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Deliverable D2.1b

“Criteria for the definition of relevant assessment scales for the pelagic habitat”

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Coordinator

Dr Kalliopi Pagou

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Contact author: Janja Francé (Janja.france@nib.si)

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Scope

The condition of the habitat type is taken into account as a whole for its biotic and abiotic properties, as well as its functions in the Criterion for Pelagic Habitat (D1C6, Descriptor 1, 2017/848/EU). 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. There is currently no coherence among the eight Mediterranean MSs, according to a study of the GES definitions for pelagic habitat (Varkitzi *et al*., 2018). GES was mostly defined on a conceptual basis, just 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 fulfil 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 ABIOMMED Activity 2, the scope was to explore the use of different components of the plankton assemblage (phytoplankton and zooplankton) to assess the biodiversity status 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 were oriented towards the development of common methodologies and indicators in GES assessment of pelagic habitat using phytoplankton component. Previous studies revealed a necessity to address the issue of proper assessment scales, also for the pelagic habitats of the Mediterranean Sea, which hosts very different environments in a relatively small area. Apart from the importance of the proper determination of the spatial scales, also the temporal/seasonal aspect in determining the phytoplankton diversity was recognized as important in the view of biodiversity assessment. Therefore, one of the objectives of the Subtask 2.1.2 *Define relevant assessment scales based on pressures and prevailing natural conditions* was to identify relevant spatial scales for pelagic habitat assessment in the Mediterranean Sea emphasizing the connection between phytoplankton variability and hydrodynamics/biogeochemistry and utilizing data products available through Copernicus Marine Service. Besides, the objective was also to identify temporal scales are relevant for the assessment of the pelagic habitat.

Executive Summary

The concept of Good Environmental Status (GES) for pelagic habitats involves a holistic definition encompassing both biotic and abiotic characteristics and functions. GES is to be assessed across various pelagic habitat types within Marine Reporting Units, accommodating additional types based on (sub)regional cooperation needs. Plankton communities in pelagic habitats, despite connectivity, exhibit greater differences between areas within (sub)regions than between pristine and impacted sites in close proximity. The GES assessment considers the extent of adversely affected habitat in square kilometres or as a percentage of the total habitat type, contingent on representative data and comprehensive monitoring. Pelagic habitats face diverse pressures from natural factors (hydrological and meteo-climatic conditions) and human activities (fishing, mariculture, climate change, etc.), making it challenging to categorize stressors in terms of severity or assess cumulative effects. Magliozzi *et al.* (2023) argued for a revision of the GES Decision's division of pelagic habitat types, considering the high spatial and temporal variability of biotic and abiotic characteristics. The work done in the framework of the ABIOMMED Activity 2 - Subtask 2.1.2 Define relevant assessment scales based on pressures and prevailing natural conditions contributed to defining relevant assessment scales (both spatial and temporal) for the Mediterranean pelagic habitat, utilizing differences in phytoplankton composition, abundance, and distribution, identifiable through satellite observations.

Various studies, including D'Ortenzio and Ribera d'Alcalà (2009) and Ayata *et al*. (2017), have defined characteristic areas in the Mediterranean Sea based on phytoplankton variability, using methods such as SeaWiFS satellite measurements and ocean colour products coupled with physical-biogeochemical models. These approaches have led to the identification of trophic regimes, clusters, and ecoregions characterized by different phytoplankton size classes (PSCs) and phytoplankton functional types (PFTs) dominance, providing valuable insights into the dynamic ecological structure of the Mediterranean Sea at both temporal and spatial scales. The research within Subtask 2.1.2 focused on the Mediterranean Sea, aiming to utilize open-source data and presents a case analysis exploring the temporal and spatial dimensions of phytoplankton functional types. The study aligns with the broader goal of GES assessment for pelagic habitats.

We utilized satellite data from the Copernicus Marine Service (CMEMS) to analyse the surface distribution of phytoplankton in the Mediterranean Sea. The dataset, "Mediterranean Sea Reprocessed Surface Chlorophyll Concentration and Phytoplankton Functional Types from Multi Satellite observations," provided daily data on three PSCs and six PFTs in the period 1998-2020, from which overall and seasonal averages were calculated for the whole period, for the first decade (1998-2007) and for the last decade (2011-2020). K-means clustering was applied to identify aggregations in the 6- and 3-dimensional space for PFTs and PSCs, respectively, based on Euclidean distance, revealing ten classes/clusters were selected as the best match for the regionalization of the Mediterranean Sea. Differences between the clusters' coverage in the two decades, for the overall period and for the seasons, were calculated and confronted with change in sea surface temperatures (SST).

Both clustering on PSCs and PFTs a yielded similar and meaningful discrimination of different areas, reflected a trophic gradient in chlorophyll-a biomass and contributions of PFTs or PSCs. Subsequently, the focus of the results was given on PFT clustering. Clusters exhibited geographical patterns aligning with well-known trophic conditions in the Mediterranean Sea, demonstrating a gradient of chlorophyll-a biomass from west to east and north to south. The clusters nearest to the coasts presented the highest biomass and were dominated by the microphytoplankton

size class (mainly by diatoms); the adjacent clusters presented a medium phytoplankton biomass and were dominated by nanophytoplankton size class (mainly by haptophytes). Together these clusters formed a “coastal belt”, which was wider in the Alboran Sea (stretching from the Strait of Gibraltar toward east), along the coasts of NW Mediterranean, in the northern and western Adriatic, in the Aegean Sea, Gulf or Gabes and southern Levantine coasts. On the other hand, picophytoplankton size class (mainly cyanobacteria) dominated the largest part of the Mediterranean open sea. Dinoflagellates exhibited a relatively stable biomass across all clusters.

The study then focused on the decadal differences (1998-2007 vs. 2011-2020) in cluster coverage, which revealed significant changes mostly in the western and northern parts of the Mediterranean Sea and provided insights into the dynamic spatial and temporal variations in phytoplankton composition and biomass, as well as their relationship with SST. Changes in cluster distribution over two decades were the most notable in the western and northern parts of the Mediterranean Sea. Almost all shifts comprised widening of the most oligotrophic clusters with the dominance of picophytoplankton (clusters “0” and “1”), only in the areas with a steep gradient of clusters (highly productive coastal areas) these changes comprised also other clusters. The northern and western Adriatic Sea behaved differently, as here shrinking of more oligotrophic clusters occurred while clusters with nanophytoplankton dominance gained areal coverage.

On the seasonal scale, the largest areas were subject to changes during winter, when the most oligotrophic clusters in the eastern basin gained in areal extension, while in the western basin and the Adriatic Sea the changes were mostly reversed. On the contrary, the most substantial differences in spring and autumn occurred in the western part of the Mediterranean Sea, where the more oligotrophic clusters with the highest proportion of smaller phytoplankton cells also showed an enlargement. In summer, which is the period of the weakest west-east chlorophyll-a gradient, a more uniform distribution of the most oligotrophic cluster across the basin was observed with just minor changes between the decades along the coasts.

The observed cluster shifts align with rising sea surface temperatures, suggesting potential connections to climate change. However, the distinction between anthropogenic influence and natural variability remained challenging. Moreover, changes in cluster distribution, particularly the shrinking of clusters with larger cells, may indicate a decrease in trophic efficiency, aligning with previous research.

This work underscores the significance of spatial and temporal scales for understanding processes in the pelagic habitats of the Mediterranean Sea, but on the other hand emphasizes the often-overlooked picophytoplankton component. The picophytoplankton component is rarely considered, especially at the large basin-wide scale or in national monitoring programmes dedicated mainly to monitoring micro-phytoplankton and to a limited extent also nanophytoplankton in Mediterranean coastal and, less commonly, offshore waters. To overcome these disparities, we recommend a diversified in situ data collection using techniques like flow cytometry, microscopy and size-fractionated filtration in addition to high-performance liquid chromatography (HPLC). This diversified data collection strategy will be essential for the refinement and calibration of algorithms for phytoplankton community structure in ocean colours, and thus enhancing pelagic habitat assessments. Importantly, the resources should be also allocated to the study of pelagic food webs, where important consequences of changes at the primary producer level should be easier to detect.

The present work corroborates the previous findings about the complexity of the Mediterranean, which hosts different trophic regimes in a relatively small spatial extension. Using the distribution of chlorophyll-a biomass in

PSCs and PFTs as given by satellite product turned to be a useful way to characterize these regimes, their spatial distribution and temporal variability. The notable changes that have occurred in recent decades need to be considered in future assessments taking into account various temporal scales and incorporating climate regimes into the assessment process. This aspect gives great importance to long-term studies that preserve plankton data series and can identify important trends or regime shifts in the pelagic habitat while also allowing to study the food webs. We therefore strongly support the maintenance of long time series, LTER stations and the integration of these data into studies that involve the use of satellite data and modelling. In addition, appropriate sampling frequency is important for the adequacy of long-term data on plankton assemblages, and we consider monthly sampling to be most appropriate.

1 Introduction

GES for pelagic habitat (criterion D1C6 of Descriptor 1 - Biodiversity) should be conceptually defined as a whole of its biotic and abiotic characteristics and its functions (Commission Decision 2017/848/EU). GES has to be defined and assessed for pelagic broad habitat types (variable salinity, coastal, shelf and oceanic/beyond shelf) within Marine Reporting Units, while also allowing for more habitat types if their need is established through (sub)regional cooperation. Despite their connectivity, the plankton communities of the pelagic habitats cannot be directly compared, and the differences between areas within the (sub)regions are frequently greater than the differences between pristine and impacted sites that are located in close proximity to one another (Francé *et al.,* 2021).

It is worthy to stress that the GES for pelagic habitats has to be assessed as extent of habitat adversely affected in square kilometres or as a percentage of the total extent of the habitat type (Commission Directive (EU) 2017/845). This is predicated on the assumption that either the available data are completely representative of the area under assessment, or that the sampling and monitoring of pelagic habitats covers the significant area of each habitat type and is able to capture departures from good environmental status at a significant scale (Magliozzi *et al*., 2023).

Magliozzi et al. (2023) also point to the time dimension of the requirement posed by the GES decision; namely the six-years cycles of assessments and measures set by the MSFD, which in turn necessitates high frequency monitoring in order to not underestimate the changes that are occurring over shorter time scales. Defining the timescales of assessment for pelagic habitat is this not an easy task, since the outcomes of such assessments can vary over short, medium and long timescales (Bedford *et al*., 2020). For example, low frequency monitoring, even while it covers extended periods of time, is likely to produce an underestimate of the change occurring over shorter timelines because many changes in pelagic environments appear over short times (Bedford *et al.,* 2020). Nevertheless, maintaining long term time-series of pelagic habitat data is of extreme importance for understanding the drivers of change which operate at longer temporal scale, such as climatic drivers (Edwards et al., 2010). Also, non-linear trends are more easily identified if the time series is longer (Giron-Nava *et al*., 2017).

Pelagic habitats are undoubtedly exposed to a variety of pressures that cause changes at multiple scales. The principal natural factors that affect plankton communities are hydrological and meteo-climatic conditions such as water mixing, precipitation regimes, air temperature), while human activities that can directly or indirectly affect the pelagic habitat are numerous, including fishing, mariculture, introduction of non-indigenous species, climate change and ocean acidification, pollution from urban, industrial and agricultural effluents, and dams-influenced changes in runoff regimes. Following this rationale, Magliozzi *et al.* (2021) classified four main categories of pressures/drivers that interact with environmental status of pelagic habitats: i) hydro-meteorological factors, ii) biological changes, iii) contaminants and litter inputs; and (iv) human physical interventions. It is however difficult to categorize the stressors in terms of the severity of their impact on habitat condition, to assess their cumulative effects or trends in time (Halpern *et al.,* 2015). Furthermore, differentiating between the influence of human activity and natural variability is a challenging task for many researchers because both factors affect changes in the pelagic realm at similar timescales, whether long- or short-term (e.g., Bedford *et al.,* 2020; Magliozzi *et al.,* 2023). Although evidence showed that the Mediterranean Sea is a very highly impacted basin (Coll *et al*., 2011), it recently experienced a decrease in cumulative human impact, along with some other areas like North Sea and East China Sea (Halpern *et al*., 2015).

According to Magliozzi *et al*. (2023), the division of pelagic habitat types into four broad pelagic categories as defined by the GES Decision requires revision to account for the high spatial and temporal variability of biotic and abiotic characteristics of pelagic habitats, to consider the large spatial extent of the marine assessment units and to accommodate the practical and realistic sampling plans within monitoring programs. A step towards implementing this was done also within the frame of SPA/RAC multidisciplinary group of experts for pelagic habitat experts nominated by the Contracting Parties of Barcelona convention (UNEP/MAP/SPA/RAC, 2023), which elaborated on the List of Reference of Pelagic Habitat Types in the epipelagic layer (from surface to 200m depth) of the Mediterranean Sea (UNEP/MAP, 2013; UNEP/MAP, 2021).

While accounting on prevailing natural conditions, anthropogenic pressures and their trends in time, the objective of the Subtask 2.1.2 was to contribute to the definition of relevant assessment scales for pelagic habitat in the Mediterranean Sea. Within this the specific objectives were to:

i) identify spatial scales that are relevant for the assessment of the Mediterranean pelagic habitat, and
ii) identify temporal scales that are relevant for the assessment of the pelagic habitat in the Mediterranean Sea.

