
Brazilian shrimp farms for *Litopenaeus vannamei* with partial and total recirculation systems

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Abstract

The alterations caused during water recirculation are little known in Brazil, especially in relation to the dynamics of the water and phytoplankton in a cultivation setting. The aim of the present study was to characterize the quality of the water and phytoplankton on shrimp farms raising *Litopenaeus vannamei* in northeastern Brazil that use either partial or total recirculation systems. Collections were performed in the supply, distribution and drainage channels at both low and high tides. Analysis of variance, followed by Tukey's test, was used for the statistical analysis, with the level of significance was set at $P < 0.05$. On the farm with partial water recirculation, the response variables total ammonium ($1.81 \pm 0.78 \text{ mg.L}^{-1}$), nitrite ($0.10 \pm 0.03 \text{ mg.L}^{-1}$), phosphate ($2.12 \pm 0.5 \text{ mg.L}^{-1}$), Bacillariophyta ($520.625 \pm 159.983 \text{ cells.mL}^{-1}$), Chlorophyta ($936.250 \pm 416,309 \text{ cells.mL}^{-1}$) and Pirrophyta ($10.078 \pm 2.762 \text{ cells.mL}^{-1}$) were higher in the drainage channel. On the farm with total water recirculation, total ammonium ($1.59 \pm 0.01 \text{ mg.L}^{-1}$) and Pirrophyta ($27.046 \pm 435 \text{ cells.mL}^{-1}$) were higher in the drainage channel. The water recirculation systems (partial and total) achieved satisfactory levels for the cultivation of *Litopenaeus vannamei*.

Key words: ammonium, nitrite, nitrate, phosphate, phytoplankton.

Introduction

Shrimp farming is currently one of the most important aquaculture activities in Brazil. Since 1997, producers, investors and

researchers have concentrated their efforts on farming the exotic species *Litopenaeus vannamei*, for which there has since been ever-increasing production. In 2003, Brazilian

shrimp production was 90,190 tons in a cultivated area of 14,824 ha, with mean productivity of 6084 kg ha⁻¹ cycle⁻¹. However, with the occurrence of infectious myonecrosis virus and the antidumping process, the activity decreased and, by 2004, production had dropped to 75,904 tons in an area of 16,598 ha, with mean productivity of 4573 kg ha⁻¹ cycle⁻¹ (Rodrigues et al. 2005). From 2005 to 2007, annual shrimp production remained at the same level, then, increased to 84,000 tons in 2008 (Carvalho and Lemos, 2009).

Aquaculture, especially shrimp farming, has been the target of constant pressure from non-governmental organizations and environmental agencies, which report that the activity imposes considerable harm to coastal environments. However, adequate management strategies with regard to feeding, water quality and soil quality have contributed to make the activity sustainable (Brito and Olivera, 2010).

Effluents from shrimp farms have better physical and chemical qualities in comparison to treated household discharge (Nunes, 2002; Alonso-Rodriguez and Páez-Osuna 2003). Studying the discharge of nutrients from shrimp farms in the cities of Sinaloa, Nayarit and Sonora on the Gulf of California (Mexico), Páez-Osuna et al. (1999) reported that aquaculture contributed 2.8 and 2.2 percent of the emission of nitrogen and phosphorus, respectively, whereas agriculture contributed 50 and 37 percent, rivers 4 and 55.4 percent and cities 3.2 and 5.4 percent, respectively.

All over the world, there is a growing effort to make shrimp farming a sustainable activity, as evidenced by new technologies that are currently in the experimental phase and will soon be employed by

the productivity sector. In Brazil, the Brazilian Association of Shrimp Breeders has created a "diet fund" to finance applied research toward making shrimp farming sustainable (Olivera and Brito, 2005).

The aim of the present study was to characterize the quality of the water and phytoplankton at shrimp farms raising *Litopenaeus vannamei* and operating with either a partial or total water recirculation system in northeastern Brazil.

Materials and Methods

Shrimp farm with partial recirculation system

The marine shrimp farm is located in northeastern Brazil (04° 10' 28" S and 038° 09' 02" W). The property has 70.49 ha of wetland divided into 12 ponds. The farm operated with a partial recirculation system. The water came from the Choró River and entered the system at high tide through the main channel to the pumping station that supplied the adduction channels using two underwater centrifuge pumps that subsequently supplied the 12 ponds. At low tide, the water was drained through the main channel and returned into the river. Thus, there was a single connection between the farm and estuary, which was used for both supply (high tide) and drainage (low tide). The density on the farm was 70 shrimp/m², and a paddle-wheel was used as aerator (5 Hp/ha). The shrimp were fed on a commercial ration containing 35% crude protein, through a tray system (40 trays/ha).

