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Seasonal and spatial variation in phytoplankton community structure of an estuary in Northeastern Brazil

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Abstract: A combination of environmental conditions affects the phytoplankton distribution and composition. This study analysed spatio-seasonal variation of phytoplankton in relationship to environmental variables (salinity and water clarity) in Passos River estuary (Brazil) which has a good water quality and thus this research is important as a basis for future sustainable management. Phytoplankton and environmental variables were sampled monthly at the upstream and downstream zones from August 2009 to July 2010. Rainfall data were obtained from the long-term monitoring station located in the Passos river watershed. Phytoplankton abundance, frequency, diversity, and evenness were calculated and subjected to canonical correspondence analysis to determine the relationship between species and variables. The majority of species were tychoplanktonic due to the shallow depths and hydrodynamism. *Johannesbaptistia* sp. dominated at upstream zones and in the rainy season. Diatoms were spatially affected by salinity and occurred throughout the year, as occurs in most estuaries in the world.

Resumen: La distribución y la composición del fitoplancton son afectadas por una combinación de condiciones ambientales. Este estudio analizó la variación espacio-temporal del fitoplancton en relación con variables ambientales (salinidad y transparencia del agua) en el estuario del río Passos (Brasil), el cual tiene una buena calidad del agua y por lo tanto esta investigación es importante como base para la gestión sostenible en el futuro. Se hizo un muestreo mensual del fitoplancton yde las variables ambientales en las zonas situadas aguas arriba y aguas abajo, de agosto de 2009 a julio de 2010. Los datos pluviométricos se obtuvieron de la estación de monitoreo a largo plazo ubicada en la cuenca del río Passos. Se calcularon la abundancia, la frecuencia, la diversidad y la equitatividad del fitoplancton y estas variables fueron sometidas a un análisis canónico de correspondencia para determinar la relación entre las especies y las variables. La mayoría de las especies fueron ticoplantónicas debido a la poca profundidad y el hidrodinamismo. *Johannesbaptistia sp.* fue dominante en las zonas de aguas arriba y en la temporada de lluvias. Las diatomeas fueron afectados espacialmente por la salinidad y estuvieron presentes a lo largo del año, como ocurre en la mayoría de los estuarios del mundo.

Resumo: Uma combinação de condições ambientais afecta a distribuição e a composição do fitoplâncton. Este estudo analisou a variação espaço - estacional de fitoplâncton em relação às variáveis ambientais (salinidade e transparência da água) no estuário do Rio Passos (Brasil), que tem uma boa qualidade da água. Esta pesquisa é, assim, importante como base para a futura gestão sustentável. O fitoplâncton e as variáveis ambientais foram amostrados

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mensalmente nas zonas a montante e a jusante entre agosto de 2009 a julho de 2010. Os dados pluviométricos foram obtidos a partir da estação de monitoramento de longo prazo localizada na bacia hidrográfica do rio Passos. A abundância do fitoplâncton, frequência, diversidade e regularidade foram calculados e submetidos à análise de correspondência canónica para determinar a relação entre as espécies e as variáveis. A maioria das espécies foram ticoplanctónicas devido às pequenas profundidades e hidro-dinamismo. A *Johannesbaptistia sp.* dominava nas zonas a montante e na época das chuvas. As diatomáceas foram espacialmente afetadas pela salinidade e ocorriam ao longo do ano, tal como ocorre na maioria dos estuários do mundo.

Key words: Biodiversity, cyanobacteria, diatoms, estuarine dynamics, salinity, seasonality, water clarity.

Planktonic estuarine species show a relationship with biotic and abiotic variables, such as predation, temperature, salinity, nutrients, and rainfall (Canini *et al.* 2013; Perbiche-Neves *et al.* 2012; Verity & Borkman 2010). The combination of these factors shapes the seasonal and spatial changes in the composition and distribution of species (Cloern & Dufford 2005).

