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Biofilm feeding by postlarvae of the pink shrimp *Farfantepenaeus brasiliensis* (Decapoda, Penaidae)

Verónica E. Viau[1], Diego Moreira de Souza[2], Enrique M. Rodríguez[1], Wilson Wasielesky Jr[2], Paulo C. Abreu[2] & Eduardo L. C. Ballester[2,*]

[1]Department of Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Buenos Aires, Argentina

[2]Institute of Oceanography, Federal University of Rio Grande, Rio Grande do Sul, Brazil

Correspondence: E M Rodríguez, Department of Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Ciudad Universitaria, Pab. II, C1428EHA Buenos Aires, Argentina. E-mail: enrique@bg.fcen.uba.ar

***Present address**: Shrimp Culture Laboratory, Federal University of Paraná, Campus Palotina, Pioneiro 2153, Palotina, Paraná, CEP, 85950-000, Brazil

Abstract

The effect of biofilm was assayed for *Farfantepenaeus brasiliensis* postlarvae fed with commercial pellets. Indoor tanks in a zero water exchange system were used, considering: shrimp fed with biofilm and commercial feed (B+F), and shrimp fed only with commercial feed (F); both receiving polyethylene sheets as artificial substrates. For B+F, sheets were placed 15 days before the trial into a heterotrophic medium (containing diatom *Thalassiosira weissflogii*, commercial feed, molasses and wheat bran in a 20 C:1 N ratio) to promote biofilm development. For F, clean sheets were used and daily replaced to avoid biofilm formation. Biofilm chlorophyll *a* concentration, micro-organisms density and water quality were determined weekly. Also, a stomach content analysis was made. An increase in chlorophyll *a* concentration was observed in biofilm during the experiment, characterized mainly by pennate diatoms, filamentous cyanobacteria, flagellates, ciliates, nematodes and rotifers. Most of these items were found in the stomach of shrimp; however, no significant differences in growth were detected between treatments. Survival was significantly higher and nitrite concentrations were significantly lower when biofilm was present. The results indicate that the use of biofilm could be considered a good tool during *F. brasiliensis* nursery phase, mainly by improving survival through the maintenance of water quality.

Keywords: *Farfantepenaeus brasiliensis*, pink shrimp, biofilm, artificial substrate, water quality, nursery

Introduction

Crustacean culture is one of the main activities developed in the world aquaculture. Currently, 50% of consumed shrimp and specifically 70% of marine penaeid shrimp commercialized are cultured in farms (FAO 2008). Although the shrimp culture in Brazil is performed with the exotic species *Litopenaeus vannamei*, native species of shrimp as *Farfantepenaeus brasiliensis* have shown potential for the culture (Lopes de Alcantara, Wasielesky, Ballester & Peixoto 2009) and the production in alternative low cost structures such as cages and pen enclosures that require little investment (Wasielesky 2000). Culturing of indigenous shrimp for either restocking programmes or bait is other interesting possibility for native species (D'Incao, Valentini & Rodrigues 2002; Preto, Pissetti, Wasielesky, Poersch & Cavalli 2009).

During the production of marine shrimp, the implementation of a nursery phase (intermediate culture between the production of postlarvae and final growth-out), could have several benefits including more robust, healthy and uniform shrimp juveniles at harvest; optimize farming infrastructure; increase level of biosecurity and even improve tolerance of shrimp to environmental fluctuations and presence of predators (Samocha, Cordova, Blancher & de Wind 2000; Yta, Rouse & Davis 2004).

Furthermore, during the last few years researchers have been developing alternative production methods for shrimp culture based on the enhanced natural productivity in zero water exchange

systems (McIntosh, Samocha, Jones, Lawrence, McKee, Horowitz & Horowitz 2000; Browdy, Bratvold, Stokes & McIntosh 2001; Moss, Arce, Argue, Otoshi, Calderon & Tacon 2001; Burford, Thompson, McIntosh, Bauman & Pearson 2003; McAbee, Browdy, Rhodes & Stokes 2003; Browdy & Moss 2005; Wasielesky, Atwood, Stokes & Browdy 2006). Moreover, the implementation of artificial substrates to provide sites for the development of microbial community showed benefits for the culture (Bratvold & Browdy 2001; Shanker & Mohan 2001; Burford, Smith, Tarbrett, Coman, Thompson, Barllay & Toscas 2004; Moss & Moss 2004; Ballester, Wasielesky, Cavalli & Abreu 2007). In this way, biofilm is defined as a community of microorganisms composed by both autotrophic and heterotrophic organisms associated to an extra-cellular matrix mainly produced by bacteria adhered to a submersed surface. This matrix is composed by polysaccharides, proteins, nucleic acids and other polymers, as well as by compounds derivate from them (Ramesh, Shankar, Mohan & Varghese 1999; Davey & O'Toole 2000).

