



A method to establish marine bio-regions in the pelagic ecosystem based on phytoplanktonic communities. Application to the southern Spanish coast

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ARTICLE INFO

Keywords:
Regionalization
Bioregions
Phytoplankton
Pelagic ecosystem
Time series
Cluster analysis

ABSTRACT

Bioregions in the pelagic ecosystem are frequently established on the basis of remotely sensed properties of the sea surface, such as sea surface temperature or sea surface chlorophyll concentration. Those works dealing with the regionalization of the marine ecosystem by means of the use of properties of the water column are less frequent, and even less those that obtain the data from periodic *in situ* monitoring programs, which are scarce. In this work we use time series of micro, nano and pico-phytoplanktonic abundances in the upper 100 m of the continental shelves of the Gulf of Cadiz and the Alboran Sea from the projects STOCA and RADMED (southern coast of Spain, Western Mediterranean). The use of times series allows us to estimate the median phytoplanktonic abundances of several phytoplanktonic groups along the water column. These statistics differ substantially from those abundances obtained for one particular campaign, reflecting the large seasonal and inter-annual variability of phytoplanktonic communities. These median profiles, estimated for the four seasons of the year and for several phytoplanktonic groups characterize each of the locations sampled in the aforementioned monitoring programs and are used for establishing the similarity between them. Then, these locations are grouped using a cluster analysis. Using some simulations from numerical experiments we determine which metrics and methods of analysis are the more suitable ones for the regionalization of the area of study. A bootstrap method is also used to determine which differences among bioregions can be considered as statistically significant. Despite the existence of a fast current that connects the Gulf of Cadiz and the Alboran Sea, our results show that the outer part of the Gulf of Cadiz shelf, and that of the Alboran Sea, can be considered as two differentiated bioregions. The latter region shows a higher productivity with a higher abundance of large cells such as diatoms, and the dominance of *Synechococcus* bacteria over *Prochlorococcus* ones.

1. Introduction

Due to the rise in consumer demand as the population continues to increase, the need for resources has heightened, pushing for the industrialisation of the world's oceans. This need will continuously increase as time progresses and the key offshore marine sectors including offshore gas and oil, coastal tourism, and renewable energy (wind and tide), among others will be exploited (Kalogeri et al., 2017; Smith,

2000). The oceans resources are limited, pushing the need for more integrated coastal management and Marine Spatial Planning (MSP) in order to implement sustainable use of the diverse marine ecosystems. To achieve this, many models have been proposed for the management of coastal areas based on the regionalization of the environment and its primary productivity. Many of these models focus on the oceans at the macroscale to provide a broad overview of possible regions to be considered for protection (Reygondeau and Dunn, 2019; Sayre et al.,

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<https://doi.org/10.1016/j.ocecoaman.2023.106930>

Received 3 July 2023; Received in revised form 2 November 2023; Accepted 8 November 2023

Available online 18 November 2023

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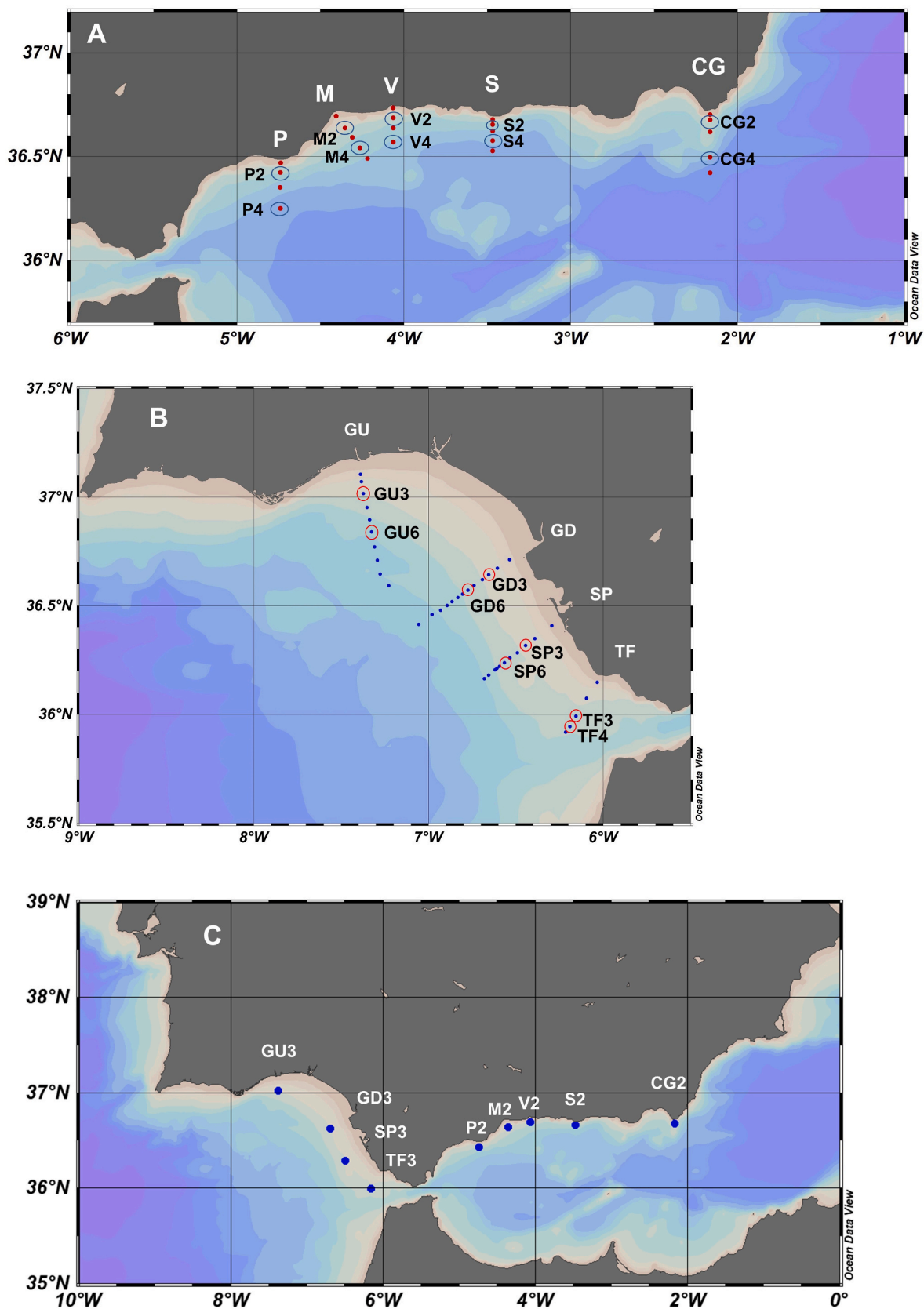


Fig. 1. Blue dots in Fig. 1A are the oceanographic stations visited on a three-monthly basis in the Alboran Sea (RADMED project). Red circled stations are those where micro and/or nano and pico-plankton analyses are carried out. Fig. 1B is the same, but for the Gulf of Cadiz (STOCA project). Fig. 1C shows the stations where both micro and nano and pico-plankton samples are taken and will be the focus of the present work.

2017; Longhurst, 2007).

Because of its reduced dimensions and semi-enclosed nature, the Mediterranean Sea is among those regions considered to be especially vulnerable to climate change and other anthropogenic stressors (Ali et al., 2022). Therefore, it is of paramount importance to establish bioregions and protection plans for them based on the sound knowledge of the ecosystem's properties and functioning's (Manea et al., 2020).

Most attempts to establish bioregions both on a regional and global scale are based on the use of remotely sensed data (Reygondeau and Dunn, 2019; Oliver et al., 2004) and in very few cases such regionalization is accomplished by means of data collection throughout the water column, such as temperature, salinity, dissolved oxygen and nutrient concentrations obtained from international data bases (Sayre et al., 2017). Sutton et al. (2017) defined bioregions in the mesopelagic ecosystems of the world oceans based on the collation of expert knowledge on water mass distributions, the location of minimum oxygen zones, discontinuities in faunal communities, etc.

The methodologies used to analyse the available information is also highly diverse, and include the use of cluster analysis (Sayre et al., 2017), neural networks (El Hourany et al., 2019, 2020), or a combination of both methodologies (Marchese et al., 2022). Berline et al. (2014) used a circulation model for establishing bioregions based on the connectivity of different geographical areas. Gómez-Jakobsen et al. (2022) used satellite data of sea surface chlorophyll concentration (SSChl) and cluster analysis to define different bioregions within the Spanish Mediterranean waters (Western Mediterranean). Muñoz et al. (2015) analysed both Sea Surface Temperature (SST) and SSChl data by means of Principal Component Analysis (PCA) to carry out a regionalization of the Alboran Sea and the Gulf of Cadiz.

To our knowledge, no attempts have been made to establish bioregions in the pelagic ecosystem based on the composition and abundance of phytoplanktonic groups along the water column, obtained from *in situ* sampling. According to Roberson et al. (2017), most of these regionalizations are based on benthic habitats, whereas much less attention is paid to pelagic ecosystems. These authors also point out that the knowledge and protection of offshore pelagic ecosystems is a major gap in marine protected areas. Filling this gap is of paramount importance, especially given the essential role of phytoplankton in marine ecosystems: Phytoplanktonic organisms are responsible for the 90 % of the marine primary production (Boyce and Worm, 2015) and provide food, directly or indirectly, for all the other marine organisms (Falkowsky, 2012). Primary producers in shelf waters support the 90 % of the world's fisheries catches and, therefore, phytoplankton information should be included for the management of protected areas (Tweddle et al., 2018).

In order to help filling this gap, we present in this work a method to establish bioregions in the pelagic ecosystem based on the use of time series of micro, nano, and pico-phytoplankton abundances from two monitoring programs in the Gulf of Cadiz and the Spanish Mediterranean waters. The phytoplanktonic community of each sampling station was characterized using the statistical properties of the seasonal distributions of different phytoplanktonic groups. Considering the large amount of data generated, we used two different methods to reduce the dimensions of the data set. Finally, the different stations were grouped by means of a cluster analysis. A numerical simulation was used to identify which metrics would produce the best results and a bootstrap analysis was then used to identify the number of clusters (bioregions) that could be established with a high level of confidence. However, this work should be considered as a first step. Despite the central role that phytoplankton plays in pelagic ecosystems, (as already explained), a better regionalization should take into account other variables along the water column, such as temperature, salinity, concentrations of oxygen and nutrients, etc. The methodology presented in this work could be easily extended to such variables, and this will be the subject of future works.

The main goal of this work is simply to develop the methodology and to apply it to the case study of the Gulf of Cadiz and the Alboran Sea.

These locations presented an especially interesting case. On one side, phytoplanktonic blooms have been associated to turbidity plumes in the Gulf of Cadiz (Caballero et al., 2014), and the primary productivity of the waters surrounding the Guadalquivir river plays a fundamental role in the anchovy fishery in this area (Ruiz et al., 2006). In the case of the Alboran Sea, Ramírez et al. (2021) have described several mechanisms capable of supplying nutrients to the photic layer in the northern Alboran Sea, sustaining a primary productivity higher than that of other Mediterranean regions (García-Martínez et al., 2019a, 2019b). These two regions, connected by the Strait of Gibraltar, have very energetic dynamics that modulate phytoplanktonic populations. Previous works had revealed that the swift Atlantic Current that flows through the Strait of Gibraltar could establish a certain connectivity between both regions in relation to planktonic organisms that drift passively with currents (García-Lafuente et al., 2021), including eggs and larvae of fish species of commercial interest (Nadal et al., 2021). Such connectivity raises the question of the similarity or difference between both geographical areas.

This work is organized as follows. Section 2 presents the data used from the monitoring programs and the methodology, including the statistical analysis of the phytoplankton time series, the method used to reduce the dimensions of the data set, and those methods used for determining the optimal choice of the cluster analysis. Section 3 shows the results, and finally section 4 presents a discussion and the conclusions.

