OPTIMIZATION OF STOCKING RATIOS OF THE GIFT STRAIN OF NILE TILAPIA (*Oreochromis niloticus*) AND FRESHWATER PRAWN (*Macrobrachium rosenbergii*) IN PERIPHYTON-BASED SYSTEM

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Abstract

The production performance of GIFT (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) in a periphyton-based system were studied in farmers' ponds at Mymensingh, Bangladesh. Fifteen ponds (area of 200-300 m^2 and maximum depth of 1.5 m) were used to compare five stocking ratios in triplicate: T1 - 100% GIFT, T2 - 75% GIFT plus 25% prawn, T3 - 50% GIFT plus 50% prawn, T4 - 25% GIFT plus 75% prawn and T5 - 100% prawn. Ponds were stocked at a density of 20,000 ha^{-1}. Bamboo poles (mean diameter 6.2 cm and 5.5 m^{-2} in length) were posted in pond bottoms vertically as periphyton substrate. Significant differences were found in some water quality parameters such as secchi depth, DO, pH, and NO_3-N. Periphyton biomass in terms of ash free dry matter and chlorophyll *a* were significantly higher in ponds stocked with prawn alone than in ponds with different combinations of GIFT and prawn. Survival of GIFT was significantly lower in ponds stocked with 100% GIFT (monoculture) whereas, that of prawn was significantly higher in monoculture ponds indicating detrimental effects of GIFT on prawn's survival. However, individual weight gains for both species were significantly higher in polyculture than in monoculture. The highest total fish and prawn yield (1,623 kg GIFT and 30 kg prawn ha^{-1}) over 125 days culture period was recorded in ponds with 75% GIFT and 25% prawn followed by 100% GIFT alone (1,549 kg ha^{-1}), 50% GIFT plus 50% prawn (1,114 kg GIFT and 68 kg prawn ha^{-1}), 25% GIFT plus 75% prawn (574 kg GIFT and 129 kg prawn ha^{-1}) and 100% prawn alone (157 kg ha^{-1}). Therefore, a stocking ratio of 75% GIFT plus 25% prawn appeared to be the best stocking ratio in terms of biomass production for a periphyton-based polyculture system.

Introduction

Periphyton-based aquaculture system is a modern and eco-friendly approach in the global pond aquaculture. Periphyton is very preferable natural food for herbivorous and omnivorous fish species especially for Indian major carp (Azim *et al*., 2002; Keshavanath *et al*., 2002), tilapia (Legendre *et al*., 1989; Keshavanath *et al*., 2004) and freshwater giant prawn (Cohen *et al*., 1983). Periphyton-based aquaculture systems offer the possibility of increasing both primary production and food availability for fish (Legendre *et al*., 1989; Hem and Avit, 1994; Guiral *et al*., 1995; Wahab *et al*., 1999; Huchette *et al*., 2000; Azim *et al*., 2001), which is an important consideration in resource-constrained countries. Many trials have demonstrated that fish production from ponds provided with substrate for periphyton is higher than that of substrate free ponds (Hem and Avit, 1994; Wahab *et al*., 1999; Ramesh *et al*., 1999; Azim *et al*., 2001). However, although tilapia is known to be a periphyton grazer (Dempster *et al*., 1993; Azim *et al*., 2003), its productions in periphyton-based systems were highly variable from no contribution of periphyton (Shrestha and Knud-Hansen, 1994) to several fold increase in production (Hem and Avit, 1994; Keshavanath *et al*., 2004).

In recent years, substrate-based systems were tested in freshwater prawn culture (Tidwell, 1999; 2000) and gave positive results. The purpose of using substrates in prawn culture was mainly to provide shelter rather than growing periphyton for food. It was also uncertain whether freshwater prawn utilized the periphyton as food. However, monoculture ponds with freshwater prawn might have experienced excessive algal blooms leading to water quality deterioration. The omnivorous and periphyton feeding tilapia species might be able to potentially utilize remaining food resources in freshwater prawn culture ponds. Therefore, it might be advantageous to culture tilapia and freshwater prawn together in periphyton-based ponds.

