

Article

Phytoplankton distribution in Mar Menor coastal lagoon (SE Spain) during 2017

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Abstract: The Mar Menor is a Spanish coastal lagoon of great ecological and economic interest. The agricultural and tourist activities developed in the surroundings of the lagoon, together with the modifications in its channels of connection with the Mediterranean Sea, have notably affected the quality of its waters, which is altering the natural balance of the ecosystem. In this work, an analysis of the density of phytoplankton present in the lagoon between the months of May to December 2017 has been carried out. There, it has been a notable increase in the density of organisms in post-summer samplings, following the recording of higher temperatures, and the presence of Chlorophyceae, Cyanophyceae, Chrysophyceae and nanoplanktonic Cryptophyceae stands out. The data collected indicate a significant increase in the eutrophication process of the lagoon that requires the development of management plans to reduce agricultural discharges and promote the recovery of the lagoon and its native species.

Keywords: coastal lagoon; phytoplankton; eutrophication; nanoplanktonic algae

1. Introduction

The Mar Menor is a coastal lagoon located in the Region of Murcia, Spain, connected to the Mediterranean Sea by five channels [1], through which there is a negative estuary-type water exchange [2]. Its salinity is higher than that of seawater, since the input of continental water does not compensate for the evaporation losses suffered by this lagoon [3].

In recent years, this coastal lagoon has undergone numerous modifications for the use of the environment for tourism purposes [4] which, together with the discharge of fertilizers that filter through surface and underground runoff [5] and the constant increase in temperatures, are altering the natural balance of the ecosystem, resulting in a notable loss of biodiversity [6]. One of the main changes that have been made is the extension of the Estacio canal for the construction of a marina [2], a modification that has brought the salinity conditions of the lagoon closer to those of the Mediterranean Sea [4].

Due to the interest, ecological [7], social and economic, of this inland water, it is necessary to analyse the quality of its waters and the state of the ecosystem in order to know its current state, be able to predict its evolution and plan conservation strategies.

To understand the ecological status of a coastal lagoon such as the Mar Menor, it is necessary to measure abiotic parameters such as temperature, salinity or dissolved oxygen, as well as biotic parameters [8]. To comprehend the influence of physicochemical parameters on the living organisms present in the lagoon, we can use the study of the variations suffered by a specific group of organisms that could be used as biomarkers, the phytoplankton. The group includes all those photosynthetic microorganisms adapted to life in suspension in the water column [9]. Being organisms sensitive to

changes, with short life cycles and easy to manipulate, they are perfect as bioindicators of water quality [10] and have been used in numerous studies [11].

Phytoplankton undergoes seasonal changes due to the usual variations in weather conditions [12,13]. These changes are part of the natural dynamics of the ecosystem. However, the global increase in average annual temperatures and the anthropogenic modification of the channels [2] and the environment can affect the usual parameters of water temperature, dissolved oxygen or salinity among others, which can affect its distribution in the water column [14].

The spatial distribution of phytoplankton also depends on the distribution of dissolved nutrients that can be found in the water column [15], with the presence of dissolved nitrogen and phosphorus in the water being especially important for their proliferation [16]. Many studies have shown that the presence of dissolved nitrogen and nitrogen products in large quantities favors the proliferation of algae [17], both macroscopic and microscopic, as is the case with phytoplankton.

With regard to the accumulation of these nutrients, in 2001, the Government of the Region of Murcia declared the Mar Menor as a "sensitive area to eutrophication" according to Directive 91/271/EEC [18] due to the increase in agricultural activity in the whole area known as the Campo de Cartagena. This increase was produced from the construction of the Tagus-Segura transfer [19] and has favored the filtration of irrigation water with a high content of fertilizers and phytosanitary products into the aquifer located below the Campo de Cartagena area and which, through underground runoff, makes the greatest contribution of fresh water to the lagoon. This type of product also contributes a high content of nitrogen and phosphorus through surface runoff [20]. This is the reason why the Mar Menor lagoon was declared a "vulnerable area to pollution by nitrates from agricultural sources" on the basis of Directive 91/676/EEC in 2002 [21].

The amount of nutrients and particles entering the Mar Menor lagoon in the period from September 2002 to October 2003 were studied [22]. The total annual discharge of nutrients into the lagoon was estimated at 27.4 hm³ /year, of which 73.5% passed through Rambla del Albuñón gully. As well as, for that period, the total input to the lagoon from the Rambla del Albuñón gully and its drainage effluents was 10,142 tons/year of suspended sediments (SS), 390 tons/year of particulate organic matter (POM), 2010 tons/year of dissolved inorganic nitrogen (DIN) (93% as NO₃-) and 178 tons/year of soluble reactive phosphorus (SRP).

With the increase in agricultural activity in recent years, these figures have increased and promotes in the lagoon the proliferation of phytoplankton, so that the turbidity of the water increases, and this increase prevents the penetration of sunlight to the bottom of the lagoon. When not receiving sunlight, macrophytes present at the bottom of the lagoon die, which decreases the fixation of nutrients to the substrate, and increases the suspended dead organic matter. This causes an uncontrolled increase in phytoplankton populations in the lagoon, a process known as eutrophy [23].

While some species proliferate notably, they invade the niche of others, so the process of eutrophication results in a significant loss of biodiversity. Nanoplanktonic species [24] and bacterioplankton species are often favored [25]. One of the most obvious effects of eutrophication processes is the massive proliferation (especially of dinoflagellate species) that results in the formation of algal blooms, that are commonly known as "red tides" [16] or "green soup". These blooms, in addition to the ecological risks they pose, can be formed by species that can produce toxins and be harmful to the flora and fauna of the lagoon [26].

The aim of this study is to evaluate the current status of the Mar Menor lagoon, to compare the data collected with previous studies [1,27] and thus be able to draw up action plans to promote the recovery of the lagoon and try to reverse the negative effects of the anthropogenic activities that have been carried out in the lagoon and its surroundings, especially over the last fifty years.

2. Materials and Methods

Regional Government carry out the measurement of the environmental variables weekly in some points with a multiparametric profiler CTD Seabird SBE-19. Variables measured are temperature, turbidity, chlorophyll-a concentration, dissolved oxygen, and salinity. For the biological sampling, the first sampling was carried out in May 2017 and the last one in December 2017. Due to

logistical problems, this samples could not be collected regularly, so we only have them of the months of May, June, September, October (month in which two samples were taken) and December. During the months of July, August and November there was not available boat to carry out the samplings. All the sampling started from the Tomás Maestre harbor in La Manga in the morning and each sampling station was marked with UTM (Universal Transversal de Mercator) coordinates. These points are represented in the following map (figure 1).

