons. The highest quality eggs were obtained 2 to 3 days after new and full moon cycles. These results were used to predict maturation and schedule induced spawning trials with captive broodstock. Wild snook egg samples were collected over a 4-year period and analyzed for fatty acid (FA) composition. ARA concentrations in wild eggs were higher than those seen in other marine species, which may be related to parental diet or environmental conditions. The PUFA profile changed over the spawning season and egg quality was best in May, June and July. High DHA levels were significantly correlated with higher fertilization and hatch rates, and with larval survival. FA were analyzed for cultured larvae ranging from 1 to 80 days-post-hatch (DPH). A significant decrease in DHA occurred in the first 6 DPH. Additionally, large numbers of larvae failed to initiate feeding and mortality appeared to be due to starvation. Histology revealed that 2 DPH snook larvae develop their digestive and eye system sufficiently to locate, capture and digest prey. Differences in the initial design of the feeding apparatus are thought to have direct consequences for first feeding and mortality. Studies to identify morphological constraints to feeding demonstrated that 3 DPH snook larvae have a poorly developed feeding apparatus, which limits their ability to consume certain prey (i.e., rotifers) at first feeding. First feeding larvae primarily consumed ciliates, tintinnids, dinoflagellates and small copepod nauplii. Additional studies examined the influence of salinity (15, 25 and 35 ppt) on larval survival and growth. Growth and survival to 14 DPH was highest in salinities at or near full-strength seawater (35 ppt). Research is underway to determine a safe concentration and exposure time (no negative effects on survival and hatch rate) of hydrogen peroxide (H_2O_2) for surface disinfection of snook eggs. Following metamorphosis snook fingerling culture issues that must be resolved include high incidence of lordosis and cannibalism. The effect of diet and water current on both of these variables needs to be determined.

FEMINIZACIÓN DE JUVENILES DE ROBALO BLANCO (*Centropomus undecimalis*) EMPLEANDO 17- β ESTRADIOL EN LA DIETA.

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El robalo blanco *Centropomus undecimalis* en un pez protándrico hermafrodita con un alto potencial de cultivo en México. Diversos estudios indican que las hembras son más grandes y aparentemente su tasa de crecimiento es mayor que en machos. El objetivo del presente estudio consistió en evaluar el efecto del esteroide 17-β estradiol (E2) en la proporción de sexos de esta especie. En este estudio se evaluaron seis periodos de exposición con E2 (7, 14, 21, 28, 35 y 42 días) usando alimento biencapsulado e impregnado con 50 mg de E2. Después del periodo de exposición, los peces fueron cultivados con dieta sin E2 por 198 días adicionales, determinándose la proporción de sexos, crecimiento y supervivencia. Los peces alimentados con dieta enriquecida con E2 por 21 y 42 días muestran mayor proporción de hembras (93 %, Fig. 1 y 2a), mientras que el grupo control presentó 100 % de machos (Fig. 2b). El mayor crecimiento en longitud y peso se obtuvo en los peces expuestos por 21 días a la administración de E2 (193.11 ± 1.83 mm y 28.56 ±0.63 g) comparado con el resto de los tratamientos. La supervivencia varió entre 92 y 98 % en todos los tratamientos.

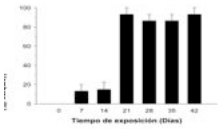

Figura 1. Promedio del porcentaje hembras de juveniles de *C. undecimalis* (± EE) por tratamiento del Experimento II. Letras iguales indican tratamientos que no fueron estadísticamente diferentes entre sí (X^2 P > 0.05). El Numero () indica el tamaño de muestra por tratamiento.

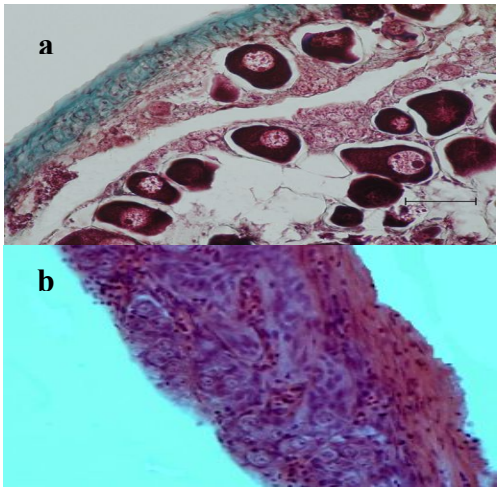

Fig. 2. Gónadas de juveniles de *C. undecimalis*. a) Ovario, b) Testículo

Esta investigación fue financiada por la Universidad Juárez Autónoma de Tabasco y por The Collaborative Research Program Support (CRSP) en colaboración con la Texas Tech University.

ADVANCES ON DIGESTIVE PHYSIOLOGY OF COMMON SNOOK (*Centropomus undecimalis*) IN TABASCO, MEXICO

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Common snook *Centropomus undecimalis* is one of the most important commercial native marine species in Southeast region of Mexico. However, this species has been highly over-exploited, for this reason the research that allows to implement its culture has become a priority. In this sense, is necessary to study the digestive physiology that allows developing its biotechnology. The objective of this project is to evaluate the digestive capacity using biochemical, histological and molecular tools during larval and juvenile stages.

Several samples of larvae and juveniles of *C. undecimalis* were sacrificed to study the digestive enzymatic ontogeny using colorimetric biochemical reactions and SDS-PAGE (for alkaline proteases) and PAGE (for acid proteases) zymograms. Also, samples of stomach and intestine were use for two purposes 1) the characterization of digestive proteases using pH and temperature as factors and the use of specific inhibitors, and 2) the in vitro degree of hydrolysis determination of several protein ingredients using pH-STAT system. Additionally, samples of the digestive system of juveniles were used for traditional H-E technique to describe the anatomical characteristics. Finally, some wild juveniles were sacrificed and stomach, intestine and pancreas were freeze in RNA later buffer for the study of the expression of trypsin and lipase digestive enzymes.

Our results showed that during the ontogeny trypsin, chymotrypsin, L-aminopeptidase, carboxypeptidase A, lipase, amylase, and phosphatase were detected from yolk absorption (2 days post-fertilization, dph) onwards, increasing their activities between 12 and 25 dph. Pepsin was detected from 34 dph onwards.

The alkaline protease zymogram showed two bands, the first (26.1 kDa) at 25 dph, and the second (51.6 kDa) at 36 dph. The acid protease zymogram showed two bands (0.32 and 0.51 rf´s) at 34 dph. On the other hand, acid protease activity in the stomach was optimum at pH 2.0 and a temperature of 75 ºC; it was inhibited in 86% with Pepstatin A. The stability for pH and temperature for acid proteases showed a wide range (2-8 pH and 25-55ºC). Alkaline proteases showed two peaks of pH maximum activity at 7 and 11, and the optimum temperature was obtained at 65ºC. The stability to different pH and temperature for alkaline proteases showed a wide range of values (4-10 pH and 35-65ºC). These proteases were inhibited with PMSF (60%), ovoalbumin (59%), SBTI (41%), TLCK (68%), and TPCK (17%), that indicate the presence of serine proteases (trypsin and chymotrypsin), between these two, trypsin had the highest activity. With EDTA metal proteases were inhibited 40% and 85% using 1-10 phenantrolin. Degree of hydrolysis for protein ingredients showed that blue crab meal, sardine meal, pork meal and beef blood meal had the higher values, additionally the maximum total

amino acid release was obtained for sardine meal in the acid stage, while, for the alkaline stage the maximum amino acid release was detected for the fish hydrolyze. The digestive tract in the common snook is formed, as in many species of Gnathostomata, by the, esophagus, stomach, intestine and accessory glands, liver and exocrine pancreas, and four intestine caecae. The digestive organs show the tissular layers; mucosa, submucosa, and smooth muscle. Finally, we amplified the gen of trypsin and lipase in pancreas of juveniles, these genes showed a similarity with other trypsin and lipase genes higher than 85% detected for other fishes.

INCORPORATION OF THE NATIVE CICHLIDS TENGUAYACA, *PETENIA SPLENDIDA*, AND CASTARRICA, *CICHLASOMA UROPHTHALMUS* INTO SUSTAINABLE AQUACULTURE IN CENTRAL AMERICA IMPROVEMENT OF SEEDSTOCK QUALITY AND SUBSTITUTION OF FISH MEAL USE IN DIETS

Indigenous Species Development/ Experiment/ 07IND02UA

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INTRODUCTION

To achieve fish culture at a commercial scale, basic and applied research need to be carried out in order to allow the closure of the cycle of life of the species. However, in Mexico the focus for this activity has been for introduced species such as tilapia and trout, where its technology is already known (Rojas y Mendoza, 2000). For this reason the research for the culture of native species has reached only a minimum scale of production. In this sense, Southeast of Mexico has a lot of native species including cichlids which represent a potential resource for human consumption. The most important species are the castarrica (*Cichlasoma urophthalmus*) and tenguayaca (*Petenia splendida*) which have high potential for culture (Steeffens, 1987; Luna, 1997).

The studies with these species began in the 80's, and now allows implementation of their culture, although only in an extensive scale in the region (Martínez-Palacios and Ross, 1994).

Some of the most important investigations for *C. urophthalmus* were conducted by Martínez-Palacios (1987) that include topics as nutrition and culture method and the handle of broodstock management and larval quality (Mendoza and Navarro, 1994). We have also started studying the acceptance of commercial feeds and growth performance of this

species. So far we have compared growth performance of fry fed *Artemia* nauplii against fry fed commercial feed. We have observed a significant difference in growth if the fish are fed nauplii during the first 30 days of feeding even though fry accepted inert food. Fish fed 30 days with nauplii grew more than twice that those that received nauplii. Empirical information indicates that males grow faster and larger than females. On the other hand, Real (2003) obtained 95 to 100% of male monosex juveniles feeding larvae with *Artemia* nauplii enriched with a concentration of 15 mg/L of 17 α-Methyl testosterone.

For *P. splendida* several studies that include ecology, habitat, feeding content and distribution were conducted (Reséndez and Salvadores, 1983; Ferreira et al., 1988; Caro et al., 1994; Domínguez and Rodiles, 1998). For culture, the advances include, García (2003), Jiménez (2004), and Chan (2004), whom determine the maximum interval of temperature resistance (30.68 and 31.95 Celsius), and the thermal preferendum (28-30 ºC) for the best metabolism and growth of juveniles. Contreras (2003) and Vidal-López (2004), obtained 95 to 100% mono sex population feeding larvae with trout commercial diet and Artemia nauplii enriched with 17 α-Methyl testosterone using a concentration of 30 mg/L for 45 days. For larviculture the morphological development was described (Martínez, 2004). In the case of nutrition Uscanga (2006) determined the protein requirement for masculinized and non-masculinized juveniles using semi-purified diets.

This information is promising; however, although the culture of these species are already known, at the moment a commercial food for trout has been used. This food contains a high quantity of protein and lipids, which increases the cost for large-scale production. In addition the ingredients of the formula are unknown, for this reason we consider that the growth and the survival of the species is not optimum.

During the formation of broodstock it is fundamental to incorporate the highest genetic diversity as possible, both at the intra- and inter-population level. This is particularly important when planning genetic selection programs. Family structure is also an important issue that may affect inbreeding and consequently affect productivity.

Genetic improvement in fish has had a large impact during the last years (Martínez y Neira, 1995; Hulata, 2001; Gjedrem, 2005; Martínez, 2005) focusing on growth enhancement, diseases resistance, transfer of desirable characters, combination of benefic characters from two species, avoid unwanted reproduction, improving food conversion rates, and tolerance to cold and bad quality water (Gjedrem, 1985, Bartley y Hallerman, 1995; Hallerman y Kapuscinsky, 1995) and applying different techniques such as selection, intra and inter-specific breeding, sex inversion, chromosomal manipulation and polyploidy (Foresty, 2000; Hulata, 2001).

Artificial selection consists of selecting organisms with the best traits and potential application allowing modification of the genetic pool of one population in respect to one or more characters in such a way that generation after generation, the groups move towards the desired goals (Kirpichnikov, 1981; Rodríguez-Gutiérrez y Marañón-Herrera, 1993; Gjedrem, 2005). The main objective is to produce stocks that are more efficient in economic terms improving growth and viability under culture conditions (Álvarez-Jurado, 1987; Gjedrem, 1997).

We have seen genetic improvement as an option for the culture of the native cichlids *P. splendida* and *C. urophthalmus* which have demonstrated high potential for inclusion in

aquaculture; however, no information exists about how these species may respond to a selection program or current differences among wild populations that may enhance the selection program.

METHODS AND MATERIALS

Objective 1. To improve Seedstock quality based on a genetic improvement program for P. splendida and C. urophthalmus.

Experiment 1. Genetic improvement of *Petenia splendida,* and Castarrica, *Cichlasoma urophthalmus* Using Total Length and Condition Factor.

This experiment was initiated late due to two consecutive flooding events in Tabasco. The first one occurred in 2007 and the second one in 2008. In both cases our facilities were not severely affected; however, our investigations were delayed due to fish loss or systems damaged.

This experiment was divided in two parts. The first one consisted in the capture of adult organisms from the wild, acclimation to captivity and fry production; the activities were conducted at the Laboratorio de Acuicultura Tropical; División Académica de Ciencias Biológicas. The second part was the grow-out of fish in hapas and floating cages the activities were conducted at the Laboratorio de Acuicultura, División Académica de Ciencias Agropecuarias at Universidad Juárez Autónoma de Tabasco.

Fish Capture and acclimation to captivity

Adult castarricas were collected from three different localities of Tabasco: Centla, Centro and Comalcalco. These localities were selected based on the historical presence of the species in the area. Forty-five adult fish were caught from each locality, then transferred to the laboratory and kept in quarantine. A prophylactic treatment was performed the first day of arrival. From each locality, fish in best conditions were selected as broodstock: 36 fish from Centla (24 females and 12 males); 24 from Centro (16 females and 8 males) and 24 from Comalcalco (16 females and 8 males). All fish were kept in 2000-L plastic tanks connected to a recirculating system equipped with a sand filter, a biofilter and a centrifugal pump that returned the water to the system. Fish were fed three times per day, providing 3% of the total biomass using pelleted trout food “El Pedregal” (Silver Cup™) containing 45% protein.

Adult tenguayacas were collected from three different localities of Tabasco: Nacajuca, Centro, and Centla; and one locality from Chiapas: Mal Paso. These localities were selected based on the historical presence of the species in the area. However, due to overfishing, forming the lot of broodstock for this species was more difficult. From each locality, fish in best conditions were selected as broodstock: 36 fish from Nacajuca (24 females and 12 males); 36 from Centro (24 females and 12 males); 12 from Centla (8 females and 4 males); and 21 from Mal Paso, Chiapas (14 females and 7 males).

All fish were individually sexed, weighed, measured and identified using a microchip injected intra-muscularly. Morphometric measurements were taken (total length, standard length, height, perimeter, length of dorsal, pectoral and pelvic fins, height and length of caudal peduncle, head length, superior jaw length, mouth and eye diameter) as well as meristics (number of scales in the lateral line, superior and inferior, number or rays in the caudal and dorsal fins, and number of spines in dorsal and anal fins). Condition factor of each fish was also calculated.

Hematologic measurements
To evaluate fish health, the following parameters were evaluated: Microhematocrit, hemoglobin, total plasmatic protein, red blood cell count and white blood cell count. Hematologic procedures followed procedures proposed by Hesser (1960) and Houston (1990). Fish were first anesthetized with Tricaine methanesulfonate (MS-222) and blood drawn from the caudal vein using heparinized syringes.

Reproduction
Gender proportion used in all tanks was 1:2 (male:female) at a density of 12 fish per tank (4 males : 8 females). Each 2000-L tanks was equipped with 8 plastics sheets placed forming an angle against the tank wall to serve as refuge and potentially as nest. Water quality in each tank was monitored daily and 50% water exchange was performed every other day.

Fry collection and rearing
Once spawning occurred, both parents were captured and identified reading the pit tag. Both fish were placed back into the tank to allow parental care of the fertilized eggs. Once hatching occurred, all the fry were siphoned-out of the spawning tanks and placed in a 100-L rearing tank. All rearing tanks were connected to a recirculation system equipped with a sand filter, a biofilter and a centrifugal pump that returned the water to the system. Fry were kept in the rearing tanks for 60 days and fed five times per day with 1.5 hours difference between feeding times. Artemia nauplii were supplied during the first 15 days and trout commercial feed afterwards (Silver Cup™) containing 45% protein.

Grow-out phase
After the rearing period, families were moved to our facilities located in the Agronomical Sciences Division, each family was placed in a 2 m^3 mosquito mesh hapa (2x1x1.20 m) using a density of 150 fish/. m^3. Biometric measures were taken every 15 days (weight and Total length) in each hapa. After 120 days of grow-out, fish were moved to 2 m^3 floating cages (2x1x1.20 m) with ½ inch of mesh size and kept there until first selection was conducted.

Line Selection
The inter- and intra-family selection method was used. After 225 days of culture, fish were separated by sex and total length and weight measured. 26% of the fish with the best weight were selected; 40 males and 40 females were stocked in 2 m^3 floating cages (2x1x1.20 m) with 1 inch of mesh size. A control group from each family was formed using fish randomly selected (40 males and 40 females). During final grow-out, fish growth was evaluated every 15 days.

Water quality in ponds
Water quality parameters were measured daily or weekly. Temperature and Dissolved Oxygen were measured using a multifunction DO meter (YSI 55™). pH was measured using a portable pH meter (CONDUCTRONIC™). Ammonia, Nitrates and Nitrites were measured weekly using the multiparametric colorimeter (HANNA™).

Statistical Analyses
A descriptive analysis was performed to all hematologic data. Relations between morphometric and meristics variables were analyzed using a discriminate analysis. Relationships between condition factor (k), weight of the female and number of eggs

produced were determined using a multiple regression analysis. Differences in weight between families were detected using a one-way ANOVA. All statistical procedures were conducted using the statistical package STATGRAPHICS Plus ® V5, and statistical differences were indicated using a 95% confidence level.

Objective 2. To determine the effect of the substitution of fish meal for poultry meal on growth, survival, apparent digestibility and chemical composition of* P. splendida *and* C. urophthalmus *juveniles.

Experiment 1: Effects of the substitution of fish meal with pork meal on P. splendida and C. urophthalmus juveniles.

This study was conduced at the Laboratory of Tropical Aquaculture at UJAT, Tabasco, Mexico. Growth of *P. splendida* and *C. urophthalmus* juveniles was evaluated using practical isocaloric and isoproteic diets containing 0, 25, 50, 75 and 100% substitution of fish meal with pork meal.

Masculinized juveniles were selected from a group of 2,000 fry produced in the Laboratory of Aquaculture at UJAT. The production of masculinized juveniles consisted on feeding larvae with 17 α-Methyltestosterone (20 mg/L) enriched-artemia nauplii for 15 days and 17 α-Methyltestosterone (60 mg/Kg of diet) enriched commercial trout diet (Silver Cup) for 30 additional days. From this batch, 270 fish were selected and randomly distributed among the 18 experimental units at a density of 15 fish/tank. Water for grow-out was recirculated using bio-filters, 25% of the volume was exchanged twice a week.

Sampling schedule for Tenguayaca and Castarrica juveniles consisted in collecting all fish in each tank at the beginning of the experiment and every 14 days. Total length and weight were measured to the nearest 0.001 mm or g. Mortality was recorded daily. Growth and food quality indexes were calculated at the end of the experiment: Feed conversion rate (FCR), specific growth rate (SGR), condition factor (CF), protein efficiency rate (PER), and percentile weight gain (WG %). Mortality was recorded daily. Feces recollection for the measure of apparent digestibility was conducted daily. Samples of fish were taken at the beginning and the end of the experiment to determine chemical composition in whole fish (AOAC, 1995). Experimental treatments were as follows:

1) Tenguayaca and castarrica juveniles fed with a diet containing 0% fish meal and 100% pork meal: (0%FM-100%PorM).
2) Tenguayaca and castarrica juveniles fed with a diet containing 25% fish meal and 75% pork meal (25%FM-75%PorM).
3) Tenguayaca and castarrica juveniles fed with a diet containing 50% fish meal and 50% pork meal: (50%FM-50%PorM).
4) Tenguayaca and castarrica juveniles fed with a diet containing 75% fish meal and 25% pork meal: (75%FM-25%PorM).
5) Tenguayaca and castarrica juveniles fed with a diet containing 100% fish meal and 0% pork meal: (100%FM-0%PorM).
6) Tenguayaca and castarrica juveniles fed with a trout commercial diet (CD).

Each treatment was run in triplicate. First feeding juveniles were fed with experimental diets four times a day (8:00. 12:00, 16:00, and 20:00 h). Fish received daily rations containing 10 percent of the total biomass in the tank. Daily rations were estimated using a spread-sheet constructed with previous growth data for each species.

Growth data were compared using a one-way ANOVA for each species. Survival was compared using a Chi-square test.

Experiment 2: Effects of the substitution of fish meal with poultry meal on P. splendida and C. urophthalmus juveniles.
All experimental conditions and measurements were similar as those used in experiment one. Treatments were as follows:

1) Tenguayaca and castarrica juveniles fed with a diet containing 0% fish meal and 100% poultry meal: (0%FM-100%PouM).
2) Tenguayaca and castarrica juveniles fed with a diet containing 25% fish meal and 75% poultry meal: (25%FM-75%PouM).
3) Tenguayaca and castarrica juveniles fed with a diet containing 50% fish meal and 50% poultry meal: (50%FM-50%PouM).
4) Tenguayaca and castarrica juveniles fed with a diet containing 75% fish meal and 25% poultry meal: (75%FM-25%PouM).
5) Tenguayaca and castarrica juveniles fed with a diet containing 100% fish meal and 0% poultry meal: (100%FM-0%PouM).
6) Tenguayaca and castarrica juveniles fed with a trout commercial diet (CD).

RESULTS

***Objective 1. To improve Seedstock quality based on a genetic improvement program for* P. splendida *and* C. urophthalmus.**

Experiment 1. Genetic improvement of *Petenia splendida,* and Castarrica, *Cichlasoma urophthalmus* Using Total Length and Condition Factor.
Acclimation to captivity took more time for *P. splendida* than for *C. urophthalmus*. It is possible that the carnivorism of *P. splendida* may have played a significant role on this, delaying significantly adaptation to foods provided in the laboratory. Despite early capture of adults of this species in the year, first spawnings were obtained until August of 2009; therefore, evaluation of growth is still ongoing in this species.
In *C. urophthalmus*, comparison of morphometric and meristic characters among localities showed a similar pattern indicating that no significant differences were found among the adults captured for establishing our broodstock lots. The discriminant analysis indicates that the number of scales in the superior lateral line, condition factor, length of dorsal and pectoral fins, total length and length of the caudal peduncle may allow separation of the three populations sampled with an 86.08% percent of cases correctly classified. Fish from Centla had the best condition factor (K; 2.42±0.30) when compared against Comalcalco (2.25±0.52) and Centro (1.99±0.16). No statistical differences were found ($P>0.05$; table 1) for the hematologic variables from the adults. However, the highest values for microhematocrit, hemoglobin, total plasmatic protein, red blood cell count and white blood cell count were found in the fish obtained in Comalcalco, while Centla showed the lowest values. These types of comparisons were not possible with *P. splendida*, because fish were not collected at the same time.

Reproduction of Castarrica was evaluated from January to November 2009. During this time, Castarricas from Centla had the largest amount of spawnings (53) and a total of 260,554 fry. Fish from Centro had 30 spawnings with a total of 96,833 fry and 21

spawnings were obtained from Comalcalco with a total of 86,845 fry. This species actively spawned between March and August. No significant differences in the average number of fry per spawning event were found between Centla (4825.07 ±1402.77) and Comalcalco (4342.25 ±1871.75) but these values were significantly higher ($p < 0.001$) than the average number of fry obtained for Centro (3339.07 ±993). Same results were obtained when the average number of fry was estimated per gram of female: Centla (21.47 fry/g), Comalcalco (18.95 fry/g) and Centro (12.23 fry/g). These results are interesting since females from Centro had larger average weight (263.83 g ±38.65) than those from Centla (229 g ± 64.38) and Comalcalco (218.27 g ± 52.33).

In the spawning tanks, water quality conditions were maintained optimal, average temperature, dissolved oxygen and ph were 29 (±2˚C), 7.5 (± 0.15) and 7.8 (± 0.12), respectively. Average values for ammonia, nitrates and nitrites were 0.30 mg/L (± 0.04), 1.3 mg/L (± 0.03) and 0.03 mg/L (±0.003), respectively.

For line selection, families that hatched within a lapse of time of 2 weeks were used to avoid differences caused by age and/or environmental conditions. Eleven families were used (5 from Centla, 3 from Centro and 3 from Comalcalco). Average fry weight at hatching was 5.5 mg with 7.0 mm in total length. Castarrica fry accepted artemia nauplii during the first days and weaning was successfully achieved feeding them with artificial food in few days. Survival at this stage was 97% reaching 41.1 mg and 35.89 mm in 60 days.

No significant differences were found between families or locations during the grow-out phase ($P>0.05$; Fig. 1). During selection, all fish were separated by sex. Females from Centla showed no significant differences in weight between families (13.50g ±4.88; $P>0.05$), but differences in length were detected (9.00cm ±0.93; $P<0.05$). Males from this locality had significant differences between families both in weight and in length averaging 19.10 g (±5.62) and 11.10 cm (±0.89; $P<0.001$), respectively. Females from Centla had significant differences in weight and length between families (average: 12.20g ±4.88 and 8.75cm ±1.03; respectively). The same was observed for males which had significant differences between families both in weight and in length averaging 14.13g (±5.18) and 9.33cm (±1.13; $P<0.001$), respectively. Fish from Comalcalco also had significant differences between families for females and males. In females fish averaged 13.20g (±5.18) and 9.10cm (±1.13) in length. Males averaged 20.45g (±5.43) and 11.2cm (±0.87; $P<0.001$).

When females from the three localities were compared, no significant differences were found in weight or length ($P>0.05$). However, highly significant differences were found between males from the three localities both for weight and length ($P<0.001$). Significant differences ($P<0.001$) were found between all families selected when compared against the control group (Figs. 2, 3 and 4).

Objective 2. To determine the effect of the substitution of fish meal for poultry meal on growth, survival, apparent digestibility and chemical composition of* P. splendida *and* C. urophthalmus *juveniles.

Experiment 1: Effects of the substitution of fish meal with pork meal on P. splendida and C. urophthalmus juveniles. The analysis of variance indicated that exist statistical significant differences in weight and total length ($P<0.05$) between treatments for *P.*

splendida after 42 days of feeding (Fig. 1a and b). At day 84, fish fed 100%SM-0%PorM and 75%SM-25%PorM substitution were larger than the rest of the treatments (Fig. 1c and d). Food conversion rate (FCR), weight gain (WG%) and survival (Sur) showed significant differences ($P<0.05$) between treatments. For survival, fish fed with 100%HS-0%PorM, CD, 0%FM-100%PorM, and 25%FM-75%PorM had higher values compared with 50%FM-50%PorM and 75%FM-25%PorM. Fish fed with 100%FM-0%PorM, CD, 50%FM-50%PorM, and 0%FM-100%PorM had statistically higher FCR compared with the other treatments. Finally, fish fed with 100%FM-0%PorM were significantly different to fish fed 25%FM-75%PorM and 75%FM-25%PorM (Table 1).

For *C. urophthlamus*, no significant differences ($P>0.05$) were detected at any time for weight, total length and food quality indexes (Fig. 2 and Table 2).

Experiment 2: Effects of the substitution of fish meal with poultry meal on P. splendida and C. urophthalmus juveniles. For *P. splendida*, significant differences in growth and total length were detected ($P<0.05$) after 56 days of experimentation (Fig. 3a and b). After 84 days of feeding significant differences ($P<0.05$) were detected for fish fed 75%FM-25%PouM and CD compared with the rest of the treatments (Fig. 3c and d). On the other hand, survival showed statistical differences ($P<0.05$) for fish fed CD and 0%FM-100%PouM compared only with 100%FM-0%PouM. For specific growth rate (SGR) and weight gain (WG%) significant higher value ($P<0.05$) were detected only for fish fed 75%FM-25%PouM compared with fish fed 100%FM-0%PouM (Table 3).

For *C. urophthalmus*, significant differences were detected ($P<0.05$) after 42 days of experimentation for weight and total length (Fig. 4 a and b); at the end of the experiment, the highest weight and total length were detected only for fish fed with CD compared with the rest of the treatments (Fig. 4 c and d). For daily fed intake (DFI) and daily protein intake (DPI) significant differences ($P<0.05$) were detected only for fish fed with 0%FM-100%PouM with the highest values compared with fish fed with 25%FM-75%PouM (Table 4).

DISCUSSION

According to our results, morphologic and meristic characters did not show significant differences in *C. urophthalmus*; however, fish from Centro and Comalcalco showed more affinity among themselves, than fish from Centla. Cheng *et al*. (2005) compared morphometric characters of *Coilia* (a teleost) from four populations, observing that Only four of the characters measured helped identifying closely related populations at a 88-100% accuracy level. Sumantadinata and Taniguchi (1990) found morphologic differences among adult carps, indicating that morphologic and meristic values provide valuable information that can be useful for genetic improvement in aquaculture.

Average values obtained for microhematocrit, hemoglobin, protein concentration in blood and red and white cells counts in *C. urophthalmus* found in this study differ from those reported by Güemez y Sima (1998) y Pelaez (2009) for this species under culture conditions. These differences can be attributed to different factors such as: origin of the fish -mainly due to differences in environmental conditions- (Atencio-García and Genes, 2007) as well as nutritional status, sexual maturation, and even differences between sexes Ranzani-Paiva, 1991; Fernández *et al.,* 2002).

In a period of time of 10 months of evaluation, fish from Centla had the highest Condition factor, number of spawnings events and fry produced than those from Centro and Comalcalco. Morgan (2004), did not find significant differences when comparing Condition factors from three populations of *Hippoglossoides platessoides*, but did find a relationship between the condition of the fish and reproductive activity. On the other hand, we found no relationship between fecundity and weight of the females, particularly due to the fact that small females from Centla spawned more times and produced more eggs than those from Centro, which presented larger sizes. Age, nutrition and environmental conditions at the place of origin may play a rule in these results, since several researchers have reported that fecundity decreases with age in fish (Musa and Salam, 2007). Adult fish from Centro presented the lowest Condition Factor and were the group that lasted longer in initiating reproduction in captivity (approximately six months). These fish were captured in a lagoon with a very large population of castarricas, but impacted by urban development.

During the first stage of evaluation, all families performed similarly; however, significant differences have been found once the fish are in the grow-out phase. Regarding to this, different authors have reported similar results during the early stages of growth evaluation (Mair, *et al*., 2004; Liti *et al*., 2005) who did not find differences in growth in tilapia lines. These authors suggested that food availability may play an important role at this age. So far we have found significant differences among the fish used as controls for each family (randomly selected) and the selected fish. Another interesting result is that no differences have been found so far for females from the different localities, but males have very different growth patterns between localities. In few months, we should be able of selecting the fish that will form our broodstock for the firs selected line of *C. urophthalmus*. More time will be needed for *P. splendida* due to the late start in the selection.

The results obtained with the diets experiments demonstrate that the fish meal can be replaced in a practical diets by the pork and poultry by-product meals up to 25%, without adverse effects on growth and survival for *Cichlasoma urophthalmus* and *Petenia splendida* which is in agree with results obtained for Hasan et al. (1997) who obtained a substitution of 20% of the fish meal by hydrolyzed feather meals in the carp *Labeo rohita.* Warith et al. (2001) were able of replacing 40% of fish meal by poultry by-product meal in juveniles of African catfish *Clarias gariepinus.* In the same way, Millanema (2002) and Van and Yu (2003) reported satisfactory results in growth with an 80% substitution of the fish meal by meat and bone meal in *Epinephelus coioides* and *Pangasianodon hypophthalmus* respectively. In another study, Brown (2005) reduced the cost of food production by 20% with a substitution of 45% of meat and bone meal in black bass *Micropterus salmoides*. Peters et al. (2006) and Zhang et al. (2006) substituted 20% of fish meal with meat and bone meal with favorable growth in *Carassius auratus gibelio* and *Oreochromis sp.* juveniles. Qinghui et al. (2006) were able to replace a 45% fish meal protein by meat and bone meal (MBM) in yellow croaker *Pseudosciaena crocea*. Additionally, Shapawi et al. (2007), reduced 50% of fish meal with poultry by-product meal in *Cromileptes altiveli*, and Zhang et al., (2008), replaced 26% of the fish meal with a mix of vegetable and animal protein meal with good results in growth and survival of *P. crocea*. Finally, Menghong et al. (2008) reported that only 6% fish meal substitution could be done with combinations of the poultry by-product, meat-bone and blood meals.

Best food quality indexes for *C. urophthalmus* and *P. splendida* were obtained when 25% fish meal was substituted for pork and poultry meals. Similar values were obtained by Warith et al. (2001), who obtained and FCR=1.63, SGR=3.56 and PER=1.72 substituting

40% of the fish meal by poultry byproducts meal when feeding *Clarias gariepinus*. These authors found very good growth, even higher than the values obtained here for *C. urophthalmus*. Millamena (2002), studied inclusion of 80% bone and blood meals in diets for *Epinephelus coioides*, obtaining values of 1.04 for FCR and 2.92 for SGR. These values are similar to the ones obtained in our study.

Other studies have reported bette results with other species than the ones obtained by us in this study. Zhang et al. (2006) obtained higher values for WG% (155.16) when studying inclusion of 20% bone and blood meals in diets for tilapias (*Oreochromis sp*). Ávalos (2006), determined higher values for Nile tilapia, *Oreochromis niloticus* (FCR=1.91; SGR=3.18; DPI=386.3; PER=1.64; DFI=0.19; WG=494.1%) using a 35% substitution of fish meal for pork meal. Tilapias as omnivorous fish may adapt easily to different ingredients in the diets while *C. urophtalmus* is considered opportunistic carnivorous and *P. splendida* is carnivorous (Chávez et al*.,* 1989). In a different study, Zhang et al. (2008) reported a SGR of 3.5 when replacing 6% of the fish meal by a mixture of protein sources for *P. crocea*.

Survival in our study was good, reaching 95% for *C. urophthalmus* and 77 - 100% for *P. splendida*. Best results were obtained when 25% of the fish meal was replaced by either pork or poultry meal. Almeida (2008) obtained 96% survival for *P. splendida* when using 25% wheat gluten indicating that some mortality is present when changes to the amount of fish meal used are made. Since *P. splendida* is a carnivorous species, its ability to digest diets containing vegetal ingredients is low. This situation is clear in our study since the diet with 100% FM had the highest survival. More research is needed to determine other options for replacing fish meal without compromising survival.

In general, partial or total substitution of fish meals in diets for fish or crustaceans - particularly carnivorous- for alternative ingredients has a series of advantages such as diversification of the industry when products that may be considered waste by-products are used. Some of these by-products are currently generated in the pork, bovine and poultry meat industry. The current way of processing this products are designed to be highly efficient and pathogen free; therefore, the by-products are high-quality from the sanitary point of view as well as from the quality of the meals obtained (El-Sayed, 1999). Fish meal used in diets for aquaculture is a very efficient ingredient with high protein and excellent amino acid profiles has become a highly valued ingredient with a price that continuously rise. This situation has created disadvantages for using fish meals since the price of diets are making aquaculture not affordable in several countries (Zaldívar, 2002). The constant fluctuation of fish meal quality has become another important issue; the fact that most of the fish meal is made of sardine, herring and hake, made the industry strongly dependant of the wild captures for these species. Environmental conditions and stock fluctuations severely impact commercial captures and therefore the price, quality and availability of fish meals. The use of alternative ingredients (such as pork, poultry and bovine by-products) will be adequate, either trough national or international enterprises that certify quality of the fabrication procedures and the meals obtained. Impacts to the feeds used in aquaculture will be substantial since an important decrease in the price of the feeds will be achieved by decreasing the use of fish meals (Robaina, 1998; Allan et al., 2000).

Previous results regarding nutrient requirements, practical diet evaluation and digestive capacity for *C. urophthalmus* (Martínez-Palacios and Ross, 1994; López, 2008) indicate that this species is an opportunist carnivorous making feasible the substitution offish meal

in the diets. However, this species requires a high amount of protein (45%) which can be easily achieved using pork meal. Pork meal has amino acid profiles similar to the one found in fish meals, with the difference that lipids and ashes are higher in pork meals. These characteristics may make pork meal difficult for fish to digest.

