Aquaculture Research

Aquaculture Research 2014, **45**, 1600–1610 doi: 10.1111/are.12106

Effects of shrimp pond water on phytoplankton: importance of salinity and trophic status of the receiving environment

Alessandro Pereira Cardozo & Clarisse Odebrecht

Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil

Correspondence: A Pereira Cardozo and C Odebrecht, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Av. Itália km 8, Caixa Postal 474, CEP 96201-900, Rio Grande, RS, Brazil. E-mail: ocalessandro@yahoo.com.br

Summary

Aquaculture generates a large load of effluents rich in organic matter and nutrients that may be introduced into the environment. This study aimed to assess in a microcosm experiment, the effect of shrimp pond water mixed with Patos Lagoon estuary water on phytoplankton chlorophyll *a* and primary production, simulating two salinities. Chlorophyll *a*, dissolved inorganic nutrients and primary production were measured in two experiments. In Harvest I, salinity of shrimp pond and environment water was similar, and chlorophyll *a* showed different trends over time, according to the amount of nitrogen available. In Harvest II, with different salinities and high nutrient concentrations in environment water, chlorophyll *a* levels showed a similar increasing trend over time in all mixtures. Net primary production showed differences among treatments in the first sampling in Harvest I, but not in the second, whereas no differences were observed among treatments in Harvest II. We conclude that shrimp pond effluent can lead to short-term variations in chlorophyll *a* and primary production levels, with similar salinities. Salinity differences result in lower chlorophyll *a* and primary production values than expected according to the nutrient input. Differences in salinity can be an important management strategy to choose the best harvest period.

Keywords: Chlorophyll, Nutrients, Patos Lagoon estuary, Phytoplankton, Primary Production, Shrimp

Introduction

The development of shrimp culture in several countries has become an important economic activity over the past few years, generating profits in short periods. However, negative impacts of this activity are common in coastal areas, especially where mangroves and marshes are present (Primavera 1997). The main impact of shrimp farming is generated by pond effluents, which are released into the surrounding environment (Paéz-Osuna, Gracia-Gasca, Flores-Verdugo, Lyle-Fritch, Alonso-Rodríguez, Roque & Ruiz-Fernández 2003).

Shrimp pond effluent presents high levels of organic matter and dissolved nutrients, and low dissolved oxygen concentration (Marinho-Soriano, Morales & Moreira 2002). Much of the suspended organic matter can be removed by providing a settling area, which leads to partial recovery of the effluent before its input to the adjacent environment (Teichert-Coddington, Rouse, Potts & Boyd 1999). The high concentrations of dissolved nutrients, especially nitrogen and phosphorus (Burford, Costanzo, Dennison, Jackson, Jones, McKinnon, Preston & Trott 2003), result from the surplus of food supplied to the shrimps and fertilizers, and this nutrient loading has the potential to eutrophicate coastal waters (Jackson, Preston & Thompson 2004).

The increased discharge during the shrimp harvest period reduces the residence time of this water in the settling area, generating a greater input of nutrients and suspended matter in the receiving environment. Especially in areas of low hydrodynamics, the input of aquaculture effluents may overcome the environmental self-purification capacity, and a proportion of the nutrients accumulate in the sediment. The resulting oxygen depletion in the water (Alongi, Lindsay & Trott 1999) and anoxic conditions in the sediment (Trott, McKinnon, Alongi & Burford 2004) alter

the structure of the benthic community; areas with increased water circulation are, however, less affected when compared with shallower areas with low physical circulation (Páez-Osuna 2001).

In the estuary of Patos Lagoon, most of the commercial activity related to marine shrimp culture is located in shallow areas, where the action of winds induces water exchange and reduces the residence time, when compared with deeper areas of the estuary (Möller, Lorrenzzenti, Stech & Mata 1996). In late summer, during the shrimp harvesting period, the water level is generally low and higher salinity prevails (Fernandes, Dyer & Möller 2005), thus enhancing the potential impact of the effluent in the estuarine area close to the shrimp farms. This study aimed to evaluate the effect of water from a shrimp culture pond (*Litopenaeus vannamei*) when mixed with environmental water on dissolved inorganic nutrients and chlorophyll *a* concentrations and primary production rate, simulating two conditions: a period of high salinity and another with lower salinity.

Materials and methods

To simulate the effect of the effluent from shrimp [*Litopenaeus vannamei* (Boone 1931)] harvesting two microcosm experiments were performed at the Marine Aquaculture Station (EMA), Federal University of Rio Grande (FURG), Rio Grande, Brazil, using environmental water in different salinities (35 and 16, Harvest I and II respectively). The following treatments were previously tested with three replicates: 100% environment water (P0), 75% environment water –25% pond water (P25), 50% environment water –50% pond water (P50), 25% environment water –75% pond water (P75), 100% pond water (P100). In microcosms P75 and P100, a quick deterioration of water was observed in less than 5 days, especially due to ammonium increase, and these two treatments were excluded from subsequent experiments.

The microcosm experiments were run for 5 days between March 23 and 27, 2009 (Harvest I) and between March 30 and April 03, 2009 (Harvest II). Autoclaved plastic bags (60 L useful volume) were pre-washed with tap water (two times) and distilled water (once). Two bags were used in each unit to avoid disruption and sample loss. Bags were sewn to PVC rings (30 cm diameter) and attached to a floating structure on the pond surface. A minimum height of 15 cm was maintained between water level and the upper extremity of microcosm, to prevent water spilling due to the action of wind. In the 'Harvest I' experiment, water from a shrimp culture pond was mixed with water of similar salinity from the adjacent Cassino Beach (salinity 35 ± 1), whereas in 'Harvest II', we used water from Justino Bay, located in the estuary of Patos Lagoon (salinity 16).

Temperature and salinity were measured using a mercury thermometer and refractometer respectively. Dissolved inorganic nutrients (nitrate + nitrite, ammonium, soluble reactive phosphorus -SRP and silicate) were measured on the first, third and fifth days of the experiment. For nitrate+nitrite, soluble reactive phosphorus and silicate analysis, the water was filtered through glass microfiber filters (S&S, GF-50A) and the filtrate was frozen (−20°C; 200 mL plastic Nalgen bottles). Spectrophotometric analysis followed traditional methods (Strickland & Parsons 1972). The concentration of ammonium was measured immediately after water sampling, according to UNESCO (1983) recommendations.