The annual cycle of precipitation affects the environmental conditions, and this process is a strong determinant of phytoplankton community. Some studies showed that phytoplankton richness in estuaries might increase during the high rainfall (Honorato-da-Silva *et al.* 2009). Water clarity delineates the photic zone and limits the development of the most sensitive species (Masuda *et al.* 2011). Salinity plays an important role in the spatial distribution and ecology of the species (Muylaert *et al.* 2009). Over a wide range of salinities, diatoms are the main component of the flora (Resende *et al.* 2005). Other groups, such as chlorophyceans, cyanobacteria, and dinoflagellates, are favoured by tidal and riverine dynamics (Fujita & Odebrecht 2007).

Therefore, the aim of this study was to investigate the spatial and seasonal changes in the phytoplankton community in the Passos River estuary, relating the patterns of these organisms to environmental variables. It was expected that salinity would influence the spatial distribution of different algal compositions on a seasonal scale.

The Passos River estuary is located in Northeast Brazil, within the national grid lines (08° 37' 53" S and 35° 05' 3" W; 08° 40' 50" S and 35° 06' 46" W). Together with the Ariquindá and Formoso Rivers, the Passos River composes an important ecosystem with great marine influence (mesotidal) from the Atlantic Ocean (Fig. 1). While the majority of estuaries have strongly water conflicts, the Passos River estuary is little modified by anthropogenic activities, mainly with overfishing, areas of aquaculture and soil erosion (CPRH 1999). Thus, research in this ecosystem is important to establish baseline conditions for monitoring and can serve as a basis for conservation and future sustainable management.

Phytoplankton (n = 48) and the environmental variables were sampled monthly at low spring tide, from August 2009 to July 2010, encompassing both the dry (September 2009 to February 2010) and rainy (August 2009; March to July 2010) periods. Four stations were established: in the upper (S1 and S2), middle (S3), and lower (S4) reaches of estuary (Fig. 1). The upper stations were in a shallow area (average depth of 2.5 m) and located 9.7 m from the shore. The middle reach was the deepest on average (4.1 m) and 6.8 m offshore, while the lower reach station was 3.0 m deep on average and 3.3 m offshore.

The tide height was obtained from the National Oceanographic Database of the Hydrography and Navigation Board of the Brazilian Navy (DHN 2012). Monthly rainfall data were obtained from the long-term monitoring station from the Pernambuco Institute of Technology (ITEP 2012). During each sampling event, a Kitahara bottle (1 L) was used to sample the water surface and salinity was estimated with a refractometer. The water clarity was measured with a Secchi disk.

Phytoplankton were sampled through subsuperficial horizontal hauls with a plankton net of 64 µm mesh size for approximately three minutes at each sample station. The choice of mesh size was based on previous studies and because during the low spring tide Northeast Brazilian estuaries have high levels of suspended particulate matter

