Partial Replacement of Fish Meal with Mangrove Based Plant Ingredients and its Effect on Water Quality, Growth Performance and Length-Weight Relationship of *Macrobrachium rosenbergii*

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Abstract: Partial replacement of 30% fish meal was done with 10% plant ingredients comprised of macroalgae (*Enteromorpha intestinalis*) and saltmarsh grass (*Porteresia coarctata*) to prepare freshwater prawn (*Macrobrachium rosenbergii*) feed. The floral based feed was applied to culture *M. rosenbergii* in Indian Sundarbans region. The water quality parameters (temperature, salinity, pH, DO, nutrient concentrations, chlorophyll a, transparency, soil organic carbon) and growth performance (average initial weight, average final weight, DGR, SGR, survival rate, FCR, total yield) were monitored throughout the culture period. The pond health was best where diet 3 was applied compared to the other ponds. The condition state was also monitored based on Length-Weight Relationship (LWR). The growth was uniform and maximum in cultured species fed with diet 3 compared to that of other formulated diets (diet C, 1 and 2). LWR ($W = 0.008 \times TL^{2.9486}$) indicated isometric growth of *M. rosenbergii* fed with diet 3. The trial experiment thus demonstrates the efficacy of diet 3 (*P. coarctata* based feed) in maintaining the overall growth performance and condition state of the cultured species, as well as the environmental health of the culture pond.

Key words: Fish meal, *E. intestinalis*, *P. coarctata*, water quality, growth performance, culture pond, growth, length-weight relationship, *M. rosenbergii*

INTRODUCTION

Freshwater prawn (*M. rosenbergii*) is one of the most commercially important species in the fisheries sector of India as a source of animal protein and foreign exchange earner. The species is widely distributed in freshwater, as well as brackishwater bodies like ponds, rivers, canals and estuaries (Ahmed, 1957). The species is the largest of the freshwater prawns and has been classified as a benthophagic omnivore based on analysis of foregut contents (Weidenbach, 1982; Cohen and Raanan, 1983). It has recently been identified as an important shellfish species for culture in South Asian countries after significant losses of penaeid shrimp culture in mid-1990s due to viral diseases (Paez-Osuna, 2001; Moss *et al.*, 2001). In India, 36,640 ha are under scampi culture with an annual production of about 39,000 ton from monoculture and as one of the components in polyculture. The scampi

are fed with farm-made or commercial feed in order to boost up the production volume. While prawns can receive substantial nutritional benefit from natural food at relatively low biomass densities of <1000 kg ha^{-1} (Tidwell *et al.*, 1997), at higher stocking densities individuals may be more dependent on prepared diets (Tidwell *et al.*, 1999). For achieving successful production in prawn culture, a great deal of consideration is generally given to feeding and management. Feed costs constitute 40-60% of operational costs in production of the freshwater prawn (D'Abramo and Sheen, 1994). Fish meal makes up a major part of the formulated feed for any carnivorous fish species as a protein source. However, the recent scarcity, rising cost and uncertain consistency in supply of fish meal have increased the efforts to search for the alternative protein sources to fish meal in aquaculture by the fish nutritionists and feed manufacturers. For the purpose of producing low cost

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effective diets, high protein feedstuffs for fish meal substitution have been investigated throughout the world (Hasanuzzaman *et al.*, 2009).

Indian Sundarbans (world's largest mangrove delta, a world heritage site and a biosphere reserve) located in the lower Gangetic region of the Indian sub-continent at the apex of Bay of Bengal (within latitude 21°13'-22°40'N and longitude 88°03'-89°07'E) offers a congenial environment in terms of salinity and other hydrological parameters for the growth and culture of scampi (Mitra and Banerjee, 2006). The deltaic complex sustains some 34 true mangrove species and 65 mangrove associate floral species (Mitra, 2000). A considerable percentage (73%) of people living in the deltaic region have opted pisciculture as one of the important livelihoods. However, there is scarce concept of scientific feed preparation and water management in the region due to which the aquaculture sector often gets devastated with disease problems. Deterioration of pond water quality due to inclusion of trash fish in the diet of cultured species is also an associated problem with the prawn culture system in this region (Mitra, 2000). Finding alternative, traceable, high quality protein to fishmeal is a key area of organic aqua feed research. Research into organic formulations has been steadily increasing over the last several years.