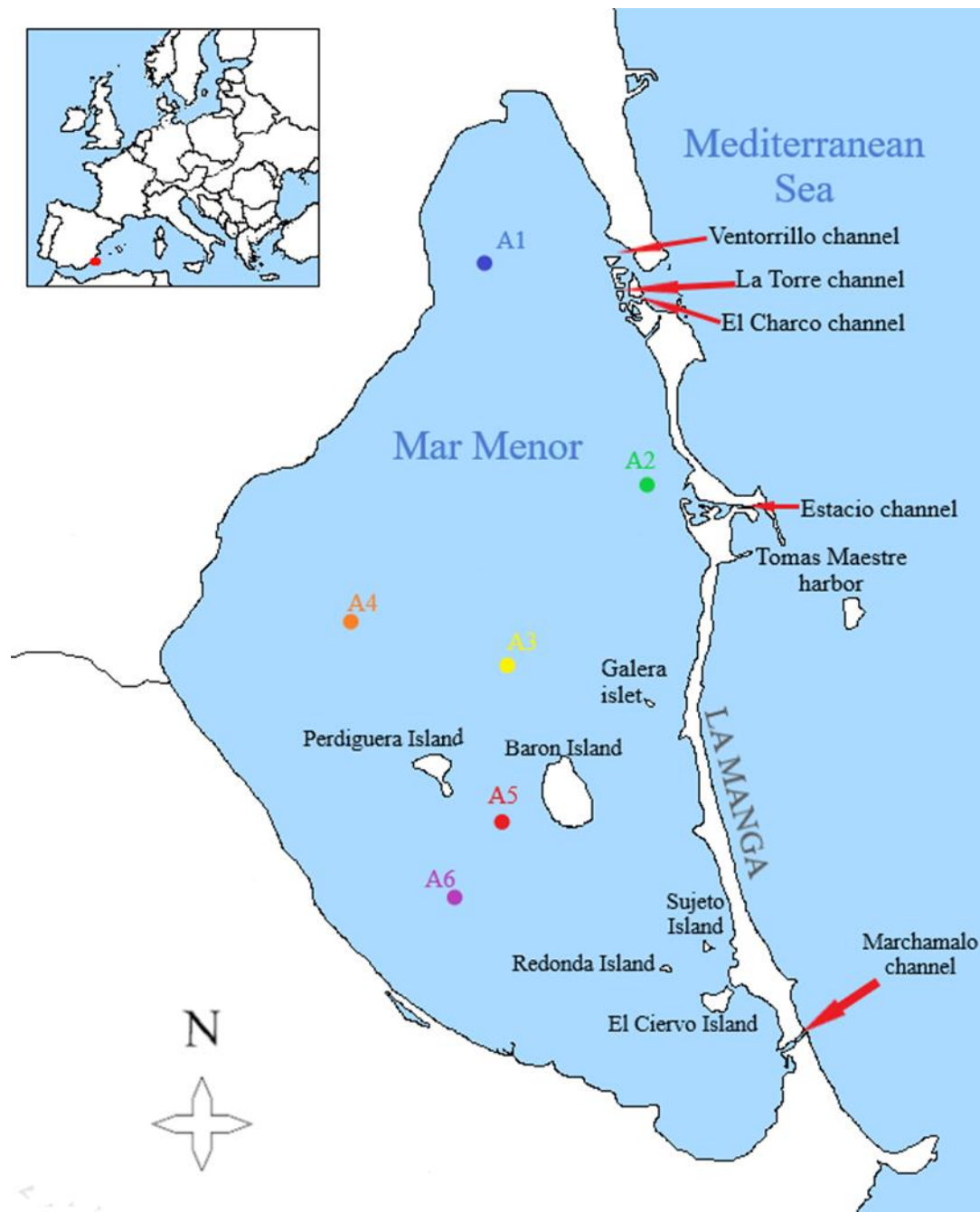


Figure 1. Map of the Mar Menor lagoon with the location of the sampling points, on a scale of 1/152,000. [Own elaboration].

Temperature and depth are measured "in situ" at each sampling point with the Turner C3 TD Temperature Deep fluorometer which also contains chlorophyll and phycocyanin fluorimeters (measured in $\mu\text{g/l}$) and a turbidity meter (measured as Formacine Turbidity Units, FTU). These measurements were taken by manually submerging the device, so that measurements are taken every

four seconds to produce a vertical profile of each of the variables to be measured. The transparency is measured using the Secchi disc depth (30 cm diameter).

The spatial distribution of chlorophyll-a concentration (Chl-a) in the lagoon and its relationship to phytoplankton density has been studied by the Ocean and Land Colour Instrument (OLCI) images. The OLCI sensor is installed at Sentinel-3 satellite. The Chl-a maps have been obtained by means of the SNAP software (Brockmann Consult, Germany) and the optical processing by means of the Sentinel-3 Toolbox using the C2RCC algorithm for OLCI sensor.

Composition and abundance phytoplankton have been studied through water samples that were collected at a depth of about 4 m using a 2.5 L Ruttner hydrographic bottle. This depth was selected because this point was the maximum of chlorophyll-a measured with fluorometer in situ. Samples was used to fill, at each sampling point, a 250 ml topaz glass bottle, fixing the sample with Lugol at 5 % in a ratio of 1:100 with a Pasteur pipette for post-analysis in the laboratory.

The analysis of phytoplankton is carried out using inverted microscope for samples collected by hydrographic bottle (phytoplankton count) and the Utermöhl method collected in CEN TC 230/WG 2/TG 3/N83 [28] has been followed.

For counting, 50 ml of the sample bottles fixed with Lugol were homogenized and poured into one of the chambers with a sedimentation column. This sample is left to settle for approximately 24 hours to allow the algae to settle at the bottom of the chamber. Then, the column with the supernatant of the sample is removed laterally, leaving in the chamber the phytoplankton content of 50 ml of sample concentrated in a few milliliters. This facilitates counting in samples with low organism density [29]. If the sample contains a high density, it is just place a specific volume (slightly less than the capacity of the chamber), it is complete until it is filled with distilled water [30] and allow it to settle.

An inverted Nikon Eclipse Ti-U microscope with 10x eyepieces is used to observe and count the sample, one of which includes an ocular micrometer [30]. Most observations were made using the 60x objective, although some were made with the 40x and 20x objectives perfectly calibrated. For more detailed observations, the Nikon camera attached to the microscope in clear field or the Nomarski differential contrast (figure 2) was used to identify specimens.

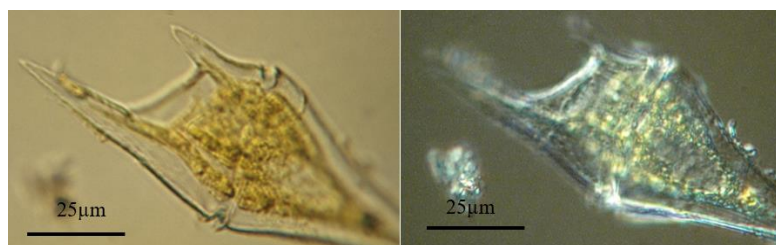


Figure 2. Microscopic images of a specimen of *Tripos furca*. Left, normal vision; right, Nomarski differential contrast.

Numerous guides and web resources [31-42] were used to identify the species. The count of organisms was carried out until we had a representative percentage of specimens for each preparation in order to reduce as much as possible the standard deviation of the obtained data. With a few exceptions, a minimum of 100 fields per camera were counted to obtain the most reliable cell density per milliliter (cel./ml) [30].

Statistical analysis of the data was performed using PAleontological STatistics (PAST) version 3.25 [43]. A descriptive analysis of the data was carried out to obtain the total of algae per sample, minimum and maximum density values, mean and standard deviation of the taxa found. We also calculated the species richness (S) of each sample, the diversity according to the Shannon-Weaver index (H') and the equitability (J) in the presence of each species [44,45]. In order to be able to compare between samples the diversity value according to the Shannon-Weaver index (H'), the program calculates the maximum diversity value by making the logarithm of the richness (S) of each sample.

From these values, the value of the equitability (J) is obtained which oscillates between 0 to 1 (or between 0 % and 100 %) where 1 indicates that all the species are in the same proportion in the sample [46].

From the obtained data, the distribution of phytoplankton by samples and classes is presented by means of maps, where, in each sampling point, circular graphs were included to show the density and proportion in which each class of phytoplankton is found in the different places. On the phytoplankton density matrix, a Principal Component Analysis (PCA) was performed. First, the data were standardized by transforming them with neperian logarithmic, eliminating those species or taxa of phytoplankton that were found only in one sample and with a density below 3 cells/ml. From the correlation matrix a clustering was made by the UPGMA method.

3. Results

3.1. Changes in environmental variables

The irrigated agricultural area has increased considerably since 1981 (when the first studies were carried out [1]) until today. According to data of Regional Agriculture Statistic the irrigated agricultural area in Campo de Cartagena, increased from 24,357 ha in 1981, to 37,296 ha in 2003 and in 2017 to 47,431 ha.