The use of biofilm in zero water exchange culture systems can be considered environmentally friendly due to decrease wastewater discharge, prevent potential spread of disease on the culture and minimize water use (McIntosh *et al.* 2000; Samocha, Lawrence, Collins, Emberson, Harvin & Van Wyk 2001; Weirich, Browdy, Bratvold, McAbee & Stokes 2002; Burford *et al.* 2003). Among the environmental benefits, the utilization of biofilm has shown positive effects on both survival and growth of white-shrimp *L. vannamei* (Bratvold & Browdy 2001; Moss & Moss 2004), as well as the pink shrimp *Farfantepenaeus paulensis* during the nursery rearing (Thompson, Abreu & Wasielesky 2002; Preto, Cavalli, Pissetti, Abreu & Wasielesky 2005; Abreu, Ballester, Odebrecht, Wasielesky, Cavalli, Granéli & Anésio 2007; Ballester *et al.* 2007), indicating that biofilm was carried out as complementary food source proving nutritional benefits for shrimp, and was responsible for water quality maintenance.

Studies on natural diet of Penaeidae have evidenced that shrimp ingest a variety of items being described as opportunistic omnivores, detritivores, carnivores and important predators (Stoner & Zimmerman 1988; Dall, Hill, Rothisberg & Staples 1990; Preston, Burford, Coman & Rothlisberg 1992; Cartes 1995). Bailey-Brock and Moss (1992) observed that postlarvae and shrimp juveniles consume both animal and plant matter, including microalgae, detrital aggregates, microfauna associated to macrophytes and small invertebrates. As shrimp grow, ontogenetic changes in food preference take place, and small invertebrates are replaced by larger invertebrate prey. Studies on feeding habit of *F. brasiliensis* and *F. paulensis* showed that their diet have a higher diversity of items grazing mainly on the periphytic microbial communities attached to the surface of aquatic macrophytes and superior plants (Albertoni, Palma-Silva & de Assis Esteves 2003; Soares, Wasielesky, Peixoto & D'Incao 2005).

The lack of studies on the pink shrimp *F. brasiliensis* concerning with the use of biofilm on its culture under experimental conditions, as shown in others penaeids shrimp, led us to evaluate the potential benefits of biofilm to the survival and growth of the species and on the water quality using artificial substrates in zero water exchange culture system during the nursery phase.

Materials and methods

Culture conditions

The experiment was conducted at the Marine Aquaculture Station FURG, Rio Grande, Rio Grande do Sul, Brazil (32°03′S, 52°12′W), during 30 days on May 2009. Six indoor circular tanks of polyethylene (0.38 m^2 of bottom surface) were employed and filled with 180 L of sea water, first filtered through a sand filter (1 mm grain size) and then through a CUNO® microfiltre (5 μm pore size). A zero water exchange system was carried out during the experimental period without renovation water, with the aim of eluding any perturbation for the accurate development of the biofilm; only dechlorinated freshwater was added to compensate the evaporation losses to keep salinity around 35 psu. The tanks were continuously and strongly aerated with air lifts pumps, the temperature was maintained at 27 ± 1°C by electric heaters and the photoperiod was 12:12 (L:D, natural light). All tanks were stocked with 43 postlarvae corresponding to a density of 100 shrimp m^{-2}, with overall mean of 0.053 ± 0.018 g, and distributed into two treatments with three replicates: (i) biofilm and feed treatment (B+F), where shrimp were fed with biofilm and commercial feed, and (ii) feed treatment (F), where shrimp were fed only with commercial feed, without biofilm.

Animals were fed three times a day with commercial diet (Supra®; 38% of crude protein) in

especially designed feeding trays (Wasielesky *et al.* 2006). Initial feeding rate was 15% of biomass/day/tank, adjusting it daily qualitatively according to the feed consumption observed in trays to avoid mortality on the cultured shrimp by the stress caused for successive biometrics.

All tanks received two polyethylene sheets (62 × 50 cm, wide and high respectively, mesh size 1 mm) as artificial substrates to allow the development of the biofilm, and additionally 15 thin polyethylene sheets (3 × 50 cm, wide and high respectively, mesh size 1 mm) used as samplers for determination of biofilm composition during the experiment, providing a total area submerged of 0.68 m^2 per tank for biofilm attachment. Both substrates and samplers were placed vertically in the water column (40 cm depth), being fixed to a line in the upper and to plumb ballast line in the bottom of each tank. Before deployment in the B+F tanks, polyethylene sheets and samplers were placed into a culture tank containing heterotrophic medium, 15 days before beginning the experiment to allow biofilm development.

