Mediterranean Marine Science

Vol 25, No 3 (2024)

Mediterranean Marine Science

Seasonal variability of epipelic microphytobenthos community in two subtidal areas of the Northern Adriatic Sea

APURVA MULE, FRANCESCA NERI, TIZIANA ROMAGNOLI, STEFANO ACCORONI, FRANCESCA CARIDI, ALESSANDRA CAMPANELLI, MARIKA UBALDI, DIANA SARNO, CECILIA TOTTI

doi: 10.12681/mms.35785

To cite this article:

MULE, A., NERI, F., ROMAGNOLI, T., ACCORONI, S., CARIDI, F., CAMPANELLI, A., UBALDI, M., SARNO, D., & TOTTI, C. (2024). Seasonal variability of epipelic microphytobenthos community in two subtidal areas of the Northern Adriatic Sea. *Mediterranean Marine Science*, *25*(3), 650–665. https://doi.org/10.12681/mms.35785

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.35785

Seasonal variability of epipelic microphytobenthos community in two subtidal areas of the Northern Adriatic Sea

Apurva MULE[1*], Francesca NERI[1*], Tiziana ROMAGNOLI[1], Stefano ACCORONI[1,2], Francesca CARIDI[1], Alessandra CAMPANELLI[3], Marika UBALDI[1,4,5], Diana SARNO[6] and Cecilia TOTTI[1,7]

[1] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy
[2] Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies (FMC), viale Adriatico 1/N, 61032 Fano, Italy
[3] Consiglio Nazionale delle Ricerche, CNR-IRBIM, largo Fiera della Pesca, 2, 60125 Ancona, Italy
[4] Dipartimento di Scienze della Terra e del Mare, Via Archirafi, 22, 90123 Palermo, Italy
[5] National Biodiversity Future Center, Piazza Marina 61, 90133 Palermo, Italy
[6] Dipartimento di Ecologia Marina Integrata, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy
[7] Consorzio Interuniversitario per le Scienze del Mare, CoNISMa, ULR Ancona, Ancona, Italy

Corresponding author: Francesca NERI; f.neri@pm.univpm.it

Contributing Editor: Adriana ZINGONE

Received: 02 November 2023; Accepted: 09 October 2024; Published online: 14 October 2024

Equal contribution: These authors contributed equally to this work

Abstract

We investigated the biodiversity and seasonality of subtidal benthic diatoms at two sites of the Northern Adriatic Sea differently affected by anthropogenic inputs. Sediment samples were collected seasonally, and diatom cells were then separated from the sediment using the density gradient centrifugation method. The total abundance of benthic diatoms ranged between 4,409 ± 1,638 and 77,663 ± 30,415 cells cm^{-2} and the biomass between 0.41 ± 0.22 and 3.66 ± 2.01 µg C cm^{-2}. At both stations, the benthic diatoms showed a marked seasonal pattern, with maximum abundance, biomass and biodiversity in spring and minimum in summer. Motile life forms, such as *Navicula, Nitzschia, Fallacia,* and *Psammodictyon,* dominated in terms of abundance at both stations throughout the study period, while plocon (centric diatoms mainly belonging to Biddulphiaceae) increased under mixing conditions, when they represented the largest contributor to biomass. At both stations and in all seasons, the Si:N:P ratio highlighted the strong P limitation, typical of the Adriatic Sea. The ammonium concentration was the highest component of DIN in spring and summer when we observed the highest and lowest MPB abundance respectively. The preference of diatoms for ammonia, coupled with the increased daylight period, may have enhanced the spring growth, whereas in summer the hypoxic conditions may have caused a decrease. Despite the different environmental conditions, the two stations exhibited similar species compositions and seasonal trends, highlighting relative stability against anthropogenic pressures of a different nature.

Keywords: Benthic diatoms; Adriatic Sea; diatom life forms; subtidal; epipelon; microphytobenthos.

Introduction

Microphytobenthos (MPB) include microalgal communities living on benthic substrata. They are composed of unicellular eukaryotic algae (mainly diatoms) and cyanobacteria (MacIntyre *et al.*, 1996; Underwood & Barnett, 2006; Hope *et al.*, 2019). MPB play a key role in marine food webs due to their significant primary production (globally *ca*. 500 million tons C $year^{-1}$) and contribution to biogeochemistry in aquatic ecosystems (Cahoon, 1999; Migné *et al.*, 2009; Pinckney, 2018).

Diatoms are the most important group of eukaryotic microalgae and include around 200,000 species (Graham *et al.*, 2016), with benthic species representing around 90% of the total species number. Benthic diatom communities have traditionally been grouped based on their associated substrata. In particular, epipelon, lie on the surface of deposit sediments, either mud or sand, where they can actively move; epipsammon are single cells living attached to single sand grains; epilithon colonise rocks and artificial hard substrata; epiphyton live on algae and plants and epizoon live on animals (Round, 1971).

Epipelic MPB biofilms play an important role in the habitat ecology, contributing to sediment stabilization (Miller *et al.*, 1996; Underwood & Paterson, 2003; Fagherazzi *et al.*, 2014) and facilitating the exchange of oxygen, silicon, and carbon between sediment and water column (Armbrust, 2009). Several studies have addressed the influence of environmental parameters, such as temperature, oxygen saturation, silicate concentration, and salinity on epipelic MPB abundance, biomass, community composition and seasonal cycle (Underwood & Barnett, 2006; Cochero *et al.*, 2015). Compared to phytoplankton, epipelic diatom assemblages are less influenced by seasonal rhythm (Underwood & Paterson, 2003; Facca & Sfriso, 2007), particularly in subtidal areas where environmental conditions are more stable than in the intertidal ones. In the Mediterranean Sea, annual peaks of MPB biomass were reported in spring and summer (Welker *et al.*, 2002; Cibic *et al.*, 2012).

The majority of studies regarding the epipelic communities focus on intertidal areas where it is easier to take samples during low tide (Barranguet *et al.*, 1998; Underwood & Barnett, 2006). In these zones, high abundances and biodiversity of MPB are commonly reported because of the optimal irradiance level. However, even in the subtidal areas, where light or photosynthetically available radiation (PAR) often represents the most relevant limiting factor, many studies have reported high MPB biomass values (Totti, 2003; Ní Longphuirt *et al.*, 2006, 2007). Regarding nutrients, some studies have highlighted that their concentrations in the water column could limit MPB growth, even though they are present with high concentration in interstitial sediments. For example, in the Northern Adriatic Sea, the abundance and community structure of the MPB have been influenced by the plume of the Po River (Totti, 2003). In the Gulf of Trieste, silicate and phosphate concentrations appeared to co-limit MPB abundances, while MPB biomass was inversely proportional to total nitrogen (Blasutto *et al.*, 2005; Cibic *et al.*, 2007; Franzo *et al.*, 2015).On the other hand, a positive correlation was found between the MPB abundance and ammonium, suggesting the importance of ammonium for MPB growth (Welker *et al.*, 2002). Sediment texture also affects the community structure and biomass production, with lower biomass production reported in sandy sediments compared to muddy ones, due to different nutrient concentrations (Barranguet *et al.*, 1998).

The Northern Adriatic Sea (NAS) is highly influenced by riverine waters which contribute to the high content of organic and inorganic nutrients. Hence, it is one of the most productive areas in the Mediterranean Sea (D'Ortenzio & Ribera D'Alcalà, 2008; Campanelli *et al.*, 2011; Cozzi & Giani, 2011). Planktonic communities have been extensively studied in the NAS through analysis of long-term datasets (Marić *et al.*, 2012; Cerino *et al.*, 2019; Totti *et al.*, 2019; Vascotto *et al.*, 2021; Bernardi Aubry *et al.*, 2022; Neri *et al.*, 2022, 2023), and are regularly monitored by both research institutions and environmental agencies. On the contrary, despite their ecological importance in marine ecosystems, MPB communities are studied less and for shorter periods, with only a few LTER sites involving MPB in the Mediterranean Sea (e.g., the C1 LTER site in the Northern Adriatic (Franzo *et al.*, 2018)), thus limiting knowledge in terms of seasonal cycle, interannual variability, and trends related to climate changes.

Benthic diatoms have been traditionally classified into growth forms (Round *et al.*, 1971, 1990): motile, adnate, erect, tube-dwelling and plocon. Motile diatoms comprise biraphid pennates that can actively move on benthic substrata (e.g., *Navicula* and *Nitzschia*). Adnates includes both monoraphid (e.g., *Cocconeis*) and biraphid (*Amphora*) diatoms living attached to the substratum by one valve face and having limited motility. Erect diatoms are attached to surfaces by means of mucilage pads, stalks or peduncles exuding from their apical pore fields or apical rimoportulae and include mainly araphid (e.g., *Gomphonema*, *Licmophora*), but also raphid pennates (e.g., *Achnanthes*, *Cymbella*). Tube-dwelling diatoms are small naviculoid or nitzschioid species living in their own mucilage tube, that appear as filaments. Plocon identifies a category of centric diatoms lying on substrata because of their frustules, which are too heavily silicified for a planktonic existence (e.g., *Biddulphia*, *Paralia*) (Round, 1971; Round *et al.*, 1990).

In the Adriatic Sea, several studies have investigated the composition, abundance and biomass of the MPB communities of epipelic (Facca *et al*., 2002a,b; Welker *et al.*, 2002; Totti, 2003; Cibic *et al.*, 2007, 2012; Franzo *et al*., 2015, 2018), epilithic (Munda, 2005; Totti *et al.*, 2007; Pennesi & Danovaro, 2017; Car *et al.*, 2020, 2021) and epiphytic (Accoroni *et al.*, 2016) communities, but only a few have focused on the ecological drivers affecting the seasonal trend of growth forms.

This study aims to investigate the seasonal variability of the epipelic diatom communities in two subtidal areas in the northern Adriatic Sea that are differently affected by anthropogenic pressure. The specific objectives are: (i) to describe MPB abundance, biomass and taxonomic composition in relation to environmental factors (ii) to assess whether there are temporal and/or spatial differences in the MPB morpho-functional composition, (iii) to assess whether the communities in the two areas show differences that could reflect dissimilar environmental/anthropogenic pressures.

