



## Composition and structure of the benthic diatom community from salt marshes of southern Brazil and their relationship to environmental variables

Composição e estrutura da comunidade de diatomáceas bentônicas de marismas do sul do Brasil e sua relação com variáveis ambientais

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**Abstract: Aim:** This study focused on the spatiotemporal variation of the benthic diatom community structure in salt marshes from the Patos Lagoon estuary and their relationship with environmental variables. **Methods:** Samplings were carried out in the winter of 2010 and summer of 2011 (during El Niño and La Niña) in sites with different sediment granulometry, salinity, and distances from the Atlantic Ocean. The surface sediment was collected using a core (10 cm in diameter and 2 cm in depth) and the benthic diatoms were removed following the Trapping method, allowing the observation of live diatoms. **Results:** The richness values (18 and 48 taxa), evenness (0.41 and 0.68), and Shannon diversity indices (2.02 and 3.31 bits/ind.) variations were not significant between the sites and seasons, although temperature and salinity differed significantly between winter and summer. However, the diatom's composition and distribution were related to temperature, salinity, and sediment particle size. Mainly the species *Hippodonta hungarica*, *Luticola simplex* *Navicula cf. cryptotenelloides*, *N. erifuga*, *N. jacobii*, *Nitzschia filiformis* var. *conferta*, *Planothidium frequentissimum* and *Tryblionella calida* were associated with lower temperature and lower salinity in the winter. The species *Navicula cf. cryptocephala*, *N. phylleptosomaformis*, *Nitzschia pusilla*, *N. frustulum*, *N. scalpelliformis* and *Pseudostaurosiropsis geocollegarum* were associated with higher temperature and higher salinity in the summer. Birraphid diatom taxa, mainly species of the genus *Navicula* and *Nitzschia*, were frequent or abundant in sandy sediments, and monoraphids, such as *Planothidium frequentissimum*, were frequent in sites where silt and clay predominated.



**Conclusions:** The composition of the benthic diatoms revealed variation between the sites in the winter and summer seasons, during El Niño and La Niña episodes. In winter, high rainfall and freshwater runoff maintained oligohaline condition in the marshes, while in summer, flooding with meso-poly-euhaline waters changed the diatom composition. The granulometry was an important factor in explaining the distribution of the birraphid and monorhaphid taxa.

**Keywords:** estuarine system; microphytobenthos; spatiotemporal variation; substrate types.

**Resumo: Objetivo:** Este estudo focalizou a variação espaço-temporal da estrutura da comunidade de diatomáceas bentônicas, em marisma do estuário da Laguna dos Patos e sua relação com variáveis ambientais. **Métodos:** Amostragens foram realizadas no inverno de 2010 e verão de 2011 (durante o El Niño e La Niña) em locais com diferentes granulometrias do sedimento, salinidades e distâncias do Oceano Atlântico. O sedimento superficial foi coletado utilizando um core (10 cm de diâmetro e 2 cm de profundidade) e as diatomáceas bentônicas foram removidas, seguindo o método Trapping, que permite a observação das diatomáceas vivas. **Resultados:** As variações dos valores de riqueza (18 e 48 taxa), uniformidade (0.41 and 0.68) e índice de diversidade de Shannon (2.02 and 3.31 bits/ind.) entre os locais e estações não foram significativos, embora a temperatura e salinidade diferiram, significativamente, entre o inverno e verão. Entretanto, a composição e distribuição das diatomáceas estiveram relacionadas à temperatura, salinidade e tamanho de partícula do sedimento. Principalmente as espécies *Hippodonta hungarica*, *Luticola simplex*, *Navicula cf. cryptotenelloides*, *N. erifuga*, *N. jacobii*, *Nitzschia filiformis* var. *conferta*, *Planothidium frequentissimum* and *Tryblionella cálida*, estiveram associadas às mais baixas temperatura e salinidade no inverno. As espécies *Navicula cf. cryptocephala*, *N. phylleptosomaformis*, *Nitzschia pusilla*, *N. frustulum*, *N. scalpelliformis* and *Pseudostaurosiropsis geocollegarum* estiveram relacionadas as mais altas temperatura e salinidade no verão. Diatomáceas birrafídeas, principalmente espécies do gênero *Navicula* e *Nitzschia* foram frequentes ou abundantes em sedimentos arenosos e, monorrafídeas, tais como *Planothidium frequentissimum* foram frequentes em locais, onde predominaram silte e argila. **Conclusões:** A composição das diatomáceas bentônicas revelou variação entre os locais nas estações de inverno e verão, durante os episódios de El Niño and La Niña. No inverno, alta pluviosidade e alto escoamento de água doce manteve condição oligohalina nas marismas, enquanto que, no verão, a inundação de água meso-poly-euhalina mudou a composição das diatomáceas. A granulometria foi um fator importante para explicar a distribuição dos táxons birrafídeos e monorrafídeos.

**Palavras-chave:** microfítobentos; sistema estuarino; variação espaço-temporal; tipo de substrato.

## 1. Introduction

Salt marshes are coastal wetlands periodically flooded with saltwater and covered mainly by salt-tolerant herbaceous plants. They are dynamic systems, responding to changing environmental conditions, with changes in relative sea level and tidal range of specific critical importance for their biotic community (Adam, 2002). In the salt marshes from the extreme south of Brazil, the diurnal tidal range is minimal (astronomical tides, less than 0.5 m) and fluctuations of water level and salinity are irregular (Costa, 1988b; Möller et al., 2001). Wind action, river flow, and marine currents are the main factors that drive the hydrodynamics in this system (Möller Junior et al., 2008; Costa & Möller, 2011).

The sediments of shallow coastal environments can sustain large populations of benthic microalgae (microphytobenthos), dominated by mobile diatom species (Underwood & Paterson, 2003). Diatoms help stabilize these sediments by producing extracellular polymeric substances (Adam, 2002).

They are also essential in the biogeochemical cycles at the sediment/water interface (or sediment/air) through oxygenic photosynthesis (Kromkamp et al., 2006; Brotas et al., 2007; Trobajo & Sullivan, 2010). Worldwide, benthic diatom studies in salt marshes are scarce, with most studies focusing on community distribution related to tidal zones (Round, 1960; Laird & Edgar, 1992; Zong & Horton, 1998; Sawai et al., 2017); salinity gradients (Sherrod, 1999); food web interactions (Galván et al., 2008); community structure (Sullivan, 1975, 1976, 1978; Sullivan & Currin, 2002; López-Fuerte & Siqueiros-Beltrones, 2006); biodiversity (De Sève et al., 2010; Wachnicka & Gaiser, 2007), and water quality bioindicators (Van Raalte et al., 1976; Della Bella et al., 2007; Chintapenta et al., 2018). Research on benthic diatoms from salt marshes in southern Brazil is few and recent, and primarily focus on floristic inventories and taxonomic studies (Silva et al., 2010; Garcia & Talgatti, 2011; Talgatti et al., 2014a, b, c; Pacheco et al., 2016; Bertolli et al., 2016, 2019, 2020).

