

Temporal succession, spatial dynamics and diversity of phytoplankton in the meromictic Laguna de la Cruz: the role of stratification patterns

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ABSTRACT

Temporal succession, spatial dynamics and diversity of phytoplankton in the meromictic Laguna de la Cruz: the role of stratification patterns

Laguna de La Cruz is a small meromictic karstic lake located in Cuenca (Central Spain), whose stratification and thermal mixing patterns largely determine the structure and composition of its phytoplankton populations. In this lake, the succession in the oxic zone of the water column starts, with the autumnal mixing, with some diatom species, such as *Cyclotella delicatula*, of the functional codon **B**, dominating the community throughout the oxic water column during the winter. This species persists in spring, but then linked to deeper layers. In spring, at the beginning of the thermal stratification, picocyanobacteria, cryptophytes, and dinoflagellates develop in upper layers, corresponding, respectively, to the functional coda **Z**, **Y**, and **Lo**, with the first two being progressively restricted to the oxycline as the stratification advances. As stratification advances, dense populations of chlorophytes belonging to the functional groups **F**, **X**, and **J** develop in the epilimnion and the metalimnion, while towards the end of the summer *Cosmarium* species, belonging to the functional group **NA**, are selected and become dominant until the autumnal mixing favors again the diatoms. As a whole, the strong stratification of the water column allows for the appearance of different niches with a differential vertical distribution of the phytoplankton during the stratification periods, which favors its diversity within the whole water column. However, several types of disturbances, such as episodes of heavy rainfall, horizontal movements of water bodies and, in particular, the whitening that occurs every year towards the middle of summer, alter the successional pattern to some extent.

Key words: Phytoplankton, succession, functional groups, stratification, meromictic lakes

RESUMEN

Sucesión temporal, dinámica espacial y diversidad del fitoplancton en una laguna meromíctica, la Laguna de la Cruz: el papel de los patrones de estratificación

La Laguna de La Cruz es un pequeño lago cártico meromíctico situado en Cuenca (Centro de España), cuyos patrones de estratificación y mezcla térmica determinan en gran medida la estructura y composición de sus poblaciones de fitoplancton. En este lago la sucesión en la zona óxica de la columna de agua se inicia con la mezcla otoñal, tomando protagonismo las diatomeas, con algunas especies como *Cyclotella delicatula*, del tipo funcional (codón) **B**, dominando la comunidad en toda la columna óxica de agua durante el invierno. Esta especie persiste en primavera, pero ligada entonces a capas más profundas. En primavera, al principio de la estratificación térmica, se produce el desarrollo en las capas superiores de picocianobacterias, criptofíceas, y dinoflagelados, correspondientes a los grupos funcionales **Z**, **Y**, y **Lo**, quedando las dos primeras progresivamente recluidas en la oxiclina a medida que la estratificación avanza. Con el avance del periodo de estratificación se desarrollan densas poblaciones de clorofíceas de los grupos funcionales **F**, **X**, y **J** en el epilimnion y el metalimnion, mientras que hacia final del verano se seleccionan especies de *Cosmarium*, pertenecientes al grupo funcional **NA**, que pasan a ser dominantes hasta que la mezcla otoñal vuelve a dar paso a las diatomeas. En su conjunto, la fuerte estratificación de la columna de agua permite crear diferentes nichos con una distribución vertical diferencial del fitoplancton durante los periodos de estratificación, lo que favorece su diversidad en el conjunto de la columna de agua. Sin embargo, diversos tipos de perturba-

ciones, tales como episodios de fuertes lluvias, movimientos horizontales de masas de agua y, en especial, el fenómeno de blanqueamiento que ocurre todos los años hacia mitad del verano, alteran en cierta medida el patrón sucesional.

Palabras clave: *Fitoplancton, sucesión, grupos funcionales, estratificación, lagos meromíticos*

INTRODUCTION

The seasonal composition of the phytoplankton assemblages follows regular patterns within the annual cycle (Padisák *et al.*, 1998; Morabito *et al.*, 2003; Salmaso & Padisák, 2007). Relatively few species are dominant at any given time, and seasonal species substitution occurs according to the contrasting strategies adopted by the different species (r-K Margalef, 1979, Sommer, 1986; R-C-S Reynolds, 1997) The process is mainly governed by changes in light and nutrient availability (Margalef, 1979; Reynolds, 1984) in a recurrent annual sequence. Reynolds *et al.* (2002), and many other authors thereafter (e.g. Kruk *et al.*, 2002; Morabito *et al.*, 2003; Huszar *et al.*, 2003; Padisák *et al.*, 2009; Salmaso *et al.*, 2015), have developed a phytoplankton classification into functional groups, whose associations are mainly determined by environmental conditions such as the physical properties of the water column and the trophic status. This functional classification of freshwater phytoplankton is based on their preferences on features related to seasonal stratification and mixing changes, but adds the trophic characteristics of the system and its different strata, as well as the vertical phytoplankton distribution.

After studying the phytoplankton abundance and biomass dynamics, the present work analyses the characteristic phytoplankton succession at the Laguna de La Cruz, a meromictic lake in Cuenca (Central-Eastern Spain), both at taxonomic and functional levels. Special attention is paid to the time-course of diversity as a descriptive parameter for the progression of succession. The establishment of both temporary and permanent pycnoclines in this lake is of paramount importance as this creates a marked stratification of the water column, with an anoxic monimolimnion present all year long, and a microaerobic or anoxic hypolimnion developing over the monimolimnion during the stratification period. Our study analy-

ses the differences in phytoplankton succession in the different strata (mixolimnion during mixis, and epilimnion, metalimnion and oxycline during stratification). In order to better visualise the main pattern of seasonal succession and to clarify the factors determining the spatio-temporal distribution of phytoplankton assemblages in this lake, several multivariate statistical analyses were carried out, aimed at describing the dynamic patterns of phytoplankton.

This was the last manuscript Prof. Maria Rosa Miracle was working on before she passed away. Here, she was making an effort to synthesise long-term observations that our group made in Laguna de La Cruz, one of her best studied lakes. With this manuscript her collaborators have tried to conclude her task, aiming to find the ultimate pattern of phytoplankton succession in one of her preferred study lakes.

MATERIAL AND METHODS

Study area

Laguna de La Cruz (Lake La Cruz) is a meromictic sink hole lake located in the south of the “Serranía de Cuenca”, at an altitude of 1032 m a.s.l. Several holomictic lakes are also found in the sink holes (“torcas”) within this area, known as the “Torcas de Cañada del Hoyo” (UTM30SWK9627) (Roca *et al.*, 2000; Rodrigo *et al.*, 2000, 2001). Laguna de La Cruz occupies the lower half of the frustoconical sink hole. During the study period, its surface covered around 1 Ha, with a maximum depth between 21 m and 22 m. This lake is mostly fed by groundwater, and presents a monimolimnion rich in calcium bicarbonate, delimited by a permanent chemocline located between 16 m and 18 m of depth, that is defined by a conductivity gradient. In addition to the chemical stratification, the lake presents a marked thermal stratification beginning by middle spring, with a thermocline deepening as season advances. This process allows the forma-

