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EFFECTS OF ENVIRONMENTAL FACTORS ON SUCCESSION OF MICRO-PHYTOPLANKTON COMMUNITY IN A MARINE SHRIMP POND AND ADJACENT AMAZON ESTUARY

Denise Cristina de Souza Ribeiro[1]
Glauber David Almeida Palheta[1]
Fábio Campos Pamplona[1]
Igor Guerreiro Hamoy[1]
Maria de Lourdes Souza Santos[1]
Nuno Filipe Alves Correia de Melo[1]

[1]Universidade Federal Rural da Amazônia – UFRA, Instituto Socioambiental e Recursos Hídricos – ISARH, Programa de Pós-graduação em Aquicultura e Recursos Aquáticos Tropicais, Avenida Presidente Tancredo Neves, 2501, CEP 66077-830, Belém, PA, Brasil. E-mail: nunomelo@uol.com.br (corresponding author).

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ABSTRACT

This study aims to evaluate the effects of the environmental factors and *Litopenaeus vannamei* farm effluents on succession and structure of the micro-phytoplankton community and water quality in the shrimp pond and adjacent estuarine waters along the two intensive shrimp production cycles. The surface water samples for quali-quantitative analyses were monthly collected in five points, in the shrimp pond (3) and the adjacent estuary (2), using a plankton net (64 µm) and polyethylene containers, during in the rainy season, and in the dry period. The micro-phytoplankton community was composed by 205 taxa. The environments were different in relation to the total abundance, richness and species indicators with the estuary presented the highest values in relation to the shrimp pond. The diatoms were predominant mainly in the estuary, with the higher abundances and the great majority of indicator species, while in the shrimp pond, the number of indicator diatoms was much lower allowing the establishment euglenophytes and cyanophytes as also indicator species. In the shrimp pond the main drivers of micro-phytoplankton community structure were the higher concentrations of ammoniacal nitrogen, nitrite, nitrate and phosphate, mainly in relation to the feed accumulation, while in the estuary, the main driver factor was the seasonality.

Key words: seasonality; nutrients; *Litopenaeus vannamei*.

EFEITOS DOS FATORES AMBIENTAIS NA SUCESSÃO DA COMUNIDADE MICROFITOPLANCTÔNICA EM UM VIVEIRO DE CAMARÃO MARINHO E ESTUÁRIO AMAZÔNICO ADJACENTE

RESUMO

Este estudo tem como objetivo avaliar os efeitos dos fatores ambientais e efluentes da fazenda de criação de *Litopenaeus vannamei* sobre a sucessão e estrutura da comunidade microfitoplanctônica e a qualidade da água no viveiro de camarão e em águas estuarinas adjacentes ao longo dos dois ciclos intensivos de produção de camarão. As amostras de águas superficiais para as análises quali-quantitativas foram coletadas mensalmente em cinco pontos, no viveiro de camarão (3) e no estuário adjacente (2), utilizando uma rede de plâncton (64 µm) e recipientes de polietileno, durante as estações chuvosa e seca. A comunidade micro-fitoplanctônica foi composta por 205 taxóns. Os ambientes foram diferentes em relação à abundância total, riqueza e espécies indicadoras sendo que o estuário apresentou os maiores valores em relação ao viveiro de camarão. As diatomáceas predominaram principalmente no estuário, com as maiores abundâncias e a grande maioria das espécies indicadoras, enquanto no viveiro, o número de diatomáceas indicadoras foi muito menor, permitindo o estabelecimento de euglenófitas e cianófitas também como espécies indicadoras. No viveiro, os principais responsáveis pela estrutura da comunidade micro-fitoplanctônica foram as maiores concentrações de nitrogênio amoniacal, nitrito, nitrato e fosfato, principalmente em relação ao acúmulo de ração, enquanto no estuário o principal fator determinante foi a sazonalidade.

Palavras-chave: sazonalidade; nutrientes; *Litopenaeus vannamei*.

INTRODUCTION

Shrimp farming is one of the fastest growing economic activities in several countries around the world (Araújo et al., 2012). However, from an environmental point of view, uncontrolled implantation of cultures can generate serious problems for the environment and the cultures themselves (Poersch et al., 2006; Barbieri et al., 2014b), especially regarding the generation of effluents from this activity, since most projects discarded this in the water without previous treatment.

The discharge of effluents rich in organic matter from marine shrimp farms can cause hyper-nitrification, eutrophication, sedimentation and change in the productivity and structure of the adjacent biotic community, modifying the natural characteristics of the environment (Wainberg, 2000).

In addition to these changes in water ecosystems, there can also be increases in nitrogen and phosphorus concentrations in the water column and accumulation of organic matter in the sediments (Bardach, 1997; Midlen and Redding, 1998; Barbieri et al., 2014a). These higher nutrient concentrations tend to favor an increase in abundance of the phytoplankton community in the receiving environment.

Phytoplankton plays an important role as a primary producer in water ecosystems, since they constitute the base of the food web. These microorganisms are extremely sensitive to environmental changes, reflecting the lowest chemical and physical changes in water. For this reason, they are excellent indicators of water quality (Padisák, 1993; Burford, 1997).

In this sense, several studies on the composition, diversity, abundance and dominance of phytoplankton and their relationships with nutrients in the environment provide fundamental information on ecosystems and their variations due to pollution (Ludwig and Reynolds, 1988; Branco and Senna, 1996; Huszar et al., 2000; Havens et al., 2003).

The study of the succession and structure of micro-phytoplankton community is extremely important in all phases of shrimp cultivation, since they are good indicators of environmental quality and a good food source for shrimp larvae. Thus, due to the implantation and development of marine shrimp farming in the Amazon, the aim of this research was to evaluate the effects of the environmental factors and *Litopenaeus vannamei* farm effluents on succession and structure of the micro-phytoplankton community and water quality in the shrimp pond and adjacent estuarine waters along the two intensive shrimp production cycles.

MATERIAL AND METHODS

The marine shrimp farm is located at the Vila de Caratateua (00º 40’ 0” S and 47º 45’ 0” W), Curuçá city, on the Northeast of the Pará state, Brazil. It has four shrimp ponds (V1, V2, V3 and V4), with an equal area (1.0 ha each) of water depth and a sedimentation basin of 0.5 ha, associated to a biological filter. The cultivated shrimp is of the exotic species *Litopenaeus vannamei*. The type of cultivation system adopted is intensive, with a stocking density of approximately 55 post-larvae m^{-2}, being that the ponds maintain specimens from the age post-larvae (PL) to 20 days of life (PL20).

A total of eight collections were carried out in 2011, with approximate intervals of 30 days, in two hydrological and production cycle periods: in the rainy season (January, February, March and April), corresponding to the first production cycle, and, in the dry period (August, September, October and November), the second production cycle.

To assess the cultivation environment and the adjacent estuary (water collection and disposal site), five collection stations were selected: P1 (inlet water channel) and P2 (effluent discharge channel). At these points, the collections were carried out during flood and ebb tides. The other points were sampled from only one pond (V3) and were called P3 (water inlet in the shrimp pond), P4 (middle of the shrimp pond) and P5 (water exit) (Figure 1).

The temperature (ºC), the pH, the salinity and the turbidity (NTU) were evaluated on site, with the aid of a multiparameter probe (HANNA, model HI9828). The dissolved oxygen (mg L^{-1}) was determined by the Winkler Method described in Strickland and Parsons (1972). The nutrients nitrite, nitrate, ammoniacal nitrogen, silicate and phosphate (all measured in mg L^{-1}) followed the methodology described on the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). The rainfall data (mm) were obtained from the National Water Agency (*Agência Nacional das Águas* – ANA) and the historical monthly average of the period from 1982 to 2011 was calculated (ANA, 2016).

