NUTRIENTS BIOCHEMISTRY IN STEPPE SALINE LAKES FROM "LA MANCHA HÚMEDA" BIOSPHERE RESERVE (CENTRAL SPAIN)

Ph.D. Thesis

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NUTRIENTS BIOCHEMISTRY IN STEPPE SALINE LAKES FROM "LA MANCHA HÚMEDA" BIOSPHERE RESERVE (CENTRAL SPAIN)

BIOQUÍMICA DE LOS NUTRIENTES EN LAGOS SALINOS ESTEPARIOS DE LA RESERVA DE LA BIOSFERA DE LA MANCHA HÚMEDA (ESPAÑA CENTRAL)

Ph.D. Thesis

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Doctoral thesis entitled: "**Nutrients biochemistry in steppe saline lakes from "La Mancha Húmeda" Biosphere Reserve (Central Spain)**", carried out by **Maykoll Corrales González** to obtain the degree of Doctor in Biodiversity and Evolutionary Biology, supervised by **Prof. Dr. Antonio Camacho González** Full Professor in Ecology, Department of Microbiology and Ecology of the Faculty of Biological Sciences (University of Valencia) and **Dr. Carlos Rochera Cordellat**, Researcher from the Cavanilles Institute of Biodiversity and Evolutionary Biology (University of Valencia).

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“Todo parece imposible hasta que se hace”

Nelson Mandela

Abstract

La Mancha Húmeda Biosphere Reserve is one of the most important lakes districts in southern Europe. Shallow saline lakes are among the most representative ecosystems of this region. They experience different levels of cultural and natural eutrophication, in such way that most of them deserve urgent conservation and/or restoration actions. In this thesis, 17 representative lakes from the site were selected to study the seasonal patterns of nutrient dynamics (carbon, nitrogen and phosphorus) and its relationship with the ecological functioning. These lakes nearly represent the range of limnological conditions found in this Biosphere Reserve. A seasonal limnological survey was conducted, showing that these lakes ranged from freshwater to hypersaline (average range: 1.75-136.73 mS cm^{-1}) and displayed trophic status from oligo-mesotrophic (2.45±2.76 µg Chl-a L^{-1}) to hyper-eutrophic (185.14 ± 201.15 µg Chl-a L^{-1}).

The studied lakes showed an endorheic behavior, most of them temporary, with a hydroperiod mainly controlled by rainfall and evaporation. Results demonstrate the highly sensitivity of these lakes to process occurring in the catchment because its endorheic nature. Accordingly, both environmental and human-produced disturbances have a direct impact in the stoichiometric relationships of nutrients as well as in the carbon-related metabolisms (i.e., photosynthesis and respiration). The inputs from runoff and wastewaters, as well as evapo-concentration processes, cause a variable accumulation of nutrients within the basins of the lakes, which triggers an uneven increase of the internal load. The multivariate analysis of the variance (MANOVA) conducted with limnological data showed that the nutrient source (that varied from natural to anthropic) was the factor with higher significant influence over the stoichiometry of nutrients as well as on the ecosystem metabolism. The lakes with wastewater inputs, as well as those lying on volcanic lithology in the Campo de Calatrava area, experience phosphorus enrichment both in water and surface sediments.

A principal component analysis (PCA) was also conducted with data that, in accordance with the above, revealed the existence of two

contrasting trophic scenarios associated to a different ecological functioning of the planktonic compartment. This functional aspect was assessed by measuring photosynthesis and aerobic respiration rates of plankton using the standardized oxygen-exchange method. The seasonal time course of plankton populations was assumed to be highly dynamical and sensitive to the environmental variations, thus being useful to describe the environmental forcing to the biological community. Hence, photosynthetic and respiration rates varied accordingly to the trophic status of the lakes. Considering their seasonal averages, they ranged, among lakes, 8.12-158.41 and 3.58-93.87 µmoles O_2 L^{-1} h^{-1} produced or consumed respectively. One of the trophic scenarios depicted by the PCA corresponded to lakes highly affected by both current point-source and/or past (internal load) pollution. Different examples are the lakes Longar, Larga de Villacañas and Camino de Villafranca, but also others ranging from meso- to eutrophic status such as Lake Manjavacas. A phosphorus enrichment was evident in these lakes and explained the enhancement of the photosynthetic rates in the planktonic compartment. The respiration activity was, in these cases, highly coupled with the photosynthetic rates, denoting a major role of autochthonous algal-derived DOM sustaining the secondary production. However, in periods when there are more catchment inputs (i.e., seasonal water recharge), contributions of allochthonous inputs of organic matter likely occur that would explain the relative uncoupling observed between these metabolic activities. Instead, the other trophic scenario relates to well preserved lakes keeping a high salinity and its natural temporal hydroperiod (e.g., Alcahozo, Tirez, and Salicor). These lakes kept low to moderate plankton metabolic rates during all season. The ratio between photosynthetic and respiration rates, in this case, was significantly lower compared to polluted lakes, which would represent a higher support of allochthonous inputs of organic matter. A natural and gradual accumulation of nutrients also occurs in these lakes because its marked endorheic nature, with high concentrations of likely recalcitrant DOC. Along hydroperiod, organic carbon in these oligotrophic lakes increases proportionally more than the other nutrients, which intensifies the stoichiometric imbalance. In the whole range of studied lakes, the production-to-respiration (P:R) ratio obtained from the oxygen-exchange measurements was net autotrophic, ranging as from 1.35 to 5.64. However, considering that photosynthesis cannot occur at night, a net heterotrophic balance could be also conceivable for some of these lakes.

The limnological survey also involved the measure of the internal loads of nutrients in the sediments, which denoted the historical role that wastewaters have played in deteriorating the ecological status of these lakes. To go deeper in the consequences of this internal load, 11 of the lakes were selected to assess phosphorus burial, fractionation and mobilization in their sediments. Results showed how wastewater inputs favored the accumulation of all phosphorus sedimentary fractions, which mainly occurred via precipitation with divalent cations (i.e., calcium or magnesium) and as buried organic matter, both representing relatively occluded P forms, although they can also be released. The condensed P, which occurs when the excess P is taken up by microorganisms and stored as polyphosphate, was also important in sediments. The immobilization of P would be partially restrained in the volcanic lakes of the region, making them more prone to eutrophication. The seasonal survey indicated that the natural drying and re-flooding of lakes replenishes the availability of labile-P in water, and often contributes to the lakes' productivity. However, artificial flooding alters redox conditions and thereby enhances a reductive dissolution of P, suggesting that the internal P supply might not drop in the polluted lakes after external inputs shortcomings, thus counteracting restoration measures.

An optical study of the dissolved organic matter (DOM) by applying absorbance and fluorescence spectroscopy was also carried out to identify its sources and degradability. In addition to the pivotal role in the carbon cycling, this DOM may also impact the water quality and the nutrients cycling since its decomposition controls the release of inorganic nutrients. This optical analysis provided the seasonal evolution of parameters, both from absorbance spectra and emission/extinction matrices that are surrogates of the origin, composition, and reactivity of DOM. The results obtained complemented the functional study described previously. The spectral slope obtained from filtered water over the wavelength range 275–295 nm ($S_{275-295}$) significantly correlated with DOC concentrations, indicating a high proportion of aged and photobleached DOM. Accordingly, lakes showed a wide-ranging pattern with a decrease in DOM aromaticity as the water retention and the evaporative concentration of solutes increased, which moreover was intensified seasonally. This involved a high occurrence of allocthonous humic-like DOM with a high absorptivity during the autumn-winter flooding events, when the runoff towards the lakes was the highest. Regression analysis indicated that this allochthonous DOM does not accumulate in lakes, which suggest a certain reactivity. The optical study also revealed a greater amount of photobleached DOM of low molecular weight during the seasonal re-flooding of the non-polluted and more saline lakes, which could partially sustain the low-to-moderate observed respiration rates. This partially labile carbon might originate from the photo-oxidation of the organic matter remaining in the lake basin during the summer period, when these lakes are dried, as well as via runoff during the re-flooding. The allochthonous inputs of organic matter also occur in the eutrophic lakes as they show catchments with similar characteristics. However, they are in this case complemented, and even exceeded, by algal derived autochthonous DOM as deduced by the optical parameters.