The ultimate objective of this experiment was to test the technical viability of a periphyton based polyculture with GIFT and freshwater prawn. If the technique was successful, it can be used as a viable means of rural fish production for poverty alleviation and nutritional security in resource poor countries like Bangladesh. Farmers can feed their family year round with less expensive tilapia and sell valued prawn in the market as cash crop. The present study was designed to compare yields from different stocking ratios of GIFT and freshwater prawn in polyculture as well as monoculture of either species in substrate-based system.

Materials and methods

Study area and experimental design

The 125 days experiment was carried out in 15 earthen farmer's ponds of Montola-Goneshampur area under Sadar Upazilla in Mymensingh district. The ponds were rectangular in shape with area ranging from 200 to 300m^2 and average maximum water depth of 1.5 m. Ponds were rain fed, well-exposed to sunlight and without inlet and outlet. The experiment consisted of five stocking ratios in triplicate: 100% GIFT alone (T_1), 75% plus 25% prawn (T_2), 50% GIFT plus 50% prawn (T_3), 25% GIFT plus 75% prawn (T_4) and 100% prawn alone (T_5). Ponds were stocked at a fixed stocking density of 20,000 juveniles or postlarvae ha^{-1}. Ponds were randomly assigned to the treatments.

Pond preparation and fish stocking

Prior to the trial, ponds were renovated, aquatic vegetation removed and predators and fishes were eradicated by using rotenone. Bamboo poles (mean length 2.0 m and mean diameter 6.2 cm) were planted vertically into the bottom mud as artificial substrate. Substrates were installed in an area, leaving a 2-m perimeter free of poles in each pond. The bamboo substrate (5.5 poles/m^2) added an effective surface area of about 60% in each pond. Ponds were treated with lime ($CaCO_3$, 250 kg ha^{-1}) (Day 1). On Day 6, cow manure, urea and triple super phosphate (TSP) was applied at 3,000, 100 and 100 kg ha^{-1}, respectively. On Day 12, PL-28 of *Macrobrachium rosenbergii* was released into the ponds in numbers according to the design. After 15 days, juveniles of GIFT (*Oreochromis niloticus*, 1.97 ± 31g) were stocked according to the design. The GIFT juveniles and prawn PL were collected from Bangladesh Fisheries Research Institute, Mymensingh and stocking was done in the morning, care being taken for acclimatization of fish to the pond conditions.

Post stocking management and fish sampling

All ponds were subjected to the same regime of feeding and fertilization. Commercial pelleted feed procured from the market was applied to the ponds at a rate of 5% of total stocked biomass for the first two months and 3% of the total biomass for the rest of the experimental period. GIFT and prawn were sampled at monthly intervals using lift net after removing some bamboo poles. Length and weight of 10% fish and prawn were measured separately to assess their growth and feed requirement. All ponds were fertilized with urea and triple super phosphate (TSP) at a rate 50 kg ha^{-1} of both at fortnightly intervals.

Water quality monitoring

The measurement of water quality parameters was performed on a fortnightly basis between 0900 and 1000 h in each sampling date. Temperature (Celsius thermometer), dissolved oxygen (Lutron, DO-5509), Secchi depth (secchi disc) and pH (CORNING pH meter 445) were determined at sampling site. Water samples were collected and brought to the Water Quality and Pond Dynamics Laboratory to determine chlorophyll *a* and nutrient concentrations. Water samples were filtered prior to nutrient analysis. Nutrients total ammonia nitrogen (TAN), nitrate nitrogen (NO_3-N) and phosphate phosphorous (PO_4-P)) were analyzed using a Hach Kit (model DR 2010) and following standard methods (APHA, 1992). Chlorophyll *a* was determined spectrophotometrically using acetone extraction following Boyd (1979).

