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Seasonal Dynamics and Environmental Drivers of Phytoplankton in the Albufera Coastal Lagoon (Valencia, Spain)

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Abstract: The Albufera of Valencia is a hypereutrophic, oligohaline lagoon that has experienced significant changes in phytoplankton composition and state in recent decades due to human activities. These activities affect phytoplankton biomass and community structure, which are key indicators of ecosystem health. In this study, phytoplankton samples from the lagoon were analyzed to identify dominant groups and genera, and their seasonal cycles were determined using biovolume measurements with the Utermöhl method. Various environmental variables were also measured. Diversity was assessed using richness, equitability, and the Shannon–Wiener index. Canonical Correspondence Analysis (CCA) and Pearson correlation revealed that temperature and phosphorus significantly influence phytoplankton abundance. A species that exhibited seasonal abundance, resulting in a change in the lagoon's color from green to brown, was identified. Water quality was assessed using the trophic state index, indicating that the lagoon is in poor condition and hyper-eutrophic. Cyanobacteria were the most dominant group, peaking in November, contrary to previous studies, followed by Chlorophyceae and Bacillariophyceae. Phytoplankton are vital bioindicators for assessing ecosystem health, underscoring the need for further research in this area.

Keywords: microalgae; cyanobacteria; Chlorophyceae; eutrophication; water quality

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1. Introduction

Phytoplankton is an important primary producer on a global scale, responsible for 45% of the Earth's net primary productivity [1] and 70% of atmospheric oxygen [2]. It is, therefore, important to understand how phytoplankton communities are organized because. Despite constituting only 1% of the Earth's photosynthetic biomass [3], it is a vital component of marine and freshwater ecosystems.

It also contributes to different global cycles, notably its importance in the global carbon cycle and oxygen production, to regulate climate and maintain the balance of gases in the atmosphere. This makes aquatic ecosystems an important sink for atmospheric CO_2, as they absorb a similar amount of CO_2 as terrestrial ecosystems and remove almost a third of anthropogenic CO_2 emissions from the atmosphere [4].

Phytoplankton, on the other hand, are the basis of aquatic food webs and are globally important for ecosystem functioning and services [3]. The dynamics of these photosynthetic organisms are linked to annual fluctuations in temperature, water column mixing, resource availability, and nutrient uptake [4].

These functions are threatened by physiological impacts on individual organisms that are related to climate and the large-scale distribution of plankton communities [5]. Such is this impact that the Water Framework Directive includes, among its biological quality indicator, elements of the composition, abundance, and biomass of phytoplankton. The importance of Ecological Potential Index (EPI), which is key to this directive and uses phytoplankton as an indicator element through four of its indicators (such as the Index of Algal Groups, chlorophyll-a (Chl-a), biovolume and percentage of cyanobacteria), also stands out [6]. On the other hand, there is the Trophic State Index [7], which takes variables such as water transparency, total phosphorus (TP), and Chl-a concentration, and measures water quality in lakes, determining their eutrophic condition.

Shallow lakes present different states of equilibrium [8], two of which predominate: a 'clear' phase and a 'turbid' phase, the former characterized by the dominance of aquatic vegetation and the latter by the dominance of algal biomass. Both phases are separated from each other by a transition period known as hysteresis, referring to a phenomenon in which the state of an ecosystem does not return to its original state even after the environmental conditions that caused a change have been reversed [9].

These transitions may be observable even after a single significant anthropogenic or natural impact that distorts its initial state [9]. For example, if a lake runs into some increase in pollution, temperature, or eutrophication, among others, it may experience these various alternative states and reach a tipping point where the ecosystem destabilizes and changes significantly, from a 'clear' to a 'dark' phase. At this point, even if pollutant levels, nutrients, or temperature drop, the lake may not return to its initial state due to changes in biological community composition, habitat structure, and other factors [10].

This phenomenon may have important implications for the management and conservation of aquatic ecosystems, as it shows the need to take precautionary measures to prevent ecosystems from reaching critical points where hysteresis may occur, because, once the tipping point is reached, complete ecosystem restoration may be difficult or even impossible [8].

Hypereutrophic systems are those that have become enriched to the point where large populations of algae and/or macrophytes appear and major changes in ecosystem structure occur [11]. This eutrophication leads to harmful algal blooms, fish kills, and many related problems both in freshwater and in seas near areas with large human populations [12]. This phenomenon occurs due to increasing inputs of phosphorus and nitrogen in human sewage, livestock excrement, and synthetic fertilizers applied to agricultural land [13].

The case of the Albufera of Valencia is no exception. Some authors [14] stated in the last century that this lagoon was under immediate threat from its growing eutrophication. This was caused by the input of organic matter of urban, agricultural, and industrial origin. It went from oligotrophic conditions in 1970 to hypereutrophic in 1980 [15].

In the middle of the 20th century, the trophic state of the lagoon began to be altered. This led to the situation of a turbid hypereutrophic lagoon that is known today as the dark phase [16].

In the 1990s, a slight recovery took place, but without reaching a clear phase. During this recovery, the presence of phytoplankton in the lagoon decreased for about two weeks at the end of winter, and the abundant cyanobacteria were replaced by diatoms and other microscopic algae [17]. At present, the lagoon is a hypereutrophic system due to excessive inputs of external organic material and inorganic nutrients, especially nitrogen and phosphorus compounds [18]. This leads to excessive growth of cyanobacteria throughout the year [19]. While it is true that some clear phases have been detected in late winter since 1996, the last one dates to March 2010 [20].

Some authors [21] argue with clear evidence that temporal changes in phytoplankton biomass are governed by, among other things, physical variations in the growth conditions of the upper water column such as light, nutrients, and temperature. There is no single factor that determines phytoplankton seasonality, but rather a combination of factors such as nutrients, loss resistance, motility, and photosynthetic physiologies. Other authors [22] have conducted studies that similarly show that high nutrient concentrations determine phytoplankton growth and seasonality. These studies found annual patterns with two peaks in phytoplankton biomass in spring and autumn. In addition, blooms are usually characterized by the dominance of one or two of the groups comprising phytoplankton, such as cyanobacteria or diatoms [23].

The Albufera is under enormous anthropogenic pressure, as its hydrological cycle is closely associated with the annual cycle of cultivation and harvesting of rice that has been produced for more than two centuries in the surrounding area and waterfowl hunting in winter [15]. The system depends on water management, which depends on the needs of rice cultivation.

Long-term studies under ambient conditions are needed to assess the possible implications of steadily increasing atmospheric CO_2 concentrations on the growth and toxicity of cyanobacteria [24]. In addition, warmer temperatures in water also increase outbreaks of toxic algal blooms and their toxicity to other organisms [25].

The literature reflects the paucity of recent descriptive studies at this site or that are too comprehensive. Existing studies to date have tended to be conducted over several years, with more occasional sampling rather than month-by-month monitoring. Moreover, as this is a dynamic system, it should be studied more frequently.