Finally, an additional seasonal survey was conducted in lakes Alcahozo and Manjavacas but with a higher sampling frequency. These lakes are close each other and belong to the same lacustrine complex, thereby experience similar weather conditions. This higher temporal resolution demonstrated the uncertainty and the non-linearity of dynamical processes that take place in these lake-catchment systems. These are characterized by pulses in the availability of nutrients and, more particularly, of labile organic material supporting productivity, either consisting of aged photodegraded or autochthonous freshly formed DOM. A flow cytometry analysis showed bacterioplankton populations to be responsive to both situations, although in a different way, which revealed a complex response to distinct DOM environments. Bacterial numbers were significantly higher in the eutrophic Lake Manjavacas compared to the regularly oligotrophic Lake Alcahozo. However, the bacterial populations in Alcahozo were showed as metabolically more active attending to the higher proportion of bacterial cells with a high DNA content, a trend based in the fluorescence intensity detected by the flow cytometry analysis. A comprehensive study of the taxonomic and functional composition of these bacterial communities could help to find out whether they show a physiology adaptation to these contrasting DOM environments.

In summary, this thesis provides a comprehensive description of pressures and their outcomes in the saline lakes from Mancha Húmeda, which commonly involve alterations of the length of the flooding period, desalinization, and wastewater inputs. The trophic status, carbon-metabolism, DOM features, and stoichiometric relationship of nutrients observed for the most unimpaired lakes can be considered as reference values to assess deviations caused by anthropic impacts. This benchmark values should contemplate the particular phosphorus enrichment observed for the volcanic lakes from the Campo de Calatrava area. Naturally occurring high concentrations of soluble phosphorus and water turbidity are both distinctive features of these volcanic lakes that greatly predetermine its ecological functioning. Some threats such as the occurrence of nutrient pollution have been partially addressed with the improvement of wastewater treatment before being poured. Still, we propose to adopt additional countermeasures such as extending the use of tributary slow flow channels and/or artificial wetlands, which would act as tertiary treatments. Other threats that may potentially contribute to increase the availability of nutrients are the desalting by wastewater inputs, which could promote solubilization of nutrients from sediments, as well as the long-term changes the regional climate, that can exacerbate the evaporative concentration of nutrients. These are concerns that should also be appraised by management strategies, which can adopt the findings and approaches provided by this thesis. These measures could contribute to a reduction in the impact on the livelihoods and economic development of the local population as advocated by the UNESCO program, thereby preventing potential conflicts that the management of a natural conservation area may bring about.

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Chapter I: General Introduction

I.1. Main ecological characteristics of Mediterranean shallow saline lakes

Shallow lakes provide a sort of ecosystem services and are exceptionally valuable ecosystems for biodiversity conservation (Strayer and Dudgeon, 2010). They typically occur in land depressions and often display seasonal flood regimes (Dodds and Whiles, 2010). Water bodies with a maximum depth of around 5-7 meters can be considered as shallow lakes, although in the Mediterranean region they usually do not exceed 3 meters depth. In this Mediterranean region, shallow lakes are often temporary and show a polymictic mixing pattern without thermal stratification (Dodds and Whiles, 2010). This continuous mix and the shallowness of these waterbodies enhances the interrelation between the sediment and the water column. In many cases these systems are also endorheic, thus being important sinks of matter coming from the surrounding catchment. This involves inputs of nutrients, which are higher when cultivated landscapes are part of the catchment (Dodds and Whiles, 2010). Accordingly, shallow lakes are usually more productive compared to deeper lakes (Scheffer, 1998; Strayer and Dudgeon, 2010).

Among shallow lakes, saline lakes occur in arid and semiarid environments within closed-drainage basins. They are reservoirs of microbial, animal and plant species (Scheffer, 1998; Attayde et al., 2022). The topographic and climatic characteristics of the Iberian Peninsula allows the formation of such shallow saline lakes (Figure 1.1). The negative hydrologic balance from spring to summer and the catchment's flows convergence are characteristic of these endorheic basins. The catchment geology and evapo-concentration processes lead to a high accumulation of salts and dissolved organic matter in these saline lakes. Salinity also changes seasonally. It increases during warmest and drought periods because of evaporation, whereas dilution occurs at the cold rainy season (García-Ferrer et al., 2003). The saline lakes occurring in La Mancha Húmeda Biosphere Reserve (Central Spain) represent the main example of this type of environment within the Iberian Peninsula.

Figure 1.1. *Landscape images showing some of the saline lakes from Central Spain which has been studied in the present thesis. From top-left to bottom-right, lakes Quero, Peñahueca, Longar and Camino de Villafranca.*

I.2. The study case of La Mancha Húmeda Biosphere Reserve: threats, vulnerabilities, and scientific relevance

La Mancha Húmeda Biosphere Reserve is an area internationally recognized by the UNESCO's Man and the Biosphere Program since 1980. This UNESCO's program aims to propose ideas and support practices for reconciling nature conservation with economic development. Wetlands in "La Mancha Húmeda" are generally classified as shallow, temporary, steppe lakes, with a small watershed (Alonso, 1998). As mentioned previously, La Mancha Húmeda differs from other lake districts from the Iberian Peninsula in its large surface area, showing a high density and diversity of saline waterbodies. Such lakes represent unique ecosystems in the Western European context, although they experience different levels of degradation (Florín and Montes, 1999). Several scientific studies have been conducted in these lakes during the last decades (Garcia-Ferrer et al., 2003; Prieto-Ballesteros et al., 2003; Montoya et al., 2013; Castillo-Escrivà et al., 2015; García and de la Cruz, 2016; Sanchez-Ramos et al., 2016; Camacho et al., 2017), however, the information on the biogeochemistry of nutrients, including aspects such as the phosphorus and organic carbon cycling, is very scarce. Still, this scientific background represents a worthy basis to choose new research objects and sites, some of which have motivated this thesis.

From a functional point of view, these lakes from La Mancha Húmeda are fundamental elements for the maintenance of some ecological processes within the region (García and de la Cruz, 2016). But different threats such as habitat loss and/or fragmentation, the intensive overgrazing and drainage to promote soil drying have transformed their functionality (García and de la Cruz, 2016). Some of these lakes are very close to populated areas (Sanchez-Ramos et al., 2016). An example is the lake complex of Alcázar de San Juan, which is also studied in this thesis (Figure 1.2). These disturbances are generally associated with an excessive input of nutrients and desalinization process (Camacho et al., 2017). This situation makes necessary to establish management and conservation plans conducted to improve the ecological status of these lakes (Cirujano et al., 2010), which requires to increase the knowledge on the particular biogeochemistry of these lakes.

Figure 1.2. *Image showing the proximity of the lake complex of Alcázar de San Juan to the urban area. From right to left, lakes Veguilla, Camino DE Villafranca and Las Yeguas.*

A particular type of shallow lakes in “La Mancha Húmeda” occur in the volcanic field of Campo de Calatrava, corresponding to the sites of hydromagmatic activity (Ortiz et al., 2011), considered as maar-type wetlands. An example of these lakes is Lake Almodovar (Figure 1.3). On the other hand, an interesting aspect of lakes from "La Mancha Húmeda" is their partial analogies with other saline aquatic systems located in other parts of the planet, such as the Great Salt Lake (Jones et al., 2009), Tuz Lake (Mutlu et al., 2008), Dead Sea (Bodaker et al., 2009; Lonescu et al., 2012), Mono Lake (Humayoun et al., 2003), and the Ethiopian Soda Lakes (Lanzén et al., 2013), though saline lakes in La Mancha lakes are all shallow and smaller. Some of these extreme environments are part of the Exobiology and Evolutionary Biology program of agencies such as NASA (Earth's Early Biosphere and its Environment- Evolution, Environment and the Limits of Life), and the scientific work carried out in them, particularly those related to their biogeochemistry and microbiology, are published in journals of high scientific impact, such as some of the examples cited above.