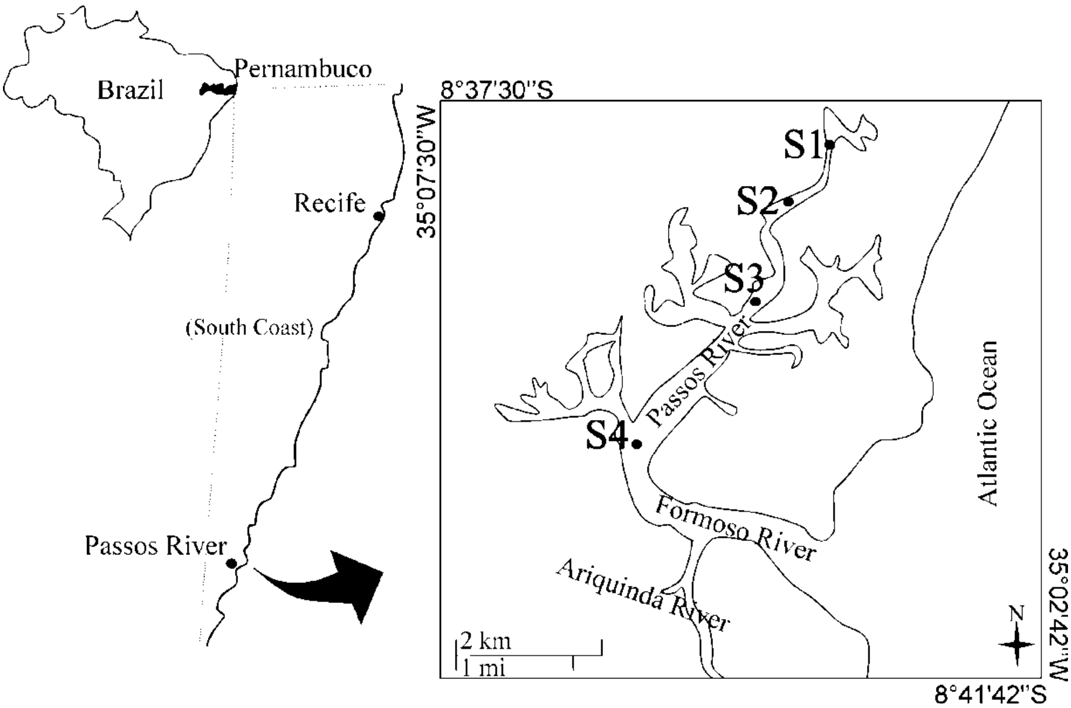

Fig. 1. Passos River estuary, Brazil, and the three sampling areas: upper (S1 and S2), middle (S3), and lower (S4) reaches.

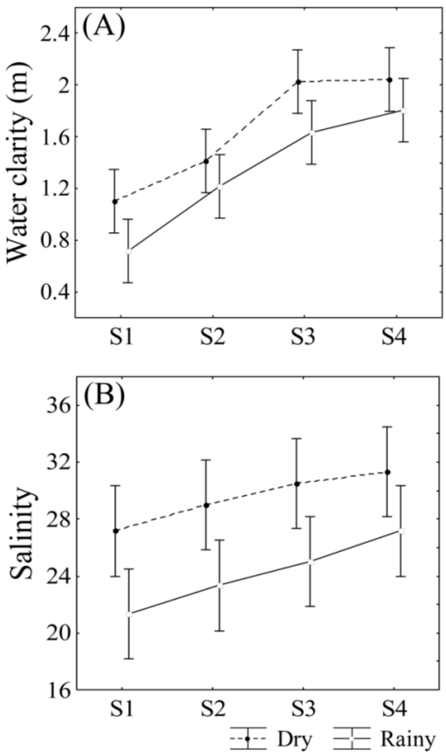

Fig. 2. Spatial and seasonal variation of the mean values of (A) water clarity (m) and (B) salinity in the Passos River estuary.

that rapidly saturate the plankton net (Bastos *et al.* 2011; Honorato-da-Silva *et al.* 2009). The samples were fixed with 4 % neutralised formaldehyde. An optical microscope coupled with a phase-contrast system was used to identify the taxa present in a subsample of 0.5 ml. Taxonomic classification followed Guiry & Guiry (2011). For a better identification of the diatom frustule ornamentations, Carr *et al.* (1986) were referenced.

Species richness was calculated as the number of species per sample. The relative abundance was calculated, with a dominant species defined as accounting for over 50 % of the total number of organisms in the sample (Lobo & Leighton 1986). Based on their frequency of occurrence (Mateucci & Colma 1982), the species were classified into four categories: very frequent (with values greater than 70 % of occurrence), frequent (between 69 and 40 %), infrequent (between 39 and 10 %), and sporadic (less than 10 %). Subsequently, Shannon-Weiner index was calculated (Shannon 1948), with the data reported in bits.cel^{-1}, where 1 bit is equal to a unit, and evenness was calculated following Pielou (1967) using Bioestat 5.0 software (Ayres *et al.* 2007). To verify the normality of the environmental variables, the Shapiro-Wilk test was applied, and all of the data had a normal distribution. To investigate the spatial (S1 to S4 sites

Table 1. Very frequent species in the Passos River estuary with the code of the names used in the CCA analysis and the ranges of salinity and water clarity that co-occurred with these species.

