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# Distribution of Nutrients and Changes in Phytoplankton Composition in a Tropical Mesotidal Estuary, Northeastern Brazil

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## Abstract

Abiotic parameters and phytoplankton were collected during 2010 and 2011 with the purpose of describing the phytoplankton distribution and the environmental characteristics. The diatoms were the most representative group in terms of species richness; in terms of density, the Cyanobacteria were more representative. Diversity and equitability were generally low in the estuary due to the dominance of *Microcystis aeruginosa*, an opportunistic and potentially toxic species of Cyanobacteria. The estuarine region is strongly impacted by high pollutant loads, especially nitrogen and phosphate compounds. Historical series of Apparent Oxygen Utilization (AOU) showed negative trends associated with changes in the estuarine system. The main biological components changed through 1999-2011 period. The dominance of the species changed from the Bacillariophyta in previous studies to the Cyanobacteria in our study. The species *Microcystis aeruginosa*, through its high density, dominance and frequency of occurrence, was the key species in the area.

## Keywords

Coastal Environment, Nutrients, Biodiversity, Phytoplankton, Tropical Estuary

## 1. Introduction

Estuaries are dynamic systems characterized by gradients of salinity, turbidity, temperature, nutrient concentration and organic matter [1]. Approximately 60% of the large cities distributed around the Earth are located near estuarine regions, making these environments of great importance for the planet. These regions are

the main suppliers of nutrients to the coastal regions, since they receive and concentrate the material originating from their drainage basin and can receive significant contributions from anthropic action [2]. The spatial connection with this region is the continental shelf, which acts as a final recipient of water and materials from the continent that are transported by the discharge of rivers and estuaries. The meeting of low-salinity continental waters with coastal waters defines regions where there are high gradients of properties, the so-called front zones [3].

Currently, the coastal zones, due to population and urban growth, are the areas most impacted by anthropic action and consequently are the areas most subject to algal blooms due to eutrophication processes resulting from domestic effluents and increases in organic matter [4]. The ability to respond quickly to spatial and temporal fluctuations of environmental conditions makes the phytoplankton community a good bioindicator of changes in environments arising from natural causes or as a result of human actions [5].

The contamination of an aquatic ecosystem is manifested in phytoplankton populations by the development of two inverse and simultaneous phenomena: on the one hand, the emergence and proliferation of selective species and on the other hand, the disappearance of part or all of the original population of the environment. To determine the biological quality of water, the populations can be used as a reference frame in which the presence or absence of organisms is fundamental or to verify the existence of organisms that are indicative or characteristic of some type of contamination [6]. In estuarine ecosystems, planktonic populations are known to be influenced by spatio-temporal variations in physico-chemical parameters and tidal dynamics [7].

Phytoplankton is the main primary producer of the coastal environments, being responsible for the beginning of the flow of matter and energy of the trophic network of these environments; contributing to their fertilization; and directly supporting the herbivores and indirectly the animals of the higher trophic levels, including economically important species [8].

Works about phytoplankton in estuarine ecosystems in the northeastern region of Brazil, and especially in the state of Pernambuco, are well known in [9]-[22]. Work in coastal areas is still insufficient ([23] [24] [25] [26]) and, specifically for the coastal areas adjacent to the estuary of the Jaboatão River, are non-existent.

The Jaboatão River Basin, located mostly in the Metropolitan Region of Recife (RMR), presents problems common to Brazilian urban water basins: the degradation of natural resources through inadequate land occupation and use; pollution caused by the release of domestic wastes and industrial effluents; and high population density. This estuary represents one of the most vulnerable areas in the RMR to the degradation provoked by the increase of urban pressure and real estate (1100 inhabitants/km$^{-2}$) [19]. These factors constitute a high degree of risk to the continuity of the existence of this environment. The present work aimed to identify the phytoplankton community, correlating its density and environ-

mental characteristics (nutrients, DO) in the estuary of the Jaboatão River as well as in its adjacent platform in the area under the influence of the estuarine plume.

## 2. Materials and Methods

### 2.1. Study Area

The Jaboatão watershed, located in northeastern Brazilin Pernambuco State (8˚00'S - 8˚14'S and 34˚50'W - 35˚15'W), is 413 km$^2$ in area and 75 km in length. The river crosses the RMR, and the mouth is on the Atlantic Ocean (**Figure 1**).

The climate is typically tropical, hot and humid. The air temperature is 26˚C ± 2.8˚C, and the mean annual precipitation and evaporation are approximately 1.5 and 1.2 m·yr$^{-1}$, respectively [27]. The rainfall regime is subdivided into two well-defined periods: the dry season (September-February), when precipitation is exceeded by evaporation; and the rainy season (March-August), when rainfall dominates evaporation (**Figure 2**). The estuary extends for approximately 13 km$^2$, with an average depth of 2.6 m [19] [28] [29] (**Figure 1**). The drainage basin includes areas originally covered by the Atlantic Rain Forest and is presently occupied by sugar cane and high-density populated areas (1100 inhabitants km$^{-2}$) [19] [30]. Despite the deforestation of the margins and the large volume of industrial and domestic effluents it receives, the estuary itself is surrounded by relatively well-preserved and highly productive mangrove forests. Organic matter

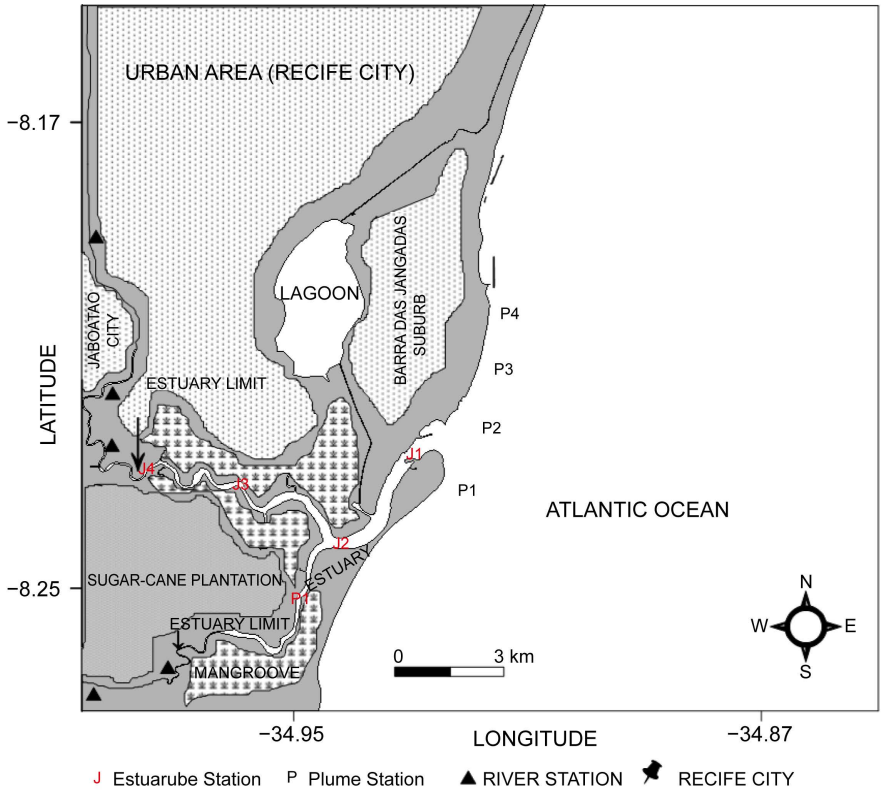


**Figure 1.** Sampling stations located in the estuary and plume of the Jaboatão River (northeastern Brazil).

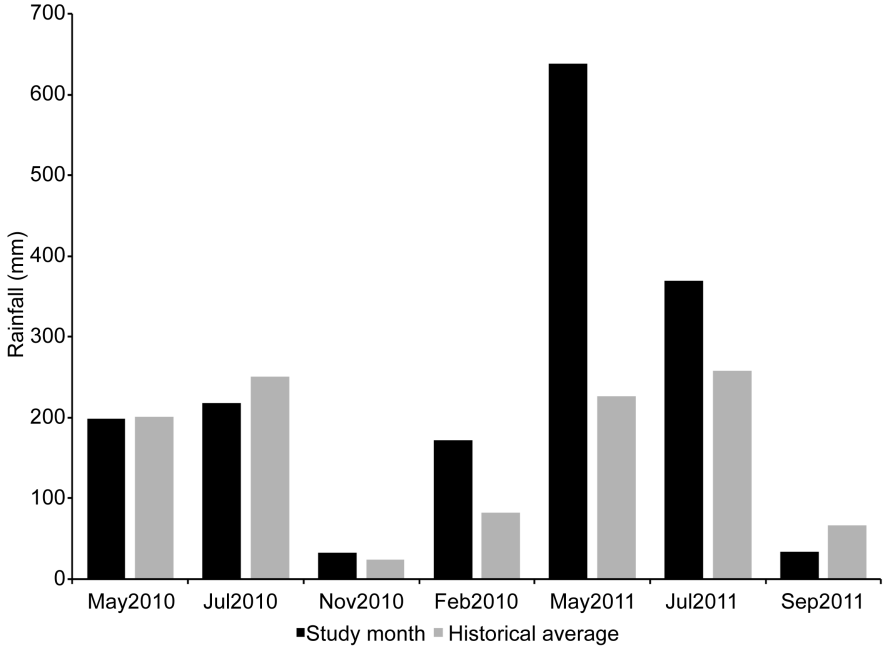


**Figure 2.** Climatological series of precipitation (1981-2011) for the study area (source: INMET).

pollution by the sugar-cane agro industry increases substantially during the harvest and milling season, which is from September to February. Environmental state agency reported a high BOD of 69.6 $mg \cdot l^{-1}$ in the harvest periods [29]. The polluting organic load sources are represented mainly by domestic sewage in the Jaboatão River (14.46 t BOD $d^{-1}$) [31]. Algal blooms are now more frequent during the year and consist of several species of Cyanophyceae, *Oscillatoria* sp. and *Euglena* sp. (Euglenophyta), suggesting some degree of permanent impact on the environment [28]. The river runoff is strongly controlled by rainfall (**Figure 2**), with an average discharge of 2 - 10 $m^3 \cdot s^{-1}$ (annual average) [29] [32] [33]. The tidal regime is semidiurnal, with mean amplitude of 1.3 m (neap tides) and 1.8 m (spring tides) [27]. The estuary is well mixed, being classified as type 1 with an absence of vertical stratification [27] [29].

## 2.2. Sampling and Analysis

The samples were collected in a longitudinal profile in the Jaboatão River estuary, covering the marine area (estuarine plume) through four stations in the estuary and four in the plume (**Figure 1**). Samples were taken during the dry (November 2010, February 2011 and September 2011) and the rainy seasons (May 2010, July 2010, May 2011 and July 2011), comprising a total of seven campaigns. For this study, we divided the estuarine region into 8 segments (every 5 units of salinity) based on the longitudinal saline gradient classification proposed by [34]. The estuarine limit with the plume region was calculated based on the average saline in the plume samplings.

The temperature and salinity (conductivity) were measured using a CTD (Sea-Bird Electronics SBE911plus; Sea-Bird Scientific Inc.®). The salinity was also

verified against the chlorinity, which was determined using $AgNO_3$ titration [35]. The local depth was determined by digital echo sounder, mark LCD-resolution: 0.1 m) and depth of visual disappearance of the Secchi disk (water transparency).

Water samples were collected with Van Dorn bottles for further analysis of dissolved oxygen (DO) and nutrients. The pH was measured on the NBS scaleon board after sample collection using a pH/ion analyser 350 and a Ross combination electrode (Orion®). The precision and the accuracy of the pH measurements were ±0.005 units and 0.1%, respectively. DO was analysed by the Winkler method [36], with a precision of ±1.3 µM. The relative oxygen saturation (%) in the water was calculated using the following equation for temperatures between 0˚C and 40˚C and salinities between 0 and 40:

$$\% = \frac{DO}{DO^*} \times 100 \quad (1)$$

where DO is the oxygen concentration in the sample and DO* is the oxygen solubility in the water at the same temperature and salinity using the UNESCO tables [37].

Apparent oxygen utilization (AOU) was calculated according to [38].

