

# Phytoplankton community as monitoring tool in the terminal stretch of a micro-tidal estuary facing the Tyrrhenian Sea

Francesco Bolinesi<sup>1</sup>, Emanuela Serino<sup>1</sup>, Angelo Carotenuto<sup>1</sup>, Silvia Fanina<sup>1</sup> and Olga Mangoni<sup>1,2</sup>

<sup>1</sup>Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Naples; <sup>2</sup>NBFC, National Biodiversity Future Center, Palermo, Italy

## ABSTRACT

Mediterranean coastal systems, particularly those characterized by river mouths, have played an important role in the history of human civilization, thanks to unique species assemblages, a high biodiversity, and complex ecological interactions. The overlapping of multiple cross-scale effects, triggered by the ongoing climate change, makes these systems particularly important sites for ecological studies. Here, we have investigated the phytoplankton community structure in the terminal stretch of the Sele River (Tyrrhenian Sea) under two different hydrological regimes: i) with river mouth occluded by sediments preventing water exchange with the sea; ii) with river mouth completely opened, which allowed the maximum water mass exchanges between the river and the sea. The strong variations in physical-chemical

properties of the water column, the point-distribution of total phytoplankton biomass, and the dominance of cryptophytes and cyanophytes under occluded and opened river mouth, respectively, indicate the presence of strong allogenic pressures acting on the system. Our data suggest that the monitoring of phytoplankton communities in the terminal stretch of micro-tidal estuaries, could be a useful tool for studying the extent of climate change underway in Mediterranean coastal marine areas.

Corresponding author: Olga Mangoni, Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, via Cinthia 21, 80126, Naples, Italy.  
E-mail: [olga.mangoni@unina.it](mailto:olga.mangoni@unina.it)

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## Introduction

Mediterranean micro-tidal environments are transitional systems characterized by a tidal range <2m, where salt-wedge intrusion along the final stretch of rivers plays an important role in shaping biological communities (Boothroyd, 1978; Davies, 1964; Kiriaki *et al.*, 2010; van der Tuin, 1991). The extent of this process depends on several geomorphological and physical factors, such as, for example, the river mouth configuration or man-made barrier-like dams (Haralambidou *et al.*, 2010; Lichter *et al.*, 2011).

From a geomorphological point of view, a fennel-shaped river mouth can be defined as an estuary, in which the mixing between seawater and freshwater generates strong salinity gradients and the presence of a typical brackish water layer (Perillo, 1995; Poulos *et al.*, 1993; Valle-Levinson, 2010). These systems are particularly vulnerable to climate change, since strong seasonality and variability in precipitation produce large inter-annual fluctuations in river flow and water mass exchange with the sea (Gasith and Resh, 1999; Kennish, 2002, 2021; Newman *et al.*, 2005; Ustin *et al.*, 2014; Verri *et al.*, 2018;). In the last decade, in fact, a reduction of net rainfall of ~33% of the annual mean was reported for southern Italian regions, mostly due to a reduced contribution of winter rainfall (Caloiero *et al.*, 2021; Polemio and Casarano 2004, 2008). The effects of the drought period and human interventions like land reclamation, construction of river embankments, and water extraction activities significantly contributed to altering the ecological profiles of these areas (Dugdale *et al.*, 2012; Hearn and Robson, 2001; Kennish, 2021; Newman *et al.*, 2005; Richard *et al.*, 2018;). The combined effect of salt-water intrusion and river discharge produces significant changes in nutrient loads, since the extent to which nutrients are retained in rivers and then made available during the biomass development

seasons depends on the water retention time (Lancelot and Muylaert, 2011; Lichter *et al.*, 2011; Newman *et al.*, 2005; Pinckney *et al.*, 1999;). Many authors state that changes in river water regime from lotic to lentic are accompanied by an increase in allogenetic phytoplankton biomass (Lampert and Sommer, 1997; Reynolds, 1984, 1999, 2003; Reynolds and Descy, 1996; Waylett *et al.*, 2013) generating blooms under optimal environmental conditions dominated by species able to grow at intermediate salinity as well as tolerant to large salinity fluctuations in brackish waters of terminal stretches (Jackson *et al.*, 1987; Muylaert *et al.*, 2009; Roubeix and Lancelot, 2008). Being passively transported by currents, phytoplankton at the source of the river would require a very rapid replication time so that they can generate blooms. This occurs when net specific growth rates of species exceed the residence time of the water (Lucas *et al.*, 2009; Reynolds, 1984). Hence, phytoplankton represents a significant component of primary production only in slow-moving rivers in lowland regions. The responses of the phytoplankton community to variations in water flow rate, nutrient loads, light availability, and temperature are species-specific and highly dependent on the whole environmental context, differing from time to time within each segment of an estuary (Artigas *et al.*, 2014). Several studies report that diatoms are the dominant phytoplankton group in rivers, with changes in size spectra composition and species associated with changes in nutrient concentration, turbidity, and flushing rate (Genkal 1997; Reynolds *et al.*, 1994b). For example, Schuchardt and Schirmer, (1991) and Muylaert *et al.*, (2000) report the dominance of diatoms in turbid estuaries throughout the year under high nutrient availability. Marshall *et al.*, (2006) report the dominance of diatoms in spring in estuaries where turbidity is low, when light levels are relatively low and stratification does not occur. Among other groups, dinoflagellates and cyanobacteria are typically more abundant in summer during periods of low water discharge, long water residence time, minimal flushing rates, and low turbidity levels (Lancelot and Muylaert, 2011). Tavernini *et al.*, (2011) report the dominance of diatoms, chlorophytes, and cyanophytes in the terminal stretch of the Po River, with changes in seasonal species assemblages showing an increase of cryptophytes in late July and of cyanophytes at high water discharge rates, from the end of April to mid-June. Within the problematic framework described above, we have analyzed the phytoplankton community structure through the detection of diagnostic pigments (Ansotegui *et al.*, 2001; Mangoni *et al.*, 2017) in the final stretch of the Sele River (Tyrrhenian Sea) under two distinct hydrological regimes: during a severe drought period in July 2017, in which, for the first time in living memory, the mouth of the river was completely occluded by sediments, and in May 2021, in presence of a high water flow rate and a completely open river mouth. Our aim was to understand to what extent the strong environmental variations influenced the structure of the phytoplankton community in the terminal stretch of one of the most important river Southern Italy.

## Materials and Methods

### Study area

The Sele River is the second largest river in southern Italy in terms of average water volume after the Volturno. It is located within a wide alluvial coastal plain, with a drainage basin

of 3235 km<sup>2</sup> and a solid flow of 500,000 m<sup>3</sup> y<sup>-1</sup> (Cocco *et al.*, 1989). 64 km long and tributary of the Tyrrhenian Sea (Di Paola *et al.*, 2014), it has the highest mean annual flow discharge among the rivers of Southern Italy (*i.e.*, ~69.4 m<sup>3</sup> s<sup>-1</sup>) (Magliulo *et al.*, 2021). It has been an important site for ancient populations, with the first archaeological settlements dating back to the second millennium B.C. (Ferrara *et al.*, 2010). The climate of its basin is characterized by prolonged warm and dry summers and wet and mild winters, with mean annual precipitation between 700 to 2000 mm, (average of 1180 mm) showing a marked spatial variation (Diodato *et al.*, 2011). Over the past 150 years, the Sele estuary coastline has been affected by erosion. From 1870 to 1984, the coastline gradually receded, with the highest erosion rates occurring in the main areas around the river mouth. In the last 150 years, the Sele River and its catchment area have also been affected by increasing human activities that exerted a significant influence on the evolution of the area. The construction of an artificial drainage channel (Canale di Bonifacimento) (Alberico *et al.*, 2012a, b), with a dense net of artificial drainage channels and the presence of the Dam of Persano strongly enhanced coastal erosion and altered the river environmental conditions (Alberico *et al.*, 2012a, 2012b; Arienzo *et al.*, 2020; Pappone *et al.*, 2011;). The Dam of Persano, located 16.2 km from the river mouth, built between 1929 and 1932, created a basin of 1.5 million m<sup>3</sup>, which affected the downstream sediment deposition causing the consequent retreat of the coast (D'Acunzi *et al.*, 2008; Freeman *et al.*, 2007; Magdaleno *et al.*, 2018). In most recent years, the significant geomorphological alterations of the Sele estuary and the presence of pollutants and conspicuous loads of organic matter deriving from anthropogenic activities have been documented, along with a pronounced accumulation of allogenetic substances under low flow conditions caused by rainfall deficit (Arienzo *et al.*, 2020; Benassai *et al.*, 2015; De Rosa *et al.*, 2022; Montuori *et al.*, 2022;).