Very frequent species	CCA Code	Salinity	Water clarity (m)
Johannesbaptistia sp.	Johane	24-27	0.9-1.8
Lyngbya sp.	Lyng2	26-33	0.9-2.2
Pseudanabaena sp.	Pseud1	17-20	0.8-1.2
Heterocapsa sp.	Hetero	34-35	1.2-1.7
Actinoptychus splendens (Shadbolt) Ralfs	Aslp	25-30	1.3-2.1
Amphora sp.	Amph	15-29	1.2-2.4
Bacillaria paxillifera (O. F. Müller) Hendey	Bpax	20-30	1.8-2.1
Chaetoceros muelleri Lemmermann	Cmuel	20-30	1.8-2.1
Chaetoceros subtilis Cleve	Csub	25-27	1.2-1.9
Coscinodiscus centralis Ehrenberg	Ccent	25-27	1.5-1.8
Cylindrotheca closterium (Ehrenberg) Lewin & Reimann	Ccost	14-27	1.0-1.6
Gyrosigma balticum (Ehrenberg) Rabenhorst	Gbalt	20-28	0.6-1.8
Nitzschia lorenziana Grunow	Nloren	28-30	1.7-2.1
Odontella mobiliensis Grunow	Omob	26-32	1.5-2.2
Pennales (identified only to the Order level)	Penn1	20-27	0.4-1.8
Synedra sp.	Syned	23-30	0.6-1.0
Thalassionema nitzschioides (Grunow) Mereschkowsky	Tnitz	27-35	1.0-2.3

locations) and seasonal (dry and rainy periods) variations of the environmental parameters, a two-way ANOVA was used, with Tukey's multiple comparison post hoc tests and Bartlett's test to verify homogeneity of variances (Zar 1996), using Statistica 7.0 software (Statsoft 2004). The relationship between the environmental variables (rainfall, salinity, and water clarity) and the very frequent species was tested with canonical correspondence analysis (CCA) using Canoco 4.5 software (ter Braak & Smilauer 2002). The significance of the correlation was evaluated using the Monte Carlo permutation procedure, with 999 unrestricted permutations, on the first two CCA axes.

The minimum rainfall during the study was 22 mm (November 2009) and the maximum was 592.5 mm (June 2010). The water clarity varied spatially and seasonally ($F_{3,40}$ = 30.27, P < 0.01 and $F_{1,40}$ = 12.09, P < 0.01, respectively; Fig. 2A). The highest mean water clarity (2.0 m) was in the lower estuary during the dry season, while the lowest (70 cm) was during the rainy season at the upstream station. The salinity also varied spatially and seasonally ($F_{3,40}$ = 3.73; P < 0.01 and $F_{1,40}$ = 22.73; P < 0.01, respectively; Fig. 2B). The highest salinity occurred during the dry season in the lower estuary (mean of 31.3), and the upstream zone had the lowest mean salinity during the rainy season (21.3). Thus, both the water clarity and salinity increased in the downstream direction.

The phytoplankton community was characterised by 129 taxa, overwhelming dominated by diatoms (Ochrophyta), which formed 73 % of the total. This group was followed by blue-green algae (Cyanobacteria, 18 %), chlorophyceans (Charophyta, 4 %), dinoflagellates (Myzozoa, 4 %), and euglenophyceans (Euglenozoa, 1 %).

Three types of marine species were collected; 32 % of the total were tychoplanktonic, followed by oceanic (28 %) and neritic (24 %) plankton. The majority of the species were tychoplanktonic due to the shallow depths and high hydrodynamism, which resuspends the organisms. Marine planktonic species (neritic and oceanic) are carried into the estuary by the tides (Masuda *et al.* 2011). The freshwater species (10.6 %) were mainly cyanobacteria and chlorophyceans, benefited by riverine inflow. Three diatoms were typical estuarine species (5.3 %; *Gyrosigma balticum* (Ehrenberg) Rabenhorst, *Entomoneis alata* Ehrenberg, and *Surirella striatula* Turpin).

