The use of microorganisms as food source for *Penaeus paulensis* larvae

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Abstract

Three experiments were conducted to test the usefulness of microorganisms as food source for the *Penaeus paulensis* larvae. Larvae fed only bacteria survived longer (3 days) than those cultured in filtered (< 1.0 μm) seawater. However, they grew better when fed flagellates and ciliates, reaching the protozoa II stage after 8 days. Gut content analysis showed that the ciliate *Cristigera minuta* was heavily grazed by the larvae. The addition of microalgae and *Artemia* sp. nauplii besides microorganisms did not result in increased survival. However, all larvae that received supplementary food in the form of flagellates and ciliates showed larger cephalotorax length than those in the control treatments.

Keywords: Microorganisms; Shrimp; Larvae; Food

1. Introduction

The culture of marine shrimp has shown an exceptional increase worldwide, mainly due to improvement of methods and techniques related to hatching and rearing of larvae (Barnabé, 1990). Among these, the use of antibiotics assured higher production of larvae due to the suppression of pathogenic bacteria (Garriques and Arevalo, 1995). However, after some time, resistant microorganisms appear, demanding increasing amounts of antibiotics for its elimination (Dixon, 1994).

In addition to the use of antibiotics, water exchange is also applied in order to reduce the abundance of pathogenic bacteria and maintain water quality. Nevertheless, this contributes to the lowering of microorganisms responsible for nutrient recycling in the

system, deteriorating the water quality, and leading to the use of intensive water treatments and manipulations (Maeda, 1994). More recently, studies have tested the use of microorganisms in the control of microbial infections in rearing larvae with promising results (Maeda, 1989; Moriarty, 1990; Nogami and Maeda, 1992; Maeda, 1994; Austin et al., 1995; Gil, 1995). The addition of selected bacteria (probiotics) in tanks or ponds may control deleterious forms through: (a) competitive exclusion of pathogenic bacteria; (b) enhanced nutrition of larvae by supplying essential enzymes; and (c) production of antibiotic substances that inhibit the growth of undesired cells (Garriques and Arevalo, 1995). Similarly, the use of microbially matured water selects non-opportunistic bacteria and thus protects larvae from the proliferation of pathogenic bacteria (Skjermo et al., 1997).

Aside from the properties listed above, bacteria can also act as a food source for larvae either by direct consumption, or by fueling aquatic and benthic foodwebs in aquaculture systems. Such features have been well documented for natural ecosystems, following the seminal papers of Pomeroy (1974) and Azam et al. (1983) who showed the importance of microorganisms in the transfer of matter and energy through aquatic food chains. However, few studies have been conducted in order to evaluate the importance of microorganisms as food source for marine larvae (Moriarty et al., 1983; Maeda, 1989; Moriarty, 1990; Douillet and Langdon, 1994; Moriarty, 1997).

In this study, we tested the hypothesis of whether bacteria and protozoan (flagellates and ciliates) enhance the survival of the critical first stages of the larvae of *Penaeus paulensis* (Pérez-Farfante, 1967). Our objective was to verify if the type and size of microorganisms could influence the survival and growth of *P. paulensis* larvae.

2. Material and methods

Three experiments conducted during this study used larvae (nauplii VI) of the shrimp *P. paulensis* (Pérez-Farfante, 1967). The larvae were obtained in the laboratory using adults from wild broodstock captured along the Southern Brazilian coast (Santa Catarina and Rio Grande do Sul, 28°S–48°W). Maturation of the female was induced by unilateral ablation of the eyestalk, and manipulation of environmental conditions (Marchiori and Boff, 1983). The larvae, used in each experiment came from the same spawn, and were kept in tanks with controlled temperature (27°C) for 2 days until the nauplii VI stage was reached. During this period, there was no food supply, and the larvae subsisted on their own reserves. The experiments were begun with an initial density of 100 nauplii VI/l, in systems without water exchange, but with constant aeration. The seawater used in the experiments was previously filtered through a cartridge filter system (Cuno filters -1.0 μm pore size).

2.1. Experimental design (Fig. 1)

2.1.1. Experiment 1

Experiment 1 was conducted to verify if *P. paulensis* larvae could survive by feeding only on bacteria. The experiment was composed of two treatments, in duplicate (15 l

Experiment 1	
A	B
filtered (1 μm) seawater (filt. sw)	filt. sw + C:N:P = 1060:2:2

Experiment 2				
A	B	C	D	E
filt. sw	filt. sw + C:N:P = 530:80:5	filt. sw + C:N:P = 1060:160:10	½ filt. sw + ½ Enriched and Aged Seawater (EASW)	EASW

Experiment 3		
A	B	C
filt. sw + *Chaetoceros calcitrans* + *Artemia* sp.	½ filt. sw + ½ EASW + *C. calcitrans* + *Artemia* sp.	EASW + *C. calcitrans* + *Artemia* sp.

Fig. 1. Experimental design of the three experiments.

each). Treatment 1A (control) consisted of filtered seawater, while treatment 1B received an addition of nutrients (glucose—$C_6H_{12}O_6$, ammonium—NH_4Cl and phosphate—$Na_2HPO_4 \cdot 2H_2O$) making a C:N:P ratio of 1060:2:1.

