

## **Status of pelagic habitats within the EU-Marine Strategy Framework Directive: proposals for improving consistency and representativeness of the assessment**

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1 **Abstract**

2 Anthropogenic activities have transformed the pelagic habitat in the last decades with profound implications for its  
3 essential functions. While the EU-Marine Strategy Framework Directive 2008/56/EC and the Commission Decision (EU)  
4 2017/848 have set criteria and methodological standards for the assessment and determination of Good Environmental  
5 Status (GES) for pelagic habitats in EU waters, there is strong evidence that Member States have not yet harmonized the  
6 pelagic GES assessment across EU marine waters. Today, pelagic habitats are assessed by evaluating whether good status  
7 is achieved by each of the pelagic indicators, but this approach fails to observe the high variability of the pelagic  
8 environment. To this end, GES is not estimated at pelagic habitats scale but only for each individual indicator. This paper  
9 synthesises the latest developments on pelagic habitats assessment and identifies the main factors limiting the  
10 consistency of the assessment across Member States: i) coarse spatial and temporal scales of sampling effort as regards  
11 to the pelagic habitat dynamics, ii) little consideration of the whole range of plankton (and, to some extent, of  
12 zooplankton) size and trophic spectra, iii) lack of integrated hydro-biogeochemical and biological studies and  
13 collaboration among experts from different scientific fields, iv) limited availability of pressure-based indicators, and v)  
14 lack of integration methods of the pelagic indicators' status for the GES determination. This analysis demonstrates the  
15 importance of maintaining a consistent sampling frequency and a spatially extensive network of stations across the  
16 gradient of anthropogenic pressures, where spatial environmental data can help objectively extrapolating field data.

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18 *Keywords:* good environmental status MSFD, plankton indicators, marine water column, satellite

## 1. Introduction

Marine waters contain a diversity of habitats and organisms that provide a range of important ecosystem services [1]. In the pelagic realm, many of these habitats and species have been altered by human activities, either directly (e.g., fishing, deep-sea mining, mariculture, ballast-spread invasive species) or indirectly by means of atmosphere-ocean (e.g., climate change and ocean acidification) and the land-ocean (e.g., urban, industrial and agricultural effluents, dams-influenced changes in runoff regimes) interactions [2,3]. Moreover, these pressures operate at different temporal and spatial scales causing cumulative impacts [4,5] and hampering the identification of causality [6]. These changes of pelagic physical and biological dynamics cause ecological, hydrological and environmental impacts that propagate horizontally (coast-offshore) and vertically (surface-seabed) [4]. An important step to informed management for reducing anthropogenic impacts on marine biodiversity consists of evaluating the response of pelagic systems to direct and indirect pressures. The Member States (MS) of the European Union (EU) assess the impacts and pressures on pelagic habitats under the Marine Strategy Framework Directive [7]. It remains challenging however to disentangle the anthropogenic contribution from natural variability on the functional and structural characteristics of pelagic habitats.

Pelagic habitats are naturally dynamic systems due to the interactions of multiple hydrological and anthropogenic drivers [8]. The assessment of these multi-scale systems is a policy priority of Descriptor 1 (i.e., Biodiversity, criterion D1C6) of the MSFD, and also linked to other pressure Descriptors (i.e., Descriptor 2: Non-Indigenous Species, Descriptor 5: Eutrophication, Descriptor 8: Contaminants). For the MS of the EU, the MSFD has the role to ensure that the biotic and abiotic structure and functions of pelagic habitats are not adversely affected by anthropogenic pressures and remain in Good Environmental Status (GES) (Commission Decision (EU) 2017/848 [9], herein referred to as GES Decision). GES is defined by Article 1 (3) of the MSFD by the degree to which marine resources are used at a sustainable level to ensure their continuity for future generations (GES Decision). The GES determination should consider changes in the spatial heterogeneity of regional sea characteristics [10], and therefore depends on the spatial and temporal characteristics of the monitored and assessed marine area. As a recommendation, the GES assessment of pelagic habitats in the MSFD needs common determination of GES, evaluation criteria, and consistent methods across the European Seas (e.g., data type and frequency, indicators and analysis) to achieve comparable results for the policy requirements[9,11].

Previous studies have highlighted the challenges of determining GES for pelagic habitats (e.g., [12,13]), and how pelagic abiotic and biotic characteristics change over time in response to different factors (e.g., climate change, [14]). For example, long-term (centennial to decadal) changes in hydro- meteorological conditions (e.g., water mixing, precipitation, temperature) have been identified as dominant drivers of pelagic processes such as primary production [15]. Planktonic organisms respond to changes in the characteristics of water masses and hydrographic processes according to their lifespan, growth rate and size [16].

Research has shown that plankton dynamics (e.g., changes in biomass, diversity) in the North Sea and Northeast Atlantic have strongly changed compared to decades ago due to large-scale climatic drivers (e.g., sea surface temperature, water transparency, salinity [14,17]). Other types of changes also occur over seasonal and short (episodic) timescales as a result of nutrient dynamics in coastal systems with high levels of land-based sources [18,19]. For example, impairment of metabolic and reproductive functions of aquatic organisms and alterations in food web topology can occur over annual to decadal timescales due to the uptake of persistent organic pollutants and trace metals [20], or over the course of a few months as a result of eutrophication and hypoxia [21]. In temperate areas (comprising all EU Member State jurisdictional waters), the abundance and phenology of phytoplankton communities at inter-annual scale is modulated by the intensity and duration of winter water mixing and summer stratification [22]. These changes, which occur over different timescales, are difficult to capture through specific driving factors and

44 processes in the context of the reporting obligation of the MSFD. Yet, often because of lack of data spanning multiple  
45 timescales, the pressure-state relationships between human activities and pelagic dynamics are difficult to establish,  
46 as well as the importance of the short-term (days to months) relative to long term changes (years to decades).  
47 Moreover, the pelagic habitats in the MSFD, i.e. D1C6 criterion, must be assessed as extent of habitat adversely  
48 affected in square kilometres or as a percentage of the total extent of the habitat type [23], assuming that available  
49 data are fully representative of the pelagic habitat and that the assessment will not be biased by the selected  
50 indicators nor the sampling strategy. The MSFD requires MS to update their marine strategy every six years (MSFD  
51 Article 17(2)), which could lead MS to establish the six-years cycle as the timescale for the pelagic assessment (GES  
52 Decision). As the observed changes in pelagic habitats are strongly short time-dependent, limiting the MSFD pelagic  
53 assessment to a timescale that is not relevant to capture this variability can generate biased results, with long-term  
54 measurements at low frequency underestimating the total change that occurs over shorter timescales [24].  
55

56 A series of indicators that address interacting pelagic processes have been adopted by MS to monitor the pelagic  
57 habitat [24–26]. These indicators include both general (i.e., abundance, biomass) and plankton-specific metrics (i.e.,  
58 taxonomy diversity) that are based on different sampling strategies and methodologies, to address changes in the  
59 status of pelagic habitats [10]. These indicators typically target groups of pelagic communities that are associated  
60 with specific spatio-temporal scales, and anthropogenic pressures that are mostly not captured with traditionally  
61 applied sampling strategies [27]. Also, to date, the data collection for pelagic indicators is limited by the extent of the  
62 pelagic habitats. Therefore, there is a need for inter-regional consistency and explicit consideration of the relevant  
63 timescales for each indicator to capture and evaluate the spatio-temporal extent of human impacts on pelagic  
64 habitats [24]. To achieve this goal in a conceptually harmonised and coordinated way at the EU level, the MSFD  
65 Common Implementation Strategy [28] has developed a step-by-step approach that ranges from the selection of  
66 habitat characteristics to indicator identification and the setting of relevant thresholds. Given the differences in  
67 pelagic habitat affecting the physical and biological characteristics in space and time across regional seas, the  
68 development of indicators can be region- and subregion-specific (GES Decision). Accordingly, the GES Decision  
69 highlights the need for MS to cooperate at EU, regional or subregional level for selecting indicators that ensure the  
70 assessment is based on reliable data and functionally comparable methods. So far, MS assess pelagic habitat status  
71 by evaluating whether good status is achieved at a pelagic indicator level without integrating the indicator results for  
72 the GES assessment at habitat scale [11]. As a consequence, the assessment is fragmented, and the GES status is not  
73 consistent across MS or inconclusive. To this end, the selection of common abiotic and biotic characteristics of pelagic  
74 habitats across regional seas would facilitate the adoption of common indicators and their integration in the GES  
75 determination and assessment [27].