As regards nutrient concentrations, the 2003 studies show that the figures in the waters of the two main gullies (Albujón and Alcázares) were between 10 and 55 mg/l for nitrate, while those for total phosphorus (TP) were between 2 and 12 $\mu\text{g/l}$ [27]. Today, values for 2017 were 170 to 240 mg/l for nitrate and for TP between 6-10 $\mu\text{g/l}$.

Weekly variation data for central sampling point (A3 site, figure 1) has been obtained for the five environmental variables considered in the continuous sampling during the year 2017, the result of which is shown in figure 3. It should be noted that the dissolved oxygen values have presented the lowest values from the beginning of August to mid-September with an average value of 4.6 mg/l, and the highest values during January and February with an average of 7.9 mg/l, also coinciding with the maximum values of chlorophyll-a, which on 12 January was 37.8 $\mu\text{g/l}$ and presented a downward trend until reaching 5.1 $\mu\text{g/l}$ on 8 March; from mid-May to the end of August the average value was 1.5 $\mu\text{g/l}$; between September and October the average was 5.0 $\mu\text{g/l}$ and was falling to values close to 2 $\mu\text{g/l}$ at the end of the year. Turbidity has followed a similar pattern to chlorophyll-a, with a minimum of 1.2 FTU in June and a maximum of 6.5 FTU in September. Finally, salinity has followed an upward trend, from average minimum in March with 40.4 PSU to maximum in October-November, with an average value of 44.6 PSU.

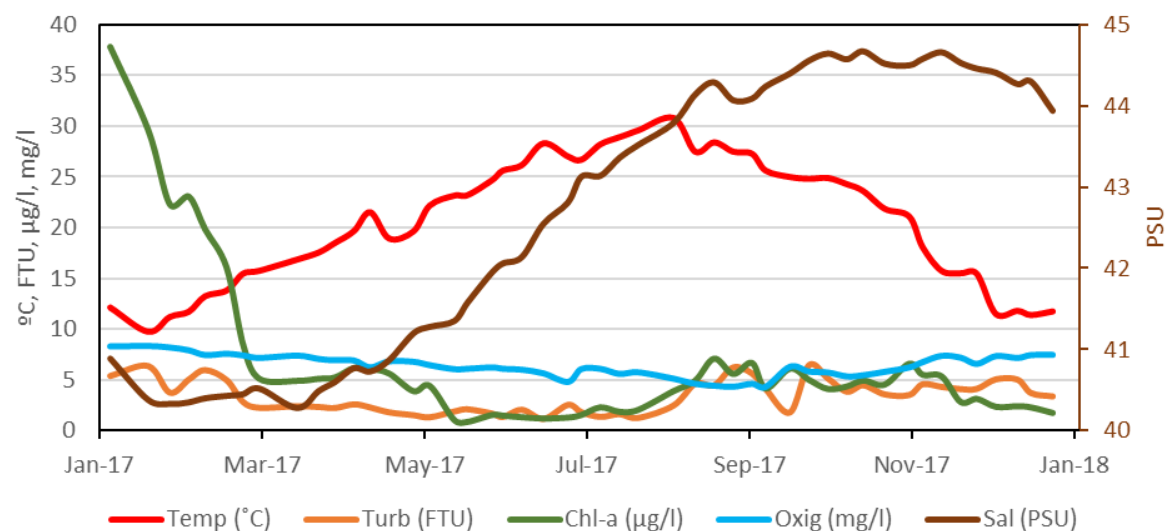


Figure 3. Annual evolution during 2017 at central site A3 of environmental variables: Temperature (Temp, °C), Turbidity (Turb, FTU), Chlorophyll-a concentration (Chl-a, $\mu\text{g/l}$), Dissolved Oxygen (Oxig, mg/l) and Salinity (Sal, PSU).

Sentinel-3 satellite OLCI images for five dates up to four days near to the field works were downloaded and processed. Results are according to the data measured in situ at A3 site sampling during the year (Fig. 3). The maximum values in the lagoon are in September and the minimum between May and June (Fig. 4). There is a bottom effect in the margins of the lagoon, because the low depth and the vision by satellite of the subaquatic vegetation appearing some pixels in red color. The concentration of chlorophyll-a is homogeneous throughout the lagoon in the five images studied, with variations of 2 $\mu\text{g/l}$. On the west coast there are always lower values. The image also shows that the Mediterranean Sea always presents low values, between zero and one, and a distribution on the near coast linked to the water currents.

From the data of each sample of phytoplankton (see supplementary data), we obtained their densities, and a descriptive analysis was made by date and by sample (table 1). In addition, the indices of species richness (S), diversity (H') and equitability (J) were included.

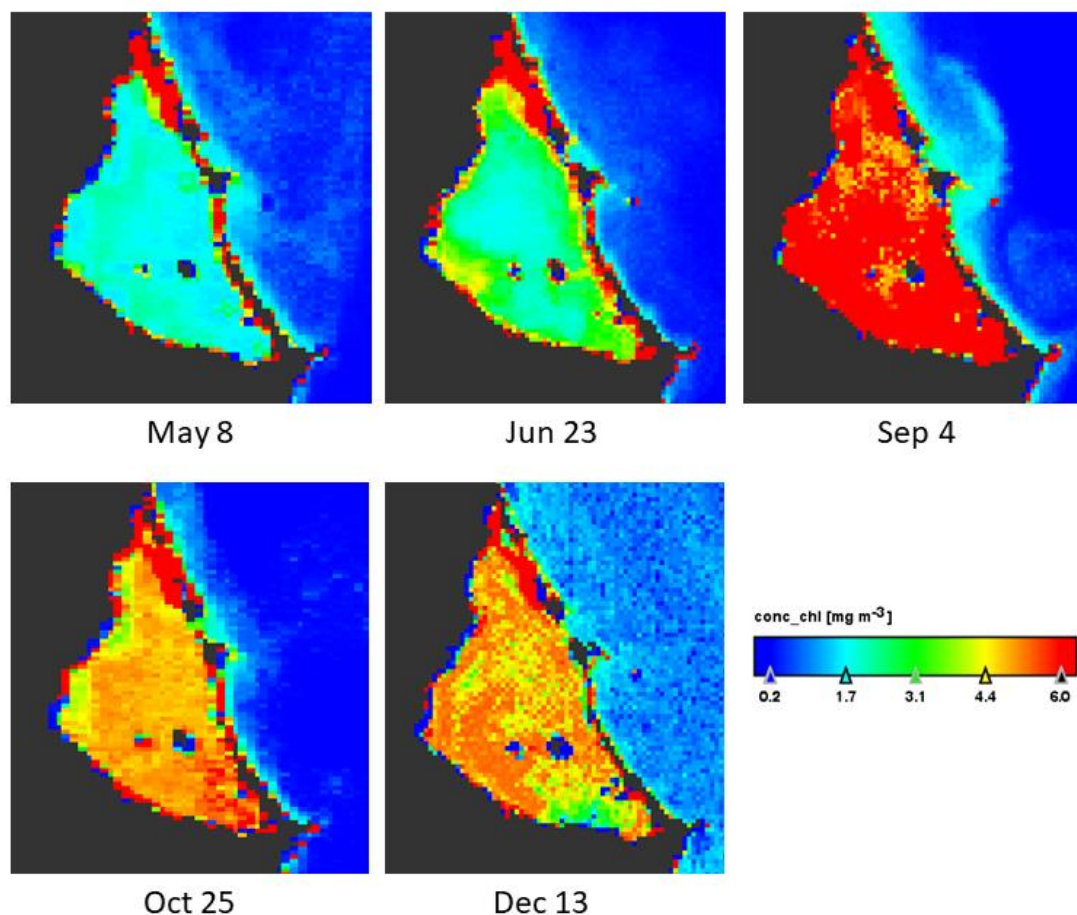


Figure 4. Thematic maps of Chlorophyll-a concentration ($\mu\text{g/l}$) in surface of Mar Menor lagoon in the dates of 2017 indicated below each image. Note that red area in NE corresponds to the salt evaporation ponds of San Pedro del Pinatar.