The dissolved inorganic nutrients, ammonia + nitrite + nitrate (DIN), phosphate (DIP), were analysed according to [36] after filtration of the samples using Whatman® GF/C 0.47-mm glass fibre filters. The precision was ±0.02 µmol for nitrate, ±0.02 µmol for nitrite, ±0.02 µmol for ammonia, and 0.01 µmol for phosphate. The accuracy was ±2% for DIP, ±3% for nitrate and nitrite, and ±5% for ammonia.

The samples for the phytoplankton study were collected with Niskin oceanographic bottles and later fixed with Lugol solution. The analyses were performed according to the sedimentation method of Utermöhl [39] [40] [41], and counts were performed under a ZEISS Axiovert inverted microscope. Additional information on the phytoplankton identification methodology can be seen in the supplementary material.

## 2.3. Meteorological Data

The rainfall data were obtained through the website of the Pernambuco State Agency for Water and Climate (APAC) and the National Institute of Meteorology (INMET).

## 2.4. Statistical Analyses

The similarity between the biological samples was evaluated based on the Bray-Curtis coefficient, using the data based on the relative abundance, transformed into the fourth root, with amalgamation method by the group mean. The similarity between the abiotic samples was also evaluated with the relative abundance data transformed into the fourth root, by the mean of the group but based on the Euclidean distance. The Principal Components analysis (PCA) was based on the

hydrological parameters and the phytoplankton cell density, applying the Pearson's moment-to-product correlation coefficient, with the self-value of the main components and the auto-vector being extracted. The trend of the time series was obtained through the Mann-Kendall test and Linear Regression. For the two tests, the programs PRIMER 6® (Plymouth Routines in Multivariate Ecological Research) and XLSt at 2010® were used, respectively.

## 3. Results

### 3.1. Climatology and Physical Factors

The total monthly rainfall ranged from 32.4 mm in November 2010 to 638.6 mm in May 2011. The typical dry season months had the lowest indexes, according to the historical values of the study region (**Figure 3(a)**). The study period did not show significant differences with the values recorded historically for the same months (*t*-test; *p*: 0.38; *α*: 0.05).

The salinity in the estuary ranged from 0.04 to 27.54, from freshwater to polyhaline, presenting the highest values mainly in the dry period (**Figure 3(b)**). In the dry period, the variability was lower in relation to the rainy season, showing significant differences between the two periods (*t*-test, *p*: 0.05, *α*: 0.05).

In the estuary, the thermal amplitude and its average value were 2.5˚C and 28.0˚C ± 1.0˚C, respectively, while in the plume region, the amplitude was 3˚C and the average 28.5˚C ± 0.8˚C. The studied area showed significant differences (*t*-test, *p*: 0.04; *α*: 0.05), which can also be observed in **Figure 3(c)** and **Figure 3(d)** through the salt gradient. Seasonal analyses also showed statistically significant differences between the dry and the rainy season (*t*-test; *p*: 0.0001; *α*: 0.05).

As shown in **Figure 3(c)** and **Figure 3(d)**, the estuarine gradient also showed thermal variations throughout the year, which are characteristic in tropical coastal areas. The mean amplitude between the climatic periods was 1˚C (**Figure 3(c)**: 28.8˚C; **Figure 3(d)**: 27.8˚C).

The local depth in the estuary presented an average value of 3.0 ± 2.0 m, while in the plume region, the mean depth was 11.5 ± 2.0 m. The water transparency was also lower in the estuary (0.53 ± 0.2 m) than in the plume region, where the Secchi disc recorded an average value of 2.23 ± 1.4 m (**Figure 3(e)** and **Figure 3(f)**). In the estuary, the highest values of transparency occurred in the dry period (September to February), a season of lower fluvial contribution and lower values of rainfall. The values observed between the dry and rainy periods did not show significant differences (*t*-test; *p*: 0.37; *α*: 0.05).

### 3.2. Chemical Factors: pH and Oxygen

The pH values recorded in the estuarine area showed a range of 6.41 to 8.36, while in the plume region, the values ranged from 7.76 to 8.92. Statistical analysis showed significant differences between the two regions (*t*-test; *p*: 0.0001; *α*: 0.05). The pH values were always lower in regions near the river and did not

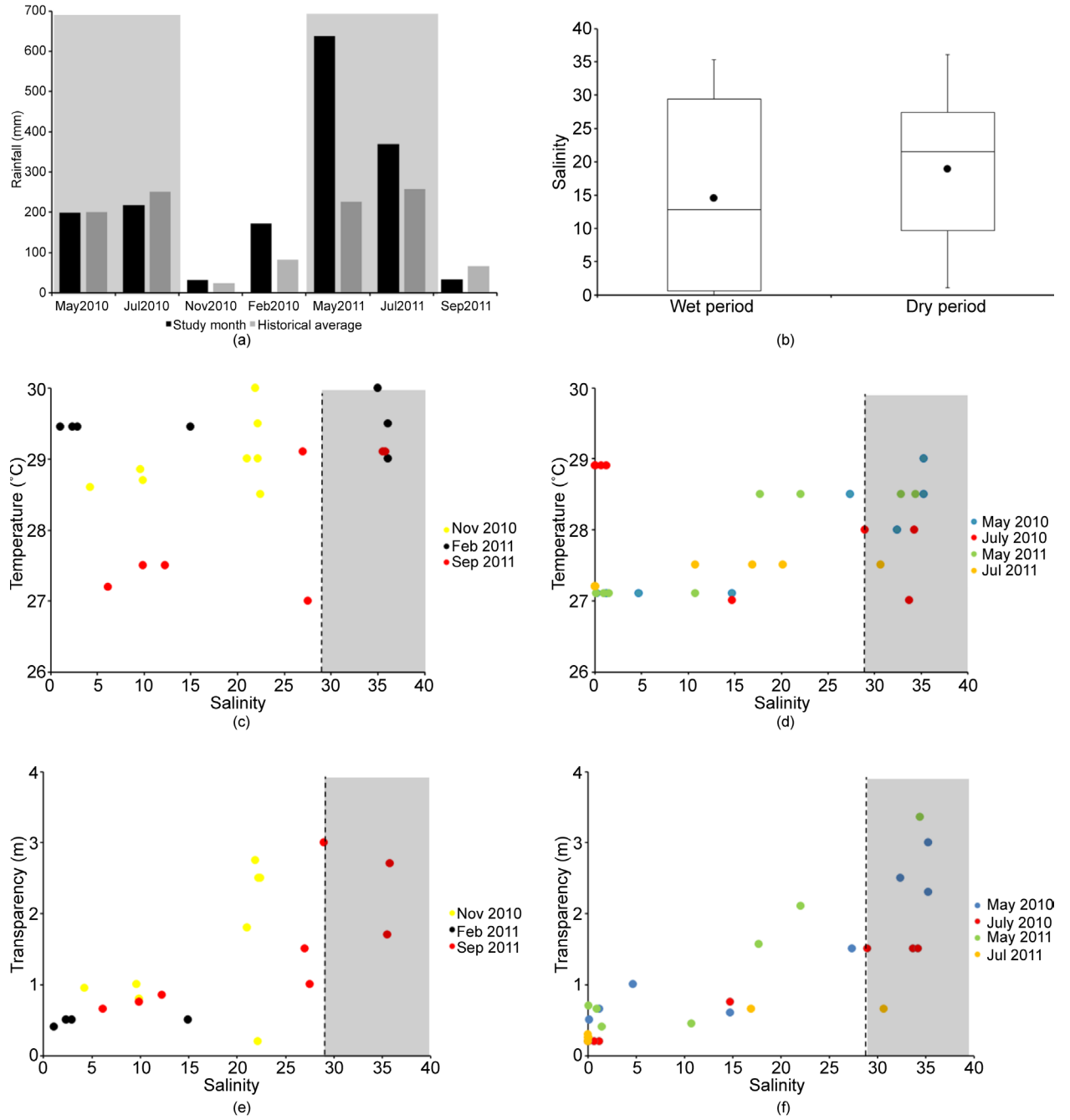


**Figure 3.** Rainfall (study period and historical average). Grey region indicate wet period (a); salinity (b); surface temperature (˚C) of the water through the salt gradient (c)-(d) and transparency (m) in the estuary and plume of the Jaboatão River (e)-(f). The dotted line indicates the mean salinity value for the plume region. In **Figure 3(b)**, black circles indicate the mean and the horizontal line indicates the median.

show significant differences (*t*-test; *p*: 0.82; *α*: 0.05) between the dry and the wet period (**Figure 4(a)** and **Figure 4(d)**).

DO in the estuary presented low levels, with a minimum of anoxia and a maximum of 4.1 ml·L$^{-1}$; the mean value in the estuarine area was 1.6 ml·L$^{-1}$, while in the plume region, the mean value was 4.4 ml·L$^{-1}$. DO concentrations showed significant differences between the estuarine area and the plume region

(*t*-test; *p*: 0.0001; *α*: 0.05). During climatic periods in the study region, 50% of the samples showed concentrations below the limit indicated by CONAMA (3.5 ml·L$^{-1}$; law decree 357, 2005) [42] (**Figure 4(c)**, **Figure 4(d)**; red line). The oxygen saturation (%) followed the same pattern as DO, with a minimum of 0%, a maximum of 85.65%, and an average of 30%. In the plume region, the oxygen saturation showed an average of 95.45%, with a minimum of 78.46% and a maximum of 110.5%.

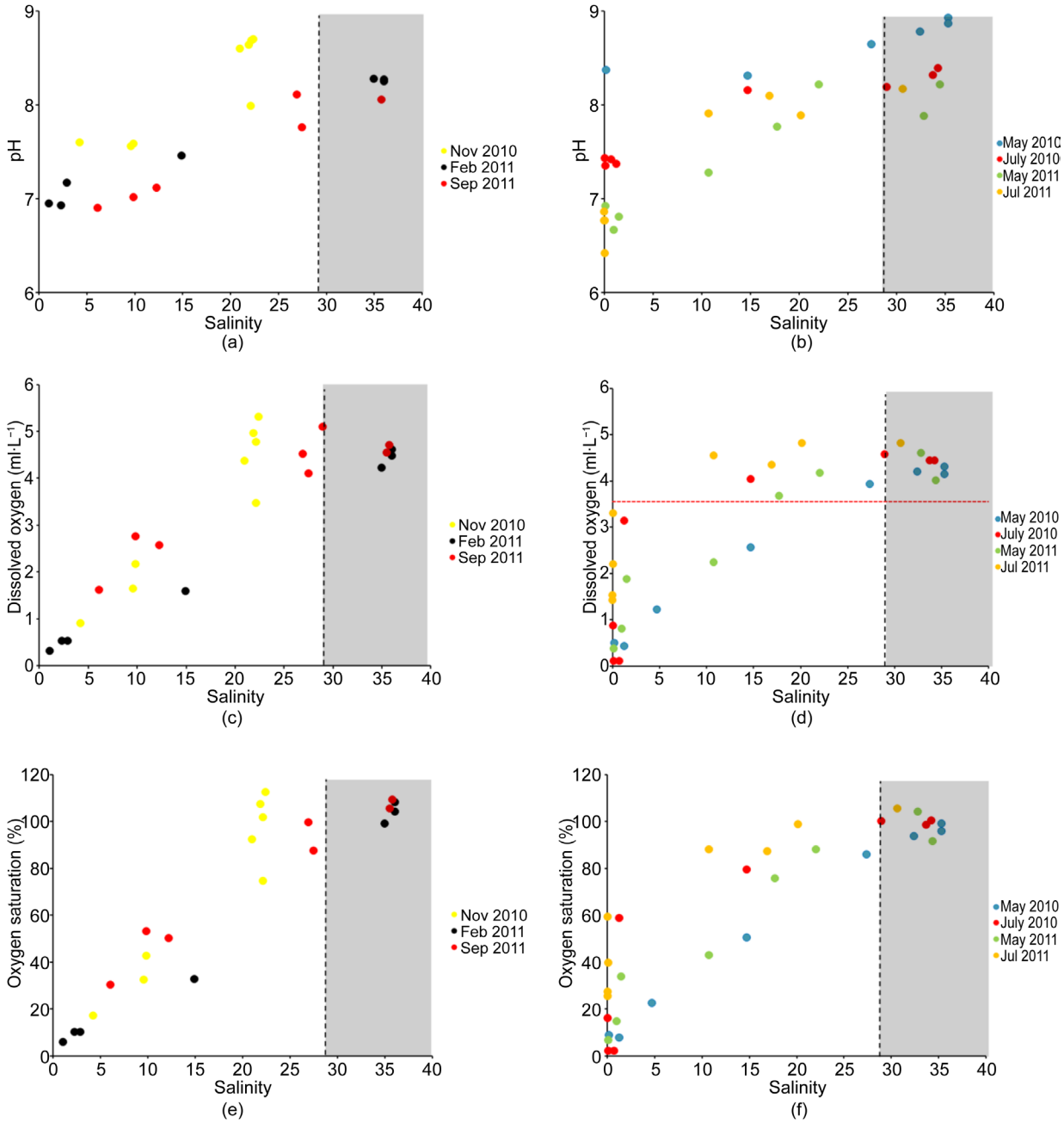


**Figure 4.** Surface pH of the water through the salt gradient (a)-(b), dissolved oxygen (c)-(d) and oxygen saturation rate (e)-(f) in the estuary and plume of the Jaboatão River. The red line indicates the limit established by the CONAMA 357 legislation. The dotted line indicates the mean value of salinity in the pen region. Grey area corresponds to plume region. Average and standard deviation for the dry and wet period are also shown.