2.1.2. Experiment 2

This experiment, comprising of five treatments (3 l in triplicate), was intended to test the survival of *P. paulensis* larvae feeding on larger microorganisms (flagellates and ciliates). Treatment 2A (control) consisted of filtered seawater. Treatments 2B and 2C received nutrients (glucose, ammonium and phosphate) making C:N:P ratios of 530:80:5 and 1060:160:10, respectively. Treatments 2D and 2E were composed of enriched and aged seawater (EASW), i.e., 3 days before the beginning of the experiment glucose, ammonium and phosphate were added to a tank containing seawater (C:N:P = 530:80:5). It was kept under constant temperature (27°C) and with aeration allowing the growth of bacteria but mainly flagellates and ciliates. Maximum densities of 3443 flagellates/ml and 810 ciliates/ml were reached during this period. Treatment 2D received half EASW and half filtered seawater by volume, whereas treatment 2E received only EASW.

2.1.3. Experiment 3

This experiment was designed to check the influence of bacteria and protozooplankton addition to standard larviculture practices, where microalgae and *Artemia* sp. nauplii

are normally supplied as the main food sources. The treatments (3 l volume in triplicate) were designed as follows. Treatment 3A consisted of filtered seawater. Treatment 3B received half EASW and half filtered seawater (by volume), whereas treatment 3C received only EASW. All treatments received the microalgae *Chaetoceros calcitrans* from the beginning to day 6, maintaining an abundance of 10^5 cells/ml in the tanks. Estimates of this microalgae abundance were made daily using Neubauer chambers and light microscope. After the fourth day newly hatched *Artemia* sp. nauplii (continental strain, Prime Artemia, UT, USA) were offered at a density of 5 organisms/ml.

2.1.4. Physical and chemical analysis

Water temperature, salinity, and pH were monitored daily using mercury thermometer (± 0.1°C), optical refractometer Atago (± 1) and pH meter (Digimed; ± 0.01). Samples for dissolved inorganic nutrients (ammonia and phosphate) were collected at the beginning and end of each experiment. Ammonia was measured according to UNESCO (1983) and phosphate as described in Strickland and Parsons (1972).

2.1.5. Enumeration of bacteria and protozooplankton

For the determination of bacterial, flagellates and ciliates abundance, the treatments were sampled each 36 h in experiment 1, and daily during experiments 2 and 3. Aliquots of 20 ml were fixed with Lugol (Throndsen, 1978) and kept in stoppered glass flasks. For bacterial and flagellates counts, volumes of 0.5–1.0 ml were stained with the fluorochrome Acridine Orange and visualized under epifluorescence microscope (Zeiss Axioplan) (Hobbie et al., 1977). The lugol present in the water was removed using one to two drops of 3.0% w/v sodium thiosulfate solution (Nishino, 1986). Ciliates abundance was determined by scanning the entire bottom of 10 ml Utermöhl chambers using inverted microscope (Utermöhl, 1958). Ciliate species was identified according to Kahl (1933), Curds et al. (1983) and Carey (1992).

2.1.6. Larvae

Larval survival was estimated by enumeration of organisms at the beginning and at the end of the experiments. The larval stage was determined, using light stereoscope microscope, according to Iwai (1978). In the third experiment the length of cephalotorax of larvae was estimated in 90 organisms of each treatment.

In all experiments the stomach contents of nine larvae per treatment, were observed using Acridine Orange and epifluorescence microscope. The larvae were fixed with a 4% v/v formalin solution. After this, their surface was cleaned by first submerging them in a 25% v/v ethanol solution for 10 min, followed by sonication (ultra-sound 20 kHz, 30 s, Cole Parmer Ultrasonic Homogenizer 4710). They were later washed three times in filtered 4% v/v formalin solution and placed over a darkened Nuclepore polycarbonate filter (0.2 μm pore size) settled on a glass slide, to create a dark background. Each larvae was covered with 2–3 drops of a 0.1% w/v solution of Acridine Orange before dissection. The dissection was conducted under a stereoscope microscope using two dissection needles. Two cross sections were made close to the mouth and at the end of the abdomen. The stomach content extruded when a cover slip was pressed on the larvae. After 10 min, the extruded material was stained by the Acridine Orange and readily visible under epifluorescence microscope.

Table 1
Temperature (°C), salinity, pH, ammonia and phosphate concentration (μM) values in each treatment during the three experiments