Understanding the structure and dynamics of the diatoms community in the salt marsh from the Patos Lagoon estuary is important, especially due to the impacts of climatic change. El Niño and La Niña are recurring phenomenon that causes sea surface temperatures in the equatorial Pacific Ocean to fluctuate, affecting the climate of South America and precipitation patterns (Paes & Moraes, 2007). High rainfall associated with El Niño events significantly increases runoff and freshwater discharge in the Patos lagoon estuary changing the salinity and water circulation patterns. Low precipitation in the drought period, associated with La Niña, decreases the freshwater outflow from the rivers and allows the inflow of marine water from the Atlantic Ocean and the highest frequency of marine species. These variations influence the ecological functioning of coastal aquatic ecosystems, with changes in the microalgae and fish communities (Odebrecht et al., 2005; Odebrecht et al., 2010; Garcia et al., 2003, 2004).

Sediment-inhabiting diatoms are typically classified into two distinct groups based on their characteristics: epipsammic diatoms, which are small and exhibit slight mobility on the sand, and epipelagic diatoms, which are larger and move more freely through finer sediments such as silt and clay. The size of sediment particles, as noted by Round (1971, 1979) and Sabbe & Vyverman (1991), is generally a determining factor in the species composition and distribution of diatoms.

In this study, we investigate the species richness, evenness, diversity, and composition of benthic diatom assemblages in salt marshes from the Patos Lagoon estuary and their relationship to influencing factors (wind, precipitation, temperature, salinity, pH and granulometry). During the study, periods of precipitation anomalies associated with El Niño and La Niña events were registered, so this study may provide baseline information of changes in the community structure related to these events. Based on the difference of the temperature and salinity in Patos Lagoon estuary, between the winter and summer, we expected variations in diatom community structure. Considering the different sediment granulometry between the sites we also expected a spatial variation of the composition distribution of the diatom species.

## 2. Material and Methods

### 2.1. Study area

The salt marshes are located on the shores around the Patos Lagoon estuary (Rio Grande do Sul, Brazil)

(31°50' 32°09'S, 52°00' 52°15'W) (Costa, 1998a). The Patos Lagoon estuary is composed of shallow bays (< 1.5 m depth) with subtidal and intertidal unvegetated and vegetated soft bottoms, and artificial hard substrates, as well as intermediate to deep areas and channels (Seeliger, 2001; Barbosa & Lanari, 2022). The region is classified as humid subtropical (Cfa) according to Köppen's climate classification (Alvares et al., 2013). The marshes present a spatial heterogeneity with mud planes, vegetated planes, channels, and tide pools, related to the topographic height and consequent time of submersion/exposure of the sediment (Costa, 1998a; Costa et al., 2003). Vascular plant communities are distinct due to the vertical flood gradient and horizontal salinity gradient. The freshwater marshes are dominated by *Juncus* spp., while the estuarine margins with higher salinity levels are dominated by *Spartina densiflora* Brong, *Spartina alterniflora* Loisel, and *Bolboschoenus maritimus* (L.) Palla.

Three sites with progressive distances from the Atlantic Ocean were established: Saco do Silveira - SS (31° 52' 48"S, 52° 14' 38"W, 36 km from the estuary mouth); Ilha da Pólvora- IP (32° 01' 14"S, 52° 05' 59"W, 18 km from the estuary mouth) and São José do Norte - SJN (32° 06' 49"S, 52° 04' 22"W, 9 km from the estuary mouth with *Spartina alterniflora* Loisel (Figure 1).

### 2.2. Sampling, preparation, and diatom analysis

Surface sediments (18 samples in total) were collected in winter (w) 2010 and summer (s) 2011 using a core (PVC pipe, 10 cm diameter and 2 cm deep). Three core samples were taken at each site (IP A, IP B, IP C, SS A, SS B, SS C, SJN A, SJN B, and SJN C).

The Trapping method was used which allows the observation of living and moving benthic diatoms. The surface sediments obtained from the sampling sites were placed in Beckers and dark acclimated for at least 7 hours. Afterward, the supernatant was removed and the samples were homogenized and placed in Petri dishes. Subsequently, dual cellulose tissue quadrats (2 cm × 2 cm – Whatman 105) were placed on top of the samples. The dishes were covered and kept for 24 hours for natural illumination. After the tissues were removed and dissolved in 3 mL of acetic Lugol and glycerol 4% to release the attached diatoms. This volume was used for oxidation and analysis (Eaton & Moss, 1966; Laudaes-Silva & Cimardi, 1989).

The samples were oxidized with nitric acid in a test tube at a 1:1 ratio, and the slides were mounted using Naphrax® resin (IR = 1.74). The slides are deposited in the Herbarium HAS of the Natural Science Museum, Secretary of Environment and Infrastructure of Rio Grande do Sul. The diatoms were identified using both light microscopy (LM Zeiss Axioplan, with Axiocam ERc 5s coupled, 100x) and scanning electron microscopy (SEM JEOL JSM 6060) following specialized literature (Hustedt, 1930, 1955, 1985; Pégallo & Pégallo, 1897-1908; Hendey, 1964; Patrick & Reimer, 1966; Navarro, 1981, 1982; Krammer & Lange-Bertalot, 1986, 1988, 1991; Witkowski et al., 2000; Metzeltin et al., 2005).