In 1984, Miracle et al. [26] analyzed the spatial and temporal variability of phytoplankton, observing notable changes in some months. In the same year, García et al. [27] highlighted the dominance of cyanobacteria throughout the year, especially in autumn and winter. In 1987, Miracle et al. [28] found that light and nutrients influenced the high spatial heterogeneity of phytoplankton, affected by water quality and water management. In 2003, Villena and Romo [29] related cyanobacterial peaks to temperature and nutrients. Romo et al. in 2008 connected pesticide use and water cycle management with phytoplankton dominance and loss of submerged plants. Finally, in 2020, Soria-Perpinyà et al. [30] used remote sensing to map cyanobacterial blooms, validating the data with direct observations. There is a gap of twenty years from 2004 to make new studies about phytoplankton in this lagoon.

The main objective of the present study is to deepen the temporal evolution of phytoplankton in the Albufera de Valencia throughout the year during the period from 2 February 2023 to 4 January 2024. In addition, the results will be compared with previous studies to check whether the state of the lagoon has undergone changes in recent years. Ultimately, this will allow the quality of the lagoon to be assessed by using quality indices.

2. Materials and Methods

2.1. Study Area

The Albufera of Valencia is a hypereutrophic and oligohaline lagoon, near the Balearic Mediterranean Sea with an average depth of 1.2 m, covering 21 km^2. The area surrounding the lagoon, of great ecological and cultural value, has been the object of various protections: it was declared a Natural Park in 1986, included on the Ramsar list in 1989, and designated a Special Protection Area for Birds in 1991. It has also been part of the Natura 2000 Network since 2006 and is proposed as a Biosphere Reserve. However, the lagoon has suffered significant degradation due to agriculture, urbanization, and industrialization, which has led to severe eutrophication and deterioration of its ecosystem.

2.2. Sampling and Data Collection

With respect to phytoplankton sampling in the Albufera lagoon, 18 samples were taken in a central point of Albufera between February 2023 and January 2024, which made it possible to complete an annual cycle. Samples and data were taken at the location called "center", at geographical coordinates 39.34600 N & −0.33660 W, as shown in Figure 1.

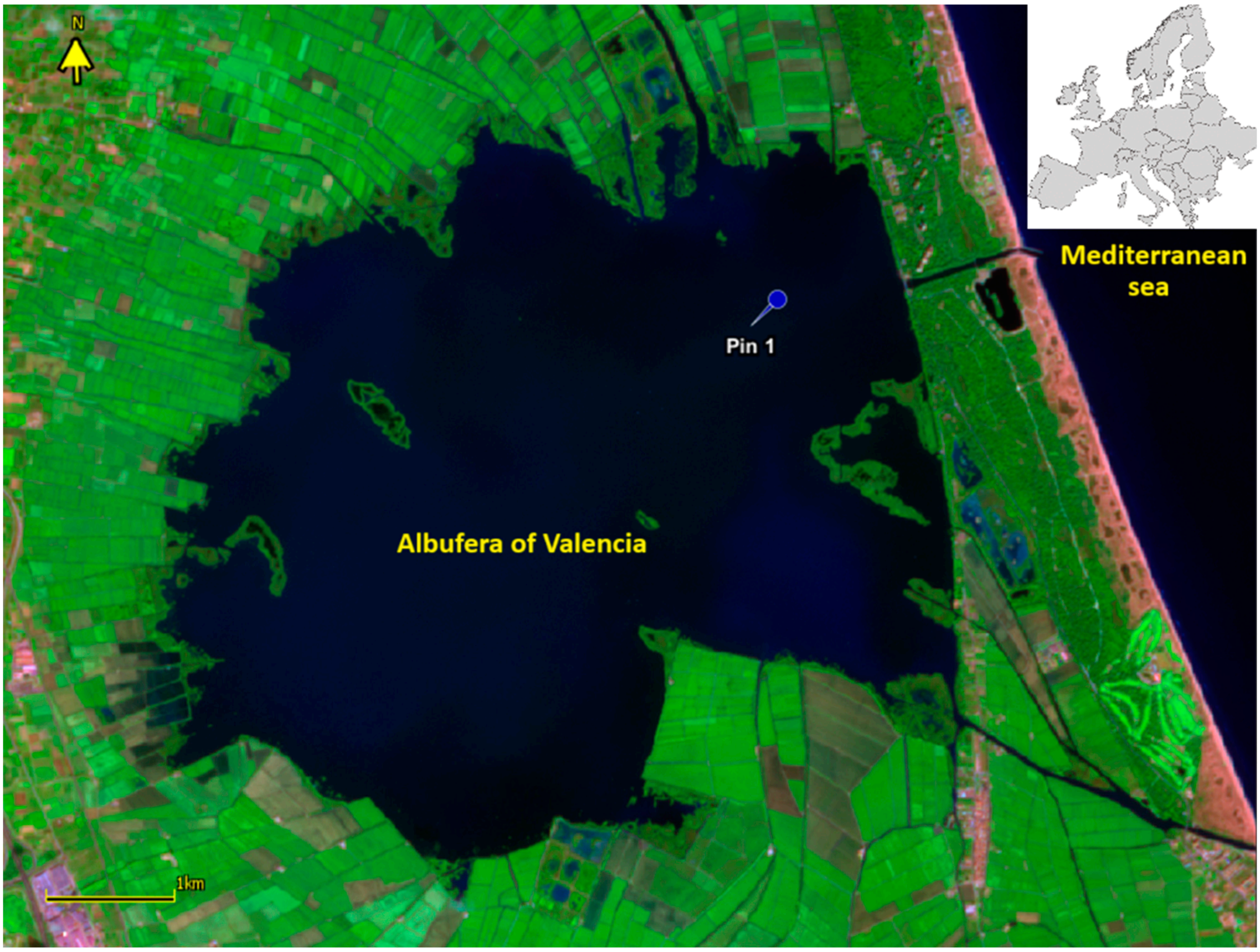

Figure 1. Location of the sampling point used during the samplings conducted (39.34600 N & −0.33660 W) in Albufera lagoon. Sentinel-2 image of 17 September 2023 in enhanced vegetation color mode.

A water sample integrating 0–50 cm column depth was taken with a 250 mL glass bottle, which was then fixed with an acid solution of Lugol's solution, thus allowing the preservation of phytoplanktonic organisms in conditions of approximately 4 °C temperature and darkness. In this way, their study could be carried out by optical microscopy in the laboratory in, ideally, the following 2–4 weeks [31].

Furthermore, physicochemical variables, including Secchi Disk (ZSD), conductivity (measured using the HI98311 Hanna conductivity meter), temperature, and pH (measured using the pHep®4 pH meter), were recorded in order to facilitate a comprehensive analysis of the water conditions. Total Suspended Solids (TSMs) were evaluated using a Whatman 934-AH fiber-glass filter following the gravimetric method described by other studies [32].

Measurements of the concentration of photosynthetic pigments such as Chl-a, carotenes (Car), and phycocyanin (PC) were also taken. PC was measured in situ using a fluorometer (Turner C3™ Submersible Fluorometer, Turner Designs, San Jose, CA, USA). Regarding Chl-a and Car, samples were filtered through Whatman GF/F fiber-glass discs, extracting pigments from cells with a 1:1 solution of 90% acetone and dimethyl sulfoxide, following the Shoaf and Lium method [33], and the pigment concentration was determined by spectrophotometer method using the calculation equations of Jeffrey and Humphrey [34] for the Chl-a, and Strickland and Parsons formula [35] for Car.

