Hydrobiologia (2005) 544:77–88
DOI 10.1007/s10750-004-8335-9

Primary Research Paper

Diatom ecological preferences in a shallow temperate estuary (Ria de Aveiro, Western Portugal)

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Received 3 June 2004; in revised form 24 September 2004; accepted 28 December 2004

Key words: diatoms, ecological preferences, diversity, environmental parameters, multivariate analysis

Abstract

The study of the diatom ecological preferences was conducted from January 2002 to June 2003 in Canal de Mira, Ria de Aveiro, Western Portugal. Three sampling stations along a salinity gradient were sampled monthly, in new moon, at high and low tide. Salinity, temperature, pH, dissolved oxygen and nutrient contents were measured for each sampling station; chlorophyll *a* and diatom diversity and abundance were also evaluated. Canonical correspondence analysis was used to identify the environmental variables governing the composition and structure of diatom assemblage. The variation in the species data among the different reaches was strongly determined by the salinity spatial gradient and by the temperature temporal gradient. The lower reaches were dominated by marine species (e.g. *Auliscus sculptus*, *Chaetoceros densus*, *Fallacia forcipata*, *Licmophora flabellata*, *L. grandis*, *Surirella comis*), while in the most upstream station typical freshwater species dominated (e.g. *Caloneis permagna*, *Cymatopleura solea*, *Cymbella tumida*, *Gomphonema longiceps*, *Pinnularia stommatophora*, *Stauroneis smithii*). Weighted averaging was used to estimate optima and tolerances of some diatom *taxa* for the most influential variables. It was possible to establish groups of *taxa* with defined and distinctive salinity and temperature preferences.

Introduction

Diatom studies of an applied nature in estuaries and shallow coastal waters have been few in number, especially when compared to the situation in freshwaters and the ocean (Sullivan, 1999). Diatoms are valuable indicators of environmental conditions, since they respond directly and sensitively to many physical, chemical and biological changes that occur in these systems. Moreover, a number of species have fairly narrow ecological ranges that makes them useful indicators of environmental changes for parameters such as salinity, pH, temperature and nutrient concentrations. The species-specific sensitivity of diatom eco-physiology to many habitat conditions is manifested in the great variability in biomass and species composition of diatom assemblages (Snoeijs, 1999). The fact that each diatom species has a specific optimum and tolerance for some environmental parameters, including pH, salinity, temperature, nutrients and light availability, makes them particularly useful indicators in estuarine systems (Lim et al., 2001).

Among unicellular microalgae, diatoms probably represent one of the most diverse groups, with a number of species estimated to be between 10 000 and 100 000. Therefore, they constitute an ideal group to study biodiversity and to understand the factors controlling it. The composition of diatom communities reflects an entire complex

of ecological parameters at a particular site (van Dam et al., 1994).

There are a variety of diatom habitats within an estuary. Freshwater diatoms may be brought in by river flow and marine species may be transported into brackish areas by tidal action, while estuarine species flourish in the productive mixing zones. Samples generally give a clear picture of diatom community structure, including benthic and planktonic species (Cooper, 1999).

The purpose of this study was to investigate the diatom ecological preferences along the Canal de Mira, Ria de Aveiro. The relationship between diatom assemblage and the environmental parameters governing their composition and structure was also focused in this study using the canonical correspondence analysis (CCA) multivariate analysis. The ecological optima and tolerances were estimated for the parameters and diatom species that stood out in the CCA biplot.

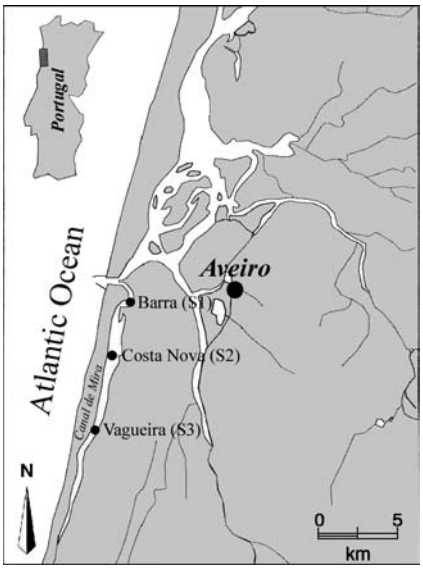

Figure 1. Map with indication of the sampling places in Canal de Mira – Ria de Aveiro. S1 – Barra (40°38′ N; 08°44′ W); S2 – Costa Nova (40°36′ N; 08°44′ W); S3 – Vagueira (40°33′ N; 08°45′ W).

Material and Methods

Study site and sampling strategy

Ria de Aveiro is a mesotrophic shallow estuarine system, also described as a coastal lagoon, on the Northwest coast of Portugal (40°38′ N; 8°44′ W) (Cunha et al., 2003). According to the estuaries classification proposed by Pritchard (1989), Ria de Aveiro can be considered a bar-built estuary. This well-mixed estuarine system has a maximum length of 45 km and width of 10 km and is connected to the Atlantic Ocean through a narrow channel (Fig. 1). The tidal range varies from 0.6 m in neap tides to 3.2 m in spring tides, with an average of about 2 m (Dias et al., 2000).

