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Phytoplankton community structure defined by key environmental

variables in Tagus estuary, Portugal

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7 Abstract

8 In this work we analyze environmental (physical and chemical) and biological 9 (phytoplankton) data obtained along Tagus estuary during three surveys, carried out in 10 productive period (May / June / July) at ebb tide. The main objective of this study was 11 to identify the key environmental factors affecting phytoplankton structure in the 12 estuary. BIOENV analysis revealed that, in study period, temperature, salinity, silicate 13 and total phosphorus were the variables that best explained the phytoplankton spatial 14 pattern in the estuary (Spearman correlation, $\rho=0.803$). A generalized linear model 15 (GLM) also identified salinity, silicate and phosphate as having a high explanatory 16 power (63%) of phytoplankton abundance. These selected nutrients appear to be 17 consistent with the requirements of the dominant phytoplankton group, 18 Baccilariophyceae. Apparently, phytoplankton community is adapted to fluctuations in 19 light intensity, as suspended particulate matter did not come out as a key factor in 20 shaping phytoplankton structure along Tagus estuary.

Keywords: Phytoplankton structure, nutrients, salinity, spatial scale, productive period,
estuaries.

23

24 **1. Introduction**

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25 Phytoplankton composition is considered as a natural bioindicator because of its 26 complex and rapid responses to fluctuations of environmental conditions (Livingston, 27 2001). The main environmental factors recognized as controlling community structure 28 of phytoplankton are physical, (mixing of water masses, light, temperature, turbulence 29 and salinity) and chemical (nutrients). Regulation of phytoplankton in estuarine systems 30 is more complex due to the interaction of freshwater inputs and tidal energy (Alpine and 31 Cloern, 1992). Further, the increasing human population densities and industrial 32 development have contributed to the eutrophication of these ecosystems.

33 Tagus estuary, the major Portuguese estuarine system, represents an important 34 ecological system, still subjected to considerable urban and industrial pressures. 35 Regarding phytoplankton community of Tagus estuary, Cabrita and Moita (1995) 36 conducted studies on biomass (chlorophyll a), Cabeçadas (1999) investigated in lower 37 estuary photosynthetic response of phytoplankton and Oliveira (2000, 2003) studied 38 algal composition and abundance along the estuary. More recently Gameiro et al. (2004) 39 focused on the annual variability of phytoplankton composition and biomass, as well as 40 physical and chemical parameters, in the restricted upper estuary. A screening model 41 developed by Ferreira et al. (2005) derived some general features relating phytoplankton 42 species composition to hydrology of a number of estuarine systems including Tagus 43 estuary, giving an indication of the respective potential biodiversity. On the basis of 44 physical, chemical and phytoplankton biomass a recent study, carried out in Tagus 45 estuary, allowed distinct habitats to be identified in winter and summer. In the latter 46 period the increment of productivity was particularly noticeable in mid-estuary region 47 (Brogueira and Cabeçadas, 2006).

Environmental and phytoplankton data presented in this work were obtained duringthree surveys along the Tagus estuary carried out in the productive period, providing an

50 opportunity to explore potential reasons for differences in community composition. 51 Specifically, the aim of this study is to evaluate the phytoplankton dynamics and the 52 environmental variability along Tagus estuary and investigate the main environmental 53 factors affecting phytoplankton structure.

54

55 2. Materials and methods

56 2.1 Area description and sampling

57 Tagus estuary is one of the largest estuaries in the western European coast, covering an area of 320 km². The estuary is meso-tidal and its shallow upper part is occupied by 58 59 extensive tidal flats and salt marshes (Fig. 1). The main freshwater source is Tagus river, having an annual average flow of approximately $400 \text{ m}^3/\text{s}$, although its discharge 60 varies greatly from summer to winter between approximately 30 m³/s in a dry summer 61 62 and 2000 m³/s in a wet winter (SNIRH, 2006). Tagus river is the main nutrient source to 63 the estuary, but Sorraia and Trancão rivers also represent significant nutrient 64 contributors to the system. Although organic loadings to the estuary have been reduced 65 in recent years, partially treated or untreated effluents from greater Lisbon still enter the 66 estuary, particularly from southern shoreline, contributing approximately 30% for total 67 organic nitrogen and 10% for nitrate (IST, Maretec, 2002).

