ABIOMMED

SUPPORT COHERENT AND COORDINATED ASSESSMENT OF BIODIVERSITY AND MEASURES ACROSS MEDITERRANEAN FOR THE NEXT 6-YEAR CYCLE OF MSFD IMPLEMENTATION

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"A list of selected phytoplankton indicators, their strengths and weaknesses, and specific criteria used by each MS in determining GES for criterion D1C6"

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Coordinator

Dr Kalliopi Pagou

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Affiliation: [1] NIB, [2] IZOR, [3] HCMR, [4] CSIC, [5] Univ. of Roma, [6] UNIME, [7] ISPRAMBIENTE, [8] Univ. of Urbino, [9] Univ. Politecnica delle Marche,

Contact authors: Janja Francé (Janja.france@nib.si), Sanda Skejić (sanda@izor.hr)

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Scope

According to the Criterion for the pelagic habitat (D1C6, Descriptor 1, 2017/848/EU), the condition of the habitat type is considered as a whole for its biotic and abiotic characteristics and its functions. GES has to be defined for pelagic broad habitat types (variable salinity, coastal, shelf and oceanic/beyond shelf), and it allows for more habitat types if their need is established through (sub)regional cooperation. The comparison of the GES definitions for pelagic habitat showed that the level of coherence among the eight Mediterranean MSs is currently low (Varkitzi *et al*., 2018). GES was mostly defined on a conceptual basis, in some of the MSs, directly in relation to pelagic habitats (Varkitzi *et al*., 2018). To define tailored GES for pelagic habitats and in this way fulfill the first general objective of the call (Support for the (sub)regional assessment of the extent to which GES has been achieved), phytoplankton and zooplankton communities as biotic components of the pelagic habitat have to be included as relevant indicators.

Within the Activity 2, the scope is to explore the use of different components of the plankton assemblage (phytoplankton and zooplankton) to assess the biodiversity status, to set threshold values for those components in relation to ecologically relevant assessment areas, and to improve the coherence of GES definition for the MSFD next implementation cycle across the Mediterranean. In the first task (Task 2.1) the activities are oriented towards the development of common methodologies and indicators in GES assessment of pelagic habitat using the phytoplankton component. The work has to rely on best practices from different European regional seas (such as HELCOM and OSPAR areas), national approaches and on the previous work done in the framework of previous projects (such as DEVOTES, ACTIONMED, MEDCIS and MEDREGION). Therefore, one of the objectives of the Subtask 2.1.1 *Selection of available phytoplankton indicators for testing and further development* is to review the existing methods/approaches for determining phytoplankton status. With the identification of existing phytoplankton indicators, we created a comparative catalogue of possible phytoplankton indicators with strengths and weaknesses, specific criteria used by each MS and identified major challenges for a successful (re)definition of setting the GES for pelagic habitat in the Mediterranean Sea.

Executive Summary

While a variety of assessments of the environmental status of the Mediterranean Sea that use phytoplankton parameters can be found in the scientific and other literature, there are still several constraints that prevent an operational use of these indicators. In this report, we critically assessed the possible use of phytoplankton indicators with the stress on indicators related to diversity, reviewed the state of the art in the GES definition among the Mediterranean Member States and explore the difficulties posed for the operational use of such indicators for the pelagic habitat in the Mediterranean region.

The review of indicators within the area covered by the OSPAR Regional Sea Convention, i.e., the North-East Atlantic, revealed that marine phytoplankton and zooplankton community indicators for the assessment of the Environmental Status of Pelagic Habitats are currently still under development. In OSPAR Intermediate Assessment 2017, the pelagic habitat was assessed with three common indicators that consider plankton communities at different organizational levels. Pelagic Habitat indicator 1 (PH1) “Changes in phytoplankton and zooplankton communities” uses the relative changes in abundances of lifeform pairs based on functional traits to indicate ecological change and thus assess the pelagic habitat at an intermediate organizational level. Pelagic Habitat indicator 2 (PH2) “Changes in Phytoplankton Biomass and Zooplankton Abundance” provides an indication of temporal deviations in total phytoplankton biomass or total copepod abundance from the assumed natural variability (OSPAR, 2017b). Pelagic Habitat indicator 3 (PH3) “Changes in plankton diversity” identifies changes in the community structure using taxonomic diversity indices.

In the Baltic Sea, HELCOM is currently carrying out the third holistic assessment, according to which the pelagic habitat is assessed by different indicators for the open and coastal sea areas. For the open sea areas, three indices are applied: “Zooplankton mean size and total stock” as biodiversity core indicator, “Chlorophyll-a” as eutrophication pre-core indicator and “Cyanobacterial Bloom Index” as eutrophication pre-core test indicator. “Chlorophyll-a” is also applied in the coastal areas together with “Phytoplankton biovolume”.

In the Mediterranean Sea, no operational indices except “Chlorophyll-a” are in wider use to assess the status of the pelagic habitat, although there have been several studies at the sub-regional or local levels in which diverse indicators were tested. Some of the indicators’ groups have been proposed for further testing, such as size-related metrics, diversity and dominance metrics and metrics based on bloom frequency. Under the EcAp and IMAP umbrellas of Barcelona convention, two common indictors for assessing the pelagic habitat of the Mediterranean Sea are proposed (Habitat distributional range and Condition of the habitat's typical species and communities), for which a common reference list of pelagic habitat types have to be first agreed.

In the analysis of the strengths and weaknesses of phytoplankton indicators, several obstacles have been highlighted in the process of developing an operative assessment system, like the necessary but difficult step of linking the often non-linear response of plankton communities to human pressures, and the setting of the reference conditions. However, a successful assessment system would benefit from many advantages of using diversity indices, like the ease of calculation, provision of additional information and high sensitivity of an index.

Besides all the above, the development of an assessment system for the pelagic habitat should take into account the specific needs of Member States for determining GES for D1C6, although at this stage GES is still mainly provided at the general level addressing habitat characteristics and diversity of pelagic organisms at a broad sense.

An additional challenge is posed by the fact that the status of pelagic habitat has to be assessed as the extent of habitat adversely affected in km^2 or as % of the total extent per habitat type, which is hardly supported by the monitoring data at present.

The deliverable highlights the steps and challenges towards a definition of GES for pelagic habitats in the Mediterranean Sea, tackled also by the wider scientific community at the EU scale. Future work will have to (re)consider spatial and temporal scale-dependency of interactions between pelagic habitat conditions and communities, identify magnitude, direction, and uncertainties in the pressure-response relationships and overcome the difficulties in establishing baselines and reference conditions. As a promising alternative to deal with these difficulties, a system developed by OSPAR for assessing the biodiversity status with categories was taken into consideration, which uses either indicator thresholds or just the temporal change of the indicator linked to the impacts based on expert judgement. Such categories could be a good point to define better the GES related to pelagic habitats even in the absence of thresholds.

1 Introduction

Regional Sea conventions (OSPAR, HELCOM, Barcelona and Bucharest Conventions) have long considered phytoplankton as a key element for integrated environmental assessment systems. Different phytoplankton characteristics, as abundance, biomass, community composition, and frequency and intensity of blooms can be used for such assessment purposes and were made mandatory for the assessment of coastal and transitional waters by WFD (2000/60/EC). Also regarding MSFD, in the Commission Decision (EU) 2017/848 for the assessment of eutrophication (Descriptor 5) the secondary criterion D5C3 was included focusing on Harmful algal blooms in the water column (number, spatial extent and duration of harmful algal events). However, at the Mediterranean level, no other phytoplankton parameters except chlorophyll *a* are officially in use for the assessment of the ecological status of coastal water bodies. Altogether, chlorophyll *a* still remains the most extensively used indicator mostly due to its time saving, cost-effective and reproducible analytical methods, but it can hardly be related to the biodiversity status.

In general, phytoplankton can be considered as a suitable indicator as they form the basis of the marine food web, are mostly commercially unexploited and are susceptible to environmental pressures. Phytoplankton, for their characteristics, such as: high growth rate and seasonal variability, do not respond to the same anthropogenic pressures as benthos and not in the same temporal frame (Margiotta *et al*., 2020). For this, phytoplankton has been defined as 'the indicator without memory' (Camp *et al.,* 2016) that can be used as a quality indicator only in relation to well-defined environmental conditions. In contrast to bulk phytoplankton biomass, expressed as chlorophyll *a*, the assessment of the taxonomic composition of the phytoplankton community could provide upgraded information about the whole community, which should, however, include also pico- and nanophytoplankton beside those belonging to the micro size class that are encompassed by usual microscopy techniques (Domingues *et al*., 2008).

A variety of ways to use phytoplankton parameters for the environmental status assessment can be found in the scientific literature, webpages, different projects' reports and deliverables, which have been developed and/or used at the Mediterranean Sea level (Varkitzi *et al*., 2018a). There are, however, several constraints that still prevent wider use of these indicators, especially at the operational level. While plenty of studies oriented towards the investigation of phytoplankton community related indices in relation to biodiversity status and anthropogenic pressures, very few phytoplankton diversity indicators are actually used for assessment purposes. In this report, we critically assessed the possible use of phytoplankton indicators with the stress on indicators related to diversity, review the state of the art in the GES definition among the Mediterranean Member States (MS) and explore the difficulties posed for the operational use of indicators for assessing the pelagic habitat in the Mediterranean region. This will facilitate the choice of suitable indicators to be tested in case studies.