### Sampling and environmental parameters

Water sampling was carried out in the terminal stretch of the Sele River, from the mouth up to 3.5 Km upstream, under two distinct flow-rate conditions (Figure 1a-c). The first sampling was carried out in July 2017 (Arienzo *et al.*, 2020) with the river occluded by sediments, a condition that persisted for 1 week, and the second sampling was carried out in May 2021, with the river's mouth completely opened. Stations were located taking into consideration the presence of drainage channels flowing into the river (Table 1).

At each station, 5 L of water were collected with a Niskin bottle and successively subsampled for the analyses of biological and chemical parameters, as described below.

For the determination of total phytoplankton biomass (Chl a), 50-500 mL of water (depending on the presence of particles in the samples) were drawn from the Niskin bottle and filtered onto GF/F Whatman glass-fiber filters (25-mm diameter) immediately cryopreserved in liquid nitrogen until the analyses in the laboratory.

At each station, water temperature and salinity were determined using Idromar XMAR212 (2017) and Sbe 19 plus (2021) CTD probes interfaced with a GPS (Garmin Map 78S, Garmin, Olathe, USA).

### Total phytoplankton biomass and chemo-functional groups

For the determination of total phytoplankton biomass (Chl a), 50-500 ml of water (depending on the presence of particles in the samples) were drawn from the Niskin and filtered on GF/F Whatman filters (25-mm diameter) immediately cryopreserved in liquid nitrogen until the analyses in laboratory (Holm-Hansen *et al.*, 1965; Lorenzen and Jeffrey, 1980; Yentsch and Menzel, 1963). Chlorophyll-a and phaeopigments were determined, after extraction with 90% acetone, with a spectrofluorometer (Mod.RF-6000; Shimadzu Corporation, Kyoto, Japan), with calibration curves checked daily with fresh Chl a standard solutions (Sigma-Aldrich, St. Louis, USA) (Holm-Hansen *et al.*, 1965). The pheo:chl a ratio was used as a proxy of grazing activity on phy-

**Table 1.** Sampling station with coordinates and bottom depth.

Station	Latitude N	Longitude E	Depth (m)
1	40.4814990	14.9448934	3.037
2	40.4812454	14.9488489	6.017
3	40.4810787	14.9506658	3.819
4	40.4812777	14.9538731	3.221
5	40.4829086	14.9582883	3.333
6	40.4850757	14.9623601	3.479
7	40.4877350	14.9655997	3.157
8	40.4902284	14.9688533	3.306
9	40.4920247	14.9718688	3.414
10	40.4962250	14.9724679	2.732
11	40.4997458	14.9718300	3.559



**a**



**b**



**c**

**Figure 1.** Final stretch of the Sele river with sampled stations (a). Mouth of the river in July 2017 (b) and May 2021 (c).

toplankton cells (Shuman and Lorenzen, 1975). For the determination of the pigmentary spectra, 1.5 L of water was filtered on GF/F Whatman filters (47-mm diameter) and the filters were cryopreserved as for Chl a. Frozen filters were homogenized and re-suspended in 100% methanol and analyzed by High-Performance Liquid Chromatography (HPLC) (1100 Series, Hewlett Packard, Palo Alto, USA) in a reverse phase (C8 column 3  $\mu$ m Hyperloop MOS) (Vidussi *et al.*, 1996). The use of HPLC in the study of phytoplankton communities has been largely demonstrated to be a useful tool for the estimation of phytoplankton community composition through the analyses of photosynthetic pigments (Jeffery and Vesik, 1997; Wright *et al.*, 1996). The method is based on the analysis of accessory pigments, in addition to Chlorophyll-a (Chl-a) or the modified divinyl-Chl-a found in all phytoplankton species, and on the evidence that some of these accessory pigments are taxon-specific (Brunet and Mangoni, 2010; Millie *et al.*, 1997; Wright and Jeffrey, 2006). This technique allows to detect and identify microscopically overlooked or undetermined ultraphytoplankton species (Ansotegui *et al.*, 2003; Antajan *et al.*, 2004; Garibotti *et al.*, 2003; Saggiomo *et al.*, 2023), providing reproducible results. For the determination of chlorophylls and carotenoids, a spectrophotometer with a diode array detector was set at 440 nm, making it possible to determine the absorption spectrum of the 350–750 nm interval for each peak to check the purity of single pigments. The column was calibrated using different pigment standards (chlorophyll a, chlorophyll b, chlorophyll c<sub>1</sub>+c<sub>2</sub>, chlorophyll c<sub>3</sub>, MgDVP, alloxanthin, diatoxanthin, prasinoxanthin, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, peridinin, zeaxanthin, neoxanthin, violaxanthin, b-carotene). Standards were provided by the International Agency for 14 C Determination, VKI Water Quality Institute, Copenhagen, Denmark. Quantification was based on the absorbance at 440 nm and the factor response value (peak area/pigment concentration) for each pigment, as described by Mantoura and Repeta (1997). The contribution of main phytoplankton groups to the total Chl a was estimated by CHEMTAX 1.95 software as indicated by Latasa (2007), using an iterative process to find the optimal pigment:Chl a ratios. The chemotaxonomic groups identified include cyanophytes (Cyano), chlorophytes (Chloro), prasinophytes (Prasino), euglenophytes (Eugleno), cryptophytes (Crypto), diatoms (Diat), pelagophytes (Pelago) haptophytes (Hapto), dinoflagellates (Dino), xanthophytes (Xanto).

### Inorganic nutrient concentrations

For the determination of inorganic nutrient concentrations (N-NO<sub>3</sub>, P-PO<sub>4</sub>), 10 mL water were filtered on 0.2  $\mu$ m cellulose acetate filters, and stored in HDP vials at -20°C. The analyses in the laboratory were conducted using a discrete sampling analyzer (EasyChem Plus, Systea, Anagni, Italy) equipped with a UV-VIS spectrophotometer following the procedure described by Hansen and Grasshoff (1983) adapted to current instrumentation, using a standardized method to detect nitrate (EPA - Ref. National Environmental Methods Index 9171 Nitrate via V(III) reduction), and orthophosphate (ISO 15923 - Ref. International Standard Organization 15923-1 Water quality).

### Statistical analyses

The spatial distribution of physical variables (temperature, salinity) was plotted using ODV 5.6.3 software.

To estimate the pairwise dissimilarity between the two contrasting water flow conditions in a low-dimensional space, a non-metric multidimensional scaling based on a distance matrix computed with the Bray-Curtis dissimilarity has been performed and represented using a bi-plot in which the correlation coefficients between environmental variable and the NMDS scores were presented as vectors from the origin based on algorithm proposed by Taguchi and Oono (2005). The significance of differences between the two putative conditions of river's water flow was tested by ANOSIM (Clarke, 1993). All statistical analyses were performed using the PAST 1.95 software.

## Results

### Environmental parameters

Values of salinity are reported in Figure 2. In 2017 the distribution of salinity showed the presence of a strong water stratification, with a net halocline between 1.5-2 m (station 2) and 2-2.5 m (station 9) whose depth increased moving toward the inner part of the river (Figure 2a). In general, values ranged between 39.8 and 0.7. At station 1, near the sandbar occluding the river mouth, salinity reached values up to 39 originating a 'bubble-like' front probably related to the seawater percolation or episodic waves intrusion from the adjacent sea. The isohalines between 5 and 8, which have been the subject of dispute and debate over time (Deaton and Greenberg, 1986; Khlebovich, 1968, 1969), took place between 1 m (station 2) and 1.8 m depth at station 9, with a slightly deepening moving from the mouth to the inner part of the river. Station 1 was the only one with salinity >8 in the entire water column. In 2021, with the river mouth completely opened, the entire sampling area was still characterized by a net water stratification, with a 5-8 salinity boundary cline placed between 1.5 and 2.5 m depth, deepening moving from the mouth (station 1) to the inner part of the river (station 11) (Figure 2b). In the entire area, salinity ranged between 36.2 and 0.01. All stations presented two distinct layers: a freshwater one, between 0 and 1.5 m, and a marine one, below 2.5 m. The only exception was station 10, where values reached maximum salinity of 10 on the sea bottom, and the freshwater layer reached its maximum thickness.