Figure 1.3. *Lake Almodovar, a volcanic lake located in volcanic field of Campo de Calatrava. This is one of the lakes studied in the present thesis as representative of this volcanic area.*

I.3. Nutrients cycling in shallow lakes and the role of sedimentary phosphorus

Major nutrients (i.e., carbon, nitrogen and phosphorus) sustain the productivity of aquatic ecosystems. They occur in the water and sediments either as dissolved or particulate forms (Champion and Currie, 2000; Kalinkina and Tekanova, 2022; Liu et al 2022). Examples of transport and transformation processes of these nutrients are biodegradation, photolysis, hydrolysis, gasification, adsorption, precipitation and/or sedimentation (Chen et al., 2022). The mobilization of nutrients in lakes by these processes can be conceptually synthesized in an input/output model. Transport vectors of nutrients can be biotic (e.g., birds, humans, other animals; Cottenie and de Meester, 2004) and abiotic (e.g., atmospheric transport, aquifers, point and diffuse runoff, etc.; Cáceres and Soluk, 2002). In lakes from La Mancha Húmeda, diffuse inputs of nutrients mainly originate from groundwater upwelling and surface runoff (Ballesteros et al., 2018), increasing these inputs as the agricultural use of the catchment does. Lakes receiving wastewater from nearby towns also occurs in the region, which produce an extra amendment of nutrients.

The process of eutrophication in lakes can be either natural or anthropogenic. This refers to an excessive enrichment of nutrients (nitrogen and phosphorus), which result in the massive growth of phytoplankton and the detrimental development of submerged aquatic vegetation. The productivity and ecological status of lakes also depends on the stoichiometric balance of these nutrients (Scheffer, 1998). On the other hand, the seasonal variation is also important for the consequences of eutrophication. Hence, environmental factors such as the temperature have been shown to greatly influence the efficiency of the phosphorus use (i.e., chlorophyll-a per unit of phosphorus relationships) by phytoplankton (Liu et al., 2022). Reversing the symptoms of nutrient enrichment requires the quantification of the external amendments of these nutrients, but also knowing the amount and dynamics of internal loads stored in sediments (Conley et al., 2009; Jilbert et al., 2020; Abell et al., 2022).

Phosphorus enters to the lakes from diverse sources, both natural and anthropogenic, associated with processes in the surrounding watershed. In lake systems, and particularly in calcium-rich alkaline waters, phosphorus accumulates mostly in sediments (Granéli, 1999; Søndergaard et al., 2003). These sediments can be considered as a reservoir for immediate, moderate and low available phosphorus, depending on its mobility from sediments to the water. Similar to organic carbon, both reactive and recalcitrant phosphorus fractions can be clearly distinguished. On the other hand, chemical processes regulating phosphorus desorption are oxygenation, water mineralization, temperature, and pH. All these factors, except perhaps pH, may vary notably in the lakes studied in this thesis, either during the seasonal cycle (e.g., temperature) or during the day/night cycle (e.g., oxygen concentration).

I.4. Dissolved organic matter (DOM) transformation in aquatic ecosystems

The dissolved organic matter (DOM) is an important component of the global carbon cycle and a large reservoir of carbon in aquatic ecosystems (Hedges, 1992; He et al., 2016). The DOM is a mixture of compounds that can originate from both natural and anthropogenic sources. Main natural sources in lakes are: 1) autochthonous sources, being important contributors the phytoplankton and the submerged aquatic vegetation (Baines and Pace, 1991; Kritzberg et al., 2005; Wang et al 2007; Zhang et al., 2009), and 2) allochthonous inputs coming by runoff from terrestrial vegetation, also including the derived humic substances (Thurman, 1985; Kritzberg et al., 2005; Garcia et al., 2015).

The DOM can be quantitatively assessed as the concentration of dissolved organic carbon (DOC), although it involves a heterogeneous mixture of compounds, mostly carbohydrates, proteins, lipids, phenolic compounds, and humic substances. The DOM is related with key metabolic processes that have cascade effects on the food webs such as, the control of nutrients availability (Schindler et al., 1992; Bao et al., 2023), the attenuation of solar radiation, or the fueling of heterotrophic microbial communities (Williamson et al., 1999 and references therein). The transformation of this DOM is a key process in the functioning of lacustrine ecosystems (Hansen et al., 2016).

DOM degradation is carried out by microorganisms, with more or less efficiency (Catalán et al., 2013; Berggren et al., 2018), which in any case leads to the formation of refractory DOM after being partially degraded (e.g., humic acids). Due to its low biodegradability, this refractory fraction can almost or totally leave the biogeochemical carbon cycle and become a carbon sink with a prolonged residence time. A relevant aspect for the present study is that the reactivity and biotransformation of DOM can be regulated by factors such as the water mineralization (Mopper et al., 2006) and photooxidation (Carlson and Hansell, 2015), which are both chemical and physical processes, respectively, with a high relevance in Mediterranean saline lakes. Moreover, processes such as flocculation or adsorption by mineral particles can lead to the accumulation of this organic matter in sediments. This settled organic matter can contribute to the regulation of phosphorus (Boers et al., 1984; Kim et al., 2003; Tammeorg et al., 2022) or nitrogen (Anuradha et al., 2011) availability.

A part of the DOM pool presents in the water column, which often is related with refractory compounds, is referred as CDOM (fraction absorbing light) or FDOM (fraction of CDOM emitting fluorescence) depending on its optical characteristics (Figure 1.4). The determination of the optical properties of DOM, both using UV-Visible absorption and fluorescence spectroscopies, has been extensively used to assess its lability and origin (Fellman et al., 2010; Hansen et al., 2016). These analytical methods have been useful to assess photooxidation and microbial degradation processes in highly saline ecosystems (Osburn et al., 2011).

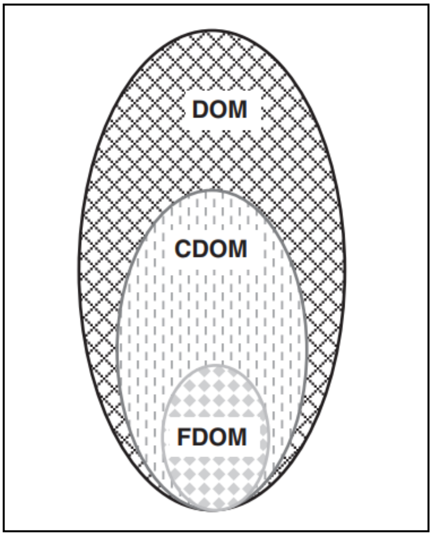


Figure 1.4. *Schematic of the overlap between dissolved organic matter (DOM), chromophoric dissolved organic matter (CDOM) and fluorescent dissolved organic matter (FDOM). Modified from Coble et al. (2014).*

I.5. Metabolic regime of lakes as an assessment of ecosystem functioning

The global metabolism in aquatic ecosystems represents a balance between carbon fixation (gross primary production) and biological carbon oxidation (ecosystem respiration). It is assumed to be closely related with the transit of nutrients and their biogeochemical cycles (Pacheco et al., 2014). It is also seasonally regulated by key environmental factors such as light, temperature and flooding (Alfonso et al., 2018). The photosynthetic and respiration rates indicate the intensity of both carbon-related metabolic processes, whose behavior can provide a good description of the global functioning of the system. These metabolic processes are expected to respond differently depending on the trophic condition of the lake. However, differences in water salinity may also have an impact in these rates (Sudhir and Murthy, 2004). A better understanding of the metabolic dynamics has the potential to strengthen predictions of how changes in some environmental stressors (e.g., climate, desalinization, eutrophication) may affect the functioning of the lakes studied in this thesis.