2 Updated review of phytoplankton indicators

2.1 OSPAR area

Within the area covered by the OSPAR Regional Sea Convention, i.e., the North-East Atlantic, marine phytoplankton and zooplankton community indicators are currently still under development to assess the Environmental Status of Pelagic Habitats (OSPAR, 2017a; McQuatters-Gollop *et al.,* 2022). In the Quality Status Report 2010 (https://qsr2010.ospar.org/en/index.html), only ten ecological quality objectives developed for the North Sea were presented, which focused mainly on the interactions between mobile species and human pressures. Since 2010, 18 indicators have been developed to assess the state of biological diversity across the OSPAR Maritime Area, including indicators that can help to assess pelagic habitats and their communities, as well as food webs. These indicators were assessed for the first time in the Intermediate Assessment 2017 (https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/).

The pelagic habitat was assessed with three common indicators (https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/biodiversity-status/habitats/) that consider plankton communities at different organizational levels:

1. Pelagic Habitat indicator 2 (PH2) **"Changes in Phytoplankton Biomass and Zooplankton Abundance"** provides an indication of temporal deviations in total phytoplankton biomass or total copepod abundance from the assumed natural variability (OSPAR, 2017b). PH2 aims to assess the plankton at the broadest organizational level, so it does not tackle changes in plankton diversity.
2. Pelagic Habitat indicator 1 (PH1) **"Changes in phytoplankton and zooplankton communities"** uses the relative changes in abundances of lifeform pairs based on functional traits to indicate ecological change and thus assess the pelagic habitat at an intermediate organizational level (Tett *et al*., 2008; McQuatters-Gollop *et al.,* 2015; OSPAR, 2017b). For example, in the pairing of diatoms and dinoflagellates the dominance of the latter could indicate eutrophication resulting in less desirable food or changes in the relative abundance of microphytoplankton and non-carnivorous zooplankton could indicate changes in energy flow through the pelagic food web (McQuatters-Gollop *et al.,* 2019).
3. Pelagic Habitat indicator 3 (PH3) **"Changes in plankton diversity"** identifies changes in the community structure using taxonomic diversity indices (OSPAR, 2017c) acting at the finest level of organization, if possible, down to the species level.

PH2 Changes in Phytoplankton Biomass and Zooplankton Abundance

Total phytoplankton biomass is assessed using chlorophyll-a or Phytoplankton Colour Index as a proxy, whereasfor zooplankton abundance the total copepods abundance is used. The methodology can be applied to fixed-monitoring station time series and to large-scale spatio-temporal data sets, such as the Continuous Plankton Recorder (CPR) data or satellite data. For the latter, a semi-quantitative measurement of phytoplankton biomass is possible by using the so-called Phytoplankton Colour Index (PCI), a method applied on the CPR data.

Detail on the use of indicator can be found at https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/biodiversity-status/habitats/plankton-biomass/. Here is presented only briefly: both chlorophyll-a and PCI are used in this assessment as they represent the two types of data regularly monitored in many areas. First, data are fitted to the correct geographic scale and then averaged per month over the whole time series. A basic, robust time series analysis is then run with the identification of anomalies representing deviations from the assumed natural variability of the time series. Annual and monthly anomalies are produced, with the former being more useful for decision makers. For the best representation, anomalies are categorized based on percentiles into *small change* (anomalies within the 25–75 percentile range), *important change* (anomalies within the 2.5–25 percentile range and 75–97.5 percentile range) and *extreme change* (anomalies within the 0–2.5 percentile range and 97.5–100 percentile range). Anomalies within the *small change* category represent the scenario least likely to represent significant shifts at the plankton community level and thus to impact on the marine ecosystem. Anomalies within the *important change* and *extreme change* categories have increasing potential to represent significant modification of the plankton community and thus to impact on the marine ecosystem. However, it is important to stress that the categorisation of the anomalies is strongly dependent on the length of the time series. This initial categorization will be further discussed in the future, with potential changes and improvements. In the future, the methodology could be strengthened by identifying clear regime shifts, in addition to anomalies. Furthermore, future assessments could examine deviation from a reference period, which definition requires knowledge of environmental and human pressure data.

Several challenges and knowledge gaps are stressed for the PH2 indicator: i) there is a need to include additional existing data sets, both at the large geographic scale and for coastal stations and further division of some areas into ecohydrodynamic zones; ii) the assessment lacks the link with environmental variables (such as temperature and salinity) and human pressures; and iii) reference periods have to be defined for each assessment unit in relation to environmental and human pressures data, and to scientific knowledge of the area. (see: Buttay *et al.*, 2015).

PH1 Changes in phytoplankton and zooplankton communities

Indicators based on plankton lifeforms (i.e., organisms with the same functional traits) can be used to reveal plankton community responses to factors such as nutrient loading from human activities and climate-driven change. When examined in pairs with an ecologically-relevant relationship, changes in the relative abundance of two lifeforms together (called a lifeform pair) can indicate change in key aspects of ecosystem function, including links between pelagic and benthic communities, energy flows and pathways, and food web interactions. Lifeforms are based on traits such as size, trophic cascades, motility, and other key biological features. Plankton lifeform pairs therefore consist of two contrasting and ecologically-relevant plankton lifeforms, eight of this pairs being currently in use (For ecological rationale and references see McQuatters-Gollop *et al.,* 2019):

1. diatoms / dinoflagellates
2. pelagic / tychopelagic diatoms
3. large (≥ 20 µm diameter) / small (< 20 µm diameter) phytoplankton
4. microphytoplankton / non-carnivorous zooplankton
5. small (< 2 mm) / large (≥2 mm) copepods - adult body length
6. holoplankton / meroplankton
7. crustaceans / gelatinous zooplankton
8. gelatinous zooplankton / fish larvae and eggs

Both abundance and biomass data can be used to inform lifeform pairs, depending on the lifeform in question and data availability from monitoring programs.

Because this is a new Indicator Assessment in the first phase of development, no assessment value exists. Instead, the years 2004 to 2008 are used as compared to the last 6-year period (2009 to 2014) to examine changes in lifeform pairs. Such an approach was used to assess UK marine waters (McQuatters-Gollop *et al*., 2019). To address the challenge of a large quantity of UK plankton data, gathered with different sampling methods, with variable levels of taxonomic identification and different enumeration methods and provide a holistic view of the UK plankton, an indicator based on plankton lifeforms was developed which allows the use of all plankton datasets, regardless of differences in sampling or analysis techniques. To identify temporal change within plankton lifeform pairs, the approach called "Plankton Index" (PI) is used to quantify the change of life-form pairs from a starting (reference) period to a new (assessment) period. In the process of defining PI, first an envelope is drawn around the point representing monthly samples of several years (e.g., 5 years). Then, monthly averaged data from subsequent/assessment periods are plotted in the same plot space. PI is calculated as the proportion of new points falling within the reference envelope, with PI values approaching 1 indicating no difference in plankton communities and PI values approaching 0 indicating a substantial change in plankton communities between the two time periods.

As this approach was used for the evaluation of GES for pelagic habitats under the MSFD in UK marine waters (McQuatters-Gollop *et al.,* 2019), it revealed that some of the plankton lifeforms used in the assessment displayed spatially variable changes during the past decade. Bedford et al. (2020) assessed these changes across the North-West European shelf over multiple decades. Results revealed multi-decadal, whole-region-scale change in plankton lifeforms over the North-West European shelf indicating shifts in the functional balance of plankton communities. A few examples are: i) CPR data revealed a significant increasing trend in meroplankton in the Greater North Sea while holoplankton showed a significant decreasing trend indicating a changing balance of benthic and pelagic fauna; ii) increasing trend of dinoflagellates inshore and contrasting multi-decadal decreasing trend offshore; iii) diatoms showed an increasing trend in the Greater North Sea and a decreasing trend in the Celtic Sea.

An important advantage of these plankton indicators is that the proposed concepts are relatively easily transferable to other European regional seas (Gowen *et al*., 2011; Rombouts *et al.,* 2013). Additionally, plankton lifeforms are aggregations of taxa and so are less likely to experience the extreme seasonal fluctuations of single species indicators. Finally, because lifeforms consist of multiple taxa with a similar functional role, spatial intercomparability is increased, as even though the target taxa fulfilling a functional role may vary, the corresponding lifeform is often regionally ubiquitous (McQuatters-Gollop *et al.,* 2019).

From its initial use in the Intermediate Assessment 2017 the PH1 indicator has been further developed to form the common indicator for assessing pelagic habitat (D1) and food webs (D4) - *PH1/FW5 Change in plankton communities*. In September 2022, the OSPAR Commission released an amended OSPAR CEMP Guideline on the Common indicator: PH1/FW5 Change in plankton communities (OSPAR, 2022).

