### **ORIGINAL ARTICLE**



# Effects of droughts, sea level rise, and increase in outfall discharges on phytoplankton in a temperate estuary (Tagus Estuary, Portugal)

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### Abstract

The effects of climate change on the estuarine environment are not fully understood. In temperate regions, droughts are expected to increase in frequency and severity, due to lower precipitation, and the mean sea level is expected to rise. This study aimed to assess how the estuarine environment will be affected by river flow's reduction, mean sea level rise, and the increase in nutrients discharged from anthropogenic sources. Seven scenarios were simulated and analyzed in the Tagus estuary (Portugal), using the hydrodynamic and biogeochemical model SCHISM: (i) reference scenario, (ii) 10% increase of the wastewater treatment plant (WWTP) outfall's discharge, (iii) 25% reduction of the river flow, (iv) 50% reduction of the river flow, (v) sea level rise of 0.5 m, (vi) sea level rise of 1 m, and (vii) the combination of 0.5 m of sea level rise and 25% reduction of the river flow. Both the reduction of the river flow and mean sea level rise led to higher salinities and lower nutrients and chlorophyll-a concentrations in the mid and upper areas of the estuary. The reduction in riverine nutrients in the estuary may increase the importance of nutrients from anthropogenic sources (e.g. WTTP discharges) in shaping the spatial variability of the phytoplankton communities in the future.

Keywords Tagus Estuary · Sea level rise · River discharges · Phytoplankton · Climate change · Modeling

# Introduction

Climate change effects, intensity, and consequences may vary at different latitudes and ecosystems (Gallego-Álvarez et al. 2011, Obregon et al. 2011; EEA 2022). Assessing these effects using a case-by-case approach is crucial. Estuaries are extremely dynamic with great spatial and temporal variability. Thus, the evaluation of the effects of climate change

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in these systems is a complex task. Sea level rise (SLR) is expected to increase the coastal influence in the estuarine waters on a global scale (Khojasteh et al. 2021). SLR will also contribute to increasing the salinity of estuarine waters, changing the nutrient and turbidity dynamics (van Maanen and Sottolichio 2018). Additionally, it will likely increase the tidal effect in the Tagus Estuary Bay channels due to higher tidal amplitude in these areas (van Maanen and Sottolichio 2018).

The Mediterranean-climate region is expected to register reduced precipitation and river flow in the next decades, as well as intense and more frequent heat waves (EEA 2022). Such changes may alter salinity and turbidity gradients in estuaries and, consequently, promote changes in the biological communities, including phytoplankton. However, given the capacity of biological communities to cope with environmental changes and due to the complexity of interactions with the environment (Hallegraeff 2010), the effects of climate change on phytoplankton communities cannot be fully understood. The increase in droughts' frequency and intensity may reduce the freshwater input into the estuaries, influencing the estuarine communities by changing the estuary morphology (Statham 2012) and promoting saltwater intrusion (Rodrigues et al. 2016, 2019). In Portugal, reduced precipitation and increased air temperature are expected, which may increase the frequency and intensity of droughts (Costa and Soares 2009, Gallego-Alvarez et al. 2011, EEA 2022,). Vicente-Serrano et al. (2014) estimated that precipitation has already decreased by 15.6% since 1960, while the mean annual air temperature has increased by 1.5 °C in the Iberian Peninsula, in comparison with the annual mean. Regarding the mean summer temperature, the same study estimated an increase of 2.1 °C for the same period. Concerning SLR, Antunes et al. (2019) estimated a mean rate of 1.94 mm/year for the Portuguese coast (Cascais) from 1920 to 2000. SLR rates higher than 2 mm/year were observed from 2000 onwards (Antunes, 2019). SLR is expected to continue in the future, which will likely promote the increase in both the estuarine area depth of the water column.

The freshwater input is one of the main sources of nutrients (nitrogen (N), phosphorus (P), and dissolved silica (DSi)) to estuaries (Saraiva et al. 2007; Kaiser et al. 2013). Hence, the reduction in the freshwater inflow is likely to cause a reduction in the nutrients reaching the estuary. Of these, N and P are also directly loaded from anthropogenic sources with higher percentages of organic matter and NH<sub>4</sub><sup>+</sup> (e.g. through discharges of wastewater treatment plants-WWTP). A shift in the available nutrients from inorganic to organic forms may lead to great shifts in the community composition, favouring flagellate forms instead of Bacillariophyta (Glibert 2016). DSi, which is mainly originated from the erosion of the soils in the river basin (Treguer et al. 1995; Garnier et al. 2002), is essential for diatoms—the typical dominant group in healthy estuaries (Cloern 2001; Kaiser et al. 2013; Cereja et al. 2021, 2022a). A decrease in DSi may lead to a shift in the phytoplankton community towards groups that are not dependent on DSi, such as Cryptophytes (Domingues et al. 2008). The human population inhabiting the surroundings of an estuary can also influence the nutrient budget, which can indirectly affect the phytoplankton community and the estuarine ecology. An increase in the population usually corresponds to higher anthropogenic nutrient input, both through the river flows and from outfalls discharging directly into the estuary (Bricker et al. 2008). The increase in nutrients may lead to a reduction in the estuarine water quality and eventually eutrophication (Poikane et al. 2019).