The concentration of total chlorophyll *a* was estimated daily on glass microfiber filters (Whatman GF/F, 0.7 μm pore size), after filtering 15–50 mL of water from the microcosms. A second water sample was size fractionated by filtering through a 20 μm pore nylon mesh, and the filtrate was retained on glass microfiber filters (Whatman GF/F). The filters were stored deep-frozen (−20°C) in the dark for 2 days, 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 (Sunnyvale, CA, USA).

Primary production was estimated on the first and fourth days using the dissolved oxygen method and the Winkler technique (Strickland & Parsons 1972). Water from each microcosm was transferred to light and dark BOD bottles and one was fixed to determine the initial dissolved oxygen concentration. The bottles were incubated (for 4–5 h) in an aquarium at a controlled temperature (25 ± 1°C), illuminated using six fluorescent lamps (350 μW cm^{-2}), followed by titration. Gross and net primary production and respiration rates were estimated, as was the productivity by biomass (Strickland & Parsons 1972). For comparison, we also used a factor of 0.04 for the chlorophyll *a* to carbon ratio. This ratio ranges from 0.01 to 0.05

(Cloern, Grenz & Videgar-Lucas 1995; Behrenfeld, Boss, Siegel & Shea 2005) according to light and nutrient availability, among other factors (Geider, Macintyre & Kana 1997; Wang, Behrenfeld, Le Borgne, Murtugudde & Boss 2009). The differences in and among the treatments were tested using a two-way analysis of variance (two-way ANOVA), followed by the post-hoc comparison; when necessary to preserve the precepts of normality and homoscedasticity, data were mathematically transformed (Zar 1999).

Results

The shrimp pond water used in the experiments 'Harvest I' and 'Harvest II' presented low initial values of nitrate + nitrite (3.3–3.7 μM) and high values of soluble reactive phosphorus (9.6–14.5 μM), whereas ammonium concentration was relatively high in Harvest I (9.1 μM) compared with Harvest II (1.2 μM**).** Silicate was low in both experiments (see Table 1).

The average values of temperature, salinity, dissolved inorganic nutrients and chlorophyll *a* observed in both experiments are presented in Table 2. In Harvest I, ammonium values were lower in P25 and P50 (0.7–0.8 μM) than was observed in Harvest II (1.8–12.7 μM). Nitrate+nitrite values were in the same range in both experiments (4–7 μM), except the higher value in P25, Harvest II (12.4 μM). Soluble reactive phosphorus, silicate and chlorophyll *a* presented higher average values in Harvest II, except for chlorophyll *a* in P50. In both experiments, chlorophyll *a* fraction <20 μm was responsible for approximately 80% of the total chlorophyll.

Table 1 Dissolved inorganic nutrients and total chlorophyll *a* levels in shrimp culture pond and environment water used in the experiments Harvest I, Cassino Beach water (March 23 to 27, 2009) and Harvest II, Justino Bay (March 30 to April 03, 2009)

	Harvest I (μM)	Cassino	Harvest II (μM)	Justino
Nitrate+nitrite (μM)	3.27	9.30	3.74	7.35
Ammonium (μM)	9.09	3.86	1.19	2.05
SRP (μM)	9.56	1.52	14.45	8.09
Silicate (μM)	2.27	43.61	1.25	105.44
Chlorophyll *a* (μg cl-*a* L^{-1})	120.3	0.11	88.7	30.11

Harvest I

Salinity remained fairly constant over time in all treatments (34–35), whereas water temperature showed daily variations, with morning minimum values (21.3 ± 0.1°C) and maxima (27.4 ± 1.2°C) in the afternoon. The high level of ammonium at P0 on March 23 (3.86 ± 0.87 μM) was reduced to 1.38 ± 0.52 μM on March 27, similar to the values observed during the experiment in P25 and P50 (Fig. 1a). The treatment P0 also showed a significant decrease ($P < 0.05$) in nitrate+nitrite values from the first day (9.3 μM) until the end of the experiment (5.2 μM), whereas P25 presented values close to 4–5 μM during the experiment (Fig. 1b), and P50 increased significantly on March 27 when compared with the initial values. On March 23, P0 was significantly higher ($P < 0.05$) than P25 and P50; on March 25, all treatments differed significantly, with higher and lower values at P0 and P50 respectively. On the last day, all treatments presented similar concentrations of nitrate+nitrite.

Soluble reactive phosphorus concentration was also higher ($P < 0.05$) on the first day at P0 (1.52 ± 0.54 μM), unlike other treatments and dates without statistical differences ($P < 0.05$) between treatments over time (P25 0.55 ± 0.1 μM; P50 0.81 ± 0.1 μM) (Fig. 1c). For silicate, P0 showed the highest values on March 23 and 25 (44.07 ± 1.94 μM) and a significant decrease ($P < 0.05$) on March 27 (33.9 ± 2.83 μM), always significantly higher $P < 0.05$) when compared with the treatments P25 and P50. The latter two treatments were statistically different from each other on March 23 only. P25 and P50 recorded a significant decrease ($P < 0.05$) between March 23 and 25 that lasted until March 27 in P25, whereas in P50, a significant increase was observed on March 27 (Fig. 1d). The mean atomic nitrogen to phosphorus ratio (N:P) was always less than 11, except for the first value observed in P25 (14.3 ± 0.2), which was significantly higher than P50 (Fig. 1e). The mean atomic N:Si ratio was below 0.3 in all treatments, with a significant reduction ($P < 0.05$) at P0 through time and an increase in P50 on March 25 (Fig. 1f).