Planktonic bacteria play an important role in aquatic ecosystems as organic matter decomposers, recyclers of nutrients, greenhouse gases producers and consumers, as well as intermediaries for energy and matter flows in food webs (Gasol and Del Giorgio, 2000; Karayanni et al., 2019). Altogether, bacterial activity can have a large impact on ecosystem metabolism either in the ecosystem balance between production and respiration, in the turnover of organic carbon and the global carbon cycle, as well as in DOC transformations (Carlson and Hansell, 2015). Accordingly, tracing the dynamics of bacterial abundances and activity may be useful as a diagnostic tool to assess lake functioning. Flow cytometry may result a practicable and useful assessment approach to trace the dynamics of these bacterial populations, which can reflect changes in the dynamics of the organic matter availability and nature.

I.6. Objectives of the thesis

The main objective of this thesis is to unveil the patterns of nutrients cycling and transformation in representative saline lakes from La Mancha Húmeda Biosphere Reserve that show a contrasting trophic status, then relate them with some functional aspects of the planktonic community. This was made with the of establishing scientific foundations for an adequate management of these aquatic ecosystems, which are unique in the Western Europe context. Accordingly, specific objectives of the thesis are:

1. To characterize hydrological, physical-chemical, and functional patterns of the saline lakes from La Mancha Húmeda, relating them with the conservation status of the lakes, as well as with diagnostic parameters associated with the resource limitation and the carbon-metabolism of the plankton communities. This specific objective is addressed in Chapter III.

2. To evaluate the relationship between phosphorus bioavailability and the productivity of the lakes based on phosphorus-chemical fractionation in sediments, which involves assessing the burial and mobilization processes of this nutrient. This specific objective is addressed in Chapter IV.

3. To perform an optical characterization of the dissolved organic matter (DOM) from these lakes, to obtain information on its sources, degradative suitability, and its influence in the dynamics of bacterioplankton populations. This specific objective is addressed in Chapters V and VI.

I.7. Structure of the thesis

The thesis is structured in 6 specific chapters. **Chapter I** includes a general introduction. **Chapter II** compiles a description of the study sites, as well as that of the general material and analytical methods used. **Chapter III** assesses the hydrogeochemical patterns of lakes considering the impact generated by key environmental factors (i.e., water salinity, seasonality and the origin of nutrient inputs). This chapter includes the description of the metabolic activity of plankton populations as a diagnosis of ecosystem functioning. **Chapter IV** is concerned with the water-sediment phosphorus exchange processes. In this case, the relationship between phosphorus bioavailability and the productivity of the lakes is evaluated based on the analysis of P-sedimentary fractionation. In this chapter it is also considered the role of main environmental factors affecting the dynamics and availability of phosphorus. **Chapters V** and **VI** involves an optical characterization of the dissolved organic matter (DOM) present in the water column of these lakes, seeking to identify its sources, degradative suitability, as well as the influence of DOM on bacterioplankton dynamics. Finally, this thesis ends by offering **Final remarks** and the main **Conclusions** derived from the results.

Chapter II: Material and analytical methods

II.1. Study site

Studied lakes are located at the UNESCO La Mancha Húmeda Biosphere Reserve (Central Spain) (Figure 2.1). The reserve has an extension of more than 400.000 hectares and represents the largest wetland district inside the Iberian Peninsula (Alonso, 1998). This reserve is a special bird protection area (SPAs) and wetland of international significance under the Ramsar convention. The reserve is located in the upper basin of the Guadiana River, which is a large area of steppe wetlands with climate values similar to some wetland areas in North Africa and Central Asia (Margalef, 1947). The study area is also characterized by high density and diversity of lakes, lithology dominated by marls and limestones and quite flat topography with low drainage, for which it is believed that the origin of this wetland district was endorheic (Dantin, 1932, 1940).

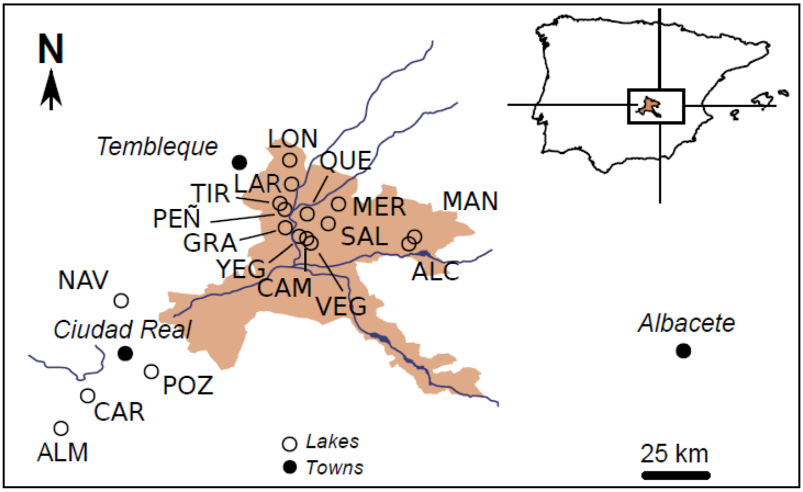


Figure 2.1. *Location of La Mancha Húmeda Biosphere Reserve in the central Spain.*

II.1.1. Geomorphology, hydrology, and climate

The landscape of the study area is dominated by agricultural crops. It is formed by horizontal tertiary deposits, in which the main rivers overflows, flooding, endorheic lakes and aquifer upwellings constitute the essence of this wetland zone (Jerez, 2010). Many geomorphological processes have been described as responsible for the formation of the lakes basin, highlighting among them karst processes, fluvial processes, tectonics and hydromagmatic processes, wind deflation and interaction with groundwater. The contribution of precipitation, runoff and groundwater flow for each lake is also variable, giving rise to different types of feeding (Florín et al., 1993). The volcanic area of Campo de Calatrava, located in the center of the province of Ciudad Real, is particular due to its origin within the region. In this case, the formation of Maars as a result of phreatomagmatic eruptions have led to the formation of a large number of temporary lakes. Most lakes lie on contact boundaries between Triassic marls and alluvial quaternary deposits, although the Tertiary limestone outcrops are also frequent (Florín et al., 1993). This geological landscape is rich in evaporite rocks and alkaline basalts whose solubilization may result in a high concentration of dissolved salts (Florín et al., 1993) and, for the latter, particularly P-release (Cebriá and López-Ruiz, 1995). Depending on the contact with these substrates, water bodies have naturally range from freshwater to hypersaline.

The lakes average altitude is 600 meters above sea level. The climate of the area is continental and Mediterranean with average rainfalls close to 400 mm per year, the maximum values are recorded in autumn and the minimum in winter, being the summer the driest season. Temperatures ranging from -15 ºC to 43 ºC, depending on the season (Jerez, 2010) being in average 15 ºC. The study area presents a marked seasonality that makes summers warm with average temperatures ranging between 23 ºC and 27 ºC and cold winters ranging between 6 and 8 ºC.

II.1.2. Wetland vegetation

The typical vegetation of the lakes is composed by helophytes. Thus, when the salinity is not high, species such as *Thypha dominguensis*, *Phragmites australis*, *Schoenoplectus tabernaemontani* and exceptionally *Cladium mariscus* can be found. Halophilic plants can also be found such as *Limonium spp*., *Salicornia europaea* or species of reed with a more halophilic character (*Juncus subulatus*). With respect to submerged vegetation, under oligotrophic conditions and depending on of water salinity and transparency, these lakes may exhibit submerged macrophytes populations (Hepatics and Charophytes) whose maximum development occurs during the spring period. This type of aquatic vegetation can play an important role in the nutrient cycling within these lakes (Battle and Mihuc, 2000).