2. Data and methods

2.1. Phytoplankton data

RADMED and STOCA are monitoring programs maintained by the Instituto Español de Oceanografía (IEO; Spanish Institute of Oceanography) in the Spanish Mediterranean waters and in the Gulf of Cádiz respectively. Both projects are made of a large set of oceanographic stations distributed along transects which extend on the on-offshore direction. Although the monitoring is multidisciplinary, including physical, chemical and biological variables, we focus in this work on the phytoplanktonic populations and its use for the regionalization of the Alboran Sea and Gulf of Cádiz.

All the transects in both monitoring projects are visited on a tri-monthly basis. RADMED project started in 2007, although some data from the previous Ecomálaga project in the area of Málaga Bay extend from 1992 and will be used in this work. STOCA project was initiated in 2009, but the phytoplankton sampling started in 2014 or 2016, depending on the transect. Table S1 in supplementary material shows the dates of all the campaigns carried out until 2021. These are the data used in the present study.

For the sake of clarity, Fig. 1A shows only the RADMED stations corresponding to the Alborán Sea, which is one of the areas analysed in this work. These stations correspond to the transects P (Cape Pino), M (Málaga), V (Vélez), S (Cape Sacratif) and CG (Cape Gata). However, the RADMED transects extend from Málaga to Barcelona, including the Balearic Islands and can be seen in Fig. S1A in the supplementary material and in López-Jurado et al. (2015). Also for the sake of clarity, Fig. 1B only shows the transects of STOCA project where the stations analysed in this work are located. These transects are GU (Guadiana river), GD (Guadalquivir river), SP (Sancti Petri), and TF (Cape Trafalgar). The complete sampling design for STOCA is presented in Fig. S1B in supplementary material and in Sánchez-Leal et al. (2020). Water samples for micro-phytoplankton analysis were taken at one of the continental shelf stations of all the transects and all the campaigns. These stations were labelled as 2 in RADMED stations and as 3 in STOCA project. Water samples for the analysis of nano and pico-phytoplankton were taken at two stations of each transect, one in the continental shelf and another one on the continental slope. These stations were labelled as 2 and 4 in RADMED, and as 3 and 6 in STOCA project (Fig. 1A and B) with the only exception of TF transect where the sampling was carried

out at stations 3 and 4 (Fig. 1B). The objective of this work is to analyse both micro, nano, and pico-phytoplankton populations in both the Alboran Sea and the Gulf of Cadiz. For this reason, the stations analysed were stations 2 from the Alboran Sea, and stations 3 from STOCA where both micro and nano and pico-plankton were sampled. These stations are shown in Fig. 1C. Notice that the continental shelf of the Gulf of Cadiz has a width larger than 40 km in our region of study (see Fig. 1B and García-Lafuente et al., 2006). Hence, sampling stations in the Gulf of Cadiz, located in the central part of the shelf can be far from the coast. On the contrary, the Spanish shelf of the Alboran Sea is very narrow with a width ranging between 2 and 10 km (See Fig. 1A or Parrilla and Kinder, 1987) and the sampling stations are close to the coast.

Water samples were taken at 0, 10, 20, 50, 75, and 100 m depth in the RADMED stations, and at 5, 25, 50, 75, and 100 m depth in STOCA stations when the station depth reached 100 m. In some cases, the maximum depth was 75 m. 125 mL water samples were taken for micro-phytoplankton samples, and 5 mL cryovials for nano and pico-phytoplankton. Micro-phytoplankton samples were treated with an acidified Lugol's iodine solution and nano and pico-plankton samples were treated with glutaraldehyde solution and immediately frozen in a liquid nitrogen container. Micro-phytoplankton samples were examined and analysed by inverted microscopy (DMi1 Leica) prior to a sedimentation procedure (Üthermöhl, 1958). Nano and pico-phytoplankton abundances were analysed with a flow cytometer (FACScalibur Becton and Dickinson; Gasol, 1999).

The micro-phytoplankton samples were analysed at genus level in most cases, and to species level when possible. Nevertheless, in order to generate long-term series which can be used as proxies of the environmental state of the phytoplanktonic community, these series were grouped into three main groups: diatoms, dinoflagellates, and small flagellates (Tomas, 1997). Cocolithophorids are not analysed because they require a different preservation methodology on board and would duplicate the number of samples. This is not possible at present taking into account the vessel and personal availability (García-Martínez et al., 2019b). A fourth group was generated adding the preceding three to evaluate the total amount of micro-phytoplanktonic cells. This group will be referred to hereafter as total micro-phytoplankton. Nano and pico-plankton was divided into four groups: eukaryotic nano and picoplankton, and autotrophic bacteria of the genera *Prochlorococcus* and *Synechococcus*. Finally, for each oceanographic station and for each campaign, we obtained eight discrete profiles corresponding to the abundances of diatoms, dinoflagellates, small flagellates, total micro-phytoplankton, eukaryotic nanoplankton, eukaryotic picoplankton, *Prochlorococcus*, and *Synechococcus* bacteria. Abundances of micro-phytoplankton will be expressed hereafter in cel/mL and nano and picoplankton abundances in $10^3 \times \text{cel/mL}$.

2.2. Calculation of climatological profiles

As already commented, we obtained eight discrete profiles corresponding to the eight phyto-planktonic groups defined above for each oceanographic campaign and for each of the nine oceanographic stations of the continental shelves of the Alboran Sea and the Gulf of Cadiz that will be analysed in this work (Fig. 1C). These vertical profiles were interpolated with a 1 m depth interval with a shape preserving cubic interpolation scheme (pchip) from the sea surface to 75 m depth. Including the sea surface, these profiles were made of 76 data points. Although some stations reached the 100 m depth, we limited our analysis to the first 75 m to allow comparison between stations of different depth.

In order to characterize the phytoplanktonic population of each location, it is not appropriate to use those data corresponding to one single campaign, as they can vary strongly from one survey to another. For this reason, an average or climatological profile should be calculated for each oceanographic station and phytoplanktonic group using all the available campaigns. Furthermore, each of the phytoplanktonic groups

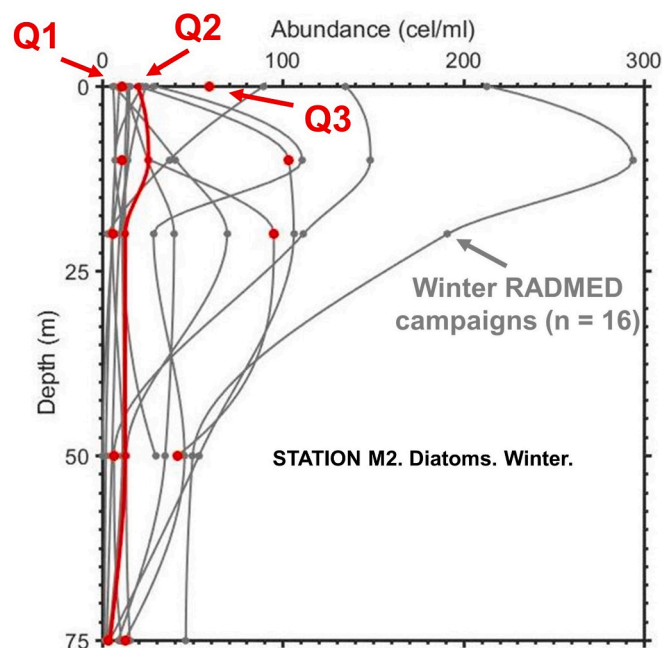


Fig. 2. Example of the methodology used for characterizing the phytoplanktonic communities of each oceanographic station. In this example, grey dots are the discrete vertical profiles corresponding to the winter abundance of diatoms from all the oceanographic campaigns carried out at station M2 (expressed in cel/mL). Grey lines are the interpolated abundance profiles. The red line and small red dots show the median values and interpolated median profile from this data set. Large red dots show the Q1 and Q3 quartiles for each discrete depth level. In these cases, the interpolated quartiles have not been included for the clarity of the plot.

is expected to have a different behaviour for each season of the year. For this reason, for each oceanographic station, and for each phytoplanktonic group, we grouped all the profiles corresponding to the same season of the year. To clarify this, Fig. 2 shows, by way of example, all the diatom profiles corresponding to station M2 for the winter season. The grey dots correspond to the discrete values obtained from the microscopy analysis of water samples, and the grey continuous lines correspond to the interpolated profiles. Following García-Martínez et al. (2019a; 2019b) and Vargas-Yáñez et al. (2019), an average profile was calculated using all the data from all the different campaigns. However, the distribution of these abundances was largely asymmetrical with frequent extreme values. For this reason, we elected to calculate the median value for each depth level. The median profile was used as a climatological profile for characterizing the behaviour of each phytoplanktonic group at each location and for each seasonal period. To illustrate this, the red line labelled as Q2 in Fig. 2 shows the median profile corresponding to the winter diatom population at station M2. As well as the median, we calculated the first (Q1) and third (Q3) quartiles to characterize the inter-annual variability of phytoplanktonic populations.

2.3. Characterization of oceanographic stations

Each oceanographic station could be characterized by the seasonal cycles of the eight phytoplanktonic groups defined in our monitoring design. The temporal resolution of RADMED and STOCA is three-monthly, therefore we have four median profiles corresponding to the four seasons of the year for each of the following groups: diatoms, dinoflagellates, small flagellates, total micro-phytoplankton, nanoplankton, picoplankton (both eukaryotic), *Prochlorococcus*, and *Synechococcus* bacteria. In this way, the seasonal evolution of the phytoplanktonic community at each location is characterized by 32

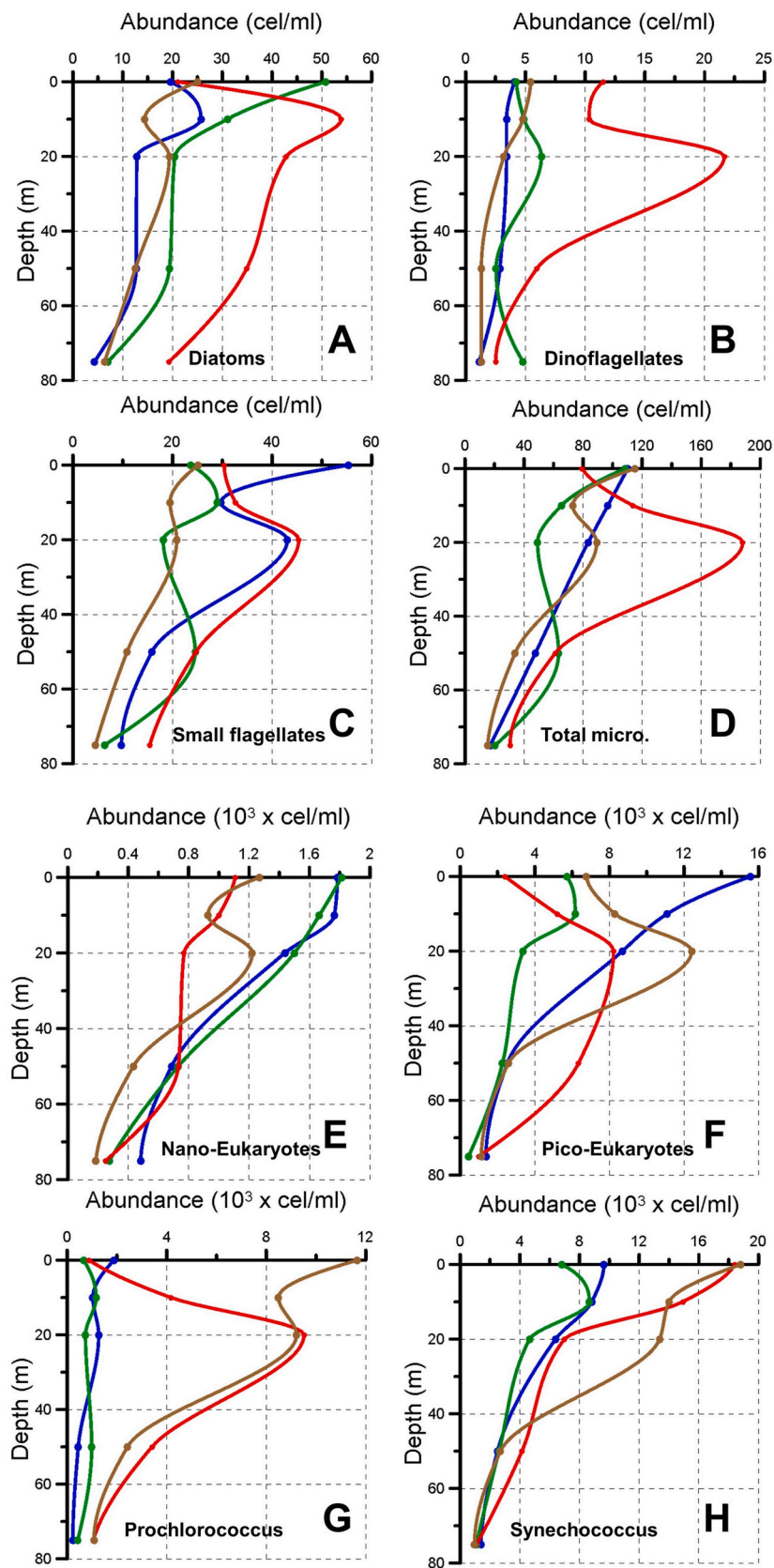


Fig. 3. Seasonal median profiles for the abundances of diatoms (3 A), dinoflagellates (3 B), small flagellates (3C), total micro-phytoplankton (3D), nano-eukaryotes (3 E), pico-eukaryotes (3 F), Prochlorococcus (3G) and Synechococcus (3H) at station M2. Blue corresponds to winter, green to spring, red to summer and brown to autumn.