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Tabla 1. Average values for hematologic parameters measured in adult castarricas (C. urophthalmus) from the different localities.

	Centla	**Comalcalco**
Microhematocrit (%)	22.03 ± 4.26	28.55 ± 3.88
Hemoglobin (g/100 mL	4.10 ± 0.93	4.78 ± 0.77
Total protein in plasma (g/dl)	4.98 ± 2.98	6.04 ± 1.58
Erythrocytes (cel/mm^3)	1.16 ± 0.43	1.65 ± 1.09
Leucocytes (cel/mm^3)	31.85 ± 14.01	31.13 ± 21.38

Tabla 2. Fry production during January-October 2009 from females used as broodstock .

Females	**Weight (g)**	**Jan**	**Feb**	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**	**Oct**	**Total # Fry**
J1: Centla	159				5,702	6,758	7,540					**20000**
J2: Centla	179	4,332		5,500		6168						**16000**
J3: Centla	206	2,544			4,927		3349	3,727	2200			**16747**
J4: Centla	281	2,238			4,833	5,500	4607	4,000	2,822			**24000**
J5: Centla	309			5,823	6,813	6,738	5,933	6,275	5764			**37346**
J6: Centla	141				6,190		5,810					**12000**
J7: Centro	267				4,555		3,954					**8509**
J8: Centro	240			3,857	3,714	3,074		3,489	2,979	2,637	2,197	**21947**
J9: Centro	252				3,947	4,710		4,510	3,923		2,645	**19735**
J10: Comalcalco	300				7,927							**7927**
J11: Comalcalco	222	2,283	2,667		3,050							**8000**
J12: Comalcalco	227				5,589				5,850	5,261		**16700**
J13: Comalcalco	181			5,750		7,150						**12900**

Table 3. Food quality indexes and survival of *P. splendida* juveniles feed with diets substituting fish meal for pork meal.

	Substitution					
Indexes	**0%SM-100%PorM**	**25%SM-75%PorM**	**50%SM-50%PorM**	**75%SM-25%PorM**	**100%SM-0%PorM**	**CD**
FCR	1.71 ± 0.70a	2.44 ± 0.38b	1.49 ± 0.45a	2.33 ± 0.40b	0.84 ± 0.25a	1.41 ± 0.45a
Sur	100.00 ± 0.00a	95.56 ± 3.85a	86.67 ± 6.67b	88.89 ± 10.18ab	97.78 ± 3.85a	100.00 ± 0.00a
SGR	3.14 ± 0.28	2.65 ± 0.29	3.08 ± 0.20	3.03 ± 0.56	3.69 ± 0.22	3.25 ± 0.42
CF	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.00	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
DFI	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.00	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
DPI	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
PER	1.52 ± 0.81	0.92 ± 0.15	1.58 ± 0.44	1.32 ± 0.96	1.87 ± 1.03	1.70 ± 0.56
WG%	814.02 ± 165.79ab	550.33 ± 137.92b	901.67 ± 154.30ab	578.91 ± 289.51b	1241.51 ± 292.25a	900.56 ± 308.25ab

Table 4. Food quality indexes and survival of *C. urophthalmus* juveniles feed with diets substituting fish meal for pork meal.

	SUBSTITUTION					
Indexes	**0%FM-100%PorM**	**25%FM-75%PorM**	**50%FM-50%PorM**	**75%FM-25%PorM**	**100%FM-0%PorM**	**CD**
FCR	2.94 ± 0.82	2.17 ± 0.29	2.72 ± 1.04	2.43 ± 0.84	3.11 ± 0.79	2.40 ± 0.07
Sur	100.00 ± 0.00	100.00 ± 0.00	97.78 ± 3.85	97.78 ± 3.85	97.78 ± 3.85	100.00 ± 0.00
SGR	1.25 ± 0.19	1.53 ± 0.11	1.44 ± 0.32	1.59 ± 0.35	1.22 ± 0.09	1.19 ± 0.15
CF	1.81 ± 0.09	2.07 ± 0.24	1.76 ± 0.02	1.76 ± 0.09	1.67 ± 0.06	2.03 ± 0.26
DFI	0.19 ± 0.01	0.19 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.00	0.17 ± 0.01
DPI	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
PER	0.79 ± 0.19	1.04 ± 0.14	0.93 ± 0.46	1.02 ± 0.43	0.74 ± 0.17	0.93 ± 0.03
WG%	146.51 ± 36.19	192.61 ± 23.96	161.49 ± 81.50	183.03 ± 72.25	121.56 ± 38.15	130.93 ± 23.82

Table 4. Food quality indexes and survival of *P. splendida* juveniles feed with diets substituting fish meal for poultry by-product meal.

	Substitution					
Indexes	**0%FM-100%PouM**	**25%FM-75%PouM**	**50%FM-50%PouM**	**75%FM-25%PouM**	**100%FM-0%PouM**	**CD**
FCR	0.87 ± 0.12	0.95 ± 0.20	1.39 ± 0.44	0.83 ± 0.29	1.43 ± 0.40	0.85 ± 0.18
Sur	100.00 ± 0.00a	86.67 ± 0.00ab	95.56 ± 3.85ab	97.78 ± 3.85ab	77.78 ± 10.18b	100.00 ± 0.00a
SGR	3.69 ± 0.02ab	3.81 ± 0.27ab	3.47 ± 0.10ab	3.97 ± 0.09a	3.25 ± 0.32b	3.85 ± 0.09ab
CF	1.13 ± 0.08	1.10 ± 0.04	1.05 ± 0.01	1.08 ± 0.02	1.07 ± 0.09	1.23 ± 0.06
DFI	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
DPI	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
PER	2.60 ± 0.33	2.42 ± 0.54	1.72 ± 0.60	2.87 ± 0.83	1.65 ± 0.50	2.71 ± 0.67
WG%	1221.41 ± 15.73	1352.09 ± 258.06	1038.86 ± 82.61	1513.07 ± 104.22	875.77 ± 196.15	1378.82 ± 89.91

Table . Food quality indexes and survival of *C. urophthalmus* juveniles feed with diets substituting fish meal for poultry by-product meal.

	SUBSTITUTION					
Indexes	**0%FM-100%PouM**	**25%FM-75%PouM**	**50%FM-50%PouM**	**75%FM-25%PouM**	**100%FM-0%PouM**	**CD**
FCR	2.29 ± 0.16	2.14 ± 0.35	1.96 ± 0.20	1.78 ± 0.35	1.99 ± 0.19	1.33 ± 0.13
Sur	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	97.78 ± 3.85	97.78 ± 3.85	100.00 ± 0.00
SGR	2.27 ± 0.26	2.12 ± 0.09	2.19 ± 0.15	2.26 ± 0.21	2.33 ± 0.12	2.71 ± 0.07
CF	1.80 ± 0.11	1.75 ± 0.12	1.92 ± 0.29	1.97 ± 0.28	1.83 ± 0.23	1.84 ± 0.22
DFI	0.20 ± 0.00a	0.19 ± 0.00ab	0.18 ± 0.00ab	0.17 ± 0.01b	0.19 ± 0.00ab	0.18 ± 0.01ab
DPI	0.09 ± 0.00a	0.08 ± 0.00ab	0.08 ± 0.00ab	0.08 ± 0.00b	0.09 ± 0.00ab	0.08 ± 0.00ab
PER	0.97 ± 0.07	1.05 ± 0.16	1.14 ± 0.12	1.28 ± 0.25	1.12 ± 0.11	1.68 ± 0.15
WG%	395.36 ± 93.19	341.52 ± 27.94	366.25 ± 50.72	388.86 ± 69.99	411.77 ± 42.97	567.35 ± 31.21

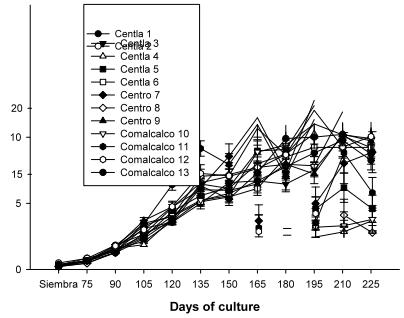


Figure1. Average weight from 13 castarricas (*C.urophthalmus*) families from the three localities evaluated.

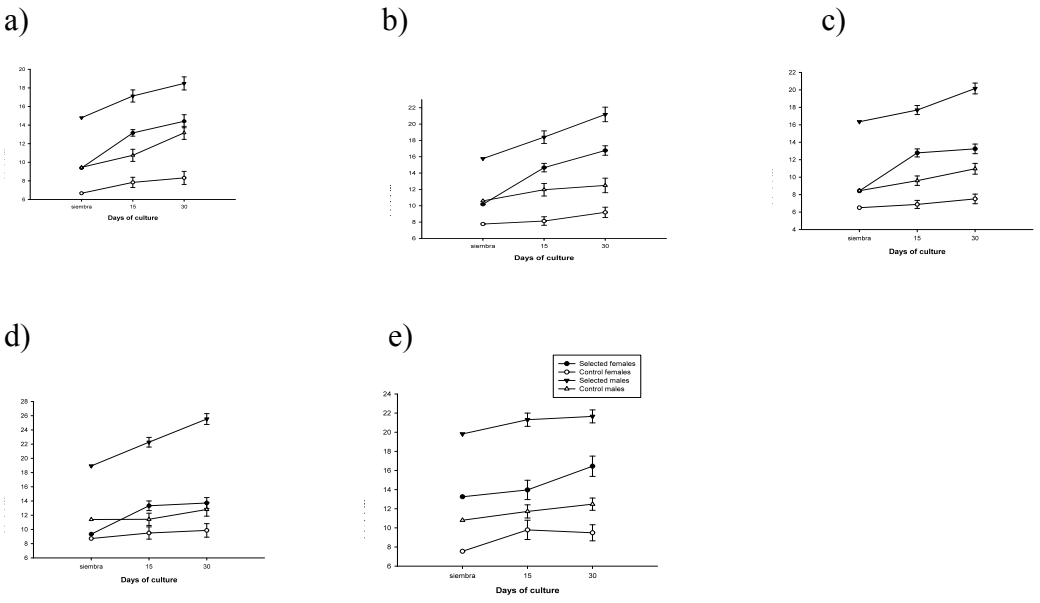


Figure 2. Average growth in weight (g) of males and females selected compared against control groups of *C. urophthalmus* families from Centla: a) Family 1; b) Family 2; c) Family 3; d) Family 4 and e) Family 5.

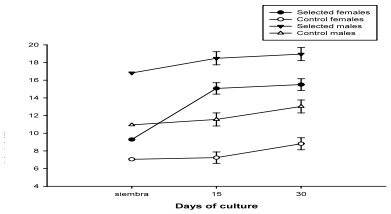
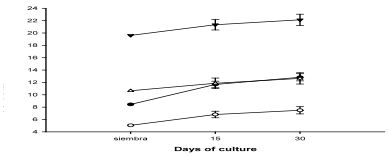
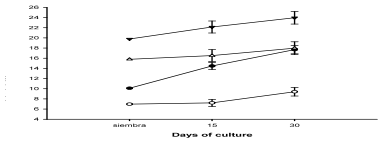


Figure 3. Average growth in weight (g) of males and females selected compared against control groups of one *C. urophthalmus* family from Centro

a)



b)



c)

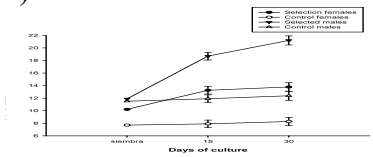


Figure 4. Average growth in weight (g) of males and females selected compared against control groups of *C. urophthalmus* families from Comalcalco: a) Family 1; b) Family 2 and c) Family 3.

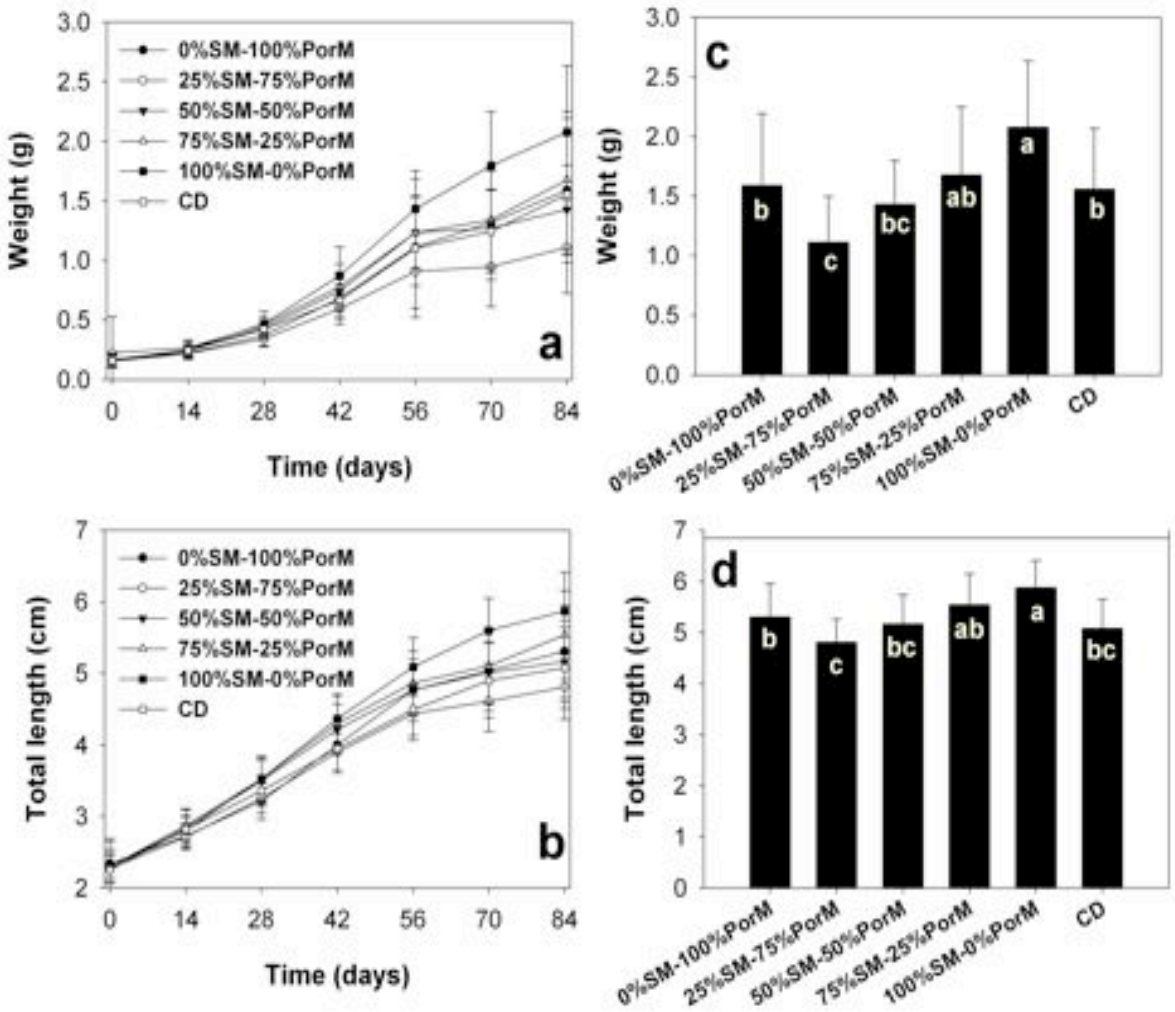


Figure 5. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *P. splendida* juveniles feed with diets substituting fish meal for pork meal.

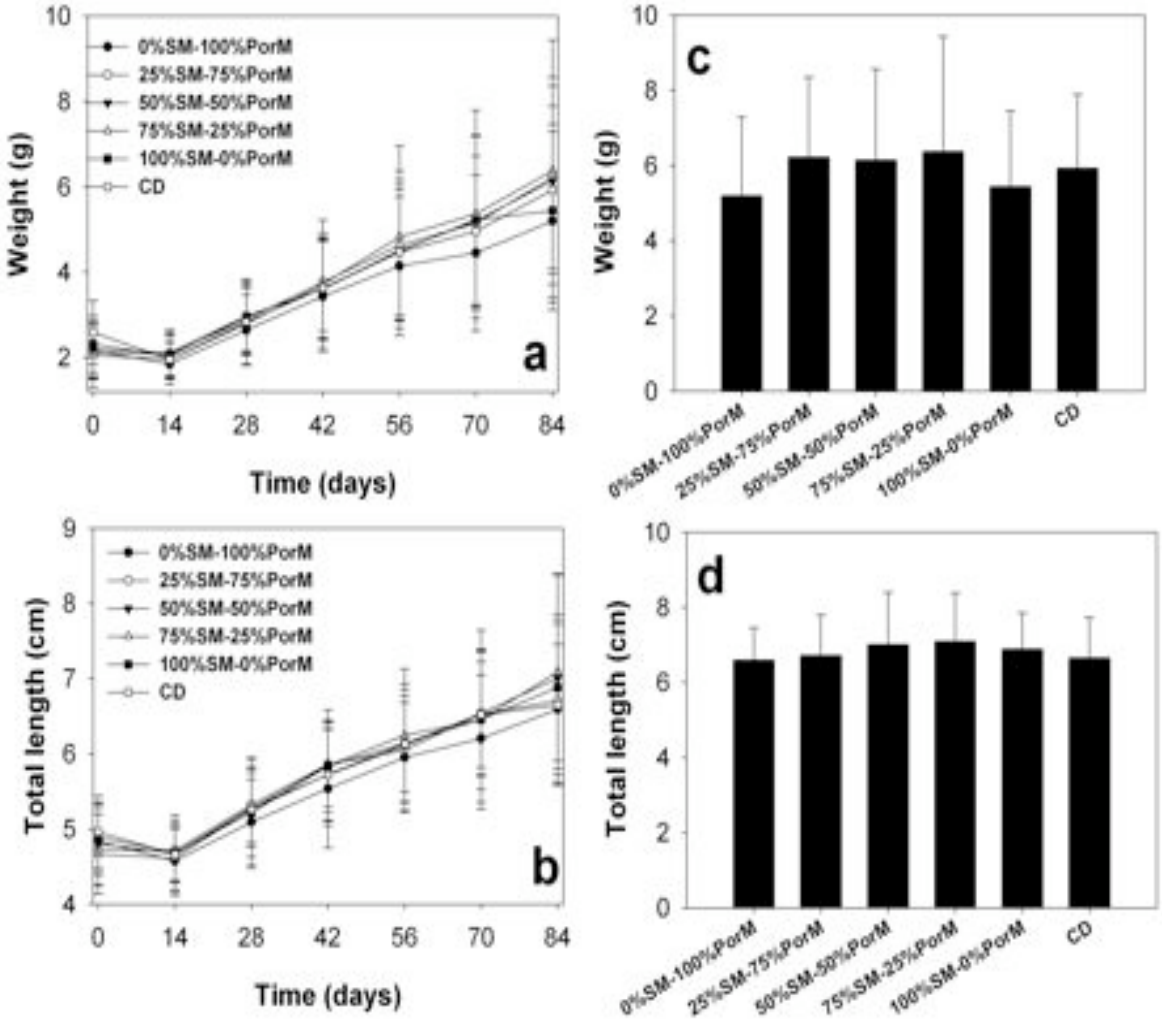


Figure 6. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *C. urophthalmus* juveniles feed with diets substituting fish meal for pork meal.

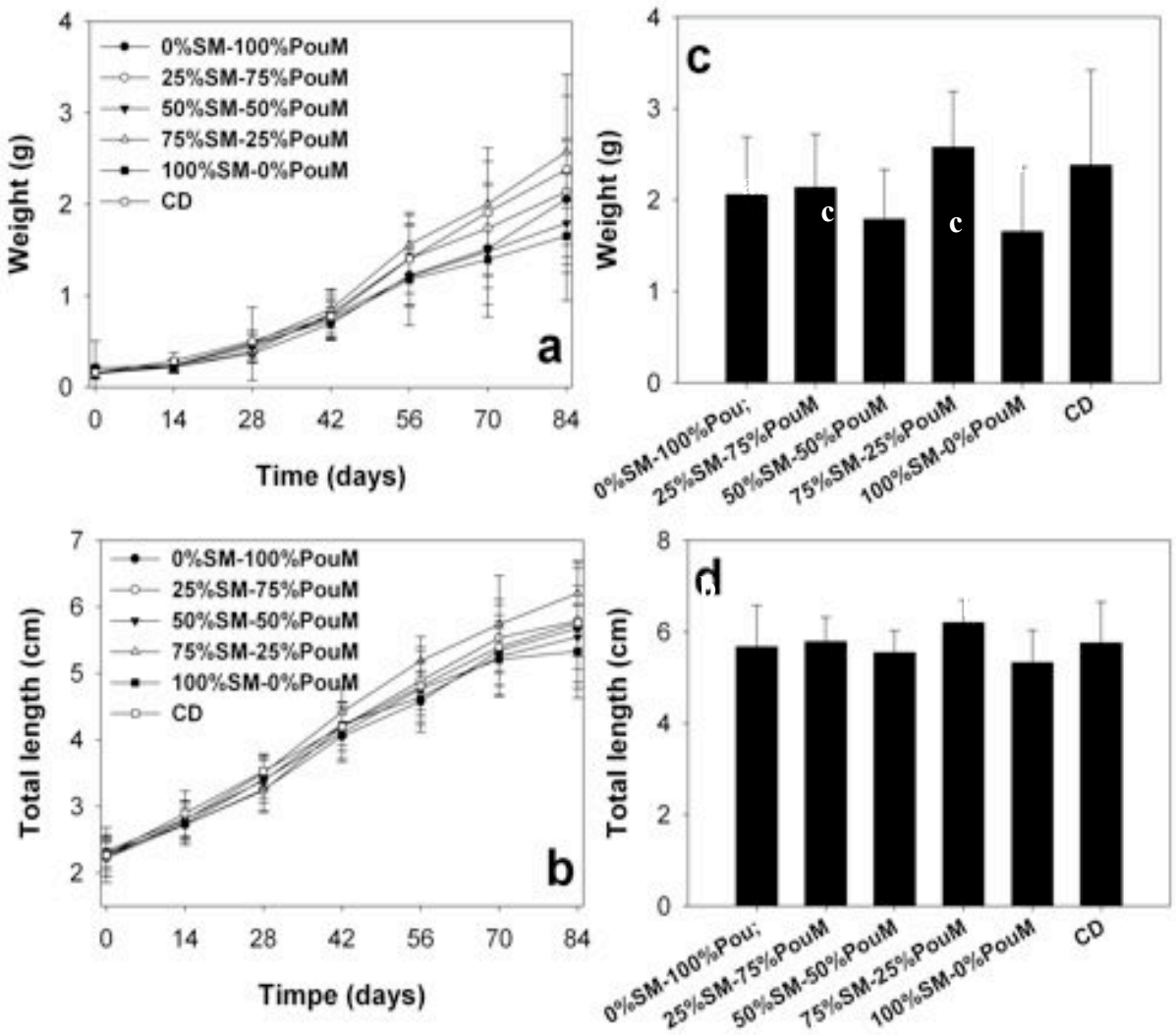


Figure 7. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *P. splendida* juveniles feed with diets substituting fish meal for poultry by-product meal.

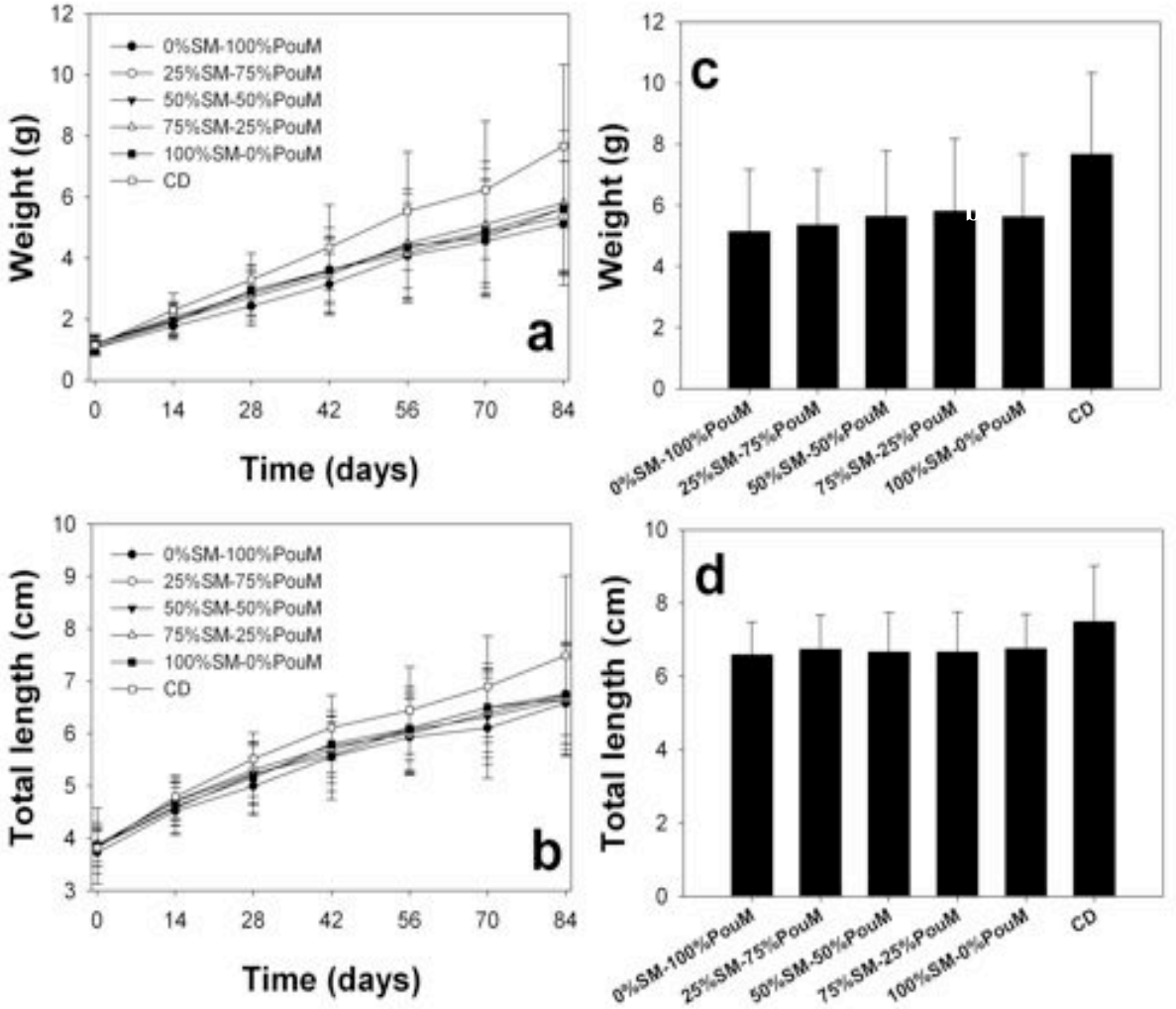


Figure 8. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *C. urophthalmus* juveniles feed with diets substituting fish meal for poultry by-product meal.

SPAT COLLECTION, GROWTH RATES AND SURVIVAL OF THE NATIVE OYSTER SPECIES, CRASSOSTREA CORTEZIENSIS AT SANTA MARIA BAY, MEXICO INDIGENOUS

Species Development/ Experiment/ 07IND03UH

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ABSTRACT

A trial was conducted to determine whether Crassostrea corteziensis spat could be obtained in sufficient numbers and successfully cultured during the early nursery stages at Santa Maria Bay, Sinaloa, Mexico, to test the feasibility of culturing this native species instead of C. gigas. Although spat was abundant, it did not exhibit significant growth during the early stages of the trial (October-February) but then grew more rapidly from March to May. Slow growth may be a function of low temperatures and an unusual lack of productivity in the Bay, postulated to be correlated to the El Niño phenomena. Although sufficient numbers of spat can be readily obtained, survival was also low (53%). Repetition of the trial during the summer months and when El Niño is not occurring. The need for bivalve hatcheries which can supply regional farmers is also indicated.

INTRODUCTION

Oyster culture on the coasts of the Gulf of California, Mexico is primarily based on an introduced species, the Pacific Oyster (Crassostrea gigas). A few exceptions exist where the native "Pleasure oyster" (Crassostrea corteziensis) is cultured, such as at Boca de Camichin, Nayarit. In the State of Sinaloa the federal government has been promoting oyster culture as one way to reduce pressure on the shrimp fishery, which is considered to be in crisis. As part of this initiative, C. gigas has been cultured for 8 years in Altata Bay, for 4 years at Santa Maria Bay, and other oyster culture programs are planned for other areas of the State. Although market studies have indicated that there is a strong local preference for the Pleasure Oyster over the Japanese oyster, culture of the former has not taken off, primarily due to the difficulty in obtaining seed, either through spat collection or hatchery production. C. gigas seed is more easily obtained from large hatcheries in the U.S., hence the dependence on this species in the Gulf of California.

In Nayarit where the Pleasure Oyster has been cultured for over 30 years, it grows to commercial sizes of 2-3 inches in length in about 9 months. It is also more abundant than in Sinaloa, and the Nayarit industry is able to depend on wild spat collection due to the abundant spat fall. In the more northern state of Sinaloa, the Pleasure Oyster is commonly

found on the roots of mangroves in coastal lagoons of Sinaloa, but does not obtain large sizes, presumably due to competition for space and possibly due to the high densities in which it settles on the roots. Rodriguez-Dominguez and Perez-Gonzalez (2009) found that Pleasure Oysters which detach from mangrove roots and establish themselves on the estuary bottom grew faster than those which remained attached. The Sinaloa estuaries also tend to be higher in salinity and become colder in the winter than those in Nayarit where the Pleasure Oyster is abundant. Hence there was some question of whether C. corteziensis was an appropriate culture species for these more northern areas with different climatic and hydrological conditions. Spat collection had also not been attempted in the northern areas.

The range of C. corteziensis is from northern Mexico to Peru (Hertlein, 1951), being found most commonly found in the intertidal zone of estuaries and coastal lagoons attached to mangrove roots and other solid substrates (Stuardo and Martinez, 1975). It is found in salinities of 3 to 39 ppt, with mortalities occurring in lower salinities. Larvae have been reported in salinities between 21 and 37 ppt, although higher larval growth rates are found between 23-28 ppt. This species appears to tolerate temperatures ranging between 16-32oC, with optimal growth occurring between 28-30oC. Mortality reaches one-hundred percent when the temperature exceeds 34 oC (Caceres-Puig, et. al. 2007).

The growth rate for C. corteziensis varies with temperature, salinity, food availability and age. Chavez Villalba et al (2005) reported higher growth rates in spring and summer (0.063 mm/day to 0.266 mm/day) than in the autumn and winter (0.016 mm/day to 0.159 mm/day). Chavez Villalba et al (2008) found that growth rates were 0.222 to 0.234 mm/day in coastal lagoons with higher salinity levels, and increased to 0.304 mm/day in areas with aquaculture farm discharges and lower salinity (25 ppt). The same authors reported that daily weight gain was 0.153 g/day in areas where particulate organic matter (POM) was 31 g/L and was fell to 0.077 g/day where POM was 9.7 g/day. In some cases, this relationship between POM and bivalve growth rates may be not be observed since in coastal habitats POM consists of bacteria, detritus, nano-zooplankton, but generally only phytoplankton is the major source of nutrients for filter feeding bivalves (Dame 1996).

Growth rates in Nayarit for C. corteziensis have been reported to be rapid, with daily growth rates of 0.345 mm/day during the first seven months post-set. Growth rates are particularly high during the first two months (0.666 mm/day) of a study by Stuardo and Martinez, 1975) but were reduced to 0.333 mm/day during the following six months. In the Agua Brava Lagoon, Nayarit, it was found that oysters growing on mangrove roots had lower instantaneous growth rates (K=0.04 monthly) had than those which grew on the lagoon bottom (K=0.89 monthly) (Rodriguez Dominguez and Perez Gonzalez, 2009). In Navalato, Sinaloa (close to the study site), Gongora Gomez et al (2006) found growth rates of 0.195 mm/day and 0.138 g/day at temperatures of 21.3 oC and 31 oC and salinities of 31.7 ppt and 39 ppt, respectively.

Chavez Villalba et al (2005) calculated the following parameters using the von Bertalanffy model: $L\infty = 114$ mm, K= 1.1 per year and tO = 0. A later study (Chavez Villalba et al 2008) used the same model to derive: $L\infty = 132.5$ mm, K= 1.08 per year and tO = 0.

The objective for this study was to determine if C. corteziensis spat could be collected from mangrove roots in sufficient numbers to support small-scale farming which is often conducted by women of lower socio-economic levels who may not have the resources to purchased eyed-larvae or spat. Additionally, the feasibility of farming this species in this

northern bay with higher salinities and lower salinities than the Boca de Camichin area would also be evaluated by tracking growth and survival rates. Training in oyster farming methods was also incorporated into the extension assistance provided during the research efforts.

METHODS

Study site

The Santa Maria Bay-Playa Colorado complex is a coastal lagoon comprised of three bays; Playa Colorado in the north, Santa Maria Bay in the central area and Calcetin Bay in the south. This is the largest lagoon (53,000 ha) in the State of Sinaloa and is bordered by the municipalities of Angostura and Navolato. It is located between 24° 25' and 25° 30' north and between 107° 35' and 108° 25' west (Figure 1).

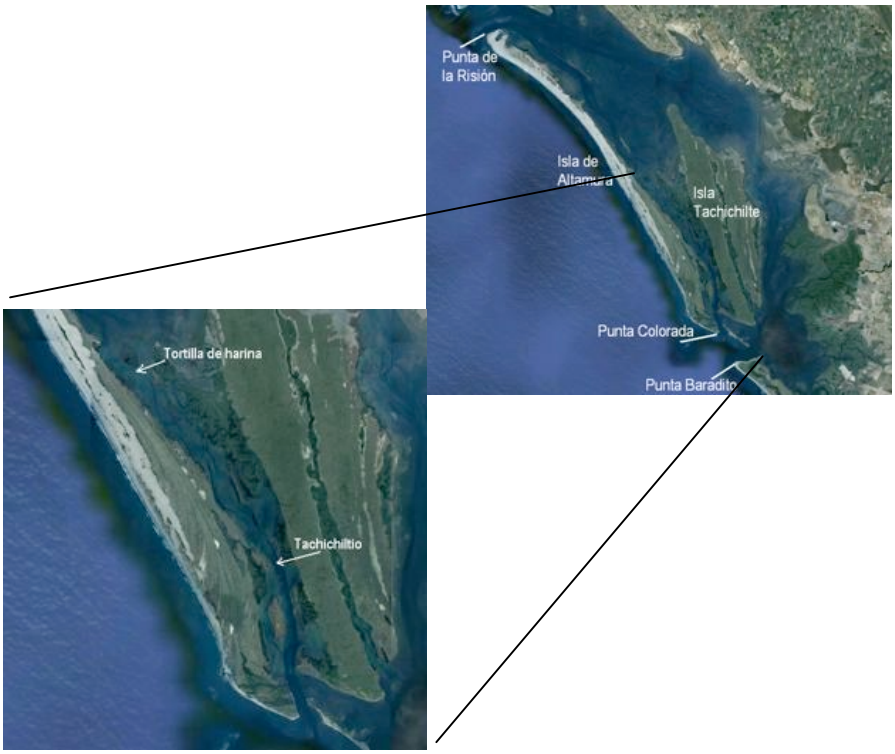

Figure 1. Location of oyster culture sites in Santa Maria Bay, Sinaloa, Mexico.

In the extreme north and south of the system are extensive areas of mangroves, as well as isolated patches that border the Talchichilte, Saliaca, de los Pajaros and Altamura islands. The total mangrove area is 2118 ha (De la Fuente and Carrera, 2005), making this one of the largest concentrations of mangrove in Mexico.

Santa Maria Bay is separated from the Sea of Cortez by an extensive sand barrier island (Altamura) which has three channels, two towards the northern end and one at the south. The northern channels are between Punta Perhihuete, Islas del Rancho and Punta la Rision, forming two mouths which are approximately 3.5 km wide. In the south, the channel is between Punta Colorado and Punto Baradito with a width of approximately 3.5 km. These channels lend themselves to a nearly constant exchange of water between the bay and the Sea of Cortez. Tachichilte Island is located in the interior of the Bay and has a series of shallow sand flats. There is a channel between Tachichilte Island and Altamura Island where currents coming from the northern and southern mouths meet. This area is called "Tortilla de Harina" by the local fishers. The oyster culture site is located slightly south of

Tortilla de Harina. The strong currents in this area provide for constant water exchange in the oyster farming area.

