Role of microalgae *Thalassiosira fluviatilis* in weight gain and survival of the shrimp *Farfantepenaeus paulensis* reared in indoor nursery tanks

Luciano V. Jensen; Wilson J. Wasielesky; Eduardo L. C. Ballester; Ronaldo O. Cavalli and Marcos S. Santos

(LVJ, WJW, ELCB, ROC, MSS) Fundação Universidade Federal do Rio Grande, Departamento de Oceanografia, Laboratório de Maricultura, Caixa Postal 474, CEP 96201-900, Rio Grande, RS, Brasil.
(WJW) E-mail: manow@mikrus.com.br

Abstract

The present work investigated the effects of the use of the microalgae *Thalassiosira fluviatilis* in the survival and weight gain of *Farfantepenaeus paulensis* post-larvae. Shrimp were reared in indoor nursery tanks in a density of 1000 PL_{26}/m^2. The diatom was added to the treatment tanks in a density of 3.10^4 cells/ml while in control tanks no diatom was added. All tanks received artificial substrates in the form of polyethylene screens and 50% of water was exchanged daily and. At the end of the trial no significant differences in shrimp weight and survival were detected among treatments. The results indicate that the use of *T. fluviatilis* during *F. paulensis* nursery phase did not bring any benefit for the shrimp; furthermore the introduction of the microalgae in the culture tanks increased the level of nitrogen and phosphorous compounds.

Key words: *Farfantepenaeus paulensis, Thalassiosira fluviatilis,* pink shrimp, microalgae, nursery.

Introduction

In the last few years interest in penaeid shrimp culture has increased worldwide due to the growing market demand and high commercial value achieved by this product. As a result, Brazilian production of penaeid shrimps has increased from 3,600 metric tons in 1997 to 90,190 metric tons in 2003 (Rocha *et al.* 2004). One of the strategies commonly employed by farmers to improve shrimp yields is the segmentation of the production cycle into nursery and grow-out phases (Rocha *et al.* 2004). Culture of laboratory-reared post-larvae (PL) in nursery tanks prior to their release into grow-out ponds is considered advantageous as it facilitates the evaluation of PL quality, prevents disease outbreaks and makes better use of artificial diets. Stocking grow-out ponds with larger and more resistant shrimp juveniles not only results in higher survival and growth rates but also reduces the time to commercial size (Apud *et al.,* 1983).

In temperate areas where shrimp culture is limited to warmer months, the use of indoor nursery tanks allows a 3-4 weeks head start as 1-g juveniles may be transferred to grow-out at the beginning of the warm season (McAbee *et al.,* 2003). Similarly, Kumlu *et al.* (2001) suggest that to achieve commercial viability in temperate areas extension of the grow-out period by 1-2 months is possible through the use of intensive nursery systems operated indoors, thus two crops could be achieved or shrimp at larger size could be attained over a longer culture period.

The shrimp *Farfantepenaeus paulensis* (Pérez-Farfante, 1967) is considered a cold tolerant species which is naturally found from Mar del Plata, Argentina, to Ilhéus, Brazil (D'Incao, 1995). In southern Brazil, where low winter temperatures are a limiting factor for shrimp culture, this species has

demonstrated good potential for culture both in pen enclosures (Wasielesky *et al.,* 2001) and earthen ponds (Peixoto *et al.,* 2003).

Several studies dealing with the culture of *F. paulensis* during the nursery phase have been carried out. Speck *et al.* (1993) assessed the effect of stocking density on survival and growth, while the important role of natural food items (Hennig and Andreatta, 1998; Jensen *et al.,* 2004) and the biofilm attached to submerged surfaces on shrimp performance and maintenance of water quality (Thompson *et al.,* 2002; Ballester *et al.,* 2003) were also demonstrated. Optimal rearing conditions in terms of temperature (Hennig and Andreatta, 1998; Tsuzuki *et al.* 2000), salinity (Corleto *et al.* 1993; Tsuzuki *et al.* 2000) and ammonia (Wasilesky *et al.,* 1994) were also determined. Results from some of these studies and our own observations indicate that lower survival and growth rates are usually observed when nursery rearing of *F. paulensis* is carried out under laboratory conditions rather than in outdoor (earthen ponds, pen enclosures and cages) conditions. This is probably due to the higher availability of natural food items and better water quality found in the natural environment. Nevertheless, variable climatic and physicochemical water characteristics may reduce survival and growth of laboratory-reared PL in grow-out structures placed in the natural environment. For instance, Tsuzuki *et al.* (2000) found that *F. paulensis* PL_{25} (25 days after metamorphosis to the post-larvae stage) and older could tolerate salinity and temperature variations that normally occur in estuarine areas.

