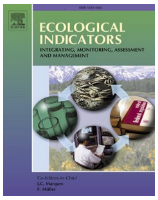
Ecological Indicators 126 (2021) 107630

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Large-scale testing of phytoplankton diversity indices for environmental assessment in Mediterranean sub-regions (Adriatic, Ionian and Aegean Seas)

Janja Francé[a,*], Ioanna Varkitzi[b], Elena Stanca[c,d], Francesco Cozzoli[c,e], Sanda Skejić[f], Nicola Ungaro[g], Ivano Vascotto[a,h], Patricija Mozetič[a], Živana Ninčević Gladan[f], Georgia Assimakopoulou[b], Alexandra Pavlidou[b], Soultana Zervoudaki[b], Kalliopi Pagou[b], Alberto Basset[c]

[a] *National Institute of Biology, Marine Biology Station Piran, Fornače 41, 6330 Piran, Slovenia*
[b] *Hellenic Centre for Marine Research HCMR, Institute of Oceanography, 19013 Anavyssos, Attica, Greece*
[c] *University of Salento, Department of Biological and Environmental Sciences and Technologies, S.P. Lecce-Monteroni – Ecotekne, 73100 Lecce, Italy*
[d] *CRM-Arpa Puglia, Corso Vittorio Veneto 13, 70126 Bari, Italy*
[e] *Research Institute on Terrestrial Ecosystems (IRET) - National Research Council of Italy (CNR), via Salaria km 29.3 – 00015, Monterotondo Scalo (Roma), Italy*
[f] *Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia*
[g] *ARPA Puglia, Corso Trieste 27, 70126 Bari, Italy*
[h] *Jozef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia*

ARTICLE INFO

Keywords:
Phytoplankton
Diversity indices
Pelagic habitat
Marine strategy framework directive
Good environmental status
Adriatic Sea
Ionian Sea
Aegean Sea
Mediterranean

ABSTRACT

According to the methodological standards established by Marine Strategy Framework Directive, the assessment for the pelagic habitat under the Biodiversity Descriptor should be carried out at the regional or sub-regional level. In the case of Mediterranean Sea, the sub-regional assessment seems optimal to take into account biogeographic differences in species composition and functional characteristics. Previous research has shown that phytoplankton diversity indicators are efficient for reliable environmental assessments, although more effort has been recommended to test these indicators on a wide spatial scale to cover wider gradients of natural and anthropogenic pressures. In this work, a set of eight diversity indices was tested against the pressure levels within a common data set of the structure and abundance of phytoplankton communities from the Adriatic, Ionian and Aegean Seas. Expert knowledge was used to define four categories of impacts that take into account partial pressures, such as point and non-point pollution, industry, ports and fisheries. At the level of the common data set, most of the diversity, evenness and dominance indices could only distinguish between the highest level of impact and the rest of impact categories. These indices maintained the distinction between two levels of subsequently dichotomised impacts (no to low impact vs. high impact) across latitudinal and longitudinal gradients. On average, the indices were less sensitive to impacts in the northernmost and westernmost areas than in the southernmost and easternmost areas, although they still showed a significant response. The results also suggest that phytoplankton communities become more uniform and less dominated by a single taxon as sampling depth increases at sites with low impact, while evenness and dominance at impacted sites remain similar at all depths. In order to establish meaningful definitions of good environmental status and targets for pelagic habitats in the Mediterranean Sea, it is necessary to establish spatially specific thresholds by additional examination of indices of good performance.

---

[*] Corresponding author.

E-mail addresses: janja.france@nib.si (J. Francé), ioanna@hcmr.gr (I. Varkitzi), e.stanca@arpa.puglia.it (E. Stanca), francesco.cozzoli@unisalento.it (F. Cozzoli), sanda@izor.hr (S. Skejić), n.ungaro@arpa.puglia.it (N. Ungaro), ivano.vascotto@nib.si (I. Vascotto), patricija.mozetic@nib.si (P. Mozetič), nincevic@izor.hr (Ž. Ninčević Gladan), gogo@hcmr.gr (G. Assimakopoulou), aleka@hcmr.gr (A. Pavlidou), tanya@hcmr.gr (S. Zervoudaki), popi@hcmr.gr (K. Pagou), alberto.basset@unisalento.it (A. Basset).

https://doi.org/10.1016/j.ecolind.2021.107630
Received 8 December 2020; Received in revised form 14 March 2021; Accepted 16 March 2021

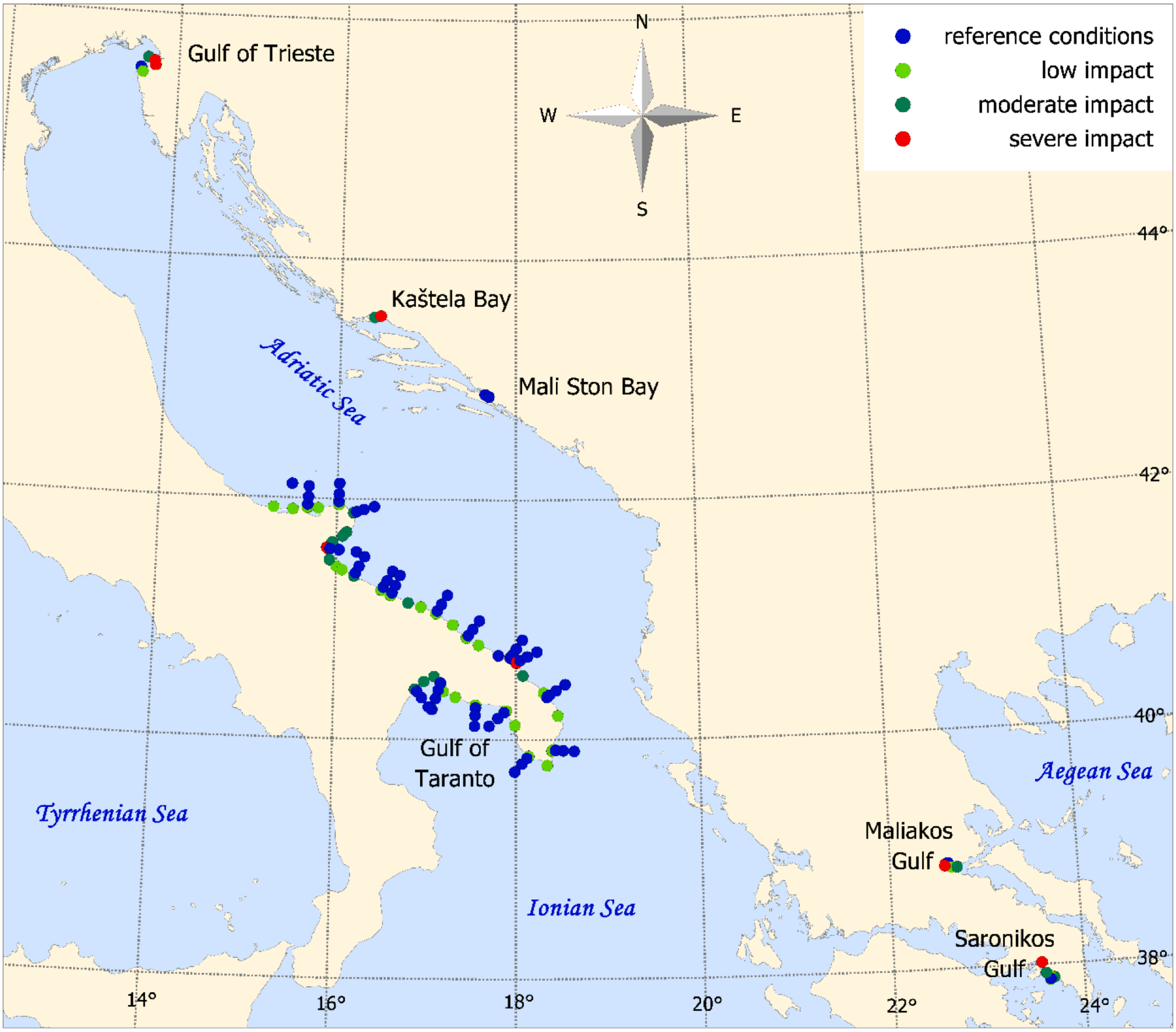


Fig. 1. Map of the study areas with the distribution of the sampling sites. Blue circles indicate sites with reference conditions (impact level 0), light green circles indicate sites with low impact (impact level 1), dark green circles indicate sites with moderate impact (impact level 2) and red circles indicate sites with severe impact (impact level 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1. Introduction

Mediterranean Member States do not yet have a uniform approach to the assessment of good environmental status (GES) in accordance with the requirements of Marine Strategy Framework Directive (MSFD; 2008/56/EC). The number of plankton indicators developed and/or catalogued for European Seas varies for the different components of biodiversity. While some indicators are available for phytoplankton, indicators for zooplankton are much less numerous and almost non-existent for prokaryotes (Teixeira et al., 2016; Varkitzi et al., 2018a). These differences can be attributed to several factors, but one important reason is certainly the fact that phytoplankton is listed as a key biological element in Water Framework Directive (WFD; 2000/60/EC), whereas other groups of pelagic organisms are not (Barbone et al., 2014). Nevertheless, specific, operational phytoplankton indicators that relate exclusively to the biodiversity aspect of Mediterranean Sea are rarer and their use is mainly restricted to certain areas (e.g., Facca et al., 2014; Ninčević Gladan et al., 2015; Pachés et al., 2012; Romero et al., 2013). To bridge this “never-ending” gap between the individual case studies and a common approach, there is an urgent need for a summary case study that would cover areas on a larger spatial scale with different structural and functional characteristics of the pelagic habitat.

In the electronic catalogues of Cozzoli and Basset (2017) and DevoTOOL (Teixeira et al., 2014, 2016) a relatively small number of operational phytoplankton indicators are found, which are valid for Mediterranean Sea. Of all the indicators in European Seas, the most popular are those that include the biomass of phytoplankton in terms of chlorophyll-a concentrations or other biomass measures, followed by indices with the abundance of species or different taxa groups. There are also several diversity indices in the catalogues, but it is extremely difficult to know how many of them are operational. In their review, Varkitzi et al. (2018a) proposed a subset of indicators for each pelagic biodiversity component to be tested for use in the GES determination of the water column habitat in Mediterranean Sea. Besides chlorophyll-a, the following assessment methods were proposed for phytoplankton: i) Size-related metrics, such as the multi-metric index of size spectra sensitivity ISS-phyto (Vadrucci et al., 2013), for its high accuracy, low uncertainty and relatively simple sample processing (Cozzoli et al., 2017); ii) diversity and dominance metrics, such as Shannon-Wiener’s Diversity Index (Shannon, 1948) for high accuracy and Berger-Parker’s

Dominance Index (Berger and Parker, 1970) for its high accuracy, low uncertainty and simple focusing only on the most abundant taxa (Cozzoli et al., 2017); and iii) Bloom frequency index (Facca et al., 2014) to measure the dominance of a species during algal bloom.

In contrast to the repealed European Commission Decision 2010/477/EU, which defined the criterion "Habitat condition" with three indicators in Biodiversity Descriptor, Decision 2017/848/EU simplified the definition in a merged criterion D1C6, which is the subject of this study. The methodological standards related to criterion D1C6 for pelagic habitats provide for assessment at regional or sub-regional level, which would reflect biogeographic differences in species composition. By comparing a number of ecological indices calculated on the basis of data sets on the composition of the phytoplankton community, we address the biodiversity, structure and function of pelagic habitats in Mediterranean Sea, all stated by criterion D1C6. The selected data sets in this large scale case study cover three sub-regions of Mediterranean Sea: 1) Adriatic Sea, 2) Ionian Sea and Central Mediterranean Sea, and 3) Aegean-Levantine Sea (according to the report on the MSFD Marine Reporting units, WG DIKE_16-2017-03), which all share the pelagic broad habitat type of coastal waters. This addresses biogeographic differences in the phytoplankton community at the mesoscale level (~100 km), as different case study areas are distributed along a gradient with different trophic conditions: from the mesotrophic northernmost part of Adriatic Sea to the oligotrophic coasts of the eastern Adriatic (central and southern Adriatic) and from the meso- to oligotrophic conditions around the coasts of Italian Apulia (southern Adriatic Sea and northern Ionian Sea) to the Greek oligotrophic coastal waters (Aegean Sea). Furthermore, within each case study area, gradients of different pressures and eutrophication regimes were carefully represented, covering waters from eutrophic to oligotrophic characteristics on a small scale (submesoscale, ~10 km). The Member States that contributed the data sets were Croatia, Greece, Italy and Slovenia.

In this work, a set of eight diversity indices calculated on the basis of the common data set of the structure and abundance of the phytoplankton community was tested against four levels of anthropogenic pressures derived from a set of partial pressures, such as point and non-point sources of pollution, industry, ports and fisheries. The scope of the study was to assess the discrimination power of the tested indices to identify the status of pelagic habitat biodiversity. In addition, we aimed to reveal this distinctiveness along spatial (longitudinal and latitudinal, depth and distance from coasts) and temporal (seasonal) gradients.

2. Materials and methods

2.1. Study areas and datasets preparation

The data sets were prepared according to the LifeWatch metadata and data templates provided by the University of Salento. The map of the study areas with the distribution of sampling sites in coastal (up to 1 NM off the coast) and open sea waters (more than 1 NM off the coast) is shown in Fig. 1.

The data sets are of varying length: from one to twelve years in the period 2001–2018, and the data were collected with monthly, bimonthly or seasonal frequency. The degree of anthropogenic impact was assessed for each sampling site by expert judgement. Different levels of impact were categorized as low (marked 1), moderate (marked 2) and severe impact (marked 3), while stations with no or minimal impact were classified as reference conditions (marked 0) (Fig. 1). In order to ensure maximum coherence between the categorisation of pressures and impacts of sampling sites in different sub-regions, a common matrix of pressure categories (Appendix, Table A1) was established as defined by Lugoli et al. (2012) and Simboura et al. (2016).

2.1.1. Aegean study area

The dataset for Greece includes data from a total of 13 sampling stations in coastal waters. Samples were collected at 2 or 4 standard depths: at the surface and near the bottom, or at the surface, at 20 m, at 50 m, and near the bottom. The data from Saronikos Gulf cover the geographical area of the sub-region Mediterranean Aegean Levantine-Central Aegean and the data from Maliakos Gulf cover the geographical area of the sub-region Mediterranean Aegean Levantine-North Aegean. Saronikos Gulf is a coastal area near the Athens metropolitan area and the port of Piraeus, which communicates with the Aegean Sea to the south. The Bay of Elefsis (northern Saronikos) is on average 90 m deep, with limited water exchange, low freshwater inflows and therefore with strong seasonal stratification and low oxygen distribution. These characteristics, together with industrial and shipping activities, lead to the trapping and accumulation of nutrients and organic matter (Pavlidou et al., 2014, 2019). The inner part of the Gulf is located near the port of Piraeus and receives the treated sewage of ~ 5 million people in the north. The southern inner part communicates with the outer part of Saronikos Gulf and receives the influence of the open Aegean waters. Fishing and aquaculture are common practices in Saronikos Gulf.

Maliakos Gulf is a shallow semi-enclosed coastal area (~200 km^2) in central Greece. It is separated by two headlands into an inner-western part (max. depth 25 m) and an outer-eastern part (max. depth 50 m). In the west it receives the inflows of the Spercheios River, while in the east water masses flow in from the open oligotrophic Aegean Sea (Christou et al., 1995). The area has a high phytoplankton biomass and blooms of potentially harmful microalgae with occasional fish killing outbreaks (Kormas et al., 2003; Varkitzi et al., 2018b). Maliakos Gulf supports an important quota of the national production of fisheries and aquaculture. However, it is exposed to various pressures from point and non-point sources of pollution: river inflows, urban and industrial sewage and agricultural runoff (Akoumianaki et al., 2013; Markogianni et al., 2017).

