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RESEARCH PAPER

Periphyton-Based Jaraqui (*Semaprochilodus insignis*) Culture with Two Types of Substrates at Different Densities

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Abstract

Influences of two types of substrates, natural (macrophyte, *Pistia stratiotes*) and artificial (plastic screen) were evaluated at 3 different densities (10, 20, 30%) on periphyton development, water quality and growth performance of jaraqui (*Semaprochilodus insignis*). Fish of average initial weight 1.46-1.69 g and length 4.15-4.26 cm stocked at 1/m^2 in 46 m^2 masonry tanks were grown for 120 days. The tanks were fertilized with urea, triple superphosphate and wheat bran. The types and densities of substrates tested did not drastically influence water quality. Natural substrate harboured higher periphyton biomass (1.48±0.09 mg/cm^2) as well as species diversity (28 genera) than the artificial substrate (0.84±0.12 mg/cm^2, 20 genera). Fish in 10% and 20% artificial substrate and 20% natural substrate treatments showed better mean values of body weight (24.64, 23.86, 29.26 g respectively) on termination of the experiment. Fish survival was the lowest in 30% natural substrate (84.1%) and highest in 10% artificial substrate (94.2%). Higher fish biomass was recorded in 20% natural and 10% artificial substrate treatments (25.05 and 21.71 g/m^2), which amounts to a four-month yield of 250 and 217 kg/ha in the two treatments.

Keywords: Aquaculture, jaraqui, substrates, growth, water quality.

Introduction

Aquaculture is increasingly being considered as the answer for food security issues the world over, in the face of declining marine capture fisheries. At the same time, there is a great concern on the possible environmental impacts of aquaculture. Among the various strategies adopted to increase fish production from inland waters, periphyton-based fish culture is a viable option, since it also addresses environmental concerns. Periphyton-based specific systems with no additional feeding have long been practiced in the Africa (Hem and Avit, 1994) and Asia (Wahab and Kibria, 1994), mainly using bamboo and other locally available natural substrates. Most truly herbivorous fish species feed on larger, benthic, epilithic or periphytic algae, rather than phytoplankton (Horn, 1989). A positive effect of substrate introduction and consequent periphyton development on the production of the target species and on water quality has been observed (van Dam *et al*., 2002). Much of the periphyton-based fish culture research has been carried out comparing the growth of the target organism with and without substrates, providing no feed supplementation (Ramesh *et al*., 1999; Keshavanath *et al*., 2001; Milstein *et al*., 2003; Rai *et al*., 2008). Studies conducted in fish ponds comparing the effect of food supply versus periphyton have found that the provision of substrates can reduce the need for artificial food and can be an alternative to commercial food in the culture of herbivorous fish and prawn (Azim *et al*., 2002; Keshavanath *et al*., 2002, 2004; Uddin *et al*., 2008, 2009; García Gonzalez *et al*., 2011). Milstein *et al*. (2013) reported that under low density tilapia farming as required in organic aquaculture, the use of substrates equivalent to 40-50% of the pond surface allowed a 30-40% reduction in food input, without negatively affecting fish growth. This approach can be an ideal alternative in resource-limited regions in Asia, Africa and Latin America, where small-scale rural fish culture is commonly practiced (El-Sayed, 2006). In periphyton-based ponds, periphyton is used as additional natural food, substrate as shelter to minimize territorial

effects and improve water quality through trapping of suspended solids, organic matter breakdown and enhanced nitrification (Haque *et al*., 2014). The success of periphyton-based fish production system depends on factors like the type and density of the substrate, amount and quality of periphyton, food habit of the cultivated species, stocking density, seasonality, availability of non-periphytic food sources, etc. (Gangadhar and Keshavanath, 2008). Studies aimed at determining the efficiency of substrate type in the production of periphyton have tested biodegradable substrates such as bamboo, sugarcane bagasse, rice straw, palm leaf, coconut leaf and arecanut leafsheath as well as non-biodegradable substrates like tyre, ceramic tile, PVC pipe and glass slide (Mridula *et al*., 2005; Gangadhar and Keshavanath, 2008; Keshavanath *et al*., 2012, 2015).

Macrophyte *Pistia stratiotes*, found abundantly in the Amazon region of Brazil, is a suitable natural substrate in fish farming due to the extensive root system with mycelia capable of absorbing organic and inorganic nutrients and encouraging periphyton development (Pott and Pott, 2000). Artificial substrate plastic screen, being resistant when exposed to sunlight and water and relatively cheaper, is a desirable periphyton substrate. Jaraquis (*Semaprochilodus* spp.) that are iliophagous scavengers are considered appropriate for substrate-based culture system, since they consume periphyton (Santos *et al*., 2006). Further, they play an important social role by catering to the needs of low-income population in the Amazon, accounting for approximately 50% of fish landings in the port of Manaus (Thomé-Souza, 2007; Gandra, 2010).

The present experiment was carried out to compare the water quality, abundance of periphyton and growth and survival of jaraqui (*Semaprochilodus insignis*) in a plankton-based system vs. two periphyton-based systems using *P. statiotes* and plastic screen respectively, as substrates.

Materials and Methods

Area of Study and Facilities

The experiment was conducted at the Balbina Fish Culture Station, Department of Fisheries and Aquaculture of the State of Amazon (SEPA), located in the complex of Balbina hydropower unit, municipality of Presidente Figueiredo, AM. Apart from the tank and laboratory facilities of Balbina Fish Culture Station, facilities of Limnology Laboratory belonging to the Biodiversity Coordination (CBIO) at the headquarters of the National Institute of Amazon Research (INPA), Manaus were also used.