As far as the temperature is concerned, it showed clear differences between the two sampling periods (Figure 3). In July 2017 water temperature exceeded 28.5 °C in the first meter of the water column, generating a marked thermocline at ~1.3 m depth in the overall sampling area (Figure 3a). The bottom layer was characterized by the presence of the coldest water reaching the minimum of 24.59 °C at station 2. The surface layer showed a slight increasing trend of temperature moving from the mouth to station 11, with values ranging between 28.5°C and 31.34°C. In May 2021, the water column appeared to be rather homeothermic (Figure 3b). In general, values decreased moving from the mouth to the station 11, where temperature reached the minimum of 19.3 °C. A weak hermos-stratification was present in the first 30 cm between stations 1 and 5, where the water retention time reached its maximum. Nevertheless, a slightly increased temperature in the bottom water layer was observed in vertical profiles between stations 5 and 9, in accordance with the increase of salinity linked to seawater inflow.

The concentration of N-NO<sub>3</sub> and P-PO<sub>4</sub> in July 2017 led to

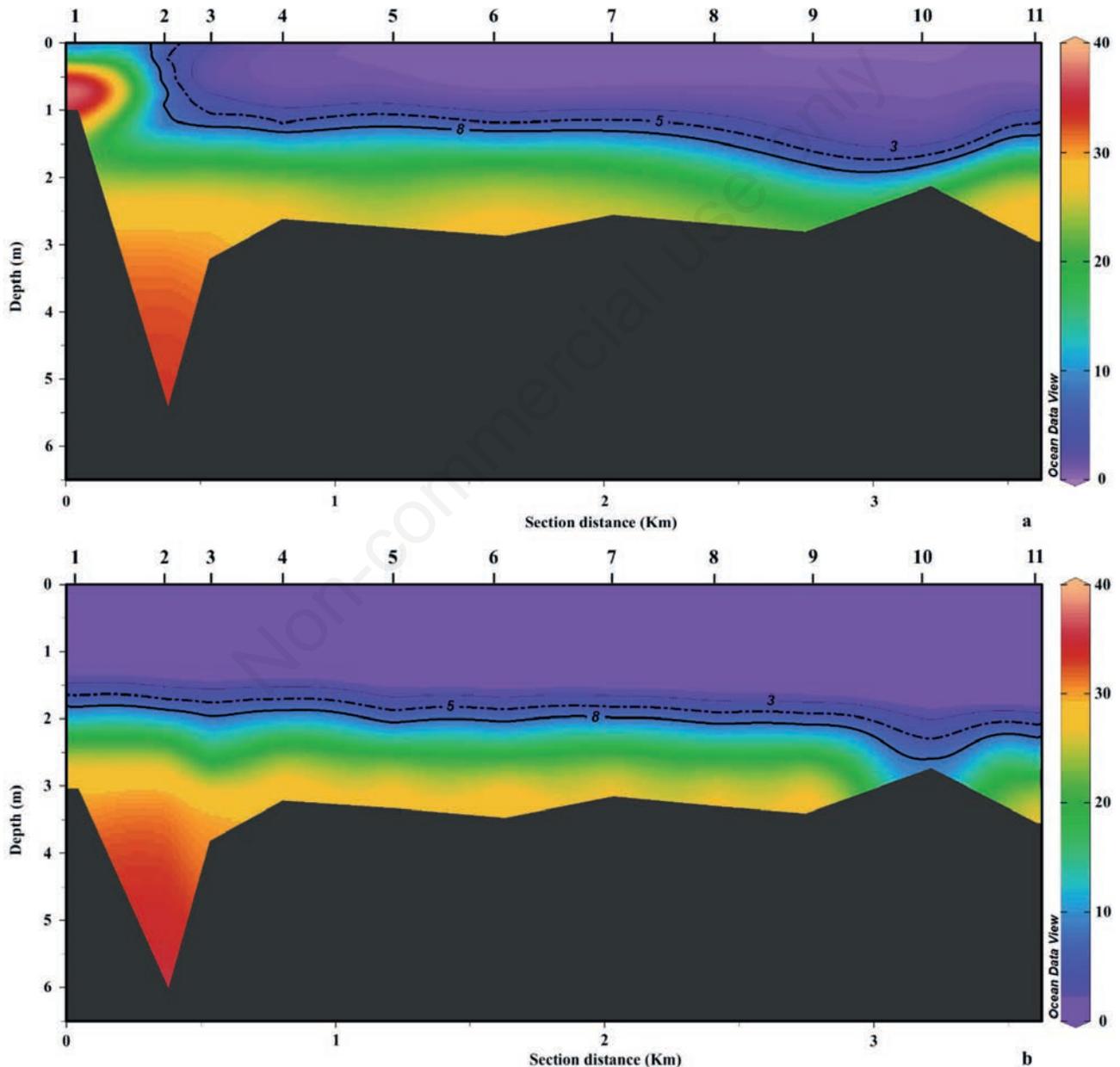
a mean N:P ratio of 12.12 in the entire area, showing a point-distribution depending on the influence of drainage channels (Arienzo *et al.*, 2020). In particular, N-NO<sub>3</sub> ranged between 12.85  $\mu\text{mol L}^{-1}$  (station 5) to 23.80 (station 10) with a mean concentration of 18.2  $\mu\text{mol L}^{-1}$ ; P-PO<sub>4</sub> ranged between 0.81  $\mu\text{mol L}^{-1}$  (station 3) and 8.39  $\mu\text{mol L}^{-1}$  (station 10) with a mean concentration of 2.84  $\mu\text{mol L}^{-1}$  (Figure 4a).

In May 2021, values of N-NO<sub>3</sub> showed a mean concentration of 15.76  $\mu\text{mol L}^{-1}$  ranging between 4.57  $\mu\text{mol L}^{-1}$  (station 2) and 23.51  $\mu\text{mol L}^{-1}$  (station 9), and displaying a point-shaped distribution, as observed in the first sampling (Figure 4b, c). P-PO<sub>4</sub> were characterized by very low concentrations, with a mean

of 0.2  $\mu\text{mol L}^{-1}$  and values ranging between 0.08  $\mu\text{mol L}^{-1}$  (station 2) and 0.39  $\mu\text{mol L}^{-1}$  (station 6). These aspects led to a mean N:P ratio of 83, a value far from what was observed in 2017 and from the Redfield one. In particular, the N:P ratio showed values ranging between 148 (station 3) and 30 (station 5), with the highest values in the surface layer.

### Phytoplankton biomass and chemo-functional groups

The distribution of total phytoplankton biomass (Chl a) in July 2017 showed the presence of eutrophic condition in the