On this background, the present study aims to evaluate the effects of partially replacing the ingredients of animal origin of conventional prawn feed with macroalgae (*E. intestinalis)* and saltmarsh grass (*P. coarctata*) powder. The protein levels of *Enteromorpha* spp. generally range between 5-20% of dry weight and is abundantly available in saline water bodies of Sundarbans (Mitra and Banerjee, 2006). Because of high protein content, Protein Concentrates (PC's) of seaweeds have become more important for the food industry, especially in developed countries (Wong and Cheung, 2001). The recent utilization of macroalgae as shrimp feed is also gaining momentum in deltaic ecosystem of Indian Sundarbans (Banerjee *et al.*, 2010). The salt marsh grass, *P. coarctata* is another cheapest source of protein (18%), widely available in the intertidal mudflats of Sundarbans (Mitra and Banerjee, 2006).

MATERIALS AND METHODS

Collection and processing of macroalgae and saltmarsh grass: Live and healthy *E. intestinalis* and *P. coarctata* were collected from the intertidal mudflat in the mangrove belt of Kakdwip region (21°52'35.7"N latitude and 88°11'55.0"E longitude) during low tide condition and washed thoroughly with double distilled water to remove epiphytes and other extraneous matter. Collected materials were sun dried and ground to make a fine powder. The 3 categories of powder were prepared:

- Category 1: *E. intestinalis* powder
- Category 2: *P. coarctata* powder
- Category 3: Equal mixture (1:1) of *E. intestinalis* and *P. coarctata* powder

Diet preparation: The 4 different types of diets were formulated viz. control diet (diet C) and three experimental diets (diet 1-3). The major source of protein in diet C was from the fish meal. The fish meal protein was partially replaced in the experimental diets at a rate of 10% of feed with the powder of *E. intestinalis*, *P. coarctata* and mixture (1:1) of both powder. The diet C was provided to pond 1 (treated as control) and subsequently diets 1-3 were provided to ponds 2-4, respectively (treated as experimental). All the ponds had three replicates each. Formulation of control and experimental diets are presented in Table 1. The proximate chemical composition of the diets after preparation was done according to AOAC (1980) as provided in Table 2.

Experimental prawn farming: The 8 months culture experiment of *M. rosenbergii* was conducted during February to September, 2011 in farm condition at Kakdwip region of Indian Sundarbans (Fig. 1). Total 12 ponds of different size were selected for trial of experimental and control diets in triplicate. Ponds were prepared following standard procedure. Seeds were procured from local hatchery and stocked at a rate of 2 individual m^{-2} with average initial length 1.0 cm and 0.02 g initial average body weight in each pond. The mean stocking weight was determined from a sample of 100 prawn seeds that were

Table 1: Ingredients (%) of control diet (C) and experimental diets (1-3)

Ingredients	Diet C	Diet 1	Diet 2	Diet 3
Soybean meal	10	10	10	10
Mustard oil cake	34	34	34	34
Rice bran	17	17	17	17
Wheat bran	7	7	7	7
Vitamin+mineral mixture	2	2	2	2
Fish meal	30	20	20	20
Powder of *Enteromorpha intestinalis*	-	10	5	-
Powder of *Porteresia coarctata*	-	-	5	10