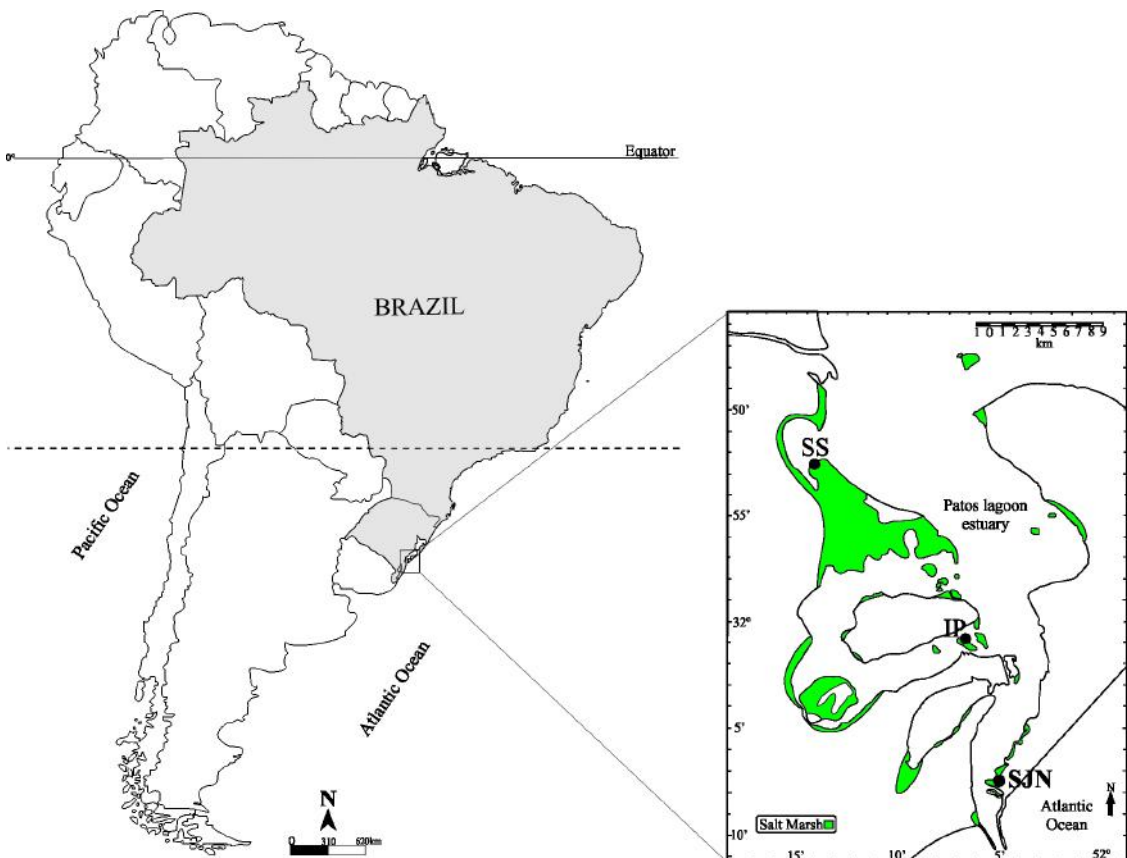
Quantification of the taxa was carried out using two permanent slides per sample, aiming to attain a minimum sample efficiency of 80% following Pappas & Stoermer (1996). Species abundance was determined according to Lobo & Leighton (1986). Frequent species were determined as those that occurred more than 50% in the season or sampling site. Richness (S) was estimated from the

number of specific taxa, and diversity and evenness indices were based on Shannon-Weaver (Shannon & Weaver, 1949).

Concomitantly to sediment sampling for diatoms, we measured temperature, pH, and salinity in the interstitial water with a YSI Model 30. The salinity zones were based on the Venice system (Association for the Sciences of Limnology and Oceanography, 1958). The sediment granulometric analysis was performed by sifting and sedimentation (Stokes law) according to Suguio (1973) and the sand-clay-silt classification was based on Shepard (1954). Wind and precipitation data were obtained from the National Institute of Meteorology (INMET, 2022).

### 2.3. Statistical analysis

Normality and homogeneity of variances were not met for all variables. Thus, tests for significant differences between seasons were evaluated by non-parametric Mann-Whitney, and among sampling sites, by the Kruskal-Wallis test ( $p < 0.05$ ), using Jamovi software (The Jamovi Project, 2021).



**Figure 1.** Study area and sampling sites in the Patos Lagoon estuary (southern Brazil): SS = Saco do Silveira, IP = Pólvora Island, and SJN = São José do Norte [modified from Costa (1998b)]. The dotted line is the Tropic of Capricorn.

A correlation analysis and a Principal Component Analysis (PCA) were undertaken to investigate the relationships between the environmental variables, granulometry, and community indices. As variables were measured in different units, a correlation (normalized var-covar) matrix for the PCA was used, which implies normalizing all variables and dividing the raw values by their standard deviations.

A Canonical Correspondence Analysis (CCA) was performed to explore the relationship between the environmental variables, granulometry, and community structure, with the data from the granulometric analyses, temperature, salinity, pH, and species counts. Only taxa with relative abundance of at least 5% were included in the analysis. PCA and CCA analyses were carried out using PAST software (Hammer et al., 2001).

### 3. Results

#### 3.1. Environmental variables

The descriptive statistics for the environmental variables are summarized in Table 1. The analysis of the selected environmental variables of the interstitial water from salt marshes showed that temperature

varied between 15.4 and 21.5 °C in winter and 25 and 29 °C in summer (Figure 2a-2b). Salinity (Figure 2c-2d) varied between 0.1 and 3.4 ppt (limnetic-oligohaline zone) in winter, and between 8.0 and 35 ppt (meso-euhaline zone) in summer. São José do Norte presented the highest salinities in winter (1.2-1.9 ppt) and summer (15-35 ppt). Saco do Silveira had the lowest salinities (0.1-0.3 ppt) in winter, while Pólvora Island in summer (8-19.5 ppt). The pH (Figure 2e-2f) presented higher variability in summer (5.6-8.6) than in winter (6.8-8.8). The highest pH was measured at Saco do Silveira in winter (8.8) and the lowest at Pólvora Island in summer (5.6).

Temperature and salinity differed significantly ( $p < 0.001$ ) in winter and summer. None of the three analyzed environmental variables were significantly different among the sampling sites (Tables 2 and 3).

The sediment from Pólvora Island and Saco do Silveira sites were mainly composed of sand (IP 67% - 91%, SS 68% - 100%), on the other hand, the São José do Norte site was mainly composed of silt sediment (SJM 59% - 66%), except at site SJN C in winter, in which sand also predominated (55%) (Figure 3).

**Table 1.** Mean, maximum and minimum values, standard mean errors, and standard deviation for salinity, temperature, and pH split by seasons.