Shrimp farm with total recirculation system

The marine shrimp farm is located in northeastern Brazil (5°, 11" S and 37°, 20"W). The property has 62.4 ha of wetland divided into 24

ponds. The farm operated with a total recirculation system. The water came from the Apodi River and entered the system at high tide through a sedimentation basin to the pumping station that supplied the adduction channels using two underwater centrifuge pumps that subsequently supplied the 24 ponds. The density on the farm was 75 shrimp/m², a paddle-wheel was used as aerator (5 Hp/ha). The shrimp were fed on a commercial ration containing 35% crude protein, through a tray system (40 trays/ha).

Experimental design

Water collection points were established on the farms, from which samples were collected weekly at high and low tide at nights with moonlight. A total of 112 samples were collected from each recirculation system. The type of channel (supply, adduction, drainage) from which the samples were taken was recorded.

The following mathematical model was employed:

$$Y = M + F_1 + F_2 + (F_1 + F_2) + E$$

in which Y is the mean of the variable; M is the mean of the experiment; F₁ is the effect of channel type; F₂ is the effect of tide type; and E is the experiment error.

Water quality

Water samples were collected weekly for chemical analysis. Samples were collected using a bottom sampler placed above the sediment. A specific plastic bottle was employed, with no contamination from other liquids. Chemical analysis of the water involved the determination of different nutrients using the respective methodologies: nitrite (NO₂) (mg/L) based on Golterman (1978); nitrate

(NO₃²⁻) (mg/L) based on Mackereth et al. (1978); total ammonia (NH₄⁺) (mg/L) based on Koroleff (1976); orthophosphate (PO₄⁻) (mg/L) with persulfate digestion (K₂S₂O₈) based on APHA (1995). Water temperature and dissolved oxygen concentration were recorded *in situ* using a YSI model 550A oxygen meter daily. Water data were collected at the pond bottom at 7 a.m., 12 a.m., 3 p.m. and 8 p.m.. Water salinity and pH were measured at the pond surface weekly at 5 p.m. using an ATC RTS-101 refractometer and a digital PH-1700 pH meter, respectively.

Phytoplankton community

For the collection of phytoplankton, vertical sampling was performed using plastic bottles with a volume of 600 mL. The water was filtered through a cylindrical-conical net (mesh size: 15 µm) to 15 mL, providing a 40-fold concentrated sample. The phytoplankton was fixed with formalin (4%), buffered with borax (1%) and stored in 10-mL plastic recipients.

Sedgewick-Rafter chamber and binocular optical microscope (OLYMPUS CH30) with a magnification of 800× were used for the qualitative and quantitative analyses through the identification and quantification of the microalgae samples, respectively. The samples of the channels were analyzed from three different sub-samples. The phytoplankton density was expressed as individuals per milliliter (cells.mL⁻¹), estimated based on the sample preparation methodology represented by the following formula:

$$PD = [(nm / nq) \times 1000] / F$$

in which PD is phytoplankton density; nm is the number of microalgae found in the chamber; nq is the number of quadrants analyzed in the chamber;

and F is the filtering (40) and dilution (60) correction factor.

The main taxonomic microalgae groups which were identified were as follows: Bacillariophyta, Chlorophyta, Cyanophyta and Pirrophyta (Cupp, 1943; Prescott, 1954; Bicudo and Bicudo, 1970; Needhan and Needhan, 1982; Strebler and Krauter, 1987; Silva-Cunha and Eskinazi - Leça, 1990; Hoek et al.1995).

Statistical analysis

The Kolmogorov-Smirnov test demonstrated the normal distribution of the data (Zar, 1984), allowing the use of parametric statistics. When statistically significant differences were detected during the analysis of variance, Tukey's mean contrast test was used to identify the differences, with the level of significance set at 95% ($P < 0.05$). The statistical analysis was carried out with the help of the Statgraf 7.0 software.