The culture tank consisted in an enclosed raceway system filled with filtered sea water (as above mentioned) continuously aerated with air lifts, photoperiod 12:12 (L:D, natural light), temperature 27 ± 2°C, salinity of 35 psu and total ammonium below 1 mg L^{-1}. To promote the development of the heterotrophic medium, the tank was inoculated with diatom *Thalassiosira weissflogii* (2 × 10^4 cells mL^{-1}) and fertilized with commercial shrimp diet (Supra®; 38% of crude protein), molasses and wheat bran. The nominal C:N ratio of the daily organic matter additioned to the tank was 20:1, according to previous studies (Chamberlain, Avnimelech, McIntosh & Velasco 2001; McIntosh 2001; Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz & Brock 2007). No water exchange was carried out during the formation of the heterotrophic medium. Once the biofilm was developed, the polyethylene sheets were transferred to each experimental tank assigned to B+F group.

On the other hand, with the purpose to simulate similar experimental conditions, clean polyethylene sheets were placed in the tanks of the F treatment at the beginning of the experiment. Nevertheless, polyethylene sheets were daily replaced throughout the 30 days period to avoid biofilm formation; as is well known, biofilm formation and bacterial colonization occur immediately after the immersion of any surface in the water (Whal 1989). Extreme care was taken during changing artificial substrates to minimize shrimps stress.

Biofilm sampling and monitoring

Biofilm dry weight, chlorophyll *a* concentration and micro-organisms density were determined weekly from three 3 × 3 cm samplers, removed 25 cm below the water surface from every tank assigned to the B+F treatment.

Biofilm dry weight was determined by difference in the sampler weight before and after exposition in the water. Sampler was dried at 60°C until constant weight. For determination of chlorophyll a concentration each sampler was placed into vials with 10 mL of acetone 90% v/v (Merck®, Whitehouse Station, NJ, USA) and stored for 24 h in the darkness at −12°C. Concentration of pigment was then determined in a fluorometer (Turner TD 700, Sunnyvale, CA, USA), according to Strickland and Parsons (1972). For micro-organisms density determination, each sampler was placed into vials with 20 mL of sterilized saline water and fixed with Lugol 2%. Biofim was detached from the sampler sheet using an ultrasonic homogenizer (4710 Series; Cole Parmer Instrument Co., Vernon Hills, IL, USA), by applying three pulses of 10 s duration at 20 kHz with intervals of 10 s to prevent an increase in temperature and to avoid disruption of the micro-organisms (Thompson *et al.* 2002). Then, samples of the detached biofilm were analysed under binocular microscope (Olympus® BX 51, 100 to 1000× magnification) to identify the main groups of micro-organisms presented, considering filamentous cyanobacteria, diatoms, flagellates, ciliates, rotifers and nematodes. Additional samples of the detached biofilm were diluted with distilled water, transferred to sedimentation chambers and left there for 24 h for determination of micro-organisms density, according to Utermöhl (1958). For this purpose, a minimum of 30 randomly selected fields were counted in each chamber, using an inverted light microscope provided with phase contrast (Olympus® IX 51 TL 14, 100 to 1000× magnification). For filamentous cyanobacteria, totally filamentous observed in each field were considered while for flagellates, both autotrophic and heterotrophic organisms were regarded for counting. Finally, both dilution (between 0.1 and 1 mL) and microscope magnification (above mentioned) were considered for determination of the density for each micro-organism presented in the samples.

Shrimp monitoring

At the end of the experimental period, shrimp survival and body weight (nearest 0.01 g) were recorded for each treatment, and weight gain was then estimated. Furthermore, at days 15 and 30, five shrimp juveniles from each tank for both treatments were sampled and fixed in formalin 5% for stomach content analysis. The pro-ventricle of each shrimp was carefully dissected and its content was spread on a glass and observed in the inverted microscope (above mentioned) to characterize and count the different items (Thompson *et al.* 2002).

Water quality monitoring

Water quality was monitored throughout the experimental period. The following variables were daily determined: temperature (mercury thermometer, precision ±0.5°C), pH (Digimed™ digital pHmeter; Digimed, São Paulo, Brazil precision ±0.01), light intensity (Chauvin Arnoux CA810 Digital Luxmetre 20000 lux (Chauvin Arnoux, Paris, France) range, precision ±3%), dissolved oxygen (Digimed™ digital oximeter; Digimed, precision ±0.01) and salinity (Atago™ optic rephractometer; Atago, Tokyo, Japan, precision ±1 psu). Three times a week, a water sample was taken from each tank to determine the concentrations of ammonium (Unesco 1983), nitrite (Bendschneider & Robinson 1952) and phosphate (Aminot & Chaussepied 1983). Moreover, water samples taken at day 1, 15 and 30 of the experiment, and were used to determine the water chlorophyll *a* concentration, according to Strickland & Parsons (1972).

Statistical analysis

Statistical differences between treatments of survival, body weight and weight gain were analysed using the Student's *t*-test (Sokal & Rohlf 1995). In the case of percentages, the angular transformation was previously applied. For determination of differences in biofilm dry weight, chlorophyll *a* concentration and micro-organisms, the non-parametric Friedman test (for non-independent samples) was used, followed, when necessary, by the Dunn test for multiple comparisons between means.