76 This paper presents the progresses and challenges of the MSFD pelagic habitat assessment and discusses the potential  
77 contribution to the harmonised evaluation of pelagic habitat GES, while taking into consideration the differences and  
78 similarities between and within the marine regions. This analysis particularly focuses on three issues that emerged from  
79 the latest MS reports [11]. First, the four broad habitat types (i.e., variable salinity, coastal, shelf, and oceanic beyond  
80 shelf) defined by the GES Decision require revision to depict the spatio-temporal variability of pelagic habitats and to  
81 consider the large extent of the assessment units, the broad oceanographic characteristics, and the low data sampling  
82 (i.e., scaling effect of data collection) (Sections 2 and 3). Second, there is a general lack of agreed indicators and GES-  
83 related thresholds at sub-regional and regional scale to ensure comparable and harmonised assessments (Sections 3  
84 and 4). Third, the paper recommends a more ecologically relevant and adaptive process for the assessment and  
85 monitoring of the impacts and pressures of pelagic habitats (Section 4).  
86

## 2. Current pelagic indicators and monitoring in the Marine Regions

According to the GES Decision preamble, MS need to “build upon standards stemming from Union legislation or, where do not exist, upon standards set by Regional Sea Conventions (RSCs) or other international agreements”. For example, when threshold values are not yet established for GES, MS can refer to existing ones of the RSCs (e.g., [29,30]). To this end, the following section summarizes the pelagic indicators that are currently used by the MS for the pelagic habitat GES, defined at national level or in the framework of RSCs. Also, it illustrates the data collection frequency for the main indicators’ parameters across Marine Regions.

A thorough understanding of the effect of pressures and their interactions in the marine realm is key to building robust pressure indicators and ensuring consistency of MSFD assessment between marine regions. However, the current indicators that have an EU-wide scale of applicability have regionally-specific thresholds, when thresholds exist (Table 1). None of the indicators in Table 1 quantify alone D1C6 as “extent of habitat adversely affected in square kilometres (km<sup>2</sup>) and as a proportion (percentage) of the total extent of the habitat type [9]”, nor to direct anthropogenic pressures (Table 1). The analysis of the MS MSFD official reports (2012-2018, [11]) showed that the D1C6 assessment is carried out at indicator level (i.e., good status of the indicator) and not by broad habitat types (i.e., variable salinity, coastal, shelf, oceanic beyond shelf), and it lacks of integration methods among indicators to support GES as “achieved”. The ability of these *in-situ*-based indicators to detect changes in pelagic habitat status is nevertheless relevant for setting quantitative threshold values.

**Table 1** Link between indicators currently used by Member States to assess D1C6 and anthropogenic direct-indirect pressures across Marine Regions (Baltic Sea (BAL), North East Atlantic Ocean (NEA), Mediterranean Sea (MED), and Black Sea (BLK)). An expert-based confidence score (1 to 5, 5 is high) is provided to indicate the pressure-response relationship of the indicator and its application to the marine region. The confidence score was obtained from the evaluations of pelagic habitat experts during the Joint Research Centre workshop held online on the 9<sup>th</sup> and 10<sup>th</sup> of March 2021 [10], and it is expressed only for those indicators used at regional level for the assessment of D1C6.

Indicator	Pressure	BAL	NEA	MED	BLK	Scale of application	Threshold Value	Threshold definition	Where	Unit
Chl-a ( <i>in-situ</i> and satellite)	Eutrophication	5		5	5	EU	[31]	REGIONAL	MED	µg/l, mg/m <sup>3</sup>
Seasonal succession of dominating phytoplankton groups		3		1		EU	0.58 to 0.74	REGIONAL	BAL	weight biomasses (µg/l) of major functional or dominating phytoplankton groups over a sampling year
Phytoplankton abundance				1	3	EU	BLK: by broad habitat type [51], MED: at national level	REGIONAL, NATIONAL	BLK, MED	cells/l , taxa cell count l <sup>-1</sup>
Phytoplankton biomass				3	3	EU	BLK: by broad habitat type [51], MED: at national level	REGIONAL, NATIONAL	BLK, MED	mg/m <sup>3</sup>

Zooplankton abundance				4	4	EU	BLK: by broad habitat type [51], MED: at national level	REGIONAL, NATIONAL	BLK, MED	taxa number individual m <sup>-3</sup>
Zooplankton biomass				4	4	EU	BLK: by broad habitat type [51], MED: at national level	REGIONAL, NATIONAL	BLK, MED	mg/m <sup>3</sup>
Copepoda biomass				4	4	EU	BLK: by broad habitat type [51], MED: at national level	REGIONAL, NATIONAL	BLK, MED	%, mg/m <sup>3</sup>
Common indicator PH1/FW5: changes in plankton functional types (life form) index Ratio	Eutrophication, Climate change		2, 5	- , 2		EU	inexistent	REGIONAL	NEA	Plankton abundance or biomass (per species/genera/taxa)
PH2: plankton biomass and/or abundance			3, 4	- , 2		EU	inexistent	REGIONAL	NEA	Plankton abundance or biomass (per species/genera/taxa)
PH3: changes in biodiversity index(s)			1, 2	- , 2		EU	inexistent	REGIONAL	NEA	Plankton abundance or biomass (per species/genera/taxa)
Zooplankton Mean size and Total Stock	Eutrophication, Overfishing	3, 3		2, 2		EU	Mean size: 5.0 -23.7; Total stock: 55 -220	REGIONAL	BAL	mean size (µg wet weight ind-1) / total stock (mg/m <sup>3</sup> )
phytoplankton & zooplankton biodiversity and evenness indices	Cumulative impacts (e.g., eutrophication, overfishing, climate change)			2	3	REGIONAL	inexistent	REGIONAL	BLK, MED	[48]

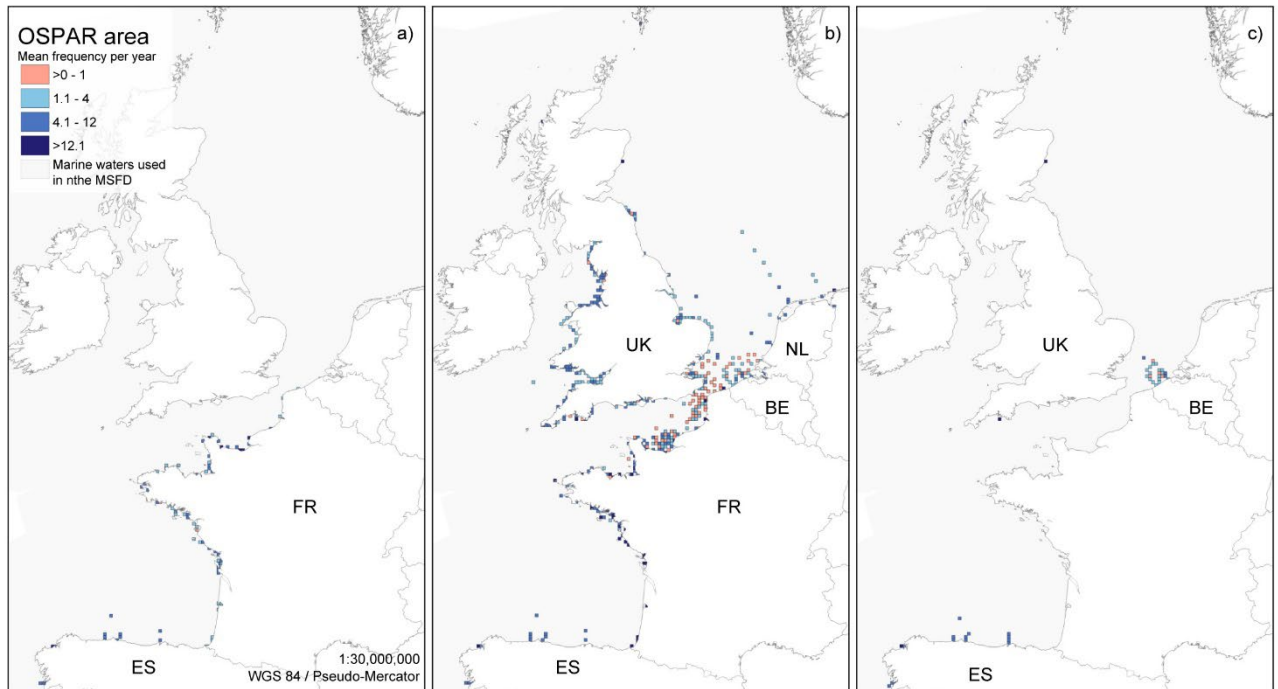
In the Northeast Atlantic region (OSPAR area), a suite of complimentary plankton indicators, providing insight into different aspects of the plankton community, are used for MSFD assessment and reporting [32,33]. The indicators are informed by data covering a spectrum of taxonomic information, from bulk information such as chlorophyll concentration to species abundance data (Figures 1, 2). This flexible approach makes best use of the wide variety of plankton data available (e.g., Table 2), regardless of their sampling and analysis methodologies, with varying taxonomic specificity, in the OSPAR region.

**Table 2:** Summary of the variables collected across Marine Regions, Member States (MS) and Regional Seas Conventions (RSCs) for the MSFD assessment. Time period refers to the available information at source. The density sampling of parameters in the latest MSFD assessment period (2012-2017) were displayed in the maps (Figures 1-5). For Greece, Slovenia and Croatia there are additional national monitoring programs that mostly focus on ‘hotspot’ coastal areas with heavy anthropogenic pressures, such as treated urban sewage water, industrial activity, construction works, which are not considered in this table. See supplementary material for country abbreviations. Note: the U.K.-wide MSFD framework is still effective despite the UK is no longer part of the EU and continues developing its marine strategy through the OSPAR Convention.

