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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

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Abstract :

At present there is no consistent approach for the definition of Good Environmental Status (GES) and targets in the Mediterranean Sea, especially for Biodiversity Descriptors, according to the Article 12 of the Marine Strategy Framework Directive (MSFD). The use of plankton indicators in the Mediterranean Sea refers mostly to pelagic habitats in coastal waters and to case studies connected with environmental pressures, e.g. in the Adriatic, Aegean etc. The aim of this review is to study the existing biodiversity indicators for different plankton groups in order to compare GES definitions for the Biodiversity Descriptor and identify the relevant gaps and priority needs to improve coherence for the MSFD implementation across the Mediterranean. For these purposes, we focus on plankton indicators for phytoplankton, zooplankton and prokaryotes. Regional conventions (OSPAR, HELCOM, Barcelona and Bucharest Conventions) have long considered phytoplankton as a key element for integrated assessment systems. Phytoplankton biomass, community composition, abundance, frequency and intensity of blooms are used for such assessment purposes. Chlorophyll a still remains the most widely used indicator mostly thanks to its time saving, cost-effective and reproducible analytical methods that provide easily comparable datasets. Despite some integrated indices proposed for phytoplankton in the literature at the Mediterranean level, a number of constrains still prevent their wide use. Regarding zooplankton communities, commonly used indicators have a taxonomic base while recently size structure and biomass can provide a valuable index of zooplankton population dynamics and ecosystem production. Jellyfish blooms' occurrence and frequency are also considered important zooplankton indicators in specific areas, e.g. North Adriatic. Concerning the prokaryotes, so far MSFD takes into account only their pathogenic component. The revision of MSFD GES definitions shows that all Mediterranean MSs have defined GES at the Descriptor level (e.g. D1 Biodiversity), but our comparison of approaches shows a low level of coherence in GES related to pelagic habitats and plankton communities. Gaps mostly focus on the lack of thresholds and baselines for many biodiversity indicators, and on the scarcity of common and consistent methodological approaches for biodiversity assessment by the MSs. Suggestions to fill these gaps and inconsistencies among MSs include: integration of EU legislation and Regional Agreements and Conventions; targeting on priority species and habitats; testing of existing biodiversity indices with good performances in case studies; coordination and intercalibration actions for the establishment of threshold values and baselines; determination of common methodologies; undertaking of regular monitoring programs and impact assessment studies at regional and sub-regional levels.

Highlights

► Low coherence for GES definitions in Mediterranean plankton communities was found. ► Phytoplankton is a key element for assessment systems of MSFD and Regional Conventions. ► GES targets are based only on Chlorophyll *a* thresholds in the Mediterranean so far. ► Quantitative GES targets for zooplankton in Mediterranean exist only in Slovenia. ► Gaps mostly focus on lack of thresholds and baselines for many biodiversity indicators.

Keywords : Plankton indicator, s Pelagic habitats, Biodiversity, Mediterranean sea, MSFD, GES assessment

1 Introduction

The ancient Greek word *Pelagos,* found in Homer's epics, refers to the open sea. The pelagic realm spans through the whole water column and it is the largest ecosystem on Earth (Kaiser et al., 2011). It can be subdivided by the water depth and the distance from shore to the neritic zone, defined as the ocean part within the continental shelf, and to the oceanic zone off the continental shelf. However, the term "pelagic habitat" as used by the Marine Strategy Framework Directive (MSFD 2008/56/EC), relates to the whole pelagic realm, as also delineated by Würtz (2010) in his overview of the Mediterranean pelagic habitats. The Annex 1 of the guidelines for reporting under the MSFD (European Commission, 2012) includes the reference and term lists, which represents a simplified version of the EUNIS classification for the category "water column habitats", with the following divisions: i) Reduced salinity water; ii) Variable salinity (estuarine) water; iii) Marine water: coastal; iv) Marine water: shelf; and v) Marine water: oceanic.

The Mediterranean is the largest European semi-enclosed sea. It has heterogeneous topography, with narrow continental shelf, average depth of approx. 1600 m and highly complex water circulation (Bergamasco and Malanotte-Rizzoli, 2010). Although it is a shallow sea compared to the oceans, a large part of the Mediterranean can be considered as a deep sea, given that several areas reach and exceed 4000 m depth (Coll et al., 2010). The Mediterranean pelagic realm is thus a highly variable four-dimensional structure (Würtz, 2010). All these peculiarities of the Mediterranean pelagial are reflected in the structure and dynamics of the plankton communities (Siokou-Frangou et al., 2010).

The Mediterranean Sea is generally oligotrophic, with increasing nutrient limitation from west to east, mostly as phosphorus limitation. This feature leads to a heterogeneous distribution of primary production and to a decreasing west-east gradient in chlorophyll *a* concentrations (D'Ortenzio and Ribera d'Alcalà, 2009). There are, however, some areas with higher chlorophyll *a* concentrations, which are in coastal waters generally related to river inputs (e.g., western part of the Northern Adriatic, Mangoni et al., 2008; Zoppini et al., 2010, 1995), while there are more connected to air-sea interactions in the open seas. The Mediterranean Sea is generally well oxygenated, which is true also for its deep layers (Siokou-Frangou et al., 2010).

The biodiversity of the Mediterranean Sea is very high, reflecting the wide range of climatic and hydrological conditions that allowed for the survival of both temperate and subtropical organisms, primarily originating from the Atlantic Ocean, and with a high percentage of endemic species (Coll et al., 2010). An important bulk of species diversity is attributed to the prokaryotic (Bacteria and Archaea) and eukaryotic (Protists) marine microbes (as reviewed in Luna, 2015 and Sunagawa et al., 2015). Diversity of several microbial groups can be accurately and readily recognized under the optical microscope (e.g. diatoms, dinoflagellates, coccolithophores and silicoflagellates among phytoplankton, and tintinnids, foraminifers and radiolarians among microzooplankton)(Kršinić, 2010; Kršinić and Kršinić, 2012), however the taxonomic determination of a plethora of marine microorganisms requires application of culture-independent molecular-based methods. Much less is known about groups of auto- and heterotrophic nanoflagellates and picoplankton species (Coll et al., 2010). Molecular methods and next generation sequencing tools/platforms, which are growingly applied to uncover microbial diversity, are promising tools that will help to assess the status of the pelagic habitats in a more accurate, rapid and on a long term even less expensive manner also in the Mediterranean Sea in the nearby future.

In the last decades, plenty of Mediterranean Sea plankton investigations were first oriented towards phyto- and zooplankton biomass and structure of plankton communities (species composition, abundance and seasonal distribution), and later included also the heterotrophic components of the pelagic food web and biological processes (reviewed by Siokou-Frangou et al., 2010). Literature oriented towards the assessment of the environmental status with the use of plankton indicators is far scarcer, especially those related to the open waters of the Mediterranean Sea.

So far plankton indicators mostly refer to Mediterranean coastal waters with specific case studies, e.g. in the Adriatic and the Aegean, and their development is always connected to environmental pressures (Markogianni et al., 2017; Ninčević Gladan et al., 2015; Spatharis and Tsirtsis, 2010; Varkitzi et al., in press). The environmental status of the pelagic habitat is addressed in the Biodiversity Descriptor 1 (D1) of the MSFD, for which the new Commission Decision 2017/848/EU sets one primary Criterion (D1C6). The condition of this habitat type is considered as a whole of its biotic and abiotic characteristics and its functions. In this review, we focus on the plankton indicators drawing attention to phytoplankton, zooplankton and prokaryotes. The aim of this study is to review the existing biodiversity indicators for different plankton groups in order to compare the Good Environmental Status (GES) definitions for the Biodiversity Descriptor and identify the relevant gaps and priority needs to improve coherence for the MSFD implementation across the Mediterranean. We also refer to other European Seas for reasons of comparison of available plankton indicators.

2 Existing approaches for the determination of GES and targets

There is no consistent approach for the definition and assessment of GES and targets in the Mediterranean in relation to MSFD Descriptors' (Fig. 1), and this is most obvious in the case of biodiversity descriptors (Paramana et al., 2017). Altogether, the number of biodiversity indicators catalogued for European Seas by the Devotes project (DEVOTool, Teixeira et al., 2016, 2014) is quite high for phytoplankton, benthic invertebrates and fish. However, a high number of those phytoplankton indicators remain at a non-operational level (in conceptual phase or under development) or without any status assigned, despite the fact that most of them were expected to be operational already, as they were parts of the Water Framework Directive (WFD) assessment (Teixeira et al., 2014).