A total number of 19 stations were sampled in July 2001, May 2002 and June 2003along Tagus estuary (Fig. 1), always during ebb tide.

Surface water samples were collected with a Niskin bottle for measurement of dissolved oxygen (DO), nutrients (nitrate+nitrite referred as NO₃, ammonium (NH₄), phosphate (PO₄), silicate (Si(OH)₄), total nitrogen (TN), total phosphorus (TP), suspended particulate matter (SPM), and phytoplankton identification and abundance determination.

75

76 2.2. Physical and chemical measurements

Temperature (T) and salinity (S) were measured <u>in situ</u> utilizing an Aanderaa probe (CTD sensor), calibrated for salinity with an Autosal salinometer. Samples for DO determination were collected and analyzed according to Winkler method as described by Aminot and Chaussepied (1983). Water samples for nutrient determinations were immediately filtered through AcetatePlus MSI (MicroSeparation Inc.) filters and placed in a freezer. Samples for TN and TP measurements were preserved with sulfuric acid (4M) and maintained at 4°C until analysis (maximum 48h).

84 Nutrient analysis was carried out using a TRAACS autoanalyser following colorimetric 85 techniques outlined by the manufacturer. Estimated precision (10 replicates) are the 86 following: 2.6% for NO₃, 2.9% for PO₄, 0.2% for Si(OH)₄, 2.0% for NH₄ at middle 87 scale concentrations. Accuracy of nutrient measurements was maintained by using CSK 88 Standards (WAKO, Japan). TN and TP were determined, after simultaneous oxidation 89 according to the ISO methodology (1997) based on Koroleff method (1983), also 90 automatically. SPM was determined gravimetrically by filtering water samples through 91 pre-combusted Whatman GF/F filters and drying at 70 °C.

92

93 2.3. Phytoplankton

94 Phytoplankton samples (500 mL) were preserved immediately after collection with 95 acidified Lugol. Identification was performed using an Axioscop Zeiss microscope with 96 phase contrast. Counting was done according to Utermohl (1958) and Lund et al. (1958) 97 with an Invertoscop Zeiss IM 25. Species richness (S), expressed as species number per 98 sample (Pielou, 1975), was estimated considering all species identified during the study 99 period. Species diversity was calculated according to Shannon and Wiener (1963)

100 (H'= $p_i \log p_i$, $p_i=N_i/N$, N_i number of cells of species i and N total number of 101 cells/sample) and Evenness (J') as H'/log S (Pielou, 1975), using \log_2 in both 102 formulations.

103

104 2.4 Statistical Analysis

105 2.4.1 Multivariate Analysis

106 Multivariate statistical analysis was applied to environmental and phytoplankton data in 107 order to identify spatial trends or discrete groups and correlations between 108 environmental and phytoplankton variables. These analyses were undertaken using 109 PRIMER (v.6) software (Plymouth Routines in Multivariate Ecological Research, 110 Plymouth Marine Laboratory, Plymouth, U.K; Clarke and Gorley, 2006).

111 ANOSIM test

The analysis of similarity, ANOSIM (Clarke, and Green, 1988; Clarke 1993) was performed to test statistical differences in environmental and phytoplankton data among sampling months and sampling stations. We used a two-way crossed test, based on Bray-Curtis similarities (phytoplankton abundances, fourth-root transformed) and Euclidean distance matrix (environmental data, log(x+1) transformed) as our study focused on a fixed set of sites, sampled at several occasions.

118 PCA, MDS and BIOENV analysis

Environmental data (log(X+1) transformed) was handled using correlation-based
Principal Component Analysis (PCA), on the basis of standard Euclidean distance
between samples to define their dissimilarity.