For the qualitative analyses of the micro-phytoplankton, horizontal trawling was carried out with a plankton net (64 µm), while for the quantitative analyses, 200 mL of water was collected directly from the water surface with the aid of polyethylene containers (20 L). Buffered formalin was added to both samples, with final concentration of 4% in all samples. Water samples were collected in 1-L plastic bottles for the analysis of chlorophyll *a* (mg m^{-3}), and were refrigerated until the time of filtration, and calculated as described by Strickland and Parsons (1972).

The qualitative samples were analyzed with an Olimpus CX 21 microscope, and the micro-phytoplankton abundance was evaluated by counting the organisms per liter (org L^{-1}) through the Utermöhl (1958) method, using a Zeiss inverted microscope, with phase contrast at magnification of 400X. The micro-phytoplankton were identified with basis on specialized references, and were identified to the lowest taxonomic level possible.

The criterion adopted to calculate the frequency of occurrence was the same used by Matteucci and Colma (1982). The following categories were established: very frequent (F>70%), frequent (40% < F ≤ 70%), infrequent (10% < F ≤ 40%) and sporadic (F ≤ 10%).

Species richness (S) was estimated by counting the number of taxa per sample. The Shannon diversity index was calculated for each sample (Shannon and Weaver, 1949) and the equability (J’) was calculated from the Shannon index (H’) applied to the abundance data.

The Shapiro-Wilk test was performed to verify the normality of environmental (physical-chemical and biological) data in the comparisons with spatial (shrimp pond and estuary), seasonal (rainy and dry) and tidal (estuary only) factors. Since the environmental data did not present normality, even after transformations, the non-parametric Mann-Whitney and Kruskal-Wallis tests – which correspond to the parametric analyses t-test and ANOVA, respectively – were used to compare them under the influences of the aforementioned factors. Spearman’s correlation analysis (rs) was performed to compare the correlations between environmental

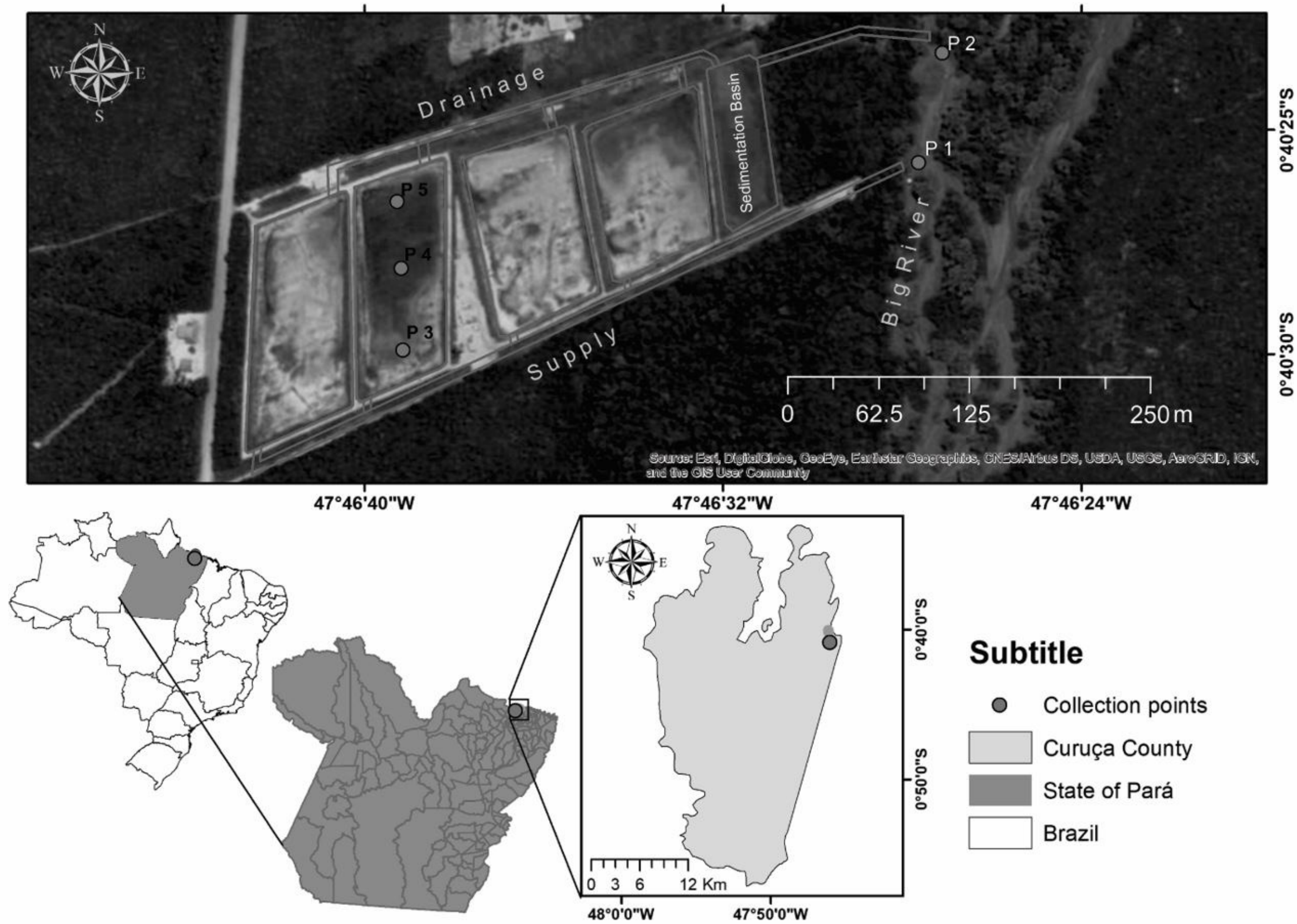

Figure 1. Location of the study area and collection points in the estuary and shrimp pond. Estuary: Inlet water channel (P1), effluent discharge channel (P2). Shrimp pond: Water inlet in the shrimp pond (P3), middle of the shrimp pond (P4) and water exit (P5), Curuçá-PA.

parameters. All the analyses were performed with the aid of the Past statistical software (Hammer et al., 2001).

The indicator species for the estuary and shrimp pond were determined using the Individual Indicator Value (IndVal) (Dufrêne and Legendre, 1997), which combines abundance of the species with its frequency of occurrence in several groups of sites and through 10,000 permutations (Monte Carlo method), the significance of the IndVal values of each species ($p < 0.05$) was evaluated through the software PCORD 5 (McCune and Mefford, 2011). The taxa selected for each environment through IndVal, in turn, composed the biological matrix and were associated to the 12 abiotic variables in the RDA described below.

Finally, to evaluate the influence of the abiotic variables on the micro-phytoplankton community, a Canonical Redundancy Analysis (RDA) (Ter Braak and Šmilauer, 2002) eliminating the interference of the different units of measure in the matrix of environmental variables through standardization by Ranging (Legendre and Legendre, 2012). A Hellinger transformation was carried out for the biological data matrix (Legendre and Legendre, 2012).

The explanatory variables were selected with the RDA to determine which of them significantly explained the distribution of the associations of micro-phytoplankton taxa in the collection environments (estuary and shrimp pond) and in the collection periods. The program used to carry out the RDA was CANOCO 4.5.

RESULTS

Abiotic parameters

The average rainfall variation had a significant seasonal difference ($p < 0.01$), with the highest rainfall values in the rainy season. In 2011, it was observed that a negative anomaly pattern occurred in most months, except for the months of January and July, which reached rainfall concentrations above the historical average (Figure 2).

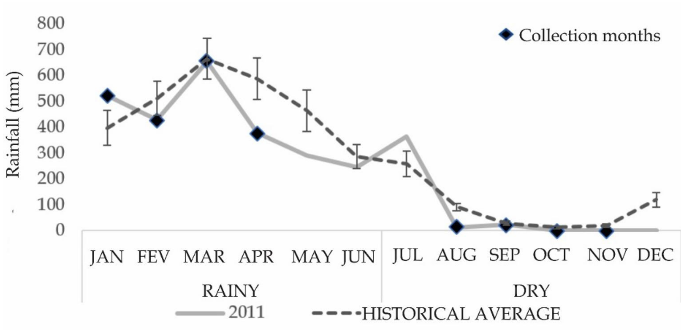

Figure 2. Historical monthly average (1982-2011) and average rainfall (mm) in 2011. Source: National Water Agency (ANA, 2016).