Experimental Design and Tanks

The experiment was conducted for four months from mid-March to mid-July 2013, in 21 masonry tanks of dimension 3.70 m width and 12.60 m length, totaling a water surface area of 46.62 m² each. The average depth of the tanks was 0.70 m. The two substrates, *P. stratiotes* and plastic screen, were used to cover 10%, 20% and 30% each of the tank area. The pond area covered at these 3 densities was 4.62 m^2, 9.24 m^2 and 13.86 m^2 respectively. The treatments were randomly assigned to triplicate tanks. Three tanks without substrate served as the control.

Preparation of the Experimental Tanks

The preparation of the experimental tanks was as follows. They were drained, washed, disinfected and dried. Lime (CaO) was applied at 30 g/m² to the tank bottom. Then inorganic fertilizer triple superphosphate (186 g), urea (372 g) and wheat bran (100 g) were applied in each tank. The tanks were filled with water up to an average depth of 0.70 m and substrates were added in accordance with the type and density. The free floating macrophyte was confined to one end of the tanks in 1-3 lots (covering 10, 20 and 30% area respectively in the 3 treatments) with the help of circular rubber frames, while square pieces of plastic screen (covering 10, 20 and 30% area respectively in the 3 treatments) were fixed vertically along half the length of the tanks, using a frame around them. After 10 days of fertilization, fry of jaraqui collected from Lake Catalan, Iranduba near Manaus and acclimated to local conditions at Balbina Fish Farm were stocked at a density of 1/m^2. The initial average weight and length of the fish was in the range 1.46$\pm$0.21 to 1.69$\pm$0.08 g and 4.15$\pm$0.09 to 4.26$\pm$0.16 cm respectively.

Water Quality Monitoring

The quality of water in the culture tanks was monitored daily through evaluation of various physico-chemical parameters (dissolved oxygen, temperature, pH and electrical conductivity) by sampling at 30 cm depth in the water column. Water samples were collected between 07:00 and 09:00 hr. Samples for the determination of alkalinity, NO_2, NO_3, NH_3 and PO_4 were collected once every15 days, also at 30 cm depth. Water temperature (°C) and dissolved oxygen (mg/L) were measured with a portable AT-160 Alfakit. Electric conductivity ($\mu S/cm^2$) was measured by a portable conductivity meter and pH by a portable pH meter. Alkalinity (mg $CaCO_3$/L) was analyzed by volumetric titration method. Nitrogen (nitrite NO_2, nitrate NO_3, ammonia NH_3) and phosphorus (orthophosphate PO_4) were measured using a digital photocolorimeter model AT-100 P.

Periphyton Biomass

Periphyton from natural (*P. stratiotes*) and artificial (plastic screen) substrates were collected

from an area of 5x5 cm^2 in triplicate in each tank at 15-day intervals. From plastic screen, collection was made by scraping with a thin spatula and the material was mixed in 50 ml of distilled water. Periphyton samples from *P. stratiotes* were collected from three randomly selected plants in each tank, by cutting their roots separately, arranging them in a 5x5 cm^2 area and carefully scrapping with the help of a needle and a fine brush. Subsequently, each periphyton sample was distributed in 50 ml of distilled water. The periphyton solutions were transferred individually to previously weighed falcon tubes and centrifuged for 15 minutes. The supernatant was discarded and the tubes were kept at 60 °C for a period of 12 hours. Thereafter, each tube was reweighed and the periphyton biomass (dry) was determined by the formula (weight of falcon tube with periphyton) - (weight of falcon tube without periphyton). The mean biomass of each experimental tank was determined by summing up the three measurements taken in each tank and the mean biomass of the treatments was calculated from the sum of values of the three experimental units.

Taxonomic Composition

The periphyton sampling for determining the taxonomic composition was made at monthly intervals, following the methodology described by Bicudo and Bicudo (1970). After extraction, the samples were preserved in Transeau solution (6:3:1 water:alcohol:formaldehyde) in polyethylene bottles. Taxonomic identification was performed using keys as per Bicudo and Menezes (2006).

Proximate Composition of Periphyton

The periphyton sample collection from substrates to determine the biochemical composition was done as described earlier, except that the area sampled was 6 times larger. The samples collected at monthly intervals were analyzed in triplicate to determine the percentage of moisture, protein and ash according to the standards established by AOAC (1975) and adopted by the Instituto Adolfo Lutz (1985). Total lipid was determined by the method of Bligh and Dyer (1959). NFE fraction was calculated by subtracting the sum of the percentages of moisture, ash, lipid and protein from 100.

Biometry

Biometry was performed at the beginning of the experiment and every thirty days until termination. Fifteen fish from each replicate was weighed on an electronic balance (accuracy 0.1 g) and standard length was measured. In addition to weight and length, the following growth performance parameters were determined at the end of the 120-day experiment.

Survival

Survival was determined as the difference between the initial and final fish counts. The percentage of survival was calculated using the following formula;

$$S\,(\%) = \frac{N_{final}}{N_{initial}} \times 100$$

where S is survival, N_{final} is the number of fish counted at harvest and $N_{initial}$ is the number of fish stocked.

Gain in Biomass

Biomass gain was calculated by the equation:

$$GB(g) = B_{final}(g) - B_{initial}(g)$$

where GB is the gain of biomass, B_{final} is the biomass estimated at the end of the experiment and $B_{initial}$ is the biomass estimated at the time of stocking the tanks.

