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

“Mar Menor” is the name given to the marine lagoon (or coastal lagoon) of 135 square kilometers and with a depth of up to 7 m approximately, it is located on the coast of the Region of Murcia (Spain), located at the SE of the Iberian Peninsula, between the parallels 37º 38’ and 37º 50’ North latitude and the meridians 0º 43’ and 0º 57’ West longitude, which stands out for its ecological and economic importance. It is separated from the Mediterranean Sea by a sandy strip about 22 km long and between 100 and 1500 m width, known as “La Manga del Mar Menor”. This barrier does not completely close the lagoon due to the existence of five canals that connect it with the Mediterranean Sea [1]. The lagoon maintains higher salinity levels than the sea (figure 1). It is due to the fact that freshwater inputs from the channeled continental drainage and surface and underground runoff [2] do not compensate for evaporation losses, so it maintains a negative estuarine structure due to water exchange currents with the Mediterranean through the canals [3].
In recent years, the area and its surroundings have undergone various anthropogenic modifications for exploitation. The most noteworthy modifications are the urbanization of La Manga and other towns adjacent to the lagoon for tourist exploitation [4] and the agricultural exploitation of the Campo de Cartagena area [5]. It is worth mentioning the extension of the Estacio channel and the construction of a marina [2], which has meant an increase in the exchange of water with the Mediterranean Sea and an alteration in the natural balance of the lagoon, which may affect the flora and fauna present in the lagoon [6]. Since 1979, farming has been favored by the construction of the Tagus-Segura transfer, which allowed the use of intensive irrigation [7].

From an economic and social point of view, coastal lagoons are a great source of raw materials and food, they are of great importance as recreational places, tourist attractions and cultural assets, so they are a significant source of income for the inhabitants of nearby towns, especially during the summer. In recent years, they have also been the subject of studies on climate change as indicators due to the important modifications they undergo with small variations in temperature and general climatic conditions [8]. It is precisely the tourist use that is mainly responsible for the great deterioration that the Mar Menor has suffered in recent decades. The mass tourism of the area, its use for bathing and other recreational activities and the massive urbanization of the area (especially La Manga), has resulted in an excessive accumulation of waste, discharges from neighboring populations and an alteration of the natural dynamics of the area. Specifically, the Mar Menor has undergone a strong process of eutrophication that has increased significantly since the 1970s mainly
due to the large contributions of nitrogen and phosphorus derived from the most developed activities in the area: the tourism and the urban development, and the discharges of agricultural origin [9].

The eutrophication process is produced from the increase in the concentration of nutrients in the lagoon, mainly nitrogen and phosphorus, which in many cases can act as limiting factors [10], dragged by surface and underground runoff from nearby crop areas. This excess of nutrients favors, together with the increase in temperatures in the summer months [11], both the proliferation of macrophytes, such as *Caulerpa* proliferates, and of phytoplankton. This proliferation of phytoplankton increases the turbidity of the waters and prevents sunlight from penetrating to the bottom of the lagoon, which decreases the population of macrophytes that fix the nutrients in suspension to the substrate. In this way, the presence of nutrients in suspension is favored and a greater proliferation of this phytoplankton [12], in particular nanoplanktonic species such as cyanobacteria of the genus *Synechococcus* [13] or picoeukaryotes species [14,15] proliferate. This large growth of phytoplanktonic populations can trigger a process known as Harmful Algal Blooms (HAB) or "red tides". This phenomenon occurs after excessive growth of phytoplankton in an aquatic ecosystem, especially those species that belong to the dinoflagellates class, so that the lagoon acquires a greenish or slightly reddish color [16]. These phytoplanktonic explosions are indicators of the advanced state of eutrophication of the lagoon. For this reason, the Government of the Region of Murcia declared the Mar Menor as a "zone sensitive to eutrophication" in 2001 following Directive 91/271/EEC [17] and in 2002 the entire Campo de Cartagena area surrounding the lagoon was declared a "zone vulnerable to pollution by nitrates of agricultural origin" following Directive 91/676/EEC [18].

