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Temporal variability of plankton and nutrients in shrimp culture ponds vs. adjacent estuarine water

ALESSANDRO PEREIRA CARDOZO[1] ,VANESSA OLIVEIRA BRITTO[2] & CLARISSE ODEBRECHT[2]

[1]*Universidade Federal do Rio Grande, FURG, Pós-Graduação em Oceanografia Biológica, Av. Itália km 8, 96201-900, Rio Grande, RS, Brazil. E-mail: ocalessandro@yahoo.com.br*
[2]*Universidade Federal do Rio Grande, FURG. Instituto de Oceanografia, Av. Itália km 8, 96201-900, Rio Grande, RS, Brazil.*

Abstract. Water quality, phytoplankton and zooplankton were evaluated in a shrimp pond (*Litopenaeus vannamei*) and the adjacent Patos Lagoon estuary in summer from November/2007 to February/2008. Every two weeks, temperature, salinity, dissolved inorganic nutrients, total chlorophyll *a* and size fractions (>20µm; 1-20µm; <1µm) and phytoplankton and zooplankton densities were analyzed. Excepting temperature, significant differences were observed between both environments. In the estuary, salinity variations (1-21) were related with water exchange while, in the pond, increased salinity (7-20) probably resulted from evaporation. Silicate (51.08-100.73µM) was always higher in the estuary, while the levels of ammonium (0.71-5.37µM), phosphate (0.24-2.43µM) and suspended matter (10.57-78.63mg.L^{-1}) were higher in the pond. High nitrate/nitrite (18.67µM) levels correlated with inorganic fertilization in the pond. In November/2007, the level of chlorophyll *a* in the pond was lower (5.7-16.4µg.L^{-1}) than in the estuary (20.0-32.9µg.L^{-1}) where diatoms always prevailed. In the pond, flagellates and cyanobacteria <1µm were the main organisms responsible for the chlorophyll *a* increase (30.6µg.L^{-1}) in January/2008. Zooplankton, mainly the copepod *Acartia tonsa*, presented higher concentrations in the pond during the first month (238 org.L^{-1}) and probably exerted important grazing pressure reducing chlorophyll *a*. After that, zooplankton decreased significantly releasing the grazing pressure on phytoplankton and indicating the importance of top-down control. In conclusion, changes in chlorophyll *a* levels were mainly related with water exchange in the estuary while the nutrient availability and top-down control in the pond, influenced significantly the size structure and composition of phytoplankton.

Keywords: Phytoplankton, chlorophyll, zooplankton, nutrients, shrimps, estuary

Resumo. Variabilidade temporal do plâncton e nutrientes em viveiro de cultivo de camarão vs. águas estuarinas adjacentes. A qualidade da água, fitoplâncton e zooplâncton foram analisados em um viveiro de camarão (*Litopenaeus vannamei*) e em área adjacente do estuário da Lagoa dos Patos no verão de novembro/2007 a fevereiro/2008. A cada duas semanas, temperatura, salinidade, nutrientes, clorofila *a* total e frações de tamanho (>20µm; 1-20µm; <1µm), e densidade de fitoplâncton e de zooplâncton foram analisadas. A exceção da temperatura, diferenças significativas foram observadas entre os dois ambientes. No estuário, a variação de salinidade (1-21) esteve relacionada com a circulação da água, enquanto no viveiro o aumento de salinidade (7-20) foi provavelmente devido a evaporação. Valores de silicato mais altos (51.08-100.73µM) foram observados no estuário, enquanto que os de amônia (0.71-5.37µM), fosfato (0.24-2.43µM) e material em suspensão (10.57-78.63mg.L^{-1}) foram mais altos no viveiro. Altos valores de nitrato/nitrito (18.67µM) apresentaram relação com as fertilizações no viveiro. Em novembro/2007, os valores de clorofila *a* total foram menores no viveiro (5.7-16.4µg.L^{-1}) do que no estuário (20.0-32.9µg.L^{-1}), onde sempre foi observada a predominância de diatomáceas. No viveiro, flagelados e cianobactérias autotróficas <1µm foram os principais organismos responsáveis pelo aumento de clorofila *a* (30.6µg.L^{-1}) a partir de Janeiro/2008. Ao longo do primeiro mês no viveiro, altos valores de densidade do zooplâncton, composto principalmente pelo copépode *Acartia tonsa* (238 org.L-1), provavelmente aumentaram a pressão de predação e reduziram o teor de clorofila *a*. Após, houve uma

redução do zooplâncton com efeito na menor predação sobre o fitoplâncton, indicando a importância do controle *top-down*. Concluindo, variações de clorofila *a* no estuário estiveram mais relacionadas com a circulação da água enquanto que, no viveiro, a disponibilidade de nutrientes e o efeito *top-down*, influenciaram a estrutura de tamanho e composição do fitoplâncton.

Palavras chave: Fitoplâncton, clorofila, zooplâncton, nutrientes, camarões, estuário

Introduction

Recently, shrimp culture-related businesses were established in the Patos Lagoon estuary following the increasing worldwide trend for aquaculture (FAO 2008). However, shrimp culture is a controversial activity due to the cultivation of exotic species in many cases and also because the effluent that originates from the ponds and is discharged in the adjacent environment. Such discharge is the result of daily enrichment of the ponds with large amounts of formulated food and regular fertilization, which aim to stimulate the primary production and induce the growth of the entire trophic web.

In general, shrimp culture ponds exhibit higher levels of chlorophyll *a* and nutrients than do adjacent environments (Casillas-Hernández *et al.* 2007). The increased levels of chlorophyll *a* and nutrients are due to the release of large amounts of nitrogen and phosphorus from formulated food and fertilizations that are used to stimulate phytoplankton growth (Funge-Smith & Briggs 1998). Changes in the structure of the phytoplankton community are observed in ponds with low water renewal (Burford 1997); small-sized cells (<10µm) dominate when the levels of ammonium are high, enhancing the growth of microzooplankton (Burford *et al.* 2003). Casé *et al.* (2008) verified that enhanced nutrient input affected plankton composition and density in shrimp farms of northeastern Brazil, indicating that the plankton structure may be used as an indicator of water quality in tropical shrimp culture ponds. The dominance of diatoms and copepods was replaced by cyanobacteria, protozoan and rotifers as nutrients increased through the final period of culture. Zooplankton represents an important food item for the growth of post-larval shrimp during their first days of culture (Anderson *et al.* 1987, Chen & Chen 1992). Studies carried out in Australia and in the Patos Lagoon estuary in Brazil have shown that the dominance of copepods in shrimp ponds reflects the composition of the adjacent environment (Coman *et al.* 2003, Cardozo *et al.* 2007).

The impact of pond effluents on the adjacent ecosystems is variable and depends on various factors as the magnitude of the discharge, the chemical composition of the pond effluents and the characteristics of the environment that receives the discharge, such as circulation and dilution rates (Páez-Osuna 2001). Studies conducted in Australia demonstrated the accelerated eutrophication of the adjacent Muddy Creek estuary in Queensland, which received effluents with a high content of organic matter from a local culture farm (McKinnon *et al.* 2002a). However, the effluent did not result in adverse environmental effects in other areas of the same estuarine zone (Trott & Alongi 2000). Another Australian estuary, Morris Creek in Queensland, was shown to have significant increases in particulate nutrients and total suspended solids during periods of effluent discharge (McKinnon *et al.* 2002b). In northeastern Brazil, it was shown that shrimp farming is responsible for the largest emission of nitrogen to six estuaries, and for a smaller input of phosphorus (Lacerda *et al.* 2006).