Biomass was calculated by multiplying the average total weight by fish number, as shown in the equations below.

$$B_{final}(g) = PT_{final}(g) \times N_{final}$$

$$B_{initial}(g) = PT_{initial}(g) \times N_{initial}$$

where PT_{final} is the total average weight of the fish at the end of the experiment and $PT_{initial}$ is the total average weight of the fish at the time of stocking the tanks.

Condition Factor

It was calculated as the ratio of the total weight of the fish (g) and the standard length (cm), according to the following equation.

$$Kn = \frac{PT\ (g)}{CP(cm)^b}$$

where Kn is the condition factor, PT is the total weight, CP is the standard length and b is the slope coefficient of the length weight equation, estimated by the least squares method as shown below.

$$PT = a.CP^b$$

Specific Growth Rate (SGR)

SGR was calculated by the product of one hundred into the difference between the logarithms of the initial and final weight, divided by the time in days as given below.

$$SGR\% = \frac{100(\ln B_{final} - \ln B_{initial})}{Time(days)}$$

Weight Gain

The weight gain (%) was calculated by the product of one hundred into the difference between the initial and final weight divided by the initial weight.

$$\text{Weigt gain (\%)} = \frac{100(B_{final} - B_{initial})}{B_{initial}}$$

Statistical Analyses

One-way ANOVA (P<0.05) as described by Chambers *et al*. (1992) was used to check the effect of treatments on water quality variables, periphyton taxonomic composition and fish growth parameters. Multiple comparison of means was carried out by the q Tukey test as described in Miller (1981) and Yandell (1997), aided by the algorithms of Piepho (2004), Hothorn *et al*. (2008) and Bretz *et al*. (2011). The analyses were performed using the statistical software R (R Core Team, 2014).

Results and Discussion

Water Quality

Electrical conductivity (P=0.063), alkalinity (P=0.524), nitrite (P=0.506), nitrate (P=0.294) and ammonia (P=0.857) showed no significant difference in values in natural and artificial substrate treatments. However, dissolved oxygen (P=0.005), pH (P=0.005), temperature (P=0.035) and orthophosphate (P=0.004) values showed significant differences (Table 1). Water quality data suggests that the parameters monitored were within acceptable limits for fish farming (Kubitza, 1997; Bhatnagar *et al*., 2004). Dissolved oxygen (DO) decreased with increase in *P. stratiotes* density, while there was no difference in artificial substrate treatment at different densities. The lower DO concentration with increase in macrophyte density can be related to oxygen consumption by plants during night time and death and decay of some of their parts (Howard-Williams and Junk, 1976). Several authors have reported a reduction in oxygen level in tanks where substrates are installed, because they affect surface aeration. Further, plant substrates increase the biological oxygen demand, contributing to DO reduction in culture tanks (Dharmaraj *et al*., 2002; Joice *et al*., 2002; Mridula *et al*., 2003; Keshavanath *et al*., 2004). For most tropical fish species, the minimum DO concentration should be higher than 4.0 mg/L (Tavares, 1994); in the present study the average values remained above 5.0 mg/L. Keshavanath *et al*. (2001) recorded the highest DO levels in tanks with PVC pipes (mean 6.4 mg/L), intermediate values in tanks with bamboo (mean 4.9 mg/L) and low concentrations in tanks with sugarcane bagasse (mean 1.3 mg/L). In sugarcane bagasse added tanks, Umesh *et al*. (1999) also found similar DO values (1.3 mg/L).

pH of the water was alkaline ranging from 7.93 to 8.94. According Zaniboni Filho *et al*. (2002), juveniles of *Prochilodus lineatus* (species from the same family of *Semaprochilodus*) survive in a pH range of 4.08 to 9.84. Gangadhar and Keshavanath (2012) reported good production of farmed rohu (*Labeo rohita*) in alkaline pH (8.39 to 9.82). The values of electrical conductivity in the natural substrate treatment were marginally higher which may be related to the increase in organic matter due to the decomposition of parts of the macrophyte. Martins and Pitelli (2005) observed that increased electrical conductivity results from the release of nutrients during the process of decomposition of plants. Tavares (1994), who recorded variation of 23-71 $\mu S/cm^2$ in fish ponds, observed that EC values can be used as a reference to assess the availability of nutrients in nurseries. Keshavanath *et al*. (2002) reported that the temperature of water in fish tanks is influenced by the presence of substrates as they promote shading of the water column and hence reduce the temperature. In the present study also,

Table 1. Physico-chemical parameters (mean ± SD) of water in the two substrate treatments at different densities

Parameter	Control	Substrate type					
		Natural			Artificial		
		Substrate density (%)					
		10	20	30	10	20	30
DO	7.19±0.26^{a}	6.71±0.46^{a}	5.74±0.29^{b}	5.32±0.04^{b}	6.90±0.25^{a}	7.02±0.13^{a}	6.79±0.36^{a}
pH	8,66±0.16^{ab}	8.68±0.37^{ab}	8.18±0.23^{bc}	7.93±0.22^{c}	8.65±0.05^{ab}	8.88±0.09^{a}	8.94±0.25^{a}
EC	57.56±3.62^{a}	60.15±2.52^{a}	60.35±2.82^{a}	62.78±3.98^{a}	53.73±10.25^{a}	56.12±6.37^{a}	52.38±1.38^{a}
Temp	29.85±0.38^{a}	29.68±0.23^{a}	28.79±0.16^{b}	28.01±0.39^{b}	29.96±0.12^{a}	29.86±0.24^{a}	29.88±0.20^{a}
AL	23.56±1.33^{a}	24.30±4.68^{a}	22.26±2.63^{a}	23.48±1.61^{a}	26.52±1.48^{a}	25.19±1.58^{a}	23.11±1.94^{a}
NO_2	0.01±0.00^{a}	0.02±0.01^{a}	0.03±0.00^{a}	0.03±0.01^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}
NO_3	1.21±0.22^{a}	0.95±0.20^{a}	1.32±0.12^{a}	1.16±0.28^{a}	1.35±0.31^{a}	1.35±0,.20^{a}	1.54±0.09^{a}
NH_3	0.38±0.18^{a}	0.21±0.12^{a}	0.20±0.14^{a}	0.29±0.00^{a}	0.33±0.22^{a}	0.24±0.15^{a}	0.33±0.16^{a}
PO_4	1.42±0.51^{b}	1.99±0.20^{b}	2.90±0.24^{a}	3.42±0.25^{a}	1.32±0.35^{b}	1.47±0.30^{b}	1.41±0.29^{b}