II.1.3. Selection of study sites

Currently La Mancha Húmeda Biosphere Reserve is delimited into seven sectors: 1) the lakes of the southeast of Toledo, 2) Mota del Cuervo, 3) Sierra de Altomira, 4) Las Tablas de Daimiel and surrounding wetlands, 5) Campo de Montiel (including las Lagunas de Ruidera), 6) the volcanic lakes of Campo de Calatrava and 7) the lakes of eastern La Mancha. This delimitation was based on the interaction with aquifer systems, local relief, linkage between lakes and drainage networks, lithology and the origin of the watersheds and basins (Florín et al., 1993).

For this study, a total of 17 lakes were selected based on a gradient of water mineralization, including both polluted and unpolluted lakes (Table 2.1) (Figure 2.2), trying to cover different sectors mentioned above. This selection was made to embrace the range of conditions observed in the region. They are all shallow, and range from subsaline to hypersaline (sensu Hammer, 1986). Water of subsaline lakes display mainly calcium bicarbonated hard waters, whereas more saline waters are sulphated and clorurated. In the later lakes, the Mg^{2+} and Na^{2+} are more abundant than Ca^{2+} (Corrales-González et al., 2019). Benthic compartments vary notably among lakes. A few decades ago, some of them showed well-structured microbial mats dominated by the cyanobacteria *Microcoleus chtonoplastes* (Guerrero and de Wit, 1992). The general shallowness of the lakes and the frequent winds cause the sediment re-suspension. The later contributes to the presence of tycoplankton in the water column and a high turbidity that is occasionally observed.

Table 2.1. *Name, code, location, size and saline type of studied lakes. Symbols + and – in wastewater column correspond to presence and absence of wastewater inflow. Salinity classification of lakes was made according to Hammer (1986).*

Lake	Code	UTM-X (m)	UTM-Y (m)	Surface (ha)	Saline type	Wastewater discharges
Alcahozo	ALC	510761	4360263	70.00	Mesosaline	-
Almodovar	ALM	398552	4284836	20.15	Hyposaline	-
Camino de Villafranca	CAM	477983	4362388	142.66	Mesosaline	+
Caracuel	CAR	407550	4298128	66.16	Subsaline	-
Grande de Villafranca	GRA	471198	4366857	135.58	Hyposaline	-
Larga de Villacañas	LAR	473257	4384801	88.02	Mesosaline	+
Longar	LON	472617	4394746	93. 31	Hypersaline	+
Manjavacas	MAN	512248	4363047	230.40	Mesosaline	+
Mermejuela	MER	488154	4376557	9.57	Hypersaline	-
Nava Grande	NAV	418431	4337216	118.80	Subsaline	-
Peñahueca	PEÑ	471026	4374447	113.87	Hypersaline	-
Pozuelo	POZ	427889	4307876	48.76	Hyposaline	+
Quero	QUE	478126	4372535	79.43	Hypersaline	-
Salicor	SAL	484891	4368474	58.15	Hypersaline	-
Tirez	TIR	469503	4376897	92.48	Hypersaline	-
Veguilla	VEG	479352	4360529	79.15	Subsaline	+
Yeguas	YEG	475539	4363272	66.75	Hypersaline	+ (in the past)

All the studied water bodies are originally temporary. The hydroperiod starts with the autumn rains and the maximum flood occurs during winter and early spring. By middle spring, evaporation starts to exceed recharge and the lakes become dry by early summer, except those receiving wastewater from nearby wastewater treatment plants (WWTP). Usually, these WWTP perform a secondary treatment as a final step consisting of a removal of suspended solids and a biological treatment to degrade the organic matter, though they do not display nutrient removal tertiary treatment. The partially cleaned wastewater is then poured to the lakes as a way of water reclamation. Surrounding belts or spots of seasonally developing common reed (*Phragmites australis*) usually develop in lakes receiving these wastewaters. By contrast, the halophytic vegetation dominates the shores of those maintain more natural conditions. The absence of submerged macrophytes is a general trend in these lakes. The high salinity and the anthropic pressure are factors mainly limiting its development

(Camacho et al., 2017). Benthic microalgae or microbial mats may yet occur in the more saline lakes (Cabestrero et al., 2018), but these communities remain generally deteriorated by the anthropic pressure. All these circumstances impose in most cases a higher role to the planktonic community compared to the benthos.

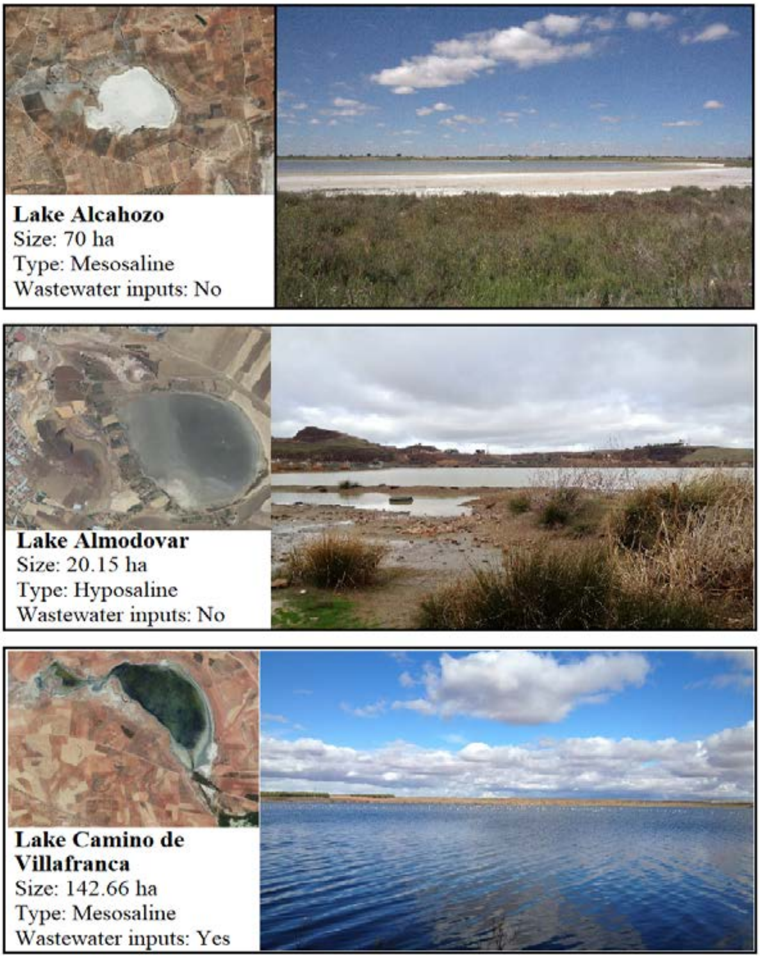


Figure 2.2. *Images of the studied lakes, satellite images of the surrounding area and some characteristics of the waterbodies.*