Periphyton sampling and analysis

The periphyton biomass growing on bamboo substrate viz. dry mater (DM) and pigment concentration (chlorophyll *a* and pheophytin *a*) were determined fortnightly following standard methods (APHA, 1992), beginning from the 15th day of the substrate installation and continued at monthly intervals. From each pond, three poles were selected by random number tables and two 3x2 cm^2 samples of periphyton were taken at each of four depth (25, 50, 75 and 100 cm below from the water surface) per pole. At the time of periphyton collection, care was taken not to remove any of the substrate itself. After sampling, the poles were replaced in their original positions, marked and excluded from subsequent samplings.

One of the two periphyton samples was used to determine total DM and ash content. The materials from each pole were collected on pre-weighed and labeled pieces of aluminum foil, dried at 105^0C until constant weight (24 h in a Memmert stove, Model UM/BM 100-800), and kept in a dessicator until weighed (BDH 100A; precision 0.0001g). Dry samples from depth, poles and ponds per treatment were pooled, transferred to a muffle furnace and ashed at 450^0C for 6 h and weighed. The DM, ash free mater (AFDM) and ash content were determined by weight differences (APHA, 1992).

The remaining second sample (3x2 cm^2) was used to determine chlorophyll *a* and pheophytin *a* content following standard methods (APHA, 1992). Collected materials were immediately transferred to labeled tubes containing 10 ml 90% acetone, sealed and stored overnight in a refrigerator. The following morning, samples were homogenized for 30 sec with a tissue grinder, after refrigerated for 4 h, the samples were centrifuged for 10 min at 2000-3000 rpm. The supernatant was carefully transferred to 1 cm glass cuvettes and absorption measured at 750 and 664 nm using a spectrophotometer (Milton Roy Spectronic, model 1001 plus). Samples were then acidified by addition of three drops of 0.1 *N* HCl and absorbance measured again at 750 and 665 nm after 90-sec acidification. Chlorophyll *a* and pheophytin *a* concentrations were calculated using the equation given in APHA (1992).

Harvesting

At the end of the experiment, bamboo poles were removed, water was pumped out of the ponds, all fish were collected, weighed (Denver-xp-3000; precision=0.1 gm) and measured (measuring board; precision=1 mm). Weight gain per fish was calculated by deducting the average initial weight from the average final weight. Specific growth rate (SGR) was estimated as:

SGR =[Ln(final weight) - Ln(initial weight) x 100]/cultured period (days).

Statistical analysis

For statistical analysis of data, a one-way ANOVA was carried out using statistical package, SPSS version 10.0. Significance was assigned at the 0.05% level.

Results

Fish yield parameters

Yield parameters of GIFT and prawn under five different stocking ratios are shown in Tables 1 and 2, respectively, and in Figure 4. Survival of GIFT was significantly lower in monoculture ponds (T_1) than in polyculture with prawn (T_2-T_4). Individual weight gains increased with lowering stocking density.

Table 1. Comparisons of means (and ± S.D., N=3) of yield parameters of GIFT in different stocking ratios by ANOVA.

Yield parameters		Treatments				ANOVA Result
		T1	T2	T3	T4	
Survival (%)	Mean	56.99[b]	65.62[a]	64.64[a]	63.88[a]	*
	±S.D.	0.64	3.22	4.20	1.20	
Stocking Length (cm)	Mean	4.76	4.54	4.70	4.51	NS
	±S.D.	0.63	0.52	0.57	0.42	
Harvesting length (cm)	Mean	19.01[c]	19.94[b]	20.20[b]	20.85[a]	*
	±S.D.	0.82	0.61	1.02	0.84	
Stocking Weight (g)	Mean	2.16	1.81	2.05	1.76	NS
	±S.D.	0.86	0.69	0.78	0.53	
Harvesting weight (g)	Mean	135.95[d]	164.92[c]	172.66[b]	179.67[a]	*
	±S.D.	19.09	13.71	16.85	22.90	
Individual weight gain (g)	Mean	133.79[d]	163.1[c]	170.6[b]	177.9[a]	*
	±S.D.	2.7	3.5	3.56	1.81	
SGR (% bw d^{-1})	Mean	3.20[b]	3.47[a]	3.42[a]	3.56[a]	*
	±S.D.	0.17	0.07	0.15	0.19	
Yield (kg ha^{-1} 125 d^{-1})	Mean	1549.44[d]	1622.53[c]	1114.35[b]	574.07[a]	*
	±S.D.	31.18	50.70	57.62	12.56	