Marine Region	MS/RSCs	Parameters	Type	Time period	source
Baltic Sea	HELCOM	Chlorophyll- a frequency	fixed stations	2013-2015	<u>HELCOM Map &amp; Data Service</u>
		Phytoplankton frequency (diversity)		2013-2020	
		Zooplankton frequency (abundance, biomass, body size?)		2011-2016 2015-2020	
Northeast Atlantic Ocean	BE, DE, NL, U.K. (including Wales)	Phytoplankton community abundance	fixed stations	2012-2020, 1999-2019, 2000-2019, 1969-2020	OSPAR
		Phytoplankton biomass			
		Zooplankton community abundance			
	ES	Chlorophyll- a concentration	fixed stations	1989-2021, 1991-2021, 1992-2021, 1994-2020, 2001-2021, 2007-2021, 2009-2021, 2013-2021	RADIALES STOCA
		Phytoplankton diversity			
		Zooplankton biomass			
		Zooplankton diversity			
	FR	Phytoplankton diversity	fixed stations	1992-2016	REPHY, SRN, SOMLIT/ PHYTOBS, ARCHYD
		Chlorophyll- a concentration			
		Chlorophyll- a concentration			
OSPAR	Phytoplankton community abundance	transects	1958- 2018	Continuous Plankton Recorder	
	Zooplankton community abundance				
	Phytoplankton biomass				
Black Sea	BG	Chlorophyll- a concentration	fixed stations	2012-2017	ANEMONE project
		Zooplankton (species diversity, abundance, biomass)			
		Phytoplankton (species diversity, abundance, biomass)			
	RO	Chlorophyll- a concentration		2012-2021	ANEMONE project, National Monitoring programme
		Zooplankton (species diversity, abundance, biomass)			
		Phytoplankton (species diversity, abundance, biomass)			
Mediterranean Sea	EL	Chlorophyll-a concentration	fixed stations	2012 – 2021	<u>HCMR</u>
		Phytoplankton (abundance, species diversity)		2018-2021	HCMR, Fisheries Research Institute (FRI)
		Zooplankton (biomass, abundance, species diversity)		2018-2021	
	ES	Chlorophyll- a concentration	fixed	1992-2021, 1994-2021,	RADMED, ESMARES

		Phytoplankton diversity	stations	2007-2021, 2010-2021	
		Zooplankton biomass			
		Zooplankton diversity			
	FR	Phytoplankton (abundance)	fixed stations	1987-2020	<u>REPHY</u>
		Chlorophyll- a concentration			
	HR	Chlorophyll- a concentration	fixed stations	2017-2021	IZOR
		Phytoplankton (abundance, species diversity)			
		Zooplankton (species diversity)			
	IT	Chlorophyll- a concentration	fixed stations	2015-2017	<u>ISPRA</u>
		Zooplankton (species diversity, abundance, biomass)			
		Phytoplankton (species diversity, abundance, biomass)			
	SI	Chlorophyll- a concentration	fixed stations	2012-2021	NIB
		Phytoplankton (species diversity)			

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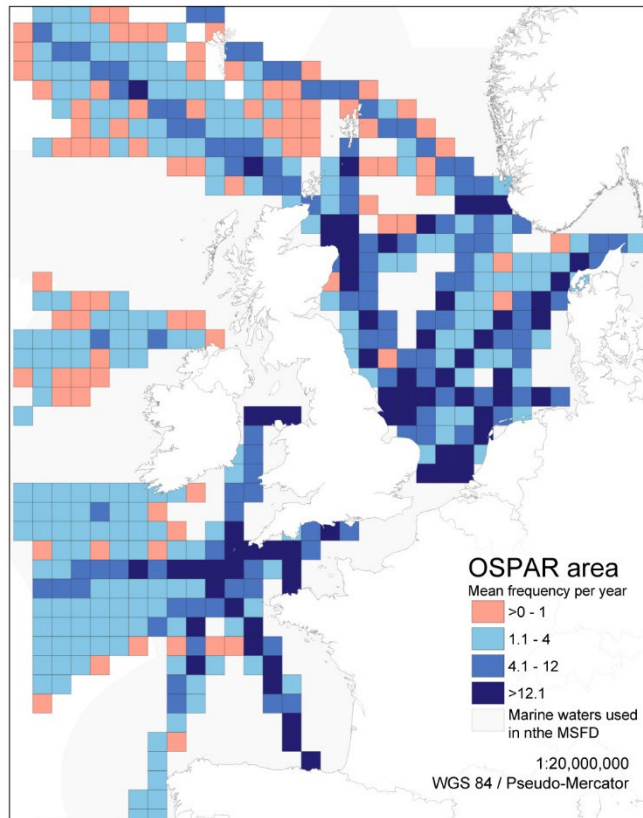
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**Figure 1:** Mean annual frequencies of *in-situ* monitoring of (a) surface concentration of chlorophyll-a, (b) phytoplankton (abundance or biomass or diversity) and (c) zooplankton (abundance or biomass or diversity) in the Northeast Atlantic Ocean, OSPAR area, on a 15 km by 15 km cell grid between 2012 and 2017. ‘Marine waters’ indicates the delimitation of Member States’ marine waters used in the MSFD 2012-2018 and 2018-2024 reporting cycle ([WISE Marine](http://www.eea.europa.eu/legal/copyright), Copyright to European Environment Agency, <http://www.eea.europa.eu/legal/copyright>). Contributing countries to the OSPAR area are (a) France and Spain, (b) U.K., Netherlands, Belgium, France, and Spain, and (c) U.K., Belgium, and Spain (Table 2). In (c), the star symbol indicates the area mapped in Figure 6. See Supplementary Material for details on the data source and analysis.

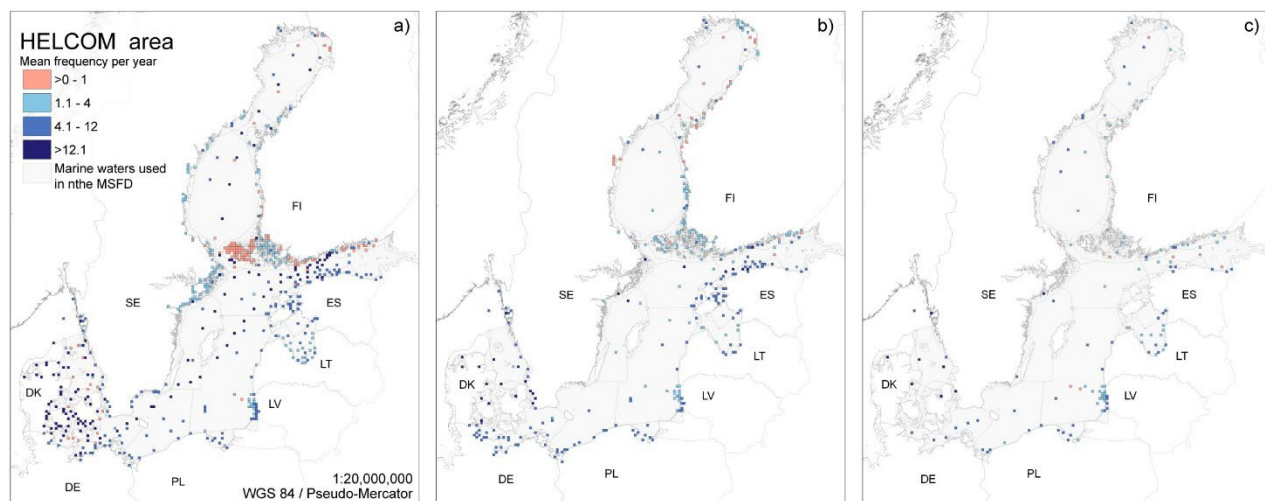




**Figure 2:** Mean annual frequencies of *in-situ* monitoring of phytoplankton (abundance or biomass or diversity) and zooplankton (abundance or biomass or diversity) in the Northeast Atlantic Ocean, OSPAR area, using the ship-of-opportunity Continuous Plankton Recorder (CPR), on a 50 NM by 50 NM cell grid between 2012 and 2017. ‘Marine waters’ indicates the delimitation of Member States’ marine waters used in the MSFD 2012-2018 and 2018-2024 reporting cycle ([WISE Marine](http://www.eea.europa.eu/legal/copyright), Copyright to European Environment Agency, <http://www.eea.europa.eu/legal/copyright>). See Supplementary Material for details on the analysis