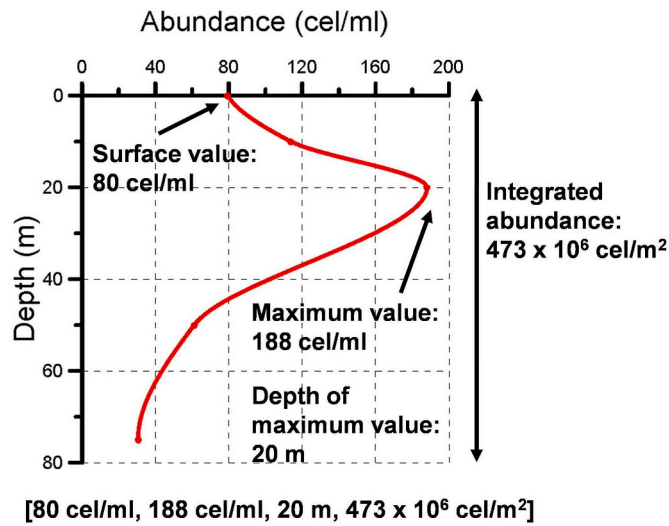


Fig. 4. Red dots and line are the median profile for the diatom abundance during summer at station M2. The values of the surface abundance (cel/mL), maximum abundance (cel/mL), the depth where the maximum abundance was found (m), and the value of the vertically integrated abundance ($10^6 \times \text{cel/mL}$) are included. The insert at the bottom shows the four indices that characterize this particular profile.

median profiles. For example, Fig. 3 shows such profiles for the M2 station at Málaga Bay (see Fig. 1C for the location). All the seasonal median profiles for all the phytoplanktonic groups and all the oceanographic stations are provided in supplementary material.

It is important to note that the length of the time series used for the calculation of the median profiles is different for each oceanographic station. Such time series extend from 1994 to 2021 in the case of the westernmost stations of the Alboran Sea, but they cover a much shorter period of time in the case of the Gulf of Cadiz stations (June 2014–August 2021, or March 2016–August 2021, depending on the transect; see Table S1 in supplementary material). For this reason, the median values calculated from the longest time series would be closer to the true median values of their distributions in one cases than in others. These shortcomings will be reduced as the lengths of the time series increase.

Each interpolated median profile is made of 76 data points or depth levels. Therefore, the complete evolution of the seasonal cycle of the phytoplanktonic community at each location (for instance, M2 at Fig. 3) is characterized by $4 \times 8 = 32$ median profiles which are made of $4 \times 8 \times 76 = 2432$ data points. The similarity between each pair of locations (oceanographic stations) should be estimated by comparing the two sets of 2432 points corresponding to both stations. However, this could be considered an excessive number of data and it would be desirable to reduce it. Two different methods were used for such reduction. These methods, explained in detailed below, were implemented by means of matlab codes that are available in supplementary material, together with a guide for their application to the seasonal median profiles that are also provided in supplementary material.

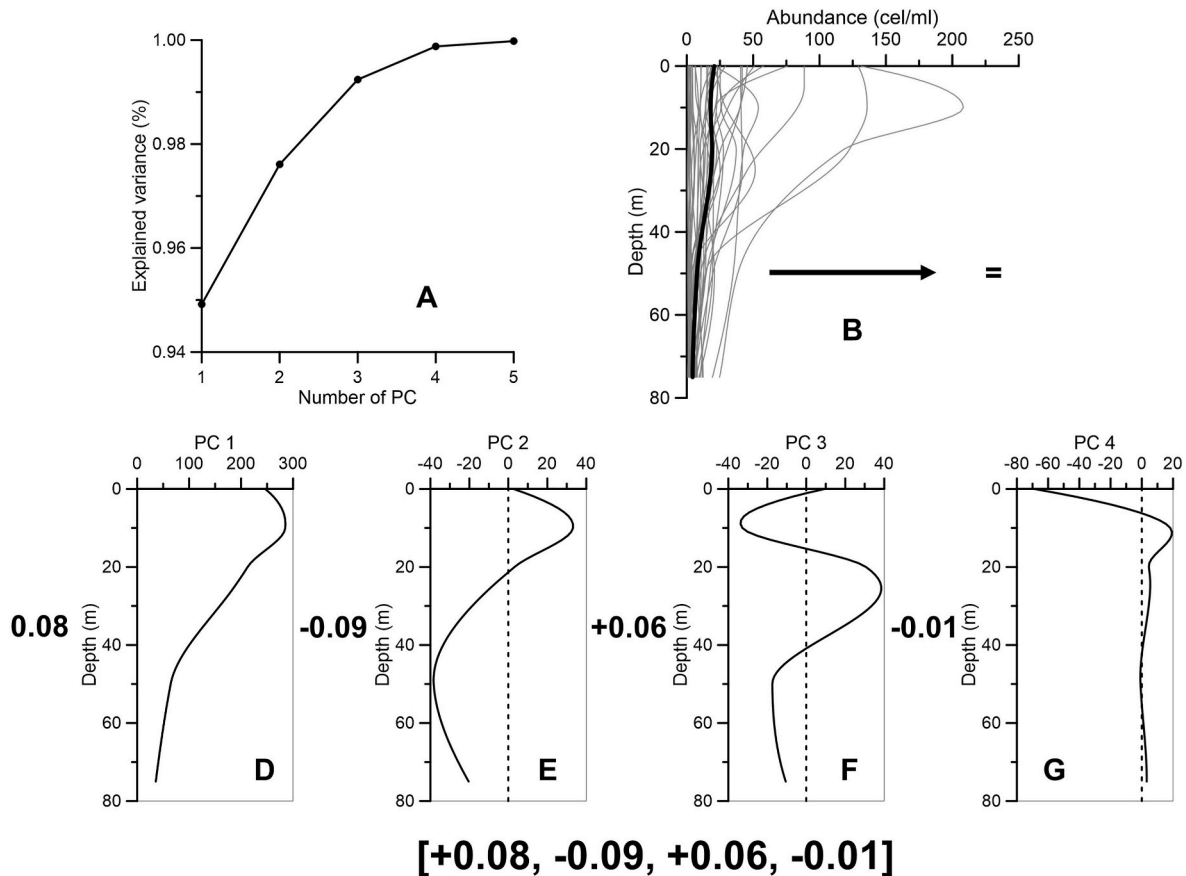


Fig. 5. Fig. 5A shows the percentage of variance of the original median profiles represented by the use of 1–5 PCs. Fig. 5B shows the 36 median profiles for diatom abundances for the four seasons of the year and the 9 oceanographic stations analysed in this work. The thick black line corresponds to the winter median profile from station CG2. This particular profile is decomposed as a combination of the first 4 PCs. Fig. 5D to G shows these 4 PCs and the factors which represent their contribution to the winter CG2 profile.

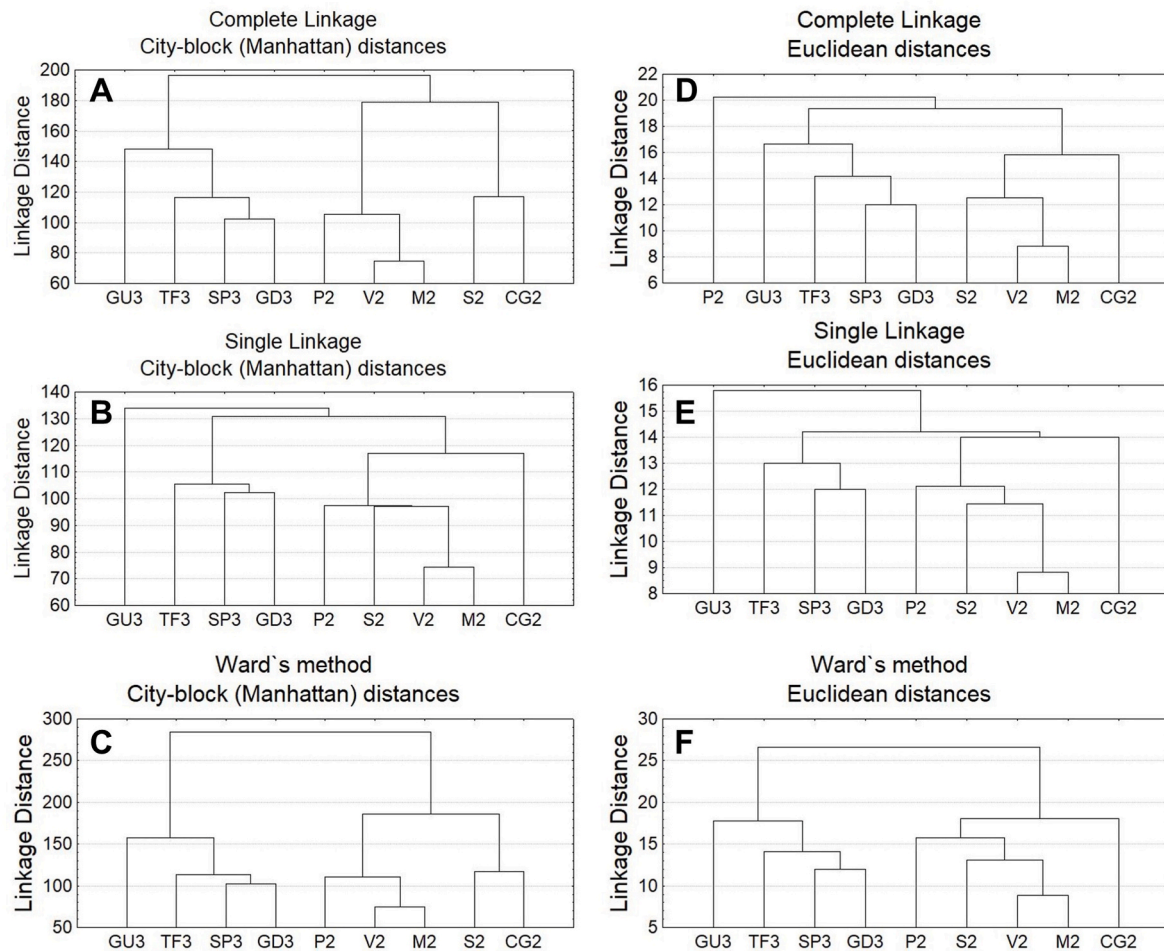


Fig. 6. Different results from the cluster analysis of the M1 data matrix corresponding to different metrics and methods for the calculation of the distance between clusters (see titles in each figure).