Materials and Methods

Study area

Sediment samples were collected in the northern Adriatic Sea at two stations along the coast of the Marche region: Senigallia (SG) station is the coastal station of the eLTER Senigallia-Susak transect (43.7550° N, 13.21050° E) located 1.2 nM from the coast (bottom depth of 12 m), close to the mouth of the Cesano River. This station has been sampled monthly since 1988 for both meteorological, and water column physical, chemical and biological parameters. Portonovo (PN) (43.60335° N, 13.61175° E) station is located 1 nM from the coast (bottom depth of

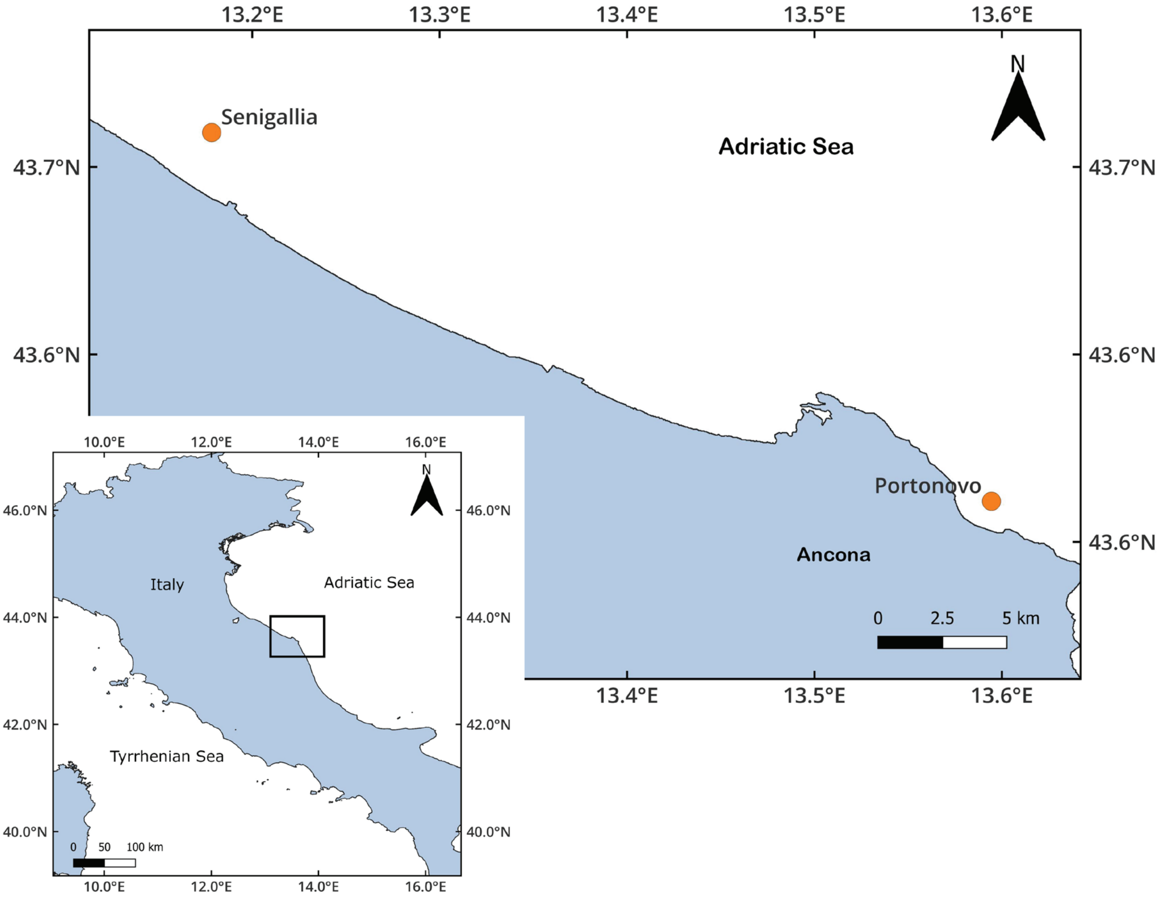

Fig. 1: Map of the study area. Senigallia and Portonovo sampling stations are represented by the orange circles.

15 m) (Fig. 1). PN station has been sampled since 2007 for physical, chemical and biological parameters. Both stations are crossed by the Western Adriatic Current, which conveys nutrient-richer waters from the northern areas southwards, mainly from the Po River (Artegiani *et al.*, 1997). The main winds are Bora and Scirocco, and largely affect the circulation in the area (Russo & Artegiani, 1996). The two stations are differently impacted by anthropogenic pressure: SG is more affected by riverine input than PN, which is a tourist area, with significant boat traffic during summer.

Sampling

Sampling was carried out with seasonal frequency in spring (28/05/2020), summer (20/07/2020), autumn (19/10/2020) 2020, and winter 2021 (19/01/2021). Temperature, salinity, chlorophyll-a (chl-a, derived from fluorescence data using the conversion factors provided by the agency during periodical calibration), turbidity and oxygen data were acquired by a CTD SeaBird Electronic SBE 911plus unit. Water samples for determination of dissolved inorganic nutrient analysis – nitrites (NO_2^-), nitrates (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{-2}) and orthosilicate $Si(OH)_4$ – were collected by Niskin bottles at the surface (1 m) and close to the bottom (12 m and 15 m for Senigallia and Portonovo, respectively), immediately filtered (GF/F Whatman, 0.7-µm porosity) and stored at −22 °C in polyethylene vials until analysis.

Sediment samples were collected using a grabber. In each station, the bottom grabber was lowered three times to collect samples in 3 replicates. For each grab, the top 1 cm of sediment was collected from an undisturbed part of the surface (about 10 ml of sediment) with a syringe with a 2-cm diameter mouth. The sediment samples were placed into a sterile 50 ml plastic Falcon tube and stored at +4 °C until the diatom extraction procedure.

Grain size analysis

Grain size and soil texture analysis was carried out using the Mastersizer 3000 (Malvern Instruments Ltd.). The grain size results were classified according to Friedman & Sanders (1978) grain-size scale.

Nutrient analysis

Nutrients were analysed spectrophotometrically (Parsons *et al.*, 1984) with a quAAtro autoanalyser (AxFlow; Seal Analytical GmbH, Germany). The Dissolved Inorganic Nitrogen (DIN) concentration is intended as the sum of NO_2^-, NO_3^- and NH_4^+ concentrations.

Microphytobenthos extraction

Diatom cells were separated from sediment using the density gradient centrifugation method with Ludox HS-40 Colloidal silica (Méléder *et al.*, 2007). A mixture composed of 30 ml of Ludox and 5 ml of sediment was vigorously shaken, ultra-sonicated for 10 min, to detach diatom cells from sand grains and centrifuged to suspend diatom cells in the supernatant. The supernatant was then rinsed with distilled water and centrifuged 4-5 times to remove the Ludox. Finally, the pellet (containing cells) was suspended in 10 ml distilled water, fixed with 0.8% formaldehyde and stored at + 4 °C for the purpose of: (i) counting under the light microscopy (LM) and (ii) cleaning treatment and Scanning Electron Microscopy (SEM) analysis.

Identification and counting

To identify and quantify the microalgae, fixed samples were homogenized, and subsamples (volume varying between 0.1 and 4 ml) were then gently injected with a micropipette into a 10-ml composite Utermöhl chamber previously filled with a solution of 0.2% formalin in filtered seawater. This method allows homogeneous distribution of subsamples in the counting chamber (Totti *et al.*, 2004). The samples were allowed to settle overnight and were thereafter observed with an inverted microscope (Zeiss Axiovert 135) equipped with phase contrast. Counting was carried out at 400x magnification on 30 random fields. Biomass was estimated by cell biovolumes measured during counting, following Menden-Deuer & Lessard (2000).

The whole chamber bottom was then observed at 200x for a more correct estimation of larger and rarer species, which significantly affect the biomass value.

Identification was made at the lowest possible taxonomical level, using the available literature, e.g. Van Heurck (1880-1885), Peragallo & Peragallo (1897-1908), Van der Werff & Huls (1957-1974), Hustedt (1985), Patrick & Reimer (1966, 1975), Cardinal *et al.* (1984), Poulin *et al.* (1987, 1990), Bérard-Therriault *et al.* (1986), Simonsen (1987), Round *et al.* (1990), Hasle & Syvertsen (1997), De Stefano *et al.* (2000), Riaux-Gobin & Romero (2003), and Sar *et al.* (2003). In the case of uncertain identification, cells were assigned to undetermined pennate 1, pennate 2 and so forth, until the SEM analysis was carried out (see below), which allowed the identification of many taxa unidentified through LM analysis.

Diatom taxa were annotated according to their growth form: adnate, plocon, motile and erect following Round (1971), Round *et al.* (1990), Romagnoli *et al.* (2007, 2014), Totti *et al.* (2007, 2011), D'Alelio *et al.* (2011). For the purpose of simplification, with the exception of *Amphora*, biraphid taxa were assigned to the motile growth form. Only *Cocconeis* (*sensu lato*) and *Amphora* (*sensu lato*) were assigned to adnate. Araphid (or more rarely monoraphid) presenting the apical pore field were assigned to the erect form. Tychopelagic centric genera, usually characterized by heavily silicified frustules, were assigned to the plocon form.

Frustule cleaning and SEM analysis

To improve the species identification, all samples were processed for SEM observations, after frustule cleaning. For each sample, a 1-ml subsample was acid-cleaned according to the von Stosch's method (Hasle & Syvertsen, 1997). Samples were centrifuged with distilled water to remove salt. Next, the pellet was resuspended in 1 ml distilled water, containing HNO_3 and H_2SO_4 (1/4 v/v) to remove organic material and then rinsed with distilled water. A drop of the cleaned material was placed on a stub and sputter coated with a thin layer of gold-palladium for observation with a Zeiss Supra 40 FE-SEM (Carl Zeiss AG, Oberkochen, Germany).

Statistical analysis

Phytoplankton taxa included in the taxonomic list were excluded from statistical analyses as their presence in the benthos was not native but was due to settling from the water column. One taxon belonging to the epipsammon community (*Rhaphoneis* sp.) was observed only once in a single replicate and with negligible abundance and was hence excluded from further analysis.

To identify the taxa that characterized the different seasons, the Indicator Value (IndVal) was applied, as it combines the relative abundance of a species with its relative frequency of occurrence in a given period (Dufrêne & Legendre, 1997). The INDSPANA software (version 1.1) was used to calculate the IndVal.

The R software (R version 4.1.1, R Core Team, 2021) was used for the following analyses.

Two-sample Wilcoxon tests and Kruskal-Wallis Rank Sum test were used to check for significant differences between the two stations and among seasons, respectively, using the wilcox.test and kruskal.test functions in the stats package (R Core Team, 2021). Non-Metric Multidimensional Scaling (NMDS) was performed to highlight the relationship between seasonal abundances of diatom growth forms and environmental parameters in the two stations. Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences among seasons and stations in the NMDS. The metaMDS (setting the autotransform as true), envfit and adonis2 functions from the R vegan package were used for the NMDS, the fitting of the environmental variables and for the PERMANOVA (Oksanen *et al.*, 2022).

For each season, the Shannon diversity index (H') (Shannon, 1948) was calculated using the diversity function available in the R vegan package (Oksanen *et al.*, 2022). To test for significant differences between the two stations, the two-sample Wilcoxon test was performed using the wilcox.test function in the stats package (R Core Team, 2021).

Co-occurrence analysis was performed on presence-absence data using the R cooccur package (Griffith *et al.*, 2016), where statistically significant pair-wise species co-occurrences are classified as positive, negative or random associations, using a probabilistic model (Veech, 2013), based on comparison of observed and expected co-occurrences (Veech, 2013; Griffith *et al.*, 2016).

Results

Grain size

At both stations the sediment was very fine sand, with a relatively consistent sand content (87.58% and 83.88%, in Portonovo and Senigallia respectively).