To measure the concentration of TP, the digestion method [36] and molybdenum blue were used, measuring the absorbance of 882 nm [37].

2.3. Phytoplankton Observation and Indices Calculation

For phytoplankton counts, the Ütermohl method [38] was followed since it is the most used in phytoplankton studies of this type [28,32,39–41]. Due to high cell concentration, 1 mL of the sample to be analyzed was placed in a 2.8 mL sedimentation column with the remaining 1.8 mL of distilled water allowed to decant for a minimum of twenty-four hours to facilitate the visibility of the organisms, and this was placed on a Nikon Eclipse Ti-U inverted optical microscope (Nikon Corp., Tokyo, Japan). Species were identified, when possible, and genera were found by making use of the guides of the following: Bourelly [42], Streble and Krauter [43], and Hickel et al. [44]. The total number of cells of the species and genera identified that appeared in a number minimum of 10 fields was counted randomly. Due to the high concentration of specimens in the samples, this method was determined to provide a representative estimate of the percentage of the species present.

Subsequently, the cell density (number of cells/mL) was obtained and the algal biovolume was calculated by multiplying the number of cells of each specimen by the average volume they have according to their taxon.

The algal biovolume of the different taxa was estimated according to geometric approximations [45]. For this purpose, between 25 and 100 individuals (cells or colonies) of each species were measured and the average measurements were calculated according to the method of Romo et al. [40]. In addition, these biovolumes were grouped by classes to facilitate future statistical analyses relating each group to the different environmental or physicochemical variables. The percentage of cyanobacteria was also calculated from biovolume, except for the order Chroococcales, but considering *Microcystis* sp. This is in accordance with the legislation stipulated by the WFD when applied in Spain. The value for good/moderate ecological quality is 28.5%, values greater than 48.5% are deficient and the limit for bad quality is >68.5%.

The idea of categorizing phytoplankton to describe variations in lake composition has a long history [46]. Reynolds [47] was the first to propose a classification based on factors such as eutrophication and nutrient availability. This proposal grouped species with similar traits into "narrowly defined functional groups" [48], differentiating them by specialized adaptations. However, this classification may be inaccurate and lose information on diversity and ecological interactions. Therefore, John et al. [49] proposed taxonomic groups to preserve such information.

2.4. Data Analysis

Descriptive statistics were performed using Microsoft Excel software [50]. Species richness was calculated as the number of species per sample. The Shannon-Wiener diversity index [51] and evenness index [52] were also calculated from the abundance of species using the tool incorporated in the statistical software PAST 4.11 [53]. It should be noted that the taxonomic level used to calculate these indices was species level, and the unknown cells were considered a single species. This index was used instead of the Margalef index [54] since both this and Simpson's index [55] consider proportions and not absolute numbers. For the Margalef index, there is an underestimation of the index; for that reason, it is considered that this index will more easily lead to errors in its calculation.

On the other hand, with the previously normalized data using the Napierian logarithm, several Pearson correlation analyses between phytoplankton species density and physicochemical variables were carried out also with the statistical software PAST [53]. In addition, a classical Canonical Correspondence Analysis (CCA) was used to evaluate the relationships between the different physicochemical variables and the abundance of the phytoplankton groups throughout the year.

To determine the water quality of the lagoon, the index created by Carlson [7] was used to determine the degree of eutrophication. It comprises four ranges: "oligotrophic" (0–40), "mesotrophic" (40–55), "eutrophic" (55–70) and "hypertrophic" (>70). Using the equations of Carlson [7], the Trophic State Index (TSI) of the lagoon was calculated. Each of the equations made use of one of the three physicochemical variables measured in this study: transparency according to the depth reached by the Z_{SD} (m), Chl-a (mg/m^3), and TP (μg/L).

Multivariate analysis also included a Cluster Analysis by the Unweighted Pair Group Method with Arithmetic (UPGMA) mean according to Sokal and Michener. These calculations are made with PAST 4.11 Software [53].

3. Results

3.1. Physicochemical and Biological Variables

A total of 18 samples were analyzed on the dates indicated and the results obtained are shown below (Table 1).

Table 1. Physicochemical variables of the samples taken on each date, values of temperature, conductivity, Secchi disk depth (Z_{SD}), total suspended matter (TSM), organic solids (LOI), and total phosphorus (TP).

Date	Temperature (°C)	Conductivity (μS/cm)	Z_{SD} (m)	TSM (mg/L)	LOI (mg/L)	TP (mg/L P)
02 Feb 2023	12.4	1639	0.25	81.5	53.1	0.19
10 Feb 2023	18.4	1287	0.43	49.5	27.8	0.25
22 Mar 2023	13.6	1244	0.24	90.9	31.8	0.25
03 Mar 2023	13.4	1710	0.18	102.5	27.5	0.11
23 Mar 2023	18.3	1618	0.20	81.4	41.9	0.04
14 Apr 2023	18.0	1500	0.20	91.0	45.2	0.02
01 Jun 2023	24.6	1682	0.13	177.6	95.3	0.34
13 Jun 2023	26.3	1680	0.14	158.3	75.4	0.32
29 Jun 2023	30.6	1964	0.12	139.6	74.4	0.39
11 Jul 2023	32.0	1848	0.17	126.4	74.6	0.34
09 Aug 2023	28.5	1990	0.25	98.4	75.6	0.18
12 Sep 2023	24.0	1601	0.24	84.0	44.8	0.18
17 Sep 2023	21.2	1449	0.18	75.8	60.2	0.22
27 Oct 2023	17.7	1690	0.12	177.2	85.9	0.31
14 Nov 2023	15.2	1890	0.13	104.0	72.7	0.31
01 Dec 2023	13.7	1848	0.17	107.6	76.7	0.30
18 Dec 2023	11.2	1963	0.19	60.4	52.5	0.26
04 Jan 2024	10.3	2032	0.22	71.2	52.9	0.32
Mean	19.1	1702	0.19	104.3	59.3	0.24
Max	32.0	2032	0.43	177.6	95.3	0.39
Min	10.3	1244	0.12	49.5	27.5	0.02
St. Dev.	7.0	232	0.07	37.6	20.4	0.10

We found the maximum temperature as expected in July with 32.0 °C and the minimum in January (10.3 °C). The maximum conductivity was 2032 μS/cm and the minimum was 1244 μS/cm. Water transparency values measured with the Z_{SD} ranged from 0.12 to 0.43 m, typical values for highly eutrophicated lakes. TSM reached their maximum near the maximum biovolume corresponding to the spring and autumn bloom (177.63 mg/L), and the organic fraction measured as loss of ignition (LOI) is 95.26 mg/L in the same date sampling. TP value is between 0.02 and 0.39 mg/L.

Regarding the biological indices calculated, the concentration of photosynthetic pigments found in the different dates, the Shannon–Wiener index [51], and the three forms of the Carlson index [7] are shown in Table 2.

Table 2. Results of biological variables, including photosynthetic pigments, species diversity and richness, Trophic State Index, and % of cyanobacterial density. Chl-a = chlorophyll-a; Car = carotenoids; PC = phycocyanin; TSI = Trophic State Index; Z_{SD} = Secchi disk depth; TP = total phosphorus.