Characteristic areas in the Mediterranean Sea have previously been defined by differences in variability in phytoplankton composition, abundance, and distribution in distinct places. These differences, which may be related to variability in hydrodynamics and biogeochemistry, can be identified using ocean modelling products and satellite data. For example, D’Ortenzio and Ribera d’Alcalà (2009) characterized different trophic regimes in the Mediterranean Sea using SeaWiFS satellite surface chlorophyll measurements. Based on typical annual dynamics and concentration ranges, they grouped various regimes into seven clusters, ranging from "non-blooming" and "blooming" clusters to "intermittently blooming" clusters. They predicted that the temporal changes in these clusters could be seen as an effective macrocosm to track changes in the structure of the food web in response to variations in external forcing. Indeed, Mayot et al. (2016) upgraded this study with interannual variability and identified new trophic regimes. A step forward is to use satellite data-based ocean colour products to resolve different ecoregions within the Mediterranean Sea. Recently, such product yielding four phytoplankton functional types (PFTs) was used and coupled to a physical-biogeochemical model by Ciavatta *et al*. (2019). In this way they divided the Mediterranean Sea into three ecoregions characterized by different PFTs dominance: the largest one dominated by picophytoplankton in oligotrophic open waters, the region of nanophytoplankton dominance in and the smallest region dominated by micro-phytoplankton in the most productive, coastal areas.

Ayata *et al*. (2017) synthesized nine different studies on the regionalization of the Mediterranean Sea, including the above-mentioned study by D’Ortenzio and Ribera d’Alcalà (2009). In the studies used by Ayata *et al*. (2017), Mediterranean ecoregions were defined based on various characteristics, including climatological and modelled data on ocean currents, physical and chemical parameters and the distribution of organisms, with chlorophyll concentration data being the most commonly used. In addition to four heterogeneous regions with highly dynamic conditions and intense mesoscale activity as the main feature, they identified eleven consensus regions with relatively homogeneous conditions and nine consensus frontiers, which agree well with the hydrodynamic characteristics of the Mediterranean Sea.

To effectively manage the pelagic realm, a vertical resolution of assessment units should also be given. For example, a vertical delimitation of pelagic habitats would possibly consider the whole water column from surface to seabed in seas characterized by seasonal thermocline (like Mediterranean Sea) or just part of the water column from surface to the hypoxic layer in areas with permanent halocline (like the Baltic and Black Seas) where the hypoxic sub-layer is considered resilient to measures taken in the time scale of the MSFD cycles of 6 years (Magliozzi *et al*., 2021). One study to delineate the vertical dimension was undertaken by Sammartino *et al*. (2018). They utilized an Artificial Neural Network to generate a 3-D Chlorophyll-a field in the Mediterranean Sea, extrapolating from surface satellite estimates, thereby enhancing the resolution of finer spatial and temporal scales compared to traditional climatological methods. Importantly, this model reproduced the subsurface Chlorophyll-a maxima (i.e., deep chlorophyll maximum – DCM) very well, a feature that was recognized as important for some oligotrophic regions of the Mediterranean Sea also by the multidisciplinary group of experts (UNEP/MAP/SPA/RAC, 2023). Namely, their new tentative list of habitat types in the epipelagic layer down to 200m of depth acknowledge the existence of DCM in the proposed pelagic habitat type “Marine water: oceanic - very low surface CHL (<0.1 mg/m^3) with deep CHL maximum”.

Temporal scales are important for defining reference conditions/thresholds or assessing the significance of any change, for example, when compared to extreme events. Bedford *et al.* (2020) demonstrated the significance of the time dimension in the evaluation of real pelagic habitat indicators utilised in the North East Atlantic. In this aspect, an interesting upgrade to the spatial distribution of pelagic habitat was done by Tew Kai *et al*. (2020) with the inclusion of a dynamic temporal dimension for the delineation of coastal-shelf seascapes in the North-East Atlantic area. With a three-dimensional ocean circulation modelling approach, they used twelve key oceanographic and derived variables from operational coastal oceanography to produce a time series of categorical maps that were combined with MSFD marine sub-region to build seascapes.

Building on these foundations, the research conducted within Task 2.1, Subtask 2.1.2 aimed to contribute to the delineation of relevant assessment scales that consider both anthropogenic influences and prevailing natural conditions within Mediterranean pelagic habitats. We also aimed at utilizing readily accessible open-source data acquired at a high frequency, covering the entire area of interest. Our study presents a case analysis delving into the temporal and spatial dimensions of phytoplankton functional types (PFTs) using data derived from the Copernicus Marine Service product titled "Mediterranean Sea Reprocessed Surface Chlorophyll Concentration and Phytoplankton Functional Types from Multi-Satellite Observations." This product relies on regional empirical algorithms showing the relationships between the total Chlorophyll-a concentration and seven accessory pigments considered diagnostic for the main phytoplankton groups (Di Cicco *et al.,* 2017).

2 Mediterranean assessment scales

2.1 Satellite data preparation

The satellite data was obtained from the Copernicus Marine Service (CMEMS) web portal (https://marine.copernicus.eu/, accessed on January 2023). The product titled: "Mediterranean Sea Reprocessed Surface Chlorophyll Concentration and Phytoplankton Functional Types from Multi Satellite observations" (DOI: https://doi.org/10.48670/moi-00112) was used for the analysis. The latter provides daily L3 data on surface distribution of three phytoplankton size classes (PSCs) and six phytoplankton functional types (PFTs) expressed as Chlorophyll-a concentration in sea water (mg/m^3) for the whole Mediterranean Sea.

L3 are the daily composite products interpolated to a regular grid (1 km resolution) as obtained by merging all the ocean satellite passages. This multi-sensor product is based on MODIS-AQUA, NOAA20-VIIRS, NPP-VIIRS and Sentinel3A-OLCI data. The product provides estimates of biomass expressed as Chlorophyll-a concentrations for 9 phytoplankton groups: 3 size classes (micro-phytoplankton, nanophytoplankton, picophytoplankton) and 6 taxonomic groups (diatoms – abbreviated as diato, dinoflagellates – abbreviated as dino, cryptophytes – abbreviated as crypto, haptophytes – abbreviated as hapto, green algae and prochlorophytes – abbreviated as green and prokaryotes – abbreviated as prokar (Di Cicco *et al.,* 2017). In this classification, "micro" consists of diatoms and dinoflagellates, "nano" includes cryptophytes and haptophytes, and "pico" is referred to cyanobacteria, green algae and prochlorophytes (Di Cicco *et al*., 2017).

Daily PFT concentration values were downloaded from the CMEMS for the 1998-2020 period. The NCO (NetCDF Operators) software toolkit was used to calculate mean values for the whole period, seasonal averages for the whole period, mean and seasonal values for the first decade (1998-2007) and mean and seasonal values for the last decade (2011-2020). The seasons were defined as winter (January-March), spring (April-June), summer (July-September) and autumn (October-December).

The CMEMS product titled: "Mediterranean Sea - High Resolution L4 Sea Surface Temperature Reprocessed" (DOI: https://doi.org/10.48670/moi-00173) was used for sea surface temperature (SST) analysis. This product provides daily gap-free SST in 0.05° resolution. The NCO software toolkit was used to calculate mean and seasonal values for the first decade (1998-2007) and mean and seasonal values for the last decade (2011-2020).

2.2 Data analysis

In depth analysis was performed in Python programming language and PyTorch an open source machine learning framework. K-means clustering was used, to identify aggregations in the 6- and 3-dimensional space for PFTs and PSCs, respectively, based on Euclidean distance. Altogether 1,956,210 data points were used in the analysis.

Coastal points were removed from the dataset due to large uncertainty. Clustering was performed for 4, 6, 10 and 12 classes (clusters) on mean PFTs chlorophyll concentration values for the whole period (1998-2020) and the results were compared to literature data. Ten classes were selected as best match to existing regionalisation of the Mediterranean Sea. These classes were used in all further analyses. Each point in the decadal and seasonal averages was assigned to one of the beforementioned classes. Cluster centroids for biomass of size classes are shown in Figure 1, while those for biomass in PFTs are shown in Figure 3.

2.3 Spatial and temporal scales

2.3.1 Mapping of the Mediterranean Sea in 10 clusters

Although the clustering was done with Chl-a biomass in phytoplankton functional types (PFTs) and in phytoplankton size classes (PSCs), we mainly present the result based on PFTs, since both clustering produced very similar results. The clusters are characterised by the average biomass of 6 PFTs 3 PSCs expressed as Chl-a concentration (mg/m^3) in the period 1998-2020. The clustering level that gave the most meaningful result in terms of discrimination of different areas was the one with 10 clusters.

For the PSCs clustering, the average total Chl-a biomass in clusters ranged from 0.05 mg/m^3 in cluster “0” to 3.95 mg/m^3 in cluster “9” (Figure 1). The majority of clusters are located near the coasts (clusters “9” to “4”) and present very high (“9”; 3.95 mg Chl-a/m^3) to medium (“4”; 0.73 mg Chl-a/m^3) phytoplankton biomass (Figs. 1 and 2). The open sea clusters cover the largest part of the Mediterranean Sea and present low (“3”; 0.42 mg Chl-a/m^3) to very low biomass (“0”; 0.05 mg Chl-a/m^3) (Figs. 1 and 2). The clusters with the highest biomass are dominated by the microphytoplankton size class: 55.7% in cluster ”9”, 50.8% in cluster “8” and 46.6% in cluster “7” (Figs. 1 and 2). The clusters with high to medium biomass are dominated by nanophytoplankton size class: 42.6% in cluster ”6”, 44.0% in cluster “5”, 45.2% in clusters “4” and “3”, and 43.8% in cluster “2” (Figure 3). On the other hand, picophytoplankton size class dominates the clusters with very low biomass: 41.4% in cluster “1” and 55.0% in cluster “0” (Figure 3). The “coastal belt” with clusters very high to medium biomass is wider in the Alboran Sea (stretching from the Strait of Gibraltar toward east), along the coasts of NW Mediterranean, in the northern and western Adriatic, in the Aegean Sea, Gulf or Gabes and southern Levantine coasts (Figure 2). Cluster “0” covers the most part of the eastern Mediterranean Sea and the Tyrrhenian Sea, while cluster “1” covers most of the central and southern Adriatic Sea and the Western Mediterranean (Figure 2).

	micro	nano	pico
9	2.2	1.4	0.35
8	1.6	1.2	0.35
7	1.1	0.94	0.32
6	0.69	0.72	0.28
5	0.42	0.51	0.23
4	0.23	0.33	0.17
3	0.12	0.19	0.11
2	0.052	0.096	0.071
1	0.021	0.047	0.048
0	0.0075	0.017	0.03

Figure 1: Cluster centroids (averages) for biomass of size classes (in mg Chl-a/m^3) for the whole study period (1998-2020). Micro – micro-phytoplankton, nano – nanophytoplankton, pico – picophytoplankton.

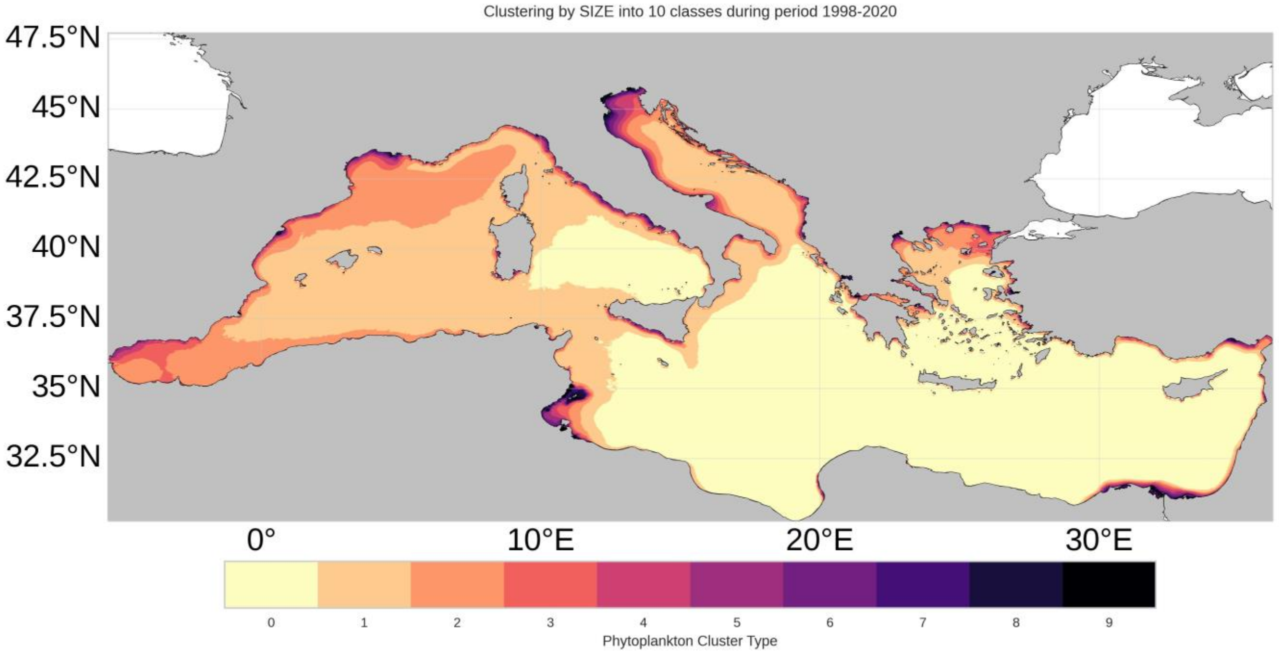


Figure 2: Map of the Mediterranean Sea with the distribution of 10 clusters based on biomass in different phytoplankton size classes (PSCs) for the whole study period 1998-2020. (for the definition of clusters see Figs. 1 and 3)