Environmental parameters

Vertical profiles of physico-chemical parameters in the water column are reported in Figure 2, while their values at the bottom layer are shown Table 1. At both stations, the temperature vertical profile showed the typical seasonal trend of the temperate areas, with high values (around 20-21 °C in the bottom layer of both stations) and water stratification in summer, and low values (around 8 and 11 °C at the bottom in SG and PN, respectively) and mixed water column in winter (Fig. 2A). The autumn and spring had mild temperatures with values between 15 and 18 °C.

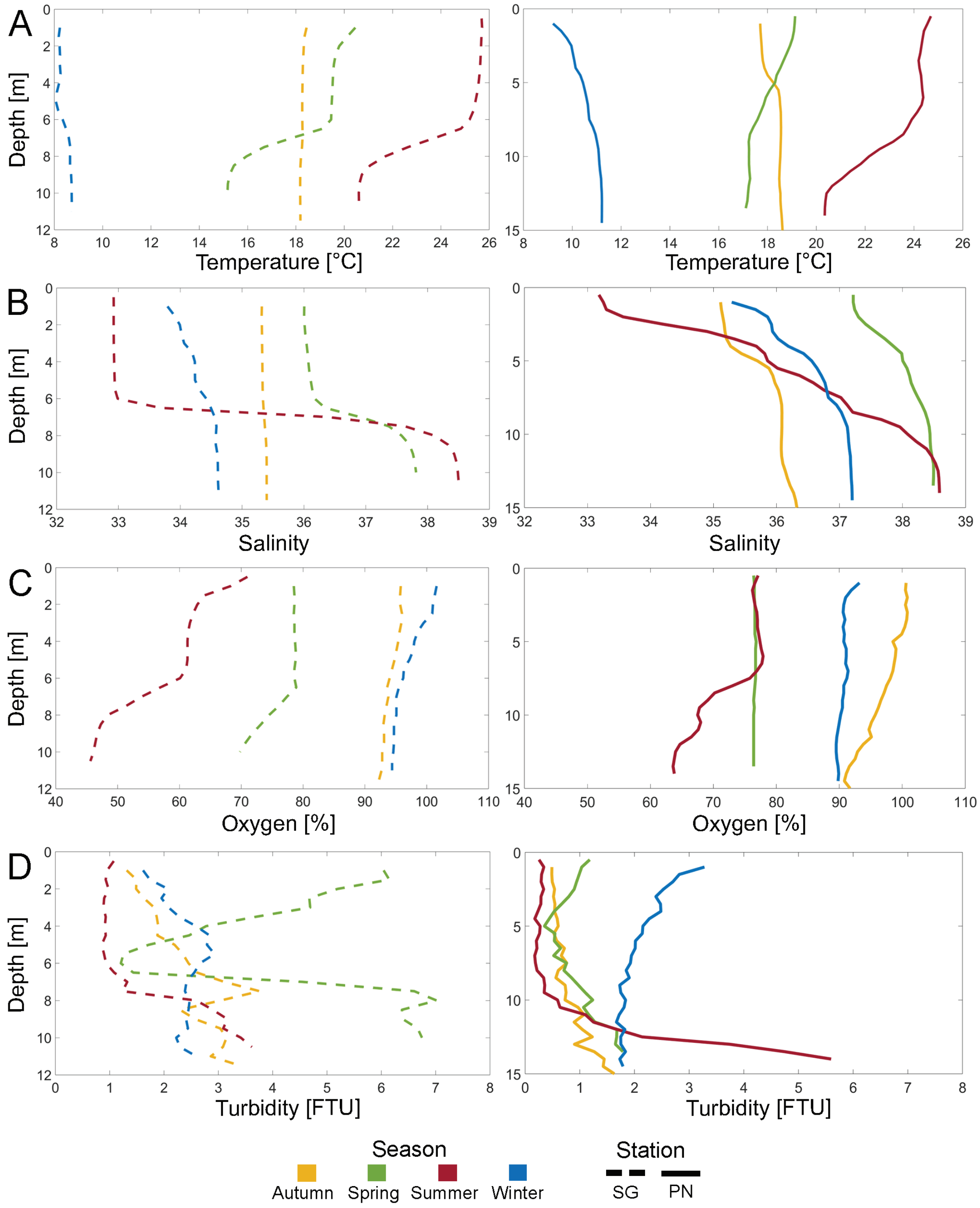

Fig. 2: CTD profiles of temperature (°C, A), salinity (B), oxygen saturation (%, C) and turbidity (FTU, D) in autumn (yellow), spring (green), summer (red), winter (blue) at Senigallia (SG, dashed line) and Portonovo (PN, continuous line).

Table 1. Seasonal values of temperature (°C), salinity, oxygen saturation (%), turbidity (FNU), chl-a (mg m^{-3}), DIN, Si(OH)$_4$ (µM), PO$_4$ (µM) at Portonovo (PN) and Senigallia (SG) at the bottom.

Station	Season	Temperature	Salinity	Oxygen	Turbidity	Chl-a	DIN	Si(OH)$_4$	PO$_4$
PN	Spring	17.23	38.49	76.4	1.68	0.28	9.67	8.54	0.07
PN	Summer	20.65	38.52	64.7	1.69	0.47	13.41	7.56	0.10
PN	Autumn	18.49	36.1	93.8	1.05	1.99	5.70	5.64	0.07
PN	Winter	11.19	37.17	89.5	1.83	2.16	10.64	8.09	0.13
SG	Spring	15.16	37.81	69.8	6.75	0.71	2.11	9.19	0.06
SG	Summer	20.60	38.49	46.1	3.41	1.69	8.97	19.11	0.06
SG	Autumn	18.18	35.40	92.8	3.15	2.48	7.72	17.30	0.31
SG	Winter	8.72	34.61	94.6	2.22	2.96	18.04	4.90	0.14

Overall, salinity values measured throughout the water column at both stations ranged between 32.9 and 38.5 (Fig. 2B). At the bottom, the values recorded at PN were higher than at SG in winter (37.2 and 34.6 in PN and SG, respectively), spring (38.5 and 37.8 in PN and SG, respectively) and autumn (36.3 and 35.4 in PN and SG, respectively), whereas in summer similar values (around 38.5) were recorded.

In the bottom layer, oxygen saturation (Fig. 2C) showed hypoxic values during summer, with values lower at SG (45.6%) compared to PN (63.8%), intermediate values in spring (69.8-76.4%) and higher values (> 90%) in winter and autumn. Turbidity showed a high seasonal variability; the highest values were observed at the bottom in spring at SG and in summer at PN (6.7 and 5.6, respectively). At the bottom layer, a chlorophyll-a maximum was observed in winter (3.08 mg m^{-3} at SG and 2.16 at PN).

The seasonal cycle of DIN (Fig. 3) at the bottom layer differed significantly between the two stations: at SG station the DIN showed the minimum value (2.11 µM) in spring and the maximum (18.04 µM) in winter, whereas at PN the minimum DIN concentration (5.70 µM) was observed in autumn and the maximum (13.41 µM) in summer. At both stations, nitrates represented the higher fraction of DIN, with the exception of spring and summer when ammonia prevailed.

The phosphate concentration at SG station was at its maximum in autumn (0.31 µM) and at its minimum in spring and summer (0.06 µM), while at PN the highest values were in summer and winter (0.1 and 0.14 µM, respectively), and the lowest values in the spring and autumn (0.07 and 0.08 µM, respectively). The silicates showed higher values at SG in summer and autumn (19.11 and 17.30 µM, respectively) and at PN in spring (8.54 µM). The lowest values were observed in winter at SG (4.90 µM) and in autumn (5.64 µM) at PN.

N:P ratios reached their maxima in summer (140.41 at SG and 138.22 at PN) and minima in autumn (25.07 at SG and 76.04 at PN) at both stations. The Si:N at SG

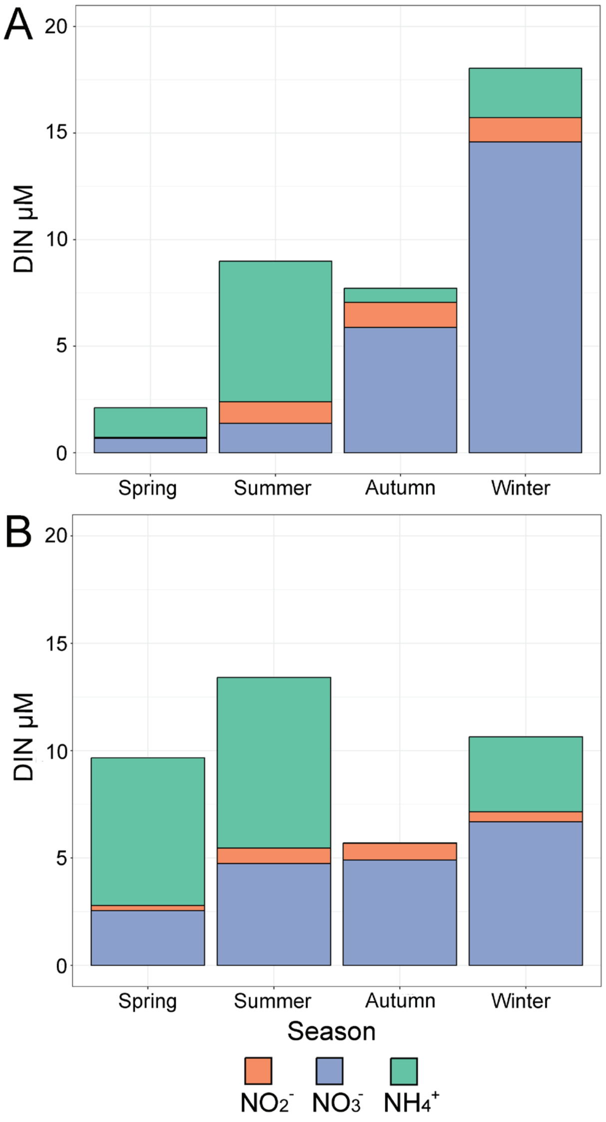

Fig. 3: Dissolved Inorganic Nitrogen (DIN) concentrations and compositions in terms of nitrite (NO$_2^-$), nitrate (NO$_3^-$) and ammonium (NH$_4^+$) near the bottom at Senigallia (SG, A) and Portonovo (PN, B) in the different seasons.

reached its maximum in spring (4.4) and minimum in winter (0.3), while at PN the maximum was observed in autumn (1) and the minimum (0.6) in summer.

Taxonomic composition

The main problems encountered during identification were that: (i) the SEM analysis did not always provide sufficient details for the identification at the species level; (ii) taxa observed at the LM were not always encountered during SEM analysis; (iii) a number of taxa that were found under the SEM were not found in literature.

The full list of identified diatom taxa is presented as supplementary material (Table S1). Overall, 116 diatom taxa were retrieved, of which 101 benthic and 15 planktonic. Fifty-one taxa were identified at the species/subspecies level.

At both stations, Naviculaceae, Bacillariaceae and Catenulaceae were the most represented families, and *Amphora, Navicula* and *Nitzschia* were the most represented genera. Genera such as *Cocconeis, Fallacia* and *Odontella* were also observed, although their species diversity was lower. Regarding the growth forms of the benthic taxa, 72 species were motile, 14 adnate, 8 erect and 7 plocon.

