

COMPOSITION, DENSITY AND BIOMASS OF ZOOPLANKTON IN CULTURE PONDS OF *Litopenaeus vannamei* (DECAPODA: PENAEIDAE) IN SOUTHERN BRAZIL

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ABSTRACT

Cardozo, A.P.; Bersano, J.G.F. & Amaral, W.J.A. 2007. Composition, density and biomass of zooplankton in culture ponds of *Litopenaeus vannamei* (Decapoda: Penaeidae) in southern Brazil. *Braz. J. Aquat. Sci. Technol.* 11(1):13-20. ISSN 1808-7035. *Litopenaeus vannamei* is the most cultivated shrimp species in Brazil, being fed basically on high protein formulated diets. However, some studies have shown that the natural zooplankton found in shrimp ponds can be part of the diet of this crustacean. In this study, the zooplankton composition, density and biomass were evaluated for two shrimp ponds from a shrimp farm in the Patos Lagoon estuary, in southern Brazil (32°00'S, 51°59'W) between 11/09/2005 and 01/20/2006. The two ponds were sampled over three consecutive months using a zooplankton net of 150 cm total length, 30 cm mouth diameter and 140 µm nylon mesh size. All the zooplankton samples were preserved in formaldehyde solution at a final concentration of 4% and transported to the laboratory, where the composition, density and biomass (wet and dry weight) were assessed. Copepoda and Cladocera were the most frequent groups, while the most abundant species were *Acartia tonsa*, *Pseudodiaptomus richardi* and *Moina micrura*, species commonly found in the Patos Lagoon Estuary. The density values obtained in the ponds were higher than those usually found in the estuary during periods of maximum production, reaching 278 org.L⁻¹ in 11/18/2005 (pond 1), and 277 org.L⁻¹ in 12/08/2005 (pond 2), suggesting that the zooplankton grow well in the shrimp ponds. Zooplankton biomass in the ponds was also relatively high, ranging from 0.15 to 13.28 g.m⁻³ of wet weight and 0.01 to 2.72 g.m⁻³ of dry weight, following the same tendency of density. These results clearly indicate that the natural zooplankton occurring in the shrimp ponds represents a potential food source for the shrimp larvae and juveniles during the first months of culture.

Keywords: Copepoda, Cladocera, Abundance, Shrimp

INTRODUCTION

In recent decades, shrimp farming has been attracting considerable attention due to its remarkable growth worldwide (FAO, 2006). The main cultivated species are *Penaeus monodon*, *Fenneropenaeus chinensis* and *Litopenaeus vannamei*, the latter alone being responsible for 80% of the world production (FAO, 2006). In Brazil, *L. vannamei* is the most cultivated species and the Brazilian production has been increasing at a remarkable rate of about 40 to 80% a year, representing nowadays 5% of the world production (FAO, 2006).

L. vannamei is a marine species from the Pacific Ocean more specifically from the west coast of Mexico to the coast of Peru, showing great resistance to varying culture conditions, tolerating a wide range of temperatures and salinities (Bray et al., 1993; Ponce-Palafox et al., 1997). These characteristics are especially attractive because they reduce

entrepreneurial risks and increase the chances of profitability. This explains why *L. vannamei*, although exotic, is the main cultivated species in Brazil, given that other native penaeid shrimps are less tolerant to varying culture conditions (Brito et al., 2000).

During the first months of culture in earthen ponds the feeding base of this species is composed partially of the natural food produced in the pond (Anderson et al., 1987; Nunes et al., 1997; Martinez-Cordova et al., 1997; Rothlisberg, 1998), zooplankton for instance, and partially by supplementary feeding with commercial formulated feeds.

Chen & Chen (1992) recorded that *P. monodon* juveniles are efficient zooplankton predators, and that predation efficiency is related to size, since larger shrimp juveniles ingested higher amount of prey. For the blue shrimp *Litopenaeus stylirostris*, higher values of growth and food conversion rates in ponds have been found where the phytoplankton production was stimulated

through out fertilizations with urea and triple super phosphate (Martinez-Cordova *et al.*, 1997).

Martínez-Córdova & Pena-Messina (2005), on comparing semi-intensive monoculture and polyculture of *L. stylirostris* and *L. vannamei*, found lower zooplankton densities in ponds with monospecific cultures of *L. vannamei*, suggesting that this species is an efficient zooplankton predator.