* P < 0.05; N.S, not significant

Table 2. Comparisons of means (and ± S.D., N = 3) of yield parameters of prawn in different stocking ratios by ANOVA.

Yield parameters		Treatments				ANOVA Result
		T2	T3	T4	T5	
Survival (%)	Mean	28.38[c]	30.48[c]	39.83[b]	48.24[a]	*
	±S.D.	2.99	3.60	1.23	5.98	
Stocking length (cm)	Mean	1.09	1.08	1.08	1.07	NS
	±S.D.	0.13	0.15	0.15	0.15	
Harvesting length (cm)	Mean	11.59	12.01	11.93	10.87	NS
	±S.D.	2.49	3.34	2.97	1.95	
Stocking weight (g)	Mean	0.01	0.01	0.01	0.01	NS
	±S.D	0.005	0.005	0.005	0.005	
Harvesting weight (g)	Mean	19.36[a]	22.16[a]	21.59[a]	16.42[b]	*
	±S.D.	3.02	3.51	0.79	2.04	
Individual weight gain (g)	Mean	21.14[a]	22.14[a]	21.58[a]	16.41[b]	*
	±S.D.	3.02	3.52	0.79	2.04	
SGR (% bw d^{-1})	Mean	5.88[a]	5.92[a]	5.91[a]	5.69[b]	*
	±S.D.	0.11	0.13	0.03	0.1	
Yield (kg ha^{-1} 125 d^{-1})	Mean	29.67[d]	68.19[c]	129.12[b]	156.87[a]	*
	±S.D.	1.40	17.10	8.64	10.55	

* P < 0.05; N.S, not significant

However, specific growth rates (SGR) were significantly higher in polyculture treatments than in monoculture one. Net yields of GIFT were also significantly different among different stocking ratios, with the highest mean value in T_2 followed by T_1, T_3 and T_4.

As opposed to GIFT, survival of prawn was significantly higher in monoculture ponds (T_5) than in polyculture with GIFT. However, individual weight gains and SGR were significantly lower in monoculture ponds than in polyculture ponds. Net prawn yield varied significantly among treatments with higher mean value in T_5 followed by T_4, T_3 and T_2. The combined net yields of the two species were 1549, 1653, 1183, 703 and 157 kg ha^{-1} in treatments T_1, T_2, T_3, T_4 and T_5, respectively for a culture period of 125 days.

Water quality parameters

Mean values (and range) of fortnightly water quality parameters are shown in Table 3. There were no significant differences in some water quality parameters such as temperature, ammonia nitrogen, phosphate phosphorus and chlorophyll *a* but there were significant difference in secchi depth, DO, pH and nitrate nitrogen. Dissolved oxygen concentration was higher in T_5 and never fell below 1.9 mg l^{-1} in any pond, on the other hand pH was higher in T_3 and never fell below 5.8 in any pond.

Table 3. Fortnightly water quality parameters recorded from different stocking ratio treatment. Values are means of 10 sampling dates and three ponds (N=30).