Water quality data were compared using a two-way ANOVA (time and treatment). The same statistical test was used for comparing the abundance of the items found in stomachs of shrimp at days 15 and 30. Homogeneity of variance was previously checked by the Cochran test, whereas normality was verified by the Kolmogorov–Smirnov test; transformation of data was eventually made. Tukey test was used for multiple comparisons between means. A significance level of 5% was always considered.

Results

Biofilm sampling

For the biofilm developed on the artificial substrate used in the B+F treatment, a significant ($P < 0.05$) increase in chlorophyll *a* concentration was noted during the experiment reaching its maximum value at day 30, whereas dry weight showed a slight increment through time, but no significant ($P > 0.05$) differences were determined (Fig. 1). Composition of the biofilm formed on the artificial substrates showed the following items: pennate diatoms, filamentous cyanobacteria, flagellates, ciliates, nematodes and rotifers. The statistical comparison in the mean number of micro-organisms per cm^2 of sampler sheet among sampling dates indicates a significant ($P < 0.05$) increment of pennate diatoms, filamentous cyanobacteria and flagellates throughout the experiment, whereas no differences ($P > 0.05$) were observed concerning ciliates, nematodes and rotifers (Figs 2–7).

Shrimp production and biofilm as food

No significant ($P > 0.05$) differences were detected for final body weight nor weight gain between treatments, whereas survival of shrimp maintained

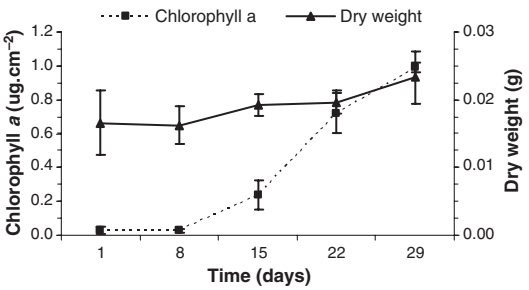

Figure 1 Chlorophyll *a* concentration and dry weight (±SE) of biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis.*

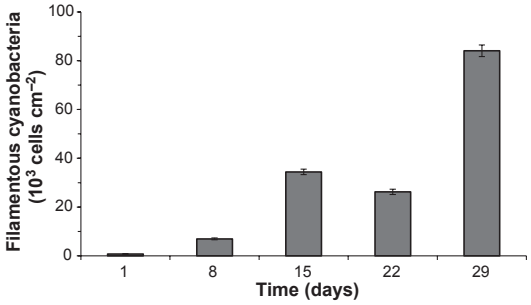

Figure 2 Mean number (±SE) of filamentous cyanobacteria per cm² in biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis.*

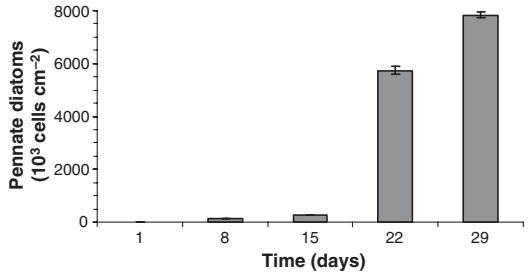

Figure 3 Mean number (±SE) of pennate diatoms per cm² in biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis*

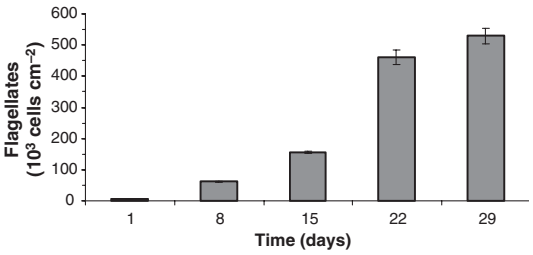

Figure 4 Mean number (±SE) of flagellates per cm² in biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis.*

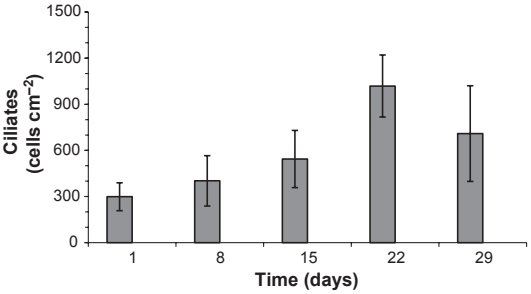

Figure 5 Mean number (±SE) of ciliates per cm² in biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis.*

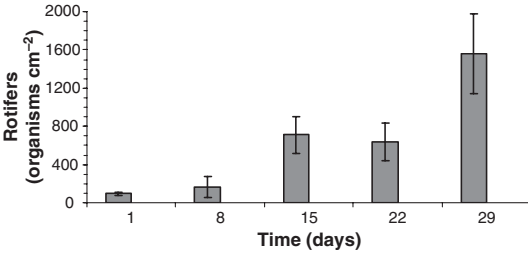

Figure 6 Mean number (±SE) of rotifers per cm² in biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis.*