The Ria has a very irregular and complex morphology, with four main arms spreading from the mouth towards different directions, forming a multi-estuarine ecosystem (Almeida et al., 2002). In this study we chose Canal de Mira, which is an elongated shallow arm, with 20 km length and an average depth of 1.5 m. This channel is independent of the other arms and can be considered a small estuary in itself. Is characterized by a clear longitudinal salinity gradient influenced by the tides and seasonal cycles and offers advantages of accessibility. Three sampling sites were established in Canal de Mira, along the salinity gradient, designated by S1 – Barra, S2 – Costa Nova and S3 – Vagueira (Fig. 1). Sampling was performed monthly, during a period of 18 months (January 2002 to June 2003: two spring seasons were covered), always in new moon, in low tide (LT) and high tide (HT), at the water subsurface.

Environmental parameters

At each site pH, salinity, water temperature and dissolved oxygen were measured, *in situ*, with a WTW MultiLine P4 portable meter. Water samples for chemical analyses and chlorophyll *a* quantification were collected and immediately stored in the dark and at low temperature (4 °C), until further processing was possible. In the laboratory these water samples were filtered through GF/C filters (1.2 μm pore diameter), for quantification of photosynthetic pigments. Filtrates were used for determination of nutrient contents. Nitrate concentration was measured by the sodium salicilate method, according to Rodier (1984). Nitrite concentration was determined by the sulfanilic acid and α-naphtylamine method according with Rodier (1984). The analysis of ammonia was

performed by the indophenol blue technique, following the recommendations and procedures of Hall & Lucas (1981). For the phosphate, in the form of orthophosphate, was used the stannous chloride method (APHA, 1992). Silica was determined by the molibdosilicate method (APHA, 1992). Chlorophyll *a* was determined spectrophotometrically at 665 and 750 nm, before and after acidification (Strickland & Parsons, 1972) and calculated according to Lorenzen's (1967) monochromatic equation.

Biological sampling

For qualitative diatom study were used *in vivo* samples, taken from the water subsurface with short tows of a 25 μm mesh-size plankton net. These samples allowed a preliminary taxonomic survey, using a bright-field microscope. Samples for taxonomic and quantitative study were cropped with a glass bottle (1 l capacity) at the water subsurface (in 'well-mixed' estuaries, as it is the case of Ria de Aveiro, the vertical distribution of the salinity is uniform) and immediately preserved with Lugol 1% (iodine/iodide potassium). In the laboratory, samples were concentrated by settling during 8 days. After preparing the appropriate concentration (which depends on the cell density in the initial sample), samples for taxonomic and quantitative study were submitted to a digestion process with concentrated nitric acid and potassium dichromate, in order to digest any organic matter (present in large amounts in many samples). This process took place during approximately 3 days, at the end of which samples were rinsed with distilled water. A small sedimentation chamber was prepared by adhering (with silicone) an acrylic ring to a coverslip. A fixed volume (1 ml) of each detritus-free sample was then pipetted into the chamber and the liquid was evaporated at room temperature (20–25 °C), away from dust particles. When these samples were dry and shown to have a homogeneous distribution, the ring was removed and the coverslips were mounted on a glass microscope slide with Naphrax®. Similar methodologies were successfully employed in the diatom quantification by other authors (e.g. Almeida & Gil, 2001; Lim et al., 2001). The taxonomic study was performed on a bright-field microscope Olympus CX 31 and, when necessary, on a Scanning Electron Microscope (SEM) JEOL JSM-6301 F; in this case, samples were washed by centrifugation and an aliquot of each sample was transferred, with a micropipette, to an aluminium stub and air-dried. The whole 1-ml aliquot was always counted. At least 400 diatom valves were enumerated on each slide. Diatom species were identified using the standard floras of Peragallo & Peragallo (1897–1908), Germain (1981), Hustedt (1985), Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Round et al. (1990), Sims (1996), Tomas (1996) and Witkowski et al. (2000).

Data analysis

Diatom diversity (H′) was estimated using the Shannon–Wiener's index. A three-way analysis of variance (ANOVA) without replication was used to assess the differences between sampling stations, tides and months, for density and diversity (Zar, 1996). Prior to testing, normality and homoscedascity of data were checked. Differences at $p \leq 0.05$ level were accepted as significant.

The relationship between diatom densities and environmental variables was investigated by means of Canonical Correspondence Analysis (CCA) using the CANOCO version 4.0 package (ter Braak & Smilauer, 1998). CCA extracts synthetic gradients from the biotic and environmental matrices, which are quantitatively represented by arrows in graphical biplots (ter Braak & Verdonschot, 1995). The length of the arrow is relative to the importance of the explanatory variable in the ordination, and arrow direction indicates positive and negative correlations. Prior to analysis, diatom abundances were log transformed and environmental variables were standardized. Downweighting of rare species was performed. A forward selection procedure was performed on the set of environmental variables. A Monte Carlo test using 199 permutations ($p \leq 0.05$) was performed to test the significance of the correlations between the environmental factors and the species distributions. Only the significant variables were included in the model (ter Braak & Verdonschot, 1995).