Abundance and biomass of benthic diatoms

The abundance and biomass of benthic diatoms at the two stations are shown in Figure 4. At both stations, the highest abundance was found in spring (mean ± STD = 77,663 ± 30,415 and 68,247 ± 29,000 cells cm^{-2} at PN and SG, respectively), and the lowest in summer (mean ± STD = 4,409 ± 1,638 cells cm^{-2} at PN and 6,890 ± 3,280 cells cm^{-2} at SG) (Fig. 4A,B). A second slight peak was observed in autumn. However, only at PN was total abundance significantly higher in spring than in summer (Kruskal-Wallis test, $p < 0.05$; Dunn post-hoc test, $p < 0.05$).

Regarding the diatom biomass, the highest values at both stations were found in spring (mean ± STD = 3.34 ± 1.42 and 3.66 ± 2.01 µg C cm^{-2} at PN and SG stations, respectively) and the minima in summer (mean ± STD = 0.53 ± 0.17 and 0.41 ± 0.22 µg C cm^{-2} at PN and SG stations, respectively) (Fig. 4C,D), although differences between those seasons were not statistically significant (Kruskal-Wallis test, $p > 0.05$).

No significant differences were found between the two stations in terms of total abundance and biomass in each season (Wilcoxon rank sum test, $p > 0.05$).

Community composition

The community composition in terms of growth form abundances at both stations is shown in Figure 4A, B. Motile growth forms were the most represented at both PN (66, 57, 85, 87% in spring, summer, autumn and winter, respectively) and SG (51, 82, 88% in spring, autumn and winter, respectively), followed by adnate, with the exception of SG in summer, when adnate diatoms were dominant (72%). At both stations, the importance of adnate diatoms decreased in autumn and winter, when motile forms were dominant. The temporal distribution of the motile diatoms paralleled that of the total diatoms, reaching the maximum in spring and the minimum in summer. The most abundant motile taxa were *Diploneis* and *Navicula*, while *Amphora* was the most important adnate taxon. Plocon forms were mainly represented by Biddulphiaceae, the contribution of which was lowest in spring at both stations, yet higher than that of adnate at PN station in autumn and winter. At both stations, the presence of erect diatoms (*Tabularia*, *Rhaphoneis* and *Achnanthes*) was very low.

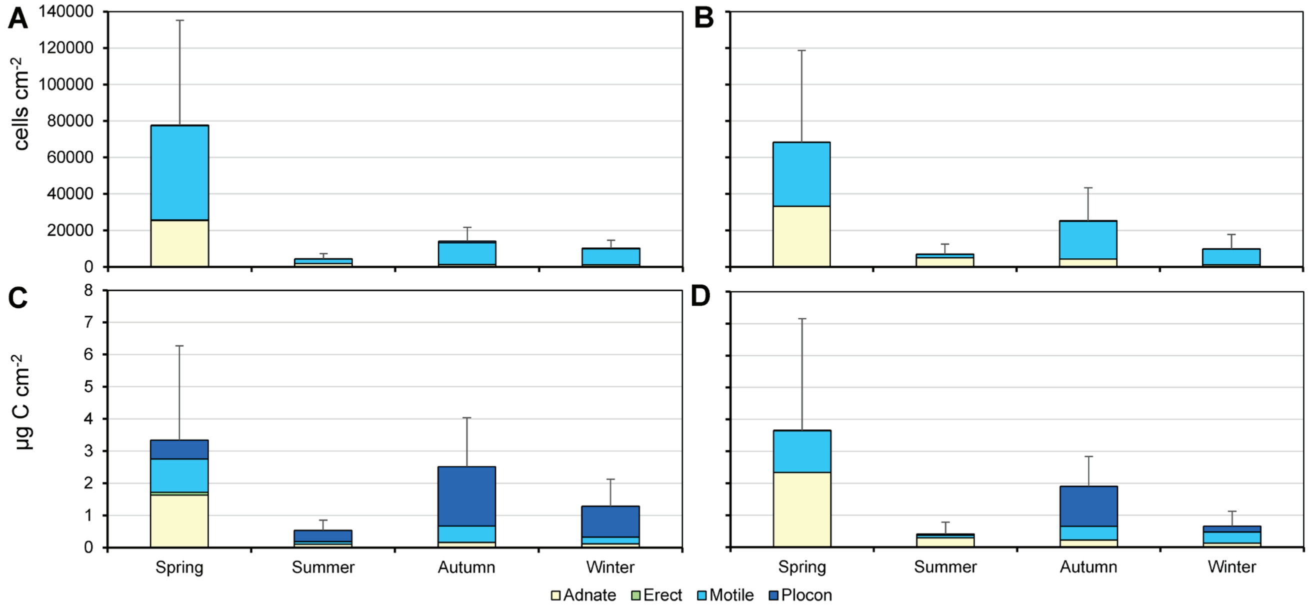

Fig. 4: Abundance (cells cm^{-2}, A, B) and biomass (µg C cm^{-2}, C, D) of epipelic diatoms at Portonovo (A, C) and Senigallia (B, D) stations in the different seasons, with the contribution of each growth form. Bars indicate standard deviation (of the total).

At SG, the abundance of adnate forms was significantly higher (Kruskal-Wallis test, $p < 0.05$; Dunn post-hoc test, $p < 0.05$) in spring (mean ± STD = 33,159 ± 22,750 cells cm^{-2}) than in winter (mean ± STD = 1,081 ± 944 cells cm^{-2}). Moreover, higher values of plocon were found in autumn (mean ± STD = 406 ± 106 cells cm^{-2}) than in spring (mean ± STD = 11 ± 7 cells cm^{-2}). At PN, the abundance of motile forms was significantly higher (Kruskal-Wallis test, $p < 0.05$; Dunn post-hoc test, $p < 0.05$) in spring (mean ± STD = 51,646 ± 33,463 cells cm-2) than in summer (mean ± STD = 2,491 ± 722 cells cm^{-2}). (Kruskal-Wallis test, $p > 0.05$). Comparing the two stations, no significant difference was observed in terms of adnate, erect, motile and plocon form abundances (Wilcoxon rank sum test, $p > 0.05$).

In terms of biomass, in spring, the community was dominated by adnate forms (mainly *Amphora*) at both stations followed by motile (*Diploneis*, *Navicula*) and plocon (Biddulphiales) forms (Fig. 4C, D). During summer, the biomass percentage of plocon forms increased at both stations although their contribution was higher at PN (Fig. 4C, D). In autumn, plocon forms reached the maximum biomass percentage at both stations, followed by motile and adnate forms. In winter, plocon forms were still the largest contributor to the biomass at PN station, while at SG station plocon and motile forms showed comparable values. At both stations, erect forms contributed slightly to diatom biomass (Fig. 4C, D).

At PN, the biomass of motile forms was significantly higher in spring (mean ± STD = 1.03 ± 0.64) than in summer (mean ± STD = 0.09 ± 0.04) (Kruskal-Wallis test, $p < 0.05$; Dunn post-hoc test, $p < 0.05$). The same was found in SG (mean ± STD = 1.30 ± 1.17 and 0.07 ± 0.05 for spring and summer, respectively) (Kruskal-Wallis test, $p < 0.05$; Dunn post-hoc test, $p < 0.05$). Furthermore, at SG station, a significantly higher biomass of plocon forms was observed in autumn (mean ± STD = 1.25 ± 0.30 μg C cm^{-2}) than in spring (mean ± STD = 0.01 ± 0.02 μg C cm^{-2}) (Kruskal-Wallis test, $p < 0.05$; Dunn post-hoc test, $p < 0.05$).

Species composition: Indicator Value analysis and Diversity indexes

Indicator Value analysis (IndVal) was performed to identify the taxa that were indicators of each season regardless of the stations (Table 2). Several taxa were indicators of spring: motile forms such as *Navicula distans* (Fig. 5F-H), *Nitzschia longissima*, *Fallacia* cf. *forcipata* (Fig. 6A-E), Pennate sp. 10, several *Navicula* species, and *Psammodictyon panduriforme*; adnate forms like *Amphora* cf. *copulata* (Fig. 6A), *Amphora* cf. *proteus* (Fig. 6B, C), and one plocon (*Biddulphia* sp. 1). No significant indicator species was observed in summer. *Ralfsiella smithii*, (plocon) and *Gyrosigma* cf. *balticum* (motile) were found as indicators of autumn, while *Cocconeis*

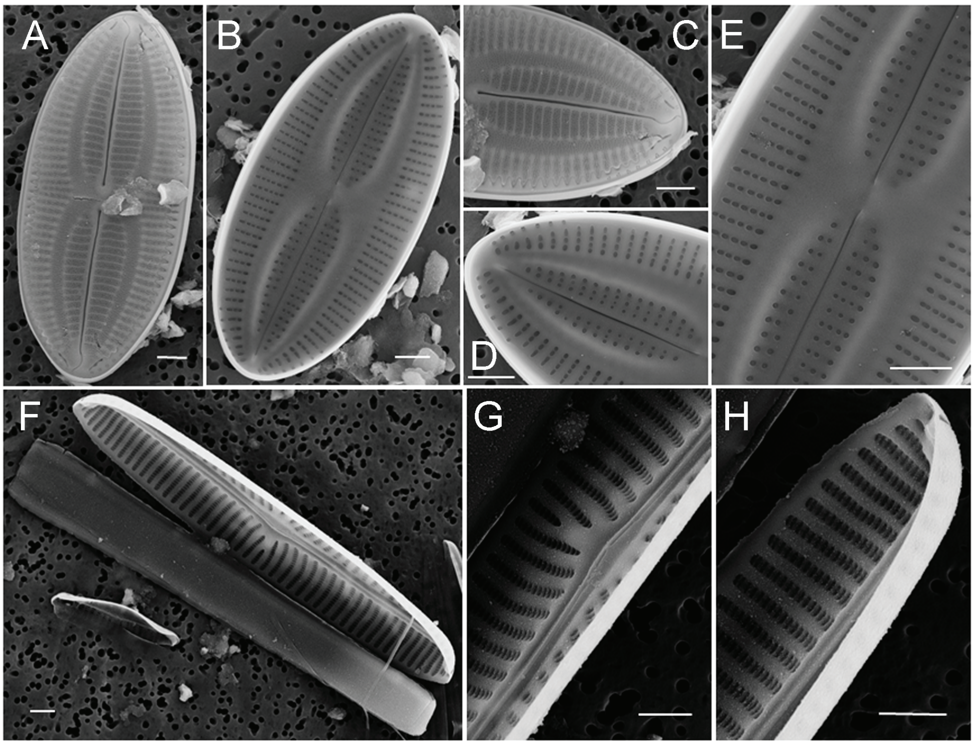

Fig. 5: SEM images of: A-E= *Fallacia* cf. *forcipata*, A= external valve view showing ‘H’ shaped lyre, B= internal valve view showing blank ‘H’ shaped lyre and areolae lines around raphe canal, C= enlargement of a part of external valve showing distinct ‘conopea’ at the end of the cell, D= enlargement of the internal valve showing raphe endings showing a slight deflection towards the other side; E= enlargement of the internal valve view showing deflected central raphe endings and round areolae, F-H= *Navicula distans*, F= internal and side view of the valve with plain girdle band, G= enlargement of the internal view showing uplifted central raphe endings and missing striae in the centre, H= enlargement of the internal apical part showing terminal end of the raphe. Scale bars: H-O: 2 μm.