In the Guideline, the double role of PH1/FW5 indicator is stressed: lifeform pairs are useful for detecting changes in the annual cycle of ecologically linked lifeforms, while assessing lifeforms separately is more suitable for evaluating gradual change over time. As found by Bedford *et al*. (2020), it appears that correlating lifeform abundance and environmental variables gives more interpretable result than correlating environmental variables to already calculated index of lifeform pairs. For this, a method has been developed to find potential links between environmental pressures and change in lifeform abundance over time using a robust nonparametric test.

PH3 Changes in plankton diversity

This indicator assesses changes in plankton diversity at its finest organizational level; ideally, this would represent species level. An important theoretical consideration behind the development of PH3 refers to ecological information delivered by such indicator. To describe community structure and change, three aspects of diversity indices are of highest interest: i) the number of taxa, ii) overall abundance and iii) evenness in the community. Recently, all three aspects received an increased interest in issues of environmental management, especially if combined with each other (Buckland *et al.,* 2011).

To fulfil these requirements, Rombouts *et al.* (2019) developed a multi-metric indicator compliant with the common OSPAR indicator PH3, which includes alpha diversity (structure of the phytoplankton community) and beta diversity (to assess the change in community structure from one sampling unit to another along a spatial or temporal gradient, e.g., years). The pilot study included the testing of the efficiency and the performance of several existing diversity indices on data from three time-series in the coastal areas of the Western Channel and northern part of Bay of Biscay (North Atlantic, France). The alpha diversity indices tested were richness, Margalef's and Menhinick's among those based on number of taxa, and Shannon's Simpson's, Hulburt's, Berger-Parker's, Brillouin's, Pielou's and geometric mean of relative abundance (G; Buckland et al., 2011) indices among those assessing evenness and dominance. As for beta diversity, Local Contribution to Beta Diversity (LCBD; Legendre and De Cáceres, 2013) index was used that indicates how much each sample contributes to total community variance in time. Values of LCBD can be interpreted as follows: value near to zero would indicate a temporal window (for example a year) with an average species composition, while a larger LCBD value may indicate degradation in terms of decline in species richness and diversity or special ecological conditions resulting from a disturbance event. For years having a significantly high LCBD values, the importance of taxa in the community was calculated with Importance Value Index (IVI; Curtis, 1959) as the sum of relative density and the relative frequency of the taxonomic units in the community. All indices were calculated at the genus level. Based on the results, authors assessed the complementary of ecological information provided by each of the indices, the strength of the relationship with environmental factors and the relative ease of interpretation for stakeholders. Among alpha diversity indices, the best scores for these criteria were obtained for the Menhinick Index (D) and the Hulburt Index (δ) describing genus richness and dominance, respectively.

For a pilot assessment that served as a proof of concept in OSPAR Intermediate Assessment 2017, the selected alpha and beta diversity indices were calculated at five sites: four in French waters and one in Spanish waters. The extent of temporal variation in the phytoplankton community was also assessed to identify years with significant changes/shifts in taxa composition. The results of the pilot assessment showed that diversity indices are useful to describe the structure of the phytoplankton community and also its variability. Thus, PH3 informed on different aspects of phytoplankton diversity from a community to a genus level but can at the current stage of development and knowledge, lacking a straight connection to GES, serve only as a "surveillance" indicator.

Further work was needed from Initial Assessment in 2017 to integrate all pelagic habitat indicators (PH1, PH2 and PH3) since an integrated assessment would give more confidence in the evaluation of plankton changes, and in their integration with indicators of other ecosystem components, in particular food-web indicators. Progress at the level of the OSPAR region has been significantly boosted through the European Commission's funding of the EcApRHA (Applying an Ecosystem Approach to (sub) Regional Habitat Assessment) project, which has fed into the assessments presented in Intermediate assessment 2017. EcApRHA created opportunities for policy and science representatives to interact and so ensure that project results are fit for OSPAR purposes. Among other objectives, EcApRHA aimed to explore the potential integration and aggregation options for pelagic, benthic habitats and food webs (https://www.ospar.org/work-areas/bdc/ecaprha/about-ecaprha). As found also during Mediterranean project (e.g., MEDCIS, MEDREGION, ABIOMMED), one of the main difficulties encountered by EcApRHA when trying to integrate the indicators were the differences in their defined spatial and temporal resolution and because of potential differences in their responses to pressures.

However, these indicators are currently still under development (OSPAR, 2022). They are assessed in parallel but for a more robust future assessment of the pelagic habitat, the indicators should be considered simultaneously to i) understand changes and dynamics within the plankton community, ii) reduce the uncertainty in the assessment and iii) to understand the links with anthropogenic pressures (OSPAR, 2022). In the last assessment made by McQuatters et al. (2022), the work done on assessment in 2017 was merged into a holistic semiquantitative evaluation of the state of Northeast Atlantic marine biodiversity across marine food webs, pelagic and benthic habitats, and NIS. For the pelagic habitat, PH1/FW5, PH2 and PH3 indicators were used as explained above. Added value of the work of McQuatters et al. (2022) was further development of the assessment methodology through application of classification categories to classify indicator change within the wider ecosystem context. As this approach is important also for the work in ABIOMMED Activity 2, it is described more in detail below (Chapter 4.3). However, although for some of the ecosystem components the results of the analysis were marked with high confidence, the status assessment of the pelagic habitat (among others such as food webs and NIS) remained uncertain due to gaps in data, unclear pressure-state relationships, and the non-linear influence of some pressures on biodiversity indicators (McQuatters et al., 2022). The next assessment is due in 2023, as OSPAR's Quality Status Report 2023 (QSR 2023).

To aid the biodiversity assessment for the QSR 2023, the EU-funded project NEA PANACEA ("North-East Atlantic project on biodiversity and eutrophication assessment integration and creation of effective measures") gathered 8 partners from 5 OSPAR Contracting Parties (Germany, France, the United Kingdom, Spain and the Netherlands) to collaborate to deliver biodiversity assessments specifically on pelagic habitats, benthic habitats, food webs and marine birds (https://www.ospar.org/about/projects/nea-panacea). Project partners aim to develop new biodiversity indicators and to improve existing ones, working on existing gaps such as data flow, indicator operability, expansion of geographical coverage or the development of threshold values. As a follow up of the EcApRHA project, NEA PANACEA also addresses best ways to integrate multiple indicators to deliver a single integrated assessment of a specific ecosystem component, in our case the most interesting is pelagic habitats.

Activity 1 of NEA PANACEA project is dedicated to pelagic habitats. Apart delivering the assessment of pelagic habitats, the NEA PANACEA expert group aims to increase the spatial scale and both temporal and taxonomic resolution of the indicators PH1, PH2 and PH3, improve indicator operability and provide improvements of the data ingestion protocols involved. Activity 1 is comprised of the following tasks: i) Task 1.1 - Expanding data coverage and developing data tools to support robust assessment; ii) Task 1.2 - Refinement, operationalisation, and assessment of PH1/FW5; iii) Task 1.3 - Refinement, operationalisation and assessment of PH2 and PH3; iv) Task 1.4 - Integration within and across pelagic indicators; and v) Task 1.5 - Linking pelagic indicators with food web indicators and their connection to other ecosystem components and MSFD-descriptors. At current, the NEA PANACEA experts are comparing temporal trends of the pelagic habitat indicators, however no thresholds have been set up to now (Lisette Enserink, personal communication).

2.2 HELCOM area

Monitoring is a well-established function of the Helsinki Convention. Coordinated monitoring of physical, chemical and biological variables of the open sea of the Baltic Sea has been carried out since 1979. The HELCOM Monitoring and Assessment Strategy (MAS) was adopted in 2013 to support an indicator-based monitoring and assessment approach and a regionally coordinated implementation of the Baltic Sea Action Plan (BSAP) and the MSFD. The objective of MAS is to establish a Joint Monitoring System, which enables to use regionally agreed and coordinated principles in national monitoring programmes to achieve a high degree of coordination, cooperation, sharing and harmonization of monitoring activities and data.

The monitoring activities are organized under 12 monitoring strategies where ecosystem elements or anthropogenic pressures are grouped thematically (https://helcom.fi/wp-content/uploads/2020/10/Introduction-to-the-HELCOM-Monitoring-Manual.pdf). Monitoring of phytoplankton as an ecosystem element is included in monitoring strategy “Water column habitats”.

HELCOM is currently carrying out the third holistic assessment (HOLAS III) of the Baltic Sea, covering the period 2016-2021. The results are expected to be published in 2023. In the Thematic assessment of biodiversity 2011–2016 (HELCOM, 2018a) made for HELCOM Second Holistic Assessment of the Ecosystem Health of the Baltic Sea (HOLAS II) the pelagic habitat was assessed by the following indicators:

Open sea areas:

- **“Zooplankton mean size and total stock”** (Biodiversity core indicator. Applied in open sea.)
- **“Chlorophyll-a”** (Eutrophication core indicator reflecting total pelagic primary production. Applied in the open sea.)
- **“Cyanobacterial Bloom Index”** (Eutrophication pre-core indicator included as test. Applied in open sea.)