Weighted averaging was used to estimate optima ($\hat{u}_k$) and tolerances (t_k) of salinity and temperature for the *taxa* that stood out in the CCA diagram. Because *taxa* had unequal occurrences,

were used the recommendations of Birks et al. (1990) and the number of occurrences were used to adjust the tolerance assigned to each *taxon*.

Formulae were as follows:

$$\hat{u}_k = \sum_{i=1}^{n} y_{ik} x_i / \sum_{i=1}^{n} y_{ik},$$

$$t_k = \left[\sum_{i=1}^{n} y_{ik} (x_i - \hat{u}_k)^2 / \sum_{i=1}^{n} y_{ik} \right]^{1|2},$$

where $\hat{u}_k$ is the optimum value of the *taxon k*, t_k is the tolerance of the *taxon k* for the parameter in cause, y_{ik} is the abundance of the *taxon k* in sample *i* and x_i is the value of the parameter in sample *i*.

Results

Environmental parameters

The range of the environmental variables (retained by a forward selection procedure) is presented in Table 1. During the study period a clear longitudinal salinity gradient was observed between station S1 and station S3, varying from 0.0 g l^{-1}, in S3, in April 2003, to 36.9 g l^{-1}, in S1, in October 2002. The water temperature exhibited the typical seasonal pattern of temperate estuaries, reaching maximum values in summer and minimum values in autumn/winter months. The lowest temperature (9.5 °C) was recorded in February 2003, in S2 and the maximum attained was 21.8 °C, in June 2003, in S3. During the study period gentle fluctuations were observed for pH.

The highest concentrations of ammonia were registered in S1, where a large peak was verified in September (0.444 mg l^{-1} N-NH_4^+). The highest values were registered in autumn/winter, decreasing in spring/summer months. The phosphate concentration ranged from a minimum value of 0.001 mg l^{-1} P-PO_4^{3-} registered in December 2002 to a maximum value of 0.227 mg l^{-1} P-PO_4^{3-} achieved in November 2002. In general, the highest values of phosphate were registered in July, September and October 2002.

Diatom assemblages

The three most abundant species of the diatom assemblage were *Pseudo-nitzschia seriata* (10%), *Navicula radiosa* (8%) and *Paralia sulcata* (6%). The marine species *Biddulphia tridens*, *Campylodiscus fastuosus*, *C. intermedius*, *Chaetoceros didymus*, *C. socialis*, *Fallacia forcipata* and *F. tenera* were only found at station S1. On the other hand the freshwater species *Caloneis permagna*, *Cymatopleura solea*, *Cymbella tumida*, *Gomphonema longiceps*, *Pinnularia stommatophora*, *Stauroneis smithii* and *Surirella elegans* were restricted to S3. Station S2 is a sampling station with typically brackish characteristics, which are also reflected in the diatom assemblage.

Diatom densities varied from 1.3×10^3 cells l^{-1} in June 2003 at station S1 to 5.6×10^5 cells l^{-1} in September 2002 at station S1 (Fig. 2). Significant differences of density between the two tides were found (Table 2), being quite higher in LT but no seasonal pattern was observed. A decrease of species density towards S3 was verified. Regarding diatom diversity (Fig. 3), significant differences

Table 1. Ranges of environmental parameters during the study period, in the three sampling places

	Barra			Costa Nova			Vagueira		
	Min	Max	Average ± SD	Min	Max	Average ± SD	Min	Max	Average ± SD
Sal (g l^{-1})	17.6	36.9	31.9 ± 4.5	1.0	36.7	24.8 ± 11.0	0.0	33.7	15.1 ± 10.8
T (°C)	11.2	19.7	15.7 ± 2.2	9.5	20.7	15.7 ± 2.6	10.2	21.8	16.6 ± 3.3
pH	6.39	8.31	8.03 ± 0.45	6.83	8.30	8.00 ± 0.31	7.50	8.45	8.05 ± 0.25
NH_4^+ (mg N l^{-1})	0.003	0.444	0.060 ± 0.088	ND	0.146	0.042 ± 0.031	ND	0.132	0.043 ± 0.035
PO_4^{3-} (mg P l^{-1})	0.001	0.227	0.036 ± 0.051	0.003	0.150	0.038 ± 0.040	0.011	0.168	0.055 ± 0.042

ND-undetermined value. (Sal – salinity; T – water temperature; NH_4^+ – ammonia; PO_4^{3-} – phosphate). Only variables retained by a forward selection procedure (Monte Carlo permutation test) are presented.