Figure 3: Average contribution of phytoplankton size classes to total biomass in different clusters for the whole study period (1998-2020). Micro: micro-phytoplankton, nano: nanophytoplankton, pico: picophytoplankton

For the PFTs clustering, the total Chl-a biomass in clusters resulted in almost the same averages that ranged from 0.05 mg/m^3 in cluster “0” to 3.90 mg/m^3 in cluster “9” (Figure 4). Also, the distribution of clusters in the Mediterranean Sea was very similar to those based on PSCs (Figs. 2 and 5). The clusters with the highest biomass were dominated by the diatoms: from 51.3% in cluster ”9” to 37.2% in cluster “6” (Figure 6). The clusters with medium and low biomass are dominated by haptophytes: from 33,9% in cluster ”5” to 38,6% in cluster “1”, while the cluster with the lowest biomass was dominated by prokaryotes/cyanobacteria (Figure 6). The contribution of diatoms and cryptophytes to total biomass was diminishing from cluster “9” to cluster “0”: from 51,3% to 9,3% and from 20% to 2,6%, respectively. Contrary to this, the relative biomass of prokaryotes/cyanobacteria was rising from 4.1% in cluster “9” to 43.7% in cluster “0” (Figure 6). Similarly, the contribution of haptophytes was rising from 15% in cluster “9” to 38,6% in cluster “1” and was again a little lower in cluster “0” (36,4%). Green algae and prochlorophytes had the highest contribution to total biomass in clusters with medium to low biomass (max 10.3% in cluster 3”). On the other hand, dinoflagellates relative biomass was more or less stable in all clusters – 4.1% to 5.3% (Figure 6).

	diato	dino	crypto	hapto	green	prokar
9	2	0.16	0.77	0.58	0.23	0.16
8	1.5	0.13	0.55	0.65	0.2	0.13
7	1	0.099	0.38	0.62	0.17	0.11
6	0.66	0.075	0.25	0.54	0.15	0.1
5	0.4	0.054	0.15	0.42	0.12	0.095
4	0.22	0.037	0.083	0.29	0.081	0.084
3	0.11	0.022	0.039	0.17	0.047	0.068
2	0.043	0.012	0.015	0.085	0.021	0.05
1	0.015	0.006	0.005	0.045	0.0086	0.037
0	0.0051	0.0024	0.0014	0.02	0.002	0.024

Figure 4: Cluster centroids for Chl-a biomass of PFTs (in mg/m^3) for the whole study period (1998-2020). Diato – diatoms, dino – dinoflagellates, crypto – cryptophytes, hapto – haptophytes, green – green algae and prochlorophytes, prokar – prokaryotes

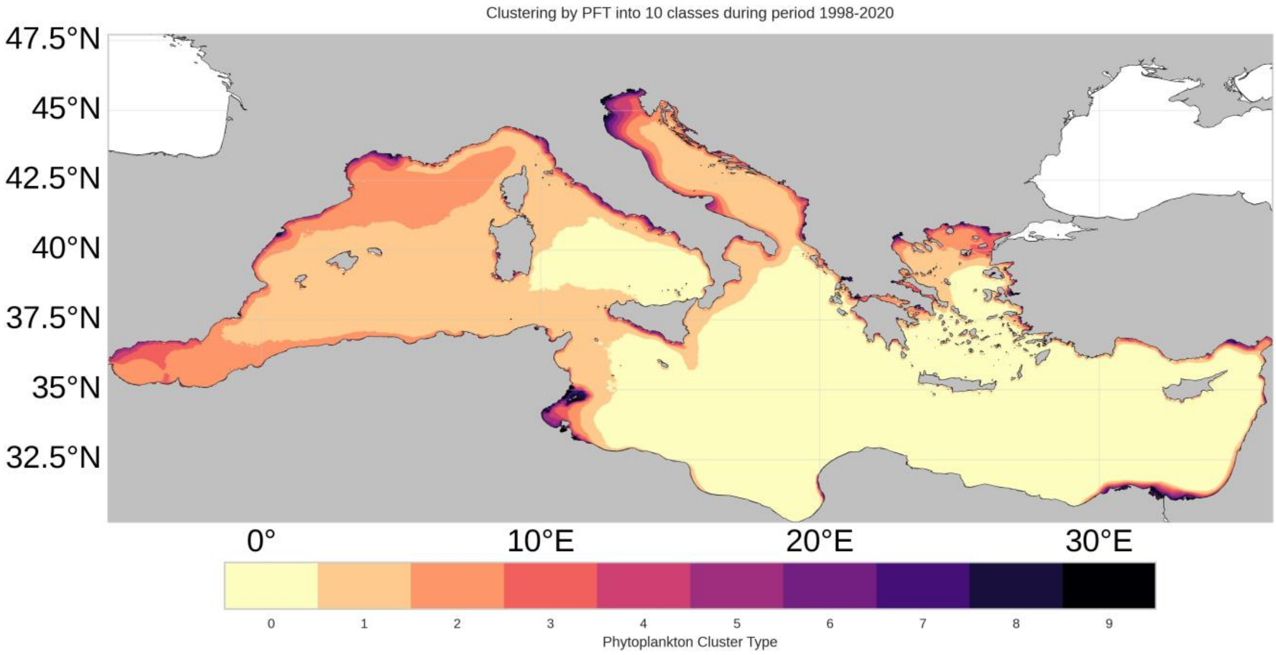


Figure 5: Map of the Mediterranean Sea with the distribution of 10 clusters based on biomass in different phytoplankton functional types (PFTs) for the whole study period (1998-2020). (for the definition of clusters see Figs. 4 and 6)

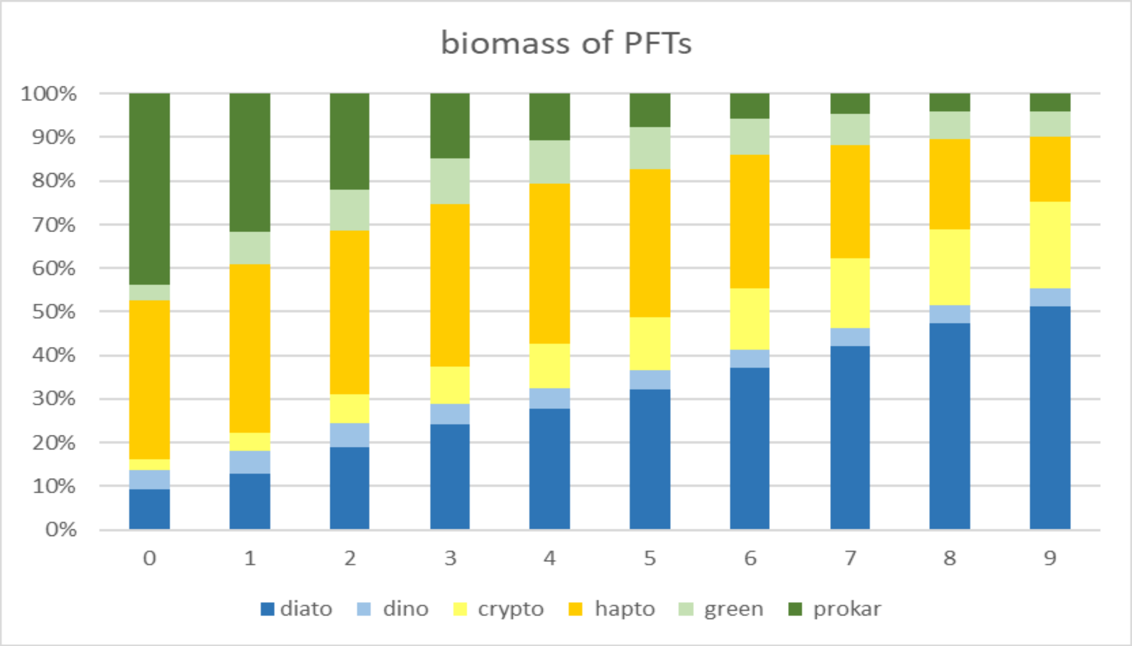


Figure 6: Average contribution of phytoplankton functional types (PFTs) to total biomass in different clusters for the whole study period (1998-2020). Diato – diatoms, dino – dinoflagellates, crypto – cryptophytes, hapto – haptophytes, green – green algae and prochlorophytes, prokar – prokaryotes.

2.3.2 Decadal differences in PFTs clustering

Here onwards, only the results of the PFTs clustering are presented as those on PSCs gave very similar results. Clustering was computed first at a general annual level for the two decades: 1998-2007 and 2011-2020 and differences between the areas covered by different clusters were drawn. Differences were confronted with change in sea surface temperatures (SST) for the same two decades. The same procedure was then repeated for every season, as different characteristics were expected for different seasons.

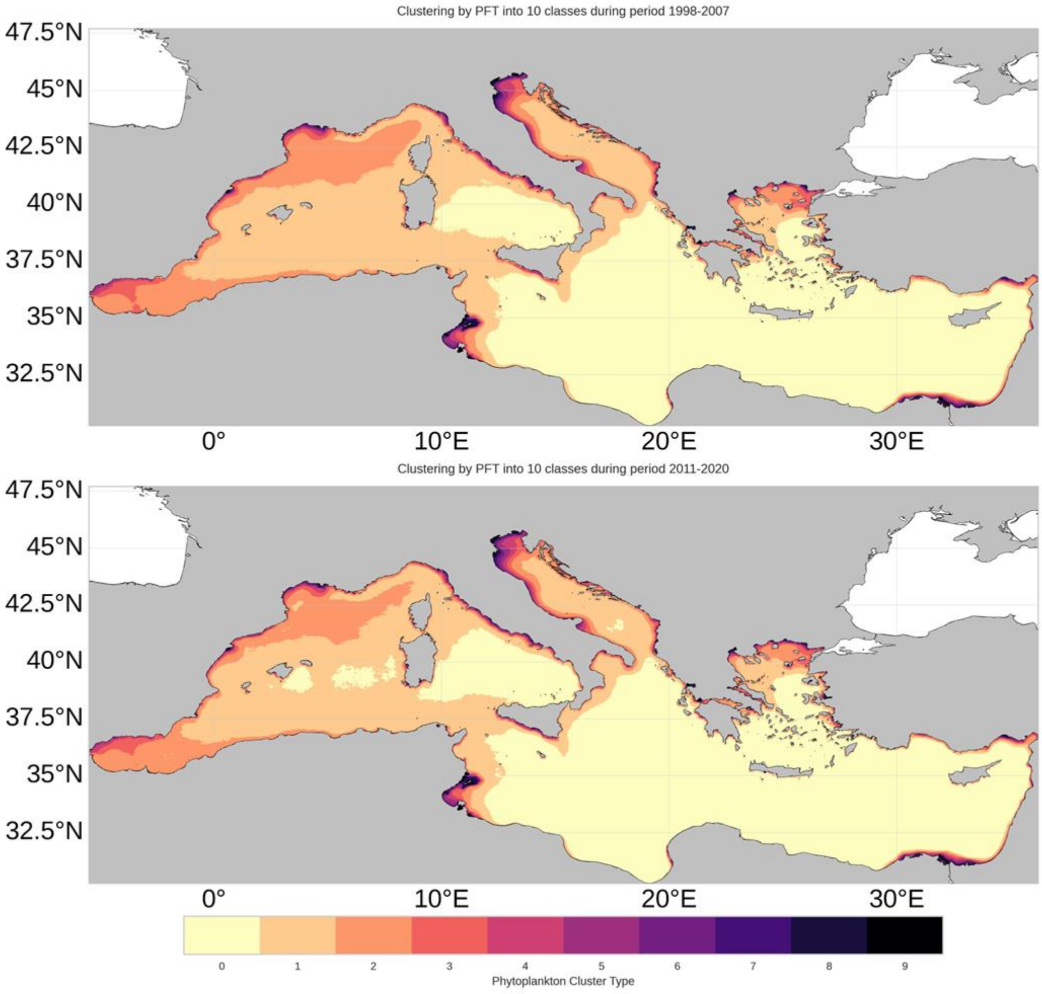
Decadal differences at the average annual level

General picture of the differences in the cluster's coverage revealed that no major differences occurred in the eastern and southern part of the Mediterranean Sea, which was covered by the cluster "0" in both periods (Figures 7 and 8). On the other hand, major changes at the annual level occurred in the western part of the Mediterranean Sea, northern Aegean Sea, and Adriatic Sea. Changes mainly refer to enlargement of the cluster "0", which means that larger areas were characterised by the lowest Chl-a biomass and the highest contribution of prokaryotes/cyanobacteria. The biggest area of the cluster "0" in the eastern and southern Mediterranean enlarged at its edges, as marked by the blue colour in Figure 8. Also, the patch of cluster "0" in the Tyrrhenian Sea has enlarged towards north and west, and of new areas of cluster "0" emerged in the western Mediterranean Sea between Balearic Islands and Sardinia (Figures 7 and 8). Moreover, a small area of cluster "0" appeared in the southern Adriatic Sea. There were also some changes in the coverage of clusters "1" and "2", mainly in the Ligurian Sea where a part of cluster "2" area shrunk towards west resulting in a narrower belt of cluster "2" in the NW

Mediterranean. Same transition between clusters “2” and “1” occurred in the northern Aegean Sea and in Alboran Sea (Figures 7 and 8).