Table 2. List of MPB taxa characterised by the highest IndVal for each season (irrespective of stations) calculated for the top layer of sediment. Significant p values are expressed as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). The shades of colour are proportional to the IndVal values, from dark green to white, in decreasing order.

Taxa name	Spring	Summer	Autumn	Winter
Navicula distans	***96.57***	0.46	1.36	0.46
Nitzschia longissima	***93.96***	0.01	3.53	2.03
Fallacia cf. *forcipata*	***87.00***	1.27	4.61	7.12
Pennate sp. 10	***83.33***	0.00	0.00	0.00
Psammodictyon panduriforme	***82.99***	4.62	2.23	6.61
Amphora copulata	***82.26***	10.29	6.33	1.11
Navicula sp. 4	*82.12*	0.01	0.38	0.32
Navicula sp. 2	***81.17***	1.83	6.43	1.07
Amphora proteus	***80.38***	8.63	7.81	3.18
Biddulphia sp. 1	*56.44*	5.11	0.00	0.00
Navicula sp. 5	***50.00***	0.00	0.00	0.00
Nitzschia cf. *sigma*	43.27	0.26	19.00	3.87
Tryblionella cf. *marginulata*	41.87	0.48	5.86	1.04
Biddulphia sp. 2	11.62	25.58	0.00	0.00
Ralfsiella smithii	1.81	2.81	***63.13***	20.41
Gyrosigma cf. *balticum*	0.00	0.00	*55.56*	27.78
Surirella sp.	6.54	21.29	*50.10*	18.79
Pleurosigma cf. *latum*	20.41	0.50	46.66	32.18
Campylodiscus sp.	3.91	3.13	45.57	7.81
Caloneis sp.	0.00	2.17	40.22	1.09
Halamphora coffeiformis	28.03	0.02	35.91	0.00
Gyrosigma cf. *fasciola*	0.00	0.00	29.17	6.94
Entomoneis alata	0.00	0.00	28.70	2.32
Diploneis weissflogiopsis	9.08	7.00	21.98	11.03
Cocconeis sp.	4.87	0.93	2.11	*67.6*
Amphora cf. *graeffei*	8.82	0.00	0.21	22.89
Diploneis sp. 3	12.50	4.17	0.00	0.00

sp. was an indicator of winter.

The Shannon diversity index (Fig. 7) showed no significant differences between the two stations in any seasons (Wilcoxon rank sum test, $p > 0.05$), but lower values were found in winter compared to other seasons.

The NMDS plot (Fig. 8) shows how the seasonal abundances of the four growth forms were related to environmental factors. In particular, adnate taxa were positively related to salinity and turbidity, while plocon were positively related to oxygen and nutrients, but negatively with turbidity. Motile forms showed a negative relationship with temperature, while erect forms showed no clear relationship as they were not well represented in the community. The two stations did not differ. However, spring and summer were clearly separated from the other seasons. These results were confirmed by the PERMANOVA, which only showed significant differences among seasons (PERMANOVA, $p < 0.001$).

Species co-occurrence

In this analysis, 48.95% and 45.29% of the total species pair combinations of SG and PN respectively were removed from the species pairing total, because of the low pair combination (<1), which means that the pair-

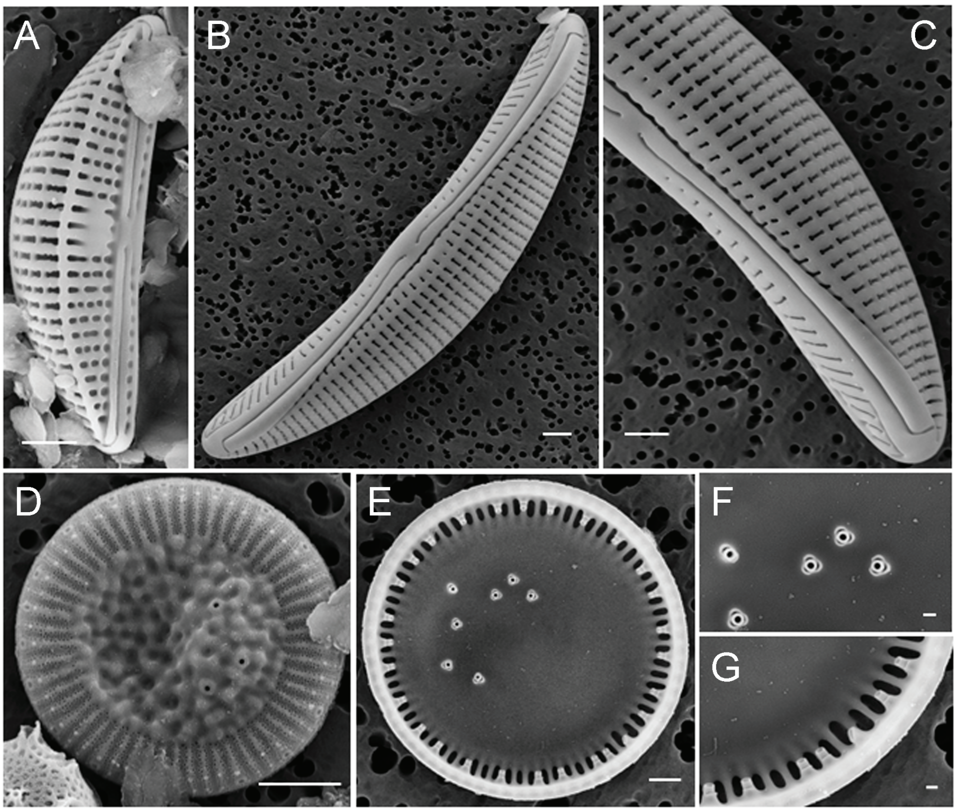

Fig. 6: SEM images of: A= *Amphora* cf. *copulata*, external valve view showing elongated areolae; B-C= *Amphora* cf. *proteus*, B= external valve view showing curved terminal raphe endings at centre and distal end, C= enlargement of the cell that shows curved raphe endings and the particular T-shaped areolae; D-G= *Cyclotella choctawatcheeana*, D=external valve view, E=internal valve view, F= inside valve with central fultoportulae surrounded by three auxiliary pores, G= internal valve showing the rounded tube of marginal rimoportulae on every second or third interstriae and a fultoportulae with a slit opening. Scale bars: A, B, C, D= 2 µm, E= 1 µm, F, G= 200nm.

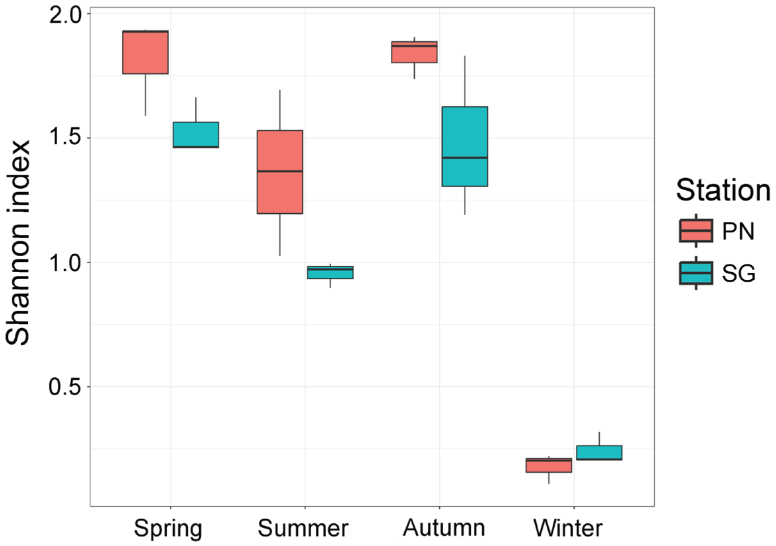

Fig. 7: Shannon's index (H') in the four seasons at the Senigallia (SG) and Portonovo (PN) stations.

ing was not relevant. At SG station (Fig. 9A), 91.76% of co-occurrences were random, 5% unclassifiable, and the remaining 3.24% non-random (1.47% and 1.76% for positive and negative co-occurrences, respectively). At PN station (Fig. 9B), 96.36% of interactions were random, 2.43% unclassifiable and 1.21% non-random (0.61% for both positive and negative co-occurrences, respectively).

Discussion

This study is a first investigation of the seasonal abundance and biomass of epipelic diatoms in two distinct sites of the Adriatic coastal area, Senigallia and Portonovo. The two sites have a similar granulometric composition, the same depth, and likely comparable light conditions, although light penetration data were not available.

The two stations differed in terms of environmental

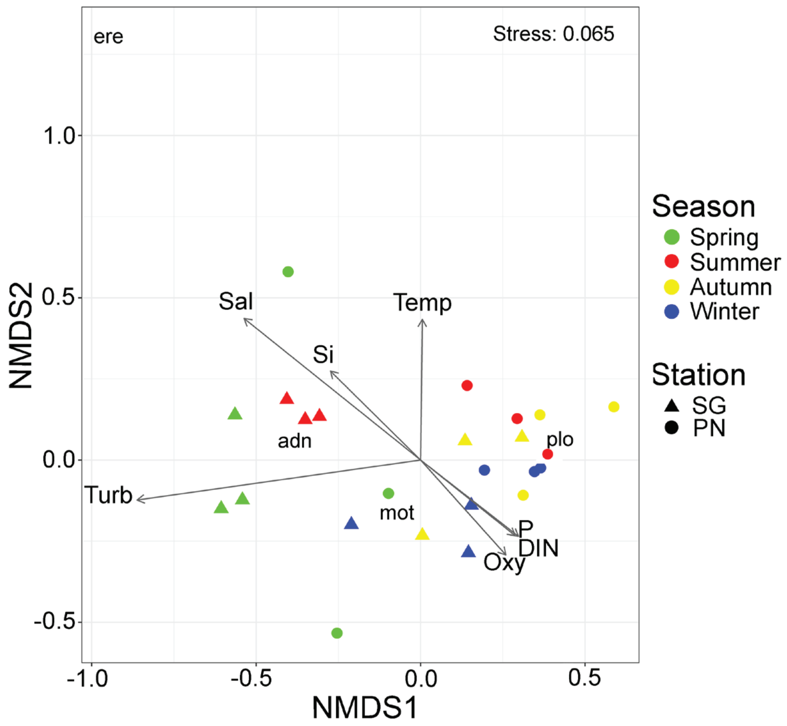

Fig. 8: NMDS performed on the seasonal abundances of the four growth forms (adn: adnate, ere: erect, mot: motile, plo: plocon) and environmental parameters (Temp: Temperature, Sal: Salinity, DIN: Dissolved Inorganic Nitrogen, P: Phosphate, Si: Silicates, Turb: Turbidity, Oxy: Oxygen saturation) for the two stations (circle for Senigallia and triangle for Portonovo). The points represent the seasonal abundances at the two stations, while the arrows represent the environmental parameters fitted on the NMDS.