Date	Chl-a (µg/L)	Car (µg/L)	PC (µg/L)	Diversity	Richness	TSI (Z_{SD})	TSI (Chl-a)	TSI (TP)	% Cyanobacteria
02 Feb 2023	166.9	72.8	223.4	2.29	38	80	81	80	42.3%
10 Feb 2023	186.4	74.8	272.0	2.75	59	72	82	84	49.6%
22 Mar 2023	177.1	90.1	251.8	2.81	44	81	81	84	41.2%
03 Mar 2023	190.0	93.0	169.1	2.78	36	85	82	72	63.8%
23 Mar 2023	206.4	97.3	310.0	2.52	46	83	83	57	47.9%
14 Apr 2023	333.1	151.6	361.7	2.73	31	83	88	47	76.5%
01 Jun 2023	281.4	134.8	363.0	2.80	45	89	86	88	35.4%
13 Jun 2023	222.5	104.3	372.0	2.74	40	88	84	87	64.5%
29 Jun 2023	180.7	89.8	344.4	2.32	42	91	82	90	47.8%
11 Jul 2023	165.4	81.4	304.6	3.00	43	86	81	88	54.1%
09 Aug 2023	130.9	97.3	355.5	3.14	32	80	78	79	75.1%
12 Sep 2023	158.3	175.1	307.8	3.15	37	81	80	79	66.7%
17 Sep 2023	222.4	307.2	351.8	1.64	34	85	84	82	80.2%
27 Oct 2023	268.4	421.6	689.5	1.46	39	91	85	87	83.2%
14 Nov 2023	327.8	483.7	980.0	1.84	26	89	87	87	84.3%
01 Dec 2023	213.5	395.0	670.0	2.45	33	86	83	86	67.9%
18 Dec 2023	273.9	482.7	770.1	2.39	37	84	86	84	73.8%
04 Jan 2024	178.6	334.3	394.6	2.48	42	82	81	87	70.3%
Mean	215.8	204.8	416.2	2.51	39	84	83	81	62.5%
Max	333.1	483.7	980.0	3.15	59	91	88	90	84.3%
Min	130.9	72.8	169.1	1.46	26	72	78	47	35.4%
St. Dev.	58.4	152.6	214.9	0.48	7	5	3	11	15.5%

The lowest recorded Shannon–Wiener diversity index was 1.46 on 27 October 2023. The highest recorded Shannon–Wiener diversity index, which occurred on 12 September 2023, also corresponded to the end of summer. However, this was due to high temperatures and the renewal period of waters in the lagoon, which was caused by the emptying of rice fields for harvest.

The Trophic State Index (TSI) values calculated from chlorophyll-a concentration (Chl-a) and Secchi disk depth (Z_{SD}) consistently exceed 70 for all dates analyzed, thus classifying the system as hypertrophic. Conversely, the TSI calculated from total phosphorus (TP) also indicates hypertrophy on all dates, except for two instances: 23 March (TSI-TP = 57) and 14 April (TSI-TP = 47), which correspond to the eutrophic and mesotrophic categories, respectively. However, on these same dates, the TSI based on Chl-a and Z_{SD} still indicates hypertrophic values (83 on 23 March; 83 [Chl-a] and 88 [Z_{SD}] on 14 April). This finding indicates that, despite lower phosphorus concentrations being recorded in the water column on these dates, the trophic state of the system had not improved, but rather remained characteristic of a hypertrophic system due to high concentrations of phytoplankton biomass and limited water transparency.

The minimum percentage of cyanobacteria recorded was 35.4% on 1 June, indicating that on all dates assessed, this value was well above the 28.5% threshold set by the Water Framework Directive (WFD) to classify a water body as having moderate status in terms of ecological potential. Only four samples according to this indicator are classified as moderate, six as deficient and seven as bad ecological potential.

As illustrated in Figure 2, a graphical representation is provided of the sum of all pigments, along with their differential distribution, thus facilitating the visualization of the changes in the lagoon. The values ranged from 130.9 to 333.1 mg/m^3 in Chl-a, from 72.8 to 473.7 mg/m^3 in Car, and between 169.1 and 980.0 mg/m^3 in PC.

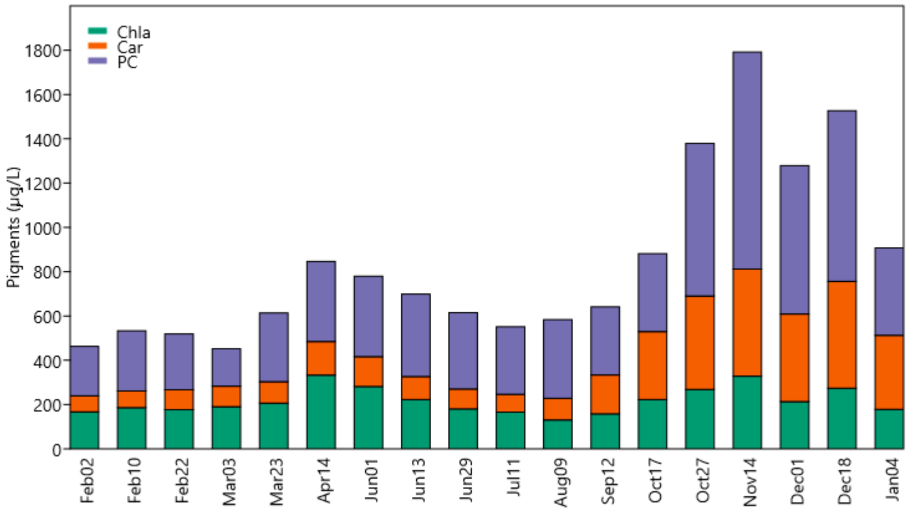

Figure 2. Stacked bar diagram of temporal evolution of photosynthetic pigments (µg/L) over the study period. Chla = chlorophyll-a; Car = carotenoids; PC = phycocyanin.

Figure 2 clearly shows an annual photosynthetic pigments cycle as described in the literature, with the PC always abundant, above Chl-a and Car, and two peaks in the months of May and November, which also coincide with the peaks of phytoplankton biovolume. It has been observed that between September 2023 and January 2024, coinciding with the autumn peak, Car surpassed Chl-a. Furthermore, a visual analysis of the lagoon revealed a change in its colour from green to brown during these months.

This could be related to the increase of filamentous cyanobacteria, especially the increase of the species *Jaaginema lanceiforme* corrig. (Kalbe 1963) Anagnostidis 2001 and another specimen not yet identified that seems to be cyanobacteria, similar to *Oscillatoria acutissima* Kufferath 1914 with a fusiform shape, which we publish in the table of results as “Unknown algae”. The Car/Chl-a ratio reached its maximum in the autumn season, and, with respect to PC; it increased in the month of November, due to high temperatures and prolonged winds, which caused mixing in the water column.