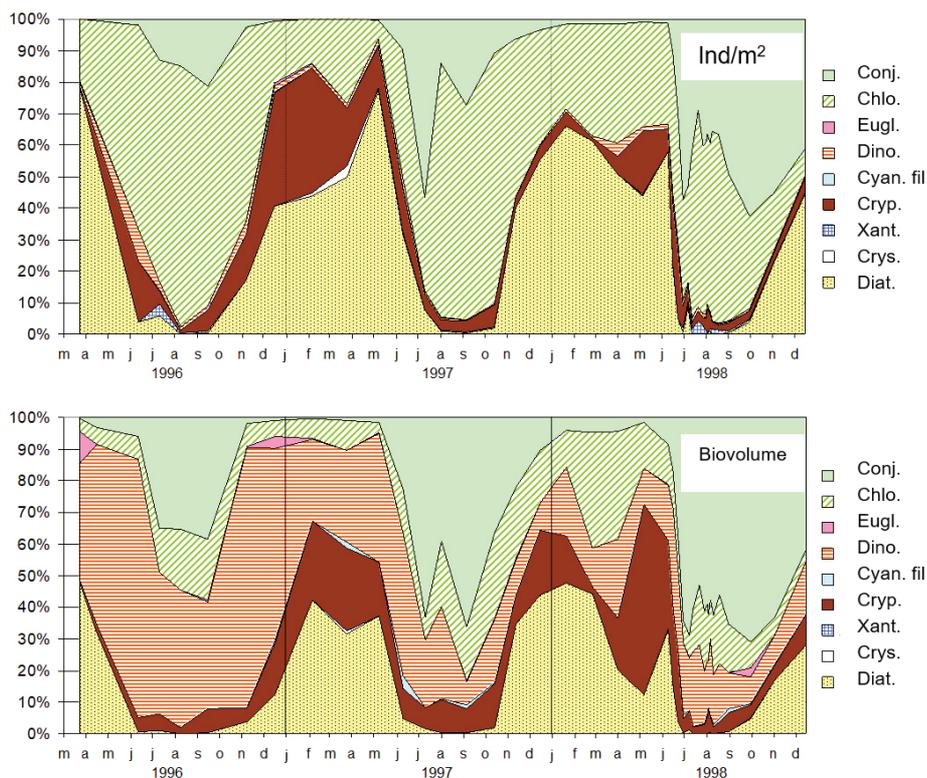


Figure 1. Relative contribution (%) of the different nano- and micro-phytoplankton groups integrated for the whole oxic water column of Laguna de La Cruz throughout the study period. The results are given as a percentage (%) of the individuals/m² (above) and of biovolume (below). *Contribución relativa (%) de los diferentes grupos de nano- y microfitoplancton integrados para toda la columna de agua óxica de la Laguna de La Cruz a lo largo del periodo de estudio. Los resultados se proporcionan como porcentaje (%) de los individuos/m² (arriba) y como biovolumen (abajo).*

tion of an anoxic hypolimnion which, in turn, increases in volume to reach the lower limit of the metalimnion in summer months. In summer, the oxic-anoxic interphase is located at around 11 m (Camacho *et al.*, 2017). Limnological information about the characteristics of this lake dates back to studies performed from 1980s onward (e.g. Vicente & Miracle, 1988; Miracle *et al.*, 1992; Rodrigo *et al.*, 2001). However, a broader and detailed survey of the temporal and spatial patterns of temperature, light penetration, chlorophyll-*a* concentration, and picocyanobacterial abundance, was performed monthly from 1996 to 1998 (Camacho *et al.*, 2003a). Conversely, however, almost no data on nano- and microphytoplankton have been available for these years, though hundreds of samples were collected. We here cover this gap, by analysing these samples, in

order to unveil the community patterns of phytoplankton in Lake La Cruz. This is among the soundest studies so far performed on the phytoplankton community of a meromictic lake (Zadereev *et al.*, 2017).

Sampling and analytical and statistical methods

Sampling was done monthly at the lake's central point, beginning in March 1996 and ending in December 1998. In addition to monthly sampling, additional samples were collected with a weekly frequency in the summer 1998, to better investigate the usual whitening event, caused by tumultuous calcium carbonate precipitation, repeatedly occurring every summer (Camacho *et al.*, 2017).

The samples used to study phytoplankton were collected with a double cone fine-layer

sampler (Miracle *et al.*, 1992) or a Ruttner bottle depending on the stratification status, then pooled into glass topaz 250 ml bottles and immediately fixed with Lugol (Wetzel & Likens, 2000). Different depths of the vertical profile covering the different strata were sampled. At each sampling event the number of samples varied according to strata thickness: 2-3 samples from the epilimnion, 3-7 samples from the metalimnion, 1-4 from the oxic hypolimnion and 1 sample from the oxic-anoxic interphase. In order to determine the sample collection depths, the corresponding vertical profiles of water electrical conductivity and temperature (WTW LF-191 conductivity meter with a temperature probe), dissolved oxygen (WTW digital meter, model OXI-91), light penetration (Li-Cor model Li-1000), and pH and Eh (with two electrodes ORION model 81-02SC and ORION model 96-78, respectively), were obtained *in situ* at the sampling point. Simultaneously, samples were collected to analyse chlorophyll-*a*, nutrients and dissolved salts concentrations. All these data, as well as the results of the picocyanobacterial counts, can be found in Camacho *et al.*, 2003a.

For phytoplankton quantification, the Utermöhl sedimentation method (1958) was followed by subsequent counting with a Zeiss inverted microscope (CARL ZEISS, Germany 473012-9902). Phytoplankton species were identified mainly following Huber-Pestalozzi (1968), Förster (1982), Komárek and Fott (1983), Krammer and Large-Bertalot (1986-1988-1991a-1991b) and Popovský and Pfiester (1990). In order to calculate algal biovolumes, the formulae of the volumes of the nearest geometric forms, or their corresponding combinations, were applied according to Rott (1981) and Hillebrand (1999). Phytoplankton species were assigned to different functional groups following Reynolds *et al.* (2002) and Padisák *et al.* (2009). The point samples data were integrated per square meter of the oxic water column as follows:

$$T_i = \sum_{k=1}^n X_{ik} \left(\frac{Z_{k+1} - Z_{k-1}}{2} \right)$$

where:

T_i = total individuals of the species *i* in a water column of one square metre section, ranging from the surface to the oxygen extinction depth.

k = each sampling depth

Z_{k+1} = next depth below depth k

Z_{k-1} = previous depth above depth k .

X_{ik} = number of individuals per cubic metre of species *i* that was measured at depth Z_k .

Diversity was computed using the Shannon diversity index (Shannon & Weaver, 1963; Margalef, 1979) for each individual sample depth as well as for the phytoplankton abundances of all species integrated within the oxic water column. The heterogeneity of the phytoplankton community within the water column (Margalef, 1979), D_β , was expressed as the ratio between the effective number of species, i.e., $\exp(H')$ (Jost, 2006), which resulted from the diversity data of the integrated water column and the mean of those corresponding to the different sampling depths. H' is the Shannon-Wiener entropy, as follows:

$$H' = - \sum_{i=1}^S p_i \ln p_i \text{ (Shannon \& Weaver, 1963).}$$

Statistical analyses were run using SPSS for Windows v11 and MVSP v3 software (Multivariate Statistical Package for Windows, Kovach, 1998). A Principal Component Analysis (PCA) was performed for the primary ordination of phytoplankton assemblages to unveil the successional pattern. Thereafter, a Canonical Correspondence Analysis (CCA) was performed to relate the main environmental explanatory variables with the phytoplankton assemblages. Normalisation ($\log x+1$) was performed in both cases when convenient after checking data distribution.

RESULTS

Seasonal variation of the different algal groups

When considered in terms of algal abundance, a clear alternation occurred during the annual cycle between diatoms and chlorophytes. Diatoms were the most abundant phytoplankton group during the mixing periods, whereas chlorophytes were the most abundant during summer stratification

mixing period. Euglenophytes, appearing mainly in autumn, and filamentous Cyanobacteria, occasionally present in summer, were also very scarce, and their modest appearance always occurred close to the oxyline. Contrastingly, picocyanobacteria (*Synechococcus*) accounted for an important part of the primary producers, though they are not detailed in this paper since their dynamics was previously described by Camacho *et al.* (2003a).