DO: Dissolved oxygen (mg/L); pH: Hydrogen ion concentration; EC: Electric conductivity ($\mu S/cm^2$); Temp: Temperature (^oC); AL: Alkalinity (mg/L); NO_2: Nitrite (mg/L); NO_3: Nitrate (mg/L); NH_3: Ammonia (mg/L); PO_4: Orthophosphate (mg/L). Values with the same superscript in each row are not statistically different (P>0.05).

temperature values in the natural substrate treatment showed an inverse trend to increased vegetation cover. According to Boyd and Tucker (1998), fish grow well within a temperature range of 25° to 35°C. The optimal range of thermal comfort for growth of *Prochilodus* sp. is between 20° and 30 °C (Proença and Bittencourt, 1994; Ostrensky and Boeger, 1998). In the present study, the mean temperature varied between 28.01 to 29.96 °C and there was no observable adverse effect of this variable on the performance parameters of jaraqui. Alkalinity influences fish growth, since it affects the availability of nutrients by interfering with the organic productivity system (Verani, 1985). In fish farming, the desirable value of alkalinity is above 20 mg/L and values between 200 and 300 mg/L result in higher fish production (Tavares, 1994). In this study, despite being relatively low, alkalinity was within the acceptable limits for the culture of jaraqui (mean value 24 mg/L). According Rojas *et al*. (2001), *Prochilodus liniatus* shows better growth rate in water with alkalinity of around 30 mg $CaCO_3$/L. Levels above 30 mg/L generally indicate an effective pH buffering system (Proença and Bittencourt, 1994).

Nitrogen is essential for living bodies because it is a major constituent of proteins. In the environment, inorganic nitrogen is found in a range of oxidation categories as nitrite (NO_2), nitrate (NO_3), ion ammonia (NH_3) and molecular nitrogen (N_2). Nitrite is toxic to fish when its concentration in water exceeds 0.5 mg/L (Ostrensky and Boeger, 1998). At these concentrations, it oxidizes hemoglobin of the blood to methemoglobin, causing death by asphyxiation (Spotte, 1979; Ostrensky and Boeger, 1998). Exposure to sublethal concentrations of nitrite (0.3-0.5 mg/L) can lead to a reduction in the growth and disease resistance. In freshwater fish, concentrations above 0.7 mg/L can cause severe mortality. In the present study, both the treatments showed concentrations well below the toxicity threshold, ranging from 0.01 to 0.03 mg/L. The concentrations of nitrate (NO_3) showed no definite pattern of variation. Nitrate is the least toxic ammonium compound (Jensen, 1995). Concentrations of 5.0 mg/L and above make nitrate lethal (Tavares, 1994). The concentrations of ammonia in this study were within the acceptable standards for fish farming (Table 1) (Kubitza, 1998). Ammonia levels above 0.5 mg/L are considered harmful in fish farming (Tavares, 1994; Kubitza, 2003). Orthophosphate levels greater than 3 mg/L can be stressful for fish (Bhatnagar and Devi, 2013). In the present study, only 30% natural substrate treatment had concentrations above the maximum tolerable limit. This could be due to increased supply of dead organic matter (parts of weed) to aquatic microbial fauna, and its intensified mineralization, resulting in the release of ions such as PO_4 (Howard-Williams and Junk, 1976; Wetzel, 2001).

Periphyton Biomass

Fluctuations in periphyton biomass were observed in both substrate treatments at all the 3 densities, mean values being higher with natural substrate. Significant differences were observed between the substrates only on days 60 (P=0.0001), 90 (P=0.0001) and 105 (P=0.0033), when periphyton biomass on the natural substrate was statistically superior to that of artificial substrate at all densities (Table 2). It is possible that ecological succession within the community occurs differently on the two substrates. The biomass of periphyton is highly variable, depending on the habitat, taxonomic composition, fertilization level and substrate type (Azim *et al*., 2004; Azim and Asaeda, 2005). In this respect, the choice of substrate is important as it needs to provide conditions conducive to the establishment of a periphytic community with sufficient quantity and nutritional quality to meet the needs of farmed organisms (Sibbing and Witte, 2005). Both the substrates tested facilitated periphyton development, yet macrophyte showed higher biomass. This may be associated with the nature and complexity of the structural roots of the natural substrate providing better attachment surface. The amount of periphyton

Table 2. Periphyton biomass (mg/cm²) (mean ±SD) on the two types of substrates at different densities