The Tagus Estuary (Portugal) is a mesotidal temperate estuary, with an average area of 320 km<sup>2</sup>. The dynamic tide reaches up to 80 km upstream (near the village of Muge) from the city of Lisbon (Vale and Sundby 1987; Gameiro et al. 2007) with the freshwater part of the estuary extending for around 30 km (between Vila Franca de Xira and Muge). The estuary houses around 2.3 million habitants (INE.pt) in its margins. Such population increased by 7% in the last decade (2011-2021 period, INE.pt and PORDATA.pt n.d.) and is expected to continue increasing (INE 2020)-in an opposite trend to what is predicted the total population in Portugal (UNCTAD.org n.d.). The population increase can lead to higher amounts of N and P to be discharged into the Tagus Estuary. Currently, there are 14 domestic WWTP, with secondary treatment or higher, discharging directly into the Tagus Estuary, and many more WWTP discharging in the surrounding channels and affluent streams (adp.pt). The Tagus River is the main tributary of the Tagus Estuary, with a mean flow of 230.9 m<sup>3</sup>/s between 2008 and 2019 (Cereja et al. 2021, SNIRH.pt). This flow has been decreasing at a rate of -4.3 m<sup>3</sup>/s per year in the mentioned period (Cereja et al. 2021, SNIRH.pt). The estuary is well-mixed, but stratification can occur during high river discharges and neap tides (Vale and Sundby 1987; Neves 2010; Rodrigues and Fortunato 2017). The lower estuary, where the water depth is higher, presents in general higher stratification (Neves 2010; Rodrigues and Fortunato 2017; Rodrigues et al. 2020). In the Tagus Estuary, both riverine and anthropogenic discharges have an important contribution to the nutrient concentrations, mainly to N compounds (NH<sub>4</sub><sup>+</sup>,  $NO_2^-$ , and  $NO_3^-$  – Saraiva et al. 2007, Gameiro et al. 2014, Cereja et al. 2021, 2022a, b). In terms of suspended sediments, concentrations up to 130 mg/L were observed at the surface of the Tagus Estuary and the turbidity maximum is located about 40 km upstream of the inlet (Vale and Sundby 1987; Cereja et al. 2022b). Hence, the expected climatic and anthropogenic-induced alterations together or individually may have implications in the water quality status of the Tagus estuary environment.

Changes in the physical and chemical parameters may strongly influence the phytoplankton community of the Tagus Estuary. This community presents a clear seasonal pattern, with chlorophyll-a concentrations up to 20  $\mu$ g/L (Gameiro et al. 2004, 2007, 2011; Cabrita 2014; Brito et al. 2015). The phytoplankton community also presents great interannual variations, with both unimodal and bimodal patterns through the years (Brotas et al. 2016; Cereja et al. 2021). The Tagus Estuary is dominated by diatoms at least since the 1980s. Cryptophytes are also a relevant group, as well as green algae, of which Prasinophyceae is the most important group in the estuary (Brito et al. 2015; Brotas et al. 2016; Tracana and Brotas 2019).

The estuarine dynamics and its response to climate change can be evaluated using numerical models (e.g. Cole and Cloern 1987, Chao et al. 2017). This allows the integration of freshwater and coastal forcings, and the estuarine internal circulation. Recent models, which include ecological dynamics (e.g. the interaction between the nutrients, primary producers, and grazers), can enhance the simulation capabilities for such variables (Liu et al. 2018; Wang et al. 2021). Several studies have used numerical models

to simulate estuaries' response to climate change effects, such as droughts, sea level rise, tidal amplification, and saltwater intrusions (e.g. Knowles and Cayan 2004, Levinton et al. 2011, Cheng et al. 2015, Delgado et al. 2017, Eslami et al. 2019, Rodrigues et al. 2019). Numerical models have been used to describe the Tagus Estuary hydrodynamics for over 40 years (e.g. ADCIRC – Fortunato et al. 1997, 1999; MOHID - Portela and Neves 1994; ELCIRC - Vargas et al. 2008; SIMSYS – Dias and Valentim 2011, Guerreiro et al. 2015). However, numerical studies focused on how climate change may affect temperate estuaries' water quality, and in special the Tagus Estuary phytoplankton community is still scarce. Understanding how the estuary is expected to respond to changes in the main drivers is important to ensure adequate environmental assessments and the potential impacts of such changes in the overall quality of the environment, as discussed by Cereja et al. (2022a, b), Brito et al. (2012), Costa et al. (2020), and Rodrigues et al. (2020).

The main objective of this study is to evaluate how climate change and population variations may influence the estuarine environment and the water quality in the Tagus Estuary, and to discuss potential effects on the phytoplankton community. To reach this objective, a numerical modeling approach was used and a set of scenarios were evaluated, namely: 1 - one scenario of increase in the nutrient input, due to outfalls; 2 - two different levels of sea level rise; 3 - two different scenarios of reduction in freshwater inputs; and 4 - 1 scenario combining the previous scenarios, using both the climate predictions made for this region and the historical data to setup the scenarios. Considering these scenarios, the variations induced by climate change, droughts, and increase in WWTP discharges in several parameters (i.e. nutrient and chlorophyll-a concentrations, water temperature and dissolved oxygen) will be assessed.