Method 1. We followed an approach similar to that used by Lavigne et al. (2015) for the definition of the different types of chlorophyll profiles in the Mediterranean Sea. Each of the 32 profiles corresponding to each location were characterized by four numbers or indices which take into account both the shape of the profile and the total amount of phytoplanktonic cells within the water column. These four indices were the abundance at the sea surface, the maximum abundance along the profile, the depth at which the maximum abundance was found, and the total abundance of phytoplanktonic cells which was obtained through the vertical integration of the profile. These numbers were expressed in cel/mL, cel/mL, meters, and $10^6 \times \text{cel}/\text{m}^2$ respectively for the micro-phytoplanktonic groups, and in $10^3 \times \text{cel}/\text{mL}$, $10^3 \times \text{cel}/\text{mL}$, meters, and $10^9 \times \text{cel}/\text{m}^2$ for the nano and pico-planktonic groups. To illustrate this method, Fig. 4 shows these indices for the median summer profile for total micro-phytoplankton in the M2 station.

Considering the phytoplanktonic community of each location as made of 8 phytoplanktonic groups (diatoms, dinoflagellates, small flagellates, etc.), each group has four seasonal profiles, and now each profile is characterized by 4 indices (as opposed to 76 data). Therefore, each location or oceanographic station is characterized by 4 seasons \times 8 phytoplanktonic groups \times 4 indices = 128 data points. This is a considerable reduction if compared with the 2432 data that would arise from the use of the complete profiles.

Method 2. In this case, we reduced the dimensionality of our data set using Principal Component Analysis (PCA). Each oceanographic station and each season of the year is characterized by a different median profile for each phytoplanktonic group, which simply reflects the spatial and

temporal (seasonal in our case) variability of environmental conditions and the adaptation to such conditions of the different phytoplanktonic groups. If we consider one particular group, let's say, diatoms, we have 4 seasons \times 9 locations = 36 different diatom median profiles. The grey lines in Fig. 5B show these 36 median profiles. Using PCA we can obtain a new set of 36 orthogonal profiles. Each one of the 36 profiles can be decomposed as a linear combination of the new orthogonal profiles, which are the Principal Components (PC). However, just a few of these PCs are needed for reproducing almost all the variance of the original profiles (Fig. 5A). We finally considered four PCs (Fig. 5C–D). In the example of Fig. 5, the winter median profile from the station CG2 is highlighted with a thick black line. This particular profile is decomposed as a linear combination of the first four PCs. The coefficients of this combination indicate the contribution of each PC to the winter diatom profile in CG2. Obviously, this procedure was applied to each of the 36 diatom median profiles corresponding to all the oceanographic stations and seasons of the year (all the grey profiles in Fig. 5B). Each profile had a different shape, and therefore, had different contributions (coefficients) from the first four PCs.

This procedure was followed for each phytoplanktonic group. As in the case of Method1, we now characterize each oceanographic station by 4 seasons \times 8 phytoplanktonic groups \times 4 coefficients = 128 data points.

2.4. Cluster analysis. Selection of the method

There are many possible methods to establish bioregions in the ocean, depending on the variables used to describe each geographical

Table 1

Values of the parameters m (depth of the maximum value) and c (increase in the integrated value) for the 5 coloured profiles presented in Fig. 7. Each pair of parameters defines a type of vertical profile.

Profile	M	C
1 (black)	0	0.25
2 (blue)	25	0.5
3 (red)	50	0.75
4 (green)	75	1
5 (purple)	100	1.25

area, and the method used to group those areas with similar values of the chosen variables. In the present work we have used the distribution of eight different phytoplanktonic groups along the water column. Concerning the method for establishing geographical areas with common characteristics, we followed a hierarchical agglomerative cluster analysis. In this analysis we considered 9 cases corresponding to the 9 oceanographic stations in the Alboran Sea and the Gulf of Cádiz (Fig. 1C). For each case we had 128 variables corresponding to the 128 variables that characterized each station. These 128 variables could be those corresponding to Method1, or to Method2. In the case of Method1, the depth of the maximum abundance, or the integrated abundance had very different variances from the surface or maximum abundances. For this reason, these variables were standardized prior to the cluster analysis. This standardisation was not needed when Method2 was used.

Several methods could be used for calculating the distance between cases: Euclidean, city block, Chebyshev, Mahalanobis, etc. (see any text book on multivariate analysis). Different methods could also be used to calculate the distances between clusters (simple linkage, complete linkage, Ward's method, etc.). In a first exploratory analysis, we prepared data matrices made of 9 cases and 128 variables for both Method1 and Method2 and carried out a cluster analysis using the commercial software STATISTICA 7. In this exploratory analysis we used different metrics and methods for calculating the distances between clusters. Different methods yielded different results and therefore, according to their phytoplanktonic communities, we could establish different bioregions depending on the method used (Fig. 6). As this is not an acceptable situation, a numerical simulation was performed for determining the best cluster metric.

We generated five profiles of a simulated variable. These five profiles had a different shape and different integrated values, in order to resemble the real situation presented by phytoplanktonic profiles. These five profiles obeyed the following equation:

$$f_i(z) = c_i + \exp\left[-\frac{(z - m_i)^2}{2L^2}\right] \quad (1)$$

L is a decaying length which was set as 25 m in this example, m_i is the position of the maximum of the profile within the water column, and c_i is included to increase the integrated value of f_i . Table 1 shows the five pairs of values of c and m that generated the 5 profiles presented as thick coloured lines in Fig. 7A.

From each of these five profiles we generated three new profiles adding a white noise (thin lines in Fig. 7A) with standard variances 0.14, 0.06, and 0.05 respectively. Profiles P1-3 are presented in Fig. 7A as black thin lines. These profiles are simply perturbations of the type-1 profile, and therefore they all have a similar shape. Profiles P4-6 (thin blue lines) are perturbations of type-2 profile (thick blue line), etc. In this case, it is clear that a cluster analysis should primarily establish 5 clusters made of profiles P1-3, P4-6, P7-9, P10-12, and P13-15. The 15 profiles were characterized using Method1 (four indices) and Method2 (PCA). Then, cluster analyses were carried out using different metrics and methods for calculating the distances between clusters. A metric and method would be considered as appropriate if it was able to group the 15 profiles within the 5 groups of three profiles that were defined.

2.5. Detection of statistically significant differences between oceanographic stations

In an agglomerative cluster analysis, we start with as many clusters as cases (9 in the present work), and we finish with one single cluster. As opposed to continuing to our analysis until we obtain one single cluster, we could ask the question, with how many clusters should we stop our analysis? There are different methods for this, but a frequent criterion is to stop when there is a jump in the distance between clusters. In our case, we posed the opposite problem: when can two oceanographic stations be considered as different according to their phytoplanktonic communities? This question has important implications for the present work and for the project management. On the one hand, data from two stations which are not distinguishable should be treated jointly, increasing the number of data available for the description of the different phytoplanktonic groups and increasing the accuracy of the estimations of statistics such as the median, mean, quartiles, etc. On the other hand, long-term monitoring programs should be kept as simple as possible reducing the number of stations. It is not worth sampling two stations with such similar properties that cannot be differentiated from a statistical point of view, especially considering the high economical cost of oceanographic campaigns.

Even in the case that we compared one oceanographic station with itself, using profiles of abundances sampled at slightly different dates for the comparison, the distance would be different to zero, despite the fact that both sets of data come from the same population. This distance is obviously due to chance and comes from the fact that the sample median will be different from the true median. We used a bootstrap method for determining the critical value or threshold that the distance between stations should overcome to consider them as statistically different. First, we considered one station from our monitoring program (we joined all the data from M2 and V2 stations in this numerical experiment to construct a fictional station with a large number of samples). For each of the eight phytoplankton groups, and for each season of the year, we have as many abundance profiles as oceanographic campaigns have been carried out. We followed an iterative method with n iterations. For each of the iterations we took nine sub-samples (simulating the 9 oceanographic stations) of n_{sub} profiles (with repetition) simulating the development of n_{sub} campaigns. Then we calculated the median profiles and the 128 variables that characterize each of the nine simulated stations, and calculated the distance matrix. We obtained the maximum of such matrix. Therefore, this is a value that was not surpassed by any of the distances between the nine stations (which in fact came from the same population). Then, we repeated this process for $n = 500$ times and calculated the 95 percentile. This means that in the 95 % of the iterations, the distances between stations did not surpass this value that was considered as the critical value. This procedure was repeated for Method1 and Method2.

Once a regionalization of our area of study was established, we calculated median profiles for the four seasons of the year and for each phytoplanktonic group for each of the bioregions. We also calculated vertically integrated abundances of such phytoplanktonic groups. The significance of the differences between these integrated abundances at different bioregions or between different seasons of the year was assessed by means of Kruskal-Wallis tests, when several groups were compared, and by means of U Mann-Whitney tests, when two groups were compared. In both cases, we worked at the 95 % confidence level and STATISTICA 7 software was used.

3. Results

3.1. Results of cluster analysis

A cluster analysis was carried out with the 15 simulated profiles (Fig. 7A) following the same Method1 used for the analysis of phytoplanktonic data, that is, the 15 profiles were characterized by means of

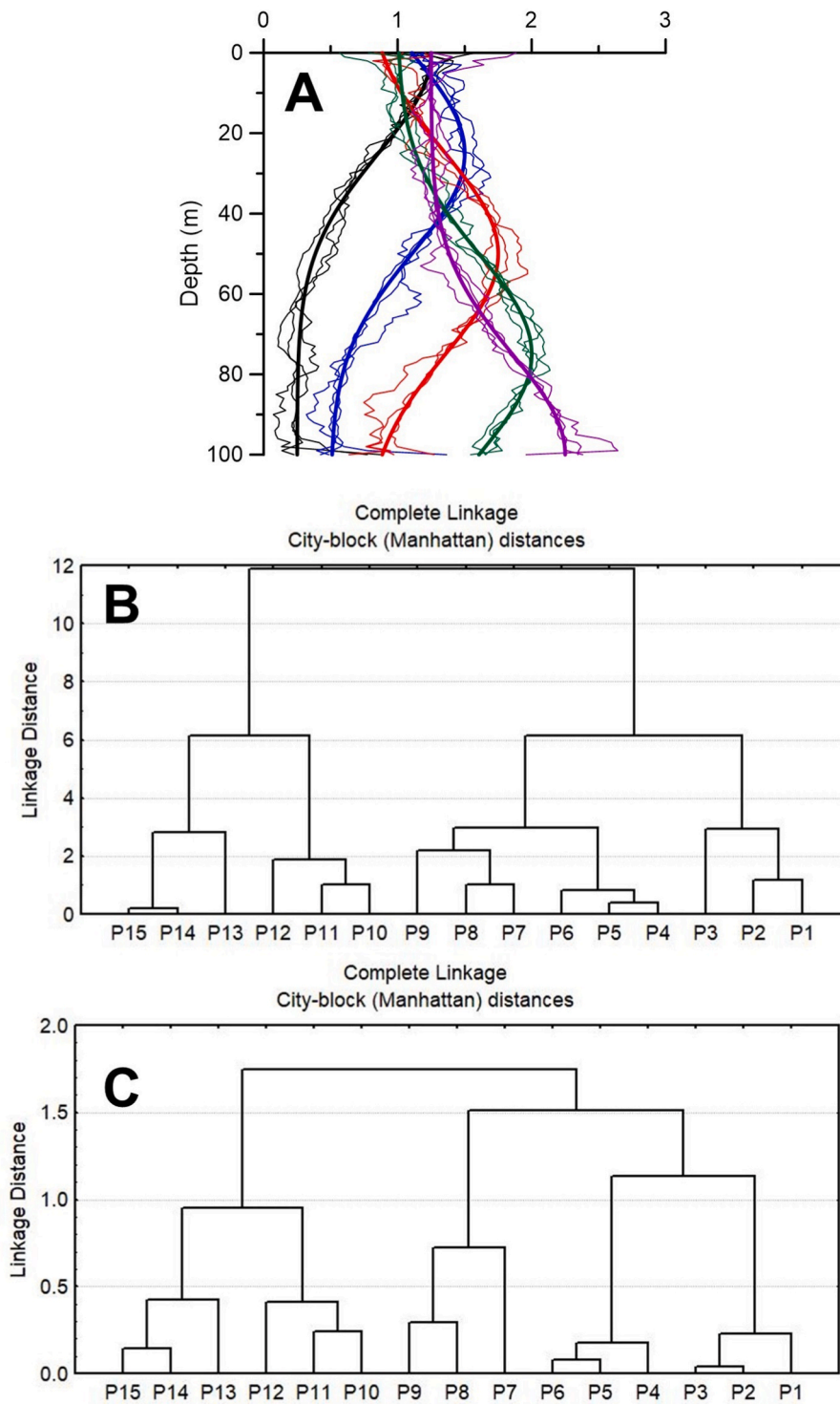


Fig. 7. Thick coloured lines in Fig. 7A are the five different types of vertical profiles generated by means of expression (1) and the five pairs of parameters in Table 1. Thin coloured lines are the three profiles generated from each type of profile adding a noise of different variance. Finally, the thin lines in Fig. 7A show 15 profiles corresponding to 5 different types. Fig. 7B shows the result of the cluster analysis using Method1 with city-block distance and complete linkage, and Fig. 7C shows the result of the cluster analysis using Method2.