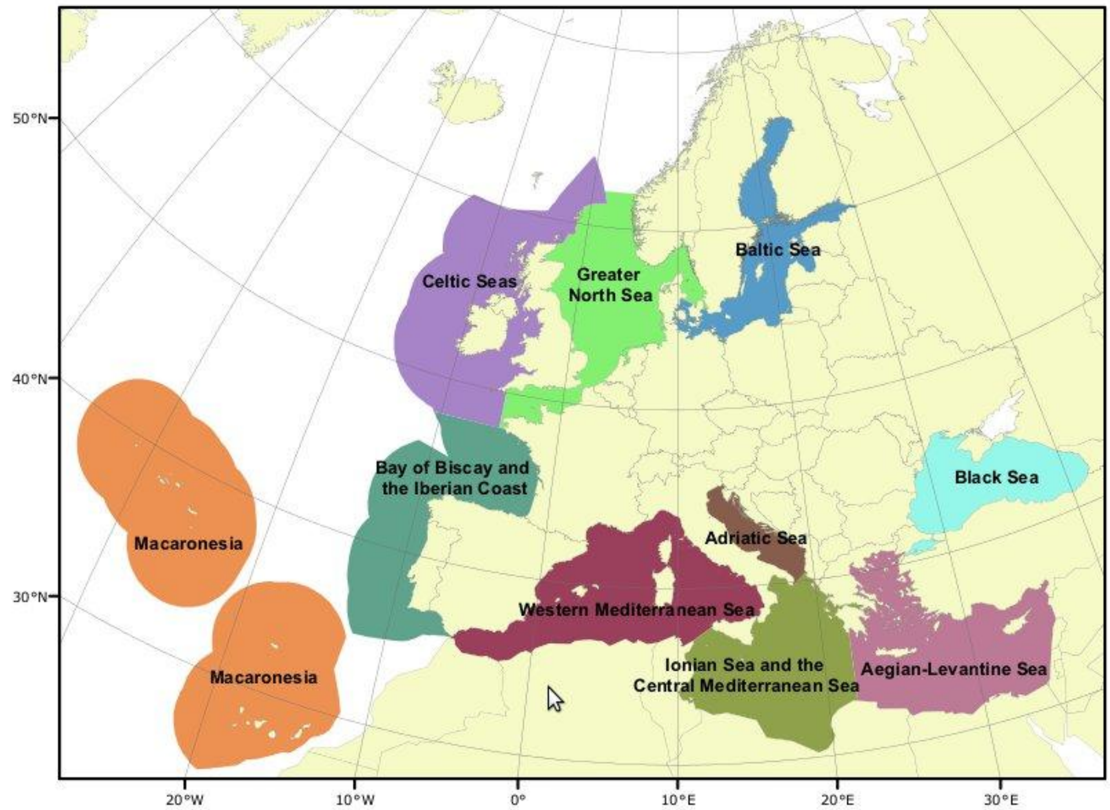


Fig. 1. Sub-regions in the Mediterranean and other European seas which are covered by the Marine Strategy Framework Directive (source: www. emodnet-chemistry.eu).

2.1 Review of phytoplankton indicators

The WFD (2000/60 EC), which establishes a framework for the protection of all European waters, was the first to have addressed systematically and Europe-wide the biotic components of water habitats. In the case of coastal waters, the biological quality element used for the assessment of the ecological status in the pelagic habitat is phytoplankton. Phytoplankton parameters to be used for this assessment are biomass, community composition and abundance, as well as frequency and intensity of blooms. Several attempts have been made to develop an integrated assessment of ecological quality of coastal waters based on more than one of these attributes in different European regions, where phytoplankton has long been considered in the assessment systems required by regional conventions, such as OSPAR, HELCOM, Bucharest and Barcelona conventions.

A variety of phytoplankton indicators can be found in the scientific literature, web-pages, different projects' reports and deliverables, which have been developed and/or used at the Mediterranean Sea level, all aiming to assess the status of the marine environment. The use of a combination of multiple phytoplankton related parameters is encouraged by the scientific community and it has been made mandatory in European Directives. Although some integrated indices have been proposed in the literature by different groups of experts at the Mediterranean Sea level (e.g. Pachés et al., 2012; Romero et al., 2013; Spatharis and Tsirtsis, 2010), a number of constrains still prevent the wide use of these assessment systems, especially at the operational level. The methods used to analyse phytoplankton communities are mainly based on time consuming cell counts. This dictates a trade-off between the number of samples in a monitoring plan and the financial budget, the available personnel and the response time (Cozzoli et al., 2017).

Phytoplankton community composition in coastal waters is in general seasonally and inter-annually highly variable, lacking a direct relationship to nutrient status of the water body (Pugnetti et al., 2007). Taking into account the wide range of cell dimensions, phytoplankton biomass can be greatly underestimated or overestimated. To overcome this constrain, carbon biomass or biovolume can be used (Cozzoli et al., 2017) although data-series containing one of these parameters are extremely rare in coastal waters.

On the other hand, the use of chlorophyll *a* as a status indicator has a lot of advantages: both spectrophotometric and fluorimetric analytical methods are time and cost-effective and reproducible, while the results are easily comparable among datasets (Domingues et al., 2008); the sensitivity of chlorophyll *a* to nutrient concentrations in the water column is well documented (Håkanson and Eklund, 2010; Harding et al., 2013; Mozetič et al., 2012). It is therefore not surprising, that chlorophyll *a* datasets are among the most widely used in the assessment systems (Giovanardi et al., 2018; Höglander et al., 2013). However, there are also some disadvantages of the use of chlorophyll *a*, e.g. the non-linear relationship between chlorophyll *a* concentration and species biomass expressed in carbon content due to environmental factors and interspecific differences (Kruskopf and Flynn, 2006) or shifts in the baselines due to other pressures related to global change (Carstensen et al., 2011).

Biodiversity core indicators program of HELCOM evaluates the status of the Baltic Sea as reflected by plankton communities along with other biological parameters (benthic biotopes, fish etc). The core indicators are commonly agreed tools to follow the progress towards the Baltic Sea Action Plan overall goal of achieving GES in the Baltic by 2021. In the Baltic region, chlorophyll *a* is the only phytoplankton parameter taken into account together with nutrient concentrations, water transparency and oxygen debt in the Eutrophication Assessment Tool (HEAT 3.0), that complies with WFD requirements (Fleming-Lehtinen et al., 2015). In the recent HELCOM pre-core indicator report for Biodiversity, the Diatom/Dinoflagellate index (Dia/Dino index) was included as a test indicator for the purposes of the mid-2017 'State of the Baltic Sea' report (HELCOM 2017a). This phytoplankton indicator reflects the dominance patterns in the phytoplankton spring bloom, it is connected with the food pathway into pelagic or benthic food webs and it is applicable in all coastal and open sea assessment units with few exceptions. It is primarily a descriptive trend indicator for changes in the food web but it may also indicate the eutrophication effect of silicate limitation. This indicator's results are to be considered as in intermediate progress and the threshold values are yet to be commonly agreed in HELCOM. Other phytoplankton biodiversity indicators under development by HELCOM are "Seasonal succession of functional phytoplankton groups" (candidate), "Phytoplankton community composition as a food web indicator" (candidate), "Phytoplankton species assemblage clusters based on environmental factors" (candidate) and "Phytoplankton taxonomic diversity (candidate)" (HELCOM 2017b).

Two phytoplankton parameters are used to assess the status of waters with regard to anthropogenic eutrophication in the North-East Atlantic region using OSPAR Comprehensive Procedure: chlorophyll *a* concentrations and elevated levels of HAB indicator species (Foden et al., 2011). In the Black Sea region all WFD recommended phytoplankton parameters are in use for the assessment of the ecological status of Romanian and Bulgarian coastal waters with Integrated Biological Index (IBI) – Phytoplankton (Carletti and Heiskanen, 2009), which was based on the work of Spatharis and Tsirtsis (2010).

In the WFD Mediterranean Geographical Intercalibration Group (Med-GIG) there has been a wide discussion on the use of different phytoplankton attributes for the assessment of the ecological quality of coastal waters. Finally, the European Commission (EC) Decision 2013/480/EU considered chlorophyll *a* as the only classification criterion for Biological Quality

Element (BQE) Phytoplankton. Reference conditions and boundaries between the Good and Moderate Ecological Classes have been based on chlorophyll *a* concentrations in different coastal water typologies, and they are presented in Table 1. This classification system has been incorporated in the recent EC Decision 2018/229/EU, which repeals the previous EC Decision 2013/480/EU and establishes the values of the MS monitoring system classifications as a result of the intercalibration exercise, pursuant to Directive 2000/60/EU. The MSs that participated in the Med-GIG and currently follow this classification system are Croatia, Cyprus, Greece, France, Italy, Slovenia and Spain.

Table 1: Water types, reference conditions and boundaries in the Mediterranean coastal waters for chlorophyll *a* concentrations (parameter used as a proxy of phytoplankton biomass) according to the WFD Mediterranean Geographical Intercalibration Group (Med-GIG). (Source: ANNEX to the European Commission Decision 2018/229/EU).

Coastal water types	Coastal water typology criteria	Reference conditions of Chl-a ($\mu g\ L^{-1}$)		Good/Moderate Boundaries of Chl-a ($\mu g\ L^{-1}$)	
		geometric mean	90-percentile	geometric mean	90-percentile
Type I	Type I: highly influenced by freshwater inputs, salinity<34.5	1.40	3.33-3.93	6.30	102.00-17.73
Type II-FR-SP (France-Spain)	Type II: not directly affected by freshwater inputs, 34.5<salinity<37.5	-	1.90	-	3.58
Type II-A Adriatic Sea	"	0.33	0.8	1.50	4.00
Type II-A Tyrrhenian Sea	"	0.32	0.77	1.20	2.90
Type III-W Adriatic Sea	Type III: not affected by freshwater inputs, salinity>37.5	-	-	0.64	1.70
Type III-W Tyrrhenian Sea	"	-	-	0.48	1.17
Type III-W FR-SP (France-Spain)	"	-	0.90	-	1.80
Type III-E	"	-	0.20	-	0.53
Type Island-W	"	-	0.60	-	1.20-1.22

At the regional level, the number of phytoplankton indicators is the lowest for the Mediterranean Sea as compared to other European Seas (Teixeira et al., 2016; 2014). Higher numbers of phytoplankton indicators mainly reflect larger research efforts and data collection in the North-East Atlantic Ocean (twenty two indicators addressing only phytoplankton or more biodiversity components; Uusitalo et al., 2016) and/or greater development and implementation of indicators in more eutrophied seas, such as Baltic and Black Seas. There are three operational indicators in the Baltic Sea, addressing exclusively phytoplankton and eight operational that include phytoplankton with other components. In the Black Sea most of the biodiversity indicators are relevant to phytoplankton (seven are operational) and benthic invertebrates as well.

A search for the Mediterranean Sea within the DEVOTool catalogue (Teixeira et al., 2016, 2014) shows that there is one biodiversity indicator exclusively addressing phytoplankton under development and one without status in the Mediterranean Sea, while there are seven that address phytoplankton together with other biodiversity components (five are operational, one is under development and one is without status). For Eastern Mediterranean there is only one operational phytoplankton indicator with the biodiversity component, i.e. Pielou evenness Index.