Regarding the zooplankton, studies carried out in Australia have shown that copepods are dominant in marine shrimp ponds (Coman *et al.*, 2003), generally reflecting the composition of the environment from where the water is pumped. In these studies reductions of zooplankton biomass after the introduction of some post-larvae species, such as *Penaeus japonicus* (Coman *et al.*, 2003) and *P. monodon* (Preston *et al.*, 2003), were observed, indicating their predation on zooplankton.

Besides the importance of zooplankton as a food source for shrimp in growth ponds, the use of these organisms, mainly copepods, as live food in marine aquaculture has been receiving great attention in the last years (Delbare *et al.*, 1996). These organisms are rich in phospholipids, highly unsaturated fatty acids (HUFA) and natural antioxidants, being nutritionally superior to rotifers and *Artemia* nauplii (Watanabe *et al.*, 1983; Sargent *et al.*, 1997; Stottrup & Nosker, 1997).

Several studies have shown that copepods have a higher nutritional value, when compared to *Artemia* and rotifers (Helland *et al.*; 2003), and also the increase in the success of some larvicultures when copepods are used (Payne *et al.*, 1998; Schipp *et al.*, 1999; Payne & Rippingale, 2000).

In the southern region of Rio Grande do Sul state in southern Brazil, shrimp culture farms situated in the Patos Lagoon estuary (PLE), have been operating since 2000. The main cultivated species is *L. vannamei*, due to the availability of larvae and also because of its tolerance to salinity variations. In the PLE, flood and ebb tide regimes are controlled mainly by winds and rainfall in the draining basin (Garcia, 1997), leading to great salinity variations, with high salinities associates with periods of flood and lower salinities related to periods of ebb tide (Niencheski & Baumgarten, 1997).

The zooplankton of the PLE is dominated mainly by copepods (Montú, 1980), however, frequent salinity variations observed in the area, can affect the zooplankton composition. During periods of flood tide, organisms of marine origin such as Calanoida copepods can be dominant, while cladocerans and Cyclopoida copepods are commonly associated with low salinities during ebb tide periods (Montú *et al.*, 1997).

In general the shrimp farms in the region pump water directly from the PLE, and thus the zooplankton composition in the ponds is expected to be similar to that found in the PLE at the moment of pumping.

However, a substitution of species could possibly occur in the ponds, according to temporal salinity changes.

In Brazil, there are no studies that evaluate the growth potential of zooplankton in marine shrimp culture ponds, as well as its role as a live food source. In this context, the aim of this study was to evaluate the composition, density and biomass (wet and dry weight) of zooplankton in culture ponds of the white shrimp *Litopenaeus vannamei* in the PLE southern Brazil.

MATERIAL AND METHODS

The present study was carried out at Carcibrás shrimp farm in São José do Norte - RS, Brazil (32°00'S, 51°59'W). Two earthen ponds, pond 1 (P1) (area of 2.2 hectares) and pond 2 (P2) (area of 3,8 hectares) both with an average depth of 0.8 m were used and prepared in a similar way before being flooded.

Lime was added for correction of soil pH and disinfection. After that, the bottom of the ponds was ploughed with the use of a tractor in order to mix lime with the soil. The flooding was started with water being pumped directly from the PLE to a higher ground reservoir and then distributed by means of channels made of masonry and filtered through a net of 600 µm mesh size to avoid the presence of possible predators, such as juvenile fish and small crustaceans.

After a period of seven days the total flooding of the P1 was completed, and the zooplankton sampling program was initiated in 11/09/2005. For P2, due to its larger size, was more time required to complete the flooding process and the zooplankton sampling began in 11/16/2005.

Other than the size difference between the two ponds, they were also different in their soil composition. Both were characterized by sandy soil, but P1 was situated on lower ground and had a higher amount of organic matter due to its proximity to the PLE.

During the sampling period no water renewal was carried out in the ponds, only replacements of the losses due to evaporation and infiltration of the slopes, resulting in the introduction of about 10 % of the total volume of the ponds per week. Five fertilizations took place in P1 on 16 November, and 16, 26 and 27 December 2005 and 05 January 2006, with the addition of urea and triple super phosphate in a ratio of 10:1. No fertilizations took place in P2.