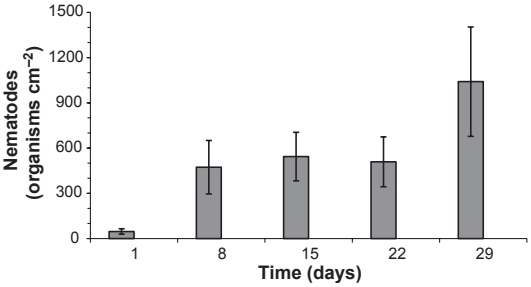

Figure 7 Mean number (±SE) of nematodes per cm² in biofilm developed on the artificial substrate used in the B+F treatment during the experiment with shrimp–postlarvae of *F. brasiliensis.*

in the B+F treatment was significantly ($P < 0.05$) higher than that observed in the F treatment (Table 1).

The stomach content of shrimp sampled at days 15 and 30 in the B+F treatment showed presence of flagellates, pennate diatoms, filamentous cyanobacteria and rotifers as main items. The same items were observed in the F treatment at day 30, but only filamentous cyanobacteria and flagellates were seen at day 15 (Figs 8 and 9). Although a significant ($P < 0.05$) higher quantity of flagellates was registered at day 30 in comparison to day 15 for both treatments, the incidence of this item was significantly ($P < 0.05$) higher in stomach of the shrimp sampled from the B+F compared with the F group, for both sampling dates (Fig. 9).

Table 1 Growth response of shrimp postlarvae of *F. brasiliensis* cultured for 30 days in two different feed conditions (with or without biofilm)

Treatment	Initial weight (mg)	Final weight (mg)	Gain weight (%)	Survival (%)
B+F	53.53 ± 0.89[a]	157.57 ± 9.56[a]	195.09 ± 39.42[a]	53.49 ± 2.69[a]
F	52.36 ± 1.70[a]	160.61 ± 6.20[a]	208.13 ± 36.73[a]	40.31 ± 3.38[b]

Mean values of initial and final body weight, gain weight and survival (±SE for three replicates) with different superscripts indicating a significant difference ($P < 0.05$) between treatments: B+F, substrates with biofilm; F, substrates without biofilm.

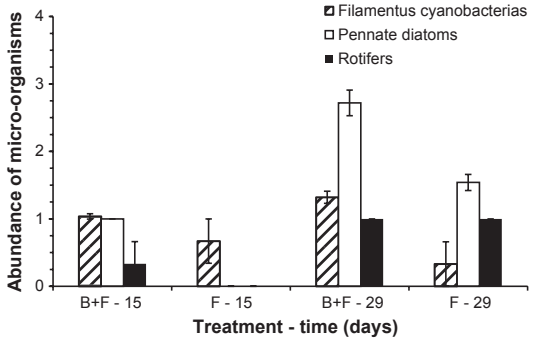

Figure 8 Mean number (±SE) of micro–organisms (filamentous cyanobacteria, pennate diatoms and rotifers) in the stomach of shrimp postlarvae of *F. brasiliensis* fed with (B+F) or without (F) biofilm during the 30 days experiment.

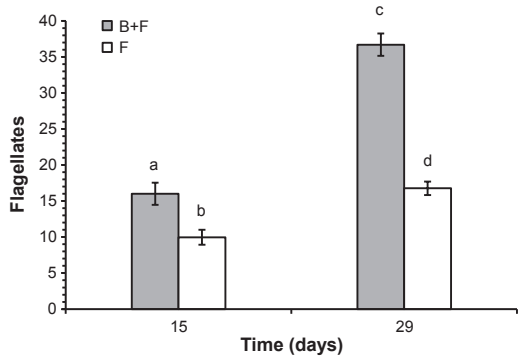

Figure 9 Mean number (±SE) of flagellates in the stomach of shrimp postlarvae of *F. brasiliensis* fed with (B+F) or without (F) biofilm during the 30 days experiment.

Water quality

Mean values of abiotic parameters monitored during the experiment are shown in Table 2. Temperature, pH, light intensity, dissolved oxygen, salinity, phosphate and ammonium did not show any significant difference ($P > 0.05$) between treatments at any sampling time. However, from day 15 up to the end of the experiment, nitrite concentration was significantly ($P < 0.05$) higher in the F treatment compared with B+F in which the nitrite was maintained at low levels throughout the experiment. On the other hand, from day 15 up to the end, water chlorophyll *a* concentration in the water was significantly ($P < 0.05$) lower in F treatment.