Phytoplankton studies in coastal and open waters of the Mediterranean Sea present high heterogeneity in terms of frequency, study area, sampling strategy, methodology and organisms addressed. Therefore, it is very difficult to compare and conclude about large scale seasonal and spatial patterns and cycles. Another aspect to be considered is the connection of phytoplankton indicators with the associated habitat types, as also for zooplankton, macroalgae, angiosperms and benthic invertebrates (MSFD Task Group 1 Report, Teixeira et al., 2014). In the context of the keystone species approach (according to the MARBEF definition and Menge et al. 2013), taxon specific indicators are considered important (at genus or species level). However, there are no taxon specific indicators for phytoplankton (together with microbes), unlike zooplankton (biomass of ctenophore *Mnemiopsis leidyi*), phytobenthos (depth distribution of *Posidonia oceanica*) and other biological elements (Smith et al., 2014).

In the frame of the WISER and ActionMed projects, Cozzoli et al. (2017) tested some commonly used indices/metrics for the description of phytoplankton communities and showed that some metrics are strongly dependent upon the sampling effort (as number of enumerated individuals per sample), while others are relatively independent. Actually they found a large heterogeneity in metrics response to the sampling effort intended as number of counted individuals per phytoplankton sample. Size-related metrics (IVD_{mean}', *ISS-phyto'*), being characterised by high precision and low uncertainty, could generally provide greater accuracy than taxonomic metrics to describe the community and are able to reach an acceptable accuracy at sample sizes lower than 200 counted individuals. Size-related metrics have also the advantages of being sensitive to environmental stress (Lugoli et al., 2012; Sabetta et al., 2008; Vadrucci et al., 2013), minimising the problem of taxonomic expertise required, and allowing quantitative inter-calibration procedures (Carvalho et al., 2013).

Dominance metrics present similar accuracy to that of the size-related metrics (Cozzoli et al., 2017). In particular, the Berger-Parker's dominance index proved to be an efficient metric, with special focus only on the easily identifiable most abundant taxa and, as other dominance indices (Facca and Sfriso, 2009) it is sensitive to environmental conditions. Therefore, Cozzoli et al. (2017) suggest that the use of size (as it is in *ISS-phyto'*) and dominance (as it is in *MPI'*) metrics, alone or combined in multimetric indices, could be an efficient approach for operational monitoring implementation, able to maximize the precision and minimize the uncertainty of estimates. According to this work, these metrices can produce reliable environmental assessments by using a sampling effort (in terms of counted individuals per sample) 50% lower than the 400 cells required from the most used international standard.

2.2 Use of Harmful Algal Blooms (HABs) in assessment systems

Some species of phytoplankton are considered as key elements to monitor the marine environment in certain cases, e.g. when they produce harmful algal blooms (HABs). In the European context, Ferreira et al. (2011) recommended that, if, but only if, HAB frequency, amplitude, or toxicity increase in response to nutrient inputs, then HABs should be treated as one of the MSFD indicators of eutrophication.

Phytoplankton blooms play a central role as ecological/environmental status assessment traits of high policy importance for WFD and MSFD. However, one of the main challenges in their practical application is the need of data with frequency corresponding to the spatial and temporal scales of phytoplankton variability. So far there are no operational indicators for HABs related to D1 in the Mediterranean MSs (Cozzoli et al., 2016). In parallel to the approach of Ferreira et al. (2011), HAB related indicators could only be operational for D1 if occurrence and extent of HAB species prove to be connected in a significant manner with the status of biodiversity in a certain habitat.

The open waters of the Mediterranean Sea, being of oligotrophic character, are not at risk for important HAB events, but during the seasonal phytoplankton peaks they may occasionally contain potentially toxic algae, such as *Pseudo-nitzschia* spp. (Garcés and Camp, 2012). On the contrary, blooms of (potentially) harmful algae are occurring frequently in Mediterranean coastal waters. Garcés and Camp (2012) list some "hot spot" regions, such as Alboran, Ligurian, Adriatic and Aegean Seas. These can be high biomass blooms or toxic blooms, which can both have deleterious effects of coastal waters biodiversity. These authors suggest one possible connection of HABs to D1, related to habitat change, for example the substantial modifications of the Mediterranean Catalan coastline have created more confined waters and led to the increase of HAB events over the last 50 years. Substantial degradation or alteration of coastal habitat may thus alter the biodiversity also through changes in HAB phenomena.

In a recent study that deals with port environments across the Adriatic Sea, Mozetič et al. (2017) found 52 HAB taxa, among them also some toxigenic non-native phytoplankton species with possible invasive character (*Pseudo-nitzschia multistriata*, *Ostreopsis* sp. and *O.* cf. *ovata*). Given the species found most frequently (*Dinophysis caudata*, *D. sacculus*, *D. fortii*, *Phalacroma rotundatum*, *Alexandrium* species, *Lingulodinium polyedrum* and *Pseudo-nitzschia* species), accumulation of toxins in seafood is the most expected harmful effect in the Adriatic Sea, but fish killings and high biomass blooms are also expected to occur (Mozetič et al., 2017). In coastal areas of Eastern Mediterranean, toxic microalgal Dinoflagellates of the "*Dinophysis acuminata* complex" have been mostly responsible for *Mytilus galloprovincialis* intoxications over the last fifteen years (Thermaikos Gulf, north Greece, see Koukaras and Nikolaidis, 2004; Varkitzi et al., 2013). *Pseudo-nitzschia delicatissima* and *P. multiseries* are frequent potentially toxic Diatoms, *Alexandrium tamarense*, *A. minutum* and *Dinophysis caudata* are other most frequent potentially toxic Dinoflagellates, and *Chaetoceros* spp., *Skeletonema costatum* and *Prorocentrum micans* are among the frequent high biomass producers (Maliakos Gulf, central Greece, see Varkitzi et al., in press). These coastal areas are affected by riverine inputs, high pollution levels and eutrophication.

Similar harmful species checklists resulted also from a study of HABs in the Tyrrhenian Sea (Zingone et al., 2006), from reviews of HABs in Greek coastal waters (Ignatiades and Gotsis-Skretas, 2010) and NW Mediterranean Sea (Vila and Maso, 2005). In some areas there is also a considerable impact of high biomass blooms, which cause discoloration of waters. They are mainly caused by dinoflagellates (*Noctiluca scintillans*, *Prorocentrum* and *Alexandrium* species), *Phaeocystis* species, *Vicicitus globosus* and some Prasinophytes (e.g. Ignatiades and Gotsis-Skretas, 2010, Zingone et al., 2006).

Within the scope of the OSPAR Eutrophication Strategy for the North-East Atlantic, nine Contracting Parties applied the OSPAR Common Procedure in 2017, using data from 2006 to 2014 (OSPAR, 2017). The elevated levels of nuisance/toxic phytoplankton indicator species and the increased duration of blooms were used as eutrophication assessment parameters by seven out of nine Contracting Parties. The nuisance species included the foam-forming species *Phaeocystis* or the dense surface blooms of *Noctiluca* as indicator species. The abundance of *Phaeocystis* spp. had already been developed as an OSPAR common indicator for the south-eastern North Sea in a previous stage. However, the Contracting Parties have assigned different importance to phytoplankton indicator species and algal toxins as indicators for eutrophication, and this has led to differences in their use. The application of toxic phytoplankton species was questioned, suggesting that the link to anthropogenic nutrient enrichment has been found to be insufficient to guarantee continuous use in some cases, e.g. in the Sea of Scotland and the southern North Sea HABs have been linked to large hydrodynamic movements and climatic conditions (Davidson et al., 2014; Gieskes et al., 2007). Therefore a more general approach is favored by the United Kingdom, involving the use of an index for application in WFD assessments, instead of using single phytoplankton species.

There is a list of over 60 species of potentially harmful phytoplankton in the Baltic Sea with connection to toxicity, mechanical disturbance, bloom formation and water coloration (Ojaveer et. al., 2010). However, there are no biodiversity indicators related to nuisance/toxic phytoplankton species that are currently being developed by HELCOM. Instead, there is the Cyanobacterial bloom index (HELCOM 38-2017; Kaitala and Hällfors, 2008) that integrates the rank abundance of only the two main bloom forming and nitrogen fixing cyanobacteria *Aphanizomenon flos-aquae* and *Nodularia spumigena* during the whole growth season in the Baltic Sea (Laamanen and Kuosa, 2005). There are also three other indices developed to compare cyanobacteria blooms between different years, i.e. the bloom normalized duration (T), extent (A) and intensity (I) (Hansson and Hakansson, 2007; Öberg, 2016). In the Black Sea about 20 species are listed as potentially toxic, but only few cases of toxicity have been reported (Alexandrov et al., 2012; Bargu et al., 2002; Vershinin et al. 2005). The harmful effects are associated mainly to hypoxic conditions during bloom events there (Black Sea Commission, 2008).

2.3 Review of zooplankton indicators

Information on the zooplankton communities, including the species composition/distribution and seasonal/geographical variability, provide a relevant contribution to the definition of GES for various MSFD Descriptors (e.g. D1, D2 and D4). There is considerable scientific and practical interest in understanding how the biological components of marine systems respond to both single and multiple stressors. The response of zooplankton to environmental conditions is of particular interest due to the central and mediating role that this group occupies as a trophic link between planktonic primary producers and larger consumers. Consequently, any variation in zooplanktonic biomass and species composition has implications on biogeochemical cycling, trophic dynamics, fisheries and other ecosystem services. For example, target zooplankton organisms are important trophic links to many commercially and recreationally important fish species, as demonstrated in several case studies from NW Med, SW Med, Adriatic Sea and Aegean sea (E Med) (Bacha and Amara, 2009; Borme et al, 2009; Nikolioudakis et al., 2012; Palomera et al, 2007).

Zooplankton as GES indicator can include various levels of complexity, ranging from rather reductionist to holistic indicators, integrating a broad range of environmental information. In general, in marine coastal ecosystems, the plankton community is often characterized by a pronounced degree of unpredictability, a feature that hinders the definition of the baselines necessary to identify a Threshold Value for the definition of GES (HELCOM, HOD 48-2015). Long term changes in total abundance, biomass, species composition and community structure can be used as representative of environmental changes in the pelagic compartment and of potential impacts related to anthropogenic pressures, such as nutrient enrichment or oil spills (HELCOM, 2012).