Ten day old post-larvae (PL-10) were bought from a larviculture company and introduced in the ponds. In P1, 660,000 PL-10 were introduced in 11/18/2005, reaching a density of 30 PL-10.m⁻². In P2, 980,000 PL-10 were stocked in 12/08/2005, giving a final density of 25 PL-10.m⁻².

Zooplankton samplings were carried out by transversal tows made in the opposite direction of predominant winds direction, which were predominately from the NE quadrant during the study period. In each pond, three sampling points were selected, one point next to the water inflow, another in the central region of the pond, and the third point next to the water outflow. The sampling schedule was every two to four days in the first month, and an average of every 8 days during the remaining period.

After zooplankton sampling, temperature, salinity, pH and oxygen dissolved were measured in the surface waters. For temperature measurements a mercury thermometer (Incoterm 99370/02) was used, the salinity was measured with a hand refractometer (Atago S/Mill-E), pH with a manual pH measurer (ATC F-HI98127) and the oxygen dissolved with the use of an oximeter (Hanna F-HI9147K).

Sampling took place at night because both zooplankton organisms and shrimps were more active in the water column during that period. The samples were collected with a 1.5 m length cylindrical-conical net of 30 cm mouth diameter and 150 μm mesh size. A 20 cm-diameter buoy was attached to the mouth of the net to keep it next to the surface.

In the first seven days of samplings the filtered volume was estimated through the calculation that uses the covered distance and the area of the net mouth. After this period a flowmeter was connected to the net mouth to estimate the sample filtered volumes. Due to the fact that two methods were used to obtain filtered volumes, a test sample was carried out with and without the flowmeter to test for differences. But, no differences were found between the two volume estimates, probably because of the low water circulation inside the ponds.

After samples were taken, they were transferred to one-liter plastic bottles and immediately fixed in a buffered formaldehyde 4% solution. Two days after sampling the volume in the plastic bottles was transferred to glass bottles and taken to the laboratory. For the qualitative and quantitative analyses, the samples were fractionated with a Motoda-type quarter and transferred to a known volume beaker from which aliquots of 10 ml were taken with a sub-sampler (Boltovskoy, 1981). The sub-samples were transferred to Bogorov's counting chambers and examined under a stereoscopic microscope.

In order to assess the zooplankton biomass in terms of grams of wet weight, the whole sample was used. For this, the collected material was retained on a preweighed sieve with a screen aperture of 50 μm , towel-dried to remove excess water, and weighed on an analytical scale Mars model LC-5 (precision of 0.5 g).

To determine the biomass in dry weight, an aliquot was removed from the samples and immediately

filtered in previously weighed on fiber glass filters (0.45 μm). To maximize the filtering a Quimis vacuum pump, model 355B, with one HP was used. Then the filters were placed in an oven at 60 °C for 14 hours, being kept after this period in a desiccator with blue silica-gel for 24 hours. After that period, the dry weight was determined on an Gehak analytical scale (precision of 0,001 g) model AG200 according to the method of Beers (1976). To correct the weight loss (wet and dry) of the organisms due to fixation with formaldehyde 4 %, a loss of 35 % of the weight of the organisms was considered, following the recommendations of Durbin & Durbin (1978).

In order to obtain the prosome length, total length and body width of the dominant species, pictures of the organisms ($n = 30$ for each species) were taken with a video camera (Sony CCD-IRIS) coupled to a stereoscopic microscope (Olympus U – CMAD3) connected to a computer. The measurements were obtained with the image analysis program Image-Pro Plus 4.1.

The density and biomass values in the two ponds were compared with two-way ANOVA, followed by the *posteriori* Tukey's test when significant differences ($p < 0.05$) were observed.

RESULTS

Temperature and salinity values varied during the study with salinity staying around 4 between 11/09/2005 and 12/08/2005 in P1, and between 11/16/2005 and 12/01/2005 in P2 (Figs. 1-2). After this period a gradual increase in salinity was observed in the two ponds until the end of the experiment, with maximum values of 14 for both P1 (Fig. 1) and P2 (Fig. 2). The temperature did not show great variations during the study period. The lowest water temperatures recorded were 18 °C for P1 on 11/09/2005 (Fig. 1), and 23.5 °C on 12/15/2005 for P2 (Fig. 2). The maximum temperature recorded was 27.5 °C for both ponds.