Spat collection and culture

Juvenile C. corteziensis were collected from mangrove roots in the Tachichiltio area on October 19, 2008. For the experiment, 640 spat were used. These had an average weight of 9.91 g when collected and were placed in Nestier trays suspended on a long line (Figure 2). Oyster weight and survival was monitored over a 7 month period. Data on temperature, salinity and chlorophyll a were also collected on a monthly basis. The trays were cleaned regularly to keep them free of biofouling.

Figure 2. Culture and monitoring of *C. corteziensis* spat. CRSP student Saul Lopez Sanchez is shown caring for the oysters and collecting data.

RESULTS

Oyster weight did not increase significantly between October and February, varying between 9.9 and 10 g ($P > 0.05$). Oysters began to grow after February and achieved a final weight of 21.7 g in May, with an average growth rate of 0.128 g/day (Figure 3).

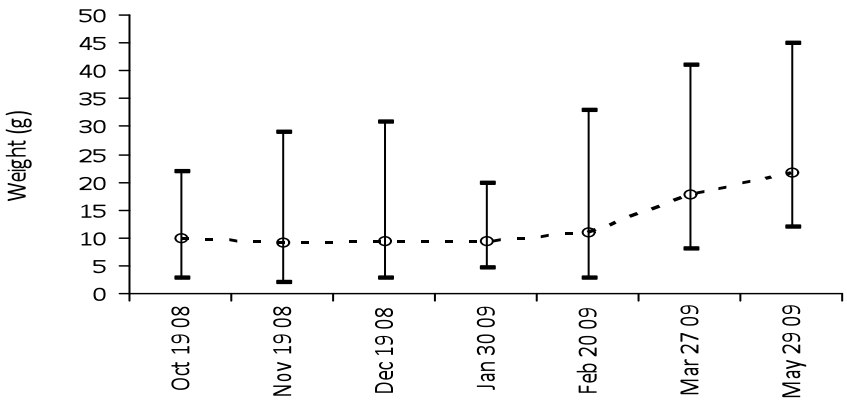

Figure 3. Growth of C. corteziensis spat cultured in Nestier trays at Santa Maria Bay, Sinaloa, Mexico.

The lack of growth between October and February coincided with a decrease in water temperature from 28.5 ^{o}C in October to 19 ^{o}C in February. The resumption in growth after February coincides with the increase in temperature which began in February (Figure 4).

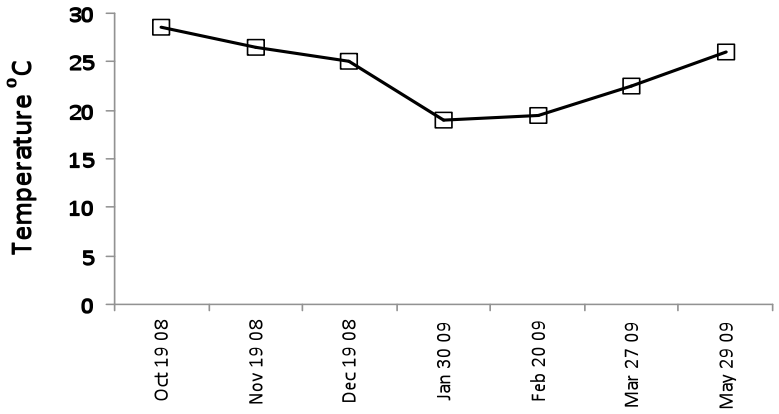

Figure 4. Surface water temperatures at oyster culture site in Santa May Bay, Sinaloa, Mexico.

During the period in which the oysters demonstrated little growth (October to February), chlorophyll a levels rose from 0.09 µg/L to 0.23 µg/L. From February to May, chlorophyll a levels decreased from 0.23 µg/L to 0.18 µg/L, at the same time that oyster growth rates were increasing (Figure 5).

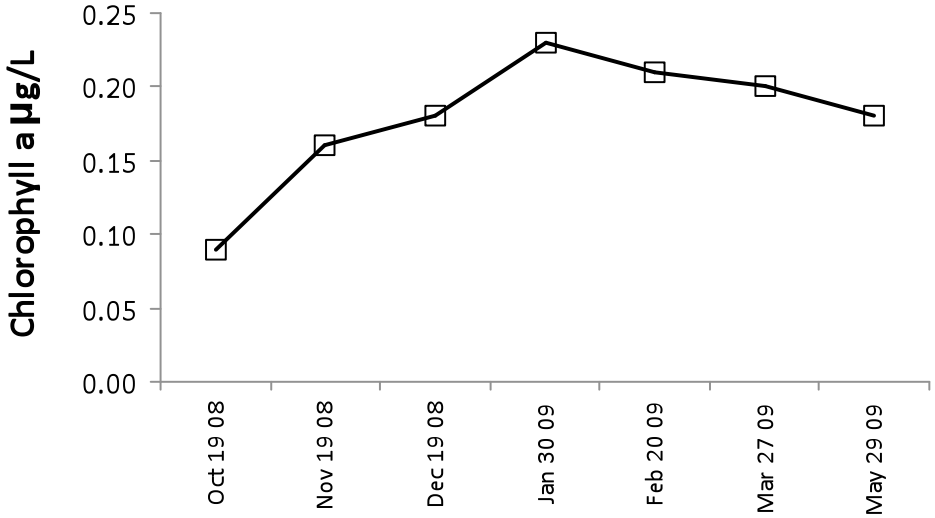

Figure 5. Chlorophyll a concentrations at oyster culture site, Bahia Santa Maria Bay, Sinaloa, Mexico.

Salinity varies between 36-36.5 ppt during the October-May period (Figure 6), indicating that some evaporation was occurring since salinity in the Bay was greater than that of the ocean.

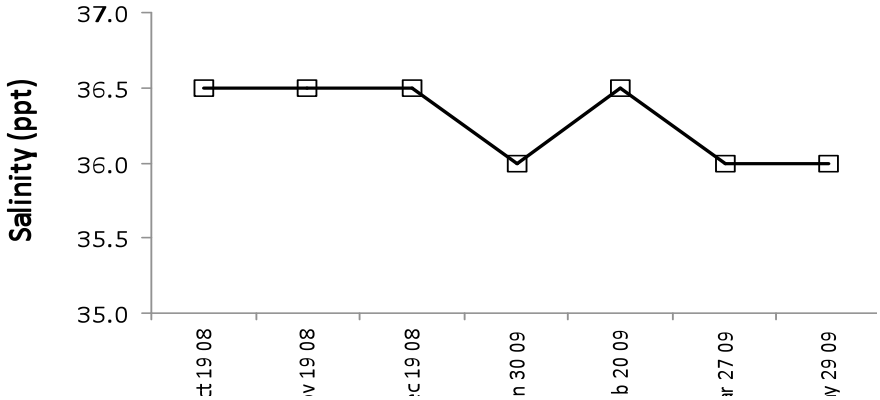

Figure 6. Salinity at oyster culture site, Santa Maria Bay, Sinaloa, Mexico.

Oyster survival at the end of seven months was 53% (Figure 7).

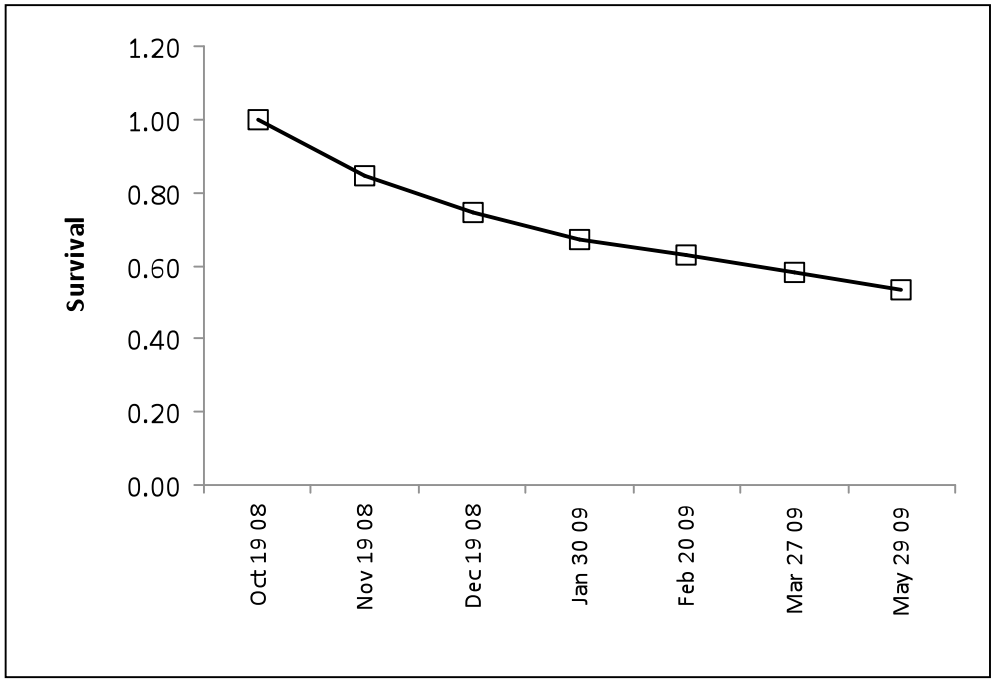

Figure 7. *C. corteziensis* spat survival at Santa Maria Bay.

DISCUSSION

During the culture period, water temperature varied between 19 oC and 28.5 oC, but the highest temperatures were during the beginning of the experiment. Subsequently, temperatures were lower than 26.5 oC which represent suboptimal temperatures for growth of this species according to the results of Caceres-Puig et al (2007) who showed that the highest growth rates are obtained between 28 oC a 30 oC. The temperatures in January (19oC) and February (19.5 oC) were close to the lethal limit for this species (16 oC).

Another factor in the slow growth may have been the low chlorophyll a concentrations (0.09 to 0.23 µg/L), whereas in other years, typical chlorophyll a levels range between 1-3

µg/L (Galindo Reyes, 2000). These adverse factors may have been related to the beginning of the El Niño phenomena which began during the summer of 2009 along the coast of Sinaloa (http://www.ciifen-int.org/). Other anecdotal information points such as a similar decrease in growth rate for farmed C. crassostrea gigas and a very poor wild shrimp harvest point to changing conditions in this usually highly productive bay.

In spite of lack of growth during the first four months, the growth rate later rose to 0.128 g/day, which is close to the growth rates reported from the Sonora coast.

The survival rate of 53% was low, and most mortality was observed during the winter coinciding with the period during which growth was also slowest.

CONCLUSION

In conclusion, it appears that in this case, culture of C. corteziensis would not have been economically feasible due to the slow growth and low survival. Given that conditions during this particular year may have been more adverse than usual given the low temperatures, low chlorophyll a levels and generally low productivity of the bay, repetition of these trials may be merited at a future date, rather than entirely discarding the idea of culturing C. corteziensis in the northern areas. Additionally, beginning nursery culture earlier in the year when temperatures are higher may increase growth rates.

Despite the disappointing results, some benefit did accrue to local oyster farmers as they received technical assistance in oyster farming that will benefit them in their work with C. gigas, and potentially with C. corteziensis in the future.

ANTICIPATED BENEFITS

The results of this work have spurred efforts to establish a bivalve hatchery at the School of Marine Sciences (FACIMAR) at the Autonomous University of Sinaloa. Even if future spat collection efforts are more productive than this trial, there is a chronic shortage of eye-larvae and spat for nearly all species, but particularly for native species. Even *C. gigas* larvae are increasingly difficult to obtain given the problems with production encountered by the large U.S. oyster hatcheries which have previously supplied stock to the Mexico oyster farmers.

Additionally, this work involved a considerable amount of training in both nursery and grow-out methods for oysters which benefited two oyster growing cooperatives, “Marine Culture of Colorado Beach” and “Fishers of La Reforma”. These cooperatives have a total of 23 members (4 women and 19 men). One CRSP student, Saul Lopez Sanchez, used this work as his research topic for his undergraduate thesis. Mr. Lopez Sanchez is originally from Santa Maria Bay and plans to return to work there after graduation.

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OYSTER-RELAYING AND DEPURATION IN AN OPEN-WATER LOCATIONS

Indigenous Species Development/Experiment/ (07IND04UH)

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ABSTRACT

Oysters (*Crassostrea corteziensis*) were harvested at Boca de Camichin, Nayarit, Mexico and relayed to two sites (La Palicienta and Pozo Chino) to test whether oysters could be depurated in this way. Fecal and total coliform bacteria and *E. coli* in water and oyster tissues were monitored for ten days during July 2008. Bacterial levels generally exceeded legal limits during most of the ten day period at both sites, although levels were lower at La Palicienta during the neap tides. Relay and depuration is therefore not feasible at these sites during this time of year, although it is known that bacteria levels are significantly lower during winter months, suggesting that this experiment should be repeated during the winter.

INTRODUCTION

Bivalves are particularly susceptible to contamination due to their filter feeding habits. Pathogens acquired in this way can subsequently infect human consumers. This may be particularly serious in areas where waste water treatment is rare, shellfish sanitation plans non-existent and consumers lack awareness of the possible risks. This is generally the case in Mexico, where the risk is exacerbated by the preference for consuming bivalves raw and lack of good post-handling methods. Shellfish-borne disease that can affect humans include: *Salmonella* spp. (typhoid fever, Salmonellosis) *Shigella* sp. (dysentary and Shigellosis), *Vibrio cholerae* (cholera), *E. coli*, enteroviruses y rotavirus (gastroenteritis), *Clostridium perfringes* (gas gangrene), *Hepatitis A* (infectious hepatitis), *Entamoeba histolytica* (amoebiasis), *Ascaris lumbricoide*s (ascariasis), *Enterobius vermicularis* (enterobiasis), *Taenia sp.* (taeniasis), *Aeromonas spp* (gastroenteritis and skin infections) (Araujo et. al, 1989; Metcalf and Eddy, 2003).

Specific tests to detect each pathogen are required, making comprehensive testing for the purpose of shellfish sanitation economically unfeasible. Coliform bacteria are therefore generally used as indicator organisms. In Mexico, Regulation NOM-031-SSA1-1993 establishes the maximum legal permissible levels for total coliforms as 70 MPN/100 ml and 14 MPN/100 ml for fecal coliforms for shellfish growing waters. The maximum permissible level for oyster tissues is 230 MPN/100 g.

Oysters also have the capacity to depurate themselves if they are kept in clean waters for specific periods of time, although not all pathogens (e.g. some viruses) can be reduced to safe levels. There are generally two methods for depuration. One is to use land based, flow-through systems with water that is free of contaminants. In these systems, the required depuration time is 24-48 hours. The other is to move oysters after harvest to an open-water setting where water quality monitoring has demonstrated that pathogen levels are below legally permissible levels. Depuration in these cases usually lasts from 7-10 days, although the length of this period is a precautionary measure.

In the Boca de Camichin Estuary, Nayarit, oyster culture using the native "Pleasure Oyster", *Crassostrea corteziensis*, has been practiced for over 30 years and is an important economic activity in this impoverished area. The oysters are sold locally and also in Tepic, Guadalajara and Mazatlan. A recent study indicated that the estuary waters have coliform levels above the legally permissible levels for an area approved for shellfish culture (Olivo-Garcia, 2007). This study only tested water, not oyster tissues.

In this study, oysters were obtained from farms in Boca de Camichin which had coliform tissue levels of 233 MPN/100g (slightly above the legal permissible level of 230 MPN/100g) and taken to two distant sites with water which was presumable cleaner than the oyster culture site, based on previous water quality testing. The water at each site and the oyster tissues were tested for coliform bacteria for ten days with the goal of determining the suitability of the sites for depuration and the rate of depuration. The sites were chosen due to their proximity at the mouth of the estuary and distance from potential sources of contamination.

Coliform bacteria counts have been utilized as indicators of fecal contamination in water (Orosco et al. 1983; Araujo et al., 1989; Barrera-Escorcia et al, 1999; Yap y Kahoru, 2001; Ravagnani et al., 2005, Ruiz García 2007, Olivo Rojas, 2007, Pérez González, 2009) and in oyster tissue (Rosas et al. 1985, Rodríguez, 1986, Leyva Castillo 1996, Barrera-Escorcia et al, 1998). Fecal coliforms are particularly appropriate indicators for recent contamination; these are distinguished using brilliant green bile broth or EC broth (Anónimo, 1987, Araujo et al. 1989). The association of fecal coliform bacteria in residential waste waters with other pathogens which cause gastrointestinal illness, bacterial infections and other disease has been amply documented (Araujo et al., 1989, Metcalf y Eddy, 2003).

Marine and estuarine waters appear to contain bacterial communities which may act to eliminate coliform bacteria so that that proliferation of coliforms appears to be low (Vallaro et al. 1950, Romero Jarero 1982 y Romero Jarero et al., 1986). In the Boca de Camichin estuary, the highest fecal coliform counts are during periods of low salinity due to input by rivers, while in Santa Maria Bay, Sinaloa, Ruiz Garcia (2007) attributed low coliform counts to high salinities and high exchange rate between the Bay and ocean.

Haws et al. (2006) noted that in Sinaloa and Nayarit waste waters do not receive adequate treatment before being released into coastal water bodies and represent a high public health risk, particularly through consumption of aquatic organisms. In Nayarit, this assertion was confirmed by Olivo Rojas (2007) who found that during the year concentrations of total and fecal coliforms were above legal permissible levels. In the case of Sinaloa, Ruiz Garcia (2007) found that total and fecal coliforms were on the average below the permissible level. For example, in Santa Maria Bay and Altata Bay, Mendez et al (1990)

reported low concentrations with a maximum of 23 MPN/100 ml in May, which is below the legal limit of 70 MPN/100 ml for oyster culture waters. In Altata Bay, Sinaloa, Perez Gonzalez (2009) found total and fecal coliform levels well below permissible levels and attributed this to the mixing of freshwater and marine water by tides.

Contamination of oyster tissue with fecal coliform has been documented in the Gulf of Mexico (Rosas et al, 1985; Rodríguez, 1986) where levels much higher than the legal limit of 230 MPN/100g for tissue. Pathogens such as *Salmonella*, *Escherichia* coli and *Plesiomonas shigelloides* were also found. These results from Mexico are in contrast to those found in other Latin American countries such as Cuba (Leyva Castillo et al., 1996) and Puerto Rico (Fontanez Barris, 2005), where coliforms and other pathogens were well below permissible levels.

Study Site

The Boca de Camichin Estuary is at the mouth of the San Pedro River and is located in the northwestern part of Nayarit State in the Municipality of Santiago Ixcuintla. The estuary is located between 24° 48' al 24°44' north and 105˚30´al 105˚29' west (Figure 1). This estuary is part of a larger coastal system known as the Marismas Nacionales (National Wetlands) which are comprised of 200,000 ha of water area including 157 coastal lagoons, barrier islands and mangroves. It is considered to be a national treasure with incomparable biodiversity and conservation value (Garcia-Carmona, 2003). Additionally, it is economically important for its fisheries and aquaculture.

The water quality of Boca de Camichin is influenced by freshwater from the Lerma Santiago River 11 kilometers to the south. Nine kilometers to the north, the San Pedro river has another narrow outlet, La Palicienta, before continuing on to form the Boca de Camichin Estuary. Additionally, the southern part of Boca de Camichin runs inland for a short distance creating a narrow water body called Pozo Chino which is isolated from the ocean by a narrow peninsula.

Figure 1. The study site-Boca de Camichin Estuary, Nayarit.

METHODS

Oysters spat is collected on oyster shells strung on lines ("sartas") which are hung from rafts and subsequently grown out in this manner. For the purposes of this experiment, six sartas with mature oysters were taken from the main farming area at Boca de Camichin and taken to La Palicienta and Pozo Chino. These are the closest possible potential depuration sites and are presumably free from contamination due to their distance from human settlements. La Palicienta is a small mouth of the San Pedro River north of Boca de Camichin with a strong oceanic influence. Pozo Chino is located to the southeast of Boca de Camichin which does not have any other freshwater influence.

Oysters were left at these sites for ten days during which total coliforms, fecal coliform and *E. coli* were measured periodically in the water and oyster tissues. The experiment began during the neap tide with a tidal range of 50-70 cm and ended with a spring tide with a range of 170 cm.

Sample collection and preparation

Water samples were taken using sterilized BOD bottles which were submerged with the lids on, and opened at a depth of 20 cm. Each bottle was wrapped in aluminum foil, placed in a plastic bag and transported in an ice chest with frozen gel packs. At each site, 15-20 oysters were collected in order to obtain a total of 50 g of oyster tissue. Each oyster was carefully brushed to remove mud and biofouling, washed with distilled water and then opened with a knife cleaned with distilled water. Tissue was removed from the shell using sterilized forceps. To make a 1:10 solution using the 50 g of tissue, 450 ml of phosphate buffer solution was added and the mixture was then macerated in a sterilized blender. The tissue solution was then stored in a sealed, sterilized flask.

Laboratory analysis

The Most Probable Number Presumptive and Confirmative tests were used.

Presumptive Test

Water samples and the flask with the tissue samples were agitated using 25 rapid up and down motions in a 30 degree arc to homogenize the samples. As each container was opened, the mouth was flamed. Ten ml and 1 ml of the sample were each used to inoculated three test tubes containing the culture media. From the tubes inoculated with 1 ml of sample, a 1 ml sample was then taken and used to inoculate test tubes containing 9 ml of media, thus obtaining a dilution of 0.1 ml. Durham vials were used for gas collection. This procedure was conducted four times for each water and tissue sample. All were incubated at 35 ^{o}C and examined after 24 hours. If gas was not observed, incubation was continued for an additional 24 hours. A standard MPN table was used to determine the concentration of total coliforms.

Confirmative Test

Samples were taken from each test tube that showed gas formation in the presumptive test and inoculated into tubes containing brilliant green lactose bile (BGLB) broth which were incubated at 44.5 ^{o}C for 24 hours. Tubes were examined at 24 hours; if no gas formation was observed, incubation was continued for an additional 24 hours. A standard MPN table was used to determine the concentration of fecal coliform.

RESULTS

The concentrations of total and fecal coliforms and *E. coli* in water and oyster tissues taken from La Palicienta and Pozo Chino are summarized in Table 1. In the case of La Palicienta, total and fecal coliforms were under the upper limit for shellfish growing waters as established by regulation NOM031-SSA1-1993. In the case of Pozo Chino, levels exceeded the legal limits.

Table 1. Geometric mean and median for coliforms and *E. coli* in water and oyster tissues from La Palicienta and Pozo Chino.

Site	Mean or median	Date	Total coliform in water MPN/100 ml)	Fecal coliform in water (MPN/100 ml)	*E. coli* in water (MPN/100 ml)	Total coliform in oyster tissue MPN/100 g	Fecal coliform in oyster tissue (MPN/100g)	*E. coli* in oyster tissue MPN/100 g
Palicienta	Mean	7/13/09	65	2	0	711	233	163
		7/15/09	145	40	33	486	251	223
		7/16/09	460	156	39	885	200	200
		7/17/09	144	114	52	1100	1100	885
		7/18/09	37	17	12	1100	1100	1100
		7/19/09	605	20	13	1100	1100	1100
		7/22/09	1100	1100	1100	1100	1100	1100
	Median	7/13/09	87	2	0	780	305	195
		7/15/09	150	43	43	460	240	240
		7/16/09	460	122	36	1100	210	210
		7/17/09	122	93	43	1100	1100	1100
		7/18/09	43	18	14	1100	1100	1100
		7/19/09	780	32	18	1100	1100	1100
		7/22/09	1100	1100	1100	1100	1100	1100
Pozo Chino	Mean	7/13/09	96	29	13			
		7/15/09	81	53	0	885	585	585
		7/16/09	263	39	32	477	87	40
		7/17/09	28	28	19	1100	1100	1100
		7/18/09	137	50	45	1100	1100	1100
		7/19/09	1100	1100	439			
		7/22/09	1100	1100	1100	1100	1100	1100
	Median	7/13/09	93	43	24			
		7/15/09	93	68	0	1100	780	780
		7/16/09	305	49	33	780	122	57
		7/17/09	33	33	19	1100	1100	1100
		7/18/09	158	43	43	1100	1100	1100
		7/19/09	1100	1100	1100			
		7/22/09	1100	1100	1100	1100	1100	1100

The variation in the mean concentration of total and fecal coliform, and *E. coli*, in water and tissue samples was statistically significant between different days of the experiment. There was also significant variation between samples as indicated by the standard deviation (Table 2).

Table 2. Mean and standard deviation for total and fecal coliforms, and *E. coli* in water and oyster samples from Palicienta and Pozo Chino.

Site		Date	7/13/09	7/15/09	7/16/09	7/17/09	7/18/09	7/19/09	7/22/09	P
Palicienta	Water	Total coliforms	108.5±106	150.75±48	460±0	342.75±507	60.75±62	725±442	1100±0	P<0.001
		Fecal coliform	2.25±2	61.25±61	199±176	172.25±193	21.25±15	27.75±19	1100±0	P<0.001
		E. coli	0	42.5±27	46.25±32	55.5±25	18.5±18	15.75±9	1100±0	P<0.001
	Tissue	Total coliforms	780±370	565±371	940±320	1100±0	1100±0	1100±0	1100±0	P=0.018
		Fecal coliform	290.75±197	272.5±132	202.5±38	1100±0	1100±0	1100±0	1100±0	P<0.001
		E. coli	223.25±177	258.25±151	202.5±38	940±320	1100±0	1100±0	1100±0	P<0.001
Pozo Chino	Water	Total coliforms	166.75±198	111.75±92	305±179	31±14	204.5±191	1100±0	1100±0	P<0.001
		Fecal coliform	45.75±36	62.5±36	51.5±38	31±14	64.75±58	1100±0	1100±0	P<0.001
		E. coli	23.5±23	0	39±27	22.5±15	62.75±60	832±536	1100±0	P<0.001
	Tissue	Total coliforms		940±320	688.25±498	1100±0	1100±0		1100±0	P<0.001
		Fecal coliform		940±320	688.25±498	1100±0	1100±0		1100±0	P<0.001
		E. coli		717.5±453	61.25±54	1100±0	1100±0		1100±0	P<0.001

Results from La Palicienta

The initial concentration of total coliform in water from La Palicienta was 65 MPN/100 ml (the legal upper limit for shellfish growing grounds is 70 MPN/100 ml), and stayed below 200 MPN/100 ml during the first six days of the experiment, with the exception of day 4 when the value increased 400 MPN/100 ml (Figure 2A and 2 D). Towards the end of the experiment, levels rose to 1100 MPN/100 ml. In contrast, the concentration of total coliform bacteria in oyster tissues was between 500 and 1000 MPN/100 g in the first four days and in following days, was above 1100 MPN/100 g (Figure 2A and 2B). The initial concentration of fecal coliform in water from La Palicienta was less than 2 MPN/100 ml, but at day four increased to between 122 (median) and 156 (mean) MPN/100 ml, then dropped to between 20 (mean) and 32 (median) MPN/100 ml on the seventh day, then the experiment ended with 1100 MPN/100 ml (Figure 2B and 2E). In contrast, concentrations of fecal coliform was between 200 and 300 MPN/100 g during the first four days and beginning on the fifth day, concentrations rose to 1100 MPN/100 g (Figure 2B and 2 E).

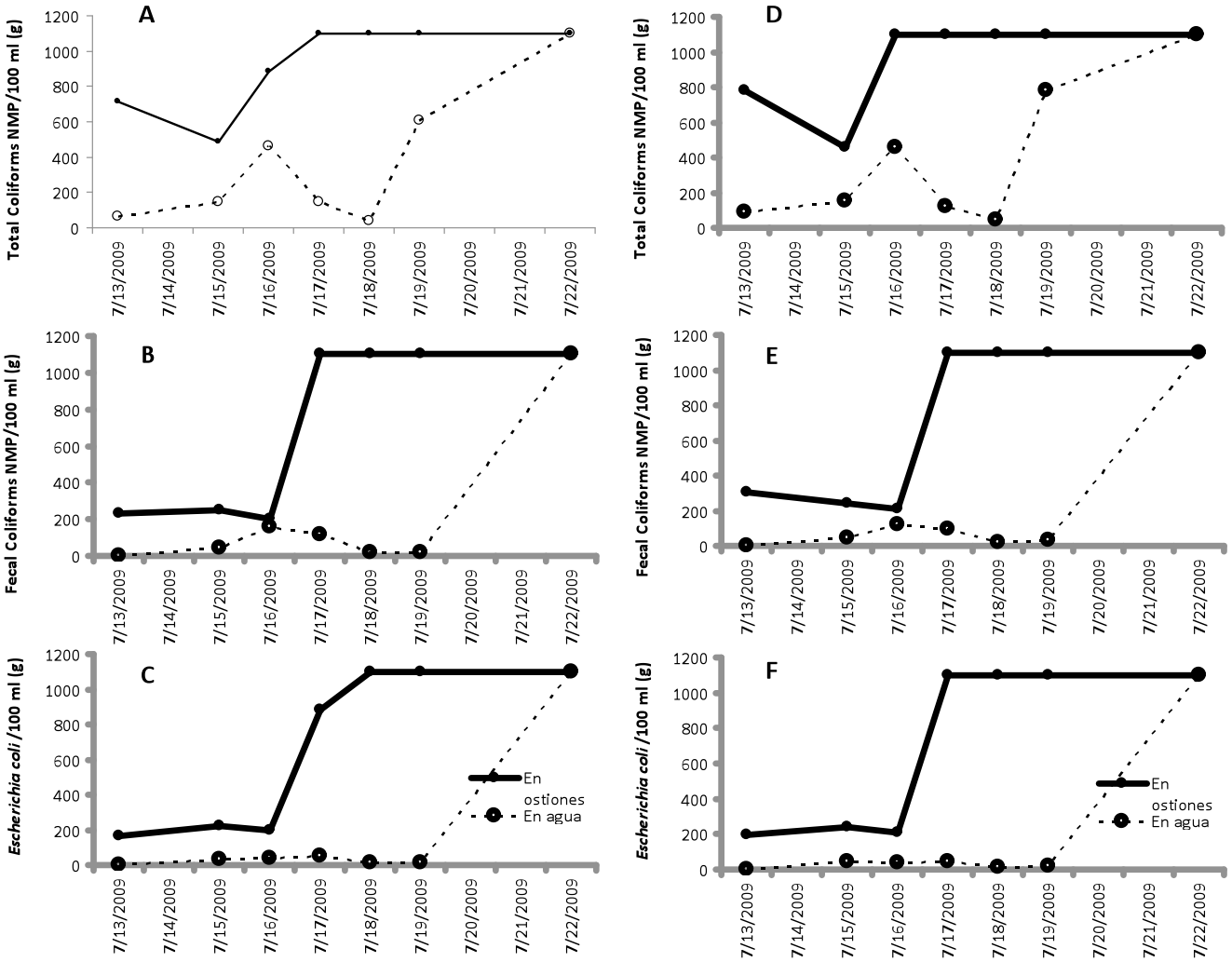

Figure 2. Geometric mean (A,B, C) and median (D,E, F) of total coliforms (above), fecal (middle) and *E. coli* (below) in water (dotted line) and oyster tissues (solid line) from La Palicienta.

At the beginning of the experiment, *E. coli* was not detected in water samples, but between the second and seventh day, the concentration varied between 12 and 52 MPN/100 ml and ended at 1100 MPN/100 ml (Figure 2C and 2F). In oyster tissues, *E. coli* was found at the beginning of the experiment at concentrations less than 200 MPN/100 g and increased in following days, reaching 1100 MPN/100 ml (Figure 2C and 2F).

From the third through fifth days, a significant amount of fresh water entered the system as shown by the low salinities between 13 and 19 ppt (Figure 3 A). This influx of freshwater coincided with the neap tides that are characterized by low tidal amplitudes and low current velocities (Figure 3B). After the sixth day, when the tides increased, the salinity also increased to 33-35 ppt indicating increased marine influence. The concentration of coliforms and *E. coli* during the first five days were associated with the predominant influence of freshwater during the neap tides. At the end of the experiment, coliform and *E. coli* levels arose during the spring tides and increases in salinity to 33-35 ppt.

The concentrations of coliforms and *E. coli* in oyster tissues stayed relatively low during the neap tides and increased to more than 1100 MPN/100 g when the spring tides occurred after the fifth day of the experiment (Figure 3).

The concentration of total coliform in water at La Palicienta stayed below 200 MPN/100 ml during the first six days of the experiment, with the exception of the fourth day when levels increased temporarily to 400 MPN/100 ml (Figure 2A and 2D). Levels later increased to greater than 110 MPN/100 ml. In contrast, oyster tissue levels stayed between 500 and 1000 MPN/100 g during the first four days and then rose to greater than 1100 MPN/100 g (Figure 2A and 2 B).

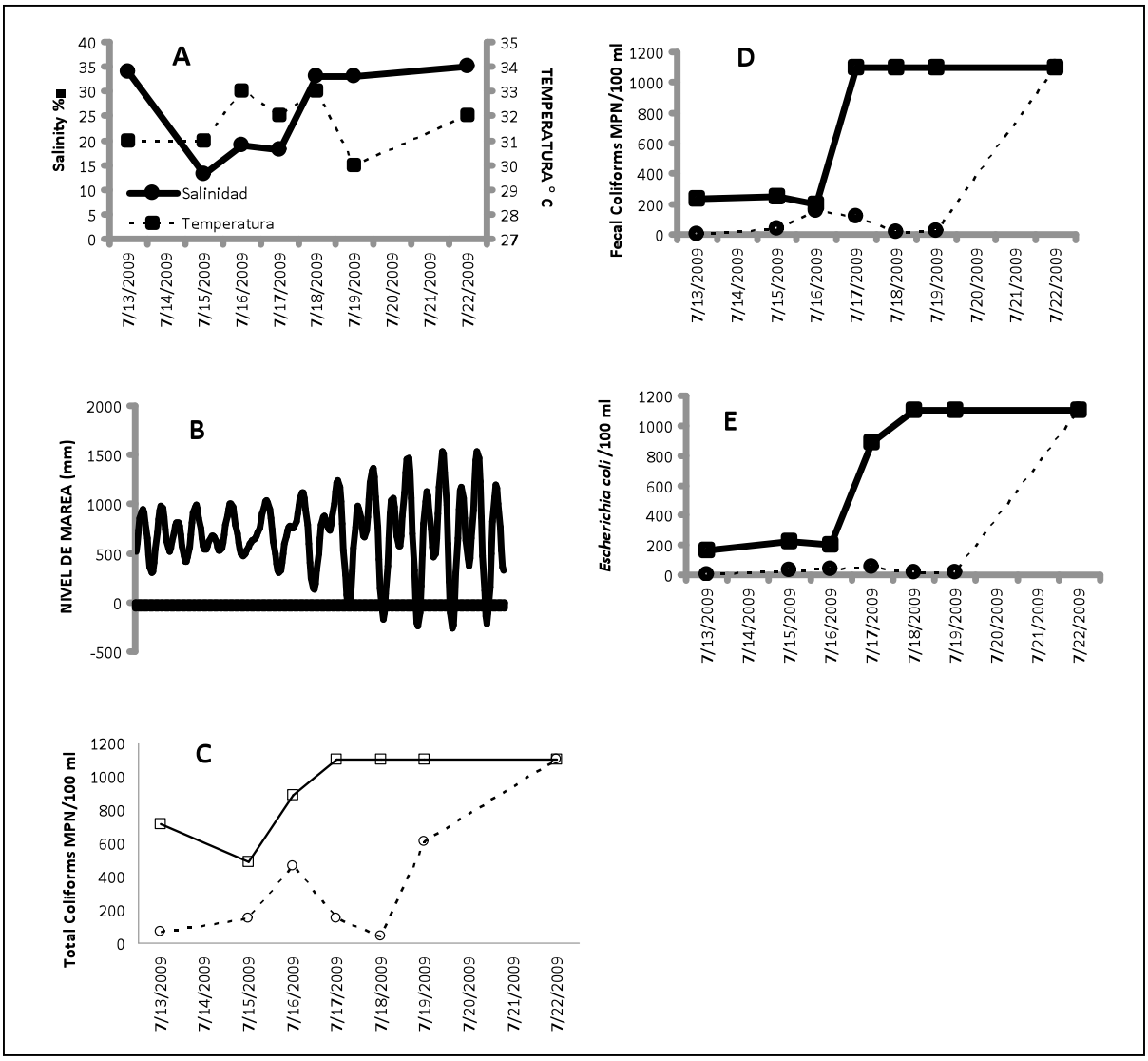

Figure 3. Temperature, salinity (A), tide level (B), total coliforms (C), fecal coliform (D) and *E. coli* in water (dotted line) and in tissues (solid line) for the experiment in La Palicienta.