In the Patos Lagoon estuary, water circulation and salinity changes are driven by the direction of the wind and the discharge of freshwater from northern portion of the lagoon, resulting in flood or ebb regimes in the channel area (Möller *et al.* 1996). In shallow areas of the estuary, predominant winds from the NE induce water exchange and lower residence time when compared to deeper central areas of the lagoon (Möller *et al.* 1996). Hydrology plays a key role in the control of chlorophyll *a* variability in the Patos Lagoon estuary at different time scales, though other factors like water temperature, light, and nutrient availability (mainly nitrogen) and mesozooplankton predation are also important (Fujita & Odebrecht 2007, Abreu et al. 2010). It was observed that short-term (i.e., hours, days) chlorophyll *a* variability is mainly controlled by winds, while long-term changes are related to the freshwater input by rainfall. Higher values of chlorophyll *a*, phytoplankton density and biovolume occur in spring/summer compared to autumn months and are mainly comprised by microplankton diatoms (20-200 µm) (Odebrecht & Abreu 1997, Odebrecht *et al.* 2005). During the spring and summer periods, higher zooplankton densities are also observed in the estuary (Montú *et al.* 1997).

Shrimp ponds located in the Patos Lagoon are supplied with estuarine water from shallow areas where circulation and wind-induced sediment suspensions influence the concentration of chlorophyll *a*. It can be assumed that the fertilization of ponds creates favorable conditions for the growth of phytoplankton and zooplankton, increasing the availability of natural food for shrimp, which exert a top-down control (Cardozo *et al.* 2007). This study aimed to assess the variations, along the shrimp production cycle in southern Brazil, of the plankton community and abiotic parameters in a fertilized culture pond of *Litopenaeus vannamei* vs. the adjacent shallow waters of the Patos Lagoon estuary.

Material and methods

The three months study was conducted from 21 November 2007 to 14 February 2008 during the culture period of the shrimp *Litopenaeus vannamei* (Boone 1931) in the Viveiros do Sul farm (São José do Norte City, Rio Grande do Sul State, Brazil - 31º56'04S, 52º00'11W). The pond (area of 3 hectares; 100 cm average depth) was flooded for five consecutive days (13 to 17 November 2007) from the adjacent shallow area of the estuary. The water was pumped during the day and the supplying channel was naturally replenished at night. Prior to flooding, the standard procedure of pH balancing the pond bottom (pH 7) was performed with calcium carbonate. Fertilization with 100 Kg of $(NH_2)_2CO$ and 20 Kg of $Ca(H_2PO_4)_2$, in order to increase phytoplankton growth since the beginning, resulted in concentrations of 6µM phosphorus and 109.5µM nitrogen (N:P ratio = 18). After allowing complete flooding of the pond, seven fertilizations were performed during the first month of shrimp culture (11/21/2007 to 12/20/2007) with 240 Kg of $(NH_2)_2CO$ and 34.5 Kg of $Ca(H_2PO_4)_2$, adding up to a total input of 1.875 µM phosphorus and 133.75 µM nitrogen (N:P = 71). Ten day-old shrimp post-larvae were stocked in the pond on November 27/2007 in an approximate density of 30 shrimps m^{-2}. The shrimps were fed with pellets (35% crude protein) three times a day: 25% in the morning, 25% in the afternoon and 50% at the evening.

Surface samples were taken in three stations every two weeks from the shrimp pond, and from three stations in Patos Lagoon estuary. Sampling started on 21 November 2007 after the pond was flooded and ended on 14 February 2008 when the shrimps were harvested; sampling was ended one month earlier in the estuary due to the water setback during the drought period that started on January 17. The average depth of the sampling points in the estuary and the pond were of 0.45 m and 0.9 m, respectively. During the sampling period, the water loss due to evaporation and soil infiltration was compensated for by water input from the estuary, approximately 8% (2,400 m^3) of pond total volume during the whole culture period.

Temperature (mercury thermometer) and salinity (refractometer) were measured *in situ*. Rainfall information was obtained using data from Brazilian Meteorological National Institute (INMET). Analysis and quantification of the levels of chlorophyll *a*, dissolved inorganic nutrients, suspended matter, phytoplankton and zooplankton were performed in the laboratory. For nutrient analysis (nitrate/nitrite, phosphate and silicate), water was filtered through glass microfiber filters (S&S, GF-50A) and the filtrate was frozen (-20ºC; 200mL plastic Nalgen bottles), following traditional methods (Strickland & Parsons 1972). Ammonium levels were measured immediately after water sampling according to the recommendations described by the UNESCO (1983). For measurement of suspended matter, 150-500 mL of water was filtered through previously dried and weighted (60°C for 24 h) glass microfibre filters (S&S GF-50A) (Strickland & Parsons 1972).

Total chlorophyll *a* level was measured on glass microfibre filters (Whatman GF/F 0.7µm pore size) after filtering 15-50 mL of water. A second water sample was size fractionated by filtering through a 20µm pore nylon mesh, and a third sample was filtered through 1µm pore polycarbonate filter and both filtrates were retained on glass microfiber filters (Whatman GF/F). The filters were stored for two days at -20ºC in the dark, and pigments were extracted (24 h) in 10 mL of 90% acetone added to each vial. Fluorescence of the acetone extract was measured without acidification (Welschmeyer 1994) using a calibrated Turner Design Fluorometer, model TD 700. The level of <1µm chlorophyll *a* was obtained directly from the fluorescence measurements, and the other fractions were estimated by subtracting the <1µm chlorophyll *a* from the <20 µm fraction, and both from the total value to estimate the >20µm fraction.

A total of 57 phytoplankton samples were collected and the density (organisms per liter) was estimated by transferring surface water to dark glass bottles, fixing with Lugol´s solution in 4% final concentration (Strüder-Kypke *et al.* 2001) and analyzing in sedimentation chambers (2-10 mL) using an inverted microscope following the Utermöhl method (Hasle 1978). Large organisms of phytoplankton were enumerated in the whole chamber under 100x magnification. Smaller organisms (20-50µm) were counted under 200x

magnification in bands or in half chamber, and the most abundant and smallest cells were counted in as many fields as necessary to reach 150 organisms at the highest magnification (400x). To enumerate <1µm cyanobacteria, 2 to 3 ml of water were filtered through 1µm pore size polycarbonate filters. The filtrate was retained on darkened polycarbonate filters (0.2µm pore size, Nucleopore), mounted on glass slides with a drop of mineral oil and coverslips, sealed and stored frozen no longer than three months. Phycoerythrin and phycocyanin fluorescing cyanobacteria were counted using a Zeiss Axioplan fluorescence microscope following the methodology described by Waterbury *et al.* (1986) with excitation wavelength of 546 nm (green filter) and 450-490 nm (blue filter). Phytoplankton biovolume was estimated based on cell measurements and geometric formulae as proposed by Hillebrand *et al.* (1999).

A total of 54 zooplankton samples were collected using a 1.5m-length cylindrical-conical net (150 µm mesh size; 30 cm mouth diameter) with a flow meter attached to the net mouth in order to obtain the filtered volume. The net was hauled at surface covering a linear average distance of 50 meters and the collected material were transferred to one-liter plastic bottles and fixed in a 4% buffered formaldehyde solution. Zooplankton was counted using a Bogorov counting chamber under a stereoscopic microscope (Boltovskoy 1981). Statistical differences between the sampling points over time were tested using a two-way analysis of variance (ANOVA) (Zar 1999).

Results

The temperature in the pond and the estuary ranged from 21ºC to 28ºC; the exception was the temperature of the estuary on the first sampling date which registered at 15ºC, the lowest value. The salinity fluctuation (S 1-21) in the estuary resulted from water exchange between freshwater discharge from the northern portion of the lagoon and the intrusion of marine waters. In contrast in the pond a gradual increase was observed due to evaporation from the initial value of salinity 7 (11/21/2007) to 20 (2/14/2008) (Figure 1). Rainfall during the study period accumulated 294 mm, the precipitation distributed in 10mm in November/2007, 58mm in December/2007, 101mm January/2008 and 125 mm in February/2008. These values are smaller than average historic monthly precipitation, with exception in January when precipitation was slightly higher than the historic monthly value.