Therefore, the development of strategies to improve the performance of indoor nursery systems for the culture of *F. paulensis* must be pursued. Among these strategies, increasing the availability of natural food items should be considered. Microalgae are known to play important roles as a source of nutrients and in maintaining good water quality during culture (Amjad and Jones, 1994). In our laboratory, the diatom *Thallassiosira fluviatilis* has been successfully cultured and used during the culture of *F. paulensis* larvae. *T. fluviatilis* has an appropriate size for shrimp consumption throughout larviculture and nursery rearing (12 to 14 μm; Alfonso and Leal, 1995), good nutritional value and digestibility, rapid growth rates, and tolerates fluctuations in temperature, light and nutrients. Therefore, among several microalgae species which were tested over the last decades, species from the genus *Thalassiosira* have gained widespread use in aquaculture (Brown, 2002).

In the present work, we evaluated the influence of microalgae *(T. fluviatilis)* on water quality and the productive performance of shrimp *(F. paulensis)* reared in an indoor nursery system.

Material and Methods

The experiment was carried out at the Laboratório de Maricultura, Fundação Universidade Federal do Rio Grande – FURG, Brazil. Six circular concrete tanks (bottom area of 10 m^2) filled with 6000 liters of sand-filtered seawater were used. Three replicate tanks were randomly assigned to the control treatment where no microalgae was added, while in the three other tanks *T. fluviatilis* was added in a density of 3.10^4 cells/ml.

All tanks received six artificial substrates in the form of 0.9 m^2 polyethylene screens (white color, 1 mm mesh size) that were placed vertically and increased the area for shrimp settlement and biofilm attachment to about 108% of the tank bottom area. Each tank was then stocked with 10.000 PL_{26} with an initial mean (± SD) weight of 5.5 (± 3.0) mg.

Every day between 8:00 and 9:00 a.m., water samples were collected from all the tanks to estimate the concentration of unidentified microalgae species and *T. fluviatilis* with a haematocymeter. Afterwards, 50% of the tank volume was exchanged and, when necessary, *T. fluviatilis* was added to the treatment tanks until reaching 3.10^4 cells/ml.

Water temperature, salinity, pH, dissolved oxygen and transparency were measured daily between 9:00 and 11:00 a.m. with a mercury thermometer (0.5°C precision), a hand-held Atago® refractometer (1 unit precision), a HandyLab® pHmeter (precision 0.01), a Handylab® oxygen meter (precision 0.01) and a Secchi disk, respectively. Additionally, every 5 days water samples were collected to determine the concentrations of total ammonia (UNESCO, 1983), nitrite and phosphate (Aminot and Chaussepied, 1983). Chlorophyll *a* concentrations of the biofilm attached to the artificial substrates and the culture water were also measured at 5 day intervals. Two 2 cm^2 pieces of substrate collected at 25 cm water

depth and 500 ml of water collected from each tank were filtered through a GF/F Whatmann® (0.45 μm pores, 47 mm diameter). Extraction of the photosynthetic pigment from the substrates and filters was done in vials filled with 20 ml of acetone 90% (Merck® PA) kept in the dark at -12°C during 24 hours. Absorbencies in the wave lengths 630 and 664 nm were determined utilizing a Micronal® spectrophotometer model B 342 II according to Strickland and Parsons (1972). The equations proposed by Jeffrey and Humphrey (1975) were used to estimate the chlorophyll *a* concentrations.