Table 2: Proximate composition of control and experimental diets

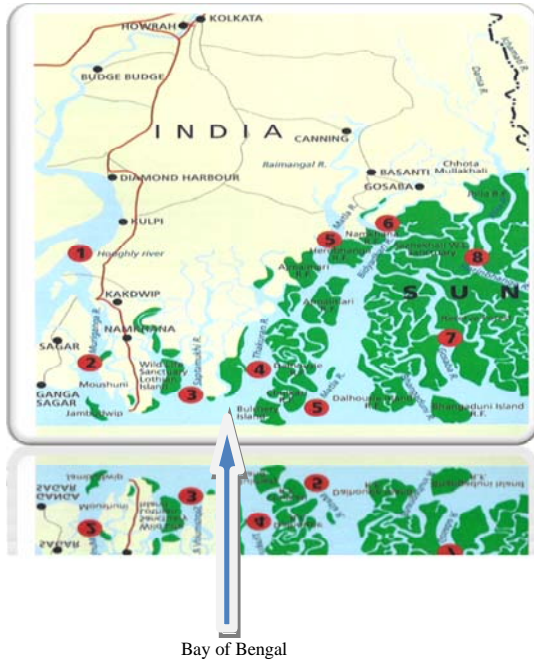| Proximate composition (%)	Diet C	Diet 1	Diet 2	Diet 3
Crude protein	33.47±1.2	34.94±1.2	34.93±1.8	36.49±1.3
Soluble carbohydrate	34.12±1.6	33.89±1.9	35.01±2.5	36.18±0.9
Crude fat	6.1±0.52	6.1±0.99	5.8±0.46	6.2±0.23
Ash	11.4±0.21	10.2±0.12	11.9±0.31	12.1±0.23
Moisture	10.4±0.22	9.5±0.11	10.1±0.34	10.2±0.99

Fig. 1: Map showing the location of culture site at Kakdwip Region of Indian Sundarbans

blotted to free from water. Before stocking all the prawn seeds are well acclimatized to avoid temperature and pH shocks (Sarver *et al.*, 1982). Prawns were fed to satiation twice a day. The feeding levels were adjusted by checking the feeding trays 2 h after serving the diets. Prawns were harvested at the end of 240 days. Total body length, weight and number of prawns from each pond were recorded.

Growth performance: The following growth parameters were measured for the cultured species from the control and experimental ponds:

- Average initial weight and final weight were measured using pan balance
- Daily Growth Rate (DGR) was calculated using the expression:

$$DGR\left(g\,day^{-1}\right) = \frac{W_f\left(Final\ weight\right) - W_i\left(Initial\ weight\right)}{t\left(days\right)}$$

- Specific Growth Rate (SGR) was calculated after the harvesting of prawns as per the expression (De Silva and Anderson, 1995):

$$SGR\left(\%\right) = \frac{\ln\left(Final\ weight\right) - \ln\left(Initial\ weight\right)}{Days\ experiment} \times 100$$

- Feed Conversion Ratio (FCR) was calculated after the harvesting of prawns as per the expression (De Silva and Anderson, 1995):

$$FCR\left(\%\right) = \frac{Feed\ consumed\left(Dry\ weight\right)}{Live\ weight\ gain\left(Wet\ weight\right)}$$

- Survival rate was calculated as per the formula (De Silva and Anderson, 1995):

$$Survival\ rate = \frac{Final\ No.\ of\ prawns}{Initial\ No.\ of\ prawns} \times 100$$

Length-Weight Relationship (LWR): The biometric measurements of the cultured species were made with graduated scale.

The measurements taken were Total Length (TL) from the tip of the rostrum up to the tip of the telson. The measurements were made to centimeter (cm) as described by FAO species identification sheets for fishery purposes (Fischer *et al.*, 1981). The specimens were also weighed using a pan balance for taking the total Weight (W). The mathematical relationship between length and weight was calculated using the conventional formula (Ricker, 1973):

$$W = a \times TL^b$$

Where:
W = Total weight
TL = Total Length
a = Proportionality constant
b = Isometric exponent

Water quality parameters: Water quality parameters such as temperature, pH, salinity, Dissolved Oxygen (DO), transparency and nutrient concentrations (nitrate, phosphate and silicate) for all the ponds were measured on monthly basis following standard procedures as stated by Strickland and Parsons (1972). Chlorophyll a, concentration was measured spectrophotometrically after acetone extraction as per APHA (1995) and soil organic carbon was analyzed by Walkley and Black (1934) method.

Statistical analysis: The data generated for all biotic and abiotic parameters were expressed as mean±SD. Significance of variations in the water quality, growth performance and length-weight relationship of the cultured species was tested by using one way Analysis of Variance (ANOVA) which was followed by Duncan's multiple range test for significant values using SPSS software. Values were considered significant at 5% level of significance (Gomez and Gomez, 1984).