Certain metrics for zooplankton communities have been emerging as valuable indices for population dynamics. Zooplankton community metrics are functions of changing natural environmental factors and respond to a gradient of mixed anthropogenic pressures. These metrics have been traditionally based on the taxonomic structure (biodiversity indices) while recently size structure and biomass are more frequently used to provide a valuable assessment of zooplankton population dynamics and ecosystem production. The development of indicators is based mainly on the following zooplankton attributes: total abundance, total biomass, copepod abundance, % copepod abundance, copepod biomass, % copepod biomass (since copepods is the most abundant group in the mesozooplankton community), microphagous species biomass, % microphagous species biomass, cladocerans/copepods ratio, rotifers+cladocerans/copepods ratio, zooplankton mean size. There are more zooplankton indicators in use in other European Seas in comparison to the Mediterranean Sea, mostly due to larger research efforts and long history of data collection, as in the case of phytoplankton (Serranito et al., 2016; Uusitalo et al., 2016).

Biodiversity indices. Given the large number of indices, it is often difficult to decide which the best method to measure diversity is. A method of selecting a diversity index is on the basis of whether it fulfils certain functions criteria - ability to discriminate between sites, dependence on sample size, what component of diversity is being measured, and whether the index is widely used and understood. The selection of indicators, namely evenness, species richness and biodiversity indices, has been made mostly according to the rational proposed by Southwood and Henderson (2000) and Magurran and Mc Gill (2011).

Size and biomass related indices. During the first reporting period of the MSFD, attempts have been made to include zooplankton and jellyfish in the assessment of the Slovenian marine waters (Orlando Bonaca et al., 2012a, b, c). The two functional groups - elements were considered in the frame of D4 Food webs and D1 Biodiversity (Criterion 1.6) of pelagic habitats for the initial assessment and GES definition. Slovenia reported quantitative baseline and thresholds for zooplankton and only qualitative for jellyfish. To assess the environmental status based on zooplankton, *the annual geometric mean of mesozooplanktonic biomass* was used as the metrics. The threshold between Good/Not Good status was defined on the basis of the multi-annual geometric mean of the reference period for the Gulf of Trieste (northern Adriatic). The reference conditions were based on analyses of the zooplankton biomass derived from surveys made by Italian, Croatian and Slovenian researchers between 1971 and 1981 (Benović et al, 1984); which is regarded to as the period before the main eutrophication period and before the overfishing of pelagic fish in the northern Adriatic Sea occurred. As for jellyfish, the initial assessment was based on the nearly 200-year data series on the presence of Scyphozoans jellyfish in the northern Adriatic, with an emphasis on data about the occurrence of the moon jelly (*Aurelia aurita*). The periodicity of jellyfish occurrence was assessed with wavelet analysis (Kogovšek et al, 2010), which was then used to define the qualitative threshold for GES.

In HELCOM region, zooplankton is also included in core indicators for biodiversity (HELCOM, 2017c). Zooplankton mean size has declined in most areas of the Baltic Sea since 1980s, as a result of both the increase of the biomass of small zooplankton taxa – as a consequence of eutrophication (Uye, 1994) – and the decrease of the copepods' biomass – as a consequence of higher predation by zooplanktivorous fish (sprat and herring) and/or altered environmental conditions, e.g. decreased salinity, increased temperature and deep water hypoxia (Gorokhova et al., 2016). To quantify these changes, the mean zooplankter size (MeanSize) presented as a ratio between the total zooplankton abundance (TZA) and total biomass (TZB), is proposed as one metrics in the core indicator for food web structure. This metrics is complemented with an absolute measure of total zooplankton stock, TZA or TZB, to provide a two-dimensional index, MSTS (Mean Size and Total Stock). MSTS represents a synthetic descriptor of zooplankton community structure (by MeanSize) and the stock size (by TZA or TZB). Indeed, abundant zooplankton with high mean individual size would represent both favourable fish feeding conditions and high grazing potential, whereas all other combinations of zooplankton stock and individual size would be suboptimal and imply food web limitations in terms of energy transfer from primary producers to higher trophic levels and poorer food availability for planktivorous fish (HELCOM, 2017b).

2.4: The Barcelona Convention for phytoplankton and zooplankton indicators

One of the important MSFD demands is that MSs cooperate and coordinate the actions using the regional sea conventions. The Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) was established in 1976 and at present comprises 22 Contracting Parties. In this way it consolidates the activities in European Community MSs and other Mediterranean countries.

In 2016 the Barcelona Convention adopted the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) (UNEP/MAP, 2017). IMAP describes the strategy, themes, and products that the Contracting Parties are aiming to deliver over the second cycle of the implementation of the Ecosystem Approach Process (EcAp process 2016-2021), in order to assess the status of the Mediterranean Sea and coast. One of the main outcomes of this process is that IMAP covers the Ecological Objectives related to Biodiversity (EO1) in accordance with D1 of MSFD. Among the existing five Biodiversity common indicators, there are only two related to pelagic habitats, namely the Common indicator 1: Habitat distributional range (E01) to also consider habitat extent as a relevant attribute, and the Common indicator 2: Condition of the habitat's typical species and communities (E01).

To provide representative sites and species to include in the monitoring programs, a reference list of species and habitats is presented in Annex 1 of the IMAP document (UNEP/MAP, 2017). The Contracting Parties need to include the monitoring of the reference list species and habitats within at least two monitoring areas in their national monitoring programmes, one in a low pressure area and one in a high pressure area from human activity. Key features from this Annex related to pelagic habitats are listed in Table 2.

Table 2: Reference list of species and habitats from Annex 1 of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) (UNEP/MAP, 2017).

Predominant habitat	Specific habitat type or species to be monitored	Additional information: specific representatives species or habitats	Assessment monitoring scale
Water column - coastal waters	Coastal waters phytoplankton communities	HABs	national/regional
Water column - coastal waters	Coastal waters zooplankton communities	cf. jellyfish population dynamics and blooms	national/ sub-regional
Water column - shelf and oceanic waters	Shelf and oceanic waters phytoplankton communities	HABs	Sub-regional
Water column - shelf and oceanic waters	Shelf and oceanic waters zooplankton communities	cf. jellyfish population dynamics and blooms	Sub-regional

For the water column – coastal waters habitat high priority has been determined, meaning that sufficient resources at national and/or joint at (sub-) regional scale should be dedicated to acquire relevant data at sufficient spatial and temporal resolution. For the water column – shelf and oceanic waters habitat the priority still needs to be defined. For all the water column habitats the only established indicator is related to chlorophyll *a* concentration, although there are several monitoring techniques developed both for phytoplankton and zooplankton, and moreover, these functional groups are included in a number of observatory stations and long term monitoring programmes across the Mediterranean Sea.

Besides in the Biodiversity Ecological Objective, phytoplankton biomass is largely considered under the EO5 Eutrophication with the Common Indicator 14: Chlorophyll *a* concentration in water column (UNEP/MAP, 2017). It is recommended that the Contracting Parties rely on the classification scheme on chlorophyll *a* concentration developed by the Med-GIG under the umbrella of WFD, taking into account the water typology mentioned above.

2.5 Suitability of prokaryotes and related variables for the definition of GES

Prokaryotes are dominant organisms in terms of biomass and diversity in marine ecosystems (Salazar et al., 2016; Sunagawa et al., 2015; Whitman et al., 1998). A large fraction of primary production in the ocean becomes dissolved organic matter by various mechanisms in the food web, and this part of primary production is almost exclusively accessible to heterothophic bacteria (Duarte et al., 2013, Jiao and Azam 2011; Robinson and Williams 2005; Williams et al., 2013), which together with cyanobacteria (e.g. *Prochlorococcus, Synechococcus*) represent the major components of marine picoplankton community. Consequently, marine microorganisms are essential for the functioning of all marine food webs, playing a pivotal role in biogeochemical cycles, carbon sequestration and abatement of pollutants in marine ecosystems (Azam and Malfatti, 2007; Carlson et al., 2007; Gasol et al., 2008; Jiao and Azam, 2011; Zoppini et al., 1995).

As microbial-organic and inorganic matter pool interactions play a key role in ecosystem functioning, changes in the activity, abundance and structure/composition of autotrophic and heterotrophic prokaryotic communities are expected to have significant implications not only in processes like primary production, organic matter re-mineralization and element cycling, but also in energy and carbon fluxes (Azam and Malfatti, 2007; Benner and Herndl, 2011; Jiao and Azam, 2011). Therefore, the suitability of prokaryotes as indicators of environmental quality are widely recognized (Caruso et al. 2004; Cochrane et al., 2010; McQuatters-Gollop et al. 2010; Pomeroy et al., 2007).

Microbial populations are considered potential sentinels of environmental changes since their community composition/structure and function is known to vary in response to environmental changes, and their high sensitivity to contaminants is recognized (Caruso et al., 2016a, b, and references there in). Prokaryotic variables can be crucial for several MSFD descriptors, in particular D1 (Biodiversity), D4 (Food webs), D5 (Eutrophication), D8 (Contaminants) and D9 (Contaminants in seafood). A preliminary screening of prokaryotic parameters that could be potentially used as indicators of environmental quality was already performed by McQuatters-Gollop et al. in 2010 and revised by Caruso and her colleagues in 2016a. For example, within the set of proposed parameters prokaryotic abundance, biomass and production were listed as potential indicators within MSFD Descriptor D4 (Food Webs). The whole list of potential prokaryotic parameters/variables that could be applied as indicators in different MSFD Descriptors is available in review by Caruso et al. (2016a).

However, despite the importance and effort for the assessment of environmental status, the MSFD takes into consideration only phytoplankton, zooplankton and large metazoa (*i.e.* benthic macrofauna and fish) and still ignores autotrophic and heterotrophic prokaryotes, which actually represent the majority of the microbial community. As pointed out by Caruso et al. (2016a), prokaryotic parameters should be viewed as complementary indicators for natural and anthropogenic impacts, in particular to investigate the function of microbial communities in different ecological zones and to explore its relationship with different pressures (*i.e.* temperature increase/decrease, physical disturbance and organic loading) in marine ecosystem.