	Substrate type					
	Natural			Artificial		
	Substrate density (%)					
Days	10	20	30	10	20	30
15	3.55±2.57^{a}	1.40±1.62^{a}	1.77±1.43^{a}	0.69±0.27^{a}	0.86±0.44^{a}	1.28±1.04^{a}
30	0.72±0.27^{a}	0.33±0.29^{a}	1.02±1.32^{a}	0.96±0.15^{a}	0.75±0.52^{a}	0.43±0.33^{a}
45	0.88±0.83^{a}	0.45±0.18^{a}	1.14±0.91^{a}	0.82±0.65^{a}	0.62±0.27^{a}	0.71±0.28^{a}
60	0.95±0.26^{a}	1.12±0.26^{a}	1.14±0.25^{a}	0.38±0.06^{b}	0.37±0.14^{b}	0.26±0.12^{b}
75	1.12±1.23^{a}	1.66±0.65^{a}	1.34±0.41^{a}	1.12±0.52^{a}	0.64±0.20^{a}	0.79±0.49^{a}
90	1.47±0.51^{ab}	1.70±0.25^{a}	1.40±0.47^{ab}	0.67±0.18^{bc}	0.77±0.29^{ac}	0.40±0.21^{c}
105	1.63±0.60^{ab}	2.92±1.68^{a}	1.28±0.29^{ab}	0.87±0.39^{ab}	0.52±0.10^{b}	0.51±0.22^{b}
120	1.53±0.49^{a}	2.03±1.66^{a}	1.80±0.88^{a}	1.17±0.39^{a}	0.78±0.15^{a}	0.79±0.16^{a}
Mean	1.48±0.09^{a}	1.45±0.65^{a}	1.36±0.59^{a}	0.84±0.12^{a}	0.66±0.13^{a}	0.65±0.06^{a}

Values with the same superscript in each row are not statistically different (P>0.05).

biomass ranged from 0.26-1.28 (artificial substrate) and 0.33-3.55 mg/cm^2 (natural substrate) respectively; the highest levels were found on day 15 in both substrate treatments.

The highest average periphyton biomass of 1.48 mg/cm^2 and 0.84 mg/cm^2 for the natural and artificial substrates respectively was reorded in 10% density treatment of the two substrates. These values were lower than those recorded by Siqueira and Rodrigues (2009) on pet bottle blades (4.0 mg/cm^2), Keshavanath *et al*. (2012) on coconut leaf (1.58 mg/cm^2) and Anand *et al.* (2013) on bamboo (3.79 mg/cm^2). However, lower values compared to that of natural substrate in the present study were reported on substrates such as PVC (0.97 mg/cm^2), glass (0.91 mg/cm^2), bamboo (0.90 mg/cm^2) by Gangadhar and Keshavanath (2008) and bagasse (1.06 mg/cm^2) by Keshavanath *et al*. (2012). The time required for the establishment of periphyton is a few weeks, with maturity and climax around 30 days (Pompeo and Moschini-Carlos, 2003). Huchette *et al*. (2000) found dense proliferation of periphyton communities after two weeks of incubation of the substrates in water. Lima (2009), who evaluated the colonization of glass slides in the rainy and dry seasons in a stream of Paraíba, noted that the abundance of periphyton varied significantly with incubation time. In the present study, periphyton biomass variations observed with the progress of the experiment are an indication of periphyton growth as well as grazing pressure.

Proximate Composition of Periphyton

The biochemical composition of the periphyton collected from different treatments is summarized in Table 3. There were no significant differences in protein (P=0.33), lipid (P= 0.93), ash (P=0.91), NFE (P=0.87) and total energy (P=0.77) values in natural as well as artificial substrate treatments. Nutritional quality of periphyton can be quite variable, depending on the taxonomic composition, type of substrate, ecosystem, fertilization and predation pressure (Azim *et al*., 2003, Keshavanath *et al*., 2012). Protein, lipid, carbohydrate and ash contents were 8-10%, 2-5%, 52-60% and 25-38% respectively in periphyton grown on granite boulders suspended in the Gulf of California (Montgomery and Gerking, 1980). An average protein content of 15% was estimated in periphyton collected from coral reef (Polunin, 1988). Dempster *et al*. (1995) reported 28-55% protein and 5-18% lipid in some periphytic algal species. Proximate composition of periphyton from different substrates varied from 9-32% protein, 2-9% lipid, 25-28% NFE and16-42% ash (Thompson *et al*., 2002; van Dam *et al*., 2002; Azim *et al*., 2005). Gangadhar and Keshavanath (2008) reported 26.06% protein, 3.08% lipid, 38.02% NFE and 17.45% ash, while Mridula *et al*. (2003) recorded 9.4%, 0.33%, 38% and 23% values for the respective parameters in periphyton from sugarcane bagasse. Azim *et al*. (2001) estimated 27.19% crude protein in periphyton developed on bamboo, 14.63% on hizol branches, 18.74% on kanchi (bamboo shoot) and 12.69% on jute sticks. Nielsen *et al*. (1997) reported values of 58% protein in biofilms collected from biofilters installed in water recycling system.

The protein requirement of freshwater fish ranges from 25 to 35% depending on age (Ashraf and Fairgrieve, 1998). Protein levels of periphyton recorded in this study varied between 26.54 and 31.50%, which resemble that of some natural food plants used in aquaculture (Hepher 1988; Yakupitiage, 1993; Dempster *et al*., 1995). Studies conducted with *Prochilodus* sp. fry indicated good growth performance with cattle feed containing 26-35% CP (Bomfim *et al*., 2005; Visbal *et al*., 2013). These values are within the range recorded for periphyton protein in this study, indicating that it is a good natural food source. Lipid levels ranged from 5.70-6.88% in periphyton from the natural substrate and 3.92 to 4.15% in that from the artificial substrate. Hepher (1988) found 7-10% lipid content in planktonic algae collected in fish ponds. Azim *et al*. (2002) reported lipid contents of 5.43%, 0.35% and 2.75% for periphyton developed on hizol, bamboo and jute stick respectively. Ash content of less than 30% in the diet can be considered acceptable for herbivorous fish (Yakupitiage, 1993). Azim *et al*. (2002) recorded ash contents of 14, 19, and 31% in periphyton from kanchi, bamboo and jute stick