The temperature in the estuary reached 29.19 ºC and in the pond 30.42 ºC (Figure 3). This variable had a significant seasonal difference, both in the estuary and in the pond, with higher average values in the dry period. It also revealed a significant difference between the collection environments, with higher values being observed in the pond (Tables 1 and 2). For each environment, the pH did not have significant seasonal differences. However, the pond showed the significant higher values in the both periods (Tables 1 and 2, Figure 3). The salinity was significantly higher in the dry period ($p < 0.05$), in both environments (Figure 3).

Dissolved oxygen presented significant differences both by season and between environments, with the highest DO values found in the pond ($U = 1$; $p = 0.0000$) (Figure 3). As for turbidity, values were significantly higher in the pond ($U = 24$; $p = 0.0061$),

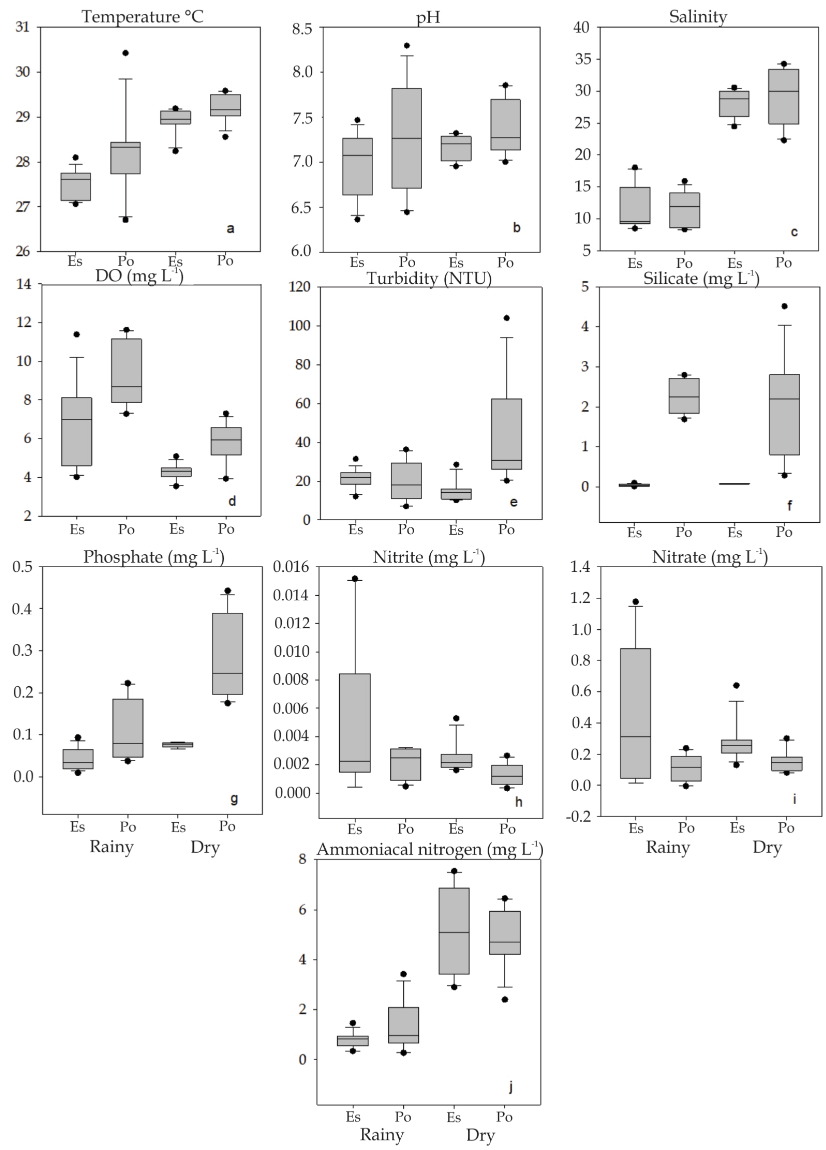

Figure 3. Box-plot graphs of water quality variables for rainy (plots at the left) and dry (plots at the right) seasons in the estuary (Es) and shrimp pond (Po), Curuçá-PA. (a) temperature; (b) pH; (c) salinity; (d) dissolved oxygen; (e) turbidity; (f) silicate; (g) phosphate; (h) nitrite; (i) nitrate; (j) ammoniacal nitrogen.

Table 1. Water quality values (means, standard deviations, maximum and minimum) of the temperature, salinity, dissolved oxygen, turbidity, silicate, phosphate, nitrite, nitrate, ammoniacal nitrogen and chlorophyll *a* in the estuary and shrimp pond, Curuçá-PA.

VARIABLES	ESTUARY			POND		
	Mean±SD	Max	Min	Mean±SD	Max	Min
Temperature (°C)	28.11±0.76	29.19	27.07	28.69±0.84	30.42	26.71
pH	7.06±0.29	7.47	6.36	7.32±0.45	8.30	6.44
Salinity	18.56±9	30.55	8.47	20.54±9.64	34.26	8.33
Dissolved Oxygen (mg L^{-1})	5.64±2.05	11.39	3.54	7.51±2.15	11.63	3.93
Turbidity (NTU)	18.73±5.92	31.50	10.10	31.12±21.77	104.00	6.99
Silicate (mg L^{-1})	4.58±1.40	6.32	0.05	2.15±0.87	4.51	0.28
Phosphate (mg L^{-1})	0.06±0.03	0.09	0.01	0.19±0.12	0.44	0.04
Nitrite (mg L^{-1})	0.004±0.004	0.02	0.00	0.002±0.001	0.00	0.00
Nitrate (mg L^{-1})	0.37±0.34	1.18	0.02	0.14±0.007	0.30	0.00
Ammoniacal-N (mg L^{-1})	2.69±2.47	7.54	0.34	3.09±2.07	6.45	0.27
Chlorophyll *a* (mg m^{-3})	7.57±4.50	27.46	2.86	90.50±85.67	268.48	5.10

Table 2. Results of the Kruskal-Wallis and Mann-Whitney tests of the water quality variables (temperature, salinity, dissolved oxygen, turbidity, silicate, phosphate, nitrite, nitrate, ammoniacal nitrogen and chlorophyll *a*) between the different factors (tides, seasons and environment type). Asterisks (*) show variables with significant differences at p-value <0.05.

VARIABLES	ESTUARY		POND	ESTUARY x POND
	TIDE	SEASON	SEASON	SPATIAL
	p	p	p	p
Temperature (°C)	0.8005	0.00004*	0.0005*	0.0017*
pH	0.696	0.4023	0.9539	0.0477*
Salinity	0.5331	0.00003*	0.00003*	0.216
Dissolved Oxygen (mg L^{-1})	0.8722	0.00009*	0.00004*	0.0118*
Turbidity (NTU)	0.3703	0.0102*	0.0061*	0.0245*
Silicate (mg L^{-1})	0.2412	0.0002*	0.8852	0.0000*
Phosphate (mg L^{-1})	0.9816	0.0150*	0.0001*	0.0000*
Nitrite (mg L^{-1})	0.4198	0.2229	0.0512	0.3629
Nitrate (mg L^{-1})	0.9633	0.4024	0.3123	0.0013*
Ammoniacal-N (mg L^{-1})	0.5657	0.0003*	0.00005*	0.0712
Chlorophyll *a* (mg m^{-3})	0.0058*	0.795	0.5067	0.0000*

during the dry period (Figure 3, Table 2). In this period an increase of turbidity occurred mainly at the end of the cultivation cycle.

In the estuary, the nutrients that presented significant seasonal differences were silicate, phosphate and ammoniacal nitrogen; in the pond, phosphate and ammoniacal nitrogen presented differences, with higher concentrations in the dry period. The nutrients that revealed spatial differences were silicate and nitrate, with higher values in the estuary, and phosphate, which presented higher concentrations in the pond (Figure 3, Table 1).