2.1.2. Central Eastern Adriatic study area

The Croatian dataset includes 4 sampling stations, two in Kaštela Bay in the central Adriatic and two in Mali Ston Bay in the southern Adriatic. In Kaštela Bay, samples were taken at 5 standard depths every 5 or 10 m, including the surface and bottom layers, while in Mali Ston Bay only two depths were sampled (surface and middle layer). Kaštela Bay is a semi-enclosed bay (area 61 km^2), which is under moderate anthropogenic influence due to urban and industrial wastewater. The bay communicates with the adjacent waters through a relatively wide (1.8 km) and deep opening (average depth ~ 40 m). The main freshwater inflow is the Jadro River. Water circulation in the bay is mainly generated by local winds (Gačić et al., 1987). In the 1980s, an increase in phytoplankton biomass and primary production in Kaštela Bay was associated with an increase in anthropogenic nutrient load (Marasović et al., 2005). After the activation of a modern sewage system in November 2004, the reduction of nutrient extremes and a decrease in bacterial abundance and production was followed by a decrease in phytoplankton biomass and the restoration of its regular seasonal cycle.

Mali Ston Bay is situated between the Pelješac peninsula and the mainland, on the south-eastern Adriatic coast. Due to the relatively low degree of urbanization and industrialization of the surrounding coasts, the anthropogenic influence is limited. A peculiarity of the bay is related to its complex hydrology, which is characterized by strong groundwater springs in the inner part and the large freshwater inflow of Neretva River in the outer part of the bay (Vukadin, 1981). The favorable hydrographic characteristics and primary production make the bay a suitable area for shellfish farming. Earlier studies (e.g. Viličić et al., 1998) classified Mali Ston Bay as a naturally moderately eutrophic system, while more recent studies, taking into account chlorophyll-a concentrations, abundance of phytoplankton and the TRIX index, classify it as an oligotrophic environment (Čalić et al., 2013; Ninčević Gladan et al., 2015; Skejić et al., 2015).

2.1.3. Northern Adriatic study area

The dataset from the Slovenian part of the Gulf of Trieste is a collection of data from 5 sampling stations. Samples were taken at 4 standard depths: at the surface, at 5 m, at 10 or 15 m and near the bottom. The Gulf of Trieste is a semi-enclosed bay at the northern end of Adriatic Sea with shallow depth (maximum ~ 25 m). Its coastal waters are affected by a variety of natural and anthropogenic processes. At the open boundary there is an intensive water mass exchange with the rest of the Adriatic Sea, which together with the freshwater inputs, largely dominated by the Soča (Isonzo) River, influences the oceanographic characteristics of the gulf. In winter the water column is mixed, while in spring intensified freshwater inputs and the warming of the surface layer together contribute to stratification, which even increases in summer (Malačič and Petelin, 2001). The Gulf of Trieste is a crossroads of human influences: there is intensive maritime traffic to and from several international ports, fisheries and aquaculture (mostly shellfish), which, together with the heavily populated hinterland, exerts considerable pressure on the coastal sea. In the past, phytoplankton blooms, which contribute to the development of hypoxia or even anoxia in the bottom layer, were the main consequence of eutrophication in this area (Malej and Malačič, 1995).

Long-term studies have shown a strong spatial and, above all, temporal variability of phytoplankton biomass and community structure, reflecting the rapidly changing hydrological and nutrient conditions in the Gulf of Trieste (Mozetič et al., 2012). However, in the recent past, a significant decrease in chlorophyll-a concentrations and changes in the structure of the phytoplankton community has been observed throughout the northern Adriatic Sea (Cabrini et al., 2012; Mozetič et al., 2012, 2010), mainly due to the decrease in nutrient concentrations, especially phosphates.

2.1.4. Southwestern Adriatic and Ionian study area

The Italian dataset covers the largest area in the Apulia region, which is surrounded by both the Adriatic Sea and Ionian Sea. It includes data from 42 sampling stations where phytoplankton data were collected for the requirements of Water Framework Directive (coastal waters, sensu WFD) and 72 sampling stations where data were collected for Marine Strategy Framework Directive (open waters). Samples were taken at the surface, in the intermediate layer and near the bottom, depending on the depth of the sampling station (maximum 50 m).

The southern part of Adriatic Sea is influenced by an almost permanent cyclonic circulation, and receives water from the northern Adriatic on the western side. These water masses, classified as Adriatic Surface Water (ASW), are colder and have low salinity (Artegiani et al., 1997; Manca et al., 2001). However, the influence of this relatively nutrient-rich coastal current on the southern Adriatic area is small and limited to spring and winter (Rivaro et al., 2004). Another source of nutrients in the basin of Southern Adriatic is the Levantine Intermediate Water (LIW), which enters the Adriatic Sea via the Otranto Channel (Zavatarelli et al., 1998). The waters of the northern Adriatic and the LIW interact locally with the almost negligible superficial runoff and with groundwater inputs of freshwater and nutrients, determining relatively weak and complex driving factors. The Fortore, Candelaro and Ofanto rivers are relatively small streams with a seasonal regime that collect water from catchments with widespread agricultural use. Although characterized by low flow intensity (Ludwig et al., 2009), these rivers have a significant impact on the water chemistry and trophic status of coastal waters, rarely extending further than 3 km from the coastline (Paparella and Martino, 2008; Porfido et al., 2011).

The northwestern Ionian Sea (Gulf of Taranto) is the transition area of different water masses, namely the ASW and Ionian Surface Water (ISW), formed by mixing with very saline Levantine surface water from Cretan Passage (Boldrin et al., 2002). The surface circulation is generally cyclonic, dominated by a coastal current flowing northwards towards the Gulf of Taranto, but with high variability and a vertical structure strongly dependent on a seasonal cycle (Boldrin et al., 2002). Along the Ionian coast, the main freshwater source is the Lato River with a very low flow regime (Barbone et al., 2014).

Both areas, the southern Adriatic and the Gulf of Taranto, are considered oligotrophic, with the southern Adriatic waters being more productive than the Ionian waters (Boldrin et al., 2002). The nutrient supply of the shelf area depends strongly on the inflows of superficial waters along the coasts, which are more abundant in the southern Adriatic area, and on the inflows of groundwater. Oligotrophy is even more pronounced in open waters, where nutrient supply to the euphotic zone depends strongly on vertical stratification and mixing processes. P-limiting conditions impose low primary productivity, even if the N:P ratio is variable (Rivaro et al., 2004). However, some areas receive stronger anthropogenic pressures, such as the area of the port of Taranto with port and industrial activities, and the adjacent inland sea Mar Piccolo Basin with intensive aquaculture (Cardellicchio et al., 2016). Urban expansion and intensive agriculture caused an increase in nutrients and organic matter in recent decades that exceeded the self-purification capacity of the basin, but in recent years a reverse process of an overall decrease in inorganic nutrient loading has also been observed (Caroppo et al., 2016; Karuza et al., 2016). In Mar Piccolo, harmful phytoplankton species still pose a threat to human health and aquaculture (Caroppo et al., 2016).

Studies have shown that phytoplankton in the nearshore areas of the Southern Adriatic and Ionian regions exhibit high spatial and temporal heterogeneity in terms of species distribution, abundance and biomass (Caroppo et al., 2006; Sabetta et al., 2008). Southern Adriatic is characterized by a phytoplankton community intermittently dominated by diatoms and phytoflagellates (Caroppo et al., 1999), whereas in Ionian Sea phytoflagellates dominate over diatoms most of the time (Caroppo et al., 2006). The structure of marine phytoplankton is characterized by high morphological variability in terms of shape, biovolume and surface-to-volume ratio (Stanca et al., 2013). These differences in morphology have a functional significance that is reflected in the adaptive strategies of phytoplankton to local gradients of environmental stress (Stanca et al., 2013).

2.2. Analysis design

Prior to calculating the indices, the individual data sets were reviewed by phytoplankton experts at the originating institution for taxonomic accuracy and current nomenclature. A thorough final check of the merged data set was then performed by a single expert using LifeWatch's taxonomic check services based on WoRMS, PESI and Catalogue of Life. In order not to exclude phytoflagellates, which are an important group in the phytoplankton community and often comprise forms that are difficult to determine, the indices were calculated including inputs from higher taxonomic levels and not only at species or genus level.

From the phytoplankton communities of more than 4000 samples and 85,584 entries, a number of different indices common in phytoplankton ecology were calculated. The indices were calculated using Index_Taverna Workflow, a tool/service of LifeWatch Phytoplankton Virtual Research Environment (http://www.servicecentrelifewatch.eu:8080/phyto-vre). While many others are available, the set of indices used in this analysis provides a complete picture and reduces redundancy in the information provided by similar indices (Cozzoli et al., 2017).

2.2.1. Richness/diversity indices

Environmental stress often tends to reduce the taxonomic diversity of affected communities by selecting only the most stress-tolerant taxa. In phytoplankton communities, eutrophication stress often promotes massive blooms of a few species that become strongly dominant. For this reason, taxonomically rich and diverse phytoplankton communities are regarded as indicators of good environmental status (Cozzoli et al., 2017).

The Taxonomic Richness is simply the number of taxa (R') found in a given sample (Fisher et al., 1943).

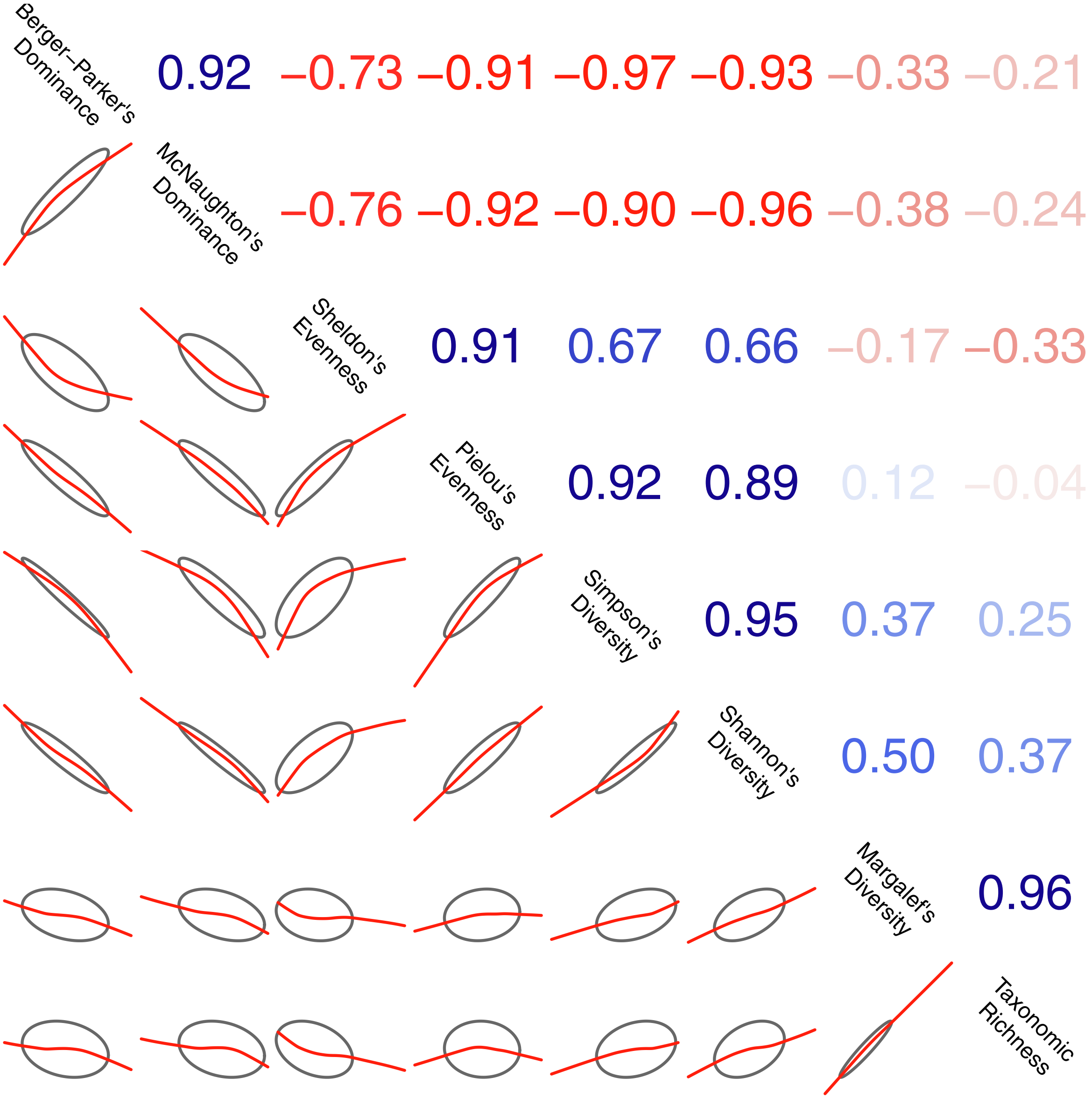


Fig. 2. Correlation between different diversity indices. The upper right panel shows the Pearson correlation coefficient between each pair of indices (shown diagonally). The red and blue colours indicate negative and positive correlation respectively. The lower left panel shows the average trend (red line) and data dispersion (95% of observations within the black ellipse). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Margalef's Diversity Index M' (Margalef, 1958) explains the relationship between the number of taxa detected (R') and a transformation of the total number of individuals counted (N):

$$M' = \frac{R' - 1}{\log N}$$

Shannon - Wiener's Diversity Index H' (Shannon, 1948) is a commonly used diversity index that takes into account both the abundance and evenness of taxa present in the community. The H' increases with the number of taxa in the community and can theoretically reach very high values. In practice, the H' for biological communities does not appear to exceed 5.0. The formula is:

$$H' = -\sum_{i=1}^{R} p_i \times \ln p_i$$

where p_i is the proportion of individuals in taxon *i* and is estimated as (n_i/N), where n_i is the number of individuals in taxon *i* and N is the total number of individuals in the community.

Simpson's Diversity Index S' (Simpson, 1949) is a measure of diversity based on the probability that any two individuals drawn at random from an infinitely large community belong to the same taxon:

$$S' = \sum_{i=1}^{R} p_i^2$$

While measuring the same community trait (richness, diversity), the above indices provide slightly different values, as they show a decreasing sensitivity to rare taxa.

2.2.2. Evenness/dominance indices

Dominance indices express the extent to which the community is monopolized by the most abundant taxon/taxa, taking into account the ratio between the abundances of these taxa and the total number of individuals counted (Cozzoli et al., 2017). Evenness indices represent the degree of equality of taxa abundances in a sample. On the contrary,

Table 1

Summary of the linear model describing the variation of the diversity indices at the different levels of anthropogenic impacts (see Fig. 3). Highly significant terms are marked in bold.

	Taxonomic Richness			Shannon's Diversity			Simpson's Diversity			Margalef's Diversity		
Predictors	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*
(Intercept)	21.30	0.27	**<0.001**	1.63	0.02	**<0.001**	0.64	0.01	**<0.001**	1.68	0.02	**<0.001**
Impact1	3.55	0.40	**<0.001**	0.02	0.03	0.465	0.01	0.01	0.465	0.33	0.03	**<0.001**
Impact2	−2.18	0.36	**<0.001**	0.01	0.03	0.831	0.01	0.01	0.208	−0.16	0.03	**<0.001**
Impact3	−1.26	0.38	**0.001**	−0.16	0.03	**<0.001**	−0.05	0.01	**<0.001**	−0.18	0.03	**<0.001**
Observations	4033			4033			4033			4033		
R^2 / adjusted R^2	0.057 / 0.056			0.016 / 0.015			0.013 / 0.012			0.078 / 0.077		

	Pielou's Evenness			**Sheldon's Evenness**			**Berger-Parker's Dominance**			**McNaughton's Dominance**		
Predictors	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***
(Intercept)	0.55	0.01	**<0.001**	0.32	0.01	**<0.001**	0.51	0.01	**<0.001**	0.68	0.01	**<0.001**
Impact1	−0.02	0.01	0.075	−0.03	0.01	**0.001**	−0.01	0.01	0.320	−0.01	0.01	0.542
Impact2	0.02	0.01	**0.006**	0.03	0.01	**<0.001**	−0.02	0.01	0.069	−0.01	0.01	0.202
Impact3	−0.05	0.01	**<0.001**	−0.06	0.01	**<0.001**	0.05	0.01	**<0.001**	0.06	0.01	**<0.001**
Observations	4031			4033			4033			4033		
R^2 / adjusted R^2	0.022 / 0.022			0.031 / 0.030			0.016 / 0.015			0.020 / 0.019		

dominance indices indicate the degree of predominance of one or a few taxa in the sample.