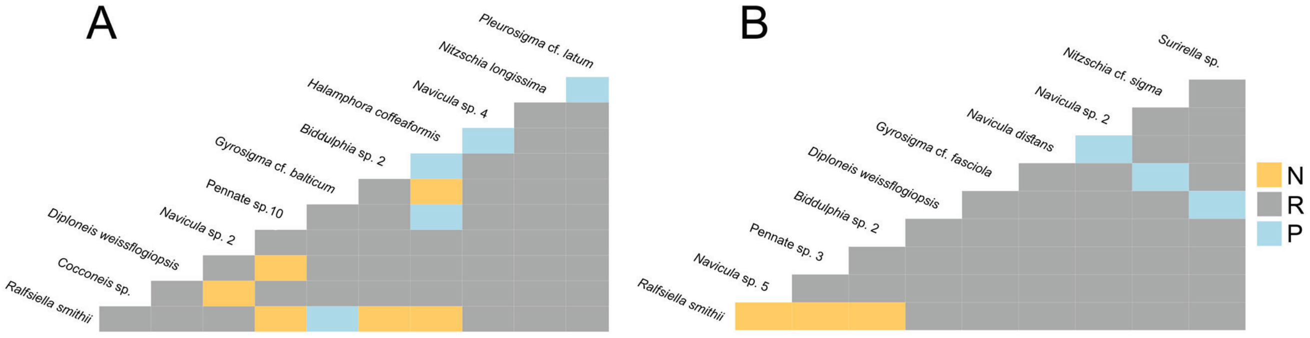

Fig. 9: Negative (N, yellow), random (R, grey) and positive (P, light blue) species co-occurrence in Senigallia (A) and Portonovo (B).

conditions (riverine input in Senigallia, reflected by the lower salinity values and presence of a strong halocline in spring-summer) and anthropogenic impact (tourist boat pressure in Portonovo), which were partly revealed by a different nutrient seasonal pattern. However, there were no important differences in the annual cycle of benthic diatom abundance, which was characterised at both stations by maximum values in spring, a summer decline and an increase in autumn.

The average abundance values observed in this study are of the same order of magnitude as those reported in previous studies in the Adriatic Sea by Tolomio *et al.* (2002), Welker *et al.* (2002), Franzo *et al.* (2015) and Rogelja *et al.* (2016). On the contrary, abundances were one-two orders of magnitude lower than the ones observed by Sdrigotti *et al.* (1999), Tolomio *et al.* (1999), Facca *et al.* (2002), Facca & Sfriso (2007) and Cibic *et al.* (2007, 2012), and one order of magnitude higher than in Totti (2003). Such differences could be explained by the different sampling depth and by the different methods of analysis used in the various studies (e.g., direct counting after dilutions of the sediment, separation of diatom cells using density gradient centrifugation or hydrogen-peroxide cleaning of diatom frustules before counting). For example, the use of the hydrogen-peroxide cleaning method (e.g., Facca *et al*., 2002, Tolomio *et al*., 2002) is expected to overestimate cell abundance because empty frustules (not considered by other counting methods) are also likely to be counted. Overestimation is also to be expected in studies based on direct counting methods (e.g., Cibic *et al*., 2007, Franzo *et al*., 2015), which require the observation of a very small aliquot of sample (10 to 20 μl) to avoid optical disturbance by the sediment. On the other hand, the density gradient separation (Totti, 2003, Méléder *et al* 2007, this study) has the advantage of processing a higher sample volume due to the removal of sediment but it may result in underestimation, as heavy cells can be lost with the sediment itself. Another possible explanation can be found in the sampling method used in this study, i.e., the grab. Although sampling was carried out very carefully, we cannot exclude a possible resuspension of a small amount of sediment causing a loss of cells.

As for the seasonal cycle, at both stations we observed the highest diatom abundance and biomass values in spring and the minimum in summer. This cycle differed partially from what was reported in other Mediterranean areas, where the maximum biomass was generally observed in spring and summer and the minimum from autumn to winter (Cibic *et al.*, 2007, 2012; Méléder *et al.*, 2007). The maximum in spring is generally related to the increasing photoperiod and light intensity, considering that light represents the most common limiting factor for MPB growth (Welker *et al.*, 2002). In most studies where the seasonal variability of MPB is addressed, a second maximum in summer is also reported, contrary to what was observed in this study. The observed summer decline could be linked to the hypoxic conditions observed at the bottom, related to both the strong stratification and high atmospheric temperature. At PN station, where the hypoxic conditions were less severe, the high turbidity suggests that light limitation could have played an important role in the biomass decrease. Moreover, although the grazing pressure was not measured in this study, we might hypothesize a higher impact of grazing by meiofauna, which is the largest MPB consumer, compared to other areas (Franzo *et al.*, 2015; Schratzberger & Ingels, 2018).

In this study, the effect of nutrient concentrations in the overlaying water column on the MPB abundance was modest, as already reported in other studies, considering that MPB have an abundant supply of nutrients from the sediment pore water (Blackford, 2002). Nevertheless, the correlation between the nutrient concentration at the bottom-overlaying water layer and the MPB abundance has been found in previous studies (Welker *et al.*, 2002; Totti, 2003). It was observed that, whenever nutrient ratios in the water column are unbalanced, the benthic diatom growth is determined by the limiting nutrient. The optimal nutrient ratio (Si:N:P) for diatoms is 16:16:1 (Brzezinski, 1985; Hillebrand & Sommer, 1997, 1999). In this study, the Si:N:P ratio at both stations and in all seasons indicates a strong P limitation, which is the typical condition of the Adriatic Sea (Grilli *et al.*, 2020). However, it is assumed that diatoms, like other microalgae, can also exploit organic P sources (Ellwood & Whitton, 2007; Yamaguchi *et al.*, 2014; Accoroni *et al.*, 2017) making the estimation of P limitation more complex. Currently, the most important sources of silicates in the Mediterranean Sea are riverine waters and groundwater discharges (Sospedra *et al.*, 2018). At SG station, the silicate concentration was higher than the DIN one in all seasons, with the exception of winter, likely because of the seasonal bloom of the planktonic diatom *Skeletonema marinoi* ($<10^6$-10^7 cells L^{-1}, Totti *et al.*, 2019) when silicates typically decrease because they are rapidly taken up by phytoplankton. At the PN station, where the impact of riverine waters is modest, DIN is higher than silicates in all seasons. Interestingly, at both stations the ammonium concentration was higher than the nitrite and nitrate ones in spring and summer, when we observed the highest and lowest MPB abundance respectively. The fact that diatoms typically prefer ammonium over nitrites and nitrates (Welker *et al.*, 2002), coupled with the increased daylight period, could have enhanced the spring MPB growth, whereas in summer the hypoxic conditions could have caused the decrease.

In terms of both taxonomic and morpho-functional composition, the structures of the MPB communities were also very similar at the two stations, indicating a high spatial stability of the MPB communities. Motile forms were dominant in all seasons, as already reported by Totti (2003) in the northern Adriatic Sea. The abundance of motile forms could be explained by their strong ability to move across the sediment in which they are the best competitors for nutrients and light (DeNicola & McIntire, 1990; Lange *et al.*, 2011). Interestingly, even though motile forms were abundant in all seasons, their contribution to biomass was low because they were mainly composed of small pennate forms. Adnate forms were the second group in terms of abundances and reached their maximum in spring, while in other seasons low values were observed. Among them, only *Amphora* species were observed, while the presence of *Cocconeis* species was negligible (with the exception of winter), as they commonly live as epiphyte (De Stefano *et al.*, 2000; Totti *et al.*, 2009), epilithic (Totti *et al.*, 2007; Car *et al.*, 2020, 2021) or epizoic (Romagnoli *et al.*, 2007, 2014; Totti *et al.*, 2011). The near negligible presence of erect diatoms was expected, as they need solid substratum to attach. Plocon diatoms represented a major contributor to diatom biomass, given the high biovolumes of the centric taxa belonging to Biddulphiaceae family. Unlike what was observed for other groups, their abundance and biomass increased with high nutrient levels, mainly in autumn and winter, in mixing conditions, as highlighted by NMDS analysis. This can be explained considering that (i), having large biovolumes, they are favoured by high nutrient concentrations, and, (ii) given their heavily silicified frustules, they are less affected by resuspension caused by the mixing conditions.

Taxa that emerge as strong indicators of spring belonged mainly to motile and adnate growth forms, while those that were indicators of autumn belonged mainly to plocon and motile forms. This suggests that the water column mixing/stratification played a role in structuring the microphytobenthos community of the study areas, favouring taxa capable of movement (motile and lesser adnate) in the seasons characterized by a stratification regime and heavily silicified taxa in the seasons when the mixed water column is more turbulent, as suggested by Franzo *et al.* (2018).

The main genera composing the MPB community

(*Navicula sensu lato*, *Nitzschia*, *Amphora sensu lato*) matched those reported for the northern Adriatic, both in the Gulf of Trieste (Welker & Nichetto, 1996; Welker *et al.*, 2002; Cibic *et al.*, 2007, 2009; Franzo *et al.*, 2015, 2018), and in the Venice Lagoon (Tolomio *et al.*, 1999, 2002; Facca *et al.*, 2002a,b; Facca & Sfriso, 2007). Notably, the absence of *Paralia sulcata*, a benthic centric diatom with a heavy frustule, represented a major component in MPB communities in studies by Franzo *et al.* (2015, 2018) in the Gulf of Trieste and by Totti (2003) in offshore areas in front of the Po River mouth and the Marche Region coast.

In both stations, the co-occurrence analysis showed that in the microphytobenthos community, the majority of interactions were random, underling the major role of environmental conditions in shaping the community. However, the presence of a number of positive and negative interactions between different diatom taxa suggests that competitive/ "supportive" mechanisms may also influence the community compositions under different environmental conditions, regardless of whether or not those taxa are indicators of a particular season or station.

Conclusions

This study provides new results in terms of the quantitative distribution (abundance and biomass), community structure and taxonomic composition of marine epipelic diatoms in the subtidal sediments of the Northern Adriatic Sea. These communities showed a marked temporal variability, with a maximum in spring and a minimum in summer but did not reveal a significant spatial variability.

The hypoxic conditions observed at the bottom layer of both stations may have been responsible for the summer decrease in MPB abundance and biomass. In terms of morpho-functional composition, the community was markedly dominated by motile forms, as expected for epipelic communities. Large benthic centric diatoms (i.e., plocon) are favoured by the mixing conditions typically observed during autumn and winter, likely because they have large and heavy frustules and therefore they are advantaged by high nutrient availability, and prevented from resuspension and mechanical damage.

Despite some differences in hydrographic characteristics and anthropogenic impact, the MPB taxonomic composition and community structure did not show significant differences between the two stations, suggesting a certain stability of the biological community.