122 Multidimensional scaling, MDS, was applied to phytoplankton abundance (4th root 123 transformed). Similarities between species are obtained by Bray-Curtis similarity 124 coefficient and the corresponding rank similarities used to construct MDS

125 configuration. Sampling points are arranged in a continuum such that points emerging 126 together correspond to sites in which species composition is similar, and points which 127 are far apart correspond to sites that are dissimilar. Stress levels of MDS representation 128 less than 0.1 indicate good representation of the data. Also on the basis of Bray-Curtis 129 similarities, the similarity percentages analysis (SIMPER) was applied to phytoplankton 130 species abundance, in order to allow the separation of every two groups of stations 131 according to phytoplankton species.

Further, patterns in community structure identified by MDS analyses were linked to environmental variables (based on Euclidean similarity index) by using the BIOENV method. This procedure allows identifying the environmental variables (individual or combined) that "best match" the patterns of community structure.

136 2.4.2. Univariate Analysis

Generalized Linear Model (GLM) was also applied to further explore the relationship between the environmental variables and phytoplankton abundance. This model, adequate for data with counts, uses the method of least squares, by linking the binary response to the explanatory covariates through the probability of either outcome, which does vary continuously from 0 to 1. The transformed probability is then modeled with an ordinary polynomial function, linear in the explanatory variables. This univariate analysis was carried out using Brodgar Software (2006).

144

145 **3. Results**

146 Based on ANOSIM analysis no significant differences among sampling months was 147 found, neither for most environmental data (ρ varying from -0.095 to 0.286, p>5%, 148 except for DO and SPM) nor for phytoplankton data. Therefore, data was averaged over 149 the three months, at each sampling site.

150

151 3.1. Environmental parameters

152 Spatial variations of environmental parameters along Tagus estuary during productive 153 period are shown in Fig. 2. Salinity mean values decreased progressively from a a 154 maximum of 34.5 at St.1 in the estuary mouth to a minimum of 0.3 at St. 14, the most 155 river-influenced site (Fig.2a). An opposite trend was observed in relation to water 156 temperature ranging from 17°C at St. 1 to 24-25 °C at Sts. 12-14 (Fig. 2b). 157 The system was well oxygenated, as oxygen saturation levels remained above 90%, 158 except at St. 15, nearby Montijo saltmarsh, where saturation decreased to 87%, 159 corresponding to 6.5 mg/l, the minimum value found in the estuarine surface water 160 (Fig. 2c). 161 Nutrient concentrations, corresponding mostly to moderate levels, decreased along the 162 estuary from riverine to marine, influenced area, reflecting in general, the main nutrient 163 discharge from Tagus river. NO₃ concentrations varied from 10 µmol/L at St.1 to 50-60 164 µmol/L at St.11-14 in the upper estuary (Fig. 2d). The same trend was observed for 165 Si(OH)₄, ranging from 4 µmol/L to 60 µmol/L (Fig. 2e), and for PO₄, ranging from 1 166 µmol/L to 3-4 µmol/L (Fig. 2f). TN distribution also reflects the major input from

167 Tagus river (maximum of 102 μ mol/L at St. 14), as well as the influence of the 168 extensive salt-marshes in the upper estuary (St. 8, 9 and 11) (Fig. 2g). TP also varied 169 from minimum values of 2-3 μ mol/L in the lower estuary (Sts. 1-3) to 6-7 μ mol/L in the 170 upper estuary (Sts. 13, 14) (Fig. 2h).

171 The existence of NH_4 point sources in the estuary is notorious, maximum value 172 (60µmol/L) being found at St. 15 (nearby Montijo saltmarsh) (Fig. 2i). However, high 173 values were also detected close to St. 16 (Seixal saltmarsh) and St. 4 (nearby Trancão 174 river discharge), respectively 18 and 13 µmol/L.