It is observed that the highest density of 67,335 cells per milliliter is reached in the sample A1 of September 1 (where 13.0 $\mu\text{g/l}$ Chl-a was registered), both in total density and in its maximum density with 65,634 in one taxa. In the samples collected on 9 December in A4 and A6 (60,219 cell/ml and 41,579 cells/ml respectively, with Chl-a values of 6.1 $\mu\text{g/l}$ and 5.2 $\mu\text{g/l}$) high densities were also observed with very high maximums and a strong standard deviation, indicating notable differences

between densities of some species and others. On the other side, we found the lowest densities in the months prior to summer, in the samples collected on 8 May in A5 and A6 (with Chl-a values of 1.2 µg/l and 2.2 µg/l) and on 25 June in A4 (Chl-a 0.1 µg/l). However, chlorophyll-a values from studies conducted in 1981 [1] ranged from 0.3 to 0.9 µg/l.

Regarding the study of taxa, in sample A1 of 1 September we also found the greatest richness (39 taxa) and the lowest richness was found in sample A4 of December (with 14 taxa). The diversity index (H') is below the usual (between 2-3) in most samples [46], being the A6 sample of May 8 the maximum value with 1.87, and being the samplings of December in the same point those that reach a lower diversity with 0.12. Finally, if we look at the equitability index (J), the trend of the diversity index (H') confirms us: most of the samples are below the percentage of normal equitability (between 50-75%), the greatest equitability is found in the A2 sample of September 1 (57%) and the lowest percentages are found in the samples collected in December (4% in A6, 9% in A4 and A5).

Table 1. Descriptive and diversity analysis. Values of chlorophyll-a (Chl-a) concentration, transparency (Secchi disk depth), phytoplankton density of the sample (Dens). For the analysis of diversity, data on the richness (S), diversity (H') and equitability (J) have been included. Sampling points as indicated in Figure 1.

Sample	Date	Chl-a (µg/l)	Secchi (m)	Dens (cells/ml)	S	H'	J
A1	Sep 1	13.0	1.3	67335	39	0.19	0,05
	Oct 28	7.0	2.3	7522	26	1,39	0,43
A2	May 8	4.5	5.3	8082	31	1,21	0,35
	Jun 25	2.4	3.6	3919	24	1,40	0,44
	Sep 1	9.0	1.3	20666	17	1,61	0,57
	Oct 28	7.9	2.1	21568	20	0,52	0,17
	Dec 9	6.2	1.9	7857	23	1,23	0,39
A3	May 8	2.0	4.4	8937	37	1,12	0,31
	Jun 25	0.3	4.5	4073	19	1,29	0,44
	Oct 28	10.7	1.6	8294	31	1,03	0,30
A4	Jun 25	0.1	4.7	3024	26	1,08	0,33
	Oct 21	7.9	1.9	7598	22	1,22	0,39
	Dec 9	6.1	1.9	60219	14	0,23	0,09
A5	May 8	1.2	3.7	2536	28	1,49	0,45
	Oct 21	7.7	1.5	12863	18	0,74	0,25
	Dec 9	4.3	1.7	36274	17	0,26	0,09
A6	May 8	2.2	3.1	2651	28	1,87	0,56
	Oct 21	8.1	1.8	11074	25	0,65	0,20
	Dec 9	5.2	1.8	41579	15	0,12	0,04

3.2. Geographical distribution of taxa

In order to show the distribution of the different classes of phytoplankton within the samples, figures 4, 5 and 6 represent the distribution of algae located at the sampling point corresponding to each sample studied. In the first sample corresponding to May 8 (figure 4), the densities of samples A2 and A3 are similar (more than 8,000 cells/ml) between them. However, Chrysophyceae are predominant in the first sample (A2), followed by diatoms and dinoflagellates (being the nanoplanktonic Chrysophyceae, *Cyclotella glomerata* and *Katodinium fungiforme*, the most abundant taxa in each class respectively), while in A3 there is a higher density of Cyanophyceae, followed by Chrysophyceae and Cryptophyceae (with *Synechococcus* sp., nanoplanktonic Chrysophyceae and *Plagioselmis lacustris* as the most abundant taxa of each class). The densities of A5 and A6 samples are similar, both in density (more than 2,000 cells/ml) and in composition. The most abundant classes are Chlorophyceae, Bacillariophyceae, Dinophyceae and algae belonging to other groups. The most abundant species are nanoplanktonic Chlorophyceae and *Phaeocystis* sp.; *Cyclotella glomerata*,

Chaetoceros muelleri and *Nitzschia longissima* in diatoms and *Katodinium fungiforme* and *Prorocentrum micans* in dinoflagellates. Outside these groups we can highlight *Scourfieldia complanata* (Pedinophyceae), which is well represented in the category "Others".

In the sample carried out on 25 June (figure 4), A2 is the sample with the lowest density (just over 500 cells/ml) and A3 and A4 have similar densities (3,024 and 4,073 cells/ml). Its composition is also very similar, with Cyanophyceae being the most abundant class, followed by Chlorophyceae and Chrysophyceae, with *Synechococcus* sp. and nanoplanktonic Chlorophyceae being the most abundant taxa of Cyanophyceae and Chlorophyceae, while in Chrysophyceae the nanoplanktonic cells, *Calycomonas* sp. and *Ollicola vangoorii* stand out. In contrast, in the A2 sample diatoms such as *Chaetoceros muelleri*, Cryptophyceae such as *Plagioselmis* sp. and dinoflagellates such as *Gymnodinium* sp. and *Prorocentrum compressum* predominate.