In this study, despite the taxonomical effort, many taxa were not identified at the species level, revealing that MPB communities host a large and unexplored biodiversity. Indeed, more data are needed to assess the seasonal cycle and the interannual variability of MPB communities. Given their ecological importance, long-term monitoring on microphytobenthic communities in subtidal areas is recommended to improve knowledge about their diversity, seasonal pattern and interannual trends.

Acknowledgements

The authors would like to acknowledge the Actea R/V crew for their support in the sampling activities. We thank Centro di Ricerca e Servizio di Microscopia delle Nanostrutture (CISMiN) of Università Politecnica delle Marche for its support in the Electron Microscopy service. The author Apurva Mule has had the support of a PhD fellowship co-funded by Università Politecnica delle Marche (Ancona, Italy) and the Stazione Zoologica Anton Dohrn (Naples, Italy). Special thanks to the two anonymous reviewers who helped in improving the quality of this ms.

References

Accoroni, S., Romagnoli, T., Pichierri, S., Totti, C., 2016. Effects of the bloom of harmful benthic dinoflagellate *Ostreopsis* cf. *ovata* on the microphytobenthos community in the northern Adriatic Sea. *Harmful Algae*, 55, 179-190.

Accoroni, S., Totti, C., Razza, E., Congestri, R., Campanelli, A. *et al.,* 2017. Phosphatase activities of a microepiphytic community during a bloom of *Ostreopsis* cf. *ovata* in the northern Adriatic Sea. *Water Research*, 120, 272-279.

Armbrust, E.V., 2009. The life of diatoms in the world's oceans. *Nature*, 459, 185-192.

Artegiani, A., Bregant, D., Paschini, E., Pinardi, N., Raicich, F. *et al.,* 1997. The Adriatic Sea general circulation. Part I: air-sea interactions and water mass structure. *Journal of Physical Oceanography*, 27, 1492-1514.

Balsamo, M., Albertelli, G., Ceccherelli, V.U., Coccioni, R., Colangelo, M.A. *et al.*, 2010. Meiofauna of the Adriatic Sea: present knowledge and future perspectives. *Chemistry and Ecology*, 26, 45-63.

Barranguet, C., Kromkamp, J., Peene, J., 1998. Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. *Marine Ecology Progress Series*, 173, 117-126.

Bérard-Therriault, L., Cardinal, A., Poulin, M., 1986. Les diatomées (Bacillariophyceae) benthiques de substrats durs des eaux marines et saumâtres du Québec. 6. Naviculales: Cymbellaceae et Gomphonemaceae. *Le Naturaliste Canadien*, 113, 405-429.

Bernardi Aubry, F., Acri, F., Bastianini, M., Finotto, S., Pugnetti, A., 2022. Differences and similarities in the phytoplankton communities of two coupled transitional and marine ecosystems (the Lagoon of Venice and the Gulf of Venice - Northern Adriatic Sea). *Frontiers in Marine Science*, 9, 974967.

Blackford, J.C., 2002. The influence of microphytobenthos on the Northern Adriatic ecosystem: A modelling study. *Estuarine Coastal and Shelf Science*, 55 (1), 109-123.

Blasutto, O., Cibic, T., de Vittor, C., Fonda Umani, S., 2005. Microphytobenthic primary production and sedimentary carbohydrates along salinity gradients in the Lagoons of Grado and Marano (Northern Adriatic Sea). *Hydrobiologia*, 550 (1), 47-55.

Brzezinski, M.A., 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. *Journal of Phycology*, 21, 347-357.

Cahoon, L., 1999. The role of benthic microalgae in neritic ecosystems. *Oceanography and Marine Biology*, 37, 47-86.

Campanelli, A., Grilli, F., Paschini, E., Marini, M., 2011. The influence of an exceptional Po River flood on the physical and chemical oceanographic properties of the Adriatic Sea. *Dynamics of Atmospheres and Oceans*, 52 (1-2), 284-297.

Car, A., Hafner, D., Ljubimir, S., Radić, I.D., Bobanović-Ćolić S. *et al.,* 2020. Colonization of bacteria and diatoms on an artificial substrate in a marine lake (eastern Adriatic Sea, NE Mediterranean). *Acta Botanica Croatica*, 79 (2), 212-227.

Car, A, Hafner, D., Dupčić Radić I., Kaleli, A., Ljubimir, S. *et al.,* 2021. Comparison of benthic diatom community structures on natural and artificial substrates in marine lake (Adriatic Sea). *Acta Adriatica*, 62 (1), 21-44.

Cardinal, A., Poulin, M., Bérard-Therriault, L. 1984. Les diatomées benthiques de substrats durs des eaux marines et saumâtres du Québec 4. Naviculales, Naviculaceae (à l'exclusion des genres *Navicula*, *Donkinia*, *Gyrosigma* et *Pleurosigma*). *Le Naturaliste Canadien*, 111 (4), 369-394.

Cerino, F., Fornasaro, D., Kralj, M., Giani, M., Cabrini, M., 2019. Phytoplankton temporal dynamics in the coastal waters of the north-eastern Adriatic Sea (Mediterranean Sea) from 2010 to 2017. *Nature Conservation*, 34, 343-372.

Cibic, T., Blasutto, O., Falconi, C., Fonda Umani, S., 2007. Microphytobenthic biomass, species composition and nutrient availability in sublittoral sediments of the Gulf of Trieste (northern Adriatic Sea). *Estuarine Coastal and Shelf Science*, 75 (1-2), 50-62.

Cibic, T., Comici, C., Bussani, A., Del Negro, P., 2012. Benthic diatom response to changing environmental conditions. *Estuarine Coastal and Shelf Science*, 115, 158-169.

Cochero, J., Licursi, M., Gómez, N., 2015. Changes in the epipelic diatom assemblage in nutrient rich streams due to the variations of simultaneous stressors. *Limnologica*, 51, 15-23.

Cozzi, S., Giani, M., 2011. River water and nutrient discharges in the Northern Adriatic Sea: current importance and long-term changes. *Continental Shelf Research*, 31 (18), 1881-1893.

D'Alelio, D., Cante, M., Russo, G.F., Totti, C., De Stefano, M., 2011. Epizoic diatoms on gastropod shells. When substrate complexity selects for microcommunity complexity. p. 345-364. In: *All flesh is grass: plant-animal interactions*. Seckbach, J., Dubinsky, Z. (Eds). Springer.

De Stefano, M., Marino, D., Mazzella, L., 2000. Marine taxa of *Cocconeis* on leaves of *Posidonia oceanica*, including a new species and two new varieties. *European Journal of Phycology*, 35 (3), 225-242.

DeNicola, D.M., McIntire, C.D., 1990. Effects of substrate relief on the distribution of periphyton in laboratory streams, I. Hydrology. *Journal of Phycology*, 26 (4), 624-633.

D'Ortenzio, F., Ribera d'Alcalà, M., 2008. On the trophic regimes of the Mediterranean Sea: a satellite analysis on the trophic regimes of the Mediterranean Sea. *Biogeosciences*, 6, 139-148.

Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 67 (3), 345-366.

Ellwood, N.T.W., Whitton. B.A., 2007. Importance of organic phosphate hydrolyzed in stalks of the lotic diatom *Didymosphenia geminata* and the possible impact of atmospheric and climatic changes. *Hydrobiologia*, 592, 121-133.

Facca, C., Sfriso, A., 2007. Epipelic diatom spatial and temporal distribution and relationship with the main environmental parameters in coastal waters. *Estuarine Coastal and Shelf Science*, 75 (1-2), 35-49.

Facca, C., Sfriso, A., Socal, G., 2002a. Changes in abundance and composition of phytoplankton and microphytobenthos due to increased sediment fluxes in the Venice Lagoon, Italy. *Estuarine Coastal and Shelf Science*, 54, 773-792.

Facca, C., Sfriso, A., Socal, G., 2002b. Temporal and spatial distribution of diatoms in the surface sediments of the Venice Lagoon. *Botanica Marina*, 45 (2), 170-183.

Fagherazzi, S., Mariotti. G., Banks, A.T., Morgan E.J., Fulweiler, R.W., 2014. The relationships among hydrodynamics, sediment distribution, and chlorophyll in a mesotidal estuary. *Estuarine Coastal and Shelf Science*, 144, 54-64.

Franzo, A., Cibic, T., del Negro, P., de Vittor, C., 2015. Spatial distribution of microphytobenthos, meiofauna and macrofauna in the north-western Adriatic Sea: a synoptic study. *Limnology and Oceanography*, 6 (1-2), 58-75.

Franzo, A., Guilini, K., Cibic, T., Del Negro, P., 2018. Interactions between free-living nematodes and benthic diatoms: insights from the Gulf of Trieste (northern Adriatic Sea). *Mediterranean Marine Science*, 19 (3), 538-554.

Friedman, G.M., Sanders, J.E., 1978. *Principles of sedimentology*. Wiley, New York.

Graham, L.E., Graham, J.M., Wilcox, L.W., Cook, M.E., 2016. *Algae 3rd edition*. LJLM Press, 689 pp.

Griffith, D.M., Veech, J.A., Marsh, C.J., 2016. Cooccur: Probabilistic species co-occurrence analysis in R. *Journal of Statistical Software, Code Snippets*, 69 (2), 1-17.

Grilli, F., Accoroni, S., Acri, F., Bernardi Aubry, F., Bergami, C. *et al.*, 2020. Seasonal and interannual trends of oceanographic parameters over 40 years in the Northern Adriatic Sea in relation to nutrient loadings using the EMODnet chemistry data portal. *Water*, 12 (8), 2280.

Hasle, G.R., Syvertsen, E.E., 1997. Marine Diatoms. p. 5-385. In: *Identifying Marine Phytoplankton.* Tomas, C.R. (Ed). Academic Press, San Diego.

Hillebrand, H., Sommer, U., 1997. Response of epilithic microphytobenthos of the Western Baltic Sea to in situ experiments with nutrient enrichment. *Marine Ecology Progress Series*, 160, 35-46.

Hillebrand, H., Sommer, U., 1999, The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnology and Oceanography* 44 (2), 440-446.

Hope, J.A., Paterson, D.M., Thrush, S.F., 2019. The role of microphytobenthos in soft-sediment ecological networks and their contribution to the delivery of multiple ecosystem services. *Journal of Ecology*, 108, 815-830.

Hustedt, F., 1985. *The Pennate Diatoms, a translation of Hustedt's "Die Kieselalgen, 2 Teil", with suppl. of N.H. Jensen.* KOELTZ Scientific Books KOENIGSTEIN: 918 pp.

Lange, K., Liess, A., Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2011. Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology*, 56 (2), 264-278.

MacIntyre, H.L., Geider, R.J., Miller, D.C., 1996. Microphytobenthos: the ecological role of the secret garden of unvegetated shallow-water marine habitats. I. Distribution abundance and primary production. *Estuaries*, 19 (2A), 186-201.

Marić, D., Kraus, R., Godrijan, J., Supić, N., Djakovac, T. *et al.,* 2012. Phytoplankton response to climatic and anthropogenic influences in the northeastern Adriatic during the last four decades. *Estuarine Coastal and Shelf Science*, 115, 98-112.