To know the current ecological status of the lagoon, the Water Framework Directive (WFD) 2000/60/EC of the European Parliament and the Council of 23 October 2000 and the corresponding transposition to Spanish Legislation in Royal Decree 817/2015, of the Ministry of Agriculture, Food and the Environment, of 11 September 2015 [19], indicate that the status of biological quality elements (including both phytoplankton and fauna and flora), chemical and physicochemical quality elements including contaminants present in significant quantities, general conditions (such as temperature, transparency, oxygen or salinity) and morphological conditions of the area should be checked. However, in this work we have focused on the study of the classes of phytoplankton present in waters of the Mar Menor as bioindicators of their quality as organisms sensitive to changes in physicochemical conditions [20,21]. If we take Reynolds [22] as a reference, we can define phytoplankton as a set of photosynthetic microorganisms adapted to life in suspension in the water column both in seas and in rivers, lakes or any other accumulation of water, to which they contribute a large part of the carbon available for pelagic food webs, being food for many animal species [23,24]. Through the study of phytoplanktonic species we can know their general composition and observe the variations that occur over time [25]. The classes of phytoplankton present in the Mar Menor and the variation in population dynamics during our sampling have allowed us to know the current state of the lagoon and to predict its future evolution from the comparison of data collected with previous studies.

2. Materials and Methods

To study the composition and the abundance of the Mar Menor phytoplankton, water samples were collected at a depth of about 4 m using a 2.5 L Ruttner hydrographic bottle. This water 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 further/post-analysis in the laboratory.

The first sampling was carried out in May 2017 and the last one in December 2017. Due to logistical problems, samples could not be taken regularly, so we have samples of the months of May, June, September, October (month in which two samples were taken) and December. During the months of July and August there was not available boat to carry out the samplings. All the sampling started from the harbor of Tomás Maestre 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 2).
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 [26] 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 [27]. If the sample contains a high density, we simply place a specific volume (slightly less than the capacity of the chamber), we complete it until it is filled with distilled water [28] 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 [29]. 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 3) was used to identify specimens.

Figure 2. Map of the Mar Menor with the location of the sampling points, on a scale of 1/152,000. [Own elaboration].
Numerous guides and web resources [30-41] 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) [29].

Statistical analysis of the data was performed using PAleontological STatistics (PAST) version 3.25 [42]. 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 [43], the diversity according to the Shannon-Weaver index (H’) and the equitability (J) in the presence of each species [44]. 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 [45].

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.

3. Results

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. It is observed that the highest density of cells per milliliter of all the sampling is reached in the sample P1 of September 1 (where 27 °C of water temperature was registered), both in total density and in its maximum density. In the samples collected on 9 December (with a water temperature of 11 °C) 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 contrary, we found the lowest densities in the months prior to summer, in the samples collected on 8 May (with temperatures of 20.5 °C) and on 25 June (28 °C, the highest temperature recorded in the sample). Regarding the study of taxa, in sample P1 of 1 September we also found the greatest richness (39 taxa) and the lowest richness was found in sample P1 of December (with 14 taxa). The diversity index (H’) is below the usual (between 2-3) in most samples, being the P1 sample of June 25 the only one that reaches this level (with a value of 2.1), and being the samplings of December those that reach a lower diversity.

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 P1 sample of 25 June (64%) and the lowest percentages are found in the samples collected in December (4% in P3).

In terms of taxa, in the samples collected in the month of May, the most abundant were nanoplanktonic Chrysophyceae, nanoplancktonic Chlorophyceae and *Synechococcus* sp., the latter being the most abundant in three of the samples collected in June (except in P1 where the most abundant was *Chaetoceros muelleri*). The richness of the May samples is between 37 (in sample P6) and 28 (in samples P4 and P5) taxa per sample with a diversity slightly below normal (1.87 being the
highest in sample P4) and an equitability between 31% and 56%. In June, the diversity index (H’)
varipigmenten 2.1 (at P1, being the highest in the sample) and 1.08, while the equitability reaches
64 % (within the usual range).