Pielou's Evenness Index E' expresses the ratio between the realized Shannon-Wiener diversity of a sample (H') and its maximum possible value (as a logarithm of R'), i.e. the expected value of H' if all taxa had an identical number of individuals (Pielou, 1975). Its formula is therefore:

$$E^{'} = \frac{H'}{\log R'}$$

Sheldon's Evenness Index Sh' (Sheldon, 1969) is also based on H' function:

$$Sh^{'} = \frac{e^{H^{'}}}{R'}$$

BergerParker's Dominance Index BP' (Berger and Parker, 1970) is the simplest measure for the numerical significance of the first most abundant species. The formula is:

$$BP^{'} = \frac{n_1}{N}$$

where n_1 is the abundance of the most abundant species and N is the total abundance of the sampled community.

McNaughton's Dominance Index McN' (McNaughton, 1967) considers the two most abundant species in a sample:

$$McN^{'} = \frac{n_1 + n_2}{N}$$

For further details on the indices, see Cozzoli et al. (2017), Appendix Section.

2.3. Statistical analyses

The correlations between the various index values were assessed using the Pearson correlation coefficient. Differences in the index responses to different variables were investigated using the linear ANOVA (for categorical variables such as impact levels or months) or the ANCOVA (for continuous variables such as latitude, longitude, depth and distance from the coast). To model and highlight the typically non-linear seasonal trends, we used the Locally Estimated Scatterplot Smoothing (LOESS) regression.

3. Results

The total number of phytoplankton taxa identified in the common data set was 849, of which the largest proportion belonged to diatoms (335) and dinoflagellates (320), while 74 taxa belonged to coccolithophores. Phytoflagellates including classes like Chlorophyceae, Prasinophyceae, Cryptophyceae, Euglenophyceae, Raphidophyceae, Dictyochophyceae and some other minor classes comprised 96 taxa whereas other non-flagellate phytoplankton included 24 taxa. Eight diversity indices were calculated on this common data set for each sampling episode and sampling depth, resulting in more than 30,000 index values.

The indices differed (Fig. 2) by anthropogenic (Table 1, Fig. 3), temporal (Table 2, Fig. 4) and spatial (Tables 3–4, Figure 5, 6, 7 and 8) gradients. The correlation matrix for the eight indices is shown in Fig. 2. Taxonomic Richness and Margalef's Diversity Index were strongly and positively correlated, while they had a weaker correlation with the other indices and were not linked to Pielou's Evenness Index. The Shannon's and Simpson's diversity indices were strongly and positively correlated with each other and with Pielou's Evenness Index. Sheldon's Evenness Index was strongly and positively correlated with the Pielou's Evenness Index, but compared to the latter it had a weaker correlation with the diversity indices. McNaughton's and Berger-Parker's dominance indices were strongly and positively correlated with each other and negatively correlated with all other richness, diversity and evenness indices.

In order to investigate how anthropogenic impacts are reflected in the diversity indices, their distribution at four levels of impact was examined with the aid of a ANOVA model (Fig. 3, Table 1). A decrease in diversity and the predominance of single or few taxa with increasing impact was clearly observed only at the highest level of impact (level 3 in Fig. 3). At stations designated as having low (level 1 in Fig. 3) and moderate (level 2 in Fig. 3) impact, the diversity indices did not differ consistently from those at reference stations (level 0 in Fig. 3). Therefore, in the further course of the analysis, the four impact levels were reduced to two levels: an impact level higher than 2 (impacted, i.e. a significant response of the phytoplankton community to anthropogenic impacts) and an impact level lower or equal to 2 (not impacted, i.e. an insignificant response of the phytoplankton community to anthropogenic impacts).

All indices showed seasonal variations (Fig. 4), with a significant peak for diversity indices and a significant minimum for dominance indices in September and October (*p*-values $<$ 0.001, Appendix, Table A2). The seasonal trend was similar at impacted and not impacted sites (red and green line, respectively, Fig. 4), with more or less constant lower values for diversity and evenness indices (or higher for dominance indices) at the impacted sites. This distinction between the two impact levels was significant throughout the year, except in a few cases, i.e. for Taxonomic Richness in most months and for the majority of the other

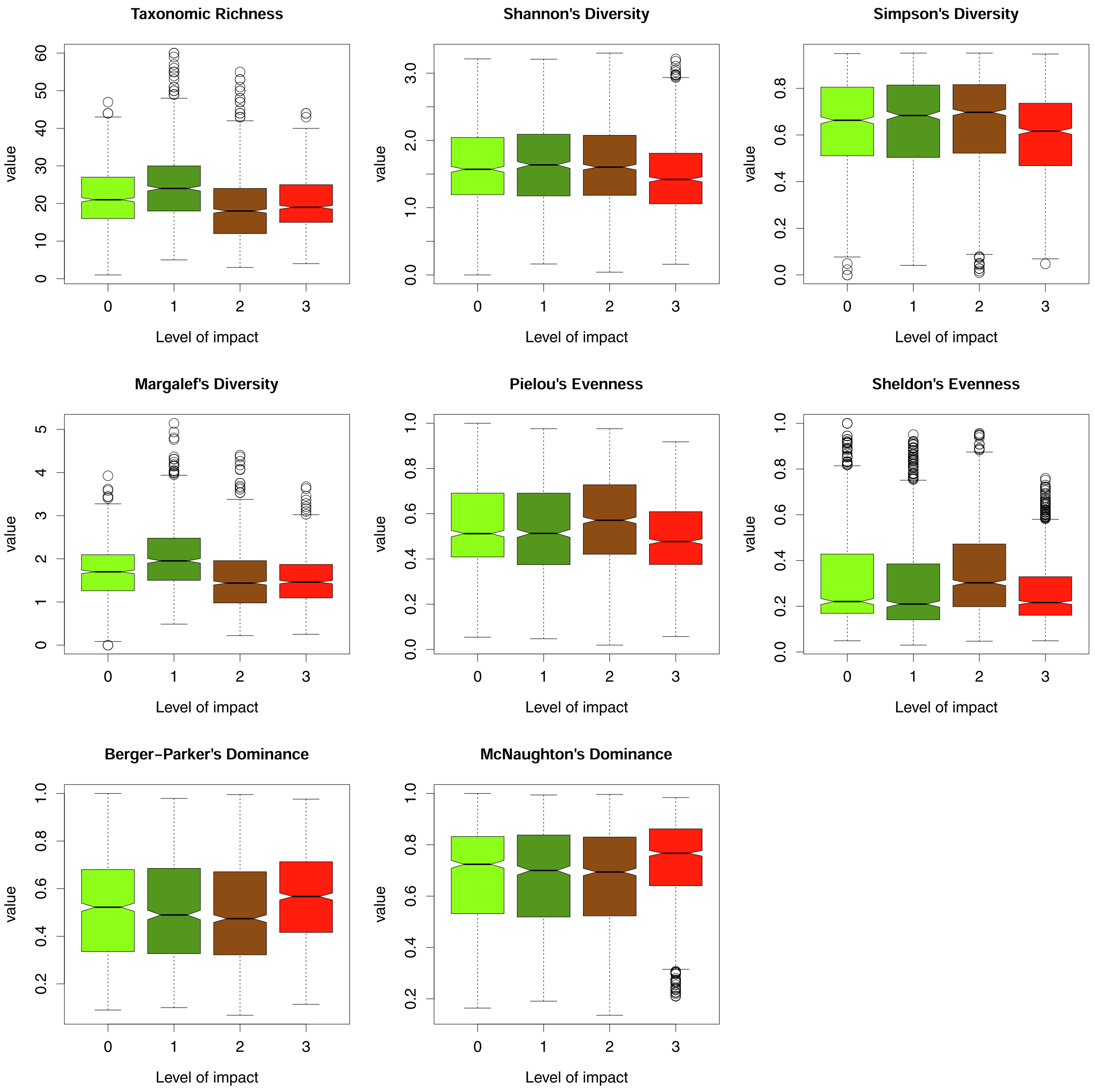


Fig. 3. Distribution of the diversity indices at the levels of anthropogenic impacts: 0 - reference condition with no or very minor impact, 1 - low impact, 2 - moderate impact and 3 - severe impact. Significant differences were assessed via ANOVA. The notches around the median represent the 95% confidence interval (CI). The absence of overlap between the notches indicates significant differences.

indices in the summer months (July–August).

The indices showed a significant variation (p-values < 0.001) along the spatial axes (Figs. 5 and 6). The southernmost and easternmost sites (Eastern Mediterranean, Aegean-Levantine sub-region) showed higher (or in the case of the dominance indices lower) index values compared to the northernmost and westernmost sites (Adriatic and Northern Ionian sub-region). The indices showed a significant (p-values < 0.05) sensitivity to impact levels over the entire spatial gradient, although their values varied widely. The sensitivity of the indices to impacts was constant along the latitudinal gradient, except for Margalef's Diversity and Sheldon's Evenness, which were significantly (p-values < 0.1 for latitude:impacted in Table 2) more sensitive at the southernmost sites (Fig. 5, Table 2). Some variations in the sensitivity of the indices for the effects along the longitudinal gradient were found for Taxonomic Richness, Pielou's Evenness, Sheldon's Evenness, and McNaughton's Dominance, which were significantly more sensitive (p-values < 0.1 for longitude:impacted in Table 3) at the easternmost sites (Fig. 6, Table 3).

The diversity indices were also examined in relation to sampling depth (Fig. 7) and coastal distance (Fig. 8), which are two important variables for the expression of anthropogenic influences on the phytoplankton community. With increasing depth, Taxonomic Richness and Margalef's Diversity decreased significantly (p-values < 0.001 for depth), independent of the level of impact (p-values greater than 0.1 for depth:impacted, Fig. 7, Table 4). The other indices, evenness and dominance indices, showed a different pattern: phytoplankton communities became more even and less dominated by a single or few taxons as depth increased in not impacted sites. However, lower evenness and higher dominance values were similar to surface values in the impacted sites (p-values < 0.1 for depth:impacted, Fig. 7, Table 4).

All indices showed a weak correlation with the distance from the

Table 2
Summary of the linear model describing the variation of the diversity indices over the latitudinal gradient and the two levels of impact (see Fig. 5). Highly significant terms are marked in bold. Non-significant variables or interaction terms have been removed by the technique of stepwise regression.

	Taxonomic Richness			Shannon's Diversity			Simpson's Diversity			Margalef's Diversity		
Predictors	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*
(Intercept)	16.04	2.37	**<0.001**	6.28	0.15	**<0.001**	2.07	0.05	**<0.001**	4.04	0.21	**<0.001**
Latitude	0.13	0.06	**0.024**	−0.11	0.00	**<0.001**	−0.03	0.00	**<0.001**	−0.05	0.00	**<0.001**
Impacted	−1.49	0.32	**<0.001**	−0.05	0.02	**0.011**	−0.01	0.01	**0.035**	−0.95	0.45	**0.033**
Latitude:Impacted										0.02	0.01	0.071
Observations	4032			4032			4032			4032		
R^2 / adjusted R^2	0.006 / 0.005			0.213 / 0.213			0.187 / 0.187			0.048 / 0.048		

	Pielou's Evenness			**Sheldon's Evenness**			**Berger-Parker's Dominance**			**McNaughton's Dominance**		
Predictors	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***
(Intercept)	2.25	0.05	**<0.001**	2.13	0.05	**<0.001**	−1.18	0.05	**<0.001**	−1.02	0.05	**<0.001**
Latitude	−0.04	0.00	**<0.001**	−0.04	0.00	**<0.001**	0.04	0.00	**<0.001**	0.04	0.00	**<0.001**
Impacted	−0.01	0.01	**0.024**	−0.27	0.11	**0.013**	0.02	0.01	**0.027**	0.02	0.01	**0.005**
Latitude:Impacted				0.01	0.00	**0.018**						
Observations	4031			4032			4032			4032		
R^2 / adjusted R^2	0.271 / 0.271			0.293 / 0.293			0.229 / 0.229			0.274 / 0.273		

coast of the not impacted sites: positive for the diversity and evenness indices and negative for dominance (green lines in Fig. 8, Table 5). Unfortunately, not enough observations in the open sea for impacted sites were available to allow an accurate estimation of the differences in scaling trend between the two impact levels (red lines in Fig. 8).

4. Discussion

The use of diversity indices to characterize a biological community is always a temptation, as they combine information on the composition and abundance of species into a single figure (Magurran, 2004) and can thus facilitate differentiation between communities with different levels of pressure. However, since biodiversity is multidimensional, the ideal combination of indices used to assess it should also cover taxonomic/genetic, structural and functional diversity (Lyashevska and Farnsworth, 2012). The importance of the diversity of autotrophic communities for ecosystem functioning has been extensively studied in recent years using various approaches (reviewed by Cardinale et al., 2011; Krause et al., 2014). However, with the assumption that the distribution of phytoplankton is not limited by their dispersion, it can be assumed that the structure of a given phytoplankton community is shaped by local environmental conditions (Ptacnik et al., 2008). In this way, the use of phytoplankton diversity attributes, as calculated by diversity indices, can be of additional value in assessing the status of pelagic habitats.

4.1. Discriminating power of diversity indices

In our study, biodiversity indices could distinguish the level of anthropogenic influences on phytoplankton communities in different coastal environments in the Mediterranean sub-regions. However, not all the indices tested showed the same performance. Within the sampling effort considered (i.e. number of cells counted per sampling episode), values of Taxonomic Richness and Margalef's Diversity oscillated randomly between impact levels, probably reflecting a strong dependence of these indices on sample size. This may be related to the fact that the accurate estimation of these indices requires a disproportionately large sample compared to the other indices (Cozzoli et al., 2017). Therefore, the performance of these indices in distinguishing impacted sites on our data set cannot be correctly assessed. This aspect is reinforced by the lack of correlation between the Richness, Margalef's Diversity and the other indices. In any case, these indices are not very helpful from a practical point of view due to the high effort required for a correct estimation.

All other indices tested were able to discriminate against communities exposed to different levels of impacts, but only when the cumulative impacts reached the highest category pre-set for analysis (i.e. severe impacts, level 3), indicating a non-linear biodiversity response to impacts. However, all these indices were significantly correlated either positively or negatively, indicating the redundancy of the information provided by the indices. Perhaps the most valuable future use in composite assessment systems would be the combination of Shannon's/Simpson's Diversity and Sheldon's Evenness, which are sensitive to impacts and less correlated with each other compared to other pairs of indices. Buckland et al. (2005) emphasize that three aspects are important in biodiversity assessments: number of taxa, abundance and evenness. The advantage of using a combination of different indices or a multimetric index to assess the state of biodiversity is that the disadvantages of one component could be overcome with the strength of the others. For example, it has been reported that evenness indices such as the Sheldon's index depend heavily on the number of individuals counted (sample size) and tend to overestimate the actual evenness (Cozzoli et al., 2017). This could be improved by the use of diversity indices that take into account the numerical proportion of taxa in a community, i.e. Shannon's and Simpson's indices, and which are highly accurate even when the number of individuals counted is small. A similar independence from sample size has been shown for the dominance indices (Cozzoli et al., 2017), which also have the analytical advantage of the necessity to recognize just one or two dominant species. The approach of using dominance-related indices has already proven successful in previous studies on assessment systems for phytoplankton biodiversity (Uusitalo et al., 2013). The disadvantages of the Shannon's and Simpson's indices are related to their limited performance in detecting changes in the abundances of taxa (van Strien et al., 2012), while dominance indices, although providing useful information for the assessment of environmental quality (Cozzoli et al., 2017; Facca et al., 2014), ignore changes in the non-dominant part of the phytoplankton community. The problem associated with dominance could also arise from situations where the level of taxonomic resolution is not high enough to resolve the co-dominance of several species in common and abundant phytoplankton genera, such as *Pseudo-nitzschia* and *Chaetoceros*. Evenness of taxa, on the other hand, is a desirable feature of the phytoplankton community, and indices that summarize this property have already been shown to be effective in distinguishing different anthropogenic influences (Karydis and Tsirtsis, 1996).