Throughout the experimental period of 30 days, shrimps were fed 5 times a day with a commercial diet (Zeigler®) at 9:00; 11:00; 14:00; 17:00 and 22:00 hours. During the first 10 days, the feeding rate was 100% of the shrimp biomass. Between the 10th and the 20th day, the feeding rate was reduced to 75% and in the last 10 days to 50% according to the growth data obtained in the weightings.

Shrimp growth was monitored by randomly sampling 100 animals from each tank at 10 day intervals. Shrimp were blotted dry and their weight was measured in a balance to the nearest 0.01 g. They were then returned to their respective tanks. Specific growth rate (SGR) was calculated using the equation proposed by Bagenal (1978):

$$SGR = [(\log_e Fw - \log_e Iw)/\Delta T] \times 100$$

Where $\log_e Fw$ and $\log_e Iw$ are the logarithm of the final and initial weight, respectively, and ΔT is the number of days of the culture.

At the end of the experimental period, remaining shrimp in each tank were individually counted.

Statistical analysis of water abiotic parameters, chlorophyll *a* concentration in the water and substrates, shrimp survival and final weight were submitted to the "t test" ($\alpha = 0.05$) (Sokal and Rohlf, 1995). Percentage data (e.g. survival) were arcsine transformed before analysis.

Results

Temperature, salinity, pH and dissolved oxygen did not show differences among treatments throughout the culture period ($P>0.05$), in all the tanks transparency was total (Table I). The concentrations of total ammonia, nitrite and phosphate were significantly different among treatments ($P<0.05$). Higher ammonia concentrations were determined in the tanks with *T. fluviatilis* at the beginning of the experiment and showed a decreasing trend through the culture; nitrite concentration grew in the first 14 days but stabilized as the ammonia concentration decreased (Fig. 1).

Mean chlorophyll *a* concentration in the water during the experiment was higher in treatment tanks than control tanks ($P<0.05$) (Table II). No significant differences were found in mean chlorophyll *a* concentration measured in the biofilm attached on artificial substrates during the experiment (Table II), either no significant differences in densities of the unidentified microalgae were detected among treatments throughout the experimental period ($P>0.05$) (Table II)

The specific growth rate of shrimps for control and treatment were respectively 10.2% and 10.1%. At the end of the experiment no significant differences were found among mean wet weight

Table I. Means (± SD) of temperature (°C), salinity, pH, dissolved oxygen (mg/L), total ammonia (mg/L TAN), nitrite (mg/L $N\text{-}NO_2^-$), orthophosphate (mg/L $P\text{-}PO_4^{3-}$) and transparency (cm) throughout the experiment.

Parameter	Without *T. fluviatilis*	With *T. fluviatilis*
Temperature	25.39 ± 1.14	25.47 ± 1.12
Salinity	28.44 ± 1.48	28.43 ± 1.50
pH	8.12 ± 0.07	8.10 ± 0.11
Dissolved Oxygen	6.92 ± 0.84	6.94 ± 0.98
Total ammonia	0.19 ± 0.08[a]	1.31 ± 0.56[b]
Nitrite	0.02 ± 0.01[a]	0.28 ± 0.24[b]
Orthophosphate	0.36 ± 0.04[a]	0.55 ± 0.20[b]
Transparency	60 cm – Total	60 cm – Total

Different letters in the same line means significant difference (P<0,05).

Table II. Means (± SD) of chlorophyll *a* in the water (μg/ml) and in the artificial substrates (μg/cm²), densities of *T. fluviatilis* and unidentified microalgae (10^4cell/ml).

Parameter	Without *T. fluviatilis*	With *T. fluviatilis*
Chlorophyll *a* in the water	0.022 ± 0.026[a]	0.099 ± 0.041[b]
Chlorophyll *a* in the substrate	22.61 ± 19.14	19.22 ± 21.17
T. fluviatilis density	0.0	3.74 ± 0.98
Unidentified microalgae density	3.26 ± 2.23	3.27 ± 2.96

Different letters in the same line means significant differences (P<0.05).

