

Water Quality, Survival and Growth Performance of *Cirrhinus mrigala* (Hamilton 1822) in Substrate Based Tanks

VIVEKANAND BHARTI^{1,2*}, PRAMOD KUMAR PANDEY³, ALAGARSAMY VENNILA⁴,
MUTHUSAMY RAJKUMAR⁵ and MALACHY NWIGWE OKECHUKWU AJIMA^{1,6}

¹Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Versova, 400 061, Andheri (W), Mumbai, India

²Fishery Resources Assessment Division, Central Marine Fisheries Research Institute, Kochi, 682 018, Kerala, India

³Central Agricultural University, Lembucherra, 799 210, Agartala, Tripura, India

⁴Division of Crop Production, Sugarcane Breeding Institute, Coimbatore, 641 007, Tamil Nadu, India

⁵Crustacean Fisheries Division, Central Marine Fisheries Research Institute, Mandapam, 623 520, Tamil Nadu, India

⁶Department of Fisheries and Aquaculture Technology, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria

Abstract

Four substrates namely, paddy straw (E1), sugarcane bagasse (E2), plastic sheet (E3) and tile (E4) were submerged in separate fibre reinforced plastic (FRP) tanks of 500 L capacity in triplicate and allowed for biofilm development for 1 month. Three tanks without substrate served as control (E0). *Cirrhinus mrigala* (Hamilton 1822) (mean weight 1.42 ± 0.05 g; length 4.12 ± 0.08 cm) were introduced 30 days after introducing the substrates in the tanks. Water quality, survival and the growth performance of *C. mrigala* were examined in all the treatments and control tanks for 90 days. Total ammonia-N and nitrite-N contents were significantly low in the treatment tanks compared to the control. At the end of the experiment, survival rate of fish was significantly higher in the treatments than that of the control. Protein content in the biofilm was 41% in E1, which was significantly ($p < 0.05$) higher than the other treatments. The study reveals that biofilms developed on the substrates helped to reduce the need for formulated feed in the culture of *C. mrigala*. The treatment tanks not only have better water quality, but fish in these tanks achieved higher survival and growth.

*Corresponding author. E-mail address: vivekanandbharti15@gmail.com

Introduction

Application of fertilisers and artificial feeds in aquaculture is a common practice to enhance the carrying capacity of culture ponds (Weimin and Mengqing 2007) and these provisions account for nearly 60% of the production cost (Babu et al. 2013; Shilta et al. 2016). In most of the feed-driven aquaculture practices, only about 30% of the feed input is converted into harvestable products (Shilta et al. 2016). Over-feeding in aquaculture leads to waste accumulation in the sediments and the water column, besides increasing the cost of production (Biswas et al. 2006a). High input cost in terms of feed and manure is considered the limiting factor for adoption of modern aquaculture techniques among poor farmers in the developing countries. The provision of substrates support aquaculture through assemblage of biofilm-based microbes, which are either consumed directly by the cultured animals or by other small organisms which are the preferred food items of the cultured species (Ramesh et al. 1999; Singh et al. 2006; Rajkumar et al. 2015). Biofilm grown on the substrates improves water quality by converting toxic ammonia through nitrification into less toxic nitrate which is utilised by autotrophs and thus increases the primary productivity in the culture system (Thompson et al. 2002; Shilta et al. 2016). Toxicity effects of nitrogenous wastes on the survival of fish have been extensively studied by Jensen (2003) and Das et al. (2004).

Substrates in aquaculture act as shelters and provide hiding places, thus reducing stress for cultured organisms (Schveitzer et al. 2013). Stress adversely affects production since it makes the animals susceptible to diseases caused by opportunistic pathogens, besides reducing their feed intake and feed conversion efficiency, resulting in decreased growth (Yarahmadi et al. 2016). Many researchers have demonstrated the use of different substrates such as bamboo for *Tor khudree* (Sykes 1839), *Labeo fimbriatus* (Bloch 1795) (Keshavanath et al. 2002), *Oreochromis niloticus* (Linnaeus 1758) and *Macrobrachium rosenbergii* (de Man 1879) (Uddin et al. 2009), plastic sheet and ceramic tile for brackishwater shrimp (Khatoon et al. 2007), rice straw mats for Nile tilapia (Shahabuddin et al. 2012) and sugarcane bagasse for *Labeo rohita* (Hamilton 1822) and *Etroplus suratensis* (Bloch 1790) (Gangadhar and Keshavanath, 2012; Shilta et al. 2016) to improve water quality, enhance growth and increase production.