Discussion

Most water quality parameters values monitored during the experiment were within the optimum range stated for marine shrimp (Van Wyk & Scarpa 1999), except for nitrite concentration at F treatment attaining levels higher than that recommend for the culture, probably contributing to the higher mortality detected in this treatment. On the contrary, the significant lower nitrite concentration found in the B+F treatment was probably caused by nitrifying bacteria and autotrophic micro-organisms associated to the biofilm developed on the artificial substrates. In this sense, the significant increase on the density of pennate diatoms, filamentous cyanobacteria and flagellates registered in the biofilm showed an effective colonization of the submersed substrates, most probably contributing to the low ammonium and nitrite concentration throughout the experiment. In addition, the high nitrification rate recorder in this group might be due to the incorporation of the nitrogenous compounds into the biomass of the suspended autotrophic organisms in water, in which, development is expected from the increased chlorophyll *a* concentration registered in the water during the assay. On the contrary, the significant low concentration in chlorophyll *a* verified in the water of the F treatment, with respect to the B+F group, could suggest that the proliferation of a suspended autotrophic community through the 30-day experiment was not sufficient to regulate

Table 2 Water quality parameters registered in tanks containing shrimp postlarvae of *F. brasiliensis* cultured for 30 days in two different feed conditions (with or without biofilm)

Parameter	Treatment	Mean	Maximum	Minimum
Temperature (°C)	B+F	26.53 ± 0.20[a]	29.1	24.57
	F	26.63 ± 0.31[a]	30.83	23.2
pH	B+F	8.44 ± 0.03[a]	8.71	7.87
	F	8.43 ± 0.04[a]	8.77	7.72
Light intensity (lx)	B+F	163.43 ± 64.29[a]	320	99
	F	165.02 ± 62.57[a]	320	103
Dissolved oxygen (mg L^{-1})	B+F	6.41 ± 0.10[a]	7.79	5.39
	F	6.44 ± 0.10[a]	7.85	5.54
Salinity (psu)	B+F	35 ± 1.5[a]	37	33
	F	35 ± 1.2[a]	37	34
Ammonium (mg L^{-1})	B+F	0.39 ± 0.09[a]	0.87	0.05
	F	0.50 ± 0.11[a]	1.06	0.07
Nitrite (mg L^{-1})	B+F	0.29 ± 0.12[a]	1.09	0.02
	F	1.43 ± 0.25[b]	3.23	0.24
Phosphate (mg L^{-1})	B+F	0.19 ± 0.02[a]	0.3	0.06
	F	0.20 ± 0.02[a]	0.3	0.07
Chlorophyll *a* (μg L^{-1})	B+F	1.36 ± 0.72[a]	3.55	0.3
	F	0.49 ± 0.29[b]	1.2	0.05

Mean values (±SE for three replicates) with different superscripts indicate a significant difference ($P < 0.05$) between treatments: B+F, substrates with biofilm; F, substrates without biofilm. The range of values observed for each parameter is indicated with a maximum and minimum value.

the uptake of the nitrogen present in the water column, which could explain the higher nitrite levels observed in this treatment. Similar results were found by Thompson *et al.* (2002) during the culture of *F. paulensis* juveniles in tanks with biofilm, explaining them by a possible relationship between low ammonium levels and the presence of autotrophic micro-organisms in the biofilm. Likewise, during the culture of *Cherax quadricarinatus* juveniles, the presence of biofilm in the tanks reduced both ammonium and nitrite concentrations through promotion of nitrification, whereas those tanks without biofilm showed increased levels of both toxic metabolites resulting in a higher mortality of the cultured juveniles (Viau, Ostera, Tolivia, Ballester, Abreu & Rodríguez 2012). Similarly, other studies carried out in aquaculture systems showed that algae periphyton assimilated ammonia and trapped organic matter from the water column to increase their biomass and thus, as algal density increased, ammonia concentration declined (Langis, Proulex, de la Noue & Couture 1988; Hargreaves 1998; Avnimelech 1999). In fact, the depuration capacity of the biofilm is a property known in water quality studies (Lowe & Pan 1996), in which some micro-organisms, such as bacteria and autotrophic algae, incorporate the nitrogen available in the water column, reducing and maintaining the ammonia levels. Also, according to Bratvold and Browdy (2001), the action of nitrifying bacteria and autotrophic organisms is more efficient if they are attached to a substrate.

In this study, during the early stage of biofilm formation, a lower density of pennate diatoms, filamentous cyanobacteria and flagellates was found with respect to previous experiments made under similar experimental conditions (Thompson *et al.* 2002). In contrast, higher densities of ciliates, nematodes and rotifers were noted during this early stage, compared with previous studies besides equal conditions (Preto *et al.* 2005; Ballester *et al.* 2007). According to Whal (1989), the colonization pattern for biofilm development strongly depends on the micro-organisms specific capacity to colonize a given surface, and on the environmental conditions determining the kind of organisms able to proliferate, suggesting that those higher size micro-organisms (such as ciliates, nematodes and rotifers) colonize substrates during a more advanced stage of biofilm formation. Moreover, the composition of the biofilm is the result of different factors, such as physical and chemical characteristics of water column and the type of substrate used for its development. In the present assay, before starting the experiment, artificial substrates were immersed in a heterotrophic system during 15 days to seed the micro-organisms for colonizing them. In this kind of system, although, the competition with bacteria for nutrients, the high water turbidity, as well the low incidence of

light inside the water column as a result of the high nutrient loading, could limit growing of autotrophic organisms (Burford *et al.* 2004), while the proliferation of ciliates, nematodes and rotifers is expected to be enhanced (Ferreira Da Silva 2009). In this regard, the conditions of the experimental tanks at the beginning of the current assay were different from those observed in the conditioning tank where biofilm was grown in terms of better light conditions and nutrient loading, among others factors, probably allowing the proliferation of autotrophic organisms (such as pennate diatoms, filamentous cyanobacteria and flagellates), and thus an increase in chlorophyll *a* concentration as shown during the 30-day experiment.