SPM varied quite irregularly from 21 to 58 mg/L all over the estuary (Fig. 2j). Besides St. 4 and St. 5 located in the vicinity of Trancão river discharge, which showed the highest levels of SPM, sites at the left margin of the estuary (Sts 15, 16, 17, 18, 19) close to extensive saltmarsh zones, exhibited considerably high variability of suspended matter levels.

180 Concerning nutrient stoichiometry, it can be observed (Fig. 3) that in the lower/middle 181 estuary (Sts. 1-8 and Sts.15-19) N:P and Si:P values were, in general, lower than 16 and 182 Si:N values lower than 1. By contrast, in the upper estuary (Sts. 9-14) N:P values were

183 in general balanced, some Si:P were above 16, while Si:N were close or above 1.

184

185 3.2 Phytoplankton Community Structure

186 *3.2.1 Composition and abundance*

187 Mean phytoplankton abundance ranged from a minimum value of 78 cells/mL at St. 18 188 in the lower/middle estuary to a maximum of 1600 cells/mL at St.11 in the upper 189 estuary (Fig. 4a). Bacillariophyceae was the most important algal group accounting for 190 87% of the total abundance (Table 1). The algal maximum occurring at St. 11 was 191 mostly due to proliferation of *Chaetoceros socialis* and *Melosira moniliformis* (Fig. 192 4b,c). Two other phytoplankton peaks were observed, one at St. 14 due to proliferation 193 of Stephanodiscus hantzschii (Fig. 4d) and the other at St. 8, mainly as consequence of 194 the development of Chaetoceros socialis and Skeletonema costatum (Fig. 4b,e). This 195 last euryhaline species, adapted to a large salinity range, is even developed at the upper 196 estuary, where the community was characterized by freshwater diatoms *Stephanodiscus* 197 hantzschii, Fragilaria crotonensis, Aulacoseira distans and A. granulata. At lower and 198 middle estuary other important Bacillariophyceae species were recorded, namely

Chaetoceros subtilis, Asterionellopsis glacialis, Cylindrotheca closterium, Detonula
pumila and Thalassionema nitzschioides.

201 Chlorophyceae attained 5% of the total algal abundance (Table1). Its maximum was 202 reached at St. 14, the most river-influenced site (salinity 0.30) being represented mainly 203 by freshwater species (*Scenedesmus acuminatus*, *S. armatus*, *Monoraphidium* 204 *contortum*, *Chlamydomonas* spp.). Cryptophyceae were important only at Sts. 4 and 6 205 (lower/middle estuary), accounting for 4% of the total algal abundance through 206 proliferation of *Plagioselmis* sp. and *Hilea fusiformis*.

The oceanic Prasinophyceae, representing 2% of total abundance, developed mainly at the estuary mouth (St. 1) (Table 1) and its principal species was *Phaeocystis pouchettii*. Euglenophyceae accounted only for 1% of total abundance, being mostly due to the development of *Eutreptiella marina* in the lower estuary. The contribution of Cyanobacteria and Dinophyceae and Ebriidea was below 1%. The most abundant dinoflagellates were nanoplanktonic *Amphidinium* species (Sts. 1 and 6) and the most common Cyanobacteria was *Merismopedia tenuissima* (St. 12).

214

215 *3.2.2. Diversity*

216 A total of 236 phytoplankton species were identified in Tagus estuary during the study 217 period. However, species richness (S) was moderate and no clear trend was apparent 218 along the estuary (Fig 5). The minimum value (25 species) was detected at Sts. 3, 18 219 and 19 in the euhaline -mesohaline zone, and the maximum (37 species) was detected at 220 St. 10 in the oligohaline-freshwater zone (Fig. 5), indicating the importance of 221 freshwater species in the estuarine community. Shannon diversity (H[']) presented high 222 values, most of them (84%) over 3.0, ranging from 1.7 (St. 11) to 4.6 (Sts. 6 and 17) 223 (Fig. 5). Evenness (J[']) was also high, and 79% of values surpassed 0.6 (Fig. 5). Changes

in J' closely mirrored changes in H', varying from a minimum of 0.4 at St. 11 to a
maximum of 0.9 at St. 17. The occurrence of a simultaneous reduction of H' and J' at
St. 11, where phytoplankton maximum was attained, reflects the strong dominance of *Chaetoceros socialis* and *Melosira moniliformis* (Fig. 4b,c).