Table 3: Effect of different diets on hydrological characteristics and soil organic carbon of culture ponds

Parameters	Pond 1 (with diet C)	Pond 2 (with diet 1)	Pond 3 (with diet 2)	Pond 4 (with diet 3)
Temperature (°C)	32.7±0.29[a]	32.76±0.15[a]	32.7±0.13[a]	32.7±0.12[a]
Salinity (psu)	0.56±0.38[a]	0.59±0.42[a]	0.41±0.44[a]	0.26±0.43[a]
pH	7.19±0.31[b]	7.62±0.04[a]	7.84±0.03[a]	7.81±0.01[a]
DO (mg L^{-1})	4.32±0.48[c]	5.06±0.11[b]	5.25±0.15[ab]	5.35±0.18[a]
Nitrate (µ gat L^{-1})	33.5±3.45[a]	27.0±3.07[b]	25.2±3.54[bc]	23.3±4.34[c]
Phosphate (µ gat L^{-1})	4.06±1.22[a]	3.43±0.49[bc]	3.35±0.52[c]	2.76±0.79[c]
Silicate (µ gat L^{-1})	59.2±6.20[a]	48.1±3.40[b]	43.1±4.16[c]	45.2±5.47[bc]
Chlorophyll a (mg m^{-3})	3.10±0.43[b]	2.89±0.31[a]	2.92±0.41[a]	3.09±0.43[b]
Transparency (cm)	18.3±2.52[b]	19.8±2.83[b]	25.7±1.37[a]	23.3±2.45[a]
Soil organic carbon (%)	4.73±1.15[a]	3.50±1.36[b]	3.19±0.47[b]	3.06±0.78[b]

The results are means±SD (Standard Deviation) and the figures with same superscripts in the same row are not significantly different (p<0.05)

RESULTS AND DISCUSSION

Water quality: The water quality of the culture ponds are given in Table 3. During the experimental period, temperature varied in the range 32.70-32.76°C, pH varied in the range of 7.19-7.84 and Dissolved Oxygen (DO) in the range 4.32-5.35 mg L^{-1}. Salinity varied in the range between 0.26-0.59 psu. All the water parameters were found to be in the range of tolerance limit of *M. rosenbergii* (New and Singholka, 1985). In general, nutrient concentrations (nitrate, phosphate and silicate) increased as culture proceeded. Nitrate concentration was found to be highest in pond 1 (33.5 µg L^{-1}) and lowest in pond 4 (23.3 µg L^{-1}). Similar trends were observed for phosphate and silicate concentrations. The indicator of primary productivity, i.e., chlorophyll a concentration also increased with time in all the culture ponds. Highest chlorophyll a level was observed in pond 1 (3.1 mg m^{-3}) and lowest in pond 2 (2.89 mg m^{-3}). Organic carbon content was highest in pond 1 showing a value of 4.73% where fish meal was used as feed ingredient. A low organic carbon value (3.06%), an indication of better pond environment is observed in pond 4 applied with diet 3. Higher water transparency was recorded from pond 3 (25.7 cm) and pond 4 (23.3 cm), respectively.

Growth performance of freshwater prawn: Growth data of the experiment are given in Table 4. The lowest survival rate of prawn was observed in pond 1 fed with diet C (63.9%) and the highest was observed in pond 4 fed with diet 3 (79.0%). The average final weight of the prawn varied from 80.83-92.87 g. The prawns fed with diet 3 showed the highest average weight throughout the 240 days of feeding trial (Fig. 2). The prawns that fed on diet 3 and 2 gained significantly higher average weight compared to the prawns fed on diet C and 1 (p<0.05). SGR for the cultured species varied between 3.45-3.69% day^{-1}. It was evident that SGR was lowest in case of diet 1 and the highest in prawns fed with diet 3. The lowest production of prawn (1071 kg ha^{-1}) in the pond condition was obtained where diet 1 was applied

Table 4: Growth performance of *M. rosenbergii* fed with mangrove based diets

Parameters	Diet C	Diet 1	Diet 2	Diet 3
Initial average weight (g)	0.02±0.01[a]	0.02±0.01[a]	0.02±0.01[a]	0.02±0.01[a]
Final average weight (g)	86.26±2.68[c]	80.83±1.31[d]	90.53±3.25[b]	92.87±2.1[a]
Daily growth rate (g day^{-1})	0.37±0.01[b]	0.33±0.01[d]	0.37±0.01[c]	0.38±0.20[a]
Specific growth rate (g day^{-1})	3.57±0.01[c]	3.45±0.05[d]	3.66±0.02[b]	3.69±0.01[a]
Survival rate (%)	63.9±2.36[d]	66.3±2.53[c]	73.7±3.53[b]	79.0±3.00[a]
Feed conversion ratio	2.02±0.02[a]	1.89±0.01[b]	1.76±0.01[c]	1.68±0.02[d]
Total yield (kg ha^{-1})	1102[c]	1071[d]	1334[b]	1467[a]