Total chlorophyll *a* values showed different trends over time: in P0, the initial concentration was low (0.1 μg cl-*a* L^{-1}) with a significant increase ($P < 0.05$) from the second day, reaching 13.0 μg cl-*a* L^{-1} on the fourth day of the experiment

Table 2 Average values (± standard deviation) of temperature (ºC), salinity, ammonium (μM), nitrate+nitrite (μM), soluble reactive phosphorus (SRP, μM), silicate (μM) and chlorophyll *a* (μg cl-*a* L^{-1}) in treatments P0, P25 and P50 in experiments Harvest I (March 23 to 27, 2009) and Harvest II (March 30 to April 03, 2009)

	Harvest I			Harvest II		
	P0	**P25**	**P50**	**P0**	**P25**	**P50**
Temperature	24.4 ± 3,6	24.2 ± 2.5	24.0 ± 1.9	23.6 ± 2.9	23.2 ± 1.7	23.5 ± 2.2
Salinity	35	34	34	16	20	24
Ammonium	2.8 ± 1.4	0.7 ± 0.3	0.8 ± 0.3	1.8 ± 0.8	12.7 ± 17.3	2.2 ± 1.7
Nitrate+nitrite	7.3 ± 0.8	5.0 ± 0.6	3.9 ± 0.5	6.0 ± 1.3	12.4 ± 3.1	6.4 ± 1.7
SRP	1.1 ± 0.4	0.5 ± 0.1	0.8 ± 0.3	6.8 ± 2.4	4.9 ± 1.7	2.8 ± 1.3
Silicate	40.7 ± 1.2	29.0 ± 7.5	23.9 ± 5.0	89.4 ± 20.3	86.6 ± 3.2	73.5 ± 8.3
Chlorophyll *a*	7.3 ± 8.1	28.8 ± 7.5	47.4 ± 21.4	37.4 ± 10.2	51.3 ± 15.9	44.4 ± 8.7

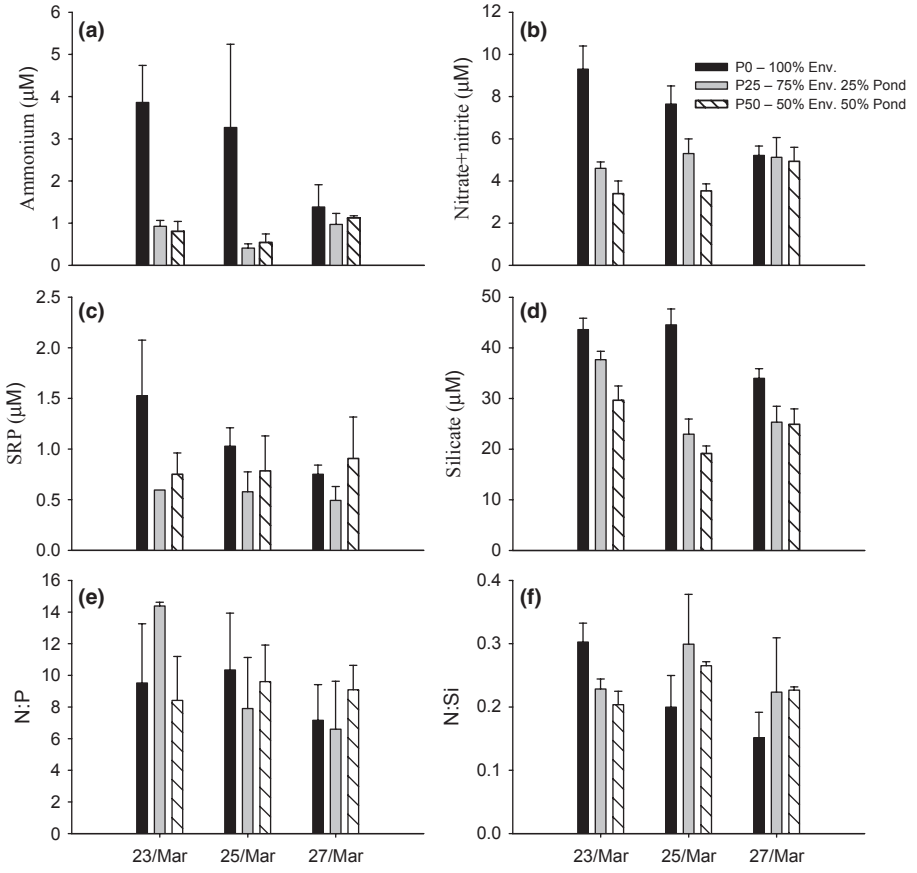

Figure 1 Concentrations of Ammonium (a), Nitrate+nitrite (b), Soluble reactive phosphorus (SRP) (c), Silicate (d) and N:P (e) and N:Si (f) atomic ratios in the treatments P0 (100% Environment water), P25 (75% Environment; 25% Pond) and P50 (50% Environment; 50% Pond) on March 23, 25 and 27, 2009 in Harvest I. ** Figures in different scales.

(Fig. 2a). In P25, the initial value was intermediate (38.0 μg cl-*a* L^{-1}) and a significant reduction ($P < 0.05$) was observed on the fourth day, recording lower concentrations at the end of the experiment (24.4 μg cl-*a* L^{-1}; Fig. 2b). In P50, the highest values were observed on March 23 and 24 (66.2 μg cl-*a* L^{-1}), followed by a significant decrease ($P < 0.05$) on the third day (40.3 μg cl-*a* L^{-1}),