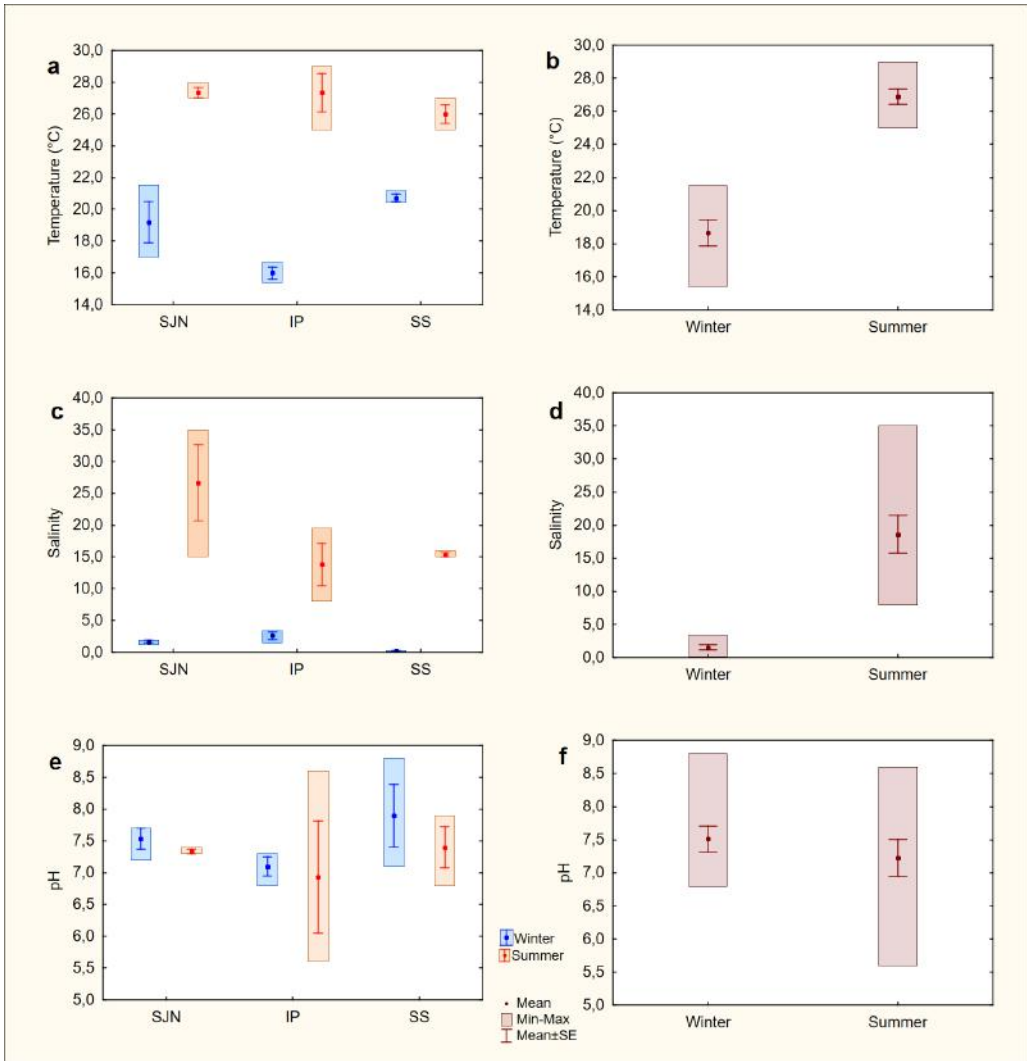
	season	salinity	T°C	pH
N	sum	9	9	9
	win	9	9	9
Mean	sum	18.6	26.9	7.22
	win	1.51	18.6	7.51
Std. error mean	sum	2.84	0.455	0.281
	win	0.393	0.802	0.195
Standard deviation	sum	8.51	1.36	0.842
	win	1.18	2.41	0.584
Minimum	sum	8.00	25.0	5.60
	win	0.10	15.4	6.80
Maximum	sum	35.0	29.0	8.60
	win	3.40	21.5	8.80

**Table 2.** Test results for significant differences between winter and summer, for the environmental variables.

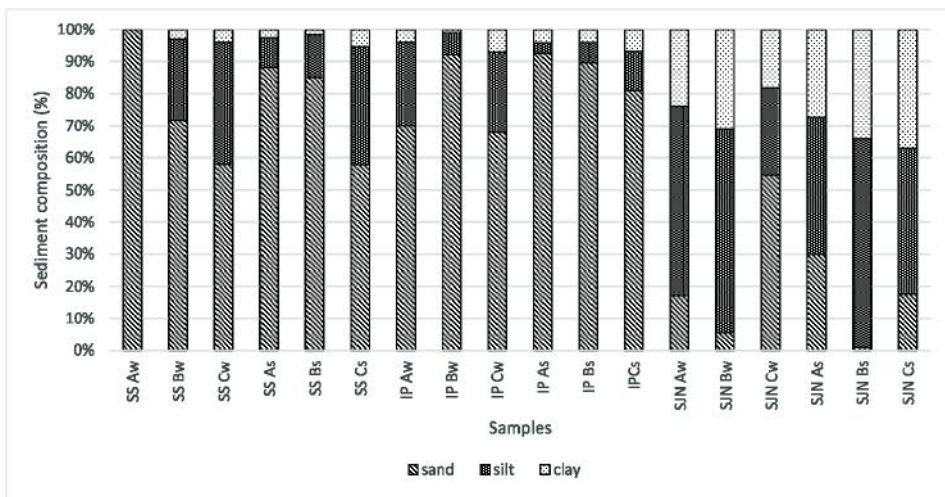
		Statistic	df	p
Salinity	Student's t	5.974 <sup>a</sup>	16	<.001
	Mann-Whitney U	0.0		<.001
Temperature	Student's t	8.940 <sup>a</sup>	16	<.001
	Mann-Whitney U	0.0		<.001
pH	Student's t	-0.846	16	0.410
	Mann-Whitney U	35.5		0.690

<sup>a</sup>Levene's test is significant ( $p < .05$ ), suggesting a violation of the assumption of equal variances.

df= degrees of freedom.



**Figure 2.** Mean, standard error (SE), minimum and maximum values of interstitial water temperature, salinity, and pH by sampling site (a, c, e) and by seasons (b, d, f), in winter 2010 and in summer 2011. SJN = São José do Norte, IP = Island of Pólvora and SS = Saco do Silveira.



**Figure 3.** Percentages of sand, silt, and clay in the sediment of the sampling sites (IP = Island of Pólvora, SS= Saco do Silveira, SJN = São José do Norte); Samples A, B, C; Seasons: s = summer, w = winter.

According to the data obtained from the meteorological station in Rio Grande (INMET, 2022), the accumulated rainfall between 1961 and 2011, was higher ( $\pm 370$  mm) in winter 2010 than in summer 2011 ( $\pm 299$  mm). The predominant wind direction was northeast in winter and southeast in summer.