In India, *C. mrigala* is one of the premier species of freshwater aquaculture. Its growth at all stages depends on the kind of food, food intake, feeding frequency and nutrient assimilating ability (Mollah and Tan 1982). It is usually cultured in manured ponds and is also governed by supplemental feeding for greater production. There is limited literature on the effects of biofilm grown on both natural and artificial substrates on the culture of *C. mrigala*. The present study was executed to assess the effect of biofilm, formed on natural (paddy straw and sugarcane bagasse) and artificial (plastic sheet and tile) substrates on water quality, survival and growth performance of *C. mrigala*.

Materials and Methods

Experimental animal

Three hundred and fifty fry of *C. mrigala* (mean weight 1.42 ± 0.05 g; length 4.12 ± 0.08 cm) were procured from Aarey Fish Farm, Mumbai and were transported to the Wet Laboratory of Central Institute of Fisheries Education, Mumbai.

They were distributed randomly in five 500 L capacity Fibre Reinforced Plastic (FRP) tanks, filled with 300 L de-chlorinated freshwater and were acclimated for 14 days. During acclimation and throughout the experimental period, the fish were fed twice daily at the rate of 2% of their body weight with a balanced formulated feed (Table1).

Table 1. Ingredients and proximate composition of formulated feed.

Feed ingredients	(g %)	Proximate composition	
Casein	33	Moisture (%)	7.79
Gelatin	7.25	Crude protein (%)	35.24
Dextrin	16.75	Fat (%)	7.84
Starch soluble	21.5	Ash (%)	7.64
Cellulose	9		
Cod liver oil	4		
Sunflower oil	4		
Vitamin and mineral	2	Digestible energy	405.71
Carboxymethyl cellulose	1.5	(kcal 100g ⁻¹)	
Betaine	0.9		
Butylated hydroxytoluene	0.1		

Experimental design

The experiment was executed in 15 FRP tanks of 500 L capacity each. All the tanks were thoroughly washed with KMnO_4 solution and then dried under the sun. Three hundred litres of de-chlorinated tap water were added to each of the experimental tanks. The tanks were fertilised with agricultural grade urea (46% N, SPIC, Tuticorin), single super phosphate (16% P_2O_5 , SPIC, Tuticorin) and potassium sulphate (50% K_2O) at the rate of 6, 9 and 1.2 g L⁻¹, respectively, once a month (Katiha et al. 2005). About 200 g of dried natural substrates viz. paddy straw (E1) and sugarcane bagasse (E2) in the form of mat as well as two artificial substrates namely plastic sheet (E3) and tile (E4), each covering an area of 200 cm² were submerged in the experimental tanks which were replicated in triplicates (Mridula et al. 2003, 2006; Shahabuddin et al. 2012; Sruthisree et al. 2015).

The control (E0), having de-chlorinated tap water without any substratum, was also replicated in triplicates. All the treatments were carried out with continuous aeration. The experiment was conducted for a total of 120 days; the daily average sunlight during the period was around 8 h varying from 7.5-8.5 h. Each tank was stocked with 10 fish (at the rate of 100,000 fish·ha⁻¹) (Mridula et al. 2003), 30 days after the addition of substrate in the tanks. There was no exchange of water throughout the experiment.