Table 1. Descriptive and diversity analysis. The mean of the densities, the maximum density of
each sample and its standard deviation are presented (the minimum density has been omitted since
in all samples it is equal to 0). For the analysis of diversity, data on the number of species (S), diversity
(H’) and equitability (J) have been included.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Total (cel./ml)</th>
<th>Max. (cel./ml)</th>
<th>SD</th>
<th>S</th>
<th>H’</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 08, 2017</td>
<td>P1</td>
<td>8,082</td>
<td>5,724</td>
<td>554</td>
<td>31</td>
<td>1.21</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>2,651</td>
<td>1,309</td>
<td>132</td>
<td>28</td>
<td>1.87</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>P5</td>
<td>2,535</td>
<td>1,491</td>
<td>148</td>
<td>28</td>
<td>1.49</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>P6</td>
<td>8,936</td>
<td>5,216</td>
<td>553</td>
<td>37</td>
<td>1.12</td>
<td>0.31</td>
</tr>
<tr>
<td>Jun 25, 2017</td>
<td>P1</td>
<td>527</td>
<td>225</td>
<td>23</td>
<td>26</td>
<td>2.10</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>3,919</td>
<td>2,086</td>
<td>224</td>
<td>24</td>
<td>1.40</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>3,024</td>
<td>2,086</td>
<td>207</td>
<td>26</td>
<td>1.08</td>
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<td></td>
<td>P4</td>
<td>4,072</td>
<td>2,086</td>
<td>232</td>
<td>19</td>
<td>1.29</td>
<td>0.44</td>
</tr>
<tr>
<td>Sep 01, 2017</td>
<td>P1</td>
<td>67,335</td>
<td>65,534</td>
<td>6,276</td>
<td>39</td>
<td>0.19</td>
<td>0.05</td>
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<tr>
<td></td>
<td>P2</td>
<td>20,666</td>
<td>7,302</td>
<td>942</td>
<td>47</td>
<td>1.61</td>
<td>0.57</td>
</tr>
<tr>
<td>Oct 21, 2017</td>
<td>P1</td>
<td>2,743</td>
<td>793</td>
<td>122</td>
<td>23</td>
<td>1.75</td>
<td>0.56</td>
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<tr>
<td></td>
<td>P2</td>
<td>7,598</td>
<td>3,622</td>
<td>456</td>
<td>22</td>
<td>1.22</td>
<td>0.39</td>
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<tr>
<td></td>
<td>P3</td>
<td>4,111</td>
<td>2,086</td>
<td>245</td>
<td>20</td>
<td>1.26</td>
<td>0.42</td>
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<tr>
<td></td>
<td>P4</td>
<td>11,074</td>
<td>9,563</td>
<td>918</td>
<td>25</td>
<td>0.65</td>
<td>0.20</td>
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<tr>
<td></td>
<td>P5</td>
<td>12,863</td>
<td>10,935</td>
<td>1,048</td>
<td>18</td>
<td>0.74</td>
<td>0.25</td>
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<tr>
<td>Oct 28, 2017</td>
<td>P1</td>
<td>21,568</td>
<td>19,394</td>
<td>1,858</td>
<td>20</td>
<td>0.52</td>
<td>0.17</td>
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<tr>
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<td>P2</td>
<td>7,522</td>
<td>4,017</td>
<td>435</td>
<td>26</td>
<td>1.39</td>
<td>0.43</td>
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<tr>
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<td>P3</td>
<td>8,310</td>
<td>4,729</td>
<td>497</td>
<td>32</td>
<td>1.31</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>8,294</td>
<td>6,231</td>
<td>604</td>
<td>31</td>
<td>1.03</td>
<td>0.30</td>
</tr>
<tr>
<td>Dec 09, 2017</td>
<td>P1</td>
<td>60,219</td>
<td>57,864</td>
<td>5,541</td>
<td>14</td>
<td>0.23</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>8,554</td>
<td>4,779</td>
<td>525</td>
<td>22</td>
<td>1.12</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>41,579</td>
<td>40,826</td>
<td>3,910</td>
<td>15</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>36,274</td>
<td>34,676</td>
<td>3,321</td>
<td>17</td>
<td>0.26</td>
<td>0.09</td>
</tr>
<tr>
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<td>P5</td>
<td>7,857</td>
<td>4,494</td>
<td>471</td>
<td>23</td>
<td>1.23</td>
<td>0.39</td>
</tr>
</tbody>
</table>

In the samples collected during the month of September, a notable increase in density is
observed, where the nanoplanktonic Cryptophyceae reach the maximum density value of the whole
sampling period (65,533 cells/ml) in the sample collected in P1, with a very low diversity index (H’)
and an equitability (J) of 5%. However, although the density of the P2 sample is also considerably
high, it reaches an equitability (J) of 57% (value considered normal in natural samples). The maximum
densities of these samples correspond to unicellular Cryptophyceae and Synechococcus sp.
respectively.