Succession of the most important phytoplankton taxa

Succession in the phytoplankton of Laguna de La Cruz started with autumnal mixing, with the development of an almost mono-specific *Cyclotella delicatula* population, corresponding to the codon B, accounting for nearly 90 % of diatom's biovolume. In the first part of the mixing period, in autumn, some pennate diatoms from genus *Fragilaria* (codon P), as well as cryptophytes like *Rhodomonas minuta*, *Cryptomonas phaseolus* and *C. obovata* (codon Y), also appeared. Species that develop at the end of summer, but also grow in autumn, were characteristic, such as *Cosmarium bioculatum* (codon N_A), *Pseudoquadrigula* sp. (codon F), *Lagerheimia* sp. (codon J) and *Peridinium umbonatum* (codon L₀).

Cyclotella delicatula also dominated winter phytoplankton assemblages, co-occurring with

Gymnodinium cf. *bogoriense*. Fast growth of *Synechococcus* sp. (Picocyanobacteria, belonging to codon Z) occurred in late winter and early spring through the oxic water column, though they were restricted to the metalimnion in summer. Some species of *Rhodomonas* (*R. minuta*, *R. lacustris*) belonging to codon X₂, and of *Cryptomonas* (*C. obovata*, *C. phaseolus*; codon Y) also developed in late winter, but they were later restricted to the oxycline, where they reached their maximum abundance during stratification. With the advent of the stratification, chlorophytes became dominant, especially *Kirchneriella irregularis*, *Pedinomonas minor*, *Scenedesmus praetervisus*, *Lagerheimia quadriseta*, *Chlorella vulgaris*, *Tetraedron minimum* and *Pseudoquadrigula* sp. Through summer, larger sized, S-strategists, algae became more abundant, such as *Ceratium hirundinella*, *Peridinium cinctum*, and species of genus *Cosmarium* (e.g., *C. abbreviatum*, *C. laeve*, *C. meneghinii* and *C. bioculatum*). Following Reynolds' functional classification, the species of *Peridinium*, mainly *P. cinctum*, belong to the codon L₀, which dominates in the epi-metalimnion of the stratified water columns along with *C. hirundinella*. The species of genus *Cosmarium* belong to group N_A, where they are usually found in association with pennate diatoms (e.g. *Tabellaria* spp.), though these pennate diatoms (in our case *Fragilaria* and *Nitzschia*) only grew significantly in the Laguna

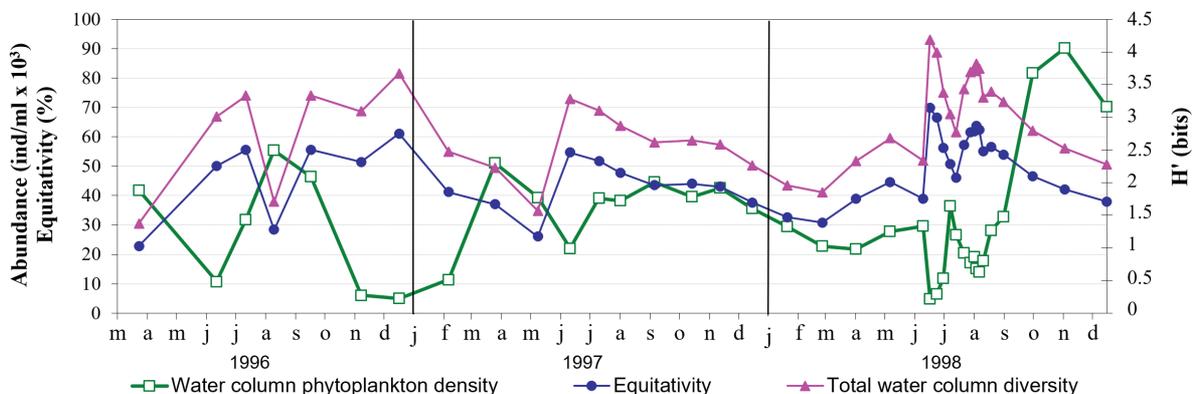


Figure 3. Phytoplankton abundance (ind/ml $\times 10^3$) in the water column of Laguna de La Cruz, Shannon diversity index (bits) and evenness (equitativity, %) of the whole water column. *Abundancia de fitoplancton (ind/ml $\times 10^3$) en la columna de agua de la Laguna de La Cruz, índice de diversidad de Shannon (bits) y equitatividad (%) en toda la columna de agua.*

de La Cruz in autumn of 1998, when the *Cosmarium bioculatum* population reached its highest values. Following the functional classification of phytoplankton by Reynolds *et al.* (2002), the successional sequence in the epi-metalimnion is:

$$B, Z \rightarrow (X, F, J) \rightarrow Lo \rightarrow N_A \rightarrow B, Z$$

In our work, phytoplankton succession in Laguna de La Cruz was differentially considered in two different strata, the epi-metalimnion and the oxycline. Succession in the oxycline advances much slower, and species of coda **Y**, **Z** and **W1**, which correspond to Cryptophyta, picocyanobacteria and Euglenophyta respectively, replace those of groups **B** and **Z** during the stratification period. The sequence at the oxycline would be as follows:

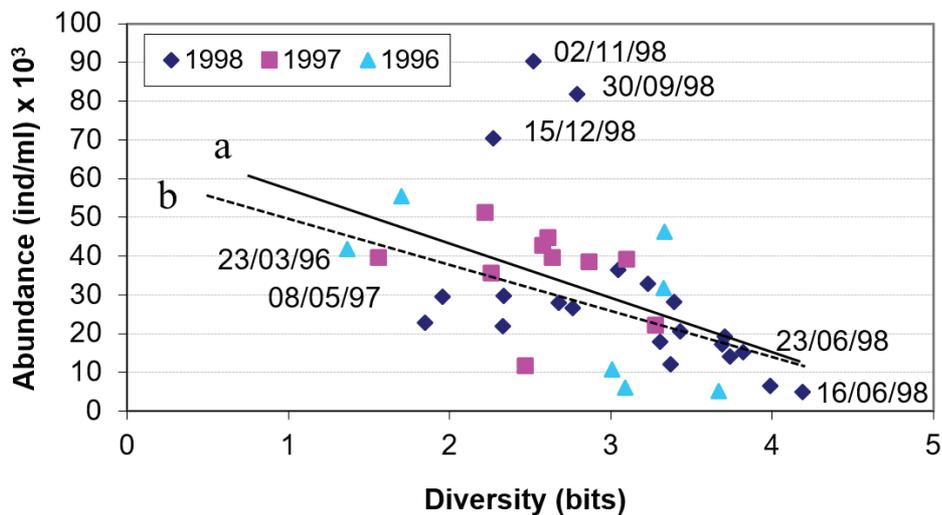
$$B, Z \rightarrow (Z, X2, \rightarrow Y \rightarrow W1) \rightarrow B, Z$$

Figure 2 shows the Mandala representing phytoplankton succession in Laguna de La Cruz

during the 1996-1998 period, along with a second Mandala representing the characteristic vertical distribution of phytoplankton during the stratification period. These mandalas show the pattern of diatoms maxima occurring in winter, that of chlorophytes in summer, conjugated algae at the end of summer and autumn, and cryptophytes in summer, though the later concentrated at the oxycline.