### 3.3. Chemical Factors: Nutrients

Ammonia showed a mean of 2.70 μmol·L$^{-1}$, with a minimum of 0.05 μmol·L$^{-1}$, presenting a peak of 14.29 μmol·L$^{-1}$ in May of 2010 and the lowest concentrations in November. Spatial variations showed significant differences between estuarine and plume regions (*t*-test; *p*: 0.0001; *α*: 0.05) (**Figure 5(a)**, **Figure 5(b)**).

Nitrate concentrations varied between 0.58 μmol·L$^{-1}$ and 32.0 μmol·L$^{-1}$, with higher values in February and September (dry period), but ~90% of the observations occurred below 9 μmol·L$^{-1}$. The spatial gradient showed significant differences between the two regions (*t*-test; *p*: 0.0001; *α*: 0.05) (**Figure 5(c)**, **Figure 5(d)**).

The nitrite varied from 0.05 μmol·L$^{-1}$ to 3.82 μmol·L$^{-1}$, with the highest concentrations in November, and showed an increasing downstream-upstream gradient (**Figure 5(e)**, **Figure 5(f)**). Spatial variations were observed between the estuarine and plume regions (*t*-test; *p*: 0.0001; *α*: 0.05).

The phosphate showed a minimum concentration of 0.27 μmol·L$^{-1}$ and a maximum of 9.46 μmol·L$^{-1}$, with an increasing downstream-upstream gradient. In the plume region, the concentrations were <1 μmol·L$^{-1}$ (**Figure 5(g)**, **Figure 5(h)**). The spatial variations showed significant differences between the estuary and the plume (*t*-test; *p*: 0.0001; *α*: 0.05).

Silicate showed higher concentrations principally in the estuarine region. The higher concentrations were registered in May (wet period), whereas the lower values were observed in February. The estuary and plume showed significant differences (*t*-test; *p*: 0.0001; *α*: 0.05).

### 3.4. Biological Factors: Phytoplankton and Apparent Oxygen Utilization (AOU)

The phytoplankton community in the studied areas consisted of 80 taxa, represented by seven phyla: Bacillariophyta (55%), Miozoa (21%), Cyanobacteria (11%), Euglenophyta (5%), Chlorophyta (4%), Charophyta (3%) and Ochrophyta (1%). The phyla Bacillariophyta and Miozoa characterized the planktonic flora in 76% of the floristic diversity. The diatoms were more representative, presenting 4 classes, 22 orders and 26 families. Dinoflagellates had the second-highest representation, with 1 class, 5 orders and 8 families, with emphasis on the genera Protoperidinium and Prorocentrum, with five taxa each. Cyanobacteria were represented by 1 class, 3 orders and 5 families (**Table S1**; **Supplementary material**).

The Bacillariophyta and Cyanobacteria groups showed different spatial distributions. Bacillariophyta did not show significant differences between the estuarine and plume regions (*t*-test; *p*: 0.24; *α*: 0.05), while Cyanobacteria showed significant differences between these regions (*t*-test; *p*: 0.0001; *α*: 0.05). Higher values were observed principally in the estuarine region (**Figures 6(a)-(d)**).

The calculated AOU showed positive values in both climatic periods. The wet period showed higher values that the dry period (average: +2.7 and +2.1 ml·L$^{-1}$,

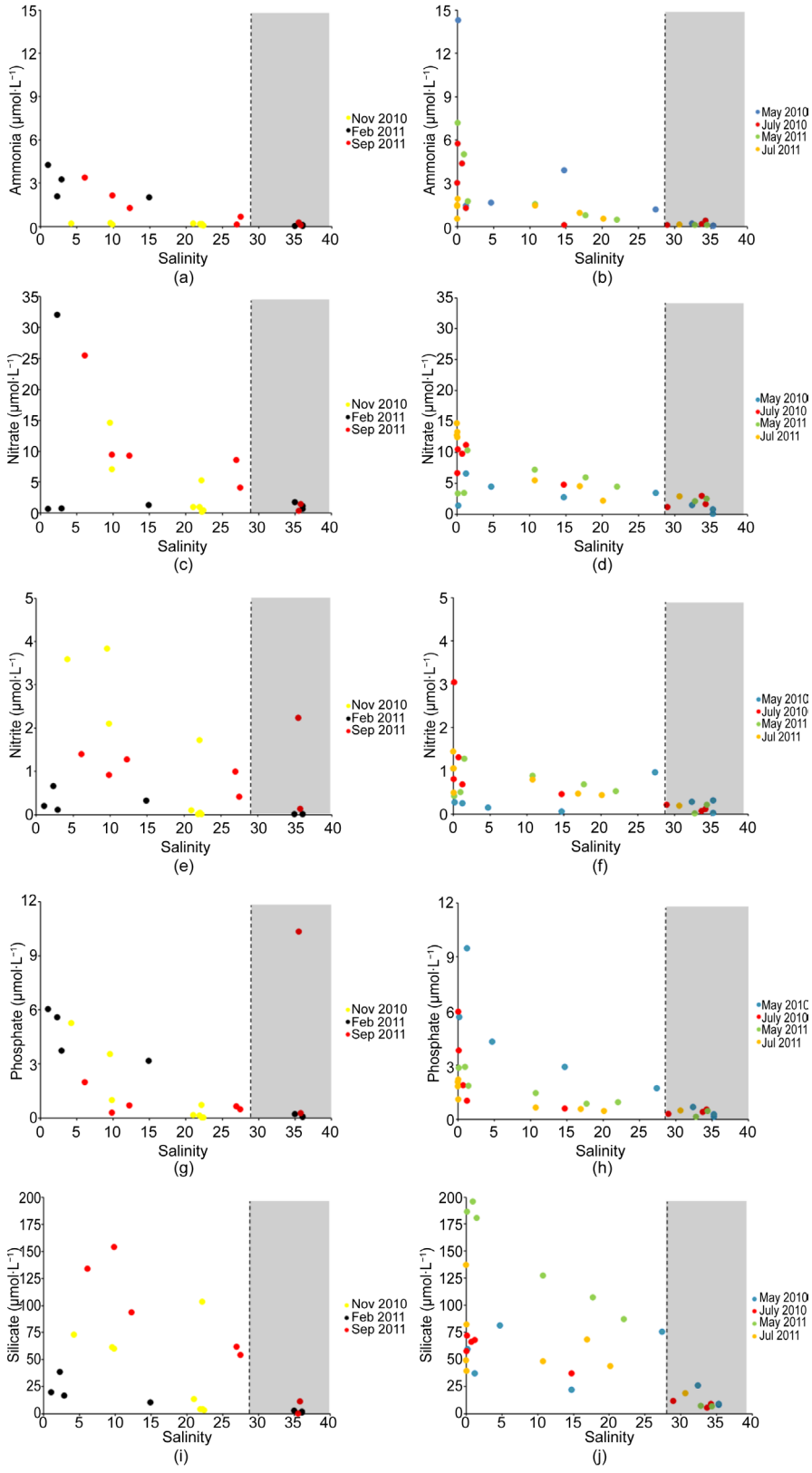


**Figure 5.** Concentrations of the dissolved nutrients in the two studied areas: (a)-(b) = ammonia, (c)-(d) = nitrate, (e)-(f) = nitrite, (g)-(h) = phosphate, (i)-(j) = silicate, during dry and rainy periods. Grey area corresponds to plume region. Average and standard deviation for the dry and wet period are also shown.

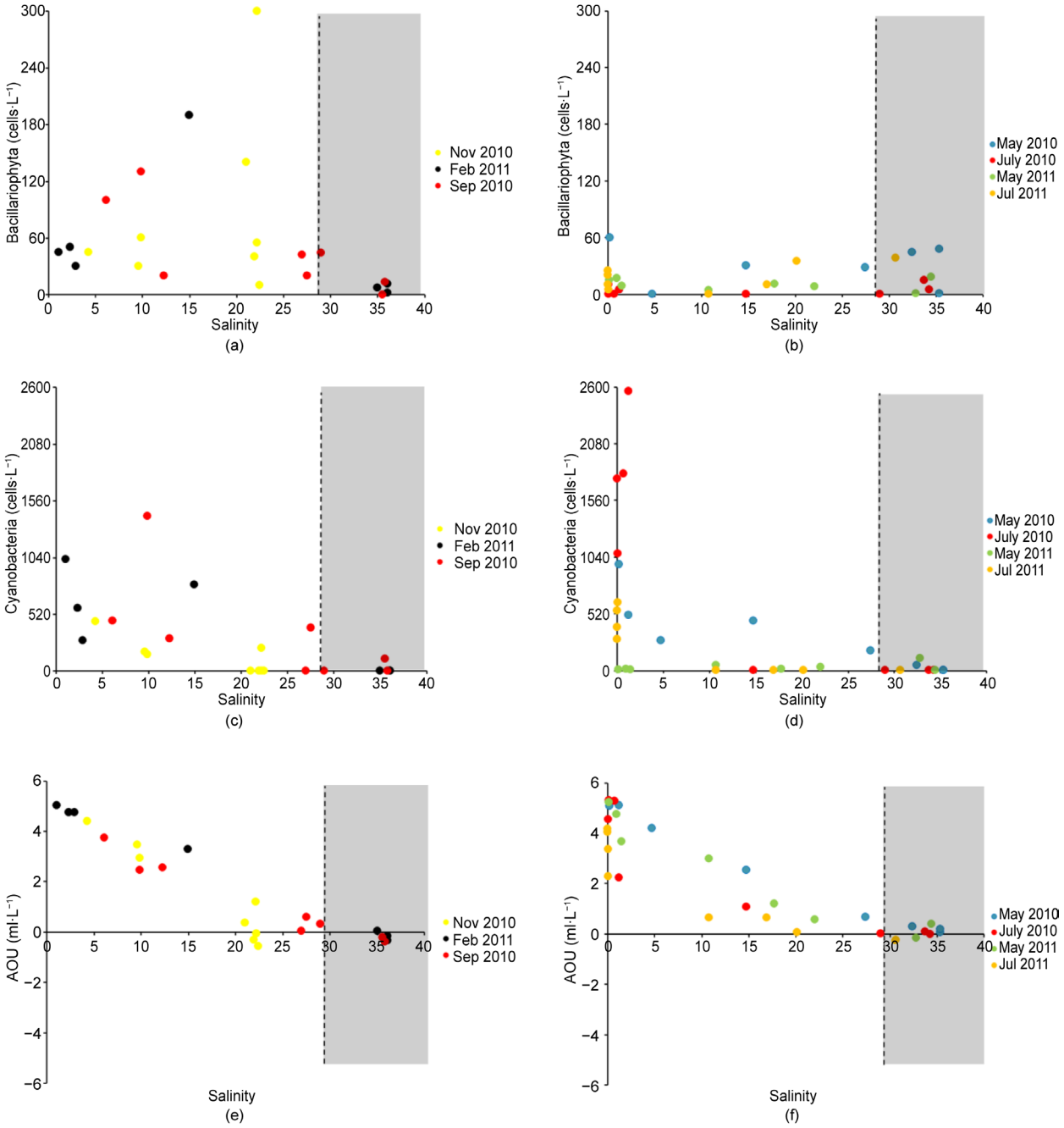


**Figure 6.** Bacillariophyta through the salt gradient (a)-(b), Cyanobacteria (c)-(d) and AOU (e)-(f) in the estuary and plume of the Jaboatão River. The dotted line indicates the mean value of salinity in the pen region. Grey area corresponds to plume region. Average and standard deviation for the dry and wet period are also shown.

respectively). Additionally, 2 samples were negatives and were recorded in the dry period, corresponding to <9% of the total samples of the period (Figure 6(e), Figure 6(f)).