Treatments	Experiment 1		Experiment 2					Experiment 3		
	1A	1B	2A	2B	2C	2D	2E	3A	3B	3C
Temp. (°C)	27.5 (±0.5)	27.5 (±0.5)	27.0 (±1.0)	27.0 (±1.0)	27.0 (±1.0)	27.0 (±1.0)	27.0 (±1.0)	27.0 (±1.0)	27.0 (±1.0)	27.0 (±1.0)
Salinity	30 (±0)	30 (±0)	34 (±0)	34 (±0)	34 (±0)	34 (±0)	34 (±0)	34 (±0)	34 (±0)	34 (±0)
pH	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)
Ammonia (μM)										
Initial	–	0.39	9.04^{a} (±1.71)	109.04^{b1} (±12.78)	244.52^{c1} (±33.56)	34.28^{abd1} (±6.48)	24.76^{ad} (±5.85)	12.85^{a1} (±0.71)	23.57^{b1} (±2.29)	32.14^{c1} (±1.79)
Final	–	1.91	9.28^{a} (±0.41)	39.52^{ab2} (±3.36)	77.38^{b2} (±17.50)	24.76^{a2} (±5.85)	14.52^{a} (±1.86)	409.28^{2} (±30.07)	262.14^{2} (±8.93)	323.09^{2} (±51.32)
Phosphate (μM)										
Initial	–	0.17	1.37^{a1} (±0.02)	6.19^{b} (±0.27)	10.5^{c1} (±0.10)	1.94^{a1} (±0.05)	2.73^{d1} (±0.05)	1.54^{a1} (±0.01)	2.17^{b1} (±0.01)	2.79^{c1} (±0.02)
Final	–	2.46	0.97^{b2} (±0.01)	4.51^{a} (±0.65)	7.52^{c2} (±0.25)	4.79^{a2} (±0.41)	4.51^{a2} (±0.02)	6.56^{a2} (±0.05)	8.11^{b2} (±0.20)	7.38^{ab2} (±0.54)

Different letters and numbers indicate statistical differences ($P < 0.05$) among treatments and time, respectively.
Mean (±SE).

2.1.7. Statistical analysis

Differences of microorganisms number among treatments in the first experiment were tested using *t*-test (Sokal and Rohlf, 1969). The results of larval survival, cephalotorax length and ammonia and phosphate concentration of experiments 2 and 3 were submitted to the ANOVA method (Sokal and Rohlf, 1969). When significant differences among treatments were found ($P < 0.05$), we applied the a posteriori Tukey test for differentiation of the groups (Sokal and Rohlf, 1969).

3. Results

Table 1 summarizes the physical and chemical results of the three experiments. Temperature, salinity and pH were stable during the assays. Ammonia concentration increased in most treatments where there was no nutrient addition, or where ammonium and phosphate were added in low concentration in comparison to glucose (1B). In experiment 2, lower ammonia concentration was measured at the end of the study period in enriched treatments, indicating a direct uptake by bacteria, while in experiment 3 there was an increase of this nutrient towards the end of the experiment, probably due to food addition and larvae excretion. Similarly, phosphate concentration was higher at the end of the third experiment in all treatments. In experiment 2, only treatments with EASW (2D and 2E) presented an increase of phosphate at the final phase, while the concentration of this element showed a reduction in the control (2A) and treatments with nutrient addition (2B and 2C). Increase in phosphate concentration was observed at the end of the study period in treatment 1B of the first experiment.

The results of experiment duration, developmental stage and percentage of surviving larvae are shown in Table 2. For experiment 3, the larvae cephalotorax length is also shown. The duration of experiments increased from the first to the last, as well as the percentage of larvae that were alive at the end of the assays. In the first experiment no larvae survived beyond the protozoea I stage, while in treatments 2D and 2E of second experiment, they reached the protozoea II stage. At the end of the third experiment all larvae in the three treatments were in the post-larvae I stage, and those larvae in treatments 3B and 3C showed larger cephalotorax length than that hatched in filtered seawater (3A) (Table 2) ($P < 0.05$).

In the first experiment the number of small free bacteria varied between 0.01 and 15.08×10^6/ml with maximum values occurring in the enriched treatment 36 h after the beginning of the study (Fig. 2). Filamentous bacteria appeared in great numbers after three days in the containers that had nutrient addition ($0.6–7.78 \times 10^4$/ml). Flagellates ($0.49–8.17 \times 10^4$/ml) and ciliates ($0–6.62 \times 10^3$/ml) showed a trend to increase towards the end of the experiment (Fig. 2). Highest increase in ciliate numbers occurred after 72 h when most larvae were dead. In this experiment, most of the larvae were impregnated with organic matter and microorganisms and, at the final period, they were observed dead in the bottom of the containers, or showing very low activity.

Abundance of small free bacteria in the second experiment showed a significant increase in most treatments after 24 h ($0.21–11.04 \times 10^6$/ml; Fig. 3). The flagellate abundance responded to the increase in bacteria, showing maximum values at day 2

Table 2
Experiment duration (days), survival (%) and development stage reached by larvae during the three experiments

Treatments	Experiment 1		Experiment 2					Experiment 3		
	1A	1B	2A	2B	2C	2D	2E	3A	3B	3C
Experiment duration (days)	1	3	4	4	4	8	8	10	10	10
Survival (%)	0	15 (±0)	0.33 (±0.99)	2.97 (±0.58)	1.1 (±0.58)	3.96 (±3.96)	2.64 (±0)	90.5 (±5.85)	79.2 (±6.56)	85.7 (±7.82)
Development stage	PZ_I	PZ_I	PZ_I	PZ_I	PZ_I	PZ_{II}	PZ_{II}	PL_I	PL_I	PL_I
Cephalotorax size (mm)	–	–	–	–	–	–	–	1.458[a] (±0.009)	1.569[b] (±0.009)	1.569[b] (±0.006)

For experiment 3, the cephalotorax size is also shown (mm). Mean (±SE).
Different letters indicate statistical differences ($P < 0.05$) among treatments.