3.2. Annual Evolution of Taxonomic Groups

According to the number of samples studied and the occurrence of each species in the different samples, the frequency has been obtained, considering the most important species that exceed 50%. The more frequent phytoplankton observed in this study (>50%) comprises the class Bacillariophyceae *s.l.* Dangeard 1933 (to distinguish from Bacillariophyceae *s.e.* D.G. Mann 1960), which included the pennate species *Nitzschia palea* (Kützing) W. Smith 1856 (89%), *Nitzschia acicularis* (Kützing) W. Smith 1853 (68%), *Nitzschia intermedia* Hantzsch ex Cleve & Grunow 1880 (63%), *Fragilaria* sp. Lyngbye 1819 (58%) and the centrics *Cyclotella meneghiniana* Kütz. 1844 (68%) and *Cyclotella meduanae* Germain 1981 (53%). In the class Chrysophyceae, only *Chrysochromulina* sp. Lackey 1939 is the most frequent, with 58%. Between Chlorophyceae the frequent species are *Desmodesmus subspicatus* (Chodat) E. Hegewald & A.W.F. Schmidt 2000 (53%), *Monoraphidium contortum* (Thuret) Komárková-Legnerová 1969 (79%), *Oocystis marssonii* Lemmermann 1898 (74%), *Scenedesmus acuminatus* (Lagerheim) Chodat 1902 (63%), and *Tetraedron minimum* (A. Braun) Hansgirg 1889 (68%). The class Conjugatophyceae is characterized by only one species *Cosmarium abbreviatum* Raciborski 1885 (58%). The class Cyanophyceae is always present (100%) in all samples with five species, both of the order Chroococcales (*Aphanocapsa incerta* (Lemmermann) G. Cronberg & Komárek 1994, *Chroococcus dispersus* (Keissler) Lemmermann 1904, *Merismopedia punctata* Meyen 1839) and Oscillatoriales, part of those known as filamentous cyanobacteria, (*Planktolyngbya contorta* (Lemmermann) Anagnostidis & Komárek 1988, *Pseudanabaena galeata* Böcher 1949); other species with important frequency are *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya & Subba Raju 1972 (58%), *Jaaginema* sp. Anagnostidis & Komárek 1988 (84%), *Merismopedia tenuissima* Lemmermann 1898 (84%), *Microcystis aeruginosa* (Kützing) Kützing 1846 (63%) and *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg 1992 (79%). Lastly, the class Cryptophyceae, of which only one species *Cryptomonas erosa* Ehrenberg 1832 (53%) appeared as frequent.

Figure 3 illustrates the annual evolution of the major taxonomic groups of phytoplankton in the Albufera of Valencia. The positioning of this figure is intended to emphasize the dominance of cyanobacteria, represented in cells per unit volume (mL). The high density of cells of these organisms (it should be noted that they are prokaryotes with a much smaller cell size than the other phytoplankton groups, which are eukaryotes) renders the other groups practically indistinguishable from one another in the figure.

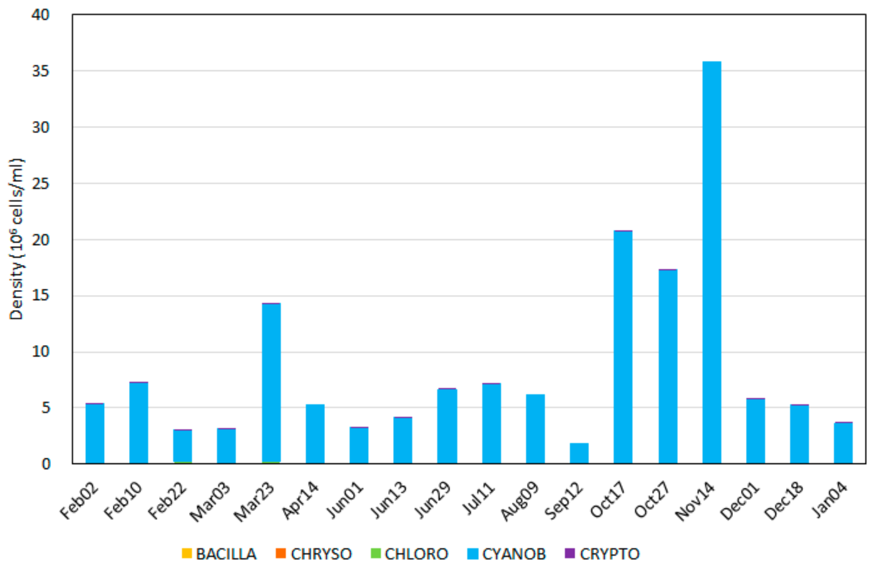

Figure 3. Annual evolution of phytoplankton density by main taxonomic groups. BACILLA = Bacillariophyceae *s.l.*; CHRYSO = Chrysophyceae; CHLORO = Chlorophyceae; CYANOB = cyanobacteria; CRYPTO = Cryptophyceae.

To correct for this effect and facilitate comparison, Figure 4 depicts the same evolution but in terms of biovolume. As can be observed in this figure, cyanobacteria continue to exert a dominant influence, although the other groups are also represented graphically, being Bacillariophyceae *s.l.* in the second position. On 10 February 2023, the second place is for Cryptophyceae and on 3 March 2023 the second place is for Chlorophyceae.

It is noteworthy that the maximum values of cyanobacterial biovolume occur in autumn, which coincides with the peaks of the two new species identified in this study and with the dates of the highest Car concentration in the lake. In this sense, the high presence of the Unknown species stands out (Figure 5), which in the month of November made it difficult to identify the rest of the species, since it hid them due to its high density in the samples.

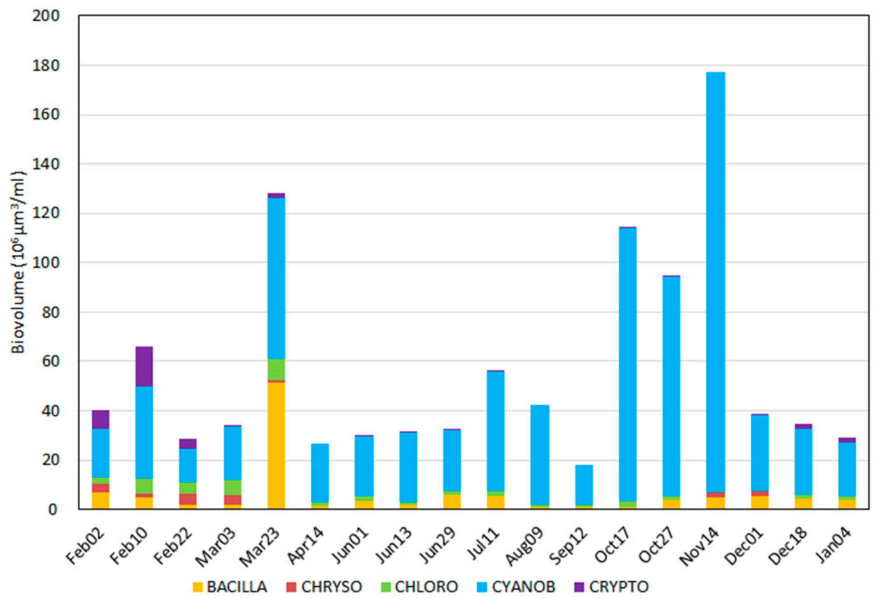

Figure 4. Annual evolution of phytoplankton biovolume by main taxonomic groups. BACILLA = Bacillariophyceae *s.l.*; CHRYSO = Chrysophyceae; CHLORO = Chlorophyceae; CYANOB = cyanobacteria; CRYPTO = Cryptophyceae.