The concept of multimetric indices was previously used to assess the status of Adriatic Sea with phytoplankton parameters. Facca et al.

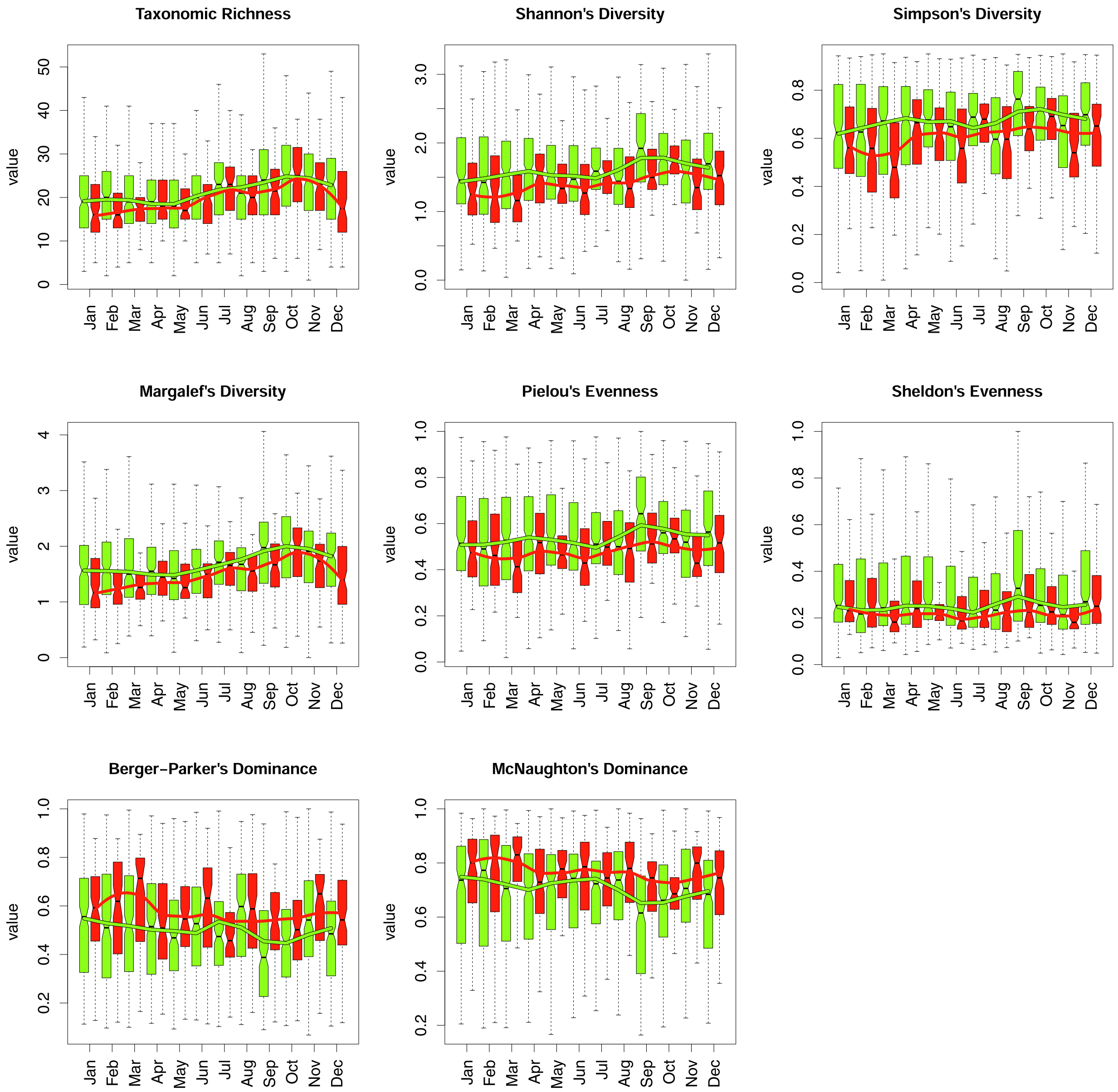


Fig. 4. Distribution of the diversity indices over months for two levels of impact (green: not impacted; red: impacted). The continuous lines show the average trend (LOESS). The notches around the median represent the 95% CI. The absence of overlap between the notches indicates significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2014) developed the Multimetric Phytoplankton Index (MPI), which combines two diversity indices (Hulburt's Dominance and Menchinick's Diversity) with bloom frequency and chlorophyll-a concentration. The MPI is currently used to assess the status of the Italian, Greek and Croatian transitional waters (European Commission Decision 2018/229 (Decision 2018)). In search of a suitable combination of indices for the assessment of coastal waters in the eastern part of Adriatic Sea, Ninčević Gladan et al. (2015) found that the Menchinick's Diversity Index best explains increased abundances of phytoplankton, but the relationship was non-linear and was therefore excluded from further consideration. In the Aegean Sea, studies showed that the Shannon's and Simpson's diversity indices are inappropriate to show a real effect along the eutrophication gradient (Karydis and Tsirtsis, 1996; Spatharis et al., 2011).

In our analysis, the cumulative impact of anthropogenic pressures had to be very high (the highest value on our categorical pressure scale) to express the change in the values of the diversity indices. This non-linear impact of the pressures on phytoplankton diversity, which is not unique to our study, makes the setting of target values (e.g. reference conditions and quality classes thresholds) a difficult task. Aggregated pressures indicators, as used in our analyzes, are relatively easy to achieve (Flo et al., 2019; Lugoli et al., 2012; Simboura et al., 2016), but we are aware that they provide a limited opportunity to assess the relevance of different pressures. Furthermore, such pressures indicators are of limited use in setting management objectives and measures for improvement in the event of failure to achieve good environmental status. To elucidate a direct pressure impact response of the phytoplankton community to anthropogenic influences, individual pressures should be tested, e.g. nutrient loads or concentrations in seawater, as in the case of chlorophyll-a (e.g., Giovanardi et al., 2018). Although many pressure parameters are routinely measured under national monitoring programs, particularly in the coastal waters of Member States, they are

Table 3
Summary of the linear model describing the variation of the diversity indices over the longitudinal gradient and the two levels of impact (see Fig. 6). Highly significant terms are marked in bold. Non-significant variables or interaction terms have been removed by the technique of stepwise regression.

	Taxonomic Richness			Shannon's Diversity			Simpson's Diversity			Margalef's Diversity		
Predictors	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*
(Intercept)	28.65	0.91	<0.001	0.28	0.05	<0.001	0.23	0.02	<0.001	1.42	0.06	<0.001
Longitude	−0.44	0.05	<0.001	0.08	0.00	<0.001	0.03	0.00	<0.001	0.02	0.00	<0.001
Impacted	−4.45	1.73	0.010	−0.13	0.02	<0.001	−0.04	0.01	<0.001	−0.19	0.02	<0.001
Longitude: Impacted	0.18	0.10	0.087									
Observations	4032			4032			4032			4032		
R^2 / adjusted R^2	0.022 / 0.022			0.171 / 0.171			0.151 / 0.151			0.021 / 0.020		

	Pielou's Evenness			**Sheldon's Evenness**			**Berger-Parker's Dominance**			**McNaughton's Dominance**		
Predictors	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***
(Intercept)	0.01	0.02	0.618	−0.29	0.02	<0.001	0.99	0.02	<0.001	1.18	0.02	<0.001
Longitude	0.03	0.00	<0.001	0.04	0.00	<0.001	−0.03	0.00	<0.001	−0.03	0.00	<0.001
Impacted	0.01	0.03	0.715	0.10	0.03	0.001	0.05	0.01	<0.001	−0.001	0.03	0.978
Longitude: Impacted	−0.003	0.00	0.094	−0.01	0.00	<0.001				0.003	0.00	0.152
Observations	4031			4032			4032			4032		
R^2 / adjusted R^2	0.251 / 0.250			0.291 / 0.290			0.183 / 0.183			0.221 / 0.221		

Table 4
Summary of the linear model describing the variation of the diversity indices over the depth gradient and the two levels of impact (see Fig. 8). Highly significant terms are marked in bold. Non-significant variables or interaction terms have been removed by the technique of stepwise regression.

	Taxonomic Richness			Shannon's Diversity			Simpson's Diversity			Margalef's Diversity		
Predictors	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*
(Intercept)	22.80	0.18	**<0.001**	1.63	0.01	**<0.001**	0.64	0.00	**<0.001**	1.79	0.01	**<0.001**
Depth	−0.15	0.01	**<0.001**	0.001	0.001	0.113	0.001	0.001	**0.002**	−0.01	0.001	**<0.001**
Impacted	−1.56	0.30	**<0.001**	−0.14	0.03	**<0.001**	−0.04	0.01	**<0.001**	−0.25	0.03	**<0.001**
Depth: Impacted				−0.003	0.001	0.096	−0.001	0.00	0.073	0.002	0.001	0.113
Observations	4032			4032			4032			4032		
R^2 / adjusted R^2	0.063 / 0.062			0.017 / 0.016			0.015 / 0.015			0.051 / 0.051		

	Pielou's Evenness			**Sheldon's Evenness**			**Berger-Parker's Dominance**			**McNaughton's Dominance**		
Predictors	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***
(Intercept)	0.54	0.001	**<0.001**	0.30	0.001	**<0.001**	0.51	0.001	**<0.001**	0.68	0.001	**<0.001**
Depth	0.002	0.001	**<0.001**	0.003	0.001	**<0.001**	−0.001	0.001	**<0.001**	−0.001	0.001	**0.001**
Impacted	−0.04	0.01	**<0.001**	−0.04	0.01	**<0.001**	0.05	0.01	**<0.001**	0.05	0.01	**<0.001**
Depth: Impacted	−0.002	0.001	**0.010**	−0.002	0.001	**0.001**	0.001	0.001	**0.038**	0.001	0.001	0.061
Observations	4031			4032			4032			4032		
R^2 / adjusted R^2	0.037 / 0.036			0.062 / 0.061			0.019 / 0.018			0.022 / 0.021		

difficult to link with data on the phytoplankton community.

4.2. Spatial and temporal aspects of the study

The definition of the boundaries is an important but difficult step in the development of an assessment system that must be scaled in space and time. As outlined by Bedford et al. (2020), the temporal scale of the assessment, i.e. the time period from which data are considered in the analysis, is very important if the assessment system requires a comparison of a particular index with the reference period. The data from different sampling sites in our analysis cover a minimum of two years (exceptionally one year) and a maximum of 12 years, almost all in the period 2007–2018. In this way, we have identified ephemeral and persistent features of the pelagic habitat (Dickey-Collas et al., 2017; Hyrenbach et al., 2000), while multi-decadal cycles that could influence the establishment of reference conditions have been avoided. This will also be the time frame for the establishment of reference conditions in our future work.

Seasonality was well covered in all sampling sites and provided interesting results. The indices reacted sensitively to seasonal fluctuations of the phytoplankton communities and showed the highest diversity and the lowest dominance in late summer and early autumn. This short time window slightly precedes the beginning of the autumn bloom of the phytoplankton in most study areas, which is mostly related to mixing of the water column. The relative difference between impacted and not impacted sites was generally maintained throughout the year for all indices, except Taxonomic Richness, which also fluctuated between the four levels of impact on the overall scale (see Fig. 3). It should be noted, however, that the majority of the other indices failed to distinguish between impacted and not impacted sites in July and August, i.e.

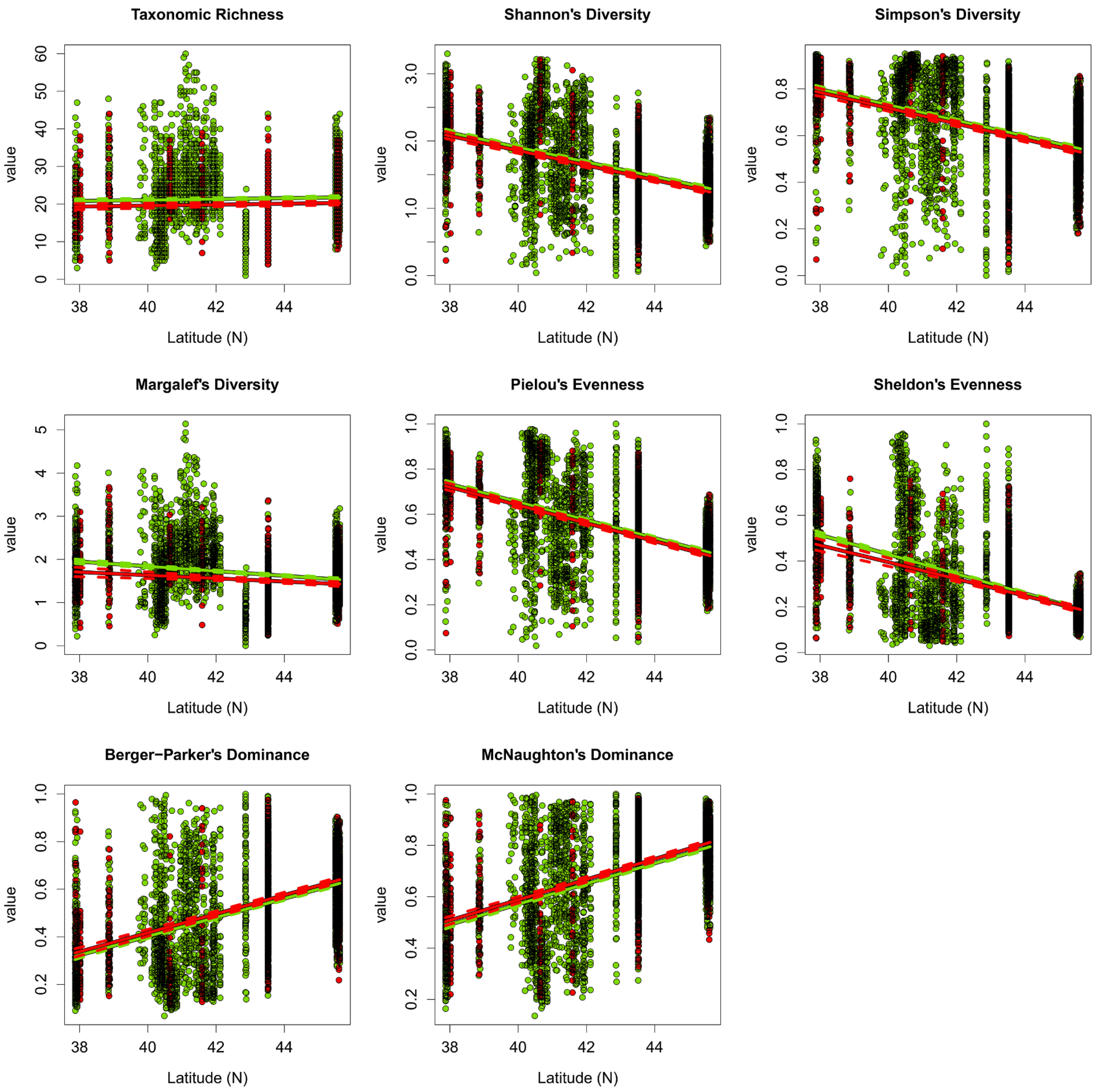


Fig. 5. Latitudinal distribution of the diversity indices (green: not impacted; red: impacted). Full lines represent the average trend, while dashed lines represent the 95% CI around the average (ANCOVA). The absence of overlap between the dashed lines indicates significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the period before the peak of diversity. This could reflect the effects of seasonal variations in environmental parameters such as nutrients. In Northern Adriatic, for example, the spatial distribution of nitrate and phosphate in summer is much more uniform compared to the distribution in other seasons, when a west-east gradient of decreasing concentrations is visible (Grilli et al., 2020). In coastal and open waters of Aegean Sea, the phytoplankton composition and function is more homogeneous during the warm period due to nutrient scarcity across different areas (Varkitzi et al., 2018b, 2020). Also on the scale of the entire Mediterranean basin, the west-east gradient of phytoplankton biomass (as chlorophyll-a concentration) is much weaker in summer compared to other periods of the year (D'Ortenzio and Ribera d'Alcalà, 2009). As phytoplankton samples collected during the midsummer period may be ineffective for the detection of environmental impacts, it would be safer and more representative to process them in combination with other samples collected during the year or to plan a different sampling strategy. This fact confirms once again that the appropriate sampling frequency is a very important parameter to be considered in monitoring routines and assessment studies of phytoplankton and pelagic habitats.