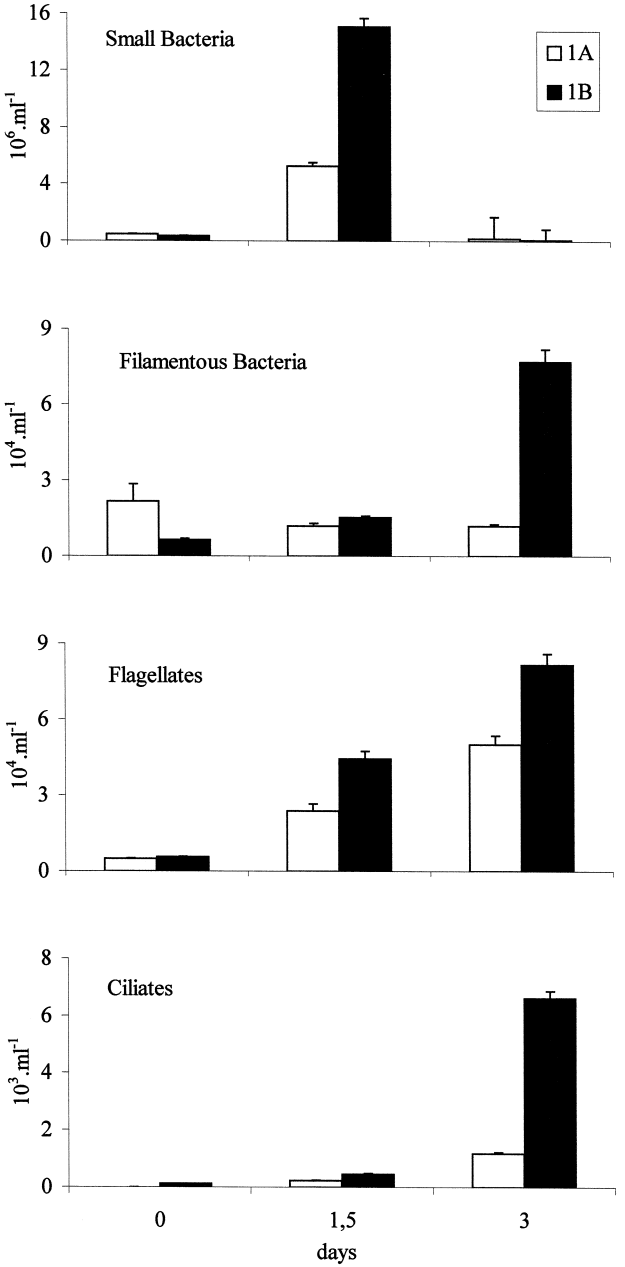

Fig. 2. Abundance of small (coccus and rod shape) and filamentous bacteria, flagellates and ciliates (Treaments 1A and 1B) during the first experiment.

(0–7.17×10^4/ml), although the number of these organisms was kept low in the treatments with EASW (Fig. 3—2D and 2E). Ciliates abundance varied between 0 and

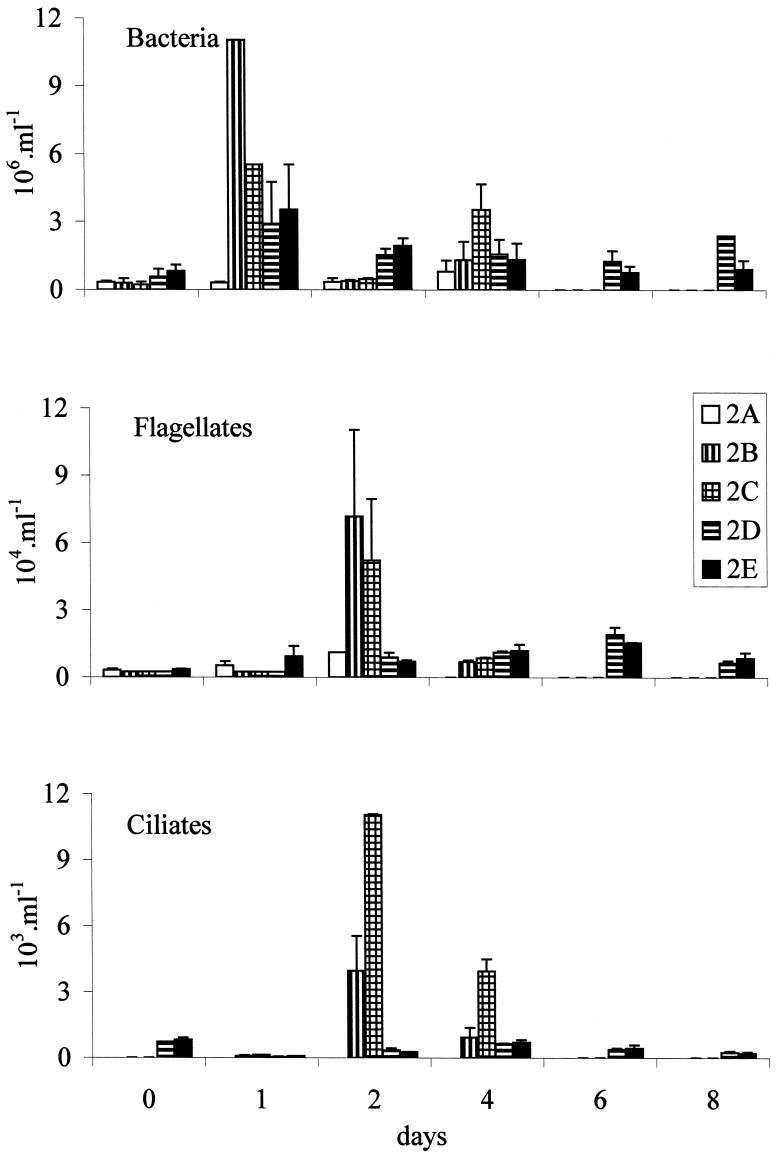

Fig. 3. Abundance of bacteria, flagellates and ciliates (Treatments 2A, 2B, 2C, 2D and 2E) during the second experiment.