Results

Water quality

On the PRS farm system, mean dissolved oxygen was highest in the adduction channel (6.92 ± 0.12 mg.L⁻¹) and lowest in the supply channel (6.34 ± 0.32 mg.L⁻¹). Mean temperature was the highest in the supply channel ($28.4 \pm 0.03^\circ$ C) and the lowest in the drainage channel ($28.1 \pm 0.11^\circ$ C). Mean pH was the highest in the supply channel (7.7 ± 0.04) and the lowest in the drainage channel (7.6 ± 0.05). Mean Salinity was the highest and the lowest in the adduction ($40\% \pm 0.35$) and in the supply channels ($37.5\% \pm 0.62$) respectively.

On the TRS farm system, the highest and the lowest dissolved oxygen were observed in the drainage

(7.61 mg.L⁻¹ \pm 0.29) and adduction channels (4.87 mg.L⁻¹ \pm 0.14) respectively. The highest and the lowest temperatures were observed in the drainage (28.6° C \pm 0.10) and the adduction channels (27.9° C \pm 0.12) respectively. Mean pH was highest in the adduction channel (7.8 ± 0.03) and lowest in the drainage channel (7.6 ± 0.06). Mean Salinity was highest in the adduction channel ($38.2\% \pm 0.68$) and the lowest in the drainage channel ($30.6\% \pm 0.12$).

On the PRS farm system, mean total ammonium was the highest in the drainage channel (1.81 mg.L⁻¹ \pm 0.78) and the lowest in the supply channel (0.59 mg.L⁻¹ \pm 0.21). Mean nitrite was the highest in the drainage channel (0.10 mg.L⁻¹ \pm 0.03) and the lowest in the supply channel (0.04 mg.L⁻¹ \pm 0.02). Mean nitrate was the highest in the adduction channel (0.92 mg.L⁻¹ \pm 0.25) and the lowest in the supply channel (0.44 mg.L⁻¹ \pm 0.16). Mean phosphate was the highest in the drainage channel (2.12 mg.L⁻¹ \pm 0.5) and the lowest in the supply channel (0.83 mg.L⁻¹ \pm 0.11). Statistically significant differences were found with regard to these nutrients in the different channels (Fig 1).

On the TRS farm system, mean total ammonium was the highest in the drainage channel (1.59 ± 0.01 mg.L⁻¹) and the lowest in the adduction channel (1.05 ± 0.13 mg.L⁻¹). Mean nitrite was the highest in the adduction channel (0.16 ± 0.01 mg.L⁻¹) and the lowest in the supply channel (0.12 ± 0.01 mg.L⁻¹). Mean nitrate was the highest in the supply channel (1.22 ± 0.06 mg.L⁻¹) and the lowest in the drainage channel (0.89 ± 0.06 mg.L⁻¹). Mean phosphate was the highest in the adduction channel (1.84 ± 0.15 mg.L⁻¹) and the lowest in the drainage channel (1.30 ± 0.09 mg.L⁻¹). Statistically significant differences were found with regard to these nutrients in the

different channels (Fig 2).

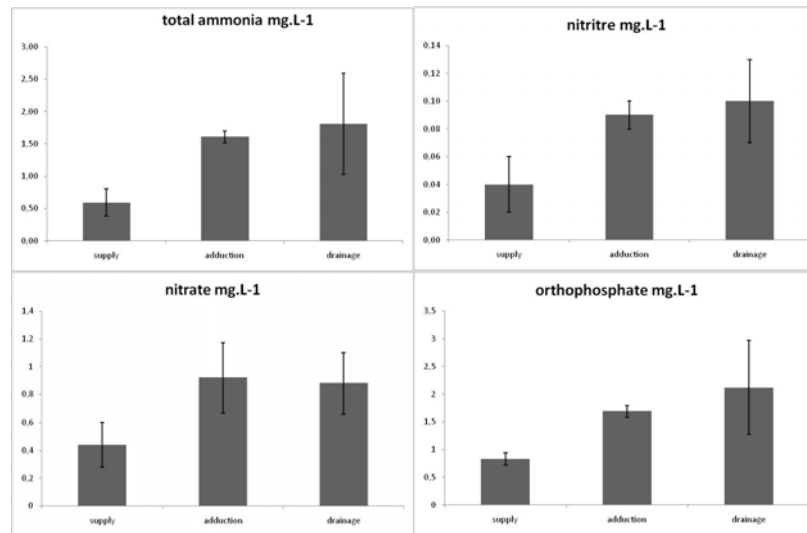


Figure 1. Total ammonia, nitrite, nitrate, orthophosphate of the water in the partial recirculation systems the *Litopenaeus vannamei* culture.

