

## Phytoplankton community in the Sado estuary

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**Abstract:** Estuaries are important coastal ecosystems and sustain some of the most productive communities of primary producers. It is essential to understand the spatio-temporal variability of environmental and biological components and to ensure the environmental quality of the whole system. This study aims to characterize the spatio-temporal variability of phytoplankton assemblages in the Sado estuary and to relate them with the main local environmental drivers. Sampling was conducted monthly (March-December) during 2018 in 4-regions of the estuary. Water samples were collected to analyze phytoplankton and physico-chemical parameters (e.g. temperature, salinity, turbidity and nutrient concentrations). Phytoplankton maximum concentration was observed upstream in May, and 1-month later downstream. Cryptophytes and other small flagellates were the dominant groups recorded upstream, although diatoms were also relevant in summer/autumn. Downstream, diatoms were dominant, except in April and October, when dinoflagellates dominated, mostly due to the presence of harmful species (respectively, *Prorocentrum cordatum* and *Gymnodinium catenatum*).

**Keywords:** environmental drivers, environmental quality, estuary, phytoplankton.

### 1. INTRODUCTION

Estuaries are among the most productive ecosystems in the world. In Portugal, the Sado estuary is the second largest estuary on the west coast. It is a well-mixed estuary (Coutinho, 2003), and supports a great variety of activities, such as industrial, urban waste disposal, harbor associated activities, agriculture, fisheries, aquaculture and recreational activities (Cabrita et al., 2020).

Phytoplankton is highly sensitive to changes in its environment, providing a good insight into water quality before eutrophication becomes excessive, and before changes become visible at higher trophic levels (Brettum and Andersen, 2005). The Sado estuary is a relatively well-studied system with several works on phytoplankton since the 60's (e.g. Oliveira e Coutinho, 1992; Sampayo, 1970). However, the research effort has not been balanced across the entire system, nor throughout seasons. To fill this gap another study was performed in the 90's evaluating the spatio-temporal variability of phytoplankton and characterizing the trophic status of the Sado estuary (Coutinho, 2003).

As part of an on-going project to assess the current trophic state of the estuary, this study aims to characterize the spatio-temporal variability of phytoplankton assemblages in the Sado estuary and to relate it with the main local physico-chemical drivers.

### 2. METHODS

The study was conducted in the Sado estuary, located in Setúbal Bay on the western Portuguese coast (Fig. 1). Four regions of the estuary were sampled: one station upstream (#1) near Alcácer channel under a strong influence of the Sado river, two in the middle region (#3 and 5, being #3 located to the north in the Marateca channel) and another one downstream near the mouth of the estuary (#7) (Fig. 1).



Fig. 1. Map of the study area with the location and identification of each study site.

To analyze the phytoplankton community, surface water samples (125 mL) were collected monthly from March to December of 2018 during high tide. Samples were field fixed with acid Lugol's solution (Thronsen, 1978). Phytoplankton identification and quantification (cells L<sup>-1</sup>) was carried out by settling 10-50 mL of water following the Utermöhl method (Utermöhl, 1958), and analyzed with an inverted microscope equipped with phase contrast and bright

field illumination (Zeiss Axiovert 200) at magnifications of 200x and 400x. To achieve a representative sample, more than 400 cells were identified and counted at the lower magnification, except for around 8% of total samples where, due to the low phytoplankton concentration, only 200 cells were identified and counted.

Several physico-chemical drivers were measured *in situ* or in lab. The Secchi depth (m) was obtained using a 30 cm wide white disk and water temperature (°C) using a multiparametric probe (Hydrolab, DS4 or DS5X models). Salinity was measured with a salinometer (Guildline Autosol8400B), pH and turbidity with laboratory meters (Metrohm 827 Lab and Merck Turbiquant 3000IR, respectively). The concentration of nutrients was obtained using a Skalar SANplus Segmented Flow Auto-Analyzer designed for saline water analysis (Si, PO<sub>4</sub>, DIN-dissolved inorganic nitrogen composed of NO<sub>2</sub>, NO<sub>3</sub> and NH<sub>3</sub>, DN-total dissolved nitrogen and DP-total dissolved phosphate, all quantified in µmol L<sup>-1</sup>). Coloured dissolved organic matter (CDOM) was obtained by spectrophotometry (UV-2600 Shimadzu), and their spectral slopes (S) were calculated between 350–450 wavelength regions.