There is nevertheless still an opportunity to include also prokaryotic components, since in the phase of MSFD initial assessment the prokaryotic community is taken into account, though only for its pathogenic component. Several marine monitoring programs are limited to the identification of few microbial pathogens, mostly cultivable (*e.g.* enumeration of indicator fecal bacteria, i.e. *Escherichia coli*, intestinal enterococci and fecal coliform bacteria), using classical culture-based approach in bathing waters and in bivalve molluscs from shellfish waters and harvesting areas. However, several studies have shown that these are not sufficient indicators of fecal pollution to protect human health (Bradshaw et al., 2016; Liang et al., 2015). Several other pathogens, such as bacteria from Vibrionaceae family, *Salmonella* spp. as well as human enteric viruses (e.g. norovirus, rotavirus, hepatitis A virus) are increasing threats for human health (Caruso et al., 2016b and references there in; Gonçalves et al., 2018). Furthermore, one should be aware that since currently widely used identification is mostly based on culture-independent approach, a large fraction of uncultured microorganisms, actually representing between 90 and 99% of total microbial community, could be missed, among which also known human and animal pathogens. Moreover in D9 the term ''contaminants'' refers to hazardous substances, such as chemical elements and compounds, with no reference to microbiological contaminants. Another gap deals with the description of the impact of microbial pathogens on the marine environment (e.g. mortality of biota, shifts in community structure due to contamination, transmission of disease etc).

3 Approaches and legislation framework for GES definition in the Mediterranean

In the following paragraphs, some interesting findings are presented from the comparison of approaches and legislation framework among the Mediterranean MSs for the definition of GES in the pelagic environment.

3.1 Comparison of approaches currently implemented for the definition of GES in pelagic habitats of the Mediterranean

The revision of the GES definitions showed that at the D1 descriptor level all 8 Mediterranean MSs have defined GES. However, not all MSs defined GES and/or environmental targets also in relation to pelagic habitat and plankton communities. The comparison of approaches in defining GES and environmental targets in relation to pelagic habitat in Mediterranean MSs is presented in Table 3. The level of coherence among Mediterranean MSs in the definition of GES related to pelagic habitat is assessed as low.

France, Spain and Cyprus defined GES for D1 at the level of Criterion 1.6 (*sensu* Commission Decision, 2010/477/EU) just for benthic habitats. Several countries set GES in a qualitative way at the Criterion 1.6 level for pelagic habitats and plankton communities, i.e. Greece, Italy, Croatia and Malta. No baselines or thresholds were provided by these countries, except for Greece that provided thresholds for chlorophyll *a* concentration in coastal waters. Malta set a particular environmental target regarding pelagic habitats, i.e. the acquisition of new knowledge through monitoring programmes. Slovenia is the only MS that provided some quantitative baselines and thresholds for plankton communities, although the GES definition is very general at the criteria level. Slovenia is also the only country that referred also to zooplankton in a quantitative way.

Table 3: Comparison of approaches in GES definitions and environmental targets (Articles 9 and 10) for 8 Mediterranean European Member States for D1 – pelagic habitats. The criteria and indicators refer to Commission Decision 2010/477/EU, followed by Commission Decision 2017/848/EU.

Country	GES definition (art. 9)	Environmental targets (art. 10)	Associated indicators (art.10)	Assessment method/Threshold/Reference conditions
Slovenia	Conceptual GES definition is provided at the general level for criteria 1.4, 1.5 and 1.6: The extent and distribution of habitats are under natural conditions. Habitats provide living space for all functional groups in accordance with the natural conditions. The diversity within functional groups is maintained. Rare and threatened habitats are properly protected and preserved.	No environmental targets were reported for the pelagic habitat.	No associated indicators were reported for the pelagic habitat.	Quantitative baselines and thresholds for pelagic habitat are defined for: • the annual geometric mean of concentrations of chlorophyll *a* in the surface water layer. • the shift in the composition of phytoplankton species or groups calculated by the index of high abundances (I_E). • by the multiannual geometric mean of dry weights of zooplankton. Additionally, a qualitative baseline is defined for the frequency of jellyfish occurrence.
Greece	Conceptual GES definition is provided for plankton communities: The effects of human activities on the structure of plankton communities are reduced to minimal levels.	Conceptual environmental target is provided: Preservation of the structure of plankton communities.	• Indicators 1.1.1, 1.2.1: Species composition and abundance of plankton communities. • Indicator 1.6.2: For the water column habitat, phytoplankton biomass as chlorophyll a concentration is considered.	Thresholds or reference conditions for D1 are not defined for plankton communities (phytoplankton, zooplankton or prokaryotes). For coastal waters, quantitative thresholds are defined for phytoplankton biomass: GES threshold is 0.53 μg/L and reference conditions threshold is 0.20 μg/L of chlorophyll a concentration. Thresholds refer to the 90th percentile of chlorophyll a values. There is a +0.03 correction coefficient for Greek waters.
Italy	Conceptual GES definition is provided for plankton communities at the Indicator 1.6.2. level:	No environmental targets were reported for the pelagic habitat.	Indicator 1.6.2 Relative abundance and/or biomass, as appropriate - considered elements :	Development in progress: The indicator 1.6.2. will be developed by means of elaborations of the data provided by routine monitoring activities at a national

	GES is considered maintained or achieved when the relative abundance of the plankton communities is compatible with the natural conditions, or it presents a slight deviation from these conditions.		pelagic habitat (expected to be developed by the year 2018).	level and from specific monitoring programmes already planned or to be defined. Metrics: direct measures of relative abundances for phytoplankton and zooplankton. Phytoplankton: Specific composition and abundance (Diversity Indices), succession, frequencies of algal blooms. Zooplankton: Specific composition and abundance (Diversity Indices), changes in the ratios between functional groups (e.g. large size/small size copepods, meroplankton/holoplankton, herbivores/non-herbivores), indicator species.
Croatia	Conceptual GES definition is provided for plankton communities at the criterion 1.6 level : Taxonomic biodiversity and abundance of plankton species is preserved in accordance with the prevailing biotic and abiotic conditions which are not significantly negatively impacted by human activities.	Conceptual environmental target is provided: Relative abundances and plankton biomass are not significantly changed from naturally occurring conditions.	• Indicators 1.1.1, 1.2.1: Species composition and abundance of plankton communities. • Indicator 1.6.2: For the water column habitat, phytoplankton biomass as chlorophyll *a* concentration is considered.	Development in progress: Some indications of the statistical methods (NMDS, PCA) and ecological indices (Bray-Curtis similarity coefficient, diversity indices, Shannon-Wiener and Pielou, number of species) to be used are provided.
France	GES definition for pelagic habitat or plankton communities was not provided.	No environmental targets were reported for the pelagic habitat.	No associated indicators were reported for the pelagic habitat.	-
Spain	GES definition for pelagic habitat or plankton communities was not provided.	No environmental targets were reported for the pelagic habitat.	No associated indicators were reported for the pelagic habitat.	-
Cyprus	Conceptual GES definition is provided at the general level of D1:	Reported environmental targets are the same as GES definition.	No associated indicators were reported for the pelagic habitat.	For D1, the assessment method is based on 19 indicators, but none of them is related to plankton.

	The marine environment of Cyprus is considered to be in good environmental status by the year 2020 if biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.			For coastal waters, quantitative thresholds are defined for phytoplankton biomass: GES threshold is 0.53 μg/L and reference conditions are below 0.20 μg/L of chlorophyll *a* concentration. Thresholds refer to the 90th percentile of chlorophyll *a* values.
Malta	Conceptual GES definition is provided at the general level of criterion 1.6: The structure and function of marine habitats ensure their long-term viability.	Conceptual environmental target is provided: To strengthen knowledge via updated data on key characteristics of the water column, including plankton communities, in order to define this habitat type in line with the requirements of the Marine Strategy Framework Directive.	Level of knowledge on water column habitat types.	Development in progress: To be established through the MSFD monitoring programme by 2018.

3.2 Comparison of European Commission Decisions 2017/848/EU and 2010/477/EU in the view of pelagic habitats

In 2017 a new Commission Decision 2017/848/EU was put in force as the evolution of the Commission Decision 2010/477/EU, after having reviewed the criteria and methodological standards on GES of marine waters, and the specifications and standardised methods for monitoring and assessment. The scope of the new Commission Decision 2017/848/EU (hereafter 2017/848/EU) is to ensure that the second cycle of implementation in the MSs further contributes to the achievement of the objectives of the MSFD and yields more consistent determinations of GES. For this, the new Commission Decision aims to deliver a clearer, simpler, more concise, more coherent and comparable set of GES criteria and methodological standards.

The Annex of 2017/848/EU sets the criteria and methodological standards for GES in marine waters, which are in Part II related to the descriptors linked to the relevant ecosystem elements, also for pelagic habitats (D1) among others. Table 4 shows the criteria and methodological standards specifically for pelagic habitats, set by 2017/848/EU.

Table 4: Criteria, including criteria elements, and methodological standards related to Pelagic habitats (from Annex, Part II of the Commission Decision 2017/848/EU)

Criteria elements	Criteria	Methodological standards
Pelagic broad habitat types (variable salinity([1]), coastal, shelf and oceanic/beyond shelf), if present in the region or subregion, and other habitat types as defined in the second paragraph. MSs may select, through regional or subregional cooperation, additional habitat types according to the criteria laid down under 'specifications for the selection of species and habitats'.	D1C6 — Primary: The condition of the habitat type, including its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), is not adversely affected due to anthropogenic pressures. MSs shall establish threshold values for the condition of each habitat type, ensuring compatibility with related values set under Descriptors 2, 5 and 8, through regional or sub-regional cooperation.	*Scale of assessment:* Subdivision of region or subregion as used for assessments of benthic broad habitat types, reflecting biogeographic differences in species composition of the habitat type. *Use of criteria:* The extent to which good environmental status has been achieved shall be expressed for each area assessed as: (a) an estimate of the proportion and extent of each habitat type assessed that has achieved the threshold value set; (b) a list of broad habitat types in the assessment area that were not assessed.
([1]) Retained for situations where estuarine plumes extend beyond waters designated as Transitional Waters under Directive 2000/60/EC.		

