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1 **Phytoplankton community structure defined by key environmental**
2 **variables in Tagus estuary, Portugal**

3
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6

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 Bacillariophyceae. 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.

21 **Keywords:** Phytoplankton structure, nutrients, salinity, spatial scale, productive period,
22 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).

48 Environmental and phytoplankton data presented in this work were obtained during
49 three 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
58 area of 320 km². The estuary is meso-tidal and its shallow upper part is occupied by
59 extensive tidal flats and salt marshes (Fig. 1). The main freshwater source is Tagus
60 river, having an annual average flow of approximately 400 m³/s, although its discharge
61 varies greatly from summer to winter between approximately 30 m³/s in a dry summer
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).

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

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

75

76 *2.2. Physical and chemical measurements*

77 Temperature (T) and salinity (S) were measured in situ utilizing an Aanderaa probe
78 (CTD sensor), calibrated for salinity with an Autosal salinometer. Samples for DO
79 determination were collected and analyzed according to Winkler method as described
80 by Aminot and Chaussepied (1983). Water samples for nutrient determinations were
81 immediately filtered through AcetatePlus MSI (MicroSeparation Inc.) filters and placed
82 in a freezer. Samples for TN and TP measurements were preserved with sulfuric acid
83 (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 $(H' = -\sum p_i \log p_i, p_i = N_i/N, N_i$ 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*

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

118 *PCA, MDS and BIOENV analysis*

119 Environmental data ($\log(X+1)$ transformed) was handled using correlation-based
120 Principal Component Analysis (PCA), on the basis of standard Euclidean distance
121 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.

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

136 *2.4.2. Univariate Analysis*

137 Generalized Linear Model (GLM) was also applied to further explore the relationship
138 between the environmental variables and phytoplankton abundance. This model,
139 adequate for data with counts, uses the method of least squares, by linking the binary
140 response to the explanatory covariates through the probability of either outcome, which
141 does vary continuously from 0 to 1. The transformed probability is then modeled with
142 an ordinary polynomial function, linear in the explanatory variables. This univariate
143 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 µmol/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 µmol/L in the lower estuary (Sts. 1-3) to 6-7 µmol/L in the
170 upper estuary (Sts. 13, 14) (Fig. 2h).

171 The existence of NH₄ 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.

175 SPM varied quite irregularly from 21 to 58 mg/L all over the estuary (Fig. 2j). Besides
176 St. 4 and St. 5 located in the vicinity of Trancão river discharge, which showed the
177 highest levels of SPM, sites at the left margin of the estuary (Sts 15, 16, 17, 18, 19)
178 close to extensive saltmarsh zones, exhibited considerably high variability of suspended
179 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

199 *Chaetoceros subtilis*, *Asterionellopsis glacialis*, *Cylindrotheca closterium*, *Detonula*
200 *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*.

207 The oceanic Prasinophyceae, representing 2% of total abundance, developed mainly at
208 the estuary mouth (St. 1) (Table 1) and its principal species was *Phaeocystis pouchettii*.
209 Euglenophyceae accounted only for 1% of total abundance, being mostly due to the
210 development of *Eutreptiella marina* in the lower estuary. The contribution of
211 Cyanobacteria and Dinophyceae and Ebridea was below 1%. The most abundant
212 dinoflagellates were nanoplanktonic *Amphidinium* species (Sts. 1 and 6) and the most
213 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

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

228

229 3.3 Statistical Analysis

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

234 Variables T, NO_3 , PO_4 , $\text{Si}(\text{OH})_4$, TN, and TP present highest positive loads to PC1
235 while S loads negatively (Table 3). This component, accounting for 59.7% of total
236 variance (Table 2) is interpreted as the gradual mixing of nutrient-enriched fresh water
237 with nutrient-poor saline water along the estuary (Fig. 6a,b). Position of most of
238 stations along this axis gives support to this interpretation, St 14 (the most riverine) and
239 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 NH_4 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
273 a dominant factor and is the single variable that is retained in all of the best 10 sets of

274 variables. SPM is still included in the best 10 results, though the respective group of
275 variables have relatively lower correlation with phytoplankton abundance ($\rho= 0.776$).