Apart some isolated patches in the coastal of the Mediterranean Sea, the only consistent shift towards clusters with a higher contribution of bigger cells was in the northern Adriatic Sea and along its western coasts (marked with red in Figure 8).

As for the average SST, the highest increase between the two decades was recorded in northern areas (Adriatic, Aegean, and Ligurian Sea), Levantine Basin and in the area SE of Sardinia (Figure 9). Almost no difference in the SST between the decades was observed in the southern part of the Mediterranean and small increase in the Alboran Sea (Figure 9).



Clustering by PFT into 10 classes during period 1998-2007

Clustering by PFT into 10 classes during period 2011-2020

Figure 7: Map of Mediterranean Sea with the distribution of 10 clusters based on biomass in different phytoplankton functional types (PFTs) for the periods: (above) 1998-2007 and (below) 2011-2020 (for the definition of clusters see Figs 4 and 6).

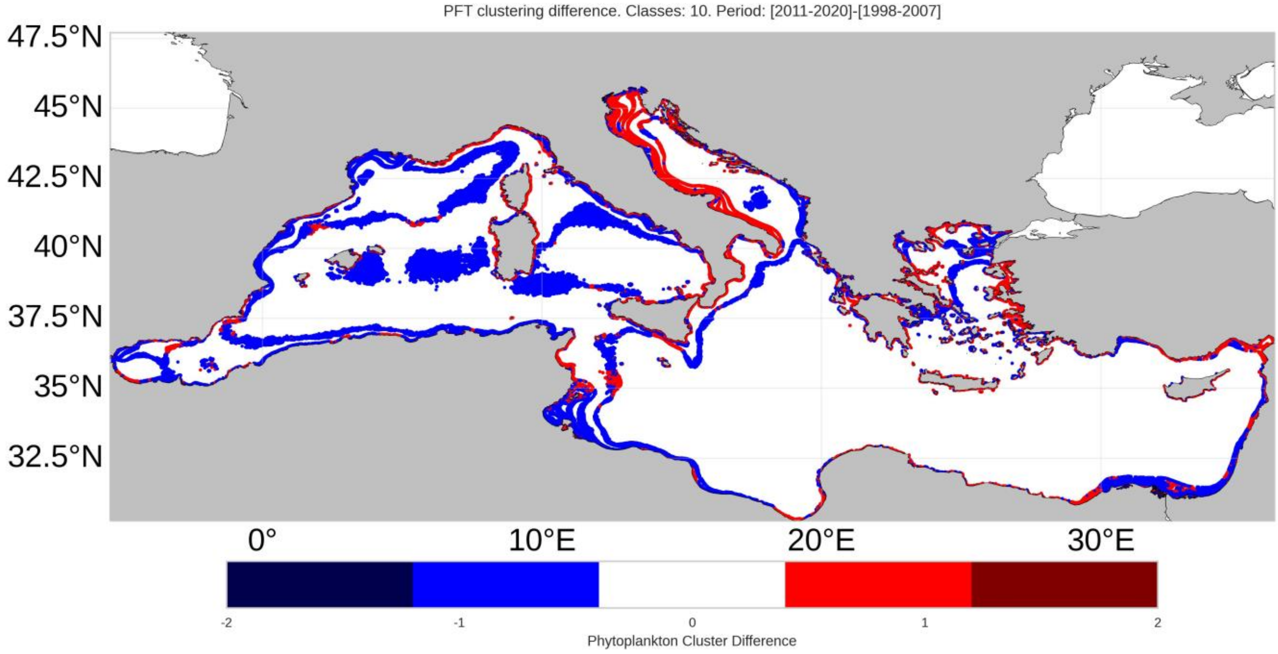


Figure 8: Map of changes in PFTs cluster areas between the periods 1998-2007 and 2011-2020: blue colours mark a change towards a cluster with a lower number, red colours indicate a change towards a cluster with a higher number, white colour indicates areas without change.

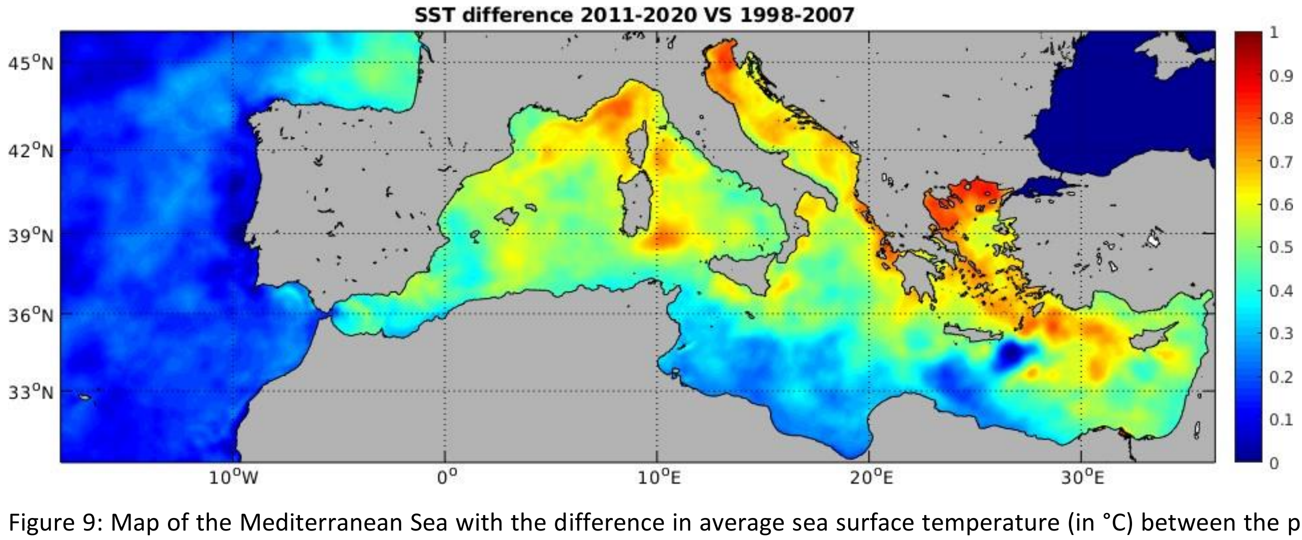


Figure 9: Map of the Mediterranean Sea with the difference in average sea surface temperature (in °C) between the periods 1998-2007 and 2011-2020

Decadal differences in winter

The distribution of PFT clusters in winter indicated a highest average Chl-a biomass and a highest contribution of bigger phytoplankton from micro size class among all seasons, since clusters “3” and “2” covered the biggest areas in western Mediterranean Sea, northern Adriatic, and northern Aegean Sea (Figure 10). On the contrary, cluster “0” covered the smallest area and restricted to the eastern Mediterranean Sea (Figure 10). Also, the major differences between the two study decades were found (Figures 10 and 11), mainly related to the expansion of cluster “0”, which in 2011-2020 covered a substantially bigger area in the eastern Mediterranean compared to previous decade. Also, other shifts between clusters in the eastern Mediterranean and Aegean Sea directed towards clusters with smaller phytoplankton and lower biomass as indicated by the dominance of blue colour in Fig. 11.

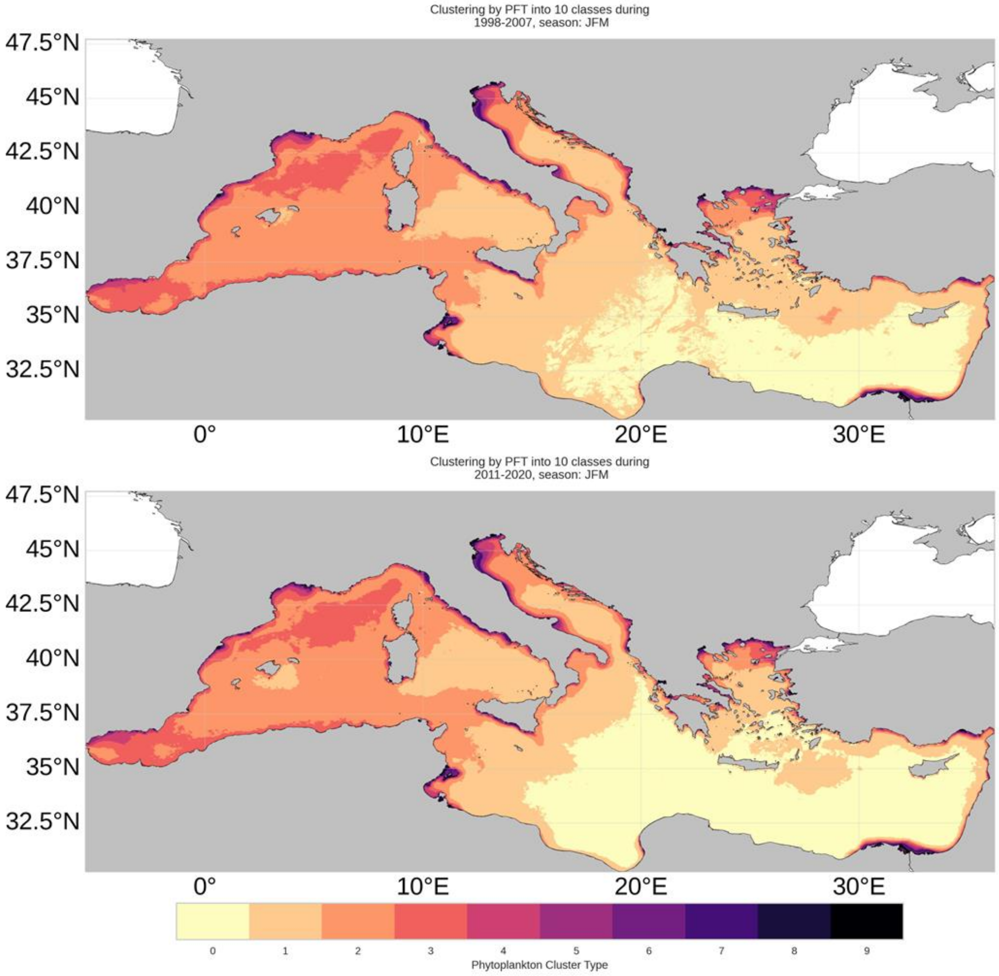


Figure 10: Winter (January, February, March) distribution of 10 clusters based on biomass in different phytoplankton functional types (PFTs) in the periods: (above) 1998-2007 and (below) 2011-2020. (for the definition of clusters see Figs. 4 and 6).

The changes between the two decades in winter season were not as uniform in the western Mediterranean and Adriatic Sea. The differences in the Alboran Sea and NW Mediterranean mainly referred to switch between clusters

“3“ and “2“ in both directions, while a small patch of cluster “1” appeared south of Balearic islands (Figures 10 and 11). The cluster “1” in the Tyrrhenian Sea expanded towards west and south. As for the Adriatic Sea, changes in the last decade mostly occurred in the direction towards clusters with bigger phytoplankton fractions, mainly enlargement of clusters “2”, “3“ and “4” (Figures 10 and 11).

The most substantial increase in winter SST occurred in the northern Adriatic, Aegean Sea, northern Levantine basin, and Ionian Sea (Figure 12). No differences in SST were observed in Alboran Sea and southern parts of the Mediterranean, while minor increases were recorded in the NW Mediterranean and Tyrrhenian Sea (Figure 12).

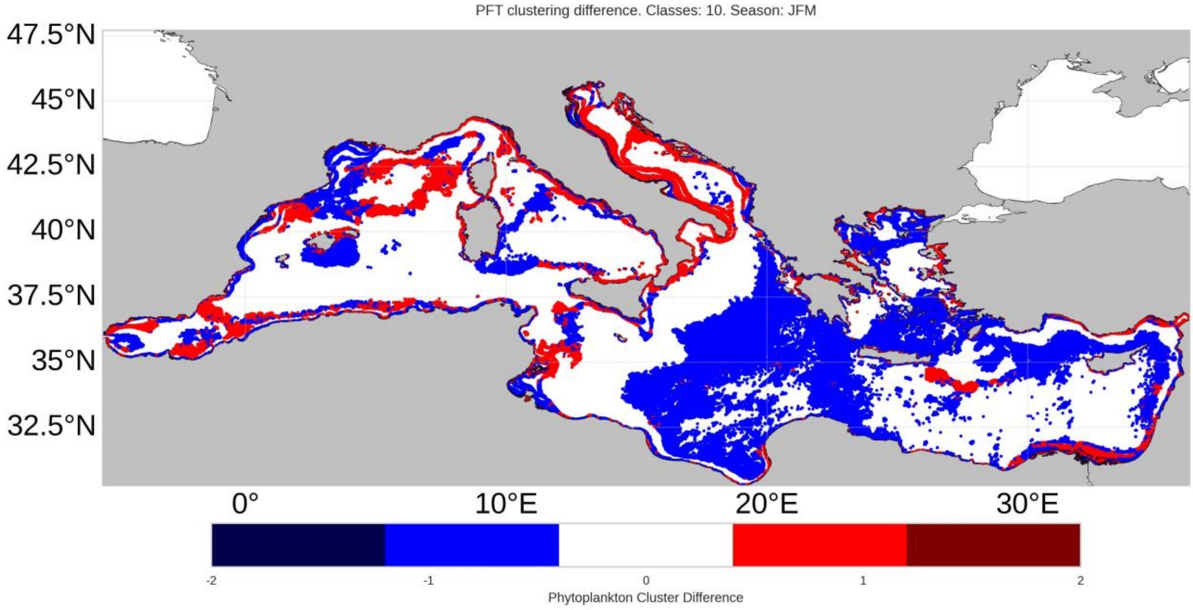


Figure 11: Map of changes in PFTs cluster areas for winter (January, February, March) between the periods 1998-2007 and 2011-2020: blue colours mark a change towards a cluster with a lower number, red colours indicate a change towards a cluster with a higher number, white colour indicates areas without change.