Firstly, at the broadest taxonomic level, indicators for phytoplankton biomass and total copepod abundance biomass provide an indication of phyto- and zooplankton productivity (*PH2: Change in plankton biomass and abundance*, [34]) The phytoplankton biomass indicator can be informed by, for example, chlorophyll data estimated from spectrophotometry or fluorometry sensors, High Performance Liquid Chromatography (HPLC) or satellite remote sensing, or the Continuous Plankton Recorder’s Phytoplankton Colour Index. The indicator of total copepod abundance instead requires a count of copepods, regardless of the taxonomic resolution to which they are identified. Secondly, at the intermediate taxonomic level, the change in plankton communities (*PH1/FW5: Plankton lifeforms*, [35]) applies the plankton lifeform indicator approach, which uses functional traits to group plankton taxa into ecologically-relevant lifeform pairs where changes in relative abundance indicate alteration in ecosystem functioning [25]. This approach makes use of taxonomic plankton data that may not be refined to species level, but because of the aggregative nature of lifeforms, data at the order, family, and genus levels can still inform the indicator. Thirdly, detailed plankton genus or species information is used in indicator *PH3: Changes in Plankton Diversity* [36] to describe community structure parameters such as species evenness, dominance, and richness [37,38]. When used together, these three indicators provide insight into plankton biodiversity by examining aspects of plankton community structure (community composition indicator (*PH3*), productivity (*PH2*) and function (functional group indicator *PH1/FW5*)). Changes in all three indicators were identified in the OSPAR 2017 Intermediate Assessment [39]. Since then, changes in some lifeforms in indicator *PH1/FW5* have been linked to climate change [40]. No thresholds exist for any of these pelagic indicators, so these indicators may reflect longer-term changes for the wider ecosystem than changes induced by the human pressures that are monitored by the MSFD.

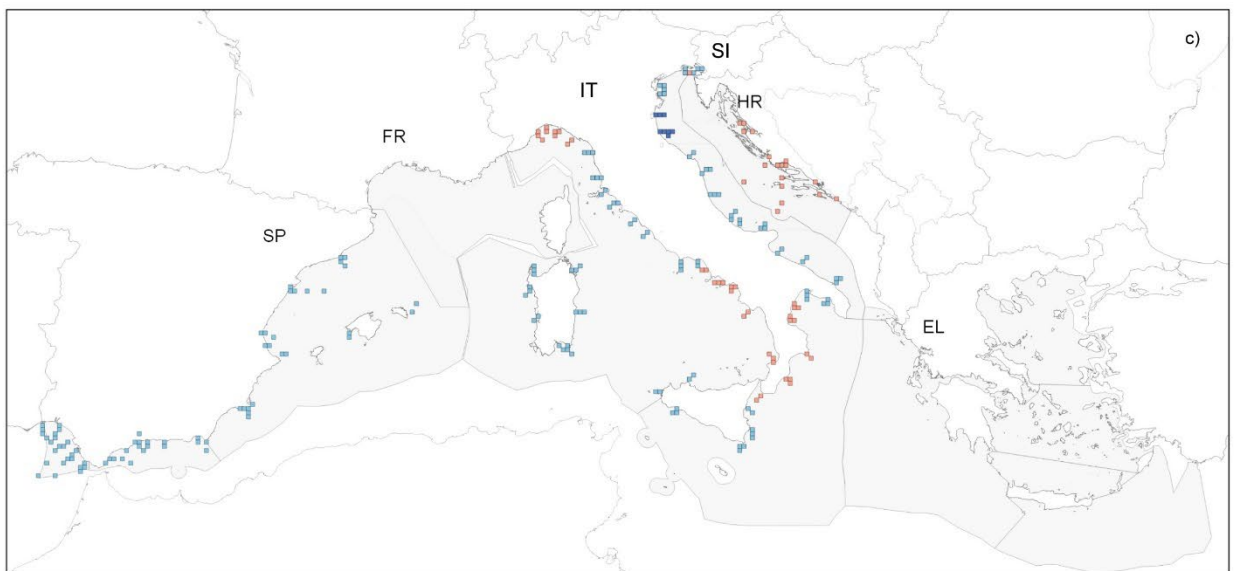
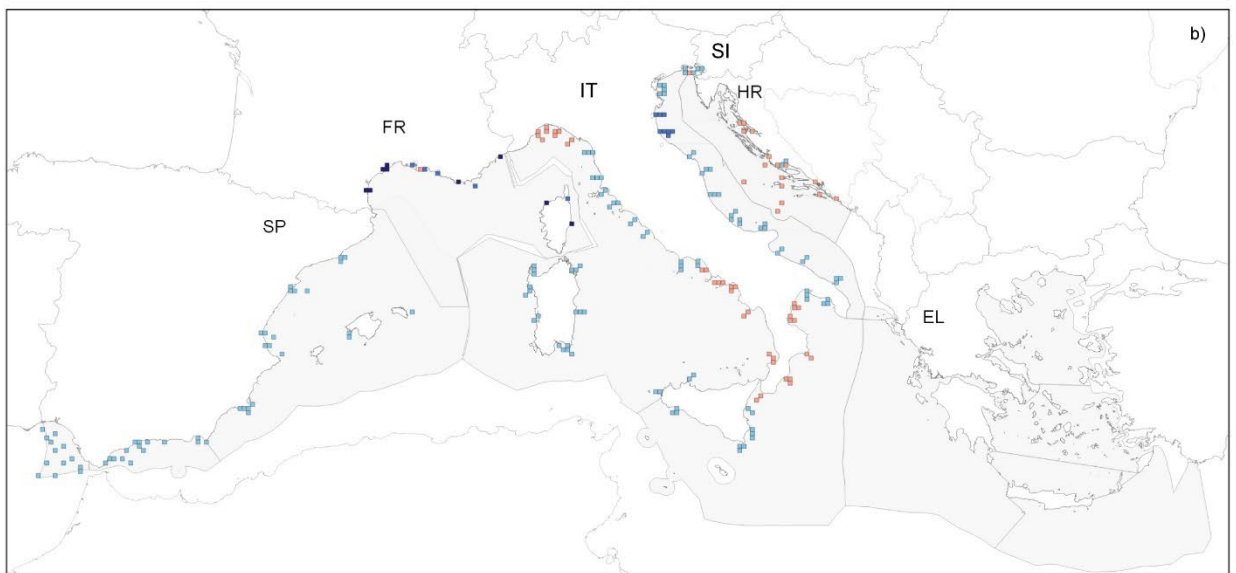
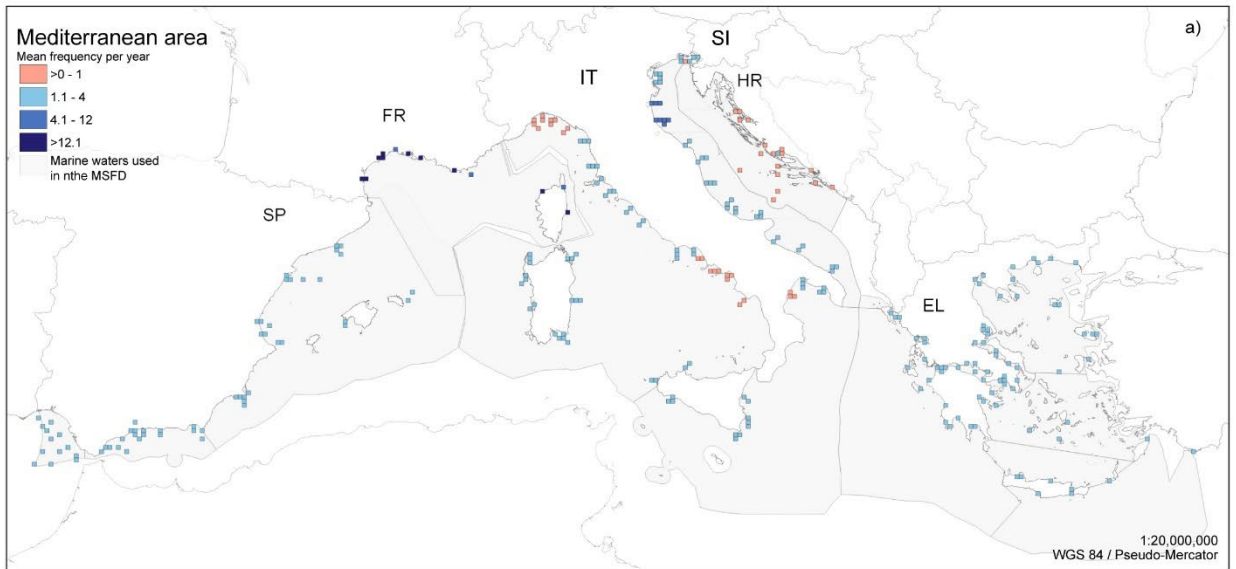
161 In the Baltic Sea (HELCOM area), two plankton indicators are used to assess pelagic habitats, addressing  
162 phytoplankton and zooplankton as plankton community components. The *Seasonal succession of dominating*  
163 *phytoplankton groups* indicator [41] is based on the taxa that are present across different sea basins (i.e.  
164 Cyanobacteria, Dinoflagellates, Diatoms and *Mesodinium rubrum*) and their seasonal succession pattern derived from  
165 the long-term data series. These are functionally diverse groups that dominate at different times of the year;  
166 therefore, the indicator considers deviations from the normal seasonal cycle (e.g. absence of dominating groups, too  
167 high or low biomass) that may affect trophic cascades and have wider implications for the sedimentation and the  
168 biogeochemical processes [41]. The indicator *Zooplankton Mean size and Total Stock* (MSTS [42]) reflects zooplankton  
169 community structure in terms of body size distribution and total zooplankton biomass [26]. Stocks of zooplankton  
170 composed of large-bodied organisms have a higher capacity for transferring energy from primary producers to fish, i.e.,  
171 high energy transfer efficiency [43]. By contrast, dominance of small-bodied zooplankton is usually associated with  
172 lower energy transfer efficiency, due to higher losses [44]. In the last holistic assessment of HELCOM HOLAS II [45],  
173 both indicators could only be applied in parts of the Baltic Sea, MSTS as a core indicator and *Seasonal succession of*  
174 *dominating phytoplankton groups* as a supporting indicator, and were, therefore, complemented by eutrophication  
175 indicators (i.e., chlorophyll-a, Cyanobacterial Bloom Index) in order to represent changes in primary producers and to  
176 provide assessment results for pelagic habitats on a regional scale for all HELCOM sub-basins. In the last holistic  
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182 HELCOM HOLAS II [41], both indicators could only be applied in parts of the Baltic Sea, MSTS as a core indicator and  
183 *Seasonal succession of dominating phytoplankton groups* as a supporting indicator, and were, therefore,  
184 complemented by eutrophication indicators (i.e., chlorophyll-a, Cyanobacterial Bloom Index) in order to represent  
185 changes in primary producers and to provide assessment results for pelagic habitats on a regional scale for all  
186 HELCOM sub-basins. Both indicators use thresholds and established reference periods for the assessment of  
187 achieving or failing GES. The reference periods are based on the long-term time-series (starting from 1980 or earlier)  
188 data collected within the established regular monitoring programme in the Baltic Sea (HELCOM COMBINE) using  
189 regionally harmonized methods for sample collection and analysis by national laboratories (Figure 3). The sampling  
190 frequency for both indicators vary between stations (from 2 to 24 samples per year), therefore both indicator  
191 assessments would benefit from regular monthly sampling (Figure 3). The data requirements for the seasonal  
192 succession of dominating phytoplankton group indicator are biomass values (wet mass) for the targeted  
193 phytoplankton groups, which is thus based on a quantitative analysis; currently, only the microscope analysis is used.  
194 Ferry-box data can be used in addition if the microscopic analysis is part of the ferry-box sampling. For MSTS, annual  
195 abundance data (although currently limited to the growth season) are needed with individual wet mass for the biomass  
196 calculation. The national, HELCOM and ICES data services are providing the access to the data annually reported and  
197 the work is on-going to adapt the data formats and extraction for the indicator calculation (Figure 3).