In the estuary, 38 taxa were identified, and 15 occurred only in that location, with the majority represented by Bacillariophyta (47%), Cyanobacteria (18%) and Miozoa (11%). In the plume region, 65 taxa were recorded, and 39 occurred exclusively in this region, following the general pattern of a majority of Bacil-

lariophyta (62%), Miozoa (23%) and Cyanobacteria (8%) (Figure 7).

The specific richness showed little difference in the estuary, ranging from seven taxa in July of 2011 to 15 in November of 2010. In the plume region, the difference was quite pronounced, with a minimum of seven taxa in July of 2010 and a maximum of 35 taxa in May of 2010. However, in both sites, no seasonal pattern was evident (*t*-test; *p*: 0.0001; *α*: 0.05). Only two species were considered to be dominant in the estuary: *Microcystis aeruginosa* (Kützing) Kützing and *Cyclotella meneghiniana* Kützing. *Microcystis aeruginosa* occurred in all seasons and months in the estuary, being dominant in 23 of 28 analysed samples and abundant in the others. *Cyclotella meneghiniana* was dominant only in station 4 (upstream) in May of 2011. Most taxa were considered rare except *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Rajuand *Oscillatoria tenuis* C. Agardh ex Gomont, which were abundant.

The plume region was dominated by *Microcystis aeruginosa, Planktothrix agardhii* (Gomont) Anagnostidis & Komárek, *Lepocinclis acus* (O. F. Müller) Marin & Melkonian, *Protoperidinium bispinum* (Schiller) Balech, *Coscinodiscus centralis* Ehrenberg, *Cyclotella meneghiniana* and *Paralia sulcata* (Ehrenberg) Cleve. *Climacosphenia moniligera* Ehrenberg, *Diploneis bombus* (Ehrenberg) Ehrenberg, *Grammatophora marina* (Lyngbye) Kützing, *Licmophora abbreviata* C. Agardh, *Melosira dubia* C. G. Kützing, *Navicula humerosa* Brébisson ex W.

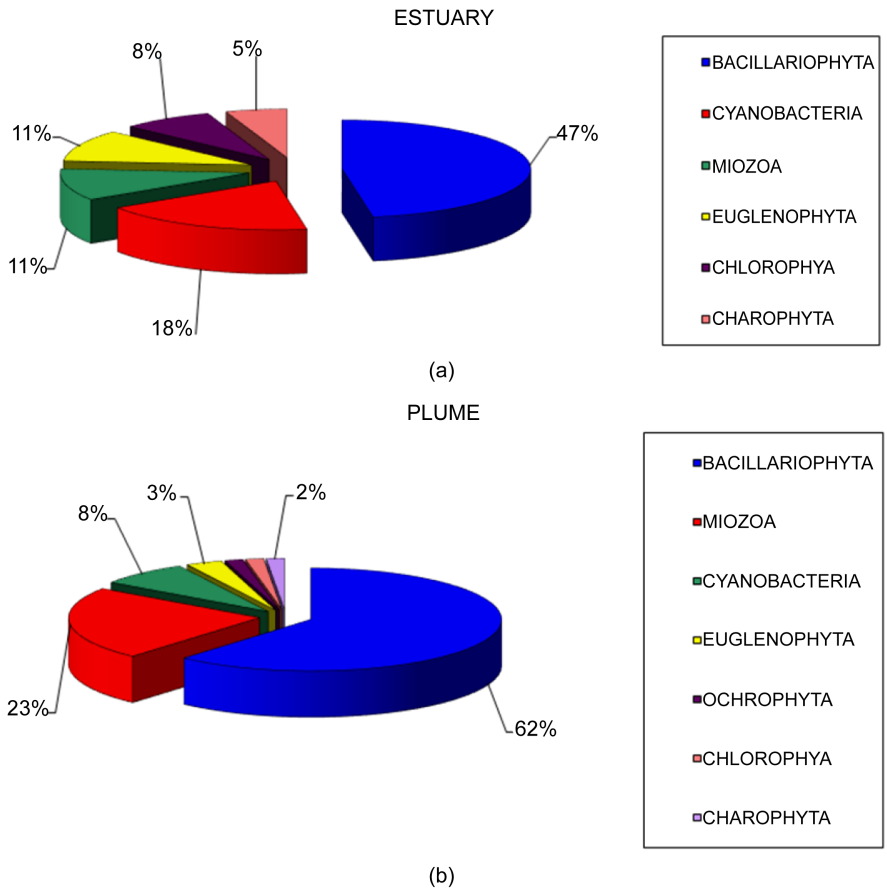


Figure 7. Percentage of occurrence of phyla in each area studied.

Smith, *Navicula* sp., *Pleuro/Gyrosigma* sp. and *Thalassiosira leptopus* (Grunow ex Van Heurck) Hasle & G. Fryxell were abundant (Figure 8).

We identified 38 taxa in the estuary, of which 63% were sporadic, 29% were uncommon, 3% were considered frequent and 5% were very frequent. The dominant *Microcystis aeruginosa* and *Cyclotella meneghiniana* were the very frequent species on the site.

In the plume, no taxon was considered very frequent; 68% were sporadic, 26% were uncommon and 6% were frequent. Among the frequent were *Microcystis aeruginosa, Coscinodiscus centralis, Navicula* sp. and *Paralia sulcata.*

In the estuary, the values of specific diversity were between the minimum of 0 bits·Cell$^{-1}$ and the maximum of 1.99 bits·Cell$^{-1}$, with 60% of the samples presenting low diversity and 40%, very low, while the equitability ranged from 0 to 0.99, with 90% of the samples showing high equitability. In the plume region, the diversity was higher, ranging from very low to medium, with 0 bits·Cell$^{-1}$ and 2.98 bits·Cell$^{-1}$, respectively. The equitability presented minimum and maximum values of 0 and 0.99, with 32% of the samples presenting medium diversity and 82% being highly equitable (Figure 9).

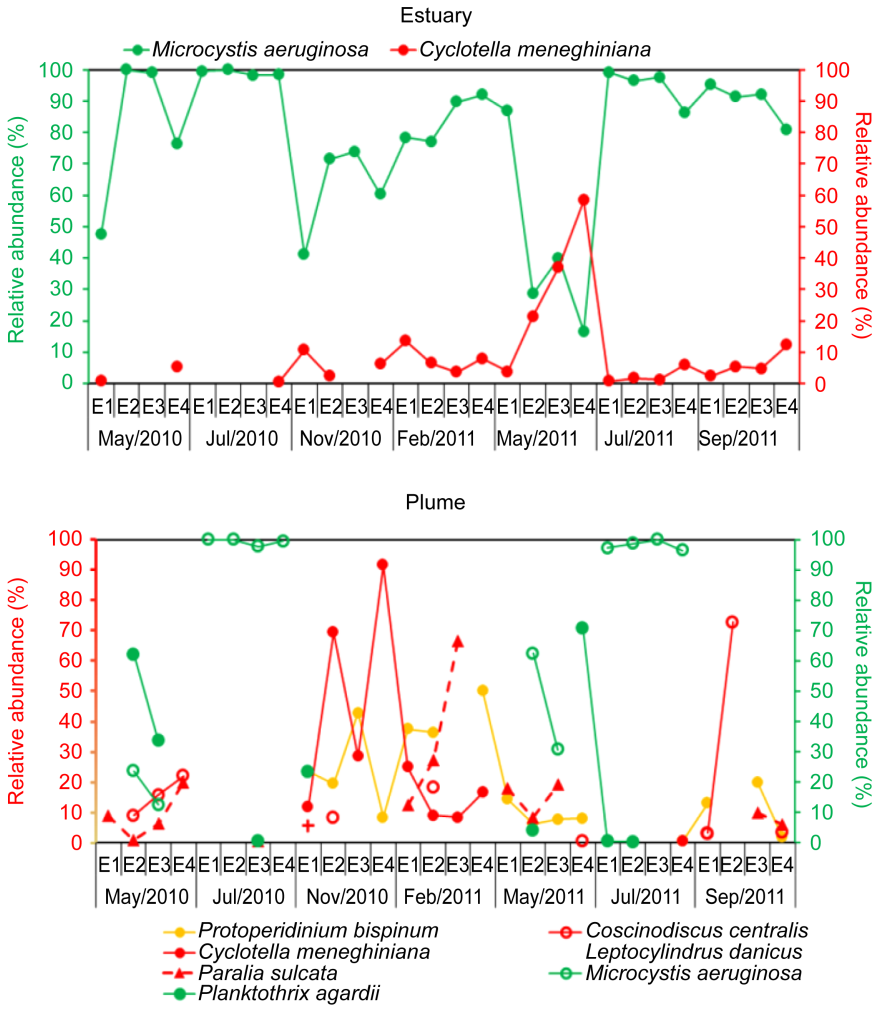


Figure 8. Relative abundance (%) of the most representative species in the studied areas.

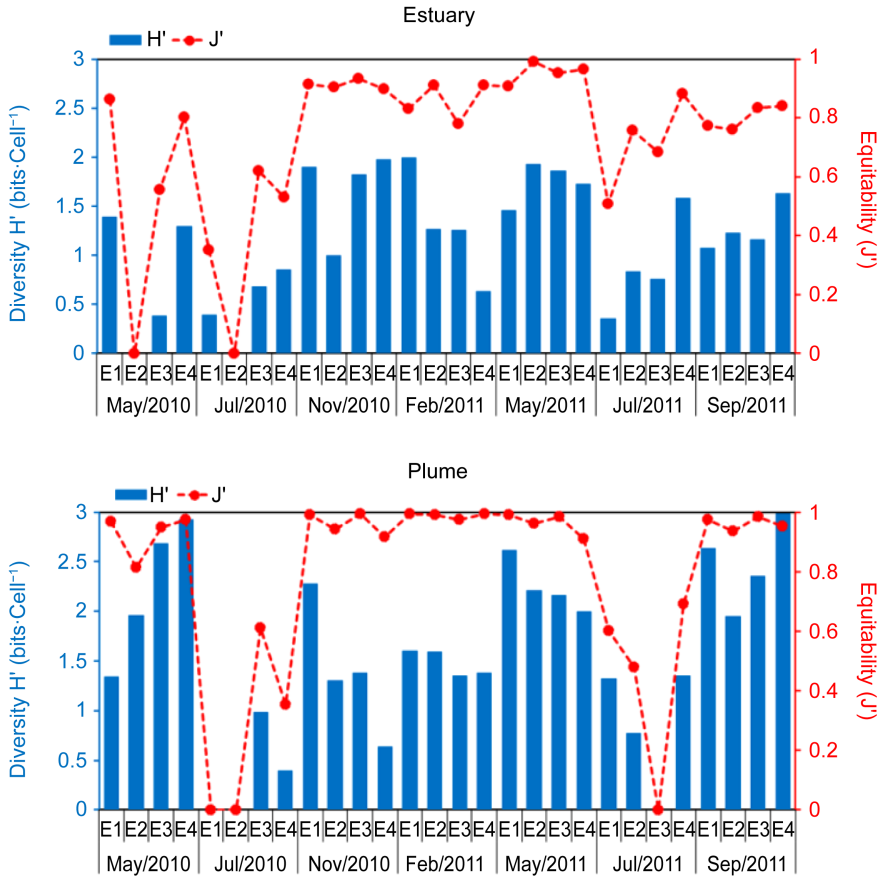


**Figure 9.** Specific diversity and equitability of the two studied areas.

In cell density, the most representative group in the estuary was the cyanobacteria, with 17548 cell·$L^{-1}$ × $10^3$, constituting 92.6%, followed by diatoms, with 1230 cell·$L^{-1}$ × $10^3$, constituting 6.5%. The rest of the groups found (Euglenaceae, Dinoflagellates, Chlorophyceae and Carophyceae) totalled 0.9% in 167 cell·$L^{-1}$ × $10^3$. In the plumeas well as in the estuary, cyanobacteria constituted 97% of the cells counted, with 13879 cell·$L^{-1}$ × $10^3$, followed by diatoms, with 740 cell·$L^{-1}$ × $10^3$, representing 2%; The remaining groups (Euglenophytes, Dinoflagellates, Chlorophyceae, Carophytes and Octylites) were 0.9%, adding 290 cell·$L^{-1}$ × $10^3$ (**Figure S1; Supplementary material**).