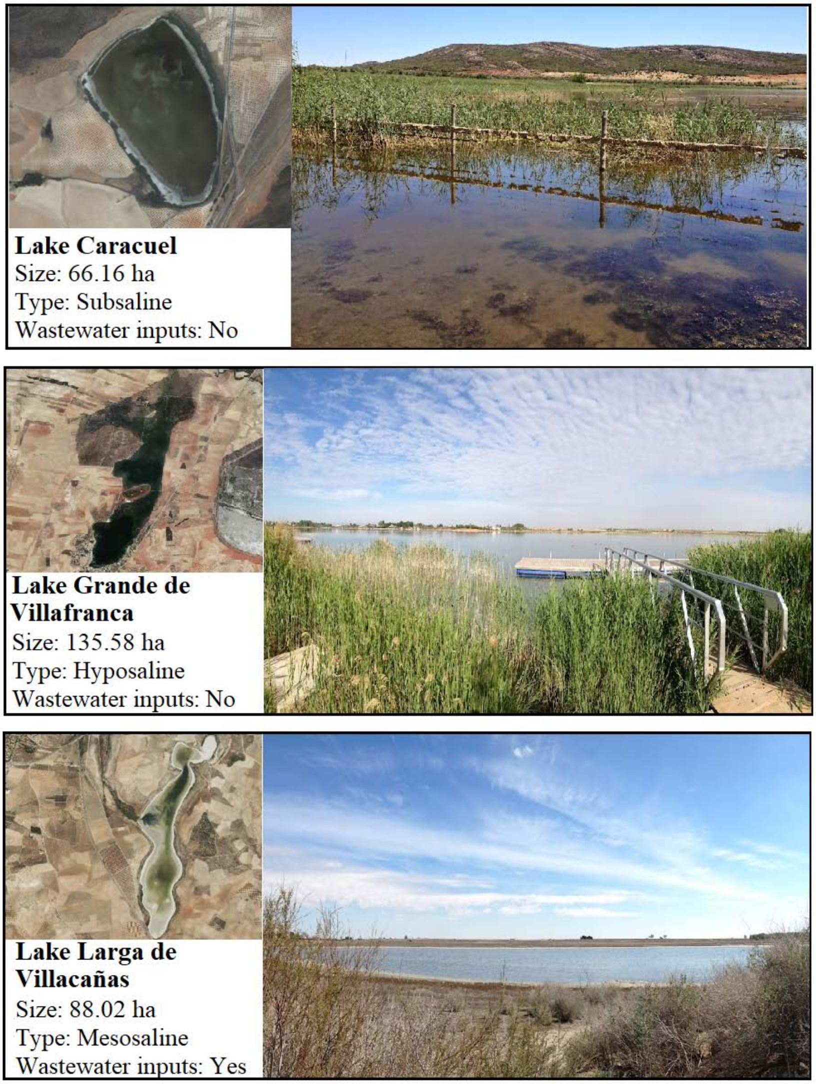


Figure 2.2. *(Continuation) Images of the studied lakes, satellite images of the surrounding area and some characteristics of the waterbodies.*

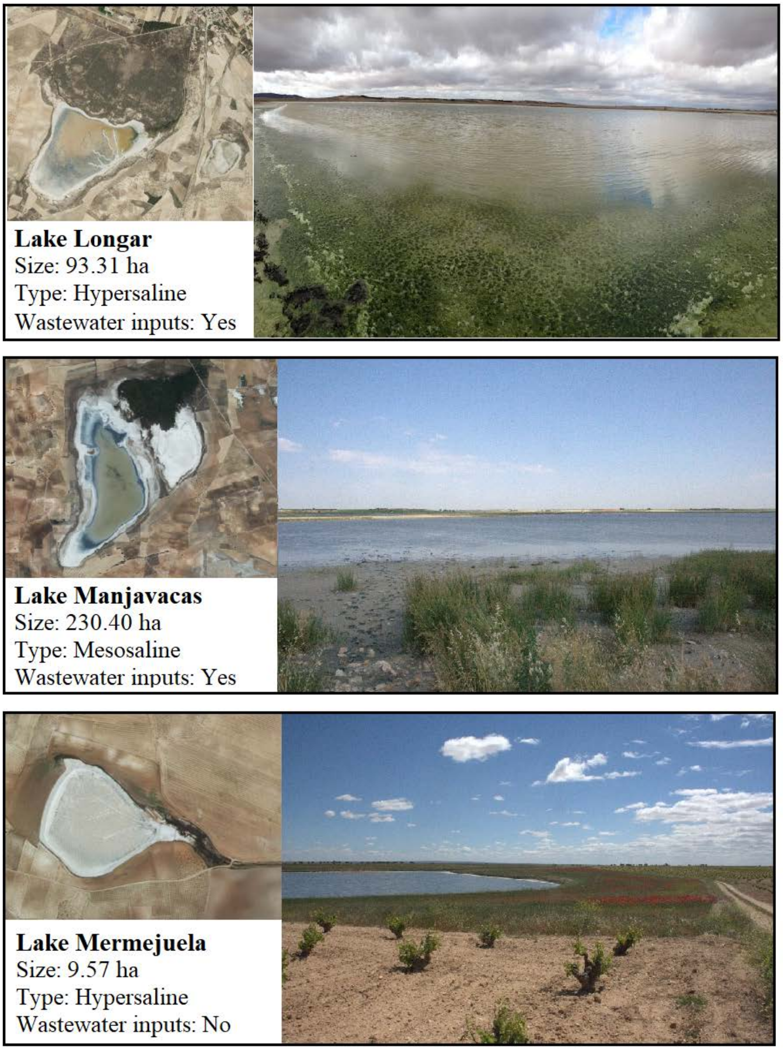


Figure 2.2. *(Continuation) Images of the studied lakes, satellite images of the surrounding area and some characteristics of the waterbodies.*

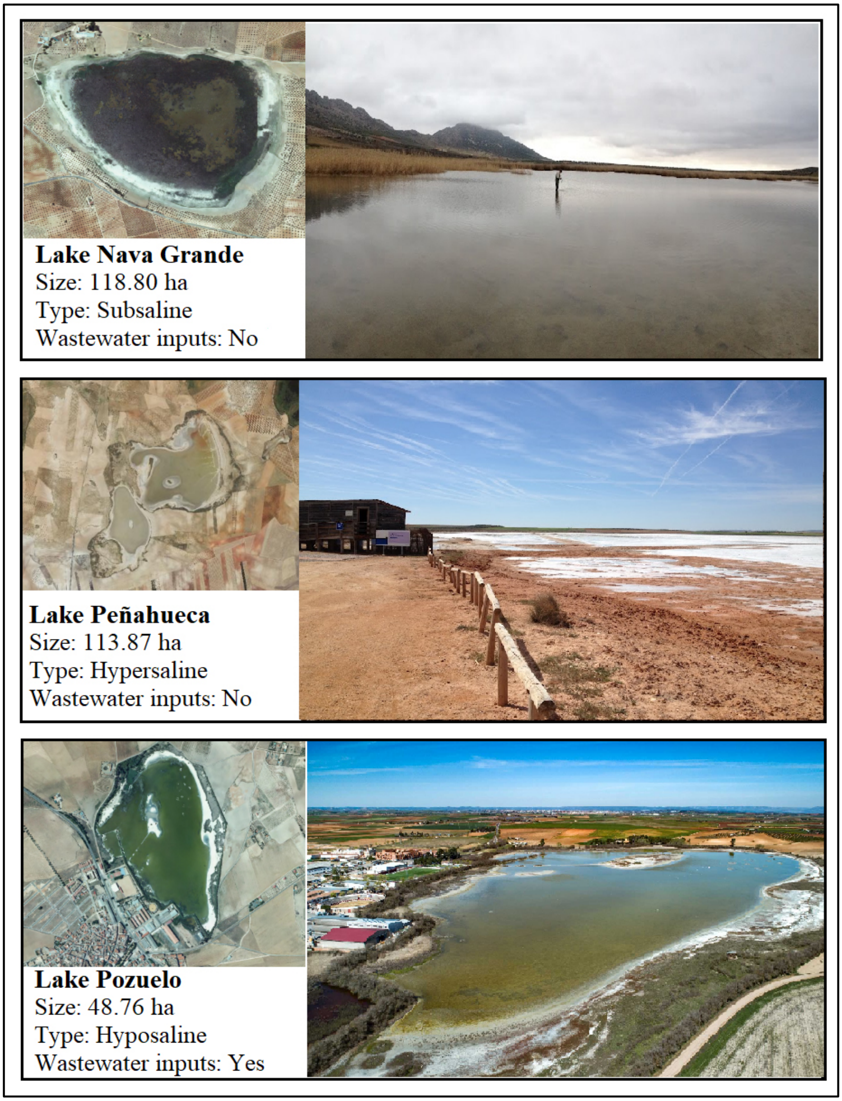


Figure 2.2. *(Continuation) Images of the studied lakes, satellite images of the surrounding area and some characteristics of the waterbodies.*