The samples collected in the month of October vary depending on the day they were collected.
While the maximum density in the samples collected on day 21 reaches 10,935 cells/ml (in P5), the
highest maximum density collected on day 28 amounts to 19,394 cells/ml (highest). In the samples of
day 21, the maximum densities correspond to Plagioselmis sp., unicellular Chlorophyceae,
Synechococcus sp. and bacterioplankton. In the samples of day 28, the maximum densities correspond
to unicellular nanoplanktonic Cryptophyceae and nanoplanktonic Chlorophyceae. The equitability (J) of day 21 samples varies from 56 % to 20 %, while the equitability (J) of day 28 samples falls slightly to percentages between 43 % and 17 %.

Finally, if we observe the maximum densities of the samples collected in the month of December, we can see a notable increase ranging from 4,494 cells/ml (in sample P5) to 57,863 cells/ml (in sample P1). These maximum densities correspond to nanoplanktonic Cryptophyceae, Flavobacterium sp. and bacterioplankton. The percentages of equitability (J) of the species present in these five samples are the lowest in the sample and range from 39 % to 4 %.

If we observe the temperature variations, we can observe that the peak of phytoplankton proliferation occurs after a temperature peak (recorded on June 25), while salinity does not seem to affect the variation in density levels, as it remains constant between September and December (around 44 PSU). A lower salinity is recorded in May (about 39 PSU) and June (about 42 PSU).

3.1. Taxa

Most of the taxa that reach the maximum concentrations in the samples collected for this study and which have been described above, correspond to nanoplanktonic specimens or species of unicellular bacterioplankton or that can be associated forming small colonies. However, in the analysis of the samples we have found species of different classes, of which we will describe the six most representative below.

**Figure 4.** Images of diatoms obtained with inverted microscope. Centric diatoms: *Coscinodiscus centralis* (a) and *Cyclotella glomerata* (b) with Nomarski differential contrast. Pennate diatom, *Nitzchia longissima* (c) and colonial diatom, *Aulacoseira* sp. (d).

- **Bacillariophyceae:** also known as diatoms. They are a class of photosynthetic and unicellular eukaryotic organisms whose most representative characteristic is the possession of a silica structure, known as frustule, which is arranged in two leaflets linked through a cingulum and covered by organic matter. The diatoms are classified in centric (as *Cyclotella glomerata* or *Coscinodiscus centralis* (figure 4), species very representative in this study) when they present a cylindrical structure, and pennate (as *Navicula* sp., *Cylindrotheca closterium* and *Nitzchia longissima*, also very abundant in this study) with an elongated shape. We can find unicellular species like *Pinnularia* sp. and colonial life like *Aulacoseira* sp. (figure 4), which is formed due to vegetative reproduction [46].
- **Chrysophyceae:** known as golden algae. They are characterized by two heterokonts (unequal) flagella. In addition to chlorophyll, they contain large amounts of fucoxanthins and in their vacuoles accumulate a polymer known as crucio laminarin. The accumulation of these pigments gives them that yellow-orange tone [47]. In the sampling, the nanoplanktonic Chrysophyceae, *Calycomonas* sp. and *Ollicola vangoorii* have been particularly abundant (figure 5).
Cryptophyceae: this class includes photosynthetic unicellular eukaryotes containing chlorophylls a and c and other accessory pigments such as alloxanthins. They are asymmetrical cells with a slightly lateral invagination from which flagella emerge. This kind of algae can be found in both continental and marine waters [48]. The species Cryptomonas marssonii, Plagioselmis lacustris, Rhodomonas salina and unicellular nanoplanktonic Cryptophaceae are the most abundant in our samples.