Table III. Means (± SD) of post-larvae wet weight (mg) throughout the experimental period, and final survival rates (%).

	Wet Weight				Survival
	Initial	10 days	20 days	30 days	
With *T. fluviatilis*	5.5 ± 3.3[a]	13.6 ± 0.6[a]	41.0 ± 5.3[b]	115.7 ± 17.6[a]	95.20±8,3[a]
Without *T. fluviatilis*	5.5 ± 3.3[a]	14.0 ± 1.1[a]	49.2 ± 9.9[a]	117.7 ± 23.4[a]	84.85±2,5[a]

Different letters in the same column indicate significant differences (P<0.05).

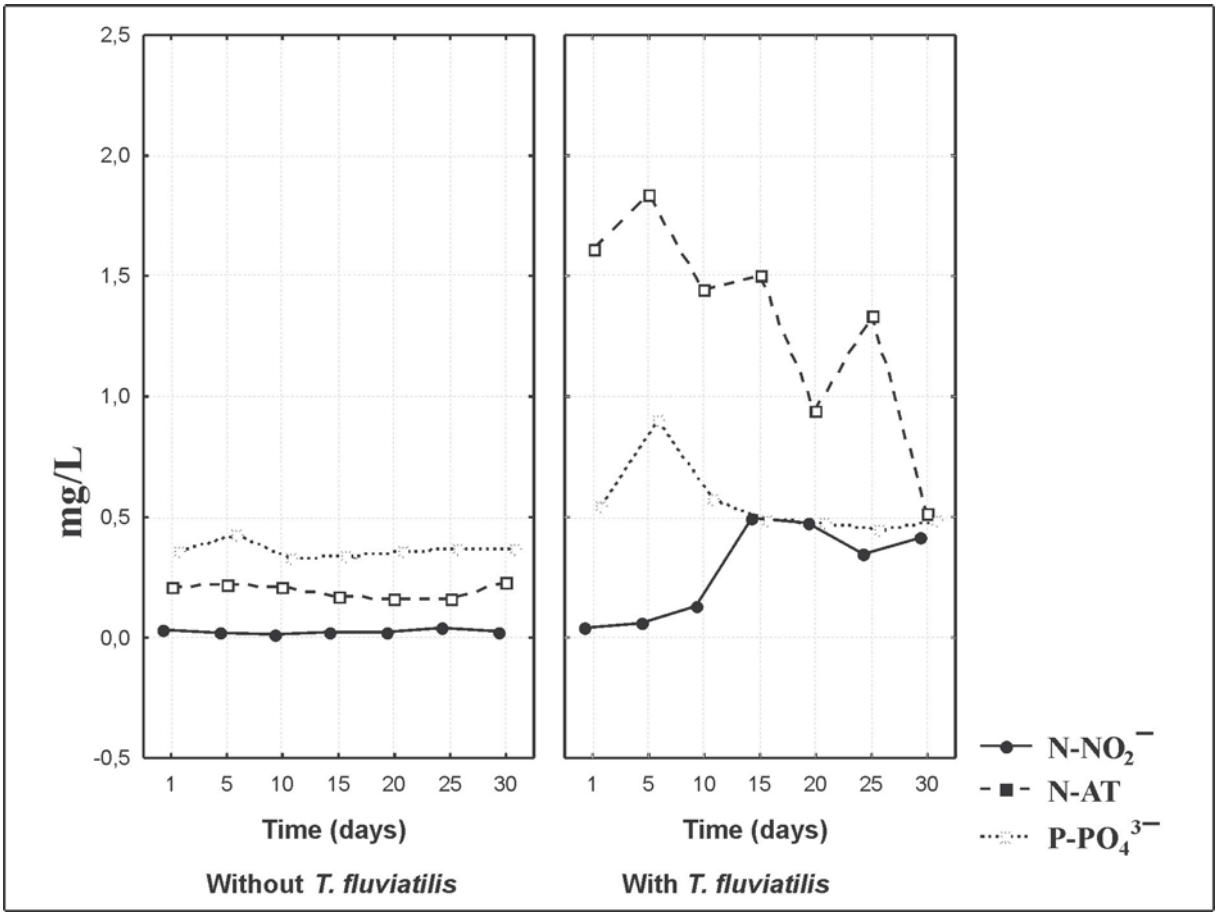

Figure 1. Variations of nitrite, total ammonia and orthophosphate concentrations of the water during the experimental period.

of shrimp from control and treatment, either the survival rate did not showed significant differences (P>0.05) (Table III).