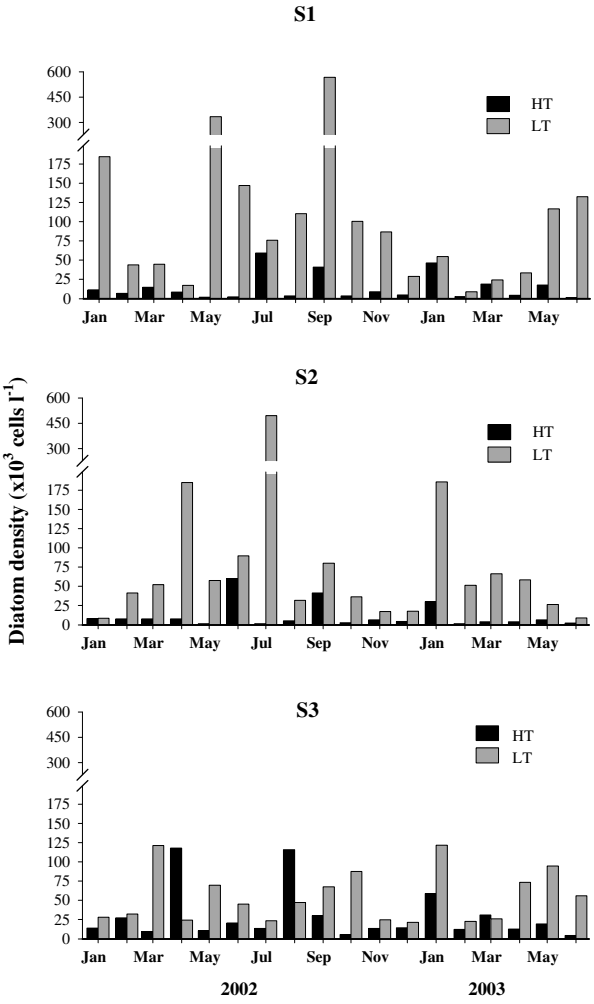

Figure 2. Variation of diatom density (cells l^{-1}) throughout the sampling period, at low tide (LT) and high tide (HT), in the three sampling stations.

Table 2. Summary of the three-way ANOVA for the biological parameters

Parameters	Source of variation	*f*	df	*p*
Diatom density	Site	0.8	2, 34	NS
	Tide	20.0	1, 34	<0.001
	Month	0.9	17, 34	NS
Diatom diversity	Site	0.1	2, 34	NS
	Tide	8.4	1, 34	0.006
	Month	5.9	17, 34	<0.001

The effect of sites, tides and months were analysed for each parameter (df-degrees of freedom; NS-non significant).

among tides and months were observed (Table 2). An increase of diversity was detected from April 2002 to June 2002. Afterwards, species diversity dropped in July 2002, when community was dominated by *Pseudo-nitzschia seriata* (4.3×10^5 cells l^{-1}), accounting for 73% of the diatom

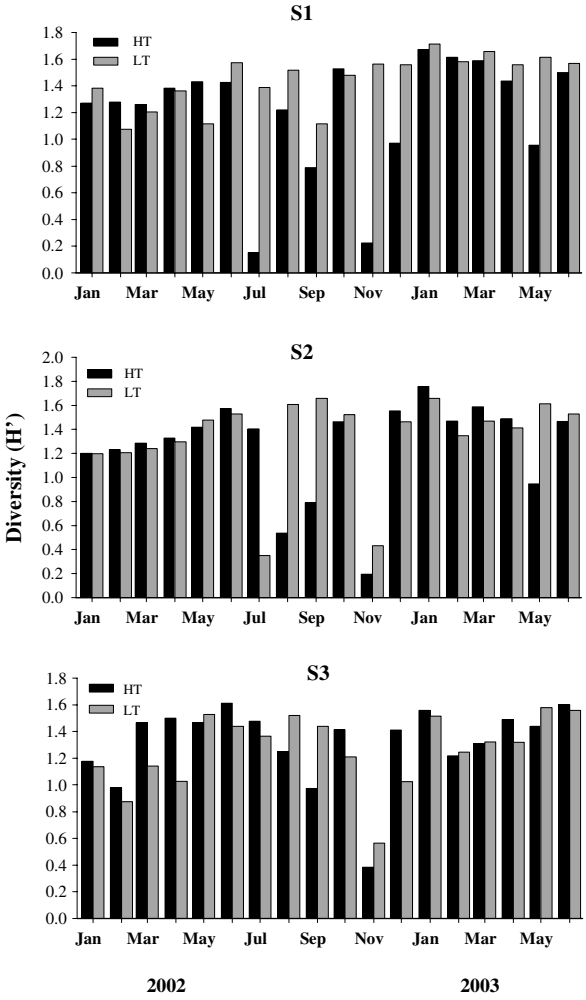

Figure 3. Variation of diversity (Shannon–Wiener's index) relative to the diatom assemblage, throughout the sampling period, at low tide (LT) and high tide (HT), in the three sampling stations.

community. In November diversity decreased drastically (except at S1, in LT), being registered the lowest values. In January 2003 an increase of the diversity was verified and the community was dominated by *Aulacoseira granulata* and *Tryblionella apiculata*. From February 2003 to June 2003 no clear pattern of diversity was observed.