The values of dissolved oxygen varied between 6.2 and 9.7 $\text{mgO}_2\cdot\text{L}^{-1}$ in P1, and between 8.6 and 10.8 $\text{mgO}_2\cdot\text{L}^{-1}$ in P2, with maximum values occurring on 12/01/2005 in both ponds. For pH, alkaline values were recorded in P2 (8.6 - 10.8) whereas in P1 the values stayed between 7 and 8.7 with maximum values registered in 12/15/2005 in P1 and 12/08/2005 in P2.

In this study 31 taxa were identified, 19 to specific level, 2 to genus level and 10 to group level. The most frequent organisms were the Calanoida copepods *Acartia tonsa*, *Pseudodiaptomus richardi* and *Notodiaptomus incompositus*, the Cyclopoida copepods *Acanthocyclops robustus* and *Microcyclops furcatus* and also, the cladoceran *Moina micrura*. Together with these organisms, Calanoida and Cyclopoida copepodites were

present in all samples in both ponds throughout the study period, being in some occasions the most abundant organisms.

For the total zooplankton mean density, the values ranged between 2 and 278 org.L⁻¹ in P1, while in P2 the values varied between 34 and 277 org.L⁻¹ (Fig. 3). Even with the occurrence of 31 taxa during the study period, only six of these taxa were the most abundant in terms of density.

Of the most abundant organisms, cladoceran *M. micrura* showed higher density values at the beginning of the study period, reaching in P2 a peak of 233 org.L⁻¹ on 11/16/05 (Fig. 4). During the periods of high abundance it was also recorded the presence of ephippia containing resting eggs of *M. micrura*.

Of all the zooplanktonic organisms, copepodites of the Calanoida and Cyclopoida orders showed the highest densities during the whole studied period, corresponding to 98 % of total zooplankton on some

occasions, with densities of 311 org.L⁻¹ (Fig. 5). The copepods *M. furcatus* and *A. robustus*, had higher densities between 11/09/05 and 12/08/05, reaching maximum values of 13 org.L⁻¹ on 11/24/05 (Fig. 6) and 8 org.L⁻¹ on 11/28/05 (Fig. 7) respectively, for P2.

As salinity increased throughout the study period, a species substitution was observed. The species of freshwater origin were gradually replaced by the copepods *A. tonsa* and *P. richardi*, species more adapted to tolerate higher salinities. *P. richardi* reached maximum densities of 32 org.L⁻¹ (Fig. 8) while *A. tonsa* reached densities of 92 org.L⁻¹ (Fig. 9).

The substitution of freshwater organisms by typically estuarine species was more markedly observed in P2, where typical salinities of the PLE were recorded for a longer period (Fig. 2). In this pond there was an increase in salinity from 12/01/05 to 12/15/05, and a clear drop in the abundance of *M. micrura* was recorded (Fig. 4), an important cladoceran of freshwater origin,

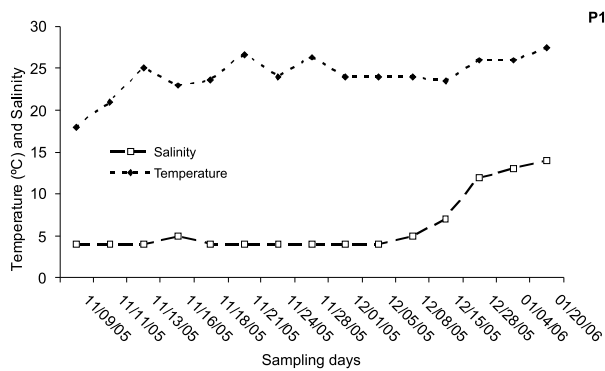


Figure 1. Temperature and salinity variation in culture pond n°. 1 (P1) of Carcibrás Shrimp Farm, São José do Norte - RS, between november of 2005 to january of 2006.