On the other hand, and as mentioned above, the conformation of the biofilm might be conditioned by the artificial substrate in terms of their physical characteristics, such as the microhabitats given by the pores and/or the texture of the mesh. In this sense, a variety of biodegradable and non-biodegradable substrates have been used in the past to enhance shrimp and crayfish production in periphyton-based aquaculture systems. Among these substrates, a commercially available fibrous synthetic material with a plant-like appearance (AquaMats® Meridian Aquatic Technology, LLC, Calverton, MD, USA) has been widely used for culture because of a high-quality biofilm production in terms of nutritional value for the cultured animals and water quality (Bratvold & Browdy 2001; Jones, Thanuthong & Kerr 2002; Moss & Moss 2004; Wasielesky *et al.* 2006; among others), but the higher cost resulted prohibitive for small-scale rural farmers. Instead, commercial polyethylene sheets used as artificial substrate have recently replaced the AquaMats® system because of their similar physical characteristics (e.g. pore size provide free space to be colonized by micro-organisms allowing a good biofilm growth, just like the AquaMats® microhabitats), and the significantly low cost, widespread availability and ease of use. The polyethylene sheets as substrate material for biofilm development have been proved with good results during the culture of *F. paulensis* (Thompson *et al.* 2002; Preto *et al.* 2005; Abreu *et al.* 2007; Ballester *et al.* 2007; between others) and *C. quadricarinatus* (Viau *et al.* 2012), as well on the contribution to the biofilm nutritional quality (Fernandes Da Silva, Ballester, Montserrat, Geracitano, Wasielesky & Abreu 2008). In the current experiment, this last material was evaluated as a potential substrate for the culture of the studied species with the aim to prove technology that enhance farm productivity and reduce manufacture costs. However, it should be considered that the quality of the biofilm will depend on the experimental conditions in which substrate will be assayed, and thus the food offering would be nutritionally different for the cultured animals. Azim, Verdegem, Khatoon, Wahab, van Dam and Beveridge (2002) compared three alternative substrates and fertilization methods for the production of three carps species under the same ponds conditions, observing differences in both the quality and quantity of the periphyton development, water quality and fish production, suggesting that such differences depends on the substrate material and on the fertilizers used in the culture.

Analysis of stomach content showed that the main consumed item for all shrimp was flagellates, followed by pennate diatoms and filamentous cyanobacteria, and only in stomach of some bigger postlarvae remains of rotifers were also seen. Nevertheless, no nematodes rests were found, probably due to their easy digestibility, as suggested by other authors (Fernandes Da Silva *et al.* 2008). Furthermore, the development of all the feeding apparatus (such as mandibular appendices and gastric mill), needed for eating and processing relatively big size zooplankton, seems to gradually complete during the ontogeny of penaeids (Bailey-Brock & Moss 1992). Actually, no studies were conducted on *F. brasiliensis* concerning food consumption during the nursery phase. Instead, a study carried out on *F. paulensis*, a related species, found that both food consumption and food preference are related to shrimp body size (Soares, Peixoto, Wasielesky & D'Incao 2005; Soares, Wasielesky *et al.* 2005), as observed in others shrimp penaeids (Hill & Wassenberg 1992; Nunes & Parsons 2000). Nevertheless, controversial results have been reported. For instance, Thompson, Abreu and Cavalli (1999) have previously shown evidences about the selectivity of *F. paulensis* juveniles for the consumption of flagellates, in accordance with the results observed in the current study. Later, Thompson *et al.* (2002) suggested that *F. paulensis* postlarvae reared in concrete tanks fed non-selectively on the biofilm. On the other hand, Abreu *et al.* (2007) provided evidence that *F. paulensis* juveniles preferentially consumed centric diatoms, as observed previously in others studies (Ballester *et al.* 2003; Preto *et al.*

2005). Besides, Pissetti (2004) and posteriorly Ballester *et al.* (2007) found that highest growth rate of *F. paulensis* juveniles were related to the consumption of biofilm colonized by nematodes. Although all these organisms are referred as being part of the diet of penaeids shrimps in the natural environment (Soares, Peixoto *et al.* 2005; Soares, Wasielesky *et al.* 2005), a careful interpretation of the results in the context of the specific experimental conditions and in the methods used to evaluate food consumption is needed. In this sense, several mentioned studies have evaluated the relevance of the biofilm as a food source for shrimp, either by gut content analysis or by changes in the number of potential preys items in biofilm during the experiment. The difficulty to estimate food consumption through analysis of stomach content, due to high trituration that food items caused by the gastric mill, was previously mentioned by Abreu *et al.* (2007). However, this kind of analysis has considered detecting several important items fed by shrimp (Soares, Peixoto *et al.* 2005). In the present study, food consumption was evaluated through gut content analysis, observing that some mouthparts of the small *F. brasiliensis* postlarvae may not be probably adapted to eat bigger organisms as nematodes, thus consuming mostly smaller items such as flagellates, pennate diatoms and cyanobacteria. Further research would be needed to elucidate this issue.