Ordination resulting from the CCA produced the species-data biplot presented in Figure 4. Forward selection of environmental variables retained seven significant variables, in decreasing importance, which influenced diatom distribution: salinity, distance to the mouth of the estuary, temperature, tide, phosphate, ammonia and pH. The seven environmental variables considered in the CCA explained 18.2% of the total variation of the diatom assemblages. The first two axes of the species–environmental variables biplot alone accounted for 11.3% of the total variability (Table 3). The spatial distribution of diatom *taxa* is clearly linked with salinity (tide and distance to the mouth of the estuary are closely related to the salinity), and the temporal distribution is mainly determined by the temperature. For example, the species *Auliscus sculptus*, *Chaetoceros densus*, *Fallacia forcipata*, *Licmophora flabellata*, *L. grandis* and *Surirella comis* typically inhabit the marine station, with high frequencies at the highest salinity values, being situated at the lower right quadrant. Several *taxa*, such as *Bacillaria*

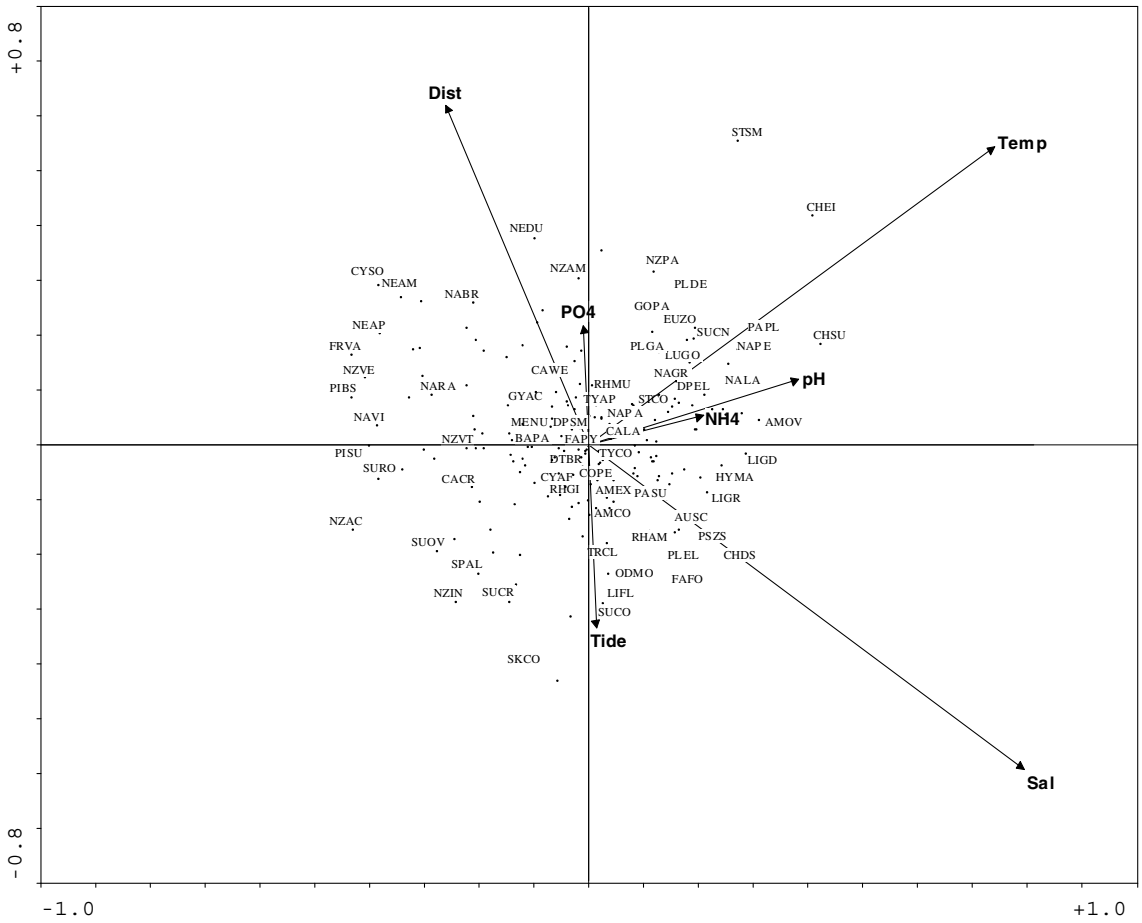

Figure 4. Results of CCA ordination: diatom *taxa* and environmental variables for the studied period. Dist – distance to the mouth of the estuary; PO4 – phosphate; Temp – water temperature; NH4 – ammonia; Sal – salinity. Points represent diatom species scores. Only selected *taxa* (see Table 4) are identified with corresponding abbreviation.

Table 3. Summary of CCA of diatom species and explanatory variables for the studied period

	Axis 1		Axis 2
Eigenvalues	0.101		0.067
Species–variable correlations	0.877		0.795
Cumulative percentage variance			
of species data	6.8		11.3
of species–variable relation	37.3		62.1
Sum of all unconstrained eigenvalues		1.498	
Sum of all canonical eigenvalues		0.272	

The statistics are only presented for the first two axes.

paxillifer, *Fallacia pygmaea*, *Gyrosigma acuminatum* and *Melosira nummuloides* have highest frequencies in the mid-salinity range while, for example, *Cymatopleura solea*, *Neidium ampliatum*, *N. dubium* and *Pinnularia brebissonii* are characteristic of the freshwater environments, being situated in the upper left quadrant of the plot, associated with low salinity values. The species