To analyze the spatio-temporal phytoplankton community structure and the underlying physico-chemical drivers, multivariate analyses were performed using PRIMER-E (version 6.1.13) with PERMANOVA (version 1.0.3) add-on software (Anderson et al., 2008; Clarke and Gorley, 2006). The taxonomic entities that occurred in less than 5 % of the total samples were excluded from the analysis. To reduce the disproportionate influence of highly abundant taxa, phytoplankton abundances were log (x+1) transformed. Based on a Bray-Curtis resemblance matrix, a permutational analysis of variance (PERMANOVA) was performed with 999 permutations for two fixed factors (study site and season) evaluated separately. Pair-wise tests for each factor were also performed. A Principal Coordinates Analysis (PCO) was used to visualize the multivariate patterns of the global phytoplankton composition (Anderson et al., 2008) and to explore the relationship between the environmental variables and phytoplankton. To this, the Spearman correlation was performed with the physico-chemical drivers and with the taxonomic class level. Only the variables with correlations higher than 0.25 were considered and for nitrogen and phosphate only the most significant form are shown. A significant level of  $\alpha = 0.05$  was considered in all the analyses.

### 3. RESULTS

During the study period, the upstream stations (#1 and 3) reached phytoplankton maximum abundance in

May (above  $40 \times 10^4$  cell L<sup>-1</sup>) (Fig. 2A and B), while the station located in the mouth of the estuary (#7) only reached the maximum abundance in June and with lower concentration (around  $30 \times 10^4$  cell L<sup>-1</sup>) (Fig. 2D). During the remaining period, phytoplankton concentration was always below  $15 \times 10^4$  cell L<sup>-1</sup> in all regions. Station #5 had always low phytoplankton concentration (Fig. 2C).

When analyzing the phytoplankton community at the class level, results showed big differences between the estuary mouth and stations located inside the estuary (Fig. 2). Cryptophytes were highly relevant in stations located inside the estuary (#1, 3 and 5) (Fig. 2A, B and C). At these stations, diatoms dominated in summer/autumn at #1, while they were almost absent at #3, and at #5 were present from spring to late-autumn. Station 5 was also characterized by the presence of dinoflagellates (Fig. 2C) showing some similarities with the downstream station (#7) (Fig. 2D). Diatoms were the dominant group near the mouth of the estuary, except in April and October when dinoflagellates dominated (Fig. 2D). Cryptophytes were also relevant in early-spring and from summer to early-winter. In this station, dinoflagellates were dominated by *Prorocentrum cordatum* in April ( $14 \times 10^4$  cell L<sup>-1</sup>) and by *Gymnodinium* spp. and *Gymnodinium catenatum* in October ( $6 \times 10^4$  cell L<sup>-1</sup>).

The PERMANOVA analysis performed for all taxonomic entities identified resulted in significant differences observed in space (sampling sites) and in time (seasons) ( $p < 0.05$ , not shown). The pair-wise tests showed that winter did not have significant differences with autumn, neither with spring ( $p > 0.05$ , not shown).

The spatial differences in the phytoplankton community were mainly explained by the following (Fig. 3): station 7, where the higher water transparency, salinity and pH were recorded (B), had a significant contribution of dinoflagellates (e.g. *Gyrodinium* spp., *Scrippsiella* group and athecate unidentified species) (A); the upstream stations (#1 and 3) were warmer, richer in nutrients and with higher turbidity (B), resulting in a high contribution of small flagellates (e.g. cryptophytes, euglenophytes, chlorophytes and others) (A). A significant contribution of diatoms also characterized the estuary: downstream with a great contribution of several chain-forming species (e.g. *Chaetoceros* spp. and *Guinardia delicatula*, and upstream mainly by pennate species (e.g. *Nitzschia* spp., *Gyrosigma/Pleurosigma* group and *Navicula* spp.) (Fig. 3A).