# Methodology

### **Numerical simulations**

The simulations of the present (reference) and future conditions in the Tagus Estuary were performed using the system of models SCHISM (Zhang et al. 2016), which has been previously calibrated and validated in the Tagus Estuary (Rodrigues et al. 2013, 2017, 2021).

# Semi-implicit cross-scale hydroscience integrated system model

Semi-implicit Cross-scale Hydroscience Integrated System Model (SCHISM n.d.) is a derivative product built from SELFE (v3.1dc; Zhang and Baptista 2008), distributed in open source, with many enhancements and upgrades including a new extension to the large-scale eddying regime and a seamless cross-scale capability from creek to ocean. SCHISM includes several modules for the simulation of waves (Roland et al. 2012), sediments (Pinto et al. 2012), oil spills (e.g. Azevedo et al. 2014), and water quality and ecological dynamics (e.g. Rodrigues et al. 2009a, b, 2011, 2012). The version v5.4.0 was used in the present application.

The hydrodynamic model calculates the free-surface elevation and the 3D water velocity, salinity, and temperature, by solving the 3D shallow water equations, which represent conservation laws for mass/volume, momentum, salt, and heat, together with the hydrostatic and Boussinesq approximations. The water quality model is based on EcoSim 2.0 (Bissett et al. 2004), and extended to account for zooplankton (Rodrigues et al. 2009a, b) and the oxygen cycle (Rodrigues et al. 2012). Besides oxygen, the model includes the cycles of carbon, N, P, DSi, and iron. The model simulates source and sink terms for several state variables, namely zooplankton groups, phytoplankton groups, bacterioplankton, dissolved and faecal organic matter, inorganic nutrients, and dissolved inorganic carbon. Detailed descriptions of the water quality model can be found in Bissett et al. (2004) and Rodrigues et al. (2009a, b, 2012).

SCHISM is based on a finite-element/finite-volume numerical scheme. The advection of salinity and temperature can be treated with a mass-conservative finite-volume upwind scheme or a TVD (Total Variation Diminishing) scheme. The domain is discretized horizontally with unstructured triangular grids, and vertically with hybrid coordinates (partly terrain-following S-coordinates and partly geopotential Z-coordinate or LSC<sup>2</sup> coordinates) (Zhang et al. 2015). Further description can be found in Zhang et al. (2015, 2016).

#### Application to the Tagus Estuary

SCHISM has been previously applied to the Tagus Estuary (Rodrigues et al. 2019, 2021). The horizontal grid covers an area between approximately 38.59° N, 9.36° W and 39.08° N, 9.75° W, from Valada (around 23 km upstream from the estuary freshwater border) to Cascais (around 10 km outside the estuary mouth). Valada is slightly upstream to the limits of the dynamic tide propagation (Vale and Sundby 1987; Gameiro et al. 2007). This grid has about 83,000 nodes and a horizontal resolution that varies between 5 m and 2 km, with a typical resolution of 15–25 m. The vertical grid is hybrid and has 39 levels (30 S terrain-following coordinates until 100 m depth and 9 Z geopotential coordinates below 100 m depth).

The numerical model is forced by (i) tides, salinity, water temperature, and water quality tracers' concentrations at the oceanic boundary; (ii) river flows, salinity, water temperature, and water quality tracers' concentrations at the riverine boundaries; and (iii) atmospheric data at the surface (air temperature, surface pressure, wind, specific humidity, and downwards longwave and shortwave radiation). Further details about the hydrodynamic model implementation can be found in Rodrigues and Fortunato (2017) and Rodrigues et al. (2021a). This model has been previously validated in the Tagus Estuary and was used to analyze the circulation, physicochemical, and chlorophyll-a dynamics in the estuary (Rodrigues and Fortunato 2017; Rodrigues et al. 2021).

### Definition of the scenarios

The scenarios for 2100 were defined using a methodological approach as described by Rodrigues et al. (2016, 2021). Two reference scenarios were considered. One in which the Alcântara outfall was included and another in which no outfalls were considered. The Alcântara WWTP was selected since it is the largest in Lisbon (serving around 800,000 people, Costa et al. 2020, adp.pt) and the one that releases the higher amount of nutrients into the Tagus Estuary. Its effect on the surrounding environment was analyzed by several studies (Costa et al. 2020; Cereja et al. 2021, 2022a). A reference scenario including the Alcântara outfall discharge was simulated to allow a correct comparison with the scenario representing the increase of this discharge (due to an estimated increase in the population of Lisbon). The estimated increase of 10% in the outfall's discharge had no significant differences relative to the reference scenario, other than a slight local alteration in nutrient concentration (please see details below in the "Influence of the increase in Alcântara outfall discharge over physicochemical parameters and chlorophyll-a" section). Thus, the remaining scenarios were simulated without the Alcântara outfall discharge and were compared with a reference scenario without outfalls. Following the procedures of Rodrigues et al. (2019), tidal conditions (fig. sm 1) and atmospheric (Dee et al. 2011, https://www.ecmwf.int/, table sm 2) forcing data from 2001 were used for the reference scenario, as this was the year closer to the mean tidal and meteorological variations for the 1990-2010 period. Due to the high computational demand,

simulations were performed only for the Spring season, corresponding to the beginning of the spring bloom (Gameiro et al. 2007; Brotas et al. 2016; Cereja et al. 2021), following a similar approach to Rodrigues et al. (2021). In each scenario, only the tested conditions were changed, while the other remained similar to the reference scenario.