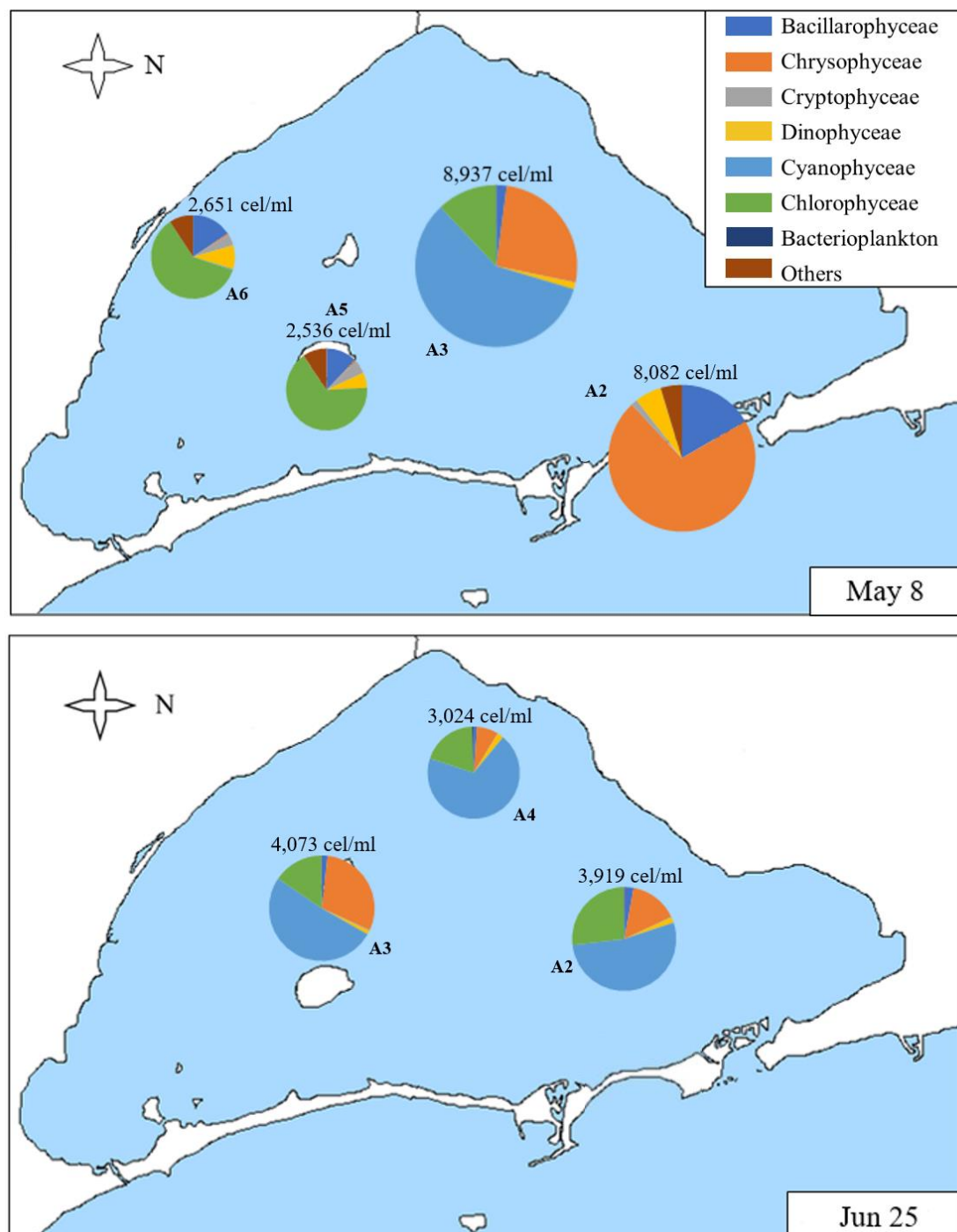


Figure 4. Map of the Mar Menor lagoon with the density graphs of the different classes of phytoplankton found in each sample on May 8 and June 25. The total density of the sample is annotated on each graph. "Others" includes the classes Conjugatophyceae, Pedinophyceae, Euglenophyceae, Diphyllleida and Thecofilosea, which appear in very low densities.

As shown in Table 1 and figure 5 the sample A1 collected on 1 September is the most abundant of all the samples (although, on average, the densities of the samples collected on 9 December are also high, as will be shown later in figure 6). In this sample, 98 % of the algae counted correspond to the Cryptophyceae group, with nanoplanktonic cells being the most abundant (with a density of 65,534 cells/ml), followed by *Plagioselmis* sp. and *Leucocryptos marina*, although in lower density.

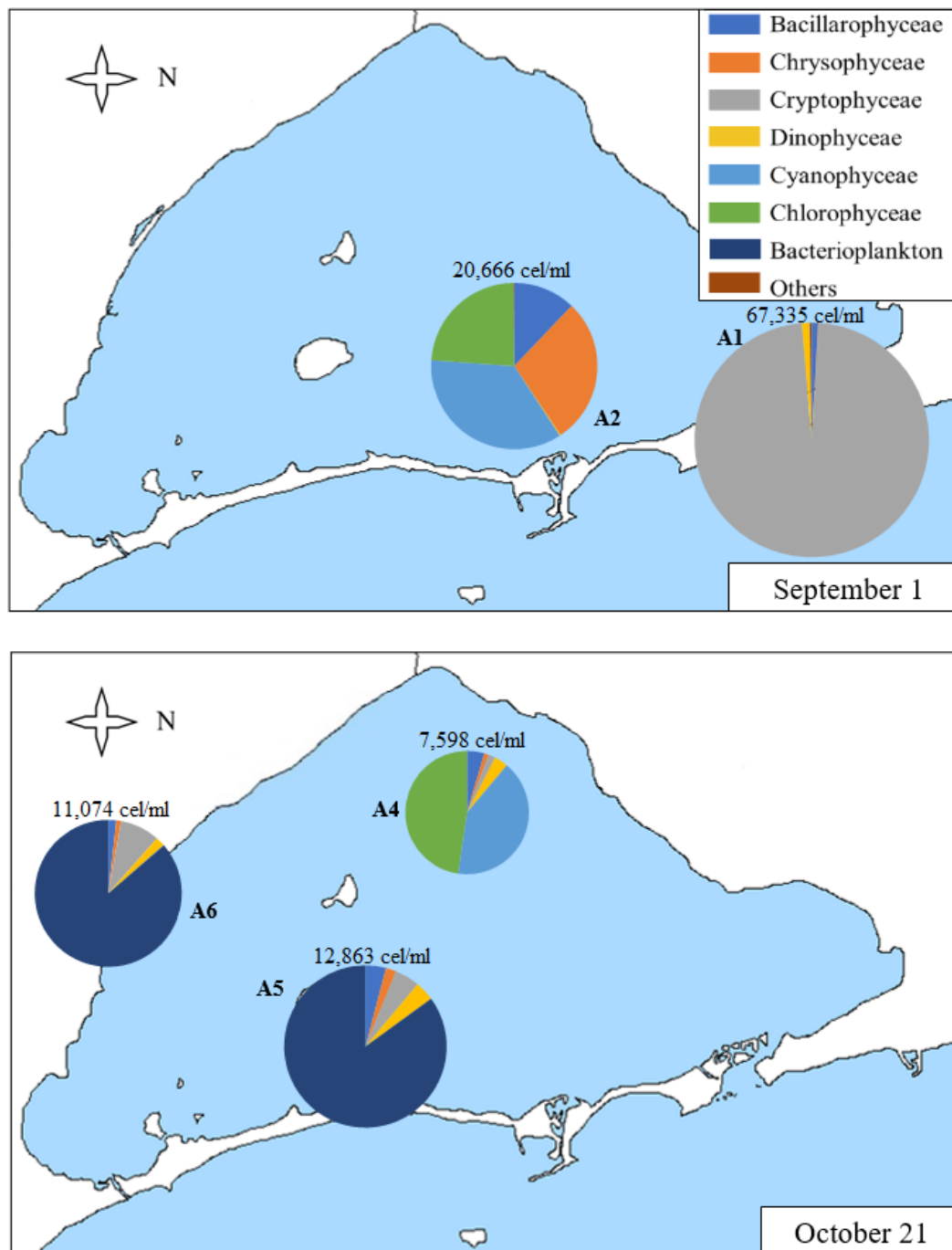


Figure 5. Map of the Mar Menor lagoon with the density graphs of the different classes of phytoplankton found in each sample on September 1 and October 21. The total density of the sample is annotated on each graph.

In percentages lower than 1 % we can find diatoms such as *Navicula* sp., *Chaetoceros* sp. and *Nitzschia longissima* and dinoflagellates such as *Gymnodinium* sp., *Protoberidinium* sp. and *Prorocentrum compressum*. The other sample collected on 1 September (A2) does not have such a high density of algae, but it is much higher than the densities of the May and June samples and, unlike A1, is much more diverse in composition. It presents Cyanophyceae, Chrysophyceae, Chlorophyceae classes and diatoms in similar proportions. The most noteworthy taxa are *Synechococcus* sp., Chrysophyceae and nanoplanktonic Chlorophyceae and diatoms of the species *Cyclotella glomerata*.