11.48×10^3/ml. The ciliate *Cristigera minuta* showed a rapid growth between days 1 and 2 in the treatments with nutrient addition (2B and 2C), and its number dropped sharply in the following 24 h. The larvae in all treatments were covered with organic matter and microorganisms, especially in the control (2A) and enriched treatments (2B and 2C). At the end of the fourth day, most larvae of these treatments were concentrated

at the bottom of the containers. However, the larvae of treatments 2D and 2E were swimming actively in the tanks, even though their bodies were covered by organic matter.

In the third experiment, a significant increase in bacterial abundance was observed after the fourth day of experiment (0.5×10^6/ml to 6.9×10^7/ml) (Fig. 4). Since there was no significant differences in bacterial number among treatments (ANOVA, $P >$

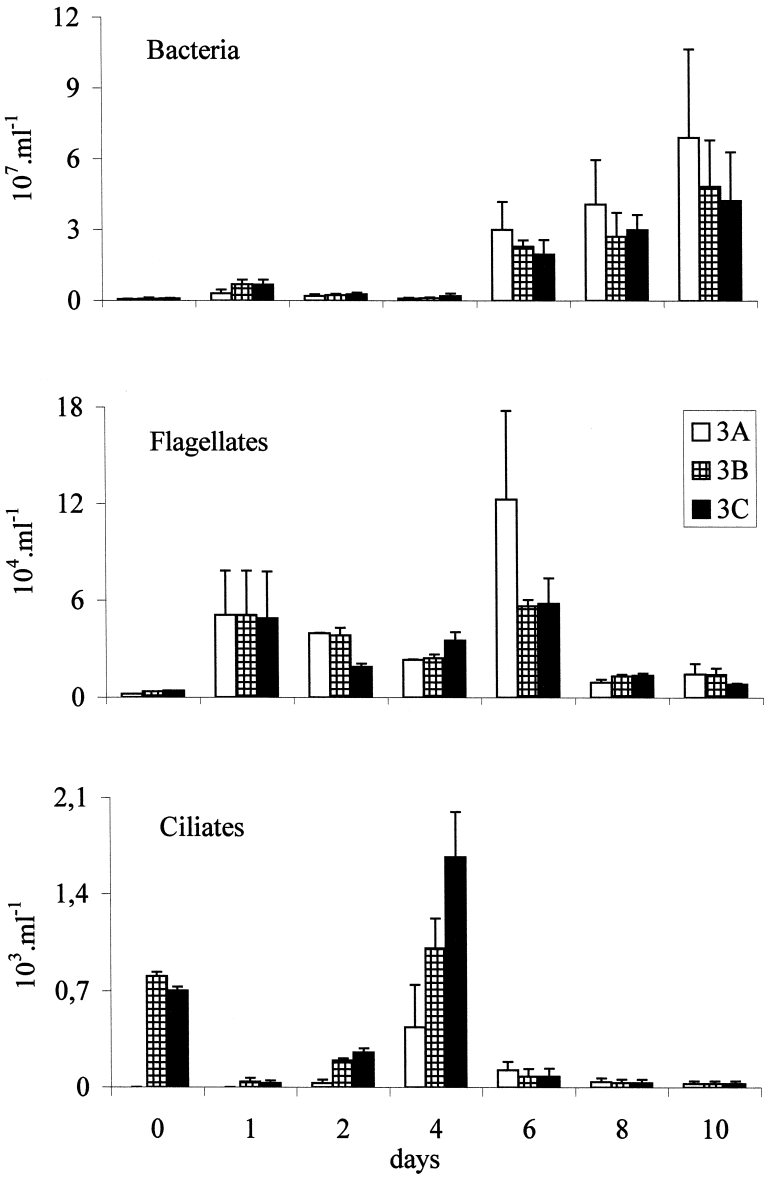

Fig. 4. Abundance of bacteria, flagellates and ciliates (Treatments 3A, 3B and 3C) during the third experiment.

0.05), and considering the fact that the rise in number occurred after adding *Artemia* to the tanks, we supposed that the water used to grow *Artemia* had a high number of bacteria. This was confirmed in later analysis of water samples from *Artemia* (3.99×10^7 bacteria/ml). Flagellates varied between 0.2 and 12.26×10^4 cells/ml, with maximum value observed in treatment 3A at day 6 (Fig. 4). Ciliates abundance ($0–1.67 \times 10^3$/ml) showed a decrease between days 0 and 1 and after the fourth day (Fig. 4), probably related to the larvae consumption. Larvae of the three treatments were initially covered with organic matter and microorganisms. However, they were completely clean after undergoing molt.