The results are means±SD (Standard Deviation) and the figures with same superscripts in the same row are not significantly different (p<0.05)
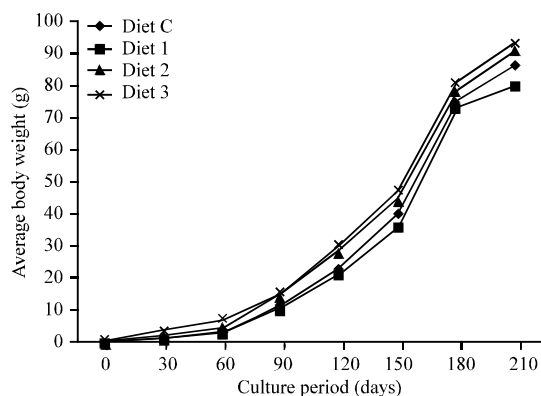

Fig. 2: Growth pattern of *M. rosenbergii* fed on different mangrove based diets

and the highest was obtained in case of prawns treated with diet 3 (1467 kg ha^{-1}). The FCR values of the different types of experimental diets varied between 1.68-2.02. The highest FCR was observed in pond 1 (provided with diet C) and the lowest in pond 4 (provided with diet 3). DGR for the cultured species varied between 0.33-0.38 g day^{-1} with highest value in pond 4 (0.38 g day^{-1}) and lowest in pond 2 (0.33 g day^{-1}).

Length-weight relationship: The mathematical relationship between length and weight of *M. rosenbergii* fed with diet C and diets 1-3 are shown in Fig. 3-6.
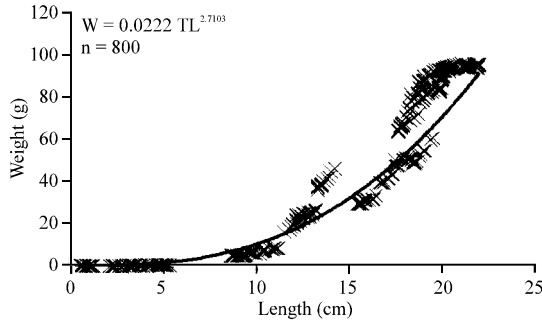
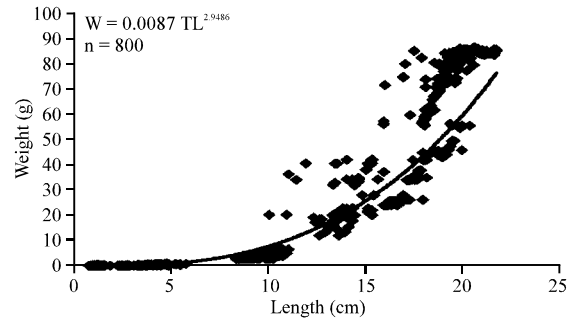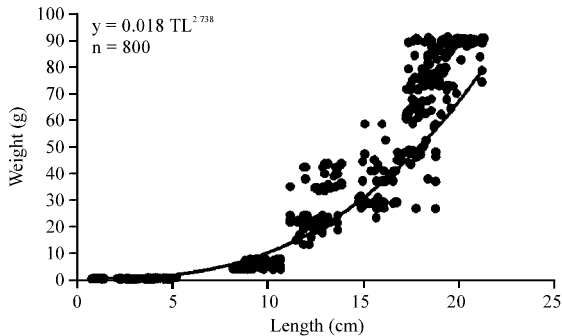Fig. 3: Length-weight relationship of *M. rosenbergii* fed with control diet (C)