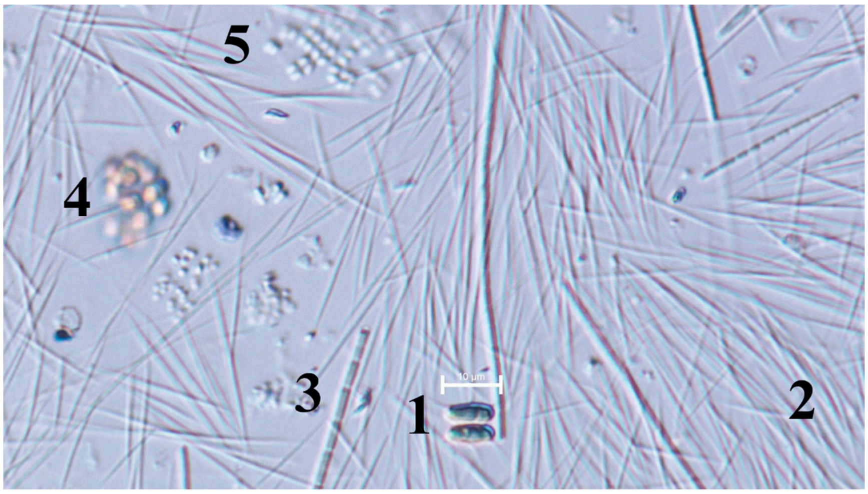

Figure 5. ×60 microscope image of *Desmodesmus* sp. (1) with abundant presence of *Unknown* sp. (2) in a sample collected on 16 November 2023. Also included are the following: *Pseudanabaena* sp. (3), *Aphanothece* sp. (4), and *Merismopedia* sp. (5).

The second most representative group is the diatoms (Bacillariophyceae *s.l.*), which reach their maximum on 23 March and their minimum on 17 October, which coincides with the highest percentage of cyanobacteria. The third group with the highest proportion was Chlorophyceae, which reached their maximum values between February and March, coinciding with the lowest values of cyanobacteria in the cycle.

The remaining groups present in the samples are Cryptophyceae, Chrysophyceae, and Xantophyceae. However, they are of lesser relevance, given that the most representative groups are the three previously mentioned. The fourth most prevalent group, Cryptophyceae, reaches its peak on 10 February, while the third most prevalent group, Chrysophyceae, reaches its peak on 22 February. It can thus be surmised that when cyanobacteria are more numerous, which occurs between summer and autumn, the other groups (except diatoms) exhibit lower values and reach their highest values in the first months of the year. Dinopyta and Euglenophyta are practically irrelevant.

On the other hand, and without downplaying its importance, the occurrence of microplastics (0.1 to 100 µm) in some of the observed samples should also be noted. While in others, larger plastic fibers of more than 150 mm were found.

3.3. Data Analysis

Figure 6 depicts the Pearson correlation matrix. This matrix illustrates the impact of the diverse physicochemical variables of the lake on the dynamics of the major phytoplankton groups. It is evident that temperature exerts a detrimental effect on the Chrysophyceae and Cryptophyceae, which reach their maximum abundance during the winter months. The conductivity has a positive effect on the diatoms, which reach their maximum in March despite the relatively low salinity. Between November and December, they also have a considerable biovolume, which coincides with the periods of higher salinity in the lake. Conversely, diatoms are negatively affected by transparency (Z_{SD}). In the case of the hypertrophic Albufera lagoon, the specific diatom species present are characteristic of poor-quality waters.

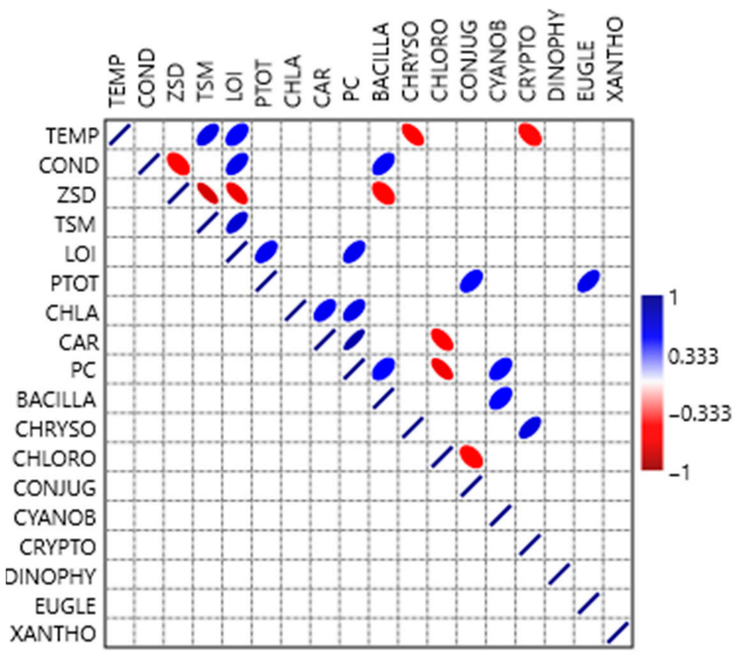

Figure 6. Pearson correlation matrix between phytoplankton density and physicochemical variables; only significant correlations are plotted. TEMP = temperature, COND = conductivity, ZSD = Secchi disk depth, TSM = total suspended matter, LOI = organic solids, PTOT = total phosphorus; CHLA = chlorophyll-a; CAR = carotenoids; PC = phycocyanin; BACILLA = Bacillariophyceae *s.l.*; CHRYSO = Chrysophyceae; CHLORO = Chlorophyceae; CONJUG = Conjugatophyceae; CYANOB = cyanobacteria; CRYPTO = Cryptophyceae; DINOPHY = Dinopyta; EUGLE = Euglenophyta; XANTHO = Xantophyceae.

Finally, a positive correlation is observed between the Cryptophyceae and the Chrysophyceae, as both groups exhibit a greater representation during the winter season.

Figure 7 illustrates the Canonical Correspondence Analysis (CCA) derived from the transformed data matrix. This representation provides a more spatially explicit depiction of the relationships previously explained.