Parameters		Treatments					ANOVA Result
		T1	T2	T3	T4	T5	
Temperature	Mean	28.40	28.17	28.05	27.90	28.40	NS
(^{0}C)	Range	27-30	27-30	27-30	26-30	25-32	
Secchi depth	Mean	42.60^a	38.43^{ab}	42.57^a	35.80^b	38.10^{ab}	*
(cm)	Range	27-67	23-59	25-68	22-50	26-57	
DO	Mean	4.89^c	5.07^{bc}	4.65^c	5.60^{ab}	6.06^a	*
(mg l^{-1})	Range	1.9-9.8	2.0-7.1	3.7-6.2	2.9-6.9	2.8-9.2	
pH	Mean	7.10^{ab}	6.81^b	9.78^b	7.27^a	6.94^{ab}	*
	Range	6.1-8.5	6.1-9.3	5.8-7.7	6.1-9.8	5.8-9.4	
Nitrate nitrogen	Mean	0.095	0.053	0.057	0.057	0.065	*
(mg l^{-1})	Range	.02-.36	.01-.23	.01-.21	.01-.17	.01-.18	
Ammonia nitrogen	Mean	0.415	0.243	0.282	0.314	0.346	NS
(mg l^{-1})	Range	.03-.81	.03-.83	.04-.74	.01-.78	.01-.69	
Phosphate	Mean	0.268	0.235	0.229	0.258	0.272	NS
phosphorus (mg l^{-1})	Range	.1-.63	.12-.38	.12-.45	.09-.63	.13-.43	
Chlorophyll *a*	Mean	165.69	172.29	161.94	173.71	152.96	NS
(μg l^{-1})	Range	70-258	8-320	69-304	54-339	54-297	