Chaetoceros eibenii, *C. subsecundus*, *Nitzschia palea* and *Stauroneis smithii*, among others, are observed in the upper right side of the biplot, associated with high temperatures, while *Nitzschia acicularis*, *Nitzschia insignis*, *Skeletonema costatum* and *Surirella robusta* are associated with low temperatures, at the lower left quadrant. Marine station (S1), especially at HT, is situated mainly in the lower right quadrant of the diagram, associated with the salinity vector (Fig. 5a). With few exceptions, the freshwater station (S3), mainly at LT, occupies the opposite side of the salinity vector. The brackish water station (S2) is principally situated in the centre of the plot, which means that it was not strongly influenced by any variable. Freshwater station (S3) is also more influenced by the nutrient concentrations, namely $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$, being situated at the top of the biplot, more or less opposing to the salinity vector. In Figure 5b it is possible to verify that most of the winter samples are situated opposite to the temperature vector. On the other hand, most of the samples collected in the spring/summer months are associated with the temperature vector.

Ecological preferences

Relationship between diatom *taxa* and their ecological preferences can be estimated by the weighted averaging. Table 4 shows the optima ($\hat{u}_k$) and tolerances (t_k) estimated for the *taxa* that stood out in the CCA, for salinity (A) and temperature (B), which were also the variables that stood out in the diagram. *Taxa* optima can be related with the environmental parameters in cause according to their proximity to the extremity of the arrow on the CCA biplot (Fig. 4). In Table 4A it is possible to identify three spatial-tidal distinct groups, according to the salinity optima and tolerances: freshwater, brackish and a marine group, already observed in the CCA diagram for the sampling stations and tides (Fig. 5a). The stenohaline species (freshwater or marine species) presented tolerances between 0.1 and 11.7, while eurihaline brackish species presented higher tolerances, ranging from 11.6 to 14.1. Table 4B presents two temporal distinct groups, in agreement with the temperature optima: a clear winter group and a second group clustering spring, summer and autumn months (already cleared in the CCA biplot for seasons) (Fig. 5b).

4. Discussion

The diatom genera found in this study resembles the genera found in other estuaries, for example, in Schelde estuary (Muylaert et al., 2000), in Urdaibai estuary (Trigueros & Orive, 2001), and in Nérvion River estuary (Urrutxurtu et al., 2003), but some differences were registered to the species level.

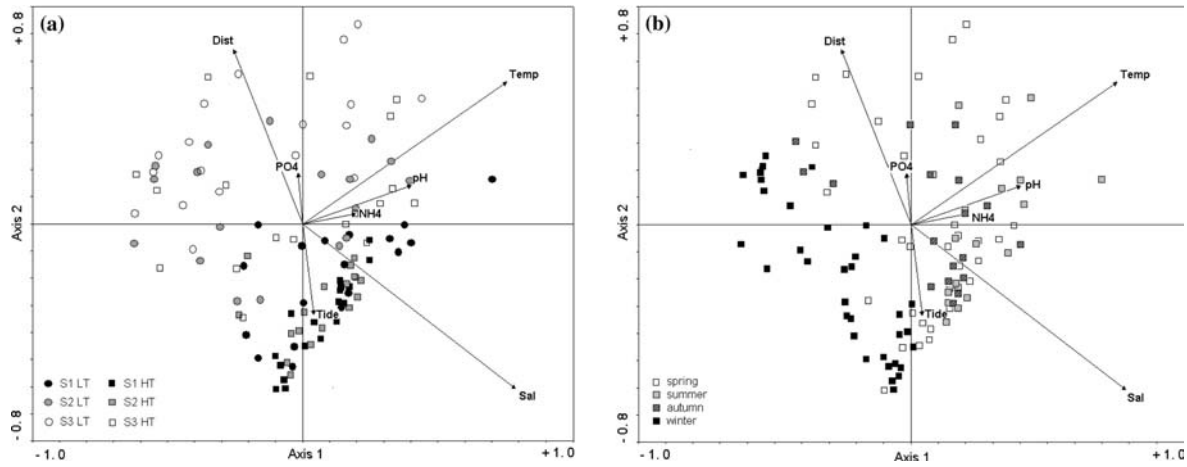

Figure 5. Results of CCA ordination: (A) biplot of sampling stations (including tide) and environmental variables; (B) sampling units according to seasons of the year and selected environmental variables.

Table 4. Optima ($\hat{u}_k$) and tolerances (t_k) for (A) salinity (g/l) and (B) temperature (°C) of the *taxa* (with codes) that stood out in the CCA diagram. (F – freshwater species, B – brackish species, M – marine species)