Coastal areas:

- “Chlorophyll-a” (National results for the metric derived from the implementation of the Water Framework Directive 2000/60/EC were used for coastal areas.)
- “Phytoplankton biovolume” (National results for the metric were used for coastal areas).

Some indicators are still under development and their results are presented descriptively for some sub-basins. These indicators include:

- **“Diatom/dinoflagellate index”**
- **“Phytoplankton biomass or biovolume”**
- **“Seasonal succession of dominating phytoplankton groups”**

Cyanobacterial bloom index (HELCOM, 2018b)

The Cyanobacterial bloom index (CyaBI) is a pre-core indicator, and its threshold values are yet to be commonly agreed in HELCOM. It is included as a test indicator for the purposes of the 'State of the Baltic Sea' report 2018. The indicator primarily describes the symptoms of eutrophication in the open sea areas, caused by nutrient enrichment and is operational in 10 open-sea sub-basins (HELCOM, 2018b). Extensive cyanobacterial blooms may also have a negative impact on the biodiversity of both the pelagic and benthic communities. CyaBI is thus used in the assessments of eutrophication and biodiversity in the Baltic Sea.

The index evaluates the accumulation of cyanobacteria in the surface waters and the biomass of cyanobacteria during summer. It uses assessment unit specific threshold values that are presented as normalized values. The indicator is based on two parameters:

1) *Cyanobacterial surface accumulation (CSA)* which combines information of volume, length of bloom period and severity of surface accumulations estimated from remote sensing observations. It relies on high-frequency data, and is optimal for describing the bloom formation at the surface. This parameter is strongly influenced by eutrophication and by climate-related variation including wind conditions. The main data source used to develop the indicator is satellite data derived from the daily algal bloom product of the Finnish Environment Institute, which is based on chlorophyll-a and turbidity products. These observations were interpreted to estimate the potentiality of surface algae accumulations in four classes (0 - no, 1 - potential, 2 – likely and 3 – evident). The spatial aggregation of daily Earth Observation from the assessment units is conducted by calculating an algae barometer value. The algae barometer (AB) value is a weighted sum of the proportion of positive algae observations in the different classes in an assessment area. Seasonal bloom characteristics were estimated using an empirical cumulative distribution function (ECDF) drawn from seasonal observations of daily algae barometer values from each assessment area. These bloom characteristics are defined as i) seasonal volume, i.e., the areal coverage above the ECDF functions; ii) length of algal surface accumulation period, i.e., the percentage of observations with algae barometer values above zero; and iii) bloom severity, i.e., the 90-percentile of the algae barometer observations (HELCOM, 2018b). The CSA index is derived by taking an average from the normalized time series of the indicative variables and grouping all the three EO-based parameters together. As the indicator responds negatively to increased eutrophication, 1 represents the best conditions and 0 the worst.
2) *Cyanobacterial biomass* in the water column analysed from in-situ observations. It supplements CSA by providing information of the actual amount of cyanobacteria in the water column. Due to less frequent monitoring, neither the status evaluation nor the threshold values of cyanobacteria biomass have sufficient confidence to stand alone as a HELCOM core indicator. Cyanobacteria biomass is analysed in water samples using microscopy techniques. The data includes biomass analyses in wet weight (µg/L) of integrated water samples. Genera included in the index are: *Nodularia*, *Aphanizomenon* and *Dolichospermum* (previously *Anabaena*). At least one sample per month must be available to allow the calculation of the seasonal average.

A threshold value is set for each parameter for each assessment unit, and the combined indicator threshold value is an average of the two. If one of the parameters is not applicable in a specific assessment unit, then only one parameter is used as the threshold value. Values that are above the threshold value indicate good status. The threshold values for the assessment units are derived separately for the two long-term datasets used for the two indicator parameters. For CSA, thresholds are based on the independent satellite-based time series on algae accumulations, while for biomass data on in-situ observations of cyanobacteria biomass are used. The threshold values are derived by combining statistical analysis of long-term data with expert judgement. The main concern in proposing threshold values is the lack of sound and consistent historical data. Since cyanobacterial blooms are a natural phenomenon in the Baltic Sea, the goal was to identify time periods with low bloom intensity although the status was already impacted by eutrophication, but the bloom intensity was low. To distinguish such periods, the shift detection method (Rodionov, 2004) was used or, if no such periods were distinguished, the averages of separate years with lower bloom intensity were calculated using the quartile method.

The confidence of the status estimate was not assessed in absence of methodology to define status confidence, so further development of this indicator is underway.

Diatom/dinoflagellate index (HELCOM, 2018c)

The Diatom/Dinoflagellate index (Dia/Dino index) is a pre-core indicator, and its threshold values are yet to be commonly agreed in HELCOM. As such, it is included as a test indicator for the purposes of the 'State of the Baltic Sea' report. Dia/Dino index reflects the dominance patterns in the phytoplankton spring bloom and has a high relevance for the pathway of the food into the pelagic or benthic food web. The relative abundance of diatoms and dinoflagellates is influenced by changes in trophic state as well as by climate change (Wasmund et al., 2017a, b).

The evaluation is based on phytoplankton data in the spring period from March to May, from the upper mixed layer (HELCOM, 2018c). Precondition for a valid calculation is a check whether the spring bloom is sufficiently represented in the data, which is achieved when the biomass of diatoms or dinoflagellates at least once exceeds a threshold of 1000 µg/L. If not, the calculation of an alternative Dia/Dino index, based on silicate consumption, may be applied, as described by Wasmund et al. (2017a). Temporal trends, showed by smoothed curves, provide additional information on the spread and variability of the Dia/Dino index.

The calculation of Dia/Dino index requires that the biomass of planktonic diatoms is divided by the biomass of autotrophic (+ mixotrophic) dinoflagellates. To set the range of this indicator between 0 and 1, the ratio is calculated as follows: Dia/Dino index = biomass of diatoms / sum of biomass of diatoms and dinoflagellates. The data requirements are: i) to have a sample representative of the upper mixed water layer, ii) data should include only the autotrophic (plus mixotrophic) components of the pelagic community, iii) the biomass should be expressed as wet weight (for explanation see the Guidelines for monitoring of phytoplankton species composition, abundance and biomass (2021) at https://helcom.fi/wp-content/uploads/2020/01/HELCOM-Guidelines-for-monitoring-of-phytoplankton-species-composition-abundance-and-biomass.pdf), iv), index refers only to the spring season (use of seasonal mean values), and v) spring biomass maxima of diatoms or dinoflagellates have to exceed a threshold. The good status is defined at a Dia/Dino index > 0.5. Authors claim that this indicator is robust, with simple and traceable calculation. The confidence of the indicator evaluation depends on the data frequency.

Phytoplankton biomass or biovolume

In some coastal areas of the Baltic Sea the direct effects of eutrophication are assessed by national WFD indicator phytoplankton biovolume, apart from the Chlorophyll-a (HELCOM, 2018d). This assessment is use also for the State of the Baltic holistic assessment. For example, in Swedish coastal waters, phytoplankton biovolume is determined from the biomass of autotrophic and mixotrophic phytoplankton and expressed as the mean value from integrated samples (0-10 m) or discrete samples (0, 5 m) if water depth is < 12m. Data from deviating sampling depths can be corrected to represent the above intervals and depths. Assessment period is June to August.

Seasonal succession of dominating phytoplankton groups (HELCOM, 2018e)

This indicator and its threshold values are yet to be commonly agreed in HELCOM, thus it is used as a test indicator for the purposes of the 'State of the Baltic Sea' report. In future, it is expected that this indicator will contribute to an overall food webs assessment, along with the other biodiversity core indicators (HELCOM, 2018e). The concept for evaluating GES using the succession of dominant phytoplankton groups is based upon the succession under reference conditions and an acceptable deviation from it. Changes in the presence of specific phytoplankton groups or the timing of their abundance/biomass peak may alter the availability of food/carbon for higher trophic levels (e.g., zooplankton) and can thus have important influence on food web structure. Moreover, the sedimentation of detritus (e.g., dead phytoplankton) can influence the microbial loop and ecosystem balance (e.g., heterotrophy-autotrophy) and the physicochemical state of the ecosystem (e.g., oxygen concentration).

The indicator evaluates the coincidence of seasonal succession of dominating phytoplankton groups over an assessment period using regionally established reference seasonal growth curves and wet weight biomass data. The indictor result value is based on the number of data points falling within the acceptable deviation range set for each monthly point of the reference growth curve and expressed as the percentage to the total number of data points. This result value is then compared to regionally relevant threshold values established to represent acceptable levels of variation. Strong deviations from the reference growth curves will result in failure to meet the thresholds set for acceptable variation, indicating impairment of the environmental status and a failure to meet good status. Since values for individual months are independent, the evaluation of status is still feasible even if some data points for some months are missing. The final evaluation is based on the average score of single dominant groups. This indicator may also be used as background data for the development of a modified lifeform approach in the monitoring and environmental assessments in the HELCOM area.