The following scenarios were simulated (see also Table 1):

- Variations in the outfall discharges one scenario: an increase of 10% in the flow discharged by the Alcântara WWTP, applied with the conditions registered in the present day. This increase in the Alcântara outfall discharge intends to represent the estimated increase of 10% of the population of Lisbon (Portuguese Statistics Institute, INE.pt).
- Reduction of the river discharges 2 scenarios: these 2. scenarios represent a reduction of 50% and 25% relative to the reference scenario in both the Tagus and Sorraia Rivers' discharges. Two different approaches were used to estimate the reduction of river discharges: (i) precipitation predictions for the 2071–2100 period in the Lisbon Metropolitan Area under the RCP 8.5 scenario of the IPCC, which suggest a 25% decrease during Spring (portaldocclima.pt). Hence, a scenario with a 25% reduction in the discharges of the Tagus and Sorraia Rivers was considered. (ii) A 50% reduction in the river discharge is predicted by extrapolating the calculations performed by Cereja et al. (2021) (considering field data of river discharge) for 2100. Such differences in estimations may result from the Tagus River flow being mostly controlled by dams, and thus dependent on management actions and human water uses. Furthermore, the Tagus River presents an extensive international basin, which is subjected to different management policies and climate forcings. Thus, a scenario with a 50% reduction in the Tagus and Sorraia Rivers' flow was also performed.
- Sea level rise 2 scenarios: two scenarios were simulated to represent SLR, namely a mean SLR of 0.5 m and a mean SLR of 1 m. Those were used as probable scenarios to occur, based on the SLR intervals estimated for the Portuguese coast and Tagus Estuary by Antunes

Table 1Summary of thescenarios evaluated. See the"Definition of the scenarios"section for a detailed descriptionof each scenario

Scenario name	Short description
WTP+10	Increase of the Alcantara's outfall discharge by 10%
RD25	Reduction of the Tagus and Sorraia Rivers' discharges by $25\%$
RD50	Reduction of the Tagus and Sorraia Rivers' discharges by $50\%$
SLR0.5	Increase of the mean sea level by 0.5 m
SLR1	Increase of the mean sea level by 1 m
RD25SLR05	Combination of RD25 and SLR 0.5 (reduction in the river dis- charges by 25% and increase of the mean sea level by 1 m)

(2019). These estimates were obtained using long-term datasets obtained from the tide gauge located at Cascais (near the mouth of the Tagus estuary).

4. Sea level rise and reduction of river discharges – 1 combined scenario: the selected scenario combines a 25% reduction in the freshwater input from the rivers and a mean SLR of 0.5 m. These were considered the less severe scenarios from each driver, and the most probable to occur as they represent less severe climatic alterations.

All simulations were performed for 45 days. The first 15 days represented the model warm-up period. The results of the last 30 days were used for the statistical analysis and the calculation of the water quality indicators, as described below.

### **Statistical analysis**

For each scenario, the following variables were extracted from the model results: water elevation (m), chlorophyll-a ( $\mu$ g/L), nutrients (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub>, PO<sub>4</sub><sup>3–</sup>, and dissolved DSi,  $\mu$ mol/L), salinity, water temperature (°C), and dissolved oxygen ( $\mu$ mol/L).

To compare the scenarios, the relative differences over the entire domain were also estimated by dividing each variable in a given scenario by the values of the same variable in the reference scenario. These relative differences were then plotted using Surfer 10 software (Fig. 2).

A multivariate hypothesis test was applied to verify the statistical significance of the differences between the scenarios. A prior analysis of these simulations detected the existence of collinearity between all nutrients (positive correlation) and between nutrients and salinity (negative correlation). Thus, only dissolved inorganic nitrogen (DIN, the sum of  $NH_4^+$  and  $NO_3^-$ ) was used as a representation of the extracted variables in the statistics test. The referred variables were extracted at 15 stations along the estuary (Table SM 1, Fig. 1A). These stations were selected for being well spread through the estuary and for being used in previous works on the Tagus Estuary. As data did not meet the normality assumption, a PERMANOVA was applied to compare the variations between several scenarios. This was done with the software primer 7 + PER-MANOVA. The analysis was performed using a Euclidean distances resemblance matrix performed with normalized data (subtracted by the mean and divided by the standard deviation). The analysis was run with a reduced model and 9999 permutations. Significant differences were considered when the *p*-value was under 0.05.

### Water quality indicators

A set of indicators was calculated at each station to assess how the different scenarios affect the water quality classification. These indicators (chlorophyll-a and nutrient concentrations) were calculated considering the metrics used in Portugal for the Water Framework Directive (WFD). Chlorophyll-a indicator was calculated following Brito et al. (2012). This indicator considers three salinity ranges (< 5, 5–25, and > 25). The indicator is calculated for each salinity class, using the samplings that are collected during the growth season (February to October) by the following equation:

$$EQR = \frac{Reference value}{90th percentile(CHLa)}$$

For each salinity class, an Ecological Quality Ratio (EQR) is calculated by dividing the reference value for that salinity by the 90<sup>th</sup> percentile of chlorophyll-a concentrations measured at the same salinity class. In the end, all salinities must be integrated by a weighted mean. Possible classifications are High, Good, Moderate, Poor, and Bad. For more information, see Brito et al. (2015) and Cereja et al. (2022a, b).