When the variables were compared considering the tidal factor, only chlorophyll showed a significant difference ($U = 37.5$; $p = 0.0058$) (Table 2), with the highest concentrations occurring at ebb tide. This variable also presented a difference between environments ($U= 58$; $p = 0.0000$), with the highest concentrations in the pond, mainly at the end of the cultivation cycle of each period.

Micro-phytoplankton community

The micro-phytoplankton community was composed of a total of 205 taxa, with a largest number of organisms (194 taxa) found in the estuary environment than in the pond (107 taxa). In the estuary, the Bacillariophyta division composed the vast majority of the taxa (160 taxa), followed by Myzozoa (15 taxa), Cyanobacteria (8 taxa), Charophyta (6 taxa), Chlorophyta (3 taxa), Cryptophyta (1 taxa) and Euglenophytes (1 taxa). In the pond, although with half the number, the Bacillariophyta division also dominated the majority of individuals (82 taxa), followed by Cyanobacteria (8 taxa), Myzozoa (8 taxa), Charophyta (4 taxa), Chlorophyta (4 taxa) and Euglenophytes (1 taxa).

In the estuary, in relation to frequency of occurrence, 10 taxa were considered very frequent: *Melosira nummuloides*, *Coscinodiscus perforatus*, *Trieres chinensis*, *Cylindrotheca closterium*, *Ditylium brightwellii*, *Pleurosigma angulatum*, *Actinoptychus annulatus*, *Rhopalodia musculus*, *Thalassionema frauenfeldii* and *Trieres regia,* which had 100% frequency in all samples. In the other categories, 20 taxa were considered as frequent, 47 infrequent and most (117), sporadic. For the pond environment, no taxa were considered as very frequent; however, only 3 taxa were considered as frequent throughout the study period, the cyanobacteria *Lyngbya* sp.1, the diatoms *Coscinodiscus perforatus* and *Trieres chinensis.* Only 17 taxa were considered as infrequent, and 87 taxa were sporadic.

Regarding to richness, the estuary had the highest number of individuals in January (114 taxa), while in March and September it had the lowest number (59 taxa). In this environment, during the rainy season, the diversity and equitability showed similar variation patterns with their minimum values (0.96 nat ind^{-1} and 0.59, respectively) in February, and maximum values (2.55 nat ind^{-1} and 0.95, respectively) in January. In the dry period, the diversity and equitability also had similar variation patterns, with their minimum values of (1.56 nat ind^{-1} and 0.67, respectively) in September, and maximum values (2.44 nat ind^{-1} and 0.88, respectively) in August.

In the shrimp pond, at the beginning of the first cultivation cycle, January also exhibited the highest richness (58 taxa), although this one was similar to the minimum value found in the estuary. Yet in relation the shrimp pond, March presented diversity values of 0.86 nat ind^{-1} and equitability of 0.89, and January, 1.41 nat ind^{-1} and 0.88. In the dry period, during the second cultivation cycle, August had a diversity value of 0.81 nat ind^{-1} however, there was a gradual decrease in diversity from the month of September (1.31 nat ind^{-1} to 0.11 nat ind^{-1}), and equitability followed the same pattern, ranging from 0.97 to 0.24, at the end of this cycle.

The total micro-phytoplankton abundance in both environments was distributed among the groups Bacillariophyta, Myzozoa, Cyanobacteria e Euglenophytes (Figures 4 and 5), with only the estuary presenting significant seasonal differences ($U = 33$; $p = 0.0262$), with higher abundance values in the dry period (maximum of 63,600 org L^{-1}). In this same period, the diatoms presented significantly higher densities ($U = 26.5$; $p = 0.0093$) in relation to the rainy season, with maximum of 26,200 org L^{-1} in August. However, this group, considering only the rainy season, also presented the highest densities in relation to the other groups, with maximum of 16,100 org L^{-1} in February, and decreasing progressively towards the end of the first cycle (April) (Figure 4).

Another micro-phytoplankton group with great prominence on the estuary was Cyanobacteria, since this group was not observed in the rainy season, however, in the dry period, these organisms had an exponential growth toward the end of the second cultivation cycle, ranging from 100 to the maximum of 1,800 org L^{-1} in October. The Myzozoa group also presented a growth, although less pronounced, at the end of this same cycle, varying from

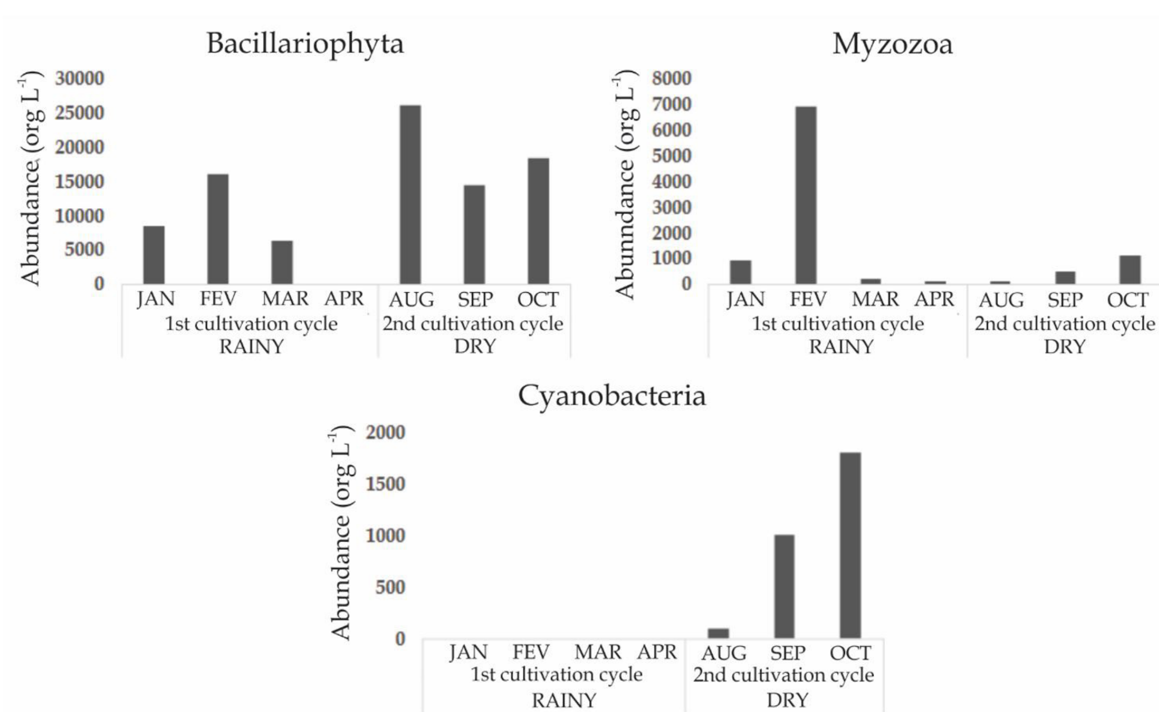

Figure 4. Total micro-phytoplankton abundance (org L^{-1}) by taxonomic group in the estuary, Curuçá-PA.