Water quality analysis

Sampling for water quality parameters was carried out during morning hours at weekly intervals. Water temperature was measured using mercury filled Celsius thermometer (G H Zeal Ltd., London, England). Water pH was estimated using a portable digital pH meter (Eutech Instruments, India). Water samples were titrated against standard EDTA solution in the presence of ammonium chloride and ammonium hydroxide as buffer and eriochrom black-T indicator for the estimation of hardness. Alkalinity of water sample was determined titrimetrically using standard sulphuric acid, and phenolphthalein and methyl orange as indicators. Dissolved oxygen content in water samples was estimated using Winkler's Method. The estimation of ammonia-N, nitrite-N and nitrate-N was carried out spectrophotometrically (Thermospectronic, UV 1, Cambridge, UK) using phenate method, Griess diazotization reaction (sulphanilic acid and N-alpha-naphthyl-ethylenediamine) method and copper-cadmium reduction followed by Griess diazotization reaction, respectively (APHA 2005).

Chlorophyll-a and phytoplankton analysis

Water sample from each tank was collected at fortnightly intervals and chlorophyll-a was determined for the estimation of phytoplankton biomass (Kasparzak et al. 2008). A known volume of water (100 mL) was filtered through Whatman GF/C filter papers, and chlorophyll-a concentration was determined spectrophotometrically (APHA 2005). Plankton samples were obtained by concentrating 100 mL of water samples from each treatment in a centrifuge tube and all the samples were preserved with 4% formaldehyde. Phytoplankton were identified by using taxonomic keys (Desikachary 1959; Ward and Whipple 1992).

Growth parameters

Fish from all the experimental tanks were sampled monthly to analyse weight gain in terms of percentage and specific growth rate (SGR). Feed conversion ratio (FCR) was also calculated. The weight was taken using an electronic balance (Denver instrument, MXX-412, USA with accuracy of 0.01 g). The following formulae were used to analyse fish growth parameters.

$$\text{Percentage weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Ln Final weight} - \text{Ln Initial weight}}{\text{Number of culture days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumption (dry weight)}}{\text{Body weight (wet weight)}}$$

Proximate analysis

The proximate analysis of fish carcass and biofilm was carried out by collecting the samples at the end of the experiment. Biofilm from each substrate was scraped out manually with the help of a cover slip. Due care was taken to minimise the chance of scraping out the surface of the substrate. The proximate composition of fish carcass and biofilm was estimated on dry weight basis using standard methods (AOAC 1995). The initial and final weight of samples were estimated for moisture content using a hot air oven (Modern Industrial Corporation, India) at 105 °C for 16 h. Nitrogen content in samples was estimated by distillation in a Micro-Kjeldahl unit (KEL Plus-Classic DX VA, Pelican Equipments, India), followed by titration. Crude protein was calculated by multiplication of the nitrogen content with a factor of 6.25. Fat content in samples was estimated by Soxhlet apparatus using petroleum ether (boiling point 40-60 °C) as the solvent. Ash content was analysed by taking the samples in a silica crucible and placing them in a muffle furnace at 550 °C for 16 h.

Statistical analysis

All statistical analyses were done using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). Comparison among the treatments and control was performed using one-way analysis of variance (ANOVA) at 5% level of significance. Mean separation was carried out using Duncan Multiple Range Test.

Results

Water quality parameters

Temperature, pH and dissolved oxygen varied from 25.38 to 25.57 °C, 7.74 to 7.85 and 8.16 to 8.95 mg/L⁻¹, respectively in all treatments and the control. Total alkalinity and hardness differed significantly (p<0.05) among treatments.

Total alkalinity was significantly higher in E1 and E4 than that of the control. Hardness was the highest in E1 among treatments and control. The concentration of ammonia-N and nitrite-N did not differ significantly among the treatments, but was significantly lower ($p<0.05$) than that of the control. Nitrate-N concentration in the control was significantly ($p<0.05$) lower than that of treatments; its concentration was maximum in E3. The nitrate-N contents in treatments E1, E2 and E4 were not significantly different from one another but they were significantly ($p<0.05$) higher than that of the control (Table 2).

Table 2. Water quality parameters of different experimental groups (mean $\pm$ SE; n=36).