### 3.2. Diatom community versus environmental variable

The diatom community attributes didn't show statistically significant differences either between the sites or between the winter and summer seasons (Tables 4 and 5). Nevertheless, we observed that São José do Norte (SJN As) had the lowest richness in summer (28 taxa), while Pólvora Island had the highest richness in both winter (IP Aw) and

summer (IP Bs), with 48 taxa. Saco do Silveira (SS Aw) had the lowest richness in winter (18 taxa) (Figure 4a-4b). The Shannon diversity index varied between 2.02 bits/ind. in Saco do Silveira (SS Aw) and 3.31 bits/ind. in São José do Norte (SJN Aw) in winter, contrary to summer where the minimum diversity value (2.61 bits/ind.) was observed in São José do Norte (SJN Bs) and a maximum of 3.20 bits/ind. in Saco do Silveira (SS As) (Figure 4c-4d). Evenness varied between the minimum value of 0.42 in Saco do Silveira and the maximum value of 0.68 in São José do Norte in winter. In summer, the minimum (0.41) and maximum (0.61) values were found in Saco do Silveira (Figure 4e-4f). The descriptive statistics for the community attributes are summarized in Table 6.

**Table 3.** Non-parametric one-way ANOVA (Kruskal-Wallis) results for significant differences between the three sampling sites, for the environmental variables.

	$\chi^2$	df	p
Salinity	0.989	2	0.610
Temperature	0.391	2	0.822
pH	3.007	2	0.222

df= degrees of freedom.

**Table 4.** Tests for significant differences between winter and summer, for richness (S), Shannon diversity index (H'), and Evenness (E).

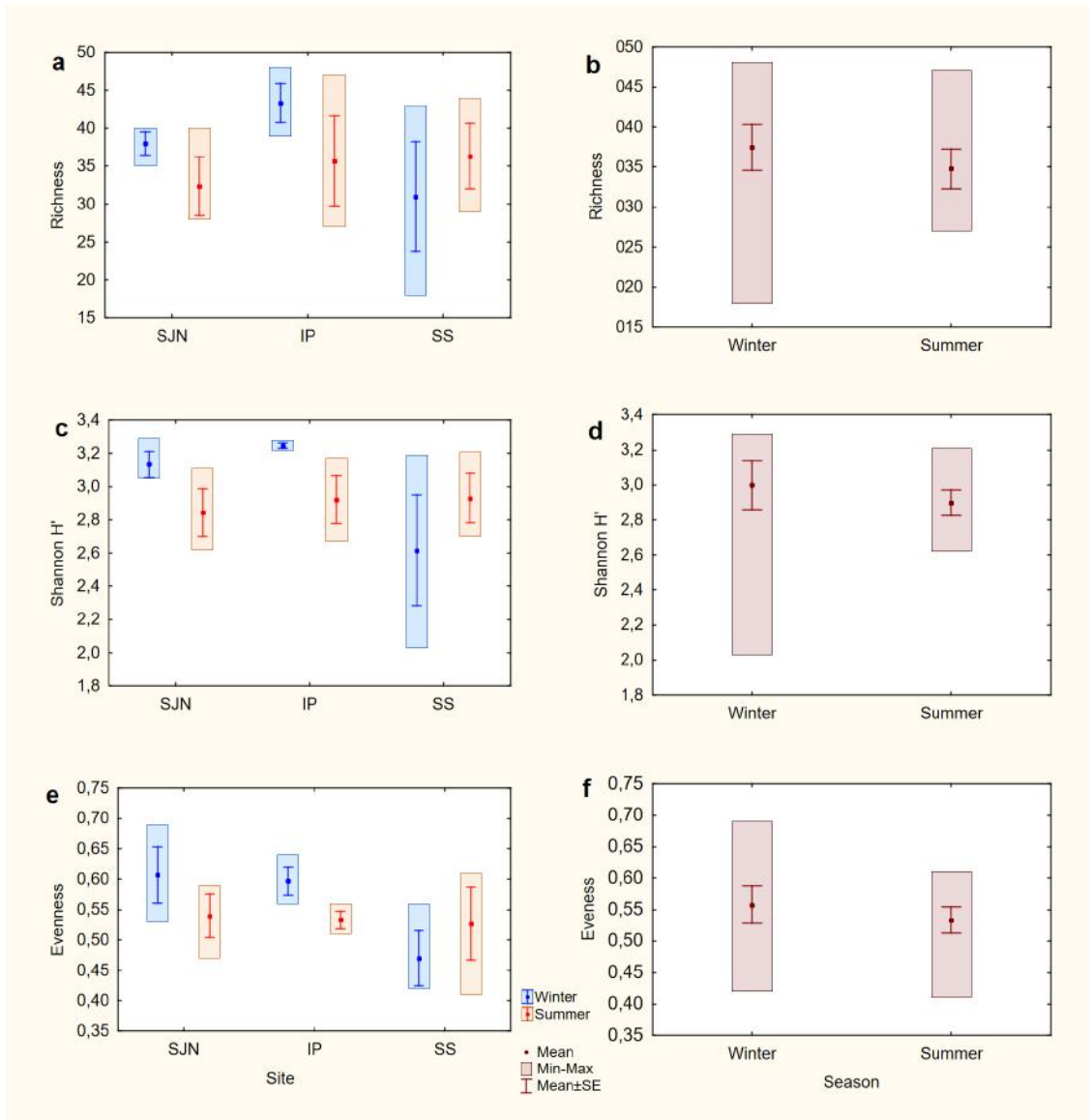
		Statistic	df	p
S	Student's t	-0.702	16	0.493
	Mann-Whitney U	30.5		0.401
H'	Student's t	-0.650	16	0.525
	Mann-Whitney U	24.0		0.161
E	Student's t	-0.707	16	0.490
	Mann-Whitney U	30.0		0.387

df= degrees of freedom.

**Table 5.** Non-parametric one-way ANOVA (Kruskal-Wallis) for significant differences between the three sampling sites, for richness (S), Shannon diversity index (H'), and Evenness (E).

	$\chi^2$	df	p
S	1.27	2	0.530
H'	2.47	2	0.291
E	1.56	2	0.459

df= degrees of freedom.



**Figure 4.** Mean, standard error (SE), minimum and maximum values of the diversity descriptors (taxa richness, Shannon diversity index, and evenness) of diatom assemblages by sampling site (a, c, e) and by seasons (b, d, f), in winter 2010 and in summer 2011. SJN = São José do Norte, IP = Island of Pólvora and SS = Saco do Silveira.