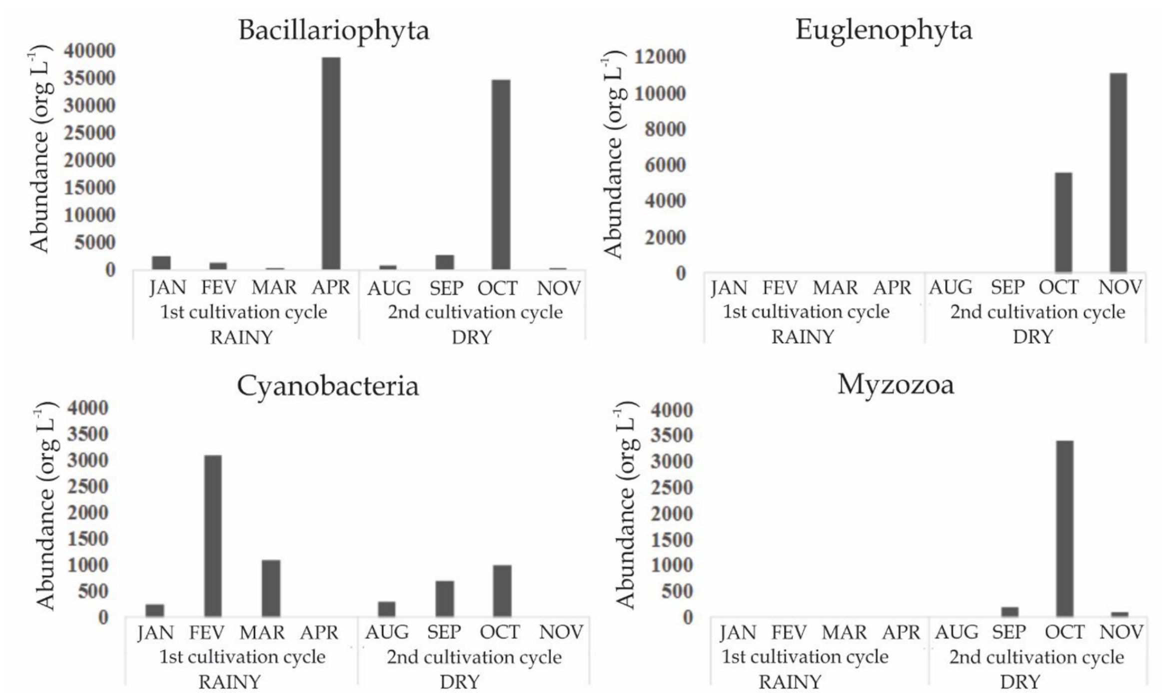

Figure 5. Total micro-phytoplankton abundance (org L^{-1}) by taxonomic group in the pond, Curuçá-PA.

100 to the maximum of 1,100 org L^{-1} (October). However, this group presented its maximum abundance in the rainy season, in an isolated peak in February (6,900 org L^{-1}) and decreasing to the end of the cycle (Figure 4). Lastly, this environment did not present a significant difference ($p > 0.05$) both in relation to the tidal factor (flood and ebb) and collection points (in water catchment and in the disposal of pond water).

The organisms that stood out with higher abundance values (> 2,000 org L^{-1}) in the estuary were the diatoms, *Coscinodiscus concinnus* (20,600 org L^{-1}), *Navicula* sp.1 (8,500 org L^{-1}), *Dimeregramma* sp (2,000 org L^{-1}) and the dinoflagellates *Tripos furca* (5,700 org L^{-1}) and *T. fusus* (2,600 org L^{-1}) during the rainy period. On the other hand, only the diatoms *Thalassionema nitzschioides* (21,400 org L^{-1}), *Thalassionema frauenfeldii* (4,900 org L^{-1}), *Thalassiosira* sp.1 (3,700 org L^{-1}), *Diploneis bombus* (3,600 org L^{-1}), *Coscinodiscus centralis* (3,100 org L^{-1}), *Cyclotella stylorum* (2,900 org L^{-1}), *Actinoptychus annulatus* (2,700 org L^{-1}) and *Diploneis weissflogii* (2,000 org L^{-1}) stood out during the dry period.

In the shrimp pond, among the identified groups, Bacillariophyta also had the highest abundance values, especially in April, in the end of first cultivation cycle in the rainy season (38,600 org L^{-1}) and in the month before the end of second cultivation cycle in the dry period (October, with 34,600 org L^{-1}) when the highest values occurred (Figure 5). This behavior was different from that observed in the estuary, which had its highest values in February and August, beginning of each cultivation cycle. Regarding the two seasonal periods, it is noteworthy that in the pond, the only group that showed significant difference was the Euglenophytes ($U = 36$; $p = 0.0070$), with great contribution to the abundance value by the taxon *Euglena* sp., only in the dry period, at the end of the cultivation cycle (November). However, when the collection points were compared within this artificial environment, there were no significant differences either in relation to the total abundance or between the micro-phytoplankton groups.

In addition, there was a significant difference between the groups over the study period ($Hc = 26.66$; $p = 0.0000$), with Bacillariophyta presenting the highest total abundance value, followed by Euglenophytes, Cyanobacteria and Myzozoa (Figure 5). The abundance pattern of the Bacillariophyta group stood out by presenting two isolated high peaks in both cultivation cycles. In the first cycle, it reached (38,600 org L^{-1}) at the end of the cycle (April), while in the second cycle, the peak was slightly lower (34,600 org L^{-1}) and occurred one month before the end of the cycle (October). Still regarding this second cycle, other groups also presented expressive growths in abundance, such as Euglenophytes (with 5,600 org L^{-1} in October 11.100 org L^{-1} in November) and the group Myzozoa (3,400 org L^{-1} in October). However, the Cyanobacteria group contributed to the abundance in both the first and second cycles, but it was observed that this group had a decline in the last month of each cycle, giving space mainly to the appearance of Euglenophytes (Figure 5).

For this environment the organisms that stood out with higher abundance values (> 2,000 org L^{-1}) in the rainy season were the diatoms *Navicula* sp.2 (24,100 org L^{-1}) and *Cylindrotheca closterium* (12,500 org L^{-1}), and the cyanobacteria *Lyngbya* sp.2 (4,200 org L^{-1}). In the dry period, the diatom *Navicula* sp.2 (33,900 org L^{-1}), the Euglenophyte *Euglena* sp (16,700 org L^{-1}) and dinoflagellate *Gonyaulax* sp (3,700 org L^{-1}).

In the comparison of environments, total micro-phytoplankton abundance was significantly higher in the estuary (U = 183.5; p = 0.0319). As for the micro-phytoplankton groups, the diatoms (U = 132; p = 0.0001) and dinoflagellates (U = 168; p = 0.0003) densities were higher in estuary environment, while the Cyanobacteria (U = 147; p = 0.0010) was higher in the pond.

Chlorophyllous biomass

In the estuary, chlorophyll *a* reached the lowest value (2.86 mg m^{-3}) in September (dry period) and highest (27.46 mg m^{-3}) in February (rainy period) during ebb tide, at the water catchment point for supplying the pond. This parameter had a constant behavior in the estuary during the study period, which did not show significant difference between the periods; however, when the tide factor was considered, the highest values occurred in the ebb tides (U = 37.5; p = 0.0058). However, in the pond, the variation in chlorophyll *a* values was much higher (minimum of 5.10 and maximum of 268.48 mg m^{-3}, in January and April, respectively) (Figure 6). It should be noted that in each period, within each cycle, chlorophyll values increased exponentially towards the end of the cycle, reaching maximum peaks of 268.48 and 233.37 mg m^{-3} in April and November, respectively. Despite this concentration variation, no seasonal differences were found in the pond.

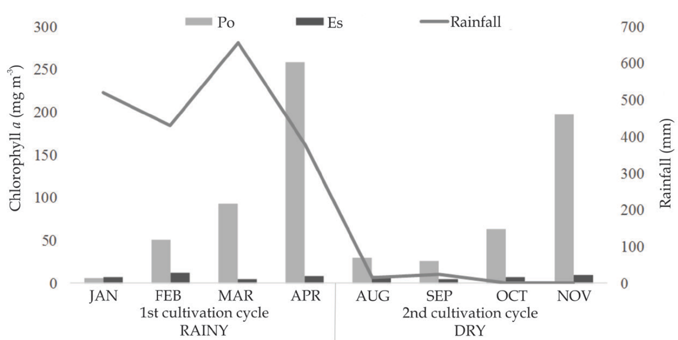

Figure 6. Micro-phytoplankton biomass (mg.m-3) throughout the study period, in the pond (Po) and the estuary (Es), Curuçá-PA.