Table 3. Proximate composition (mean ±SD) of the periphyton collected (dry weight basis) from the two substrate types at different densities

Parameter	Substrate type					
	Natural			Artificial		
	Substrate density (%)					
	10	20	30	10	20	30
Protein (%)	26.54±5.73^a	30.25±2.05^a	29.11±0.27^a	29.17±0.33^a	28.89±0.17^a	31.50±0.14^a
Lipid (%)	5.70±1.50^a	6.88±1.90^a	6.41±0.23^a	4.15±0.22^a	3.92±0.35^a	4.12±0.42^a
Ash (%)	49.97±9.99^a	48.46±2.07^a	50.18±0.26^a	47.18±0.12^a	47.29±0.33^a	47.11±0.37^a
NFE (%)	17.79±13.43^a	14.41±7.95^a	14.30±0.23^a	19.50±0.46^a	19.90±0.65^a	17.27±0.80^a
Gross Energy (kcal/100g)	228.62±10.88^a	240.54±6.36^a	231.33±1.40^a	232.03±2.61^a	228.80±2.90^a	241.11±4.09^a

Values with the same superscript in each row are not statistically different (P>0.05).

respectively, while Hepher (1988) found 27-48% ash in planktonic algae collected from fish ponds. In the present study, the ash content of periphyton was higher ranging from 48-50% in the natural substrate and 47% in the artificial substrate. According to Gangadhar and Keshavanath (2012), high ash content values may occur due to the capture of particulate matter by periphyton. Ledger and Hildrew (1998) recorded carbohydrate content ranging from 29-33% in periphyton developed on stones. However, in this study, the proportion of NFE was low (14.30-17.79 % of dry matter in the natural substrate and 17.27-19.90% in the artificial substrate). Nielsen *et al*. (1997) discovered that extra-cellular polymeric substances are responsible for 50-80% of total organic matter and therefore, the large amounts of carbohydrate in biofilms. The gross energy content varied from 228 to 240 kcal/100 g in periphyton from the natural substrate and 228 to 241 kcal/100 g from artificial substrate. These values are lower than those recorded in periphyton associated with hizol, kanchi (454 kcal/100 g) and jute stick (335 kcal/100 g) (Azim *et al*., 2002).

Taxonomic Composition of Periphyton

Most taxa recorded in this study are widely distributed in the Amazon region (Melo *et al*., 2004, 2005). In the natural substrate treatment, 28 genera of microalgae were identified among the periphyton community of which 16 (57.1%) belonged to Chlorophyta, 5 (17.9%) to Heterokontophyta, 4 (14.3%) to Cyanophyta and 3 (10.7%) to Euglenophyta. Periphyton from artificial substrate consisted of 20 genera of which 13 (65%) belonged to Chlorophyta, 3 (15%) to Cyanophyta, 3 (15%) to Euglenophyta and 1 (5%) to Heterokontophyta (Table 4). The natural substrate contained eight unique taxa. There was no statistically significant difference between treatments in terms of algal diversity (P=0.7026). Wide variation in the composition of periphyton attached to different substrates has been reported. Azim *et al.* (2002) recorded 50 genera of algae in periphyton grown on bamboo shoots (kanchi) and found that 24 of them belonged to Chlorophyceae, while 12 belonged to Bacillariophyceae. Uddin *et al.* (2007) noticed almost similar taxonomic composition in periphyton collected from bamboo substrate installed in ponds of Nile tilapia and freshwater prawn polyculture. Gangadhar and Keshavanath (2008) who evaluated different biodegradable and non-degradable substrates found dominance of Chlorophyceae (14 genera), followed by Cyanophyceae (2 genera), Chrysophyceae, Bacillariophyceae and Dinophyceae (1 genus each).

In estuarine waters, Khatoon *et al*. (2007) reported algal diversity of 15-19 genera belonging to Bacillariophyceae, Chlorophyceae and Cyanophyceae in periphyton attached to various types of substrates (bamboo, PVC, plastic plate and ceramic tile) used in shrimp farming. Santos (2012) recorded 180 taxa in periphyton developed on macrophyte *Utricularia foliosa* and nylon yarn from a lake. Here again, Chlorophyceae dominated with 66 taxa, followed by Zygnemaphyceae (47 taxa) and Bacillariophyceae (23 taxa). García Gonzalez *et al*. (2012) also reported that Chlorophyceae was predominant with 39% of the genera, followed by Bacilariophyceae (24%), Cyanophyceae (16%), and Euglenophyceae (8%). Lima (2009), evaluating the colonization of glass slides in the rainy and dry season in a stream in Paraíba, noted that the class of diatoms was the most representative during both the periods, contributing an average of 41.8% in the dry season and 84.8 % in the rainy season, followed by Oedogoniaceae (38.3%) and Cyanobacteria (8.2%) in the two seasons respectively.

Growth Performance of Fish

No significant differences in the initial biometry were observed between treatments with regard to length (P=0.3580) and weight (P=0.8040), confirming homogeneity. Length presented inversely proportional tendency to increase in natural substrate density on days 30 and 60 (Table 5). Fish weight yielded no significant differences between treatments at the end of 90 (P=0.4760) and 120 (P=0.1811) days of the experiment (Table 6).