The samples collected on 21 October (Figure 5) show lower densities than in September but are still higher than in previous months. The A4 sample predominates the classes Chlorophyceae and Cyanophyceae, followed by Bacillariophyceae, Dinophyceae and Cryptophyceae classes. The most abundant classes include *Synechococcus* sp. in Cyanophyceae and nanoplanktonic Chlorophyceae. The most abundant diatoms are *Cylindrotheca closterium*, *Cyclotella glomerata* and *Nitzschia longissima*. The A5 and A6 samples are similar, both in total density and composition. In these samples, bacterioplankton predominates. It is a group that includes both spherical unicellular bacteria (which constitute the totality of bacterioplankton in these samples) and bacteria of the genus *Flavobacterium* sp. (which only appears in this sample of December). Other taxa found are Cryptophyceae class such as *Plagioselmis lacustris* and *Rhodomonas salina*, dinoflagellates such as *Gymnodinium* sp. and diatoms such as *Chaetoceros muelleri*, *Nitzschia longissima* and *Cyclotella glomerata*.

In figure 6, we can observe that the density of the samples collected in the samples of 28 October and 9 December is higher than those of the samples collected in May and June, although somewhat lower than those of the sample collected in September.

On 28 October (figure 6), Chlorophyceae predominate in A1 and A3 samples, with nanoplanktonic Chlorophyceae predominating. However, the Cryptophyceae class predominated in the A2 sample (96% of the sample), with both nanoplanktonic Cryptophyceae and *Plagioselmis lacustris* and *Rhodomonas salina* species standing out. In the A2 sample we can also find diatoms, predominantly the species *Nitzschia longissima*, and dinoflagellates, being *Gymnodinium* sp. the most abundant species of this group. In the A1 sample, the class of Chlorophyceae are followed by Cyanophyceae class such as *Synechococcus* sp., Chrysophyceae such as *Calycomonas* sp., diatoms such as *Cyclotella glomerata* and *Cylindrotheca closterium*, *Plagioselmis lacustris* in Cryptophyceae class and *Gymnodinium* sp., *Katodinium fungiforme* and *Peridinium* sp. in dinoflagellates. In sample A3, the most abundant species are the same as in samples A1, except for the class of Cyanophyceae, which does not appear.

The last samples, collected on 9 December, are the densest of the year. The A4 sample is the most different in its composition, predominantly contained in nanoplanktonic Cryptophyceae and *Plagioselmis* sp., holding 98 % of the sample in which only 2 % is composed of diatoms such as *Chaetoceros* sp. and *Gymnodinium* dinoflagellates. The A2 sample is very similar in density and diversity, being bacterioplankton (*Flavobacterium* sp.), Cyanophyceae (*Synechococcus* sp.) and Chrysophyceae (*Calycomonas* sp.) the most abundant categories, although we can also find some *Gymnodinium* dinoflagellates. Finally, A5 and A6 samples are very similar in density and composition. They are dominated by bacterioplankton (which accounts for between 96 and 98 % of the density of the sample), followed by nanoplanktonic Chrysophyceae, dinoflagellates such as *Gymnodinium* sp. and Cryptophyceae such as *Plagioselmis lacustris* and *Rhodomonas salina*.

3.3. Multivariate analysis

Cluster analysis of similarity (Figure 7) gives first group is formed by the samples where nanoplanktonic Cryptophyceae dominate; group 2 gathers the samples dominated by bacterioplankton (Figures 5 and 6). Group 4 groups the May samples characterized by the presence of Bacillariophyceae in the three points, and with important presence of Chlorophyceae in points A5 and A6. Group 3 consists of all the other samples in which the phytoplankton density is lower than average, and their diversity is higher.

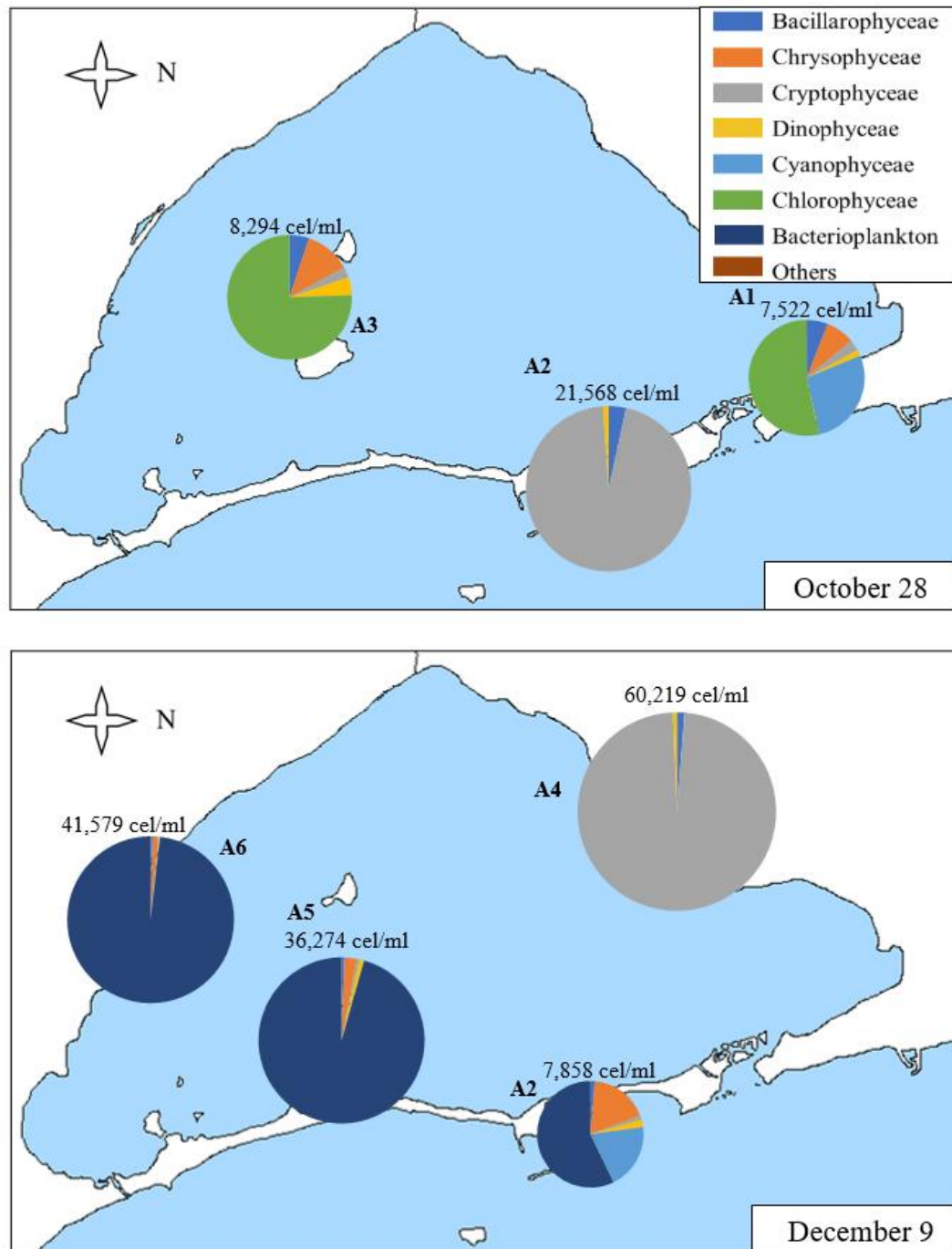


Figure 6. Map of the Mar Menor lagoon with the density graphs of the different classes of phytoplankton found in each sample on 28 October and 9 December. The total density of the sample is annotated on each graph.