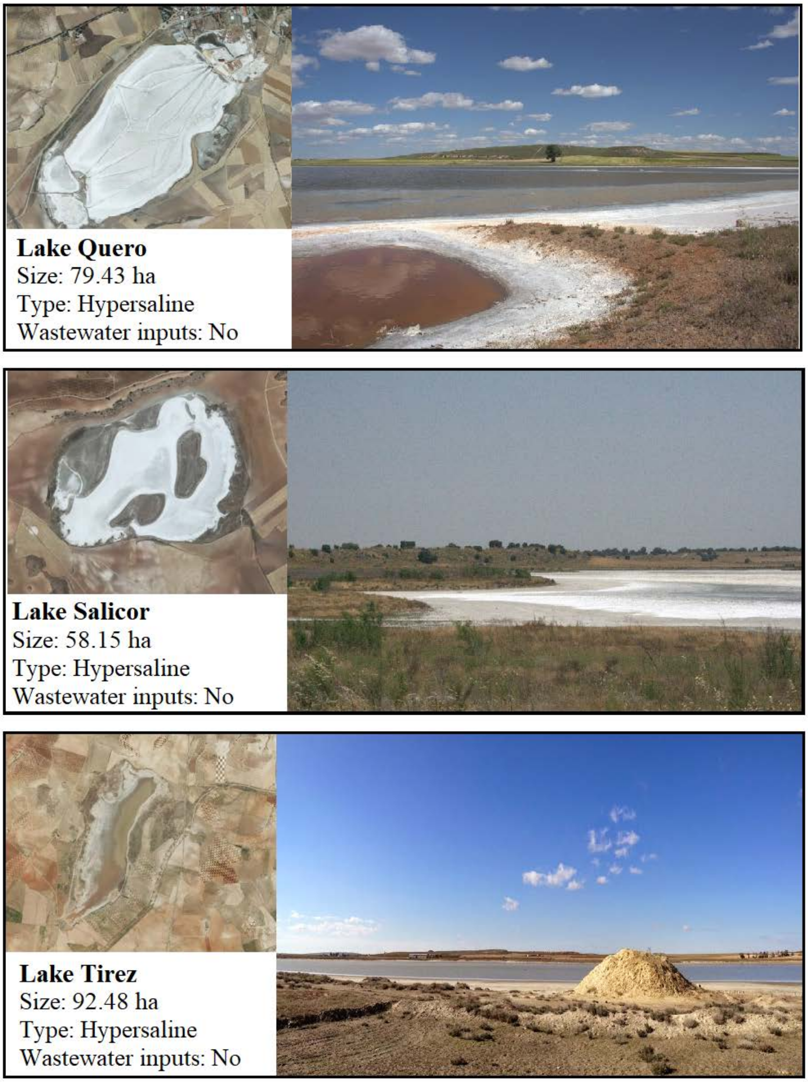


Figure 2.2. *(Continuation) Images of the studied lakes, satellite images of the surrounding area and some characteristics of the waterbodies.*

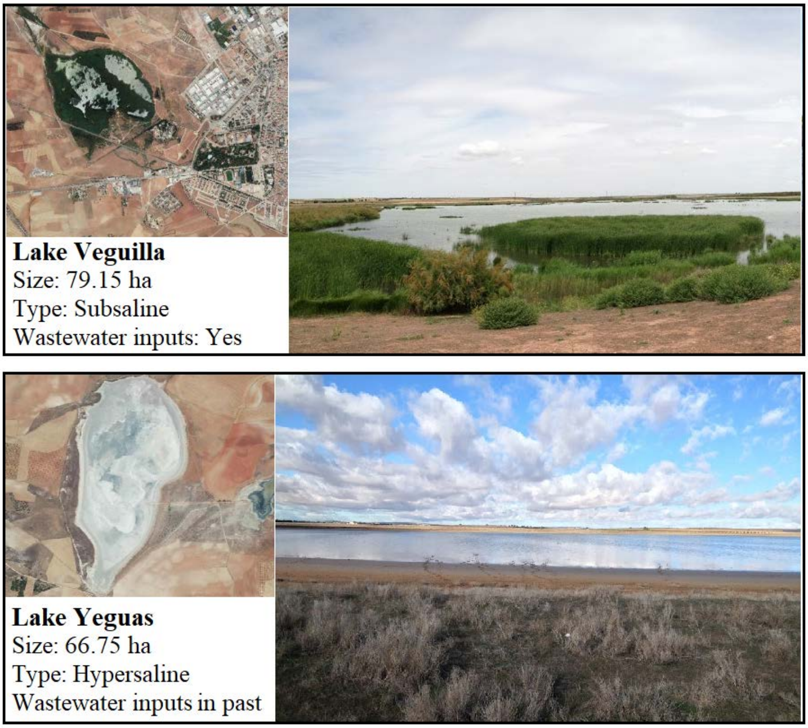


Figure 2.2. *(Continuation) Images of the studied lakes, satellite images of the surrounding area and some characteristics of the waterbodies.*

II.2. Sampling and in-situ determination of environmental variables

In the table 2.2 are summarized the lakes selected and the sampling schedule conducted to accomplish studies described in each of the chapters of the thesis. Precipitation and temperature data of the study period were obtained from the meteorological observatory of the Herencia town (JCCM, 2014). The physical-chemical determinations and sampling were performed over the deepest point of the lakes. The depth of water column was monitored by the setting up of limnimeters. The relative flooding level of the lakes (%FL) was then calculated by weighting the records of the water column depth during the study. The water electrical conductivity (EC) was determined by combining a conductivity meter LF 91 with a conductivity cell KLE 1/T (both WTW). The oxygen saturation and the dissolved oxygen (DO) measurements were measured with a WTW Multi 3410, combined with a FDO 924 IDS optical probe. A salinity correction was applied for these DO measurements. The characterization of the saline type of lakes was made based on the conductivity measures following Hammer (1986). The pH of water was measured with a portable pHmeter (Hanna Instruments).

All the containers used for sampling were previously acid washed (10% HCl) and rinsed again several times with sample water before filling. For the analyses of dissolved forms of nutrients, water was immediately filtered in the field through Whatman GF/F glass filters and kept in HDPE bottles of 125 ml. The unfiltered water samples were also collected in the same type of bottles for the analyses of the total content of nutrients. For the determination of chlorophyll *a* (Chl-*a*) concentration, samples were filtered through Whatman GF/F filters and preserved frozen until analysis. Sampling of lake sediments was also carried out monthly from December 2013 to May 2014. This was conducted by acquiring 10 subsamples per composite sample. Combined samples were then homogenized in a polyethylene container of 100 ml and filled completely. The absence of a headspace was required to minimize the alteration of samples. All samples were preserved at -20ºC until their analytical processing.

II.3. Analysis of nutrients concentrations in water and sediment samples

II.3.1. Organic carbon and organic matter content

Total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations in water were measured using a Shimadzu TOC-V CSN Organic Carbon Analyzer. The measurement method is based on a total combustion of the sample, with a subsequent detection of the CO_2 generated. To remove dissolved inorganic carbon from the samples, these were previously acidified and aerated. Total organic carbon (TOC) determination was carried out with the raw samples, whereas dissolved organic carbon (DOC) was made with samples previously filtered with glass-fiber GF/F filters, which retain particulate materials in the samples. With regards sediments, the water content was determined as weight loss after drying at 105 °C for 3 h. The organic matter and carbonate content in sediments were determined by the loss on ignition method at 460 °C/6 h (LOI460) and 950 °C/4 h (LOI950), respectively (Heiri et al., 2001).