In the estuary, the maximum density was 7255 cell·$L^{-1}$ × $10^3$ in July of 2010 and a minimum of 126 cell·$L^{-1}$ × $10^3$ in May of 2011. In the plume, the density remained below 500 cell·$L^{-1}$ × $10^3$ except in the months of July of 2010 and July of 2011, when it reached more than 7000 cell·$L^{-1}$ × $10^3$. The minimum recorded in the plume region was 37 cell·$L^{-1}$ × $10^3$ and the maximum7618 cell·$L^{-1}$ × $10^3$ (**Figure S2; Supplementary material**).

The cell density in each season showed that the estuary had the highest densities in all stations, but the difference was not significant (*t*-test; *p*: 0.07; *α*: 0.05), with densities always above 3000 cell·$L^{-1}$ × $10^3$ (**Figure S3; Supplementary material**). There was also a decreasing downstream-upstream gradient. The species *Microcystis aeruginosa* was responsible for the predominance of cyanobacteria,

both in the estuary and in the plume, dominating in 82% of the estuary and presenting a minimum density of 4 cell·$L^{-1}$ × $10^3$ and a maximum of 2560 cell·$L^{-1}$ × $10^3$. During May of 2011, the density was lower than in the other months studied, with a density of less than 10 cell·$L^{-1}$ × $10^3$.

In the plume, *M. aeruginosa* presented a minimum density of 8 cell·$L^{-1}$ × $10^3$ and a maximum of 2215 cell·$L^{-1}$ × $10^3$. This species showed seasonality in the area, occurring only in the months of the rainy season (May and Julyin both years) at well above its density in July of 2011. It was not possible to show a spatial difference (**Figure S4; Supplementary material**).

## 4. Discussion

### 4.1. Temperature and Salinity

Studies of phytoplankton and its responses to environmental variables represent important tools for understanding and diagnosing the natural and/or anthropogenic impacts of aquatic ecosystems at the level of primary producers. Natural factors such as rainfall showed a temporal distribution that agreed with historical patterns, but in July 2010 (typical rainy month), the rainfall intensity was lower than the historical average. Thus, no seasonal variations were identified for some physical and chemical factors. Within these factors, the temperature did not show significant differences spatially and temporally. This is a typical pattern for estuaries in northeastern Brazil [17] [18]. This thermal stability is typical of tropical estuarine waters, which can range from 24˚C to 30˚C, and is related to the salinity gradient that can increase the temperature by up to 4˚C, especially in the less rainy season [43]. Temperature directly influences phytoplankton, promoting an increase in reproduction and growth, especially in temperate regions. This effect is observed less noticeably in tropical waters [44] [45]. The water temperature exerts a direct influence on the physiological processes of the organisms [46], whereas salinity is an important hydrological parameter in the spatial distribution of organisms, presenting gradients that make this factor preponderant in the distribution of aquatic organisms and constitute an ecological barrier for certain species [46].

In the present study, the salinity presented seasonal variation, and rainfall did not seem to influence the values. The plume showed the highest salinity values, as expected, due to the strong marine influence. Spatial variations in the plume, from meso to euhaline, were less evident than in the estuary, where station 1 had the highest salinities andstation 4 (upstream) the lowest, ranging from freshwater to polyhaline.

### 4.2. pH, DO and Saturation

In the plume, the pH remained always alkaline, evidencing the influence of the marine waters in the area. Another author [15] observed similar values in the same area of study. The pH in the estuary ranged from slightly acidic to alkaline, with the lowest values, generally slightly <7, coinciding with the months of more intense precipitation, when they were justified by the influence of freshwater,

which is more acidic than the marine water. In tropical estuaries, the pH is generally in the range of alkalinity [17] [18] [47].

Fluctuations of the acid, neutral and alkaline pH values in the studied environment are related to the degradation processes of organic matter and photosynthetic activities [48] [49], which cause an increase in inorganic dissolved nutrients, mainly ammonia and phosphate, that indicate the presence of domestic effluents rich in organic matter [19]. The environment showed a great variation of pH due to the low DO values (average: 1.57 $ml \cdot L^{-1}$). According to [50] and [51], the decrease in pH is related to the increase of dissolved $CO_2$ concentration as a consequence of the increase in organic matter degradation and reduction of photosynthetic activities, which are the main consumers of this gas and directly influence the carbonate system and consequently the pH. The high organic load also reduced light penetration, reducing the photic layer and limiting the density and diversity of phytoplankton despite the high levels of dissolved inorganic nutrients. These factors may alter the concentration and saturation rate of dissolved oxygen, which varied significantly in comparisons of the estuary and the plume, with the latter being much more oxygenated. However, neither site showed any evidence of seasonal variation. Spatially, the estuary became evident, with the oxygen content decreasing as it entered the more internal seasons. In estuarine areas, this variation in dissolved oxygen content is common [15]. Similar values in the Recife basin were recorded by [21] (2.72 to 6.24 $ml \cdot L^{-1}$), and [26] recorded values between 1.73 and 7.78 $ml \cdot L^{-1}$ at the plume of the Capibaribe River (close to our study area).

DO is one of the most important elements for maintaining the environmental quality of aquatic ecosystems as well as being an essential element for the oxidation, decomposition and cycling of organic matter circulating in ecosystems. In 1978 [52], classified the estuarine ecosystems of northeastern Brazil in terms of water quality based on the oxygen saturation rate, creating categories for super-saturated (>100%), saturated (75% to 100%), and low saturation (below 75%), semi-polluted (25% to 50%), and polluted (<25%). Except for one point where it was considered saturated, the estuary of the Jaboatão River ranged from polluted to low-saturation zones, evidencing the low quality of its waters. The plume remained saturated to super-saturated. The higher DO contents are related to the more alkaline values of pH in the plume.

### 4.3. Nutrients

DO and pH are altered by temporal and spatial variations of nutrient inputs. The main nutrients are regulated and defined by CONAMA Resolution 357 [42]. According to the Resolution, the waters of the estuary and the plume of the Jaboatão River are considered brackish and salt I class, respectively, with the maximum limits 0.40 $mg \cdot L^{-1}$ (0.30 $\mu mol \cdot L^{-1}$) for nitrite, 0.40 $mg \cdot L^{-1}$ (1.72 $\mu mol \cdot L^{-1}$) for ammonia and 0.07 $mg \cdot L^{-1}$ (0.30 $\mu mol \cdot L^{-1}$) for nitrate. In the estuary, ammonia (average: 2.7 $\mu mol \cdot L^{-1}$), nitrite (average: 1.07 $\mu mol \cdot L^{-1}$) and nitrate (average: 8.86 $\mu mol \cdot L^{-1}$) presented mean values above those specified by the

CONAMA resolution. In the plume, ammonia (average: 0.26 μmol·L$^{-1}$) and nitrite (average: 0.27 μmol·L$^{-1}$) presented mean values below the maximum allowed, but nitrate (average: 2.4 μmol·L$^{-1}$) remained above the maximum allowed value.

The high values of nitrogen compounds and phosphate (average: 2.97 μmol·L$^{-1}$) in the Jaboatão estuary evidenced the high degree and the strong influence of the anthropic action in that environment. The same influence is not observed in the plume, where the nitrogenous compounds as well as phosphate (average: 0.39 μmol·L$^{-1}$) remained within an acceptable limit.

The concentrations of each of the nitrogen compounds are strongly influenced by the dynamic cycle of DO in the medium [19] [53]. In estuarine areas, nutrients generally originate from rivers, usually in an inverse relationship between the concentration of these elements and the salinity [54]. This process was evidenced in the estuary and plume of the Jaboatão River, which presented an inverse relationship between salinity and nutrient concentration. In the estuary, where salinity was lower, nutrient concentrations were higher. In the plume, the reverse process occurred. According to [26], in a study of the Capibaribe River plume, recorded higher values than those found in this study, except for silicate, and a defined seasonal variation, presenting higher concentrations in the rainy season.

The dissolved nutrients presented a spatial variation better evidenced in the estuary than in the plume, but it was not possible to establish a seasonal pattern in both. In estuarine plumes, the contribution is lower because the production of the estuary absorbs a good part of the nutrients, which is minimized in urban areas, where the nutrient supply is high.

### 4.4. Phytoplankton Distribution and AOU

The combined effect of the main physico-chemical factors of the pelagic environment, such as luminous intensity, nutrient concentration, temperature and salinity, determine the geographic distribution, specific composition and variability of the phytoplankton production rates [55]. Knowledge of the taxonomic composition of phytoplankton is fundamental for the study of the spatial and temporal dynamics of the community and for the characterization of functional groups [55].

In the taxonomic composition of the studied environments, the greater representativeness of the diatoms is highlighted, considering the specific richness. In the estuary, after the diatoms, it is possible to show the representativeness of the cyanobacteria, a typical group of freshwater organisms. In the plume, the second-most represented group was the dinoflagellates, a typical marine species. This relationship also occurs in other estuaries, mainly in northeastern Brazil, where the predominance of diatomshas been established [15] [18] [24] [56] [57] [58]. According to [26] also highlight the greater representativeness of diatoms in the plume of the Capibaribe River, followed by the dinoflagellates, which is the same pattern found for the plume of the Jaboatão River.

Diatoms predominate in coastal and shelf regions, gradually decreasing to-

wards the open ocean, where the contribution of dinoflagellates increases significantly [59], whereas Cyanobacteria can reach high densities in tropical marine waters, possibly constituting the group mainly responsible for primary productivity in cases of a shortage of larger phytoplankton components [60].

The cell density of the Cyanobacteria *Microcystis aeruginosa* predominated in practically all the estuarine stations, with blooms in July of 2010 and September of 2011. In the plume region, however, its occurrence was punctual, with blooms in July of 2010 and 2011.

Previous research on the estuarine ecosystem of the Jaboatão River by [13] [15] [28] considers the diatoms *Bellerochea mallleus*, *Coscinodiscus centralis* and *Cyclotella meneghiniana* to be dominant and very frequent as the key species for that ecosystem. In the present study, *Microcystis aeruginosa* is considered the key species in the studied area due to its high density, dominance and frequency of occurrence at all estuarine stations.

Previous data analysed by [14] referred to a specific diversity ranging from medium to high. A recent study by [13] and [16] revealed a marked reduction of this diversity, and in the present study, the diversity reached a value of zero, a fact that is supported by the flowering of *Microcystis aeruginosa*, demonstrating a highly compromised ecosystem.

Cyanobacteria are especially abundant in waters with high temperatures that arerich in nutrientsor in polluted waters with little oxygen where they can form a dense scum that can colour the water, forming blooms. Blooms of *M. aeruginosa* produce toxins and have been implicated in the mass mortality of aquatic animals and the destabilization of food webs [61] [62].

Additionally, the positive values shown by calculated AOU indicate higher rates of respiration (production-respiration), where DO is consumed and $CO_2$ released in the water column.

According to [63] the registration of a significant density of phytoplanktonic organisms against low species richness suggests that a habitat has received a polluting load, allowing a favourable environment for organisms that are tolerant of this condition. This was evidenced both in the plume and in the estuary, where the occurrence of other species was limited in the stations dominated by this Cyanobacteria group.