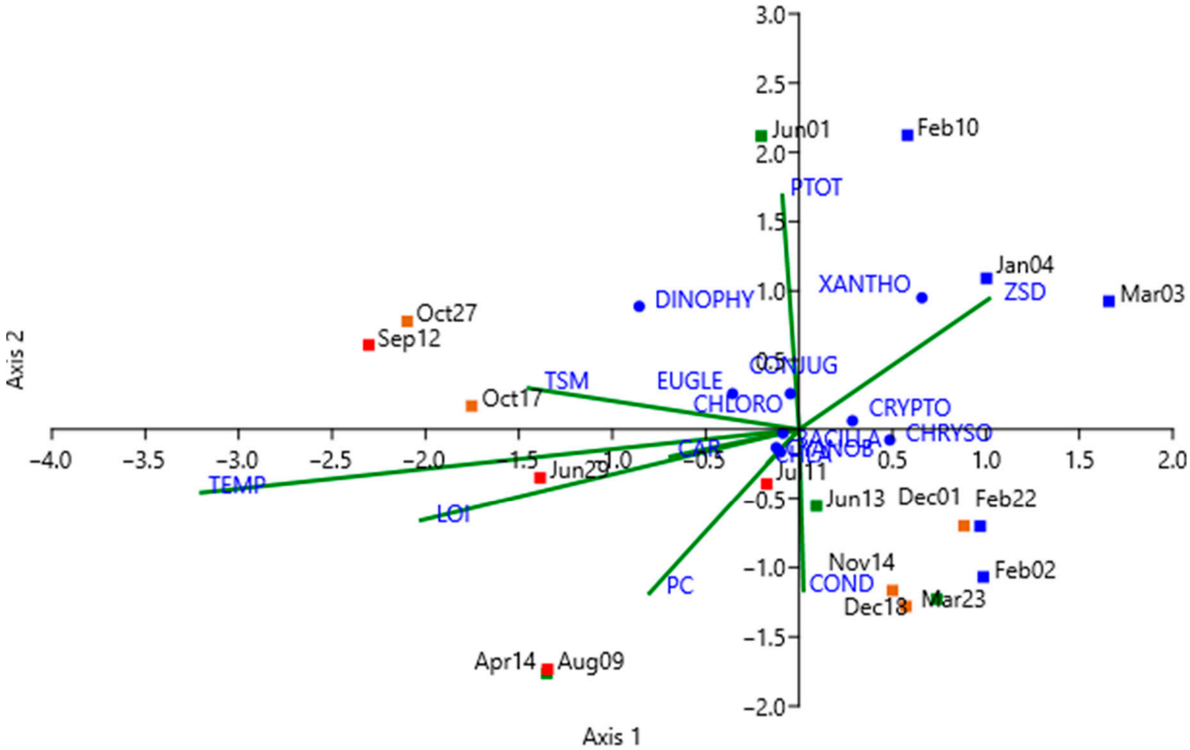

Figure 7. CCA analysis. Colors square points represent sampling dates and seasons (green = spring; red = summer; brown = autumn; cyan = winter). Blue circle = phytoplankton groups. TEMP = temperature, COND = conductivity, ZSD = Secchi disk depth, TSM = total suspended matter, LOI = organic solids, PTOT = total phosphorus; CHLA = chlorophyll-a; CAR = carotenoids; PC = phycocyanin; BACILLA = Bacillariophyceae *s.l.*; CHRYSO = Chrysophyceae; CHLORO = Chlorophyceae; CONJUG = Conjugatophyceae; CYANOB = cyanobacteria; CRYPTO = Cryptophyceae; DINOPHY = Dinopyta; EUGLE = Euglenophyta; XANTHO = Xantophyceae.

The initial observation regarding the CCA is the spatial ordering of the sample dates according to the four quadrants of the graph, which aligns almost perfectly with the seasonal variations. Axis 1 explains 39.1% of the variance and presents winter on the positive side and summer on the negative side. This indicates that the various groups exhibit seasonal patterns, with fluctuations influenced by seasonal changes in the physicochemical variables of the lake and the abundance of different groups.

In this sense, Axis 2 explains 31.3% of the variance and demonstrates a strong correspondence with COND (negatively) and Ptot and ZSD (positively), with dates of higher water quality (clearer and less saline) on the positive side and those of poorer quality on the negative side, turbid water with phycocyanin. In this regard, the groups most prevalent during the initial phase of the year, namely Cryptophyceae, Chrysophyceae, and Xantophyceae in winter, would be situated on the right side, indicating that they exhibit greater competitive ability when water quality is at its better level. Chlorophyceae and Bacillariophyceae *s.l.* appear in a central position, not related to the axis due to its presence during the annual cycle.

The same with cyanobacteria, which are predominantly distributed towards the autumn period, which coincides with the highest concentrations of photosynthetic pigments due to the extensive bloom that occurred. This also coincides with the change in watercolour to brown, when the species with accessory pigments appeared and Car surpassed Chl-a.

Dinophyta and Euglenophyta are therefore oriented towards the summer, when the concentrations of total and organic solids reach their peak, coinciding with the arrival of the westerly wind that stirs the sediment, as previously studied [56,57]. Figure 8 illustrates the cluster, demonstrating the degree of similarity between the samples and the way they would be ordered. The main variable for the similarity is the presence/absence of some classes of phytoplankton.

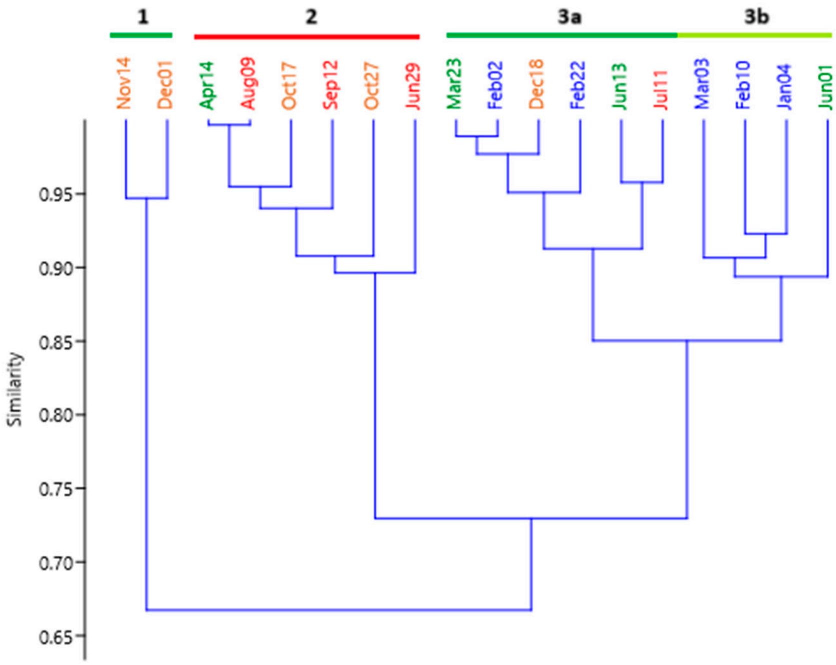

Figure 8. Cluster analysis according to the Unweighted Pair Group Method with Arithmetic (UPGMA). Colour text represents the season as in Figure 7.

The samples from left to right illustrate the most anomalous results. The first group with the November and early December samples exhibits the greatest divergence from the norm, due to the absence of Chlorophyceae and the presence of Conjugatophyceae. Subsequently, the second group presents the samples from late spring to early autumn, which were already dominated by Cyanobacteria but prior to the substantial bloom of the two brown species, are represented in the subsequent branch, and the absence of Chrysophyceae. The third group comprises samples from winter and early spring, when other groups are more representative. These include Chlorophyceae, Cryptophyceae, and Chrysophyceae in group 3a; and Xantophyceae are responsible for the separation of the 3b group of the extreme four samples. Consequently, the samples from 1 June, 13 June, and 11 July are included in this branch, where these groups are once again represented in significant quantities (see Figure 6). This further corroborates the seasonality of the fluctuations observed in the different groups of phytoplankton in the Albufera, as evidenced in the CCA (Figure 7).

4. Discussion

The importance of phytoplankton communities in aquatic ecosystems due to their high productivity is undeniable. Many authors refer to these organisms as the fundamental basis for the survival of many others on this planet, which is why their collapse could have devastating consequences.

The results obtained show a clear predominance of cyanobacteria. This is supported by numerous articles as an indicator of the state of degradation of the lake. Environmental degradation attributable to high trophic conditions is considered to favor the dominance of Cyanophyceae over less tolerant algal species [10]. It has been documented that, in more degraded ecosystems, populations become dominated by Cyanophyceae, leading to a subsequent reduction in biodiversity.