Dinophyceae: these are a group of unicellular eukaryotic organisms. Most of the species in this group have two flagella and a cover formed by plates that surrounds the nucleus. As a particularity, we can point out that some species present bioluminescence, others are parasites of marine animals and some are toxic. The accumulation of this type of algae triggers the so-called "red tides" described above [46]. In all the samples collected, a great variety of species of this group have been found (figure 6).

Cyanophyceae: prokaryotic organisms capable of oxygen photosynthesis are found in this class. They are characterized by their high content of chlorophyll and phycocyanin (green and blue pigments). They are the organisms that caused the first oxygen accumulations on the planet, they are the main producers of biomass in many of the ecosystems where they are found (there are species that develop under very different and extreme conditions) and some species have mechanisms for fixing atmospheric nitrogen [49]. We can find them in forms of free life, either as spheres or in bacilli, or forming colonies, mostly filamentous. Recently, cyanobacterial wastewater treatment methods such as Synechocystis salina and Phormidium foveolarum species are being studied to degrade the organic contaminants present [50]. In our sampling, the most abundant cyanobacteria are of the genus Synechococcus sp.: spherical cells that do not reach more than 5 µm and that appear isolated or in short chains [46].

Chlorophyceae: these are known as green algae. They can be found in marine environments as well as in continental waters, in the form of free or colonial life, perform oxygenic photosynthesis and present chlorophyll a and b not associated with other pigments. Some algae of this group can be found in terrestrial environments forming lichens associated with fungi [51]. In our samples we have found numerous nanoplancktonic Chlorophyceae, Phaeocystis sp. and, although at lower density, Pediastrum sp. (figure 7) possibly from inland waters.
3.2. Geographical distribution of taxa

In order to show the distribution of the different classes of phytoplankton within the samples, figures 8, 9 and 10 represent the distribution of algae located at the sampling point corresponding to each sample studied. In the category "Others" of the legend are included the classes Conjugatophyceae, Pedinophyceae, Euglenophyceae, Diphyllleida and Thecofilosea, which appear in very low densities.

In the first sample corresponding to May 8 (figure 8), the densities of samples P1 and P6 are very similar (more than 8,000 cells/ml) between them. However, Chrysophyceae are predominant in the first sample (P1), followed by diatoms and dinoflagellates (being the nanoplanktonic Chrysophyceae, *Cyclotella glomerata* and *Katodinium fungiforme*, the most abundant taxa in each class respectively), while in P6 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 P4 and P5 samples are very 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 *Nitzchia 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 8), P1 is the sample with the lowest density (just over 500 cells/ml) and P2, P3 and P4 have very similar densities (between 3,000 and 4,000 cells/ml). The composition of P2, P3 and P4 samples is also very similar, with Cyanophyceae being the most abundant class, followed by Chlorophyceae and Cryptophyceae, 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 P1 sample diatoms such as *Chaetoceros muelleri*, Cryptophyceae such as *Plagioselmis* sp. and dinoflagellates such as *Gymnodinium* sp. and *Prorocentrum compressum* predominate.

As shown in Table 1, figure 9 shows that sample P1 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 10). 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. In percentages lower than 1 % we can find diatoms such as *Navicula* sp., *Chaetoceros* sp. and *Nitzchia longissima* and dinoflagellates such as *Gymnodinium* sp., *Protoperidinium* sp. and *Prorocentrum compressum*. The other sample collected on 1 September (P2) 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 P1, is much more diverse in composition. It presents Cyanophyceae, Chrysophyceae, Chlorophyceae classes and diatoms in very similar proportions. The most noteworthy taxa are *Synechococcus* sp., Chrysophyceae and nanoplanktonic Chlorophyceae and diatoms of the species *Cyclotella glomerata*. 
Figure 8. Map of the Mar Menor 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. [Own elaboration].
Figure 9. Map of the Mar Menor 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. [Own elaboration].