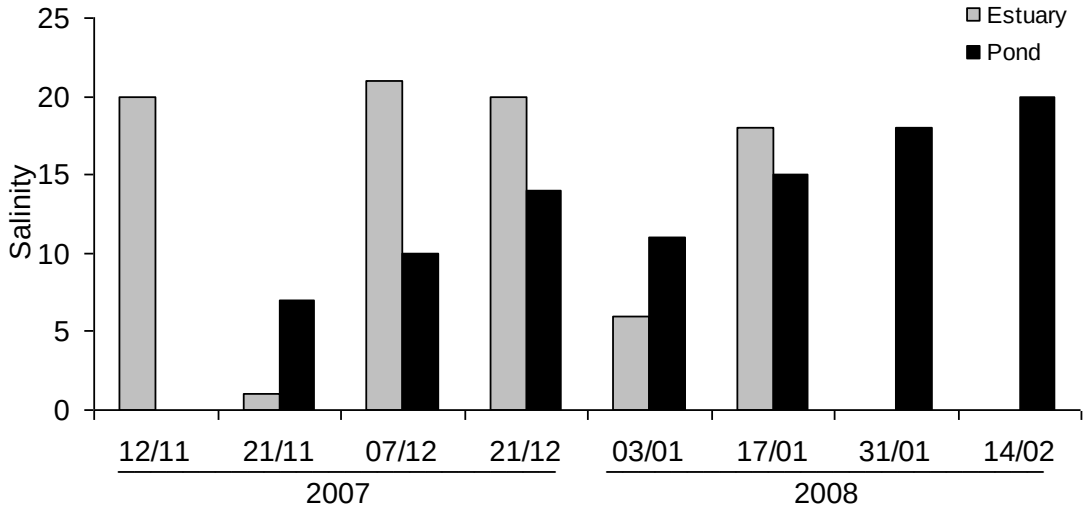

Figure 1. Salinity levels in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008

Suspended matter levels were highest (maximum 126 mg.L^{-1}) in the beginning of the study at Patos Lagoon estuary (November 12 and 21, 2007) but decreased over time (~30 mg.L^{-1}). In contrast, pond water had low levels of suspended matter (~25 mg.L^{-1}) in the beginning but increased and remained relatively high (~75-85 mg.L^{-1}) by the end of the study (January/February) (Figure 2).

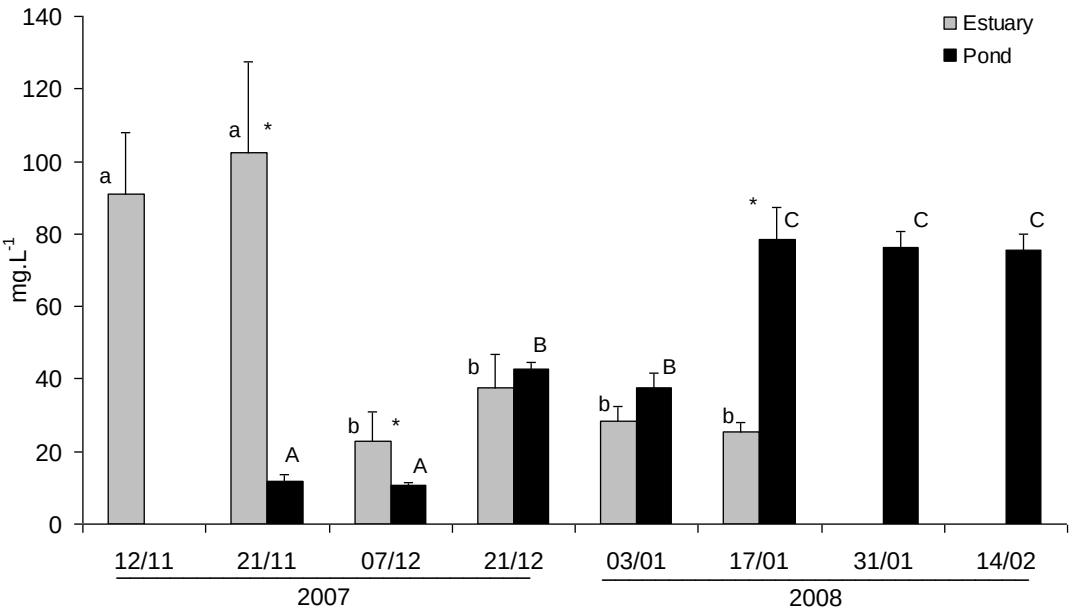

Figure 2. Suspended matter concentrations (mg/L) in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively.

The nitrate/nitrite levels in the estuary decreased from 23 µM in the first week of the study to non-detectable levels afterward (Figure 3A). In the shrimp pond, nitrate/nitrite concentrations increased up until December 21 (maximum 18 µM on December 21) but decreased below the limit of detection thereafter (Figure 3A). Ammonium levels in the estuary were always low (~2 µM) (Figure 3B). In the pond, the concentration of ammonium was relatively high (~5 µM) between November 21 to December 21 but decreased significantly afterward (<2 µM) (Figure 3B).

The concentration of phosphate in the Patos Lagoon estuary was high between November 12 and 21 (0.2 µM-1.5 µM) and low afterward (minimum on December 21) (Figure 4). In the pond, the opposite pattern was observed with respect to nitrogen elements (ammonia, nitrate and nitrite), with a significant phosphate increase at the end of the sampling period (maximum 2.4 µM) (Figure 4). Silicate levels were always high in the Patos Lagoon estuary (31-107 µM) and significantly lower in the shrimp pond (mean 10 µM) where the minimum silicate level (6 µM) was observed on December 21 (Figure 5).

Total chlorophyll *a* levels in the Patos Lagoon estuary (7.84 $\mu g.L^{-1}$-32.85 $\mu g.L^{-1}$; mean 20.45 $\mu g.L^{-1}$) decreased between the beginning and end of the study (Figure 6A), concomitant with a significant change in the size classes. In November, chlorophyll *a* <1 µm prevailed in the estuary but was replaced by larger phytoplankton chlorophyll *a* (1-20 µm; >20 µm) between December and January. Despite the high levels of chlorophyll *a* in the estuary, a relatively low level of chlorophyll *a* (5-15 $\mu g.L^{-1}$) was observed in the pond at the beginning of the study, which was followed by a significant increase in <1 µm chlorophyll *a* levels leading to total chlorophyll *a* concentration of 20-30 $\mu g.L^{-1}$ in the pond at the end of the study (Figure 6B).

The cell density of phytoplankton in the estuary was generally close to 2×10^6 $cells.L^{-1}$, except at the end of December and beginning of January (5×10^6-1×10^7 $cells.L^{-1}$) (Figure 7A). Diatoms, mainly *Skeletonema* species, and small (2-15 µm) flagellates and coccoid cells predominated, with mean frequencies of 36% and 62%, respectively (Figure 8A). In the pond, the cell density of phytoplankton was low (1×10^5 $cells.L^{-1}$) up until December 7. Afterward, a significant increase was observed, with the peak density being reached on January 3 (1×10^9 $cells.L^{-1}$), followed by a decrease on January 17 (10×10^5 $cells.L^{-1}$) and a gradual increase until the study end (Figure 7A). Small flagellates and coccoid cells (2-15 µm) were the most abundant organisms (mean of 68% over time) (Figure 9A).

Phytoplankton biovolume values in the estuary were higher than in the pond until December 21, but this trend changed afterward (Figure 7B). The higher biovolume values in the initial period in the estuary were influenced by the contribution of diatoms (Figure 8B) with the dominance of *Skeletonema* spp. In the pond, diatoms were present until December 21 and were substituted by flagellates and coccoid cells afterward (Fig 9B). In the initial period, small flagellates represented an important contribution but larger ones (flagellates >15 µm) replaced the small fraction during the last month, comprising 40% and 60% of the total phytoplankton density and biovolume, respectively (Figure 9A, 9B).