Méléder, V., Rincé, Y., Barillé, L., Gaudin, P., Rosa, P., 2007. Spatiotemporal changes in microphytobenthos assemblages in a macrotidal flat (Bourgneuf Bay, France). *Journal of Phycology*, 43 (6), 1177-1190.

Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography,* 45 (3), 569-579.

Migné, A., Spilmont, N., Boucher, G., Denis, L., Hubas, C. *et al*., 2009. Annual budget of benthic production in Mont Saint-Michel Bay considering cloudiness, microphytobenthos migration, and variability of respiration rates with tidal conditions. *Continental Shelf Research*, 29 (19), 2280-2285.

Miller, D.C., Geider, R.J., Macintyre, H.L., 1996. Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. *Estuaries* 19 (2A), 202-212.

Munda, I.M., 2005. Seasonal fouling by diatoms on artificial substrata at different depths near Piran (Gulf of Trieste, Northern Adriatic). *Acta Adriatica*, 46 (2), 137-157.

Neri, F., Romagnoli, T., Accoroni, S., Campanelli, A., Marini, M. *et al.*, 2022. Phytoplankton and environmental drivers at a long-term offshore station in the northern Adriatic Sea (1988-2018). *Continental Shelf Research*, 242, 104746.

Neri, F., Romagnoli, T., Accoroni, S., Ubaldi, M., Garzia, A. *et al.*, 2023. Phytoplankton communities in a coastal and offshore stations of the northern Adriatic Sea approached by network analysis and different statistical descriptors. *Estuarine Coastal and Shelf Science*, 282, 108224.

Ní Longphuirt, S., Leynaert, A., Guarini, J-M., Chauvaud, L., Claquin, P. *et al.*, 2006. Discovery of microphytobenthos migration in the subtidal zone. *Marine Ecology Progress Series*, 328, 143-154.

Ní Longphuirt, S., Clavier, J., Grall, J., Chauvaud, L., Le Loc’h, F. *et al.*, 2007. Primary production and spatial distribution of subtidal microphytobenthos in a temperate coastal system, the Bay of Brest, France. *Estuarine Coastal and Shelf Science*, 74 (3), 367-380.

Oksanen, J., Simpson, G.L., Blanchet, G.F, Kindt, R., Legendre, P. *et al.*, 2022. vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan

Patrick, R., Reimer, C.W. 1966. *The diatoms of United States. Vol. 1.* Monographs of the Academy of Natural Sciences of Philadelphia, 13, 688 pp.

Patrick, R., Reimer C.W. 1975. *The diatoms of United States. Vol. 2, Part 1*. Monographs of the Academy of Natural Sciences of Philadelphia, 13, 213. pp.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis.* Pergamon Press, Oxford.

Peragallo, H., Peragallo, M. 1897-1908. *Diatomées marines de France et des districts maritimes voisins*. M.J. Tempère (ed.), Grez-sur-Loing., 491 pp.

Pennesi, C., Danovaro, R., 2017. Assessing marine environmental status through microphytobenthos assemblages colonizing the Autonomous Reef Monitoring Structures (ARMS) and their potential in coastal marine restoration. *Marine Pollution Bulletin*, 125 (1-2), 56-65.

Pinckney, J.L., 2018. A mini-review of the contribution of benthic microalgae to the ecology of the continental shelf in the South Atlantic bight. *Estuaries and Coasts*, 41 (7), 2070-2078.

Poulin, M., Bérard-Therriault, L., Cardinal, A., 1987. Les Diatomées (Bacillariophyceae) benthiques de substrats durs des eaux marines et saumâtres du Québec. 7. Naviculales (les genres *Plagiotropis* and *Entomoneis*). *Le Naturaliste Canadien*, 114, 67-80.

Poulin, M., Bérard-Therriault, L., Cardinal, A., Hamilton, P.B. 1990. Les Diatomées (Bacillariophyta) benthiques de substrats durs des eaux marines et saumâtres du Québec. 9. Bacillariaceae. *Le Naturaliste Canadien*, 117, 73-101.

R Core Team, 2021. R: A Language and Environment for Statistical Computing.

Riaux-Gobin, C., Romero, O., 2003. Marine *Cocconeis* Ehrenberg (Bacillariophyceae) species and related taxa from Kerguelen’s Land (Austral Ocean, Indian Sector). Bibliotheca Diatomologica, 47. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung. Berlin. 189 pp.

Rogelja, M., Cibic, T., Pennesi, C., de Vittor, C., 2016. Microphytobenthic community composition and primary production at gas and thermal vents in the Aeolian Islands (Tyrrhenian Sea, Italy). *Marine Environmental Research*, 118, 31-44.

Romagnoli, T., Bavestrello, G., Cucchiari, E.M., De Stefano, M., Di Camillo, C.G. *et al.*, 2007. Microalgal communities epibiontic on the marine hydroid *Eudendrium racemosum* in the Ligurian Sea during an annual cycle. *Marine Biology*, 151 (2), 537-552.

Romagnoli, T., Totti, C., Accoroni, S., De Stefano, M., Pennesi, C., 2014. SEM analysis of the epibenthic diatoms on *Eudendrium racemosum* (Hydrozoa) from the Mediterranean Sea. *Turkish Journal of Botany*, 38 (3), 566-594.

Round, F.E., 1971. Benthic marine diatoms. *Oceanography and Marine Biology - An Annual Review*, 9, 83-139.

Round, F.E., Crawford, R.M., Mann, D.G., 1990. *The diatoms. Biology and morphology of the genera.* Cambridge University Press, Cambridge, 747 pp.

Russo, A., Artegiani, A., 1996. Adriatic Sea hydrography. *Scientia Marina*, 60, 33-43.

Sar, E.A., Romero, O., Sunesen, I. 2003. *Cocconeis* Ehrenberg and *Psammococconeis* Garcia (Bacillariophyta) from the Gulf of San Matías, Patagonia, Argentina. *Diatom Research*, 18 (1), 79-106.

Schratzberger, M., Ingels, J., 2018. Meiofauna matters: the roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology*, 502, 12-25.

Sdrigotti, E., Barbariol, V., Welker, C., 1999. Diatom assemblages in coastal shallow waters at the water-sediment interface (Gulf of Trieste, North Adriatic Sea). *Annales. Series historia naturalis*, 9 (2), 191-202.

Shannon, C.E., 1948. A mathematical theory of communication. *The Bell System Technical Journal*, 27, 379-423.

Simonsen, R., 1987. *Atlas and catalogue of the diatom types of Friederich Hustedt.* J. Cramer-Berlin Stuttgart, Vol. 1: 525 pp; Vol. 2: pl. 1-395; Vol. 3: pl. 396-772.

Sospedra, J., Niencheski, L.F.H., Falco, S., Andrade, C.F.F., Attisano, K.K. *et al.,* 2018. Identifying the main sources of silicate in coastal waters of the Southern Gulf of Valencia (Western Mediterranean Sea). *Oceanologia*, 60 (1), 52-64.

Tolomio, C., Moro, I., Moschin, E., Valandro, A., 1999. Resultats preliminaires sur les diatomées benthiques de substrats meubles dans la lagune de Venise, Italie (Mars 1994-Janvier 1995). *Diatom Research,* 14 (2), 367-379.

Tolomio, C., Moschin, E., Duzzin, B., 2002. Distribution des diatomées benthiques de substrats meubles dans le bassin sud de la lagune de Venise, Italie. *Diatom Research*, 17 (2), 401-414.

Totti, C., 2003. Influence of the plume of the River Po on the distribution of subtidal microphytobenthos in the northern Adriatic Sea. *Botanica Marina*, 46, 161-178.

Totti, C., De Stefano, M., Facca, C., Ghirardelli, L.A., 2004. Microphytobenthos. p. 247-266. In: *Mediterranean marine benthos: a manual of methods for its sampling and study*. Gambi, M.C., Dappiano, M. (Eds). *Biologia Marina Mediterranea*, 11 (1), 244-266.

Totti, C., Cucchiari, E., De Stefano, M., Pennesi, C., Romagnoli, T., Bavestrello, G., 2007. Seasonal variations of epilithic diatoms on different hard substrates, in the northern Adriatic Sea. *Journal of the Marine Biological Association of the United Kingdom*, 87 (3), 649-658.

Totti, C., Poulin, M., Romagnoli, T., Perrone, C., Pennesi, C., De Stefano, M., 2009. Epiphytic diatom communities on intertidal seaweeds from Iceland. *Polar Biology*, 32 (11), 1681-1691.

Totti, C., Romagnoli, T., De Stefano, M., Di Camillo, C.G., Bavestrello, G., 2011. The diversity of epizoic diatoms. Relationships between diatoms and marine invertebrates. p. 323-343. In: *All flesh is grass: plant-animal interactions*. Seckbach J., Dubinsky Z. (Eds) Springer.

Totti, C., Romagnoli, T., Accoroni, S., Coluccelli, A., Pellegrini, M. *et al.*, 2019. Phytoplankton communities in the northwestern Adriatic Sea: interdecadal variability over a 30-years period (1988-2016) and relationships with meteoclimatic drivers. *Journal of Marine Systems*, 193, 137-153.

Underwood, G.J.C., Barnett, M., 2006. What determines species composition in microphytobenthic biofilms? p. 121-138. In: *Functioning of microphytobenthos in estuaries*. Kromkamp, J., de Brouwer, J.F.C., Blanchard, G.F., Forster, R.M., Créach, V. (Eds). Royal Netherlands Academy of Arts and Sciences, Amsterdam.

Underwood, G.J.C., Paterson, D.M., 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. *Advances in Botanical Research*, 40, 183-240.

Van Der Werff, A., Huls, H., 1957-1974. *Diatomeenflora van Nederland. Deen Haag.* Afl. 1-9.

Van Heurck, H., 1880-1885. *Synopsis des Diatomées de Belgique*. 132 (Suppl. A, B, C), Anvers, 235 pp.

Vascotto, I., Mozetič, P., Francé, J., 2021. Phytoplankton time-series in a LTER site of the Adriatic Sea: methodological approach to decipher community structure and indicative taxa. *Water*, 13 (15), 2045.

Veech, J.A., 2013. A probabilistic model for analysing species co-occurrence. *Global Ecology and Biogeography*, 22 (2), 252-260.

Welker, C., Nichetto, P., 1996. The influence of mucous aggregates on the microphytobenthic community in the Northern Adriatic Sea. *P.S.Z.N.: Marine Ecology*, 17 (1-3), 473-489.

Welker, C., Sdrigotti, E., Covelli, S., Faganeli, J., 2002. Microphytobenthos in the gulf of Trieste (Northern Adriatic Sea): relationship with labile sedimentary organic matter and nutrients. *Estuarine Coastal and Shelf Science*, 55 (2), 259-273.

Yamaguchi, H., Arisaka, H., Otsuka, N., Tomaru, A., 2014. Utilization of phosphate diesters by phosphodiesterase-producing marine diatoms. *Journal of Plankton Research*, 36 (1), 281-285.