### 4.5. Principal Component Analysis (PCA)

We statistically analysed the physical, chemical, biological and rain data through a Principal Component Analysis (PCA) to obtain spatial and temporal correlations and correlations between the parameters analysed in this study (**Figure 10(a)**, **Figure 10(b)**). According to the PCA, the first 4factors explained 72.70% of the environmental variations that were correlated with the species considered to be very frequent (**Table S2; Supplementary material**). In **Figure 10(a)**, component 1 explained 40.0% of the environmental variations analysed and showed a direct correlation between water transparency (Secchi), salinity, pH and DO. These parameters had an inverse correlation with nutrients (ammonia— $NH_4^+$,

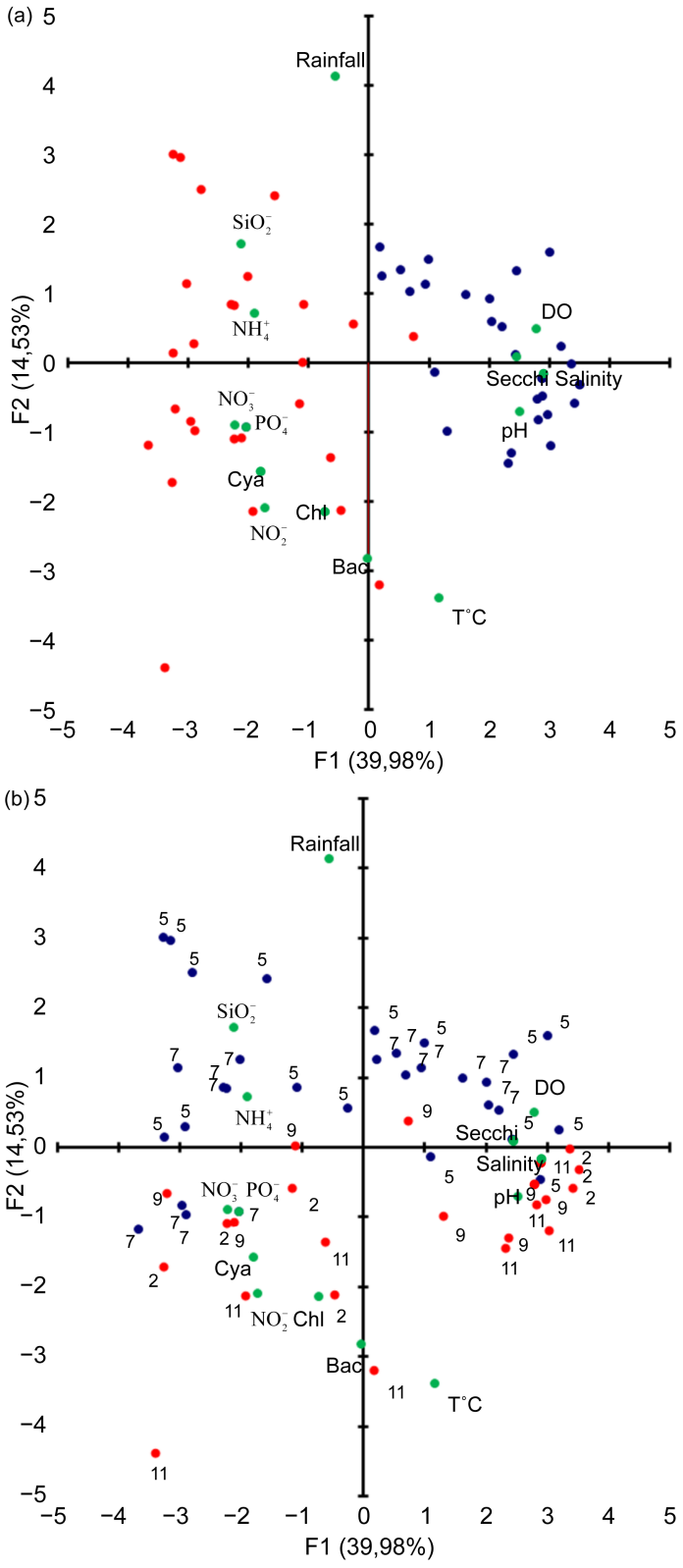


**Figure 10.** Spatial PCA of the chemical, physical and biological parameters. Green circles indicate the parameters; Red circles indicate the estuarine region; Blue circles indicate the region of the plume (a). Temporal PCA of chemical, physical and biological parameters. Green circles indicate the parameters; Red circles indicate the dry period; Blue circles indicate the rainy period (b).

nitrate— $NO_3^-$, nitrite— $NO_2^-$, silicate— $SiO_2^-$) and the Cyanophytes group.

Component 2 explained 14.53% of the variability and showed an inverse correlation between temperature (T˚C) and rainfall. Biological parameters were located in component 4 and showed an inverse correlation between Bacillariophyta (Bac) and Cyanobacteria (Cya).

We identified a clear spatial division between the estuary and the plume. A group of parameters was associated with the estuarine region (nutrients, Cyanobacteria, Bacillariophyta and Chlorophyta), while another group was associated with the plume region (DO, Secchi, salinity and pH).

A second PCA was performed to include temporal variability (months) in the biplot. We observed that salinity, pH and water transparency (Secchi) are associated with dry-season months (February and November), whereas nutrients are associated with the winter months. The biological groups of component 4 appear to be associated with the dry period (November). Temperature and rainfall did not show a defined pattern within the biplot (**Figure 10(b)**). Additionally, we included a time series of 12 years (1999 to 2011) based on similar studies carried out in this aquatic system. We used AOU calculated from the values of salinity, oxygen and temperature to compare the estuarine system with the fluvial system.

### 4.6. Trend of AOU and Change in the Phytoplankton Species

Data obtained from the CPRH database (1999 to 2011) [63] [64], were used to calculate AOU in the Jaboatão River. We used data from the fluvial station with greater geographic and data coverage. The dataset in the estuarine region was limited to 28 data (months). The results of this exercise can be seen in **Figure 11(a)**, **Figure 11(b)**. **Figure 11(a)** shows a positive AOU series with the exception of 2 months (negative values). The mean value in the river series was higher than that in the estuarine series. The calculated trend of AOU for the river was negative, whereas the estuarine series had a slight positive trend. In the estuarine region, the main biological components changed through 1999-2011 period. Bacillariophyta had been the dominant species in previous studies, while in our study between 2010 and 2011, the Cyanophyta group was dominant.

## 5. Conclusion

We can conclude that the Jaboatão estuary is strongly impacted because of the lower values of salinity and dissolved oxygen and the high concentration of nutrients, mainly the nitrogenous and phosphate components indicative of high pollutant loads. Additionally, the cyanobacteria *Microcystis aeruginosa*, an opportunistic and potentially toxic species, constitutes the dominant species responsible for the low diversity of species in recent years, as demonstrated by the observations in the temporal series. The plume, with well-oxygenated waters, high salinity and low concentration of nutrients, indicates the influence of the marine flow in the area, allowing the dominance of other species and contributing to the increase of local diversity.

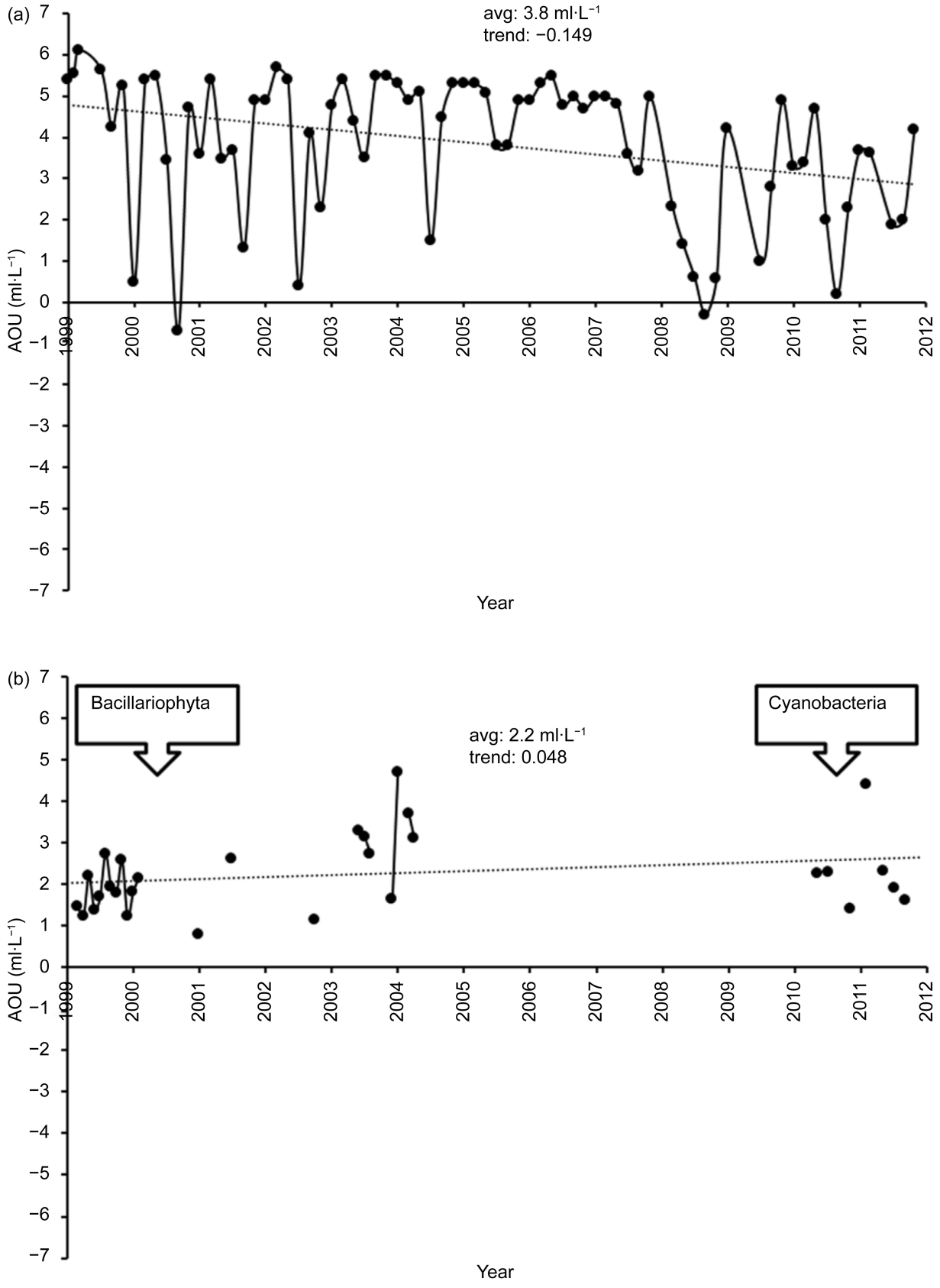


**Figure 11.** Time series (1999-2011) of the AOU calculated in the fluvial region (a) and in the estuarine region (b). The dotted line indicates the trend of the series (b).

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## Supplementary Material

### Phytoplankton

The samples for the phytoplankton study were collected with Ninskin oceanographic bottles and later fixed with lugol solution. The analyzes were performed according to the sedimentation method of Utermöhl [1] [2] [3], and counts performed under ZEISS Axiovert inverted microscope. Samples were homogenized and placed in 10 mL chambers, stained with Bengal Rose and placed to sediment for 24 hours. The counts were performed with 400X, using the technique of transection. The standardized counting of two transects was used in each chamber. In cases of sample poverty, counts covered the entire chamber. The values of phytoplankton density were expressed in cells per liter (cells·$L^{-1}$).

Taxonomic identification was done by consulting specialized literature. For the framing of taxa and checking of all scientific names, the international database was used Algaebase [4]; http://www.algaebase.org.).

The relative abundance of the taxa was determined by considering the categories: dominant, species whose numerical occurrences were greater than 50% of the total number of individuals in the sample; Abundant, species whose occurrence exceeds the average number of individuals in the sample; Rare, species whose occurrences are less than the average number of individuals in the sample. In order to calculate the frequency of occurrence, the number of samples, in which each taxon occurred, and the total number of samples were analyzed, using the formula described by [5], considering: Very common (≥70%), common (70%|−40%), infrequent (40%|−10%) or sporadic (<10%).

For the calculations of specific diversity, the Shannon index [6] was used; the values obtained were classified by Valentin classification [7], being considered high when the results were ≥ 3.0 bits.$cel^{-1}$; average, with results between 2 and 3 bits·$cel^{-1}$; low, between 1 and 2 bits.$cel^{-1}$; and very low, with result ≤1 bit·$cel^{-1}$; The equitability ($J = H'/\log S$) was calculated according to [8]. In order to calculate this index, the Plymouth Routines in Multivariate Ecological Research, PRIMER 6.0® statistical software program was used.