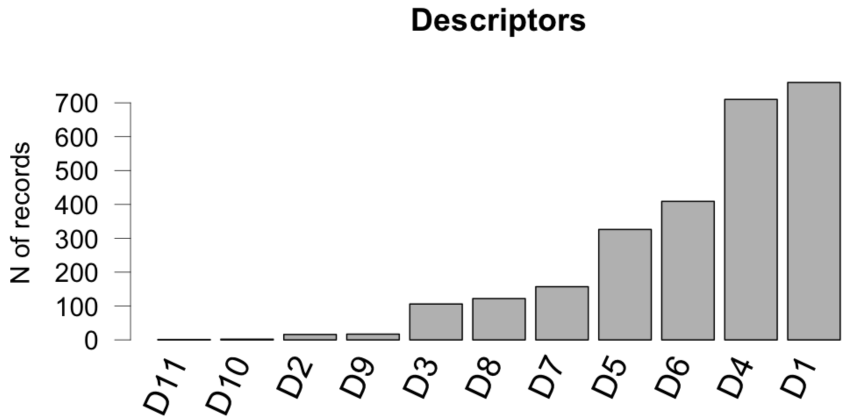
In comparison to 2010/477/EU, the new 2017/848/EU is far more precise regarding the pelagic habitat. The Criterion 1.6 "Habitat condition", that in the 2010/477/EU was based on assessment of three Indicators (1.6.1 *Condition of the typical species and communities*, 1.6.2 *Relative abundance and/or biomass, as appropriate*, and 1.6.3 *Physical, hydrological and chemical conditions*), is now combined in one primary Criterion D1C6 for pelagic habitat. In this single Criterion, the condition of the habitat type is considered as a whole for its biotic and abiotic characteristics and its functions. These habitat types are defined under the Criteria elements: broad pelagic habitat types that the MSs should take into consideration in their assessment are mandatory, and moreover, it allows for more habitat types if their need is established through (sub-) regional cooperation. In the "Specifications and standardised methods for monitoring and assessment relating to theme Pelagic habitats", the section Coastal habitat type is specified more precisely and a connection is made to Criteria from D2, D5, D7 and D8, that define pressures to the pelagic habitats. Methodological standards refer to the scale of assessment and units of measurements for the Criterion.

4 Gap analysis and priority needs to improve coherence

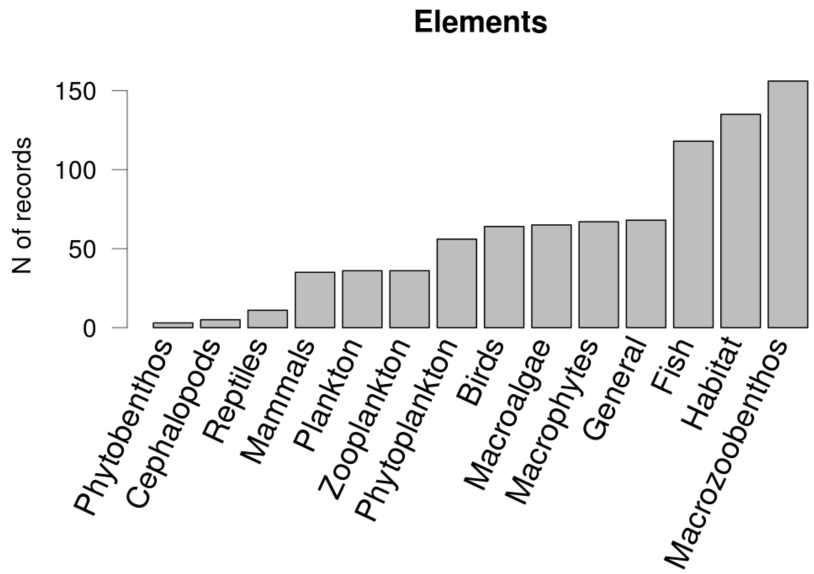
With the scope to form a basis for gap and quality analysis, for refining existing indicators and developing new, Cozzoli and Basset (2016) created an open electronic catalogue in the frame of the ActionMed EU project. This database listed published biodiversity indicators/indices/metrics, adopted also in the Mediterranean, with their information sources (related scientific publications and technical reports). In this sense, it can serve as an inventory of existing methods to assess marine environmental status in the Mediterranean Sea. A list of 91 entries in total related to phytoplankton and plankton in general can be found in this open electronic catalogue "ActionMed 1.1.2: Indicators catalogue", developed under the umbrella of ActionMed project, and accessible at http://193.204.79.93:3838/SHINY/SHINY_SERVER/ACTIONMEDCATALOGUE/.

Each record in this catalogue is a unique combination of the considered indicator/index/metric and of the number of documents in which it was published. The database includes both scientific publications and technical reports, and the number of entries in there is a proxy for the scientific knowledge and the research interest and effort devoted on that topic. Indicators for D10 (Litter) and D11 (Energy & Noise) are poorly or not represented in the catalogue. The monitoring of these descriptors is a relatively novel aspect of the MFSD that was generally not considered in previous monitoring plans, and therefore few relevant indicators have been fully developed and published at the time of the analysis.

According to this electronic catalogue, the majority of reported indicators focuses on biological elements mainly (for D1, D4, D6 of MSFD), while indicators accounting for hydrographic features (for D7) are less reported (Fig. 2a). The MSFD elements of Macrozoobenthos, Fish and Habitat appeared to be most covered in the literature (Fig. 2b). Surprisingly, phytoplankton is relatively scarcely covered, despite its importance in monitoring programmes and its strong link with D5. Zooplankton is less represented, with biomass and distributional range as most popular indicators.



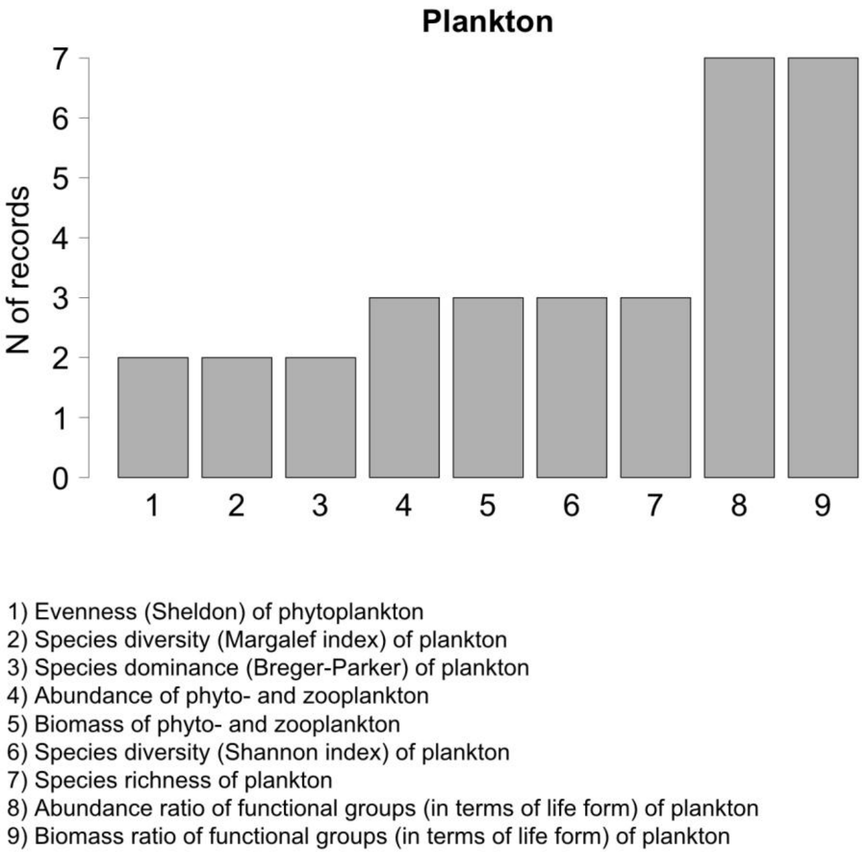
(a)



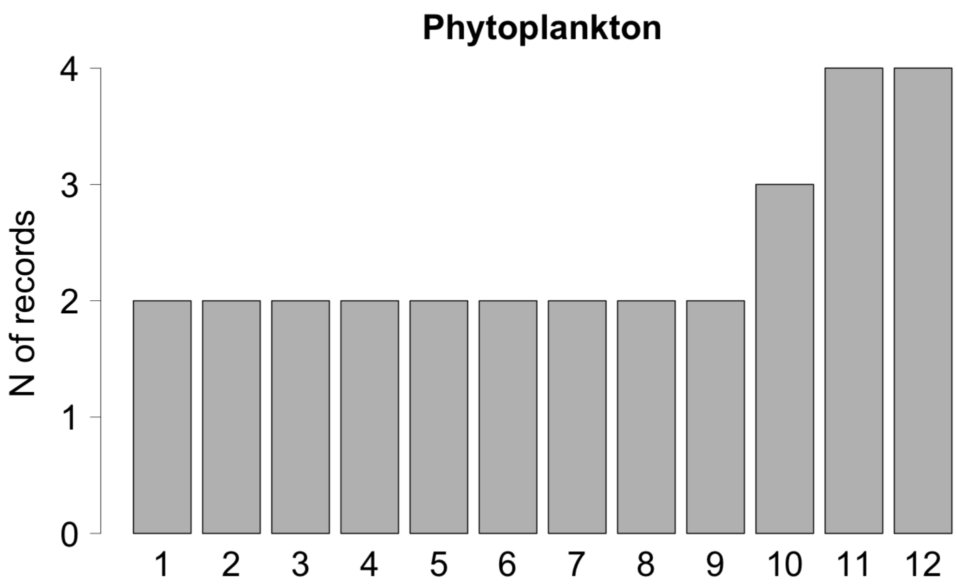
(b)

Fig. 2. Count of records in the literature which refer to a specific MSFD descriptor (a) and MSFD biodiversity element (b), as reported in the electronic catalogue of Cozzoli and Basset (2016).