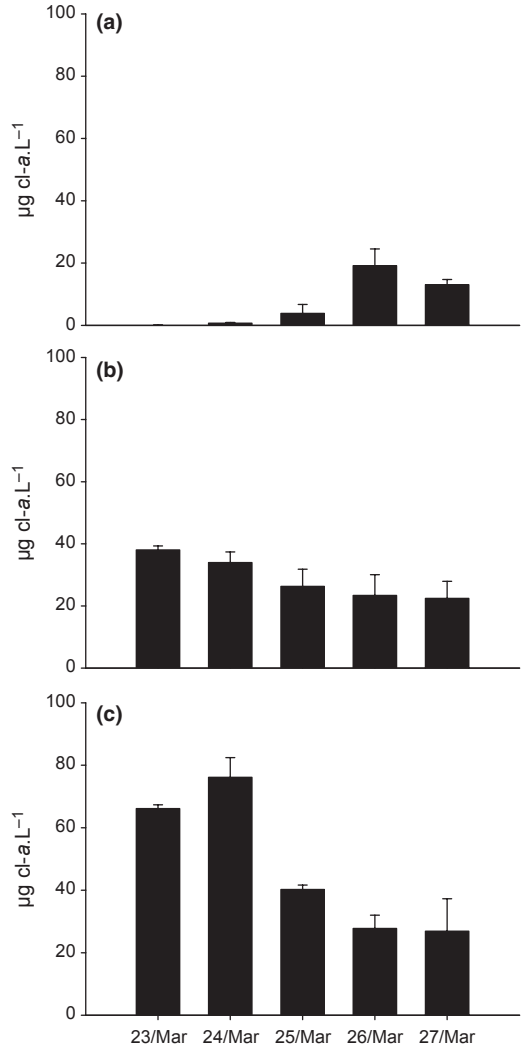

Figure 2 Levels of chlorophyll *a* in treatments P0 (100% environment water) (a), P25 (75% environment; 25% pond) (b) and P50 (50% environment; 50% pond) (c) between March 23 and 27, 2009 in Harvest I.

continuing to decrease until the end of the experiment (27 µg cl-*a* L^{-1}) (Fig. 2c), although not significantly. The chlorophyll *a* fraction <20 µm was always high, with an average contribution of 80% of total chlorophyll. In the first 3 days, significant differences ($P < 0.05$) were observed among the treatments for total and <20 µm chlorophyll. On the last sampling day, the values in P25 and P50 were significantly higher than P0, with no statistical difference among them.

The gross (Fig. 3a) and net (Fig. 3b) primary production values (mg O_2 L^{-1} h^{-1}) showed a significant difference ($P < 0.05$) between the two sampled days in P0 and P50 with opposite trends: increasing in the former and decreasing in the latter. In P25, no significant differences ($P < 0.05$) were recorded between the 2 days. Initial values of gross primary production increased in P0 from 0.03 ± 0.05 to 0.84 ± 0.35 and decreased in P50 from 1.67 ± 0.1 to 0.50 ± 0.44 between March 23 and 26. Only on March 23 were statistically significant differences observed ($P < 0.05$) among treatments. The net primary production (Fig. 3b) was negative in P0 on March 23 (-0.03 ± 0.13 mg O_2 L^{-1} h^{-1}), whereas in P50, the highest value was observed (1.37 ± 0.15 mg O_2 L^{-1} h^{-1}). Comparing the 2 days, the net production significantly ($P < 0.05$) increased in P0 (−0.03–0.73 mg O_2 L^{-1} h^{-1}) and decreased in P50 (1.37–0.28 mg O_2 L^{-1} h^{-1}; Fig. 3b). Respiration presented a lower mean value at P0 and a higher one in P50, but without significance ($P < 0.05$) between March 23 and 26 in any treatment (Fig. 3c). The net oxygen primary production, normalized by the carbon content (mg O_2 h^{-1} mg C^{-1}), was strongly negative in P0 on March 23; whereas in the other treatments, the average value was close to 1, without any significant difference (Fig. 3d).

Harvest II

In this experiment, different salinity values in the shrimp pond and environment (salinity 16) led to salinities of 20 and 24 in P25 and P50 respectively. The water temperature throughout the day ranged from the morning minima (21.15 ± 1.2°C) to the afternoon maxima (26.05 ± 0.63°C). The ammonium values did not differ significantly ($P < 0.05$) among treatments (1–3 µM), except in P25 on April 3, with extreme values (maximum 45 µM; average 34.7 ± 10.2 µM) (Fig. 4a). The concentrations of nitrate+nitrite, similar to the previous experiment, repeated the pattern of ammonium variation. All treatments showed values below 10 µM, except P25 (24 µM) on April 3 (Fig. 4b).

The soluble reactive phosphorus concentration was high at the beginning of the experiment in environment water (P0) (8.09 ± 0.54 µM), and was significantly reduced ($P < 0.05$) by approximately 50% on April 3. In contrast, P50 presented a gradual increase (1.29 ± 0.1 µM–3.9 ± 0.7 µM), whereas in P25 values varied by around 5 µM throughout the experiment. Among treatments, P0

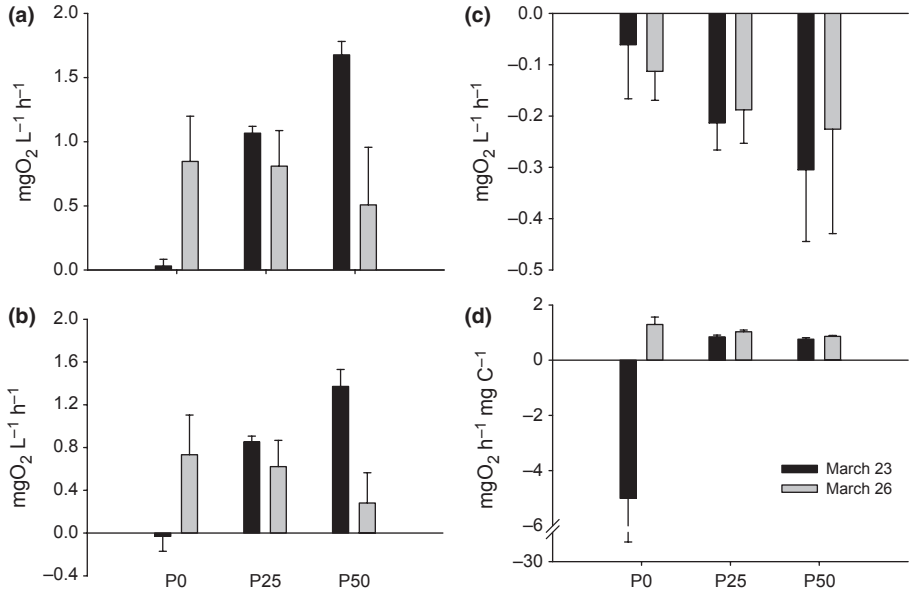

Figure 3 Gross primary production (a), net primary production (b), respiration (c), phytoplankton net productivity normalized by carbon content (d) in treatments P0 (100% Environment water), P25 (75% Environment; 25% Pond) e P50 (50% Environment, 50% Pond) on March 23 and 26, 2009 on Harvest I. ** Figures in different scales.