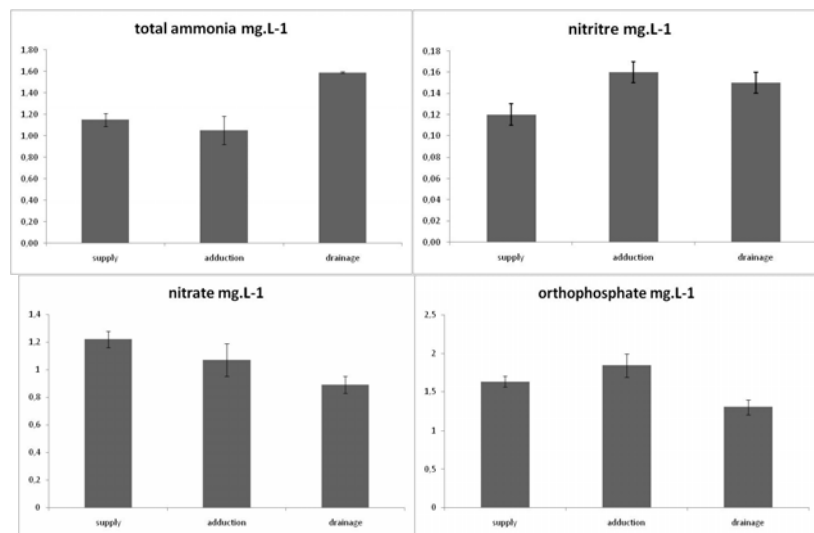


Figure 2. Total ammonia, nitrite, nitrate, orthophosphate of the water in the total recirculation systems the *Litopenaeus vannamei* culture

Phytoplankton community

On the PRS farm system, the mean Bacillariophyta density was the highest in the drainage channel ($520,625 \pm 159,983$ cells.mL⁻¹) and was the lowest in the supply channel ($219,140 \pm$

$87,394$ cells.mL⁻¹). The mean Chlorophyta value was the highest in the drainage channel ($936,250 \pm 416,309$ cells.mL⁻¹) and the lowest in the supply channel ($300,562 \pm 12,728$ cells.mL⁻¹). The mean Cyanophyta density was the highest in the adduction channel

($318,583 \pm 16,564$ cells.mL⁻¹) and the lowest in the supply channel ($88,516 \pm 10,275$ cells.mL⁻¹). The mean Pirrophyta density was the highest in the drainage channel ($10,078 \pm 2,762$ cells.mL⁻¹) and

the lowest in the supply channel ($4,063 \pm 1,328$ cells.mL⁻¹). Statistically significant differences were found with regard to these groups in the different channels (Fig 3).

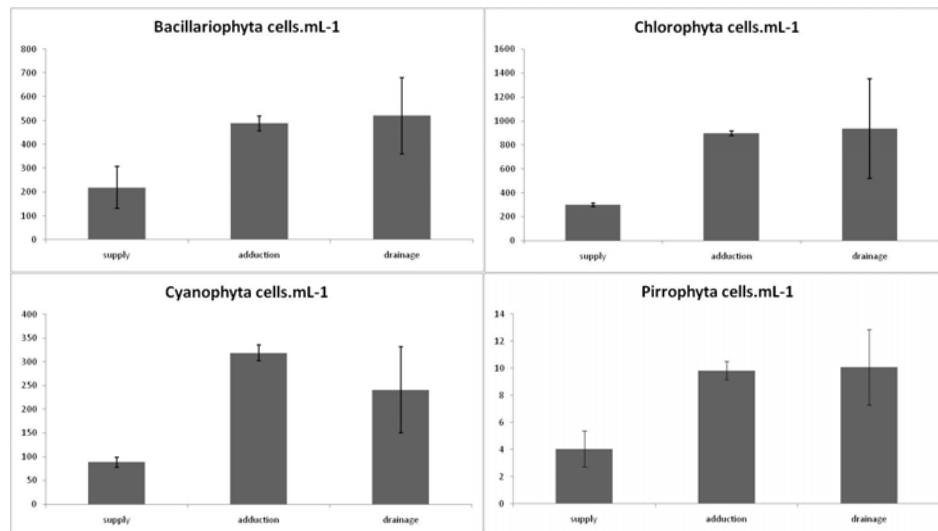


Figure 3. Phytoplankton community Bacillariophyta, Chlorophyta, Cyanophyta, Pirrophyta (cells.mL⁻¹ x 1000) in the partial recirculation systems the *Litopenaeus vannamei* culture.