Among Plankton indicators, the “Biomass ratio of functional groups” and the “Abundance ratio of functional groups” appeared to be most represented in the literature (Fig. 3a). The most popular Phytoplankton indicators are “Species diversity (Menhinick) of phytoplankton” and “Chlorophyll *a* concentration” (Fig. 3b). Despite the high sensitivity of phytoplankton to environmental impacts and especially to nutrients loads, a relatively restricted number of studies have been published about this element (56 records). The majority of published works report only total chlorophyll *a* measurements (4 records). This could be attributed to the fact that chlorophyll *a*: i) is the most commonly used parameter as a proxy for phytoplankton biomass, ii) requires easy and not expensive analyses, and iii) there were substantial efforts for the intercalibration of the assessment methodology based on chlorophyll *a* throughout the Mediterranean in the frame of WFD and MSFD, coordinated by JRC EC and by UNEP/MAP. The most popular Zooplankton indicators are “Biomass of Zooplankton” and “Distributional range of Zooplankton” (Fig. 3c). Many of these operational indicators have not been paired with thresholds in order to assess GES and accomplish legally imposed targets in the frame of the MSFD implementation. Furthermore, most of the indicators lack any measure of confidence or uncertainty associated with their assessment results (Cozzoli et al., 2017). Indicators for prokaryotes to be used within the context of the MSFD assessment were not found in the literature.

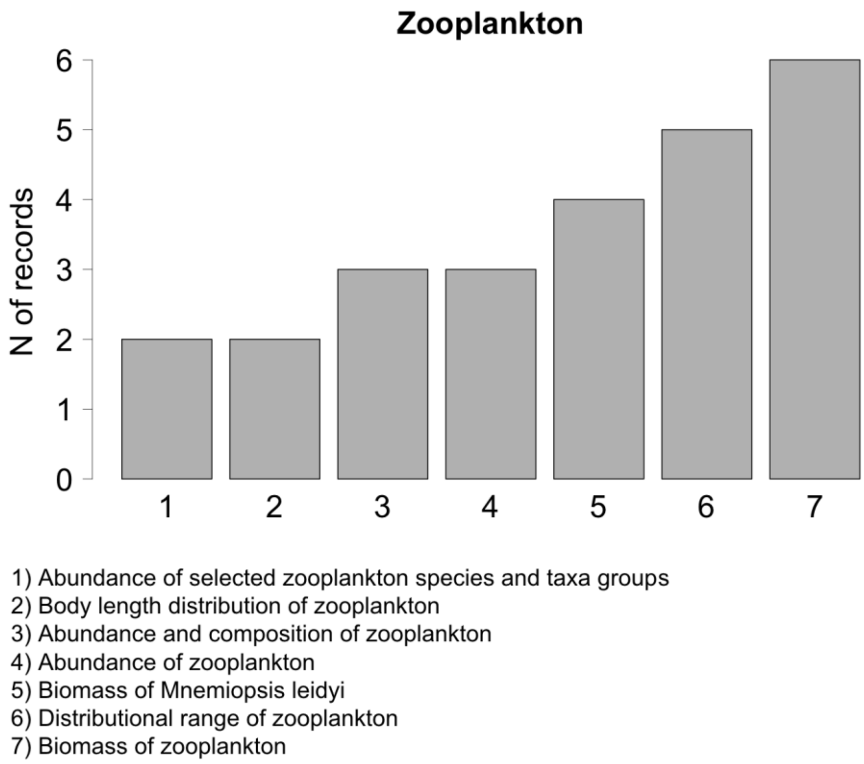


(a)



1) Abundance of phytoplankton
2) Abundance ratio of selected dinoflagellates (C-strategy species)
3) Biomass of phytoplankton
4) Distributional range of phytoplankton
5) ISS_phyto index of size spectra sensitivity phytoplankton
6) Production of phytoplankton
7) The Elevated Phytoplankton (Single Taxa) Counts Tool
8) WFD Latvian Assessment method for phytoplankton status based on chlorophyll a concentrations
9) WFD Latvian Assessment method for phytoplankton status based on phytoplankton biomass
10) Abundance of selected phytoplankton species and taxa groups
11) Concentration of Chl a
12) Species diversity (Menhinick) of phytoplankton

(b)



(c)

Fig. 3. Count of records in the literature which refer to a specific plankton indicator (a), Phytoplankton indicator (b) and Zooplankton indicator (c). Only the cases with 2 or more records are reported.

4.1 Specific remarks on gaps related to plankton communities

The most evident gaps arising from the review of approaches to define and assess environmental status by means of plankton indicators in the pelagic habitat of the Mediterranean are:

- A consistent definition of GES has not been achieved. GES definition remains general and varies a lot among Mediterranean MSs.
- The environmental targets of MSs are too general and differ considerably.
- Quantitative (operational) thresholds and baselines lack for many biodiversity indicators.
- The integration of existing EU legislation and regional sea conventions (Barcelona convention) standards is poor.
- National monitoring areas and sub-areas present a large heterogeneity in abiotic drivers and species distribution. It is therefore difficult and often inappropriate to select common thresholds for impact level.

- Common and consistent methodological approaches by MSs for biodiversity assessment are scarce.
- Scarcity or fragmentation of data, only short time series for many areas.

4.2 Priority needs for development of methodologies related to phytoplankton

The water column components phytoplankton and zooplankton are covered by fewer indicators in the Mediterranean than in other regional seas, e.g. Black Sea, Baltic Sea and NE Atlantic Ocean. However, many phytoplankton and zooplankton indicators could be used as “early warning indicators”, because of their ability to respond quickly to environmental changes and give feedback about changes happening in the food webs and ecosystems (Teixeira et al., 2014).

In general, there is a great heterogeneity of available data in terms of sampling frequency, study area, methodologies etc., in the Mediterranean. Most of the Mediterranean MSs have data from their national monitoring programmes, whether related to WFD, MSFD and/or Barcelona convention, and they are mainly limited to coastal waters. Available data from open waters are even more scarce, dispersed and heterogeneous. This scarcity or fragmentation of data creates the need for more efforts to find archival data, for example through data rescue projects.

An important aspect is to include areas with different pelagic habitat characteristics in order to be able to differentiate between structural and/or functional differences of plankton communities. D'Ortenzio and Ribera d'Alcalà (2009) propose the division of the Mediterranean Sea in areas according to the analysis of 10 years of satellite derived chlorophyll *a* data. They assume that areas with similar patterns in the seasonal time series of surface chlorophyll *a* concentration share also similar mechanisms driving the functioning of the ecosystem. Their analysis ended with seven clusters divided in four groups: coastal, blooming, intermittently blooming and no-bloom. According to their study, for example the Adriatic and the Aegean Sea belong to different clusters, mainly to coastal and no-bloom, respectively.

There is also a gap in the reporting of the frequency and intensity of pressures on biodiversity. For example, there are few indicators that demonstrate the pressure-impact relationship and include phytoplankton but they are not operational, as estimated in the frame of ActionMed project (Stanca et al., 2017). Given the above facts, it is rather impossible to get conclusions for large scale patterns on seasonal or annual basis for the Mediterranean.

Phytoplankton biodiversity indicators are mostly related to abundance, biomass, distribution, diversity and richness, and therefore cover mainly the Criteria 1.1 “Species distribution” and 1.2 “Population size”, as mentioned in the repealed Commission Decision 2010/477/EU. So far there are biodiversity indicators at the ecosystem level reported only from the NE Atlantic. It appears that in the Mediterranean there is a considerable gap for Criteria 1.6 “Habitat condition”, 1.7 “Ecosystem structure” and 1.8 “Ecosystem processes and functions”. Therefore there is a substantial need to include other components which in the new Commission Decision 2017/848/EU are part of the Habitat condition as biotic and abiotic structure of pelagic habitat and its functions (old Criteria 1.6 “Habitat condition”) and the Ecosystem related criteria included in the Food webs theme (old Criteria 1.7 “Ecosystem structure” and 1.8 “Ecosystem processes and functions”).

4.3 Priority needs for development of methodologies related to zooplankton

Until recently, the application of the zooplankton indicators for GES was mainly on a regional base and still is under development due to several difficulties. One of the difficulties in establishing indicators is the need to discriminate change due to human pressure from the considerable natural variability (in time and space) in the assemblages of species that are found in coastal and open seas. Furthermore, the great variety of species that make up plankton communities means that a large amount of information needs to be summarized. Due to the sporadic occurrence, and sometimes uncertain taxonomic affinity of all but the most common planktonic organisms, it is difficult to imagine distinguishing environmental change on a species-by-species basis. Good quality long-term zooplankton data that could provide the basis for such assessments are not only scarce, but the existing ones are often discontinued due to the financial constraints connected to the maintenance of monitoring programmes.

Plankton exhibits variability on a range of spatial and temporal scales and the assemblage of species and populations of individual species are not fixed in time and space but are dynamic. Also, a lack of zooplankton data is a key information gap. To assess the environmental status of plankton at the regional sea level, it is important that sampling stations are located in all the eco-hydrodynamic regions within each regional sea.

Detecting alterations in zooplankton and planktonic communities in general due to human pressures or climate change is not a trivial task. Any proper method should be capable of quantifying the natural dynamic variability of plankton populations and take into account the seasonal succession of some species. Furthermore, the status of zooplankton is of concern in relation to biodiversity (D1), food-web (D4) and eutrophication (D5) descriptors of the MSFD. Therefore, zooplankton indicators are required for each of these descriptors, and potentially combined with other plankton indicators, they could provide a holistic view of change in the status of the plankton community in all its aspects. This approach was tested and reported recently by Aubert et al. (2017; 2018) in the frame of the EcApRHA project for the OSPAR region in NE Atlantic Ocean.

