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Effect of paddy straw and sugarcane bagasse on growth and survival of giant freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879)

MRIDULA RAJESH, J. K. MANISSERY AND K. M. RAJESH*
College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore - 575 002 Karnataka, India
**Mangalore Research Centre of ICAR-Central Marine Fisheries Research Institute, Hoige Bazar Mangalore - 575 001, Karnataka, India*
e-mail: mridularajesh789@yahoo.co.in

ABSTRACT

Effect of artificial substrates on growth and survival of giant freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879) in the nursery and grow-out rearing was evaluated. Nursery rearing was conducted for a period of 35 days in nine 1 m^3 cement tubs with 15 cm soil base. Three tubs without substrates served as control (C) while three tubs each with 200 g of sugarcane bagasse (SB) or 200 g of paddy straw (PS) formed the treatments. Each tub was stocked with 15 post-larvae (mean weight, 0.01 g). Grow-out rearing was conducted for 90 days in six 25 m^2 cement cisterns, of which three cisterns without substrate served as control (C) and three with 5 kg sugarcane bagasse (SB) served as treatment. Each cistern was stocked with 25 juveniles (mean weight, 0.55 g). The average phytoplankton and zooplankton abundance in water as well as algae and food organisms attached on the substrate in the nursery phase was greater in bagasse than in paddy straw treatments. Addition of substrate resulted in higher growth and survival both in nursery and grow-out phase. Prawns grown in cisterns provided with substrates were of more uniform size than those in control cisterns. The study recommends the use of substrate based aquaculture for nursery and grow-out rearing of *M. rosenbergii*.

Keywords: Biofilm, Freshwater prawn, Growth, *Macrobrachium rosenbergii,* Organic substrates, Survival

Introduction

Microbial biofilm on substrates in aquatic system plays an important role in enhancing fish production (Ramesh *et al*., 1999; Umesh *et al*., 1999; Mridula *et al*., 2005; Rajesh *et al*., 2008; Gowda *et al*., 2012; Milstein *et al*., 2013). Importance of substrates in the culture of shrimp and freshwater prawn is very well recognised (Tidwell *et al*., 1999; 2000; Uddin *et al*., 2007). Periphyton based fish culture practice is developing as an alternative to feed based culture system due to high cost incurred for the artificial diets (Keshavanath *et al*., 2017). The formation of periphyton biofilm on substrates could be used for improving water quality in enriched brackishwater shrimp ponds (Khatoon *et al*., 2007). Addition of substrate "Aqua-mats™" allowed a substantial increase in density for juvenile prawn production and the increased density produced little or no decline in growth or survival (Peterson and Griffith, 1999). The addition of solid substrate produced a direct linear increase in total production (Tidwell *et al*., 2000), survival (Asaduzzaman *et al*., 2008) and net yield (Uddin *et al*., 2007) of *Macrobrachium rosenbergii* (de Man, 1879).

Even though, heterotrophic food can be produced from a variety of carbon sources, the carbohydrate rich raw materials of agricultural origin are more prominent. Use of agricultural wastes for heterotrophic food production serves two important purposes *viz*., it can be converted to protein rich microbial biomass and at the same time reduce the problem of waste disposal (Mridula, 2003).

Considering the above rationale, the present experiment was designed to evaluate the effects of two locally available organic substrates, sugarcane bagasse and paddy straw on growth and survival of giant freshwater prawn, *M. rosenbergii* in the nursery and grow-out phases.

Materials and methods

Nursery rearing

The study was carried out for a period of 35 days in 9 cement tubs of 1x1x1 m size having 15 cm of soil base filled with 80 ± 2 cm water. Three tubs without substrates served as control (C). Sugarcane bagasse (SB) and paddy straw (PS) tied in the form of bundles of 0.60-0.75 m length were suspended at the rate of 200 g tub^{-1} (2,000 kg ha^{-1}) in the water column in triplicate tubs from horizontally placed bamboo poles. All the tubs were initially manured

with 200 g (2,000 kg ha^{-1}) of poultry droppings. Each tub was stocked with 15 post-larvae (150,000 ha^{-1}) of *M. rosenbergii* (mean weight, 0.01 g) seven days after the addition of manure and substrates. Fish meal-based feed (30% protein) prepared as per Jayaram and Shetty (1981) was fed daily at 10% of the body weight in two equal meals to the prawns in all nine tubs.