276 The application of GLM analysis revealed the following relationship between
277 phytoplankton abundance and environmental variables:

$$278 \log(\text{Phyto abundance}) = 6.98815 - 0.07298\log(S) + 0.42153\log(\text{PO}_4) - 0.02145\log(\text{Si(OH)}_4)$$

279 This relationship, which includes S, Si(OH)_4 and PO_4 , has a high explanatory power
280 (63%) for phytoplankton abundance.

281

282 **4. Discussion**

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

286 The identified phytoplankton assemblages including, respectively, freshwater, estuarine
287 and freshwater, and estuarine and marine species, were dominated by
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

299 other hand, the screening model developed by Ferreira et al. (2005), using data from
300 1929 to 1998, predicted that in Tagus estuary, from a total number of 342 species, 120
301 were combined riverine and oceanic species, and only 222 were real estuarine. This
302 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.

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

346

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354

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

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Algal group	Stations																			
	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	87
Chlorophyceae	13	3	4	7	19	10	24	7	29	5	48	11	59	229	2	6	0.7	0.6	0.8	5
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	4
Cyanobacteria	0	0	0	0	0	0	0	0	3	0	0	22	0	2	3	0	0	0	0	0.3
Dinophyceae	15	4	1	5	3	16	5	3	0.2	2	0	3	0	0	4	11	0.3	0.8	0.4	0.8
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	0.2
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	1
Prasinophyceae	107	4	0	8	0.8	8	1	2	1	0.5	0	0	0	0	2	14	0	0.6	0	2

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455 Table 2

456 Eigenvalues of the correlation matrix, proportion of variance explained by each PC and

457 cumulative variation for the environmental PCA

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

458

459 Table 3.

460 Variable loads of the environmental PCA

	PC1	PC2	PC3
T	0.353	0.090	-0.036
S	-0.386	0.158	0.007
DO	0.164	-0.570	0.027
NO₃	0.396	-0.084	0.038
log (1+NH₄)	-0.091	0.652	0.256
PO₄	0.341	0.327	0.066
Si(OH)₄	0.368	-0.108	0.138
TN	0.359	0.185	0.091
TP	0.384	0.166	-0.037
SPM	0.077	0.172	-0.948

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463 Table 4.

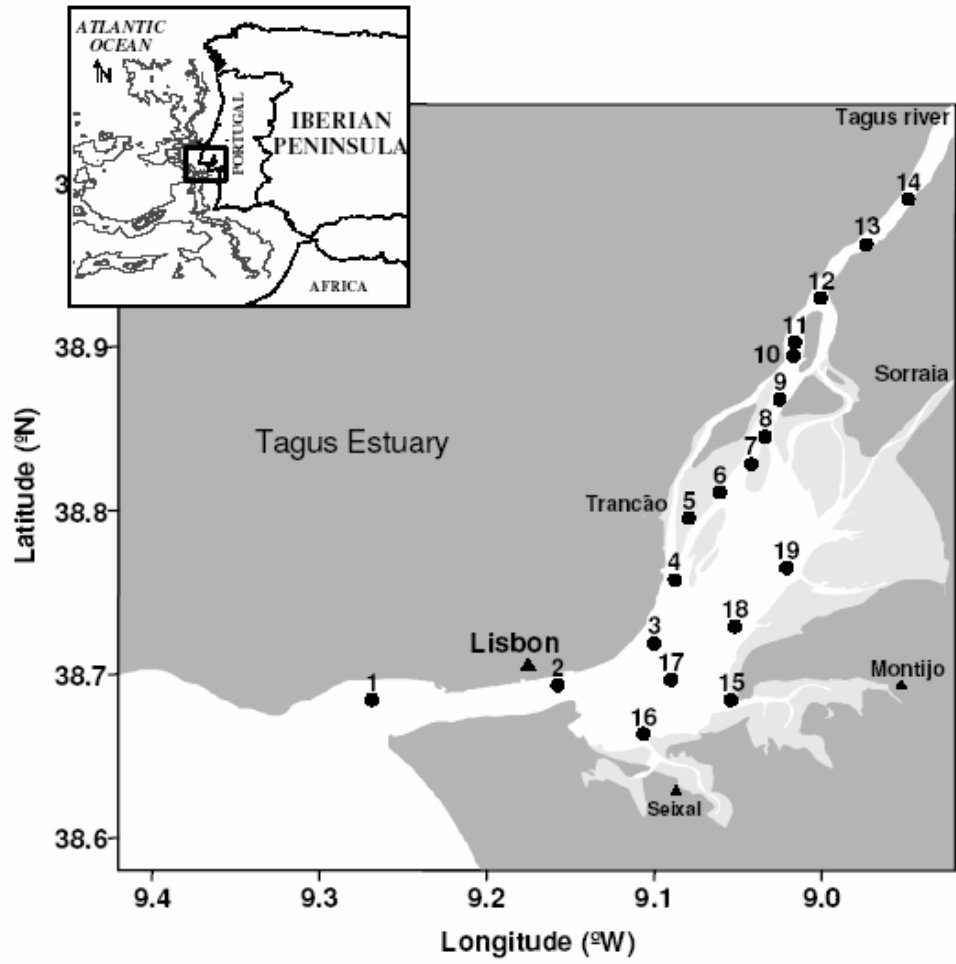
464 Summary of the 10 best results (Spearman correlation, ρ) obtained from BIOENV