Parameter	Control (E0)	Treatments			
		Paddy straw (E1)	Sugarcane bagasse (E2)	Plastic sheet (E3)	Tile (E4)
Temperature (°C)	25.38 $\pm$ 0.32 ^a	25.57 $\pm$ 0.31 ^a	25.50 $\pm$ 0.31 ^a	25.42 $\pm$ 0.32 ^a	25.47 $\pm$ 0.07 ^a
pH	7.85	7.74	7.79	7.74	7.78
Dissolved Oxygen (mg L ⁻¹)	8.95 $\pm$ 0.34 ^a	8.27 $\pm$ 0.30 ^a	8.16 $\pm$ 0.33 ^a	8.33 $\pm$ 0.23 ^a	8.31 $\pm$ 0.34 ^a
Alkalinity (CaCO ₃ mg L ⁻¹)	180.18 $\pm$ 4.35 ^a	211.9 $\pm$ 6.34 ^c	186.43 $\pm$ 4.61 ^{ab}	186.43 $\pm$ 4.61 ^{ab}	192.48 $\pm$ 4.15 ^b
Hardness (CaCO ₃ mg L ⁻¹)	154.93 $\pm$ 6.17 ^a	181.20 $\pm$ 7.24 ^c	171.34 $\pm$ 6.80 ^b	167.08 $\pm$ 6.63 ^a	173.34 $\pm$ 6.68 ^b
Ammonia-N (mg L ⁻¹)	0.18 $\pm$ 0.02 ^b	0.09 $\pm$ 0.01 ^a	0.10 $\pm$ 0.01 ^a	0.08 $\pm$ 0.01 ^a	0.10 $\pm$ 0.01 ^a
Nitrite-N (mg L ⁻¹)	0.06 $\pm$ 0.00 ^b	0.04 $\pm$ 0.00 ^a	0.04 $\pm$ 0.00 ^a	0.05 $\pm$ 0.00 ^a	0.04 $\pm$ 0.00 ^a
Nitrate-N (mg L ⁻¹)	0.79 $\pm$ 0.03 ^a	0.80 $\pm$ 0.06 ^b	0.88 $\pm$ 0.04 ^b	1.14 $\pm$ 0.09 ^c	0.93 $\pm$ 0.06 ^b

Values in the same row with different superscripts differ significantly ($p<0.05$)

Phytoplankton and Chlorophyll-a

The phytoplankton communities consisted of four groups viz. Bacillariophyceae (7 genera), Chlorophyceae (10 genera), Cyanophyceae (2 genera) and Euglenophyceae (1 genus). Chlorophyceae was the most dominant group of phytoplankton. Chlorophyll-*a* concentration of the water samples was the highest ($p<0.05$) in E1 (261.58 $\mu\text{g L}^{-1}$), while the values in treatments E2 (231.58 $\mu\text{g L}^{-1}$), E3 (207.67 $\mu\text{g L}^{-1}$) and E4 (218.08 $\mu\text{g L}^{-1}$) were significantly ($p<0.05$) higher than that of the control (174.88 $\mu\text{g L}^{-1}$) (Table 3).

Table 3. Chlorophyll-*a* in the water for different groups (mean±SE; n=18).

Treatments	Chlorophyll- <i>a</i> (µg·L ⁻¹)
Control (E0)	174.88±6.41 ^a
Paddy straw (E1)	261.58±12.54 ^d
Sugarcane bagasse (E2)	231.58±11.68 ^c
Plastic sheet (E3)	207.67±5.54 ^b
Tile (E4)	218.08±9.93 ^b

Values with different superscripts differ significantly (p<0.05)

Growth parameters

There were significant differences (p<0.05) in body weight, weight gain and SGR between the biofilm-treated groups and the control. The highest body weight was recorded in treatment E1. Significant difference (p<0.05) was observed in FCR among treatments; it was significantly (p<0.05) lower in E1 (1.50), while FCR was highest in the control (2.94). Survival rate was significantly (p<0.05) higher in the treatments than that of the control. The survival rate did not differ significantly among treatments, but it was significantly (p<0.05) lower in the control (63.33%) (Table 4).

Table 4. Fish growth indices and survival rate of experiment fish under different treatments (mean±SE; n=9).