With the natural substrate, lower body weight was recorded under 20% and 30% densities after 30 days (P=0.0003) and 60 days (P<0.0001) of culture, compared to the other treatments. Then onwards, body weight of fish increased in these treatments. On termination of the experiment, 10% and 20% densities of artificial substrate and 20% density of natural substrate showed better mean values of body weight. Interestingly, control fish achieved growth comparable to the rest of the treatments. This must be due to the use of periphyton grown on the walls of masonry tanks that acted like a hard and rough substrate. This view is supported by the presence of periphyton growth on the tank walls and visualization of its browsing by fish.

Resende *et al.* (1985) evaluated the growth potential of jaraqui (*Semaprochilodus* sp.) over 386 days in dam ponds intercropped with pigs. They observed an increase in length and weight of 10 cm and 118.6 g. In the present study, fish in 20% natural substrate treatment showed an increase in length of 6.77 cm and weight of 27.72 g over a period of four months in masonry tanks. The survival of fish in natural and artificial substrate treatments was significantly different (P=0.0006). This is associated with the lower oxygen levels recorded in treatments with natural substrate, particularly at 30% density; this treatment recorded the lowest survival of 84.1% (P<0.05). Fish survival was higher without singnificant differences (P>0.05) among artificial

Table 4. Taxonomic composition of periphyton from natural and artificial substrates

Division	Genus	Substrate	
		Natural	Artificial
CHLOROPHYTA	*Actinotaenium*	X	-
	Actinastrum	X	X
	Ankistrodesmus	X	X
	Coelastrum	X	X
	Cosmarium	X	-
	Closterium	X	X
	Desmodesmus	X	X
	Dictyosphaerium	X	X
	Eudorina	X	X
	Micrasterias	X	X
	Oedogonium	X	X
	Pandorina	X	X
	Pediastrum	X	-
	Scenedesmus	X	X
	Selenastrum	X	X
	Staurastrum	X	X
CYANOPHYTA	*Anabaena*	X	X
	Microcystis	X	X
	Oscillatoria	X	X
	Planktothrix	X	-
EUGLENOPHYTA	*Lepocincles*	X	X
	Phacus	X	X
	Trachelomonas	X	X
HETEROKONTOPHYTA (Bacillariophyceae)	*Gomphonema*	X	X
	Pinnularia	X	-
	Surirella	X	-
HETEROKONTOPHYTA (Chrysophyceae)	*Synedra*	X	-
	Mallomonas	X	-

'x' indicates presence of the genus in periphyton samples and '-' indicates absence

Table 5. Length (cm) (mean ± SD) of jaraqui over four months of cultivation under the two substrate types at different densities

		Substrate type					
		Natural			Artificial		
		Substrate density (%)					
Days	Control	10	20	30	10	20	30
00	4.25±0.09^{a} (2.19%)	4.26±0.16^{a} (3.71%)	4.18±0.11^{a} (2.54%)	4.24±0.07^{a} (1.58%)	4.15±0.10^{a} (2.38%)	4.24±0.12^{a} (2.76%)	4.15±0.09^{a} (2.10%)
30	7.24±0.66^{ab} (9.12%)	6.71±0.63^{ac} (9.37%)	5.94±0.53^{bc} (8.95%)	5.42±0.49^{c} (9.10%)	7.75±0.60^{a} (7.71%)	7.25±0.52^{ab} (7.12%)	7.20±0.77^{ab} (10.70%)
60	8.75±0.85^{a} (9.69%)	8.31±0.33^{a} (3.92%)	7.38±0.88^{ab} (11.98%)	6.51±0.24^{b} (3.76%)	8.98±0.47^{a} (5.28%)	8.71±0.51^{a} (5.83%)	8.63±0.48^{a} (5.54%)
90	9.55±0.80^{a} (8.40%)	9.59±0.17^{a} (1.77%)	9.76±1.49^{a} (15.29%)	8.06±0.29^{a} (3.66%)	9.86±0.37^{a} (3.80%)	9.58±0.25^{a} (2.64%)	9.39±0.50^{a} (5.35%)
120	9.84±0.68^{a} (6.92%)	9.82±0.13^{a} (1.30%)	10.95±1.62^{a} (14.83%)	9.22±0.54^{a} (5.80%)	10.46±0.65^{a} (6.22%)	10.14±0.54^{a} (5.35%)	9.80±0.74^{a} (7.52%)

Values with the same superscript in each row are not statistically different (P>0.05). Values in brackets are the coefficients of variation.

substrate treatments (Table 7). Resende *et al*. (1985) recorded 100% survival of jaraqui (*Semaprochilodus* sp.) reared in semi-intensive conditions in dam ponds.

Jaraqui biomass values were higher in 20% natural substrate treatment and 10% and 20% artificial substrate treatments (25.05, 21.70, 20.42 g/m^2 respectively). In the control, the yield was 18.84 g/m^2, higher than that recorded in the rest of the treatments. In the artificial substrate treatment, the biomass gain was inversely proportional to increase in substrate density (Table 7). Biomass gain in the 20% natural substrate treatment was 33% higher than that of the control, while in the artificial substrate treatment at densities of 10% and 20%, yields were 15% and 8% higher, respectively. The apparent low production of jaraqui in this study at the end of four months may be related to the types and densities of substrates. According Milstein *et al*. (2013), the use of rigid

Table 6. Total weight (g) (mean ± SD) of jaraqui over four months of cultivation under the two substrate types at different densities