Experiment in Pozo Chino

In contrast to the La Palicienta site, at Pozo Chino the initial concentration of total coliforms in water was 95 MPN/100 ml (the upper limit for shellfish growing grounds is 75 MPN/100 ml) and stayed between 80 and 140 MPN/100 ml during the first six day of

the experiment. It then increased to more than 1100 MPN/100 ml during the last four days of the experiment (Figure 4A and 4 D). Levels in oyster tissues were always above 400 MPN/100 g and rose to over 1100 MPN/100 g beginning at day five and continuing until the end (Figure 4A and 4 D).

The initial concentration of fecal coliform in water at Pozo Chino was 28 MPN/100 ml, well above the maximum legal limit of 14 MPN/100 ml. Levels stayed between 28 and 52 MPN/100ml during the first six days of the experiment and arose to more than 1100 MPN/100 ml during the last four days. In the oyster tissues, the concentration of fecal coliform was 584 MPN/100g at day 3, then fell to 87 MPN/100g on the fourth day. Beginning on day 5 and continuing until the end of the experiment, concentrations were above 1000 MPN/100 g (Figure 4 B and 4E).

E. coli was detected in Pozo Chino waters at a concentration of less than 45 MPN/100 ml during the first six days, with the exception of the third day, then rapidly rose beginning on the seventh day to 1100 MPN/ml and stayed at this level until the end of the experiment. *E. coli* was detected on day three in oyster tissues at a concentration of 584 MPN/100 g, but then fell to 40 MPN/100 g the following day. The concentration then rose on the fifth day to 1100 MPN/100 g and continue at this level until the end of the experiment (Figure 4C and 4 F).

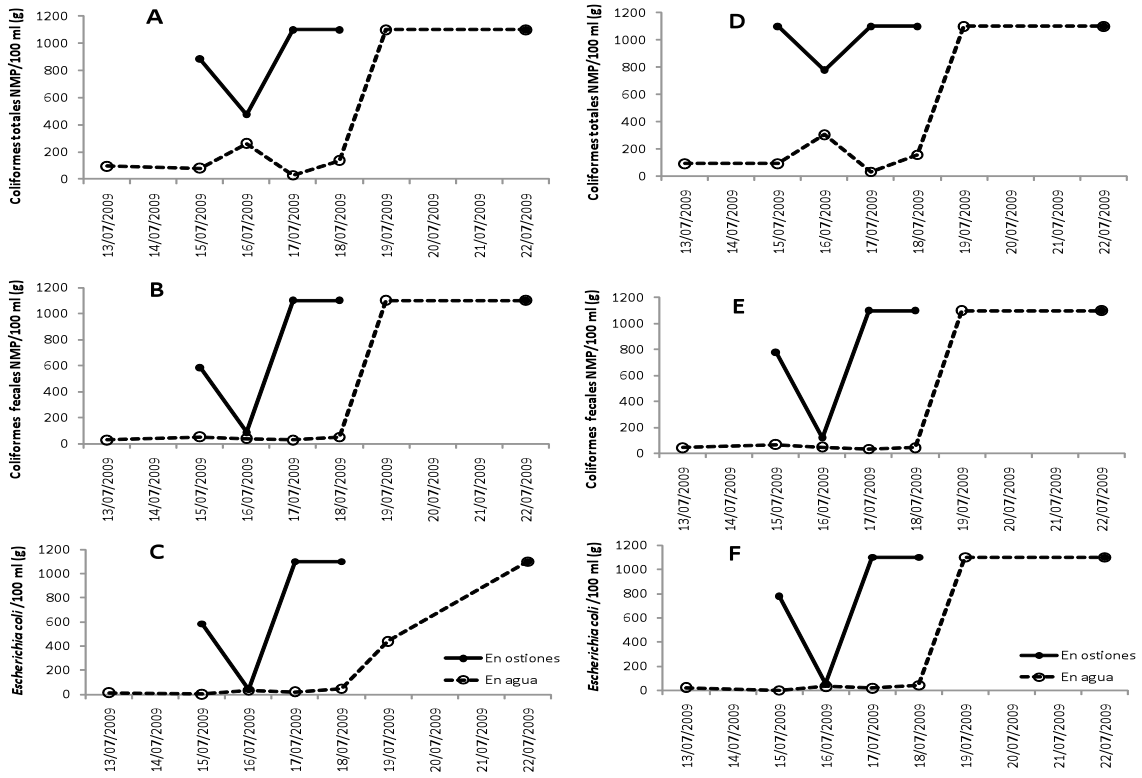

Figure 4. Geometric mean (A, B and C) and median (D, E and F) of the concentration of total coliform (above), fecal coliform (middle) and *E. coli* (below) in water (dotted line) and oyster tissue (solid line) from Pozo Chino.

The influx of freshwater at Pozo Chino is shown by the salinities of less than 35 ppt throughout the experimental period. The lowest salinity occurred on the seventh day when salinity was 26 ppt (Figure 5A). It is evident that the concentrations of total and fecal

coliforms and *E. coli* maintain relatively low levels during neap tides and then show dramatic increases on the fifth day during the spring tides (Figures 5B, C and D).

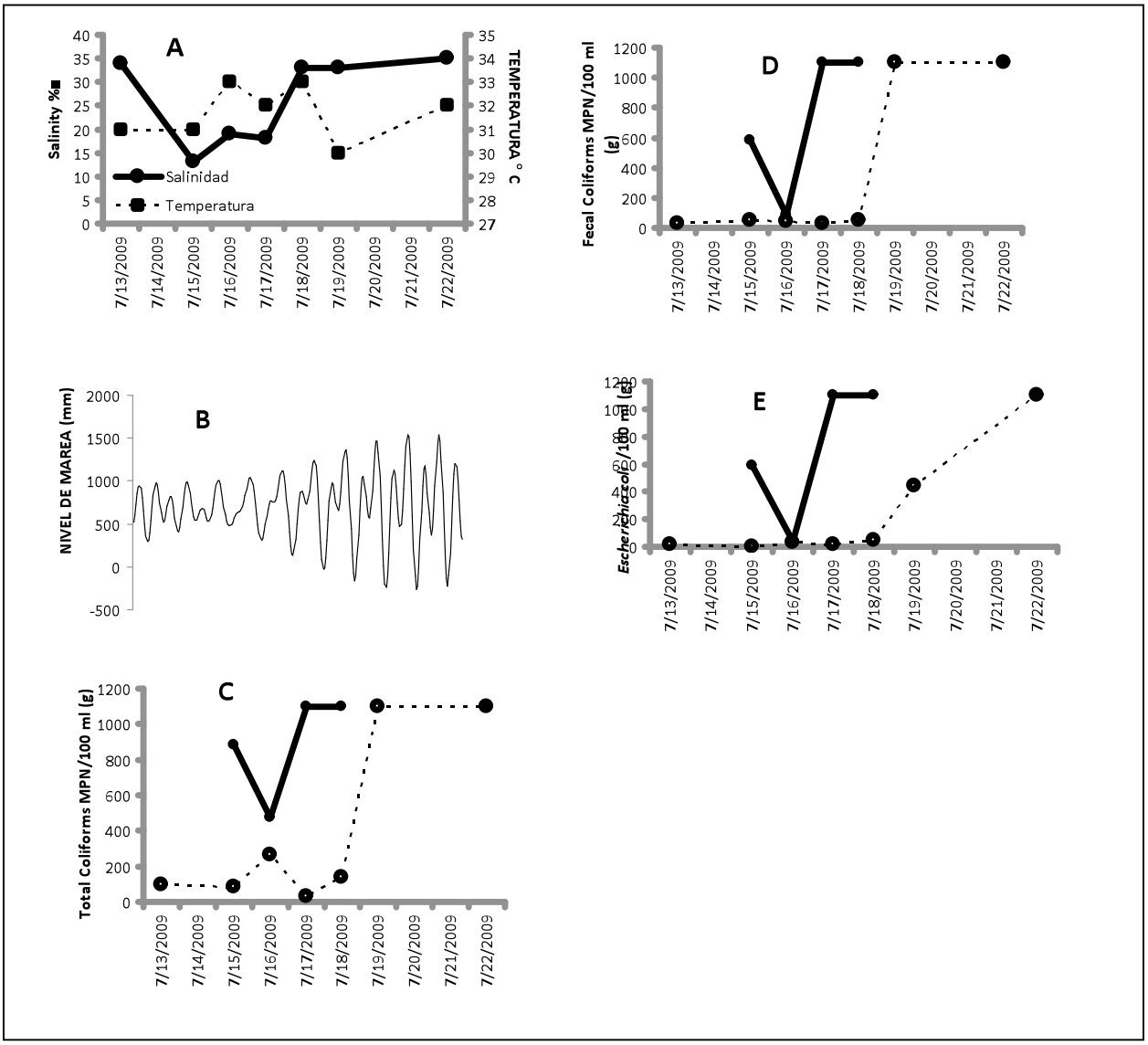

Figure 5. Temperature, salinity (A), tide level (B), total coliform (C), fecal coliform (D) and E. coli (E) in water (solid line) and oyster tissue (dotted line) at Pozo Chino.

DISCUSSION

The depuration experiment was not successful at La Palicienta since bacterial levels were not within legally permissible levels for the entire ten day period. Depuration was also not successful at Pozo Chino, although at this site, bacterial levels rose sooner than at La Palicienta

The concentration of total coliforms at both sites showed a similar pattern. Bacterial levels showed a spike at day 4, then dropped, only to rise again beginning on day 7 when they rose to high levels and remained so until the end of the experiment. The relationship between bacterial levels and environmental conditions did not show a clear correlation. For example, on day 4, the rise in bacterial levels at la Palicienta appear to be correlated with the influx of freshwater at that time, but the same occurred at Pozo Chino despite the lack of freshwater influx. The rise in bacterial levels at both sites on day 7 occurred when

salinity was dropping at Pozo Chino, but there was no change in salinity at La Palicienta at that time. The first rise in the bacterial levels coincided with the neap tides, and the second rise coincided with the spring tides, but increased more with the latter. It may be that that the stronger currents generated during the spring tides re-suspend organic material from the bottom that fosters bacterial growth. Vallaro *et al*., (1950), Araujo *et al*., (1989) y Pérez González (2009) noted that higher levels of organic matter in water tend to favor the proliferation of pathogenic and coliform bacteria. In the case of La Palicienta, the first increase in bacterial levels corresponds to an influx of freshewater, thus suggesting a riverine source of contamination.

The increases in the concentration of coliforms and *E. coli* in oyster tissues during the spring tides at both sites even though bacterial levels in the water were low suggests that oysters may be concentrating bacteria during their filter feeding. Rajagopal et al (1998) notes that increased water flows may affect oyster growth rates through increased filtration rates; thus filtration rates may be higher during the spring tides leading to an increased accumulation of pathogens during that time.

In general, the concentration of fecal coliform in oyster tissues exceeded the legal limits during most of the duration of the experiment. Only on the third day did levels fall to acceptable levels. One factor to consider is that this experiment took place during the period during which coliform levels are highest in the Boca de Camichin Estuary (Olivo Rojas 2007). It may be possible that if the experiment were to be conducted during the winter months when bacterial levels are lower, bacterial levels in tissues might be lower. It is recommended that this experiment be repeated during the cooler months.

Another option may be to investigate the use of land-based tanks for depuration, although this is complicated by the lack of electricity in areas around the estuary where water quality is highest. In a similar case in the Aserradores Estuary, Nicaragua, use of solar electric power is being tested to power a small depuration system. This may allow for use of depuration in remote areas.

CONCLUSION

The concentration of total and fecal coliform, and *E. coli* in water exhibited two cycles during the experimental period at both sites: a cycle of lower magnitude during the neap tides and one with large magnitude during the spring tides. Bacterial concentrations were relatively low during the neap tides but increased rapidly with the spring tides, possibly due to increased rates of filtration. With the exception of the first day at La Palicienta, total and fecal coliform levels always exceeded legally permissible levels. *E. coli* in oyster tissues also exceeded legal limts during the spring tides, while during neap tides, it was below legal limits at La Palicienta. At Pozo Chino, legally acceptable levels occurred only during the fourth day during the neap tide. Depuration is not therefore possible at either site during the summer months, although it may be possible during the cooler months or through use of land-based tank systems.

BENEFICIARIES

Approximately 700 people who culture oysters at Boca de Camichin benefitted from this study as well as five institutions which participated or which received the results.

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TOPIC AREA

QUALITY SEEDSTOCK DEVELOPMENT

BROODSTOCK SEED QUALITY AND FINGERLING PRODUCTION SYSTEMS REARING FOR NILE TILAPIA IN THE PHILIPPINES

Quality Seedstock Development/ Experiment/ 07QSD01NC

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ABSTRACT

Tilapia seed production in the Philippines is estimated at over 1.2 billion annually with demand expected to triple. To improve food security efforts are require to increase hatchery development and enhanced technologies for consistent and quality seed production. In a series of studies we evaluated seed production and growout performance of Nile tilapia (*Oreochromis niloticus*) as a function of broodfish age (8, 12, and 24-month old) as well as the effectiveness of different hatchery systems, namely artificial incubation units, hapas (fine mesh net pens), and ponds on survival, growth, sex conversion rate and size distribution of fry and on fingerling growout. We utilized the GIFT strain of Nile tilapia, a genetically enhanced cultivar commonly used throughout the Philippines and Southeast Asia. In the first study, we found that 8- and 12-month old broodstock had higher spawning rates, albeit statistically insignificant relative to 24-month old broodstock. Despite the older broodstock having produced the highest percentage of hatched eggs ($P < 0.05$), the youngest broodfish tended to have higher levels of total seed production as measured by the combined number of hatched-eggs, swim-up fry and fry produced ($P > 0.05$). In a second study we investigated pond growout of fingerlings produced from broodstock of different ages. We found that fingerling survival, final body weight, specific growth rate, and extraplated gross yield did not differ with broodstock age. It is concluded that broodstock ranging in age from 8 months to 2 years can be used for GIFT tilapia seed production with little impact on gross yield of marketable fish. In a third experiment, we found no significant difference on growth in hapas among fry hatched in artificial

incubation units, hapas, ponds or the combination of the three systems. However, fingerlings produced from fry hatched in artificial incubation units were found to be of two sizes only (mean wt. = 0.2-0.25 g: mean wt. = 0.3-0.35 g) while those raised from fry hatched in hapas, ponds and combination of the three systems were more variable in size. Survival rates were highest in fry-hatched from artificial incubation group and differed significantly from those derived from hapas or a combination of systems. Overall, it appears that artificial incubation unit-hatched fry had significant advantages on percent survival and size uniformity after the sex reversal treatment in hapas under the temperature range of the study (26.9 – 29.1°C). This may reflect less handling, lower stress and reduced cannibalism, factors to consider in tilapia hatchery operations. We then evaluated if the 120-day growout performance of size-matched fingerlings derived from fry reared in the different hatching systems. We found no significant differences among treatments in any of the performance variables, including survival, final weight and length, specific growth rate, feed conversion, and yield. We conclude that any of the three most common tilapia hatchery systems can be used to supply fingerlings for the growout production of Nile tilapia.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) seed production in the Philippines is estimated at over 1.2 billion annually with demand expected to triple (Bureau of Fisheries and Aquatic Resources, BFAR 2005; ADB 2005). There is a strong push and government effort to improve food security through increased hatchery development and enhanced technologies for consistent and quality seed production. The goal is to provide year-round, high-quality seed that can be widely distributed at reasonable costs to both increase income and reduce perceived risks to tilapia farmers. Fry and fingerlings either produced on farm or purchased represent a considerable investment to farmers, constituting around 15% of total cash costs for crop production, second only to feed (ADB 2005). Here, we aim to assess seed production efficiency as a function of broodstock age and different rearing production systems to evaluate better the hatchery and nursery practices intended to increase seed yield without a negative impact on fish growout performance.

It is well established that broodstock age and size, among various other factors, can affect seed quality and fecundity in farmed fish (Bromage, 1995; Green *et al*., 1997; Siddiqui and Al-Harbi, 1997). Previous studies show that small Nile tilapia broodstock produce more eggs than larger fish while larger broodstock produce more eggs per clutch than smaller ones. Work in other species, *T. zillii*, *T. tholloni*, and *S. melanotheron,* shows no direct correlation between fish size and fecundity (see El-Sayed, 2006). With regard to age, seed production per female was higher for 3-year-old compared with 1-year-old hybrid tilapia bred in concrete tanks. However, seed production as a function of broodstock biomass or density was best in 1-year-olds compared with 2-, 3-, and 4-year olds (Siddiqui and Al-Harbi, 1997). In red tilapia and *O. spilurus* 1-year old broodstock produce a greater number of seed than older age class fish (Ridha and Cruz, 1989; Smith et al. 1991). To our knowledge an evaluation of broodstock age on gamete quality (survival and growth) has never been done for the genetically improved GIFT (Genetically Improved Farmed Tilapia) strains of Nile tilapia. The GIFT strain is selectively bred for rapid growth and presently distributed by the GIFT Foundation International, Inc. It is widely used throughout the Philippines and Southeast Asia and its rapid expansion as a choice of tilapia farmers is supported by estimates that it can produce cost savings as much as 25% (Dey 2000). The GIFT Foundation is an independent organization that works with and refines genetically improved strains of Nile tilapia for food production (see Dunham et al., 2000).

These fish enjoy widespread popularity because of their enhanced growth and feed utilization parameters, but additional refinements in hatchery technology would be beneficial; for this reason our studies will evaluate the effects of age of broodstock on the viability of progeny.

Additional studies assessed the efficacy of three artificial and natural seed rearing systems (artificial incubation units, hapas and ponds) on fingerling production characteristics (survival,sex inversion, and yield) and the subsequent growout performance of tilapia. These systems encompass the growout methods being used on either family farms or small/medium-scale hatcheries in the Philippines, and there is considerable interest by farmers and state and private hatcheries on the relative impact each might have on fingerling production and subsequent growout.

OBJECTIVES

± Examine the effect of broodstock age on seed production and fingerling growout performance of genetically improved tilapia strains

± Assess the size distribution, growth and survival of fry and fingerling growout performance of tilapia seed produced from artificial incubation units, hapas, and ponds

MATERIALS AND METHODS

Study 1 - *Effect of age of broodfish on the seed production of GIFT strain of Nile tilapia*
There were three (3) treatments reflecting different broodstock ages that were replicated four times using a random block design. Treatments were as follows: Treatment I – 8 month-old broodfish, Treatment II – 1 year-old broodfish and Treatment III – 2 year-old broodfish.

Different aged broodfish of the GIFT strain (GIFT Foundation International Incorporated, GFII) were conditioned in 2.5 m x 10 m x 1 m fine mesh hapas installed in 2,500 m^2 ponds for one week prior to stocking in breeding hapas. Male and female broodfish were initially separated during the conditioning period and fed at 09:00 h and 14:00 h at 3% body weight per day on acommercial feed (30% crude protein). Animals were then weighted and 13 males and 39 females (1:3 male:female) were stocked into each breeding hapa (2.5 m x 10 m x 1 m). Water quality parameters including dissolved oxygen (mg L^{-1}) and water temperature (°C) were monitored daily at 09:00 and 15:00 h. After 14 days, eggs from mouthbrooding females as well as swim-up fry and fry were collected from each hapa. Collected eggs were pooled and were incubated in incubation jars until the swim-up fry stage. The swim-up fry were counted and included in the fry counts. The combined number of hatched eggs, swim-up fry and fry served as the index of seed production for each treatment or broodfish age.

At the end of the breeding period, the number of broodfish that spawned was recorded to determine spawning success. Spawning success of female broodfish was determined based on morphological characteristics, whereby broodfish with red genital papilla shrunken to a compressed abdomen were categorized as “had spawned”, and broodfish with a white to clear flat genital papilla and normal abdomen were categorized as “not ready to spawn” (WorldFish Center, 2004).

Differences in average seed production with broodfish age was analyzed by ANOVA followed by the Least Significant Difference test for predetermined comparisons of

means (Version 11, SPSS Inc., Chicago IL).

Study 2 - *Effect of age of broodfish on the grow-out performance of Nile tilapia fingerlings*
This study was composed of four treatments and replicated three times using a random block design. Treatments were as follows: I – sex-reversed Nile tilapia produced from 8-month old broodfish, II - sex-reversed Nile tilapia produced from 1- year old broodfish, III - sex-reversed Nile tilapia produced from 2-year old broodfish and IV – combination of sex-reversed Nile tilapia produced from different ages of broodfish.

Sex-reversed Nile tilapia fingerlings (0.192 – 0.208 gram) produced as described above from GIFT-strain broodfish of different ages were used. Fingerlings were stocked in twelve (12) 500 m^2 earthen ponds at 4 fish m^{-2} or 2000 $pond^{-1}$. Stocked fingerlings were fed twice a day^{-1} with commercial feeds at a daily rate of 20% down to 3% body weight. Fifty fish were sampled monthly for average weight and length. At the end of the 120-day culture period, 200 fish or 10% of the total fish stock were sampled for individual weight and length.

Ponds were fertilized with ammonium phosphate (16-20-0) and urea (46-0-0) at 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$ to enhance the growth of natural foods in pond water. Secchi disc visibility was maintained≤40cm. atWater quality parameters including dissolved oxygen and water

temperature were monitored weekly in the morning beginning at 09:00 h. Air temperature (°C), rainfall (mm) and photoperiod data were gathered at the local PAGASA station located at Central Luzon State University, Science City of Muñoz, Nueva Ecija.

Differences among treatment means were analyzed by ANOVA followed by Duncan's Multiple Range Test.

Study 3 - *Size distribution, sex conversion rate, growth and survival of Nile tilapia fry produced in artificial incubation units, hapas and ponds*
Four treatments were tested in triplicate using a random block design. Treatments are as follows; I – artificial incubation unit-hatched fry, II – hapa-hatched fry, III – pond-hatched fry and IV – combination of hatched fry (artificial incubation units, hapas and ponds). Tilapia breeders of the same cohort (GIFT selected generation 11; 1.5 years old) were used to produce fingerlings in artificial incubation units, hapas and ponds at the GFII facility. Prior to breeding, female and male broodstock were separated and conditioned for 7 days in hapas (2.5m x 10m x 1m) installed in a 5,000-m^2 pond. Breeders were fed at 3% body weight per day during the morning and afternoon. Prior to stocking for breeding, broodfish weights and lengths were ascertained to attain similar average broodstock sizes in the production systems.

For fry production in artificial incubation units, seven fine mesh hapas (2.5m x 10m x 1m) installed in a 5,000 m^2 pond were stocked with 48 broodfish each at a 1:3 male:female ratio. On the 10th day fertilized eggs were collected from the mouth of female breeders and incubated in round bottom jars (aritificial incubation units) for nine days until swim-up fry stage. For fry production in hapas, 7 separate hapas were stocked with breeders five days after the initial stocking of the first 7 hapas used for egg collection. Hapas were stocked with 48 breeders each at a 1:3 male:female ratio. Then 14 days later, breeders were removed and then swim-up fry were collected by scoop net. For fry production in

ponds, four 100 m^2 ponds were each stocked with 200 breeders at a 1:3 male:female ratio 6 days following initial stocking of breeders used or egg collection and fry production in artificial incubation units. After 15 days of breeding, ponds were partially drained, and tilapia fry were collected by scoop net. This schedule of breeding ensured synchronized spawning of breeders and the production of fry with uniform size and age from the 3 different hatching or fry production systems.

Following collection of fry from incubation units, hapas and ponds, animals representative of each system were pooled and initially conditioned for six hours in 1m x 2m fine mesh hapas (15,000 fry $hapa^{-1}$) prior to stocking in nursery hapas. Swim-up fry from the different sources were graded to ensure uniformity in size and then stocked and reared in twelve 2m x 4m x 1m fine mesh nursery hapas for 23 days at a density of 850 fry m^{-2} or at 6,800 fry $hapa^{-1}$. Stocking of fry from each production system was done rapidly and at the same time to minimize variation in stocking time and to prevent mortality resulting from handling and thermal stress. Fry representative of each production system were stocked in triplicate nursery hapas.

Fry were sex reversedα to produce male populations using feed impregnated with 17 -methyltestosteroneα. 17 -methyltestosterone was applied to fry mash feed at a concentration of 50 mg Kg^{-1} feed using ethanol as vehicle. The feed was mechanically mixed, air- dried and then fed to fry in nursery hapas for 23 days at a rate of 30, 20 and 10% of the fish body weight for the first, second and third week, respectively. Fry were sampled weekly for weight and length. At the end of the 23-day-sex-reversal treatment, growth, survival and size distribution of the fingerlings were determined.

Data gathered in this study included the following: size distribution, mean length and weight before and after sex reversal treatment in hapa, specific growth rate, percent survival, and some water quality parameters. Differences among the fry rearing systems were analyzed using ANOVA followed by the Least Significant Difference for predetermined comparison of means using Statistical Analysis Software (SAS) Version 9.0.

Study 4 – *Fingerling growout performance of Nile tilapia produced in artificial incubation units, hapas and ponds*

This study was composed of four treatments and was replicated three times. Treatments were as follows: I - sex-reversed Nile tilapia fingerlings derived from fry produced in artificial incubation units, II - sex-reversed Nile tilapia fingerlings derived from fry produced in hapas, III - sex-reversed Nile tilapia fingerlings derived from fry produced in ponds and IV - sex-reversed Nile tilapia fingerlings derived from fry produced in mixed sources of hatching systems (artificial incubation units, hapas and ponds).

Sex-reversed Nile tilapia fingerlings of the GIFT strain (weight range = 0.260 – 0.340 g) derived from fry produced from different hatching systems (artificial incubation units, hapas and ponds; See *Study 3*) were used in the study. Fingerlings were stocked in twelve 500 m^2 earthen ponds at 4 fish m^{-2} or 2000 fish $pond^{-1}$. The fingerlings were fed twice a day with commercial feeds at 20% of the body weight from 0-2 weeks, 10% of the body weight from 2-4 weeks, 7% of the body weight from 4-6 weeks, 6% from 6-8 weeks, 5% from 8-10 weeks, 4% from 10-12 weeks, 3% from 12-16 weeks.

Ponds were fertilized with ammonium phosphate and urea following the recommended fertilization rates of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$ to enhance the growth of natural

foods. Water quality parameters including dissolved oxygen and water temperature were monitored weekly.

Five percent or 100 fish per replicate per treatment were sampled monthly by cast net method for determination of average weight and length. Fish from each treatment and replicate were manually sexed at the end of the 120-day culture period to determine the total number of males and females. At the same time, all fish were weighed individually to determine gross fish production (yield), size distribution, survival and feed conversion ratio (FCR).

Differences among treatment means were analyzed by ANOVA followed by the Least Significant Difference test for predetermined comparisons.

RESULTS AND DISCUSSION

Study 1 - *Effect of age of broodfish on the seed production of GIFT strain of Nile tilapia*
Breeders of different ages were stocked at the same sex ratio and density. The weight of male and female broodfish of different age classes were significantly different, with weight increasing as a function of the age of broodstock. The spawning rate did not differ significantly with broodfish age (Table 1).

The number of eggs produced by the different age-class broodstock did not differ significantly, although the the youngest (8-month old) broodstock produced the highest mean number of eggs. The hatching rate increased signficantly with broodstock age (Table 2) with the 2-year age class broodstock producing eggs with the highest hatch rate of 65%. The eggs of older broodstock also appear larger. Collectively, these results cooberate that of Mair et al. (1993) where it was shown that hatch rates of larger *O. niloticus* eggs are greater than smaller eggs, possibly because older females are more experienced egg brooders.

Performance of broodfish on seed production is shown in Table 3 wherein, mean number of hatched eggs, swim-up fry and fry were recorded and analyzed. Eight-month old broodfish produced the highest number of seeds followed by 2-year and 1 year olds. ANOVA revealed no significant difference among treatments at 5% level of significance.

On seed production, no significant difference ($P > 0.05$) was found on the mean number of eggs, swim-up fry and fry, as well as on the spawning rate of the broodfish. The hatching rate in Treatment III was highest and significantly different from Treatments I and II. This shows the hatchability of the eggs produced by older broodstocks were better as compared to the eggs produced by younger broodstocks.

Water quality parameters measured during the breeding experiment fell well within the tolerance range of tilapia and well above levels that may limit production (Boyd 1984). Average dissolved oxygen was 5.48 mg L^{-1} and 7.28 mg L^{-1} in the morning and afternoon, respectively (data not shown). Average water temperature were adequate for reproduction and growth, averaging 24.48 ºC and 28.30ºC in the morning and afternoon, respectively.

Study 2 - *Effect of age of broodfish on the grow-out performance of Nile tilapia fingerlings*
Growth pattern in average body weight and total length among fingerlings derived from broodstock of different ages is shown in Figure 1. Fingerlings produced from 2 year-old broodfish (Treatment III) had the highest final weight and total length (191.458 g; 20.5 cm)

followed by those produced from a combination of broodfish ages (Treatment IV; 182.611 g; 20.2 cm), and then those derived from 8 month-old (162.855 g; 19.5 cm) and 1-year old broodfish (160.401 g; 19.4 cm). However, final weights, and other growth parameters, including specific growth rate, gross yield, feed consumption, and feed conversion were not significantly affected by the age of broodfish (Table 4). Survival rate of stocks following 120-days of culture were also not significantly altered by broodfish age. The highest survival rate of 70% was observed by fingerlings produced from a combination of different aged broodfish, followed by a 61.3% for fingerlings produced from 8 month-old broodfish, and 61% from fingerlings produced from 1 year-old broodfish. The lowest survival rate of 53.8% was in fingerlings produced from 2 year-old broodfish. Highest extrapolated fish yield of Nile tilapia fingerlings produced from different ages of broodfish was obtained in Treatment IV followed by Treatments III, II and I with mean values of 4,472.7, 4,045.3, 3,719.3 and 3,667.3 kg ha^{-1}, respectively. However, no significant differences were observed among treatments ($P > 0.05$).

During the grow-out period, the average minimum air temperature reading was 24.0°C with a maximum of 32.7 °C. Water quality parameters, including water temperature (mean range = 30.3 - 30.4°C) that fell within the optimum range for development and growth of tilapia (Balarin and Haller 1982), dissolved oxygen, and Secchi disc visibility (mean range = 31.9 - 34.6 cm) did not differ among different treatment ponds (data not shown). Dissolved oxygen readings fell within the tolerable range for tilapia, except during week 12 and beyond when DO dropped in all ponds to around 2 mg L^{-1}. Although tilapia have a high tolerance to low DO. A mild incident of mortality of similar magnitude (Treatment I - 4.05%, Treatment II - 5.95%, Treatment III – 10.70%, Treatment IV – 7.25%) was observed among the groups in this study that likely resulted from prolonged exposure to moderately low DO levels (Green and Duke 2006).

Study 3 - *Size distribution, sex conversion rate, growth and survival of Nile tilapia fry produced in artificial incubation units, hapas and ponds*
Fry were produced in either artificial incubation units, hapas, ponds or an equal combination of the three hatching systems and then grown and sex-reversed in nursery hapas for 23 days. The growth pattern of fry produced from the hatching systems was comparable during the first week (Figure 2). From the second week and beyond hapa-hatched fry had higher body weight than artificial incubation unit-hatched fry, pond-hatched fry and combination of hatched fry. Despite hapa-hatched fry having an elevated gain in length and weight and specific growth rate over the 23-day nursery period, there were no significant differences in these parameters among the groups produced from the different hatching systems (Tables 5 and 6; $P > 0.05$).

Survival of fry hatched in incubation units (92.03%) was significantly higher compared with fry hatched from hapas (65.19%) and those hatched from a combination of systems (72.81%)($P < 0.05$; Table 7). Survival of pond-hatched fry (76.50%) did not differ significantly from that of fry produced from incubation units, hapas or a combination of different hatching systems. The lower survival observed in treatments II, III and IV relative to incubation-hatched fry may have resulted from handling stress associated with collection, grading, conditioning and initial stocking of fry for the sex reversal treatment to the nursery hapas.

We also evaluated the size distribution of the fingerlings following growout using commercial graders. Generally, fry derived from the different hatching systems grew to

four different size ranges; size 24 (0.06-0.09 g), size 22 (0.10-0.14 g), size 20 (0.16 - 0.20 g), and size 17 (0.25-0.30 g). The majority of fingerlings produced fell within size 24 (Figure 3). Artificial incubation unit-hatched fry had the highest percentage (88.31%) of size 24 as compared to other treatments, and had the lowest percentage of the smaller size grades (size 22, 9.89%; size 20, 1.71%; size 17, 0.09%). Fry hatched from incubation units had the lowest variability (smallest coefficient of variation) of fish in the size 24, 20 and 17 grade relative to the other groups (Figure 3). Collectively, fry hatched in artificial incubation units showed the greatest uniformity in size and presumeably growth rate compared with fry derived from other hatching systems.

Dissolved Oxygen (DO) and water temperature were monitored during the breeding stage. Dissolved oxygen and temperature values during breeding in hapas for collection of eggs for artificial incubation units was 4.46 mg/l (DO range: 2.54-6.1) and 28.39 °C (water temperature range: 27.3-31.9), respectively. Measurements during breeding in hapas and ponds for fry collection were 3.73 mg/l (DO range: 2.61-4.79) and 28.04 °C (water temperature range: 27.2-29.1) and 3.05 mg/l (DO range: 2.72-3.56) and 28.73 °C (water temperature range: 28.03-28.83), respectively (data not shown).

Mean values of dissolved oxygen (DO) and water temperature during sex reversal and fingerling production in hapas were virtually identical among groups with an average DO of 7.39 mg/ml (range within a single group: 6.15-8.5 mg/l) and temperature of 27.76°C (range within a single group: 26.9 – 29.1°C) (data not shown).

Study 4 - *Grow-out Performance of Nile Tilapia Fingerlings From Artificial incubation units, Hapas and Ponds*

Nile tilapia fingerlings that were used in the study had an average body weight of 0.292 g, 0.308 g, 0.320 g and 0.307 g for those derived from fry hatched in artificial incubation units, hapas, ponds, and the combination, respectively. The initial stocking weight of fingerlings from the artificial incubation unit group was significantly lower relative to that of fingerlings derived from the pond-hatched fish ($P < 0.05$; Table 8). There were no other differences among the treatment groups with respect to initial weight at stocking ($P < 0.05$).

Figure 4 shows the average body weight and length of fish from the four treatment groups over the 120-day culture period. At harvest, the highest average body weight of the fingerlings occurred in fingerlings originally hatched in artificial incubation units (179.256 g) followed by those hatched in hapas (168.945 g), a combination of systems (150.042 g) and ponds (138.871 g). The same trend was observed with the daily gain in weight, average length, daily gain in length, and specific growth rate. Overall, there were no statistically significant differences in these variables among treatments ($P > 0.05$; Table 8, Figure 4).

We also evaluated the size distribution of fish at the end of the study. Except for the 251-300 g and 300 g and above size categories, all groups had representative fish in all five of the other size categories (50 g and below, 51-100 g, 101-150 g, 151-200 g, and 201-250 g) (Figure 5). The fish size category most prevalent among the groups was 101-150 g fish, while most fish produced were 101-200 g. Fingerlings derived from artificial incubation units and hapas had a greater number of fish in the three largest size categories. However, with respect to the number of fish produced per size category, analysis of variance showed no significant difference among the groups ($P > 0.05$). Thus each hatching system

evaluated is suitable for tilapia grow-out operation.

Survival rates were similar among treatment groups ranging from 56.3% to 59.3% ($P > 0.05$, Table 8). The suboptimal survival rates observed in the study, was likely a consequence of some mortality that occurred in the last month of culture, where fish showed signs of infection likely attributable to streptococcus infection (Alapide-Tendencia and de la Peña 2001). The source of infection is uncertain, but is not likely a result of poor water quality, since DO (mean range, 4.55-4.72), temperature (30.1-30.2ºC), and Secchi disk visibility (33.0-25.7 cm) were within the acceptable range for pond-culture of tilapia.

The FCRs were not significantly different among groups ($P > 0.05$, Table 8). The extrapolated fish yield for fingerlings derived from fry hatched in artificial incubation units was highest (3,890.7 kg ha^{-1}), relative to those derived from hapa (3,797.5 kg ha^{-1}), the combined hatching systems (3,489.0 kg ha^{-1}) and ponds (2,995.1 kg ha^{-1}). However extrapolated yield among groups did not differ significantly ($P > 0.05$; Table 8).