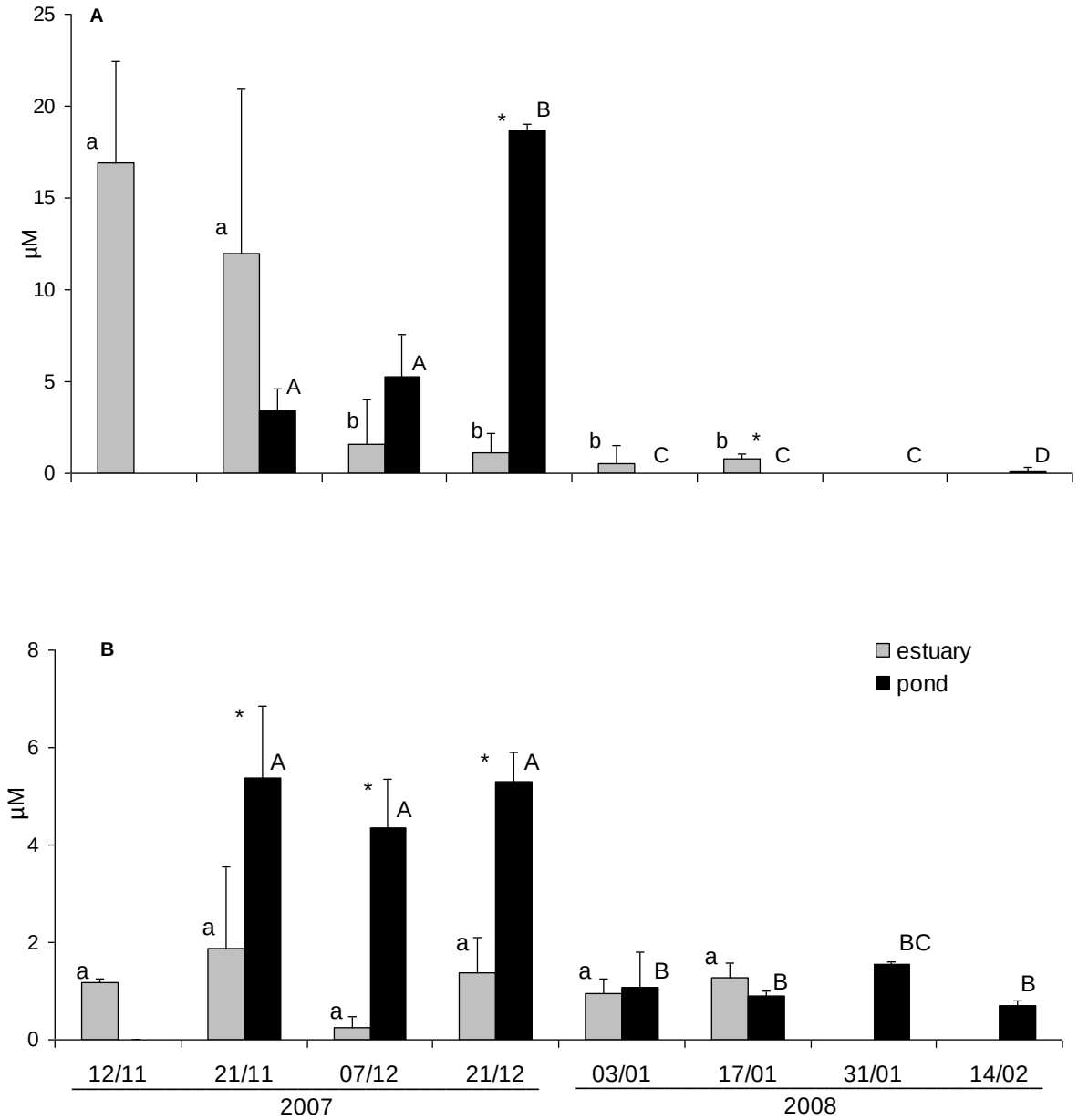

Figure 3. Concentrations of nitrate+nitrite (µM) (A) and ammonium (µM) (B) in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively.

Cyanobacteria <1 µm were found at a low density in the estuary (maximum 2.8×10^4 cells.mL^{-1}) compared to the shrimp pond where their abundance increased between December 21 (3.2×10^3 cells.mL^{-1}) and January 17 (2.2×10^5 cells.mL^{-1}) and remained high (10^5 cells.mL^{-1}) until the end of the study (Figure 10).

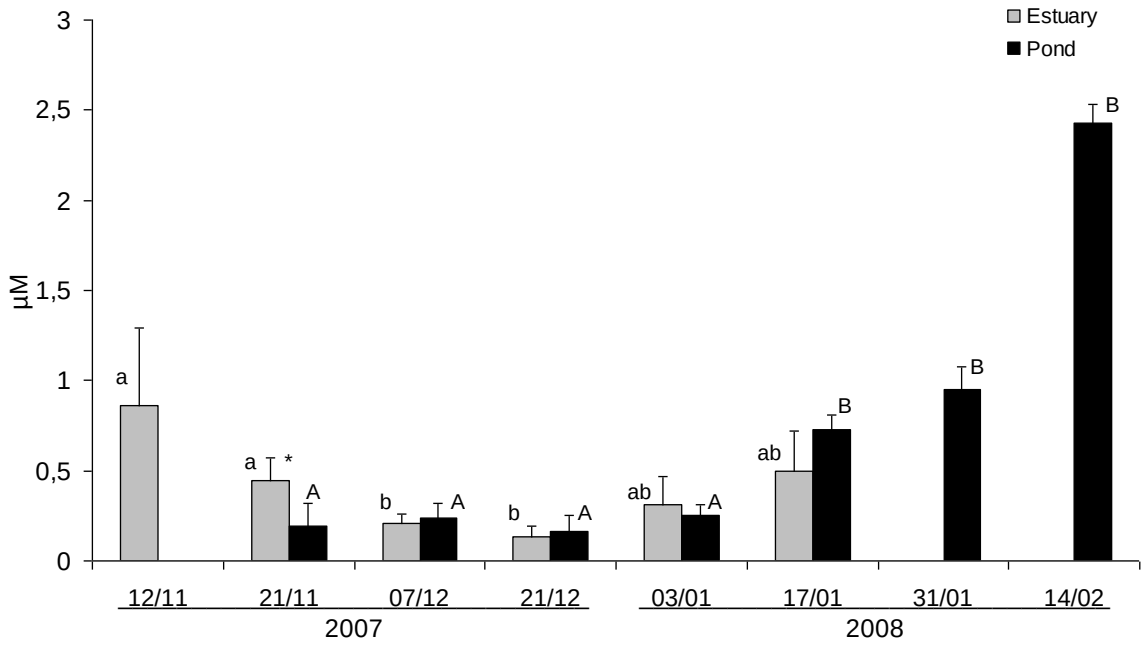

Figure 4. Concentration of phosphate (µM) in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively.

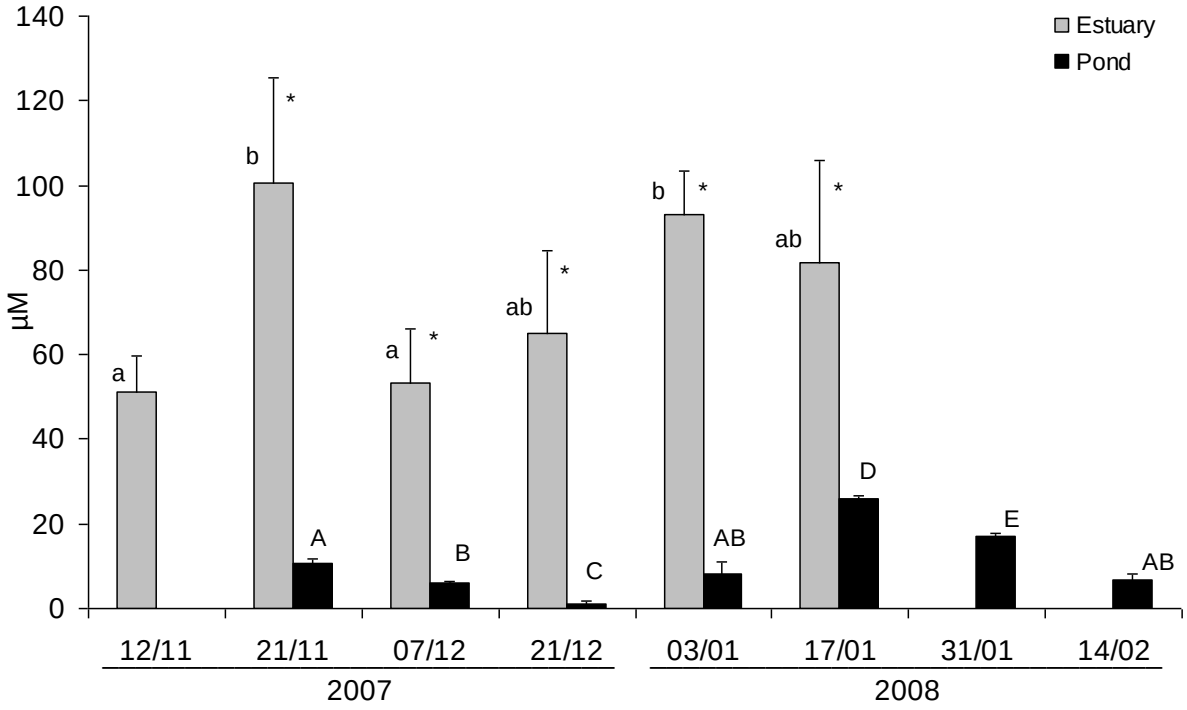

Figure 5. Silicate concentration (µM) in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively.