**Figure 3:** Mean annual frequencies of *in-situ* monitoring of (a) surface concentration of chlorophyll-a, (b) phytoplankton diversity and (c) zooplankton (abundance, biomass, body size) in the Baltic Sea, HELCOM area, on a 15 km by 15 km cell grid. 'Marine waters' indicates the HELCOM subbasins with coastal and offshore division in 2018 (available at [HELCOM Map and Data Service](#)). Countries contributing to the HELCOM area are Sweden, Finland, Estonia, Latvia, Lithuania, Poland, Germany and Denmark. See Supplementary Material for details on the data represented in the map.

In the Mediterranean Sea, the only operational plankton indicator for the pelagic habitats so far is *Chlorophyll-a (Chl-a) concentration* [47] (Figure 4a). The Mediterranean water types, reference conditions and boundaries for Chl-a concentrations were identified in MS coastal waters by the Water Framework Directive Mediterranean Geographical Intercalibration Group [31]. The MS that currently follow this classification system are Croatia, Cyprus, Greece, France, Italy, Slovenia and Spain. In the Eastern and Western basins of the Mediterranean Sea, the deeper primary production and chlorophyll maxima are important properties of the pelagic habitat, and therefore sampling along the water column is a common practice. In Greece for example, the monitoring of chlorophyll-a is sampled from seven standard depths of the water column (from 2 to 150 m) and at the Deep Chlorophyll Maximum (DCM) for each station.

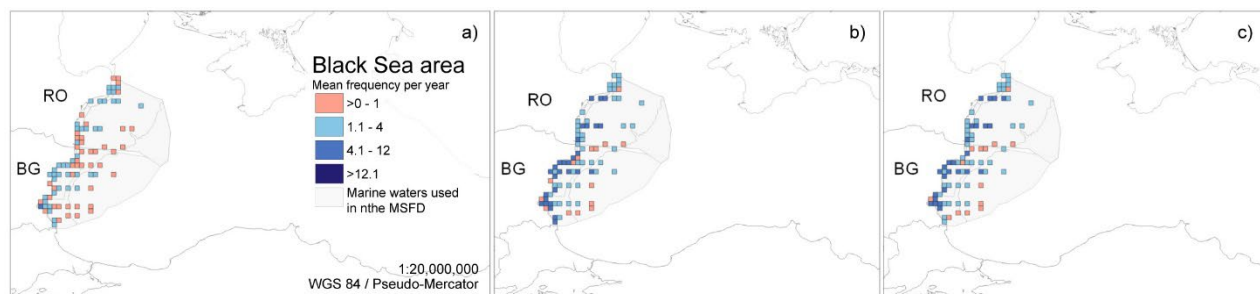
For the other phytoplankton parameters (species diversity, abundance and biomass), distinct samples are taken from five standard depths (from 2 to 100 m and DCM) per station at each sampling event. For the zooplankton parameters (species diversity, abundance and biomass) three vertical hauls are taken with WP-2 net (200  $\mu$ m mesh) at three standard depth strata (0-50, 50-100, 100-200 m) for each station. Recently, the sampling depths for phytoplankton communities, and specifically the deeper water levels, were found to reflect the cumulative impact of anthropogenic pressures [48]. The disturbances of anthropogenic pressures (e.g., both from land and coastal anthropogenic activities) on phytoplankton biodiversity indices (e.g., evenness, dominance, diversity) become more visible at higher sampling depth, where the phytoplankton communities are less dominated by a single species, and therefore are more balanced at low-impact than at high-impact sites. Many metrics for phyto- and zooplankton communities were shown to provide valuable insights on population dynamics, but they are not yet operational for the pelagic habitat assessment [13].



223 **Figure 4:** Mean annual frequencies of *in-situ* monitoring (a) surface concentration of chlorophyll-a, (b) phytoplankton (abundance, biomass, diversity) and (c) zooplankton (abundance, biomass, diversity) in the Mediterranean Sea on a 15 km by 15 km cell grid between 2012 and 2017. 'Marine waters' indicates the delimitation of Member States' marine waters used in the MSFD 2012-2018 and 2018-2024 reporting cycle ([WISE Marine](http://www.eea.europa.eu/legal/copyright), Copyright to European Environment Agency, <http://www.eea.europa.eu/legal/copyright>). Countries contributing to the Mediterranean area are Greece, Spain, France, Croatia, Italy, and Slovenia (Table 2). Sampling stations falling in the Gulf of Cádiz (ES) are mapped within the Mediterranean region to keep a clearer layout. Note that data for the mean annual frequency '>12.1' is missing in Figure 3c. See Supplementary Material for details on the data source and analysis.

231 In the Black Sea, indicators on phytoplankton (i.e., phytoplankton biomass in Romania and Bulgaria, and phytoplankton abundance only in Bulgaria) and mesozooplankton (i.e., mesozooplankton biomass (mg/m<sup>3</sup>), mesozooplankton abundance (ind/m<sup>3</sup>) and copepoda biomass (mg/m<sup>3</sup> or %) in both Romania and Bulgaria) are used for the MSFD pelagic habitat assessment (Figure 5).

236 These indicators are not yet officially agreed at regional level. Phytoplankton biomass is applied to Romanian waters following the same methodology for establishing quality classes in the WFD [49]. This indicator, its threshold values and reference periods (data from 2000 to 2010 and historical data from 1956) are based on the methodologies set by the Romanian-Bulgarian intercalibration exercise [49–51], and [52] to assess each broad habitat type. In Bulgaria, a number of statistical methods are used on phytoplankton abundance and biomass indicators to set thresholds. These are based on the signal detection theory (SDT), receiver operating characteristic curve (ROC) and combined methodology used by the Environmental Protection Agency [53], such as Regime Shift [54] and cumulative sum (CUSUM) and applied to data for the period 1961-2017 [55,56]. However, due to the lack of statistically significant outputs, there are not yet threshold values for these two indicators. For the mesozooplankton indicators, thresholds and established reference periods are used for the GES assessment. In Romania, reference conditions are based on a long-data series (1960-2002). The indicator value is compared with the average of 1960-1969 (associated with good conditions) and 1977-2002 periods (not-good conditions) for assessing GES. In Bulgaria, reference conditions are set to the period 1966-1973 using the Regime Shift [54] and the CUSUM methods [57], and indicate negligible pressure and impacts.