Simple cost and return analyses shows that fingerlings derived from fry hatched in hapas gave the highest net return with PhP 30,732.02 ha^{-1}, followed by those from artificial incubation units with PhP 28,506.50 ha^{-1}, the combined systems with PhP 19,366.88 ha^{-1}, and then ponds with PhP 7,743.16 ha^{-1} (Table 9). Overall, this basic economic analysis indicated that the grow-out rearing of Nile tilapia fingerlings derived from the different hatching systems were profitable. The cost of the different systems for producing fingerlings and labor differences were not included in the analysis, because the intent was only to compare the growout of fingerlings derived from the different hatching systems.

CONCLUSION

The GIFT Nile tilapia is the most commonly used genetically improved strain of tilapia used througout Southeast Asia, including the Philippines. The broodstock age and hatching system that best promote seed production and fingerling growout is uncertain. We assessed seed production efficiency as a function of broodstock age and different rearing production systems to evaluate better the hatchery and nursery practices intended to increase seed yield without a negative impact on fish growout performance. Our studies suggest that older broodfish produce eggs with higher hatching rates, while younger broodstock produce a greater number of seed with lower hatching rates. The growth performance, survival and yield of fingerlings cultured in earthen ponds were also not affected by broodstock age. Based on these studies, we conclude that broodstock ranging in age from 8 months to 2 years can be used for GIFT tilapia seed production with little impact on gross yield of marketable fish.

Additional studies assessed the efficacy of three artificial and natural seed rearing systems (artificial incubation units, hapas and ponds) on fingerling production characteristics (survival, sex inversion, and yield) and the subsequent growout performance of tilapia. These systems encompass the growout methods being used on either family farms or hatcheries in the Philippines, and there is considerable interest by farmers and state and private hatcheries on the relative impact each might have on fingerling production and subsequent growout. Fry hatched from artificial incubation units, hapas, ponds and the combination of the three systems showed no significant differences in specific growth rate, weight gain or length gain. However, the fingerlings produced from artificial incubation unit-hatched fry showed less variability in size after the sex reversal treatment. They also had the highest percent survival (92.03%) among

the groups. These results suggest a significant advantage of using artificial incubation unit-hatched fry to produce fingerlings with higher survival and more uniform sizes relative to the other hatching systems. Regardless of the hatching systems from which fingerlings were derived, growth performance, survival and fish yield of fingerlings was similar following a 120-day growout period. Those derived from artificial incubation units and hapas had a greater number of fish in the three largest size categories. A simple cost and return analysis also indicate that fingerling growout of fish from artificial incubation-units and hapas had the highest net profit, although this was not statistically significantly different. Although some advantages may apply to using fish hatched in artificial incubation units and hapas, our work indicates that any of the tilapia hatchery systems are suitable for supplying fingerlings for the growout production of Nile tilapia with little impact on gross yield.

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Table 1. Mean weight and spawning rate of 8-month (treatment I), 1 year-old (treatment II), and 2-year old (treatment III) broodfish used to evaluate the effects of broodstock age/size on tilapia seed production. Values shown as mean ± standard deviation (N = 4 replicates/group)

Treatment	Initial Mean Weight of Male Broodfish (g)	Initial Mean Weight of Female Broodfish (g)	Spawning Rate (%)
I – 8 month-old broodfish	116.2 ± 4.2[a]	137.6 ± 10.5[a]	41.0 ± 12.6[a]
II – 1 year-old broodfish	164.1 ± 19.3[b]	163.3 ± 3.9[b]	41.0 ± 8.1[a]
III – 2 year-old broodfish	488.2 ± 29.4[c]	331.4 ± 11.8[c]	24.4 ± 17.8[a]

There were no significant differences in variables measured among groups (P > 0.05) as indicated by similar superscript letters.

Table 2. Total number of eggs produced and hatching rate derived from 8-month (treatment I), 1 year-old (treatment II), and 2-year old (treatment III) broodfish. Values shown as mean ± standard deviation (N = 4 replicates/group)

Treatment	Number of eggs produced	Hatching rate (%)
I – 8 month-old broodfish	7040 ± 1729[a]	49.5 ± 0.0[a]
II – 1 year-old broodfish	6839 ± 869[a]	54.8 ± 0.0[b]
III – 2 year-old broodfish	6875 ± 4303[a]	65.4 ± 0.0[c]

There were no significant differences in variables measured among groups (P > 0.05) as indicated by similar superscript letters.

Table 3. Seed production (number of hatched eggs + swim-up fry + fry) derived from 8-month (treatment I), 1 year-old (treatment II), and 2-year old (treatment III) broodfish. Values shown as mean ± standard deviation (N = 4 replicates/group)

Treatment	Number of hatched-eggs	Number of swim-up fry	Number of Fry	Total seed production
I – 8 month-old broodfish	3499 ± 859[a]	4340 ± 2147[a]	2241 ± 921[a]	10079 ± 2507[a]
II – 1 year-old broodfish	3748 ± 476[a]	2229 ± 1976[a]	2305 ± 1574[a]	8281 ± 2627[a]
III – 2 year-old broodfish	4496 ± 2814[a]	3579 ± 3952[a]	1389 ± 1637[a]	9464 ±7508[a]

There were no significant differences in variables measured among groups (P > 0.05) as indicated by similar superscript letters.

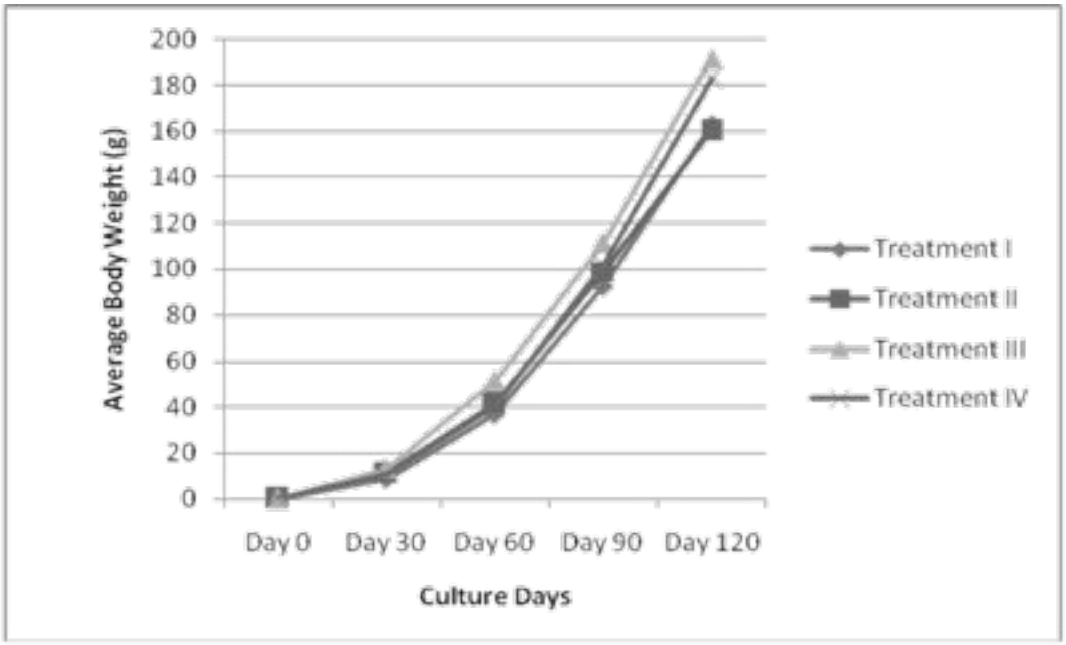


Figure 1. Mean body weight (top) and total length (bottom) of fingerlings produced from different aged broodstock and grown out for a 120-day culture period in earthen ponds. Nile tilapia fingerlings were produced from 8-month old (Treatment I), 1-year old (Treatment II), 2-year old (Treatment III), and a combination (Treatment IV) of broodstock ages (N = 3 ponds/treatment).

Table 4. Summary data of performance variables of Nile tilapia fingerlings derived from broodfish of different ages and grown out for 120 days in earthen ponds. Mean ± standard error.

Parameter	Broodstock Age 8–month old	Broodstock Age 1-year old	Broodstock Age 2-year old	Broodstock Age Combination
Initial Average Body Weight (g)	0.192	0.196	0.208	0.199
Final Average Body Weight (g)	162.855 ± 28.5	160.401 ± 1.3	191.458 ± 33.6	182.611 ± 33.5
Initial Average Total Length (cm)	2.4	2.4	2.4	2.4
Final Average Total Length (cm)	19.5 ± 1.1	19.4 ± 0.2	20.5 ± 1.1	20.2 ± 1.1
Daily Gain in Weight (g/day)	1.356 ± 0.24	1.335 ± 0.01	1.594 ± 0.28	1.520 ± 0.28
Daily gain in Total length (cm/day)	0.143 ± 0.010	0.141 ± 0.001	0.151 ± 0.009	0.149 ± 0.009
Specific Growth Rate (%)	5.611 ± 0.14	5.589 ± 0.01	5.679 ± 0.14	5.675 ± 0.16
Survival Rate (%)	61.3 ± 22.9	61.0 ± 8.2	53.8 ± 4.6	70.0 ± 14.1
Extrapolated Yield per Hectare (kg/ha)	3667.3 ± 689.3	3719.3 ± 365.5	4045.3 ± 1039.8	4472.7 ± 619.1
Extrapolated Feed Consumed per Hectare (kg/ha)	5353.2 ± 172	5506.7 ± 169	6315.4 ± 747	5692.5 ± 590
Feed Conversion Ratio (kg feed/kg weight gain)	1.5 ± 0.4	1.5 ± 0.1	1.6 ± 0.2	1.3 ± 0.1
Feed Conversion Efficiency (%)	68.7 ± 14.7	67.4 ± 6.3	63.3 ± 9.2	78.3 ± 4.4

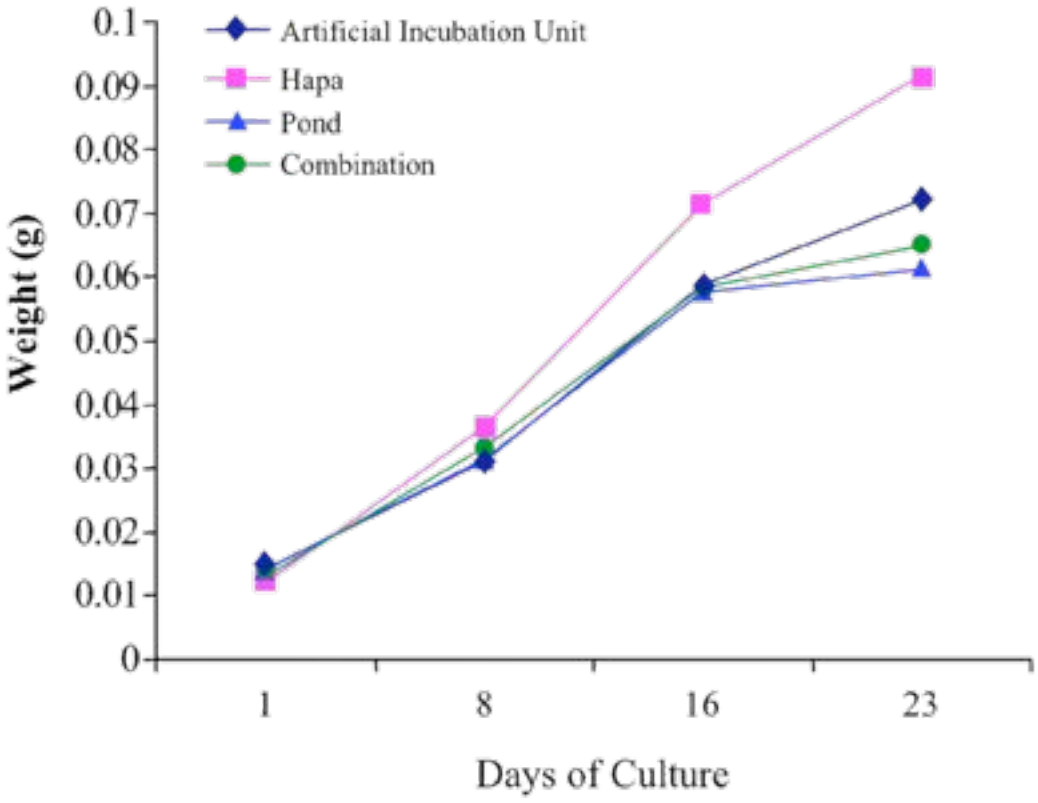


Figure 2. Changes in mean body weight of fry produced from different hatching systems and grown for 23 days in nursery hapas. Fry were produced from artificial incubation units, hapas, ponds, or a combination of the three. (N = 3 replicates/group).

Table 5. Initial and final mean length and weight of fry produced from different hatching systems (artificial incubation units, hapas, ponds, and a combination of the three) and reared for 23 days in nursery hapas. (N = 3 replicates/group).

Treatment	Initial Length (mm)	Final Length (mm)	Initial Weight (g)	Final Weight (g)
I – incubation-hatched fry	8.45	17.41	0.014	0.071
II – hapa-hatched fry	8.40	17.30	0.012	0.081
III – pond-hatched fry	8.30	17.40	0.014	0.068
IV – combination of hatched fry	8.45	17.57	0.013	0.072

Treatment groups did not differ at 5% level of significance.

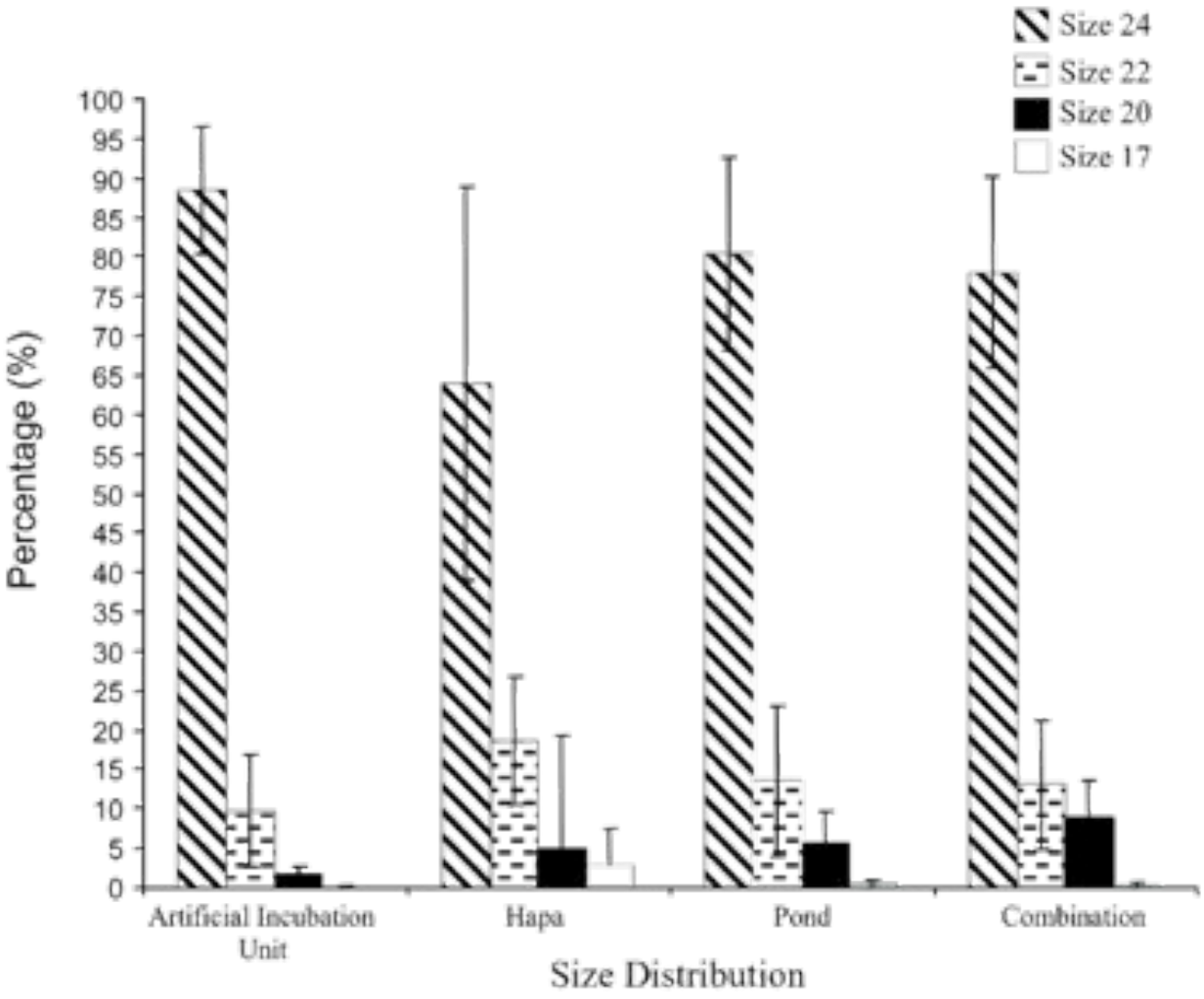
Table 6. Mean growth performances of fry produced from different hatching systems (artificial incubation units, hapas, ponds, and a combination of the three) and reared and sex reversed for 23 days in nursery hapas. Values represent mean ± standard deviation (N = 3 replicates/group).

Treatment	Gain in Length (mm)	Gain in Weight (g)	Specific Growth Rate (%)
I – incubation-hatched fry	8.96 ± 1.47	0.06 ± 0.02	6.97 ± 1.41
II – hapa-hatched fry	8.90 ± 1.80	0.07 ± 0.03	8.70 ± 1.34
III – pond-hatched fry	9.10 ± 1.52	0.05 ± 0.02	6.32 ± 1.14
IV – combination of hatched fry	9.12 ± 1.12	0.06 ± 0.01	6.92 ± 0.98

There were no differences in variables among treatment groups at 5% level of significance.

Table 7. Mean percent survival of fry produced from different hatching systems (artificial incubation units, hapas, ponds, and a combination of the three) and reared and sex reversed for 23 days in nursery hapas. Values represent mean ± standard deviation (N = 3 replicates/group).

Treatment	Survival (%)
I – incubation-hatched fry	92.03 ± 1.47^{a}
II – hapa-hatched fry	65.19 ± 1.12^{b}
III – pond-hatched fry	76.50 ± 1.80^{ab}
IV – combination of hatched fry	72.81 ± 1.52^{b}



Treatment means with the different superscript letters are significantly different from each other ($P < 0.05$).

Figure 3. Changes in mean body weight of fry produced from different hatching systems and grown for 23 days in nursery hapas. Fry were produced from artificial incubation units, hapas, ponds, or a combination of the three. Mean ± standard deviation, N = 3 replicates/group. Fry size ranges include size 24 (0.06-0.09 g), size 22 (0.10-0.14 g), size 20 (0.16 - 0.20 g), and size 17 (0.25-0.30 g).

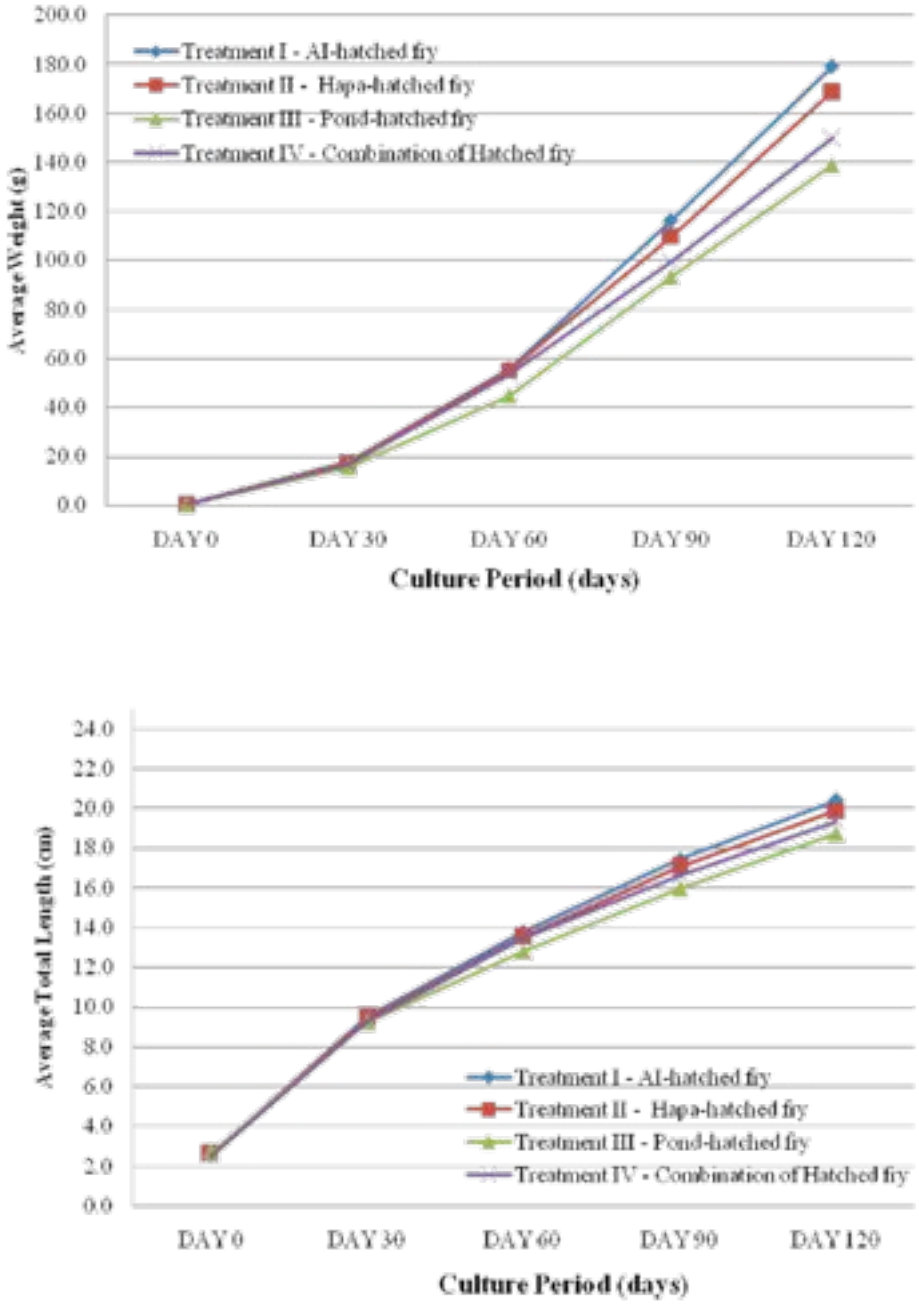


Figure 4. Mean body weight (top) and total length (bottom) of fingerlings produced from fry hatched in different systems and grown out for a 120-day culture period in earthen ponds. Fingerlings were derived from fry hatched in artificial incubation units (Treatment I), hapas (Treatment II), ponds (Treatment III), or a combination of the three (Treatment IV). (N = 3 ponds/treatment).

Table 8. Performance variables of Nile tilapia fingerlings produced from fry hatched in different systems and grown out for a 120-day culture period in earthen ponds. Fingerlings were derived from fry hatched in artificial incubation units, hapas, ponds, or a combination of the three systems. Mean ± standard deviation (N = 3 ponds/group).

Performance	**Treatment**			
	Artificial Incubation Units	Hapa	Pond	Combination
Initial Mean Weight (g)	0.292 ± 0.011^{a}	0.308 ± 0.014^{ab}	0.320 ± 0.017^{b}	0.271 ± 0.005^{ab}
Final Mean Weight (g)	179.256 ± 42.3^{a}	168.945 ± 48.6^{a}	138.871 ± 17.3^{a}	150.042 ± 14.7^{a}
Daily Gain in Weight (g day^{-1})	1.492 ± 0.352^{a}	1.405 ± 0.405^{a}	1.155 ± 0.144^{a}	1.248 ± 0.122^{a}
Initial Mean Length (cm)	2.6 ± 0.058^{a}	2.6 ± 0.115^{a}	2.7 ± 0.115^{a}	2.5 ± 0.100^{a}
Final Mean Length (cm)	20.4 ± 1.6^{a}	19.9 ± 1.8^{a}	18.7 ± 0.7^{a}	19.3 ± 0.6^{a}
Daily Gain in Length (cm day^{-1})	0.149 ± 0.013^{a}	0.144 ± 0.017^{a}	0.134 ± 0.007^{a}	0.140 ± 0.004^{a}
Specific Growth Rate (%)	5.333 ± 0.2^{a}	5.235 ± 0.3^{a}	5.058 ± 0.2^{a}	5.264 ± 0.1^{a}
Extrapolated Feed Consumed ($kg\ ha^{-1}$)	7055 ± 1258.6^{a}	6820 ± 1420.5^{a}	5853 ± 123.7^{a}	6497 ± 375.2^{a}
Feed Conversion Ratio	2.0 ± 0.153^{a}	2.1 ± 0.153^{a}	2.1 ± 0.252^{a}	2.2 ± 0.115^{a}
Feed Conversion Efficiency	50.4 ± 3.980^{a}	48.9 ± 3.686^{a}	47.3 ± 5.021^{a}	46.0 ± 2.230^{a}
Gross Fish Yield ($kg\ pond^{-1}$)	194.533 ± 30.3^{a}	189.874 ± 37.7^{a}	149.757 ± 43.2^{a}	174.448 ± 9.4^{a}
Extrapolated Gross Fish Yield ($kg\ ha^{-1}$)	3890.7 ± 605.5^{a}	3797.5 ± 754.0^{a}	2995.1 ± 864.4^{a}	3489.0 ± 187.6^{a}
Survival (%)	56.6 ± 8.1^{a}	58.6 ± 15.1^{a}	56.3 ± 22.5^{a}	59.3 ± 1.7^{a}

Treatment means with the different superscript letters are significantly different from each other ($P < 0.05$).

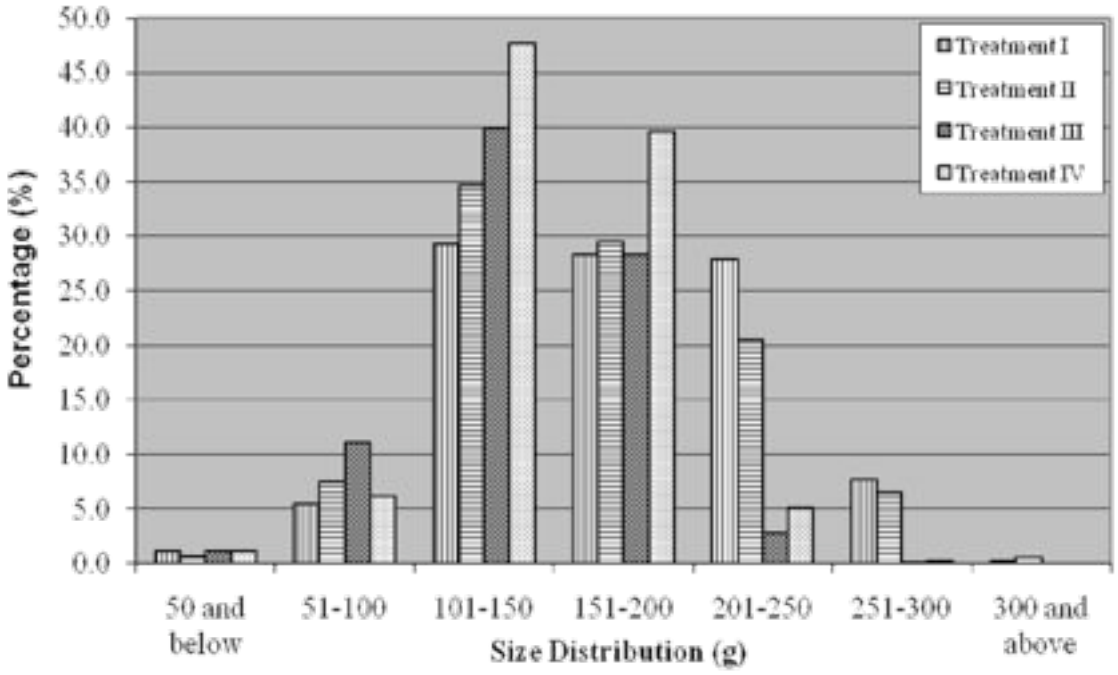


Figure 5. Size distribution of fingerlings produced from fry hatched in different systems and grown out for a 120-day culture period in earthen ponds. Fingerlings were derived from fry hatched in artificial incubation units (Treatment I), hapas (Treatment II), ponds (Treatment III), or a combination of the three (Treatment IV). (N = 3 ponds/treatment).

Table 9. Simple cost and return analysis per hectare of Nile tilapia fingerlings produced from fry hatched in different systems and grown out for a 120-day culture period in earthen ponds. Fingerlings were derived from fry hatched in artificial incubation units, hapas, ponds, or a combination of the three systems. Values are in Philippine pesos (PhP).

Item	**Treatment**			
	I	II	III	IV
Gross Return (PhP)	233,442.00	227,850.00	179,706.00	209,340.00
Cost (PhP)				
Fingerlings	18,000.00	18,000.00	18,000.00	18,000.00
Commercial Fertilizers				
Ammonium Phosphate	3,676.66	3,007.20	2,674.26	3,340.14
Urea	6,858.84	5,610.78	4,988.58	6,232.98
Commercial Feeds	176,400.00	170,500.00	146,300.00	162,400.00
Total Cost	204,935.50	197,117.98	171,962.84	189,973.12
Net Returns (PhP)	28,506.50	30,732.02	7,743.16	19,366.88
Assumptions: Stocking density	4 pcs m^{-2}			
Price of fingerlings	PhP 0.45 pc^{-1}			
Price of commercial fertilizers				
Ammonium Phosphate	PhP 35.80 kg^{-1}			
Urea	PhP 36.60 kg^{-1}			
Price of commercial feeds	PhP 25.00 kg^{-1}			
Price of marketable tilapia	PhP 60.00 kg^{-1}			

DEVELOPMENT OF SMALL-SCALE *CLARIAS* FINGERLINGS AS BAIT FOR LAKE VICTORIA COMMERCIAL FISHERIES IN WESTERN KENYA

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ABSTRACT

The artisanal fishery of Lake Victoria, Kenya has been degraded by environmental deterioration, and the stock of the African catfish (*Clarias gariepinus*) in the Lake has been drastically reduced because wild-caught catfish juveniles are used as bait. We therefore recognized that income opportunities existed for fish farmers to diversify into small-pond aquaculture to provide farm-raised catfish fingerlings as alternative source of bait. Such a development would transform existing and potential small-scale fish farmers into high-yield profitable production groups, by providing technical assistance in propagation, production, general pond husbandry, and marketing.

All selected fish farmers undertook training on mass production of *C. gariepinus*. Larval stocking densities used by the fingerling producers in simple hatcheries ranged from 100-150 fry per liter of water. They were reared for 14-21 days before being moved to protected nursery ponds or to hapas hung in nursery ponds, stocked at a density of 100 fry/m^2 and reared for another 21-30 days. Some farmers also co-stocked adult tilapia with 2-weeks old catfish fingerlings.

Preliminary work, building on the previous Kenya Business Development Services intervention, resulted in the formation of farmer clusters— registered groups of farmers with operational accounts. The Vihiga cluster specifically made excellent progress and was able to design and implement a comprehensive program where both experienced and new farmers realized the full potential of fingerling production. Sales among the Vihiga cluster showed a progressive transformation of baitfish farmers towards commercialization.

Results suggest that pricing of baitfish both at source and at end market is dependent on the supply of and demand for the baitfish. Demand is determined by the abundance of wild-caught Nile perch from the lake. Bait traders are mainly women and operate in organized groups or as individuals operating from the beaches along the Lake. A marketing plan was developed that included the current market demographics, trends, and potential for growth. The strengths and weaknesses of the farmer groups, baitfish traders, product offering, financing associated with the marketing channels were analyzed.

Some challenges faced included some farmers getting attached to selling food-size fish and thus being unwilling to sell fingerlings as bait; lack of funding to purchase feeds and seed by farmers; predation on the farm; volatile baitfish price; and lack of quality feed for Clarias fry and fingerlings.

INTRODUCTION

Kenya's aquaculture consists mainly of small-scale commercial tilapia (Oreochromis niloticus), and also catfish (Clarias gariepinus), producing only about 1,000 metric tons annually until the early 2000. In 2007, however, the government reported annual production from aquaculture of about 4,220 metric tons of fish valued at KSh 500 million. The Government of Kenya has recognized aquaculture as a sub-sector with great potential to contribute towards poverty alleviation in rural communities, dietary protein enhancement, and reducing pressure on capture fisheries. As part of the Vision 2030 Economic Stimulus Strategy, the government is supporting the industry with KSh 1.1 billion ($14.7m) under a program called 'Fish Farming Enterprise and Productivity Program.

The artisanal fishery of Lake Victoria, the largest commercial fishery in Kenya, has been degraded by environmental deterioration (water hyacinth) and a decrease in the number of smaller food species following introduction of the voracious Nile perch (*Lates niloticus*). The stock of catfish in Lake Victoria has also been drastically reduced because wild-caught juveniles are used as bait for Nile perch hooks deployed daily in the commercial fisheries. Although trawling for Nile perch was practiced by some fishers in the past, it is now illegal in the Winam Gulf, a main fishing zone in Kenya. So, some fishers have resorted to longline fishing, using fingerling-sized *C. gariepinus* as bait. The traditional supply of fingerlings is wild-caught from Lake Victoria, but this supply is intermittent and seems to be related to the extent of floating and drifting water hyacinth mats in near-shore areas, with *C. gariepinus* being numerous under the thick growth. Fishers usually use small-mesh beach seines and small seine nets to catch fingerlings for bait, but beach seining is highly destructive of the spawning habitats of native cichlids and is illegal. The Fisheries Department (FD) imposes a fishing moratorium for small seine nets from 1st April to 31st August every year thereby rendering baitfish fisher unemployed for over 4 months. Fishermen also find themselves in a difficult situation because they need the bait on a daily basis at an affordable price to be able to continue fishing. Income opportunities therefore exist for current agricultural farmers to diversification into small-pond aquaculture to provide farm-raised catfish fingerlings as alternative supply source of bait for the commercial fisheries on Lake Victoria.

The African catfish, *Clarias gariepinus*, is endemic to the Lake Victoria region. It is popular with the communities living around the lake. *C. gariepinus* is popularly farmed in polyculture with Nile tilapia (*O. niloticus*) to control unwanted tilapia populations. Catfish

are also grown in monoculture as food fish. Catfish culture is being recognized for its importance as baitfish for the Lake Victoria Nile perch fishery.

Frame Survey results for the year 2000, 2002, 2004 and 2006 showed that there were between 2.5 million and 3.0 million long line hooks operated on the Kenya side of Lake Victoria. This gives the number of boats using an average of 1,000 hooks per day to be 2,500 to 3,000 requiring a similar number of baits on daily basis. At 300 fishing days per year, it is estimated that there is an annual demand of between 750 and 900 million fingerlings at the optimum. At the reported selling price of KShs 5.00 to 8.00 per fingerling and an estimated production cost of about KShs 0.50 per fingerling, farm-based production of catfish fingerlings could be a highly profitable business for fish farmers. Despite the huge demand for catfish baitfish in the region, production has been very limited.

Spawning of *C. gariepinus* is not a major problem, but they generally have a very low survival of the juveniles. However, studies such as Ngugi *et* al. (2004, 2005) have reported successes in survival of catfish juveniles from appropriate stocking densities for fry nursed indoors in 30-L glass aquaria, as well as studies on appropriate stocking densities and varied amounts of cover provided for fry reared in hapas in outdoor ponds. Results from the studies and others suggest that additional work is needed on fingerling survival to increase catfish production for bait as well as food fish. Increased production of catfish fingerlings will raise farm income and contributing to food security in the area.

Our overall objective was to increase catfish fingerling production as bait to feed commercial fishing in Lake Victoria to reduce overexploitation of indigenous species and conserve the diversity of Lake Victoria Fisheries. We achieved the objectives through:

1. Training small- and medium-scale fish farmers as well as fisheries extension officers on hatchery technology, pond operation and management as well as business plans for catfish fingerling production.
2. Organizing fish farmers into production clusters to produce catfish fingerlings as baitfish for Lake Victoria commercial fisheries.
3. Developing the baitfish market in the Lake Victoria region for catfish fingerlings to enhance biodiversity habitats and populations.
4. Providing training for fisheries extension Officers on technology transfer mechanism to fish farmers

METHODOLOGY

Fish farmers were selected from Western Province, a high aquaculture concentration area. The area has good climate and optimum temperatures for catfish and tilapia growth. The region is very close to Lake Victoria, where catfish fingerling market can be developed for Nile perch fishery. Marketing channels would be easy to establish because of the proximity. Transportation of inputs from major town such as Kisumu city would be cheaper and transportation of produce from fish farms to the end markets can be faster.