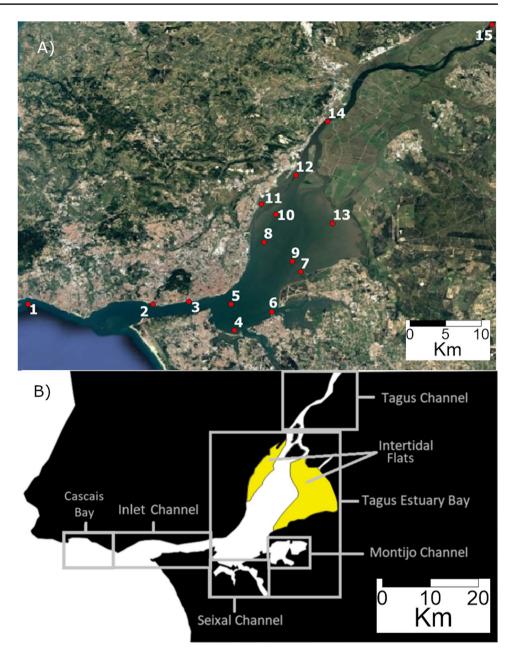
The nutrient status indicator was calculated following APA (2016). The classification is obtained by calculating the 90<sup>th</sup> percentile of  $NH_4^+$ ,  $NO_x$ , and  $PO_4^{3-}$  for each salinity class, applying one-out-all-out integration between the several nutrient species. In Portugal, this approach is also used to integrate several WFD indicators (in the present work, only nutrients and chlorophyll-a) in the final water body classification. For additional information, see APA (2016) and Cereja et al. (2022a, b).

### Results

# Physicochemical and spatial variability in the reference scenario

In general, all scenarios show a high spatial variability with significant differences between the stations (Table 2). Higher chlorophyll-a and nutrient concentrations were seen in the upper areas of the Tagus Estuary (Fig. 2). Such variability was greater in the reference scenario in comparison to the other scenarios. In the reference scenario, chlorophyll-a varied from 0.6  $\mu$ g/L at the inlet to around 8  $\mu$ g/L upstream, and, for instance, NH<sub>4</sub><sup>+</sup> varied from 0.4  $\mu$ mol/L at the inlet to 8  $\mu$ mol/L at the upper estuary, respectively. Temperature presented low variability throughout the estuary, but still lower temperatures occurred in the coastal boundary and

**Fig. 1** Locations chosen to extract data for comparison (**A**), for more information on the coordinates and location of each location, see table SM 1 and **B** Tagus Estuary Regions as seen in Cereja et al. (2022b)

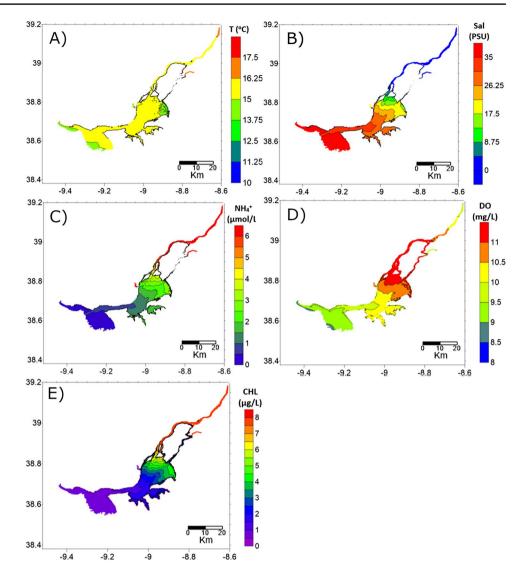


**Table 2** PERMANOVA test for the effect of enhancing the Alcântara outfall flow (WTF effect) over the stations considering all the environmental variables (multivariate analysis) and only the nutrients (for which  $NH_4^+$  was used as a proxy as all nutrients were colinear). The

table presents the degrees-of-freedom (Unique df), the sum of squares (SS), the mean squares (MS), the pseudo-F value, the p-value (P(perm)), and the number of permutations performed (perms).

Multivariate analysis						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
WTP effect	1	5.1299	5.1299	1.2886	0.2612	9954
RES	59	234.87	3.9808			
Total	60	240				
Nutrients only						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
WTP effect	1	1.6428	1.6428	5.4021	0.0224	9828
RES	59	17.942	0.30411			
Total	60	19.585				

Fig. 2 Spatial distribution of environmental parameters and phytoplankton biomass in the reference scenario without the outfall discharge during Spring. The parameters were temperature (**A**), salinity (**B**), ammonia (**C**), dissolved oxygen (**D**), and chlorophjyll-a (**E**). Please note that plots represent the temporal (30-day) average of data



higher temperatures in the river boundary. Also, the simulations presented lower temperatures at the intertidal region (constituted mainly by mudflats) in the western part of the estuary.