The method of deriving the reference growth curves of phytoplankton group is referred to the original description of Devlin et al. (2007). Water type or site-specific seasonal growth curves can be designed for each dominating phytoplankton group. In the 2011-2016 assessment period, the phytoplankton groups used were cyanobacteria, dinoflagellates and diatoms plus the ciliate *Mesodinium rubrum*. Briefly, the method of deriving the curves is: i) the transformation of phytoplankton biomass on a natural log scale; ii) overall and monthly means and standard deviations are calculated for each functional group over a reference period; iii) monthly Z scores are calculated; iv) acceptable deviations for monthly means (reference envelopes) are calculated as $z_{month} \pm 0.5$. The indicator value is based on the number of data points from the test area, which fall within the acceptable deviation range that has been set for each month of the reference growth curve. Percentage-based thresholds are established for each dominating group to determine index values for the assessment of the ecological status.

In the Baltic Sea, it is particularly difficult to find historical data from unaffected ecosystems and thus define a suitable reference period and, consequently, threshold values. Preliminarily, the proportion of observations with acceptable deviations in monthly biomass indicating normal seasonal succession of phytoplankton was set at ≥0.67 for all tested areas. Subsequently, all data were analysed to detect periods with lower total biomass and lesser year-to-year fluctuations to develop basin-specific threshold values, which yielded different reference period for each of the 12 assessment units and threshold values varying from 0.58 to 0.74. The requirement for the length of the reference period is 10 years, while the test period is commonly 5-6 years.

Where applied, the confidence of indicator status is moderate to high according to temporal and moderate according to spatial resolution. Confidence level depends on the length of the time-series and regularity of phytoplankton sampling during the growth period. This indicator should be applicable in all coastal and open sea around the Baltic Sea. To account for spatial differences in phytoplankton community composition and environmental gradients, further development and agreement related to appropriate threshold value setting is required.

The above-described indicators are used for the integrated assessment of biodiversity using the HELCOM BEAT tool. The tool integrates individual indicator results into estimates of the overall status of each ecosystem component and assessment unit (HELCOM, 2018a).

Biodiversity assessment is at the core of the HELCOM BLUES project "HELCOM Biodiversity, Litter, Underwater noise and Effective regional measures for the Baltic Sea", an EU funded project led by HELCOM and involving 14 partners from six Baltic countries. One of the projects' seven activities is dealing with improved regional assessment of biodiversity and aims, amongst other, to develop operational indicators for zooplankton and phytoplankton, including threshold values and Pan-Baltic coverage and deliver an approach for a more informative, integrated assessment of pelagic habitats. Two subtasks of the HELCOM BLUES Activity 2.3 dealt with phytoplankton (https://blues.helcom.fi/wp-content/uploads/2023/01/A2.3_Pelagic-habitat.pdf):

1) HELCOM BLUES Subtask 2.3.2 aimed at complete operationalisation of the HELCOM Seasonal succession of dominating phytoplankton groups indicator. The results: increased spatial coverage of the indicator, which is now operationalised in 10 Baltic Sea sub-basins. However, data availability hampers the operationalisation in the remaining 7 sub-basins.
2) HELCOM BLUES Subtask 2.3.4 aimed at evaluating the unified pelagic assessment approaches and developing a viable assessment in the Baltic Sea. The results: a pilot study was conducted for the use of OSPAR PH1/FW5 indicator Plankton lifeforms in three sub-basins. Main challenges that were identified: specific lifeform pairs for the Baltic Sea need to be established, lower sampling frequency as compared to OSPAR region, since winter-spring data are rare.

An additional aim of the activity (HELCOM BLUES Subtask 2.3.3) was to develop an approach to combine operationalised indicators. Here, a one out all out principle (OOAO) was decided for the biodiversity state, where Zooplankton MSTS have is one component (weight 1.0) and phytoplankton component is composed of Phytoplankton seasonal succession indicator with the weight of 0.6 and CyaBI with the weight of 0.4. In HOLAS III, the Dia/Dino index is dismissed, since it was no more sufficiently supported by the development advances (Elena Gorokhova, personal communication).

Key messages from the final conference of the HELCOM BLUES project were (https://blues.helcom.fi/wp-content/uploads/2023/01/A2.3_Pelagic-habitat.pdf): there is a need to understand the relative importance of anthropogenic pressures in comparison to climate-related ones, since there are indications of profound changes in the pelagic food web; and there is a need for indicators based on growth and production to aid a mechanistic understanding behind these changes.

2.3 Mediterranean Sea

Updated review of phytoplankton indicators

The Mediterranean Sea is the largest semi-enclosed European sea divided by MSFD into several sub-regions: Western Mediterranean, Ionian and Central Mediterranean, Adriatic Sea, and Aegean-Levantine Sea (Eastern Mediterranean) (Source: www.emodnet-chemistry.eu). The Mediterranean Sea is generally oligotrophic, with nutrient limitation increasing from west to east, mostly in the form of phosphorus limitation. It has the heterogeneous distribution of primary production and a decreasing west-east gradient of chlorophyll-a concentration (D' Ortenzio and Ribera d' Alcalà, 2009), while some coastal areas have higher chlorophyll-a concentrations, generally related to river inputs. The Mediterranean is a site of intense human activities whose impact on the marine environment is not easy to assess and quantify. The wide range of climatic and hydrological conditions in the Mediterranean Sea are reflected in the structure and dynamics of plankton communities (Neri *et al.*, 2023; Casabianca *et al.*, 2022; Francé *et al.*, 2021; Siokou-Frangou *et al*., 2010; Varkitzi *et al*. 2018a).

European Commission (EC) Decision 2013/480/EU considered chlorophyll-a as the only classification criterion for Biological Quality Element (BQE) Phytoplankton as a result of work of the intercalibration exercise of WFD Mediterranean Geographical Intercalibration Group (Med-GIG). 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, under 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.

The use of chlorophyll-a as a condition indicator has many advantages: both spectrophotometric and fluorimetric analytical methods are time- and cost-efficient and reproducible, and results are easily comparable between different data sets (Domingues *et al.,* 2008). Given the wide range of phytoplankton cell sizes, phytoplankton biomass can be underestimated or overestimated. Carbon biomass or biovolume have been proposed to overcome this limitation (Cozzoli *et al.,* 2017), although data series containing either of these parameters are extremely rare in coastal waters.

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. 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 diversity components (five are operational, one is under development and one is without status). For Eastern Mediterranean Sea, 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 are very heterogeneous in terms of abundance, characteristics of the study areas, sampling strategy, methodology, and taxonomic determination, making it very difficult to compare large-scale seasonal and spatial patterns and cycles and to draw conclusions. Another aspect to consider is the relationship between phytoplankton indicators and associated habitat types, as well as for zooplankton (MSFD Task Group 1 Report, Teixeira *et al*., 2014). Under the concept of keystone species

(Menge *et al*., 2013), taxon-specific indicators are considered important (at the genus or species level). However, there are no taxon-specific indicators for phytoplankton (along with microbes), unlike zooplankton (biomass of the ctenophore *Mnemiopsis leidyi*), phytobenthos (depth distribution of *Posidonia oceanica*), and other biological elements (Smith *et al.,* 2014).

So far, plankton indicators mostly refer to Mediterranean coastal waters with specific case studies, e.g., in the Adriatic and Aegean Seas, and their development is always associated with environmental pressures (Markogianni *et al*., 2017; Ninčević-Gladan *et al.,* 2015; Spatharis and Tsirtsis, 2010; Varkitzi *et al.,* 2018b, Francé *et al*. 2021).

As part of the WISER and ActionMed projects, Cozzoli *et al.* (2017) tested some commonly used indices/metrics and showed that some metrics are strongly dependent on sampling effort (as number of individuals counted per sample), while others are relatively independent. Size-based metrics (IVD-mean', ISS-phyto'), characterised by high precision and low uncertainty, could generally provide greater accuracy than taxonomic metrics for describing the community and are capable of achieving acceptable accuracy at sample sizes of less than 200 individuals counted. Size-based metrics also have the advantage of being sensitive to environmental stress, minimising the problem of required taxonomic expertise, and allowing for quantitative intercalibration procedures (Carvalho *et al.,* 2013).

Dominance metrics have similar accuracy to size-based metrics (Cozzoli *et al*., 2017). For example, Berger-Parker's dominance index proved to be an efficient metric that focuses only on the most easily identifiable common taxa and, like other dominance indices (Facca and Sfriso, 2009), is sensitive to environmental conditions. Therefore, Cozzoli *et al.* (2017) suggest that the use of size (as in ISS-phyto') and dominance metrics (as in MPI'), alone or combined in multimetric indices, could be an efficient approach to implement operational monitoring, able to maximise precision and minimise uncertainty in estimates. According to this work, these metrics can provide reliable environmental assessments using a sampling effort (in terms of individuals counted per sample) that is 50% lower than the 400 cells required by the most commonly used international standard.