the four indices: surface abundance, maximum abundance, depth of the maximum, and total abundance. The use of city-block distance and complete linkage yielded the expected result, grouping P1-3, P4-6, P7-9, P10-12, and P13-15. The use of city-block distance and Ward’s method also yielded the correct cluster grouping, whereas the use of any other metrics yielded wrong results as they grouped profiles from one type with those from a different one (Fig. S2 in supplementary material).

When the simulated profiles were characterized by means of Method2 (PCA), the city-block distance and the complete linkage method also provided the right grouping of the profiles. However, in this case, all the other methods also yielded the right results, with the only exception of the use of Euclidean distance and complete linkage (Fig. S3).

According to the previous simulations, when the analysis of the

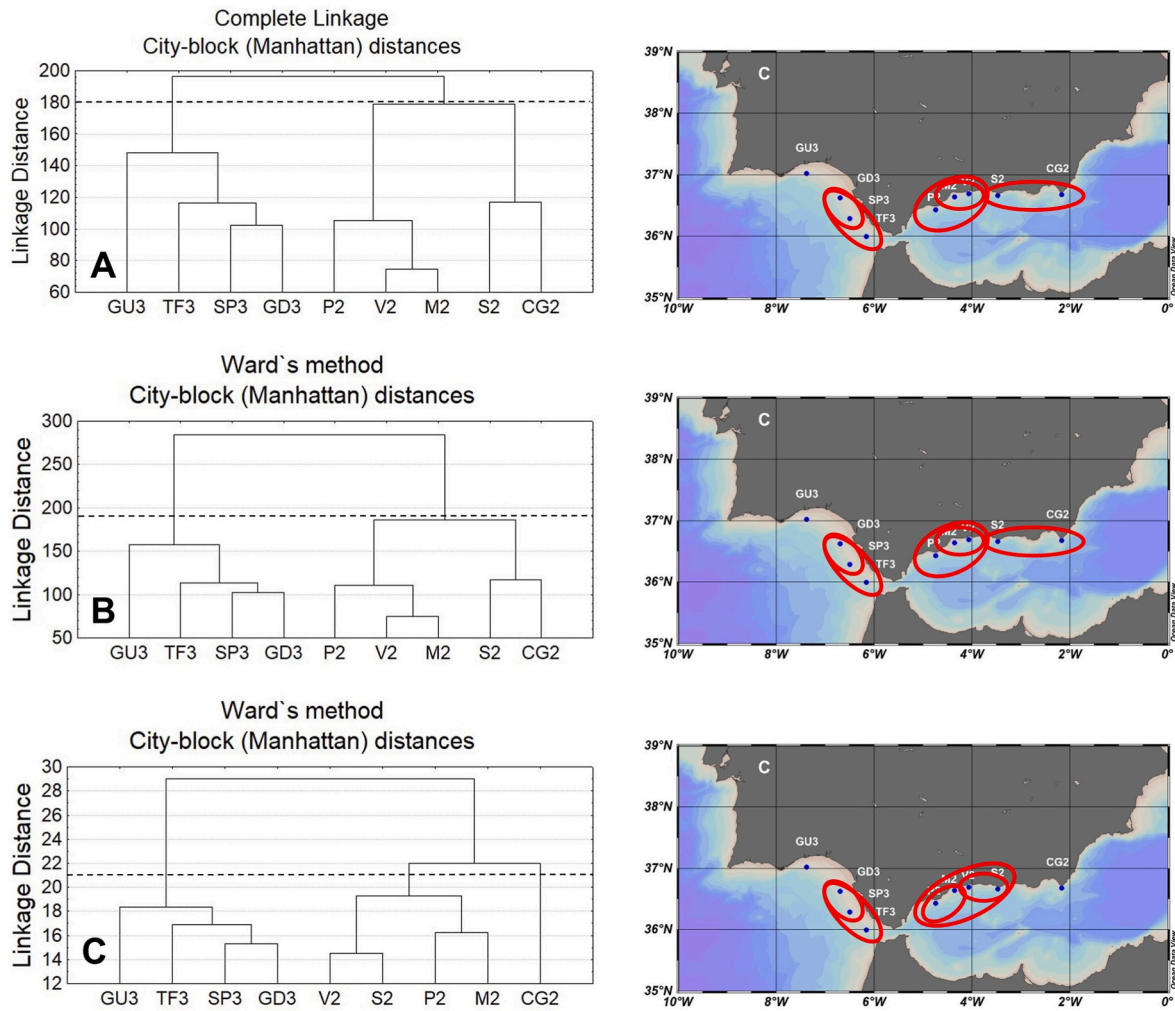


Fig. 8. A. The left panel shows the cluster analysis from M1 with the use of city-block distance and complete linkage method. The right panel shows the way in which the oceanographic stations are grouped according to the cluster analysis. B. is the same, but for the M1 method with the use of city-block distance and the Ward's method, and C. is the same for the M2 method, city-block distance and Ward's method. The horizontal dashed line shows the critic value for statistically significant distances in the 95 % confidence level.

phytoplanktonic communities was undertaken using Method1, the cluster analysis was carried out using the city-block distance with both the complete linkage and Ward's method, as these are the only methods that reproduced correctly the simulated profiles. When these two cluster methods were applied to the data of the Gulf of Cadiz and the Alboran Sea, we obtained the same results (Fig. 8A–B) and therefore we could establish unambiguously the different bioregions. When Method2 was used for analysing the 15 simulated profiles, all the metrics and methods used for the cluster analysis yielded the expected results with the only exception of Euclidean distance with complete linkage. Following this approach, we analysed the real phytoplanktonic data with all the metrics and cluster techniques that had yielded the right simulated results. However, in this case, when real data were analysed, the results were different depending on the cluster methodology. In the case of Method2 with city-block distance and the Ward's Method, the results were similar to those obtained using Method1 (Fig. 8C) and therefore these were finally the selected results (see further discussion in the discussion and conclusions section).

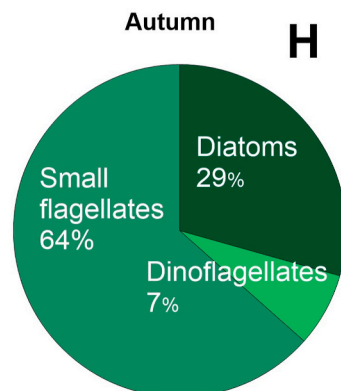
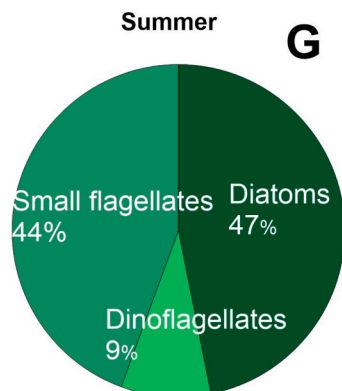
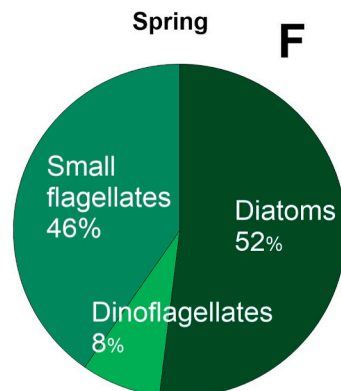
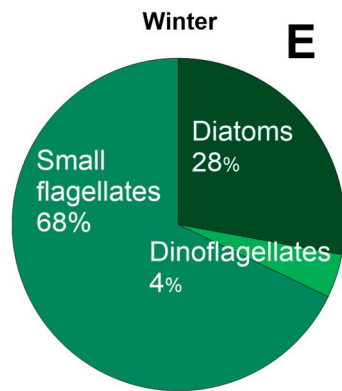
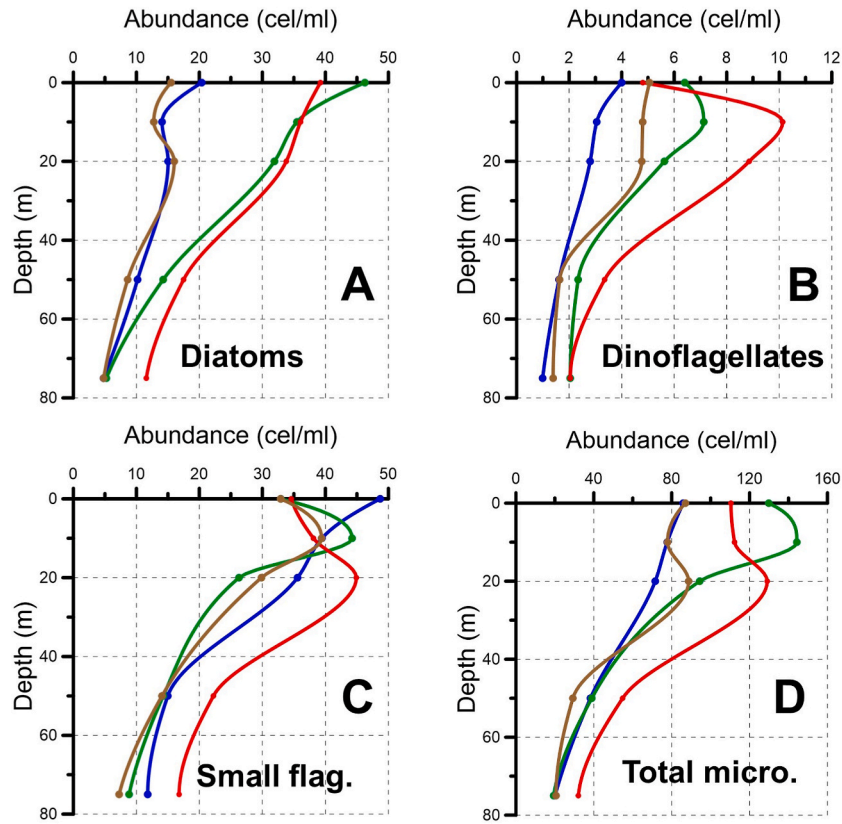
In the three cases considered in Fig. 8, the Alboran Sea and the Gulf of Cadiz were grouped into two different clusters, therefore establishing two differentiated bioregions on both sides of the Strait of Gibraltar. According to Method1, there were three different areas within the Alboran Sea. The easternmost one, including the S2 and CG2 stations,

then a second cluster formed by those stations occupying the central area (M2 and V2), and finally the westernmost station P2. The same result was obtained using the Method1 with the Ward's method and the city-block distance. A similar result, although not exactly the same, was obtained using Method2 (PCA) with the Ward's method and city-block distance. In this case, once again the Alboran Sea and the Gulf of Cadiz were grouped as two differentiated bioregions. As in the preceding cases a west-east zoning was observed in the Alboran Sea. Nevertheless, in this case, the westernmost stations P2 and M2 formed a cluster. Then the stations V2 and S2, and finally the easternmost station CG2 joined the previous clusters to complete the Alboran Sea one.