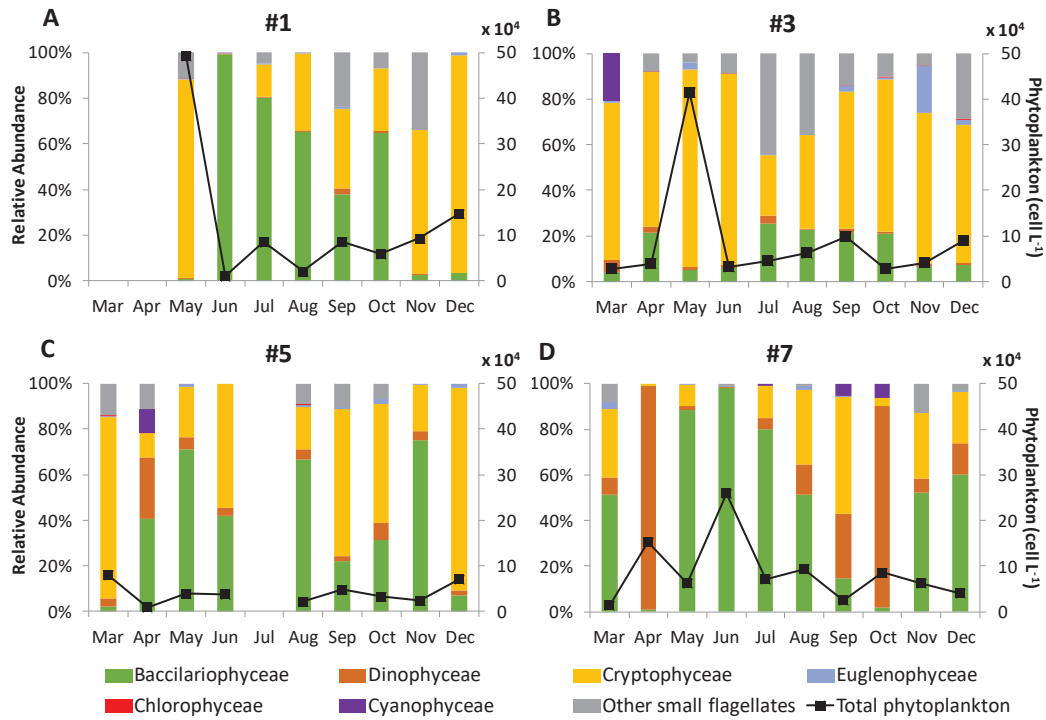


Fig. 2. Relative abundance of different phytoplankton groups (colored bars) and total concentration ( $\times 10^4$  cell  $L^{-1}$ ) (dots with connecting line) obtained for each sampling month in the different study sites (see the legend figure for the phytoplankton groups color).