In their review, Varkitzi *et al*. (2018a) proposed a subset of indicators for each pelagic biodiversity component to be tested for GES determination of water column habitat in the Mediterranean Sea. Apart from chlorophyll-a, the following assessment methods were proposed for phytoplankton:

1. size-related metrics, such as the multimetric index of size spectra sensitivity ISS-phyto (Vadrucci *et al.,* 2013), because of its high accuracy, low uncertainty, and relative ease of sample processing (Cozzoli *et al*., 2017).
2. diversity and dominance metrics such as the Shannon-Wiener diversity index (Shannon, 1948) because of its high accuracy and the Berger-Parker dominance index (Berger and Parker, 1970) because of its high accuracy, low uncertainty, and focusing on the most abundant taxa (Cozzoli *et al*., 2017).
3. Bloom Frequency Index (Facca *et al*., 2014) to measure the dominance of a species during an algal bloom.

Francé *et al.* (2021) address the biodiversity, structure, and function of pelagic habitats in the Mediterranean Sea, all defined by criterion D1C6, by comparing a series of ecological indices calculated from phytoplankton community composition datasets. The selected datasets in this large-scale case study cover three sub-regions of the Mediterranean Sea (Adriatic Sea, Ionian Sea and Central Mediterranean Sea, Aegean-Levantine Sea, all of which share the broad pelagic habitat type of coastal waters. The study addresses biogeographic differences in the phytoplankton community at the mesoscale level (~100 km), as the different case study areas are distributed along a gradient with different trophic conditions, i.e. from the mesotrophic northernmost part of the Adriatic Sea to the oligotrophic coasts of the eastern Adriatic Sea, and from the mesotrophic to oligotrophic conditions of the coasts of Apulia, Italy to the oligotrophic coastal waters of Greece (Aegean Sea). In addition, the gradients of different pressures and eutrophication regimes are represented on a small scale (submesoscale, ~10 km), that is in each case study area. The Member States contributing the datasets are Croatia, Greece, Italy, and Slovenia. The study explores the behaviour of a set of eight alpha diversity indices against four impact levels defined categorically upon expert knowledge:

1. Taxonomic Richness R’ (as number of taxa)
2. Margalef’s Diversity Index M’
3. Shannon - Wiener’s Diversity Index H’
4. Simpson’s Diversity Index S’
5. Pielou’s Evenness Index E’
6. Sheldon’s Evenness Index Sh’
7. BergerParker’s Dominance Index BP’
8. McNaughton’s Dominance Index McN’

At the level of the entire dataset, most of the indices could only distinguish between the highest level of impact and the rest of impact categories (Francé *et al*., 2023). 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 parts of the study area (i.e., northern Adriatic) than in the southernmost and easternmost areas (i.e., Aegean Sea), although they still showed a significant response. The authors conclude with emphasising the need to establish spatially specific thresholds by additional examination of indices of good performance.

Neri *et al*. (2023) tested phytoplankton indices to discriminate between coastal and offshore stations, highlighting that a combination of indices (Shannon Diversity, Pielou’s Evenness, Rarefied Richness) and descriptors (multivariate statistical analyses) is necessary for the GES assessment in different areas allowing to gain insight in the functioning of different ecosystems and forcings affecting phytoplankton communities. Furthermore, they proposed the use of other indicators, i.e., graph-network analyses and IndVal (Indicator Value analysis) to highlight the changes in the community composition and structure. Moreover, considering the marked seasonal variability of phytoplankton abundance, biomass, and community composition, the development of indicators and descriptors on a seasonal base is recommended (Neri *et al*., 2022, 2023).

Frequency of Harmful Algal Blooms (HABs)

Some species of phytoplankton cause harmful algal blooms (HABs). Phytoplankton blooms play a central role in assessing the ecological status and are of great policy importance to the Water Framework Directive (WFD) and MSFD. However, one of the major challenges in their practical application is the need for data whose frequency corresponds to the spatial and temporal scales of phytoplankton variability. To date, there are no operational indicators of HABs associated with D1 in the Mediterranean MS (Varkitzi *et al*., 2018). In the eutrophication context, Ferreira *et al.* (2011) recommended that HABs should be treated as one of the MSFD indicators of eutrophication if, but only if, HAB frequency, amplitude, or toxicity levels increase in response to nutrient inputs. Parallel to this approach, HAB-related indicators for D1 could be operational only if the occurrence and expansion of HAB species are shown to be significantly related to the status of biodiversity in a habitat.

The open waters of the Mediterranean are not threatened by significant HAB events due to their oligotrophic nature, but during 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 common in Mediterranean coastal waters, also those of *Pseudo-nitzschia* spp. (Turk Dermastia *et al*., 2020). Garcés and Camp (2012) mention some "hot-spot" regions such as the Alboran, Ligurian, Adriatic, and Aegean Seas. Although *Pseudo-nitzschia* blooms are frequently recorded and widespread, domoic acid in shellfish rarely exceeds regulatory levels (Zingone *et al.,* 2021).

In a recent study examining harbour environments throughout the Adriatic Sea, Mozetič *et al.* (2019) found 52 HAB taxa, including some toxic non-native phytoplankton species of the potentially invasive behaviour (*Pseudo-nitzschia multistriata*, *Ostreopsis* sp. and *O.* cf. *ovata*). Checklists of harmful species exist for the eastern Adriatic coast based on long-term data series from monitoring of the Adriatic Sea (Ninčević Gladan *et al*., 2020), for the Tyrrhenian Sea (Zingone *et al.,* 2006), from reviews of HABs in Greek coastal waters (Ignatiades and Gotsis-Skretas, 2010) and in the northwestern Mediterranean (Vila and Masó, 2005). Nevertheless, according to a recent review (Zingone *et al.,* 2021) toxicity-related events are not frequent in the Mediterranean Sea, and mainly consist of impacts on aquaculture, caused by the dinoflagellates *Dinophysis* spp. and *Alexandrium* spp., along with a few actual shellfish poisoning cases. In some areas, high biomass blooms that cause discoloration of water also have significant detrimental effects on coastal biodiversity. They are mainly caused by dinoflagellates (e.g., *Noctiluca scintillans*, *Prorocentrum* and *Alexandrium* species), prymnesiophytes (*Phaeocystis* spp.), *Vicicitus globosus* and some prasinophytes (e.g., Ignatiades and Gotsis-Skretas, 2010, Penna *et al*., 2015; Zingone *et al*., 2006, 2021).

UNEP MAP - Ecosystem Approach (EcAp) and its Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP)

The EU MSFD commits Member States to cooperate and integrate other conventions, such as the Barcelona Convention, which adopted the Integrated Monitoring and Assessment Programme for the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) (UNEP/MAP, 2016). The IMAP describes the strategy, issues, and products that the Contracting Parties intend to deliver in the second cycle of implementation of the Ecosystem Approach Process (EcAp process 2016-2021) to assess the state of the Mediterranean Sea and coasts. One of the main outcomes of this process is that the IMAP covers the Ecological Objectives related to biodiversity (EO1) according to D1 of the MSFD. Biodiversity common indicators, relate to two indicators for pelagic habitats, namely:

1. Common indicator 1 (CI1): Habitat distributional range (E01), to include the extent of habitat as a relevant attribute, and
2. Common indicator 2 (CI2): Condition of the habitat's typical species and communities (E01).

The prerequisite to address the CI1 is the adoption of the common reference list of pelagic habitat types, which aims to facilitate reporting of habitat data in a comparable manner, for use in nature conservation (e.g., inventories, monitoring and assessments), habitat mapping and environmental management. In the preparation of such a reference list, UNEP/RAC/SPA adopted an approach of considering the distribution of primary productivity in terms of Chl-a concentrations in combination with the reporting guidance under the MSFD (European Commission 2012), which already considers a simplification of EUNIS classification (UNEP/RAC/SPA, 2013). Such an approach is based on the assumption that the distribution of food/prey for species groups addressed by the EcAp process (marine mammals, birds and reptiles) is determining the choice of habitat by such species. Since this assumption needs to be verified and will most likely not hold for every species, UNEP/RAC/SPA is working further on defining the operable reference list of pelagic habitats in the Mediterranean. This is needed in a near future for the implementation of the EcAp roadmap and its IMAP in particular for the preparation of the Mediterranean Quality Status Report in 2023 (MedQSR 2023). The initial draft reference list of pelagic habitat types in the epipelagic layer (0 – 200 m) of the Mediterranean Sea is presented in Table 1. Except for A.6, all the habitat types listed above can be detected by satellite, which makes the proposed classification practically amenable to continuous monitoring over the whole Mediterranean region.