Seasonal variation of phytoplankton abundance, biomass and diversity

Our study clearly shows an inverse relationship between phytoplankton abundance and diversity (Figs. 3 and 4). This relationship is statistically significant, $r^2 = -0.26$, $p < 0.01$, (Fig. 4), even strongest ($r^2 = -0.506$, $p < 0.01$) when the data from autumn 1998, with moderate diversities but very high abundance of *Cosmarium bioculatum* and *Cyclotella delicatula*, were excluded from the correlation. At the beginning of spring, abundance increases and phytoplankton diversity



a: $n=38$, $r = -0.611$, $p < 0.01$, $r^2 = 0.26$

b: without autumn 1998, $n=35$, $r = -0.506$, $p < 0.01$, $r^2 = 0.37$

Figure 4. (a) Relationship between the phytoplankton abundance ($\text{ind/ml} \times 10^3$) and the Shannon diversity index (bits) of phytoplankton for the whole water column of Laguna de La Cruz for all samples of the study period, or (b) excluding the samples of autumn 1998. (a) Relación entre la abundancia de fitoplancton ($\text{ind/ml} \times 10^3$) y el índice de diversidad de Shannon (bits) del fitoplancton para toda la columna de agua de Laguna de La Cruz para todas las muestras del periodo de estudio, o (b) excluyendo muestras de otoño de 1998.

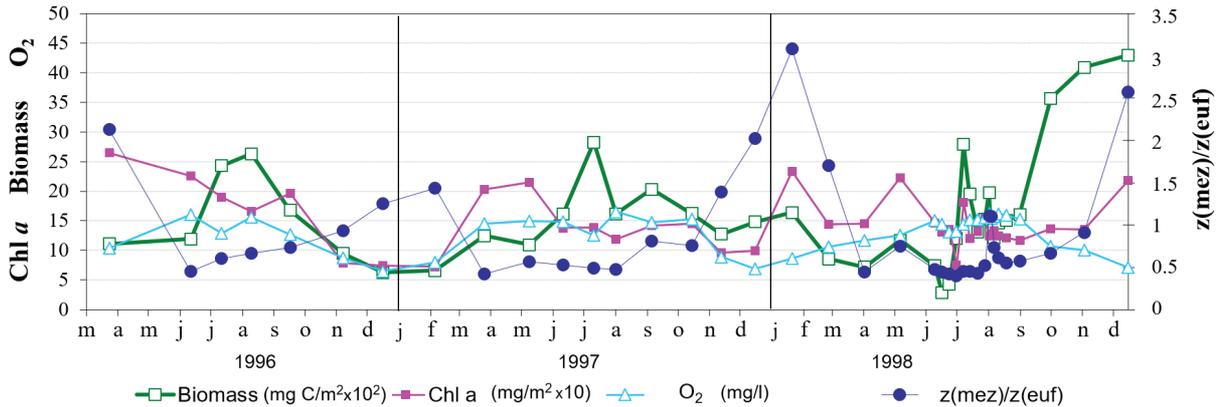


Figure 5. Integrated water column phytoplankton biomass ($\text{mg C/m}^2 \times 10^2$), integrated chlorophyll-*a* concentration ($\text{mg/m}^2 \times 10$), maximum concentration of dissolved oxygen (mg/l), and the quotient between mixing depth and the depth of the euphotic zone, during the studied period in Laguna de La Cruz. *Biomasa fitoplanctónica integrada de la columna de agua ($\text{mg C/m}^2 \times 10^2$), concentración de clorofila-*a* integrada ($\text{mg/m}^2 \times 10$), concentración máxima de oxígeno disuelto (mg/l), y cociente entre la profundidad de mezcla y la profundidad de la zona eufótica, durante el periodo estudiado, en la Laguna de La Cruz.*

drops. Yet, towards the end of spring and the beginning of summer, a sudden change occurs and the opposite situation prevails for a short period, with a drop in abundance and an increase in diversity. Conversely, halfway through summer, nutrient inputs occurred due to the horizontal transport from the shores, resulting in growth peaks accompanied by reduced diversity, although diversity decreases throughout autumn. Chlorophyll-*a* concentrations do not always match phytoplankton biomass (Fig. 5), being quite dependent on the size and relative chlorophyll content of the dominant taxa at each period, as well as on the relative contribution to the chlorophyll-*a* pool of picocyanobacteria (see Camacho *et al.*, 2003a for detailed data on picocyanobacteria abundance).

The seasonal development of phytoplankton in Laguna de La Cruz varied, being influenced by the weather conditions, in different years. Along the studied years, maximum phytoplankton abundance differed according to the intensity and depth of vertical mixing, represented by the ratio between the depth of the mixing zone (Z_{mez}) and the euphotic depth (Z_{euf}). Indeed, this ratio for the 1997-1998 mixing period (2.05) doubled that of the previous (1996-1997) hydrological cycle (1.25) (Fig. 5). The stable weather conditions in the autumn of 1998 favoured a greater stability

and coincided with higher biomass in autumn compared to the previous years, though diversity still remained high in this period (Figs. 3 and 4). During the mixing periods, especially in winter and the beginning of spring, phytoplankton diversity was lower than the average of the stratification periods (Fig. 3). Furthermore, the lowest phytoplankton diversity within any water column layer during stratification was found in the epilimnion, in such a way that the aforementioned processes, i.e., variations in diversity through sporadic nutrient inputs, were much more visible in this layer. Contrastingly, the oxic meta-hypolimnion generally presented higher diversity than the epilimnion during the well-established stratification in summer (Fig. 6), when many phytoplankton species developed at these relatively stable layers that show differential conditions at different depths, yet differentially offering contrasting niches with respect to light and nutrient availability. Nevertheless, the oxic-anoxic interphase (oxycline) also presented high values of the Shannon diversity index, partly supported by the sedimentation of several species that accumulate in this area by equalizing buoyancies to the water density gradient (see Camacho, 2006, for details).

A proxy for β -diversity, the heterogeneity of the phytoplankton community within the water

column (D_{β}), was related to the difference between the surface temperature and that of the bottom of the lake (Fig. 7 - the arrows show the seasonal pattern displayed by this relationship). All sampling events corresponding to the lake's mixing period remain in the lower left quadrant, with low temperatures linked to low heterogeneity. Autumn samplings can be divided into two groups: those before mixing (top left part of the chart) showing higher D_{β} , and those after mixing (lower left part of the chart) with lower D_{β} .

Spring and summer samplings, with large vertical differences in temperature, remain on the right-hand side of the figure, with D_{β} values above 1, except for the sampling dates that coincided with algal blooms. These blooms were caused either by the development of: i) *Lagerheimia quadriseta* in the epilimnion and *Kirchneriella irregularis* in the hypolimnion in August 1996, and to ii) the development of populations of *Cyclotella delicatula*, *Cyclotella distinguenda* and *Pedinomonas minor* at the beginning of June