Six cluster sites were selected from six districts in western Kenya. They were selected based on the availability of static ponds, suitability of catfish fingerling production, and farmers' willingness and ability to participate. A rapid needs assessment was conducted on the capabilities of cluster farmers. Some basic requirements such as minimum number of ponds and ability to source required inputs such as organic fertilizer was set based on a previous study under Kenya Business Development (USAID –KBDS project).

Production ponds for the proposed project were selected based on:

1. Farmers' interest in participating in the cluster and growing cattish fingerling to supply the baitfish market
2. Surface area per pond - should have a minimum of 200 m^2, and a maximum of 1,000 m^2 and the farmer must have not less than two ponds. Farmers willing to construct ponds to meet the above criteria were allowed to participate.
3. Farmers should be willing to work in a group and be willing to be trained.

The administrative location of each farmer for the clusters are as follows:

- Funyula Cluster: The cluster members came from four locations within Funyula Division namely Nambaku, Namboboto, Nangoshe and Nambogo.
- Matayos Cluster: Members of this cluster were drawn from two divisions, namely Matayos and Butula. Members from Butula came from the same location (Bujumba) while those from Matayos Division came from Lwanya and Matayos locations.
- Mundika Cluster: All the members of this cluster came from Matayos Division except one from Municipality Division (Township Location). Those from Matayos Disvision were shared between Busibwako and Buhayo West locations.
- Vihiga Cluster: Three members of this cluster were from Emuhaya Division and are distributed between North-West Bunyore and Wakhomo Locations. The remaining seven were from Luanda Division and all come from West Bunyore Location.
- Lurambi cluster: All members came from Lurambi dicision of Kakamega district
- The Bungoma/TransNzoia cluster: members were draw from Kiminini division of transnzoia and Kanduyi division of Bungoma

The number of ponds varied between farmers within a cluster and among clusters considerably. In Funyula and Vihiga, the number of ponds per member of the cluster varied from 1 to 8 while in Matayos, Mundika Lurambi and Transzoia, the range was from 1 to 5. The maximum and minimum total pond area for each cluster showed that Matayos and Mundika had the least total pond surface area (62 – 350 and 60 – 650 m^2 respectively) as compared to the reat (120 – 1055 and 180 – 2060 m^2). In Vihiga, maximum total surface area was almost double that of Funyula and Mundika was also almost twice that of Matayos. The minimum average pond size for Vihiga was higher than all other clusters while maximum average pond size was noted in Funyula.

All cluster groups were formed with each cluster having up to ten (10) members; one cluster (Vihiga) had 15 members. The sixty (60) fish farmers selected as cluster members were trained by Aquafish CRSP resource persons and technical staff from the Fisheries Department. Training covered topics such as enterprise budgeting, baitfish marketing, hatchery management, seed production (catfish and tilapia), applications of various hatchery production techniques including broodstock collection, fertilization and spawning techniques, incubation and hatching, egg mortality and their treatment, larval rearing and mass catfish fry production, fish nutrition and feed, fish health management, as well as transportation of live fish. Farmers also learned methods of data recording, leadership styles and communication skills. Extension officers and graduate students also participated in the training programs.

Farmers were also assisted to develop a comprehensive marketing plan that included the current marketing situation, identification of opportunities and threats, and a clearly defined marketing strategy. We developed a marketing strategy that considered market

demographics, market trends, and market potential for growth. We analyzed strengths and weaknesses of the clusters and baitfish traders, product offering, finances associated with the marketing channels and did set up a marketing monitoring and evaluation as a control plan. The plan for the baitfish market also identified buyers and sellers and explored existing market rules as well as proposing new protocols that would regulate bait fish markets.

Collaborations

A leverage fund to the tune of Kshs 4.74 Million (US$ 67,921) was provided by the local USAID Kenya Business Development Services (KBDS) under contract number A02/010/06 to develop small scale *C. gariepinus* fingerling producers in two districts in Western Kenya. The project is compatible with other donor projects addressing food security and environmental management in Kenya. These include (a) the Lake Victoria Environmental Management Project supported by the World Bank which undertakes fisheries and wetlands management research; (b) the Sustainable Agriculture Community Development Program of the UNDP Country Program Framework 2004-08 which addresses the parallel production, preservation, marketing and small-business development aspects of agriculture; (c) the UN 2004 Flash Appeal for the Drought and Food Security Crisis in Kenya which developed emergency family agriculture projects, and (d) the FAO Special Program for Food Security which identifies the need to develop small-scale aquaculture to counteract declining wild fish stocks.

RESULTS AND ACHIEVEMENTS

Formation of cluster farms and Clarias fingerlings production

Farmers are now able to evaluate and compare alternative fingerling production technologies and apply suitable technologies to produce *C. gariepinus* fingerlings for the Lake Victoria commercial fisheries. They have also learned how to keep good records regarding the operation of fish ponds to enable them assess trends in their fish production. Some farmer clusters have observed increases in fish production and revenues from fish sales. Farmers from Kisumu and Siaya who were not part of the initial training have now organized themselves into clusters from training by Lurambi and Vihiga clusters. There is an increase in cluster to cluster training to construct and manage fish ponds. There is a large spin-off of farmer-to-farmer contacts, increasing dissemination of aquaculture information and reducing reliance on government extension. Farmers are now developing enterprise budgets and business plans for the types of pond systems in use and three farmers have taken loans to increase fish production.

In particular, the Vihiga fish farmers group has become a model that is motivating the formation of other fish farmers groups. The group started off with 10 individual fish farmers as members and has grown to 25 fish farmers, including nine women. This group improved their management skills and started realizing 25 percent survival. Farmers have established a marketing link with baitfish dealers, and are consistently supplying catfish fingerlings.

The success of the fish farmers' cluster program drew the attention and financial support from the Women in Fishing Industry Project (WIFIP) based along the shores of Lake Victoria, Kenya. WIFIP helps women fish traders to identify income generating activities. WIFIP is interested in engaging women in fish farming/aquaculture to provide additional household income, and to support women during the annual fishing ban on Lake Victoria,

when income is at its lowest. WIFIP solicited the help of the some successful cluster farmers to train women in fish pond construction and catfish breeding.

Overall, the number of small-scale farmers producing catfish bait on commercial and sustainable basis has increased. Over 100 cluster farmers were trained during the study period. The level of production is currently supporting more than 20 traders handling about 2,000 fingerlings per day.

Tour of Uganda Fish Farms

Nine (9) fish farmers from Western Kenya accompanied by four (4) resource persons Uganda on a study tour of fish farms as part of the training and learning experience. The purpose of the tour was to learn alternative fingerling production technologies for both tilapia and catfish, and how Ugandan farmers kept records regarding their fish pond (farm) operations. The trip occurred from April 8 – 11, 2009. Places visited included: SUN fish farm - Kajjansi, Wakiso; Aquaculture Research Centre, Kajjansi; Tende Innovation Farm and Training Centre (TIFTC), Garuga, Wakiso; UGA Chick Fish factory and catfish farm; Umoja fish farm; and SON fish farm, Njeru.

Based on what they saw, the fish farmers from Kenya were challenged by the fact that some small scale farms in Uganda could produce up to 500,000 catfish fingerlings a month. Most of the farms visited were also using their farms as training facilities and the farm managers encouraged such integrations to help other farmers learn. The Kenyan farmers learned that they could start small and scale up their operations with little or no bank loans as was the case with some of the farmers they interacted with. They also learnt how strong the Ugandan fish farmers association was. They had the opportunity to attend one of their regular meetings. The Ugandan association has a unified voice to petition the government on matters important to fish farmers, and also work as partners in aquaculture development in Uganda. A private/ public sector partnership has allowed the government of Uganda to take a huge loan from ADB (African Development Bank) to upgrade the Kajanssi Aquaculture Centre. A fish feed extruder imported by the government is now installed at Ugachick factory to make floating pellets. The farmers are now determined to refurbish their hatcheries and turn them into commercial entities. They also commented that field visits and such tours were better methods of technology transfer than classroom teachings.

ANTICIPATED BENEFITS

- There is certainly an increase in supplies of *Clarias* fingerlings that provide Lake Victoria Nile perch fishers with a reliable source of bait.
- Fishing pressure on immature *Clarias* in Lake Victoria has been reduced although this is a long term issue. With time, reduction in beach seining will result in reduced habitat destruction on native fishes in Lake Victoria.
- The number of women taking up this activity as a proportion of males (trained and non-trained through extension) has increased.
- We are working on a steady supply of *Clarias* fingerlings that will help producers in areas where *Clarias* is gaining popularity as a cultured food fish.

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TOPIC AREA
HUMAN HEALTH IMPACTS OF AQUACULTURE

MONITORING AND REDUCING MICROCYSTINS IN TILAPIAS AND CHANNEL CATFISH CULTURED IN A VARIETY OF AQUACULTURE SYSTEMS

Human Health Impacts of Aquaculture/ Experiment/ 07HHI01UM

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ABSTRACT

This study surveyed microcystin concentrations in pond water and Nile tilapia *Oreochromis niloticus* muscles from a typical eutrophic aquaculture pond in China, then developed possible techniques for removing the microcystin-producing alga *Microcystis aeruginosa* from water. Both Nile tilapia flesh (0.10-2.21 ng/g) and pond water (0.052 - 0.134μg/L) contained considerable levels of microcystins. The pond was dominated by blue–green and green algae; Cyanophyta were 27 - 36 % of total algae, and Chlorophyta were 52 - 58 %.

In order to eliminate microcystins from the water, two coagulant treatments were tested, using chitosan- and polymeric aluminum chloride (PAC)-modified clay (Kaolin). Both laboratory-cultured and pond-collected algae were used, and both treatments removed cultured *M. aeruginosa* effectively (strain HAB 657). After treatment with clay, algae sedimented to the bottom and their cell vitality decreased noticeably. The sedimented *M. aeruginosa* cells died within a month of flocculation with chitosan-modified clay. Maximum electron transport rates (ETR_{max}), a measure of photosynthetic activity, decreased from 99.6 μmol photons cm^{-2} s^{-1} to 23.9 μmol photons cm^{-2} s^{-1} after 30 days. For those treated with PAC-modified clay, algal cells became yellowish, decayed in a week, and ETR_{max} decreased from 97.2 μmol photons cm^{-2} s^{-1} to 20.6 μmol photons cm^{-2} s^{-1} after seven days. Of the two treatments, PAC-modified clay had a quicker and slightly stronger effect.

Optimal conditions and dosages of the coagulant treatments were determined through a series of experiments. For chitosan-modified clays, the optimal pH was from 5 to 8. Optimal dosages (y_1, ml of clay solution) for removal of *Microcystis* were related to Optical Density of the sample water at 680 nm (x_1), by the regression $y_1 = 0.0349x_1 - 0.0019$ ($R^2 = 0.9972$). Optimal dosages were related chlorophyll-α concentration (x_2, mg/L) by the regression $y_2 = 0.0524x_2 - 0.009$ ($R^2 = 0.9864$). For PAC-modified clays, the optimal pH was from 5 to 9. Comparable dosage regressions were $y_1 = 0.0351x_1 + 0.0065$ ($R^2 = 0.9986$), and $y_2 = 0.0676x_2 - 0.0059$ ($R^2 = 0.9854$). These equations show that less chitosan-modified clay would be required than PAC-modified clay to mitigate the same amount of chlorophyll-*a*. Chitosan-modified clay is more environmentally friendly because it does not contain aluminum chloride like PAC-modified clay and it also is biodegradable. It can effectively remove field-reared *M. aeruginosa*. After Chitosan-modified clay treatment, chlorophyll-α decreased from 1.172 ± 0.210mg/l to 0.017 ± 0.007mg/l, a removal rate of 98.60%.

INTRODUCTION

Microcystins (MCs) are the most commonly found and poisonous of algal toxins produced by several harmful cyanobacterial species. MCs are usually associated with freshwater environments, and can be accumulated by aquatic animals such as mussels, snails, zooplankton, shrimp, frogs, and fish through ingestion of drinking water and foods with bioaccumulated toxicity (Magalhães et al., 2001, 2003; Xie et al., 2005). MCs are monocyclic heptapeptides including several variants. As a family of potent liver toxins, they may cause hepatotoxicosis, gastroenteritis, or allergic reactions and are potentially hazardous in ecosystems and to human health (Codd et al., 2005, Xie, 2006).

In China, most fresh waters have been contaminated by organic compounds, and more than two thirds of water bodies are eutrophic (Jin, 1995). As a result, toxic cyanobacterial blooms occur frequently, and algal toxins (especially MCs) are a serious concern. Microcystins are commonly found in rivers, ponds, reservoirs, lakes, water-treatment effluent, drinking water sources, and even treated drinking water (Xie, 2006). The most commonly found MCs are MC-LR, MC-RR, and to a lesser extent MC-YR (Song et al., 1999). As their molecular structure has cyclic and double bonds, MCs have considerable physiochemical stability. Existing water treatment processes such as coagulation, sedimentation, filtration, and chlorination are ineffective at removing MCs. Therefore, techniques for eliminating microcystin-producing algae from water bodies have been developed instead of direct microcystin removal during water treatment. These techniques would eliminate microcystin- producing algae, and minimize uptake by the biotic community.

Previous studies in China have found that MCs can bioaccumulate in a variety of fishes across multiple feeding habits (Xu et al., 2003; Xie et al., 2005; Chen et al., 2006; Yang et al., 2007). However, the commonly consumed Nile tilapia *Oreochromis niloticus* was not included in these studies. Because Hubei is a major producer of cultured fish, including Nile tilapia, there is a need to monitor and reduce microcystin contamination in aquaculture ponds.

Microcystis aeruginosa is the most dominant microcystin-producing cyanobacterial species. The excessive growth of toxic *M. aeruginosa* populations greatly deteriorates water quality and threatens human health. Developing safe and efficient techniques to control *M. aeruginosa* has become one of the top priorities in China today. The purpose of this study was to investigate microcystin concentrations in Nile tilapia muscle tissue and

surrounding pond water from a typical eutrophic fish pond in Hubei province, China. Furthermore, it aimed to develop removal methods for eliminating the dominant microcystin-producing species, *M. aeruginosa,* from pond water. Two types of clays were used to remove both laboratory cultured and field reared *M. aeruginosa*. In order to guide future usage, the relationships between optimal doses of each clay coagulant and algal concentrations were determined.

METHODS

Detection of microcystins in pond water and fish muscle tissues

To detect MCs, Nile tilapia muscle tissue and water samples were collected from a typical eutrophic fish pond in the Jiangxia District, Wuhan City, China. The pond was a working aquaculture pond stocked with Nile tilapia*,* common carp *Cyprinus carpio,* silver carp *Hypophthalmichthys molitrix*, bighead carp *Aristichthys nobilis*, and Crucian carp *Carassius auratus.* The pond's surface area was about 2000 m^2 and the average depth was approximately 2.5m. Samples were collected on 15 July and 17 August 2008, representing the dates of stocking and harvest.

Water samples were collected from the surface to bottom at 0.5m intervals with a water sampler (Institute of Hydrobiology, Chinese Academy of Sciences). Samples were then mixed thoroughly and a subsample was collected for water quality and microcystin tests. Water temperature, pH, conductivity, and dissolved oxygen (DO) were also measured at this time using a portable water quality analyzer (Multi 340iWTW, Germany). Water quality was analyzed following standard protocols for suspended solids, COD, nitrogen and phosphorous (APHA et al., 1998). Chlorophyll-α was extracted using acetone and measured with a spectrometer.

For identification and density analyses of algae and zooplankton species, samples were collected using plankton nets and concentrated through sedimentation. Water samples were collected from the surface to the bottom at 1-m intervals and mixed. One-liter samples from the well-mixed water were preserved immediately with 1% acid Lugol's solution. The preserved water samples were then put into sedimentation chambers for at least 24 hours. Finally, the concentrated sample was transferred into a 30 ml container, identified, and species were enumerated under a microscope.

Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC) methods were used to detect microcystin concentrations. On each sampling date, five fishes were captured by net. To analyze microcystin levels in fish, a sample of muscle tissue was removed, lypholized and stored in a freezer at -20 °C for later use. Before testing, 5 g of freeze-dried fish muscle tissue were ground and extracted twice in a well-mixed container with 100% methanol for 30 min. The methanol extract was diluted and dissolved in deionized water, which was then passed through a Sep-Pak C18 cartridge. The cartridge was rinsed with water and a 20% methanol solution. MCs were then eluted with 90% methanol in water, the methanol extract was dried, and the precipitate was dissolved in de-ionized water (Zhao et al., 2006; Xie, 2006). After extraction, analyses for MCs were performed by HPLC (Lawrence and Menard, 2001; Nicholson and Burch, 2001).

Laboratory experiments removing Microcystis aeruginosa *with modified clay*

Cultivated *M. aeruginosa* were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (strain HAB 657). Field reared *M. aeruginosa* were collected at the water surface (0m and 0.5m) with a plankton net from Guanqiao experimental pond, a

working aquaculture pond in Wuhan. The collections were conducted in July 2008 and from March to May 2009.

A laboratory experiment determined the efficacy and optimal dosages of chitosan and polymeric aluminum chloride (PAC)-modified clay (Kaolinite) in removing microcystins. For chitosan and PAC-modified clay, the ratio of chitosan or PAC to clay were both 1:10 (Divakaran and Pillai, 2001), and the concentrations of modified clays were both 11 mg clay/mL solution. There were 15 replicates at each of four algal concentrations for chitosan-modified clays (0.19, 0.27, 0.38, and 0.64 mg Chlorophyll- *a* /L) and PAC-modified clays (0.19, 0.43, 0.72, and 1.1 mg Chlorophyll-*a*/L). These 15 replicates at each algal concentration were subdivided into 5 groups of 3 replicates each which received a graded concentration of modified clay. Each replicate was placed in a 500 mL beaker filled with 400 mL of water. Algal density was estimated by spectrophotometry and added to each beaker. Algal populations were measured every other day until day seven, at which point the frequency of measurement was decreased as the algal population declined. The metrics Optical Density at 680 nm (OD_{680}) and electron transport rate (ETR) were then sampled once every month to measure algal density and photosynthetic vitality. OD_{680} was recorded using a Phyto-PAM Pulse-Amplitude-Modulation fluorometer (Heinz Waltz, GmbH, Effeltrich, Germany) for absorbance at spectrum 680nm, and ETR was measured by a Phytoplankton Analyzer (Heinz Waltz, GmbH). The OD_{680} values at each of the four algal densities were then summarized and compared. The optimal dosage was defined as the concentration at which the removal of *M. aeruginosa* was greatest. Typically this optimum dosage occurred at an intermediate concentration, allowing the isolation of one concentration as the optimum. All data analysis and statistics were completed in SPSS V13.0 (Chicago, Illinois, USA).

RESULTS

Detection of microcystins in pond water and fish muscle tissues

The experimental pond was eutrophic (Table 1) and dominated by blue-green and green algae. The density of Cyanophyta was 10.2-12 individuals/ml, contributing between 27 and 36% of the total algae by number, while the density of Chlorophyta was 17-21.8 ind./ml, contributing to between 52 and 58% of total algae (Table 2-3). Both Nile tilapia muscle tissue and pond water contained considerable microcystin concentrations, with higher concentrations of microcystins found in the tissue of fish from ponds with more dense Cyanobacteria populations (Table 4).

Laboratory experiments removing Microcystis aeruginosa *with modified clays*

After treatment with chitosan and PAC-modified clays, laboratory cultured *M. aeruginosa* were flocculated (Figure 1). The optimal dosages of coagulant were experimentally determined for each algal concentration (Table 5). The relationship between the optimal dose of chitosan-modified clay (y_1, ml) and OD_{680} (x_1) was $y_1 = 0.0349x_1 - 0.0019$ ($R^2 = 0.9972$). The relationship between optimal dose of chitosan-modified clay (y2, ml) and chlorophyll-α (x_2, mg/L) was $y_2 = 0.0524x_2 - 0.009$ ($R^2 = 0.9864$). For PAC-modified clay, the same relationships were: $y_1 = 0.0351x_1 + 0.0065$ ($R^2 = 0.9986$) and $y_2 = 0.0676x_2 - 0.0059$ ($R^2 = 0.9854$).

For optimal flocculation, pH was between 5-8 and 5-9 for chitosan- and PAC-modified clays, respectively. In these ranges, algal cells were mainly precipitated to sediments, greatly decreasing algal density in the pond, as measured by OD_{680} (Figure 2).

Within a month of treatment with chitosan-modified clays, sedimented cells were dead. An analysis of electron transport rate showed that the photosynthetic vitality decreased greatly. The maximum ETR decreased from 99.6 μmol photons cm^{-2} s^{-1} to 23.9 μmol photons cm^{-2} s^{-1} 30 days after treatment with chitosan-modified clay.

For waters treated with PAC-modified clay, algal cells became yellowish and decayed in a week, with ETR_{max} decreasing from 97.2 μmol photons cm^{-2} s^{-1} to 20.6 μmol photons cm^{-2} s^{-1} after 7 days (Figure 3). This indicated the algal cell vitality also decreased.

For field-reared *M. aeruginosa* in a laboratory setting, chitosan-modified clay was an effective coagulant (Figure 4). After clay treatment, chlorophyll-α decreased from 1.172 ± 0.210 mg/L to 0.017 ± 0.007 mg/L in 100 minutes with a removal rate of 98.60%.

DISCUSSION

Concentrations of microcystins in the sampled pond water at stocking (0.134±0.041 μg/l) and harvest (0.052±0.017 μg/l) were slightly lower than studies in other water bodies. The average concentration of microsystins from comparable lake waters were reported at 0.243 μg/l, 0.211-6.6 μg/l and 0.09 μg/l in Jiangxi, Jiangsu, and Shanghai province, respectively (Wang and Song, 1995; Zhang and Xu, 2001; Chen et al., 2002; Wu et al., 2005). For drinking water sources, the average concentration of microcystins was 0.12-14.2 μg/L. Levels varied from 0.001 - 14.188 μg/L in lakes and 0.11 - 0.24 μg/L for ditches and shallow wells (Dong et al., 1998; Mu et al., 2000; Sun et al., 2000; Xu et al., 2003).

Nile tilapia muscle tissue from the Jiangxia experimental fish pond contained lower microcystin concentrations compared to fish muscle from other water bodies. In Lake Taihu (Yang et al., 2007), the average microcystin concentrations in muscles of seven fish species were between 1.40-13.2 ng/g, with 2.68 ng/g for *Cyprinus carpio*, 1.40 ng/g for *Mylopharyngodon piceus*, 2.3 ng/g for *Ctenopharyngodon idellus*, 13.20 ng/g for *Hypophthalmichthys molitrix*, 6.08 ng/g for *Aristichthys nobilis, 3.57* ng/g *for Parasilurus asotus* and 1.7 ng/g for *Megalobrama amblycephala*.

Both chitosan and PAC-modified clays flocculated *M. aeruginosa* effectively, but PAC-modified clay had a quicker effect. The decrease in photosynthetic vitality shown by PAC-modified clay in one week was comparable to that reached after a month of treatment with chitosan-modified clay.

Considering the environmental impacts of both PAC- and chitosan-modified clays is also important as these methods are going to be tested for and potentially used in the industry. The PAC-modified clay contains aluminum, a protoplasmic poison and a destructive, persistent neurotoxin. Compared with PAC, which contains aluminum chloride that may cause side effects, chitosan contains no toxic materials and is biodegradable. While both chemicals are effective, chitosan-modified clay is recommended as the more environmentally friendly option for treating *M. aeruginosa* blooms in the field.

ANTICIPATED BENEFITS

The detection of microcystins in fish muscle tissue and pond water may draw public attention to microcystin contaminations. As *M. aeruginosa* is the dominant species that produce microcystins, controlling *M. aeruginosa* will effectively eliminate microcystin pollution. Chitosan and PAC-modified clays can both be used as promising coagulants in controlling *M. aeruginosa*. This study also provides relationships that will allow farmers to

calculate optimal coagulant dosage based on the concentration of chlorophyll-α in the water from relationships established in the laboratory. These relationships should be tested more thoroughly before being applied in the field. The chitosan-modified clay, which is more environmentally friendly, should be tested in a field setting and shows promise for future applications.

FIGURES

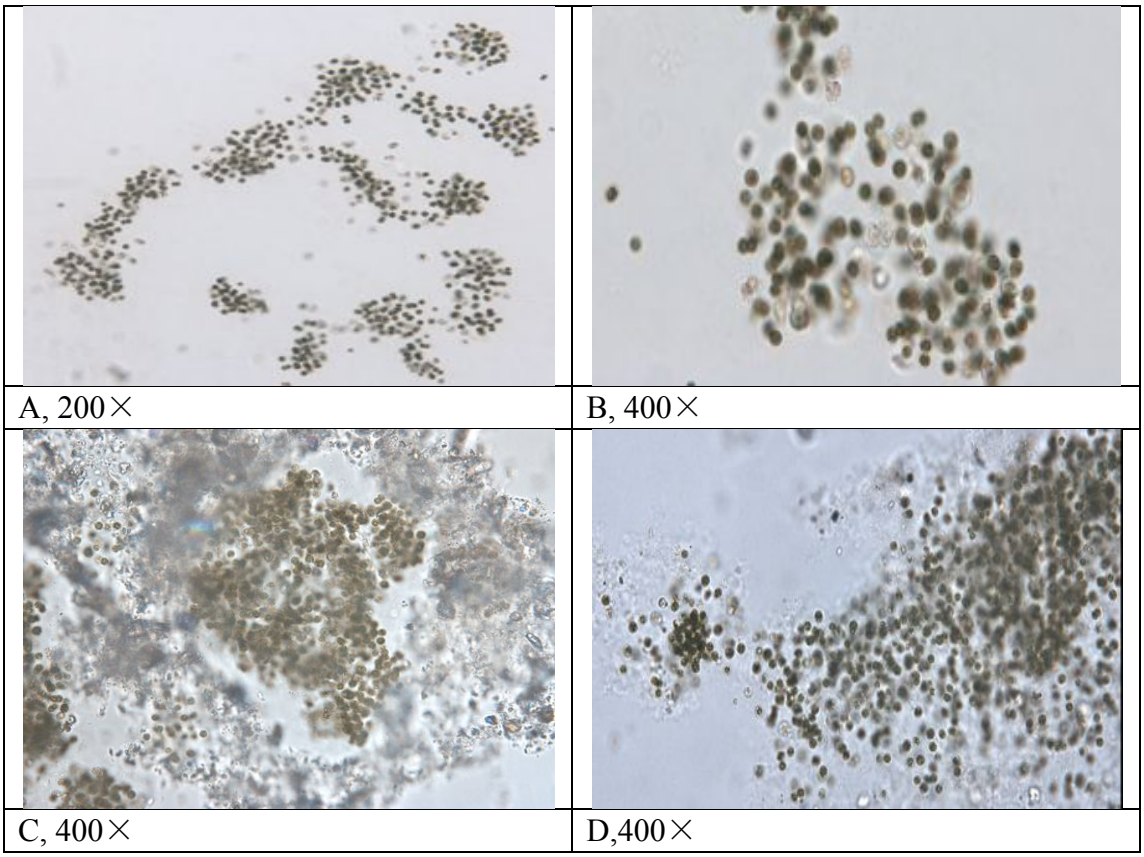
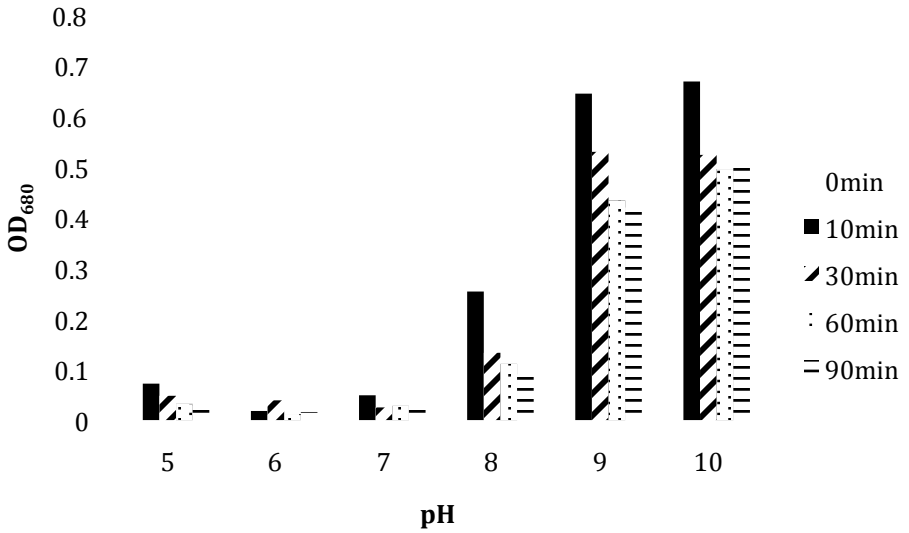


Figure 1. Microscopic pictures of laboratory cultured *Microcystis aeruginosa*, showing algal cells that were flocculated after treatment with Chitosan and Polymeric Aluminum Chloride (PAC)-modified clay. A: Algal cells before treatment, 200×; B: Algal cells before treatment, 400×; C: Algal cells flocculated after treatment with Chitosan-modified clay for 100minutes, 400×; D: Algal cells flocculated after treatment with PAC-modified clay for 100 minutes, 400×.



A

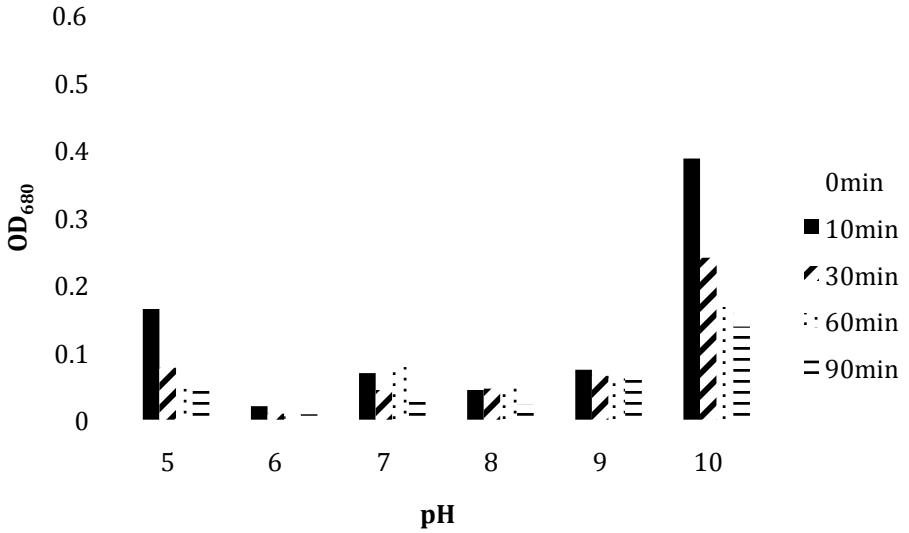


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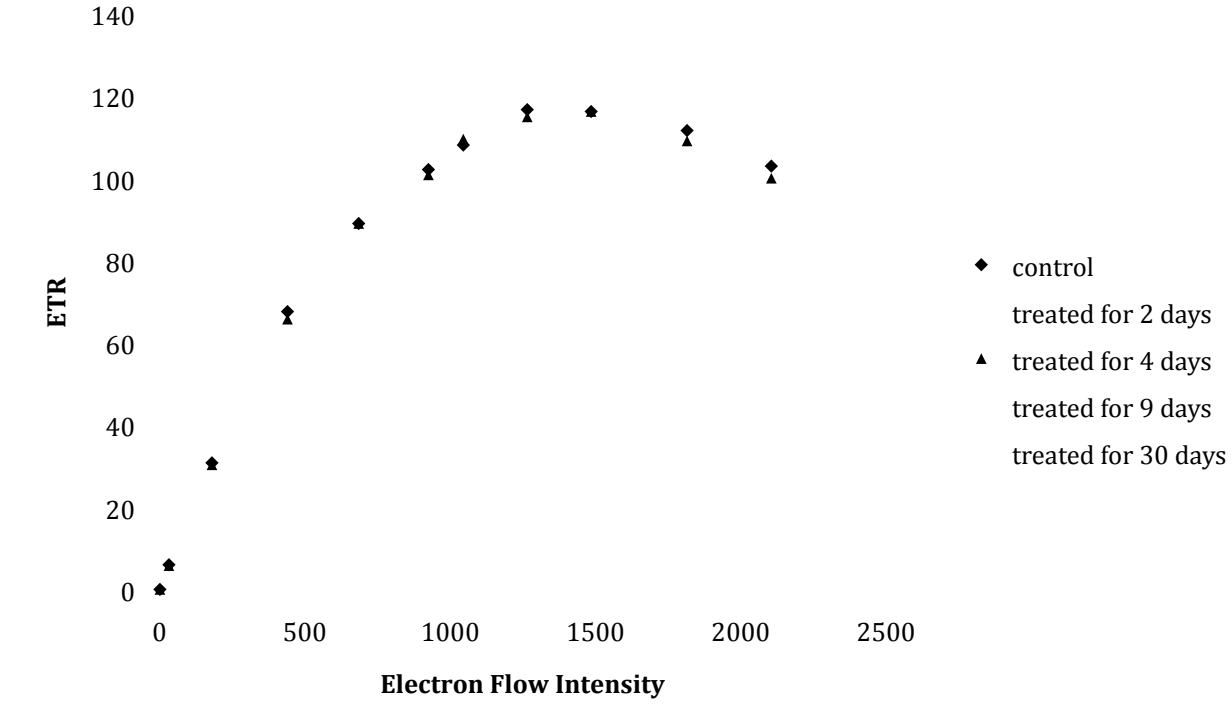
Figure 2: Relationships between pH and *Microcystis aeruginosa* concentration (OD_{680}) in the supernatant during treatment with Chitosan- and PAC-modified clays. A: treated with Chitosan modified clay; B: treated with PAC modified clay.



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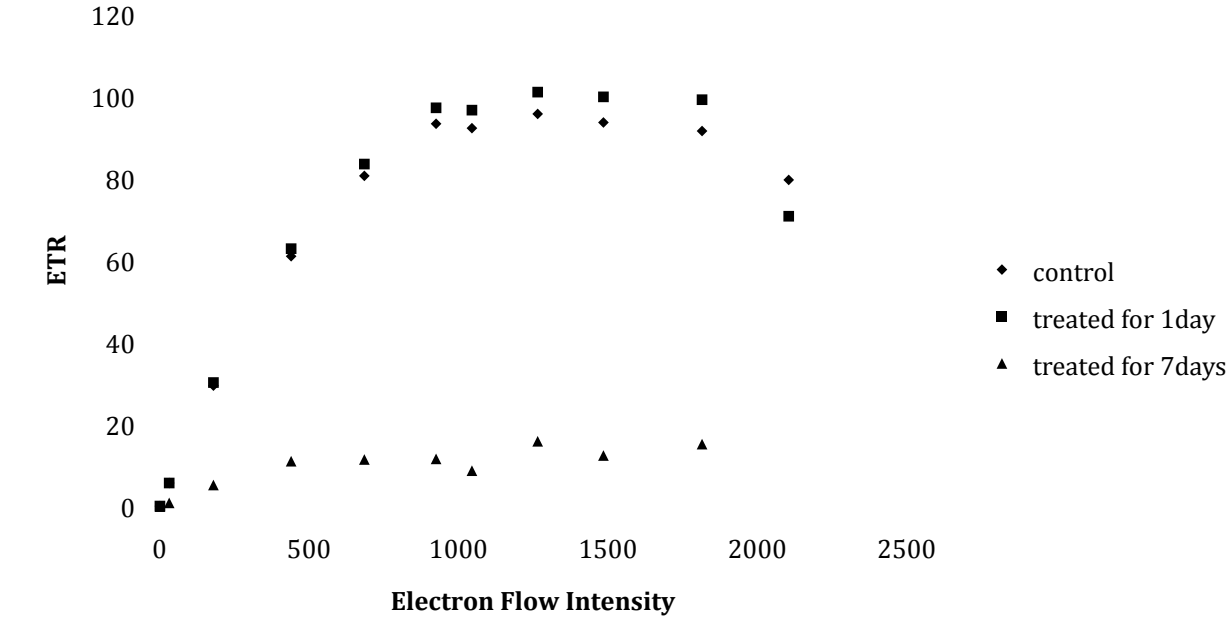
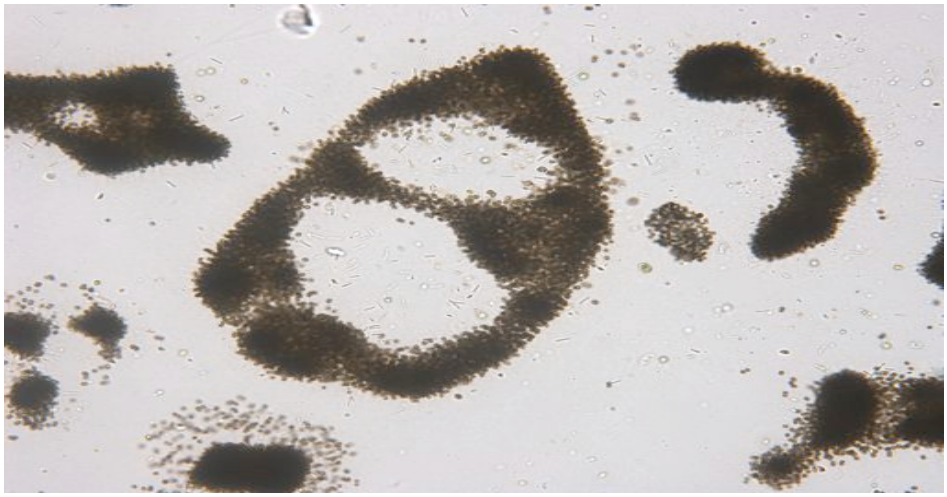


B

Figure 3: Changes in the electron transport rates (ETR) of *Microcystis aeruginosa* at different electron flow intensities (µmol photons m^{-2} s^{-1}) showing a decrease in photosynthetic vitality after treatment with coagulants. A: treated with Chitosan-modified clay, B: treated with Polymeric Aluminum Chloride-modified clay.