In all the cases the Gulf of Cadiz, stations were clustered in the same way, with a first cluster joining the central stations SP3 and GD3 which was joined, first by the easternmost station TF3, and finally by the westernmost one GU3.

The critic values obtained from the bootstrap method for determining the difference between two stations showed that the difference between the Alboran Sea and the Gulf of Cadiz clusters were significant at the 95 % confidence level (see the horizontal dashed line in Fig. 8A–B). The differentiation between the western (P2, M2, V2) and the eastern (S2, CG2) sectors of the Alboran Sea were not statistically different at this confidence level. No significant differences could be established for the Gulf of Cadiz stations. If we considered the results

Alboran Sea



(caption on next page)

Fig. 9. Fig. 9A, B, C and D show the median profiles for the four seasons of the years for diatoms, dinoflagellates, small flagellates and total micro-phytoplankton respectively in the Alboran Sea (grouping all the stations within this region). Blue lines correspond to winter, green to spring, red to summer, and brown to autumn. Fig. 9E, F, G, H show the relative contributions expressed as percentages for the vertically integrated abundances of diatoms, dinoflagellates and small flagellates. Fig. 9E corresponds to winter, 9 F to spring, 9G to summer, and 9H to autumn.

from Method2, a further sub-division could be considered statistically significant, with the easternmost station of the Alboran Sea on one side (CG2) and the rest of the stations on the other.

3.2. Description of the phytoplanktonic communities in the Gulf of Cadiz and the Alboran Sea

According to the cluster analysis, we considered that the southern Spanish continental shelf could be divided into two bioregions: The gulf of Cadiz and the Alboran Sea. Then, we proceeded to describe the characteristics of the phytoplanktonic communities for each of these two geographical areas. For each phytoplanktonic group, and for each season of the year, we combined all the available abundance profiles for each of the two regions, and obtained the corresponding median profiles.

Fig. 9A–D shows the median profiles for the four seasons of the year for the diatoms, dinoflagellates, small flagellates, and total micro-phytoplankton in the Alboran Sea.

Fig. 9E–H shows the relative contribution of the vertically integrated abundances (in percentage) of the three micro-phytoplanktonic groups (diatoms, dinoflagellates and small flagellates). Fig. 10A–D shows the median profiles for the four seasons of the year for the abundances of diatoms, dinoflagellates, small flagellates, and total micro-phytoplankton in the Gulf of Cádiz, and Fig. 10E–F presents the relative contribution of vertically integrated abundances of diatoms, dinoflagellates and small flagellates.

The micro-phytoplanktonic communities on both sides of Gibraltar showed some differences, as expected from the cluster analysis (Fig. 8). The maximum vertically integrated diatom abundance was observed during spring in the Alboran Sea, whereas such maximum was observed in winter in the Gulf of Cadiz. The surface diatom abundance reached also its maximum value in the Alboran Sea during spring while this surface maximum was observed in winter in the Gulf of Cadiz (see Figs. 9 and 10 and Table II). During winter, the diatom integrated abundance was higher in the gulf of Cadiz than in the Alboran Sea (Table II). However, a non-parametric U Mann-Whitney test did not show these differences to be significant at the 95 % confidence level. On the contrary, integrated abundances were higher in the Alboran Sea than in the Gulf of Cadiz for the rest of the year (Table II). U Mann-Whitney tests showed that these differences were significant for each of these three seasons: spring, summer, and autumn. It is also interesting to note that diatom abundances seemed to reach the maximum values at the sea surface at the Alboran Sea and no clear deep or sub-surface maxima seemed to develop throughout the year. Considering the integrated abundances, this group was the most abundant in the Alboran Sea during spring and summer, and its relative contribution kept above 28 % for the rest of the year. On the contrary, a sub-surface maximum was observed at 25 m depth during spring in the Gulf of Cadiz. Diatom abundances presented very low values along the whole water column (Fig. 10) during autumn when the integrated abundance had a very low relative contribution of 8 % (Fig. 10, Table II).

Dinoflagellates were the least abundant micro-phytoplanktonic group in both the Alboran Sea and Gulf of Cadiz (see Figs. 9 and 10 and Table II). Abundances were higher in the Alboran Sea throughout the whole year, with the only exception of winter, but U Mann-Whitney tests showed that these differences were not statistically significant.

The highest abundances of small flagellates in the Alboran Sea were reached during winter and autumn (Fig. 9 and Table II). Nevertheless, a Kruskal-Wallis test showed no significant differences among the four seasons of the year. Therefore, we can only conclude that this group

presented high abundances throughout the whole year. Similarly, no significant differences between the different seasons of the year were found for this group in the Gulf of Cadiz (Kruskal-Wallis). Comparisons between the integrated abundances of the Alboran Sea and the Gulf of Cadiz for each of the seasons of the year showed that this group was more abundant in the former region during winter and spring (Table II). They were also higher in the Alboran Sea during summer and autumn, but in these cases the differences were not statistically significant.

Fig. 11 shows the median profiles for nano and picoeukaryotes (11 A and 11 B), and for *Prochlorococcus* and *Synechococcus* (Fig. 11C–D) in the Alboran Sea. Fig. 11E–H shows the relative contribution (percentage) of vertically integrated abundances of nanoeukaryotes, picoeukaryotes, *Prochlorococcus*, and *Synechococcus*, also in the Alboran Sea during winter (11 E), spring (11 F), summer (11G), and autumn (11H), also in the Alboran Sea. Fig. 12 shows the same results, but for the Gulf of Cadiz.

Nano and picoplanktonic groups showed a tendency to form a maximum in the subsurface in the Gulf of Cadiz, whereas abundances seemed to be higher at the sea surface in the Alboran Sea, with some few exceptions as the summer median profile of *Prochlorococcus* (Fig. 11C). According to the integrated abundances, nanoeukaryotes were the less abundant group in both regions (see Table II and note the different scales in x-axis of Figs. 11 and 12). *Synechococcus* was the most abundant group in the Alboran Sea during spring and summer, followed by picoeukaryotes, being the differences between these two groups significant. *Synechococcus* was also the most abundant group in autumn, but in this case the second most abundant groups was *Prochlorococcus*. Only during winter, picoeukaryotes exceeded *Synechococcus*, but in this case the difference was not significant (Man-Whitney).

Picoeukaryotes were the most abundant group during winter in the Gulf of Cadiz, followed by *Synechococcus* and *Prochlorococcus*. However, the differences between these latter groups were not significant. Both groups of cyanobacteria showed almost the same integrated abundance during spring, whereas *Prochlorococcus* exceeded *Synechococcus* during summer and autumn.

4. Discussion and conclusions

Previous works in the Mediterranean Sea and the Gulf of Cadiz have tried to establish marine bioregions based on the properties of surface waters such as the chlorophyll concentration or the sea temperature. These works were aimed at identifying regions with different productivity, as surface chlorophyll concentration could be considered as a good proxy for the phytoplankton abundance. However, to our knowledge, no works have attempted to carry out such regionalization in the Gulf of Cadiz or the Western Mediterranean Sea on the basis of phytoplankton abundance data along the water column, let alone using the abundance of different phytoplanktonic groups that are indicative of the productivity of the pelagic ecosystem.

The use of time series of phytoplankton abundances from periodic monitoring campaigns has shown that the distribution and abundance of phytoplanktonic groups along the water column have a very large inter-annual variability. Two vertical profiles of abundances of phytoplankton collected during the same season of the year, but on different years, can differ greatly (Figs. 2–5B). This result makes it difficult to characterize the phytoplanktonic community of any location on the basis of one single campaign or a limited number of them. The analysis of RADMED and STOCA time series within the Alboran Sea and the Gulf of Cadiz revealed that the populations of phytoplanktonic groups (statistically speaking) are asymmetrical. Therefore, the central tendency of such populations (that could be used to characterize such populations) should

Gulf of Cadiz

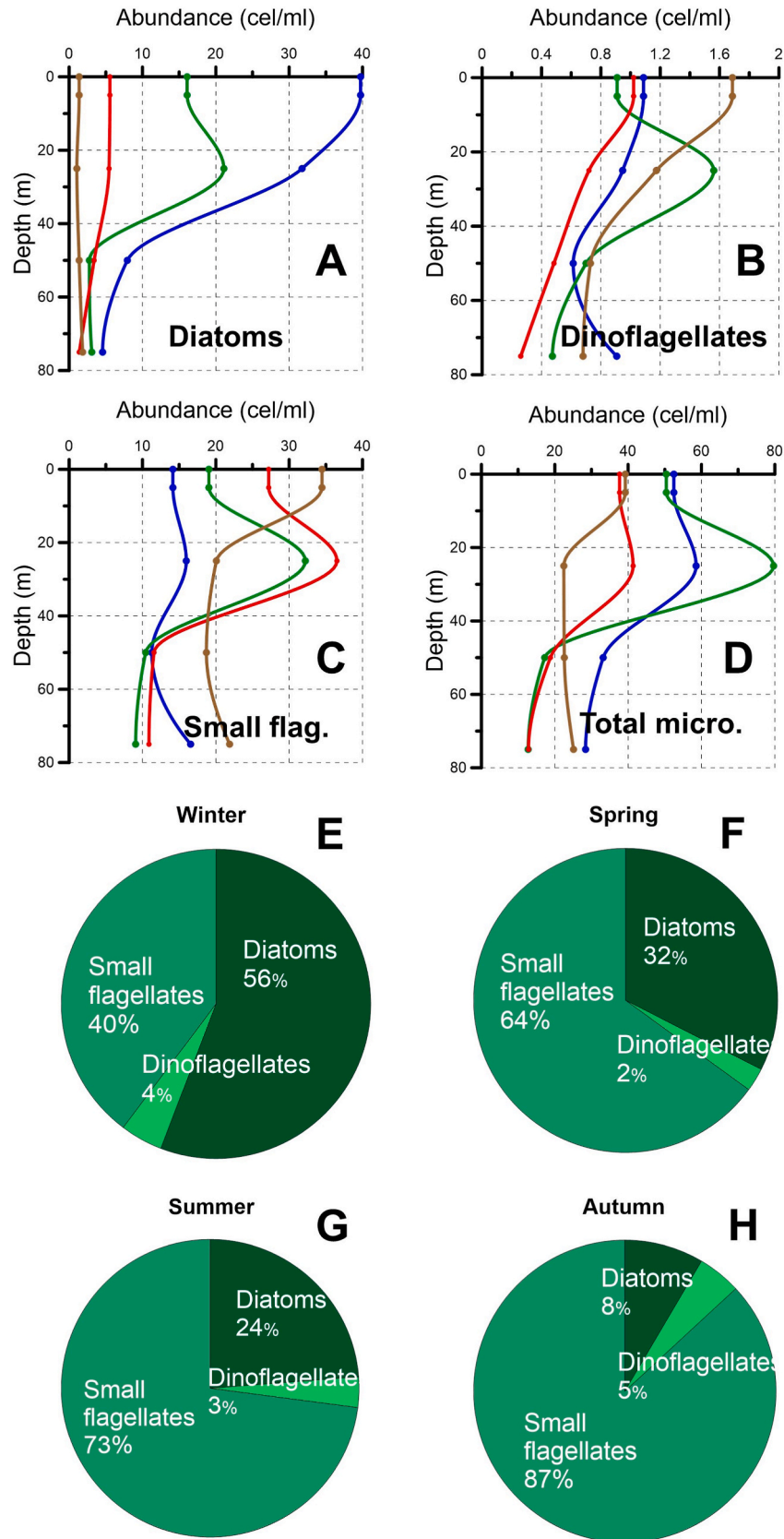


Fig. 10. The same as in Fig. 9, but for the Gulf of Cadiz.