Days	Control	Substrate type					
		Natural			Artificial		
		Substrate density (%)					
		10	20	30	10	20	30
00	1.69±0.08^{a} (4.45%)	1.59±0.35^{a} (22.31%)	1.54±0.08^{a} (5.50%)	1.48±0.13^{a} (8.49%)	1.48±0.26^{a} (17.85%)	1.60±0.26^{a} (15.99%)	1.46±0.21^{a} (14.43%)
30	9.23±2.22^{ab} (24.10%)	7.14±1.85^{ac} (25.90%)	4.95±1.40^{bc} (28.25%)	3.65±0.99^{c} (27.02%)	11.60±1.84^{a} (15.86%)	9.18±2.39^{ac} (26.07%)	9.08±2.71^{ac} (29.82%)
60	16.45±4.45^{a} (27.08%)	12.85±2.13^{ac} (16.57%)	9.20±2.94^{bc} (31.90%)	6.52±1.08^{c} (16.57%)	15.76±0.15^{a} (0.96%)	14.65±1.12^{ab} (7.61%)	14.56±1.44^{ab} (9.91%)
90	19.03±4.85^{a} (25.50%)	16.99±1.16^{a} (6.83%)	19.17±7.01^{a} (36.54%)	12.67±1.73^{a} (13.67%)	19.13±2.23^{a} (11.64%)	19.47±2.46^{a} (12.61%)	18.02±3.19^{a} (17.68%)
120	21.89±4.65^{a} (21.25%)	17.94±1.50^{a} (8.34%)	29.26±10.1^{a} (34.62%)	17.82±4.27^{a} (23.94%)	24.64±4.08^{a} (16.56%)	23.86±3.84^{a} (16.09%)	20.82±4.55^{a} (21.86%)

Values with the same superscript in each row are not statistically different (P>0.05). Values in brackets are the coefficients of variation.

Table 7. Growth performance (mean ± SD) of jaraqui over four months under the two substrate types at different densities

Parameter	Control	Substrate type					
		Natural			Artificial		
		Substrate density (%)					
		10	20	30	10	20	30
Survival (%)	93.5±2.2^{a} (2.33%)	89.9±2.5^{ab} (2.79%)	90.6±1.3^{a} (1.39%)	84.1±1.3^{b} (1.49%)	94.2±1.3^{a} (1.33%)	92.7±4.5^{a} (4.88%)	92.7±1.3^{a} (1.35%)
Biomass gain (g/m^2)	18.84±1.1^{a} (25.73%)	14.52±1.4^{a} (10.57%)	25.05±2.1^{a} (37.77%)	13.46±1.8^{a} (25.82%)	21.70±1.6^{a} (16.38%)	20.42±2.0^{a} (11.78%)	17.82±3.1^{a} (21.95%)
SGR (%/day)	2.0±0.2^{a} (10.77%)	1.9±0.2^{a} (12.01%)	2.3±0.3^{a} (14.23%)	1.9±0.3^{a} (13.27%)	2.3±0.2^{a} (6.92%)	2.2±0.1^{a} (2.66%)	2.1±0.2^{a} (8.56%)
Weight gain (%)	91.1±2.1^{a} (2.35%)	90.0±3.0^{a} (3.30%)	93.6±2.7^{a} (2.91%)	89.6±3.3^{a} (3.68%)	93.6±1.3^{a} (1.35%)	92.7±0.5^{a} (0.56%)	92.3±1.7^{a} (1.84%)
K Factor	2.3±0.2^{a} (9.43%)	1.9±0.1^{a} (5.75%)	2.2±0.2^{a} (11.40%)	2.2±0.2^{a} (7.20%)	2.1±0.1^{a} (3.17%)	2.3±0.0^{a} (0.67%)	2.2±0.1^{a} (5.98%)

Values with the same superscript in each row are not statistically different (P>0.05). Values in brackets are the coefficients of variation.

substrates with rough surfaces and white color is more effective. They suggest that the ideal density of substrate in nurseries must be equivalent to 40-50% of the pond area as it reduces the requirement of feed by 30-40%. Jaraqui production of 250 kg/ha was obtained in the present study of four-month duration, with an extrapolated production of 750 kg/ha/yr. Resende *et al.* (1985) obtained jaraqui biomass gain of 711.6 kg/0.6 ha/yr, equivalent to a yield of 1,267 kg/ha/yr. The difference in performance is associated with the differences in growing conditions and the duration of culture. While our study was conducted in masonry tanks of 46 m^2 with average depth of 0.7 m, Resende *et al.* (1985) conducted the experiment over 386 days under semi-intensive conditions in stream dam ponds, which among other factors, may have higher dietary sources available for the fish.

The specific growth rate (P=0.1560) and percentage weight gain (P=0.2510) showed no significant difference between treatments. Gangadhar and Keshavanath (2012) recorded a specific growth rate of 2.7% in a study on the effect of sugarcane bagasse density on the production of rohu (*Labeo rohita*). The condition factor, that reflects the physiological conditions of the fish, was also not significantly different (P= 0.2372) between treatments, the values ranging from 1.9 to 2.3 (Table 7). Values above one reflect satisfactory growth of fish. Higher condition factors indicate good health with isometric growth, which is desirable in fish farming (Ayode, 2011). The highest condition factor values were noticed in the control and 20% artificial substrate treatment and the lowest in 10% natural substrate treatment. These values were superior to those obtained in the cultivation of the same species in semi-intensive conditions (0.95 to 1.13) by Resende *et al*. (1985).

Conclusions

Natural substrate (*P. stratiotes*) supported higher average periphyton biomass as well as species diversity than the artificial substrate, plastic screen. Growth performance of jaraqui was better with 20% density of natural substrate, it being superior to the three densities of artificial substrate tested. The types

and densities of substrates did not drastically influence water quality.

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