* $P < 0.05$; NS, not significant

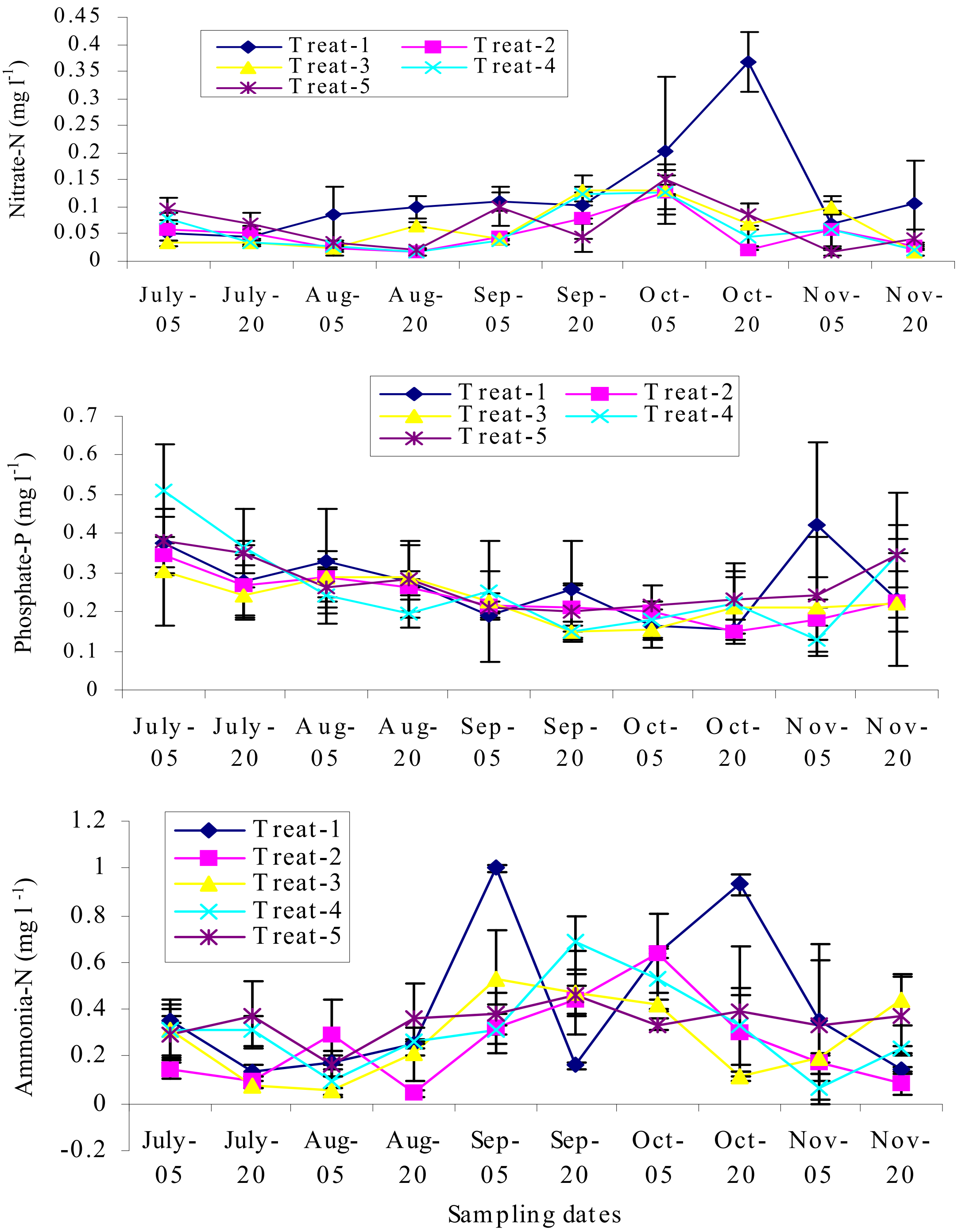

Figure 1. Fortnightly monitored water quality in different treatments through the experimental period. Values are means (± S.D.) of the three ponds (N=3) per sampling date in each treatment.

Periphyton biomass

Mean values of periphyton dry matter (DM) and ash free dry matter (AFDM) were higher in ponds stocked with prawn alone as compared to ponds with GIFT alone as well as polyculture with both species (Table 4).

Table 4. Means (and ± S.D.) of periphyton biomass and pigment parameters scraped from bamboo substrates in different treatments. Values are means of three sampling dates, four depths, three poles and three ponds (N= 108).

Parameters		Treatments					ANOVA Result
		T1	T2	T3	T4	T5	
Dry matter	Mean	2060 [b]	2191 [b]	2650 [b]	2480 [b]	3550 [a]	*
(µg cm^{-2})	±S.D.	779	910	1029	687	1283	
AFDM	Mean	1430 [b]	1530 [b]	1680 [b]	1560 [b]	2140 [a]	*
(µg cm^{-2})	±S.D.	539	501	580	437	656	
Ash(%)	Mean	29.94 [b]	29.62 [b]	34.52 [ab]	35.94 [ab]	37.17 [a]	*
	±S.D.	5.93	7.55	9.28	9.52	10.79	
Chlorophyll *a*	Mean	8.898 [b]	9.434 [b]	10.506 [b]	11.471 [ab]	15.371 [a]	*
(µg cm^{-2})	±S.D.	1.79	3.589	2.714	2.421	4.301	
Pheophytin *a*	Mean	3.08 [b]	3.07 [b]	3.20 [b]	3.18 [b]	4.03 [a]	*
(µg cm^{-2})	±S.D.	0.69	0.55	0.73	0.77	1.48	

* $P < 0.05$

Ash contents of periphyton dry matter ranged from 29 to 37% among the different stocking ratio treatments and increased as the stocking ratio of prawn was increased. The highest chlorophyll *a* and pheophytin *a* concentrations were recorded in ponds stocked with prawn alone than in ponds under other treatments.