showed higher values ($P < 0.05$) when compared with P25 and 50 in the first 2 days, and on April 3, all treatments were similar (Fig. 4c). Silicate values were higher than in Harvest I and the maximum (105.44 ± 3.4 μM) was observed at P0, where a reduction ($P < 0.05$) (61.7 ± 6.0 μM) occurred on April 3. In P25 and P50, the silicate levels ranged between 90 μM and 80 μM respectively (Fig. 4d). The atomic N:P ratio was always low, with no significant change in P0. In P50 and P25, the significantly higher N:P values ($P < 0.05$) were observed on April 1 (10.1 ± 4.99) and April 3 (13.1 ± 12.5) respectively (Fig. 4e). The atomic ratio N:Si was significantly reduced ($P < 0.05$) in P0 and P25 on April 3, whereas in P50, it increased ($P < 0.05$) on April 1 (Fig. 4f).

In contrast to Harvest I, the initial chlorophyll *a* content was high in P0 (30 ± 2.76 μg cl-*a* L^{-1}). In all treatments, a significant increase ($P < 0.05$) was observed between March 31 and April 01, when the average chlorophyll *a* content was 46.3 ± 11.1, 62.5 ± 25.6, 52.7 ± 10.7 μg cl-*a* L^{-1} in P0 (Fig. 5a), P25 (Fig. 5b) and P50 (Fig. 5c) respectively. Significant differences ($P < 0.05$) were also observed between P0 and P25 on March 31 and April 03 (Fig. 5). As in the previous experiment, the <20 μm fraction contributed approximately 80% of the total chlorophyll *a* throughout the experiment.

In all treatments, the gross primary production increased, with a significant difference ($P < 0.05$) at P0 (0.37–1.32 mg O_2 L^{-1} h^{-1}) (Fig. 6a). The average values of net primary production also increased in all treatments, although were only statistically significant ($P < 0.05$) in P0, due to high variability among the replicates (Fig. 6b). Respiration increased significantly ($P < 0.05$) in treatments P0 and P25 (Fig. 6c) The mean production normalized by the carbon content (mg O_2 h^{-1} mg C^{-1}) was always smaller than 1 and presented a significant increase ($P < 0.05$) between sampling days only in P0 (Fig. 6d).

Discussion

The release of shrimp culture pond effluents into coastal and estuarine areas often represents a greater input of nutrients, when compared with sources, such as agriculture and urban waste (Páez-Osuna, Guerrero-Galvan & Ruiz-Fernández 1999). The large load of nutrients and organic matter from aquaculture is caused by frequent pond fertilization for algal growth and the daily shrimp food. New highly productive culture technologies without water renewal reduce the culture area and the load of effluents that is released to the environment (McAbee, Browdy, Rhodes & Stokes 2003; Sowers, Gatlin, Young, Isely, Browdy & Tomaso 2005). The

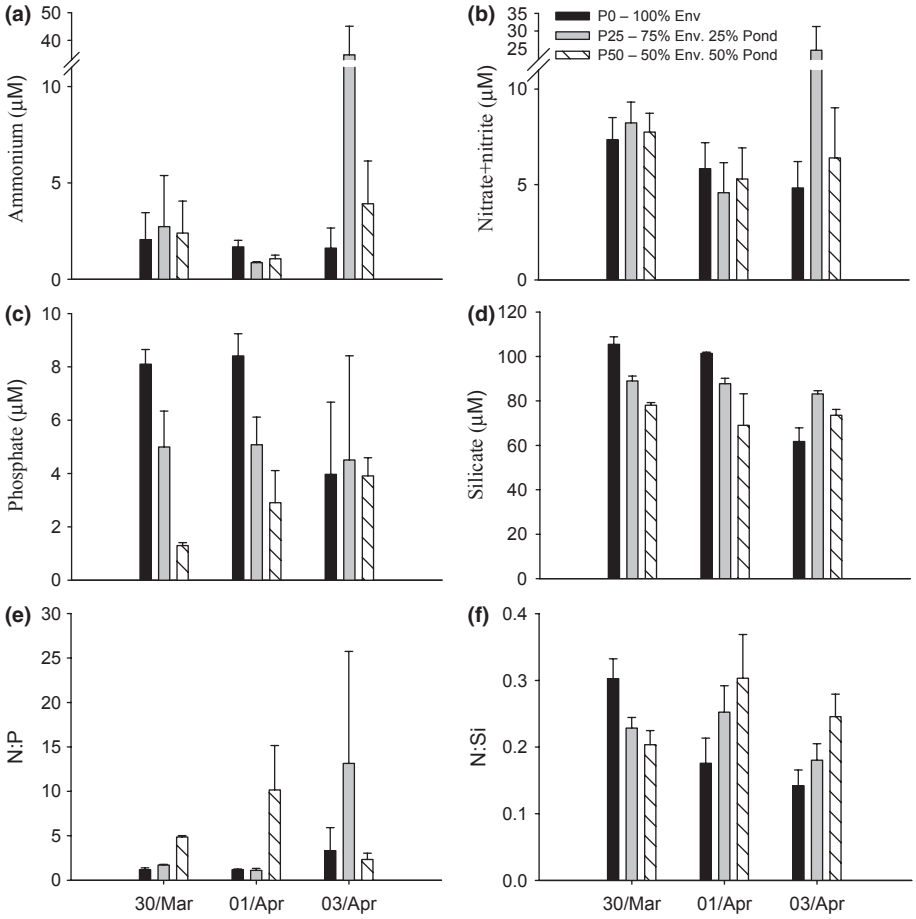

Figure 4 Concentrations of Ammonium (a), Nitrate+nitrite (b), Soluble reactive phosphorus (SRP) (c), Silicate (d) and N:P (e) and N:Si (f) atomic ratios in the treatments P0 (100% Environment water), P25 (75% Environment; 25% Pond) and P50 (50% Environment; 50% Pond) on March 30, April 01 and 03, 2009 in Harvest II. ** Figures in different scales.

concentrations of dissolved inorganic nutrients in the pond used in this study are similar to those observed in semi-extensive culture systems, where phosphate accumulation usually occurs (Burford, Thompson, McIntosh, Bauman & Pearson 2003; Peixoto, Wasielesky & JR. & Louzada L. JR. 2003; Casillas-Hernández, Magallón-Barajas, Portillo-Clarck & Páez-Osuna 2006; Cardozo, Britto & Odebrecht 2011). The results observed in the microcosms may thus simulate the environmental responses during an effluent discharge from commercial shrimp farming in the Patos Lagoon estuary.