Taxa	Code		Salinity		Taxa	Code	Temperature	
			$\hat{u}_k$	t_k			$\hat{u}_k$	t_k
(A)					(B)			
Nitzschia vermicularis	NZVE	F	1.2	0.6	*Nitzschia acicularis*	NZAC	10.7	0.8
Pinnularia brebissonii	PIBS	F	1.3	0.8	*Surirella robusta*	SURO	11.9	2.3
Fragilaria vaucheriae	FRVA	F	1.6	1.3	*Surirella ovalis*	SUOV	12.2	0.1
Cymatopleura solea	CYSO	F	1.7	0.6	*Stephanodiscus alpinus*	SPAL	12.5	0.5
Neidium ampliatum	NEAM	F	2.6	2.5	*Skeletonema costatum*	SKCO	12.5	0.8
Neidium apiculatum	NEAP	F	4.3	4.2	*Nitzschia insignis*	NZIN	12.6	0.9
Navicula brasiliana	NABR	F	4.3	3.5	*Caloneis crassa*	CACR	13.1	1.7
Neidium dubium	NEDU	F	7.6	5.7	*Surirella crumena*	SUCR	13.2	0.2
Nitzschia amphibia	NZAM	F	8.9	6.4	*Navicula radiosa*	NARA	14.4	2.4
Navicula viridula	NAVI	F	9.2	6.6	*Cymbella affinis*	CYAF	15.0	2.2
Navicula radiosa	NARA	F	17.6	11.7	*Diploneis smithii*	DPSM	15.3	2.6
Tryblionella apiculata	TYAP	B	15.0	12.8	*Ditylum brightwellii*	DTBR	15.6	2.0
Nitzschia vitrea	NZVT	B	15.3	11.9	*Amphora exigua*	AMEX	15.6	2.9
Amphora commutata	AMCO	B	18.4	12.2	*Caloneis latiuscula*	CALA	15.7	1.9
Bacillaria paxillifer	BAPA	B	19.0	14.1	*Cocconeis peltoides*	COPE	15.9	2.5
Diploneis smithii	DPSM	B	19.1	13.1	*Bacillaria paxillifer*	BAPA	16.0	2.4
Melosira nummuloides	MENU	B	19.3	11.6	*Fallacia pygmaea*	FAPY	16.3	2.7
Caloneis westii	CAWE	B	19.9	11.7	*Tryblionella coarctata*	TYCO	16.8	2.5
Rhopalodia gibba	RHGI	B	20.2	12.6	*Navicula palpebralis*	NAPA	17.6	2.3
Fallacia pygmaea	FAPY	B	21.3	11.8	*Gomphonema parvulum*	GOPA	17.7	1.8
Gyrosigma acuminatum	GYAC	B	21.7	11.9	*Staurosira construens*	STCO	17.8	0.9
Rhopalodia musculus	RHMU	B	21.9	12.9	*Paralia sulcata*	PASU	17.9	1.1
Chaetoceros densus	CHDS	M	23.3	6.4	*Placoneis gastrum*	PLGA	17.8	1.8
Trachyneis clepsydra	TRCL	M	27.9	4.1	*Navicula peregrina*	NAPE	18.1	1.3
Pseudo-nitzschia seriata	PSZS	M	29.1	3.2	*Amphora ovalis*	AMOV	18.2	0.4
Rhaphoneis amphiceros	RHAM	M	30.0	3.5	*Navicula gregaria*	NAGR	18.3	1.3
Odontella mobiliensis	ODMO	M	30.5	5.5	*Chaetoceros subsecundus*	CHSU	18.3	1.5
Surirella comis	SUCO	M	30.7	2.0	*Navicula lanceolata*	NALA	18.4	1.0
Auliscus sculptus	AUSC	M	31.5	4.1	*Planothidium delicatula*	PLDE	18.6	1.6
Hyalodiscus maculatus	HYMA	M	32.4	0.8	*Eucampia zodiacus*	EUZO	18.7	0.2
Licmophora flabellata	LIFL	M	32.5	4.8	*Surirella constricta*	SUCN	18.7	0.8
Paralia sulcata	PASU	M	32.6	5.0	*Luticola goeppertiana*	LUGO	18.7	1.5
Pleurosigma elongatum	PLEL	M	33.0	3.0	*Nitzschia palea*	NZPA	18.7	1.8
Fallacia forcipata	FAFO	M	34.1	0.1	*Parlibellus plicatus*	PAPL	19.0	1.6
Licmophora gracilis	LIGR	M	34.1	1.3	*Stauroneis smithii*	STSM	21.1	0.1
Licmophora grandis	LIGD	M	34.2	1.8	*Chaetoceros eibenii*	CHEI	21.2	0.1

During this study, and contrarily to the verified by Trigueros & Orive (2001) and Revilla et al. (2002) in Urdaibai estuary and Urrutxurtu et al. (2003) in the Nervión River estuary, no seasonal pattern of diatom density was observed. Diatom diversity revealed significantly differences among months, varying with a relative irregularity during the sampling period. Although it was not possible to establish a clear longitudinal pattern of diversity, a slight decrease from the lower to the upper

estuary was verified, basically due to the salinity gradient that diatom assemblages must support along the estuarine segment.

In the Ria de Aveiro estuary the spatial diatom assemblage composition along the different reaches was mainly controlled by the effect of the river inflow and tidal incursion. The unidirectional longitudinal salinity profile established between S1 and S3 determines the spatial structure of the diatom assemblage. In fact, differences in the composition and structure of diatom assemblage were noted between lower and upper reaches. Station S1 was dominated by marine species while in S3 dominated freshwater species. Although most diatom species in the Ria de Aveiro estuary occur within relatively broad salinity ranges, some stenohaline *taxa* were also observed. Two species (*Diploneis didyma* and *Grammatophora marina*) were observed in the three sampling stations, in the two tides and along the 18 months of sampling. These species are considered to be ubiquitous, being distributed among places and periods with very distinct physical and chemical characteristics. In what concerns to the temporal gradient, determined by the temperature, seasonal differences of distribution were registered, according to the temperature optima.