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**Table S1.** List of taxa identified in the estuary and plume of the Jaboatão River, Pernambuco, Brazil.

| TAXON | REGION | |
|---|---|---|
| | **Estuary** | **Plume** |
| CYANOBACTERIA | | |
| CYANOPHYCEAE | | |
| NOSTOCALES | | |
| APHANIZOMENACEAE | | |
| *Dolichospermum spiroides* (Klebhan) Wacklin, L. Hoffmann & Komárek | X | |
| *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & SubbaRaju | X | |
| CHROOCOCCALES | | |
| CHROOCOCCACEAE | | |
| *Chroococcus* sp. Nägeli | X | |
| MICROSCYSTACEAE | | |
| *Microcystis aeruginosa* (Kützing) Kützing | X | X |
| OSCILLATORIALES | | |
| OSCILLATORIACEAE | | |
| *Oscillatoria limosa* C. Agardhex Gomont | | X |
| *Oscilllatoria tenuis* C. Agardhex Gomont | X | |
| *Phormidium* sp. Kützing ex Gomont | X | X |
| MICROCOLEACEAE | | |
| *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek | X | X |
| *Trichodesmium thiebautii* Gomont ex Gomont | | X |
| EUGLENOPHYTA | | |
| EUGLENOPHYCEAE | | |
| EUGLENALES | | |
| PHACACEAE | | |
| *Lepocinclis acus* (O. F. Müller) Marin & Melkonian | X | X |
| *Phacus* sp. Dujardin | X | |
| EUGLENACEAE | | |

**Continued**

| | | |
|---|---|---|
| *Euglena* sp. Ehrenberg | X | X |
| *Trachelomonas* sp. Ehrenberg | X | |
| MIOZOA | | |
| DINOPHYCEAE | | |
| DINOPHYSIALES | | |
| DINOPHYSIACEAE | | |
| *Dinophysis dubia* Balech | | X |
| GONYAULACALES | | |
| GONYAULACACEAE | | |
| *Gonyaulax polygramma* Stein | | X |
| CERATIACEAE | | |
| *Tripos furca* (Ehrenberg) F. Gómez | | X |
| *Tripos teres* (Kofoid) F. Gómez | | X |
| PYROPHACACEAE | | |
| *Pyrophacus horologicum* Stein | | X |
| GYMNODINIALES | | |
| GYMNODINIACEAE | | |
| *Gymnodinium* sp. F. Stein | | X |
| PERIDINIALES | | |
| OXYTOXACEAE | | |
| *Oxytoxum scolopax* Stein | | X |
| PROTOPERIDINIACEAE | | |
| *Protoperidinium unipes* (Balech) Balech | | X |
| *Protoperidinium bispinum* (Schiller) Balech | X | X |
| *Protoperidinium cassum* (Balech) Balech | | X |
| *Protoperidinium divergens* (Ehrenberg) Balech | | X |
| Protoperidinium sp. R. S. Bergh | | X |
| PROROCENTRALES | | |
| PROROCENTRACEAE | | |
| *Prorocentrum compressum* | | X |
| *Prorocentrum gracile* Schütt | | X |
| *Prorocentrum lima* (Ehrenberg) F. Stein | X | |
| *Prorocentrum micans* Ehrenberg | X | X |
| *Prorocentrum sigmoides* Böhm | | X |
| *Prorocentrum* sp. | X | |
| BACILLARIOPHYTA | | |
| BACILLARIOPHYCEAE | | |
| THALASSIOPHYSALES | | |

**Continued**

| | | |
|---|---|---|
| CATENULACEAE | | |
| *Amphora angusta* Gregory | X | X |
| *Amphora arenaria* Donkin | X | X |
| AULACOSEIRALES | | |
| AULACOSEIRACEAE | | |
| *Aulacoseira granulata* (Ehrenberg) Simonsen | | X |
| SURIRELLALES | | |
| SURIRELLACEAE | | |
| *Campylodiscus clypeus* (Ehrenberg) Ehrenbergex Kützing | | X |
| *Campylodiscus fastuosus* Ehrenberg | | X |
| *Surirella febigerii* F. W. Lewis | X | |
| COCCONEIDALES | | |
| COCCONEIDACEAE | | |
| *Campyloneis grevillei* (W. Smith) Grunow & Eulenstein | | X |
| *Cocconeis scutellum* Ehrenberg | | X |
| RHOPALODIALES | | |
| ENTOMONEIDACEAE | | |
| *Entomoneis alata* (Ehrenberg) Ehrenberg | X | X |
| EUNOTIALES | | |
| EUNOTIACEAE | | |
| *Eunotia didyma* Grunow | | X |
| NAVICULALES | | |
| DIPLONEIDACEAE | | |
| *Diploneis bombus* (Ehrenberg) Ehrenberg | X | X |
| NAVICULACEAE | | |
| *Gyrosigma balticum* (Ehrenberg) Rabenhorst | X | X |
| *Navicula* sp. Bory | X | X |
| *Pleuro/Gyrosigma* sp. | | X |
| LYRELLALES | | |
| LYRELLACEAE | | |
| *Lyrella lyra* (Ehrenberg) Karajeva | X | X |
| *Navicula humerosa* Brébisson ex W. Smith | | X |
| BACILLARIALES | | |
| BACILLARIACEAE | | |
| *Bacillaria paxillifera* (O. F. Müller) T. Marsson | X | |
| *Nitzschia lorenziana* Grunow | | X |
| *Nitzschia sigma* (Kützing) W. Smith | X | X |

**Continued**

| | | |
|---|---|---|
| COSCINODISCOPHYCEAE | | |
| COSCINODISCALES | | |
| AULACODISCACEAE | | |
| *Aulacodiscus* sp. Ehrenberg | X | |
| COSCINODISCACEAE | | |
| *Coscinodiscus centralis* Ehrenberg | X | X |
| *Coscinodiscus oculus-iridis* (Ehrenberg) Ehrenberg | X | X |
| RHIZOSOLENIALES | | |
| RHIZOSOLENIACEAE | | |
| *Guinardia delicatula* (Cleve) Hasle | | X |
| MELOSIRALES | | |
| MELOSIRACEAE | | |
| *Melosira dubia* C. G. Kützing | X | X |
| *Melosira moniliformis* (O. F. Müller) C. Agardh | | X |
| *Melosira nummuloides* C. Agardh | | X |
| PARALIACEAE | | |
| *Paralia sulcata* (Ehrenberg) Cleve | X | X |
| MEDIOPHYCEAE | | |
| LITHODEAMIALES | | |
| BELLEROCHEACEAE | | |
| *Bellerochea horologicalisStosch* | X | X |
| EUPODISCALES | | |
| EUPODISCACEAE | | |
| *Odontella turgida* (Ehrenberg) Kützing | | X |
| *Triceratium* sp. Ehrenberg | X | X |
| TOXARIALES | | |
| CLIMACOSPHENIACEAE | | |
| *Climacosphaenia moniligera* Ehrenberg | | X |
| THALASSIOSIRALES | | |
| STHEPHANODISCACEAE | | |
| *Cyclotella styllorum* Brightwell | | X |
| *Cyclotella meneghiniana* Kützing | X | X |
| THALASSIOSIRACEAE | | |
| *Thalassiosira leptopus* (Grunowex Van Heurck) Hasle & G. Fryxell | X | X |
| *Thalassiosira subtilis* (Ostenfeld) Gran | X | |
| BIDDULPHIALES | | |
| BIDDULPHIACEAE | | |
| *Isthmiaenervis* Ehrenberg | | X |

**Continued**

| | | |
|---|---|---|
| LEPTOCYLINDRALES | | |
| LEPTOCYLINDRACEAE | | |
| *Leptocylindrus danicus* Cleve | | X |
| FRAGILARIOPHYCEAE | | |
| RHABDONEMATALES | | |
| GRAMMATOPHORACEAE | | |
| *Grammatophora marina* (Lyngbye) Kützing | | X |
| *Grammatophora oceanica* Ehrenberg | | X |
| LICMOPHORALES | | |
| LICMOPHORACEAE | | |
| *Licmophora abbreviata* C. Agardh | | X |
| *Licmoplora remulus* Grunow | | X |
| FLAGIRALIALES | | |
| FLAGILARIACEAE | | |
| *Podocystis adriatica* (Kützing) Ralfs | | X |
| THALASSIONEMATALES | | |
| THALASSIONEMATACEAE | | |
| *Thalassionema nitzschioides* (Grunow) Mereschkowsky | | X |
| OCHROPHYTA | | |
| DICTYOCHOPHYCEAE | | |
| DICTIOCHALES | | |
| DICTYOCHACEAE | | |
| *Dictyocha fibula* Ehrenberg | X | X |
| CHLOROPHYTA | | |
| CHLOROPHYCEAE | | |
| SPHAEROPLEALES | | |
| SCENEDESMACEAE | | |
| *Scenedesmus bijuga* (Turpin) Lagerheim | X | |
| *Scenedesmus quadricauda* (Turpin) Brébisson | X | X |
| TREBOUXIOPHYCEAE | | |
| TREBOUXIOPHYCEAE (ordo ircertaesedis) | | |
| TREBOUXIOPHYCEAE (incertaesedis) | | |
| *Crucigenia fenestrata* (Schmidle) Schmidle | X | |
| CHAROPHYTA | | |
| CONJUGATOPHYCEAE | | |
| DESMIDIALES | | |
| CLOSTERIACEAE | | |
| *Closterium* sp. Nitzsch ex Ralfs | X | X |
| DESMIDIACEAE | | |
| *Staurastrum* sp. MeyenexRalfs | X | |

**Table S2.** Factorial loads of the PCA analysis of first four components. In red and blue positive and negative correlations, respectively.

| Parameters | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Secchi | **0.794** | 0.015 | 0.027 | −0.309 |
| T°C | 0.377 | **−0.659** | 0.194 | −0.024 |
| Salinity | **0.941** | −0.035 | −0.089 | −0.010 |
| DO | **0.901** | 0.095 | −0.242 | 0.114 |
| pH | **0.813** | −0.138 | 0.153 | −0.140 |
| $NH_4^+$ | **−0.603** | 0.138 | 0.551 | −0.014 |
| $NO_2^-$ | **−0.552** | −0.408 | −0.527 | −0.178 |
| $NO_3^-$ | **−0.652** | −0.182 | −0.429 | 0.028 |
| $PO_4^-$ | **−0.710** | −0.176 | 0.393 | −0.301 |
| $SiO_2^-$ | **−0.676** | 0.331 | −0.371 | 0.215 |
| Cya | **−0.571** | −0.307 | 0.379 | 0.075 |
| Bac | −0.005 | −0.550 | −0.076 | **0.641** |
| Chl | −0.232 | −0.418 | −0.320 | **−0.607** |
| Rainfall | −0.176 | **0.800** | −0.086 | −0.138 |