**Table 6.** Mean, maximum, and minimum values, standard mean errors, and standard deviation for taxa richness (S), Shannon diversity index (H'), and evenness (E) split by seasons.

	season	S	H'	E
N	sum	9	9	9
	win	9	9	9
Mean	sum	34.8	2.90	0.533
	win	37.4	3.00	0.558
Std. error mean	sum	2.47	0.0741	0.0207
	win	2.88	0.139	0.0296
Standard deviation	sum	7.41	0.222	0.0622
	win	8.65	0.417	0.0889
Minimum	sum	27.0	2.62	0.410
	win	18.0	2.03	0.420
Maximum	sum	47.0	3.21	0.610
	win	48.0	3.29	0.690



### 3.3. Correlation analysis, PCA, and CCA

Temperature was significantly correlated to salinity ( $p < 0.01$ ), and these two variables can be seen as proxies for temporal variation. Also, there was a significant correlation between diversity ( $H'$ ) and temperature. Significant correlations verified between granulometric variables are expected since the values were expressed in percentage, and therefore they are complementary. For diversity descriptors, Shannon Index and evenness consider the number of taxa (richness), which explains the

significant correlation between these variables (Table 7).

The ordination of the environmental variables and diversity descriptors using PCA explained 64.4% of data variance on the first two axes (Figure 5, Table 8, Table 9). This analysis highlighted that the samples were organized according to temperature, salinity, and granulometry. The first component was positively associated with higher diversity, richness, and evenness in SJN and IP sites in the winter, and negatively associated with lower values of these descriptors in SS in the winter in silt-clay sediments.

**Table 7.** Correlation matrix (Spearman's rho) between the environmental variables and diversity descriptors (T°C: temperature, S: richness, H': Shannon diversity, E: evenness).

		salinity	T°C	pH	sand	silt	clay	S	H'	E
salinity	Spearman's rho	–								
	p-value	–								
T°C	Spearman's rho	0.621**	–							
	p-value	0.006	–							
pH	Spearman's rho	-0.187	0.124	–						
	p-value	0.457	0.625	–						
sand	Spearman's rho	-0.133	0.027	0.176	–					
	p-value	0.600	0.916	0.485	–					
silt	Spearman's rho	0.115	-0.066	-0.156	-0.984***	–				
	p-value	0.648	0.794	0.537	<.001	–				
clay	Spearman's rho	0.338	0.244	-0.216	-0.844***	0.770***	–			
	p-value	0.170	0.328	0.390	<.001	<.001	–			
S	Spearman's rho	0.060	-0.425	-0.202	-0.001	0.029	-0.085	–		
	p-value	0.813	0.079	0.422	0.997	0.908	0.736	–		
H'	Spearman's rho	-0.089	-0.576*	-0.360	-0.104	0.119	0.049	0.822***	–	
	p-value	0.725	0.012	0.142	0.680	0.639	0.848	<.001	–	
E	Spearman's rho	-0.047	-0.359	-0.372	-0.375	0.367	0.377	0.162	0.634**	–
	p-value	0.854	0.143	0.129	0.125	0.135	0.123	0.520	0.005	–

\* $p < 0.05$ . \*\* $p < 0.01$ . \*\*\* $p < 0.001$ .

**Table 8.** PCA component variance.

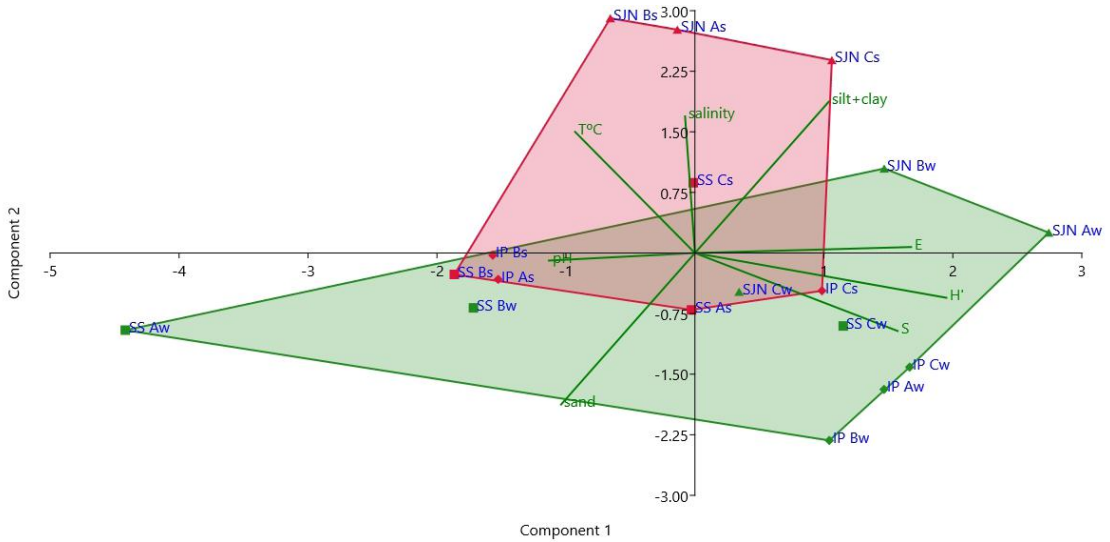
Component	Eigenvalue	% of Variance	Cumulative %
1	2.96490	37.0613	37.1
2	2.19096	27.3869	64.4
3	1.47860	18.4825	82.9
4	0.61504	7.6880	90.6
5	0.53968	6.7460	97.4
6	0.20555	2.5694	99.9
7	0.00526	0.0657	100.0
8	1.14e-5	1.43e-4	100.0