Both simulations represent realistic situations in the estuary of Patos Lagoon. In the Harvest II experiment, Justino Bay water presented lower salinity and higher soluble reactive phosphorus and silicate levels when compared with Harvest I, with seawater from the Casino Beach and slightly higher nitrate + nitrite and ammonium values. In many periods of the year, brackish waters contain large amounts of phosphorus and silicate (Niencheski & Windom 1994; Abreu, Bergesch, Proença, Garcia & Odebrecht 2010; Odebrecht, Bergesch, Rörig & Abreu 2010), whereas during drought periods, the concentration of nutrients, such as silicate and nitrate, is reduced in the estuary of Patos Lagoon (Abreu *et al.* 2010). The ammonium concentrations tend to increase during periods of reduced freshwater inflow and increasing salinity, influenced by the longer water residence time, macrophyte decomposition and the input of the domestic and industrial effluents of Rio Grande city (Baumgarten, Niencheski & Kuroshima 1995; Abreu *et al.* 2010;).

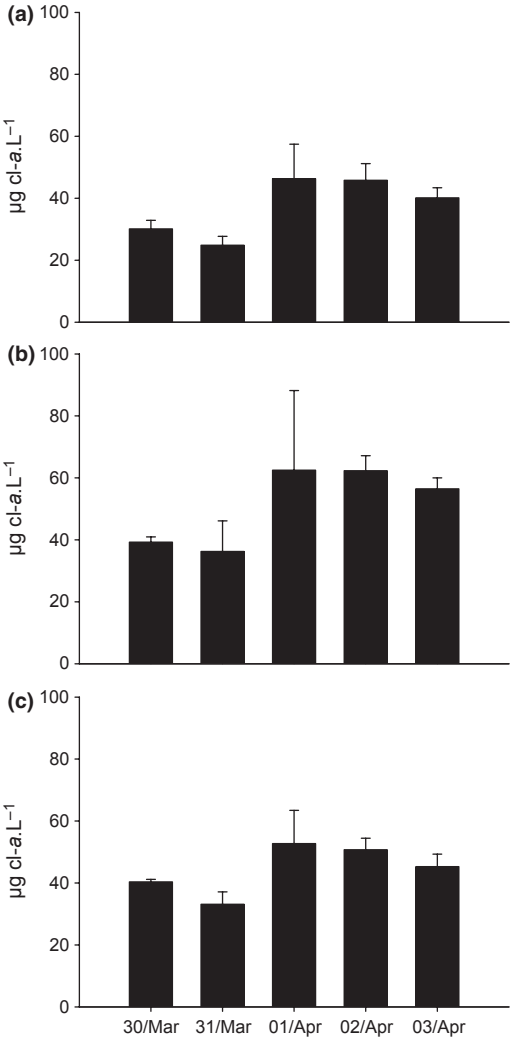

Figure 5 Levels of total chlorophyll *a* in treatments P0 (100% environment water) (a), P25 (75% environment; 25% pond) (b), e P50 (50% environment; 50% pond) (c) between March 23 and 27, 2009 in Harvest II.

The first important point is that salinity differences in approximately 10 units, and dilution up to 50% between the pond effluent and environment water, has the potential to reduce the environmental impact, as was observed in the Harvest II simulation. At first, it became obvious that the chlorophyll *a* trend was the same in all treatments, increasing on the third day and maintaining a relatively constant level thereafter. However, it is also evident that the chlorophyll *a* increase was not proportional, i.e. higher values were expected in P50 compared with P25, considering initial chlorophyll *a* in the pond (90 µg cl-*a* L^{-1}). The primary production rate was positively correlated with chlorophyll on the first day only; on April 02, no difference was observed in the 50% dilution (P50), in contrast to a large increase in environment water (P0). The effect of salinity changes and osmotic stress induces community composition changes, as was observed using water from the Darss-Zingst estuary, Germany (Pilkaitytë, Schoor & Schubert 2004). In the Harvest I, simulation without salinity differences between the environment and the pond water, primary production rates were positively correlated with the chlorophyll *a* concentrations in all treatments. Thus, we may conclude that salinity difference between the pond and environment water inhibits immediate microalgae growth and consequent biomass accumulation.

The second point is the importance of the trophic status of the receiving environment water. In the Harvest I simulation, the effect of nitrogen limitation on chlorophyll *a* and primary production became evident. The increase in chlorophyll *a* and primary production rate in the Cassino Beach water was proportional to the amount of nitrogen available. Considering the initial total inorganic nitrogen (ammonium + nitrate + nitrite 13 µM) and the Redfield ratio C:N:P of 106:16:1 (Redfield, Ketchum & Richards 1963), the maximum chlorophyll would be 20–25 µg cl-*a* L^{-1}, in agreement with the highest value observed in P0 (March 26). In P50, low absolute values of dissolved inorganic nitrogen (close to 5 µM), less than required for doubling the chlorophyll (30–40 µg cl-*a* L^{-1}) considering the Redfield ratio (Redfield *et al.* 1963), and low N:P ratio (<10), inhibited chlorophyll *a* accumulation. The fact that in the second experiment, the chlorophyll *a* values and primary production increased in all treatments reinforces the idea that nutrient availability was an important factor. In this experiment, the mean values of total nitrogen (25.2 µM and 8.9 µM in P25 and P50 respectively) and soluble reactive phosphorus (4.9 µM and 2.8 µM respectively) were higher. Production values were strongly related to the chlorophyll levels. In both experiments, despite the different water sources used for dilution, we observed an increase in primary production in the treatments with higher effluent load (P0 < P25 < P50) on the first day tested, and on the second day, a reduction in the production at P50. Without salinity difference between the effluent and the receiving environment, primary production tends