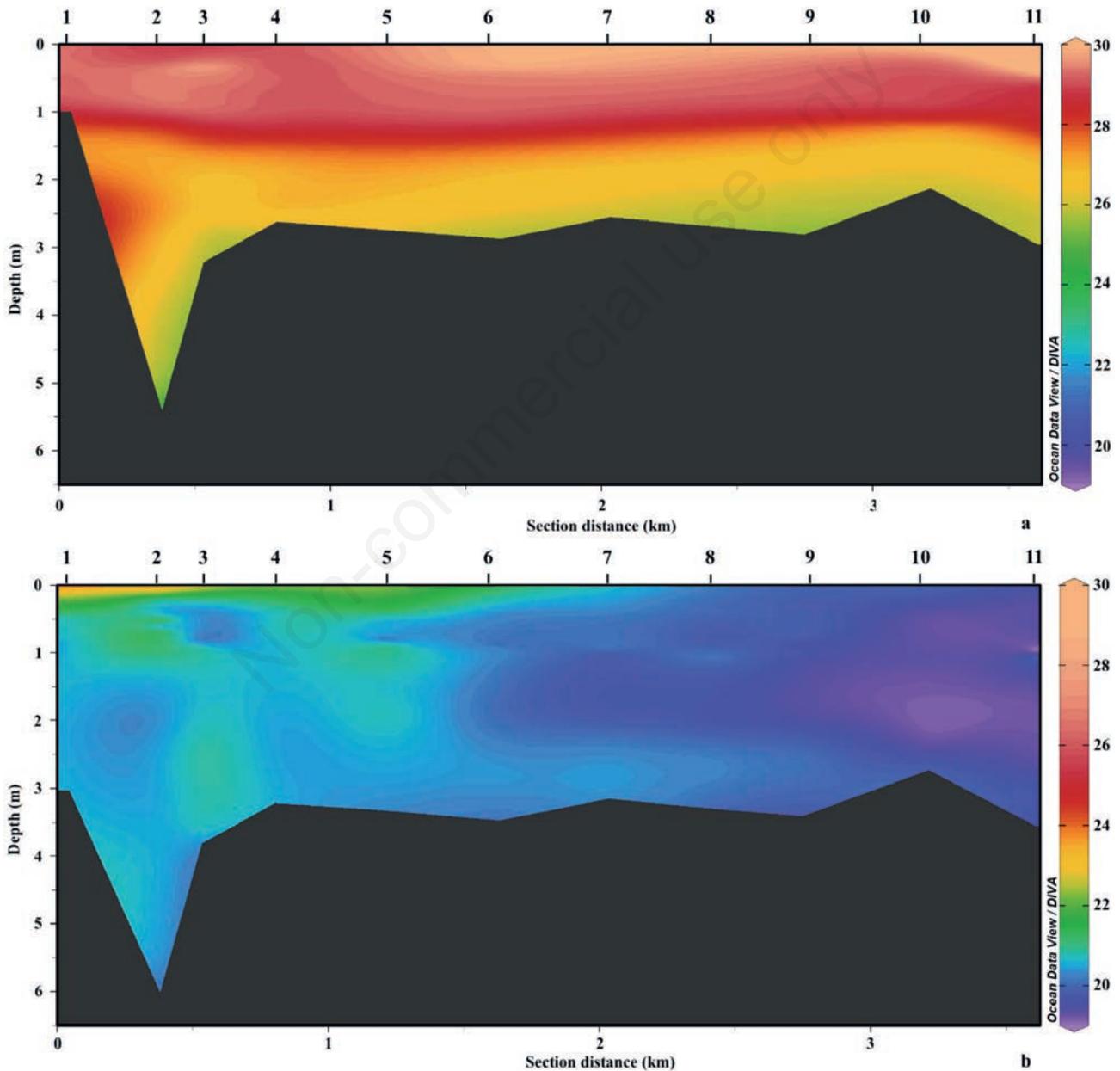


**Figure 2.** Distribution of salinity along the water column of the entire sampled area in July 2017 (a) and May 2021 (b). Y axis: depth (m); X axis: station distance; Z colored axis: salinity.

overall sampling area (Figure 5a). Values ranged between a minimum of  $15.05 \mu\text{g L}^{-1}$  at station 9, and a maximum of  $95.73 \mu\text{g L}^{-1}$  at station 10, highlighting a strong variability in the area, especially near drainage channels. This aspect was also emphasized by the fluctuation of grazing index values (Phaeo:Chl a), which showed a mean of 0.59 ranging between 0.20 (station 11) and 2.54 (station 9). The overall picture that emerged from Chl a concentration indicated the presence of an increasing trend moving from the mouth to the inner part of the river. In May 2021 (Figure 5b, c), values of Chl a were at least one order of magnitude lower compared to what was reported before, with

values usually observed in oligotrophic/mesotrophic systems. The mean value for the entire area was  $1.40 \mu\text{g L}^{-1}$ , with concentrations ranging between  $0.55 \mu\text{g L}^{-1}$  (station 10) and  $3.11 \mu\text{g L}^{-1}$  (station 11). The grazing index ratios were very high at all stations, with a mean of 1.21 and values ranging between 0.62 (station 2) and 1.87 (station 10).

As far as the pigment spectra composition (expressed as pigment:Chl a ratios) is concerned, we observed high variability in both sampling periods (Table S1). In July 2017, fucoxanthin was the dominant pigment, with a mean ratio of 0.67 and values ranging between 0.43 (station 5) and 0.97 (station 1). Alloxan-

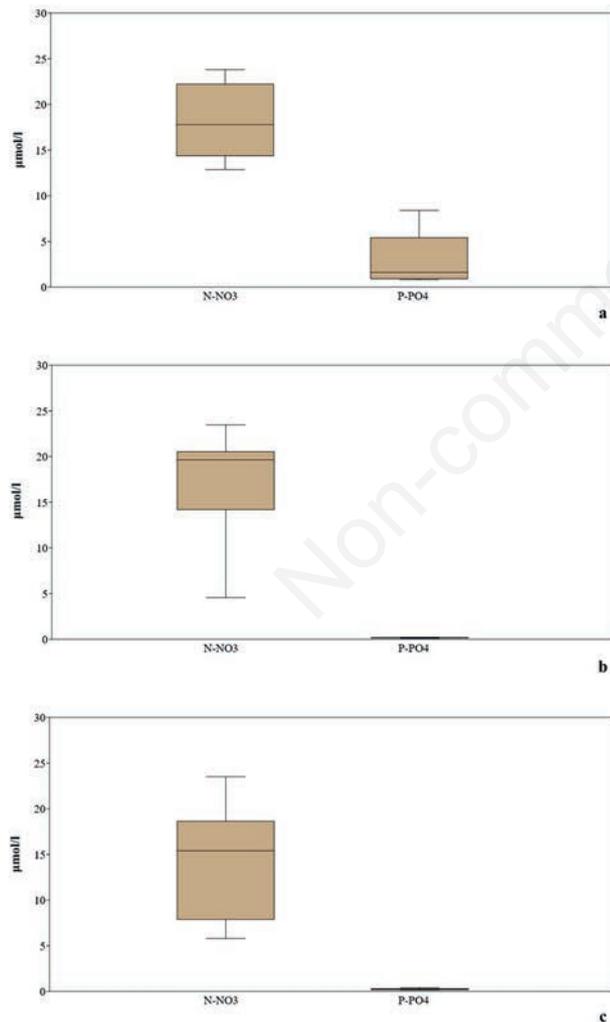


**Figure 3.** Distribution of temperature along the water column of the entire sampled area in July 2017 (a) and May 2021 (b). Y axis: depth (m); X axis: station distance; Z colored axis: temperature ( $^{\circ}\text{C}$ ).

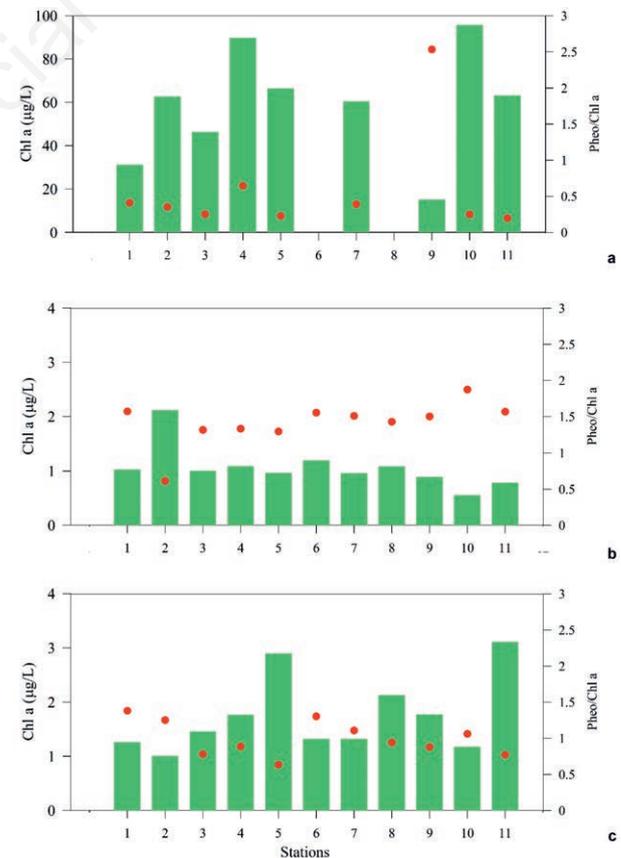
thin showed the highest ratio at stations 5 (0.64) and 10 (0.64) and, together with Chl c2 and Diadinoxanthin was among the most represented ones. Chl c3, peridinin, phaeophytin, and 19'HF were the only pigments completely absent in the sampling area. In May 2021, fucoxanthin and MgDVP were the most abundant pigments, with mean ratios of 0.49 and 0.41, respectively. aFucoxanthin ranged between 0.36 (station 2, bottom layer) and 0.56 (station 5, bottom layer); MgDVP between 0.29 (station 4) and 0.59 (station 8, bottom layer). The contribution of fucoxanthin and MgDVP to the total pigmentary spectra was higher at the bottom layer than 0 m. Neoxanthin was the only pigment to be absent at all stations. Peridinin and neoxanthin were completely absent only at surface layer, while other pigments showed weak and nonlinear changes as highlighted by colored bars. The pigmentary spectra composition described so far, determined a different chemotaxonomically composition of phytoplankton community in the two sampling periods (Figures 6a-c). In July 2017, cryptophytes strongly dominated the com-

munity in the entire area, with a mean of 58% and values ranging between 29% (station 2) and 79% (station 11). Diatoms and chlorophytes were the second most representative groups, with a mean of 16% and 11% respectively, and higher percentages at station 7. Dinoflagellates and haptophytes were completely absent, while xanthophytes were only reported with a weak percentage (2%) at station 11.