**Table 9.** Variable correlations with the first three components of the PCA.

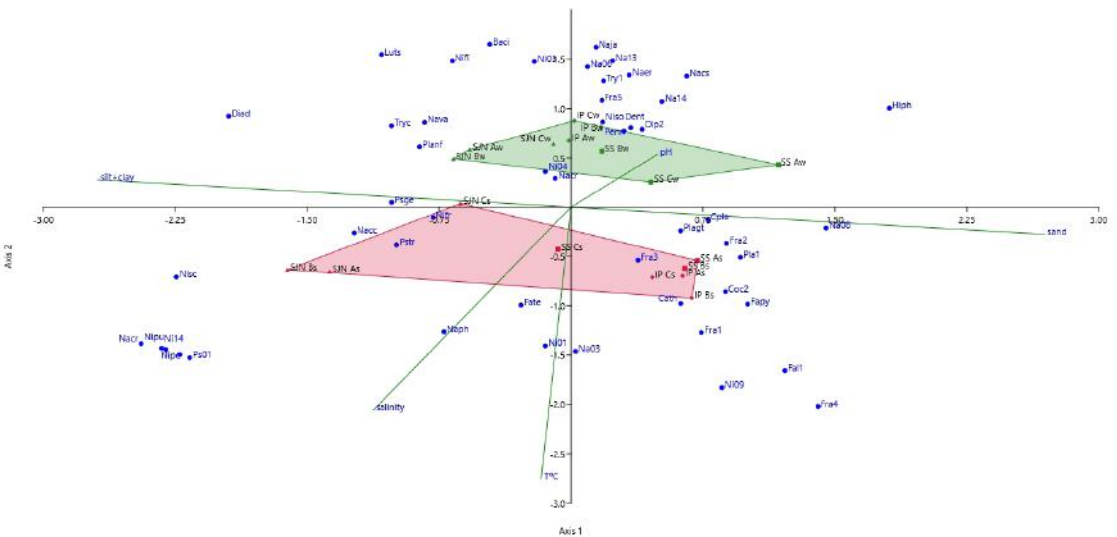
	Dim.1	Dim.2	Dim.3
Salinity	-0.0360	0.6843	0.6262
T°C	-0.4374	0.6069	0.5614
pH	-0.5329	-0.0377	-0.5316
Sand	-0.4882	-0.7586	0.3996
Silt+clay	0.4883	0.7577	-0.4012
S	0.7397	-0.3902	0.3329
H'	0.9175	-0.2240	0.2206
E	0.7890	0.0302	-0.0924

The second component was positively associated with higher values of temperature and salinity. With relation to granulometry, SJN sites were more associated with silt-clay sediment, whereas SS sites were more related to sandy granulometry. The IP sites showed a relation to sandy sites in summer and to silty sites in winter.

The evaluation of the abundant species in relation to variable data using CCA explained 64.7% of data variance on the first two axes (Figure 6, Table 10). This analysis demonstrated the relationship between the species composition with granulometry, salinity, and temperature.



**Figure 5.** Principal component analysis (PCA) ordination of environmental variables and sampling sites. Triangles: SJN = São José do Norte; Diamonds: IP = Island of Pólvora; Squares: SS = Saco do Silveira; Samples = A, B, C; red: s = summer season; green: w = winter season.



**Figure 6.** CCA Canonical Correspondence Analysis (CCA) of environmental data and abundant species in the summer (red) and winter (green) sampling. Squares, triangles and diamonds indicate the sites, and blue circles code the species. Baci: *Bacillaria paxillifera*, Cpla: *Cocconeis placentula*, Cath: *Catenula adhaerens*, Fapy: *Fallacia pygmaea*, Hiph: *Hippodonta hungarica*, Luts: *Luticola simplex*, Nacr: *Navicula cryptotenella*, Nacc: *N. cryptocephala*, Nacs: *N. cryptotenelloides*, Baer: *N. erifuga*, Naja: *N. jacobii*, Naph: *N. phylleptosomaformis*, Nava: *N. valeriana*, Nifi: *Nizschia filiformis* var. *conferta*, Nfr: *N. frustulum*, Nipu: *N. pusilla*, Nipe: *N. perindistincta*, Nisc: *N. scalpelliformis*, Plag: *Plagiogramma tenuissima* = *Ambo tenuissimus*, Planf: *Planorbidium frequentissimum*, Pstr: *Pseudostaurosira trainorii*, Psge: *Pseudostaurosiropsis geocollegarum*, and Tryc: *Tryblionella calida*.

**Table 10.** CCA eigenvalues of the first two axes.

Axis	Eigenvalue	%
1	0.46619	37.12
2	0.34708	27.64
3	0.25711	20.47
4	0.18555	14.77
5	1.7353E-10	1.382E-08

Samples from sand sediments were positive on Axis 1, ordered biraphid taxa [(*Catenula adhaerens* Mereschk, *Fallacia pygmaea* (Kütz.) A.J. Stickle & D.G. Mann, *Hippodonta hungarica* (Grunow) Lange-Bertalot et al.); monoraphid (*Cocconeis placentula* Ehrenb. and *Planothidium* sp.) and some araphid taxa (*Fragilaria* spp. and *Ambo tenuissimus* (Hust.) A. Witkoswski, Lange-Bert. & Ashworth, from SS and IP sites.

Samples from silt + clay sediments had a negative relationship to Axis 1, ordered more biraphid taxa [*Navicula* cf. *cryptotenella* Lange-Bert., *N. cryptocephala* Kütz., *Nitzschia frustulum* (Kütz) Grunow, *Nitzschia* cf. *pusilla* Grunow, *Nitzschia* cf. *perindistincta* Cholnoky, *N. scalpelliformis* (Grunow) Grunow]; a monoraphid taxon [*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot] and araphid taxa (*Pseudostaurosira trainorii* E. A. Morales, *Pseudostaurosiropsis geocollegarum* (Witkowski) E.A. Morales].

Regarding axis 2, the most important variables in determining ordination were salinity and temperature. The species *Bacillaria* cf. *paxillifera* (O. F. Müller) T. Marsson, *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski, *Luticola simplex* Metzeltin et al., *Navicula* cf. *cryptotenelloides* Lange-Bertalot, *N. erifuga* Lange-Bertalot, *N. jacobii* Manguin, *N. valeriana* Metzeltin et al., *Nitzschia filiformis* var. *conferta* (Rich.) Lange-Bertalot, *Planothidium frequentissimum*, and *Tryblionella calida* (Grunow) D.G. Mann were associated with lower temperature and salinity in the winter. The species *Navicula* cf. *cryptocephala* Kütz., *N. phylleptosomaformis* Selddon & Witkowski, *Nitzschia* cf. *perindistincta* Cholnoky, *N. pusilla*, *N. frustulum*, *N. scalpelliformis* and *Pseudostaurosiropsis geocollegarum* were associated with higher temperature and salinity in the summer.

#### 4. Discussion

The diatom community in the salt marshes of the Patos Lagoon estuary consists mainly of motile members (biraphid and monoraphid diatoms). Taxa belonging to araphid (e.g. *Fragilaria*, *Paralia*,

*Opephora*, and *Pseudostaurosira*) may assure their permanence in the benthos through attachment via mucilage to the particles and fragments of vascular plants at the sediment surface, but rarely are such forms more abundant than the biraphid forms (Sullivan, 1975, 1977, 1978; Sullivan & Currin, 2002). The trapping method used in this research showed to be efficient to capture the moving cells from sediment and to reveal the diatoms that were alive at the time of sampling. The comparative study of sampling methods in the Ratonas River estuary, Brazil (Vettorato et al., 2010) already demonstrated that this method was the most appropriate for removing the live specimens from the sediment, minimizing the problems found with the other methodologies tested.