Zooplankton abundance in the estuary was relatively low (mean 9 org.L^{-1}), except on January 3. The initial dominant copepod *Notodiaptomus incompositus* was substituted by *Acartia tonsa*, which was responsible for the density peak (63 org.L^{-1}) on January 3 (Figure 11A). In the pond, *A. tonsa* was always dominant and represented the most abundant species (238 org.L^{-1}) on December 7, while in January and until the end of the study, this species underwent a significant decrease (≤10 org.L^{-1}) (Figure 11B).

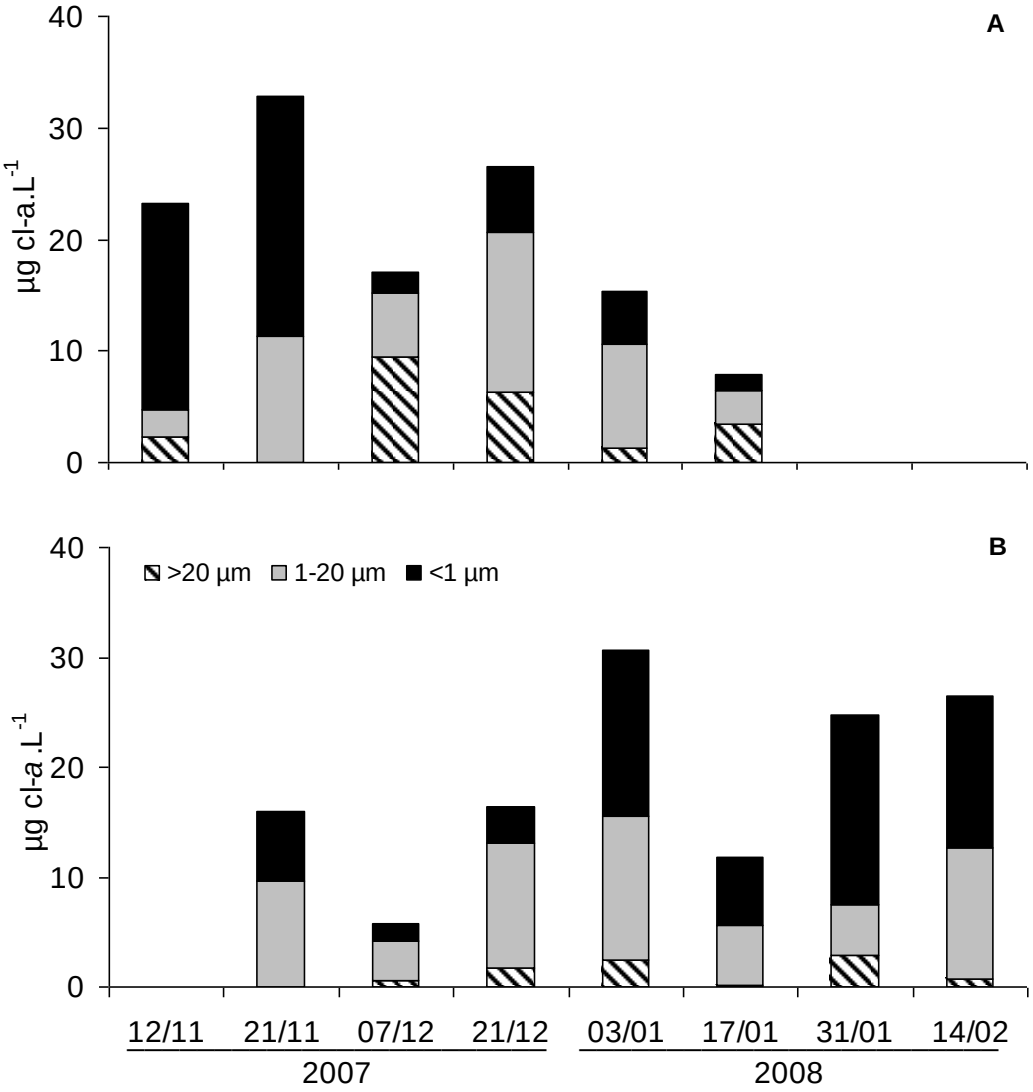

Figure 6. Size fractioned (>20 µm; 1-20 µm; <1 µm) chlorophyll *a* levels (µg chl *a*.L^{-1})) in the Patos Lagoon estuary (A) and the shrimp pond (B) during the summer production cycle from November/2007 to February/2008.

Discussion

The differences in chlorophyll *a* levels and other measured variables between the shrimp pond and the Patos Lagoon estuary may be due to (1) differences in circulation patterns between the two sites, (2) the availability of dissolved nutrients and (3) zooplankton control (microzooplankton and metazooplankton). The large fluctuation in salinity levels observed in the estuary during the first half of November indicates that water exchange was significant in this environment and played an important role in determining the differences between the estuary and pond. The estuarine circulation in summer periods is mainly controlled

by northeasterly winds that generate an outflow in all depths, reducing salinity (Castelao & Möller 2006). When the wind reduces, inflow of salt water through deeper layer elevates salinity in the estuary.