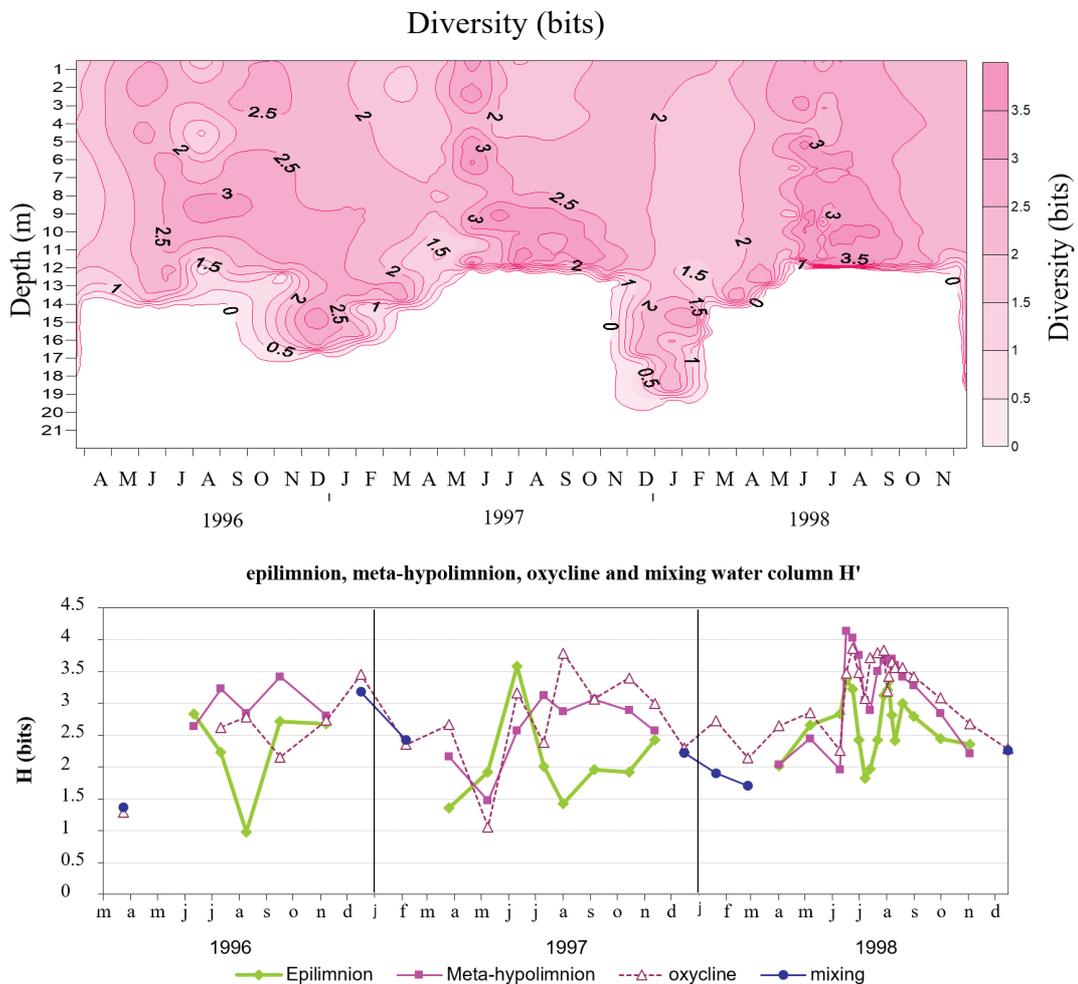


Figure 6. (Above) Isopleths showing the spatio-temporal variation of the Shannon diversity index (bits) in Laguna de La Cruz throughout the study period; and (Below) the Shannon diversity index (bits) calculated for the samples of the different layers: epilimnion, meta-hypolimnion, oxycline, and mixolimnion up to the oxycline during the mixing period. (Arriba) Isolíneas que muestran la variación espacio-temporal del índice de diversidad de Shannon (bits) en la Laguna de La Cruz durante todo el periodo de estudio; y (abajo) índice de diversidad de Shannon (bits) calculado para las muestras de las diferentes capas: epilimnion, meta-hipolimnion, oxiclina, y mixolimnion hasta la oxiclina durante el periodo de mezcla.

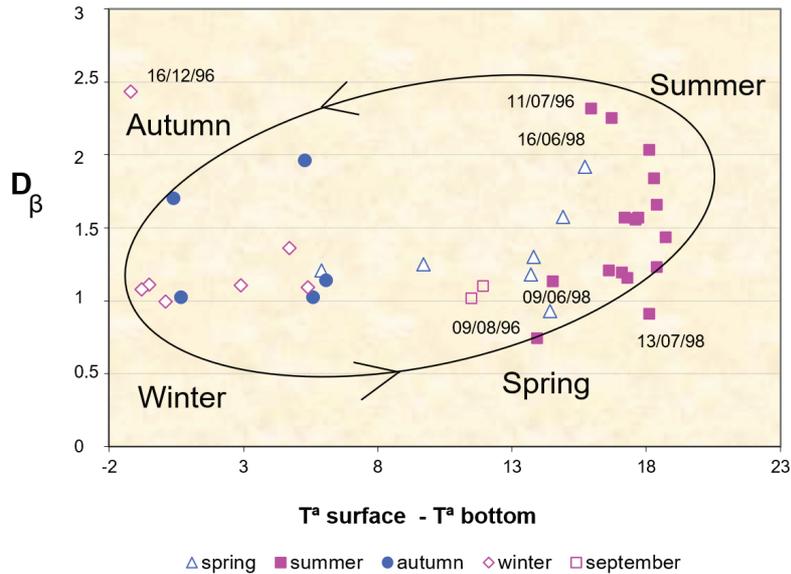


Figure 7. Relationship between the heterogeneity of the phytoplankton assemblages - D_{β} - (total column H' exponential / sample mean H' exponential), and the difference between the surface temperature and the bottom lake temperature. *Relación entre la heterogeneidad de los ensamblados de fitoplancton - D_{β} - (toda la columna H' exponencial/ muestra media H' exponencial), y la diferencia entre la temperatura de la superficie y la del fondo del lago.*

1998, and to the massive development of *Chlorella vulgaris* and *Cosmarium abbreviatum* in mid-July 1998. After the whitening event commonly occurring in August (Camacho *et al.*, 2017), the phytoplankton assemblages altered its vertical structure, being more homogeneously distributed through the water column.

Statistical analyses

In order to globally study the behaviour of the phytoplankton assemblages during the annual cycle, a principal component analysis (PCA) was performed (Fig. 8) using the abundance values (log-transformed) of the 31 most frequent phytoplankton species appearing in 1998, the sampling year that included more details (209 cases -depths- distributed within 21 sampling events). Additionally, a canonical correspondence analysis (CCA) was also done with the same data, where environmental variables were additionally considered in order to unveil the relationship between the phytoplankton assemblages and key environmental variables (Fig. 9).

The first axis resulting from the PCA explained 25 % of variance, whereas the second axis explained an additional 15 %. Samples distributed in the space defined by these two axes according to a sequence which followed the seasonal succession, (horizontal axis separated spring and autumn samples) (Fig. 8). In the ordination sequence, the summer samples corresponding to the oxycline followed those of spring. This shows the fact that some species thriving in upper layers in spring were confined to the oxycline upon stratification later on. This successional sequence also shows the summer epilimnetic samples as the closest to the autumn samples on the other side of horizontal axis, which indicates that the characteristic species of upper layers in summer remain in autumn. Within the sequence, autumn samples are followed by the samples of the mixing period, which again connect with the spring samples, showing a nice drawing of the seasonal succession of phytoplankton in Laguna de La Cruz. The vertical axis also clearly separates the samples from the mixing period, on the upper part of the chart, from those of stratification, mainly found in the lower part.