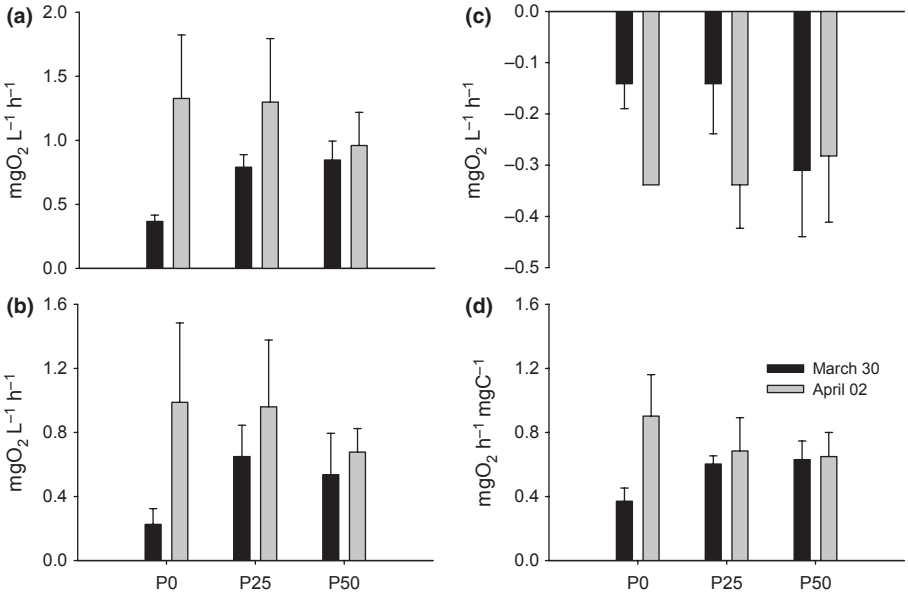

Figure 6 Gross primary production (a), net primary production (b), respiration (c), phytoplankton net productivity normalized by carbon content (d) in treatments P0 (100% Environment water), P25 (75% Environment; 25% Pond) e P50 (50% Environment, 50% Pond) on March 30 and April 02, 2009 on Harvest II. ** Figures in different scales.

to decrease gradually in a short period. When differences in salinity are observed, the delay due to osmotic stress appears to influence the production, considering that P25 exhibited a significant increase in primary production, whereas P50 maintained its primary production at the same level in both tests. Due to the high nutrient load and food added to the growing ponds, primary production values vary, with observations, for example, of maxima close to 110 mg C m^{-3} h^{-1} in shrimp ponds in Vietnam (Alongi, Dixon, Johnston, Van Tien & Xuan 1999) and 450 mg C m^{-3} h^{-1} in Australia (Burford 1997). These values are in the same range as that observed in our study (average 370 mg C m^{-3} h^{-1}; 160–720 mg C m^{-3} h^{-1}). In the Patos Lagoon estuary water, used by shrimp farmers to flood their ponds, Odebrecht, Abreu, Möller, Niencheski, Proenc and Torgan (2005) observed gross primary production ranging from 90 to 180 mg C m^{-3} h^{-1}, values much smaller than that observed in the studied ponds. In Harvest II, calculated primary productivity per biomass unit (5–10 mg C mg cl-a^{-1} h^{-1}) was in the same range as observed in Patos Lagoon estuary (4.3–15 mg C mg cl-a^{-1} h^{-1}) (Odebrecht *et al.* 2005).

On the basis of the results of this study, we conclude that the effluent discharge from shrimp culture ponds in Patos Lagoon estuary leads to short-term changes in the receiving environment. The impact varies according to the salinity difference between the environment and pond water, and to the amount of nutrients in the estuary at the time of harvest. Salinity differences result in lower chlorophyll *a* and primary production values than expected according to the nutrient input following the Redfield rate. Thus, salinity differences between ponds and the adjacent environment can be an important management strategy to be considered i.e., by choosing the best harvest period. The other critical point is to measure the nitrogen concentration in the estuary. As nitrogen is usually limiting for microalgae growth in the shrimp ponds, a reduction in primary production and chlorophyll *a* levels can be expected in the short-term if the environment is also poor in nitrogen at the time of harvesting. The use of sedimentation basins scaled correctly to the size of the shrimp culture farms will improve the retention of soluble reactive phosphorus and ammonium from the ponds, and tends to reduce a possible effect on surrounding planktonic communities.

References

Abreu P.C., Bergesch M., Proença L.A., Garcia C.A.E. & Odebrecht C. (2010) Short- and Long-Term chlorophyll

a variability in the shallow microtidal Patos Lagoon estuary, southern Brazil. *Estuaries and Coasts* **33**, 554–569.

Alongi D.M., Lindsay F.T. & Trott A. (1999) Rates and pathways of benthic mineralization in extensive shrimp ponds of the Mekong delta. *Vietnam. Aquaculture* **175**, 269–292.

Alongi D.M., Dixon P., Johnston D.J., Van Tien D. & Xuan T.T. (1999) Pelagic processes in extensive shrimp ponds of the Mekong delta. *Vietnam. Aquaculture* **175**, 121–141.

Baumgarten M.G., Niencheski L.F. & Kuroshima K.N. (1995) Qualidade das águas que margeiam o município do Rio Grande: Nutrientes e detergentes dissolvidos. *Atlantica* **17**, 17–34.

Behrenfeld M.J., Boss E., Siegel D.A. & Shea D.M. (2005) Carbon based ocean productivity and phytoplankton physiology from space. *Global Biogeochemical Cycles 19 GB* **1006**, 1–14.

Burford M. (1997) Phytoplankton dynamics in shrimp ponds. *Aquaculture Research* **28**, 351–360.

Burford M.A., Costanzo S.D., Dennison W.C., Jackson C.J., Jones A.B., McKinnon A.D., Preston N.P. & Trott L.A. (2003) A synthesis of dominant ecological processes in intensive shrimp ponds and adjacent coastal environments in NE Australia. *Marine Pollution Bulletin* **46**, 1456–1469.