Water samples from all the tanks were analysed once in every seven days between 08 00 and 09 00 hrs for different parameters. Temperature and pH were recorded using a Horiba (Japan) water quality analyser (Model U-10). Dissolved oxygen, free carbon dioxide, total alkalinity, total ammonia (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4-P) were analysed following standard procedures (APHA, 1995). Plankton (collected by filtering 20 l of water using a 60 mm net) and periphyton (scraped from 1 cm^2 area) were sampled once a week and counted using a Sedgewick Rafter Cell (Krishna and Co., Kochi, India) (Jhingran *et al*., 1969; Umesh *et al*., 1999). For the estimation of wet and dry weight of plankton, 10 l of water sample was filtered through plankton net (60 mm mesh) and the residue transferred to aluminum foils, weighed (Omori and Ikeda, 1937), dried in a hot air oven at 60°C, cooled in a desiccator and again weighed. Total plate count (TPC) of bacteria in the water and on the substrate was estimated at weekly intervals on nutrient agar plates by the spread plate method (Anwar *et al*., 1992). TPC was estimated as colony forming unit (CFU) per gram of substrate.

Finally, all the surviving prawns were harvested and their lengths and weights were recorded. Further, survival and specific growth rates (SGR in % body weight day^{-1}) were calculated. Proximate composition of biofilm was analysed following AOAC (2000) procedures.

Grow-out rearing

The experiment was carried for a period of 90 days in six cement cisterns (5x5x1 m) provided with 15 cm of sandy-loam soil base and filled with 80 ± 2 cm of water. Three cisterns without substrate served as control (C). Sugarcane bagasse (SB) tied as bundles of 0.5-0.6 m length were suspended in the water column from horizontally placed bamboo poles in three cisterns @ 5 kg per cistern (2,000 kg ha^{-1}). All the cisterns were initially manured with dry poultry manure at 5 kg per cistern (2,000 kg ha^{-1}), followed by re-fertilisation at 0.5 kg per cistern (200 kg ha^{-1}) every month. All the cisterns were stocked with 25 juveniles (10,000 ha^{-1}) of *M. rosenbergii* (mean weight, 0.55 g) each, seven days after the addition of substrate and manure. Fishmeal-based feed (Jayaram and Shetty, 1981) with 30% protein was fed daily at a rate of 3% of the body weight in two equal meals to prawns in all the cisterns.

The procedure followed for the analysis of water quality parameters, TPC of bacteria and plankton were the same as described above. For every sampling, about 50% of the prawns were collected from each cistern and their individual length and total weight recorded at an interval of 15 days. On termination, all the surviving prawns were harvested and their length and weight were recorded. Further, survival and SGR were estimated. RNA:DNA ratio of muscle and digestive enzyme activity in the gut were also estimated. The extraction and estimation of muscle nucleic acid and the enzyme activity were carried out as described by Gangadhara *et al*. (1997). Proximate composition of biofilm and prawn muscle sample was analysed following AOAC (2000) procedures.

Statistical analyses

Data obtained in the nursery and grow-out experiments were subjected to statistical analyses employing ANOVA and Duncan's multiple range test at $p<0.05$ (Duncan, 1955; Snedecor and Cochran, 1968).

Results

Nursery phase

All the water quality parameters recorded were within tolerable limits for aquaculture (Table 1). Mean pH values were slightly alkaline, ranging from 7.54 (PS) to 7.77 (SB) indicating favourable biological conditions. Mean dissolved oxygen ranged from 5.06 mg l^{-1} (C) to 5.15 mg l^{-1} (PS). Substrate-based treatments recorded lower ammonia and nitrite concentrations than control. However, nitrate concentration was higher in sugarcane bagasse and paddy straw added cisterns as compared to control cisterns. There was no significant difference ($p<0.05$) in the measured water-quality parameters between the treatments except for ammonia, nitrite, nitrate and phosphate.

The mean value of TPC of bacteria in water did not differ significantly among the treatments (Table 2). Sugarcane bagasse recorded significantly higher mean bacterial density (2.06 x 10^6 g^{-1}) than paddy straw (1.54 x 10^6 g^{-1}).

The average phytoplankton density in water was highest in SB (141 ml^{-1}) followed by PS (117 ml^{-1}) and C (87 ml^{-1}). Mean number of attached algal cells on SB was higher (419 cm^{-2}) than those associated with PS (342 cm^{-2}) (Table 2). The major groups of phytoplankton in water and attached algae on the substrate in order of abundance were: green algae> blue-green algae>diatoms.