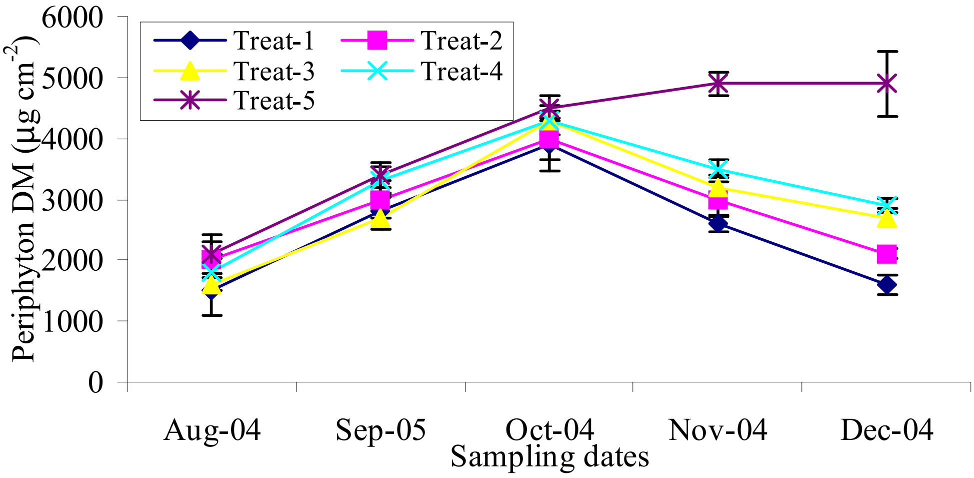

Figure 2. Quantity of periphyton dry matter per unit surface area of substrate over the experimental period. Values are means (± S.D., N=3) per sampling date in each treatment.

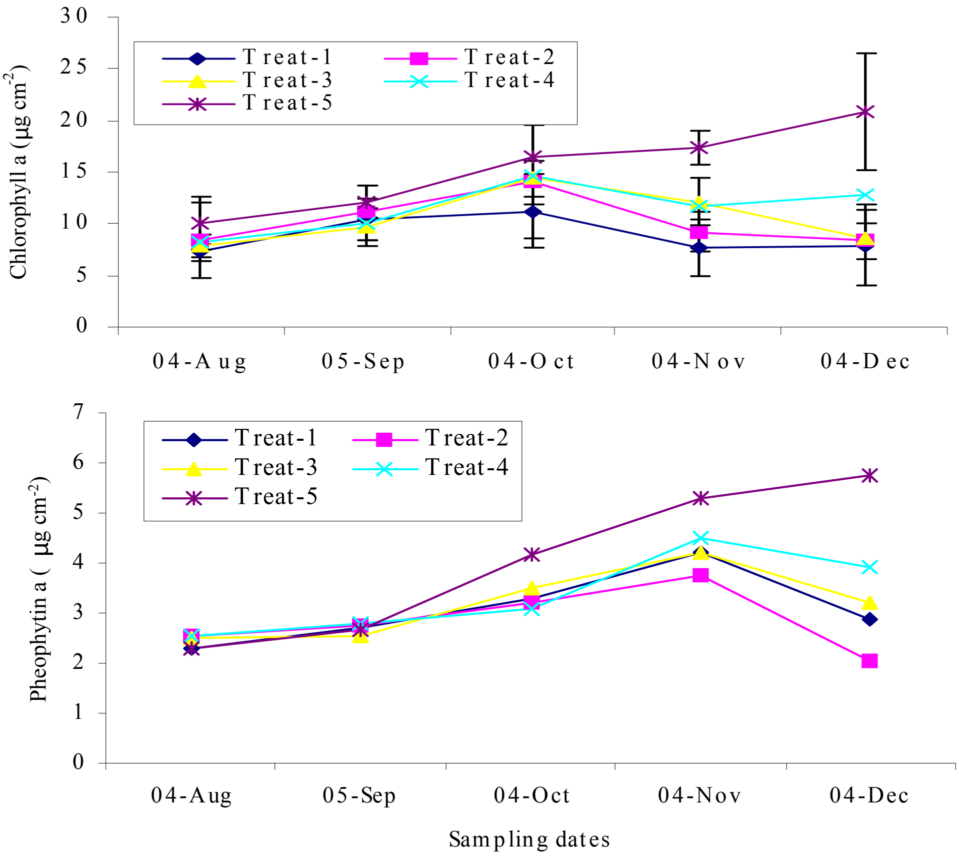

Figure 3. Monthly monitored periphyton Chlorophyll *a* and pheophytin *a* concentration in different treatment ponds throughout the experimental period. Values are mean (± S.D., (N=3) per sampling date in each treatment.