Table 1: Tentative list of pelagic habitat types in the epipelagic layer (0 – 200 m) of the Mediterranean Sea as defined by UNEP/RAC/SPA (from UNEP/RAC/SPA, 2013)

	Habitat type	**Description**
A.1.	Reduced salinity water	coastal lagoons
A.2.	Variable salinity water - high surface Chl-a (> 3mg/m^3)	estuaries, river plumes
A.3.	Marine water: neritic - medium surface Chl-a (0.5-3mg/m^3)	upwellings, re-suspension in shallow waters and outskirts of river plumes
A.4.	Marine water: oceanic - medium surface Chl-a (0.5-3mg/m^3)	upwellings
A.5.	Marine water: oceanic - low surface Chl-a (~ 0.1-0.5mg/m^3)	Chl-a fronts (whatever type of horizontal gradient of Chl-a, thus including e.g., gyres)
A.6a.	Marine water: oceanic - very low surface Chl-a (< 0.1mg/m^3) with subsurface Chl-a maximum	euphotic depth > mixed layer depth
A.6b.	Marine water: oceanic - very low surface CHL (< 0.1mg/m^3) without subsurface Chl-a maximum	euphotic depth < mixed layer depth

In addition to the tentative list of habitat types in the epipelagic domain, IMAP document provided representative sites and species to include in the monitoring programs of the Mediterranean countries (Annex 1, UNEP/MAP, 2016). Key features from this Annex related to pelagic habitats are listed in Table 2, this minimum list of monitoring requirements contain just notions to monitor phytoplankton and zooplankton communities, of which priority 1 was given to plankton communities in coastal waters, while for shelf and oceanic waters the priority still needs to be defined. However, the list of pelagic habitat types for the Mediterranean Sea is still not conclusive and needs further collaborative work.

Table 2: Minimum reference list of species and habitats for monitoring programs in the part related to pelagic habitat types of the Mediterranean Sea (from UNEP/MAP, 2016)

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

ABIOMMED partners are in contact with SPA /RAC colleagues and are looking for opportunities to link the IMAP policy to work already being done under Activity 2 on MSFD, particularly for pelagic habitats (phytoplankton and zooplankton) for EO1.

3 Strengths and weaknesses of selected phytoplankton indicators

In general, plankton communities bear the potential to display a footprint of environmental changes at an early stage, since their response is rapid due to short life cycle. Therefore, many phytoplankton and zooplankton indicators could be used as "early warning indicator" of environmental changes and as a sentinel of changes happening in the food webs and ecosystems (UNEP/MAP, 2016). In the long process of developing the environmental assessment systems with plankton diversity indicators there are several obstacles that have to be overcome. First, the necessary step of linking the response of plankton communities to human pressures is usually difficult to accomplish, since this linkage is often non-linear (Francé *et al.,* 2021; Ninčević Gladan *et al.,* 2015). Besides this, the constrains for the wider use of such indicators for the assessment of environmental status largely relate to the difficulty in establishing the reference conditions and environmental objectives for these indicators (Garmendia *et al*., 2013). Moreover, the applicability of diversity indices to assess the status of the marine environment in a management context depends on the objective of the study, their ecological relevance, the mathematical properties of a certain index, and ease of interpretation by stakeholders (OSPAR, 2017c).

On the other hand, the main advantages of using diversity indices are their advanced development within the scientific literature and their ease of calculation (OSPAR, 2017c). In the case of phytoplankton community, the diversity indices based on abundance and richness are generally calculated on the entire plankton community, which includes also heterotrophic species and can provide additional information for assessing pelagic habitats (Domingues et al., 2008) in contrast to using solely indicators based on chlorophyll-a. However, the integration of chlorophyll-a with diversity data may provide an even better understanding of environmental conditions, because the inclusion of additional metrics can increase the sensitivity of an index (Garmendia *et al.,* 2013).

In the Table 3 selected phytoplankton indicators are presented and indicator groups that are and could be used for the assessment of the pelagic habitat in the Mediterranean Sea, together with their strengths and drawbacks.

Table 3: Selected indicators and indicators groups for the assessment of pelagic habitat in the Mediterranean Sea using phytoplankton component in the Mediterranean Sea with their strengths and weaknesses (modified from Varkitzi *et al*., 2018)

Indicators	Strengths	Weaknesses	References
Chlorophyll-a	- availability of data - easy to measure - reliable data - well intercalibrated parameter - specified GES in Mediterranean MSs - possibility of using satellite remote sensing data	- provides no information on the community structure/diversity	Commission Decision 2018/229/EU
Size-related metrics, e.g., ISS-phyto	- high precision - low uncertainty - no need for taxonomic expertise	- size is rarely defined in monitoring programs - standardization needed for measurements and 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	- outcome strongly dependent on taxonomic skills of the operator - taxonomic intercalibration needed - non-linear relationship with environmental stressors	Cozzoli *et al*. (2017) Facca and Sfriso (2009); Francé *et al*. (2021); Neri *et al.* (2023)
Dominance-related metrics for algal blooms, e.g., Bloom frequency index	- high accuracy - focus only on the taxa with > 50% relative abundance	- only applicable in more eutrophic systems, where microalgal bloom tend to develop - as above	Cozzoli *et al*. (2017); Facca *et al*. (2014)
Changes plankton lifeform pairs (OSPAR PH1)	- many lifeform pairs available - both abundance and biomass data can be used - high spatial intercomparability	- questionable availability of data in the Mediterranean Sea - questionable applicability for the Mediterranean Sea	McQuatters-Gollop *et a*l., 2019; OSPAR (2022)
Changes in plankton diversity (OSPAR PH3): alpha and beta diversity indices	- identification at the genus level - accurate information on community diversity	- outcome dependent on taxonomic skills of the operator - non-linear relationship with environmental stressors	Tett *et al*. (2008); McQuatters-Gollop *et al*. (2015); OSPAR (2017b)
Seasonal succession of dominating phytoplankton groups (HELCOM)	- no need for detailed identification	- High seasonal variability may pose a difficulty to establish reference envelopes	HELCOM (2018e)

4 Recommendations for development and use of pelagic habitat indicators

4.1 MS specific criteria for determining GES for D1C6

Among Mediterranean MSs, GES is mainly provided at the general level addressing habitat characteristics and diversity of pelagic organisms at a broad. If thresholds are defined, they are only valid for individual indicators (such as Chl-a). An additional challenge is posed by the fact that the status of pelagic habitat has to be assessed as the extent of habitat adversely affected in km^2 or as % of the total extent per habitat type. MSs data hardly supports such an evaluation since monitoring covers only a limited selection of sites.

Up-to-date information on monitoring pelagic habitats in the ABIOMMED project partners' MSs are presented in Table 4.

Table 4: Pelagic habitat elements monitored in MSs and related GES definitions.

Member State	Pelagic habitat component monitored	Type of data, frequency of sampling	GES definition
Slovenia	- Chl-a concentration - Phytoplankton (species diversity and abundance)	- Fixed stations, monthly sampling	Conceptual GES definition is provided at the general level: 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.
Croatia	- Chl-a concentration - phytoplankton (species diversity and abundance) - zooplankton (species diversity)	- Fixed stations, monthly/ seasonal sampling	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.
Italy	- Chl-a concentration - phytoplankton (species diversity, abundance, and biomass) - zooplankton (species diversity, abundance, and biomass)	- Fixed stations, monthly/ seasonal sampling	Conceptual GES definition is provided for plankton communities at the indicator 1.6.2. level: 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. In this regards, long term dataset analyses are very useful to establish baselines.
Greece	- Chl-a concentration - phytoplankton (species diversity and abundance)	- Fixed stations, monthly/ seasonal sampling	Conceptual GES definition is provided for plankton communities: The effects of human activities on the structure of plankton communities are reduced to minimal levels.

Member State	Pelagic habitat component monitored	Type of data, frequency of sampling	GES definition
	- zooplankton (species diversity, abundance, and biomass)		
Spain	- Chl-a concentration - phytoplankton (abundances per groups) - zooplankton (species diversity)	- Fixed stations, monthly/ seasonal sampling	The definition of GES is based on the analysis of the temporal evolution (long-term trends and seasonality) of the proposed indicators, monitored in the different spatial domains in which it is possible to divide the pelagic environment based on physiographic and oceanographic characteristics: coastal, shelf and continental margin, oceanic, particular landscapes - estuaries, canyons and submarine mountains, epipelagic and mesopelagic.

Commentary on Table 4:

<u>Slovenia:</u> Quantitative baselines and thresholds for pelagic habitat were defined for Chl-a as the annual geometric mean of concentrations in the surface water layer and for the shift in the composition of phytoplankton species or groups calculated by the index of high abundances. The latter were defined for the assessment of environmental status according to D5 Eutrophication. Thresholds defined for Chl-a are equal to those defined for coastal waters under WFD. Based on monitoring data that is currently dismissed, thresholds were defined also for zooplankton as multiannual geometric mean of dry weight. Additionally, a qualitative baseline was defined for the frequency of jellyfish occurrence. As for current monitoring program, zooplankton is neither monitored nor its status assessed while phytoplankton community composition and abundance are followed monthly at one sampling station but currently not assessed.

<u>Croatia:</u> Quantitative baseline and threshold values for pelagic habitats were defined for Chl-a as the annual geometric mean and 90th percentile of chlorophyll-a concentrations in the surface water layer according to the WFD intercalibration technical report. These limits are used to assess ecological status with respect to eutrophication under descriptor D5. Expert assessment of ecological status is based on the species diversity of the phytoplankton community within descriptor D1 using diversity indices and the proportion of the most numerous taxonomic groups in the total abundance.