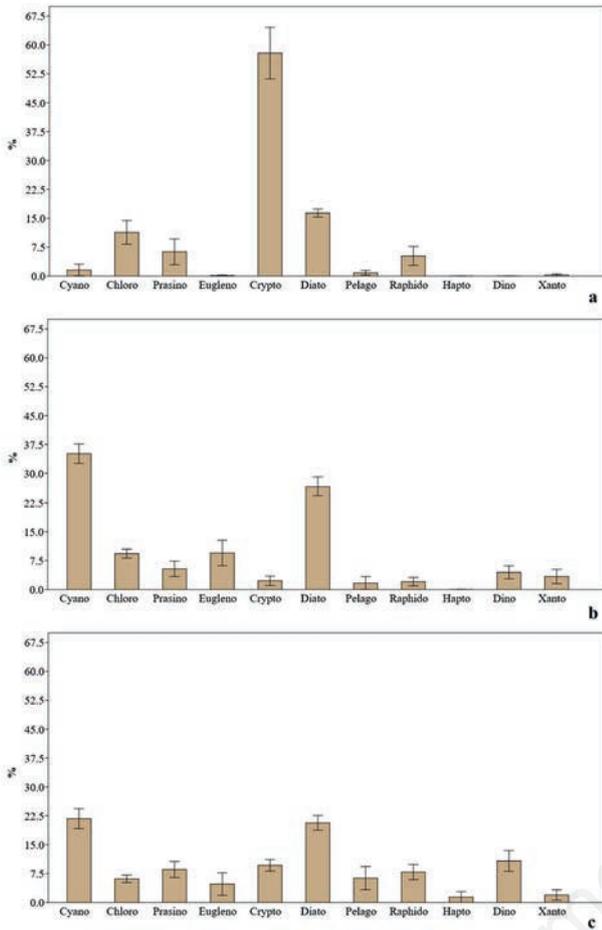
In May 2021 (Figure 7b-c), the community was dominated by cyanophytes and diatoms, with mean percentages of 28.5% and 27.3% respectively. Cyanophytes showed a strong gradient at station 4, ranging between 9% (surface layer) and 43% (bottom layer); diatoms ranged between 6% (station 3, bottom layer) and 47% (station 11). Chlorophytes and euglenophytes showed similar mean percentage (7%), with the first group that was more represented at the surface layer, and the second one more represented at deeper layer where reaches percentages up to 16% (station 11). Haptophytes was the only groups to be absent at surface layer and to be present exclusively at bottom of the station 4.



**Figure 4.** Box plots with nutrient concentrations of P-PO4 and N-NO3 in July 2017 (a); surface layer in May 2021 (b); bottom layer in 2021 (c).



**Figure 5.** Chart bar with chlorophyll a concentration (Chl a µg/L) and grazing index (Phaeo:Chl a) at each station (X axis) in July 2017 (a); surface layer May 2021(b); bottom layer in May 2021(c).



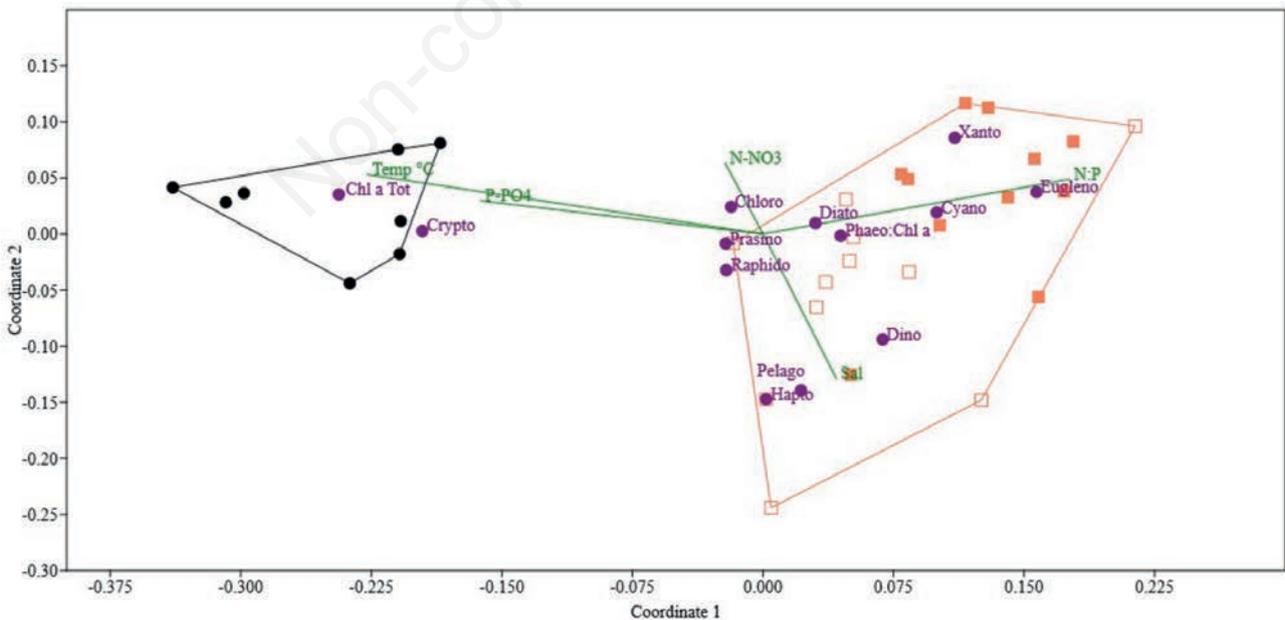
## Discussion

Mediterranean microtidal estuaries have played an important role in human civilization (Haidvogel 2018). The present study aims at providing the first insights into changes in phytoplankton community structure in the terminal stretch of the Sele River, a microtidal estuary subjected to multiple anthropogenic pressures, as the result of contrasting conditions of the river's water flow.

The reclamation works carried out in the region during the last century have profoundly altered its morphology and degree of interactions between the river, the surrounding lands, and the sea. The extent of these changes is reported today in several geomorphological studies, though with a considerable lack of information on the ecology of the area, especially regarding microalgal communities in the terminal stretch, where phytoplankton plays a pivotal role as primary producers in lentic regime waters. Our data represent the first attempt to describe the phytoplankton community structure in one of the most important hydrographic basins of southern Italy, under two distinct hydrological phases: with the mouth of the river completely occluded by sediments - for the first time in living memory - preventing the water exchange with the sea, and under a high hydrological regime with mouth completely opened.

Under two different hydrological conditions, the terminal stretch of the Sele River was characterized by the coexistence of two distinct water layers. A shallow one, showing typically fresh-water salinity whose thickness tends to be higher in the inner part

**Figure 6.** Chemotaxonomically composition of phytoplankton community in July 2017 (a); surface layer in May 2021 (b), bottom layer in May 2022 (c).



**Figure 7.** Non-metric multidimensional scaling based on Bray-Curtis index. Environmental variables are shown in green; the correlation coefficients between each environmental variable and the NMDS scores are presented as vectors from the origin. Stations of July 2017 are reported as black circles; stations of May 2021 are reported as filled (bottom layer) and empty (surface) orange squares.

of the river, and a deeper one with salinity values that typically exceeded that of the adjacent seawater, especially in deepest ponds and during summer. These thickness changes were accompanied by changes of 5-8 isohaline depth, which was absent near the mouth occluded by sediments and highest at station 10 in the inner part of the river. Salinity variation represents a key factor for the biotic component of ecosystems and the presence of brackish organisms is a typical property of estuarine environments. Changes in salinity over river mouths depends on the tides, coastal flooding, and season of the year (Reynolds, 1984; 2006), so the formation of sandy bar occluding the mouth and preventing the water exchange with the sea, can have significant effects on the functioning and ecology of such a complex system, with multi-scale effects still unknown.