Regarding the consumed items observed in the stomach of shrimp corresponding to the F treatment, it could have resulted from the natural productivity development in the water column of the culture tanks throughout the experiment, probably favoured by the accumulation of organic matter due to dead animals or exuviae, as seen in previous studies (Thompson *et al.* 1999; Decamp, Conquest, Forster & Tacon 2002; Moss & Moss 2004). In this last treatment a higher incidence of micro-organisms was detected at day 30 with respect to those sampled at the 15. Nevertheless, a quantity significantly higher of all identified items was seen in stomach of shrimp from the B+F treatment compared with those of F, both at days 15 and 30 of the experiment. This difference is positively related with the higher density of micro-organisms presented in the biofilm formed on the substrates added in the tanks of the B+F treatment, suggesting that a high consumption of the organisms forming the biofilm by shrimp is taking place.

In this sense, the bottom of the tanks of the F treatment is the only substrate on which some benthic algae and others associated organisms can settle and grow. Instead, in periphyton-based group (B+F), the substrate added increase the area in which algae, zooplankton and small invertebrates can colonize, and thus, shrimps can graze on these concentrated food items more efficiently than on planktonic foods only.

Despite of the statistically higher consumption of biofilm micro-organisms by shrimp cultured in the B+F treatment, compared with the F treatment, no significant differences in either mean body weight or weight gain were detected between both treatments. Nevertheless, a significant higher survival was detected when biofilm was provided to the culture tanks. Relative few studies were carried out on the studied species concerning the nursery phase in tanks; however, these results were partially in accordance with previous studies made on *F. paulensis* during the same phase. With regard to this, Thompson *et al.* (2002) observed for juveniles, and posteriorly Ballester *et al.* (2007) for postlarvae of this last species, that both mean body weight and survival were higher for those shrimp raised in tanks with presence of biofilm. During this study, the better survival in shrimp whose diet was supplemented with biofilm could not only be related mainly to maintenance of the water quality throughout the experiment (as above mentioned) but also to a more complete nutrition when compared with shrimp with no biofilm available. Immune function, among other physiological processes, could be enhanced by the natural food source represented by biofilm, where probiotic bacteria can proliferate (Moriarty & Decamp 2009). Several micro-organisms presented in biofilms could serve as supplemental feed source (Moss 2002; Thompson *et al.* 2002; Moss & Moss 2004; Fernandes Da Silva *et al.* 2008), suggesting that diatoms, ciliates and flagellates represent high nutritional value items for penaeid shrimp. Through stable isotope analysis, Stoner and Zimmerman (1988) showed that 20–25% of the diet of three species of the genus *Farfantepenaeus* consisted in benthonic microalgae, consumed together with detritus. Recently, Abreu *et al.* (2007) using the same method showed that micro-organisms present on biofilm can supply up to 80% of the nitrogen demands of *F. Paulensis* larvae. Particularly, flagellates have a high protein:energy ratio, being also capable to synthesize polyunsaturated fatty

acids from more simple fatty acids taken from consumed bacteria (Zhukova & Kharlamenko 1999). González-Baró and Pollero (1998) determined in *Macrobrachium borelli* that the main sources of both arakidonic (20:4n-6) and eicosapentaenoic (20:5n-3) acid was represented by either biofilm or detritus consumed by prawns in its natural environment. Fernandes Da Silva *et al.* (2008) characterized filamentous cyanobacteria, as well as heterotrophic flagellates presented in biofilm, as a relevant source of lipids, whereas diatoms could supply both protein and lipids of high nutritive value.

Conclusion

The results of the current study have evidenced the usefulness of biofilm in the production of *F. brasiliensis* during the nursery phase. Biofilm contribution represents a clear advantage for maintenance of water quality in culture tanks, as well as to a high survival of postlarvae shrimp. Hence, using biofilm in a zero water exchange system, a reduction in the cost of production could also be attained. Nevertheless, the contribution of biofilm to the cultured shrimp would be relative to the type of biofilm that is experienced. Thus, the use of biofilm as an alternative and/or complementary food source for the culture is discussed in the present study. Further research should focus to analyse the trophic evolution of the micro-organisms community that conform the biofilm to improve their nutritional value, and thus their contribution as a food source to the shrimp culture.

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