Maximum values of benthic diatom richness (48 species) and diversity (3.31 bits/ind) were lower than observed in North American salt marshes. In Delaware, New Jersey, and Mississippi salt marshes, these attributes ranged from 10 to 69 taxa (mean 2.64 - 4.68 bits/ind.), from 29 to 85 taxa (3.46 - 5.20 bits/ind.), and from 35 to 43 taxa (3.34 - 4.24 bits/ind.) respectively (Sullivan, 1975, 1977, 1978). On the other hand, the pattern observed in the Puget Sound salt marsh (USA) and Seven Estuary (UK) were similar to what we observed. In the first, the diversity ranged from 0.18 to 2.52 and from 2.12 to 3.6 and 26-40 taxa in the second. These attributes there were not also significant differences between sites (Sherrod, 1999; Underwood, 1994).

Shannon's diversity of benthic diatoms showed an inverse correlation with temperature, although not statistically significant ( $p = 0.079$ ), and richness was also inversely correlated with temperature. As the temperature can be considered a proxy for seasonality, the diversity of benthic diatoms in the sampled sites is higher in winter. We also found the highest values for richness during this season in some sites. The correlation between salinity and the diversity descriptors was very weak, but salinity differed significantly between the two seasons. Studies carried out in the salt marshes of North America also found the highest Shannon diversity values and the greatest number of species in periods of lower salinity (Sullivan, 1975, 1977, 1978).

The difference in the temperature, as well as the wind direction (northeast in winter and southeast in summer), the rainfall intensity ( $\pm 370$  mm) in the winter and ( $\pm 299$  mm) in summer affected the salinity gradient in the Patos Lagoon estuary and the spatiotemporal variations of the diatom community structure. In the winter of 2010, a moderate El Niño event was observed (Kayano et al., 2016), increasing rainfall during winter. The higher freshwater discharge from Guaíba Lake (located at the head of Patos Lagoon) and the northeastern winds promoted the retreat of seawater intrusion, keeping limnetic to oligohaline conditions in the marshes that can have favored freshwater or tolerant to low salinity species, represented on the upper quadrants in CCA (Figure 6). In the summer of 2011, the drought associated with a strong La Niña event (Kayano et al., 2016) and southeastern winds drove the water inflow from the ocean, changing the water salinity to meso-poly-euhaline zones, favoring taxa tolerant to higher salinity, plotted on lower quadrants of the CCA illustrated in Figure 6.

The sediment granulometry was another environmental variable that affected the diatom community. The diatoms from silty and clayey substrates had fewer dominant species than those from sandy sites, as evenness was positively correlated with the increase of silt/clay content of the sediment. Although not significant, evenness had an inverse correlation with the percentage of sand in the substrate. In general, biraphid taxa were more frequent in silt and clay sites, and monoraphid and araphid taxa were more frequent in sand and silt sites of Patos Lagoon salt marshes. Therefore, we could not determine whether morphological features of the sediment favored one group over the other, so controlled experiments are necessary to understand what drives the preferences of benthic diatoms to a certain substrate type. Harper & Harper (1967) measured the adhesion and movement of diatoms and found that it was essential to attach to a surface when moving. Later, Harper (1969) found that in the freshwater lentic habitat, epipsammic diatoms moved slowly in light, when compared to epipelagic species, but the rate of movement was sufficient to reach the surface. This mobility helps these organisms reach light and resources (nutrients), and for protection against desiccation (Hay et al., 1993; Witkowski et al., 2012).

In Saco de Silveira and Pólvora Island, composed mainly by sand, *Navicula* species (*N. willisiae* Seddon & Witkowski, *N. erifuga* Lange-Bertalot, *N. jacobii*, *N. valeriana*, *N. cryptotenelloides* Lange-Bertalot,

and *N. phylleptosomaformis* Seddon & Witkowski) and *Nitzschia* species [*N. lorenziana* Grunow, *N. frustulum* (Kütz.) Grunow and *N. solita* Hust.] were more frequent. In São José do Norte, composed mainly by silt/clay, *Pseudostaurosiropsis geocollegarum*, *Tryblonella granulata* (Grunow) D.G. Mann, *Planothidium frequentissimum* (Lange-Bertalot) Round & Bukhtiyarova and *Cocconeis placentula* Ehrenb. were more frequent. The composition of diatom communities has been previously reported to be related to the size of sediment particles. Studying live diatom flora from a salt marsh on the River Dee estuary (UK), Round (1960) observed differences between the marsh with silty and sandy sediments. *Diploneis didyma* (Ehrenb.) Cleve, *Navicula gregaria* Donk., *Nitzschia pygmaea* Kütz., *N. apiculata* (Greg.) Grunow and *Amphora ovalis* Kütz. were common in silt sediments, while *Pleurosigma aestuarii* (Bréb.) W. Smith, *P. elongatum* W. Smith, *Rhaphoneis surirella* (Ehrenb.) Grunow, *Navicula viridula* Kütz., and *Nitzschia panduriformis* Grunow were only found in sandy sediments. Zong & Horton (1998) also found that diatom composition varied according to marsh sediments in England. In their study, the authors observed two major groups, one consisting of sand-related species [e.g., *Cocconeis peltoides* Hust., *Plagiogramma vanheurckii* Grunow, *Delphineis surirella* (Ehrenb.) G. W. Andrews and *Opephora marina* (W. Greg.) P. Petit] and another group related to silt and clay [e.g., *Diploneis didyma* (Ehrenb.) Ehrenb., *Nitzschia sigma* (Kütz.) W. Smith and *Nitzschia obtusa* W. Smith]. Some taxa, such as *Plagiogramma tenuissimum* (= *Ambo tenuissimum*), are related to sandy sediments (epipsammic) because they adhere to sand grains and form chains (Méléder et al., 2007). Interesting to highlight that the more frequent diatom from salt marshes of southern Brazil differ from the dominant species of these environments in other countries.

## 5. Conclusion

The composition of taxa better than the richness, evenness, and diversity revealed the benthic diatoms variation between the sites in the winter and summer seasons, during El Niño and La Niña episodes. In winter, high rainfall and freshwater runoff maintained oligohaline conditions in the marshes, while in summer, flooding with meso-poly-euhaline waters changed the diatom composition. The spatial variation of the community was also associated with sediment granulometry, where biraphid taxa were more frequent in sandy sediment and monoraphids in silt and clay sites.

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