Parameter	Treatments				
	Control (E0)	Paddy straw (E1)	Sugarcane bagasse (E2)	Plastic sheet (E3)	Tile (E4)
Body weight (g)	4.66±0.08 ^a	7.76±0.09 ^d	5.55±0.05 ^c	5.25±0.02 ^b	5.22±0.07 ^b
Weight gain (%)	228.46±5.56 ^a	446.21±6.35 ^d	291.15±3.38 ^c	270.00±1.95 ^b	267.81±5.04 ^b
SGR (%)	1.17±0.02 ^a	1.66±0.01 ^d	1.34±0.01 ^c	1.28±0.01 ^b	1.28±0.01 ^b
FCR	2.94±0.12 ^c	1.50±0.09 ^a	2.02±0.11 ^b	2.20±0.10 ^b	2.30±0.08 ^b
Survival (%)	63.33±5.20 ^a	90.00±5.80 ^b	86.70±6.70 ^b	86.70±3.30 ^b	83.30±3.30 ^b

Values in the same row with different superscripts differ significantly (p<0.05)

Proximate composition of fish carcass and biofilm

Protein content in the fish carcass was significantly (p<0.05) higher in E1 and E4 compared to other treatments and control. Significantly higher (p<0.05) fat content was recorded in treatment E2 (12.37%) among all treatments. The protein content of biofilm was significantly (p<0.05) highest in the treatment E1 (41.63%). The fat content of biofilm was significantly (p<0.05) higher in natural substrates than that in artificial substrates (Table 5).

Table 5. Proximate composition of fish carcass and biofilm (% on dry weight basis) in treatments and control (mean±SE; n=3).

Treatments	Fish carcass				Biofilm			
	Moisture	Fat	Ash	Protein	Moisture	Fat	Ash	Protein
Control (E0)	78.43±0.27 ^a	6.97±1.21 ^a	10.25±1.80 ^a	49.84±0.22 ^a	-	-	-	-
Paddy straw (E1)	78.24±1.03 ^a	7.02±0.99 ^a	8.27±0.94 ^a	54.01± 1.02 ^c	86.45±3.18 ^a	7.27±0.73 ^b	28.86±2.42 ^a	41.63±2.18 ^d
Sugarcane Bagasse (E2)	76.36±0.67 ^a	12.37±2.06 ^c	8.33±0.19 ^a	50.39±0.88 ^a	72.87±5.84 ^a	5.40±0.38 ^b	24.03±2.28 ^a	31.02±1.15 ^c
Plastic sheet (E3)	77.75±0.46 ^a	9.70±2.00 ^b	8.03±0.17 ^a	49.9±0.74 ^a	88.91±1.79 ^a	2.79±0.74 ^a	32.07±3.63 ^b	26.38±3.01 ^b
Tile (E4)	75.97±0.73 ^a	7.67±1.11 ^a	8.80±0.37 ^a	52.68±2.36 ^b	83.47±3.15 ^a	3.01±0.91 ^a	31.88±6.04 ^b	24.78±2.44 ^a

Values in the same column with different superscripts differ significantly (p<0.05)

Discussion

Dissolved oxygen fluctuated from 8.16 to 8.95 mgL⁻¹ and pH varied from 7.74 to 7.85 during this study. Alkaline pH from 7.5-8.5 (Banerjea 1967) and dissolved oxygen above 5 mgL⁻¹ are considered favourable water quality for the survival and production of *C. mrigala* (Das et al. 2006). Marginally lower pH and dissolved oxygen were observed in all the treatments compared to the control. The decrease in pH in the substrate-based groups may be attributed to respiration of the higher number of fish that may subsequently increase the carbon dioxide concentration in the water (Sahu et al. 2007). Slightly lower dissolved oxygen in the substrate treatment groups compared to the control may be the result of greater demand for dissolved oxygen by cultured fish and the phytoplankton in the culture system. An inverse relationship between dissolved oxygen and density of cultured organisms has been clearly demonstrated by Biswas et al. (2006b).