Table 2

Integrated abundances (from the sea Surface to 75 m depth) for the micro, nano, and pico-planktonic groups in the Alboran Sea and Gulf of Cadiz during winter, spring, summer, and autumn. The abundances for diatoms, dinoflagellates, and small flagellates are expressed in $10^6 \times \text{cel./m}^2$, The abundances of nano and pico eukaryotes, and bacteria of the genera *Prochlorococcus* and *Synechococcus* are expressed in $10^9 \times \text{cel./m}^2$.

Alboran Sea	Winter	Spring	Summer	Autumn
Diatoms ($\times 10^6 \text{cel./m}^2$)	980	3178	2643	1022
Dinoflagellates ($\times 10^6 \text{cel./m}^2$)	150	473	492	250
Small flagellates ($\times 10^6 \text{cel./m}^2$)	2384	2467	2504	2207
Nano-eukaryotes ($\times 10^9 \text{cel./m}^2$)	88	87	80	61
Pico-eukaryotes ($\times 10^9 \text{cel./m}^2$)	501	256	414	415
<i>Prochlorococcus</i> ($\times 10^9 \text{cel./m}^2$)	88	99	373	508
<i>Synechococcus</i> ($\times 10^9 \text{cel./m}^2$)	351	595	966	751

Gulf of Cadiz	Winter	Spring	Summer	Autumn
Diatoms ($\times 10^6 \text{cel./m}^2$)	1356	901	693	196
Dinoflagellates ($\times 10^6 \text{cel./m}^2$)	108	69	96	107
Small flagellates ($\times 10^6 \text{cel./m}^2$)	966	1803	2127	2007
Nano-eukaryotes ($\times 10^9 \text{cel./m}^2$)	105	74	69	76
Pico-eukaryotes ($\times 10^9 \text{cel./m}^2$)	473	304	281	348
<i>Prochlorococcus</i> ($\times 10^9 \text{cel./m}^2$)	171	926	922	1380
<i>Synechococcus</i> ($\times 10^9 \text{cel./m}^2$)	262	932	671	673

be described by means of the median, as opposed to the mean, as used in previous works in this area (Vargas-Yáñez et al., 2019; García-Martínez et al., 2019a, 2019b). The calculation of the median profiles for each location and for each phytoplanktonic group for the four seasons of the year has allowed us to characterize the evolution of these phytoplanktonic groups along the seasonal cycle.

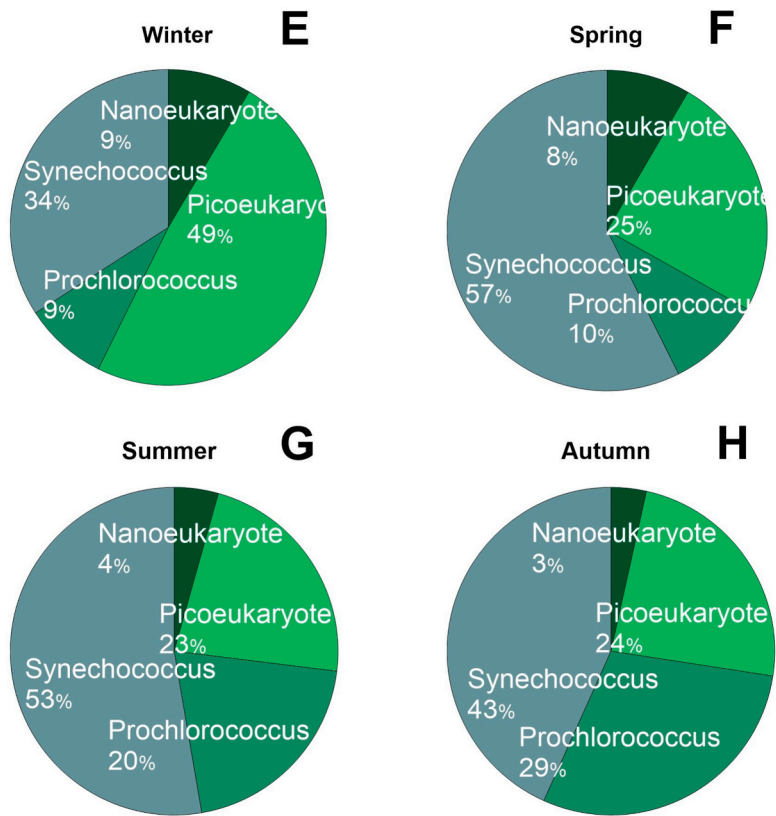
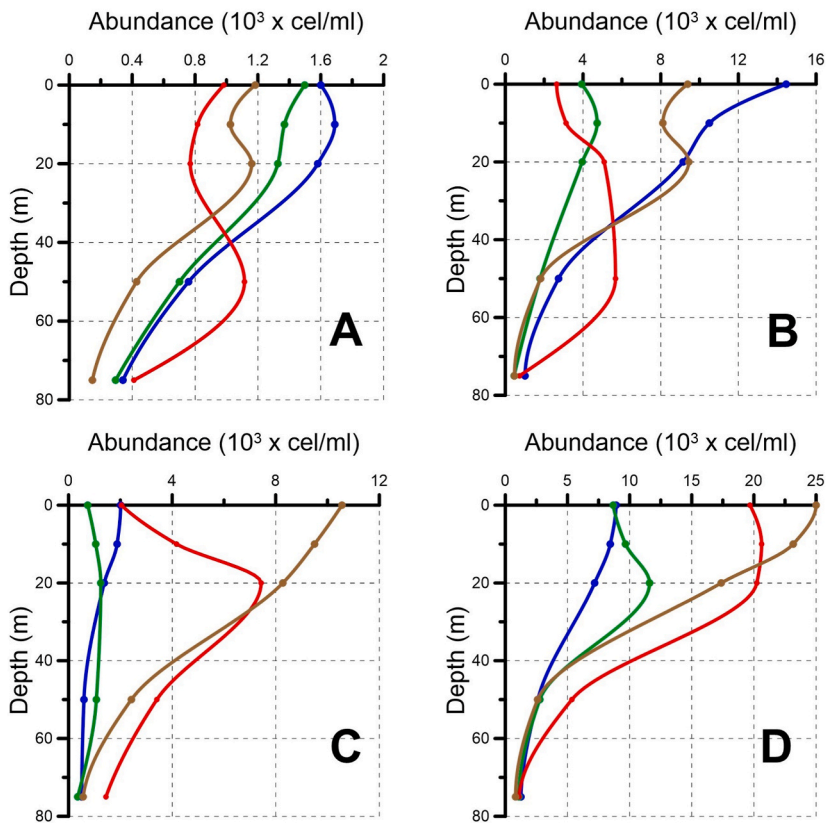
The phytoplankton analysis was carried out to the genus or even species level, but then this information was classified into eight groups belonging to micro, nano and pico plankton. Despite this important data reduction, the use of seasonal median profiles for several phytoplanktonic groups still leaves a great deal of information that should be reduced in order to make it more manageable. We developed two different methods for accomplishing such reduction. In both cases each median profile was substituted by four new variables that described both the shape and the total abundance of each profile. In the first method we characterized each profile by its surface and maximum abundances, the depth where the maximum abundance was observed, and the vertically integrated abundance. The first three variables are similar to those used by Lavigne et al. (2015) for describing different types of chlorophyll profiles within the Mediterranean Sea. The second method used PCA. The Principal Components can be understood as different types of profiles. Each median profile corresponding to a particular season of the year and a particular location can be decomposed as a mix of 4 types of profiles (4 PCs reproduced most of the original variance). Each median profile was then characterized by the proportion of each profile type contributing to it. The use of two different methodologies could be considered as redundant but has allowed us to show that our results are robust and not dependent on the methodology used to characterize the seasonal cycle of the micro, nano, and pico-phytoplanktonic groups at each location.

A similar question arises when the similarity between different locations (oceanographic stations) is considered. We can use different metrics to calculate the distance between stations. Once a distance matrix has been obtained and some clusters have been defined, we can also use different methods to calculate the distance between clusters. We could assume that different metrics and methods should yield similar results. However, we have found that this is not the case, posing the question of which combination of metrics and methods works best for our data. This question has been considered carefully in this work. If different metrics and methods for cluster analysis yielded the same result, such result would be considered as the right way of grouping our

oceanographic stations and we would be confident about the bioregions determined by the cluster analysis. On the contrary, our results showed that this was not the case and therefore we did not know which stations should be considered as belonging to the same bioregion (Fig. 6). To solve this problem, we have simulated 15 profiles of a fictitious variable. These profiles belonged to 5 different profile types. So, in this case, on the contrary to the real one, we did know a priori the results that the cluster analysis should yield (Fig. 7). Figs. S2 and S3 in supplementary material show the cluster analyses corresponding to Method1 and Method2 and the different metrics used. When the Method1 was used for characterizing the phytoplanktonic community of each station, only two cluster methodologies yielded the correct (predefined) result (city-block distance with complete linkage or Ward's method). When the phytoplanktonic community was characterized using Method2, several methods seemed to be right. Therefore, if the analysis of real phytoplankton data was carried out using Method1 to characterize the abundance profiles, the cluster analysis should be carried out using the city-block distance and the complete linkage or the Ward's method (Fig. 8A–B. Fig. S4 shows all the other cluster results using other metrics). Both analyses yielded the same result. The Gulf of Cadiz and the Alboran Sea were grouped into two different clusters. In the Gulf of Cadiz, the two central stations SP3 and GD3 were grouped in a primary cluster. Then TF3 joined this cluster, and finally the easternmost station GU3. In the Alboran Sea there was a west-east zonation with three areas formed by the westernmost station: P2, then the central stations: M2 and V2, and finally the easternmost stations S2 and CG2. However, a bootstrap analysis showed that only the difference between the two main clusters was significant. This bootstrap analysis constructed 9 stations that had exactly the same populations of the eight phytoplanktonic groups. These populations were subsampled taking 15 profiles in a random way for each season of the year and for each phytoplanktonic group. This number was chosen because it was similar to the actual number of campaigns per season available in the Alboran Sea (the number is lower for the Gulf of Cadiz). Therefore, if the median profiles were calculated for each season of the year and for each station, following the same method used for the real phytoplankton data, the distances between stations should be zero. However, this was not the case, and these distances were due to chance. The dashed lines in Fig. 8A–B shows the threshold that is exceeded only a 5 % of occasions. Hence, in the 5 % significance level we can only establish two different bioregions that would be the Gulf of Cadiz and the Alboran Sea. This does not mean that no other subdivisions could exist. The distances obtained in the bootstrap simulation arise from the fact that the median profiles calculated from the samples differ from the theoretical median profiles of the population. As the number of campaigns increases, the size of the samples in the bootstrap simulation should also increase, decreasing the 95 % threshold, allowing new bioregions to be established in a significant way.