228

229 3.3 Statistical Analysis

The application of PCA analysis to environmental data reveals that the first three PCs had eigenvalues greater than one (Table 2), indicating that all of them were significant. These components accounted for 89% of total variance, and represent a very good description of the environmental structure.

Variables T, NO₃, PO₄, Si(OH)₄, TN, and TP present highest positive loads to PC1 while S loads negatively (Table 3). This component, accounting for 59.7% of total variance (Table 2) is interpreted as the gradual mixing of nutrient-enriched fresh water with nutrient-poor saline water along the estuary (Fig. 6a,b). Position of most of stations along this axis gives support to this interpretation, St 14 (the most riverine) and St. 1 (at the estuary mouth) being more distant from each other.

240 PC2 accounted for 19% of total variance and variables NH_4 and DO are, respectively, 241 the main positive and negative contributors to this axis (Table 3). This can be 242 interpreted as a representation of "poor water quality" in specific areas as opposed to the 243 well oxygenated main body of the estuary. The isolation of St. 15, and to a minor 244 extent St. 4 and St. 16, connected with higher values of NH₄ and lower values of DO, 245 supports this interpretation (Fig. 6a,b). The remaining environmental variable, SPM, 246 contributes mostly to PC3 which still explains 10% of variation (Table 3). This 247 component is mainly associated with higher turbidity at Sts. 4 and 5, both located 248 nearby Trancão river discharge.

249

250 The MDS ordination of stations generated by phytoplankton abundance is illustrated in 251 Figure 7. Stress value associated with this 2-dimensional plot is 0.11, revealing that this 252 representation of stations is still sound. However, the 3-dimensional MDS (not shown) 253 presents a lower stress (0.07) indicating that samples are likely to fit more easily into 254 three dimensions. The successive position of the stations reveals algal communities 255 differences along the estuary. St 1 and St 14 are the most distinct sites as they differ 256 both in abundance and species composition (respectively marine and freshwater taxa) 257 According to cluster analysis (not shown) stations located in lower/middle estuary (St. 258 1-4 and 15-19) constitute a group (A) characterized by the presence of marine and 259 estuarine species (Thalassionema nitzschioides, Guinardia delicatula, Asterionellopsis 260 glacialis, Nitzschia longissima). Sts. 5-10 and 12, mostly located in the middle estuary, 261 formed another distinct group (B) with a mixed dominance of marine, estuarine and 262 some freshwater species (Melosira moniliformis, Chaetoceros subtilis, Thalassionema 263 nitzschioides, Chaetoceros socialis, Stephanodiscus hantzschii). Group C including Sts 264 11, 13 and 14 individualize the upper estuary due to higher abundance and prevalence 265 of freshwater phytoplankton species (Stephanodiscus hantzschii, Aulacoseira distans, 266 Cyclotella meneghiniana, Scenedesmus opoliensis). Consequently, Groups A and C 267 present the highest dissimilarity (81%, SIMPER analysis) while the average 268 dissimilarity between groups B-C and A-B attained respectively 65% and 62%. 269 BIOENV analysis reveals that environmental parameters have a strong correlation with 270 phytoplankton community along Tagus estuary (Table 4). The combination of variables 271 that best explained the phytoplankton pattern in the study period were T, S, $Si(OH)_4$ and

272 TP ($\rho = 0.803$) although correlation with S alone was nearly as large (0.796). Salinity is

a dominant factor and is the single variable that is retained in all of the best 10 sets of

- variables. SPM is still included in the best 10 results, though the respective group of
- 275 variables have relatively lower correlation with phytoplankton abundance ($\rho = 0.776$).
- The application of GLM analysis revealed the following relationship betweenphytoplankton abundance and environmental variables:
- 278 log (Phyto abundance) = $6.98815 0.07298\log(S) + 0.42153\log(PO_4) 0.02145\log(Si(OH)_4)$
- 279 This relationship, which includes S, Si(OH)₄ and PO₄, has a high explanatory power
- 280 (63%) for phytoplankton abundance.