Four species were dominant: a cyanobacterium *Johannesbaptistia* sp. and the diatoms *Cylindro-*

theca closterium (Ehrenberg) Lewin & Reimann, *Chaetoceros subtilis* Cleve, and *Bacillaria paxillifera* (Müller) Hendey. All four species were well represented in the rainy season. Along the upper estuary, the dominant species (between 50.0 and 75.3 % of the total abundance) were *Johannesbaptistia* sp., *C. subtilis*, and *C. closterium*. These species occurred in water clarity below 2.0 m and salinities less than 27 (Table 1). In the middle of the estuary, *Johannesbaptistia* sp. (56.2 %) and *C. subtilis* (81.3 %) dominated, and in the low estuary, the dominant species were *B. paxillifera* (67.1 %) and *C. subtilis* (75.6 %), which co-occurred with high values of water clarity and salinity. The dry season was characterised only by *C. closterium*, which dominated in the upper estuary (above 90.0 %) at high levels of salinity.

The diatoms *Amphora* sp., *G. balticum,* and *N. lorenziana* had a very frequent distribution, present in all of the samples. Other species were also very frequent (above 70 % of occurrence): *Actinoptychus splendens* (Shadbolt) Ralfs, *B. paxillifera*, *Chaetoceros muelleri* Lemmermann, *C. subtilis*, *Coscinodiscus centralis* Ehrenberg, *C. closterium*, Pennales (identified only to the Order), *Odontella mobiliensis* Grunow, *Synedra* sp., *Thalassionema nitzschioides* (Grunow) Mereschkowsky, *Lyngbya* sp., *Pseudanabaena* sp., and *Heterocapsa* sp.

C. closterium is euryhaline, tychoplanktonic, and associated with the resuspension of sediments in the water column (Ribeiro *et al.* 2003). This diatom is found elsewhere in the Atlantic Ocean (Macedo *et al.* 2001; Verity & Borkman 2010) as well as in Brazilian estuaries (Masuda *et al.* 2011). Muylaert *et al.* (2009) indicated that *C. subtilis* is commonly found in temperate zones, where annual changes of temperature, light, and rainfall have a greater effect on the species. Similarly, *B. paxillifera* was one of the main diatoms observed by Fujita & Odebrecht (2007) in the warmer months in a Southern Brazil estuary and is influenced by seasonal rainfall. This diatom was present at a high frequency in the mid-salinity range of a shallow estuary in Portugal (Resende *et al.* 2005), and the dominance of this species in the lower reaches of the Passos River estuary suggests a strong relation with high salinity and high water clarity. *B. paxillifera* was also frequently observed in the Formoso River estuary by Honorato-da-Silva *et al.* (2009) and was described as playing an important ecological role in that ecosystem. In addition to this species, *G. balticum* and *N. lorenziana* were of equal ecological importance, spatially and seasonally, with stable abundances and high frequencies of occurrence during this study.

In general, the majority of the cyanobacteria has a continental origin (Fujita & Odebrecht 2007) and are transported to estuaries through river inflow (Muylaert *et al.* 2009). However, Tebano (2008) recently described *Johannesbaptistia* sp. as dominant in saline waters, being a euryhaline species. Some studies in saline environments of South America showed that *Johannesbaptistia* sp. coexists with other filamentous cyanobacteria, such as *Trichodesmium* sp., *Lyngbya* sp., *Leptolyngbya* sp., *Phormidium* sp., and *Spirulina* sp. (Rodda & Parodi 2005; Montoya 2009), and these species were abundant in the upstream zone of the Passos River.

The Shannon-Wiener index varied from 1.23 to 1.74 bits.cel^{-1} and was low at all of the sampling stations during the study, with high evenness values (0.58 to 0.82) and a uniform spatial and seasonal distribution of species in all of the estuarine regions during the study. According to Masuda *et al.* (2011), in a Brazilian estuary, the low diversity is a consequence of few dominant species, and only four species dominate in the Passos River estuary.

The CCA indicated an association between the very frequent species and the environmental variables ($P < 0.01$). Axes 1 and 2 accounted for 47.4 % of the variance in the biotic data. The correlation between the species and environmental variables accounted for 49.7 % of the CCA analysis on the first two axes.