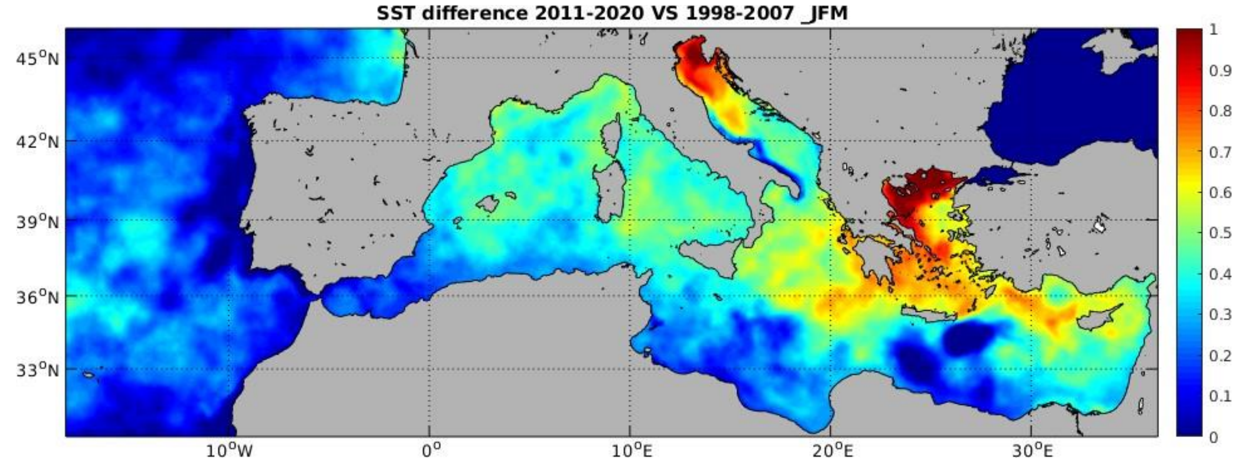


Figure 12: Difference in average winter (January, February, March) sea surface temperature (in °C) between the periods 1998-2007 and 2011-2020.

Decadal differences in spring

The cluster “0” covered the majority of eastern Mediterranean Sea, the Tyrrhenian Sea and southern part of the W Mediterranean Sea, whereas clusters “1”, “2” and “3” occupied the Alboran Sea, NW Mediterranean Sea, Adriatic and northern Aegean Sea (Figure 13). While the situation in the eastern Mediterranean remained largely the same in spring 2011-2020 compared to spring 1998-2007, the largest differences between the two decades occurred in the W Mediterranean and Adriatic Sea (Figures 13 and 14). The cluster “0” expanded from Tyrrhenian towards Ligurian Sea and replaced the cluster “1”. Clusters “1” and “2” widened in the Ligurian Sea and Balearic Sea at the expense of clusters “2” and “3”, respectively. So, in spring most of the changes toward phytoplankton community with higher contribution of smaller cells and lower biomass occurred in the western Mediterranean (Fig. 13, 14).

A contrasting pattern was observed in the Adriatic and Aegean Seas (Figures 13 and 14). Similarly to the winter season, the Adriatic Sea mainly experienced enlargement of clusters “2”, “3“ and “4“ in the northern and western part, while in the southern Adriatic Sea a patch of cluster “0” appeared in the recent decade.

The most pronounced increase in spring SST from 1998-2007 to 2011-2020 was found in the northernmost part of the Adriatic Sea, the Aegean Sea and Levantine basin, while only moderate SST increase was observed in the Balearic Sea and along the coasts of the NW Mediterranean Sea (Figure 15).

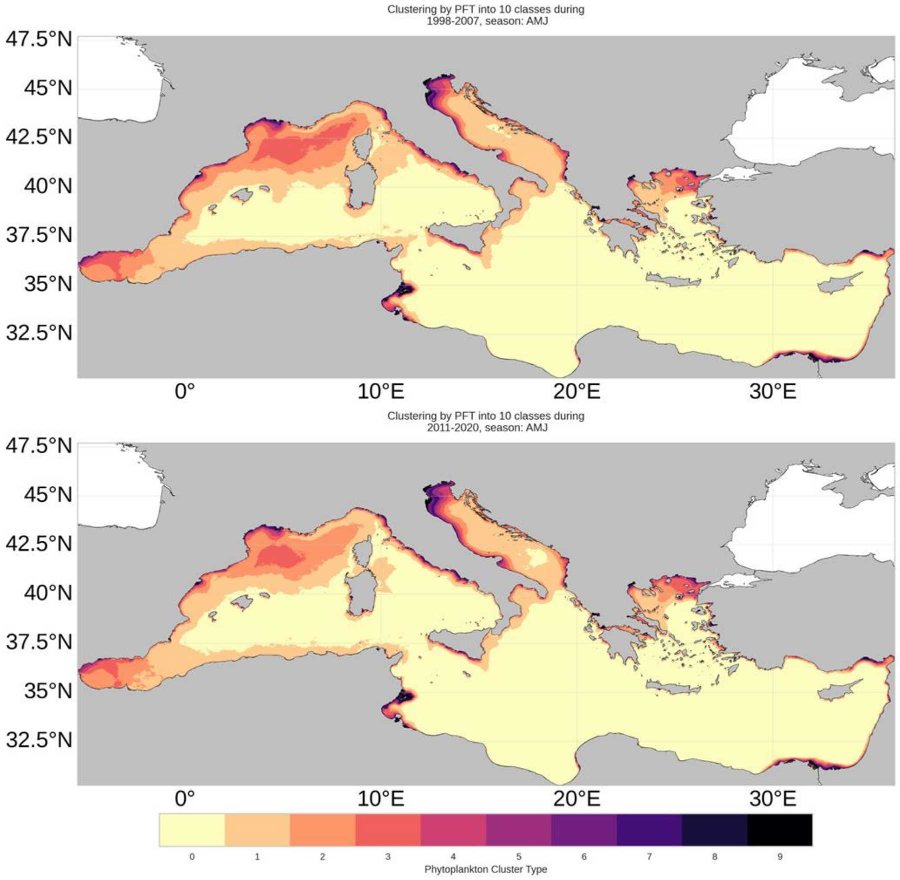


Figure 13: Spring (April, May, June) distribution of 10 clusters based on biomass in different phytoplankton functional types (PFTs) in the periods: (above) 1998-2007 and (below) 2011-2020. (for the definition of clusters see Figs. 4 and 6).

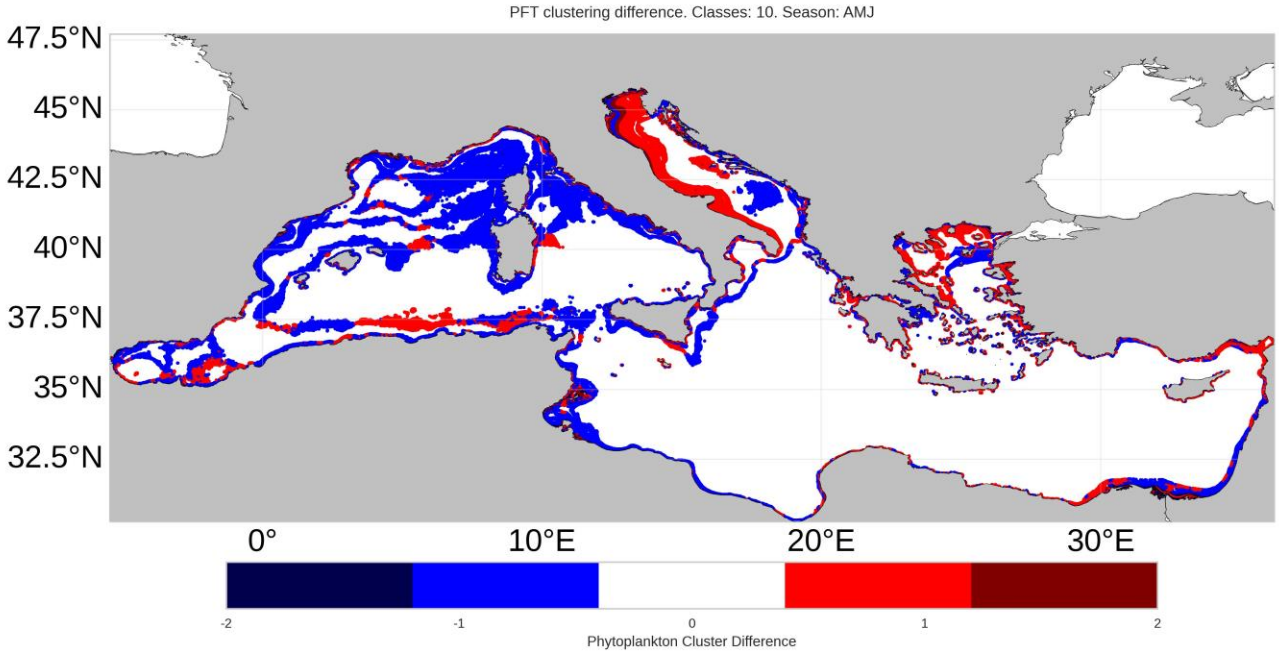


Figure 14: Map of changes in PFTs cluster areas for spring (April, May, June) between the periods 1998-2007 and 2011-2020: blue colours mark a change towards a cluster with a lower number, red colours indicate a change towards a cluster with a higher number, white colour indicates areas without change.

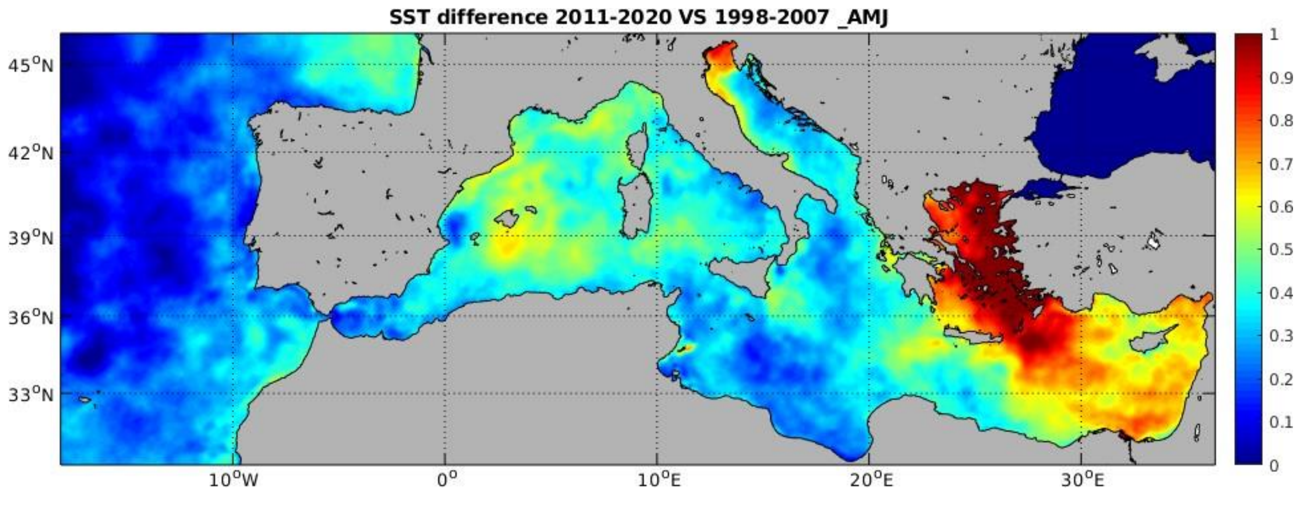


Figure 15: Difference in average spring (April, May, June) sea surface temperature (in °C) between the periods 1998-2007 and 2011-2020.

Decadal differences in summer

The summer pattern of PFT clusters showed the most uniform picture of the Mediterranean Sea, with the majority of the basin covered by the cluster “0” (Figure 16). Only the most productive coastal regions were covered by other clusters, mostly clusters “1”, “2“ and “5“, while cluster with even higher biomass were only present in the northern Adriatic and Gulf of Gabes (Figure 16). Differences between the two decades were only found in these more productive areas and were mostly oriented towards enlargement of clusters with lower biomass and smaller phytoplankton cells as indicated by the blue colour of changes in Figure 16.