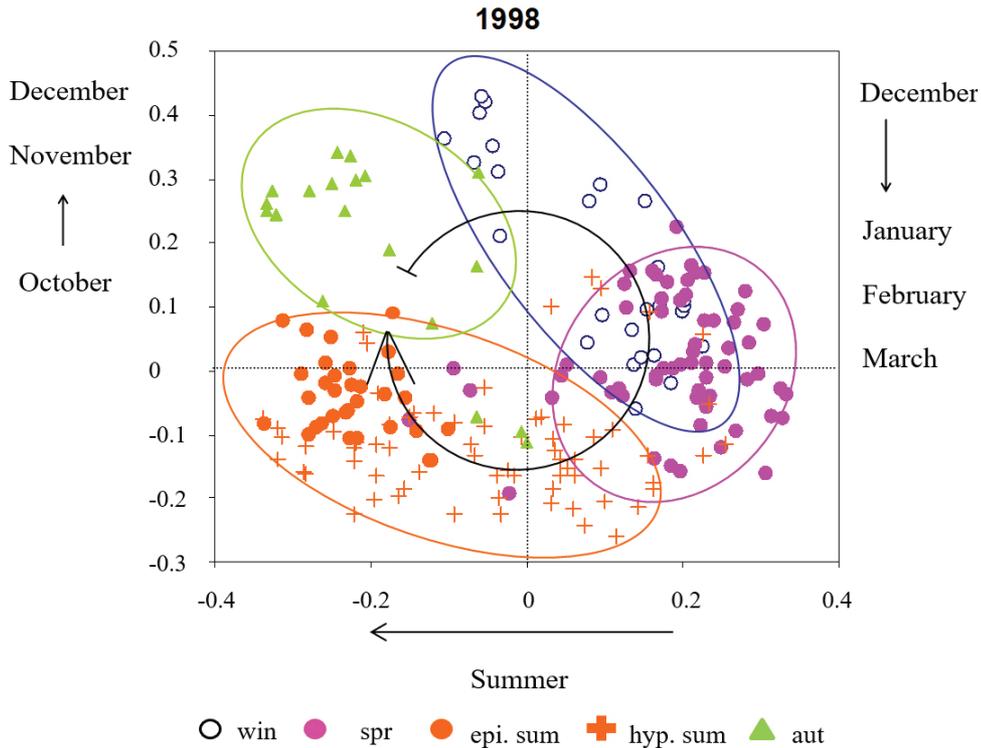


Figure 8. Situation of the phytoplankton samples taken in 1998 in Laguna de La Cruz in the space defined by the first two factors of the PCA, showing differentially the winter, spring, summer epilimnion, summer hypolimnion, and autumn samples. *Situación de las muestras de fitoplancton tomadas en 1998 en la Laguna de La Cruz en el espacio definido por los dos primeros factores de la PCA, que muestra diferencialmente las muestras de invierno, primavera, epilimnion de verano, hipolimnion de verano y otoño.*

The CCA (Fig. 9) sequentially ordered both the species (Fig. 9a) and the samples (Fig. 9b) by grouping those from winter (December, January, February and March), from early-middle spring (April, May and the first week of June), from late spring (weeks 2 and 3 of June), from summer (last week of June, and July and August) and from autumn (October and November). The CCA also separated samples from upper depths from those of the oxycline, and also counterposed samples from the mixing period to those of stratification.

From the CCA using the species data (Fig. 9a), the following phytoplankton assemblages were unveiled: 1) the characteristic species of the mixing period, *Cyclotella delicatula*, *C. distinguenda*, *Gymnodinium bogoriense* and *Kirchneriella irregularis*, which are distributed homogeneously in the water column, but are not characteristic of the oxic-anoxic interphase, and

are related mainly with nitrate, which was more abundant during the mixing period (see Camacho *et al.*, 2003a for detailed data on water chemistry); 2) *Cryptomonas erosa* and *Scenedesmus praetervisus*, whose densities were higher in spring, 3) *Scenedesmus ecornis*, *Oocystis lacustris*, *Cryptomonas* sp., *Cryptomonas obovata* and *Rhodomonas lacustris*, which, despite of being present throughout the mixing period throughout the whole water column, were relegated to the metalimnion with the advent of stratification; 4) *Rhodomonas minuta*, *Cryptomonas phaseolus* and *Ankistrodesmus gelifactum*, which are characteristic species of the oxycline, mostly developing during the stratification period. *Euglena acus* remains away as it just appeared in very few oxycline samples, which coincided with the maxima of picocyanobacteria found at these depths. On the other hand, some species arise as

characteristic of the stratification period, though no clear preference was observed by these species for a given layer when compared between the upper layers and the oxycline. These species are *Cosmarium laeve*, *C. bioculatum*, *C. meneghinii*, *Pseudoquadrigula* sp. and *Trachydiscus minutus*. Comparatively, *Chlorella vulgaris* and *Lagerheimia ciliata* appear as typical epilimnetic or metalimnetic species, respectively, whereas *Peridinium umbonatum* and *Pedinomonas minor*, however, are typical epilimnetic species, with maximum abundance in spring. *Monoraphidium subclavatum* and *Cosmarium abbreviatum* peaked both at the beginning of the summer and, later on, in autumn. Comparatively, the most ubiquitous species remain in a central position of the CCA chart (Fig. 9a).

The groups of the species resulting from both the PCA and CCA describe the succession of the most relevant phytoplankton species in Laguna de La Cruz, and coincide with the functional groups previously described.

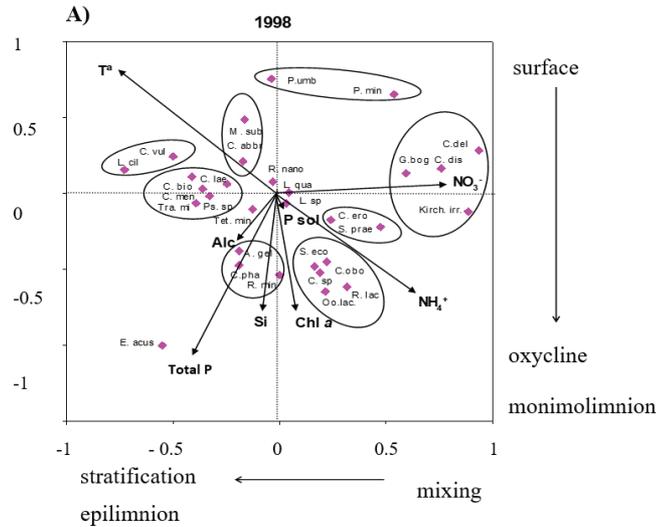
DISCUSSION

The seasonal succession of phytoplankton in Laguna de La Cruz only partly resembles that of other stratified temperate lakes (Margalef, 1979; Reynolds, 1997). As previously reported (Kiss *et al.*, 2007), phytoplankton succession in Laguna de La Cruz begins with diatoms becoming dominant during the autumn mixing period, especially *C. delicatula*. After a maximum in spring, diatoms virtually disappear, while other species replace them. Phytoplankton succession has been investigated in many lakes such as e.g., in the deep, oligotrophic and alkaline Lake Stechlin (Padisák *et al.*, 1998), where a similar pattern was reported. However, the meromictic nature of the Laguna de La Cruz, with its partial vertical mixing above the monimolimnion by the establishment of an overlying oxycline during the stratification period, allows for the occurrence of a deep stratum where diatoms remain until mid-spring, thus the succession pattern differs from other deep lakes in the vertical distribution of dominant populations. As a general framework, it is noteworthy that the density and viscosity of the water governed by temperature varia-

tion, may strongly determine the seasonal sinking behaviour of phytoplankton and determine its seasonal succession. Additionally, changes in cell or colony size also could enable some species to overcome temperature-dependent changes in water density and viscosity and adjust their sinking velocities in the different seasons (Zohary *et al.*, 2017).

The dominance of diatoms during the mixing period, when nutrients are renewed, is associated with the characteristics of many species of the genus *Cyclotella* as r-strategists that follow general phytoplankton succession patterns (Margalef, 1979; Sommer, 1986), and belong to in the C-category defined by Reynolds (1997). Following the functional phytoplankton classification, small centric diatoms, like *C. delicatula*, would belong to the codon **B**, typical of vertically mixed, mesotrophic small-medium size lakes (Padisák *et al.*, 2009), that includes species sensitive to silica deficiency and to stratification, yet tolerant to low light intensity. Hence, they can remain in the metalimnion for some time when stratification begins (Reynolds *et al.*, 2002).