281

282 **4. Discussion**

Both environmental and phytoplankton variables showed an upper-lower gradient along
Tagus estuary, despite nutrient point sources other than Tagus river being important
inputs to specific areas of the system.

286 The identified phytoplankton assemblages including, respectively, freshwater, estuarine 287 freshwater. and estuarine and marine species, and were dominated bv 288 Bacillariophyceae. The dominance of this group has been already reported by other 289 authors, either along the estuary (Oliveira, 2000; 2003) or in the upper (Gameiro et al., 290 2004) and lower estuary (Cabeçadas, 1999).

291 The total number of species (236) identified in this study was lower than that referred 292 by Moita and Vilarinho (1999). However, these authors also included phytoplankton 293 data from winter period, when Tagus river discharge is higher, and many freshwater 294 species enter the estuary. The majority of these allochthonous species do not remain as 295 components of the phytoplankton community all year round. In fact, in March 2001, 296 after a very rainy winter period, a considerable number of freshwater species (115) were 297 identified all along the estuary (Oliveira, 2003). By contrast, in July 2001 (data included 298 in this work) only 28 of these freshwater species survived at the upper estuary. On the

other hand, the screening model developed by Ferreira et al. (2005), using data from
1929 to 1998, predicted that in Tagus estuary, from a total number of 342 species, 120
were combined riverine and oceanic species, and only 222 were real estuarine. This
number compares well with the one found in our study.

303 The strong correlations revealed by BIOENV analysis support the view that, in the 304 study period, the phytoplankton in Tagus estuary, were more likely controlled by abiotic 305 factors than by biotic mechanisms, such as grazing by zooplankton. Despite data on 306 zooplankton not being available for all the study period, Monteiro (2003) reported the 307 occurrence in July 2001 of two zooplankton maxima, respectively, one at the 308 oligohaline-freshwater zone coinciding with the phytoplankton maximum, and the other 309 at the estuary mouth, with zooplankton abundance being low in the other estuarine 310 zones.

311 In fact BIOENV analysis identified salinity, temperature, silicate and phosphorus as 312 variables explaining the phytoplankton spatial pattern, although salinity alone has an 313 overriding role. Results from the GLM analysis agreed quite well with BIOENV by 314 selecting variables salinity, silicate and phosphate as explanatory variables of 315 phytoplankton abundance in Tagus estuary in the study period. The inclusion of silicate 316 and phosphorus emphasizes the nutrients requirements of the dominant algal group 317 (Bacillariophyceae), and may be related to the apparent unbalanced proportions of 318 nutrients in the estuary, revealed by shortage of Si in the lower/middle estuary and of P 319 in the upper estuary (van der Zee & Chou, 2005; Justic et al. 2005). Nevertheless, as 320 nutrient levels in the study period were higher than typical half saturation constants for 321 natural phytoplankton populations, growth of most species was unlikely to be nutrient 322 limited (Reynolds, 1999; Estrada et al., 2003).

323 Regarding the SPM variable, which varied irregularly along the estuary, concentrations 324 were above 10mg/L which represents a threshold value above which primary 325 productivity is usually inhibited (De Master et. al., 1993; Ragueneau et. al, 2002). 326 However, the apparent reduced light conditions appear adequate for Bacillariophyceae 327 growth. In fact, studies carried out by Cabeçadas (1999) on phytoplankton 328 photosynthetic responses in Tagus estuary concluded that, in the lower estuary 329 phytoplankton seemed partially adapted to high turbid conditions. It is known (Smayda 330 and Reynolds, 2001) that species of this group are characterized by lower-light 331 requirements and adaptation to fluctuations in light intensity induced by turbulent 332 mixing.