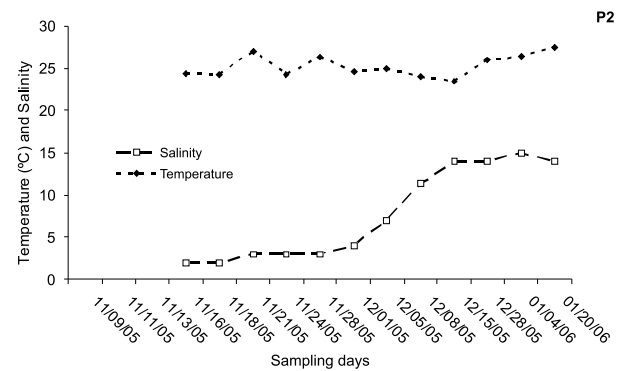


Figure 2. Temperature and salinity variation in culture pond n°. 2 (P2) of Carcibrás Shrimp Farm, São José do Norte - RS, between november of 2005 to january of 2006.

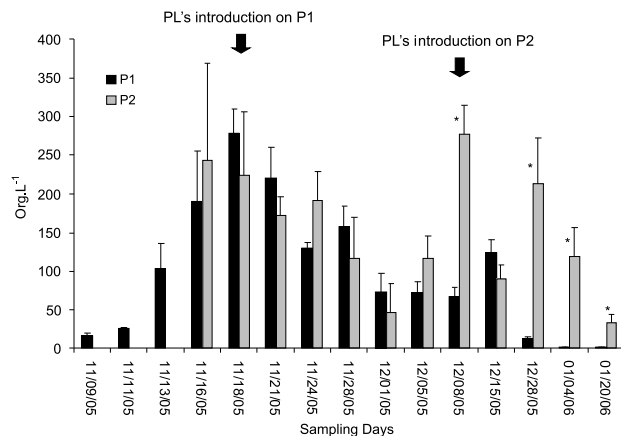


Figure 3. Total zooplankton density in org.L⁻¹ (mean ± standard deviation). * - denotes significant differences between ponds (two-way ANOVA, p < 0.05). Arrows indicate post-larvae introduction.

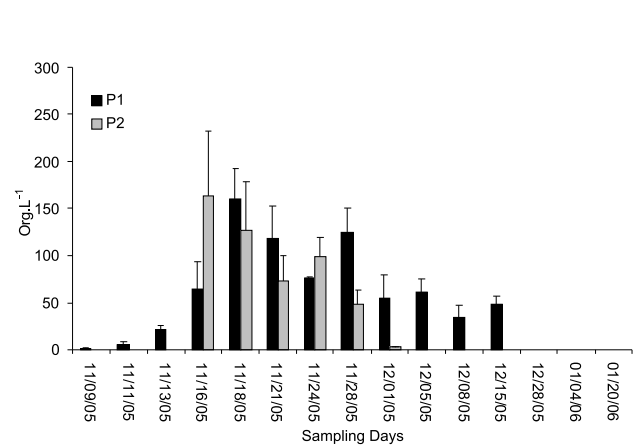


Figure 4. Mean density of *Moina micrura* in org.L⁻¹ (mean ± standard deviation).

followed by an increase in the density of the coastal and estuarine copepod *A. tonsa* (Fig. 9).

The most frequent organisms recorded during this study were distributed in different size classes, since small copepodites with 490 μm of total length (prosome + urosome), up to relatively large copepods with 1.4 mm of total length (Table 1).

Regarding the biomass, the wet weight values followed the same temporal pattern observed for the density in both ponds, with values ranging from 0.23 to 21.70 $\text{g}\cdot\text{m}^{-3}$ in P1 and from 1.28 to 20.43 $\text{g}\cdot\text{m}^{-3}$ in P2 (Fig. 10). For P1, the highest density value recorded in 11/18/05, was coincident with the highest value of wet weight. In contrast, for P2, the highest density found in P2, did not match with the wet weight peak. For this pond, the highest registered density value occurred in 12/08/05, and the peak of wet weight in 11/24/05.

The mean values of biomass in dry weight were 1.02 $\text{g}\cdot\text{m}^{-3}$ in P1, where the maximum observed value was 2.42 $\text{g}\cdot\text{m}^{-3}$, and the minimum was 0.10 $\text{g}\cdot\text{m}^{-3}$. For P2, the mean dry weight biomass during the period was

1.23 $\text{g}\cdot\text{m}^{-3}$, with a maximum of 2.72 $\text{g}\cdot\text{m}^{-3}$ and a minimum of 0.53 $\text{g}\cdot\text{m}^{-3}$ (Fig. 11).