Discussion

Several studies report the importance of microalgae use to water quality control (Amjad and Jones, 1994; Andreatta and Alfonso, 1997; Reitan *et al.* 1997; Barbieri and Ostrensky, 2001). However, results obtained in this work did not show any measurable benefit brought by the introduction of *T. fluviatilis* in the culture tanks. Conversely, the concentrations of total ammonia, nitrite and orthophosphate in the treatment tanks were higher than in control tanks without microalgae. Tamaru *et al.,* (1994) reported similar results during the larval culture of mullet *(Mugil cephalus),* these authors reported a higher concentration of un-ionized ammonia in tanks containing *Nannochloropsis oculata,* however larval survival and growth were higher in tanks provided with microalgae.

The higher values of nitrogen and phosphorus compounds found in the tanks with *T. fluviatilis* are probably a result of the introduction of residual microalgae culture medium into the nursery tanks. Nitrite concentrations remained below the safe levels (Castaño, 1997), but total ammonia concentration reached higher values than the safe level

determined by Wasielesky *et al.* (1994) for *F. paulensis* post-larvae, anyway it did not seem to negatively affect shrimp performance, as growth and survival rates were considered satisfactory. Nevertheless, present results indicate that there must be a more rigorous control of nutrients assimilation during mass microalgae culture in order to avoid introduction of high levels of nitrogen and phosphorus to shrimp's culture tank.

Specific growth rate (SGR) of shrimp in the present work (control 10.2% – treatment 10.1%) was similar to those reported in previous studies performed under indoor conditions (Speck *et al.,* 1993; Corleto *et al.,* 1993; Tzusuki *et al.* 2000), however, the stocking density (1000 PLs/m^2) applied here was more than three fold higher than in these works (300PLs/m^2). Hennig and Andreatta (1998) cultured *F. paulensis* post-larvae in an intensive system (1800 PLs/m^2) and reported survival rates ranging from 54.3 to 82.3% but the higher SGR registered was 8%. Comparisons among these studies demonstrate that in the present work a better performance of post-larvae was obtained, other factors than microalgae addition probably contributed to this result.

The presence of additional substrates enlarging the area available for shrimp distribution was probably an important feature during the culture. When shrimp are more evenly distributed within the tank, there is a relative decrease in the stocking density. Bratvold and Browdy (2001) suggested that the presence of additional substrates delays the onset of overcrowding effects such as stress. Negative influence of high stocking densities during shrimp culture was also demonstrated by Speck *et al.* (1993), Martin *et al.* (1998) and Wasielesky *et al.* (2001).

Additionally the artificial substrates provide a larger area for biofilm attachment. Experiments conducted during the nursery phase of the pink-shrimp *F. paulensis* in tanks demonstrated that the biofilm attached to the tank walls led to a reduction in the exportation of phosphorus (33% less phosphate) and a higher output of nitrogen in the forms of nitrite or nitrate instead of ammonia (Thompson *et al.,* 2002). In the same work the authors demonstrated a positive influence of biofilm as a complementary food source for the shrimps. Ballester *et al.* (2003) also suggested nutritional contribution from biofilm attached to polyethylene substrates when the cultured was performed in cages installed in an estuarine inlet. Recent results utilizing stable carbon and nitrogen isotopes confirmed that biofilm effectively contributed in the nutrition of *F. paulensis* post-larvae reared under laboratory and field conditions (Abreu *et al.,* in preparation),

Thompson *et al.* (2002) suggested that biofilm was mature when chlorophyll *a* concentration is around 5μg/cm^2, this value was related with biofilm capacity to reduce harmfull nitrogen compounds. In the present work no significant differences ($p>0,05$) were detected in chlorophyll *a* mean final concentration from biofilm attached on the substrates of treatment and control tanks, however, biofilm achieved maturity faster in control tanks (13 days) than in treatment tanks (17 days), probably because of the higher quantity of microalgae which was competing for the available nutrients.