In the CCA between the three environmental variables and the taxa (Figure 8), the first axis explains 68% of the variance and is correlated with transparency on the positive side, while turbidity and chlorophyll are on the negative side. The second axis explains 32% of the variance and shows the samples from the southern zone in the positive part while those from the northern zone remain in the negative part, indicating the spatial heterogeneity of the lagoon. Likewise, we can observe a tendency to place the samples with less diversity (H) and equitability (J) in the negative part of the

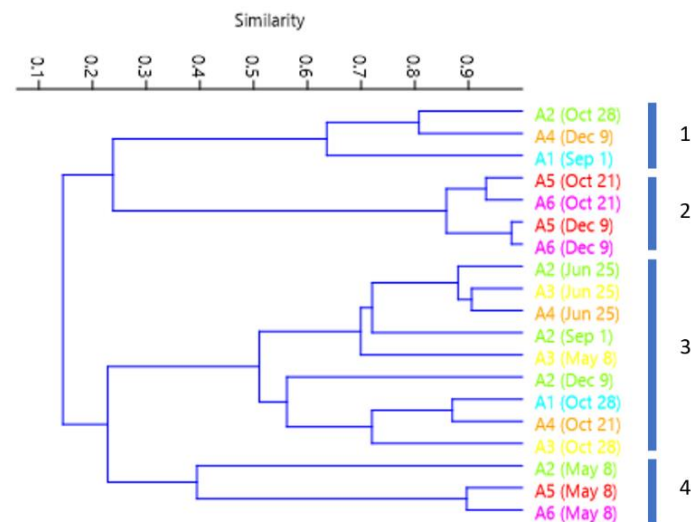


Figure 7. Cluster of correlations between site sampling points. Groups are labeled as 1 to 4. Each color corresponds to the same sampling site as Figure 1.

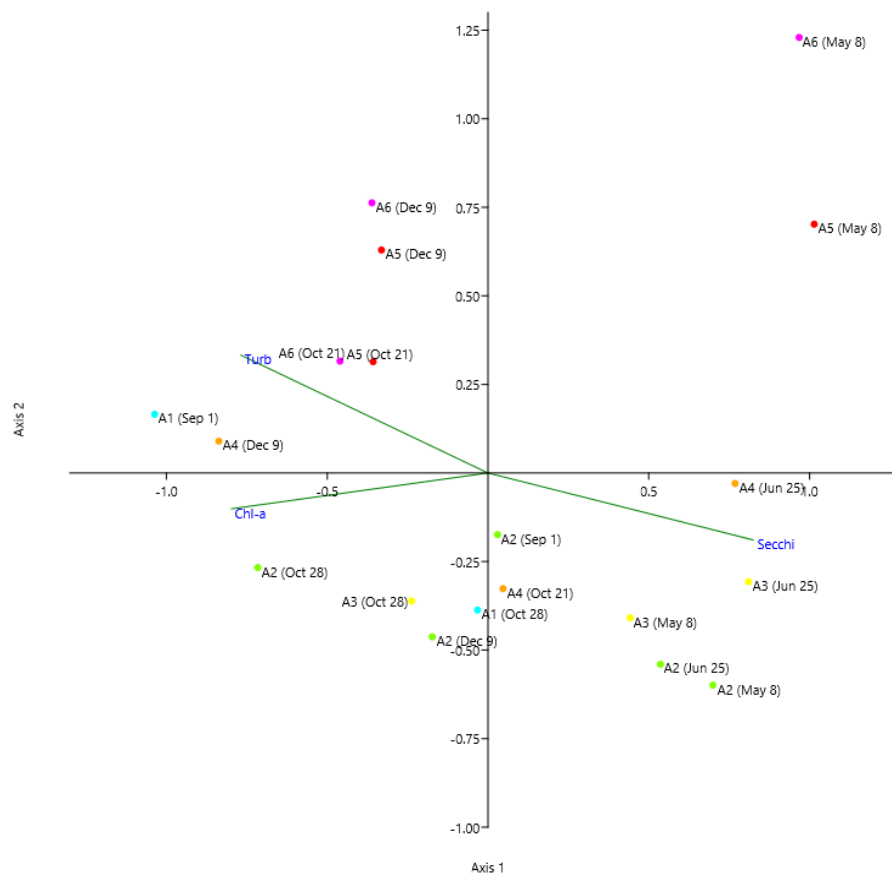


Figure 8. Scatter plot of the sampling points on axes 1 and 2, obtained with the CCA among taxa and environmental variables: Turbidity (Turb), Chlorophyll-a (Chl-a) and Transparency (Secchi). Each sampling point is identified with its location and date according to Figure 1.

first axis, while those samples in which diversity and equitability are greater tend to be grouped in the positive part (Table 1). In this sense, the first component is giving more weight to the diversity in the samples; the second component gives more weight to certain algal groups that occur in these two areas of the Mar Menor (Chrysophyceae and Dinophyceae to the north and bacterioplankton to the south).

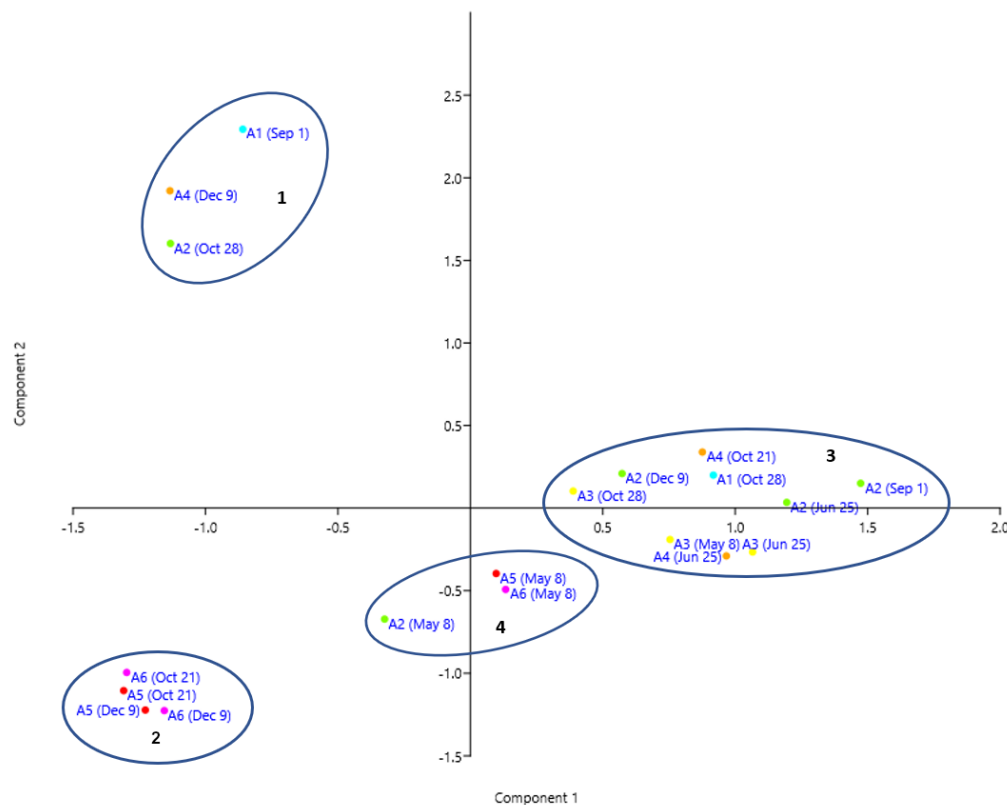


Figure 9. Scatter plot of the sampling points in the space of the principal components 1 and 2. Each sampling point is identified with its location and date according to Figure 1. Groups are labeled according to Figure 7.

The PCA among the taxa explains 50% of the variance (Figure 9). Component 1 explains 31% and component 2 explains 19%. The first component is related to phytoplankton density, ordering approximately the samples from lowest to highest (positive to negative). The taxa are distributed the presence of nanoplanktonic Chlorophyceae and *Synechococcus* sp. in the positive zone, as opposed to the presence of nanoplanktonic Cryptophyceae and bacterioplankton. These two planktonic groups are separated by the second component, the first one at the positive component 2 and at the negative part the samples with higher density of bacterioplankton (points A5 and A6 of October and December).

4. Discussion

The highest phytoplankton densities are associated with the samples in which the smaller size taxa (bacterioplankton and nanoplankton cells) have been observed. It has appeared in the samples from September to December, while the lowest densities were recorded during the samples in this year.