Fig. 4: Length-weight relationship of *M. rosenbergii* fed with diet 1 (prepared with *E. intestinalis*)
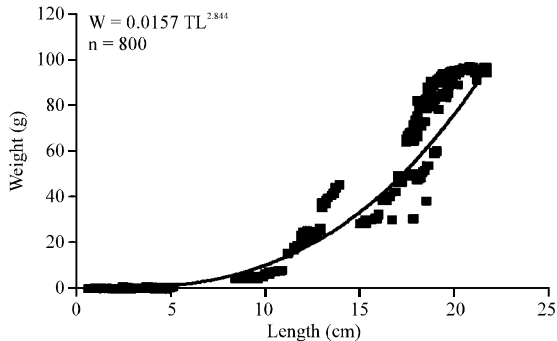

Fig. 5: Length-weight relationship of *M. rosenbergii* fed with diet 2 (prepared with equal mixture of *E. intestinalis* and *P. coarctata*)

Isometric exponent (b) values ranged between 2.71-2.95. Lowest and highest values were observed in diet 1 and 3 treated ponds, respectively. In the present study, the isometric exponent value (b = 2.95) observed in *M. rosenbergii* fed with diet 3 is an indication of isometric growth.

Water quality: Prawn growth and health are influenced by the pond bottom soil (sediment) and water quality

Fig. 6: Length-weight relationship of *M. rosenbergii* fed with diet 3 (prepared with *P. coarctata*)

parameters. Therefore, management of pond sediments and water quality is the key for successful aquaculture practice. Therefore, properties of the pond sediment and processes occuring at the bottom soil and in the soil-water interface determine the water quality. During the study period, all the hydrological parameters were found to be within the acceptable limits for culturing *M. rosenbergii* as reported in various literatures (Daniels, 1981; Sandifer and Smith, 1985; Boyd, 1990; New, 1995; D'Abramo and Brunson, 1996; Hall and Van Hamm, 1998). The experimental treatments showed better aquatic environment compared to the control treatment where conventional diet (included with fish meal) was provided. Surface water temperature in all the culture ponds had more or less parallel trend of variation throughout the study period which is reflected in the ANOVA results. This uniformity in temperature profile is due to the location of culture ponds in the same site that experience similar weather and climate. Similar observations were recorded for salinity profile which may be due to the same water source of Kalnagini River of Hugli-Matla estuarine complex. No significant variation was observed for pH due to the traditional practice of liming in lower gangetic region at regular interval of time. DO is an important component regulating the aquatic life. Its level varied significantly between ponds (p<0.05) as per the order pond 4>3>2>1. This clearly shows the role of mangrove based feed in upgrading the water quality of the culture ponds which may be attributed to the minimum deposition of organic matter at the bottom of the pond. The better aquatic environment in the experimental treatments were reflected through lowering of nutrient levels and subsequent increment of phytopigment (chlorophyll a) level. ANOVA results confirmed significant variation (p<0.05) in nutrient load between the treatments which may be attributed to leaching of feed ingredients (particularly animal component of the feed) and also the faecal matter that generates ammonia (Mitra and Choudhury, 1995). The increased water transparency in case of ponds 2-4 may be due to the unique binding

properties of the selected mangrove based plant ingredients used to prepare the feed thereby reducing the suspended particulate matter. Similar observations were recorded during culture of *Penaeus monodon* in Sundarbans through application of formulated feed prepared from the red algae *Catenella repens* (Banerjee *et al.*, 2010). The organic carbon content at the pond bottom soil exhibited a decreasing trend with time in case of experimental treatments compared to the control, thus reflecting the efficient utilization of mangrove based feed by the cultured species.