The CCA confirms that the *Cyclotella* dynamics in Laguna de La Cruz can be associated with variations in the concentration of silica, as it has been well established by other authors (e.g. Flynn & Martin-Jézéquel, 2000; Cetin & Sen, 2004) that also observed silica depletion following diatoms' peaks. Another factor that favours diatom development is turbulence, which allows them to remain in the photic zone. When turbulence decreases, as it occurs in Laguna de La Cruz with the onset of the thermal stratification, diatoms start to be replaced by other algae. Pico-cyanobacteria (Camacho *et al.*, 2003a), as well as *Rhodomonas* and small chlorophyceae, peak later than diatoms, in spring. The faster growth of these picocyanobacteria, *Synechococcus*, occurred in late winter and early spring through the oxic water column, though they were restricted to the metalimnion in summer (Camacho *et al.*, 2003a). Pico-cyanobacteria (codon **Z**), *Rhodomonas* (codon **X2**), as well as *Cryptomonas* (codon **Y**), all of them tolerating low light availability (Gasol *et al.*, 1992, 1993; Camacho *et al.*, 2001, 2003a), dominated the lower metalimnion and the oxycline during stratification. Nutrient exhaus-



A. gel: *Ankistrodesmus cf. gelifactus*; C. abbr: *Cosmarium abbreviatum*; C. bio: *Cosmarium bioculatum*; C. dis: *Cyclotella distinguenda*; C. er: *Cryptomonas erosa*; C. lae: *Cosmarium leave*; C. men: *Cosmarium meneghini*; C. sp: *Cryptomonas sp.*; C. vul: *Chlorella vulgaris*; C. del: *Cyclotella delicatula*; C. obo: *Cryptomonas obovata*; C. pha: *Cryptomonas phaseolus*; E. acus: *Euglena acus*; G. bog: *Gymnodinium cf. bogoriense*; Kirch. irr: *Kirchneriella irregularis*; L. cil: *Lagerheimia ciliata*; L. qua: *Lagerheimia quadriseta*; L. sp: *Lagerheimia sp.*; M. sub: *Monoraphidium subclavatum*; Oo. Lac: *Oocystis lacustris*; P. min: *Pedinomonas minor*; P. umb: *Peridinium umbonatum*; Ps. sp: *Pseudoquadrigula sp.*; R. lac: *Rhodomonas lacustris*

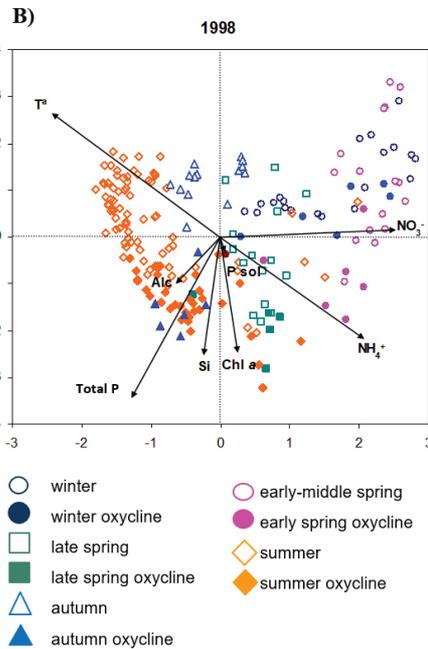


Figure 9. a) Situation of the main phytoplankton species and vectors that correspond to the different environmental variables (ammonium, nitrate, soluble phosphorus, silicate, temperature, total phosphorus and chlorophyll-a), from samples of 1998, in the space defined by the first two factors resulting from the CCA. b) Situation of the samples and vectors that corresponded to the different environmental variables from samples of 1998 in the space defined by the first two factors that resulted from the CCA. a) *Situación de las principales especies de fitoplancton y vectores que corresponden a las diferentes variables ambientales (amonio, nitrato, fósforo soluble, silicato, temperatura, fósforo total y clorofila-a), a partir de las muestras de 1998, en el espacio definido por los dos primeros factores resultantes de la CCA.* b) *Situación de las muestras y vectores que correspondieron a las diferentes variables ambientales de las muestras de 1998 en el espacio definido por los dos primeros factores que se obtuvieron de la CCA.*

tion in upper layers (Camacho *et al.*, 2003a) poses nitrogen limitation to picocyanobacteria (Camacho *et al.*, 2003b), while the drop in both the *Rhodomonas* and Chlorophyceae populations towards the end of spring could also be favoured by the grazing by the cladoceran *Diaphanosoma brachyura*, whose population considerably increases at this time (Boronat, 2003). Both zooplankton grazing and nutrient exhaustion in the epilimnion would explain the phytoplankton decrease at the end of spring, as predicted by the PEG model (Sommer *et al.*, 1986).

During the summer stratification period, small chlorophytes and species of the genus *Cosmarium* account for phytoplankton recovery in the epilimnion. With the advent of the stratification these chlorophytes became dominant, with species like *Pedinomonas minor*, *Lagerheimia quadriseta*, *Kirchneriella irregularis*, *Tetraedron minimum*, *Scenedesmus praetervisus*, *Chlorella vulgaris*, and *Pseudoquadrigula* sp. These are C-strategist according to Reynolds' (1997) classification of phytoplankton life-strategy, showing a good nutrient assimilation capacity, and a high replication ability after abrupt changes in the water column conditions. They included species belonging to the codon **F**, which groups algae tolerating low nutrient concentrations and high turbidity; to codon **X**, whose species tolerate stratification but are sensitive to nutrient shortage and feeding by zooplankton (*Chlorella*, *Tetraedron*); and the **J** group, which includes *Scenedesmus*. Throughout summer, when nutrients become increasingly scarce in surface layers and the zooplankton population increases (Boronat, 2003), larger sized algae, S-strategists, were selected; e.g., *Ceratium hirundinella*, *Peridinium cinctum* and species of the genus *Cosmarium*, (basically *C. abbreviatum*, *C. laeve*, *C. meneghinii* and *C. bioculatum*). Following Reynolds' functional classification, the species of genus *Peridinium*, mainly *P. cinctum*, belong to the codon **L0**, which dominates in the epi-metalimnion of the stratified water columns along with *C. hirundinella*. The species of genus *Cosmarium* belong to group **N_A**, and are usually found in association with pennate diatoms (e.g. *Tabellaria* spp.), though these pennate diatoms (in our case *Fragilaria* and *Nitzschia*) only grew significantly in the Laguna de La Cruz in the

autumn 1998, when the *Cosmarium bioculatum* population reached its highest values.

The autumn overturn in Laguna de La Cruz is not only due to wind-induced vertical mixing, but it is also strongly influenced by the horizontal circulation of convection currents from the littoral zone to the lake's centre (MacIntyre & Melack, 1995). These horizontal movements occur when nocturnal heat losses become very strong in late autumn, though a steep vertical density gradient is still present. Then, convective motions induced by thermal instabilities could be as important as wind mixing for the aeration and upwelling of deep water. This would partly support the growth peaks of the species with littoral affinities like those of the genus *Cosmarium* during this period. This is also shown by the interannual variations of autumnal phytoplankton development; e.g. in mid-late autumn 1998, a slightly shallower thermocline with a very steep density gradient greatly favoured blooms of *Cosmarium* species. Atelomixis, this is, partial mixing of the water column, has also a role in favouring *Cosmarium* species (Souza *et al.*, 2008), allowing that these non-motile species can reduce sinking thanks to the turbulence created by this partial mixing. This could also be important even for small species like *C. bioculatum*, which blooms under such conditions in Laguna de La Cruz.

The influence of grazing on phytoplankton species succession is evidenced in Laguna de La Cruz when observing the anti-predation features of phytoplankton species during the stratification period, when zooplankton densities are the highest, especially of rotifers and cladocerans of genus *Diaphanosma* and the species *Ceriodaphnia dubia*; (Boronat, 2003). For example, Mayeli *et al.* (2004), confirmed how both a larger colony size and spine formation in *Scenedesmus quadricauda* are effective defences against grazing by rotifers and small cladocera like *Ceriodaphnia dubia*. As the stratification period advances, larger sized phytoplankton species, which are mainly inedible for the dominating zooplankton, become more important in Laguna de La Cruz. Moreover, many small chlorophyceae that grow in this lake mostly during the stratification period present structures that are useful to avoid

zooplankton grazing, but also contribute to slow down sinking. As such, *Lagerheimia* and *Scenedesmus* display spines, *Kirchneriella* and *Pseudoquadrigula* are surrounded by a mucilage, and in some *Cosmarium* species, e.g., *C. abbreviatum* and *C. bioculatum*, a thick mucilaginous cover can also be observed. All this supports a role of grazing avoidance, additionally to the main effects of vertical stratification, in shaping the phytoplankton assemblages in Laguna de La Cruz during the stratification period.