DISCUSSION

The salinity variations recorded in the two ponds during this study possibly reflected the hydrological conditions of the water pumping region, the PLE, where daily and seasonal variations of salinity can occur (Niencheski & Baumgarten, 1997). The water renewal of 10 % per week along with evaporation losses, may have caused a gradual increase in salinity in both ponds. The small variation in water temperature observed in both ponds during this study was probably related to the constant and high temperatures that occurred during that summer.

The values of dissolved oxygen measured in the ponds, indicated that this parameter was not limiting for the organisms, since the lower values observed were close to 6 $\text{mg}\cdot\text{L}^{-1}$. With regard to the pH, the lower values

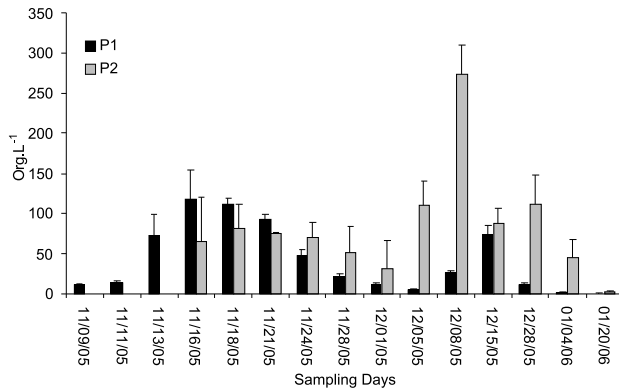


Figure 5. Mean density of copepodites in $\text{org}\cdot\text{L}^{-1}$ (mean \pm standard deviation).

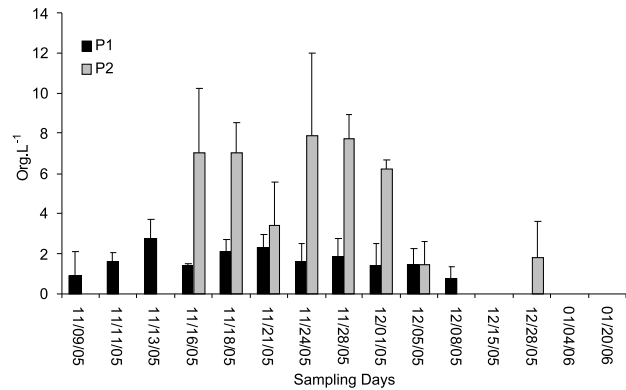


Figure 6. Mean density of *Microcyclops furcatus* in $\text{org}\cdot\text{L}^{-1}$ (mean \pm standard deviation).

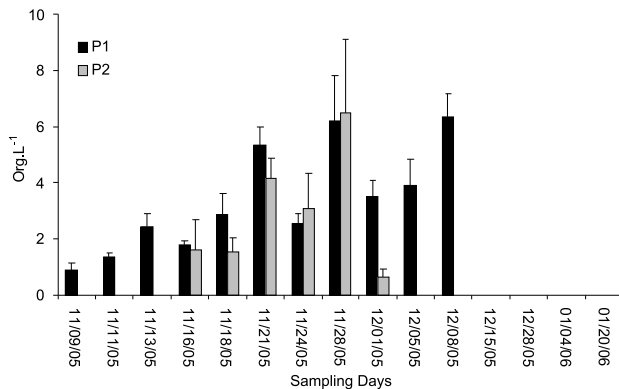


Figure 7. Mean density of *Acanthocyclops robustus* in $\text{org}\cdot\text{L}^{-1}$ (mean \pm standard deviation).

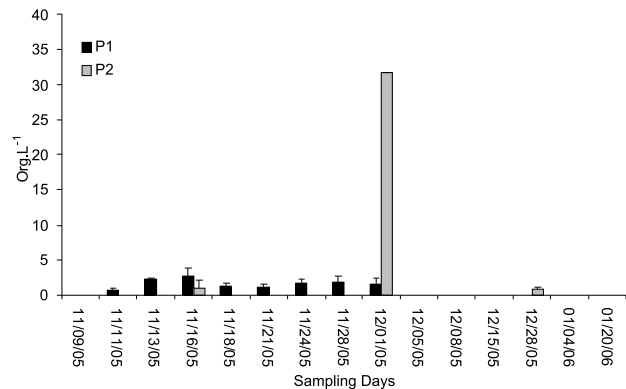


Figure 8. Mean density of *Pseudodiaptomus richardi* in $\text{org}\cdot\text{L}^{-1}$ (mean \pm standard deviation).