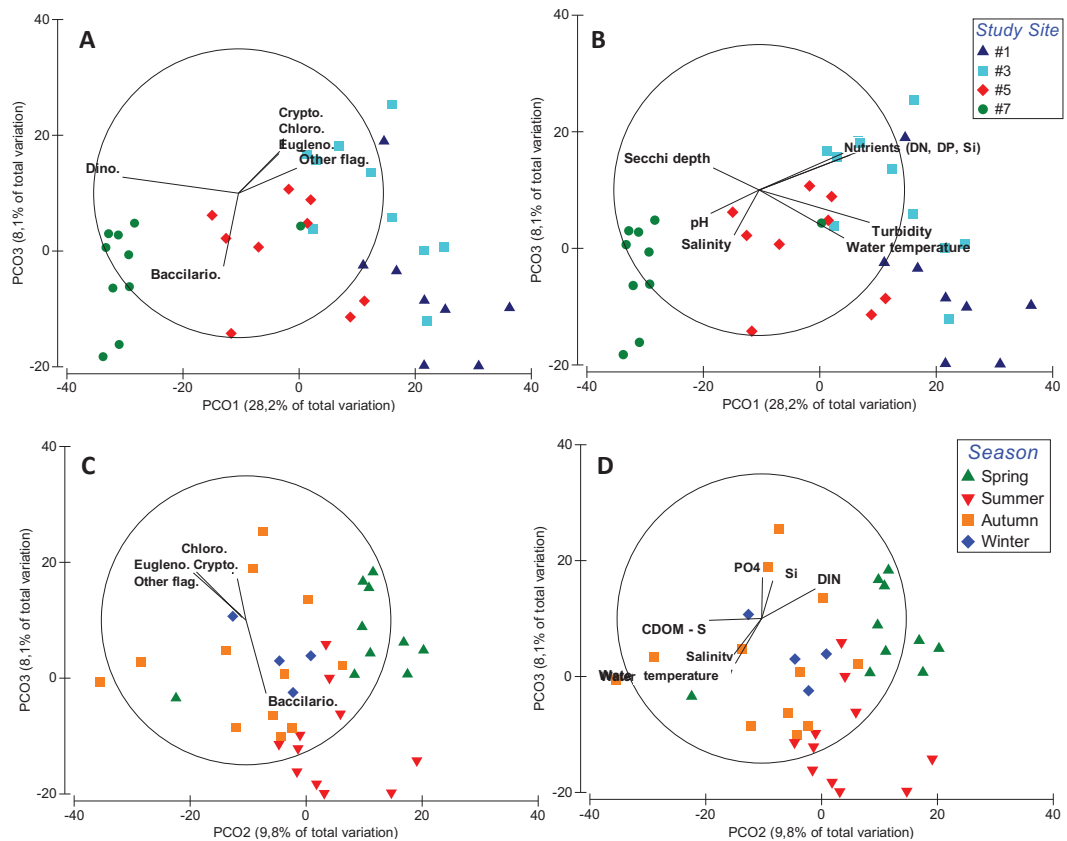


Fig. 3. Principal coordinates analysis (PCO) plots of phytoplankton structure between each study site (upper panel) and different seasons (lower panel). The left panel shows the taxonomic class level and the right panel shows the physico-chemical drivers.

At the taxonomic class level, the seasonal differences were not straightforward (Fig. 3C), and a more careful observation at a lower taxonomic level is needed (not shown). A mixed population of diatoms (mostly chain-forming species) and dinoflagellates (e.g. *Diplopsalis* and *Scrippsiella* groups, and *P. cordatum*) characterized spring. Summer and some of the autumn samples were mainly composed of pennate diatoms. Autumn had a high taxonomic variability, being characterized by a significant contribution of groups of small flagellates (e.g. chlorophytes, cryptophytes, euglenophytes), but also of small diatoms (e.g. *Skeletonema marinoi*) and dinoflagellates (unidentified species smaller than 15 µm, *Cochlodinium* spp. and the harmful species *Dinophysis caudata* and *G. catenatum*). Higher salinity and water temperature were recorded in summer and autumn, while high availability of nutrients characterized specially spring and some autumn samples (Fig. 3D). The CDOM was more significant during autumn associated with a period of increased rainfall, which suggests their utility for monitoring the influences of terrestrial inputs.

#### 4. DISCUSSION AND CONCLUSION

This study showed that different phytoplankton communities characterized the Sado estuary in the 4-analyzed regions, as well as in spring, summer and autumn. Higher phytoplankton concentrations were recorded in the upstream regions where nutrients were available in higher concentrations, similar to the results of Coutinho (2003). As in previous studies (Coutinho, 2003; Sampayo, 1970), diatoms dominated over dinoflagellates. The well-mixed nature of this estuary is known to favor phytoplankton communities dominated by diatoms. Dinoflagellates dominance was sporadic and occurred mostly downstream, near the estuary mouth, associated with higher visibility and salinity levels. This pattern suggests that some near-coastal characteristics (e.g. thermal stratification, nutrient availability by coastal upwelling) could influence the local dinoflagellates community (e.g. spring *P. cordatum* bloom). In addition, some phytoplankton species could be transported from the adjoining coastal area into the estuary (e.g. autumn *G. catenatum* bloom).

The main difference observed in this study when compared with the previous ones was the prevalence of cryptophytes over diatoms in the upstream region. This may suggest that the presence all year round of a local fast-growing cryptophyte community is favored by the high availability of nutrients observed in this region of the estuary. It is important to understand if this pattern is only related to pulses of nutrients available from internal recycling mechanisms (specific or not for this year), or if it suggests the presence of a continuous input of nutrients from an outside source of the system. To evaluate accurately the current trophic status of the Sado estuary, a longer time-series is being analyzed.

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