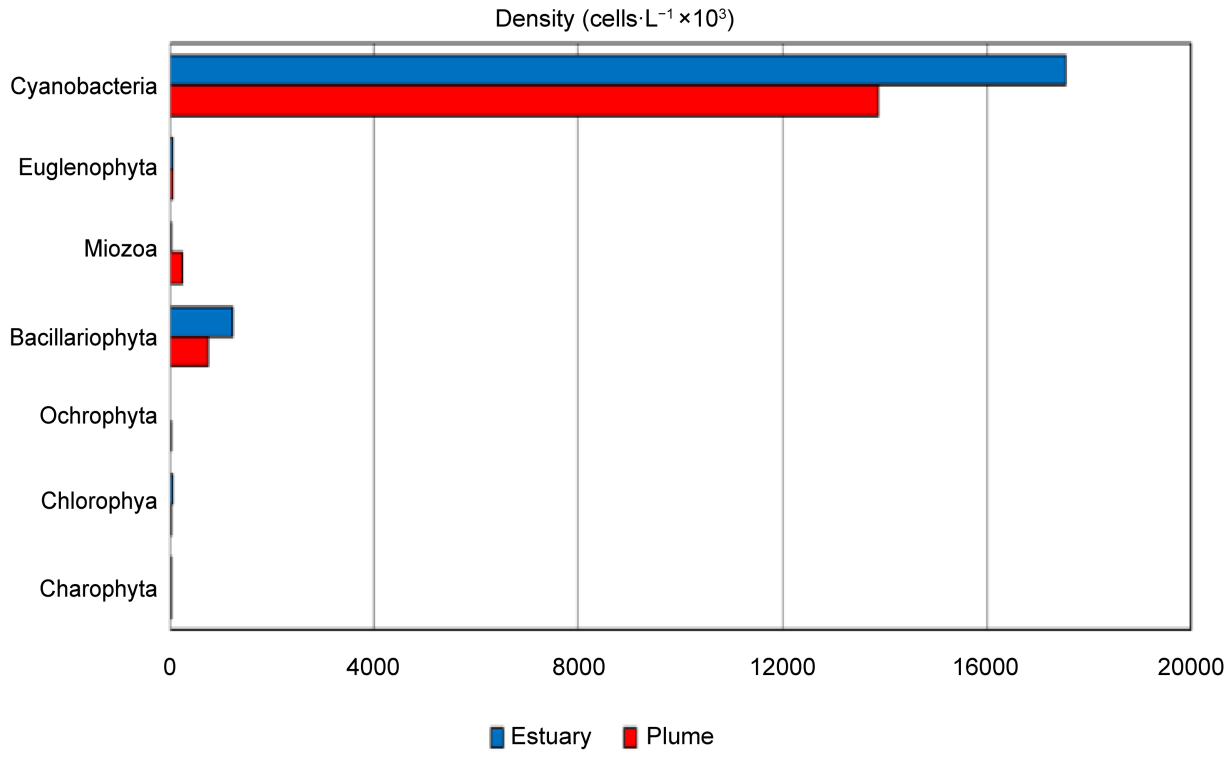


**Figure S1.** Total density of the groups in the studied areas.

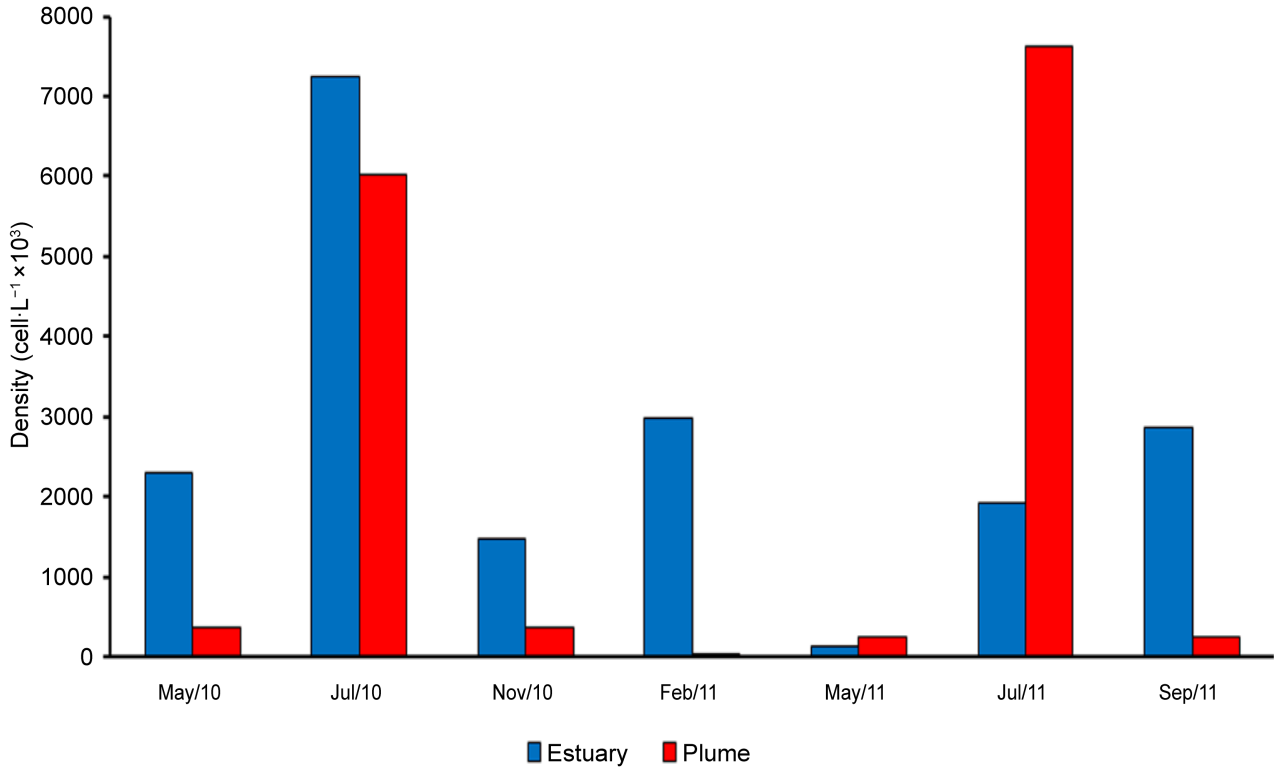


**Figure S2.** Total density for the months in the studied areas.

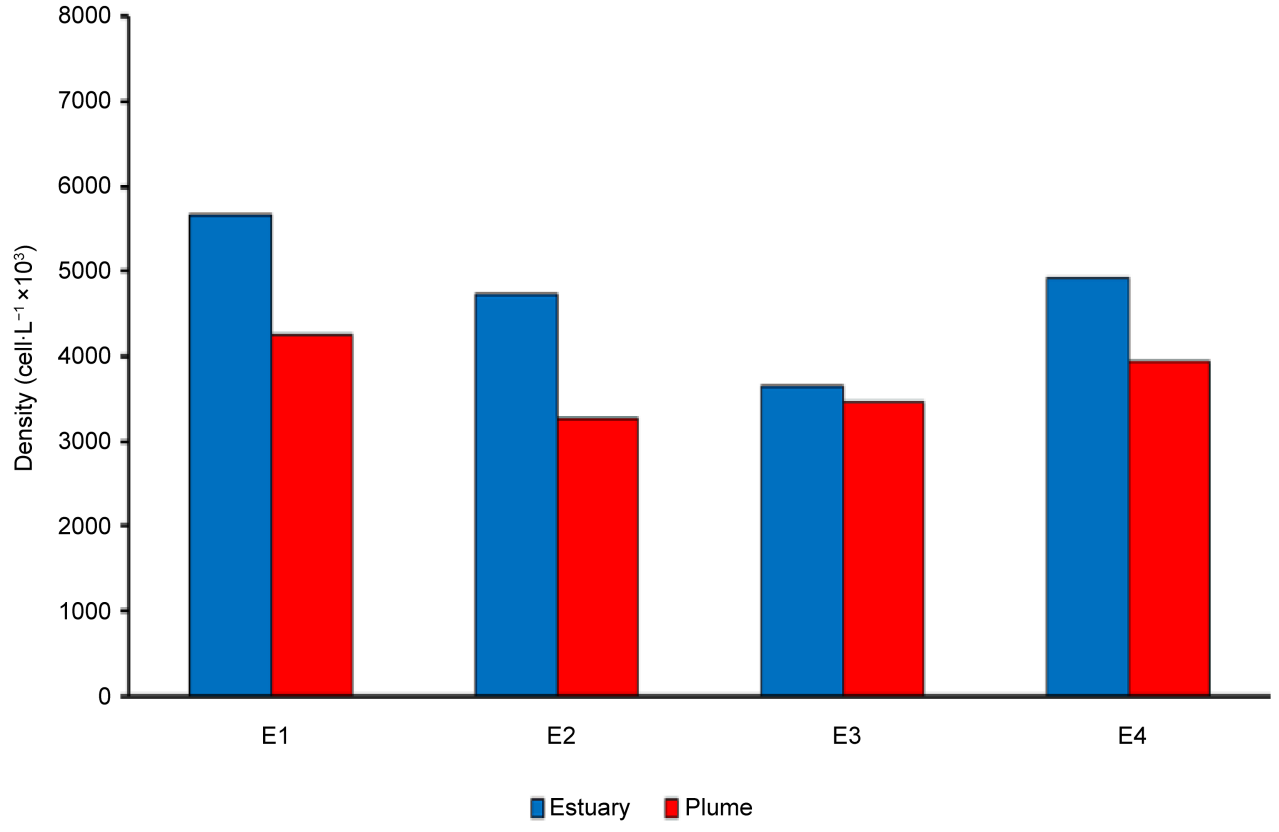


**Figure S3.** Total density for the stations in the studied areas.

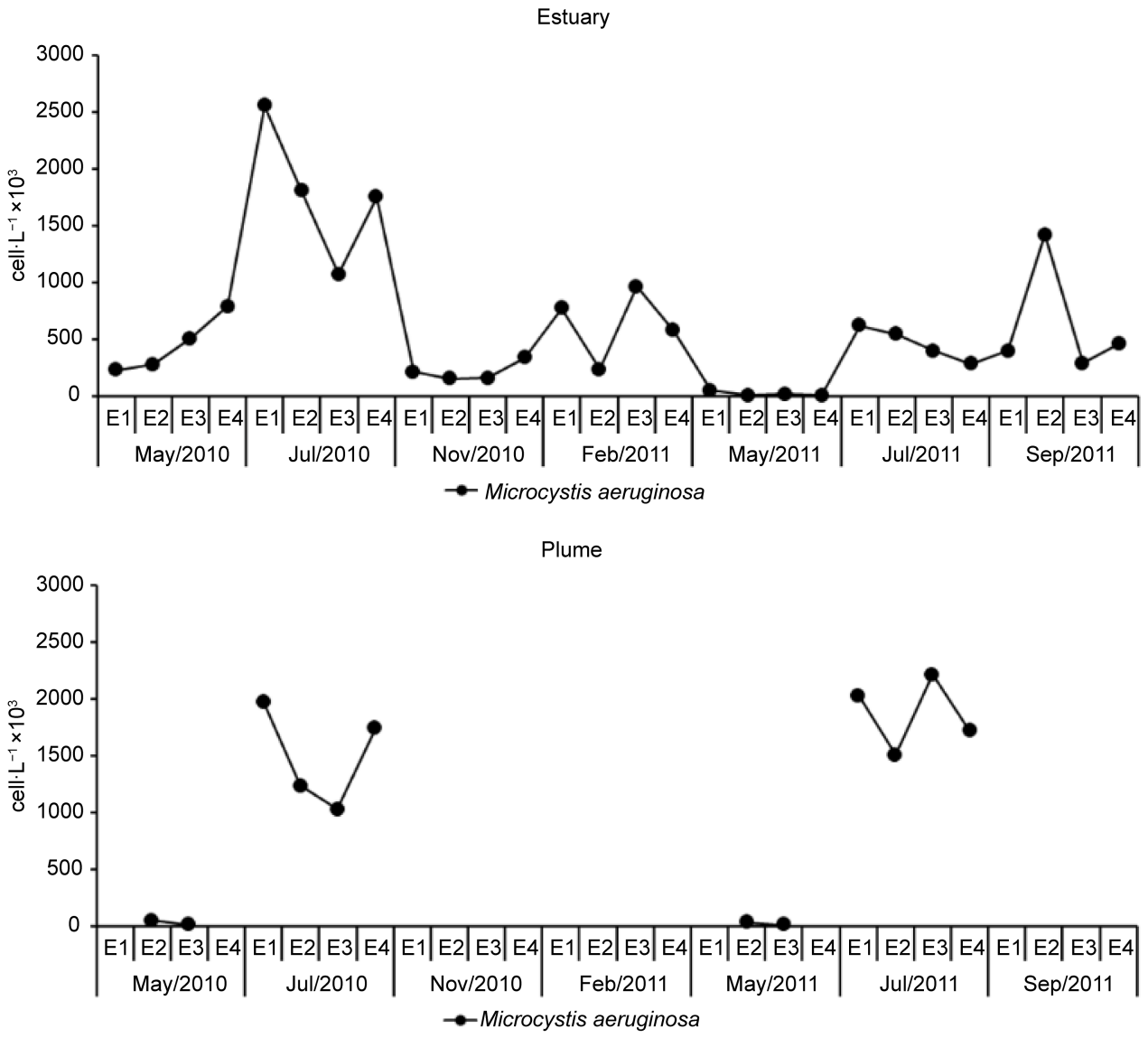


**Figure S4.** Total density of *Microcystis aeruginosa*.