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

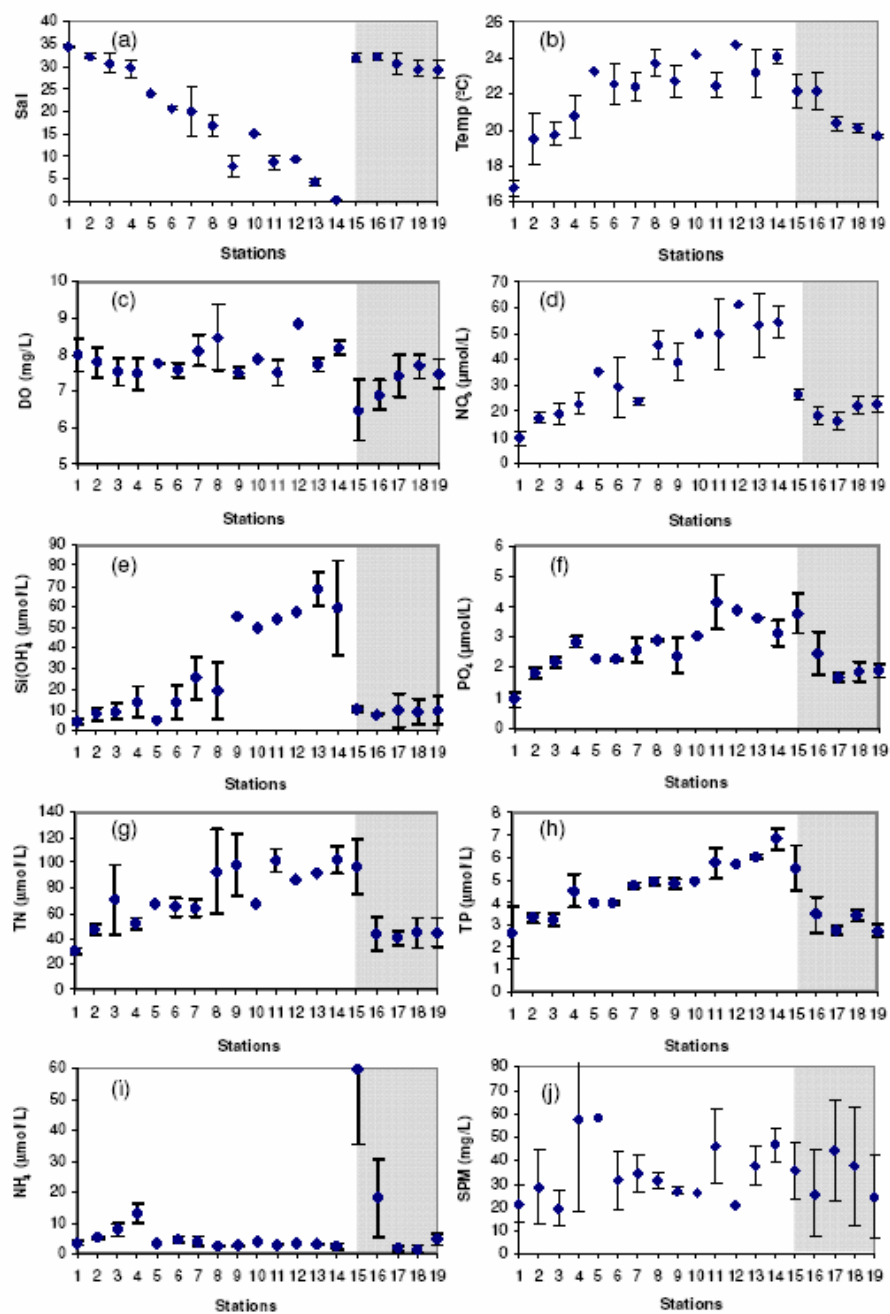
466 Note: (S in bold as appeared in all combinations)