Burford M.A., Thompson P.J., McIntosh R.P., Bauman R.H. & Pearson D.C. (2003) Nutrient and microbial dynamics in high- intensity, zero-exchange shrimp ponds in Belize. *Aquaculture* **219**, 393–411.

Cardozo A.P., Britto V.O. & Odebrecht C. (2011) Temporal variability of plankton and nutrients in shrimp culture ponds vs. adjacent estuarine water. *Pan-American. Journal of Aquatic Sciences* **6/**, 28–43.

Casillas-Hernández R., Magallón-Barajas F., Portillo-Clarck G. & Páez-Osuna F. (2006) Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: Trays and mechanical dispersal. *Aquaculture* **258**, 289–298.

Cloern J.E., Grenz C. & Videgar-Lucas L. (1995) An empirical model of the phytoplankton chlorophyll:carbon ratio – the conversion factor between productivity and growth rate. *Limnology and Oceanography* **40**, 1313–1321.

Fernandes E.H.L., Dyer K.R. & Möller O.O. (2005) Spatial gradients in the flows of Southern Patos Lagoon. *Journal of Coastal Research* **21**, 759–769.

Geider R.J., Macintyre H.L. & Kana T.M. (1997) Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll *a*: carbon ratio to light, nutrient-limitation and temperature. *Marine Ecology Progress Series* **148**, 187–200.

Jackson C., Preston N. & Thompson P.J. (2004) Intake and discharge nutrient loads at three intensive shrimp farms. *Aquaculture Research* **35**, 1053–1061.

Marinho-Soriano E., Morales C. & Moreira W.S.C. (2002) Cultivation of Gracilaria (Rhodophyta) in shrimp pond effluents in Brazil. *Aquaculture Research* **33**, 1081–1086.

McAbee B.J., Browdy C.L., Rhodes R.J. & Stokes A.D. (2003) The use of greenhouse-enclosed raceway systems for the super-intensive production of pacific white shrimp Litopenaeus vannamei in the United States. *Global Aquaculture Advocate* **6**, 40–43.

Möller O.O., Lorrenzzenti J.A., Stech J.L. & Mata M.M. (1996) The Patos Lagoon summertime circulation and dynamics. *Continental Shelf Research* **16**, 335–351.

Niencheski L.F.H. & Windom H.L. (1994) Nutrient flux and budget in Patos Lagoon Estuary. *Science of Total Environment* **149**, 53–60.

Odebrecht C., Abreu P.C., Möller O.O., Niencheski L.F., Proenc L.A. & Torgan L.C. (2005) Drought effects on pelagic properties in the shallow and turbid Patos Lagoon, Brazil. *Estuaries* **28**, 675–685.

Odebrecht C., Bergesch M., Rörig L.R. & Abreu P.C. (2010) Phytoplankton interannual variability at Cassino Beach, Southern Brazil (1992–2007), with emphasis on the surf zone diatom asterionellopsis glacialis. *Estuaries and Coasts* **33**, 570–583.

Páez-Osuna F. (2001) The environmental impact of shrimp aquaculture: causes, effects and mitigating alternatives. *Environment Management* **28**, 131–140.

Paéz-Osuna F., Gracia-Gasca A., Flores-Verdugo F., Lyle-Fritch M.L., Alonso-Rodríguez R., Roque A. & Ruiz-Fernández A.C. (2003) Shrimp aquaculture development and the environment in the Gulf of California ecoregion. *Marine Pollution Bulletin* **46**, 806–815.

Páez-Osuna F., Guerrero-Galvan S.R. & Ruiz-Fernández A.C. (1999) Discharge of nutrients from shrimp farming to coastal waters of the Gulf of California. *Marine Pollution Bulletin* **38**, 585–592.

Peixoto S., Wasielesky W. & JR. & Louzada L. JR. (2003) Comparative analysis of pink shrimp, *Farfantepenaeus paulensis,* and Pacific white shrimp, *Litopenaeus vannamei,* culture in extreme southern Brazil. *Journal of Applied Aquaculture* **14**, 101–111.

Pilkaitytë R., Schoor A. & Schubert H. (2004) Response of phytoplankton communities to salinity changes – a mesocosm approach. *Hydrobiologia* **513**, 27–38.

Primavera J.H. (1997) Socio-economic impacts of shrimp culture. *Aquaculture Research* **28**, 815–827.

Redfield A.C., Ketchum B.H. & Richards F.A.(1963) The influence of organisms on the composition of seawater. In: *The Sea v2,*(ed. by M.N. Hill), Interscience, New York.

Sowers A.D., Gatlin D.M., Young S.P., Isely J.J., Browdy C.L. & Tomaso J.R. (2005) Responses of *Litopenaeus vannamei* (Boone) in water containing low concentrations of total dissolved solids. *Aquaculture Research* **36**, 819–823.

Strickland J.D.H. & Parsons T.R. (1972) *A practical handbook of seawater analysis*. Fisheries Research Board of Canada, Ottawa.

Teichert-Coddington D.R., Rouse D.B., Potts A. & Boyd C.E. (1999) Treatment of harvest discharge from intensive shrimp ponds by settling. *Aquaculture Engineering* **19**, 147–161.

Trott L.A., McKinnon A.D., Alongi D.M. & Burford M.A. (2004) Carbon and nitrogen processes in a mangrove creek receiving shrimp farm effluent. *Estuarine, Coastal and Shelf Science* **59**, 197–207.

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

Wang X.J., Behrenfeld M., Le Borgne R., Murtugudde R. & Boss E. (2009) Regulation of phytoplankton carbon to chlorophyll ratio by light, nutrients and temperature in the Equatorial Pacific Ocean: a basin-scale model. *Biogeosciences* **6**, 391–404.

Welschmeyer N.A. (1994) Fluorimetric analysis of chlorophyll-a in the presence of chlorophyll b and phaeopigments. *Limnology and Oceanography* **39**, 1985–1992.

Zar J.H. (1999) *Biostatistical Analisys*. Prentice Hall, New Jersey.