Changes were again different in the northern Adriatic Sea where some expansion of clusters with higher biomass occurred (red coloured changes in the Figure 17).

Contrary to the spring situation, the most pronounced increase in summer SST was recorded in the Adriatic, Tyrrhenian, Ligurian and Alboran Seas, with some patches of substantial SST increase also in the Levantine basin), whereas small or no increase in summer SST observed in the Aegean Sea and in southern Mediterranean (Fig. 18).

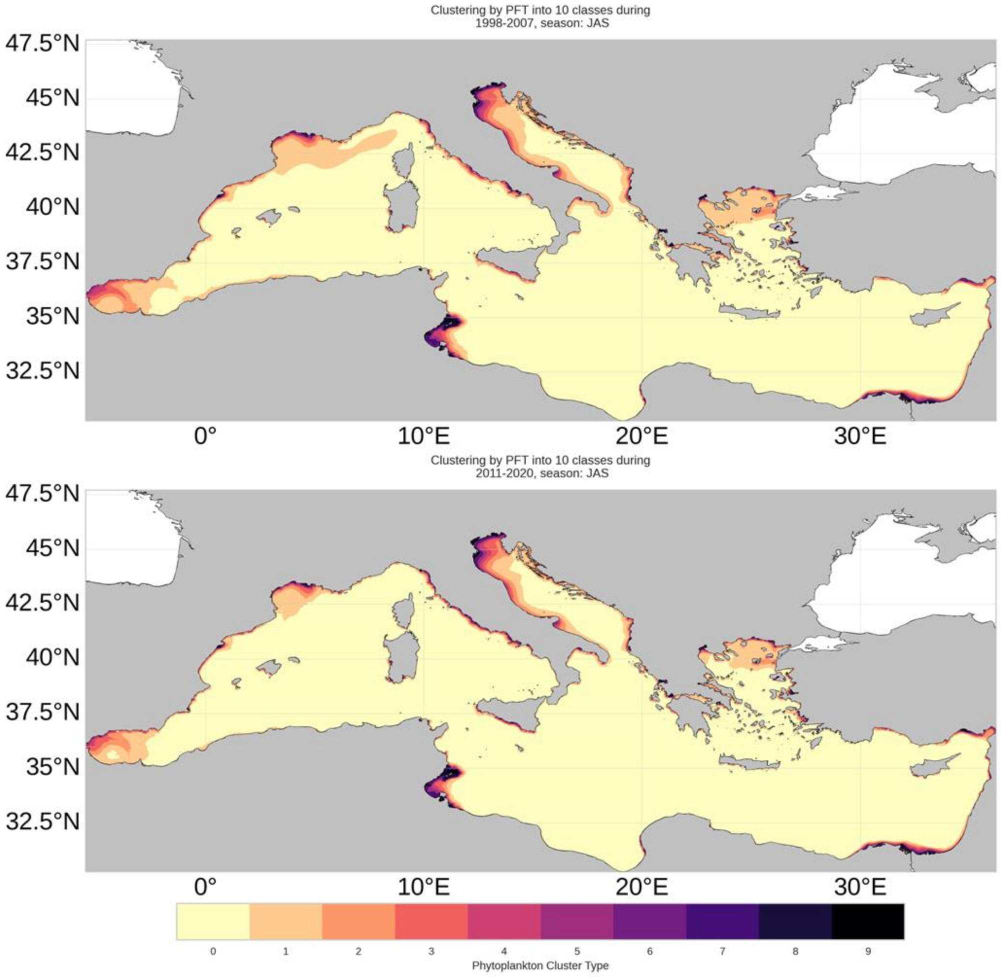


Figure 16: Summer (July, August, September) distribution of 10 clusters based on biomass in different phytoplankton functional types (PFTs) in the periods: (above) 1998-2007 and (below) 2011-2020. (for the definition of clusters see Figs. 4 and 6).

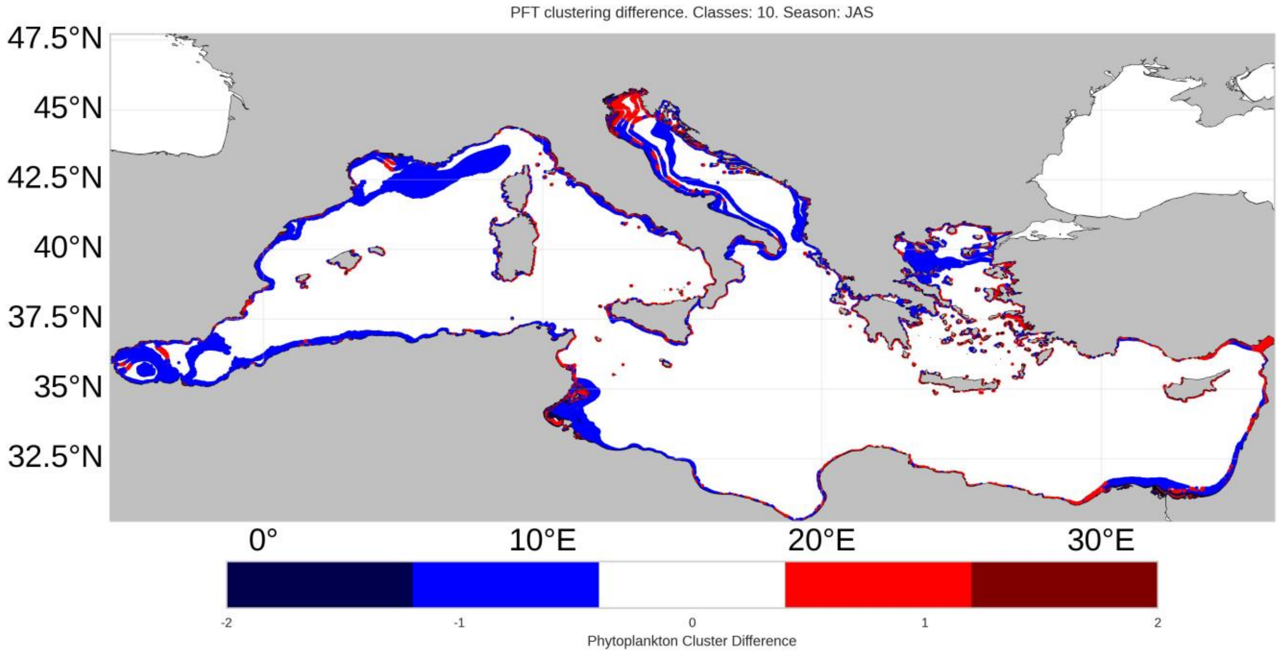


Figure 17: Map of changes in PFTs cluster areas for summer (July, August, September) between the periods 1998-2007 and 2011-2020: blue colours mark a change towards a cluster with a lower number, red colours indicate a change towards a cluster with a higher number, white colour indicates areas without change.

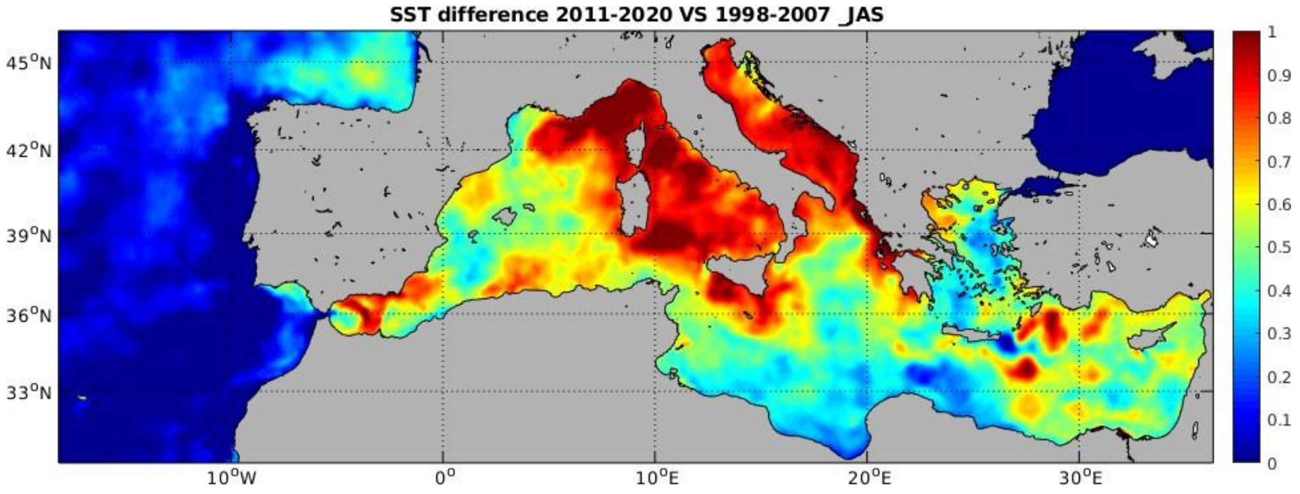


Figure 18: Difference in average summer (July, August, September) sea surface temperature (in °C) between the periods 1998-2007 and 2011-2020.

Decadal differences in autumn

After the summer oligotrophy in almost the entire Mediterranean Sea, in autumn the cluster “0” was partly replaced by cluster “1” and in some areas with other “higher” clusters (Figure 19). So, the cluster “0” in autumn covered the eastern part of the basin and the Tyrrhenian Sea. Western Mediterranean, central, southern Adriatic Sea and central Aegean Sea were mainly covered by cluster “1”, while in the Alboran Sea, Gulf of Lion, Gulf of Gabes and northern Aegean Sea there were also belts of clusters “2”, “3” and higher (Figure 19).

The largest differences between decades during autumn occurred in the western Mediterranean Sea: there was an enlargement of the Tyrrhenian patch of cluster “0” towards west and north, and the emergence of a new patch of cluster “0” between Balearic Islands, Sardinia and Corsica (Figs 19, 20). Also, cluster “1” expanded towards north, west and south in the western Mediterranean Sea. The changes towards clusters with lower biomass and smaller phytoplankton fractions occurred also in the Adriatic Sea (as marked by blue colour in Fig.20). Just isolated patches of the opposite changes (towards higher biomass and bigger phytoplankton fractions) occurred in autumn (Fig. 20).

As for the SST changes, the most pronounced increase in autumn SST was recorded in the western Mediterranean Sea, central and southern Adriatic Sea, northern Ionian Sea, and central Levantine basin (Figure 21). No SST change was observed in the southern part of the eastern basin (Figure 21).

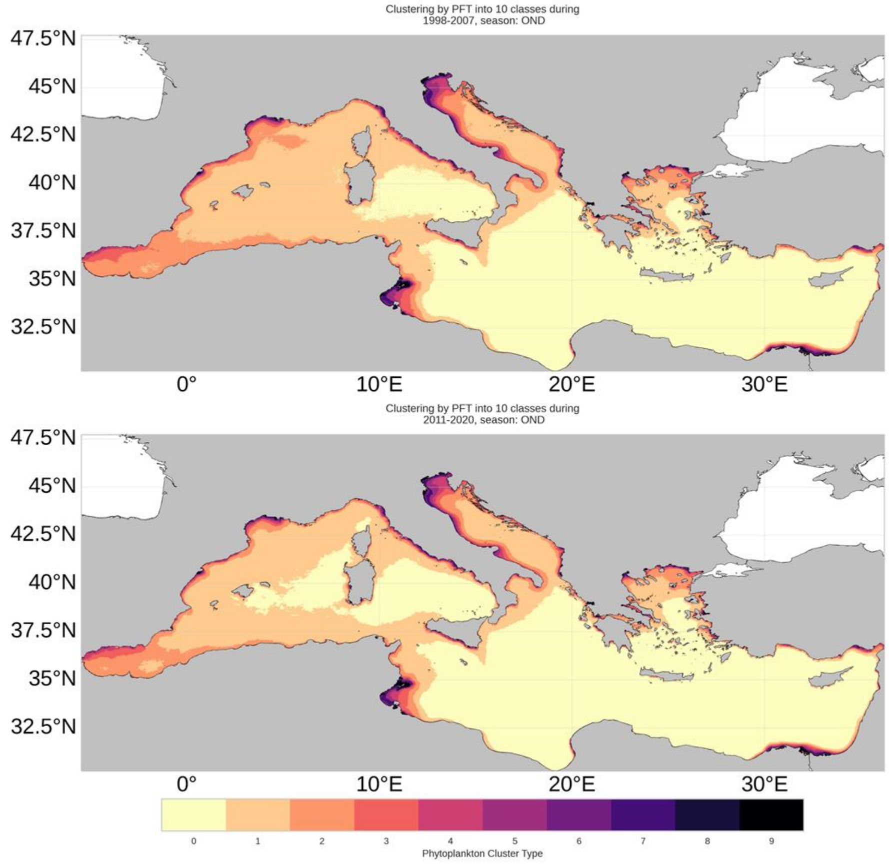


Figure 19: Autumn (October, November, December) distribution of 10 clusters based on biomass in different phytoplankton functional types (PFTs) in the periods: (above) 1998-2007 and (below) 2011-2020. (for the definition of clusters see Figs. 4, 6).