467



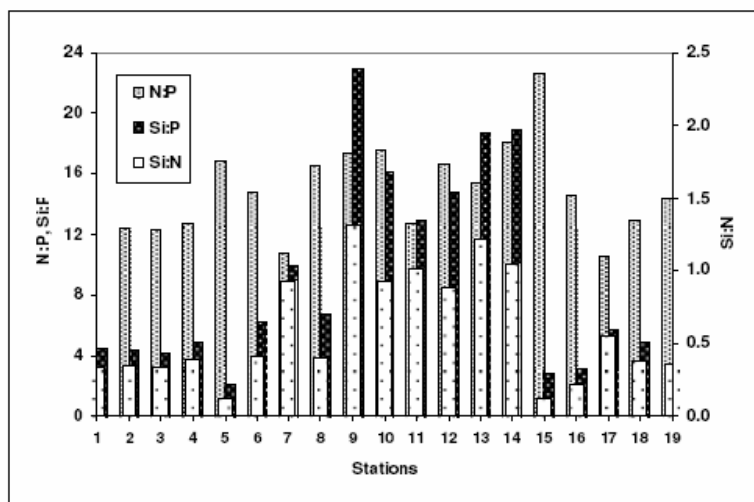
468

469 Fig. 1



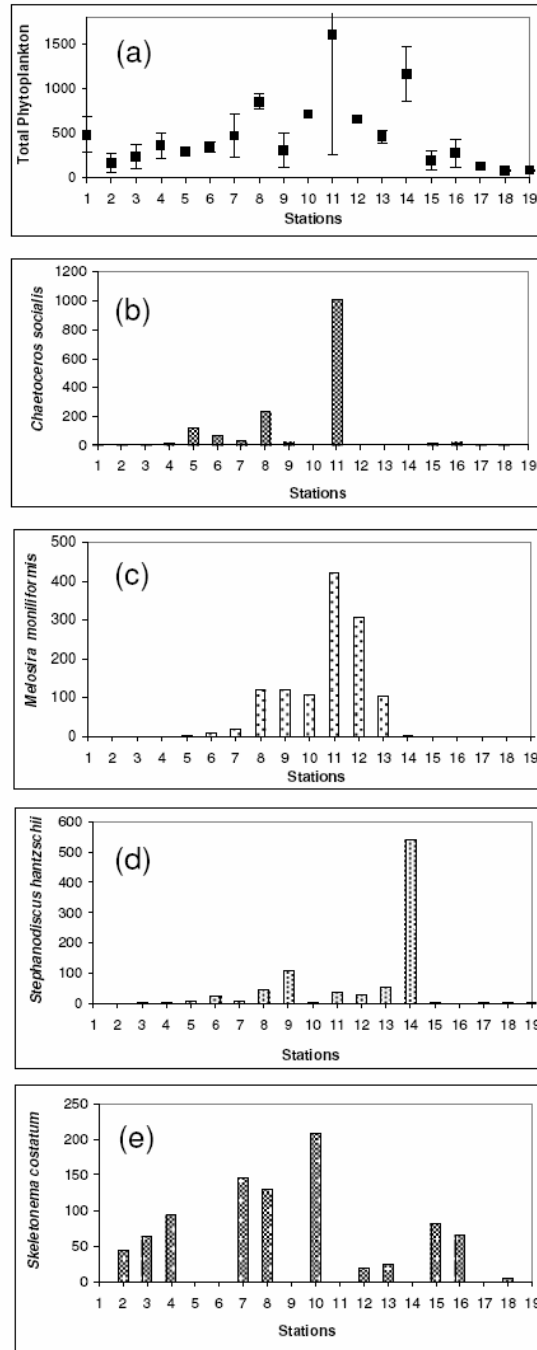
470

471 Fig. 2



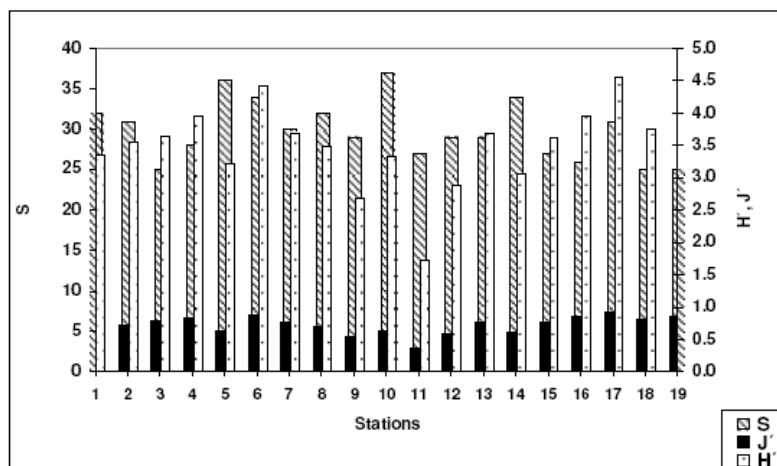
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473 Fig. 3



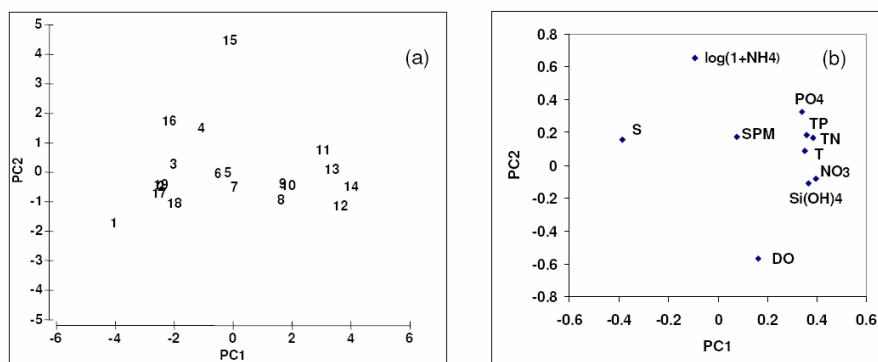
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475 Fig. 4