Growth performance of freshwater prawn *M. rosenbergii*: The present study confirms significant variation in survival rate, DGR and SGR of the cultured species between the control and experimental treatments ($p < 0.05$) which may be attributed to differences in the component and proportions of feed ingredients. The growth pattern of *M. rosenbergii* shows uniformity up to 75 days in all the culture ponds. From 75th day onwards, there is an exponential growth in the species fed with mangrove based diet. Prawns fed with diet 3 (where 30% fish meal is partially replaced by 10% *P. coarctata* powder) showed maximum average weight followed by diet 2 (where 30% fish meal is partially replaced by equal proportion of *P. coarctata* and *E. intestinalis* powder), diet C (comprised of 30% fish meal) and diet 1 (where 30% fish meal is partially replaced by 10% *E. intestinalis* powder). The FCR and SGR followed the order diet C>1>2>3 and diet 3>2>C>1, respectively indicating the efficacy of diet 3 in maintaining the pond environment and its utilization for growth of the cultured species. Significant differences in FCR were observed among the experimental treatments ($p < 0.05$). The FCR values observed for diet 1-3 during the study period are lower than the values (2.18-2.43) reported by Daniels *et al.* (1995) and Hossain *et al.* (2000) for *M. rosenbergii*. Even, higher FCR values were observed (3.7-5.6) for *M. rosenbergii* cultured in concrete tanks fed with formulated feed containing 34% protein (Siddiqui *et al.*, 1997). The highest survival rate was recorded from the prawns fed with diet 3 due to the suitable environment of the particular culture system. The unique growth performance in the present study with mangrove based feed is in accordance with the findings by Weidenbach (1980) who reported that prawns are able to adjust with the absence of feed pellets by increasing consumption of available vegetation. According to Tidwell *et al.* (1995), prawns are able to adjust to reductions in the nutritional value of prepared diets (i.e., protein source and vitamin and mineral content) by increasing predation on natural fauna (i.e., macro invertebrates) in the culture system. The highest production (1467 kg ha^{-1}) of prawns in 8 months culture period was obtained in diet 3. This is because of higher survival and weight gain observed in this treatment (Hossain and Paul, 2007). Digestibility studies in *M. rosenbergii* have indicated that the species can efficiently digest both plant and animal protein sources (Ashmore *et al.*, 1985). The overall growth performance of *M. rosenbergii* fed with diet 3 might be attributed to better nutritive balance of the ingredients especially essential amino acids (Hossain and Paul, 2007). May be the utilization of mangrove based plant ingredients in different percentage in feed have been found to be more effective than a single floral source as ingredients of fish feed (Hossain and Jauncey, 1990).

Length-weight relationship: Length-weight relationship study is an important index to measure the variation in the growth of individual prawn or group of prawns (Jayachandran and Joseph, 1988), as well as for stock assessment (Abohweyere and Williams, 2008). It also plays a significant role in studying the growth, rate of feeding, metamorphosis, fatness, onset of maturity, gonadal development and general well-being of the fish population (Le Cren, 1951; Pauly, 1993). Variation in the length-weight relationship obtained between the experimental and control treatments for *M. rosenbergii* often arises due to the variation in food composition and environmental conditions as revealed in earlier studies (Abrahamsson, 1966; Kuris *et al.*, 1987). Earlier fish biologists critically analyzed the significance of LWR and stated that when b = 3, then growth is isometric (Enin, 1994) and when it is <or >3 it is allometric (Wootton, 1992). In the present study, cultured species fed with diet 3 exibited highest b-value of 2.95 which is proximal to the standard isometric value b = 3. Based on t-test (t = 0.8097, $p < 0.05$), no significant variation is observed between the standard isometric value and the b-value obtained in the present study (Biswas *et al.*, 2011), thus indicating a proportionate increase of length and weight. The isometric exponent (b) values for diet C, diet 1 and 2 differed significantly from diet 3 ($p < 0.05$) which indicates a disproportionate length-weight relationship in cultured species (allometric growth) fed with diet C (where b = 2.71), diet 1 (where b = 2.73) and diet 2 (where b = 2.84), respectively.

CONCLUSION

The deltaic complex of Sundarbans in the lower Gangetic region offers a unique opportunity for the

culture of *M. rosenbergii*. However, the endeavor mostly becomes unsuccessful due to the use of traditional fish feed in the region. The traditional feed contains fish meal as the major ingredient that causes the deterioration of pond water quality leading to poor and non-uniform growth, low condition index values, low survival rate and high FCR. The present study reveals the upgradation of pond health and related success indicators of *M. rosenbergii* culture due to the use of mangrove based plant ingredients in preparing the feed. Among the 2 selected floral species, *P. coarctata* generated best results. Researchers conclude from this study that partial replacement of fish meal by mangrove associate floral species as feed ingredients can give better results in terms of water quality, survival, growth and condition state of the cultured species and it can be recommended to the local farmers for pond culture of *M. rosenbergii*.

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