The broad coverage of the different sub-regions of the Eastern Mediterranean ensured that our analysis covered a broad trophic spectrum, ranging from oligotrophy in the SE (Aegean Sea) to meso-/eutrophy in the NW (northern Adriatic). Only Taxonomic Richness and Margalef's Diversity did not show significant latitudinal and longitudinal variations, while all other indices expressed significant spatial variations, indicating different prevailing conditions in the sub-regions studied. These variations on both the latitudinal and longitudinal axes were greater than the variations between the two levels of impact, which were nevertheless significant (the trend lines in Figs. 5 and 6 are separate). With the combination of the two dimensions a clear gradient from NW to SE was observed with increasing diversity and evenness, and decreasing dominance, which is consistent with the known trophic gradient in the Mediterranean (Colella et al., 2016; Siokou-Frangou et al., 2010) and Adriatic Seas (Polimene et al., 2006). Apart from the level of anthropogenic pressures, the northern Adriatic is a naturally

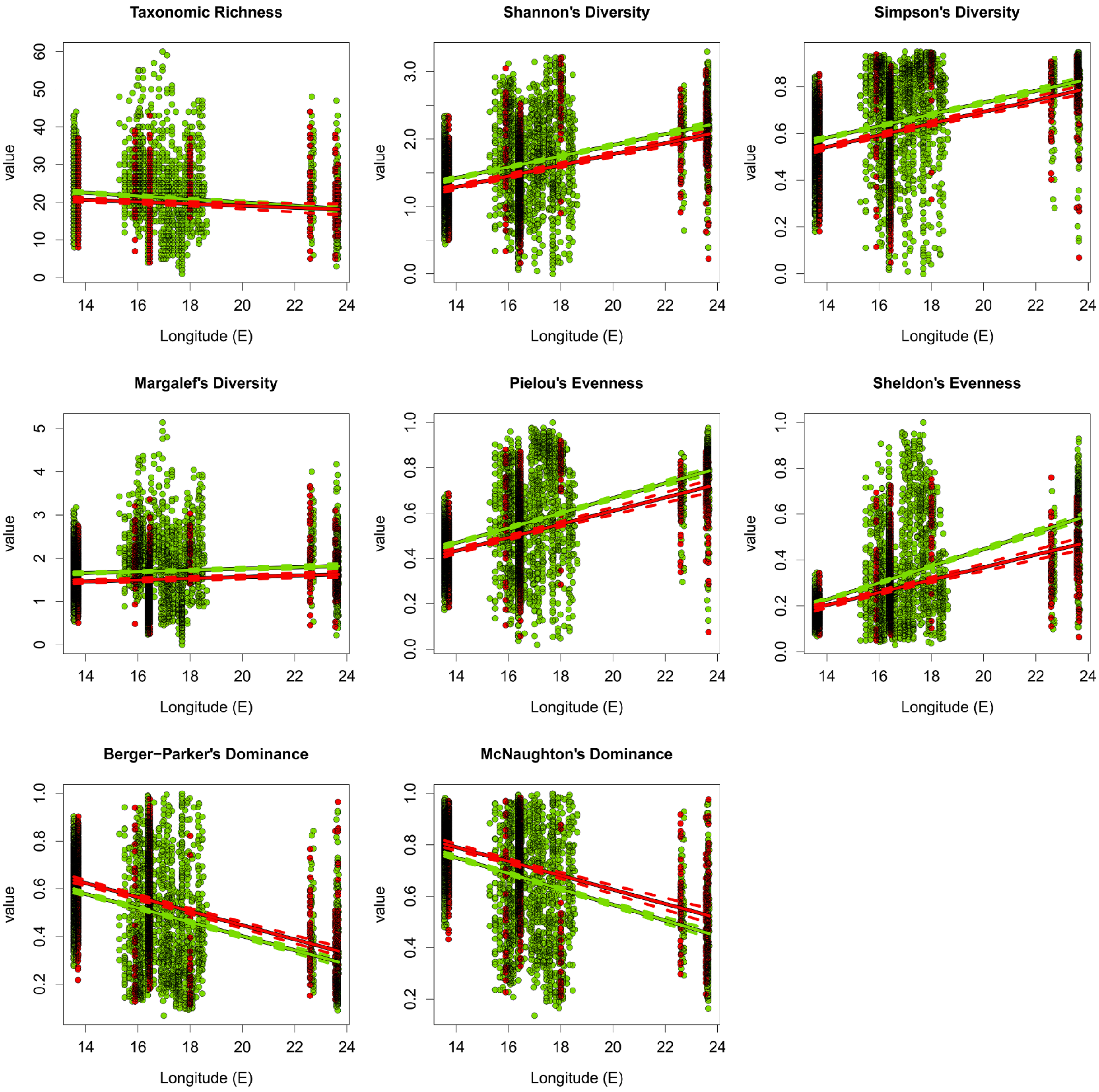


Fig. 6. Longitudinal distribution of diversity indices (green: not impacted; red: impacted). Full lines represent the average trend, while dashed lines represent the 95% CI around the average (ANCOVA). The absence of overlap between the dashed lines indicates significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

richer environment overall (Lazzari et al., 2016; Solidoro et al., 2009) compared to the southern Adriatic, the Ionian Sea or Aegean-Levantine Seas (Krom et al., 2014). Although it is known that all these environments are phosphorus-limited, the extent of this limitation varies (Cozzi and Giani, 2011; Krom et al., 2014). The relationship between phytoplankton diversity and nutrient availability has rarely been studied, but Filstrup et al. (2014) found that the richness and evenness of the phytoplankton community in the lake is negatively related to the total phosphorus concentration. There is further evidence of a positive correlation between phosphorus and phytoplankton biomass and production (Giovanardi et al., 2018; Lazzari et al., 2016). For example, chlorophyll-a was positively correlated with phosphates and silicates in the coastal waters of the Aegean Sea, while often blooming *Pseudo-nitzschia* species were strongly associated with low nitrates and silicates (Varkitzi et al., 2018b). Conversely, the ability of a phytoplankton community to use the limiting resource efficiently (i.e. resource use efficiency expressed as the ratio between phytoplankton biomass and total phosphorus) was positively correlated with diversity in terms of genus richness in a large-scale study involving Scandinavian lakes and Baltic Sea data sets (Ptacnik et al., 2008).

If more resources are limited, the diversity of phytoplankton communities is expected to be even greater (Interlandi and Kilham, 2001), compared with environments with only one limiting resource. In the Eastern Mediterranean, phosphorus limitation during the summer period may become a co-limitation of nitrogen and phosphorus. Here, in the Aegean-Levantine basin, phytoplankton diversity is known to be high and also constant over time (Varkitzi et al., 2020). In contrast, the phytoplankton diversity is lower in the northernmost of the study areas, the Gulf of Trieste. Here, the great variability of the ecosystem is also reflected in the temporal variability of phytoplankton properties, including diversity: during a drought period in 2003–2007, which led to oligotrophic conditions, phytoplankton abundance decreased while diversity increased (Cabrini et al., 2012). However, it must be stressed that today only the western part of the northern Adriatic is considered

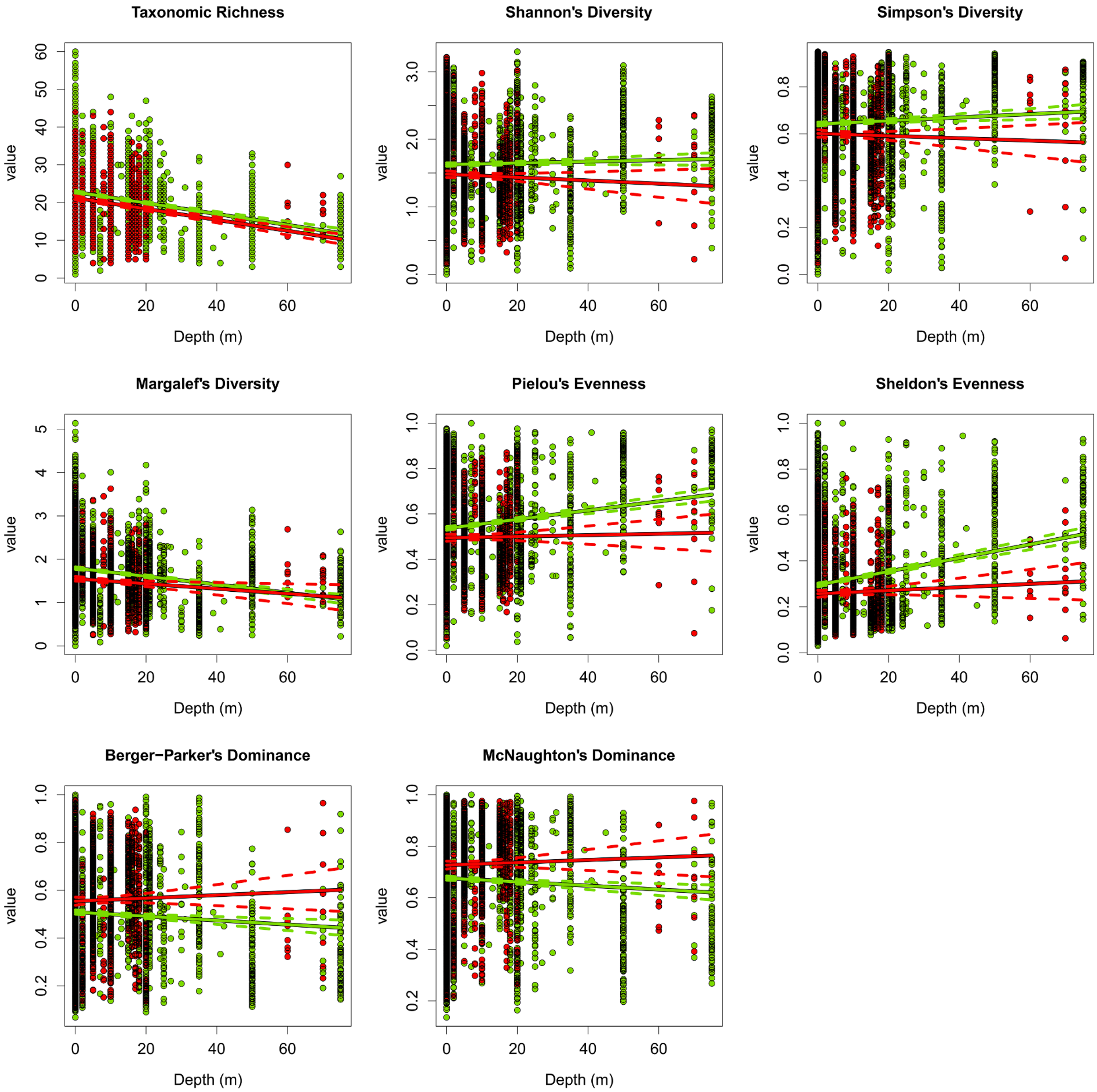


Fig. 7. Depth distribution of the diversity indices (green: not impacted; red: impacted). Full lines represent the average trend, while dashed lines represent the 95% CI around the average (ANCOVA). The absence of overlap between the dashed lines indicates significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

eutrophic (Solidoro et al., 2009), while the larger part is meso- to even oligotrophic, which is also true for the Gulf of Trieste. This underlines the need to define space-specific baselines and thresholds to assess impacts that reflect the natural variation of indicators across the Mediterranean spatial gradients.

An interesting aspect of the spatial patterns is not only the differences in the absolute value of the indices, but also the differences between impacted and not impacted sites, which were greater for SE sites than for NW sites. In SE (coastal waters of the Aegean), the phytoplankton community, which reflects the general oligotrophic character of the pelagic habitat of the Aegean as the prevailing conditions (Varkitzi et al., 2018b, 2020), is more easily disturbed by anthropogenic pressures. In the case of the impacted sites, this disturbance is therefore reflected in changes in phytoplankton diversity, which are more pronounced at southern sites compared to northern sites. In contrast, prevailing conditions in pelagic habitats are much more variable in the NW, blurring the differences in the phytoplankton community between impacted and not impacted sites. In this area the baseline conditions will be more difficult to define. Evidence of the different prevailing conditions in the areas studied can also be found in the trophic regimes, as shown by chlorophyll satellite analysis (D'Ortenzio and Ribera d'Alcalà, 2009). Almost the entire Aegean Sea is defined as a “no bloom” regime, where the uniformity of the values characterizes both a higher biomass autumn–winter period and a lower biomass spring-summer period. In contrast, the northern and western part of Adriatic Sea have a “coastal” regime with high variability (ibid.).

The comparison of the vertical distribution of phytoplankton indices and their behavior with increasing distance from the coast also provided interesting results. The effect of the sampling depth was significant only at not impacted sites, where the phytoplankton communities were more uniform and less dominated by single species in deeper layers of the water column. Thus, the difference between impacted and not impacted

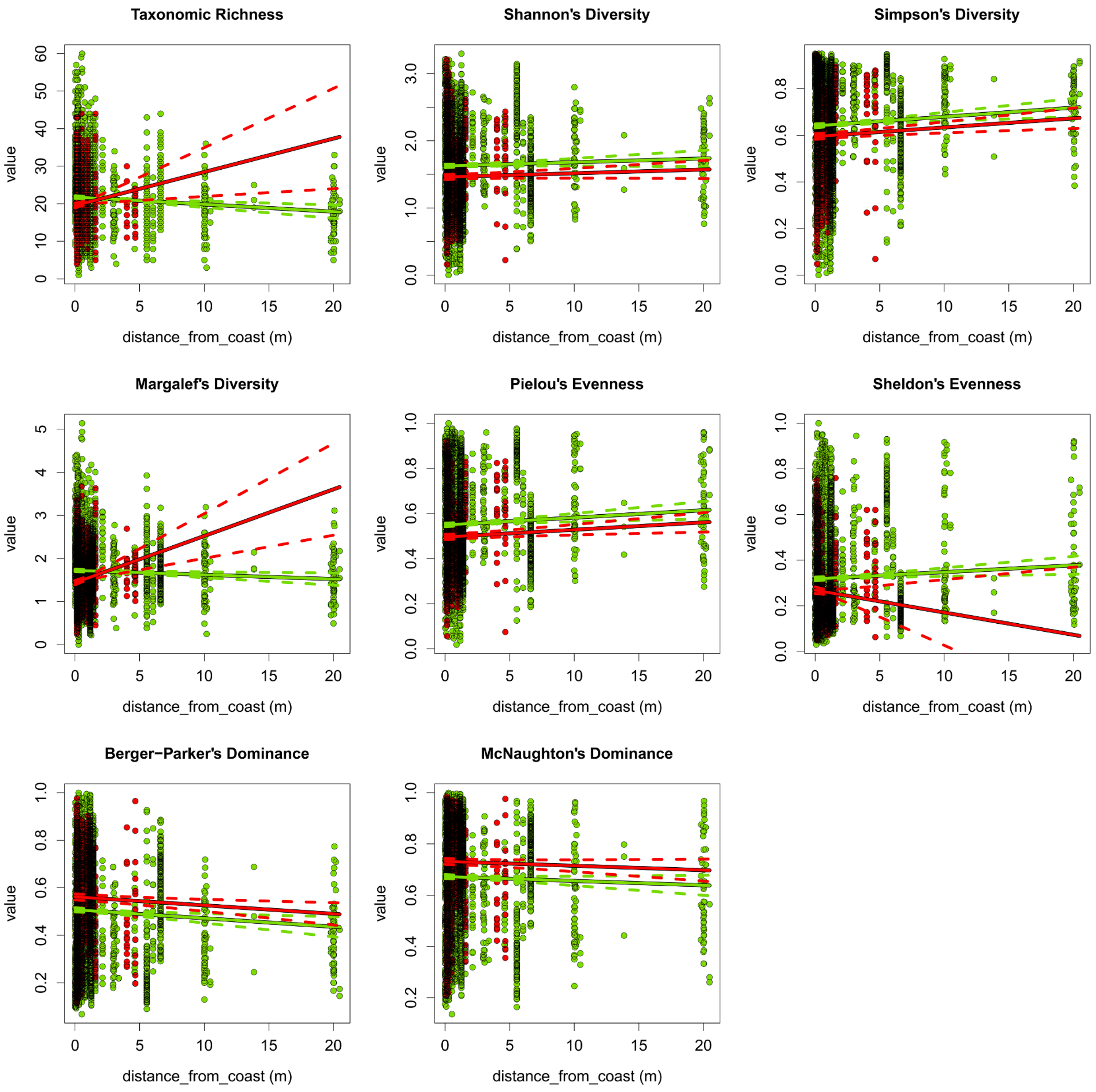


Fig. 8. Distribution of the diversity indices at different distances from the coast (green: not impacted; red: impacted). Full lines represent the average trend, while dashed lines represent the 95% CI around the average (ANCOVA). The absence of overlap between the dashed lines indicates significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sites was greater with increasing sampling depth. The fact that environmental effects are more easily detected in samples taken tens of meters below the sea surface could have important implications for the design of the sampling strategy and the assessment methodology.