Furthermore, similar to the study by Miracle et al. [28] who studied the lagoon at its maximum hypereutrophic conditions, it was found that cyanobacteria were the most abundant group throughout the year, peaking in late summer and autumn, Chlorophyceae peaked in spring, but in the present study Bacillariophyceae peaked in early spring. In the specific case of cyanobacteria, a substantial proliferation of two carotenogenic species has been recorded during the autumn bloom, coinciding with the documented transition of the lagoon's colour from its characteristic green to a reddish-brown hue. Furthermore, there is a notable increase in the ratio of carotenoids to chlorophyll, which is more pronounced and evident during the months of November and December. As demonstrated in [58], this phenomenon has been observed in other years, when the lagoon exhibited a golden-ochre hue. However, the intensity observed in 2023 was unparalleled, and the study indicates that this is associated with prolonged summer drought events lasting 2–3 months.

Conversely, Car and PC exhibit a negative correlation with Chlorophyceae. This phenomenon can be attributed to the absence of PC in these algae. It is a commonly held assumption that their Car/Chl ratio is typically much lower than that of other groups, such as red algae. Furthermore, their periods of higher biovolume coincide with the periods of lower dominance of cyanobacteria, which do contain these pigments. Nevertheless, the statistical significance of a negative correlation between Chlorophyceae and cyanobacteria could not be demonstrated by Pearson's test.

On the other hand, there is a positive correlation between cyanobacteria and diatoms, with diatoms being abundant not only during the initial months of the year but also during the final months, when cyanobacteria are at their peak. Furthermore, as previously stated, diatoms present in the Albufera are indicative of poor water quality, which is conducive to the proliferation of cyanobacteria.

Comparing the Albufera of Valencia study with similar hypereutrophic lakes, some results match. TSM peaked together with spring bloom biovolume, in line with studies in Italian lagoons [59,60]. In more degraded ecosystems, such as Lake Vela, cyanobacteria dominate throughout the year, in contrast to Chlorophyceae. The Shannon–Wiener diversity index, which is lower during population peaks, reflects the dominance of competitive species, as seen in Costa Rica [61] and in Cabras Lagoon, Italy [62]. Leruste et al. [63] showed that phytoplankton's responses to environmental changes depend on the degree of previous eutrophication.

This study highlights the importance of continuous monitoring of phytoplankton in the Albufera. Other studies [64,65] highlight the value of phytoplankton as a bioindicator due to its low cost and ease of management, especially in lagoons under agricultural pressure. The preponderance of cyanobacteria, as indicated by the percentage of samples in which it was detected, consistently exceeded the WFD threshold for deficient and poor ecological status. Furthermore, the Trophic State Index [7] consistently classified it as hypertrophic, thereby confirming the severely degraded state of water quality in the Albufera. These findings underscore the persistent nutrient enrichment and cyanobacterial dominance, highlighting the need for effective management strategies to mitigate eutrophication and improve ecological conditions, as was assessed in previous studies [14–20,26–30,40].

As for the study of Shannon–Wiener species diversity, it reached the lower values in the October–November samples, these being the dates with maximum phytoplankton biovolume values. These results are in line with the study by Camacho & Charpentier [61] in Costa Rica. This index obtained the lowest values at times of population peaks, due to the increase of few competitive species. With regard to the highest species richness, this was observed in February, due to a more equitable representation of the principal groups of phytoplankton, a lower biovolume, and an improvement in water quality.

In addition, it is important to highlight the great impact of human beings and their consumption habits (mainly of plastics) on the living beings that inhabit any type of ecosystem on the planet. As we have observed in the study, different plastic fibers have been found, most of them being secondary plastics, i.e., formed by the degradation of large plastic objects, such as bottles, bags, greenhouse tarpaulins, fertilizer bags, etc.

Previous studies, many years ago, have mainly focused on describing the evolution of phytoplankton over the years, sampling only at certain times of the year. However, this study allows us to observe in greater detail the phytoplankton cycle over the months and thus the change in environmental conditions in a much more precise way.

As in the study by García et al. [27] many years ago in Albufera lagoon, it is observed that cyanobacteria dominate throughout the cycle, but the maximum biovolume is in November instead of August. The results of this study do coincide with the cycle described [28], but the diversity in that study reaches much higher values than in this one, which could be explained by the increase of dominant species such as cyanobacteria that conquer the niches of the other groups.

What has been greatly reduced is the light penetration measured with the Z_{SD}, which in 1985 [66] peaked at 0.77 m and in this study only reached 0.43 m. This is a result of factors such as increased nutrient loading (such as nitrogen and phosphorus), algal blooms (and thus increased organic matter) as well as sediment resuspension due to the accumulation of dead matter.

Studies such [67] show that in annual studies the periods of maximum and minimum phytoplankton growth are directly related to the availability of phosphorus and other nutrients, in addition to finding a high loading coefficient in the temperature variable. In the present study, a close relationship was also observed between the annual variation of TP concentration and the growth and decline of the population of Conjugatophyceae and Euglenophytes.

This study was limited by the short time available and weather difficulties that prevented sampling at more locations and dates, which limited the ability to compare variations between areas. In the context of forthcoming research endeavors, meticulous organization of field sampling in the Albufera is imperative. Comprehensive spatial coverage, accompanied by judicious distribution of sampling points across the expanse, is to be prioritized. This approach may necessitate a reduction in temporal frequency when compared to the present study. Nevertheless, it is strongly recommended to adhere to a minimum of one sample per month, thereby ensuring the capture of pertinent temporal variations and circumventing the conventional quadrennial sampling schedule.

Caroppo et al. [65] suggest a monitoring plan to identify factors that could destabilize the ecosystem and manage nutrient loading, surface water use, and flood channel functioning. Martín et al. [68] propose flow releases before phytoplankton bloom peaks to improve water transparency and facilitate macrophyte growth, recommending predicting these peaks.

In the domain of remote sensing, future studies should concentrate on elucidating the optical properties of the various phytoplankton groups under differing trophic conditions. Furthermore, it is considered relevant to monitor the irrigation ditch gates to analyze the impact of the new irrigation cycle, as previously described twenty years ago in the work of Villena & Romo [29].

5. Conclusions

In conclusion, following approximately 20 years without studies, this is the first full phytoplankton annual cycle to be published for the Albufera of Valencia. The findings of the study demonstrate the persistent dominance of cyanobacteria, as evidenced by their percentage above the rest of phytoplankton groups throughout the year, above the WFD threshold for deficient or poor ecological status, coupled with the classification of the Albufera as hypertrophic by the Trophic State Index. These findings underscore the severe degradation of water quality in the lagoon, a condition that is further exacerbated by rising droughts and nutrient enrichment. This is indicative of the failure of current management measures to curb eutrophication effectively, underscoring the pressing need for the implementation of a more robust restoration strategy. Conversely, it can be stated that in the autumn of 2023, an exceptional occurrence was observed in the phytoplankton of the Albufera, with the emergence of previously unidentified species that have altered the hue of the lake from green to brown.

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Conflicts of Interest: The authors declare no conflicts of interest.

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