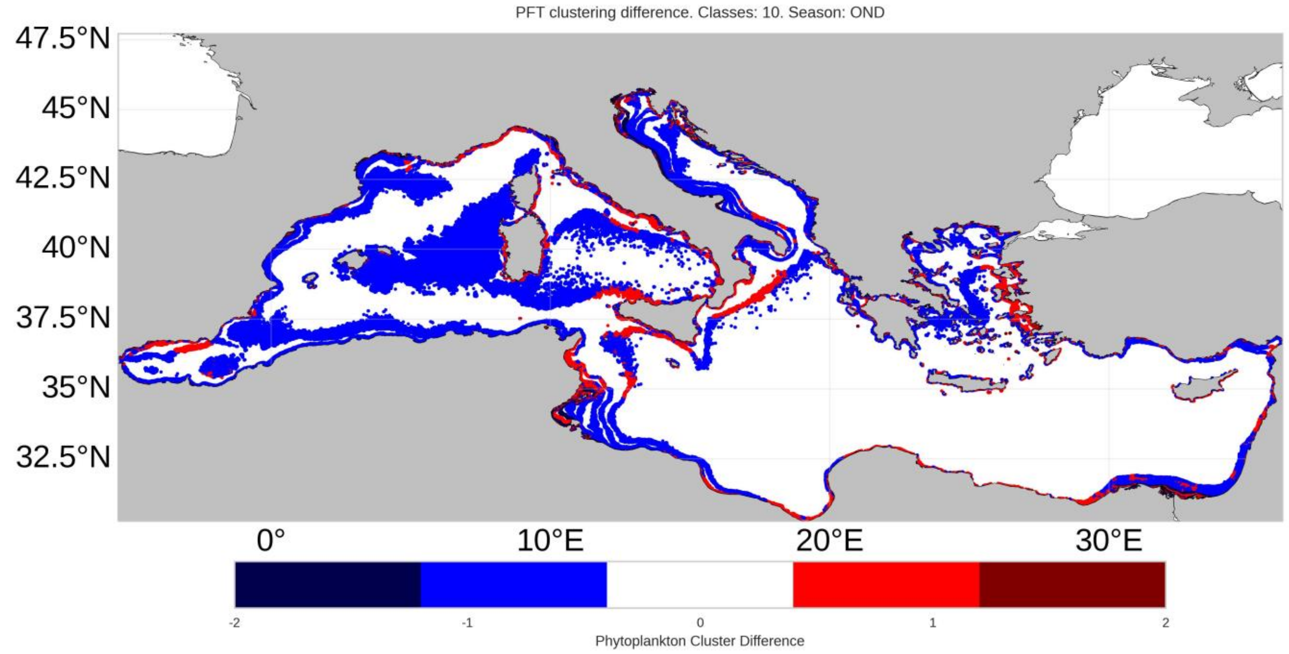


Figure 20: Map of changes in PFTs cluster areas for autumn (October, November, December) between the periods 1998-2007 and 2011-2020: blue colours mark a change towards a cluster with a lower number, red colours indicate a change towards a cluster with a higher number, white colour indicates areas without change.

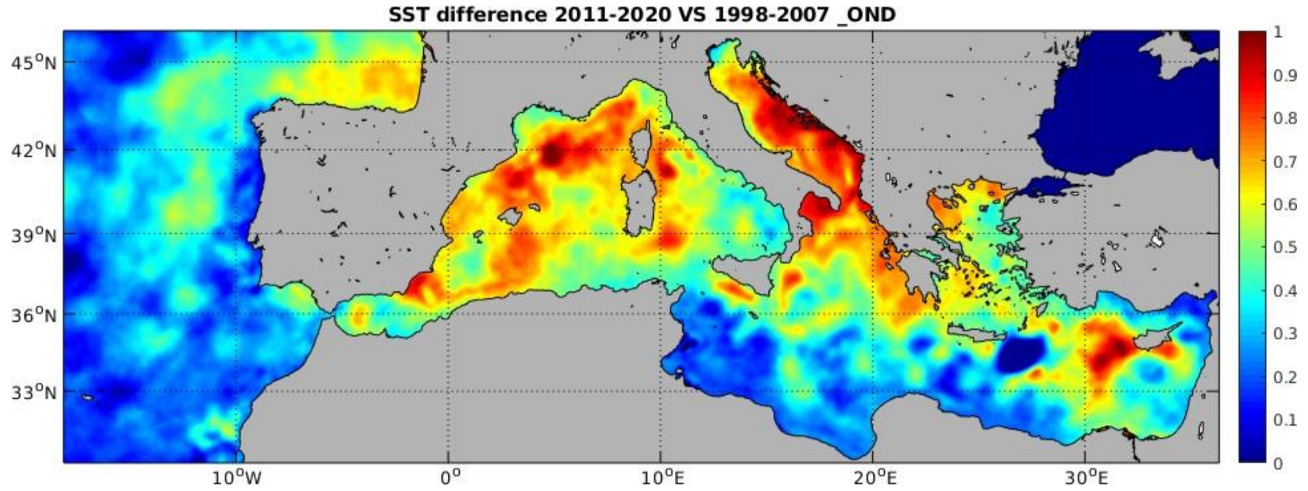


Figure 21: Difference in average autumn (October, November, December) sea surface temperature (in °C) between the periods 1998-2007 and 2011-2020.

2.4 Discussion

This work was done with the long-term data of a CMEMS product that is based on algorithms tailored to the specificities of the Mediterranean Sea (Di Cicco *et al.,* 2017). The authors justified the development of specific regional algorithms by the peculiar optical properties of the Mediterranean Sea driven, among others, different phytoplankton community structure if compared to the global ocean (Volpe *et al.,* 2007). Also, the phytoplankton diagnostic pigment ratios in the Mediterranean Sea are considered to be different from those in other global ocean regions (Sammartino *et al*., 2015). Based on this data, we tried to identify specific subregions in the Mediterranean Sea that would be characterized by peculiar phytoplankton structure (as clusters), in terms of phytoplankton size classes (PSCs) and phytoplankton functional types (PFTs). Moreover, since the data available covers more than two decades, data from the first and last ten years were compared to uncover the temporal variation of these clusters.

The limitations of using satellite data are mainly related to the intrinsic errors of ocean colour data: Algorithm errors, cloud cover and its limitation to the surface layers of the ocean (Mayot *et al.,* 2016) and may have been exacerbated in our work by errors generated by regional algorithms, which, however, according to Di Cicco *et al.* (2017), have been significantly improved compared to global abundance-based models.

Several numbers of regions/clusters can be found in the regionalization studies of the Mediterranean Sea (Ayata *et al*., 2018), ranging from 5 in Nieblas *et al*. (2014) to more than 20 and 40 identified with the use of Lagrangian trajectories in Berline *et al.* (2014) and Rossi *et al.* (2014), respectively. Ten clusters were retained in the present analysis as the best match between the discrimination of regions identified in the mentioned studies and our results. Moreover, ten clusters were enough to also identify some features in the open Mediterranean Sea that was characterised by less clusters more or less uniform.

The ten clusters were characterized by the average Chl-a biomass and the contributions of both PFTs or PSCs followed the well-known gradient of trophic conditions in the Mediterranean Sea (e.g., Siokou-Frangou *et al*., 2010; Uitz *et al*., 2012; Colella *et al*., 2016). The average Chl-a is generally decreasing from west to east, with a rather gradual and continuous decrease across the western part of the basin but a much weaker, if existent at all, decrease in the eastern part (Siokou-Frangou *et al*., 2010). This was confirmed also by the distributions of both PSC and PFT clusters in our analysis, as cluster “0” with the lowest biomass occupied the whole eastern Mediterranean with the exception of North Aegean Sea and some coastal zones affected by river outflows. In addition to this latitudinal gradient, there is also one at the north-south axis of the Mediterranean Sea, which was also evident from our results. The notorious Adriatic north-south and west-east gradient (Polimene *et al*., 2006) was also very clear from the distribution of clusters in our analysis, where the belts of clusters with diminishing biomass were evidently arranged from north-west to south-east. At the level of the average Chl-a biomass in the ten PSC and PFT clusters, the difference between the cluster “9” with the highest biomass (around 3.9 mg/m^3) and cluster “0” with the lowest biomass (around 0.05 mg/m^3) was almost 80-fold.

Our clustering of PSC gave fairly similar results to the three ecoregions defined by Ciavatta *et al.* (2019), which represented the domination of a certain size class. The three areas within the ecoregion with micro-phytoplankton domination in Ciavatta *et al*. (2019) coincided with the distribution of our clusters “7”, “8” and “9” in the northern Adriatic, Gulf of Gabez and Nile delta region. Also, the ecoregion dominated by picophytoplankton (Ciavatta *et al.* 2019) coincided well with our clusters “0” and “1” with the exception of the western and north-western

Mediterranean. In the western basin, larger area was attributed by our analysis to clusters with dominating pico-fraction (Algerian basin and area around Balearic Islands) in comparison to Ciavatta *et al*. (2019) while in the NW Mediterranean (Ligurian Sea, Gulf of Lion) nanophytoplankton dominance was identified by our clusters whereas Ciavatta *et al.* (2019) identified pico- fraction dominance. Interestingly, Ciavatta *et al.* (2019) identified a front of pico- fraction dominance surrounded by nanophytoplankton dominance in the Alboran Sea, which fairly coincided with the front dominated by larger phytoplankton by our analysis. Also here, the mesoscale dynamism of Atlantic Ocean water exchange through the Gibraltar strait plays a crucial role in the formation of fronts (D'Ortenzio and Ribera d'Alcalà, 2009). While the trophic gradient in the Adriatic was well represented by both results, larger discrepancies were observed in the Aegean Sea, where our results indicate a gradient of decreasing phytoplankton biomass and size associated with the fading of the influence of the more productive Black Sea (Varkitzi *et al*., 2020). Field data from the Aegean Sea (Varkitzi *et al.,* 2018, 2020) confirm this gradient but are not consistent with the dominance of nanoplankton in our study, except in the coastal areas that are directly affected by river discharges and anthropogenic impacts.

If we compare our cluster with the regionalization based on phytoplankton phenology (D'Ortenzio and Ribera d'Alcalà, 2009; Mayot *et al*., 2016), we can also find a lot of parallels. The authors of these studies found different representative types of phenologies, grouped in four trophic regimes displaying different amplitudes of Chl-a biomass: "No Bloom" regime characteristics of the tropical seas with no marked peak, "Bloom" regime characteristic of the temperate seas with spring peak, "Intermittently" regime with intermediate characteristics, and "Coastal" regime observed in coastal areas. Mayot *et al*. (2016) added four more types labelled as "Anomalous". More or less steep gradients of the clusters identified in this work, with dominance of larger phytoplankton size fractions in Mediterranean most productive areas (northern Adriatic Sea, coastal Gulf of Lion, Gulf of Gabes, Nile delta area) conform to "Coastal" regimes. "Bloom" and "Intermittently" regimes overlap quite well with our cluster "2" dominated by haptophytes and the nanophytoplankton size class. The "No Bloom" regimes in the most oligotrophic areas of the Mediterranean coincide with our clusters "0" and "1" absolutely dominated by pico-fraction and prokaryotes. More specifically, "No Bloom no. 1" type, which covers the eastern basin and part of the Tyrrhenian Sea matches with cluster "0", "No Bloom no. 3" type that mostly occupies the SW part of the basin matches with cluster "1", while "No Bloom no. 2" type in the Adriatic, Ionian and Aegean Sea coincides with both "0" and "1" clusters. In the study period of 1998-2014, the trophic regimes showed an interannual variability, but "No Bloom" regimes recurrently covered most of the area (Mayot *et al*., 2016) similarly to clusters "0" and "1" in our study.

Uitz *et al*. (2012) assessed the extent of primary production associated with the phytoplankton size classes in these trophic regimes. In all main regimes, nanophytoplankton is found to be the major contributor (43-50%) to total primary production throughout the year and throughout the basin (Uitz *et al*., 2012), which should hold also for the areas in our study identified as dominated by picoplankton, i.e., clusters "0" and "1". Nevertheless, picophytoplankton contributes more to primary production (32%) in these areas than microphytoplankton (22%), regardless of the season (Uitz *et al*., 2012). This also applies to other regimes, with microphytoplankton contributing more to primary production (up to 38%) than picophytoplankton (up to 27%) only during the spring peak in the "Bloom" regime (Uitz *et al.,* 2012). The contribution of phytoplankton size classes to total primary production was not assessed in the study by Uitz *et al.* (2012) for the "Coastal" regimes.

A marked difference was observed between the distribution and extent of the PSC and PFT clusters in different seasons. The highest overall Chl-a biomass was observed during winter (January to March), when also the gradient of clusters at W-E and N-S axes was the clearest. This is in line with annual maxima of almost all trophic regimes by Mayot *et al*. (2016), where biomass peaked in winter (except some cases that peak were observed in early spring or late autumn) and also with findings of Salgado-Hernanz *et al*. (2019). This gradient was still observable during spring (April to June), although the most oligotrophic cluster expanded towards west and north. The patch of cluster “4” bearing the highest percentage of nanophytoplankton in the NW Mediterranean probably forms as a consequence of wind-driven deep convection and strong upwelling of nutrients into the euphotic layer in winter , that lead to a spring bloom (Auger *et al.,* 2014). The summer minimum of Chl-a biomass is a persistent feature in all trophic regimes in the Mediterranean Sea (Mayot *et al.,* 2016), which was in our case reflected in almost total cover of cluster “0” of the whole basin except some most productive regions. Also, the Mediterranean west to east gradient of phytoplankton biomass (as chlorophyll-a concentration) is much weaker in summer compared to other periods of the year (D’Ortenzio and Ribera d’Alcala, 2009). Similarly, gradients of phytoplankton diversity which were evident in the axis northern Adriatic Sea – Ionian Sea – Aegean Sea were weaker during summer (Francé *et al*., 2021). In autumn the gradient of clusters was establishing again, but in the western part of the basin was not as strong as during winter and spring. Here, the Gulf of Lion and Algerian basin were more uniform in this period, probably due to the initiation of the biomass peak during cold months in Algerian basin, which in this way brought the biomass values closer to those in the more productive NW basin (Salgado-Hernanz *et al*., 2019).