The magnitude of negative environmental impacts may decrease significantly with increasing distance from the coast, as the impacts are mainly related to activities on land and near the coast. In our study, no sampling stations beyond 5 km off the coast were categorized as severely impacted, so the results of the investigation of the effects of distance from the coast on the diversity indices were distorted by the prevalence of near-shore stations. It is nevertheless worth noting that the sites in open waters showed slightly higher diversity and evenness (and less dominance) compared to coastal stations, regardless of the extent of the impact attributed to the sites. This confirms the need to define baselines and target values in open marine waters that are threatened by different pressures and where gaps in knowledge involve different aspects (Crise et al., 2015). However, open waters, which have been largely neglected in the assessments of pelagic habitat under the requirements of the MSFD, often suffer from a scarcity of data and/or scattered distribution over time and space (Siokou-Frangou et al., 2010). Efforts currently dedicated to pelagic habitat monitoring in some countries, e.g. Italy (N. Ungaro, personal communication), will make new data sets available for future studies of phytoplankton diversity in the Mediterranean.

5. Conclusions and future perspectives

The present study confirms that biodiversity indices can distinguish the extent of anthropogenic impacts on plankton communities in different coastal environments of the Mediterranean sub-regions. Based on the results of scaling eight different phytoplankton indices against different levels of stress and spatial and temporal gradients, we recommend to further investigate the possible use of three indices for the assessment of pelagic habitats: the Shannon's or Simpson's Diversity in combination with Sheldon's Evenness and one of the dominance indices.

The sub-regional differences, which are well covered in this study by longitudinal and latitudinal gradients, as well as differences addressed

Table 5
Summary of the linear model describing the variation of the diversity indices over the different distances from the coast and the two levels of impact (see Fig. 8). Highly significant terms are marked in bold. Non-significant variables or interaction terms have been removed by the technique of stepwise regression.

	Taxonomic Richness			Shannon's Diversity			Simpson's Diversity			Margalef's Diversity		
Predictors	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*
(Intercept)	21.78	0.19	**<0.001**	1.62	0.01	**<0.001**	0.64	0.001	**<0.001**	1.72	0.01	**<0.001**
Distance	−0.19	0.05	**<0.001**	0.01	0.001	0.102	0.004	0.001	**<0.001**	−0.01	0.001	**0.011**
Impacted	−2.25	0.39	**<0.001**	−0.16	0.02	**<0.001**	−0.05	0.01	**<0.001**	−0.28	0.03	**<0.001**
Distance: Impacted	1.09	0.35	**0.002**							0.12	0.03	**<0.001**
Observations	4032			4032			4032			4032		
R^2 / adjusted R^2	0.010 / 0.009			0.016 / 0.016			0.016 / 0.016			0.021 / 0.020		

	Pielou's Evenness			**Sheldon's Evenness**			**Berger-Parker's Dominance**			**McNaughton's Dominance**		
Predictors	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***
(Intercept)	0.55	0.001	**<0.001**	0.32	0.001	**<0.001**	0.51	0.001	**<0.001**	0.67	0.001	**<0.001**
Distance	0.003	0.001	**0.003**	0.003	0.001	**0.006**	−0.004	0.001	**0.003**	−0.002	0.001	0.117
Impacted	−0.05	0.01	**<0.001**	−0.05	0.01	**<0.001**	0.05	0.01	**<0.001**	0.06	0.01	**<0.001**
Distance: Impacted				−0.01	0.01	0.104						
Observations	4031			4032			4032			4032		
R^2 / adjusted R^2	0.019 / 0.019			0.021 / 0.020			0.017 / 0.017			0.020 / 0.019		

by sampling depth and distance to the coast, indicate that space-specific thresholds should be carefully considered in the future development of assessment systems. An interesting pattern with implications for the sampling plans of monitoring programs resulted from the seasonal distribution of the phytoplankton diversity indices, indicating that careful consideration should be given to any assessment based mainly on the summer months when the selectivity of the indices is lowest.

In previous studies, metrics relating to the size of phytoplankton cells have been proposed because they are particularly sensitive to environmental stress and are able to distinguish between different levels of anthropogenic impact (Lugoli et al., 2012; Vadrucci et al., 2013). These indices describe the trait diversity at the individual level, which, according to recent progress (Fontana et al., 2018), better reflects the differences between phytoplankton communities under different trophic regimes. The use of size and diversity metrics, alone or combined in multimetric indices, could be an efficient approach for the operational implementation of monitoring, capable of maximizing accuracy and minimizing the uncertainty of estimates (Cozzoli et al., 2017). Unfortunately, individual size-related data are difficult to obtain and are usually not included in national monitoring programs or long-term ecological research, especially to compare geographically distant communities and those thriving under different anthropogenic pressures.

However, an important step in the process of defining good environmental status for the pelagic habitat according to the normative definitions of the MSFD is first to reach a consensus on how to encapsulate all the important characteristics of the habitat, which is so different from benthic and terrestrial ones (Dickey-Collas et al., 2017), which requires further work.

CRediT authorship contribution statement

Janja Francé: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Ioanna Varkitzi:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Elena Stanca:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Francesco Cozzoli:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Sanda Skejić:** Investigation, Data curation, Writing - review & editing. **Nicola Ungaro:** Data curation, Writing - review & editing. **Ivano Vascotto:** Data curation, Writing - review & editing. **Patricija Mozetič:** Supervision, Writing - review & editing. **Živana Ninčević Gladan:** Data curation, Writing - review & editing. **Georgia Assimakopoulou:** Data curation, Writing - review & editing. **Alexandra Pavlidou:** Data curation, Writing - review & editing. **Soultana Zervoudaki:** Data curation, Writing - review & editing. **Kalliopi Pagou:** Project administration, Writing - review & editing. **Alberto Basset:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was carried out in the framework of the MEDCIS project: Support Mediterranean MSs towards Coherent and coordinated Implementation of the second phase of the MSFD, funded by the European Commission, DG Environment, Grant agreement 11.0661/2016/748067/ SUB /ENV.C2, Coordinator Dr. K. Pagou.

The authors acknowledge the financial support from the Slovenian Research Agency (research core funding No. P1-0237). Slovenian data originates from the national monitoring programmes financed by the Slovenian Environment Agency of the Ministry of Environment and Spatial Planning. The authors thank Apulian Regional Agency for Prevention and Environmental Protection (ARPA Puglia) for the data on national and regional monitoring programmes - sensu WFD and MSFD - funded by Italian Ministry of Environment and the Puglia Region.

The authors would like to thank Milijan Šiško, Mia Bužančić, Ana Bakrač and Maja Straka for their work on the microscope, Theodore Zoulias and Angelique Konstantinopoulou for sampling and laboratory support.

Appendix

Table A1
Categorisation of sampling sites into impact categories according to the type of pressures affecting the area (pressures are marked with x).

			Type of pressures	**Non-point pollution pressures**		**Point pollution sources**			**Industry**			**Port**			**Fisheries**	
Geographic area	Sampling station	**Impact category***	Partial pressures	Diffuse agricultural inputs	Freshwater inputs	Domestic discharges	Domestic, industrial and agricultural discharges	Industrial discharges	Industrial area	Water extraction	Power generation	Port activity	Navigation	Dredging	Aquaculture	Fisheries
Northern Adriatic Sea	000F	0														
	00CZ	2		x	x		x									
	00MA	1			x										x	
	000 K	3			x		x		x			x	x			
	0DB2	3		x			x		x						x	
Central Eastern Adriatic Sea	ST101	2		x	x		x		x							
	ST103	3			x		x	x	x			x				
Southern Eastern Adriatic Sea	PL105	0		x	x										x	
	FPO5	0		x	x										x	
Mediterranean Aegean Levantine_Central Aegean	S1	3			x	x	x	x	x			x	x			x
	S7	2				x		x	x			x	x			x
	S11	0											x			x
	DSS1	3				x								x		
	DSS2	3				x							x	x		
	DSS3	2				x										
	DSS4	1				x							x			
	DSS5	1				x							x			
Mediterranean Aegean Levantine_North Aegean	KR9	3		x	x	x	x	x								x
	KR10	3		x	x										x	x
	KR11	0		x												x
	KR12	1		x												x
	KR13	2		x											x	x
Southern Adriatic SeaSouthern Adriatic SeaSouthern Adriatic SeaSouthern Adriatic Sea	MC_TR01	0														
	MC_FF01	1		x	x											
	MC_FS01	1		x	x											
	MC_CA01	1		x	x							x				
	MC_FV01	1		x	x	x										
	MC_PE01	1		x		x										
	MC_VI01	2				x							x			
	MC_MI01	2											x		x	
	MC_MT01	2				x										
	MC_MN01	2		x		x										
	MC_FC01	3		x	x	x			x							
	MC_CR01	2		x	x	x										
	MC_AL01	1		x	x											
	MC_CM01	1		x	x											
	MC_FO01	2		x	x	x										
	MC_BI01	1				x							x			
	MC_ML01	1				x										
	MC_BB01	2		x		x			x							
	MC_BA01	1		x		x										
	MC_MA01	1		x		x										
	MC_MO01	1				x							x			
	MC_FR01	1		x												
	MC_VL01	1		x		x										
	MC_TG01	0		x												
	MC_PP01	0		x												

(*continued on next page*)

Table A1 (*continued*)

			Type of pressures	Non-point pollution pressures		Point pollution sources			Industry			Port			Fisheries	
Geographic area	Sampling station	**Impact category***	Partial pressures	Diffuse agricultural inputs	Freshwater inputs	Domestic discharges	Domestic, industrial and agricultural discharges	Industrial discharges	Industrial area	Water extraction	Power generation	Port activity	Navigation	Dredging	Aquaculture	Fisheries
	MC_CB01	3						x	x		x					
	MC_CC01	2		x		x										
	MC_SC01	1		x		x										
	MC_CE01	0														
	MC_FA01	1		x	x											
	MC_TC01	1				x										
	323_PUG	0														
	324_PUG	0														
	322_PUG	0														
	321_PUG	1				x							x			
	613_PUG	0														
	614_PUG	0														
	612_PUG	0														
	611_PUG	3						x	x		x					
	623_PUG	0														
	624_PUG	0														
	622_PUG	0														
	621_PUG	0														
	223_PUG	0														
	224_PUG	0														
	222_PUG	0														
	221_PUG	3		x	x											
	113_PUG	0														
	114_PUG	0														
	112_PUG	0														
	111_PUG	1			x											
	313_PUG	0														
	314_PUG	0														
	312_PUG	0														
	311_PUG	2		x	x	x										
	513_PUG	0														
	514_PUG	0														
	512_PUG	0														
	511_PUG	1		x												
	423_PUG	0														
	424_PUG	0														
	422_PUG	0														
	421_PUG	1		x		x										
	413_PUG	0														
	414_PUG	0														
	412_PUG	0														
	411_PUG	1				x										
	123_PUG	0														
	124_PUG	0														
	122_PUG	0														
	121_PUG	1				x										
	523_PUG	0														
	524_PUG	0														
	522_PUG	0														
	521_PUG	0		x												
	713_PUG	0														

(*continued on next page*)

Table A1 (*continued*)

			Type of pressures	**Non-point pollution pressures**		**Point pollution sources**			**Industry**			**Port**			**Fisheries**	
Geographic area	Sampling station	**Impact category***	Partial pressures	Diffuse agricultural inputs	Freshwater inputs	Domestic discharges	Domestic, industrial and agricultural discharges	Industrial discharges	Industrial area	Water extraction	Power generation	Port activity	Navigation	Dredging	Aquaculture	Fisheries
	714_PUG	0														
	712_PUG	0														
	711_PUG	1				x										
	213_PUG	0														
	214_PUG	0														
	212_PUG	0														
	211_PUG	2				x										
Ionian seaIonian sea	MC_PR01	1		x												
	MC_UG01	1		x												
	MC_SM01	1		x												
	MC_PC01	1				x										
	MC_CP01	1		x		x										
	MC_LS01	1		x		x										
	MC_SV01	1		x		x										
	MC_PN01	0														
	MC_FP01	2		x	x											
	MC_FL01	2		x		x										
	MC_GI01	2		x		x										
	823_PUG	0														
	824_PUG	0														
	822_PUG	0														
	821_PUG	1		x		x										
	923_PUG	0														
	924_PUG	0														
	922_PUG	0														
	921_PUG	2		x		x										
	813_PUG	0														
	814_PUG	0														
	812_PUG	0														
	811_PUG	1				x										
	913_PUG	0														
	914_PUG	0														
	912_PUG	0														
	911_PUG	0		x		x										
	723_PUG	0														
	724_PUG	0														
	722_PUG	0														
	721_PUG	1		x												

[**] Impact category: reference – 0, low – 1, moderate – 2, severe – 3.

Table A2
Summary of the linear model describing the variation of diversity indices over months and the two levels of impact (see Fig. 4). Highly significant terms are marked in bold. Non-significant variables or interaction terms have been removed by the technique of stepwise regression.