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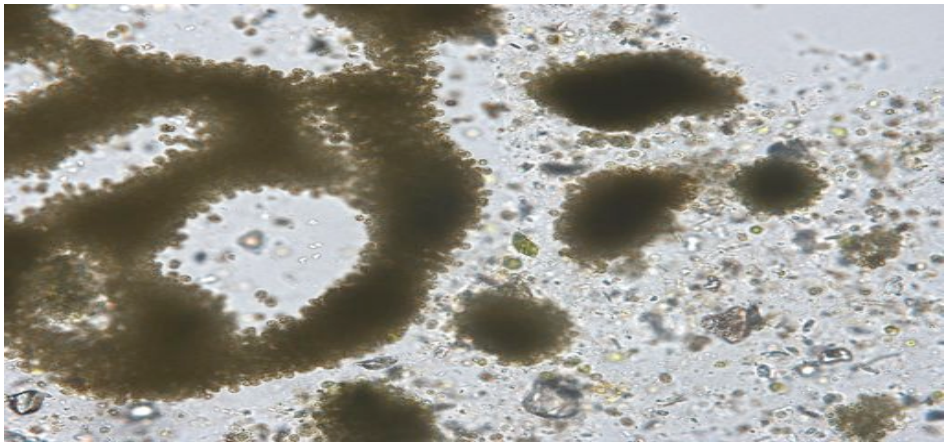

B

Figure 4: Microscopic pictures of *Microcystis aeruginosa* collected from Guanqiao experimental fish pond, Wuhan, Hubei Province, China. A: before treatment (200×); B: treated with Chitosan-modified clay for 100 minutes.

TABLES

Table 1. Physiochemical features of the experimental fish pond in Jiangxia District, Wuhan, China: Temperature (Temp., ^{o}C), pH, conductivity (Cond., mS/m), suspended solids (SS, mg/L), dissolved oxygen (DO, mg/L), chemical oxygen demand (COD, mg/L), ammonia-nitrogen (NH_3-N, mg/L), total nitrogen (TN, mg/L), and total phosphorus (TP, mg/L) were tested on two sampling dates.

	Temp. (^{o}C)	pH	Cond. (mS/m)	SS (mg/L)	DO (mg/L)	COD_{Mn} (mg/L)	NH_3-N (mg/L)	TN (mg/L)	TP (mg/L)
15 July 2008	23.47	7.85	274	14.5	7.92	2.4	0.10	0.37	0.08
17 August 2008	26.22	7.85	275	21.0	7.65	2.2	0.13	0.34	0.05

Table 2. Density (ind./ml) and percent composition of algae in the experimental fish pond, a typical eutrophic fish pond in the Jiangxia District Wuhan City, China

Algae	Algal Density and percent composition (in parentheses)	
	15 July 2008	17 August 2008
Cyanophyta	12 (36.59 %)	10.2 (27.13 %)
Pyrrophyta	3 (9.15 %)	5.2 (13.83 %)
Euglenophyta	0.2 (0.61 %)	0.2 (0.53 %)
Bacillariophyta	0.2 (0.61 %)	0.2 (0.53 %)
Chlorophyta	17 (51.83 %)	21.8 (57.98 %)
Chrysophyta	0.4 (1.22 %)	0

Table 3. The composition of Cyanophyta (%) in the experimental fish pond on the two sample dates.

Genus	July 15, 2008	August 17, 2008
Microcystis	85%	60.4%
Coelosphaerium	15%	31%
Other	-	8.6%

Table 4. Concentration and distribution of microcystins in pond water samples and Nile tilapia muscles from a eutrophic fish pond in the Jiangxia District Wuhan City, China.

	Percent Composition of Cyanobacteria	Dominant species of cyanobacteria	Microcystin concentration in pond water	Range of Microcystin concentration (ng/g) in fish muscle tissue
15 July 2008	36.59 %	*Microcystis* spp.	0. 134 ± 0.041	0. 84 ± 0.84 (0.10-2.21),

17 August 2008	27.13 %	*Microcystis* spp., *Coelosphaerium m* spp.	0.052 ± 0.017	0.68 ± 0.49 (0.33-1.49)

Table 5. The optimal dose of coagulants for maximum algal removal at different concentrations of *Microcystis aeruginosa* in a laboratory.

Algal Concentration (mg/L Chlorophyll-*a*)	Optimal dose of Chitosan-modified clay (ml)	Optimal dose of PAC-modified clay (ml)
0.19	0.5	2.0
0.27	0.8	-
0.38	2.0	-
0.43	-	4.0
0.64	5.0	-
0.72	-	8.0
1.1	-	14.0

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FOOD SAFETY STUDY OF LEAFY GREENS IRRIGATED WITH TILAPIA FARM EFFLUENTS

Human Health Impacts of Aquaculture/ Experiment/ 07HH02UA

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ABSTRACT

Aquaponics has drawn interest as a sustainable farming method merging aquaculture with hydroponics. Using the effluent from fish farming to irrigate and fertilize vegetables which filter the water so that it can be reused for fish production has many benefits but unknown risks. One potential concern would be the potential for pathogens from fish effluent splashing onto the edible portions of plants and then being passed to the human consumers of the plants.

To explore this concern, we developed an aquaponics system in a newly dedicated greenhouse and planted a lettuce crop in perforated styrofoam boards floating in a raceway filled with effluent from a tilapia production unit constituting a recirculating aquaculture system. The effluent and plant leaves were tested for bacterial counts. In an additional trial we developed a simple ultraviolet treatment system in an effort to reduce the level of bacteria in the water and on the plants.

We did detect low levels of several bacteria in the water and on the plants. The UV system did reduce these bacteria to non-detectable levels in most cases. In conclusion, we did not find any health hazard in the aquaponic system tested and we developed a simple UV treatment system that lowered the levels of the bacteria that were observed.

As an additional effort we developed an associated system utilizing tilapia and vegetable culture at the Conrado Castillo, a farming cooperative near Ciudad Victoria. The farm has been technically and economically profitable according to the farmers.

INTRODUCTION

In 2006, several nation-wide epidemics of *Escherichia coli* or related gastro-intestinal pathogens were traced to consumption of fresh vegetables (spinach, lettuce, green onions). In several of these cases the vector was thought to be contamination from human or animal wastes applied through irrigation water. As we encourage more organic farming methods and re-use of composted wastes and effluent waters from animal operations to irrigate field crops, we increase the opportunities for these types of contamination. One alternative is to increase the multiple-use of water for production of fish and use of these effluents for irrigation of vegetables that are bound for human consumption.

Across much of Asia, fish is the primary protein component of the diet and integrated fish and vegetable farming has been practiced for centuries. However, very few studies have been conducted to determine any health hazards that may result from this practice. The assumption is that fish being heterotherms (cold-blooded) and obviously not mammals, they are unlikely to be vectors for intestinal pathogens. However, fish farms do have human workers and ponds can be visited by farm animals and pets. Examination of typical aquaculture farm effluents and any residual potential pathogens that might be left on leafy vegetables irrigated with fish farm effluents, bound for direct human consumption would be an advisable precaution.

Bacteria have been identified in aquaculture systems that are considered human pathogens, such as fecal coliforms including *Escherichia coli* (Ogbondeminu, 1993; Flick, 1996; Del Rio-Rodriguez et al., 1997; Pullela et al., 1998), *Clostridium botulinim* (Pullela et al., 1998), *Pseudomonas* species (Nedoluha and Westhoff, 1995), Aeromonas hydrophila (Nedoluha and Westhoff, 1997) and *Salmonella* species (Ogbondeminu, 1993) to name a few. Some typical fish pathogens have also been known to cause illness in humans. The microflora of the fish gills, skin, and digestive tract have been shown to reflect the microflora of the water they inhabit and may also pose a threat to humans (Reilly and Käferstein, 1997; Nedoluha and Westhoff, 1997; Ogbondeminu, 1993).

Preliminary studies at the University of Arizona demonstrated that total coliforms levels of 10^4 CFU's/100ml and fecal coliform levels of 10^3 CFU's/100ml can be found in two separate recirculating systems rearing grass carp and tilapia (McKeon et al., 2000; McKeon, 1998). Samples from this system were found to contain total coliform levels as high as 10^4 CFU/100 ml. Fecal coliform levels were found to be variable over a test period of two months. Counts were as low as 1 CFU/100 ml to as high as 10^3 CFU/100 ml. These levels indicate the possible presence of human enteric pathogens that could cause illness in fish handlers and consumers if proper precautions are not observed. However, using the Colilert rapid detection test we had a negative result for E. coli presence. The source of the coliforms is unknown.

MATERIALS AND METHODS

We proposed that complementary lab and field studies be performed to examine the bacterial flora typically encountered in in-door and out-door aquaculture operations. Along with this we would grow lettuce and spinach and in greenhouses and in the field which would be irrigated with effluents from attendant fish (tilapia) production facilities. An indoor – outdoor system was evaluated in a newly dedicated research building constructed to support the AquaFish Research program. Six round fiberglass tanks containing 5,000 liters of water were stocked with 200 tilapia (*Oreochromis niloticus*), with an average weight of 140 grams each and a total length of 20 cm. The fish were fed a 30% protein diet at a rate of 5% of the biomass of fish per tank, split between three feedings by hand per day. Water temperature was maintained at 22 degrees C. during the trials. The six tanks were contained inside a building.

The effluent water from each tank was fed to a biofilter containing plastic media to capture solids and increase dissolved oxygen before the water entered the hydroponics beds. Lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) were stocked onto floating sheets of styrofoam (floating bed technique) placed on top of the water in the hydroponics bed. 287 plants were transplanted from a seed starting table to the growing boards in each

recirculation raceway for each trial (lettuce in summer and spinach in winter). The hydroponic raceways were 7,500 liters each and were constructed adjacent to the fish production building containing the tanks.

Water and plants samples were collected from the University of Tamaulipas UV system, Veterinary College, fish and plants were grown during summer, fall and winter during 2008 and 2009 at different intervals. Leaf samples are removed randomly from the growing lettuce and spinach plants. Samples were collected and stored in sterile plastic bottles and bags. They were immediately placed in an ice chest and transported to the Veterinary college water quality analysis laboratory for analysis.

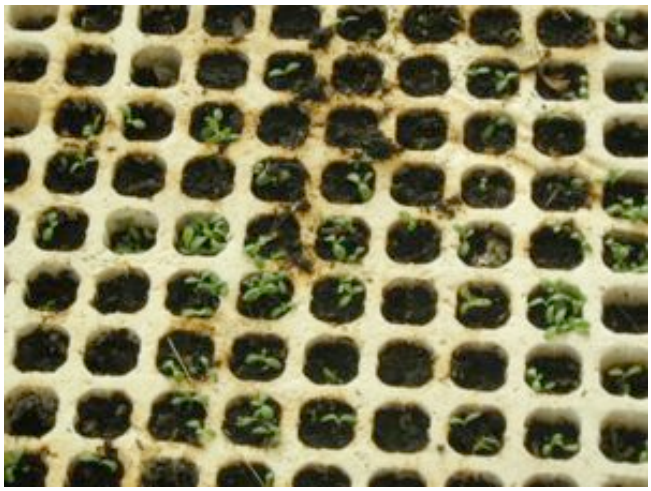


Fig. 1 Lettuce plants germinating

1. Greenhouse studies

Tilapia production facilities at the Universidad Autonoma de Tamaulipas utilize recirculation technology to rear fish. These systems incorporate biological filtration, which includes nitrifying and heterotrophic bacteria to treat water so that it can be recycled to the fish tanks for continuous rearing of fish in the same water.

Fig. 2. Student from the UAT planting lettuce in styrofoam floating on the trough of effluents.

The effluent water and a control water supply, tap water from the city water source, used for irrigation would be sampled during each irrigation event (every 3rd day for the first three weeks and 5th day for the final nine weeks). Crops cycle times for the lettuce and spinach were 7 weeks and 10 weeks respectively.

At the end of the growing cycle, leaves from the portions of the plant typically harvested for commercial sale will be collected and sampled for total fecal coliforms, E. coli, enterococci and Salmonella. Total colony forming units (CFU's) will be determined and reported on a per 100 ml basis.

2. Field Studies

Tilapia production facilities at the Ejido Conrado Castillo utilize a pond system with source water from wells passing through

two ponds stocked with fish before use as irrigation water for spinach and lettuce. These farmer cooperators agreed to provide matching support to the project and provided ponds, liners, plumbing and field preparation in addition to the care and feeding of the fish and tending the vegetables.

The field plots required more frequent irrigation in the dry field environment, with the exact irrigation schedule dependant on weather conditions. Sample schedule was not as frequent as the university lab testing but did encompass five sample dates during the irrigation schedule. At the end of the growing cycle, leaves from the portions of the plant typically harvested for commercial sale were collected and sampled for total fecal coliforms, E. coli, enterococci and Salmonella. Total colony forming units (CFU's) was be determined and reported on a per 100 ml basis.

LAB ANALYSES.

Fecal coliforms will be isolated by membrane filtration of the sample and direct plating of the filter on mFC agar according to Standard Methods (APHA, 1998). For E. coli isolation, a presumptive test involving lauryl sulfate tryptose (LST) broth will be employed. Positive presumptive tests (gas production) will be followed by the tests with E. coli (EC) broth (Hitchins et al., 1992).

Enterococci will be isolated according to Standard Methods (APHA, 1998) using membrane filtration and m Enterococcus agar for determining the presence of fecal streptococci. Confirmation will be performed after transferring typical colonies from a membrane to the surface of a brain-heart infusion (BHI) agar and incubation. A catalase test and gram stain of the BHI isolate will be done as well as observations of growth on bile esculin agar and growth in BHI broth containing 6.5% NaCl to confirm the presence of enterococci (APHA, 1998; Hartman et al., 1992).

Salmonella pre-enrichment will be accomplished using lactose broth (Flowers et al., 1992) and selective enrichment will subsequently be performed using a ducitol selenite broth. After enrichment subcultures will be performed on Salmonella Shigella (SS) agar (Difco Laboratories, 1984) and Xylose lysine desoxycholate (XLD) agar (APHA, 1998). Following incubation, an oxidase test and indole test will be performed. A short set of biochemical tests will be followed by inoculation of an API 20E strip as per directions by API (Flowers et al., 1992; APHA, 1998). Testing with Salmonella antiserum will conclude the confirmation tests (APHA, 1998).

STATISTICAL ANALYSES

We compared total fecal coliform, enterococci and Salmonella densities (Colony Forming Units per 100 ml) among treatments with a two-way ANOVA where season and irrigation treatment were the two independent factors. There were three levels for the Season factor (winter, spring, and summer) and two levels of the Irrigation-treatment factor (tap water/well water, and effluent water). Analysis for statistically different levels of potential pathogenic bacteria in the fish effluent and control water used to irrigate vegetables will be determined. Statistically different levels of potential pathogenic bacteria actually collected off the edible portions of the plants were examined. SysStat software will be utilized to facilitate analysis.

POTENTIAL IMPACTS

If levels of pathogenic bacteria in fish effluent are determined to be low enough for safe use as irrigation water, these results would be useful for developing farm management

strategies that will insure production of high-quality crops in integrated production systems based on aquaculture effluents.

The aquaculture program of the UAT (Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia "Dr. Norberto Treviño Zapata") has developed aquaculture systems which are being adapted from the US (University of Arizona models) and also incorporating ideas from Indonesia, Italy, Korea, Egypt and other Central American countries. As implementation of these systems proceeds in these locations, information about health and environmental issues have to be monitored and recorded in order to report the presence of human bacterial pathogens in the aquaculture system water and the potential for contamination and consequently, negative impact to consumers and environment.

The aquaculture systems studied in this research were conducted in both indoor closed and outdoor environments and accessible by birds and small mammals. Feces from these animals can contaminate the water with coliforms and other pathogenic bacteria. This research evaluated water and plants samples from both systems over a 2 year period to determine:

a. The presence of total and fecal coliforms, salmonella and enterococci
b. And if the UV treatment provided any significant difference.
c. And reported the number of organisms.

METHODOLOGY

The project first needed to develop and operate a new aquaponics system at UAT. The building and greenhouse addition with tanks and growing beds were built with state funds in specific support of the AquaFish CRSP grant. The system was operated for several months before the trial started in order to develop the biofilter and other biotic community in the aquaponics unit.

Standard Methods were utilized to identify and enumerate the bacteria in the system water and on the roots and leaves (APHA 1998). The specific tests were:

NOM 112-SSA1-1994 for coliforms
NOM 092-SSA1-1994 for aerobics bacteria
NOM 114-SSA1-1994 for Salmonella

In all standard techniques (SSA), the laboratory performed the presumptive portion of the multiple-tube test. This test uses 9 tubes of lauryl tryptose broth, each of which was inoculated with a different dilution of UV aquaponics system water.

The estimation of bacterial density, was made positive results from the fermentation

Table 1:

INDOORS WATER SAMPLES					
TOTAL COLIFORMS			FECAL COLIFORMS		
Tank	Mean	SD	Tank	Mean	SD
1	0.207	0.265	1	0.112	0.066
2	0.609	0.348	2	0.599	0.105
3	0.157	0.092	3	0.118	0.076
4	0.748	0.328	4	0.452	0.060
5	0.192	0.165	5	0.137	0.097
6	0.854	0.372	6	0.618	0.206

Fecal coliforms procedure was used to determine the presence and number of fecal coliforms. This test is conducted after Total coliform technique has confirmed the presence of coliforms. The presence of bacterial density is determined by the most probable numbers (MPN) reported organisms in a 100 mL, sample. The results of these methods are included in Table 2.

INDOORS Lettuce			Mean	SD	Spinach			Mean	SD
UV	Roots	TOTAL COL	0.145	0.098	UV	Roots	TOTAL COL	0.100	0.011
		FECAL COL	0.095	0.085			FECAL COL	0.090	0.016
	Leaf	TOTAL COL	0.14	0.087		Leaf	TOTAL COL	0.025	0.057
		FECAL COL	0.075	0.034			FECAL COL	0.115	0.064
NT	Roots	TOTAL COL	0.09	0.040	NT	Roots	TOTAL COL	0.115	0.077
		FECAL COL	0.105	0.075			FECAL COL	0.010	0.011
	Leaf	TOTAL COL	0.06	0.034		Leaf	TOTAL COL	0.105	0.010
		FECAL COL	0.06	0.034			FECAL COL	0.155	0.086

Fig. 4. Newly transplanted lettuce plant in aquaponics trough.

Fig. 5. Fully grown lettuce on the floating bed, ready to be harvested.

RESULTS

Figure 6. TOTAL COLIFORMS IN SYSTEM WATER

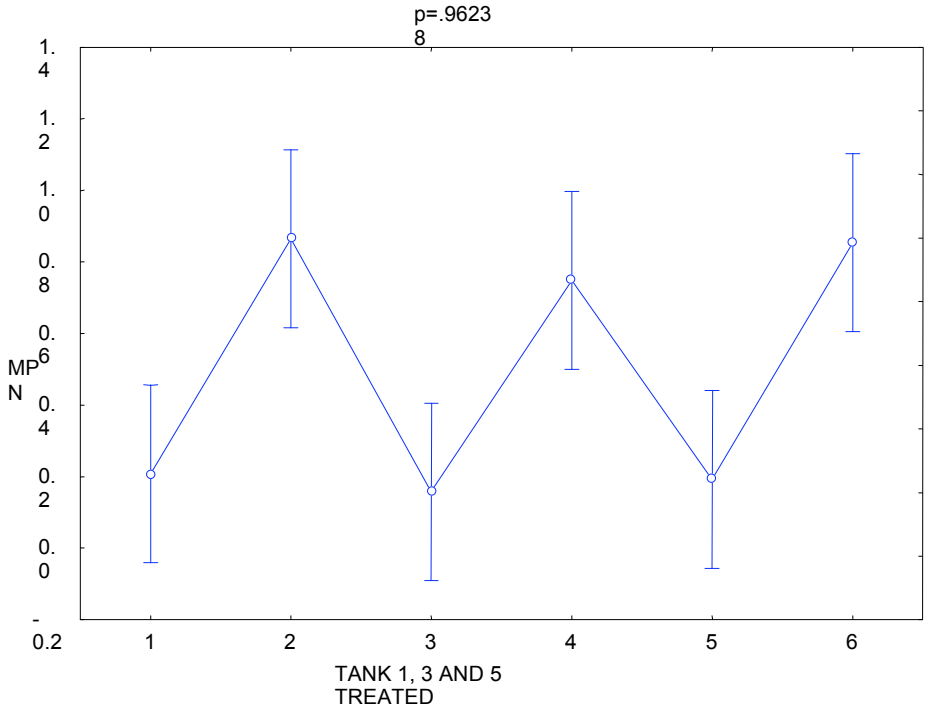


Figure 7. FECAL COLIFORMS

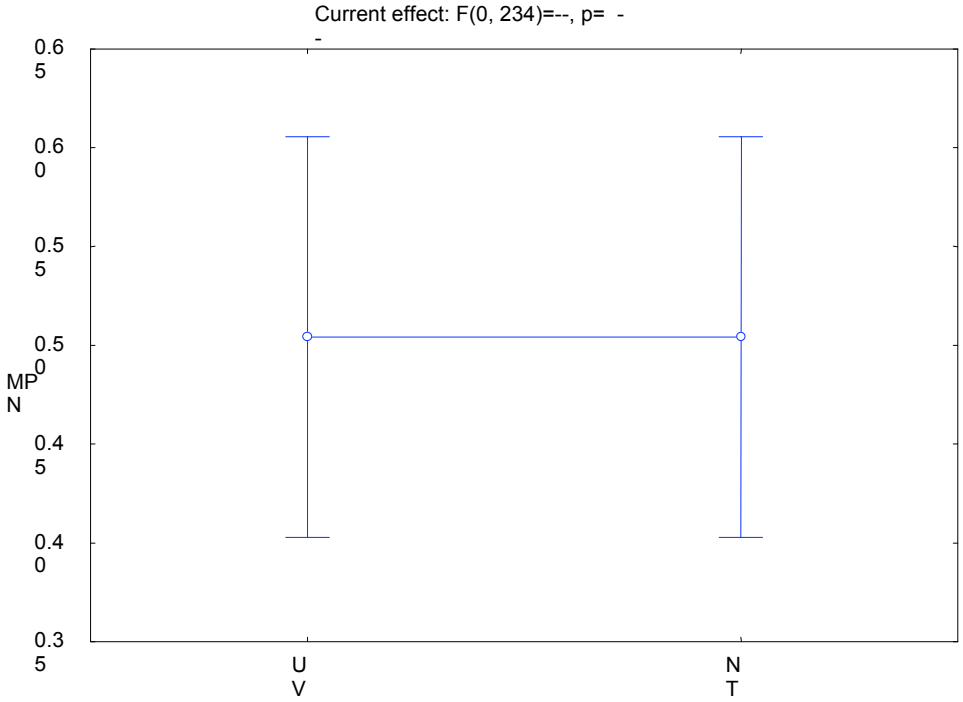


Figure 8.

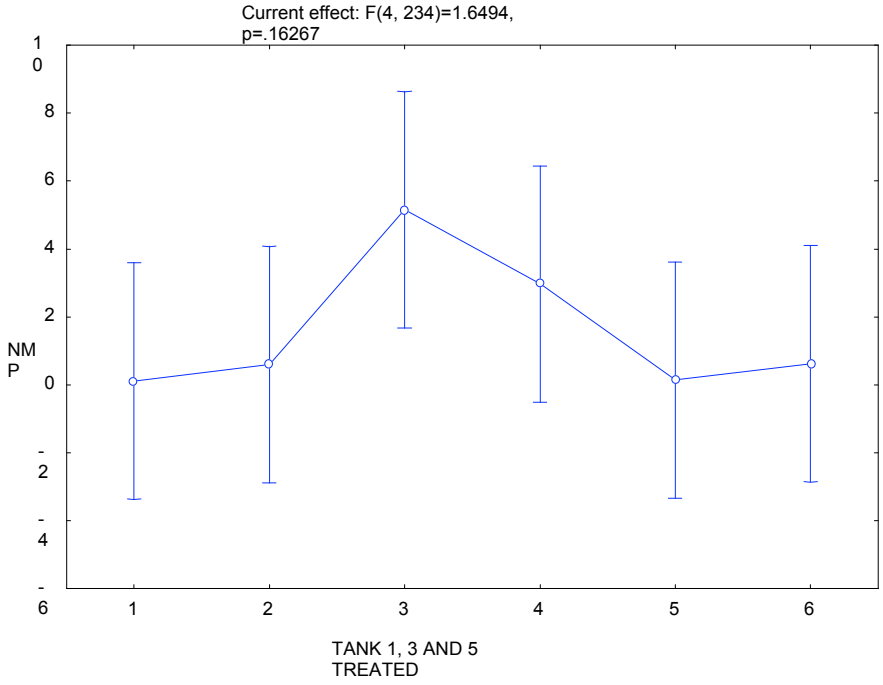
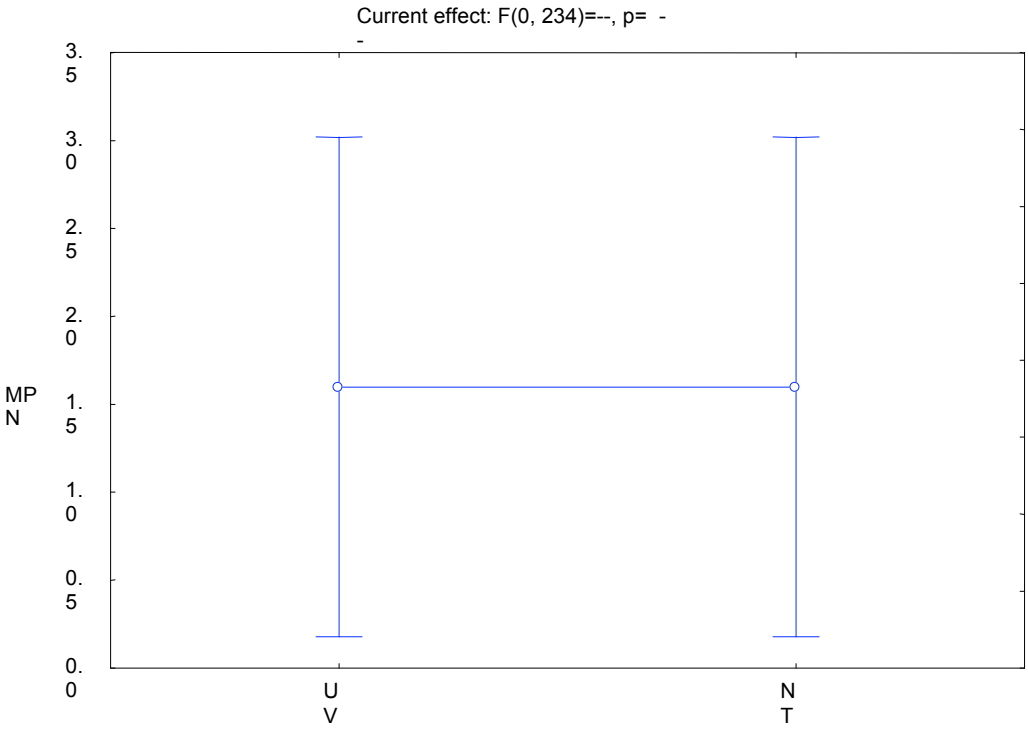
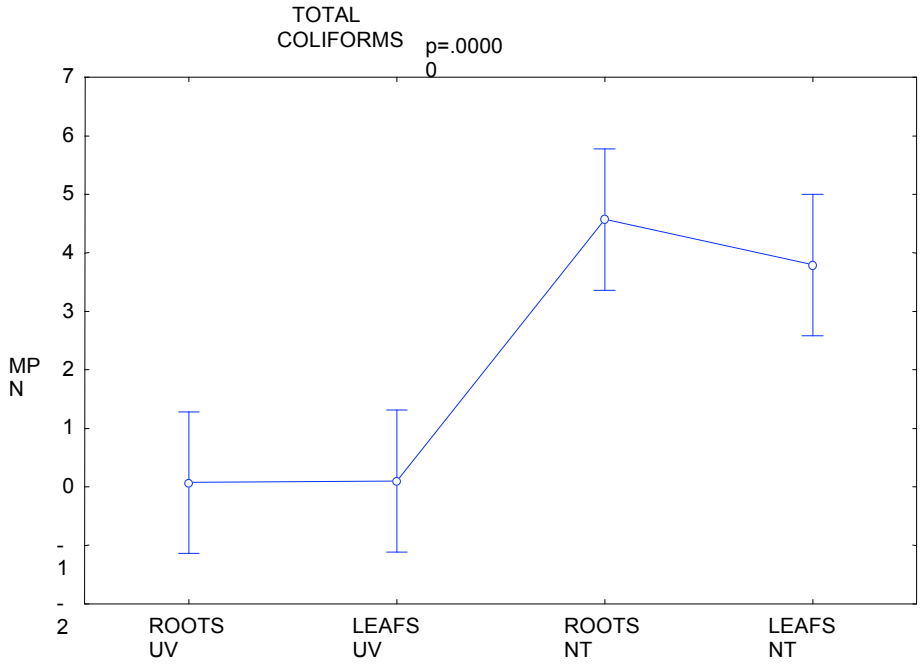


Figure 9. Coliform counts on spinach leaves.