In all the experiments, the stomach of larvae were full of particles as well as bacteria, flagellates and ciliates. In the third experiment the larvae also showed *C. calcitrans* in their stomach. Some remains of *Artemia* were also observed. It was not possible to quantify the relative importance of the different food items to the nutrition of the shrimp larvae.

4. Discussion

A major constraint in penaeid larviculture is the susceptibility to microbial infections. Live food, such as *Artemia* sp. nauplii, has been shown to be a source of potentially pathogenic bacteria (Dehasque et al., 1991; Verdonck et al., 1994). During the hatching of *Artemia* cysts, the abundance of bacteria can increase ca. 100-fold, when compared to the initial numbers. This bacterial population is hard to be removed even when nauplii are rinsed with seawater and freshwater (Verdonck et al., 1991). However, hatching of pre-disinfected *Artemia* cysts presented low bacterial number (Merchie et al., 1997). We observed that *Artemia* nauplii added to the tanks worked as a vector, aggregating more bacteria. However, we have no indication whether the added bacteria could cause any damage to the larvae, or actually function as an extra food source.

The main pathogenic effect observed in this study was the presence of bacterial epibiosis, leading to the death of most larvae in experiment 1. Infestation of *P. stylirostris* larvae by epibionts bacteria, was mainly caused by the species *Aeromonas formicans*, *Pseudomonas piscicida* and *Flavobacteria* sp. (Lewis et al., 1982). The infestation was prevented by adding some antibiotics (gentamycin, nalidixic acid and acridine) to the cultures. In our case, the supply of flagellates and ciliates (experiment 2) seems to have helped the larvae to survive and be active. Moreover, it was clear from the third experiment that the use of more energetic food (microalgae and *Artemia* sp. nauplii) allowed the larvae to molt and get rid of the microbial fouling. Similar results were obtained in the production of *P. vanamei* post-larvae (Garriques and Arevalo, 1995).

Our study clearly demonstrated that microorganisms can represent an important food source for *P. paulensis* larvae. It was shown that the larvae can survive longer by feeding only on bacteria, although much better survival and growth were obtained when larger microorganisms (flagellates and ciliates) were included in the diet.

Though bacteria represent an important food source, due to its higher N and P contents, their small size (0.5–1.5 μm length) may be a problem, since it is hardly

retained by the feeding apparatus of the larvae. To be properly consumed, bacteria must form aggregates or be attached to particles (Conover, 1982). Moreover, not all bacterial macroconsumers are equipped with bacteriolytic enzymes that allow them to digest capsules and slime produced by these microorganisms (Moriarty, 1990).

Bacteria, on the other hand, are wildly consumed by nanoflagellates and small ciliated protozoan (Williams, 1981; Sherr and Sherr, 1984), playing an important, though indirect, role in fueling benthic and planktonic food webs with energy and matter, which is transferred to higher trophic levels. Some past studies have shown the importance of bacteria for the growth of meiofauna, present in ponds, that were later consumed by penaeid shrimp (Moriarty et al., 1983; Allan et al., 1995). Others have indicated that the growth of bacteria in tanks, after nutrient addition, gave rise to the increase of protozoan, which was consumed by crab *Portunus tridentatus* larvae (Maeda, 1988).

The growth of bacteria observed in this study (before or during the experiments) clearly worked to enhance the availability of flagellates and ciliates which, in turn, improved the culture of *P. paulensis* larvae, with the production of bigger larvae. Flagellates and ciliates are an important food source for many organisms of the zooplankton, like shrimp larvae (Porter, 1984). Their significance derives not only from their elevated abundance, but also from their biochemical composition, with higher amounts of nitrogen than carbon per cell. They also represent a significant reservoir of essential nutrients like polyunsaturated fatty acids, sterols and amino acids, that are essential for the growth of penaeid shrimp (Stoecker and Capuzzo, 1990; Lim et al., 1997). Moreover, the bigger size of protozoan, as well as their ability of locomotion, probably made them more interesting prey to the *P. paulensis* larvae.

Nevertheless, the use of protozoan in larviculture is not utilized on a large scale. To our knowledge, only the study of Maeda (1989) reports the use of the ciliate *Strombidium sulcatum* as a food source for the larvae of *P. monodon*. The addition of this ciliate resulted in increasing survival and molt rates of the larvae. In this sense, we consider that the addition of the ciliate *C. minuta* found in our study, as a complementary food source, could improve the rearing conditions of *P. paulensis* larvae. This ciliate has a reasonable size (ca. 20–25 μm) and seems to be heavily consumed by the larvae, as indicated by the variability of its abundance during the experiments, and also by direct observation of the larval gut contents. Moreover, their rapid growth in quite simple conditions indicates that large scale cultivation of this ciliate could be easily obtained.

The importance of flagellates and ciliates for the feeding of shrimp larvae lead us to discuss another point related to shrimp aquaculture: the water exchange. Water renewal has been largely used in order to diminish deleterious effects of physical, chemical and biological imbalance, caused by intensive rearing conditions like high organism density and food addition (Barnabé, 1990). Water exchange has been also suggested as an alternative to reduce the number of vibrionaceae bacteria during the cultivation of larvae and juvenile stages of *P. paulensis* (Barbosa and Capra, 1994). However, recent studies have questioned this method and shown better rearing results when the water exchange rates were reduced (Hopkins et al., 1995; Vinatea and Andreatta, 1997).