<u>Italy:</u> There is a high spatial coverage along the Italian coast that is currently covered by the monitoring program. The sampling scheme covers transects of three sampling station from on-shore to off-shore (up to 12 NM from the coast). However, beside the information derived from recent monitoring, long term dataset analyses are very useful to establish baselines.

<u>Spain:</u> Spain actively participated in the report of Pelagic habitats under MSFD D1: current approaches and priorities, where the main difficulties of the proposed indicators were exposed (Magliozzi *et al*., 2021). This report emphasizes the importance to account for the spatial and temporal scales of pelagic processes to infer conclusions about dominant pressures and status expressed as a proportion of sea surface area. Among the conclusions, we emphasize that long-term species data alone are not sufficient to assess changes in the community structure as a result of a pressure. This data does not provide per se information about the direct impact of single or combined

pressures acting on different spatio-temporal scales (e.g., climate change, eutrophication). Therefore, for the species-related data, additional information on the pressure is needed. In terms of the best spatial approach for characterizing pelagic habitat, the assessment would benefit from revising the classification of 'broad' and 'other' habitat types (GES Decision). Please see General conclusions and recommendations (Magliozzi *et al.,* 2021) for further details.

4.2 Towards a definition for GES in pelagic habitats

Since the aim of the assessment using diversity indices is not only to quantify the total community composition but also to be able **to detect changes in the structure of the community on a seasonal and annual basis,** it is important to consider phytoplankton diversity at multiple timescales (OSPAR, 2017c). From a difficult step of choosing the appropriate and the most sensitive indices to quantify biological diversity, there is an even more difficult step in establishing/defining baseline and threshold values. Where GES threshold values have not yet been established, MSs can refer to those set by the RSC. There are no threshold values established for pelagic habitat assessment in the Mediterranean Sea other than those for Chl-a.

Besides the high variability of plankton communities linked to the spatial and temporal variability of hydrological and biogeochemical conditions, other methodological challenges apply in characterizing the pelagic habitat and were reviewed in Magliozzi *et al*. (2021, 2023):

- Scale-dependency of interactions between pelagic habitat conditions and communities,
- identifying magnitude, direction, and uncertainties in the pressure-response relationships for individual indicator,
- difficulty in establishing baselines and reference conditions.

<u>Spatio-temporal representativeness of pelagic habitat indicators (Magliozzi *et al.,* 2023)</u>

One of the greatest challenges in assessing GES for pelagic habitats is the incorporation of very short processes that occur on weekly to seasonal time scales (e.g., eutrophication events following a river flood) and on multi-decadal time scales (e.g., changes in community composition due to climate change) (Magliozzi *et al*., 2023). Combining these time scales for evaluation of GES is difficult because processes on the longer time scale affect the shorter ones, and the time rates of change for potential improvement are different (for GES assessment). For example, two eutrophication-related features that affect pelagic habitat could be associated with very different temporal rates of change, namely long-term (several decades) for the semi-enclosed seas with a permanent halocline (Black Sea and Baltic Sea) and short-term (6-year MSFD cycle) for the seasonally thermally stratified waters.

One way **to recognize the importance of different time scales is to define pelagic habitat vertically**. The **short-term assessment** would be associated with a vertical habitat definition from the sea surface to the seabed in seasonally thermally stratified seas (Mediterranean and Atlantic) and from the sea surface to the upper hypoxic layer in seas with permanent haloclines (Baltic and Black Sea). This short-term GES would be accompanied by short-term variability of the corresponding indicators. **Long-term determination of GES (e.g., taking climate change into account)** would be associated with low-frequency signals (multidecadal) in the deep layer in the case of a permanent halocline, or otherwise throughout the water column – this would represent the so-called *long-term assessment* (Magliozzi *et al*., 2023). One approach is to combine the determination of GES for both long- and short-term assessments to adequately account for climate change variability and other pressure effects, as well as subsurface hypoxic areas. When integrating multiple pelagic indicators for GES assessments, it is recommended to include indicators that have high sensitivity to environmental factors and anthropogenic pressures. Indicators that reflect multidecadal trends should be retained, which helps separate long-term variability from community patterns observed within the short assessment scale of the MSFD.

Due to the high cost of sampling for pelagic parameters/indicators, MSs have developed different monitoring protocols, some using **fixed point stations** and others using **transects** (Continuous Plankton Recorder). In recent years, monitoring of pelagic communities has shifted from classical sampling methods to approaches that combine optical, imaging, and molecular data and provide better taxonomic resolution of communities. **Collaboration with these scientific fields** (e.g., molecular biology, satellite remote sensing, automated optical/imaging techniques, biogeochemical modelling) is recommended to increase the amount of relevant data for GES assessment. However, these methods have often been applied to research projects at the regional scale and have not yet been used for assessment. The choice of **methods, locations and frequency of data collection** is extremely important for the development of indicators as well as for the interpretation of results in the context of natural variability and anthropogenic influences on pelagic habitat.

To improve the representativeness of the GES assessment of pelagic habitat condition in space and time, **a grid-based approach is recommended**. This approach divides assessment of broad habitats into smaller units and is based on extrapolation of in-situ indicators with spatial environmental data (e.g., satellite-based Chl-a, operational models for abiotic and biotic variables). Such a proposal could be developed for all marine regions (Magliozzi *et al*., 2023).

Expanding interregional cooperation is an important aspect of selecting representative and comparable indicators and testing methods for integrating indicators for assessment of GES. The planned exchanges between the EU-funded projects NEA PANACEA, HELCOM BLUES and ABIOMMED should help to achieve substantial progress in pelagic habitat assessment within a few years and support collaboration. Moreover, pelagic habitat assessment GES needs to consider links between diversity and other MSFD descriptors such as food web (D4) and eutrophication (D5) to ensure coherence at MSFD level.

Setting the focus to the Mediterranean Sea, here the scale-dependency of pelagic habitat conditions and diversity is even less evaluated, since the knowledge of the Mediterranean pelagic habitats is generally limited to coastal areas for which several long-term monitoring stations exist for phytoplankton. The open sea is far less studied, and no available or operational indicators have been developed in the deep Mediterranean Sea to our knowledge. UNEP/MAP (2021) therefore proposes a roadmap for defining phytoplankton and zooplankton parameters for relevant IMAP biodiversity indicators and propose a refined classification of the pelagic habitat types in the epipelagic layer (0-200 m depth). For the latter, satellite data and associated modelling chl-a regionalization are already available.

OSPAR (2022) and McQuatters-Gollop *et al.* (2022) developed a system for assessing the biodiversity status with categories, that match either indicator thresholds or just consider the temporal change of the indicator linked to the impacts based on expert judgement. These categories with the description are presented in Table 5. However, authors stress that assigning indicators to these categories has currently no formal link to any policy regulation, such as OSPAR or MSFD for GES assessments. Such categories could be a good point to define better the GES related to pelagic habitats even in the absence of thresholds.

Table 5: Biodiversity status categories based on expert interpretation of indicator change with respect to assessment thresholds (where available), links to pressures, and knowledge of indicator state (from McQuatters-Gollop *et al.,* 2022)

Not good/poor	Indicator value is below assessment threshold, or change in indicator represents a declining state, or indicator change is linked to increasing impact of anthropogenic pressures (including climate change), or indicator shows no change, but state is considered unsatisfactory
Uncertain	No assessment threshold and/or unclear if change represents declining or improving state, or indicator shows no change but uncertain if state represented is satisfactory
Good	Indicator value is above assessment threshold, or indicator represents improving state, or indicator shows no change, but state is satisfactory
Unassessed	Indicator was not assessed in a region due to lack of data, lack of expert resource, or lack of policy support.

5 Conclusions

- The review of phytoplankton indicators in other regional seas (OSPAR and HELCOM) gave a list of used indicators for pelagic habitat with varying degree of certainty and specificity.
- The review of phytoplankton indicators that have been studied, developed or applied in different areas of the Mediterranean Sea revealed that there are no established thresholds for the Mediterranean Sea due to various difficulties.
- The only indicator used so far for the assessment is the Chlorophyll-a, although the use of abundance, size, and diversity indices of phytoplankton have been proposed in various studies, but no systematic application at sub-regional/regional scale have been applied, giving the impression that the application of phytoplankton indicators in the Mediterranean basin is far behind other European seas.
- Within ABIOMMED the most appropriate indicators will be applied agreed by the partners in the defined spatial and temporal scales and try to propose indicators suitable for a common application in all subregions, based on the outcomes of the analysis of the strengths and weaknesses of the reviewed assessment system.
- The common characteristic of a variety of described pelagic habitat indicators is their constant development and/or improvement because of the acquirement of new knowledge, lack of evidence of their appropriateness, limited applicability in time and space, finding of better alternatives.
- Future work will have to (re)consider spatial and temporal scale-dependency of interactions between pelagic habitat conditions and communities, identify magnitude, direction, and uncertainties in the pressure-response relationships and overcome the difficulties in establishing baselines and reference conditions. As a promising alternative to deal with these difficulties, a categorical assessment system developed by OSPAR, which uses either indicator thresholds or just the temporal change of the indicator linked to the impacts based on expert judgement, was envisaged.

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