	Taxonomic Richness			Shannon's Diversity			Simpson's Diversity			Margalef's Diversity		
Predictors	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*	*estimates*	*std. error*	*p*
(Intercept)	19.28	0.49	**<0.001**	1.57	0.03	**<0.001**	0.63	0.01	**<0.001**	1.54	0.04	**<0.001**
month2	1.39	0.68	**0.040**	−0.05	0.05	0.307	−0.02	0.02	0.251	0.09	0.05	0.097
month3	1.01	0.66	0.127	−0.02	0.05	0.721	−0.02	0.02	0.216	0.11	0.05	**0.039**
month4	0.81	0.63	0.204	0.03	0.04	0.434	0.02	0.01	0.272	0.08	0.05	0.135
month5	−0.20	0.66	0.762	0.03	0.05	0.499	0.03	0.02	0.052	−0.03	0.05	0.573
month6	0.55	0.66	0.407	−0.01	0.05	0.774	0.002	0.02	0.906	0.03	0.05	0.511
month7	3.87	0.68	**<0.001**	0.08	0.05	0.096	0.04	0.02	**0.012**	0.26	0.05	**<0.001**
month8	1.28	0.67	0.058	−0.04	0.05	0.382	−0.03	0.02	0.078	0.09	0.05	0.086
month9	4.45	0.68	**<0.001**	0.33	0.05	**<0.001**	0.09	0.02	**<0.001**	0.39	0.05	**<0.001**
month10	5.51	0.65	**<0.001**	0.22	0.05	**<0.001**	0.06	0.01	**<0.001**	0.43	0.05	**<0.001**
month11	4.35	0.71	**<0.001**	0.01	0.05	0.850	−0.01	0.02	0.470	0.28	0.06	**<0.001**
month12	2.97	0.65	**<0.001**	0.13	0.05	**0.004**	0.04	0.01	**0.015**	0.23	0.05	**<0.001**
Impacted	−1.36	0.31	**<0.001**	−0.17	0.02	**<0.001**	−0.05	0.01	**<0.001**	−0.20	0.02	**<0.001**
Observations	4032			4032			4032			4032		
R^2 / adjusted R^2	0.051 / 0.048			0.048 / 0.045			0.042 / 0.039			0.059 / 0.056		

	Pielou's Evenness			**Sheldon's Evenness**			**Berger-Parker's Dominance**			**McNaughton's Dominance**		
Predictors	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***	***estimates***	***std. error***	***p***
(Intercept)	0.55	0.01	**<0.001**	0.33	0.01	**<0.001**	0.52	0.01	**<0.001**	0.68	0.01	**<0.001**
month2	−0.02	0.02	0.109	−0.02	0.02	0.277	0.01	0.02	0.693	0.01	0.02	0.475
month3	−0.02	0.01	0.206	−0.02	0.01	0.135	0.02	0.02	0.254	0.0001	0.01	0.982
month4	0.004	0.01	0.771	−0.01	0.01	0.544	−0.01	0.02	0.340	−0.01	0.01	0.393
month5	0.02	0.01	0.297	0.01	0.01	0.577	−0.03	0.02	**0.034**	−0.004	0.01	0.788
month6	−0.01	0.01	0.508	−0.02	0.01	0.233	0.001	0.02	0.971	0.005	0.01	0.739
month7	−0.00	0.02	0.820	−0.04	0.02	**0.015**	−0.04	0.02	**0.011**	−0.01	0.02	0.624
month8	−0.02	0.02	0.139	−0.03	0.02	**0.049**	0.03	0.02	**0.033**	0.02	0.02	0.299
month9	0.08	0.02	**<0.001**	0.04	0.02	**0.007**	−0.09	0.02	**<0.001**	−0.09	0.02	**<0.001**
month10	0.03	0.01	**0.025**	−0.01	0.01	0.537	−0.06	0.02	**<0.001**	−0.05	0.01	**0.002**
month11	−0.03	0.02	0.085	−0.05	0.02	**0.002**	0.02	0.02	0.169	0.01	0.02	0.634
month12	0.03	0.01	0.079	0.004	0.01	0.760	−0.03	0.02	0.074	−0.03	0.01	**0.025**
Impacted	−0.06	0.01	**<0.001**	−0.06	0.01	**<0.001**	0.06	0.01	**<0.001**	0.06	0.01	**<0.001**
Observations	4031			4032			4032			4032		
R^2 / adjusted R^2	0.039 / 0.036			0.032 / 0.029			0.042 / 0.039			0.040 / 0.037		

References

Akoumianaki, I., Papaspyrou, S., Kormas, K.A., Nicolaidou, A., 2013. Environmental variation and macrofauna response in a coastal area influenced by land runoff. Est. Coast. Shelf Sci. 132, 34–44. https://doi.org/10.1016/j.ecss.2012.04.009.

Artegiani, A., Paschini, E., Russo, A., Bregant, D., Raicich, F., Pinardi, N., 1997. The adriatic sea general circulation. Part II: baroclinic circulation structure. J. Phys. Oceanogr. 27 (8), 1515–1532. https://doi.org/10.1175/1520-0485(1997)027<1515:tasgcp>2.0.co;2.

Barbone, E., Pastorelli, A., Perrino, V., Blonda, M., Ungaro, N., 2014. Pattern of variation of zooplankton assemblages under the influence of river runoffs: a study case along the Apulian marine coastal waters (southern Italy, Mediterranean Sea). Mar. Freshw. Res. 65 (7), 652–665. https://doi.org/10.1071/MF13157.

Bedford, J., Ostle, C., Johns, D.G., Budria, A., McQuatters-Gollop, A., 2020. The influence of temporal scale selection on pelagic habitat biodiversity indicators. Ecol. Ind. 114, 106311. https://doi.org/10.1016/j.ecolind.2020.106311.

Berger, W.H., Parker, F.L., 1970. Diversity of planktonic foraminifera in deep-sea sediments. Science 168 (3937), 1345–1347. https://doi.org/10.1126/science.168.3937.1345.

Boldrin, A., Miserocchi, S., Rabitti, S., Turchetto, M.M., Balboni, V., Socal, G., 2002. Particulate matter in the southern Adriatic and Ionian Sea: characterisation and downward fluxes. J. Mar. Syst. 33–34, 389–410. https://doi.org/10.1016/S0924-7963(02)00068-4.

Buckland, S.T., Magurran, A.E., Green, R.E., Fewster, R.M., 2005. Monitoring change in biodiversity through composite indices. Philos. Trans. Royal Soc. B: Biol. Sci. 360 (1454), 243–254. https://doi.org/10.1098/rstb.2004.1589.

Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., Virgilio, D., 2012. Phytoplankton temporal changes in a coastal northern Adriatic site during the last 25 years. Est. Coast. Shelf Sci. 115, 113–124. https://doi.org/10.1016/j.ecss.2012.07.007.

Cardellicchio, N., Annicchiarico, C., Di Leo, A., Giandomenico, S., Spada, L., 2016. The Mar Piccolo of Taranto: an interesting marine ecosystem for the environmental problems studies. Environ. Sci. Pollut. Res. 23 (13), 12495–12501. https://doi.org/10.1007/s11356-015-4924-6.

Cardinale, B.J., Matulich, K.L., Hooper, D.U., Byrnes, J.E., Duffy, E., Gamfeldt, L., Balvanera, P., O'Connor, M.I., Gonzalez, A., 2011. The functional role of producer diversity in ecosystems. Am. J. Bot. 98 (3), 572–592. https://doi.org/10.3732/ajb.1000364.

Caroppo, C., Cerino, F., Auriemma, R., Cibic, T., 2016. Phytoplankton dynamics with a special emphasis on harmful algal blooms in the Mar Piccolo of Taranto (Ionian Sea, Italy). Environ. Sci. Pollut. Res. 23 (13), 12691–12706. https://doi.org/10.1007/s11356-015-5000-y.

Caroppo, C., Fiocca, A., Sammarco, P., Magazzu, G., 1999. Seasonal variations of nutrients and phytoplankton in the coastal SW Adriatic Sea (1995–1997). Bot. Mar. 42 (4), 389–400.

Caroppo, C., Turicchia, S., Margheri, M.C., 2006. Phytoplankton assemblages in coastal waters of the northern Ionian Sea (eastern Mediterranean), with special reference to cyanobacteria. J. Mar. Biol. Assoc. United Kingdom 86 (5), 927–937. https://doi.org/10.1017/S0025315406013889.

Christou, E.D., Pagou, K., Christianidis, S., Papathanassiou, E., 1995. Temporal and spatial variability of plankton communities in a shallow embayment of the Eastern Mediterranean. In: Eleftheriou, A., Ansell, A.D., Smith, C.J. (Eds.), Biology and Ecology of Shallow Coastal Waters. Olsen and Olsen, Fredensborg, Denmark, pp. 3–10.

Colella, S., Falcini, F., Rinaldi, E., Sammartino, M., Santoleri, R., 2016. Mediterranean ocean colour chlorophyll trends. PLoS ONE 11 (6), e0155756. https://doi.org/10.1371/journal.pone.0155756.

Cozzi, S., Giani, M., 2011. River water and nutrient discharges in the Northern Adriatic Sea: current importance and long term changes. Cont. Shelf Res. 31 (18), 1881–1893. https://doi.org/10.1016/j.csr.2011.08.010.

Cozzoli, F., Basset, A., 2017. Electronic catalogue of biodiversity indicators. In: Paramana, T., Katsouras, G., Chalkiadaki, O., Ladakis, M., Dassenakis, M., Reizopoulou, S., Basset, A., Cozzoli, F., Stanca, E., Selmeczy, G.B., Alemany, F., Deudeto, S., Alomar, C., Orlando Bonaca, M., Mavrič, B., Francé, J., Papatheochari, T., D., S., Simboura, N., Niavis, S., Coccossis, H., Varkitzi, I., Assimakopoulou, G., Drakopoulou, P., Karageorgis, A., Pantazi, M., Smith, C., Papadopoulou, N., Pavlidou, A., Panayotidis, P., Sigala, K., Katsiaras, G., Arvanitidis, C., Streftaris, N., Giannoudi, L., Pagou, K. (Eds.), Final Report on MSFD biodiversity descriptors/indicators for the Mediterranean – A comparison of targets and associated indicators & Proposal of a common set of biodiversity indicators for the Mediterranean Sea. Deliverable 1.3, ActionMed EU project, pp. 161–179.

Cozzoli, F., Stanca, E., Selmeczy, G.B., Francé, J., Varkitzi, I., Basset, A., 2017. Sensitivity of phytoplankton metrics to sample-size: a case study on a large transitional water dataset (WISER). Ecol. Ind. 82, 558–573. https://doi.org/10.1016/j.ecolind.2017.07.022.

Crise, A., Kaberi, H., Ruiz, J., Zatsepin, A., Arashkevich, E., Giani, M., Karageorgis, A.P., Prieto, L., Pantazi, M., Gonzalez-Fernandez, D., Ribera d'Alcalà, M., Tornero, V., Vassilopoulou, V., Durrieu de Madron, X., Guieu, C., Puig, P., Zenetos, A., Andral, B., Angel, D., Altukhov, D., Ayata, S.D., Aktan, Y., Balcıoğlu, E., Benedetti, F., Bouchoucha, M., Buia, M.C., Cadiou, J.F., Canals, M., Chakroun, M., Christou, E., Christidis, M.G., Civitarese, G., Coatu, V., Corsini-Foka, M., Cozzi, S., Deidun, A., Dell'Aquila, A., Dogrammatzi, A., Dumitrache, C., Edelist, D., Ettahiri, O., Fonda-Umani, S., Gana, S., Galgani, F., Gasparini, S., Giannakourou, A., Gomoiu, M.T., Gubanova, A., Gücü, A.C., Gürses, Ö., Hanke, G., Hatzianestis, I., Herut, B., Hone, R., Huertas, E., Irisson, J.O., İşinibilir, M., Jimenez, J.A., Kalogirou, S., Kapiris, K., Karamfilov, V., Kavadas, S., Keskin, Ç., Kideyş, A.E., Kocak, M., Kondylatos, G., Kontogiannis, C., Kosyan, R., Koubbi, P., Kušpilić, G., La Ferla, R., Langone, L., Laroche, S., Lazar, L., Lefkaditou, E., Lemeshko, I.E., Machias, A., Malej, A., Mazzocchi, M.G., Medinets, V., Mihalopoulos, N., Miserocchi, S., Moncheva, S., Mukhanov, V., Oaie, G., Oros, A., Öztürk, A.A., Öztürk, B., Panayotova, M., Prospathopoulos, A., Radu, G., Raykov, V., Reglero, P., Reygondeau, G., Rougeron, N., Salihoglu, B., Sanchez-Vidal, A., Sannino, G., Santinelli, C., Secrieru, D., Shapiro, G., Simboura, N., Shiganova, T., Sprovieri, M., Stefanova, K., Streftaris, N., Tirelli, V., Tom, M., Topaloğlu, B., Topçu, N.E., Tsagarakis, K., Tsangaris, C., Tserpes, G., Tuğrul, S., Uysal, Z., Vasile, D., Violaki, K., Xu, J., Yüksek, A., Papathanassiou, E., 2015. A MSFD complementary approach for the assessment of pressures, knowledge and data gaps in Southern European Seas: the PERSEUS experience. Mar. Pollut. Bull. 95 (1), 28–39. https://doi.org/10.1016/j.marpolbul.2015.03.024.

Čalić, M., Carić, M., Kršinić, F., Jasprica, N., Pećarević, M., 2013. Controlling factors of phytoplankton seasonal succession in oligotrophic Mali Ston Bay (south-eastern Adriatic). Environ. Monit. Assess. 185 (9), 7543–7563. https://doi.org/10.1007/s10661-013-3118-2.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32000L0060.

Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive) (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008L0056.

D'Ortenzio, F., Ribera d'Alcalà, M., 2009. On the trophic regimes of the Mediterranean Sea: a satellite analysis. Biogeosciences 6 (2), 139–148. https://doi.org/10.5194/bg-6-139-2009.

Dickey-Collas, M., McQuatters-Gollop, A., Bresnan, E., Kraberg, A.C., Manderson, J.P., Nash, R.D.M., Otto, S.A., Sell, A.F., Tweddle, J.F., Trenkel, V.M., 2017. Pelagic habitat: exploring the concept of good environmental status. ICES J. Mar. Sci. 74 (7), 1846–1854. https://doi.org/10.1093/icesjms/fsx158.

European Commission Decision 2010/477/EU: Commission Decision of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters (notified under document C(2010) 5956) Text with EEA relevance. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010D0477%2801%29.

European Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017D0848.

Facca, C., Bernardi Aubry, F., Socal, G., Ponis, E., Acri, F., Bianchi, F., Giovanardi, F., Sfriso, A., 2014. Description of a Multimetric Phytoplankton Index (MPI) for the assessment of transitional waters. Mar. Pollut. Bull. 79 (1), 145–154. https://doi.org/10.1016/j.marpolbul.2013.12.025.

Filstrup, C.T., Hillebrand, H., Heathcote, A.J., Harpole, W.S., Downing, J.A., 2014. Cyanobacteria dominance influences resource use efficiency and community turnover in phytoplankton and zooplankton communities. Ecol. Lett. 17 (4), 464–474. https://doi.org/10.1111/ele.12246.

Fisher, R.A., Corbet, A.S., Williams, C.B., 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. J. Anim. Ecol. 12 (1), 42–58. https://doi.org/10.2307/1411.

Flo, E., Garcés, E., Camp, J., 2019. Land uses simplified index (LUSI): determining land pressures and their link with coastal eutrophication. Front. Mar. Sci. 6 (18) https://doi.org/10.3389/fmars.2019.00018.

Fontana, S., Thomas, M.K., Moldoveanu, M., Spaak, P., Pomati, F., 2018. Individual-level trait diversity predicts phytoplankton community properties better than species richness or evenness. ISME J. 12 (2), 356–366. https://doi.org/10.1038/ismej.2017.160.

Gačić, M., Dadić, V., Krstulović, N., Marasović, I., Morović, M., Pucher-Petković, T., Sviličić, N., 1987. Near-shore transport processes induced by the wind. Est. Coast. Shelf Sci. 24 (1), 35–46. https://doi.org/10.1016/0272-7714(87)90004-7.

Giovanardi, F., Francé, J., Mozetič, P., Precali, R., 2018. Development of ecological classification criteria for the Biological Quality Element phytoplankton for Adriatic and Tyrrhenian coastal waters by means of chlorophyll a (2000/60/EC WFD). Ecol. Ind. 93, 316–332. https://doi.org/10.1016/j.ecolind.2018.05.015.

Grilli, F., Accoroni, S., Acri, F., Bernardi Aubry, F., Bergami, C., Cabrini, M., Campanelli, A., Giani, M., Guicciardi, S., Marini, M., Neri, F., Penna, A., Penna, P., Pugnetti, A., Ravaioli, M., Riminucci, F., Ricci, F., Totti, C., Viaroli, P., Cozzi, S., 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. https://doi.org/10.3390/w12082280.

Hyrenbach, K.D., Forney, K.A., Dayton, P.K., 2000. Marine protected areas and ocean basin management. Aquat. Conserv. 10 (6), 437–458. https://doi.org/10.1002/1099-0755(200011/12)10:6<437::aid-aqc425>3.0.co;2-q.

Interlandi, S.J., Kilham, S.S., 2001. Limiting resources and the regulation of diversity in phytoplankton communities. Ecology 82 (5), 1270–1282. https://doi.org/10.1890/0012-9658(2001)082[1270:lratro]2.0.co;2.

Karuza, A., Caroppo, C., Monti, M., Camatti, E., Di Poi, E., Stabili, L., Auriemma, R., Pansera, M., Cibic, T., Del Negro, P., 2016. 'End to end' planktonic trophic web and its implications for the mussel farms in the Mar Piccolo of Taranto (Ionian Sea, Italy). Environ. Sci. Pollut. Res. 23 (13), 12707–12724. https://doi.org/10.1007/s11356-015-5621-1.