4.4 Priority needs for development of methodologies related to prokaryotes

A main limitation of the MSFD is that most indicators are descriptive and they focus on species distributions / biodiversity, giving only partial information on the structure of the ecosystem with possible limitations in environmental quality assessment (Borja et al., 2008). The description of diversity and knowledge on the role of prokaryotes in ecological processes in the marine environment is poor if not completely neglected in MSFD. Knowledge improvements, methodology standardization and a holistic approach to the study of marine ecosystems are urgently needed in order to improve our understanding of the role of prokaryotes in ecosystem structure and functioning (Cochrane et al., 2010; Glockner et al., 2012). The diversity of prokaryotes in marine ecosystems is high (Pedrós-Alió, 2006; Sunagawa et al., 2015) so this component could/should be included within D1. Furthermore, new culture-independent, molecular-based approaches and novel omic techniques should be applied in monitoring programmes and can/should be used to study marine microbial populations and their variability through time and space in relation to environmental parameters. In the long term, the application of these novel approaches and techniques would bring more accurate, reliable, time-saving and even less expensive methods to describe the diversity of total microbial community/population of interest and/or of their pathogenic constituents.

With the application of enumeration techniques for prokaryotes in marine samples (such as flow cytometry and epifluorescence microscopy), prokaryotic abundance and biomass have been included in some routine monitoring programmes. As suggested by Caruso et al. (2016a), these two parameters could be applied as indicators within MSFD descriptor D4 (Food Webs) and D5 (Eutrophication). In order to evaluate the present and future abundance, biomass and distribution of prokaryotes, we need to establish standardized procedures for monitoring of these microorganisms at a large scale, high frequency and for long periods (Caroppo, 2015).

There are several different ways in which prokaryotes data can be used to estimate ecosystem properties or environmental status. The most essential is the abundance of specific microbial assemblages: pico-phytoplankton and heterotrophic prokaryotes. The ratio between the various microbial assemblages: picocyanobacteria/eukaryotic picophytoplankton can be used to indicate nutrient levels, as cyanobacteria are more likely to be abundant in low nutrient oligotrophic environments, while eukaryotes tend to dominate in high nutrient conditions (Calvo-Díaz et al., 2008). High abundance of the cyanobacterium *Prochlorococcus* or dominance of pico-eukaryotes is associated with nutrient-rich environments (Stomp et al., 2007). Since scattered light is proportional to cell size and fluorescence is proportional to pigment content, it is possible to differentiate among various groups of microorganisms according to their average cell size, types of pigments and pigment ratios (La Ferla et al., 2014; Morán et al., 2015).

On the other hand, heterotrophic bacteria are the major consumers of phytoplankton production and because of their large surface area-to-volume ratio these small but abundant organisms have the greatest ability to interact with chemical substances in seawater, of both natural and anthropogenic origin. Therefore, the greatest reservoir of carbon in the biosphere (Hansel et al., 2009) - the dissolved organic matter pool - is almost exclusively accessible to heterotrophic bacterioplankton. The uptake of organic matter by bacteria is a major carbon-flow pathway and its variability can change the overall patterns of carbon flux in the ocean (Azam 1998; Azam and Malfatti, 2007). The microbial loop channels energy and nutrients *via* bacteria to protozoa, to larger zooplankton and on to fish (Azam 1998; Azam and Long, 2001; Azam and Malfatti, 2007; Pomeroy et al., 2007). Moreover, the recently proposed concept of a microbial carbon pump (MCP) suggests that microbial metabolism of labile DOM, which is rapidly respired back to CO_2 or assimilated by diverse microbes, and trophic interactions within the microbial loop generate refractory DOM (RDOM), a persistent form of DOM that can survive for thousands of years, constituting a previously undescribed mechanism of carbon sequestration (Jiao et al., 2010). The MCP provides a conceptual framework that describes how microbes are also DOM producers and contributors to the creation of RDOM. The central questions of the MCP concern the structure-specific molecular consequences of microbe and organic matter interactions (Jiao and Azam, 2011). Therefore, not only the abundance, biomass and productivity of hetetrotrophic prokaryotes should be considered, but also their community structure and function should/could be included as indicator within several MSFD Descriptors (for example D1, D4 and D5, but even D8 and D9) to assess environmental status and set up effective mitigation measures and to address the relationship between diversity and ecosystem functioning.

5 Recommendations for improving coherence at regional/sub-regional scale for pelagic habitats in the Mediterranean

In order to improve coherence of approaches for pelagic habitats at regional and/or sub-regional scale, some general recommendations are presented:

- Establish threshold values and determine baselines to enable consistency.
- Determine common methodologies to empower comparability and consistency.
- Target on priority species and habitats.
- Regular and specific monitoring programmes, impact assessment studies and research programs should be undertaken at different levels (from MS to regional level). Consider also a regional approach in monitoring planning.
- There is a persistent need to increase funding for methodological research in national monitoring programmes.
- Integrate EU legislation and Regional Agreements and Conventions.
- Take initiatives at policy / legislation level to achieve or maintain GES.

Besides these general recommendations for plankton indicators, there are some more specific ones oriented towards the easier development of assessment methodologies and their putting in practice:

- Compilation of written guidelines for calculating and interpreting indicator values.
- Coordination of statistical evaluation of the scoring system among indicators.
- Completion of a map showing the eco-hydrodynamic regions, and development of networks of sampling stations (also based on existing sampling locations) to ensure that they are representative of the eco-hydrodynamic regions within an assessment area, including sampling of plankton parameters (phytoplankton, zooplankton and prokaryotes) in coastal and open waters.
- Substantial work is still needed to clearly define the use of planktonic communities as indicators for the assessment of GES, due to the lack of crucial data with adequate spatial and temporal coverage and the lack of established methods at the regional/subregional level.
- Implementation of simplified methods for phytoplankton size spectra assessment and related assessment metrics.
- Improved assessment of zooplankton biomass and standardization of biomass calculations are needed.
- Implementation of routine sampling for zooplankton size fractions using nets with appropriate mesh sizes (e.g. 50 μm and 200 μm), and bottle samples for microzooplankton (<50 μm).
- There is a need to begin widespread sampling of prokaryotes to address geographical gaps in the coverage of Mediterranean, about the role of prokaryotes in the biogeochemical cycles.
- Studies about the interactions between microbes and other planktonic organisms and higher trophic levels, and between microbes and organic/inorganic matter pool.

For the assessment of GES at the Mediterranean region scale, in Table 5 we suggest the use of metrics and indicators that would facilitate the comparability of biodiversity components of the water column habitat.

To summarize in conclusion, scientists need to agree on and follow common methodological approaches for biodiversity assessment in order to increase compatibility and consistency; test existing biodiversity indices with good performance in case studies among MSs; define GES thresholds and baselines for pelagic biodiversity indicators at the national and regional level, where feasible; suggest measures towards GES achievement in the pelagic environment.

Table 5: Suggested methodologies towards GES determination for different biodiversity components of the water column habitat in the Mediterranean Sea.

Biodiversity component	Methodology	Reason	Necessary steps for the development of GES targets and standards	Reference
Phytoplankton	Chlorophyll a	Availability of data, easy to measure, reliable data, remote sensing	This is a well intercalibrated paramenter with specified GES targets among MSs but not among non-european Mediterranean countries	Commission Decision, 2018/229/EU
	Size-related metrics, e.g. ISS-phyto	High precision, low uncertainty, no need for taxonomic expertise	Biovolume and biomass intercalibration in order to have same comparable measurement units, linear dimensions and formulas, e.g. through the Phyto Traits Thesaurus (Rosati et al., 2017) for standardized geometry and morphology terms, and the Atlas of shape (http://phytobioimaging.unisalento.it/en-us/products/AtlasOfShapes.aspx?ID_Tipo=0) for standardized geometric biovolume and surface areas' formulas	Cozzoli et al., 2017; Vadrucci et al., 2013
	Diversity and dominance metrics, e.g. Shannon-Wiener's diversity index and Berger-Parker's dominance index	High accuracy, focus on the most abundant taxa	Taxonomic intercalibration through basic/advanced courses and meetings in order to adopt a common line for taxonomy harmonization, especially when using and comparing phytoplankton species composition, abundance and biomass as indicators of environmental impacts	Cozzoli et al., 2017; Facca and Sfriso, 2009
	Dominance-related metrics for algal blooms, e.g. Bloom frequency index	High accuracy, focus only on the taxa with >50% relative abundance	As above	Cozzoli et al., 2017; Facca et al., 2014

Zooplankton	Diversity and abundance metrics, e.g. evenness, species richness, total abundance, copepod abundance	Commonly used	Taxonomic intercalibration (basic/advanced courses and meetings) for harmonization of zooplankton species identification and counting techniques	HELCOM, 2017c; Magurran and Mc Gill, 2011; Southwood and Henderson 2000
	Size and biomass related metrics, e.g. total biomass, copepod biomass, annual geometric mean of mesozooplanktonic biomass	Commonly used	Size and biomass intercalibration in order to have same comparable measurement units and linear dimensions	HELCOM, 2017c
	Jellyfish blooms, e.g. periodicity of jellyfish occurrence	Used in the N Adriatic	Selection of method(s) for the quantification of jellyfish and the definition of jellyfish bloom levels; participation in regional networks for jellyfish data collection	CIESM, 2001; Kogovšek et al, 2010
Prokaryotes	Abundance and biomass related metrics, e.g. total abundance and biomass of prokaryotes	Already used in some monitoring programs	Intercalibration of abundance enumeration and biomass measurements in order to have comparable methods	Caroppo, 2015; Caruso et al., 2016a, b
	Molecular metrics, e.g. new culture-independent, molecular-based approaches and novel omic techniques, e.g.	Commonly used for microbiological assays	Intercalibration for common analytical techniques	Caroppo, 2015; Caruso et al., 2016a, b

	DNA barcoding, Next Generation Sequencing, RT-qPCR			

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