These findings were confirmed by the CCA multivariate analysis, which suggested that the main environmental gradient was formed by the salinity changes along the Canal de Mira. In the CCA diagram salinity and tide are correlated, being both ordered in the lower right quadrant. In this quadrant are represented mainly the samples collected at station S1. This place is located near to the ocean and for that reason it receives a great influence of the seawater salinity. Most of the samples collected in the HT were also situated in this quadrant which was expected since the salinity was higher in this tide situation. Temperature has also an important effect. Samples of the warmer and shallower sampling station (S3) tend to cluster separately from the colder and deeper station (S1 and S2). The deeper, larger sites such as the stations S1 and S2 will take longer to heat up and cool down than the shallower S3. This is in well agreement with the study carried out by Lim et al. (2001). The species collected in the winter months, situated on the lower left quadrant of the plot, in the opposite side to the temperature vector, are clearly separated from those collected in the remaining period. NH_4^+ and PO_4^{3-} were also selected by the forward selected procedure; however, their importance is relatively reduced when compared with the other selected variables. *Taxa* with broad ecological tolerances for the measured environmental variables are all ordered near the central origin to the CCA. As is often the case in multivariate analysis of species data, a large part (81.8%) of the variation remained unexplained by other environmental variables. Such uncertainty is probably associated with grazing pressure (from zooplankton), competition for resources among species, river flow, diatom ressuspension and other factors, which might have provided further explanatory information about the phytoplankton variability. Therefore, in order to fully comprehend the intricate trophic interactions of aquatic systems, they should be integrated in such models. Another sound factor that may contribute to the high percentage of unexplained variance is the presence of many species with broad tolerances for the environmental gradient (centre of the plot), whose contribution for the CCA is practically null.

The salinity and temperature optima estimated are in well agreement with the species distribution in the CCA diagram. Comparing with the temperature optima calculated by Weckström et al. (1997) in Finlandia lakes, the values estimated for the same *taxa* encountered in Ria de Aveiro estuary were systematically higher (e.g. the temperature optima estimated for *Nitzschia palea* in Finlandia lakes was 12.4, while in this study was 18.7). This is probably due to the higher mean water temperature registered in this work (about 16 °C) in comparison to that (12.9 °C) of Weckström et al. (1997). However, these differences are not enough to allow attributing another temperature preference of these species in the temperature gradient. Due to the lack of literature on the optimal salinity and temperature for diatoms in estuaries, it is difficult to assess the reliability of the results obtained in this study. The optima and tolerances were estimated for the species found in Ria de Aveiro. However, some freshwater species were brought in by river flow and some marine species have been transported into brackish areas by tidal action. Therefore, estimating the optima and tolerances for these species in their habitat, would probably result differently. In the case of the freshwater and

marine clusters, instead of optima or ecological preferences it can be assumed that these values can correspond to the limit ecological conditions in a mixed environmental system. The brackish water cluster is, in fact, the estuarine assemblage, constituted by the resident species, suggesting that optima and tolerances were reliable in this case. This cluster revealed greater salinity tolerances than those estimated for the marine and freshwater clusters. The broad salinity tolerances of these *taxa* enable them to inhabit the estuarine environments (especially the middle reaches), submitted to high salinity fluctuations. The stenohaline species, with narrower salinity tolerances, can only occur within narrow salinity ranges (either marine or freshwater).

The difficulty in the interpretation and reliability of the estimated apparent optima and tolerances is a common problem in ecology. In some cases, a species is characterised by an individual behaviour in one system, while in another aquatic system behaves differently, thus leading to different optima and tolerances. According to Nygaard (1996) these differences are probably a consequence of the existence, within several species, of two or more types with different tolerances and environmental requests. This has obviously important consequences also on the reliability of the indicator value of species in different environments. As an example, *Navicula radiosa* is usually described as a freshwater species. However, in this study the optimum and tolerance values estimated for this species are typically values from brackish waters. Furthermore, there is a potential bias associated to the estimation of the optima and tolerances, as a consequence of the tidal and fluvial transport. Some contamination of the stations due to the passive transport has to be considered. Additionally, and contrarily to the tidal transport, the fluvial transport is not constant, showing a seasonal fluctuation. In this way, the estimated tolerances may be overestimated in some cases.

Certain *taxa* associated with the gradient extremes for salinity and temperature may be used in future studies as potential indicator species for these variables changes. For example, *Chaetoceros densus*, *Fallacia forcipata* and *Licmophora grandis* were all found at relatively high density associated with the salinity gradient extreme. On the other hand, the species *Cymatopleura solea*, *Neidium ampliatum* and *Pinnularia brebissonii* were more common at station S3, associated with lower salinity values, and were rare or absent at station S1, associated with higher salinity values.

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