The upper estuary was also the area with higher dissolved oxygen; however, the oxygen decreased in the vicinity of the river boundary (Fig. 2). The mudflat region presented higher concentrations of nutrients, chlorophyll-a, and oxygen concentrations in comparison to its vicinity, but lower than the upper estuary. The estuary mouth and the adjacent coastal waters presented lower chlorophyll-a and nutrient concentrations. The salinity presented the exact opposite trend.

### Influence of the increase in Alcântara outfall discharge over physicochemical parameters and chlorophyll-a

A slight increase in nutrient concentrations was shown only by the station located near the outfall (p=0.022, Table 2) for the scenario with an increase of 10% of the discharge from the Alcântara outfall. Nevertheless, no significant differences were seen in the estuarine environment (considering chlorophyll-a, nutrients, dissolved oxygen, and temperature), even when analyzing only the area surrounding the outfall (station 3, Table 2, www.INE.pt). Therefore, the discharge from the Alcântara outfall was not considered for the remaining scenarios.

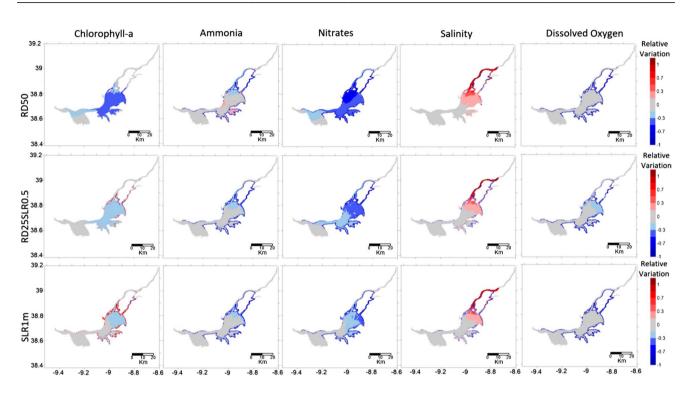
### Influence of sea level rise and river discharge

Both mean SLR and the reduction of river discharge scenarios lead to similar trends, showing a reduction in nutrient and chlorophyll-a concentrations throughout the estuary (Table 3 and Fig. 3). Significant differences (p-value=0.000) were only seen at the six stations located further upstream in the estuary (Table 3; station 1 was excluded from this analysis, as it is located outside the estuary). The influence of SLR and river flow was larger in these stations, which

Table 3 PERMANOVA test comparing the different selected loca-
tions and comparing the scenarios variations inside the locations
(nested by the location). The table presents the degrees-of-freedom

(Unique df), the sum of squares (SS), the mean squares (MS), the pseudo-F value, the p-value (P(perm)), and the number of permutations performed (perms)

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Location	14	6458.2	461.3	266.94	0.000	9879
Scenarios (location)	75	243.86	3.2515	1.8815	0.000	9769
Res	2793	4826.6	1.7281			
Total	2882	11528				



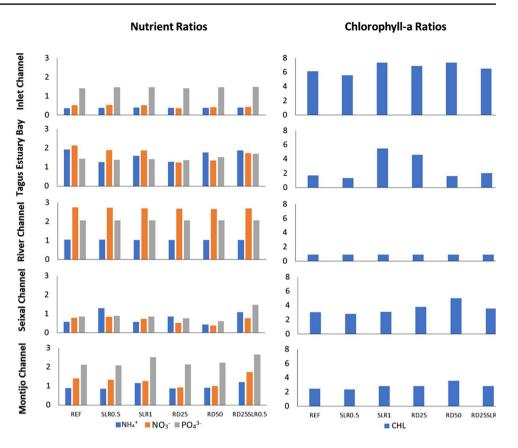
**Fig. 3** Relative comparison (% of variation) for the chlorophyll-a, ammonium ( $NH_4^+$ ), dissolved oxygen, and salinity variations between reference scenario and 50% reduction of river flow (top, RD50%), 25% reduction of river flow and sea level rise of 0.5 m (middle,

RD25SLR0.5), and sea level rise of 1 m (bottom, SLR1m) scenarios. For the results of the other parameters and scenarios not presented here, please see figure SM 2–7

represent both the upper estuary and the upper part of the mid-estuary. All these locations presented significant differences between the reference scenario and the RD50 scenario, which was the scenario that presented larger differences relative to the reference scenario. The SLR1 m and the RD25SLR0.5 scenarios also presented significant differences at the Sorraia river channel and Cala do Norte. For the RD50 scenario—50% reduction of the river flow, the effects were more evident at the medium and upper estuary: chlorophyll-a decreased by 34% (to 66% of the reference concentrations) and  $NO_3^-$  decreased by 70% (to 30% of the reference concentrations) (Fig. 3). The SLR1m scenario resulted in greater differences for (i) salinity in the upper estuary, with increases over 100%, (ii)  $NO_3^-$  in the medium estuary, with 70% reductions, and (iii) chlorophyll-a in the medium estuary, with reductions around 30% (Fig. 3).