Some of the features formed by our clusters match well with consensus regions of Ayata *et al*. (2018), i.e., areas of the Mediterranean Sea that were consistently identified as regions in regionalization studies. The patch of nanophytoplankton dominated cluster in the Gulf of Lion and part of the Ligurian Sea agrees with the Ligurian consensus region in Ayata *et al*. (2018), which is one of the most productive regions in the Mediterranean Sea (e.g., Siokou-Frangou *et al*., 2010; Uitz *et al.,* 2012). In this area, the primary productivity is arguably dominated by picophytoplankton (Ciavatta *et al*., 2019) or nanophytoplankton (Di Cicco *et al.,* 2017). Differently, areas characterized by steep gradients in our clusters such as northern Adriatic, Gulf of Gabes were also recognized by Ayata *et al*. (2018) as consensus regions, while others like NW Aegean Sea and Nile delta region were not. All these regions are known eutrophication hot spots (UNEP/MAP, 2017), but at those that were not identified as consensus regions, anthropogenic causes my prevail over natural biogeochemical and hydrodynamical conditions favouring more eutrophic status. Other features in the Mediterranean Sea identified in our study fit with the consensus frontiers between different regionalizations (Ayata *et al*., 2018). For example, the peculiar formation of clusters in the Alboran Sea can be associated with the Almeria-Oran front which is characterized by threadlike areas of higher primary production and phytoplankton biomass (Ayata *et al*., 2018).

Finally, there is also a fairy well agreement of the clusters defined by our analysis with draft pelagic habitat types defined by UNEP/MAP (2023) for the epipelagic layer, since clusters can be confronted to these habitat types by the average chlorophyll-a concentrations and other specifications. Clusters “9” and “8” with very limited coastal extension conform to A.2 habitat type with variable salinity and very high chlorophyll-a (> 3mg/m^3). Clusters “7” to “4” which also compare only in highly productive coastal regions as a gradient of clusters coincide to A.3 neritic habitat type with medium surface or subsurface chlorophyll-a (0.5-3mg/m^3). On the other side, clusters from “3” to “0” conform to oceanic waters: cluster “3” to pelagic habitat type A.4.a with medium surface or subsurface chlorophyll-a (0.5-3mg/m^3) in the NW basin, cluster “2” to pelagic habitat type A.4.b with low to medium surface chlorophyll-a (0.1-1mg/m^3), and clusters “1” and “0” to pelagic habitat types A.5 with low surface chlorophyll-a (<0.1mg/m^3) with the remark that we can not specify subtype with deep chlorophyll maximum within our clusters.

With respect to intrinsically dynamic nature of pelagic habitats that interact with multiple hydrological and anthropogenic drivers (Hunsicker *et al*., 2016) and taking into account climate change as one of the principal drivers acting at the large scale on the pelagic habitat and its biota (e.g., Behrenfeld *et al*., 2006), the differences between the distribution and extent of clusters found in our study between the periods 1998-2007 and 2010-2020 were expected. In summary, shifts in the distribution and extent of clusters occurred mostly in western and northern parts of the Mediterranean Sea. Almost all shifts comprised widening of the most oligotrophic clusters with the dominance of picophytoplankton (clusters “0” and “1”). Changes in the western Mediterranean basin were in line with the findings by Gómez-Jakobsen *et al.* (2022), who confirmed a general decrease in chlorophyll-a concentrations in Spanish Mediterranean waters over almost the same time span.

In areas with a steep gradient of clusters, the observed changes comprised also other clusters. The northern and western Adriatic Sea behaved differently, as here the shift occurred in other direction, i.e., shrinking of more oligotrophic clusters. If compared to the difference of SST between the same periods, all areas of cluster change conform well with more or less accentuated rise of SST, for example NW Mediterranean, Tyrrhenian and north Aegean Sea. In the Levantine basin, where the rise in SST was also substantial, no change could have occurred since the area was already in the cluster “0”, although a low but spatially consistent trend of chlorophyll-a concentrations was observed in the eastern Mediterranean by Salgado-Hernanz *et al*. (2019) with a decrease also in the duration of the phytoplankton growing period.

On the seasonal scale, the largest areas were subject to changes during winter, when the most oligotrophic clusters in the eastern basin increased, while in the western basin and the Adriatic Sea the changes were mostly reversed. The changes in the western basin during winter, the main growing season for this area (e.g., Mayot *et al.,* 2016), are consistent with the increasing chlorophyll-a concentrations observed by Salgado-Hernanz *et al.* (2019). In the other seasons, the eastern Mediterranean remained in cluster “0” and most changes occurred in the western part, especially in spring and autumn, when the more oligotrophic clusters with the highest proportion of smaller phytoplankton cells gained expansion. The smallest changes were observed in summer, when cluster “0” already covered almost the entire basin in the first period, corresponding to the weakening of the west-east chlorophyll-a gradient in summer (D’Ortenzio and Ribera d’Alcala, 2009). In the northern Adriatic and the belt along the western coasts of the Adriatic Sea, there was a positive trend in the shift of clusters, meaning that oligotrophic clusters were replaced by those with larger cells and higher chlorophyll-a concentrations in winter and spring in this region. Although this trend appears to be a reversal of the oligotrophication trend that occurred in the northern Adriatic at the turn of the century (Mozetič *et al*., 2010; Colella *et al*., 2016), recent studies have shown that this area has indeed recently experienced a positive trend in chlorophyll-a concentration, on an annual basis (Salgado-Hernanz *et al*., 2019) or on a seasonal basis (Grilli *et al.,* 2020).

Mayot *et al.* (2016) argued that the main climatological bioregions identified as “No Bloom”, “Bloom”, “Intermittently” and “Coastal” are sufficiently comprehensive to summarize the phenology of phytoplankton at the surface of the Mediterranean, even at an interannual scale. At the same time, Mayot *et al*. (2016) speculated that future climate change will promote the oligotrophic state of the Mediterranean (i.e., more occurrences of “No Bloom” bioregions). Our results show that remarkable changes have taken place in recent decades, especially at the level of the cover "No Bloom" regimes, confirming this assumption. Moreover, D’Ortenzio and Ribera d’Alcalà (2009) speculated that each of these blooming regimes harbours a slightly different food web. Ciavatta *et al*. (2019) calculated trophic efficiency in the regimes dominated by different phytoplankton size classes and confirmed that small areas dominated by microphytoplankton have by far the highest trophic efficiency, while most of the Mediterranean, dominated by picophytoplankton, has much less efficient trophic fluxes. If the general trend confirmed by our analysis is the shrinking of clusters with larger cells, then the trophic efficiency of the pelagic habitat will also decrease (Ciavatta *et al.,* 2019).

In our study, the analysis of changes in the distribution and extent of clusters reveals trends mirroring those observed in SST, suggesting potential connections to climate change. However, distinguishing other types of anthropogenic influence from natural or climate variability on the structural characteristics of pelagic habitats, especially at a large scale, remains a difficult challenge (Magliozzi *et al*., 2023). As depicted also by Bedford *et al*. (2020), who recommended incorporating climate regimes in the assessment process, our results point to the significance of assessing the pelagic habitats across various temporal scales.

As highlighted by D’Ortenzio and Ribera d’Alcalà (2009), regionalization based on a single variable, such as surface chlorophyll-a concentration, provides less detailed information in comparison to those elaborated on a range of parameters, but can still reveal valuable patterns (bio-regions) that significantly contribute to our understanding of the functioning of the Mediterranean ecosystem.

3 Towards definition of relevant assessment scales for the pelagic habitat

Our study has shown how important the spatial and temporal scales are for understanding processes in the pelagic habitats of the Mediterranean. But apart from this, there is also a scale that is worth emphasising, namely the size of the phytoplankton component in terms of phytoplankton size classes.

The picophytoplankton component is rarely considered, especially at the large basin-wide scale or in national monitoring programmes dedicated mainly to monitoring microphytoplankton (and to a limited extent nanophytoplankton) in Mediterranean coastal and, less commonly, offshore waters. Regionally limited studies such as those by Delpy *et al.* (2018), Mena *et al.* (2016), Denaro *et al*. (2013), Bernardi Aubry *et al*. (2006) and Šantić *et al.* (2013) provide interesting insights into the characteristics of the picoplankton community in specific areas, albeit with a limited understanding of the temporal and spatial components. In addition, picophytoplankton is studied using different methods than the other two size classes of phytoplankton, for which Utermöhl microscopy is typically used, making it difficult to compare or combine results in assessment studies. As recommended by Ciavatta *et al.* (2019), it is necessary to collect *in situ* data using alternative techniques such as flow cytometry, microscopy and size-fractionated filtration in addition to high-performance liquid chromatography (HPLC). This diversified data collection strategy is essential for the refinement and calibration of algorithms for phytoplankton community structure in ocean colours (Ciavatta *et al*., 2019), but also for the inclusion of these results in pelagic habitat assessments and comparison with micro- and nanophytoplankton results. Furthermore, the resources should be also allocated to the study of pelagic food webs, where important consequences of changes at the primary producer level should be easier to detect. At the food web level, the measures to be taken if good status is not achieved should also become more manageable and plausible.

Previous studies already discussed in this deliverable demonstrated that the Mediterranean is a complex system, which hosts different trophic regimes in a relatively small spatial extension. Using the distribution of chlorophyll-a biomass in PSCs and PFTs, as given by satellite product, is a useful way to characterize these regimes, their spatial distribution and temporal variability. Spatial scales (in terms of clusters) depicted by our study showed that notable changes have occurred in recent decades, particularly at the level of the cover of the most oligotrophic regime, which should be considered in future assessments, that should take into account various temporal scales and incorporating climate regimes into the assessment process. This aspect gives great importance to long-term studies that preserve long term data series. These studies are of utmost importance to identify important trends or regime shifts in the pelagic habitat and also allow research on changes in food webs. Therefore, we strongly support the maintenance of long time series, LTER stations and the integration of these data into studies that involve the use of satellite data and modelling. In addition, appropriate sampling frequency is important for the adequacy of long-term data on plankton assemblages, and we consider monthly sampling to be most appropriate.

4 Conclusions

- The work done in the frame of the Activity's 2 Subtask 2.1.2 built upon the assumption that the definition of the assessment scales is an essential step in the development of an assessment system for GES of the pelagic habitats.
- This study utilized long-term data from a Copernicus Marine Environment Monitoring Service (CMEMS) product with algorithms tailored for the specificities of the Mediterranean Sea, considering its unique optical properties and distinct phytoplankton community structure. The analysis aimed to identify specific subregions in the Mediterranean Sea characterized by peculiar phytoplankton structures, considering phytoplankton size classes (PSCs) and phytoplankton functional types (PFTs).
- Ten clusters were identified, which reflected a trophic gradient in chlorophyll-a biomass and contributions of PFTs or PSCs. Clusters exhibited geographical patterns aligning with well-known trophic conditions in the Mediterranean Sea, demonstrating a gradient of chlorophyll-a biomass from west to east and north to south. The clusters nearest to the coasts presented the highest biomass and were dominated by the microphytoplankton size class (mainly by diatoms); the adjacent clusters presented a medium phytoplankton biomass and were dominated by nanophytoplankton size class. Together these clusters formed a “coastal belt”, which was wider in the Alboran Sea (stretching from the Strait of Gibraltar toward east), along the coasts of NW Mediterranean, in the NW Adriatic, in the Aegean Sea, Gulf or Gabes and southern Levantine coasts. On the other hand, picophytoplankton size class dominated the largest part of the Mediterranean open sea.
- Strong agreements were found with previous regionalization studies in some regions, while discrepancies highlighted the dynamic nature of pelagic habitats.
- Temporal variability and seasonal dynamics were studied using data from two separate decades (1998-2007 and 2010-2020). Changes in cluster distribution over two decades were observed, with notable shifts mostly in the western and northern parts of the Mediterranean Sea. Seasonal dynamics revealed winter as a period of significant change, with the most oligotrophic clusters expanding, while summer showed a more uniform distribution.
- The study identified trends in cluster distribution mirroring sea surface temperature changes, suggesting potential connections to climate change. However, the distinction between anthropogenic influence and natural variability remained challenging. Moreover, changes in cluster distribution, particularly the shrinking of clusters with larger cells, may indicate a decrease in trophic efficiency, aligning with previous research.
- The study supports the recommendation to assess pelagic habitats across various temporal scales, emphasizing the importance of incorporating climate regimes into the assessment process. It also acknowledges the value of regionalization based on a single variable for providing valuable patterns in understanding the functioning of the Mediterranean ecosystem.

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