Since dramatic rainfall-decreasing trends were reported for the southern Italian Tyrrhenian coast, causing droughts during summer (Polemio and Casarano, 2004, 2008; Romano *et al.*, 2022), the results presented here could be the first sign of a new condition towards which this system will likely tend more frequently in the near future.

Contrarily to the salinity, the thermal profile of the water column showed marked differences between the two sampling periods in accordance with the season. In July 2017, a net thermocline was present at  $\sim 1.3$  m depth, with water temperature exceeding  $28.5$  °C in the first meter of the water column reaching colder values on the bottom (minimum  $24.59$  °C, at station 2). In spring 2021 the water column was rather homeothermic, with lower values in the inner part of the river and relatively warmer water on the bottom linked to the seawater intrusion. A weak stratification was observed in the first 30 cm between stations 1 and 5, and despite the fact current meters data are not available, we hypothesize that this warmer layer reflects the hydrological dynamics of the area, where the increase in water retention time favors surface waters warming. It must be noted that stations were sampled  $\sim 10$  minutes apart from each other, moving from the mouth to station 11, so sampling activities should not have influenced these data.

Many authors indicate that any potential alteration to river flows would increase the temporal and spatial variability of estuarine fronts, water stratification, and mixing, with potentially negative impacts associated with eutrophication (Reynolds, 1984; 2006). The impact of nutrient enrichment on rivers is often season specific and complicated by their dynamic nature (Newman *et al.*, 2005). Differences in nutrient concentration observed in our study, with extremely large spatial and temporal variations in N:P ratios, suggest how complex and articulated are the dynamics of nutrient supply in this area, where the presence of drainage channels (in addition to the combined effects of water retention times) can strongly influence the chemical properties of the water column (Albanese *et al.*, 2007; Arienzo *et al.*, 2020; Montuori *et al.*, 2022). Nitrates did not show drastic changes between the two sampling periods, as instead observed with the phosphate load, which led to higher N:P ratios in 2021, with values up to 148 at station 3, compared to those in 2017. The first biological effect of nutrient enrichment in pelagic environments is the growth of phytoplankton species, whose blooms extent and community structure strongly depend on local conditions, for example, the extent to which the nutrient load is retained by the receiving water (Newman *et al.*, 2005; Reynolds, 2006). This is the reason why rivers are considered highly selective environments, where flow rate and turbidity, along with temperature, are the most critical factors lim-

iting the development and structuring the phytoplankton community composition (Rojo *et al.*, 1994; Reynolds, 1994; Tavernini *et al.*, 2011). The higher the flow rate and turbidity, the lower the amount of phytoplankton can be expected: where the water retention time is longer than the generation time of the phytoplankton, large blooms can occur (Ibeling *et al.*, 1998; Lampert and Sommer, 1997; Newman *et al.*, 2005). Nevertheless, the extent to which nutrient loads trigger phytoplankton blooms in micro- and macrotidal estuaries is different, with small nutrient enrichment producing larger effects in the microtidal ones (Warwick *et al.*, 2018). In general, allogenic factors (*i.e.* inoculum of phytoplankton species transported downstream, temperature, light regime, discharge rate, turbidity) are considered the main drivers of phytoplankton succession in lowland rivers (Reynolds, 1994, 2006), with autogenic ones becoming more relevant with increasing eutrophication levels (del Giorgio *et al.*, 1991).

The distribution of total phytoplankton biomass (Chl a) in July 2017 points to a speckled distribution with an increasing trend of concentration moving toward the inner part of the river, where values are typical of eutrophic waters reaching the maximum of  $97.73$   $\mu\text{g L}^{-1}$ . The station with lowest concentrations of Chl a ( $15.0$   $\mu\text{g L}^{-1}$ ) showed the highest phaeo:Chl a ratio (2.7), indicating the presence of large amounts of degraded biomass, most likely because of the adjacent drainage channel transport. On the contrary, in May 2021, values of Chl a were at least one order of magnitude lower, with a mean of  $1.40$   $\mu\text{g L}^{-1}$ , appearing more homogeneously distributed in the entire sampling area and being characterized by very high phaeo:Chl a ratios, the latter supporting the hypothesis that much of the phytoplanktonic biomass was photosynthetically inactive. The overall picture emerging from nMDS analyses clearly shows the differences between the two samplings periods, with data from July 2017 (black points) clustering in the left side of the plot, and those from May 2021 (orange squares) in the right part. Temperature, P-PO<sub>4</sub> and the N:P ratios are among the main environmental drivers shaping the phytoplankton community, with high Chl a concentration and cryptophytes characterizing the first sampling period, and high N:P ratios characterizing the second one. In May 2021 (orange squares), euglenophytes, cyanophytes, and xanthophytes were the dominant groups, especially at the surface layer (filled orange squares). The correlation between salinity and depth highlights the role of seawater inflow in shaping the structure of the phytoplankton communities, with dinoflagellates, haptophytes, and pelagophytes dominating in the deepest and saltiest layers, and high loads of N-NO<sub>3</sub> that were instead associated to the shallow layer, because of inputs from drainage channels. A high and positive R value (0.9777) in the ANOSIM analysis ( $p=0.0001$ ) indicates the strong and significant dissimilarity between the phytoplankton communities' composition in the two sampling periods (Table 2). Altogether our data

**Table 2.** Results of the ANOSIM (ANalyses Of SIMilarities) test carried out to ascertain differences between the two sampling periods, representing contrasting water flow conditions.

ANOSIM Test – Bray-Curtis	
Permutation N	9999
Mean rank within	132
Mean rank between	334.6
R	0.9777
p (same)	0.0001

agree with the results reported by Tavernini *et al.*, (2011), who, in the Po River, observed the highest concentration of cryptophytes biovolume in late July, concomitantly with an higher contribution of cyanophytes between April and June: these patterns provide a further confirmation that the terminal stretch of the Sele River is exposed to strong allogenic pressures.

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## Conclusions

In addition to what discussed so far, we pinpoint here that the phytoplankton community represents a promising and effective monitoring tool for the study of estuarine environments, in which cross-scale interactions produce large fluctuations in physical, chemical, and biological properties. Nevertheless, the large variability of the phytoplankton community structure both in space and time, and the extremely ample variations in N:P ratios underline how much allogenic pressures can influence communities' functional traits in the terminal stretch of the Sele River and, supposedly, in other estuarine areas, with similar combinations of natural and anthropogenic pressure. The presence in both study periods of a saltier layer near the bottom highlights the role played by the seawater inflow in the ecology of the area, characterized by a strong summer thermoaline stratification, which exacerbates the difference between superficial and bottom water layers.

Although our results are not sufficient to mechanistically explain the functioning of such a complex ecosystem, the ample array of interactions among local climate, hydrodynamical and ecological variables that emerged from our study suggest that the Sele River estuary will most likely be increasingly exposed to the multiple stressors caused by the ongoing climate change and the increase of human uses of land and rivers. The presence of other estuarine environments in the Mediterranean Sea and the peculiar characteristics of the terminal stretches of rivers altogether suggest that more effort should be paid to investigate and monitor these delicate and vulnerable ecosystems. This appears to be urgently needed in order to identify new management measures of freshwater inputs in the Mediterranean Sea, one of the marine regions worldwide most prone to the consequences of climate change.