### Effects on water quality metrics

The water quality ratios (relation between the 90<sup>th</sup> percentile and the reference level) presented different trends according to the analyzed indicator and estuarine area. In general, both  $NO_3^-$  and chlorophyll-a concentrations decreased in the climate change scenarios, leading to an improvement of the water quality metrics (i.e. decrease in the nutrient ratio and an increase in the chlorophyll-a ratio). This reduction was mainly registered in the medium estuary and for the scenarios representing a reduction in the river flow (Fig. 4). The reduction in river flow also led to a decrease in the  $NH_4^+$  concentrations in the medium part of the estuary. These changes resulted in positive trends in the water quality ratios, namely for  $NO_3^-$ ,  $NH_4^+$ , and chlorophyll-a. The lower parts of the estuary presented a different trend, showing no changes in the water quality ratios for the simulated scenarios. **Fig. 4** Nutrient (left) and chlorophyll-a (right) water quality ratios for the different Tagus Estuary regions. Nutrient ratios are calculated by dividing the 90<sup>th</sup> percentile by the reference levels and therefore, higher ratios mean worse water quality. The chlorophyll-a ratios are calculated by dividing the reference by the 90<sup>th</sup> percentile of the chlorophyll-a concentrations and therefore, lower values mean worse water quality.



# Discussion

### Increase of the Alcântara outfall's discharge

The Tagus Estuary receives treated effluents from dozens of WTTP both directly and through the small streams that enter the estuary channels (adp.pt). In the present study, only the largest outfall discharging into the estuary, the outfall from the Alcântara WWTP, was simulated. The increase simulated for the discharge of this outfall (10%) would have no significant effect on the water body (chlorophyll-a, dissolved oxygen, and temperature) besides a small increase in the nutrient concentrations in the region near the discharge. This variation was so small that the induced increase in  $NH_4^+$ was lower than the natural standard deviation of the simulated month registered by field studies (Cereja et al. 2021, 2022a). The influence of the Alcântara outfall has been seen to influence the water body near the location of the discharge (Cereja et al. 2021, 2022a), but with a low impact in a medium-range distance (Cereja et al. 2022b) due to the strong currents in the area (Rodrigues and Fortunato 2017). The outfalls in the Tagus Estuary have been reported to be more important on a local scale, not affecting large areas of the estuary (Gameiro et al. 2014; Cereja et al. 2022b). However, it should be noted that the influence of the WWTP effluents discharged throughout the Tagus estuary should be further addressed in future research. There are several outfalls along the Tagus Estuary, some of them causing a larger effect on the local macrofauna communities (another WFD biologic indicator) and water salinity than the Alcantara's outfall (e.g. Xabregas outfall, Costa et al. 2020; Cereja et al. 2022b), even with equivalent treatment level and serving a much smaller population. Thus, the local effects of these point sources and their combined effects should be further addressed.

# Freshwater reduction and sea level rise effects over the estuary

The reduction in the river flow and mean SLR had a similar influence on the estuarine environment. The changes promoted by these drivers led to an increase in the contribution of coastal waters and a reduction in the freshwater influence in the estuary. This leads to higher values of salinity and lower concentrations of nutrients. The scenarios representing the reduction in the river flows generated greater variations in the tested variables than the mean SLR scenarios. In particular, the largest alterations in salinity and nutrients were shown by the scenario simulating a 50% reduction in the river flow (relative to the reference scenario).

The freshwater input is typically a main driver of variability in estuaries and in particular in the Tagus Estuary (Cabecadas et al. 1999; Gameiro et al. 2007, 2014; Cereja et al. 2021, 2022b). It is also one of the main sources of nutrients in this estuary (Cabeçadas et al. 1999; Saraiva et al. 2007; Borges et al. 2020). Hence, a reduction in river flow can deeply affect the dynamics of the Tagus Estuary, causing a reduction in nutrient and chlorophyll-a concentrations, as well as an increase in salinity, especially in the upper estuary. It is noticeable that such alterations in nutrients were mainly seen for NO<sub>3</sub><sup>-</sup> and Si concentrations, as the source of these nutrients is primarily riverine (Caetano et al. 2016; Borges et al. 2020).  $NH_4^+$  concentrations in Tagus Estuary have been reported to be associated with the discharges from WWTP outfalls, although with a local scope (Gameiro et al. 2004; Cereja et al. 2022b).  $PO_4^{3-}$  concentrations in Tagus Estuary have been related with desorption from the sediments (Cabrita and Brotas 2000). This also justifies the fact that all the variations were mainly registered in the shallow upper areas of the estuary as these are more influenced by the river discharge.

Moreover, an increase in salinity is expected to strongly affect the remaining variables. High salinity pushes the maximum turbidity upper in the estuary (Guo et al. 2017; Zhu et al. 2022). The Tagus Estuary presents high sediment resuspension and is considered a turbid estuary (Vale and Sundby 1987). Turbidity is one of the major drivers for the variability of phytoplankton communities in the Tagus Estuary (Gameiro and Brotas 2010; Gameiro et al. 2011) and thus an alteration of the turbidity patterns may alter the concentrations of chlorophyll-a. This reduction in nutrients and SPM is a possible consequence of droughts and SLR. SLR leads directly to a higher contribution of coastal water to the estuary. Coastal water is typically poorer in all nutrients when compared to freshwater; thus, an increase in the contribution from coastal waters would lead to a general reduction in nutrient concentrations. Attrill and Power (2000) reported an increase in the salinity in the upper Thames Estuary, leading to higher pH and lower suspended solids and the loss of seasonality in dissolved nitrogen. Geyer et al. (2018) also observed a reduction in nitrogen in Apalachicola Bay during droughts, based on pluriannual field data. However, no significant effect over the chlorophyll-a concentrations was observed by the above authors, suggesting that the phytoplankton community in that estuary was in an equilibrium between higher nutrient concentrations at high river discharge situations and high residence times at drought situations.