On the TRS farm system, the mean Bacillariophyta density was the highest in the adduction channel (77.437 ± 599 cells.mL⁻¹) and the lowest in the drainage channel (55.507 ± 518 cells.mL⁻¹). The mean Chlorophyta density was the highest in the supply channel (353.750 ± 8.334 cells.mL⁻¹) and the lowest in the drainage channel (314.398 ± 167 cells.mL⁻¹). The mean Cyanophyta density was highest in the supply channel (61.250 ± 1.498 cells.mL⁻¹) and lowest in the adduction channel (54.250 ± 865 cells.mL⁻¹). The mean Pirrophyta density was highest in the drainage channel (27.046 ± 435 cells.mL⁻¹) and the lowest in the adduction channel (22.166 ± 503 cells.mL⁻¹). There was a statistically significant difference among these groups in the different channels (Fig 4).

Discussion

According to Boyd (2000), the concentration of dissolved oxygen is the variable that most influences the wellbeing of aquatic organisms. In the Responsible Aquaculture Program of the Global Aquaculture Alliance (GAA), the initial norm for the minimal concentration of oxygen in effluents from marine shrimp farming is 3 mg.L⁻¹, with a goal of 4 mg.L⁻¹ (Boyd, 2002). The Brazilian National Environmental Council – CONAMA establishes that this value should not be less than 5 mg.L⁻¹ (Nunes, 2002a). The concentration of dissolved oxygen on the shrimp farms with partial and total recirculation systems were in compliance with the guidelines of both the GAA and CONAMA.

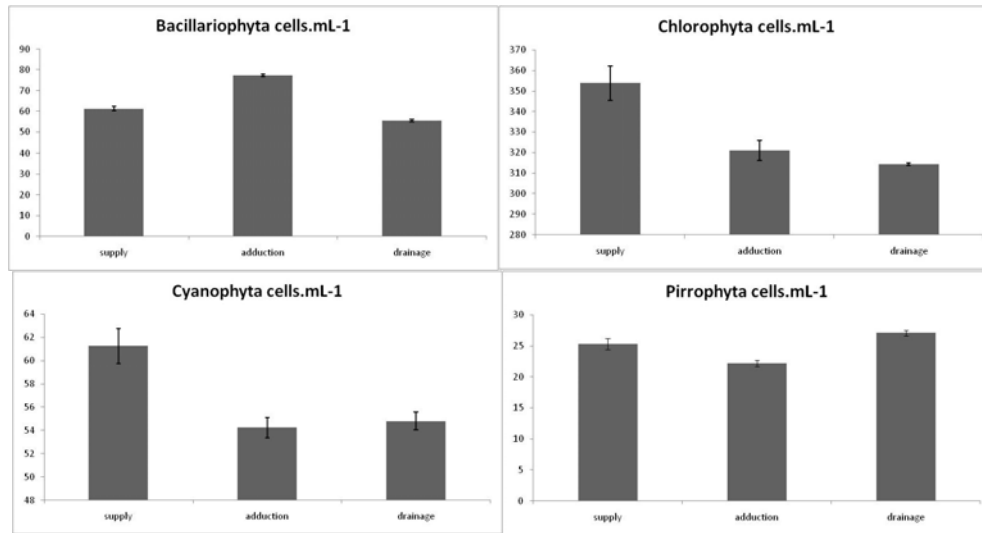


Figure 4. Phytoplankton community Bacillariophyta, Chlorophyta, Cyanophyta, Pirrophyta (cells.mL⁻¹ x 1000) in the total recirculation systems the *Litopenaeus vannamei* culture.

Despite of a reduction in temperature in winter, the northeastern region of Brazil has relatively uniform monthly temperature levels (Nunes, 2002b). The ideal temperature ranges for *L. vannamei* is reported to be between 26°C and 33°C (Nunes, *op. cit*) and 22°C and 32°C (Pillay, 1990). According to Nunes (2002b), the water temperature in shrimp farming activities is closely associated to air temperature, oscillating in a proportional fashion. Throughout the present study, the temperature at all collection sites was uniform, with a mean of 28° C.