Higher survival, dry weight, and metamorphosis rates were found for *P. paulensis* larvae grown in static water renewal conditions. This result was associated with high

bacterial abundance in the tanks (Vinatea and Andreatta, 1997). The authors considered that bacteria was probably participating in the *P. paulensis* feeding, or improving the water quality through nitrification process. No reference was made to the possible presence of protozoan. In the light of our results, we may speculate that the high shrimp survival and growth observed by Vinatea and Andreatta (1997) resulted not only from the increase in bacterial number, but also from the presence of protozoan in the tanks, if enough time was given for the growth of these microorganisms.

It is important to note that the highest values of flagellates and ciliates in our study were observed 2 to 3 days after the beginning of the experiments, while bacteria reached maximum values in 24 h. Thus, it is likely that high water renewal will allow the growth of bacteria in tanks, but not of flagellates and ciliates, that have lower growth rates. If this is true, water renewal actually works against the larviculture success, since it increases the probability of pathogenic bacterial cells to occur, but does not allow the growth of protozoan, that could control the bacterial abundance by grazing and represent a complementary food source to the larvae. This hypothesis remains to be tested in future studies.

The results of this study are preliminary, but they point out the importance of microorganisms, particularly flagellates and ciliates, in the culture of *P. paulensis* larvae. The Protozoa represents an important food complement, which may improve larvae production of commercially important species.

Acknowledgements

The authors are grateful to C. Odebrecht, B. Biddanda, W. Wasielesky and an anonymous referee for the comments on a previous version of this manuscript. We also thank the help of C. Odebrecht in the identification of the ciliate *C. minuta*. F. Thompson and P.C. Abreu were supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). This work was also supported with grants from Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

References

Allan, G.F., Moriarty, D.J.W., Maguire, G.B., 1995. Effects of pond preparation and feeding rate on production of *Penaeus monodon* Fabricius, water quality, bacteria and benthos in model farming ponds. Aquaculture 130, 329–349.

Austin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, I., Griffith, D.R.W., 1995. A probiotic strain of *Vibrio alginolyticus* effective reducing diseases by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii* (Short communication). J. Fish Dis. 18, 93–96.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257–263.

Barbosa, T.C.P., Capra, S.M., 1994. Bacteriological study of larval and juvenile stages of penaeid shrimp (*Penaeus paulensis*). In: Koop, K. (Ed.), Ecology of Marine Aquaculture. A workshop on research in aquaculture, Osorno, Chile, 17–25.

Barnabé, G., 1990. Aquaculture, Vol. 1. Ellis Horwood, New York.