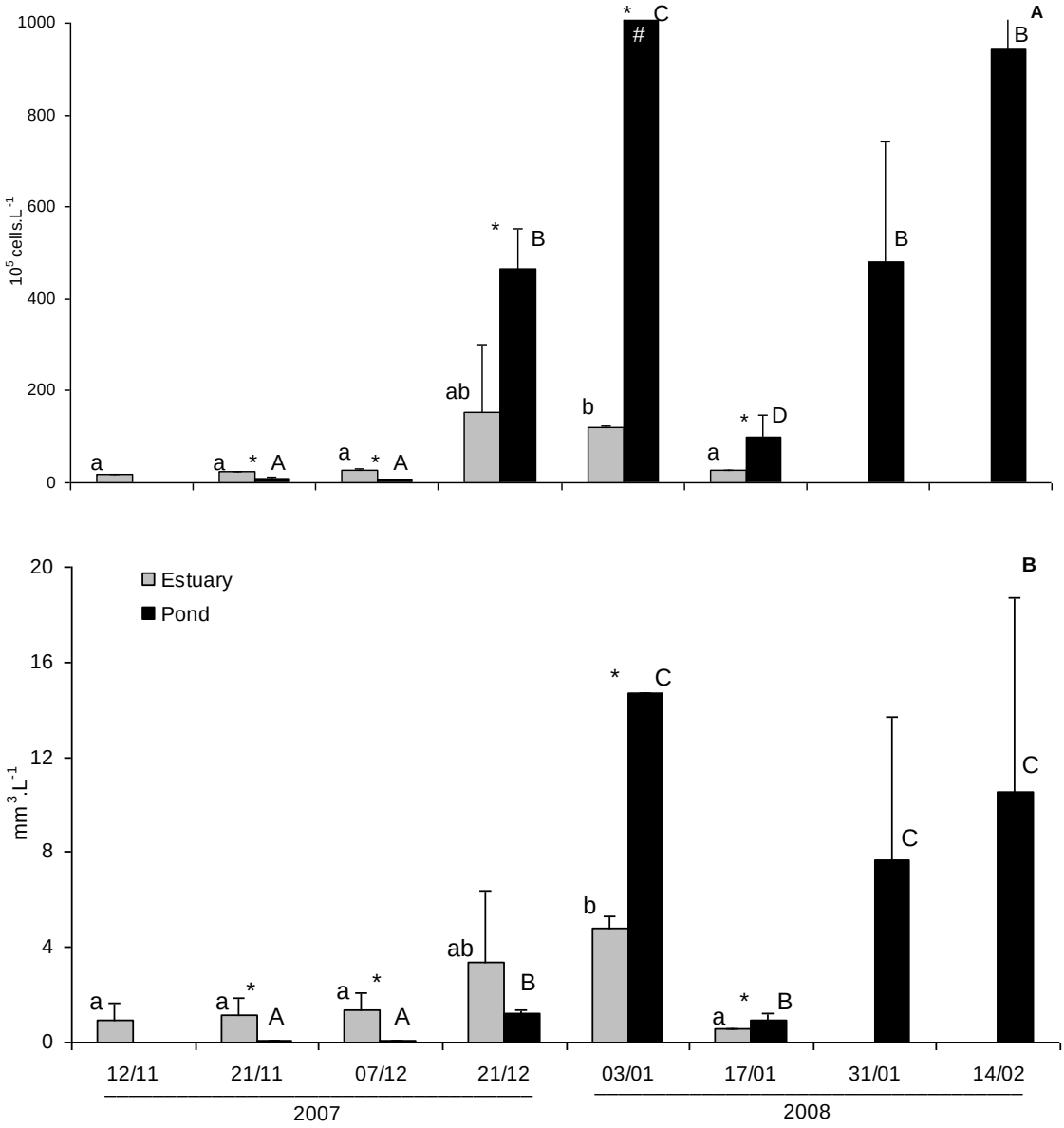

Figure 7. Phytoplankton density (10^5 cells.L^{-1}) (A) and biovolume (mm^3.L^{-1}) (B) in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively. # phytoplankton density of 10000×10^5 cells L^{-1}.

The technique used to pump the estuary water into the pond during the daytime only while at night the channel was replenished naturally, reduced the suspend matter input. In fact, on the first sampling day (November 21) both suspended matter and silicate levels were approximately eight times lower in the pond compared to the estuary. Silicate was not introduced through pond fertilization or the diet and the significant decrease in silicate levels in the pond at the beginning of the study may be associated with its adsorption on suspended matter. The slight increase observed in January could in turn be associated with the silicate release due to the increase in salinity (Liss 1976). If all dissolved inorganic silicate that entered from the estuary into the pond did so during the flooding period (~75 µM) and was taken up by phytoplankton, we would expect chlorophyll *a* levels between 50 and 60 µg chl a L^{-1} at the study beginning (November 21), according to C:N:Si:P atomic ratio of 106:16:15:1

(Redfield *et al.* 1963). The low concentration of chlorophyll *a* in the pond in comparison to the estuary at the beginning of the study resulted mainly from the reduction of chlorophyll *a* <1 µm which is an important fraction attached to suspended matter (Abreu *et al.* 1992). Thus, we can conclude that the water-pumping technique used may have strongly influenced the concentrations of suspended matter, adsorbed silicate and chlorophyll *a* in the pond at the beginning of the study. Nitrate and phosphate levels were also relatively low despite prior sediment fertilization.

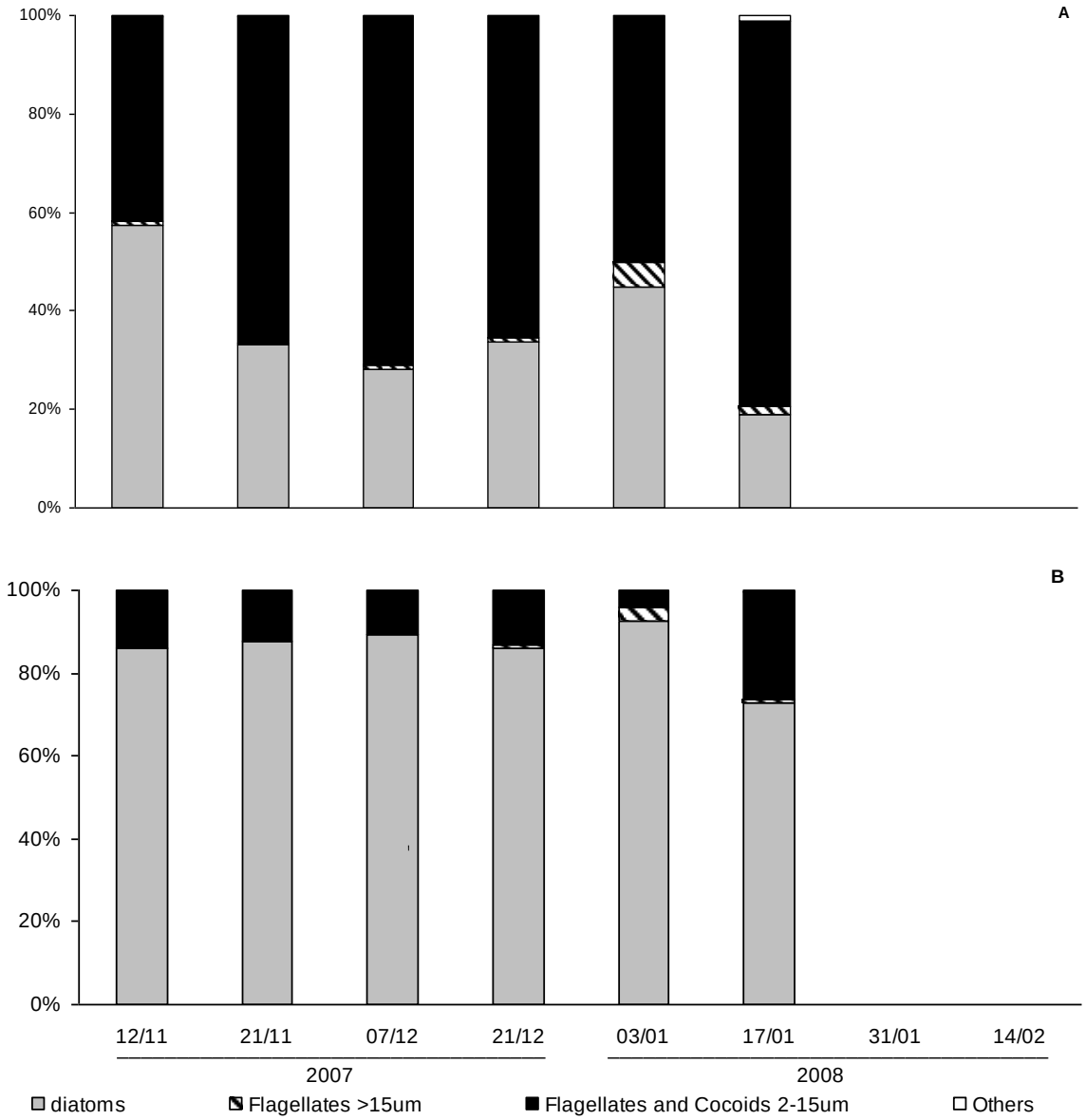

Figure 8. Contribution (%) of main phytoplankton groups to cell density (A) and biovolume (B) in the Patos Lagoon estuary during the summer production cycle from November/2007 to February/2008. Size class was determined by maximum length distance.

The concentration of ammonium in the pond doubled on November 21 compared to the concentration observed in the estuary. Benthic regeneration provides an important input of ammonium in the water column in Patos Lagoon waters (Niencheski & Jahnke 2002) and in ponds (Burford & Lorenzen 2004), especially during the first month of culture, when vegetation debris is still decomposing. During the first month of the study and until December 21, the 4-fold increase in the level of nitrate coincided with regular fertilizations, leading to the observed excess of nitrogen and low phosphate in the pond water (N:P atomic ratio of ~150). Later in the study, nitrogen and phosphate

levels reversed, nitrogen decreased while suspended matter and phosphate increased until the end of the study (February 14). The increase in suspended matter over time was probably associated with the production of shrimp (Martin *et al.* 1998), the surplus of food and the growth of phytoplankton stimulated by nutrient remobilization (Trott & Alongi 2000). In addition, natural regeneration may also influence the concentration of phosphate in the pond water, and the salinity increase favors the release of adsorbed phosphorus (Liss 1976).