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## References

- Albanese S, De Vivo B, Lima A, Cicchella D, 2007. Geochemical background and baseline values of toxic elements in stream sediments of Campania region (Italy). *J. Geochem. Explor.* 93, 21–34.
- Alberico I, Amato V, Aucelli PPC, *et al.*, 2012b. Historical shoreline change of the Sele Plain (southern Italy): the 1870-2009 time window. *Journal of Coastal Research*, 28:1638-47.
- Alberico I, Amato V, Aucelli CPP, *et al.*, 2012a. Historical and recent changes of the Sele River coastal plain (Southern Italy): natural variations and human pressures. *Rend. Fis. Acc. Lincei*, 23:3–12.
- Ansotegui A, Sarobe A, Trigueros JM, *et al.*, 2003. Size distribution of algal pigments and phytoplankton assemblages in a coastal—Estuarine environment: Contribution of small eukaryotic algae. *Journal of Plankton Research*. 25:341–55.
- Ansotegui A, Trigueros JM, Orive E, 2001. The Use of Pigment Signatures to Assess Phytoplankton Assemblage Structure in Estuarine Waters. *Estuarine, Coastal and Shelf Science*. 52:689-703.
- Antajan E, Chrétiennot-Dinet M,-J, Leblanc C, *et al.*, 2004. 19'-hexanoyloxyfucoxanthin may not be the appropriate pigment to trace occurrence and fate of Phaeocystis: The case of *P. globosa* in Belgian coastal waters. *Journal of Sea Research*. 52:165–77.
- Arienzo M, Bolinesi F, Aiello G, *et al.*, 2020. The Environmental Assessment of an Estuarine Transitional Environment, Southern Italy. *Journal of Marine Science and Engineering*. 8:628.
- Artigas ML, Llebot C, Ross ON, *et al.*, 2014. Understanding the spatio-temporal variability of phytoplankton biomass distribution in a microtidal Mediterranean estuary. *Deep Sea Research Part II: Topical Studies in Oceanography*. 101:180-92.
- Benassai G, Di Paola G, Aucelli PPC, 2015. Coastal risk assessment of a micro-tidal littoral plain in response to sea level rise. *Ocean & Coastal Management*. 104:22-35.
- Brunet C, Mangoni O, 2010. Metodologie di campionamento e di studio del plancton marino. In G. Socal, I. Buttino, M. Cabrini, O. Mangoni, A. Penna, & C. Totti (Eds.), *Manuali e Linee Guida* (Vol. 56, pp. 343–349). ISPRA.
- Caloiero T, Caroletti GN, Coscarelli R, 2021. IMERG-Based Meteorological Drought Analysis over Italy. *Climate*. 9:65.
- Clarke KR, 1993. Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology*. 18:117-143
- Cocco E, De Magistris MA, De Pippo T, *et al.*, 1989. Coastal Dynamics Along the Shores of Campania and Lucania (Southern Italy). In: *Proceedings of the Sixth Symposium on Coastal and Ocean Management*, Charleston, SC, USA, 3, pp. 2794–2807.
- D'Acunzi G, De Pippo T, Donadio C, *et al.*, 2008. Studio dell'evoluzione della linea di costa della piana del Sele (Campania) mediante l'uso della cartografia numerica. *Stud. Costieri*. 14:55–67.
- Davies BR, Walker KF, 1986. The Ecology of River Systems. *Monographiae Biologicae*, 60. 793 pp.
- Deaton LE, Greenberg MJ, 1986. There is no horohalinicum. *Estuaries*. 9:20–30.
- del Giorgio PA, Vinocur AL, Lombardo RG, Tell HG, 1991. Progressive changes in the structure and dynamics of the phytoplankton community along a pollution gradient in a lowland river—a multivariate approach. *Hydrobiologia*. 224:129–54
- De Rosa E, Montuori P, Triassi M, *et al.*, 2022. Occurrence and Distribution of Persistent Organic Pollutants (POPs) from Sele River, Southern Italy: Analysis of Polychlorinated Biphenyls and Organochlorine Pesticides in a Water–Sediment System. *Toxics*. 10:662.
- Di Paola G, Aucelli PPC, Benassai G, German R, 2014. Coastal vulnerability to wave storms of Sele littoral plain (southern Italy). *Natural Hazards*. 71:1795–819.
- Diodato N, Fagnano M, Alberico I *et al.*, 2011. Mapping soil erodibility from composed data set in Sele River Basin, Italy. *Nat Hazards*. 58:445–57.
- Dugdale R, Wilkerson F, Parker AE, *et al.*, 2012. River flow and ammonium discharge determine spring phytoplankton

- blooms in an urbanized estuary. *Estuarine, Coastal and Shelf Science*. 115:187-99.
- Ferrara B, 2010. Heraion alla foce del Sele: l'uso del territorio tra scienza e archeologia. *Scienze naturali ed archeologia: il paesaggio antico: interazione uomo /ambiente ed eventi catastrofici*. Atti Convegno Internazionale, p. 123-126.
- Freeman MC, Pringle CM, Jackson CR, 2007. Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *J. Am. Water Res. Assoc.* 43:5-14.
- Garibotti IA, Vernet M, Kozłowski WA, Ferrario ME, 2003. Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: A comparison of chemotaxonomic and microscopic analyses. *Marine Ecology Progress Series*. 247, 27-42.
- Gasith A, and Resh VH, 1999. Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. *Annual Review of Ecology and Systematics*. 30:51-81.
- Genkal SI, 1997. Problems in identifying centric diatoms for monitoring the water quality of large rivers. In: J. Prygiel, B.A. Whitton, J. Bukowska (eds), *Use of Algae for Monitoring Rivers III*. p. 182-187. Douai, France, Agence de l'Eau Artois-Picardie.
- Haidvogel G, 2018. Historic Milestones of Human River Uses and Ecological Impacts. *Riverine Ecosystem Management*. 2018:19-39.
- Haralambidou K, Sylaios G, Tsihrintzis VA, 2010. Salt-wedge propagation in a Mediterranean micro-tidal river mouth. *Estuarine, Coastal and Shelf Science*. 90:174-184.
- Hearn CJ, and Robson BJ, 2001. Inter-annual variability of bottom hypoxia in shallow Mediterranean estuaries. *Estuar. Coast. Shelf Sci.* 52:643-57.
- Holm-Hansen O, Lorenzen CJ, Holmes RW, Strickland JDH, 1965. Fluorometric determination of chlorophyll. *ICES Journal of Marine Science*. 30:3-15.
- Ibelings B, Admiraal W, Bijkerk R, et al., 1998. Monitoring of algae in Dutch rivers: does it meet its goals? *Journal of Applied Phycology*. 10:171-81.
- Jackson RH, Williams PJJ, Joint IR, 1987. Freshwater phytoplankton in the low salinity region of the River Tamar estuary. *Estuarine, Coastal and Shelf Science*. 25:299-311.
- Jeffrey SW, Vesik M, 1997. Introduction to marine phytoplankton and their pigment signatures.
- Khlebovich VV, 1968. Some peculiar features of the hydrochemical regime and the fauna of mesohaline waters. *Mar. Biol.* 2:47-9.
- Khlebovich VV, 1969. Aspects of animal evolution related to critical salinity and the internal state. *Mar. Biol.* 2:338-45.
- Kennish M, 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation*. 29:78-107.
- Kennish M, 2021. Drivers of Change in Estuarine and Coastal Marine Environments: An Overview. *Open Journal of Ecology*. 11:224-39.
- Lampert W, Sommer U, 1997. *Limnology: the ecology of lakes and streams*. Oxford University Press, New York, USA. 336 pp.
- Lancelot C, Muylaert K, 2011. 7.02 Trends in Estuarine Phytoplankton Ecology. Available from: <https://www.researchgate.net/profile/Arvind-Singh-21/post/Did-you-find-any-relationship-between-the-water-quality-and-distribution-of-phytoplankton-in-that-estuarine-waters/attachment/59d64e4379197b80779a7a31/AS%3A492057329442816%401494327017453/download/235306.pdf>
- Legendre P, Legendre L, 1998. *Numerical Ecology*, 2nd English ed. Elsevier, The Netherlands. 853 pp.
- Lichter M, Klein M, Zviely D, 2011. Dynamic morphology of small south-eastern Mediterranean river mouths: a conceptual model. *Earth Surf. Process. Landforms*. 36:547-62.
- Lorenzen CJ, Jeffrey SW, 1980 - Determination of chlorophyll in sea water. *Unesco Tech. Pap. Mar.Sci.* 35:1-20.
- Lucas LV, Thompson JK, Brown LR, 2009. Why are diverse relationships observed between phytoplankton biomass and transport time? *Limnology and Oceanography*. 54:381-90.
- Magdaleno F, Donadio C, Kondolf GM, 2018. 30 year response to damming of a Mediterranean river in California, USA. *Phys. Geogr.*, 39:197-215.
- Magliulo P, Cusano A, Russo F, 2021. Land-Use Changes in the Sele River Basin Landscape (Southern Italy) between 1960 and 2012: Comparisons and Implications for Soil Erosion Assessment. *Geographies*. 1:315-32.
- Mangoni O, Saggiomo V, Bolinesi F, et al. 2017. Phytoplankton blooms during austral summer in the Ross Sea, Antarctica: Driving factors and trophic implications. *PLoS ONE*. 12:e0176033.
- Mantoura RFC, Repeta DJ 1997. Calibration methods for HPLC. In: Jeffrey SW, Mantoura RFC, Wright SW (eds) *Phytoplankton pigments in oceanography: Guidelines to modern methods*. UNESCO, Paris, France. pp. 407.
- Marshall HG, Lacouture RV, Buchanan C, Johnson JM, 2006. Phytoplankton assemblages associated with water quality and salinity regions in Chesapeake Bay, USA. *Estuarine, Coastal and Shelf Science*. 69:10-8.
- Millie DF, Schofield OM, Kirkpatrick GJ, et al., 1997. Detection of harmful algal blooms using photopigments and absorption signatures: A case study of the Florida red tide dinoflagellate, *Gymnodinium breve*. *Limnology and Oceanography*. 42: 1240-51.
- Montuori P, De Rosa E, Di Duca F, et al., 2022. Occurrence, Distribution, and Risk Assessment of Organophosphorus Pesticides in the Aquatic Environment of the Sele River Estuary, Southern Italy. *Toxics*. 10:377.
- Muylaert K, Sabbe K, Vyverman W, 2000. Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). *Estuarine, Coastal and Shelf Science*. 50:673-87.
- Muylaert K, Sabbe K, Vyverman W, 2009. Changes in phytoplankton diversity and community composition along the salinity gradient of the Schelde estuary (Belgium/ The Netherlands). *Estuarine, Coastal and Shelf Science*. 82:335-40.
- Newman J, Anderson NJ, Bennion H, et al., 2005. Eutrophication in rivers: an ecological perspective. Technical Report. ADAS, 37 pp.
- Pappone G, Alberico I, Amato V, et al., 2011. Recent evolution and the present-day conditions of the Campanian Coastal plains (South Italy): The case history of the Sele River Coastal plain. *WIT Trans. Ecol. Environ.* 149:15-27.
- Perillo ME, 1995. Chapter 2. Definitions and Geomorphologic Classifications of Estuaries. G.M.E. Perillo (ed), *Developments in Sedimentology*. 53:17-47.