In the estuary, no significant correlations were found between the environmental variables. However, in the pond, only correlations between chlorophyll *a* and turbidity were found (rs = 0.972; p = 0.028), and they were significant and closely correlated only in the rainy period.

Among the 73 taxa that contributed to the abundance values in the estuary, only 12 taxa were indicators for this environment, with significant IndVal values ($p < 0.05$). The diatoms *Diploneis bombus* (53.6%) and *Coscinodiscus concinnus* (50%) had high indicator value in the estuary. In the pond, of the 34 taxa that contributed to the abundance values, only 5 taxa were indicators, presenting significant IndVal values ($p < 0.05$). For this environment, the diatom *Navicula* sp.1 and cyanobacteria *Lyngbya* sp.2 had the highest IndVal values (37.6 and 37.5%, respectively) (Table 3). It should be noted that most of the indicator organisms for each environment are diatoms.

Relationship between environmental variables and the micro-phytoplankton community

In the estuary, the Canonical Redundancy Analysis showed that the significant environmental variables ($p < 0.05$) concentrated 50.7% of the total variance (Figure 7), with the first two axes concentrating most of the variability (33.7%).

Table 3. Micro-phytoplankton species that presented significant IndVal (IV) values (p-value < 0.05) in relation to estuary and shrimp pond and their respective taxonomic groups: Diat (Diatoms), Dino (Dinoflagellate), Cyan (Cyanobacteria), Eugle (Euglenophyte).

Environment	Groups	Indicator Species	(IV)	p-value
Estuary	Diat	*Diploneis bombus*	53.6	0.0001
	Diat	*Coscinodiscus concinnus*	50	0.0001
	Dino	*Tripos furca*	46.4	0.0003
	Diat	*Thalassiosira* sp.1	46.4	0.0002
	Diat	*Thalassionema frauenfeldii*	42.9	0.0003
	Diat	*Actinoptychus annulatus*	32.1	0.0019
	Diat	*Diploneis weissflogii*	32.1	0.0013
	Diat	*Rhophalodia musuculus*	32.1	0.0024
	Diat	*Diploneis gruendleri*	28.6	0.0051
	Diat	*Skeletonema costatum*	28.6	0.0056
	Diat	*Dimeregramma* sp	25	0.0121
	Diat	*Tryblionella granulata*	21.4	0.0248
Pond	Diat	*Navicula* sp.1	37.6	0.0036
	Cyan	*Lynbya sp.2*	37.5	0.0007
	Eugle	*Euglena* sp	25	0.0061
	Diat	*Navicula* sp.2	25	0.0073
	Cyan	*Pseudanabaena* sp	16.7	0.0395

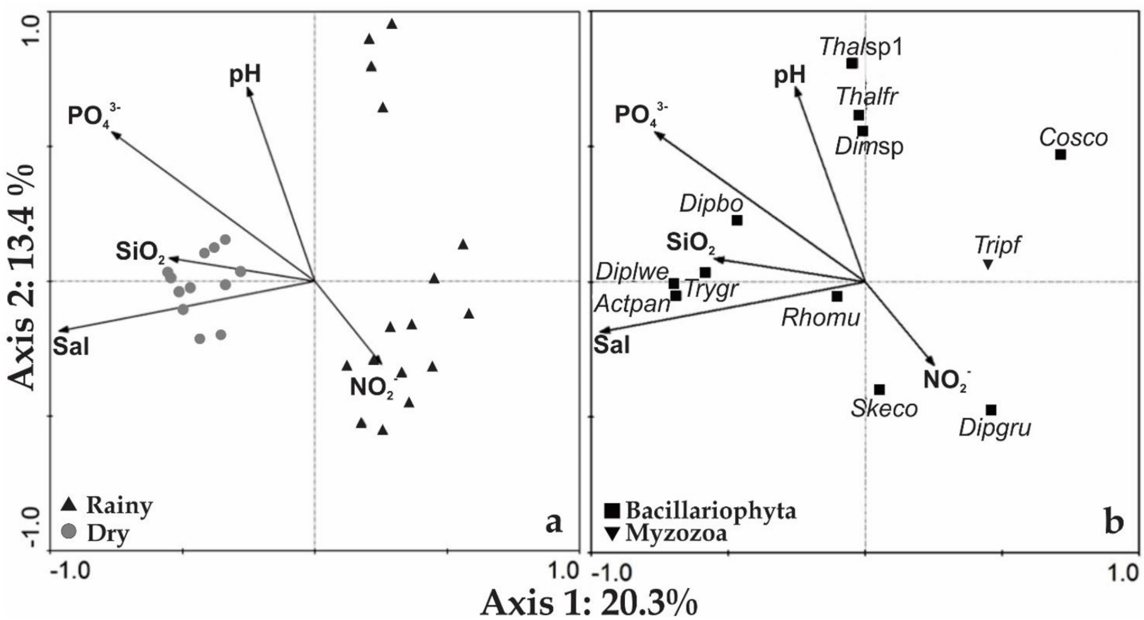

Figure 7. Redundancy analysis (RDA) ordination plots of axes 1 and 2 for estuary showing: (a) Projection of the environmental variables in relation to rainy and dry period samples; (b) Projection of the environmental variables in relation to micro-phytoplankton taxa. Only the significant variables were represented ($p < 0.05$). PO_4^{3-} (Phosphate), SiO_2 (Silicate), Sal (Salinity), NO_2^- (Nitrite). Species codes: Actpan = *Actinoptychus annulatus*; Cosco = *Coscinodiscus concinnus*; Dimsp = *Dimerogramma* sp.; Dipbo = *Diploneis bombus*; Dipgru = *Diploneis gruendleri*; Diplwe = *Diploneis weissflogii*; Rhomu = *Rhpalodia musculus*; Skeco = *Skeletonema costatum*; Thalfr = *Thalassionema frauenfeldii*; Thalsp1 = *Thalassiosira* sp.1; Tripf = *Tripos furca*; and Trygr = *Tryblionella granulata.*

Axis 1 evidences a seasonal gradient in the estuary, explaining 20.3% of the data variability. The negative portion shows the projections of the variables silicate and salinity that presented their highest values during dry period (Figure 7a). The species that were associated with these variables in axis 1 were the diatoms *Diploneis bombus, Tryblionella granulata, Diploneis weissflogii* and *Actinoptychus annulatus* (Figure 7b). On the other hand, the analysis showed that *Coscinodiscus concinnus* and *Tripos furca* were inversely influenced by salinity, since these taxa exhibited the highest abundance values in the rainy season (Figure 7b).

Axis 2 concentrated 13.4% of the data variability and was probably correlated with nutrient intake from the feed. As the main effect of this process, a slight increase in the concentration of nitrite in the rainy season is observed, occurring mainly at the end of first cultivation cycle, in April. This increase favored the diatoms *Skeletonema costatum* and *Diploneis gruendleri*. In the positive portion of the axis, under the influence of the highest pH values, the samples of March, corresponding to the month in which the highest rainfall occurred, being associated with the diatoms *Thalassiosira* sp.1, *Thalassionema frauenfeldii* and *Dimeregramma* sp. (Figure 7b).

In the shrimp pond, the physico-chemical variables contributed significantly ($p < 0.05$) to the distribution of the taxa, concentrating 76.7% of the data variability; this explanation was better than for in the estuary, evidencing that environmental variables strongly influence this environment. The first two axes of the RDA concentrated the highest variability, 48.7% (Figure 8).

The Redundancy Analysis carried out in the shrimp pond showed that axis 1 concentrated 29.8% of the data variability. Thus, the positive portion strongly correlated with the variables salinity, ammoniacal nitrogen, chlorophyll *a* and phosphate, correlating with dry period samples (Figure 8a). The diatom *Navicula* sp.2 was related to the highest values of salinity and ammoniacal nitrogen, and highlighting with higher abundance in April and October, which corresponded to the end of the first and second shrimp cultivation cycles, respectively. On the other hand, the Euglenophyte *Euglena* sp. correlated with the highest concentrations of chlorophyll *a* and phosphate, evidencing high densities of this taxon in November samples, coinciding with the end of the second shrimp culture cycle. In contrast, in the negative portion of this axis, rainfall and silicate exhibited their highest values in rainy season samples. In addition, *Lyngbya* sp.2 correlated with both these variables and periods (Figure 8b).