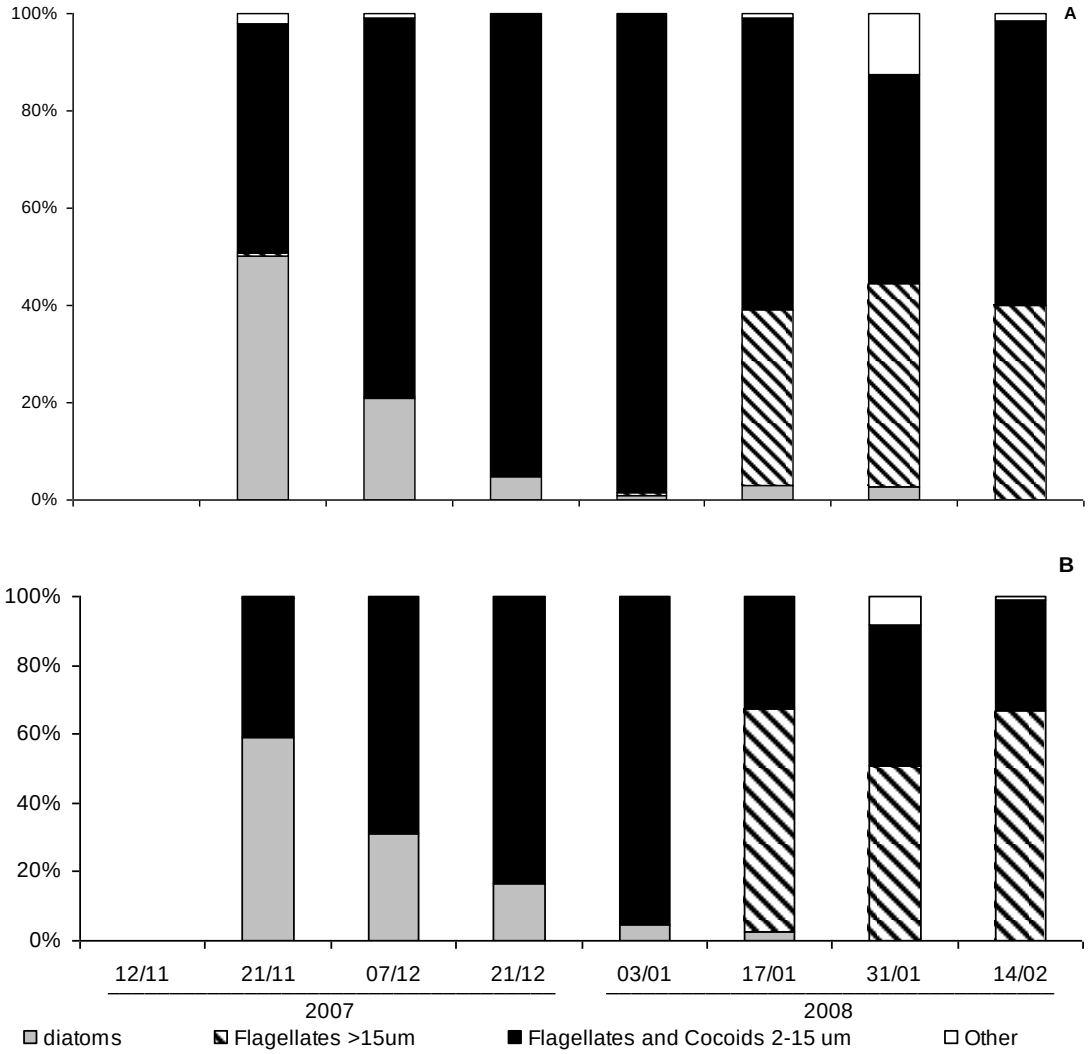

Figure 9. Contribution (%) of main phytoplankton groups to cell density (A) and biovolume (B) in the shrimp pond during the summer production cycle from November/2007 to February/2008. Size class was determined by maximum length distance.

The opposite trend between chlorophyll *a* levels and zooplankton density during the initial period of the study (December 7 to January 3) suggests some relationship between these organisms and phytoplankton occurred. The chlorophyll *a* concentration (16 µg L^{-1}) was only about half the value expected (40 µg.L^{-1}) according to the C:N:P atomic ratio of Redfield et al. (1963) and considering the amount of nitrogen nutrients (N 133.75 µM) introduced in the pond due to artificial fertilization. Zooplankton density increased between November 21 and December 7, with *A. tonsa* representing the most abundant organism, which feeds preferably on cells ranging from 5-20 µm in size (Stottrup & Jensen 1990). The significant decrease in the chlorophyll *a* (1-20 µm; <1 µm

fractions) on December 7 possibly reflects the copepod's herbivory peak. Using an estimated ingestion rate of 2.31 µg chlorophyll *a*/organism/day (Tester & Turner 1988), the zooplankton grazing removal (mainly *A. tonsa*) would be approximately 14 µg.L^{-1} d-1 chlorophyll *a*.

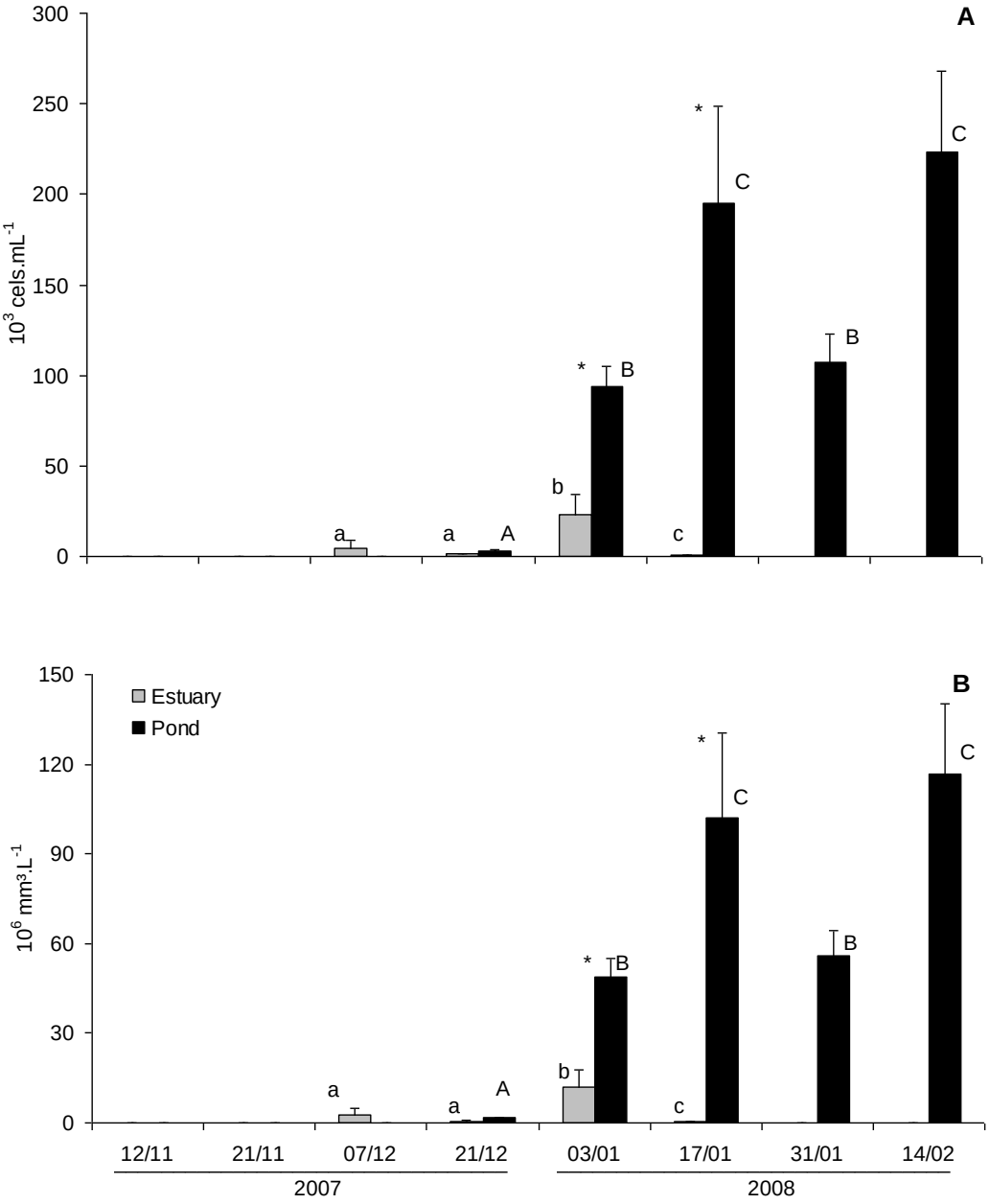

Figure 10. Density of <1 µm cyanobacteria (10^3 cells.mL^{-1}) (A) and biovolume (B) in the Patos Lagoon estuary and the shrimp pond during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively.