In the present work, it is difficult to compare the minimum concentrations found with historical data, given that the Tagus Estuary has high seasonal and interannual variability for both nutrient and chlorophyll-a concentrations, with their ranges in historical data greatly surpassing the variability caused by any of the simulations in the present work (Gameiro et al. 2007, 2011; Brito et al. 2015; Cereja et al. 2021, 2022a). Such different ranges in these parameters result from the use of mean conditions for both the tide and climate forcings in the simulated scenarios, which deeply contrast with the high interannual variability of the Tagus Estuary. Therefore, the simulated scenarios aim to represent the mean variations, rather than the large interannual variability registered in the Tagus Estuary. Additionally, external influences, such as upwelling events, are known to influence mainly the estuary mouth (Cabeçadas et al. 2010) with no available information on possible effects on the Tagus Estuary Bay.

In estuaries with relatively high residence times, such as the Tagus Estuary (Ferreira et al. 2005; Brito et al. 2012), a reduction in nutrient concentrations can lead to nutrient limitation of the phytoplankton growth. This can reduce the chlorophyll-a concentrations in the estuary and alter the phytoplankton community composition. In other Portuguese estuaries, Domingues et al. (2005, 2011) observed a decrease in the Bacillariophyta dominance due to reductions in either Dsi and N during late spring and summer in the Guadiana Estuary, and Lopes et al. (2007) observed a similar pattern in Ria de Aveiro, with a loss of Bacillariophyta dominance in favour of Chlorophyta during late spring and summer, due to the reduction in river discharge and consequent reduction of DSi. For the scenarios simulated in the present work, chlorophyll-a concentrations decreased more intensely in the medium estuary, where nutrient concentrations presented lower variability among the different scenarios. This effect may be a consequence of the higher residence times expected in the model for this region of the estuary, which is characterized by residence times that vary from 8 to 28 days, depending on the river flow and tidal amplitude (Ferreira et al. 2005; Saraiva et al. 2007; Brito et al. 2012). Hence, a reduction in river flow would lead to higher residence times in the Tagus Estuary, possibly increasing grazing, as reported for other estuarine systems (Ambler et al. 1985; Pace et al. 1992).

### Influence on the water quality indicators

Both the reduction in the river flow and mean SLR scenarios resulted in a decrease in the nutrients and chlorophyll-a concentrations, which are two of the water quality indicators used in the WFD classification. It is important to keep in mind that the water quality metrics for transition waters combine the concentrations with salinity (APA 2016; Cereja et al. 2022a). The higher salinity classes are more sensitive to increases in both nutrients and chlorophyll-a, as a consequence of lower reference values. Thus, the increase in the salinity throughout the estuary predicted in the mean SLR and river reduction scenarios could lead to a worsening in the classification results. Even so, it was possible to observe an improvement in the water quality metrics for the RD50% and the SLR1 scenarios-the ones leading to larger variations relative to the reference scenario. The estimated improvement in the water quality indicators resulted directly from a decrease in the river flow, which reduced the nutrients and chlorophyll-a input into the system. NO<sub>3</sub><sup>-</sup> was the nutrient more influenced by riverine water and thus the one presenting the most relevant decrease. This reduction, and consequent enhancement in the water quality ratios, may lead to a greater resistance of the metrics to changes in nutrients from anthropogenic sources. Even in the case of an overall improvement of water quality, the point sources of nutrients (i.e. the discharges from WWTP) may become drivers with greater importance for the estuarine ecosystem spatial variability. Moreover, the reference conditions in Portugal were defined using historical data (2000-2010 period, Brito et al. 2012, APA, 2016, Caetano et al. 2016, Cereja et al. 2022a). Thus, it is possible that this effect is already being incorporated into the assessment. Cereja et al. (2021) identified a decreasing trend in river discharges from the 1970s to the present, meaning that the freshwater input has decreased in the last 50 years, which could lead to outdated historical data-based reference values. Furthermore, a decrease in the total phytoplankton biomass per se may also deeply affect the estuarine food webs and nursery function of the estuary. Thus, it is important to re-evaluate the effects of a decrease in chlorophyll-a concentrations in the estuarine ecology and the assessment of the water quality, as currently implemented.

# Conclusions

The expected reduction in the river flow and stronger coastal water contribution to the estuarine water due to the sea level rise (SLR) will probably lead to an increase in salinity throughout the Tagus Estuary in the next decades, accompanied by a decrease in both nutrients from riverine sources and in the estuary chlorophyll-a. The increase in the flow (10%) of one of the largest outfalls discharging into the estuary had no significant effect over the majority of the analyzed variables, only leading to a small increase in nutrients in the region surrounding the discharge. However, further analyses are required to assess how such changes in a larger number of WWTP discharges throughout the estuary will affect the water quality and the environment.

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**Data Availability** All data may be provided upon request made to the corresponding author.

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