The ideal pH for the farming of *L. vannamei* ranges from 8.1 to 9.0 (Hernandez and Nunes, 2001). According to Nunes (2002a), the Responsible Aquaculture Program of the GAA proposes a pH goal for effluents between 6 and 9. In both the farms with partial and total recirculation systems, the pH was in compliance with the established norms.

According to Vinatea (1997), the main factors that affect salinity on marine shrimp farms are precipitation and evaporation. Boyd (2000) reports

that most farms are located in tropical zones with well-defined dry and rainy seasons. According to Pillay (1990), *L. vannamei* tolerates a salinity range from 0 to 50 ppt. The ideal salinity for cultivation ranges from 15 to 25 ppt (Vinatea, 1997), but this species is successfully farmed at low salinities. In the present study, the salinity was greater than that proposed by Vinatea (1997), but within the range the species tolerates (Pillay, 1990).

Total ammonium is found in the ponds water as a byproduct of the metabolism of the shrimp and by decomposition of organic matter via bacteria and can be reused by vegetal matter or nitrified to nitrate through the action of chemoautotrophic bacteria. Racotta and Herrera (2000) found a greater consumption of dissolved oxygen in the cultivation of *L. vannamei* with an increase in the concentration of ammonium in the water. According to Boyd (2001a), the Responsible Aquaculture Program of the GAA proposes a maximal total ammonium concentration of 5 mg.L⁻¹ in shrimp farming effluents, with the goal

being 3 mg.L⁻¹, whereas CONAMA establishes that this concentration should not surpass 0.4 mg.L⁻¹ (Nunes, 2002a). The values found in the present study were within the range proposed by the GAA, but were not in compliance with the CONAMA guidelines. Chen and Lin (1992) reported that 2 mg.L⁻¹ of total ammonium and 0.11 mg.L⁻¹ of non-ionized ammonium are the maximal acceptable concentrations for the cultivation of the prawn shrimp *Pennaeus monodon*. The higher concentrations of ammonium in the drainage channel indicate that it is necessary to establish a mechanism for increasing and/or accelerating the nitrification process in the system.

Boyd (2000) reports that the maximal acceptable concentration of nitrite in shrimp farm ponds is 0.3 mg.L⁻¹, whereas nitrate can range from 0.2 to 10 mg.L⁻¹. In the present study, the concentrations remained within these limits. The presence of ammonium nitrogen in effluents from a total recirculation system is highly worrisome, as it may lead to the eutrophication of the system (Boyd, 2002), especially when there is a low concentration of dissolved oxygen and inadequate fertilization, leading to an excess of ammonium during cultivation. The toxicity of nitrate in aquatic organisms does not appear to be a serious problem, but it may increase in water recirculation systems, in which high levels can be reached as a result of the nitrification of ammonium. The toxicity of this compound is due to its effects on osmoregulation and possibly the transport of oxygen (Vinatea, 1997). The recommended concentration of nitrate ranges from 0.4 to 0.8 mg.L⁻¹ (Nunes, 2001; Barbieri and Ostrensky, 2002). The values in both systems analyzed here were in compliance with the

recommended concentrations, favoring the development of Bacillariophyta and Chlorophyta, which are the main groups of microalgae for the cultivation of marine shrimp.

The Responsible Aquaculture Program of the GAA, cited by Boyd (2001b), reports that the initial norm for the maximal concentration of total phosphorus in shrimp farming effluent is 0.5 mg.L⁻¹, with the goal being 0.3 mg.L⁻¹. These concentrations are believed to be low enough to avoid eutrophication in most coastal waters. In the present study, mean concentrations of total phosphorus surpassed the values recommended by the GAA. The concentration of orthophosphate should range from 0.2 to 0.4 mg.L⁻¹ (Barbieri and Ostrensky, 2002). Concentrations below the recommended range may negatively influence the development of phytoplankton, whereas high concentrations trigger the discharge of this nutrient in effluents, which can cause ecological imbalance in the receiving environment. In aquatic systems, phosphorus is found in the form of phosphate, with orthophosphate the most common and most utilized by vegetal matter. The last 20 to 25% of the drainage of ponds contains the greatest concentration of phosphorus, with the greatest introduction of this compound in the system during the removal of the specimens from the ponds (Boyd, 2001b). In the recirculation systems analyzed here, there was a greater phosphorus concentration in the adduction channel in comparison to the drainage channel.