Axis 2 explained 18.9% of the data variability, being correlated mainly to nitrate concentrations. These were higher especially in the samples of dry period. This axis, similar to the RDA for the estuary, also reflects a gradient resulting from the nutrient accumulation from the feed during the shrimp cultivation period. The highest concentrations of nitrate were associated with cyanobacteria *Pseudanabaena* sp. and the diatom *Navicula* sp.1, this last one reaching the highest values of abundance in January and February (rainy period), as well as in August and September (dry period) (Figure 8b).

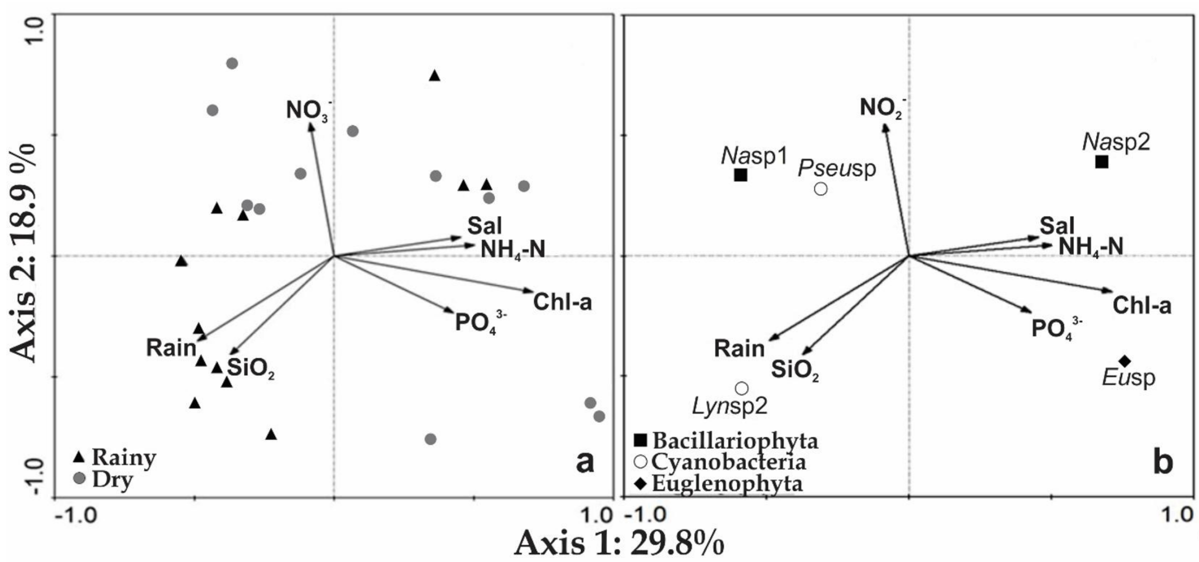

Figure 8. Redundancy analysis (RDA) ordination plots of axes 1 and 2 for shrimp pond showing: (a) Projection of the environmental variables in relation to rainy and dry period samples; (b) Projection of the environmental variables in relation to micro-phytoplankton taxa. NO_3^- (Nitrate), Rain (Rainfall), SiO_2 (Silicate), Sal (Salinity), NH_4-N (Ammoniacal nitrogen), Chl-a (Chlorophyll a), PO_4^{3-} (Phosphate). Only the significant variables were represented ($p < 0.05$). Species codes: Eusp = *Euglena* sp.; Lynsp2 = *Lyngbya* sp2; Nasp1 = *Navicula* sp1; Nasp2 = *Navicula* sp2; and Pseusp = *Pseudanabaena* sp.

DISCUSSION

The structure of the phytoplankton populations is directly related to the physical and chemical characteristics of the water and associated with other environmental factors, acting together or separately to provide the establishment of populations adapted to these variations (Phlips et al., 2002).

The variables temperature and pH showed very similar averages among different environments and these values remained within the expected range for continental waters in the Amazon region (Berrêdo et al., 2008; Silva et al., 2011) as well as for cultivation, these values were considered ideal (Boyd, 1998; Alves and Mello, 2007). The salinity values in the environments were higher during the dry period, mainly in the pond. This fact also observed in the work realized by Araújo et al. (2012) in the dry season in the same pond. The high values are attributed to evaporation due to the higher solar incidence and low rainfall, characteristics of the region. However, in the first cultivation cycle the salinity was within the recommended range (< 29). On the other hand, in the second cycle, only the month of August presented an ideal range. The dissolved oxygen in both the estuary and the pond reached the highest values in the rainy season in January. According to Boyd (1990) values considered suitable for shrimp farming range from 4 to 6 mg L^{-1}, and a decrease in shrimp growth only occur in values below 2 mg L^{-1} with organisms mortality.

In the estuary, the highest values of turbidity were recorded in the rainy season, while in the pond, they occurred in the dry season, especially in September, with the highest values (69 to 104 NTU) at the collection points near the aerator (at the exit and middle of the pond, respectively). This pattern was related to the use of mechanical aeration in the pond to increase oxygenation in the culture water, contributing to an increase in suspended material and increasing turbidity values, in addition to the contribution of micro-phytoplankton organisms and resuspension of micro-phytobenthos, mainly evidenced by the occurrence of the benthic pennate diatoms with high abundances. McGraw et al. (2001) found that there was an increase in the concentrations of suspended material proportional to the increase in the aeration rates in ponds populated with the shrimp *L. vannamei*. According to Manzolli et al. (2011) the main factors that affect turbidity are the presence of suspended solids, organic and inorganic matter, microscopic organisms and algae.

During the study period, the concentrations of dissolved inorganic nutrients such as silicate, nitrite, nitrate and ammoniacal nitrogen had the highest values in the estuary; however, phosphate had the highest concentrations in the pond. For Glud et al. (2002), the increase of phosphorus in cultivation systems comes from the types of feed offered, which are rich in nutrients and tend to increase during the cultivation cycle, a fact that was also observed in the present work. However, in the estuary, in addition to the natural nutrient input (nitrate with higher value at the catchment point during the ebb tide in the rainy season), these concentrations may also have been favored by the discharge of the shrimp effluent, since nitrite, had a higher value at the point located near of shrimp pond effluent. However, high chlorophyll values were not observed at this point, despite the large amount of nutrients. Bauer et al. (2017) conclude that the environment quickly assimilated the effluents discharge, after few days of harvesting marine shrimp. Instead, in the shrimp pond, high values of nutrients were registered at the end of the two cultivation cycles, a large increase in the values of chlorophyll a, as observed in Figure 6.

The two researched environments were similar in terms of micro-phytoplankton composition, except for Cryptophytes group, which appeared only in the estuary, in January, represented by the species *Cryptomonas marssonii*, which is characteristic of fresh water environments. Diatoms contributed with the highest number of organisms in both environments, however, the estuary presented greater dominance of this group. Leão et al. (2008) assessed the micro-phytoplankton ecology of the Igarassu River estuary in the state of Pernambuco, Brazil, verifying that the phytoplankton community presented 210 species, distributed in Bacillariophyta, with more expressive representativity (146 spp.), followed by Cyanophyte, Chlorophyte, Euglenophyte e Dinophyte (Myzozoa), evidencing that diatoms dominate estuary environments, which corroborates the present results.

Melo et al. (2010), in a study in the state of Rio Grande do Norte, found that in different marine shrimp culture systems, phytoplankton groups that contributed to the abundance values were also diatoms, cyanophytes and dinoflagellates (Myzozoa). The same was found in Mexico, by Alonso-Rodríguez and Páez-Osuna (2003), who related that in marine shrimp farms, Bacillariophyceae, Cyanophyceae, Chlorophyceae and Euglenophyceae were the groups that dominated coastal waters and shrimp farming systems.