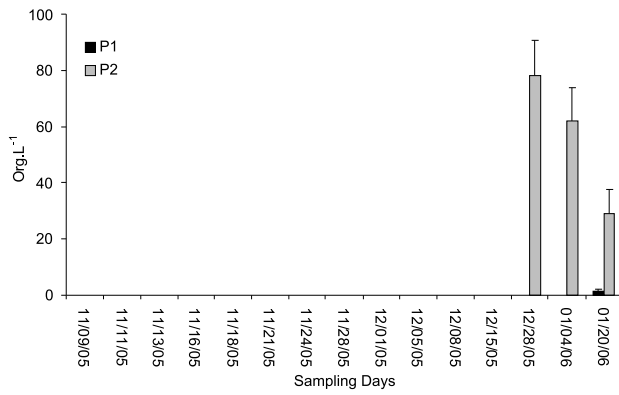


Figure 9. Mean density of *Acartia tonsa* in org.L⁻¹ (mean ± standard deviation).

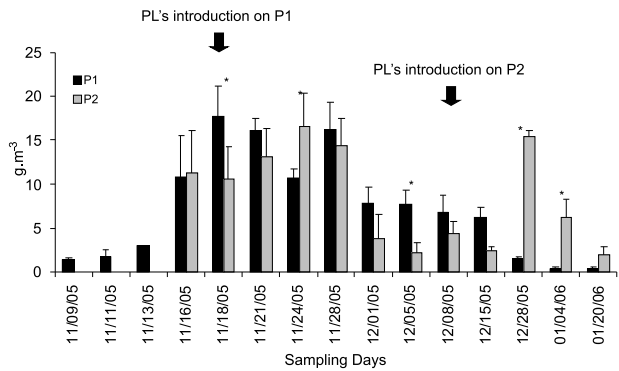


Figure 10. Wet weight biomass of total zooplankton in P1 and P2 in g.m⁻³. * - denotes significant differences between ponds (two-way ANOVA, p < 0.05). Arrows indicate post-larvae introduction.

recorded for P1 were probably a result of its soil which had great amount of organic matter due to its proximity to PLE. In P2, the values were always higher than 8 because this presented a sand sediment due to its higher quote to the PLE.

The species composition found in the ponds in the beginning of the study, presented organisms of freshwater origin, such as *N. incompositus* and *M. micrura*, and with the salinity increase, there was a substitution by species of marine origin, such as *A. tonsa* (Montú *et al.*, 1997). This species can be considered truly eurihaline, being found in the PLE at salinity values from 0 to 35 (Montú, 1980). All the species recorded in this study have been found in the PLE by Montú (1980) and Montú *et al.* (1997).

The zooplankton density values observed in the ponds were higher than the value of 40 org.L⁻¹ registered in the PLE by Montú *et al.* (1997). In this study mean densities of 96 org.L⁻¹ in P1, and 158 org.L⁻¹ in P2, indicate that in semi-closed environments as the earthen ponds, zooplankton can find favorable conditions for reproduction and growth, even considering the presence of predators such as shrimp larvae. The density values

Table 1. Size of the more frequent organisms (average ± standard deviation). For copepods TL – Total length (Prosoma + Urosome), PL – Prosoma length, W – width. * For *Moina micrura* TL correspond to the total carapace length, from the top of the head to the tip of the spine.

Groups	TL (µm)	PL (µm)	W (µm)
<i>Pseudodiaptomus richardi</i>	1.387 ± 65	924 ± 42	343 ± 24
<i>Acanthocyclops robustus</i>	1.085 ± 133	702 ± 76	415 ± 37
<i>Acartia tonsa</i>	938 ± 96	735 ± 71	249 ± 18
<i>Microcyclops furcatus</i>	828 ± 87	538 ± 78	311 ± 35
Copepodits (Calanoida e Cyclopoida)	580 ± 90	425 ± 62	172 ± 23
<i>Moina micrura</i>	716 ± 69 *	-	-