The mean density of zooplankton (Table 2) was higher in SB (134 l^{-1}) compared to C (126 l^{-1}) and

Table 1. Water quality parameters (mean±SD) during nursery and grow-out phase experiments

Parameter	Nursery phase			Grow-out phase	
	C	SB	PS	C	SB
Water temperature (°C)	26.94 ± 1.05[a]	26.76 ± 1.15 [a]	26.85 ± 1.07[a]	27.41 ± 1.73[a]	27.35 ± 1.66[a]
pH	7.64 ± 0.21[a]	7.77 ± 0.21[a]	7.54 ± 0.17[a]	7.77 ± 0.38[a]	7.72 ± 0.46[a]
Dissolved oxygen (mg l^{-1})	5.06 ± 0.82[a]	5.13 ± 0.96[a]	5.15 ± 0.94[a]	8.22 ± 0.85[a]	7.84 ± 0.92[a]
Carbon dioxide (mg l^{-1})	3.27 ± 3.69[a]	3.60 ± 4.26[a]	3.80 ±3.62[a]	2.55 ± 2.88[a]	2.28 ± 3.08[a]
Total alkalinity (mg l^{-1})	59.67 ± 8.79[a]	61.05 ± 9.69[a]	58.06 ± 9.70[a]	68.24 ± 9.54[a]	67.52 ± 8.96[a]
Total ammonia (mg at l^{-1})	7.16 ± 1.73[a]	5.19 ± 0.89[b]	5.32 ± 0.94[b]	4.82 ± 0.96[b]	3.34 ± 1.08[a]
Nitrite-nitrogen (mg at l^{-1})	1.79 ±0.33[a]	1.54 ±0.28[b]	1.52 ±0.30[b]	1.71 ± 0.28[a]	1.28 ± 0.32[a]
Nitrate-nitrogen (mg at l^{-1})	5.15 ± 0.74[a]	6.08 ± 0.46[b]	6.21 ± 0.49[b]	1.68 ± 0.52[a]	2.00 ± 0.44[a]
Phosphate-phosphorus (mg at l^{-1})	1.38 ± 0.84[a]	1.33 ± 0.68[a]	1.29 ± 0.59[b]	1.29 ± 0.64[a]	1.44 ± 0.52[a]

*Means bearing different superscripts in the same row differ significantly ($p<0.05$) for a given experiment

PS (125 l^{-1}). The mean value recorded for attached food organisms on the substrate was higher in SB (252 cm^{-2}) than PS (229 cm^{-2}). The major groups of zooplankton in water and attached food organisms on the substrate were protozoans, rotifers, ostracods and cladocerans.

The mean final weight of prawns (Table 3) was significantly higher ($p<0.05$) in SB (1.28 g) than PS (0.99 g) and C (0.83 g). The survival and specific growth rate (SGR) were significantly higher in SB than PS and C.

Grow-out phase

The mean values of dissolved oxygen were higher in C (8.22 mg l^{-1}) than SB (7.84 mg l^{-1}). Mean pH values observed were slightly alkaline; 7.72 (SB) and 7.77 (C). Total ammonia and nitrite were higher in C than SB while

Table 2. Average values and range of TPC and plankton during the nursery and grow-out phase experiments

Parameters	Nursery phase			Grow-out phase	
	Treatment				
	C	SB	PS	C	SB
TPC of bacteria in water (CFU x 10^4 ml^{-1})	6.07[a] (0.78-13.6)	6.80[a] (0.64-15.1)	6.46[a] (0.64-13.92)	9.73[a] (0.67-49.33)	11.24[b] (0.71-53.33)
TPC of bacteria on substrate (CFU´ 10^6 g^{-1})	-	2.06[b] (0.86-4.90)	1.54[a] (0.44-3.9)	-	16.18 (5.87-49)
Phytoplankton in water (no. ml^{-1})	87[a] (10-195)	141[c] (25-220)	117[b] (15-225)	111[a] (33-222)	120[a] (25 - 242)
Phytoplankton on substrate (no. cm^{-2})	-	419[b] (100-600)	342[a] (75-490)	-	267 (135 -362)
Zooplankton in water (no. l^{-1})	125[a] (15-205)	134[b] (10-210)	126[a] (10-240)	122[a] (28-198)	142[b] (13-235)
Zooplankton on substrate (no. cm^{-2})	-	252[a] (50-410)	229[a] (25-390)	-	187 (88-350)

*Means bearing different superscript letters in the same row differ significantly ($p<0.05$) for a given experiment