Carey, P.G., 1992. Marine interstitial ciliates. British Natural History Museum Publications, London, UK.
Conover, R.J., 1982. Interrelations between microzooplankton and other plankton organisms. Ann. Inst. Océanogr., Paris 58, 31–46.
Curds, C.R., Gates, M.A., Roberts, D.M., 1983. British and other freshwater ciliated protozoa, part II: oligohymenophora and polyhymenophora. In: Kermack, D.M., Barnes, R.S.K. (Eds.), Synopses of the British fauna (new series) 23. Linnean Society of London and Estuarine and Brackish Water Sciences Association, London, UK.
Dehasque, M., Verdonck, L., Sorgeloos, P., Swings, J., Léger, Ph., Kersters, K., 1991. Determination of the bacterial contamination in live food production systems in marine fish hatcheries in Southern Europe. In: Larvens, P., Sorgeloos, P., Jaspers, E., Ollevier, F. (Eds.), LARVI '91—Fish and Crustacean Larviculture Symposium, European Aquaculture Society (Special Publ.), 15, 399–402.
Dixon, B.A., 1994. Antibiotic resistance of bacterial fish pathogens. J. World Aquacult. Soc. 25, 60–63.
Douillet, P.A., Langdon, C.J., 1994. Use of probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture 119, 25–40.
Garriques, D., Arevalo, G., 1995. An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vannamei* postlarvae in Ecuador. Proceedings of the special session on Shrimp Farming. The World Aquaculture Society, San Diego, CA, USA, 53–59.
Gil, B.G., 1995. The use of bacteria as probionts in shrimp larviculture. Larvi '95—Fish and Shellfish Larviculture Symposium, Gent, Belgium,. European Aquaculture Society, (Special Publication), 24.
Hobbie, J.E., Daley, R.J., Jasper, S., 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environm. Microbiol. 3, 1225–1228.
Hopkins, J.S., Browdy, G.L., Hamilton, R.D. II, Heffernan, J.A. III, 1995. The effect of low-rate sand filtration and modified feed management water quality and production of intensive shrimp ponds. Esquaries 18, 116–123.
Iwai, M., 1978. Desenvolvimento larval e pós-larval de *Penaeus* (Melicertus) *paulensis* Pérez-Farfante, 1967. (Crustacea, Decapoda) e o ciclo de vida dos camarões do gênero *Penaeus* da região centro-sul do Brasil. PhD Thesis, Universidade de São Paulo-USP, São Paulo, Brasil.
Kahl, A., 1933. Ciliata libera et ectocommensalia. In: Grimpe, G., Wagler, E. (Eds.), Die Tierwelt der Nord- und Ostsee. Lief 23 (Teil II, c3), Leipzig, 29–146.
Lewis, D.H., Leong, J.K., Mock, C., 1982. Aggregation of penaeid larvae due to microbial epibionts. Aquaculture 27, 149–155.
Lim, C., Ako, H., Brown, C.L., Hahn, K., 1997. Growth response and fatty acid composition of juvenile *Penaeus vanamei* fed different sources of dietary lipid. Aquaculture 151, 143–153.
Maeda, M., 1988. Microorganisms and protozoa as feed in mariculture. Prog. Oceanogr. 21, 201–206.
Maeda, M., 1989. Some aspects of the biocontrolling method in aquaculture. In: Miyachi, S., Karube, I., Ishida, Y. (Eds.). Current Topics in Marine Biotechnology, Japan. Soc. Mar. Biotechnol., Tokyo, 395–398.
Maeda, M., 1994. Biocontrol of the larvae rearing biotope in aquaculture. Bull. Natl. Res. Inst. Aquaculture 1, 71–74.
Marchiori, M.A., Boff, M., 1983. Induced maturation, spawning and larvae culture of the pink shrimp *Penaeus paulensis* Pérez-Farfante. Mems. Assoc. Latinoam. Acuicultura 5, 331–337.
Merchie, G., Dehasques, M., Sorgeloos, P., 1997. DC *Artemia* lessens risk of introducing potentially pathogenic microorganisms in the culture of shrimp and fish larvae. Aquaculture Asia, Jan.–Mar. 1997, 46–48.
Moriarty, D.J.W., 1990. Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. Microbiology in Poecilotherms, 217–222.
Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333–349.
Moriarty, D.J.W., Cook, H.L., Hassan, R.B., Thanabal, M., 1983. Primary production and meiofauna in some penaeid prawn aquaculture ponds at Gelang Patah. Malay. Ag. J. 54, 37–51.
Nishino, S.F., 1986. Direct acridine orange counting of bacteria preserved with acidified lugol iodine. Appl. Environ. Microbiol. 52, 602–604.
Nogami, K., Maeda, M., 1992. Bacterial as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus*. Can. J. Fish. Aqua. Sci. 11, 2373–2376.
Pomeroy, L.R., 1974. The Ocean's food web, a changing paradigm. BioScience 24, 499–504.

Porter, K.G., 1984. Natural bacteria as food resources for zooplankton. In: Klug, M.J., Reddy, C.A. (Eds.), Current Perspectives in Microbial Ecology, Ann. Soc. Microbiol., Washington DC, 340–345.

Sherr, B.F., Sherr, E.B., 1984. Role of heterotrophic protozoa in Carbon and energy flow in aquatic ecosystems. In: Klug, M.J., Reddy, C.A. (Eds), Current Perspectives in Microbial Ecology, An. Soc. Microbiol., Washington DC, 412–423.

Skjermo, J., Salvesen, I., Øie, G., Olsen, Y., Vadstein, O., 1997. Microbially matured water: a technique for selection of a non-opportunistic bacterial flora in water that may improve performance of marine larvae. Aquacult. Int. 5, 13–28.

Sokal, R.R., Rohlf, F.J., 1969. Biometry. The Principles and Practice of Statistics in Biological Research, Freeman, San Francisco, USA.

Stoecker, D.K., Capuzzo, J.M., 1990. Predation on protozoa: its importance to zooplankton. J. Plankton Res. 12, 891–908.

Strickland, J.D.H., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis. Fish Research Board of Canada, Ottawa, Canada.

Throndsen, J., 1978. Preservation and storage. In: Sournia, A. (Ed.), Phytoplankton Manual, Unesco, Paris, 69–74.

UNESCO, 1983. Chemical methods for use in marine environmental monitoring. Intergovernmental Oceanographic Commission, Manual and Guides 12, 29–36.

Utermöhl, H., 1958. Zur Vervollkommnurg der quantitativen Phytoplankton Methodik. Int. Ver. Theor. Angew. Limnologie 9, 1–38.

Verdonck, L., Dehasque, M., Swings, J., Sorgeloos, P., Léger, Ph., 1991. The microbial environment of rotifer (*Brachionus plicatilis*) and *Artemia* production systems. In: Larvens, P., Sorgelos, P., Jaspers, E., Ollevier, F. (Eds.), LARVI '91—Fish and Crustacean Larviculture Symposium. European Aquaculture Society (Special Publ.) 15.

Verdonck, L., Swings, J., Kersters, K., Dehasque, M., Sorgeloos, P., Leger, P., 1994. Variability of the microbial environment of rotifer *Brachionus plicatilis* and *Artemia* production systems. J. World Aquacult. Soc. 25, 55–59.

Vinatea, L., Andreatta, E.R., 1997. Comparative study of continuous and static water renewal strategies in the larviculture of *Penaeus paulensis* (Pérez-Farfante, 1967) associated with high stocking densities and different water renewal rates. Aquaculture 154, 247–259.

Williams, P.J. leB., 1981. Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kieler Meeresforsch. Sonderh. 5, 1–28.