The very frequent species were influenced by salinity and water clarity at axis 1 (Fig. 3A). *B. paxillifera* had an inverse relationship with rainfall along axis 2. This environmental variable was directly correlated with *C. subtilis* and *Amphora* sp. The ordination of the samples did not indicate a clear grouping of the climate periods (dry and rainy), but pooled samplings revealed a positive relationship along axis 1 with the group of samples located more upstream and along axis 2 with the group located more downstream (Fig. 3B).

Passos River exhibits an increasing salinity gradient toward the lower reaches and has conditions ranging from mesohaline to euhaline, which influences the high abundance of marine diatom species, in accordance with previous studies (Ekpenyong 2000; Muylaert *et al.* 2009). In this study, as observed in other estuaries in tropical areas (Masuda *et al.* 2011; Resende *et al.* 2005), water clarity influenced the presence of diatoms and may be dependent on the rainfall

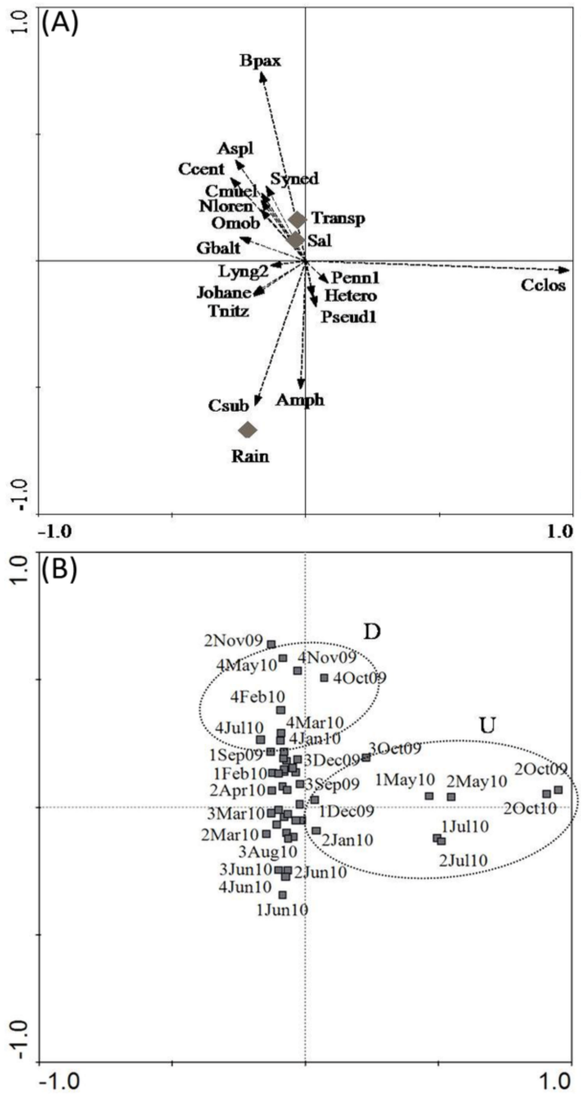

Fig. 3. (A) Ordination of the CCA between the very frequent species and environmental variables in the Passos River estuary, Brazil. Abbreviations: Sal = salinity; Transp = water clarity; Rain = rainfall. (B) Sample ordination, in which the samples are identified by the number of the sampling station, initials of the months, and number of the year (9 = 2009; 10 = 2010) grouped by downstream (D) and upstream (U).

pattern. Other environmental conditions could also affect the phytoplankton, such as nutrient availability, water temperature, light, and dissolved oxygen levels (Macedo *et al.* 2001), and could account for the remaining variability in the data.

Thus, the phytoplankton community was influenced by salinity and water clarity, with spatial and seasonal changes. The diatom species occurred throughout the year, as occurs in most estuaries in the world. The distribution of cyanobacteria *Johannesbaptistia* sp. was an exception to this configuration, with this genus being dominant at the upper and middle zones as well as in the rainy season.

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