333 Despite nutrient discharges from point sources in specific areas of Tagus estuary, no 334 evidence of poor water quality relationships with changes in phytoplankton structure 335 could be found. In fact, Cyanobacteria and Dinophyceae, which are dominant in high 336 trophic state ecosystems, as well as Cryptophyceae whose predominance during the 337 summer is known to be an indicator of eutrophication (Moncheva et al. 2001), were all 338 minor contributors to the algal community. Indeed, they represented only 1-2 % of the 339 phytoplankton total abundance, suggesting that, at present, cultural eutrophication is not 340 a problem in this estuary. In addition, the high values of Shannon-Wiener index and 341 Evenness confirm that most phytoplankton communities are at equilibrium in Tagus 342 estuary.

This work can be valuable for the outlining of water quality monitoring programs, as it allows to take into account a reduced number of key environmental parameters able to characterize the phytoplankton community structure in Tagus estuary

346

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354

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Algal group								Statio	ons											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Bacillariophyceae	332	147	223	224	238	206	432	833	269	672	1562	612	400	934	165	218	116	71	73	
Chlorophyceae	13	3	4	7	19	10	24	7	29	5	48	11	59	229	2	6	0.7	0.6	0.8	
Cryptophyceae	15	10	8	110	0.4	97	5	3	0.8	28	0.2	8	0.4	0	11	25	1	0.6	8	
Cyanobacteria	0	0	0	0	0	0	0	0	3	0	0	22	0	2	3	0	0	0	0	
Dinophyceae	15	4	1	5	3	16	5	3	0.2	2	0	3	0	0	4	11	0.3	0.8	0.4	
Ebriidea	0	0.1	0	4	0	4	2	0.1	0.8	1	1	0.2	0	0.1	0	0.1	1	3	1	
Euglenophyceae	0.6	1	0.7	1	36	34	2	9	4	1	0.6	0	0.1	0	0	1	4	2	0.7	
Prasinophyceae	107	4	0	8	0.8	8	1	2	1	0.5	0	0	0	0	2	14	0	0.6	0	

Table 1 Dominant algal groups (cells/mL) along Tagus estuary in the productive period.

454

- 455 Table 2
- 456 Eigenvalues of the correlation matrix, proportion of variance explained by each PC and

PC	Eigenvalues	% Variation	Cum. % Variation
1	5.970	59.7	59.7
2	1.900	19.0	78.8
3	1.010	10.1	88.8

457 cumulative variation for the environmental PCA

459 Table 3.

	460	Variable	loads of	the enviro	nmental PCA
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	PC1	PC2	PC3
Т	0.353	0.090	-0.036
S	-0.386	0.158	0.007
DO	0.164	-0.570	0.027
NO ₃	0.396	-0.084	0.038
log (1+NH ₄)	-0.091	0.652	0.256
PO ₄	0.341	0.327	0.066
Si(OH) ₄	0.368	-0.108	0.138
TN	0.359	0.185	0.091
ТР	0.384	0.166	-0.037
SPM	0.077	0.172	-0.948

463 Table 4.

- 464 Summary of the 10 best results (Spearman correlation, ρ) obtained from BIOENV
- 465 analysis of combined environmental variables matching phytoplankton abundance

Number of Variables	ρ	Variables
4	0.803	T, S , Si, TP
1	0.796	S
3	0.794	T, S , Si
5	0.789	T, S , NO, Si, TP,
4	0.784	T, S, NO, Si
3	0.781	S, NO, Si
2	0.779	S, Si
2	0.778	S, NO
5	0.776	T, S , Si, TP, SM
2	0.776	S, TP

466 Note: (S in bold as appeared in all combinations)



468



471 Fig. 2



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