249 **Figure 5:** Mean annual frequencies of *in-situ* monitoring (a) surface concentration of chlorophyll-a, (b) phytoplankton (abundance, biomass, diversity) and (c) zooplankton (abundance, biomass, diversity) in the Black Sea on a 15 km by 15 km cell size between 2012 and 2017. 'Marine waters' indicates the delimitation of Member States' marine waters used in the MSFD 2012-2018 and 2018-2024 reporting cycle ([WISE Marine](http://www.eea.europa.eu/legal/copyright), Copyright to European Environment Agency, <http://www.eea.europa.eu/legal/copyright>). Countries contributing to the Black Sea area are Romania and Bulgaria (Table 2). Note that data for the mean annual frequency '>12.1' is missing in the figure. See Supplementary Material for details on the data source and analysis.

### 3. The assessment of Good Environmental Status

The definition of good status for each indicator is so far depending on regional characteristics and data availability (Section 2). However, this definition must be consistent with the overall need to track the condition of the pelagic habitats relative to the main pressures adopting an approach that is functionally similar and consistent at the EU level (GES Decision). The GES determination is given by the assessment of each broad habitat type and MS must provide (MSFD requirement) a GES outcome in a form of agreed set of indicators, thresholds, and integration methods (GES Decision), which could be combined or deviate from the RSCs approaches. At RSCs level, GES assessment is not necessarily following the MSFD approach. For example, OSPAR provides thematic assessment on pelagic habitats status based on single indicators (i.e., Biodiversity Status Intermediate Assessment 2017), while HELCOM uses biological quality ratios [29], also based on single indicators, that are then integrated for the GES assessment of pelagic habitats.

GES for the pelagic habitats relies upon the habitat definition and spatio-temporal consistency of assessment areas, and is therefore influenced by three main sources of uncertainty: i) no commonly agreed operational definition of GES exists for pelagic habitat types in the MSFD, including an agreed set of indicators [11]; ii) integration methods for regional indicators to support a harmonised and comparable GES assessment, as required by the MSFD, are not yet specifically developed for D1C6 (i.e., exception in HELCOM HOLAS III, [29]); and iii) no estimate exists on the bias of the sampling strategy selection used to report GES as fraction of surface area in percentage or square kilometres (GES Decision).

The following paragraphs inform on how good status at indicator level is calculated and whether integration approaches are foreseen.

At the OSPAR level, no agreed thresholds or vision of GES exist for pelagic indicators. Instead, the focus has been given for the pelagic habitat on state or surveillance indicators, whose change can be used to interpret changes in other food web indicators [58]. This approach is undergoing further development with the 2023 indicator assessments linking change in plankton indicators to pressures and with the construction of ecosystem component 'Thematic Assessments' for the upcoming round of OSPAR reporting in the Quality Status Report (QSR). Thematic Assessments formally link changes in indicators to pressures and management measures, through evidence-based narrative. The UK, however, has a different approach for pelagic habitat indicators, which are measured against the following target: 'Pelagic habitats are in GES if observed changes are not caused by anthropogenic pressure'. For the 2018 UK assessment, pelagic habitats were found to be in a state of 'GES uncertain' due to lack of evidence supporting links between pressures and indicator change.

In the Baltic Sea, the indicators of MSTs and *Seasonal Succession of Dominating Phytoplankton Groups* are applied to assess the status of pelagic habitats using specific threshold values. The assessment of pelagic habitats for the upcoming holistic assessment of HELCOM HOLAS III will build on the indicator reports [41,42] and be part of the biodiversity chapter of the Thematic Report linked to prevailing pressures. The concept of GES for the MSTs indicator is related to an efficient food web, meaning both favourable fish feeding conditions and a high potential for efficient use of primary production [26]. For this reason, zooplankton mean size and total biomass are combined in the assessment concept and both specific threshold values need to be achieved to reach GES. Other combinations with at least one threshold not met would imply limitations of the food web in terms of energy transfer and productivity. Threshold values are set using reference periods with good fish feeding conditions (based on data for clupeid fish using body condition indices) and periods when eutrophication effects are low (defined as 'acceptable chlorophyll-a concentration' as used in the eutrophication assessment) [42]. The concept of GES for the Seasonal Succession of Dominating Phytoplankton Groups is built on a reference status succession and acceptable deviation from region-specific reference seasonal growth curves. Strong deviations from reference curves outside the acceptable variation



301 indicate impairment of the environmental state and correspond to a failure of GES. This assessment approach has some  
302 similarities with the OSPAR lifeform approach as it identifies changes. Since the establishment of thresholds and  
303 reference periods is dependent on available monitoring data and time-series length, reference periods do not reflect  
304 pristine or historical conditions, but rather times that have already been influenced by anthropogenic pressures.  
305 Further work is needed to properly classify changes from the established reference values in relation to actual  
306 improvements or deteriorations compared to the 'reference' state of the environment as well as the role of climate-  
307 induced alterations in these indicators. The integration of the two pelagic indicators in the Baltic Sea will be carried  
308 out in the Biodiversity Assessment Tool (BEAT) for the pelagic habitat assessment (HOLAS III, [59]), with the inclusion  
309 of eutrophication indicators (e.g., Chlorophyll-a, Cyanobacterial Bloom Index and water clarity) and weighting of the  
310 different indicators. Linkages with various pressures and abiotic and biotic drivers of change continue to be  
311 investigated and should be considered in principle when assessing the GES of pelagic habitat, but will probably only be  
312 addressed qualitatively in a descriptive manner rather than quantitatively in the upcoming assessments.

313 In the Mediterranean Sea, a recent revision of approaches for GES definitions and environmental targets (MSFD  
314 Articles 9 and 10) for the eight Mediterranean MS showed that not all MS have yet defined GES for plankton  
315 communities and pelagic habitats [13]. MS are considering GES in a qualitative way for plankton communities so far  
316 (e.g., the phytoplankton species high abundance corresponds to not-good status in Greece, Italy, Croatia and Malta), as  
317 thresholds exist only for Chlorophyll-a concentration in coastal waters. The combined use of multiple biodiversity indices of  
318 phytoplankton and zooplankton (evenness and dominance also) with linkages to regional scale pressures is under  
319 evaluation in the Mediterranean Sea [48,60]. A way forward could be to combine general pressure indicators of  
320 phytoplankton/zooplankton communities (such as Chlorophyll-a, jellyfish blooms, anomalous presence of non-indigenous  
321 species) with species-specific functional traits or others status indicators in order to evaluate deviations with respect to  
322 pelagic communities where anthropogenic pressures are considered as not significant.

323 In the Black Sea, the phytoplankton and zooplankton indicators have all thresholds values. In Romania for  
324 phytoplankton biomass, the indicator value for GES is obtained by calculating the 90<sup>th</sup> percentile value of the summer  
325 season values (June - August). This value is then compared with the averages of the reference periods 1956-1960 (GES)  
326 and 1980-1988 (no GES). For the mesozooplankton indicators (biomass, abundance), GES is established by the statistical  
327 analysis of data from 1960-2002 and expert knowledge. For each broad habitat types, GES is obtained by calculating the  
328 90<sup>th</sup> percentile of the indicator value from the cold and warm seasons (full set of data in six years) in each marine unit.  
329 These values are then compared with the average of 1960-1969 period (GES) and 1977-2002 (no GES). The final GES is  
330 quantified by interpolating (Inverse Distance Weighted method) the indicators outputs across the assessment area.  
331 The GES is achieved when 90% of the assessment area is in good status. In Romania, the pelagic indicators were  
332 integrated in the Black Sea Integrated Monitoring and Assessment Programme (BSIMAP), which was approved by the  
333 BSC at the end of 2016. Its adoption is a positive step as it contributes to the harmonization of the reporting format  
334 across countries and provide the basis for comparing general environmental trends of the Black Sea marine  
335 environment. National assessments refer to or reuse regional assessments as they are, and complement them with  
336 additional elements, whilst seeking harmonization with neighbouring countries.

337  
338 The indicators examples illustrate that long-term observations are essential to define reference conditions of pelagic  
339 habitats (e.g., Black Sea). Long-term data on species-specific metrics can indicate tipping points and/or trends within  
340 the sampled area. However, and besides the varying spatial and temporal sampling issue, the interpretation of their  
341 patterns is often not straightforward and requires additional information on pressures to make definitive conclusions  
342 about GES. The approach for integrating these species-specific pelagic indicators would need to account for their link  
343 with direct or indirect anthropogenic pressures (Table 1). Since the last MSFD assessment (2012-2017), several MS  
344 increased the number of monitoring stations or the sampling frequency per year (Table S4 Supplementary Material). In  
345 conclusion, the calculation of good status based on species-specific metrics alone (such as phytoplankton species

346 abundance or biomass) may often require further insights into links to pressures and therefore should be further  
347 investigated in the upcoming assessments.