Dzierzbicka-Głowacka study [47] indicates that phytoplanktonic populations are affected by the effect of solar energy on the surface of the lagoon, the dynamics of water movement and the distribution of nutrients in the water column. If we observe temperature variations, we can see that, as in our work, temperature increases favor the proliferation of phytoplankton [48,49]. These increases tend to occur in the summer months, so that in subsequent months the highest phytoplankton densities are usually recorded [50] and, in the transition between spring and summer, a significant increase in the density of Cyanophyceae and diatoms with respect to other times of the year should be noted [51]. An example of this effect can be found in the research of Siegel and Gerth [52] where a significant increase in the density of Cyanobacteria was recorded in August 1997, one of the warmest of the 20th century. This is not always the case. In the study of Alves de Souza et al. [53] we find that, in the Chilean Reloncaví fjord, while the species *Protoceraium reticulatum* has a peak

maximum density in warmer years (coinciding with the El Niño phenomenon), the species *Dinophysis acuminata* predominates in the colder years (coinciding with the La Niña phenomenon).

On the other hand, the Campo de Cartagena constitutes an important system of aquifers in the Mediterranean basin, in which most of the water is used for irrigation and other agricultural activities in the area. This agricultural activity causes the aquifers to be recharged, firstly, with useful rainwater (without taking into account that which is lost through surface runoff and that which evaporates) and, secondly, with the water that has been used in the irrigation of crops, whether extracted from the aquifer itself or from the Tagus-Segura transfer. Recently, desalinated water has begun to be used. This use has favored a reduction in the pollution of the aquifer system, especially the Quaternary aquifer, by nitrates and other compounds used in agriculture [54]. It should be noted that while the actual concentrations of TP reaching the Mar Menor remain at similar values to those of 2003, the concentration of nitrate has increased significantly by a factor of four, reaching values above the recommended environmental maxima.

Due to the importance of nitrogen in the nutrition of plant species, when these compounds reach the waters of the lagoon they favor the massive proliferation of plant species (especially phytoplankton, as we can see in the results of our study) so that there is an increasingly eutrophy process of the lagoon, as reflected in the dynamic model of Martínez and Esteve [54]. The renewal of the aquifer waters is a slow process (progress through the pores is a few meters a day) until they reach the Mar Menor, so the accumulation of nitrates is very favored [5]. This increase in the nutrients in suspension is also due to the loss of meadows of macrophytes and aquatic plants that fix these nutrients to the soil [55], so the average density of phytoplankton in the Mar Menor is increasing [1].

Regarding the composition of the samples, we can say that the predominant phytoplankton groups are Chrysophyceae, Cryptophyceae, Chlorophyceae and Cyanophyceae. We can also highlight bacterioplankton in four of the samples collected on December. However, these are not the groups with the greatest number of species. Of a total of 109 identified taxa, the groups with the highest number of species are dinoflagellates (Dinophyceae) with 45 registered taxa and diatoms (Bacillariophyceae) with 28 registered taxa, similar to the work of Ros et al. [28]. If we observe their distribution in the maps (figures 4, 5 and 6), we can see that we find higher density of diatoms in samples taken closer to channels of west side, while dinoflagellates are distributed in central open areas. The diatoms lack structures that allow them to move unlike the dinoflagellates that possess flagella, so they tend to proliferate in areas where water movements favor the renewal of nutrients and dissolved oxygen [56]. We can also find 8 species of Chrysophyceae and 8 others of Cryptophyceae, followed by 6 taxa of Cyanophyceae, 5 of Chlorophyceae and 2 groups of bacterioplankton. The other 5 species recorded correspond to other classes such as Conjugatophyceae, Pedinophyceae, Euglenophyceae, Diphyllleida and Thecofilosea. Of particular note is the increase in density of the dinoflagellates *Gymnodinium* sp., a genus characterized by the production of toxins from some of its species [57] and which favors the formation of the so-called "red tides". Also of concern is the possible accumulation of toxic metabolites due to the proliferation of green and blue algae [58].

Finally, we can observe that the phytoplankton taxa that have the greatest proliferation are those that can be included in the category of nanoplankton with a high reproduction rate, such as the cyanophytes of the genus *Synechococcus* sp. or any of the identified nanoplanktonic organisms (see supplementary data). Apart from these groups, we can highlight the diatoms *Cyclotella glomerata*, *Cylindrotheca closterium* or *Nitzschia longissima*. *Cyclotella glomerata* is characterized by being a freshwater species [40] and appears, surely, from surface waters draining over the Mar Menor; *Cylindrotheca closterium* is a species typical of marine environments [1] and *Nitzschia longissima* can be found in hypersaline environments [59]. With the data from these species we can see that, although the Mar Menor receives considerable amounts of fresh water, its waters maintain a salinity that is specific to or superior to that of a marine ecosystem.

Our results have been compared with the studies conducted at the same point in 1981 [1], with salinity between 43 and 46 PSU and in our study period very similar, between 41 and 45 PSU. However, there is a big difference in the density of phytoplankton, since the values in 1981 ranged from 10 to 500 cells/ml and currently these values are between 220 and 5700 cells/ml. In this values

we have not considered the counts of small cells, because we note that in that year the sample was collected with a plankton net and was a lack of ability in detection small pico and nanoplankton and not was counted [1]. The maximum density values were in winter and now they are in autumn. There is a coincidence in the dominance of Chrysophyceae in the spring. But the rest of the year the abundance of the nanoplankton reduces the importance in terms of density of the other groups such as Bacillariophyceae and Dinophyceae. The presence of certain taxa is coincidental in the two studies. We point out among them *Cyclotella*, *Nitzschia*, *Calycomonas*, *Prorocentrum*, *Gymnodinium* and *Peridinium*. However, we now highlight the presence of Cryptophyceae and nanoplankton, which were not observed in 1981. This is an indicator of moving the trophic state from mesotrophy to eutrophy as observed in Lesina lagoon (Italy) [60] and Toulon bay (France) [61]. The increase of nitrate concentration in the inflow freshwater to Mar Menor arises from mean value of 32 mg/l in 2003 [62] to 210 mg/l in 2017.

These results show that the main ecologic problems of the lagoon are the nitrogen and phosphorus discharges derived from the contamination of the aquifers of the zone due to the agriculture of the surroundings and the discharges coming from the tourist zone. To remedy this situation, it is essential to design and implement environmental policies and strategies, especially those that focus on limiting suspended nutrients to regulate the massive proliferation of phytoplankton [63]. In the integral report on the ecological state of the Mar Menor in 2017 [64], different methods of water purification are described to reduce its nitrogen content, such as anionic exchange, reverse osmosis, electrodialysis, bioelectrogenesis and denitrification (whether chemical, catalytic or biological). On the other hand, in works such as Dimitrieva and Semenova [65] we can see that the proliferation of phytoplankton can trigger a proliferation of zooplankton, which may be useful in future plans for the recovery of the lagoon.

5. Conclusions

The results of our study indicate a significant increase in phytoplankton density in the marine lagoon compared to previous data, according to the eutrophication pressure favored by agricultural dumping and waste derived from touristic exploitation in which the lagoon is involved.

In terms of composition, the greatest diversity of species is found in the spring and early summer months. However, the notable increase in density in the summer and autumn months occurs in species such as *Synechococcus* sp. and groups of Chlorophyceae, Chrysophyceae and nanoplanktonic Cryptophyceae, while species diversity drops significantly. In spring we find more diversity with the presence of diatoms and dinoflagellates, among other groups.

Comparing the evolution of the lagoon with previous works, we can conclude that this eutrophication process could increase. To avoid this, it would be necessary to implement control measures for the use of fertilizers in nearby growing areas and to study water treatment techniques so that phytoplankton densities can be reduced.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Main table of density (cells/ml) of the phytoplankton in all the samples studied.

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