Karydis, M., Tsirtsis, G., 1996. Ecological indices: a biometric approach for assessing eutrophication levels in the marine environment. Sci. Total Environ. 186 (3), 209–219. https://doi.org/10.1016/0048-9697(96)05114-5.

Kormas, K.A., Nicolaidou, A., Thessalou-Legaki, M., 2003. Variability of environmental factors of an eastern Mediterranean Sea river influenced coastal system. Mediterranean Mar. Sci. 4 (1), 67–77. https://doi.org/10.12681/mms.242.

Krause, S., Le Roux, X., Niklaus, P.A., Van Bodegom, P.M., Lennon, J.T., Bertilsson, S., Grossart, H.-P., Philippot, L., Bodelier, P.L.E., 2014. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Front. Microbiol. 5 (251) https://doi.org/10.3389/fmicb.2014.00251.

Krom, M., Kress, N., Berman-Frank, I., Rahav, E., 2014. Past, present and future patterns in the nutrient chemistry of the Eastern Mediterranean. In: Goffredo, S., Dubinsky, Z. (Eds.), The Mediterranean Sea: Its history and present challenges. Springer, Netherlands, Dordrecht, pp. 49–68.

Lazzari, P., Solidoro, C., Salon, S., Bolzon, G., 2016. Spatial variability of phosphate and nitrate in the Mediterranean Sea: a modeling approach. Deep Sea Res. Part I 108, 39–52. https://doi.org/10.1016/j.dsr.2015.12.006.

Ludwig, W., Dumont, E., Meybeck, M., Heussner, S., 2009. River discharges of water and nutrients to the Mediterranean and Black Sea: major drivers for ecosystem changes during past and future decades? Prog. Oceanogr. doi:10.1016/j.pocean.2009.02.001.

Lugoli, F., Garmendia, M., Lehtinen, S., Kauppila, P., Moncheva, S., Revilla, M., Roselli, L., Slabakova, N., Valencia, V., Dromph, K.M., Basset, A., 2012. Application of a new multi-metric phytoplankton index to the assessment of ecological status in marine and transitional waters. Ecol. Ind. 23(0), 338–355. https://doi.org/10.1016/j.ecolind.2012.03.030.

Lyashevska, O., Farnsworth, K.D., 2012. How many dimensions of biodiversity do we need? Ecol. Ind. 18, 485–492. https://doi.org/10.1016/j.ecolind.2011.12.016.

Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell Science Ltd, Oxford, U. K.

Malačič, V., Petelin, B., 2001. Gulf of Trieste, In: Cushman-Roisin, sod. (Eds.), Physical Oceanography of the Adriatic Sea. Kluwer Academic Publishers, Nederlands, pp. 167–216.

Malej, A., Malačič, V., 1995. Factors affecting bottom layer oxygen depletion in the Gulf of Trieste (Adriatic Sea). Ann. Ser. Hist. Nat. 5 (1), 33–42.

Manca, B., Franco, P., Paschini, E., 2001. Seasonal variability of the hydrography in the Adriatic Sea: water mass properties and circulation. In: Faranda, F.M., Guglielmo, L., Spezie, G. (Eds.), Mediterranean Ecosystem Structures and Processes. Springer-Verlag, Milano, Italy, pp. 45–60.

Marasović, I., Ninčević, Ž., Kuspilić, G., Marinović, S., Marinov, S., 2005. Long-term changes of basic biological and chemical parameters at two stations in the middle Adriatic. J. Sea Res. 54 (1), 3–14. https://doi.org/10.1016/j.seares.2005.02.007.

Margalef, R., 1958. Information theory in ecology. Gen. Syst. 3, 36–71.

Markogianni, V., Varkitzi, I., Pagou, K., Pavlidou, A., Dimitriou, E., 2017. Nutrient flows and related impacts between a Mediterranean river and the associated coastal area. Cont. Shelf Res. 134, 1–14. https://doi.org/10.1016/j.csr.2016.12.014.

McNaughton, S.J., 1967. Relationships among functional properties of Californian Grassland. Nature 216, 168. https://doi.org/10.1038/216168b0.

Mozetič, P., France, J., Kogovšek, T., Talaber, I., Malej, A., 2012. Plankton trends and community changes in a coastal sea (northern Adriatic): Bottom-up *vs.* top-down control in relation to environmental drivers. Est. Coast. Shelf Sci. 115, 138–148. https://doi.org/10.1016/j.ecss.2012.02.009.

Mozetič, P., Solidoro, C., Cossarini, G., Socal, G., Precali, R., France, J., Bianchi, F., De Vittor, C., Smodlaka, N., Fonda Umani, S., 2010. Recent trends towards oligotrophication of the northern Adriatic: evidence from chlorophyll a time series. Estuar. Coasts 33, 362–375. https://doi.org/10.1007/s12237-009-9191-7.

Ninčević Gladan, Ž., Bužančić, M., Kušpilić, G., Grbec, B., Matijević, S., Skejić, S., Marasović, I., Morović, M., 2015. The response of phytoplankton community to anthropogenic pressure gradient in the coastal waters of the eastern Adriatic Sea. Ecol. Ind. 56, 106–115. https://doi.org/10.1016/j.ecolind.2015.03.018.

Pachés, M., Romero, I., Hermosilla, Z., Martinez-Guijarro, R., 2012. PHYMED: an ecological classification system for the Water Framework Directive based on phytoplankton community composition. Ecol. Ind. 19, 15–23. https://doi.org/10.1016/j.ecolind.2011.07.003.

Paparella, P., Martino, G., 2008. Variazioni spazio-temporali delle condizioni trofiche delle acque costiere pugliesi dal fiume Candelaro a Brindisi (1998–1999). Thalassia Salentina 31, 53–66. https://doi.org/10.1285/i15910725v31p53.

Pavlidou, A., Kontoyiannis, H., Zarokanelos, N., Hatzianestis, I., Assimakopoulou, G., Psyllidou-Giouranovits, R., 2014. Seasonal and spatial nutrient dynamics in Saronikos Gulf: the impact of sewage effluents from Athens sewage treatment plant. In: Ansari, A.A., Gill, S.S. (Eds.), Eutrophication: Causes, Consequences and Control. Springer Science+Business Media, Dordrecht, pp. 111–130.

Pavlidou, A., Simboura, N., Pagou, K., Assimakopoulou, G., Gerakaris, V., Hatzianestis, I., Panayotidis, P., Pantazi, M., Papadopoulou, N., Reizopoulou, S., Smith, C., Triantaphyllou, M., Uyarra, M.C., Varkitzi, I., Vassilopoulou, V., Zeri, C., Borja, A., 2019. Using a holistic ecosystem-integrated approach to assess the environmental status of Saronikos Gulf, Eastern Mediterranean. Ecol. Ind. 96, 336–350. https://doi.org/10.1016/j.ecolind.2018.09.007.

Pielou, E.C., 1975. Ecological Diversity. John Wiley & Sons, New York.

Polimene, L., Pinardi, N., Zavatarelli, M., Colella, S., 2006. The Adriatic Sea ecosystem seasonal cycle: validation of a three-dimensional numerical model. J. Geophys. Res. Oceans 111 (C3). https://doi.org/10.1029/2005jc003260.

Porfido, A., Barbone, E., La Ghezza, V., Costantino, G., Perrino, V., Ungaro, N., Blonda, M., 2011. The GIS approach to detect the influence of the fresh water inflows on the marine-coastal waters: the case of the Apulia Region (Italy) through standard monitoring data. In: Cafarelli, B. (Ed.), Proceedings of the 2nd Spatial Data Methods for Environmental and Ecological Processes, Foggia, Italy. http://hdl.handle.net/10446/26450.

Ptacnik, R., Solimini, A.G., Andersen, T., Tamminen, T., Brettum, P., Lepistö, L., Willén, E., Rekolainen, S., 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. Proc. Natl. Acad. Sci. 105(13), 5134–5138. 10.1073/pnas.0708328105.

Rivaro, P., Ianni, C., Massolo, S., Ruggieri, N., Frache, R., 2004. Spatial and seasonal variability of dissolved oxygen and nutrients in the Southern Adriatic coastal waters. Chem. Ecol. 20 (4), 279–307. https://doi.org/10.1080/02757540410001670191.

Romero, I., Pachés, M., Martínez-Guijarro, R., Ferrer, J., 2013. Glophymed: an index to establish the ecological status for the Water Framework Directive based on phytoplankton in coastal waters. Mar. Pollut. Bull. 75 (1–2), 218–223. https://doi.org/10.1016/j.marpolbul.2013.07.028.

Sabetta, L., Vadrucci, M.R., Fiocca, A., Stanca, E., Mazziotti, C., Ferrari, C., Cabrini, M., Konjka, E., Basset, A., 2008. Phytoplankton size structure in transitional water ecosystem: a comparative analysis of descriptive tools. Aquat. Conserv. 18, S76–S87. https://doi.org/10.1002/aqc.954.

Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Techn. J. 27 (3), 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.

Sheldon, A.L., 1969. Equitability indices: dependence on the species count. Ecology 50 (3), 466–467. https://doi.org/10.2307/1933900.

Simboura, N., Pavlidou, A., Bald, J., Tsapakis, M., Pagou, K., Zeri, C., Androni, A., Panayotidis, P., 2016. Response of ecological indices to nutrient and chemical contaminant stress factors in Eastern Mediterranean coastal waters. Ecol. Ind. 70, 89–105. https://doi.org/10.1016/j.ecolind.2016.05.018.

Simpson, E.H., 1949. Measurement of diversity. Nature 163, 688. https://doi.org/10.1038/163688a0.

Siokou-Frangou, I., Christaki, U., Mazzocchi, M.G., Montresor, M., Ribera d'Alcalá, M., Vaqué, D., Zingone, A., 2010. Plankton in the open Mediterranean Sea: a review. Biogeosciences 7 (5), 1543–1586. https://doi.org/10.5194/bg-7-1543-2010.

Skejić, S., Vilibić, I., Matijević, S., Jozić, S., Ninčević Gladan, Ž., Morović, M., Marasović, I., Prelesnik, H., 2015. Long term regulating mechanisms of phytoplankton biomass in a traditional shellfish aquaculture area. Fresen. Environ. Bull. 24 (9), 3001–3013.

Solidoro, C., Bastianini, M., Bandelj, V., Codermatz, R., Cossarini, G., Melaku Canu, D., Ravagnan, E., Salon, S., Trevisani, S., 2009. Current state, scales of variability and decadal trends of biogeochemical properties in the Northern Adriatic Sea. J. Geophys. Res. 114, C07S91. https://doi.org/10.1029/2008JC004838.

Spatharis, S., Roelke, D.L., Dimitrakopoulos, P.G., Kokkoris, G.D., 2011. Analyzing the (mis)behavior of Shannon index in eutrophication studies using field and simulated phytoplankton assemblages. Ecol. Ind. 11 (2), 697–703. https://doi.org/10.1016/j.ecolind.2010.09.009.

Stanca, E., Cellamare, M., Basset, A., 2013. Geometric shape as a trait to study phytoplankton distributions in aquatic ecosystems. Hydrobiologia 701 (1), 99–116. https://doi.org/10.1007/s10750-012-1262-2.

Teixeira, H., Berg, T., Fürhaupter, K., Uusitalo, L., Papadopoulou, N., Bizsel, K.C., Cochrane, S., Churilova, T., Heiskanen, A.-S., Uyarra, M.C., Zampoukas, N., Borja, A., Akcali, B., Andersen, J.H., Bauchard, O., Berzano, M., Bizsel, N., Bucas, M., Camp, J., Carvalho, S., Flo, E., Garcés, E., Herman, P., Katsanevakis, S., Kavcioglu, R., Krause-Jensen, D., Kryvenko, O., Lynam, C., Mazik, K., Moncheva, S., Neville, S., Ozaydinli, M., Pantazi, M., Patricio, J., Piroddi, C., Queirós, A.M., Ramsvatn, S., Rodriguez, J.G., Rodriguez-Ezpeleta, N., Smith, C., Stefanova, K., Tempera, F., Vassilopoulou, V., Verissimo, H., Yilmaz, E.C., Zaiko, A., Zenetos, A., 2014. Existing biodiversity, non-indigenous species, food-web and seafloor integrity Gens indicators. Deliverable 3.1, Devotes EU project, p. 198.

Teixeira, H., Berg, T., Uusitalo, L., Fürhaupter, K., Heiskanen, A.-S., Mazik, K., Lynam, C. P., Neville, S., Rodriguez, J.G., Papadopoulou, N., Moncheva, S., Churilova, T., Kryvenko, O., Krause-Jensen, D., Zaiko, A., Veríssimo, H., Pantazi, M., Carvalho, S., Patrício, J., Uyarra, M.C., Borja, À., 2016. A catalogue of marine biodiversity indicators. Front. Mar. Sci. 3 (207) https://doi.org/10.3389/fmars.2016.00207.

Uusitalo, L., Fleming-Lehtinen, V., Hällfors, H., Jaanus, A., Hällfors, S., London, L., 2013. A novel approach for estimating phytoplankton biodiversity. ICES J. Mar. Sci. 70 (2), 408–417. https://doi.org/10.1093/icesjms/fss198.

Vadrucci, M.R., Stanca, E., Mazziotti, C., Umani, S.F., Georgia, A., Moncheva, S., Romano, A., Bucci, R., Ungaro, N., Basset, A., 2013. Ability of phytoplankton trait sensitivity to highlight anthropogenic pressures in Mediterranean lagoons: a size spectra sensitivity index (ISS-phyto). Ecol. Indic. 34, 113–125. https://doi.org/10.1016/j.ecolind.2013.04.013.

van Strien, A.J., Soldaat, L.L., Gregory, R.D., 2012. Desirable mathematical properties of indicators for biodiversity change. Ecol. Ind. 14 (1), 202–208. https://doi.org/10.1016/j.ecolind.2011.07.007.

Varkitzi, I., Francé, J., Basset, A., Cozzoli, F., Stanca, E., Zervoudaki, S., Giannakourou, A., Assimakopoulou, G., Venetsanopoulou, A., Mozetič, P., Tinta, T., Skejic, S., Vidjak, O., Cadiou, J.F., Pagou, K., 2018a. Pelagic habitats in the Mediterranean Sea: a review of Good Environmental Status (GES) determination for plankton components and identification of gaps and priority needs to improve coherence for the MSFD implementation. Ecol. Ind. 95, 203–218. https://doi.org/10.1016/j.ecolind.2018.07.036.

Varkitzi, I., Markogianni, V., Pantazi, M., Pagou, K., Pavlidou, A., Dimitriou, E., 2018b. Effect of river inputs on environmental status and potentially harmful phytoplankton in a coastal area of eastern Mediterranean (Maliakos Gulf, Greece). Mediterranean Mar. Sci. 19 (2), 326–343. https://doi.org/10.12681/mms.14591.

Varkitzi, I., Psarra, S., Assimakopoulou, G., Pavlidou, A., Krasakopoulou, E., Velaoras, D., Papathanassiou, E., Pagou, K., 2020. Phytoplankton dynamics and bloom formation in the oligotrophic Eastern Mediterranean: field studies in the Aegean, Levantine and Ionian seas. Deep Sea Res. Part II 171, 104662. https://doi.org/10.1016/j.dsr2.2019.104662.

Viličić, D., Jasprica, N., Carić, M., Burić, Z., 1998. Taxonomic composition and seasonal distribution of microphytoplankton in Mali Ston Bay (eastern Adriatic). Acta Bot. Croat. 57, 29–48.

Vukadin, I., 1981. Hydrographic properties of Mali Ston Bay and the adjacent Sea. In: Roglić, J., Meštrov, M. (Eds.), Symposium on Mali Ston Bay. Dubrovnik, Croatia, pp. 52–65.

Zavatarelli, M., Raicich, F., Bregant, D., Russo, A., Artegiani, A., 1998. Climatological biogeochemical characteristics of the Adriatic Sea. J. Mar. Syst. 18 (1–3), 227–263. https://doi.org/10.1016/S0924-7963(98)00014-1.

Commission Decision (EU) 2018/229 of 12 February 2018 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise and repealing Commission Decision 2013/480/EU (notified under document C(2018) 696)Text with EEA relevance.