#### 4. Recommendations for the spatio-temporal representativeness of pelagic habitat indicators

The GES Decision sets the level of assessment of pelagic habitats to broad habitat types within Marine Reporting Units. This GES definition for pelagic habitats is a challenge since, as these water bodies are fluid in movement that are characterised by much higher spatial and temporal variabilities than the Reporting Units and the *in-situ* sampling strategy can address. The approaches to defining Marine Reporting Units also vary between regional seas, MS and descriptors. This section details why the use of Marine Reporting Units and broad habitat types, as defined in the GES Decision, shows challenges for a pragmatic and effective spatio-temporal assessment of pelagic habitats.

##### 4.1 The time scales

One of the main challenges for assessing pelagic habitat GES is to include processes that may act at weekly to seasonal time scales (e.g., eutrophication event after a river flood) and at multi-decadal time scales (e.g., phytoplankton community composition due to climate change or deep layer anoxia in permanent halocline areas). Mixing these two time scales in the GES assessment is difficult because the longer time scale processes influence the shorter ones and the time rates of change for potential GES improvement are different. Two eutrophication-related characteristics affecting the pelagic habitat status are associated with highly different time rates of change, i.e., long-term (multi-decadal) for the semi-enclosed seas with a permanent halocline (Black and Baltic Seas) and short-term (about the 6-years MSFD cycle) for the seasonally thermally-stratified waters. A way to acknowledge the relative importance of these different time scales is to vertically define the pelagic habitat. The short-term assessment (e.g., 6 years cycle 'short assessment') would be associated to a vertical habitat definition from the sea surface to the seabed in seasonally thermally-stratified seas (the Mediterranean Sea and Atlantic area), and from the sea surface to the upper hypoxic layer in permanent halocline areas in the corresponding semi-enclosed seas (the Baltic and Black seas, [27]). This short-term GES would be associated with short-term variability of the related indicators. A longer-term GES determination (e.g., considering climate change 'long assessment') would be linked to low frequency signals (multi-decadal) within the deep layer in case of permanent halocline (and eutrophication-related) or within the entire water column otherwise (and climate change-related using trends of e.g., SST, PH2: *Change in plankton biomass and abundance*, PH3: *Changes in Plankton Diversity*). Adopting this approach would result in two assessments, i.e., considering the short-term processes (current MSFD cycle), and the long-term phenomena that include the effects of climate change (e.g., multi-decadal temperature increase) and the geomorphologically-induced hypoxia of the bottom layer (areas with permanent halocline and low water renewal time) [27]. Because these permanently hypoxic layers are exposed to eutrophication, the time scale for substantial improvement is longer than the 6-years MSFD cycle, therefore a parallel pelagic habitat assessment associated with longer time scales for improvement would allow showing relevant trends [27]. The differentiation of 'short assessment' and 'long assessment' would allow evaluating both time scales and the effective accounting of human-induced climate change effects.

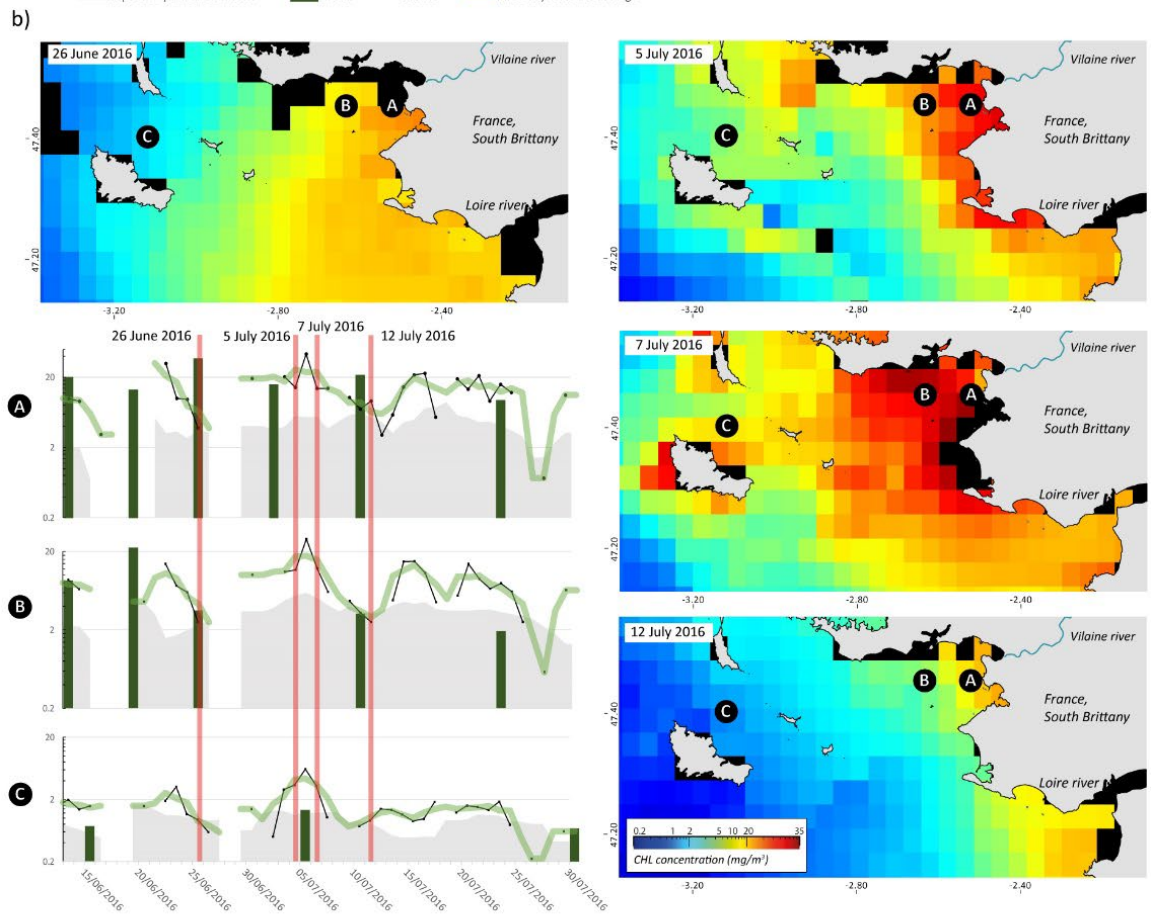
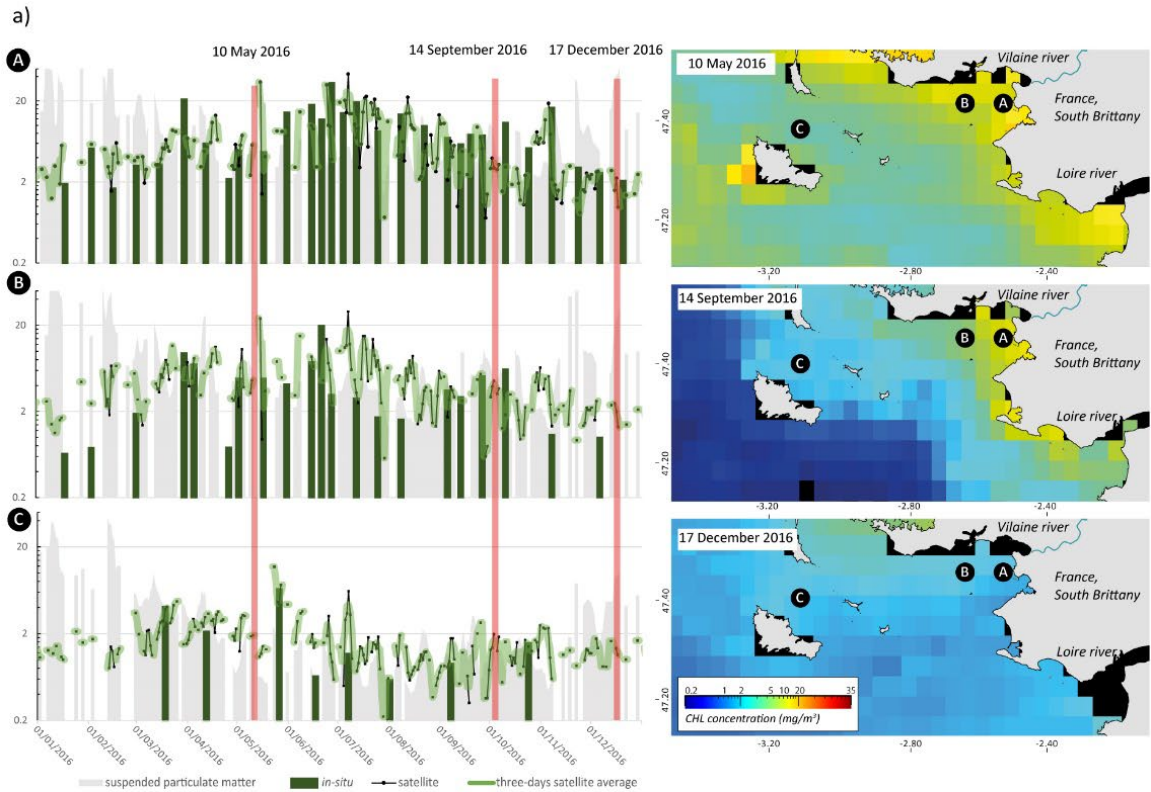
##### 4.2 The spatial scales