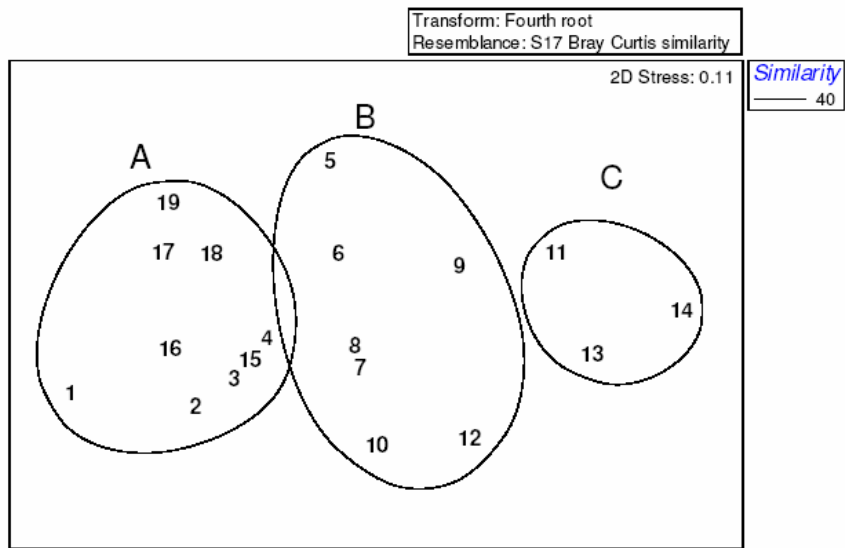
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477 Fig. 5



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479 Fig. 6



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481 Fig. 7

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