The samples collected on 21 October (Figure 9) show lower densities than in September but are still higher than in previous months. The P1 sample has the lowest density and it is dominated by Cryptophyceae class, diatoms and dinoflagellates, with *Plagioselmis* sp., *Nitzchia longissima* and *Gymnodinium* sp. being the most noteworthy taxa in each of these categories. Although the P2 sample has a higher density than the P3 sample, its composition in percentage is similar, predominantly the classes Chlorophyceae and Cyanophyceae, followed by Bacillarophyceae, 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 *Nitzchia longissima*. The P4 and P5 samples are similar, both in total density and
composition. In this sample, bacterioplankton predominates. It is a group that includes both spherical unicellular bacteria (which constitute the totality of bacterioplankton in this sample) and bacteria of the genus *Flavobacterium* sp. (which do not appear in this sample). In the rest of the sample, we found Cryptophyceae class such as *Plagioselmis lacustris* and *Rhodomonas salina*, dinoflagellates such as *Gymnodinium* sp. and diatoms such as *Chaetoceros muelleri*, *Nitzchia longissima* and *Cyclotella glomerata*.

![Map of the Mar Menor 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.](image)

**Figure 10.** Map of the Mar Menor 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. [Own elaboration].

In figure 10, 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 10), Chlorophyceae predominate in P2, P3 and P4 samples, with nanoplancktonic Chlorophyceae predominating. However, the Cryptophyceae class predominated in
the P1 sample (96% of the sample), with both nanoplanktonic Cryptophyceae and *Plagioselmis lacustris* and *Rhodomonas salina* species standing out. In the P1 sample we can also find diatoms, predominantly the species *Nitzchia longissima*, and dinoflagellates, being *Gymnodinium* sp. the most abundant species of this group. In the P2 and P3 samples, 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 P4, the most abundant species are the same as in samples P2 and P3, except for the class of Cyanophyceae, which does not appear.

The last samples, collected on 9 December, are the densest of the year. The P1 sample is the most different in its composition, predominantly contained in nanoplanktonic Cryptophyceae and *Plagioselmis* sp., occupying 98% of the sample in which only 2% is composed of diatoms such as *Chaetoceros* sp. and Gymnodinial dinoflagellates. The P2 and P5 samples are 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 Gymnodinial dinoflagellates. Finally, P3 and P4 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*.

### 4. Discussion

Once we observe the results of this study, we can locate a maximum point of phytoplankton density in the month of September, just after reaching the highest temperature values of the water. This maximum density decreases little by little during the autumn-winter months and the densities recorded during the sampling of the months of May and June are the lowest recorded in the study.

Dzierzbicka-Głowacka study [52] 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 [53,54]. These increases tend to occur in the summer months, so that in subsequent months the highest phytoplankton densities are usually recorded [55] 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 [56]. An example of this effect can be found in the research of Siegel and Gerth [57] 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 work of Alves de Souza et al. [58] we find that, in the Chilean Reloncaví fjord, while the species *Protoceratium 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 [59]. 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 Martinez and Esteve [59]. 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 [60], 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 Cyanophyceae, Chrysophyceae, Cryptophyceae and Chlorophyceae. 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 (Bacillarophyceae) with 28 registered taxa, similar to the work of Ros et al. [28]. If we observe their distribution in the maps (figures 8, 9 and 10), we can see that we find higher density of diatoms in samples taken closer to channels, while dinoflagellates are grouped in more protected areas from currents. The diatoms lack structures that allow them to move unlike the dinoflagellates that possess flagella, so they tend to proliferate in areas of currents that favor the renewal of nutrients and dissolved oxygen [61]. 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, Diphylleida 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 [62] 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 [63].

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 Nitzchia longissima. Cyclotella glomerata is characterized by being a freshwater species [39] and appears, surely, from surface waters draining over the Mar Menor; Cylindrotheca closterium is a species typical of marine environments [51] and Nitzchia longissima can be found in hypersaline environments [64]. 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.

Once these results have been reviewed, we can see that the fundamental 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 [65]. In the integral report on the ecological state of the Mar Menor in 2017 [66], 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 [67] 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/saline lagoon compared to previous data. This shows the eutrophication pressure in which the lagoon is located, favored by agricultural dumping and waste derived from tourism.

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, Table S1: Main table of density of the phytoplankton in all the samples studied.

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