The limited spatio-temporal representativeness of the pelagic habitats using the Marine Reporting Units is generally caused by a systematic undersampling of the highly variable pelagic processes. The high costs and limited availability of means-at-sea reflects this aspect in the data collection strategies (e.g., [16])(Table 2). When looking at the last MSFD reporting cycle, for example, MS have developed different monitoring protocols to measure, e.g., plankton abundance and biomass, by using fixed point stations (Figure 1a) or transects (i.e., merchant ships Continuous Plankton Recorder (CPR), Figure 2) mostly at sub-surface waters to limit costs (Table 2 and Supplementary Material). The choices for the data collection of the frequency, method, and locations are key when developing the indicators and interpreting the assessment results in the context of natural variability and anthropogenic impacts on pelagic habitat. The GES Decision does not include methodological standards for the sampling frequency and spatial resolution of biotic and abiotic parameters (most of the times fortnightly or monthly, Figures 1, 3, 4), which is rarely adapted to the local variability. This is key to detect the relevant natural and anthropogenic changes and their impacts

392 on pelagic habitat (Figure 6). The understanding of the area monitored and its pressures affecting GES would  
393 substantially be improved using a grid-based approach dividing the assessment units of broad habitats into smaller  
394 units of regular sizes. A regular distribution of sampling sites with weekly effort within each of the broad habitat type  
395 would be ideal but, in practice, sampling sites are restricted to specific areas with often a much lower frequency (e.g.,  
396 river plume areas, Figure 6). It is thus unlikely that the monitoring results are indicative of the whole assessment unit.  
397 In order to unlock most of this major limitation, data from satellite observations (e.g., surface chlorophyll-a – this  
398 paper, harmful algal blooms - <https://www.s3eurohab.eu/node/1>) and operational models (e.g., nutrient  
399 distributions from Copernicus Marine Services related to the risk for harmful algal blooms) at daily time scale can be  
400 used to extrapolate the in-situ observations/indicators within the gridded approach to better depict the spatio-  
401 temporal variability of the pelagic habitat (e.g., algal bloom events in the Bay of Vilaine, France, Figure 6). The cell size  
402 of the grid should reflect the scale used for most of the available input data and observed processes (about few  
403 kilometres). Variables from monitoring stations such as surface chlorophyll-a can be extrapolated in space, and  
404 eventually at short time scales, using satellite-derived estimates to reflect the extent of locally-detected events and  
405 their potentially adverse effects (Figure 6). Specificities of the pelagic habitats across the European Seas are taken  
406 into account for the planning of monitoring programs and sampling strategies, as in the case of the well documented  
407 deep chlorophyll-a maxima in the open waters of the Eastern and Western Mediterranean Sea basins, mentioned in  
408 *Section 2*.

409 To quantitatively improve the representativeness in space and time of pelagic habitat status and related pressures, a  
410 grid-based approach using spatio-temporal environmental data from satellites and/or operational models is therefore  
411 recommended. For example, the satellite-based chl-a indicator could be used across marine regions to i) locally  
412 identify suitable sampling frequency and station locations for optimizing in-situ data collection; ii) spatially extrapolate  
413 of *in-situ* chl-a levels using the horizontal gradients of satellite-derived chl-a, thus using the relative chl-a values  
414 derived from Earth observation, iii) detect the extent and duration of river-induced eutrophication events for  
415 increasing the representativeness of pelagic habitat GES (Figure 6); and iv) investigate eventual relationships amongst  
416 indicators, which form the basis of determining threshold values for adverse effect on habitat.

417 Finally, in the integration of different pelagic indicators for the assessment of GES, it is recommended to include  
418 indicators that have high sensitivity to environmental factors and to anthropogenic pressures. To this end, retaining  
419 indicators that depict multidecadal trends (e.g., linked to climate change) will help to disentangle long-term variability  
420 from the community patterns observed within the shorted assessment scale of the MSFD.



422 **Figure 6:** Timeseries of *in-situ* and daily satellite-derived estimates of surface chlorophyll-a concentration at the location of three  
423 monitoring stations in the Bay of Vilaine (south Brittany, western France) that enhance the variability a) at the seasonal scale and b)  
424 at the scale of river-induced event during summer. Panel a) details for 2016 the effective sampling frequency and levels of surface  
425 chlorophyll-a of the three stations (A, B, C) from the Vilaine river mouth to offshore (dark green bars) in comparison to the values of  
426 the satellite estimates (daily estimates as green dots and lines, 3-days moving average in thick light green, multisensory from  
427 Copernicus CMEMS at 1/24° resolution [61], levels of satellite-derived total suspended matter are indicated in light grey), and in  
428 comparison to the distribution maps of satellite-derived levels during the spring bloom (May 10), autumn (September 14) and winter  
429 (December 17) (see red bars in the timeseries plot). Panel b) is the same as panel a) but focusing on a summer bloom event (45 days  
430 for the timeseries, 15 days for the maps) generated by the Vilaine river from about the end of the previous event on June 26, to the  
431 peak on July 5 and 7, and the end on July 12. Note that a substantial part of the difference between *in-situ* and satellite-derived  
432 chlorophyll-a levels may arise from the sampled size, about 1 litre versus 3.4 km by 4.6 km, respectively. The satellite information  
433 may efficiently be used to reasonably extrapolate the extent and duration of the *in-situ*-derived GES estimate of pelagic habitat.  
434

## 5. Conclusions and summary of recommendations

Monitoring data remain expensive and limited in space and time so that the Marine Strategy should optimize sampling to best explore the pressure-response relationships and spatial representativeness of GES assessment. The current sampling network raises two main problems: sampling gaps due to regional habitat variability and specificities, and lack of structural organization for the monitoring of pelagic habitats.

The first issue is the coarse interpolation of the GES assessment for broad habitat types (Figures 1-5) and within Marine waters that does not ecologically reflect the variability of pelagic habitats. This lack of representativeness is due to the limited network of sampling stations and sampled data. For example, a coastal area experiencing a harmful phytoplankton bloom event may be missed by a bi-monthly sampling strategy during the riskiest season due to the possible shortness of events and the spatial heterogeneity. Similarly, the identification of relevant GES information requires evaluating the frequency of anthropogenic-induced blooms at a specific coastal location. These examples emphasize the need of consistent sampling frequency and a network of sampling stations encompassing different sources of anthropogenic pressures. A gridded approach based on the extrapolation of *in-situ* indicators using spatial environmental data (e.g., satellite-derived chl-a, operational models for abiotic and biotic variables) is recommended to improve the spatio-temporal representativeness of GES assessment. Such proposal could be set up across all marine regions. An approach is to combine the GES determination for both the long- and the short- assessments to suitably accounting for the variability of climate change and other pressure effects and within subsurface hypoxic areas.

The second issue relates to the lack of agreed indicators at sub-regional and regional scale and characterized by sampling bias on biological communities. Over the last years, monitoring of pelagic communities has shifted from classic sampling technologies to approaches combining optical-image-molecular data that allow improving the taxonomical resolution and to consider the whole size-trophic spectra of biological communities [16]. However, these methods have often been applied to research projects at regional scale and not yet to improve the spatial and temporal resolution of data sampling for the MSFD GES assessment. Collaboration with these scientific fields (e.g., molecular biology, satellite remote sensing, optical/imaging automated techniques, biogeochemical modelling) is recommended to increase the volume of relevant data for the GES assessment.

From a policy perspective, the question of inter-regional cooperation is absolutely central. Ultimately, the success of any management action rests on cooperation, e.g., selection of representative indicators and testing methods of indicators integration for the GES assessment at the scale of the habitat. The present pelagic assessment has many weaknesses but the foreseen exchanges between the new EU-funded projects, the [NEA PANACEA](#) (North East Atlantic region), [HELCOM BLUES](#) (Baltic Region) and [ABIOMMED](#) (Mediterranean region) should support this level of collaboration. Finally, addressing pelagic habitat GES requires accounting for linkages of diversity with other MSFD descriptors, such as food web and eutrophication to ensure consistency at MSFD level. Substantial progress is needed before the assessment of pelagic habitats becomes effective and comparable across the EU seas. However, expansion to new methods, data source and collaborations, as presently recommended, should contribute to make substantial progress within a few years on the GES assessment of pelagic habitats.

472 **Credit authorship contribution statement**

473 C.M. wrote the first draft of the manuscript, coordinated and reviewed the manuscript. M.P. analysed the data,  
474 produced the maps and reviewed the manuscript. J.N.D. and A.P. coordinated the work and reviewed the manuscript.  
475 M.G.A, V.I., G.Q.R., G.E., H.B., B.L. and A.L.F. provided the monitoring data and reviewed the manuscript. All authors  
476 have approved the final article.

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