In the general context, the concentrations of total ammonium, nitrite, nitrate and phosphorus behaved similarly on the farms with partial and total recirculation systems, with lower mean concentrations in the supply channel and a gradual

increase in the drainage channel. With regard to the tides, the concentrations of compounds were lower at high tide, as the large amount of effluent water had a dilution effect.

There is a large variety of data in the literature on the quantity and composition of phytoplankton on shrimp farms. Clifford (1992) and Cabrera (1996) suggested that, for semi-intensive farming, the phytoplankton density should remain between 80,000 and 120,000 cells.mL⁻¹. On the other hand, Clifford (1994) and Nunes (2001) suggested that for semi-intensive shrimp farming, the density of total algae should be between 80,000 and 300,000 cells.mL⁻¹. According to Chien (1992), in intensive farming in Taiwan, total phytoplankton density is between 100,000 and 10,000,000 cells.mL⁻¹. In the present study, total density of algae was well above that suggested by the authors cited.

The desirable groups of algae in shrimp ponds are Bacillariophyta and Chlorophyta. Nunes (2001) recommended a minimum Bacillariophyta density of 20,000 cells.mL⁻¹ and a minimum Chlorophyta density of 50,000 cells.mL⁻¹. The concentrations in the present study were well above these recommended values, indicating eutrophication of the system due to enhanced levels of nutrients.

Cyanobacteria and Pirrophyta are undesirable phytoplankton groups in marine shrimp ponds, as these groups create problems result from toxins and the depletion of dissolved oxygen. Nunes (2001) suggested densities of the Cyanobacteria and Pirrophyta of 40,000 and 500 cells.mL⁻¹, respectively. In the present study, densities far higher than the recommended values were found. In the first report of infectious myonecrosis virus (IMNV) in Brazil, a strong correlation was found between

Cyanobacteria blooms and the intensity of the mortality caused by the virus (Nunes et al. 2004). Water samples collected in May, July and August 2003 on farms in the states of Piauí (where the IMNV first appeared) and Ceará had a high concentration of Cyanobacteria belonging to the genera *Pseudanabaena* (150,000 cells.mL⁻¹) and *Limnospira* (280,000 cells.mL⁻¹) as well as the species *Scilpiella trochoidea* (123,000 cells.mL⁻¹), whereas the maximum acceptable density of Cyanobacteria on shrimp farms is 40,000 cells.mL⁻¹ (Nunes et al. 2004).

Studying the mortality of *Penaeus monodon* in ponds in Australia, Smith (1996) found the main cause to be Cyanobacteria blooms. Studying the toxicity of the Cyanobacteria *Shizothrix calcicola* in ponds of *Litopenaeus vannamei* in Mexico, Pérez-Linares et al. (2003) detected a severe disorder in the digestive tract tissues, with a consequent negative effect on the assimilation and absorption of nutrients. Considerable densities of Cyanophyta in shrimp ponds and a scarceness of Bacillariophyta lead to deficient shrimp growth (Alonso-Rodríguez and Páez-Osuna, 2003). Cyanophyta is the main cause of the loss of water quality, as these organisms reduce water transparency and oxygen levels in both the water and sediment. This group of microalgae has the ability to absorb atmospheric nitrogen in situations when nitrogen in the aquatic environment is scarce, leading to undesirable blooms.

Massive Pirrophyta blooms have been reported in different areas of shrimp farming in Asia and Latin America (Alonso-Rodríguez and Páez-Osuna, 2003). According to Yan et al (2003), one of the obstacles to Chinese mariculture is the blooms of noxious algae, such as some species of Pirrophyta. Factors such as

inadequate fertilization and environmental conditions (temperature and salinity) are responsible for the undesirable blooms of Pirrophyta and Cyanophyta. According to Paredes and Salaya (1998), the main problem with administrating the results of phytoplankton composition is the enormous diversity of sizes, forms and quality of the algae and that not all are beneficial to the farms and adjacent coastal ecosystems, such as the case with Pirrophyta, as many species produce toxic substances that, in large quantities, can be harmful to the cultivated shrimp and even human. Shumway (1990) reported that an increase in the concentration of Pirrophyta has been observed in recent years in seas throughout the world.

As ponds are dynamic systems that undergo significant ecological variations, the administrators of shrimp farms should plan the management of water (fertilization, liming) and feeding based on the availability of natural food, thereby permitting a reduction in production costs and effluents.

In water recirculation systems, dissolved nutrients and high natural productivity can be reutilized within the system itself, thereby reducing costs on fertilization and artificial feeding.

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