Discussion

Water temperature was within the limits and was suitable for fish production. DO concentrations were generally suitable for fish culture throughout the experimental period and never crossed the lethal lower limit. All water quality nutrients were within the limit and were suitable for fish culture.

Periphyton biomass in terms of DM and chlorophyll *a* increased with decreasing the GIFT: prawn ratio indicating avoidance of periphyton by prawn and preference of periphyton by GIFT as food. The visual observation confirmed this result. The higher ash contents of periphyton were collected from ponds stocked with prawn alone. This might be due to trapping of more sediment particles within the periphytic mats because of less grazing pressure.

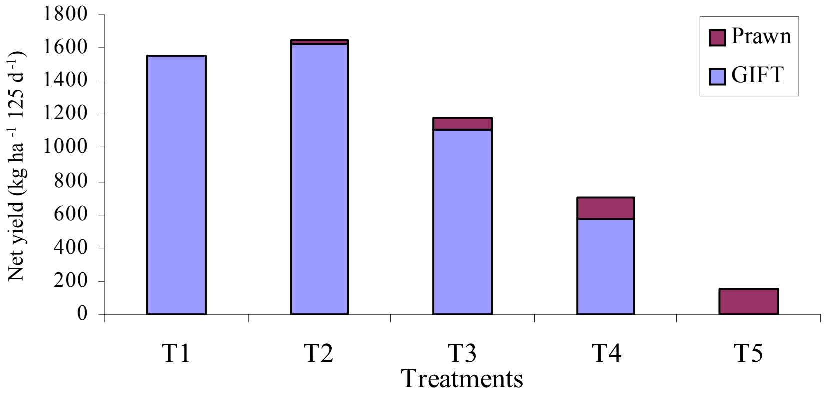

Figure 4. The relative contribution of GIFT and freshwater giant prawn to total net yield in different stocking ratio treatments.

Survival of GIFT was significantly higher in polyculture but that of prawn was significantly higher in monoculture. This might be because of intra- and inter-specific antagonistic behaviours of the former for food and space. Especially, GIFT might have affected prawn survival during molting. Although survival of prawn was not affected by their own stocking density, individual weight gain was significantly lower in ponds stocked with the highest number of prawns. However, individual weight gain of GIFT was significantly increased by decreasing their stocking density, due to the less competition for food.

The total harvesting yield was found highest in T_2 (1,623 kg GIFT and 30 kg ha^{-1} prawn) over 125-days experimental period. Other reported yields include:1,427 kg ha^{-1} of fish in 155 days with bamboo substrate (Azim *et al.*, 2004); a maximum tilapia biomass equivalent to 8 metric tones ha^{-1} y^{-1} in accadja-enclosed in the Ebrie Lagoon, Ivory Coast, with natural recruitment of fingerlings (Hem and Avit, 1994); and 713 kg ha^{-1} of calbaush

(*Labeo calbasu*) in 120 days in ponds with kanchi (scrap bamboo) as substrate (Wahab *et al*., 1999).

The total yields of GIFT were higher in the stocking ratio 75% GIFT plus 25% prawn than those of monoculture of GIFT or polyculture of GIFT and prawn with other combinations. This experiment indicated that where a combination of 75% GIFT and 25% prawn is used, synergistic benefits compensate for any inter-specific or intra-specific dietary competition. Nevertheless, alternative approach should be developed to minimize the antagonistic behaviour of GIFT on its co-inhabiting species. Caged GIFT in freshwater prawn culture or caged prawn in GIFT culture might be an option. In the latter case, artificial feed can only be provided to prawn whereas, GIFT can depend on natural food. Performance based on biomass production has been assessed, however, economic scenario might be more helpful in farmers decision making process.

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