- Pinckney JL, Paerl HW, Harrington MB, 1999. Responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments. *Journal of Phycology*. 35:1455-63.
- Polemio M, Casarano D, 2004. Rainfall and drought in southern Italy (1821-2001). IAHS-AISH publication. 286:217-27.
- Polemio M, Casarano D, 2008. Climate change, drought and groundwater availability in southern Italy. In: W. Dragoni and B.S. Sukhija (eds), *Climate Change and Groundwater*. The Geological Society Special Publications. 288:39-51.
- Reynolds CS, 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. *Freshwater Biology*. 14:111-42.
- Reynolds CS, 1994a. The long, the short and the stalled: on the attributes of phytoplankton selected by physical mixing in lakes and rivers. *Hydrobiologia*. 289:9-21.
- Reynolds CS, 1994b. The role of fluid motion in the dynamics of phytoplankton in lakes and rivers. In: P.S. Giller, A.G. Hildrew, D. Raffaelli (eds), *Ecology of Aquatic Organisms: Scale, Pattern, Process*. Blackwell Scientific Publications, Oxford, UK, p.141-87.
- Reynolds CS, 1999. Modelling phytoplankton dynamics and its application to lake management. *Hydrobiologia*. 395:123-31.
- Reynolds CS, 2003. Planktic community assembly in flowing water and the ecosystem health of rivers. *Ecological Modelling*. 160:191-203.
- Reynolds CS, 2006. *The Ecology of Phytoplankton*. Cambridge University Press, Cambridge, UK 535 pp.
- Reynolds CS, Descy JP and Padisák J, 1994. Are phytoplankton dynamics in rivers so different from those in shallow lakes? *Hydrobiologia*. 289:1-7.
- Reynolds CS, Descy JP, 1996. The production, biomass and structure of phytoplankton in large rivers. *Archiv für Hydrobiologie, Supplementband Large Rivers*. 10:161-87.
- Romano E, Petrangeli AB, Salerno F, Guyennon N, 2022. Do recent meteorological drought events in central Italy result from long-term trend or increasing variability? *International Journal of Climatology*. 42:4111-28.
- Royo C, Cobelas MA, Arauzo M, 1994. An elementary, structural analysis of river phytoplankton. *Hydrobiologia*. 289:43-55.
- Roubeix V, Lancelot C, 2008. Effect of salinity on growth, cell size and silicification of an euryhaline freshwater diatom *Cyclotella meneghiniana* Kutz. *Transitional Waters Bulletin*. 1:31-8.
- Saggiomo M, Bolinesi F, Brunet C, *et al.*, 2023. A CHEMTAX-derived phytoplankton community structure during 12-year observations in the Gulf of Naples (LTER-MC). *Marine Ecology*. 2023:e12745.
- Schuchardt B, Schirmer M, 1991. Phytoplankton maxima in the tidal freshwater reaches of two coastal plain estuaries. *Estuarine, Coastal and Shelf Science*. 32:187-206.
- Shuman F, Lorenzen R, Carl J, 1975. Quantitative degradation of chlorophyll by a marine herbivore. *Limnology and Oceanography*. 20:580-6.
- Taguchi Y-H, Oono Y, 2005. Relational patterns of gene expression via non-metric multidimensional scaling analysis. *Bioinformatics* 21:730-40.
- Tavernini S, Pierobon E, Viaroli P, 2011. Physical factors and dissolved reactive silica affect phytoplankton community structure and dynamics in a lowland eutrophic river (Po river, Italy). *Hydrobiologia*. 669:213-25.
- Ustin SSM, Hestir E, Khanna S, *et al.*, 2014. Developing the capacity to monitor climate change impacts in Mediterranean estuaries. *Evolutionary Ecology Research*. 16:529-50.
- van der Tuin H, 1991. Guidelines on the study of seawater intrusion into rivers. International Hydrological Programme, UNESCO, 138 pp.
- Valle-Levinson A, 2010. Definition and classification of estuaries. Available from: [https://assets.cambridge.org/97805218/99673/excerpt/9780521899673\\_excerpt.pdf](https://assets.cambridge.org/97805218/99673/excerpt/9780521899673_excerpt.pdf)
- Verri G, Pinaroli N, Oddo P, *et al.*, 2018. River runoff influences on the Central Mediterranean overturning circulation. *Clim. Dyn.* 50:1675-703.
- Vidussi F, Claustre H, Bustillos-Guzman J, *et al.*, 1996. Determination of chlorophylls and carotenoids of marine phytoplankton: separation of chlorophyll a from divinyl-chlorophyll a and zeaxanthin from lutein. *Journal Plankton Research*. 18:2377-82.
- Warwick RM, Tweedley RJ, Potter IC, 2018. Microtidal estuaries warrant special management measures that recognise their critical vulnerability to pollution and climate change. *Marine Pollution Bulletin*. 135:41-46.
- Waylett AJ, Hutchins MG, Johnson AC, *et al.*, 2013. Physicochemical factors alone cannot simulate phytoplankton behaviour in a lowland river. *Journal of Hydrology*. 497:223-33.
- Weisse T, 2008. *Limnoecology: The Ecology of Lakes and Streams*. *Journal of Plankton Research*. 30:489-90.
- Whitton BA, 1975. *River Ecology*. Vol. 2. University of California Press, Berkeley, USA. 725 pp.
- Yentsch CS, Menzel DW, 1963. A method for the determination of phytoplankton chlorophyll and phaeophytine by fluorescence. *Deep Sea Res.* 10:221-31.
- Wright SW, Jeffrey SW, 2006. Pigment markers for phytoplankton production. *Marine Organic Matter: Biomarkers, Isotopes and DNA*. 2005:71-104.
- Wright SW, Thomas DP, Marchant HJ, *et al.*, 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: Comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the "CHEMTAX" matrix factorisation program. *Marine Ecology Progress Series*. 144:285-98.

Online supplementary material:

Table S1. Pigments: *Chl a* ratios at each station in July 2017 (a); surface layer May 2021(b); bottom layer in May 2021(c).