Reduction in ammonia-N and nitrite-N in all substrate-based treatments compared to the control may be the result of their nitrification by the bacteria attached to the substrates, generating nitrate-N as the end product that helps in autotroph proliferation in the water column (Pan et al. 2016). Nitrifying bacteria are known to improve water quality by converting highly nitrogenous toxins such as ammonia and nitrite into nitrate (Rajkumar et al. 2015; Viau et al. 2015). In this study, high proliferation of Chlorophyceae and Bacillariophyceae was observed in the presence of substrates which may be due to nitrate enrichment by biofilm developed in the culture system (Khatoon et al. 2007; Viau et al. 2012). Higher chlorophyll-*a* concentration in the water samples of the substrate-based treatments compared with the control may be attributed to acceleration in biochemical cycling of nutrients in the culture system (Moss and Moss 2004; Viau et al. 2015).

The substrates in the culture system enhanced specific growth, weight gain and survival rate of fish (Umesh et al. 1999; Mridula et al. 2006). Survival of *C. mrigala* was significantly ($p < 0.05$) higher in all the substrate based treatments in comparison with the control (63.33%), the highest survival being recorded in paddy straw treatment. The highest survival rate in paddy straw treatment might have been achieved due to the presence of significantly ($p < 0.05$) high concentration of chlorophyll-*a* linked phytoplankton biomass in water and high load of nutrient rich organic debris in biofilm acting as single cell protein source (Anupama and Ravindra 2000; Kasprzak et al. 2008) and also due to the low ammonia and nitrite nitrogenous waste (Azim et al. 2002; Thompson et al. 2002; Mridula et al. 2006). Further, significantly lower ammonia-N (0.09-0.10 mgL⁻¹) in all substrate-based treatments compared to the control (0.18 mgL⁻¹), might have greatly improved the survival of *C. mrigala* in substrate based treatments (Viau et al. 2012). The highest weight gain (7.76 g) of *C. mrigala* was in paddy straw treatment.

Besides maintaining favourable water quality in the aquaculture system, biofilm must have supported fish growth by acting as a source of dietary nutrients. It was noticed that natural substrates favoured higher growth in *C. mirgala* in comparison with artificial substrates, which might be due to the high protein content in the biofilm from paddy straw (41.63%) and sugarcane bagasse (31.02%) (Renukaradhya and Varghese 1986; Seenappa and Devaraj 1995). Significantly ($p < 0.05$) higher fat content in paddy straw (7.27%) and sugarcane bagasse (5.40%) biofilm might have also facilitated higher growth of fish in natural substrate treatments than artificial substrate treatments (Seenappa and Devaraj 1995). Values varying from 23 to 30% protein, 2 to 9% lipid and 16 to 42% ash have been reported in periphyton grown on different substrates (Thompson et al. 2002; Anand et al. 2013). Higher total nitrogen content in paddy straw (1.09%) than that in sugarcane bagasse (0.67%) might be one of the causes for the significantly ($p < 0.05$) higher protein in the biofilm formed on paddy straw than that on sugarcane bagasse (Ramesh et al. 1999; Umesh et al. 1999). The proximate composition of fish carcass ranged from 75.97-78.43% moisture, 49.84-54.01% protein, 6.97-12.37% fat and 8.03-10.25% ash, which are supported by the findings of Paul et al. (2004). The higher protein and fat content in the carcass of the treated fish indicates that there was favourable effect of biofilm on the proximate composition of *C. mirgala*.

Conclusion

This study indicated that higher body weight (7.76 g) and survival (90%) of *C. mirgala* were achieved in the presence of biofilm developed on paddy straw. Biofilm in different treatments reduced nitrogenous ammonia ($0.08\text{--}0.10\text{ mg L}^{-1}$) and increased nitrate content in the culture of *C. mirgala*. The presence of paddy straw in the culture system resulted in higher chlorophyll-a content than that of the control and other treatments. Higher protein contents in biofilm on paddy straw (41.63%) and sugarcane bagasse (31.02%) were recorded. Similarly, biofilm formed on paddy straw (7.27%) and sugarcane bagasse (5.40%) showed higher fat content. The study reveals that the biofilm developed on the substrates helped to reduce the need of formulated feed in the culture of *C. mirgala*, apart from maintaining the favourable water quality, an essential factor for achieving high survival and growth of the cultured organisms. The higher protein and fat content in the carcass of the treated fish indicate that there was favourable effect of biofilm on the proximate composition of *C. mirgala*.

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