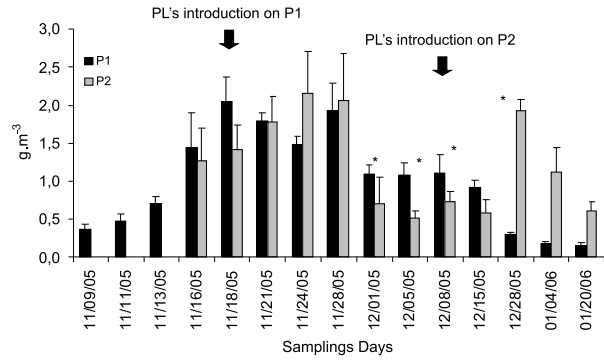


Figure 11. Dry weight biomass of total zooplankton in P1 and P2 in g.m⁻³. * - denotes significant differences between ponds (two-way ANOVA, p < 0.05). Arrows indicate post-larvae introduction.

recorded in this study are the highest observed for the PLE region until now.

The observed drop in zooplankton density in P1 right after the shrimp PL introduction, represents a strong indication of predation on zooplankton, since at that time, salinity, one of the parameters that regulates the zooplankton composition and density, did not show great variations. The introduction of shrimp PL in P2, also seems to have led to a reduction in the total zooplankton density, giving support to the idea that in the first days in the ponds, zooplankton is an important alimentary item for the PL (Martinez-Cordova *et al.*, 1997; Rothlisberg, 1998; Martinez-Cordova *et al.*, 2002).

Besides this evidence, the post-larvae seem to have exerted selective predation upon zooplankton, given that for both ponds a marked reduction in the numbers of copepodits (580 µm in size) was observed after the introduction of the post-larvae, suggesting a preference for organisms in this size class. This fact was more evident in P2, where the copepodits represented more than 90 % of total zooplankton, and after the introduction of post-larvae showed a density reduction of 50 %.

However, it is important to highlight the fact that at the time of the PL introduction in P1, the P2 also showed a considerable decrease in the zooplanktonic

density, even with the absence of PL. In this in case, the factor causing the zooplankton decline, was probably the overpopulation of cladocerans, since one of the most abundant organisms in this pond, the cladoceran *M. micrura*, showed the formation of ephippia, which are resting eggs that indicate unfavorable conditions for population growth.

When environmental conditions deteriorate due to overcrowding, lack of food, oxygen depletion and variations of other environmental conditions, cladocerans populations tend to produce the ephippia through sexual reproduction (Margalef, 1983; He *et al.*, 2001). Given that great variations in the physical-chemistry parameters were not observed during the period of dominance of the cladocerans, their population collapse in P2 may have occurred due to the exhaustion of adequate food resources caused by overcrowding.

Therefore, variations in zooplankton abundance in shrimp ponds must be carefully interpreted, because besides the predation pressure exerted by the post-larvae, the reductions and increases in zooplankton density can also be related to natural processes of succession in recently colonized environments.

Opportunistic species can show population explosions that lead to a fast exhaustion of the system support capacity, occurring then a collapse of the population. Before reaching stable conditions, a system can endure many processes of succession that over time can culminate with a climax community (Odum, 1986). Concerning the zooplankton from shrimp ponds around the Rio Grande region, a climax situation would be unlikely to happen given the frequent salinity fluctuations recorded in the area and the short time duration of the ponds operation (~ five months).

Even though shrimp ponds seems to favor the growth of zooplankton, the values of density and biomass of natural zooplankton produced in the ponds will probably vary according to geographical and regional conditions. On comparing to the results of this study with literature values, some authors have recorded lower zooplankton densities (Preston *et al.*, 2003; Coman *et al.*, 2003), while others have found higher values in shrimp ponds (Coman *et al.*, 2006), including some extreme density peaks (~ 840 org.L⁻¹) such as that registered by Martínez-Córdova & Peña-Messina (2005).

This is probably related to the amount and quality of the available food for zooplankton in the ponds. Other than the intrinsic characteristics of each area, the production of zooplankton in culture ponds can be stimulated through fertilizations that increase phytoplankton productivity (Barbieri & Ostrensky, 2002). In addition the use of organic inductors, can promote an increase in ciliate and flagellate communities in the ponds that stimulate the growth of zooplankton (Martinez-Cordova *et al.*, 2002).

In conclusion, the results of this study indicate that in semi-closed environments with little water renewal, such as shrimp ponds, high zooplankton density and biomass can be reached. Thus, the natural zooplankton occurring in shrimp ponds represents a potential food source for the shrimp post-larvae and juveniles during the first months of culture in southern Brazil.

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