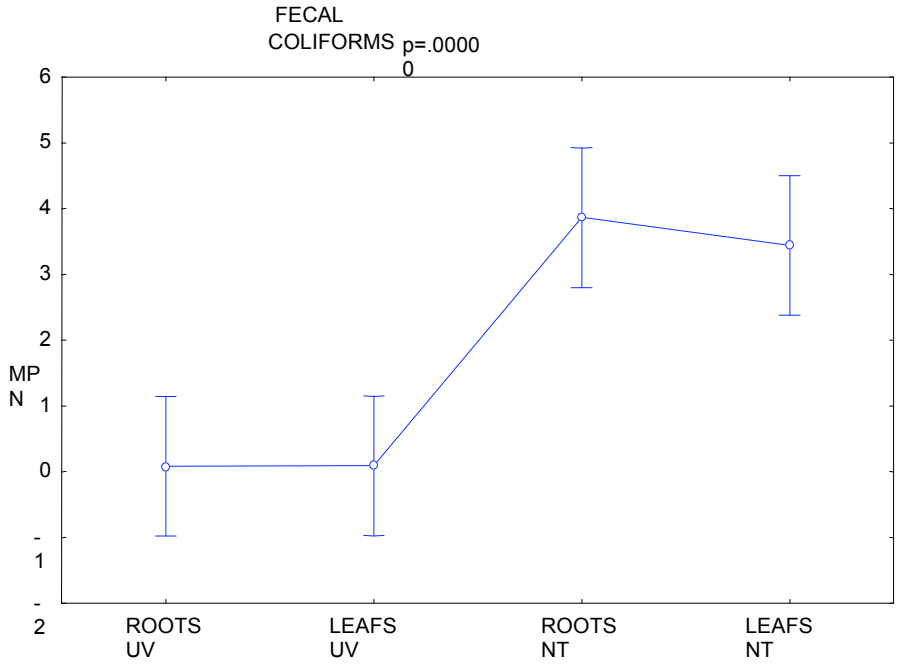
SPINACH - TOTAL COLIFORMS

Figure 10. Total COLIFORMS



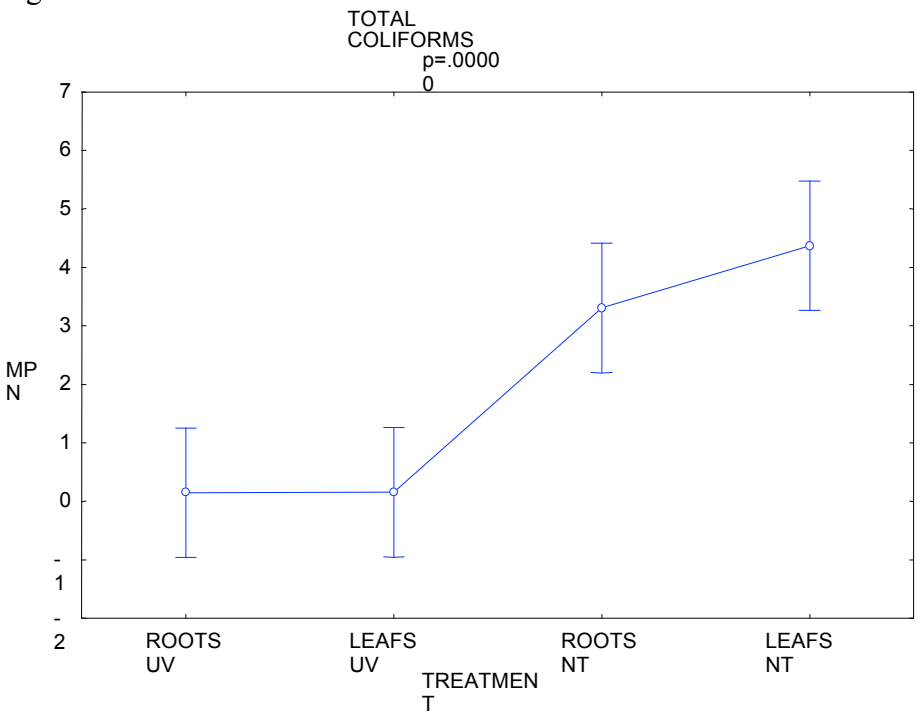
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	364.2311	1	364.2311	49.21482	0.000000
TRATAMIENTO	341.8653	3	113.9551	15.39759	0.000000
Error	562.4640	76	7.4008		

Figure 11. FECAL COLIFORMS



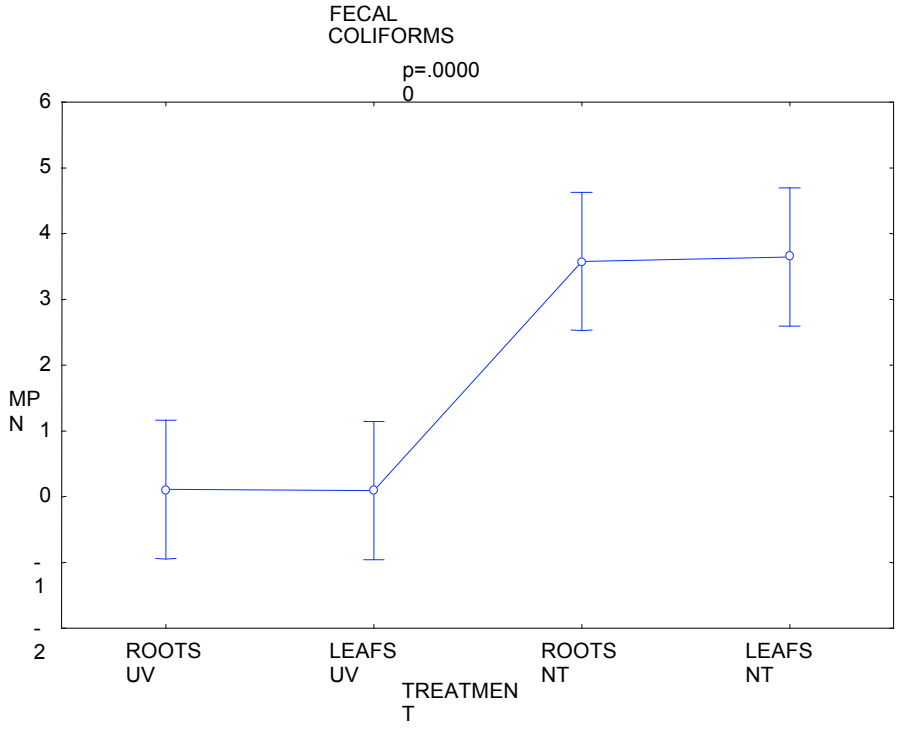
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	279.4903	1	279.4903	49.18566	0.000000
TRATAMIENTO	256.0980	3	85.3660	15.02300	0.000000
Error	431.8588	76	5.6824		

Figure 12. LETTUCE - TOTAL COLIFORMS



Effect	SS	Degr. of Freedom	MS	F	p
Intercept	318.5217	1	318.5217	51.60458	0.000000
TRATAMIENTO	283.4483	3	94.4828	15.30742	0.000000
Error	469.0988	76	6.1724		

Figure 13. FECAL COLIFORMS



Effect	SS	Degr. of Freedom	MS	F	p
Intercept	276.4333	1	276.4333	49.70261	0.000000
TRATAMIENTO	246.6937	3	82.2312	14.78514	0.000000
Error	422.6927	76	5.5617		

Figure 13. TOTAL COLIFORMS

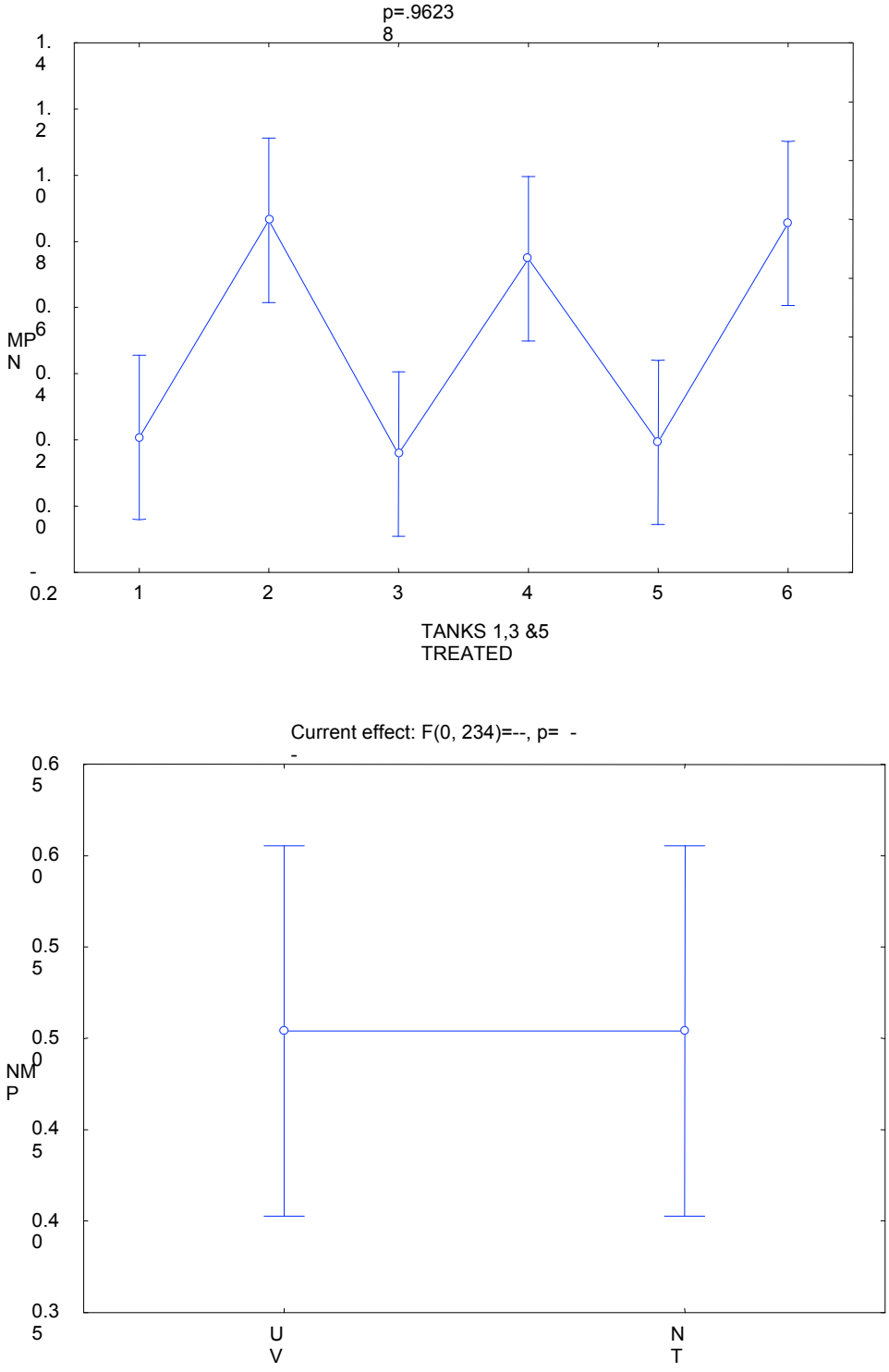
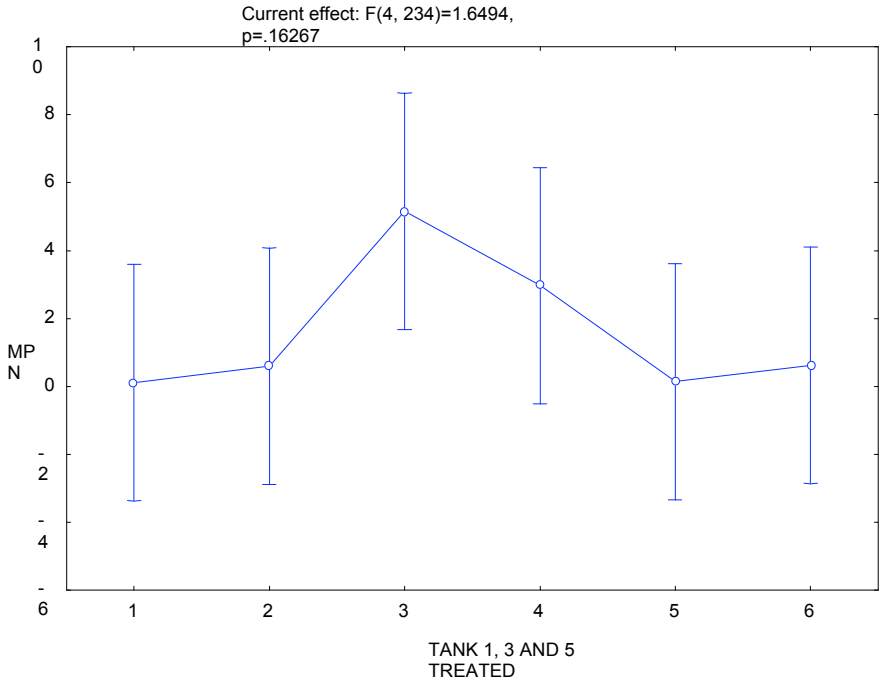


FIGURE 14. FECAL COLIFORMS



PRINCIPAL FINDINGS AND CONCLUSIONS

In the aquaponics system constructed at the UAT, the system was found to be technically feasible with good growth of fish, lettuce and spinach. Water samples from each sampling date and each system found total, and fecal coliforms in measurable numbers. However, Salmonella, E. coli, and Enterococci were negative.

The levels of the coliforms were all in the range of typical background numbers found in nature and not indicative of contamination. However, as any level could be seen to be undesirable, a system to lower the level was devised. This UV Treatment provided a significant reduction of one order of magnitude in total coliform counts in the recirculating aquaponics system. The second system constructed at the Ejido Conrado Castillo was also technically successful. The farmers reported producing commercial quantities of tilapia and vegetables in addition to their household consumption.

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INTERNATIONAL WORKSHOP FOR AQUACULTURE SANITATION

Human Health Impacts of Aquaculture/ Activity/ 07HH103UH

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ABSTRACT

An international workshop was held in Culiacan, Sinaloa and Santiago Ixcuintla, Nayarit for thirty-nine participants including small-scale farmers, government officials, NGO's, researchers and extension agents. The purpose of the workshop was to increase technical capacity in the areas of bivalve culture and sanitation, and provide a venue for development of collaborative opportunities. This was the fourth annual international event sponsored by CRSP and included representatives from Mexico, Nicaragua and the U.S.

INTRODUCTION

Bivalve mollusk culture is a priority for aquaculture development throughout LAC. In the case of Mexico, state governments (e.g. Nayarit, Sinaloa, Sonora) and the federal government have prioritized shellfish culture for development for nearly ten years. The Autonomous University of Sinaloa and its numerous partners in aquaculture development have recently worked together in an integrated effort to accelerate development of the shellfish industry with long-term support from CRSP/USAID. In the case of Latin America, there is wide spread recognition of the potential for shellfish aquaculture, but progress towards realizing its potential has been slow until recently. The Central American University (UCA), has been promoting shellfish culture and management of the shellfish fisheries for over ten years with support from multiple donors, including CRSP. This workshop was the fourth in a series of international workshops sponsored by CRSP that have provided an international venue for exchanging knowledge and lessons learned related to shellfish culture and sanitation. Additionally, the topic of increasing extension capacity is addressed in these workshops.

The objectives of this training workshop were to:
Present the results of research and development efforts in three countries (U.S., Mexico and Nicaragua);

- Increase technical capacity among farmers, researchers, NGO's and government officials both within Mexico and with international colleagues;
- Increase extension capacity and partnerships;
- Provide a net working opportunities for small-scale Mexican farmers; and
- Develop strategies for current and future collaborative efforts.

METHODS

The workshop was held in two parts. The first component was held at UAS in Culiacan, Mexico on Sept. 22-24, 2008 and included two days of conferences with 19 presentations, and one day of field visit to a pilot site where shellfish polyculture (oyster, pen shell, shrimp) is being demonstrated in conjunction with a Santa Maria Bay community. Thirty-six persons participated in this part of the workshop. This area of the Bay (Altata) is now targeted for shellfish growing water classification by the State of Sinaloa and the Mexican Federal Government. The second component was conducted in Nayarit State in Santiago Ixcuintla. One day of presentations (11) were held the first day. Thirty-nine persons participated in this part of the workshop. Two field visits were made; the first on September 26, 2008 to a major oyster growing area, Pozo Chino. This area is one which is now projected for shellfish growing water classification by the State of Nayarit and the Mexican Federal Government. The second field visit on September 27, 2008 was to another major oyster growing area, Boca de Camichin.

Workshop organizers included: UAS, Sinaloa Institute of Aquaculture, Sinaloa State Aquaculture Sanitation Committee, CIAD, National Polytechnical Institute (CIIDIR-IPN), Autonomous University of Nayarit and University of the Coast. Dr. John Supan from LSU also participated. An industry volunteer from the U.S., Mr. David Nisbet, owner of Goosepoint Oyster Company, also attended and provided technical input. Erick Sandoval, CRSP Collaborator and Microbiologist at UCA-Nicaragua, attended the workshop and made several presentations. He also visited several microbiology and public health laboratories in Culiacan and Nayarit. The US PI, Maria Haws, also participated in organizing the workshop and made several presentations.

RESULTS AND CONCLUSION

Training and exchange of lessons learned included participation for thirty nine HC and four U.S. participants. The workshop was also video-taped and DVD's with the video and powerpoint presentations were delivered to the participants after conclusion of the workshop.

BENEFITS

There was a high level of satisfaction among the trainees and several have since put the acquired knowledge and skills to use. Participants in this workshop were either farmers, extension agents or government officials with direct involvement in aquaculture development or its regulation who have since either become integrated into the CRSP network in Mexico or have attended subsequent trainings (Regional workshop on shellfish culture and sanitation ,07HH104UH and Training in BMPs for the production of mollusks in Nayarit and Sinaloa, 07BMA04UH). One particularly notable partnership that has emerged from the collaborative training efforts is the partnership between CESASIN and UAS. CESASIN (Sinaloa State Aquaculture Sanitation Committee) originally focused on regulating biosecurity for shrimp farmer and providing extension services to them, representing the principal extension corps in the State. Since the CRSP work began, they have expanded to providing coverage and services to shellfish farmers and have been

strong partners in the CRSP efforts. The on-going exchanges between UAS and UCA (Nicaragua) has also been beneficial in establishing ties between scientists from these countries. Additionally, the materials that have been produced during the workshops have been used extensively in three countries for other training and education purposes.

ACKNOWLEDGEMENTS

The LSU, UHH, UAS and UCA teams are grateful to the Aquaculture and Fisheries CRSP which provided funding and support for this effort, as well as to CESASIN for their long-term collaboration with the CRSP efforts.

REGIONAL WORKSHOP ON SHELLFISH CULTURE AND SANITATION

Human Health Impacts of Aquaculture/ Activity/ 07HH104UH

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ABSTRACT

A regional workshop was held in Culiacan, Sinaloa on September 28-29, 2009 for thirty-six participants including small-scale farmers, government officials, NGO's, researchers and extension agents. The purpose of the workshop was to increase technical capacity in the areas of bivalve culture and sanitation, and provide a venue for development of collaborative opportunities. The workshop audience drew upon the principal bivalve aquaculture stakeholders in the Pacific Region of Mexico (North Baja California, South Baja California, Sonora, Sinaloa and Nayarit). This was the second regional event sponsored by CRSP.

INTRODUCTION

Bivalve mollusk culture is a priority for aquaculture development throughout LAC. In the case of Mexico, state governments (e.g. North Baja California, South Baja California, Nayarit, Sinaloa, Sonora) and the federal government have prioritized shellfish culture for development for nearly ten years. The Autonomous University of Sinaloa and its numerous partners in aquaculture development have recently worked together in an integrated effort to accelerate development of the shellfish industry with long-term support from CRSP/USAID. This workshop was the second in a series of regional workshops sponsored by CRSP that have provided a venue for exchanging knowledge and lessons learned related to shellfish culture and sanitation. Additionally, the topic of increasing extension capacity is addressed in these workshops. An important aspect of the regional workshops is to provide an opportunity for an exchange of lessons learned between the various stakeholders groups.

The objectives of this training workshop were to:

- Present the results of research and development efforts by stakeholders from the Northern/Central Pacific Coast of Mexico;
- Increase technical capacity among farmers, researchers, NGO's and government officials both within Mexico and with international colleagues;
- Increase extension capacity and partnerships;
- Provide a net working opportunities for regional stakeholders; and
- Develop strategies for current and future collaborative efforts.

METHODS

The workshop was designed and planned as a collaborative effort between HC institutions (UAS, CESASIN, CIAD) and U.S. Universities (LSU and UHH). The workshop was held on September 28-30, 2009. The first two days were dedicated to presentations and discussion sessions. On the third day, a field tour was held in which the participants visited a shellfish polyculture project in Navolato, Sinaloa. The workshop was opened with remarks from the UAS Rector, two university department heads and five distinguished government officials from the major institutions with an interest in aquaculture and sanitation.

Presentations included:

- Experiences of shellfish producers in Sonora (producers and extension agents)
- Experiences of shellfish producers in Nayarit (producers and extension agents)
- Experiences of shellfish producers in South Baja California (producers and extension agents)
- Experiences of shellfish producers in Baja California (producers and extension agents)
- Experiences of shellfish producers in Sinaloa (producers and extension agents)
- Possible risks to shellfish sanitation by agrochemicals (Guadalupes Llanes Ocana, School of Physical Mathematics, UAS)
- Traceability in bivalve mollusk culture (Dr. Omar Calvario Martinez, CIAD)
- Mollusks as biotoxin vectors in the coastal zone (Rosalba Alonso Rodriguez, UNAM)
- The importance of mollusks as bioindicators of environmental alterations (Celia Vazquez Boucard, CIBNOR)
- Trends in bivalve consumer preferences in Sinaloa and implications for sanitation (Dr. Francisco Javier Martinez, CIAD)
- The importance of bivalve mollusk sanitation from the nutritional perspective (Marcela Vegara Jimenez, Nutrition/UAS)
- Habitat impact on the sanitary quality of oysters: experiences from the central region of Sinaloa (Magdalena de Jesus Uribe Beltran, UAS)
- Presence of coliforms and *E. coli* in water and oyster tissue in Nayarit and Sinaloa (Guillermo Rodriguez, FACIMAR/UAS)
- Culture trials with the Japanese Oyster, *Crassostrea gigas* in Navolato, Sinaloa (Dr. Andres M. Gongora Gomez, CIIDRI-IPN)

RESULTS AND CONCLUSION

Thirty-nine stakeholders participated in the workshop. The workshop was also video-taped and DVD's with the video and powerpoint presentations were delivered to the participants after conclusion of the workshop.

BENEFITS

There was a high level of satisfaction among the trainees and several have since put the acquired knowledge and skills to use.

ACKNOWLEDGEMENTS

The LSU, UHH, UAS and UCA teams are grateful to the Aquaculture and Fisheries CRSP which provided funding and support for this effort, as well as to CESASIN for their long-term collaboration with the CRSP efforts.

MICROBIOLOGICAL QUALITY OF BIVALVE GROWING WATERS AND TISSUES

Human Health Impacts of Aquaculture/ Experiment/ 07HHI05UH

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ABSTRACT

Black cockles, (*Anadara similis* and *A. tuberculosa*) are an important fisheries resources throughout Latin America. Women, children and the poor are particularly dependent upon bivalve collection for food and income. Developing shellfish sanitation plans that including water quality monitoring and relay and depuration strategies are key elements of an on-going integrated bivalve fisheries management effort in Nicaragua. *E. coli*, *Salmonella* sp. and *Vibrio parahaeolyticus* levels were monitored monthly over a one year period in the Aserradores Estuary, Nicaragua. *E. coli* levels were highest during the rainy season (May-October) at three out of six stations for cockle tissues and water samples. *Salmonella* sp. was found sporadically, mostly commonly during the dry season (November-April). This indicates the need for on-going water quality monitoring to assure food safety. Depuration in the laboratory and at an open water site indicated that *E. coli* levels are reduced to legally permissible levels in cockle tissues in 8-12 hours. *Vibrio parahaemolyticus* was always within legally permissible levels both in water and tissue samples. Relay and depuration proved to be technically simple with minimal cost, suggesting that cockle gatherers may use this strategy to improve cockle safety, and potentially, product value.

INTRODUCTION

This work had three objectives:

- Monitor the presence of *Salmonella* sp., *Echerichia coli* and *Vibrio parahaemolyticus* in the waters of the Aserradores Estuary at six stations and in the tissues of *Anadara tuberculosa* (black cockle) taken from the estuary; and
- Establish a depuration site in the estuary and conduct controlled depuration trials in the laboratory for two species of *Anadar*a cockles.

Black cockles are an important fisheries resource throughout Latin America hold aquaculture potential as well. The most common species of black cockle are *Anadara similis*, *A. tuberculosa and A. grandis.* These inhabit mangrove ecosystems and are widely

distributed along the Pacific Coast of Latin America, ranging from Laguna Ballenas in Baja California to Tumbes, Peru (Keen, 1971). Cockles are generally found in muddy, or sandy-muddy substrates (Cruz and Jimenez, 1994). *A. similis* and *A. tuberculosa* now comprise the bulk of the fishery since the large *A. grandis* is only rarely found due to overfishing. Fishing is primarily conducted by women and children, in part because the cockles are a resource they can access without boats or gear. Cockles are an important daily and emergency food source for inhabitants of poor coastal communities, with women and children being particularly reliant upon bivalve collection to supply basic protein and income needs. Between 1600 and 2000 people gather cockles on a regular basis. Approximately 30 million *A. tuberculosa* and *A. similis* are harvested from Aserradores and three nearby areas, Kilaca, Padre Ramos and El Realejo (CIDEA 2005, 2009). Some of this product is exported to El Salvador and Honduras.

Throughout the LAC region, management of the cockle fishery is a chronic issue. Cockle populations have been overfished through most of their range to the extent that some countries have a permanent ban on cockle collection, although this rarely effectively enforced. Nicaragua has a closed season for cockles from April to July, coinciding with the months of highest demand, rather than any biological basis. Given that enforcement is relatively ineffective, a ban on fishing during times of peak demand has not resolved the fisheries issues. Additionally, since cockles are one of the most important resources for coastal communities, particularly since they are a daily food for many of the poor, when authorities do try to enforce the law, it poses hardship for many coastal residents. One result of enforcement is that the poorer collectors can not commercialize their product, although larger vendors manage to more successfully evade the law.

Since 2004, CIDEA and international partners have employing multiple approaches to addressing the fisheries management issues for cockles in Nicaragua as well as in Tanzania under USAID funding under the SUCCESS program (Sustainable Coastal Communities and Ecosystems) and since 2007, CRSP has provided additional support to this effort. To date, establishment of community-based no-take zones has proven effective in increasing cockle abundance and size in the estuary, including areas outside of the no-take zones.

At the same time, efforts were made to find ways to improve both the safety and value of the cockles for the benefit of consumers and fishers. Although highly nutritious, black cockles, like most filter feeding bivalves, can also represent a serious health issue. Filter feeding bivalves are able to pump water over the gills and mantle tissue, and remove food particles from the water column (Cantelmo et al. 1992). Pathogens that cause human disease may be present in the water, primarily due to contamination by sewage, and these may be ingested as the bivalve filter feeds (up to 50 liters of water per day) (Fernandez et al., 1971; Martinez et al., 1991; Wanke and Guerrant, 1987). Shellfish-borne diseases present serious public health issues around the world (Dsenclos, 1991). Among the most serious and potentially fatal illnesses that can result from eating contaminated bivalves are gastrointestinal illness associated with *E. coli*, *Salmonella*, *Hepatitus* and several types of Vibrios, including *Vibrio vulnificus* and *V. parahaemolyticus*.

This work had two objectives: 1) quantify the depuration rate for cockles in the laboratory to have a baseline for comparison with depuration in the field; and 2) test relay and depuration methods in the field. If inexpensive and simple depuration methods are developed that villagers can use, it would have multiple benefits including reducing the risk associated with consuming cockles, increase stakeholder engagement in the broader

management efforts and potentially add value to the product. Additionally, the water quality monitoring not only would allow identification of possible depuration sites and high risk sites, it could inform future management decisions about where to establish no-take zones for the cockle fisheries management efforts. Ideally, the no-take zones could be re-located in the most contaminated areas to both reduce human health risks but also increase the probability of community cooperation with this management strategy.

In cases where shellfish growing grounds are contaminated to the extent that consuming bivalves gathered from these areas is hazardous to human health, three options exist. In some cases, contamination is seasonal and thus shellfish may be safely harvested periodically from the area. In the case of Asserradores, this option was judged not to be viable due to the short period of time in which cockles could be harvested and because the ability to constantly monitor water quality throughout the large estuary was not assured. Depuration is another option; this involves keeping contaminated bivalves in clean water until they can purge themselves of pathogens. Depuration can reliably remove bacteria, but not all viruses, although virus load may be reduced. Two depuration options could have been feasible in Asserradores. One would be to use land-based tanks to hold the cockles during the depuration period. A second option is to conduct the depuration in an area of the estuary which has been shown to be clean and which can easily be monitored on a regular basis to assure that its water quality remains at an acceptable level. In this case, the latter option was chosen as the being the least expensive, most viable for the community to maintain with minimal technical assistance and because larger numbers of cockles could be depurated at any one time. The process of harvesting cockles from a contaminated area and conducting depuration at another site is referred to as, "relay and depuration". This is a common practice in other shellfish fisheries, for example, in the Gulf of Mexico oyster industry.

Generally *E. coli* is used as an indicator organism for shellfish sanitation since its presence is correlated with contamination from human or animal wastes (Fernández and Brunker 1977, McNeely 1979). Additionally, levels of *V. parahaemolyticus* and Salmonella sp. were measured in this study. Although several species of cockles are fished, only *A. tuberculosa* was studied due to its size and importance in the fishery.

MATERIALS AND METHODS

I. Baseline analysis of the presence of pathogens in cockle tissues and growing ground waters

Six sampling stations were established within the Aserradores Estuary for collection of water samples and cockle tissue samples

Figure 1. Location of sampling stations in the Aserradores Estuary

Stations	Coordinates (UTM)	
Station 1	UTM	0462964
		1393996
Station 2	UTM	0465682
		1393984
Station 3	UTM	0464418
		1396710
Station 4	UTM	0463631
		1397458

Station 5	UTM	0466130
		1396366
Station 6	UTM	0465416
		1399132

Cockles (*A. tuberculosa*) and water samples were collected from each station monthly from August 2008 to August 2009. Cockles and water samples were placed in sterilized plastic bags after collection and transported to the laboratory. The water samples were immediately stored on ice and transported at a temperature of <10ºC.

Analysis of *E. coli* levels in water was conducted using the Most Probable Number (MPN) method according to standard protocols (Standard Methods, 2005). The same methods was used for the tissue analysis as specified by the FDA (U.S. Food and Drug Administration) (BAM, 1998). Permissible *E. coli* levels were established by the EPA (1976) and are the same as those established by the Nicaraguan Government (CEPIS, 1986), i.e. < 43 MPN/100 ml water. Permissible levels for *E. coli* in molluscan bivalves have been established as $<1.0x10^2$ MPN/g (NTON, 2008). *Salmonella* spp. analysis was conducted by a isolation method, a modification of the standard method specified by the FDA (BAM, 1998). Permissible levels of *Salmonella* spp. in frozen or fresh seafood are zero (NTON, 2008). Dectection of *V. parahaemolyticus* in water and cockle tissue was conducted using TCBS agar plates with 5% NaCl. The permissible level established by NTON is $<1.0x10^3$ UFC/g. The CIDEA/UCA laboratory is certified by the National Accreditation Office under regulation NTN 04 001 05 which is the equivalent of ISO/IEC 17025-05.

II. Comparison of cockle depuration rates in the field and laboratory

This study was conducted using 504 cockles collected from the Aserradores Estuary. To monitor depuration rates in the laboratory, 6-24 gallon aquaria were used. The water supply was recirculated through a sand filter and was monitored to assure bacteriological quality. Twenty-four cockles were placed in each aquarium and left for periods of 4, 8, 12, 24, 48 and 72 hours respectively before being removed for bacteriological analysis of their tissues. Assays were conducted as noted above for *E. coli*, *V. parahaemolyticus* and *Salmonella* spp.

The field depuration site was selected based on previous water quality monitoring which demonstrated that E. coli levels were well below permissible levels. Cockles were suspended in net bags at this site at 1 meter depth. In order to also evaluate whether the number of cockles in a net bag affected the depuration time, comparisons were made between bags with 12 or 24 cockles. Cockles were kept at the site for 4, 8, 12, 24, 48 and 72 hours before being removed for bacteriological analysis.

RESULTS

I. Baseline analysis of the presence of pathogens in cockle tissues and growing ground waters

All sampling stations except for Station 1 were located within the internal branches of the estuary. Station 1 was located at near the mouth of the estuary. Cockle for tissue analysis were not collected at this point due to the difficulty in obtaining sufficient numbers for the analysis.

Six water and sixty cockle samples (randomly selected from among the cockles collected) were analyzed each month for a total of 72 water samples and 720 cockles over the year long monitoring period.

Figure . *E. coli* levels in water samples (MPN/100 ml).
Highlighted data indicates levels above legally permissible levels.

	Station					
Sampling period	**1** **Estuary mouth**	**2** **Los Tornos**	**3** **Rio Viejo**	**4** **Rio Viejo**	**5** **La Chanchera**	**6** **La Chanchera**
August 2008	<1.8	4.5	79	17	33	11
September 2008	7.8	17	14	7.3	130	<20
October 2008	920	0	540	50.4	130	170
November 2008	2	25	45	80	8	<20
January 2009	4	13	49	49	4	<20
February 2009	<1.8	23	2	17	6.8	<20
March 2009	<1.8	6.8	7.8	4.5	4.5	<20
April 2009	2	2	<1.8	2	2	20
May 2009	7.8	33	4.5	13	13	20
June 2009	46	33	49	1600	49	<20
July 2009	2	6.8	17	23	49	20
August 2009	11	23	2	11	6.8	20

Figure 2. *E. coli* levels in tissue samples (MPN/g)
Highlighted data indicates levels above legally permissible levels.

	Station					
Sampling period	**1** **Estuary mouth**	**2** **Los Tornos**	**3** **Rio Viejo**	**4** **Rio Viejo**	**5** **La Chanchera**	**6** **La Chanchera**
August 2008	NA	330	80	<20	20	50
September 2008	NA	<20	20	80	80	<20
October 2008	NA	<20	20	50	130	170
November 2008	NA	<20	<20	<20	<20	<20
January 2009	NA	<20	<20	<20	<20	<20
February 2009	NA	20	<20	<20	<20	<20
March 2009	NA	<20	<20	<20	<20	<20
April 2009	NA	3500	<20	20	<20	20
May 2009	NA	<20	<20	<20	<20	20
June 2009	NA	<20	20	700	20	<20
July 2009	NA	20	<20	<20	<20	20
August 2009	11	20	<20	40	<20	20

Figure 3. Presence or absence of *Salmonella* spp.
ND= not detected, W= in water samples; T= in tissue samples

	Station					
Sampling	**1**	**2**	**3**	**4**	**5**	**6**

period	Estuary mouth		Los Tornos		Rio Viejo		Rio Viejo		La Chanchera		La Chanchera	
	W	T	W	T	W	T	W	T	W	T	W	T
August 2008	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
September 2008	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
October 2008	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
November 2008	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
January 2009	ND	NA	ND	ND	ND	ND	ND	X	ND	ND	ND	ND
February 2009	ND	NA	ND	ND	ND	ND	X	ND	X	X	X	ND
March 2009	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	X	ND
April 2009	ND	NA	X	ND	ND	ND	X	ND	ND	ND	ND	ND
May 2009	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
June 2009	ND	NA	ND	ND	ND	ND	ND	ND	X	X	ND	ND
July 2009	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	X
August 2009	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

V. parahaemolyticus was either not detected or levels were always within permissible levels for both tissue and water samples from all stations during all sampling periods.

II. Comparison of cockle depuration rates in the field and laboratory

Laboratory depuration

Table 1 summarized the results of cockles held in aquaria with recirculating, filtered seawater for varying periods of time. Initially cockle tissues exhibited high levels of fecal coliform at 330 MPN/g and *E. coli* at 330 MPN/g. Salmonella was not detected and *V. parahaemolyticus* was below permissible levels at < $1.0x10^3$ UFC/g. After 12 hours of depuration, both fecal and total E. coli levels fell to permissible levels.

Table 1; Bacteriological results for cockle tissues held in laboratory depuration system. Highlighted text indicates reduction to legally permissible rates for coliforms.

Time in aquarium	Fecal coliform (MPN/g)	*E. coli* (MPN/g)	*Salmonella* sp. UFC/ 25 g	*Vibrio parahaemolyticus*
0 hrs	330	330	Ausencia	< $1.0x10^3$ UFC/g
4 hrs	78	78	Ausencia	< $1.0x10^3$ UFC/g
8 hrs	78	78	Ausencia	< $1.0x10^3$ UFC/g
12 hrs	20	20	Ausencia	< 1.0x103 UFC/g
24 hrs	< 20	< 20	Ausencia	< $1.0x10^3$ UFC/g
48 hrs	< 20	< 20	Ausencia	< $1.0x10^3$ UFC/g
72 hrs	< 20	< 20	Ausencia	< $1.0x10^3$ UFC/g

Relay and depuration

A depuration site was selected across from the coast of Aserradores Island. Water quality at this site was monitored for three months prior to initiation of the tests. Coliform levels were always lower than the permissible levels. Cockles were put into 2 containers with wood frames and mesh sides with 408 cockles in the first container, and 240 cockles in the second container. Both containers were submerged to a depth of 1 meter. Samples from each container were removed at 4, 8, 12, 24, 48 and 72 hours. At the end of 72 hours, samples were transported back to the CIDEA laboratory for bacteriological analysis.

Table 2. Bacteriological analysis of cockle tissues from relay and depuration trials (408 cockles/container)

Depuration tiem	Fecal coliform (MPN/g)	*E.coli* (MPN/g)	*Salmonella* sp UFC/ 25 g	*Vibrio parahaemolyticus*
0 hrs	170	130	Re-testing	$< 1.0x10^2$ UFC/g
4 hrs	350	130	Re-testing	$< 1.0x10^2$ UFC/g
8 hrs	50	20	Re-testing	$< 1.0x10^2$ UFC/g
12 hrs	20	20	Re-testing	$< 1.0x10^2$ UFC/g
24 hrs	<20	<20	Re-testing	$< 1.0x10^2$ UFC/g
48 hrs	65	50	Re-testing	$< 1.0x10^2$ UFC/g
72 hrs	170	<20	Re-testing	$< 1.0x10^2$ UFC/g

Similar to the depuration rates observed in the laboratory, the field trials showed adequate depuration rates after 8 hours.

DISCUSSION

The results of this study indicate the need for caution in harvesting black cockles because of high levels of pathogens at some stations during certain months, but also indicates that they can be gathered during certain time and from specific locations with minimal risk to the consumer. *E. coli* levels in waters were highest during the rainy season (May-November), most likely due to run-off from neighboring residential and cattle grazing areas. Earlier studies indicated that the most contaminated areas are closest to cattle grazing areas (SUCCESS, 2008). Contamination was also found in some areas during the June-July period, thus suggesting that some risk is present during all times of year. *E. coli* levels in cockle tissues, however, only exceeded permissible levels three times during the year indicating that *E. coli* levels in tissues and water from the same site are not necessarily correlated. Most shellfish sanitation programs however, monitor only *E. coli* in water and prohibit collection of shellfish at particular sites when *E. coli* levels exceed the legal standard as a precautionary measure. Although several more years of data may be necessary to reliably confirm temporal patterns in *E. coli* presence and concentrations, it would be reasonable to exercise more caution in designating areas where shellfish could be collected during the rainy season. At the same time, the monitoring did reveal that several areas appear to be relatively safe for shellfish collection over the entire year, particularly Stations 1 and 6. Shellfish can also be moved to these areas for depuration.

Salmonella was detected in water at Stations 2, 4, 5 and 6 but in tissues only at Stations 4, 5 and 6. Given the zero tolerance in regulations for seafood in the case of *Salmonella* and its potential lethality, caution would be indicated in these areas. *Salmonella* occurrence exhibited a different pattern than *E. coli*, being most commonly found during the dry season. The alternation of high levels of *E. coli* and *Salmonella* during the year suggested that in some sites, it may be unsafe to harvest shellfish at any time of year.

V. parahaemolyticus was either not detected or was within permissible levels at all stations at all sampling periods and may therefore not be a pathogen of concern in this estuary.

CONCLUSION

This work indicates that cockles can be depurated rapidly enough in both the laboratory and field that either tank systems or an open water depuration site could help improve cockle safety. A tank depuration system is being tested in 2010 using solar electric power funded by a grant from the European Union. The field experiment also demonstrated that

as long as technical assistance is provided to routinely water quality, the Aserradores community can use open water depuration sites to improve cockle safety with a minimum of delay and cost. Although a longer period of monitoring is required for validation of the water quality results, this work along with a previous year's data of water quality monitoring in the Aserradores Estuary is assisting researchers to develop a database that will allow prediction of annual patterns of contamination. Aside from the obvious benefits to human health, this will also allow for improved site selection for no-take zones to support cockle fisheries management since ideally the no-take zones will coincide with the most heavily contaminated areas.

ANTICIPATED BENEFITS

Approximately 700 people in the Aserradores Estuary community collect or consume cockles. This work lays the ground work for efforts to improve human health, protect the cockle resource and improve the economic status of community residents.

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