The current results demonstrated that the addition of *T. fluviatilis* provided no advantage for *F. paulensis* post-larvae cultured in the indoor nursery system; however more research is needed in order to determine if higher densities of microalgae or other microalgae species could provide an effective contribution. The presence of substrates and biofilm probably helped to maintain good water quality and to provide a complementary food source for the shrimps, as the provision of substrates is cheaper than microalgae production, it appears like a promising alternative to the use of microalgae during the nursery phase *F. paulensis* of culture.

Acknowledgements

This work was possible through funds provided by FAPERGS, Polo Pesqueiro and SCT-RS. L. Jensen acknowledges the receipt of a CAPES scholarship. Wilson Wasielesky Junior and Ronaldo Cavalli are research fellow of CNPq (Proc. 301354/01-4 and 300131/01-1, respectvely).

References

Alfonso. E. and Leal, S. 1995. Tópicos básicos para criação e manutenção de um cepário de microalgas. São Paulo: UNESP, 17p.

Aminot. A. and Chaussepied, M. 1983. Manuel des analyses chimiques en milieu marine. Brest: ANEXO. 395 pp.

Amjad, S. and Jones, D. A. 1994. Role of microalgae in conditioning water in penaeid larval culture. Pakistan Journal of Marine Sciences, 3(1): 41-51

Andreatta, E. R. and Alfonso, E. 1997. Produção de pós-larvas de camarão marinho. Florianópolis: UFSC, 193p.

Apud, F. D.; Primavera, J. H. and Torres, P. L. 1983. Farming of prawns and shrimps. Extension Manual 5. Iloilo, Philippines: SEAFDEC Aquaculture Department, 1983. 67p. (Extension Manual, 5).

Bagenal, T. B. 1978. Methods for assessment of fish production in fresh waters. London: Blackwell, 365p.

Ballester, E. L. C.; Wasielesky Jr., W.; Cavalli, R. O.; Santos, M. H. S. and Abreu, P. C. 2003. Influência do biofilme no crescimento do camarão-rosa *Farfantepenaeus paulensis* em sistemas de berçário. Atlântica, 25(2): 37-42.

Barbieri, C. JR. and Ostrensky, 2001. Camarões Marinhos: reprodução, maturação e larvicultura. Vol. 1. Aprenda Fácil Editora. Viçosa, MG. 243p.

Bratvold, D. and Browdy, C. L. 2001. Effects of sand sediment and vertical surfaces (Aquamats™) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture, 195: 81-94.

Brown, M. R. 2002. Nutritional value and use of microalgae in aquaculture. In: Cruz-Suárez, L. E., Ricque-Marie, D. Tapia-Salazar, M., Gaxiola-Cortéz, M. G., Simoes, N. (Eds). Avances en Nutrición Acuícola VI. Memorias de VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

Castaño, C.S. 1997. Toxicidade aguda do nitrito sobre o camarão rosa *Penaeus paulensis,* cultivados em diferentes salinidades. 25p. Monografia de graduação, curso de Oceanologia, FURG, Rio Grande, RS.

Corleto, F.; Cavalli, R.O. and Marchiori, M.A. 1993. Crescimento de pós-larvas de *Penaeus paulensis,* Perez-Farfante, 1967 em diferentes salinidades. In: Encontro Rio-Grandense de Técnicos em Aqüicultura, 4., Porto Alegre, 1993. Anais. Porto Alegre: Ed. UFRGs, 1993. p.31-39.

D'Incao, F. 1995. Taxonomia, padrões distribucionais e ecológicos dos Dendrobranchiata (Crustacea: Decapoda) do Brasil e Atlântico Ocidental. Doctoral thesis. Universidade Federal do Paraná, Curitiba, Paraná, Brazil.