In the depths near the oxycline, when silica is still available, *Cyclotella* establishes first at the onset of the stratification, but it is progressively displaced by species that appear in spring in upper layers, such as those of the genera *Synechococcus*, *Rhodomonas* and *Cryptomonas*, which later on remain confined in the nutrient-rich oxycline stratum. These species of coda **Y**, **Z** and **W1**, which correspond to cryptophytes, picocyanobacteria and euglenophytes respectively, replace those of groups **B** and **Z** in deep layers during the stratification period. Nanophytoplankton succession no longer progresses in the oxycline, keeping the characteristic structure of a microbial plate established on the density gradient in the oxycline-redoxcline zone of such type of meromictic lakes (Zadereev, 2017). In Laguna de La Cruz, this is formed by a dense multilayer structure dominated, from top to the bottom of the oxycline, by picocyanobacteria accompanied with some species of chlorophytes, cryptophytes, and euglenophytes, overlying the anoxic layers where sulphur bacteria -Chromatiaceae and Chlorobiaceae- develop (Vicente & Miracle, 1988; Miracle *et al.*, 1992; Dasí & Miracle, 1991, Rodrigo *et al.*, 2000; Camacho *et al.*, 2017). In the upper zone of the oxycline, where nutrients are available, but where both oxygen concentrations and light availability are low, the dominant nanophytoplankton includes species of *Rhodomonas* and *Cryptomonas* which, as the picocyanobacteria, hold phycoerythrin as a photosynthetic adaptation to harvest light at low intensities, but mainly centered on a certain range of the spectrum that can be harvest by this pigment (Gervais, 1998, Camacho *et al.*, 2001, 2003a). The ability of harvesting the dim light reaching these depths, together with its mixotrophic capac-

ity and motility, also explains the development of *Cryptomonas* spp. in the chemocline of Laguna de La Cruz (Camacho *et al.*, 2001).

Our data corroborate previous statements (Dasí & Miracle, 1991) that phytoplankton diversity in Laguna de La Cruz inversely correlates with cell abundance. This negative correlation is a general feature commonly found in many natural lakes, like the neighbouring Laguna del Tejo (Morata *et al.*, 2003), as well as in reservoirs (De León & Chalar, 2003). The lowest diversity was found when phytoplankton abundance peaked, with minimum values obtained in the two samplings when *Cyclotella delicatula* reached a relative abundance of up to 75 % along the water column. This is favoured by the absence of thermal gradients that differentially support environmental heterogeneity that could favour different algal groups at different depths. Contrastingly, higher diversity was found at the beginning and end of summer given the vertical heterogeneity of the water column produced by the thermal stratification, which explains the higher diversity when the water column was considered as a whole (Margalef, 1979; Tilman, 1994). However, in this period, drops in diversity were also observed, especially in the epilimnion, particularly those linked to the whitening event due to tumultuous calcium carbonate precipitation occurring every year around the end of July and the beginning of August. This causes a strong settling of phytoplankton and photosynthetic bacterial cells both by co-nucleation with calcite crystals and by the shadow effect of these crystals (Miracle *et al.*, 2000; Camacho *et al.*, 2003a; Romero-Viana *et al.*, 2006, 2011; Camacho *et al.*, 2017). The differential weather conditions in autumn 1998 (greater difference between the average maximum temperature and the average minimum temperature and the low rainfall compared to previous autumns) favoured greater stability compared to previous years, which comparatively enabled the development of higher biomass still maintaining a relatively high diversity in a heterogeneous water column (Fig. 6). The more intense, and slightly shallower, temperature gradient in the autumn of 1998, would explain the horizontal transport of nutrients and phytoplankton from the littoral zone to the pelagic area (MacIntyre & Melack, 1995).

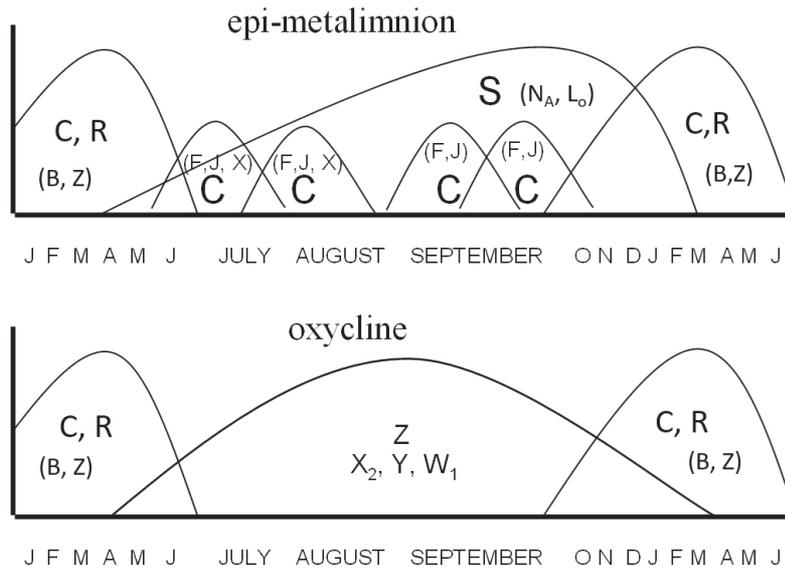


Figure 10. Phytoplankton annual sequence in the epi-metalimnion and the oxycline of Laguna de La Cruz according to the strategies proposed by Reynolds (1997), R, C and S strategists. *Secuencia anual del fitoplancton en el epi-metalimnion y la oxiclina de la Laguna de La Cruz de acuerdo con las estrategias propuestas por Reynolds (1997), estrategias R, C y S.*

Depending on the strategies of the different species, and following Reynolds (1997), the yearly phytoplankton sequence in the epilimnion-metalimnion of the Laguna de La Cruz (Fig. 10) partly coincides with the ideal sequence for a warm monomictic lake. However, in this lake, during stratification, there is no simple replacement of R-strategy species by C-strategy species followed by S-strategy species. Instead, there is also a replacement among C-strategists species when they dominate. As a consequence of the main disturbance, the whitening event, the autogenic succession pattern typically resulting from a period of stratification with stability is not well fitted. Whitening promotes a strong decrease in transparency, in such a way that most phytoplankton biomass can not be sustained and sinks, as demonstrated by the sedimentation patterns (Miracle *et al.*, 2000), as well as by the drop in biomass and chlorophyll-*a* concentrations following the whitening event (Fig. 5). This is a very severe disturbance that, in contrast with intermediate disturbances, causes a drop both in biomass and diversity as strongly affecting the whole community, and this explains the concomitant drop in diversity (Sommer *et al.*, 1993). This,

together with other disturbances such as storms, is why diversity does not follow a simple increasing pattern with plankton's annual succession until autumnal mixing, but instead rises and falls during summer as a consequence of the differential effects of these disturbances (Naselli-Flores *et al.*, 2003). This is especially clear in the epilimnion, where these effects are stronger, as also documented for deep alpine lakes by Dokulil and Teubner (2003). Our work confirms that, as for other lakes (Padisák *et al.*, 1998; Becker *et al.*, 2010; Xiao, 2011; Martinet *et al.*, 2015) functional groups represent a useful way to describe the succession of phytoplankton.

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