Regarding micro-phytoplankton abundance, the three groups that contributed the highest abundance ratios were Bacillariophyta, Cyanobacteria and Myzozoa, in addition to the Euglenophytes group, which contributed only to the pond during the dry period. The study showed that some groups alternated in dominance along the hydrological periods and cycles of production, as shown in (Figures 4 and 5), due to factors and ideal conditions for this to occur, such as the presence of certain nutrients. In a study in the Brazilian Northeast, Casé et al. (2008) verified that high densities of phytoplankton resulted in blooms of some species in shrimp ponds, relating to high levels of nutrients, such as Cyanophytes, which was generally responsible for these blooms, as reported by the author, especially the species *Pseudanabaena* cf. *limnetica*, which reached values above 600,000 cells mL^{-1}.

In the present work it was also verified that *Pseudanabaena* sp. contributed to the abundance values in the pond, mainly in the dry period. However, the numbers were around 500 org L^{-1}. For this same environment, high values of the diatom *Navicula* sp.2 (58,000 org L^{-1}) were found at the end of the two crop cycles, and lower values of silicate were verified in the last months, suggesting an increase in the abundance of this diatom through the consumption of this nutrient.

During the study, favorable conditions were observed for the development of some taxa, characterizing a specific dynamic for each environment. This was shown in the multivariate analysis, where it was possible to determine that some environmental factors were determinant in the composition of the micro-phytoplanktonic organisms in the estuary and in the pond. The RDA evidenced a seasonal variation in the estuary, and in this environment, the micro-phytoplankton community, especially the two main groups (diatoms and dinoflagellates), were strongly influenced by the rainfall factor, which favored a decrease of the salinity in the estuary. In turn, this environment was also influenced by the water discarded from the marine shrimp farm.

The main groups represented were Bacillariophyta, standing out as indicator species in the estuary: *Diploneis bombus*, *Coscinodiscus concinnus*, *Thalassiosira* sp.1, *Thalassionema frauenfeldii*, *Actinoptychus annulatus*, *Diploneis weissflogii*, *Rhopalodia musculus*, *Diploneis gruendleri*, *Skeletonema costatum*, *Dimeregramma* sp, *Tryblionella granulata*, typical of marine environments. The exception was the species *R. musculus*, characterized by being from estuary environments. These species dominated in terms of abundance throughout the study. The other main group was Myzozoa (dinoflagellate), with *Tripos furca*, which contributed for the abundance value only with this taxon, during the rainy period, in January and February.

In the estuary, according to the redundancy analysis, the species that correlated positively were *C. concinnus* and *T. furca,* typical of marine environments, but these reached the highest values of abundance in February to March, period of higher rainfall and consequently lower values of salinity, a parameter that is affected mainly by climate factors and is greatly influenced by the tides (Phlips et al., 2002). This pattern of occurrence indicated that these taxa support great variations of this variable. According to Macêdo et al. (2004), the seasonal variations of salinity are well evidenced in estuary regions, with higher records in periods of less rainfall.

The availability of phosphate and silicate favors the development of diatoms, especially silicate, used to form its frustules (Tréguer et al., 1995; Brzezinski et al., 2003). In the Brazilian tropical marine waters, diatoms usually comprise more than 80% of total phytoplankton (Eskinazi-Leça et al., 2000). In addition, the great development of diatoms is related to their rapid growth rate in turbulent and nutrient-rich regions and to the suspension of benthic forms and resistance spores in the water column (Karentz and Smayda, 1984) and have a high volume/surface ratio, making the organisms less dense, facilitating their buoyancy and permanence in the water column for longer (Margalef, 1978).

Regarding the shrimp pond, the indicator species for this environment were the pennate diatoms *Navicula* sp.1 and *Navicula* sp.2, the cyanobacteria *Lyngbya* sp.2 and *Pseudanabaena* sp. and the Euglenophyte *Euglena* sp. In october had the lowest concentration of silicate and the highest value in abundance of the Bacillariophyta group in this environment. This was shown by the RDA, which found inverse patterns for these parameters, evidencing that probably there was consumption of this silicate, stimulating the reproduction and consequently an increase of the abundance of these siliceous microalgae, especially the pennate diatom *Navicula* sp.2, which had the highest abundance value among the diatoms that contributed to this environment. However, these higher abundances of benthic pennate diatoms (like *Navicula* sp.) can be related to shrimp predation, because shrimp exhibited a selective consumption of centric diatoms, like demonstrated by Martínez-Córdova et al. (2015), or also due to its resuspension caused by of the mechanical aeration.

According to Barbieri and Ostrensky (2002), the presence of diatoms is always desirable in ponds, as this is the most important microalgae group for shrimps. The diatoms present a cell wall composed of silica, which is more vulnerable to digestive enzymes than those composed of cellulose, as is the case of other groups of microalgae. For Boyd (1989), diatoms have better growth than cyanobacteria; in many cultivated farms, a high diatom rate in the phytoplankton community is preferable than other groups.

Gómez-Aguirre and Martínez-Córdova (1998) mention that in shrimp farms the main contribution of phytoplankton in the sub-adult and adult stages is through the food chain and that the shrimp also consume phytoplankton when they are attached to the debris. For Alonso-Rodríguez and Páez-Osuna (2003), the occurrence of some species may be seasonal or may last for a long time, and sometimes blooms last a short period. However, a high abundance of one or some species may alter the shrimp growth due to depletion of oxygen at night. In this sense, pond management is essential in a productive shrimp farm, where adequate levels of nutrients will allow the biomass and the correct structure of phytoplankton.

The present study verified that the taxa *Euglena* sp. dominated the end of the second production cycle, in October and November. Melo et al. (2010) evidenced that *Euglena* sp. occurred in higher concentrations in intensive cultivation system of marine shrimp, due to the highest organic matter levels. It should be stressed that in the same shrimp pond and in the same study period, Palheta et al. (2012) classified the shrimp pond as a hypereutrophic environment, based on the Trophic State Index (TSI), resulting in high values of total phosphorus (above 1 mg L^{-1}), when compared with reference values (0.2 to 0.69 mg L^{-1}) for intensive shrimp farming system described by Alonso-Rodríguez and Páez-Osuna (2003). In these systems has very higher stocking density of marine shrimp, and consequently, a higher metabolic levels and organic matter production, with increase nitrogen compounds, mainly ammoniacal nitrogen. Indeed, in the present study were observed that ammoniacal nitrogen in the end of cycle, with mean 3.09 mg L^{-1}. Xavier et al. (1991) observed that ammonia values ranged from 0.77 to 1.58 mg L^{-1} in fish-breeding tank, favored *Euglena sanguinea* blooms. For Hodgkiss and Ho (1997), some studies find that high levels of nutrients in cultivation systems may be the most important regulating factor for phytoplankton communities.

CONCLUSION

In general, Bacillariophyta was the dominant group, in terms of richness and abundance, in the estuary and the marine shrimp pond. The indicator species for both environments were better represented by the diatoms, however, in the estuary, the great majority of indicator species were diatoms, and while in the shrimp pond the number of indicator diatoms was much lower, allowing that euglenophytes and cyanophytes are also indicator species.

The RDA ordination analysis allowed to identify strong seasonal influence on the estuarine environment, while in the shrimp pond, this one was reduced. In the shrimp pond the main drivers of micro-phytoplankton community structure were ammoniacal nitrogen, nitrite, nitrate and phosphate, mainly in relation to the feed accumulation. This nutrient accumulation allows the establishment of the more adapted organisms, such as *Euglena* sp., this group develops mainly under conditions of high nutrients availability, which favored the higher abundance values at the end of the second cultivation cycle in the dry season.

This study reinforces the importance of knowledge the micro-phytoplankton community in aquaculture shrimp ponds and in the environment where the effluents from this activity are discharged, to assess the community structure of these organisms, and its relationships to the environmental factors. In this sense, studies that follow the monitoring of water quality in this growing economic activity, which is greatly relevant for food production, should be encouraged.

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