The extremely low nitrate levels observed in January and February following fertilizations is probably related to phytoplankton uptake, as indicated by the increase in chlorophyll *a* observed during this period. In culture ponds, 56-71% of nitrogen and 51% of phosphorus is introduced with the food (Funge-Smith & Briggs 1998, Páez-Osuna *et al.* 1999), stimulating the growth of microalgae and zooplankton. In our study the drastic decline in zooplankton (96%) observed from January through the end of the study may be explained by the top-down control exerted by growing shrimp larvae in

the pond, once that the dominant copepod *A. tonsa* is eurihaline (Montu *et al*, 1997) and less affected by salinity changes that occurred in the pond. In previous studies Martinez-Cordova *et al.* (1997) and Cardozo *et al.* (2007) showed that zooplankton levels decreased up to 50% after the introduction of post-larval shrimp into rearing ponds. Thus, it seems that in the current study, the grazing pressure of shrimp on zooplankton favored the growth of flagellates >15 µm after January 17, when *A. tonsa* had its density reduced, also reducing the predation pressure on the flagellates.

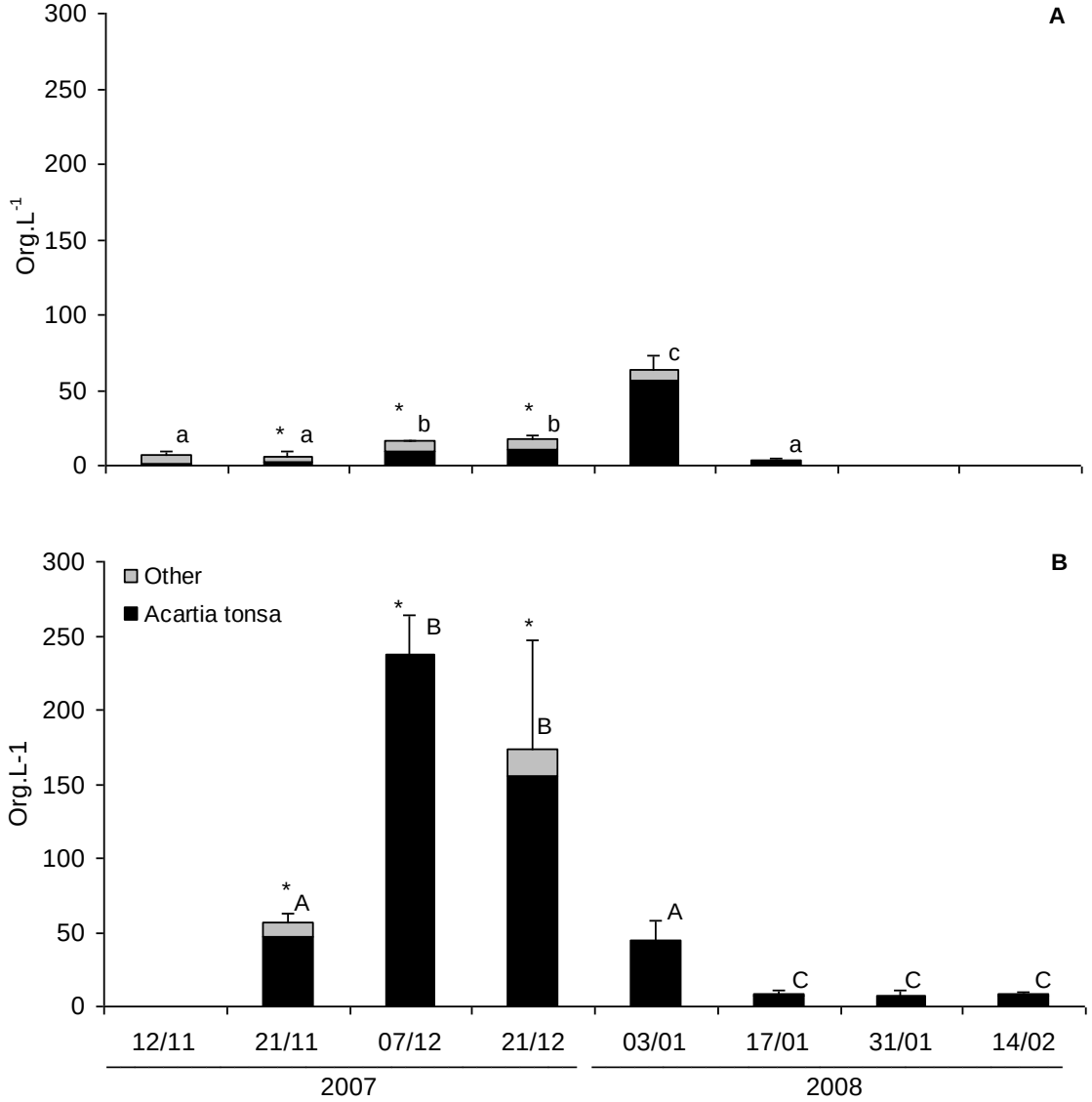

Figure 11. Total zooplankton density (org.L^{-1}) in the Patos Lagoon estuary (A) and the shrimp pond (B) during the summer production cycle from November/2007 to February/2008. * denotes differences between estuary and pond, lowercase and uppercase letters indicates singular differences in the estuary and pond, respectively.

In January and February, the high concentration of chlorophyll *a* <1 µm coincided with increased cell density of cyanobacteria <1 µm in the pond. During these months, the low concentration of dissolved inorganic nitrogen probably hampered the growth of larger microplankton cells and favored small-sized organisms (Ning *et al.* 2000). Reduced light and increased suspended matter may also stimulate an increase in picoplankton (Burford 1997). In the estuary, chlorophyll *a* and zooplankton levels were similar to those found in other shallow areas of the Patos Lagoon estuary (Montú *et al.* 1997, Abreu *et al.* 2010). We observed a great dominance of diatoms in terms of cell density and mainly biovolume, contributing to microplankton and nanoplankton chlorophyll *a*. The increase in

microplankton chlorophyll *a* levels during the spring and summer has been linked to salinity, the availability of nutrients and to the growth of *Skeletonema* species in previous years (Bergesch & Odebrecht 1997, Bergesch *et al*. 2009, Abreu *et al*. 2010).

In conclusion, with the exception of temperature, significant differences were observed between the shrimp pond and the estuary. The initial reduction in suspended matter, phosphate and silicate in the pond resulted from culture management; the increase in phosphate during the final period of the study reflected phosphate regeneration and the rise in salinity which released phosphate into the water column. Until the end of December, phytoplankton cell abundance and biomass were low in the pond probably due to zooplankton grazing, while in January and February a drastic decrease in zooplankton (96%) was probably related with the consumption of post-larval shrimps. During this period, small sized flagellates, coccoid cells and cyanobacteria <1 µm reached high levels in the pond, while in the estuary larger cells were present in lower concentrations. In the estuary, water exchange resulted in temporal fluctuations of salinity and was probably the main controlling factor, while in the pond the results suggest that top-down control was the factor that influenced the planktonic community, first by zooplankton grazing on phytoplankton and later by the predation of shrimp on zooplankton, releasing the grazing pressure on phytoplankton.

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