Table 3. Length-weight, survival and specific growth rate (mean ± SD) of *M. rosenbergii* in the nursery and grow-out phase experiments

Treatment*	Average length (cm)	Average weight (g)	Survival (%)	SGR (%/day)
Nursery phase				
C	4.79 ± 0.14[a]	0.83 ± 0.06[a]	62.22 ± 7.7[a]	6.85 ± 0.10[a]
SB	5.59 ± 0.30[b]	1.28 ± 0.11[c]	86.67 ± 13.3[b]	7.52 ± 0.12[c]
PS	5.08 ± 0.10[c]	0.99 ± 0.07[b]	80.00 ± 11.5[b]	7.12 ± 0.11[b]
Grow-out phase				
C	10.10 ± 0.75[a]	10.43 ± 0.91[a]	65.33 ± 1.53[a]	1.42 ± 0.84[a]
SB	11.40 ± 0.27[b]	12.60 ± 0.44[b]	85.33 ± 0.58[b]	1.51 ± 0.36[a]

*Means bearing different superscripts in the same column differ significantly ($p<0.05$)

nitrate and phosphate were higher in SB than C. However, no significant differences were ($p>0.05$) observed for all the water quality parameters, except for total ammonia (Table 1).

The introduction of manure and substrate resulted in a rapid increase in bacterial biomass (CFUs ml^{-1}) both in water and on the substrate. The overall average value of TPC of bacteria in water was higher in SB (11.24 x 10^4 ml^{-1}) than C (9.73 x 10^4 ml^{-1}) and TPC on the substrate (SB) was very high (16.18 x10^6 g^{-1}) (Table 2). There was a rapid increase in the density of phytoplankton in water and attached algae on substrate after initial fertilisation and addition of substrates. The average phytoplankton density in water was higher in SB (120 ml^{-1}) than C (111 ml^{-1}) (Table 2). The phytoplankton recorded were mainly blue-green algae, green algae and diatoms. The average number of attached algae on substrate was 267 cm^{-2} (Table 2). The major groups of attached algae encountered on the substrate were green algae, blue-green algae and diatoms.

The average density of zooplankton in water recorded was 122 l^{-1} in C and 142 l^{-1} in SB (Table 2). The average density of attached zooplankton on the substrate was 187 cm^{-2}. The major groups of zooplankton observed were protozoans, rotifers, ostracods and cladocerans.

The final mean length and weight of prawn recorded was higher (11.4 cm and 12.6 g) in SB than (10.1 cm and 10.43 g) C (Table 3). The survival rate of prawn was significantly higher in SB (85.33%) than C (65.3%). The computed prawn yields were 257.06 g 25 m^{-2} 90 $days^{-1}$ for SB and 161.36 g 25 m^{-2} 90 $days^{-1}$ for C.

The percentage of protein recorded in the prawn muscle was higher in SB (17.36) than C (14.18) (Table 4). Fat and nitrogen free extract (NFE) levels were almost similar in both C and SB. Significantly higher RNA, DNA, and RNA:DNA ratio (Table 5) were recorded in SB (14.92, 4.03 and 3.71 mg g^{-1} respectively) compared to C (8.75, 3.33 and 2.64 mg g^{-1} respectively).

The activity of protease was higher ($p<0.05$) in prawn intestine and hepatopancreas from SB than in those from C (Table 5). However, there was no significant difference in lipase and amylase activities in the intestine and hepatopancreas of prawns harvested from the two treatments.

Table 4. Proximate composition of muscle of *M. rosenbergii* (expressed on wet weight basis) and biofilm grown on sugarcane bagasse (expressed on dry weight basis) in grow-out phase experiment

Treatments	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Crude fibre (%)	*NFE. (%)
Prawn from C	72.40	14.18	7.84	2.96	-	2.62
Prawn from SB	74.85	17.36	7.55	2.42	-	2.18
Biofilm on bagasse	-	16.36	1.78	19.28	25.13	37.45

*N FE - Nitrogen free extract

Table 5. Mean RNA, DNA, RNA/DNA ratio in the muscle and specific activities of gut digestive enzymes in *M. rosenbergii* from grow-out phase experiment