Henning, O. L. and Andreatta, E. R. 1998. Effect of temperature in an intensive nursery system for *Penaeus paulensis* (Pérez-Farfante, 1967). Aquaculture, vol. 164, p.151-166.

Jeffrey, S. W. and Humphery, G. F. 1975. New spectrophotometric equations for determining chlorophyls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pfalanzen., 167: 191-194.

Jensen, L. V.; Wasielesky, W. J.; Cavalli, R. O.; Peixoto, S.; Santos, M. H. S. and Ballester, E. 2004. Growth and Survival of pink-shrimp *(Farfantepenaeus paulensis)* post-larvae in cages and pen enclosures. Scientia Agricola, 61(3): 332-335.

Kumlu, M.; Eroldogan, O. T. and Saglamtimur, B. 2001. The effects of salinity and added substrates on growth and survival of *Metapenaeus monoceros* (Decapoda: Penaeidae) post-larvae. Aquaculture, 196: 177-188.

McAbee, B. J.; Browdy, C. L.; Rhodes, R. J. and Stokes, A. D. 2003. The use of greenhouse-enclosed raceway for the superintensive production of pacific white shrimp *Litopenaeus vannamei* in the United States. Global Aquaculture Advocate, 6(4).

Martin, J-L. M.; Veran, Y.; Guelorget, O. and Pham, D. 1998. Srimp rearing: stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds. Aquaculture, 164: 135-149.

Peixoto, S.; Wasielesky Jr., W. and Louzada Jr., L. 2003. Comparative analysis of pink shrimp, *Farfantepenaeus paulensis* and pacific white shrimp *Litopenaeus vannamei,* culture in extreme southern Brazil. Journal of Applied Aquaculture, 14(1/2): 101-111.

Reitan, K. I., Rainuzzo, J. R. G. and Olsen, Y. 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture, 155: 207-221.

Rocha, I. P.; Rodrigues, J. and Amorim, L. 2004. A carcinicultura brasileira em 2003. Revista da ABCC. 1: 30-36.

Speck, R. C.; Cavalli, R. O. and Marchiori, M. A. 1993. Efeito de diferentes densidades de estocagem sobre o crescimento e a sobrevivência de pós-larvas de *Penaeus paulensis* (PÉREZ-FARFANTE, 1967) em sistema de berçário. In: Encontro Rio-Grandense de Técnicos em Aqüicultura, 4., Porto Alegre, 1993. Anais. Porto Alegre: Ed. UFRGs, 1993. p.31-39.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry. Principle and practices of statistics in biological research. W. H. Freeman and Co., New York. 889p.

Strickland, J. D. H. and Parsons, T. R. 1972. A practical handbook of seawater analysis. Ottawa: Fishery Research Board Canada, 310p.

Tamaru, C. S.; Murashige, R. and Lee, C-S. 1994. The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L. Aquaculture, 119: 167-174.

Thompson, F. L.; Abreu, P. C. and Wasielesky Jr., W. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture, 203: 263-278.

Tsuzuki, M. Y. and Cavalli, R. O. 2000. The effects of temperature, age and, acclimation to salinity on the survival of *Farfantepenaeus paulensis* post-larvae. Journal of the World Aquaculture Society, 31(3): 459-468.

UNESCO. Chemical methods for use in marine environmental monitoring. Paris, France: Intergovernamental Oceanographic Commission, 1983. (Manual and guides, 12).

Wasielesky, W. J.; Marchiori, M. A. and Santos, M. H. S. 1994. Efeito da amônia no crescimento de pós-larvas do camarão rosa, *Penaeus paulensis,* Pérez-Farfante, 1967 (Decapoda: Penaeidae). Nauplius, Rio Grande, 2: 99-105.

Wasielesky Jr., W.; Poersh, L. H.; Jensen, L. and Bianchini, A. 2001. Effect of stocking density on pen reared pink shrimp *Farfantepenaeus paulensis* (Pérez-Farfante, 1967) (Decapoda, Penaeidae). Nauplius 9(2): 163-167.

Received: May 2005
Accepted: October 2006