When Method2 was used, several metrics yielded the right result for the 15 simulated profiles. For this reason, all of them were used for the analysis of real data from RADMED and STOCA (see Fig. S5 in supplementary material). In this case different bioregions (clusters) were obtained depending on the metric used, and more importantly, some of them were different from those obtained using Method1. On the contrary, the cluster analysis corresponding to city-block distance and Ward's method yielded a similar result to that from Method1, with two main bioregions formed by the Gulf of Cadiz and the Alboran Sea. The clusters within the Gulf of Cadiz were the same obtained with Method1 and those within the Alboran Sea, while not being exactly the same, were very similar, with a west-east zonation (Fig. 8C). In this case the distance between the Gulf of Cadiz and the Alboran Sea was significant, and a further division between the CG2 station, at the easternmost sector of the Alboran Sea, and the central and western sections was also significant. Hence, Method1 with city-block distance and complete linkage and Ward's method on one hand, and Method2 with city-block distance and Ward's method, on the other, yielded almost the same

Alboran Sea



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Fig. 11. Fig. 11A, B, C and D show the median profiles for the four seasons of the years for nanoeukaryotic phytoplankton, picoeukaryotic phytoplankton, *Prochlorococcus* and *Synechococcus* respectively in the Alboran Sea (grouping all the stations within this region). Blue lines correspond to winter, green to spring, red to summer, and brown to autumn. Fig. 11E, F, G, H show the relative contributions expressed as percentages for the vertically integrated abundances of nanoeukaryotic phytoplankton, picoeukaryotic phytoplankton, *Prochlorococcus* and *Synechococcus*. Fig. 11E corresponds to winter, 11 F to spring, 11G to summer, and 11H to autumn.

regionalization of the Gulf of Cadiz and the Alboran Sea, and all these three methodologies were appropriate according to the analysis of simulated profiles. The coherence of these results made us consider them as the right form of establishing bioregions in our area of study. However, we admit that the present results would have been more robust if the different metrics used together with method2 had produced the same clusters. This is a limitation of our present work on which further research is needed.

The existence of two differentiated bioregions in the Gulf of Cadiz and the Alboran Sea can be considered a robust result because it was obtained using both Method1 and Method2 and complete linkage and Ward's method. The distance between these two clusters is significant for both Method1 and Method2. It is very likely that other bioregions will be established when the number of campaigns increases. Despite the lack of significance, the west-east zonation within the Alboran Sea could be a real feature that simply reflects the trophic gradient existent in the Alboran Sea from the more eutrophic waters close to the Strait of Gibraltar (Reul et al., 2005), to the more oligotrophic ones in the eastern Alboran Sea (Vargas-Yáñez et al., 2019; García-Martínez et al., 2019a, 2019b).

The analysis of the aggregated phytoplanktonic communities for each of the two bioregions revealed a higher abundance of total micro-phytoplankton in the Alboran Sea than in the Gulf of Cadiz. The most abundant micro-phytoplanktonic group in both regions was small flagellates, which was more abundant in the Alboran Sea than in the Gulf of Cadiz. These results could indicate a higher productivity of the Alboran Sea waters. Diatoms usually bloom under conditions of high nutrient concentrations. This group was also more abundant in the Alboran Sea throughout most of the year. Besides the total abundance of each micro-phytoplanktonic group, the relative contribution of large and small cells is also another indicator of the productivity of marine waters (Rodríguez et al., 2001). Diatoms were the dominant group in the Alboran Sea during spring and summer and maintained a relative contribution higher than 28 % during winter and autumn. This phytoplanktonic group was the most abundant in the Gulf of Cadiz only during winter and had a very small contribution in autumn.

This trophic gradient on both sides of Gibraltar is also supported by the different abundances of *Prochlorococcus* and *Synechococcus*. Visintini et al. (2021), Flombaum et al. (2013), Partensky et al. (1999) have shown that *Synechococcus* is more abundant in cold waters with mesotrophic conditions, whereas *Prochlorococcus* shows a preference for warm oligotrophic waters. Moore et al. (2002) showed that *Prochlorococcus* could use ammonium and urea, whereas *Synechococcus* was better adapted to nitrate-rich waters. Latasa et al. (2016) analysed the distribution of these two lineages in the Northwestern Mediterranean and Northeastern Atlantic and also found that *Synechococcus* were more abundant in mesotrophic waters and *Prochlorococcus* in oligotrophic ones. Similar results were obtained in the Gulf of Cadiz where *Synechococcus* were more abundant in surface well-mixed waters, with high nitrate concentrations, whereas *Prochlorococcus* showed higher abundances in deeper layers in poor and stratified waters (González-García et al., 2018; Anfuso et al., 2013). The analysis of these two pico-planktonic groups revealed that *Synechococcus* was more abundant than *Prochlorococcus* in the Alboran Sea, while the opposite situation stood for the Gulf of Cadiz.

This trophic gradient between both sides of Gibraltar had already been shown by Echevarría et al. (2009) and Prieto et al. (2009). Reul et al. (2005) had also shown that the northwestern sector of the Alboran Sea was a highly productive area. There are several explanations for the

high productivity of the northern Alboran Sea. On one hand, there is an intense mixing of Atlantic and Mediterranean waters within the Strait of Gibraltar favoured by the strong velocity shear between both currents and the large vertical displacements associated to internal tides (Bolaño-Penagos et al., 2023). This mixing is able to fertilize the Atlantic Waters flowing into the Alboran Sea, enhancing the primary production of the surface waters that flow in a northeast direction into the Alboran Sea, approaching its northern coast (Macías et al., 2007, 2014). On the other, two cyclonic circulation cells close to the northern coast of the Alboran Sea would favour the upwelling of sub-surface waters, injecting nutrients at the euphotic layer (Vargas-Yáñez et al., 2019; García-Martínez et al., 2019a, 2019b).

The analysis of phytoplankton data from the stations sampled in RADMED and STOCA projects shows clearly that two differentiated bioregions can be identified on both sides of the Strait of Gibraltar, and the abundances and composition of the different phytoplanktonic groups at these two bioregions suggest that the northern Alboran Sea waters are more productive, with higher abundance of total micro-phytoplankton as well as of diatoms. The predominance of *Synechococcus* over *Prochlorococcus* in the Alboran Sea, and the opposite situation in the Gulf of Cadiz also support this hypothesis. However, we should be very careful about this conclusion. This result seems to be a robust and reliable one considering those stations used in this work. The criteria used to select their positions was to sample stations with a depth around 100 m, located in the central part of the continental shelf. This central position is far from the shore in the case of the Gulf of Cadiz because of its large width and therefore would not be directly affected by river discharges (mainly Guadalquivir and Guadiana). Gomiz-Pascual et al. (2021) have shown that the Guadalquivir river has an average discharge of 116.5 m³/s, and Caballero et al. (2014) evidenced that there could be important phytoplanktonic blooms associated to the turbidity plumes of these rivers. Ribas-Ribas et al. (2013) and Huertas et al. (2005) calculated the transports of phosphates, silicates and dissolved organic matter associated to the river discharges, and Ruiz et al. (2006) and Caballero-Huertas et al. (2022) showed that the fertilizing effect of the rivers would affect not only to the phytoplanktonic abundance, but also to the zooplankton one and to the spawning and body condition of small pelagic fishes such as anchovy and sardine. Therefore, it seems clear that river discharges in the Gulf of Cadiz are the cause of an onshore-offshore trophic gradient (González-Ortegón et al., 2018; González-García et al., 2018; Prieto et al., 2009) and the inshore waters of the Gulf of Cadiz would be much more eutrophic than those waters sampled in this work and located in the offshore waters of the continental shelf. On the contrary, the central part of the Northern Alboran shelf is close to the shore, because of its narrow character, and there are no important rivers draining into its waters. All these circumstances make us conclude that the Northern Alboran shelf, and the outer shelf of the Gulf of Cadiz constitute two different bioregions being the former more productive than the latter, but a different result could be obtained if the inshore area of the Gulf of Cadiz were considered. We speculate that if coastal stations were sampled, they would constitute a third bioregion, with the same, or even higher productivity than the Alboran Sea.

In conclusion, the analysis of time series of phytoplankton abundance and composition along the water column is a very valuable tool for assessing the definition of bioregions in the pelagic ecosystem. The use of numerical simulations has helped us to establish the best methods for carrying out a cluster analysis and for establishing different bioregions in a robust and reliable way. The Gulf of Cadiz and the Alboran

Gulf of Cadiz

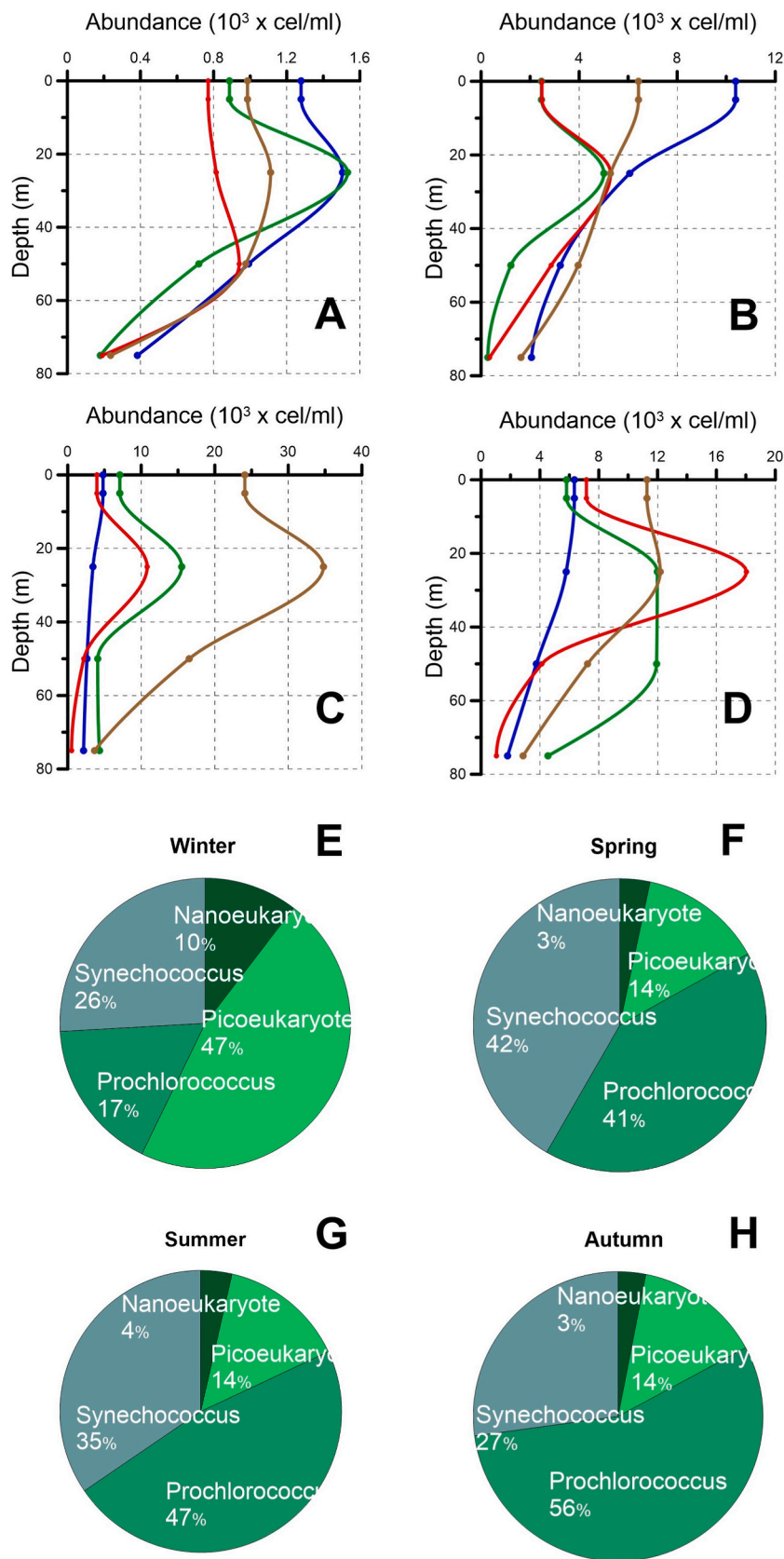


Fig. 12. The same as in Fig. 11, but for the Gulf of Cadiz.

Sea have been identified as two differentiated bioregions, based on the properties of their phytoplanktonic communities. Nevertheless, as time series get longer, a more detailed description of both geographical areas will be possible, defining other bioregions within each of these two large regions. Finally, it is necessary to sample the phytoplanktonic community at the inshore waters of the Gulf of Cadiz where the influence of river discharges could be responsible for the existence of another bioregion characterized by a productivity higher than that of the offshore continental shelf.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ocecoaman.2023.106930>.

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