Parameter	Treatments**	
	C	SB
RNA (mg g^{-1})	8.75^a	14.92^b
DNA (mg g^{-1})	3.33^a	4.03^b
RNA:DNA ratio	2.64^a	3.71^b
*Protease		
Intestinal	27.75^a	49.05^b
Hepatopancreatic	16.95^a	25.98^b
*Amylase		
Intestinal	82.87^a	83.99^a
Hepatopancreatic	40.88^a	40.62^a
*Lipase		
Intestinal	0.55^a	0.57^a
Hepatopancreatic	0.83^a	0.88^a

* µ moles of product liberated h^{-1} mg tissue $protein^{-1}$

**Means bearing different superscript in the same row differ significantly ($p<0.05$)

Discussion

The lowest values of dissolved oxygen recorded in both nursery and grow-out rearing during the first week of the experiment in all the treatments are characteristic of water with predominant heterotrophic food production (Moriarty, 1997). Ammonia, nitrite, nitrate, phosphate and alkalinity values recorded in the present study were generally within the suitable limits for fish culture (Azim, 2001; Mridula *et al*., 2005). Substrate-based treatments recorded lower ammonia and nitrite and higher nitrate concentrations than the control. Development of biofilm on substrates in aquaculture systems can act as *in situ* biofilter for reduction of harmful ammonia load (Joice *et al*., 2002; Rajesh *et al*., 2008; Bharti *et al*., 2016). Langis *et al*. (1988) recorded lower ammonia in aquaria harbouring bacterial biofilm on glass panels.

Addition of substrate had a substantial effect on prawn growth. Increasing availability of surface area through the

addition of artificial substrate, produced a direct linear increase in total production of *M. rosenbergii* (Tidwell *et al*., 2000). The higher growth recorded in substrate based treatments in both nursery and grow-out experiment could be due to the higher production of phytoplankton, zooplankton and microbial biofilm. Further, prawns grown in tubs and cement cisterns provided with substrates were significantly larger and had a more uniform size than those from cisterns without substrate.

The increased surface area due to substrate provides increased benthic habitat for the growing algal and bacterial colonies upon which prawns could graze and find protection during molting. Thompson *et al*. (2000) confirmed that the shrimp directly consume biofilm after the examination of its gut contents. Several reports are already there on significant increase in total production of marine and freshwater prawns in substrate-based systems (Ra Anon *et al*., 1984; Daryl, 2001; Uddin *et al*., 2007; Khatoon *et al*., 2007; Asaduzzaman *et al.,* 2008). The addition of substrates to ponds allowed for an increase in prawn production by 14% and mean size by 13%. Tidwell *et al.* (1998) evaluated the effect of added substrates on prawns stocked at relatively lower density (59,280 ha^{-1}), and found that the production and mean size increased by 20 and 23% respectively. Increase in survival from 62.8 to 72% and increase in the net yield by 23% was recorded in ponds added with substrates (Asaduzzaman *et al*., 2008). The higher percentage survival recorded in the substrate based treatment could be attributed to the provision of additional shelter by substrates and the positive effect of biofilm on water quality in terms of ammonia.

The nucleic acid content was higher in prawns harvested from substrate-based cisterns compared to those from control cisterns. This could be due to the provision of extra protein from biofilm which reflected on the muscle protein of prawn harvested from the substrate added treatments. High protein content in the gut of the shrimps fed on biofilm was recorded by Tidwell *et al*. (2000). It has been reported that RNA : DNA ratios in body tissues can be used to monitor protein synthesis and growth in fish (Bulow, 1970; Wright and Martin, 1985; Khan and Zafri, 1991). In the present study, higher RNA was recorded in prawns harvested from the substrate based treatments indicating higher protein synthesis and also higher growth. Protease activity was the highest in prawn from substrate-based treatment than control. In general, protease activity in the intestinal segments is higher than that in hepatopancreas. Bazaz and Keshavanath (1993), Manjappa (1999) and Mridula *et al.* (2003, 2005) reported similar observations in *Tor khudree*, *Catla catla*, *Labeo fimbriatus* and *Labeo rohita* respectively. Dietary protein content is known to effect protease activity (Mukhopadhyay *et al.,* 1978; Phadate, 1987; Gangadhar *et al*., 1997; Mridula *et al.,* 2003, 2005).

Results of the present study clearly demonstrated that the addition of paddy straw and sugarcane bagasse resulted in improvement of water quality by lowering ammonia and led to good and uniform growth, survival and higher muscle protein content in *M. rosenbergii.* Hence substrate-based aquaculture can be effectively adopted for the nursery and grow-out rearing of *M. rosenbergii*. Use of easily available and biodegradable agricultural plant residues as substrate helps in proper bio-conversion.

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