II.3.2. Nitrogen forms

The nitrate (NO_3) concentration in water was measured following the second derivative UV/Visible spectroscopy method (method 4500-NO3-C; APHA 2005; Ferree and Shannon 2001) using a Beckman DU-7 UV-Visible spectrophotometer. For this, the 2nd derivative at 224 nm is obtained from a 194-254 nm scan of filtered water samples, which is proportional to the concentration of NO_3 in the sample. The Mili-Q water blank and standards were treated the same as the samples. The ammonium (NH_4) concentrations were obtained following the Indophenol blue modified method (Verdouw et al., 1978). This method is based in the formation of indophenol blue by the reaction of ammonia with phenol and sodium salicylate. Color development is proportional to the NH_4 concentration in the water. On the other hand, total dissolved nitrogen (TDN) and total nitrogen in water (TN_w) were measured with a Shimadzu TOC-V CSN analyzer, which has a nitrogen determination module. The proportion of dissolved organic nitrogen (DON) present in the TDN was calculated by difference from TDN minus the sum of the inorganic forms (i.e., nitrate and ammonia) as described by Bronk and Gilbert (1991).

The content of total nitrogen in sediments (TN_s) was quantified as nitrate after the sediment samples digestion using an alkaline persulphatic oxidizer (NaOH 6 g L^{-1} and $K_2S_2O_8$ 6 g L^{-1} final concentration). Previous measurement of the second derivative of absorbance at 224 nm, to corroborate whether the levels of TN were within the range of the method, when the levels were high, dilutions of the samples were carried out (Golterman, 2004).

Table 2.2. *Main target, lakes visited, number of hydrological cycles, period, and sampling rates corresponding to each of the chapters of the thesis.*

Chapter	Lakes included	Hydrological cycles	Date	Sampling rates	Target
III	Alcahozo, Almodovar, Camino, Caracuel, Grande de Villafranca, Larga de Villacañas, Longar, Manjavacas, Mermejuela, Nava Grande, Peñahueca, Pozuelo, Quero, Salicor, Tirez Veguilla and Yeguas	2	From 12/2012 to 08/2014	Monthly	Stoichiometric and nutrient balance
IV	Alcahozo, Almodovar, Caracuel, Grande de Villafranca, Larga de Villacañas, Longar, Manjavacas, Salicor, Tirez Veguilla and Yeguas	1	From 12/2013 to 05/2014	Monthly	P-chemical fractionation
V	Alcahozo, Almodovar, Camino, Caracuel, Grande de Villafranca, Larga de Villacañas, Longar, Manjavacas, Mermejuela, Nava Grande, Peñahueca, Pozuelo, Quero, Salicor, Tirez Veguilla and Yeguas	2	From 12/2012 to 08/2014	Monthly	DOM optical characteristics
VI	Alcahozo and Manjavacas	1	From 11/2014 to 04/2015	Weekly	DOM dynamics and bacterioplankton activity

II.3.3. Phosphorus forms

The soluble reactive phosphorus (SRP) in water was determined by the phosphomolibdic acid-ascorbic method following the Standard Methods (APHA, 2005). In acid conditions the orthophosphate reacts with ammonium molybdate and potassium antimonyltartrate producing phosphomolybdic acid. When this compound is reduced with ascorbic acid it takes an intense blue coloration.

Total phosphorus in water (TP_w) and sediments (TPs) was determined on unfiltered water after performing an autoclave persulfate oxidation in an acidic matrix (135 °C for 2 h) to hydrolyze any P-form to orthophosphate. Determination of released orthophosphate was carried out as described for SRP after the pH-neutralization of samples (APHA, 2005). Total phosphorus (TP_w) and total dissolved phosphorus (TDP) in water were determined on unfiltered and GF/F filtered samples, respectively, after performing an autoclave persulfate oxidation in an acidic matrix (135 °C for 2 h) to hydrolyze any P-form to orthophosphate. Released orthophosphate in both digestions was quantified as described for SRP after the pH-neutralization of samples. The dissolved organic phosphorus in water (DOP) was estimated as the difference between TDP and SRP. Likewise, particulate phosphorus in water (PP) was obtained by subtracting the TDP from the TP_w.

II.4. Water mineralization

The calcium (Ca^{2+}) concentration in water was measured by titration with EDTA (APHA, 2005). The concentration of total iron (Fe^{2+} + Fe^{3+}) was determined after reaction with ferrospectral (3-(2-pyridyl)-5,6-bis (4-phenylsulfonic acid)-1,2,4-triazine) using a Beckman DU-7 spectrophotometer (De Vries and Ouboter, 1985). Total iron (Fe), magnesium (Mg) and manganese (Mn) concentrations were measured by ICP-MS (Agilent,model 7900). Sulfate (SO_4^{2-}) was determined by nephelometry, which consisted of a turbidimetric quantification upon precipitation of SO_4^{2-} as a barium salt. Alkalinity was measured after titration with HCl, using a pH shift indicator (phenophtalein) to the equivalence end point pH. Calculations for the dissolved inorganic carbon (DIC) speciation (CO_3^{2-} + CO_3H^- + CO_2) were performed using the hydrochemistry software Aqion (http://www.aqion.de/), which uses the USGS software PhreeqC as an internal numerical solver (Parkhurst and Appelo, 1999), by computing data of alkalinity, pH and temperature.

II.5. Optical characterization of the dissolved organic matter (DOM)

Ultraviolet-visible absorbance and fluorescence analyses were conducted all at once with samples warmed to room temperature. Absorption spectra were obtained between 240 and 750 nm on a Beckman DU-700 spectrophotometer using a 1-cm path length cuvette. Milli-Q water was used to remove the background. Sample absorbance at 750 nm was assumed to not be affected by CDOM and then it was subtracted from the spectra to correct baseline fluctuations. The Napierian absorption coefficient at 320 nm was obtained as follows:

$$a(\lambda)=2.303A(\lambda)/L$$

Where, $a(\lambda)$ is the absorption coefficient (m^{-1}) at a wavelength, 320 nm in this study, A is the absorbance at 320 nm and L is the cuvette length in meters. Data of coefficient a(320) normalized to DOC concentration were used as a measure of DOM aromaticity (Ortega-Retuerta et al., 2007), in such a way that the increase of this coefficient in proportion to the DOC indicates that this latter is more enriched in chromophores (Figure 2.3). In addition to single-wavelength measures, spectral slopes were also determined for the intervals of 275–295 nm (S275-295) and 350-400 nm (S350-400) by fitting the absorption spectra to linear regression on log-transformed spectra following Helms et al., (2008). These slope coefficients represent a measure of how the absorption decreases in relation to wavelength (Figure 2.3). Slopes S275-295 and S350-400 are used as surrogates of DOM molecular weight, low and high respectively (Helms et al., 2008). The slope ratio (Sr) was calculated as the ratio of S275–295 to S350–400.

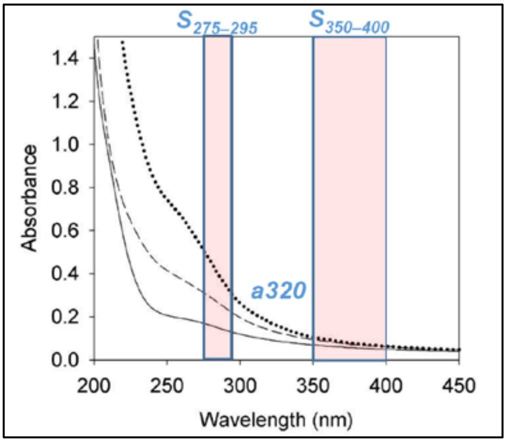


Figure 2.3. *Chromophoric fraction of DOM (CDOM) can be characterized by observing spectral slope regions and absorption coefficients. The increase of S275–295 relative to S350–400 is related with photochemically induced alterations and a decrease of molecular weight of DOM. The absorption coefficient a320 is usually used as a proxy of total CDOM.*

In Table 2.3 are summarized the DOM chromophores and fluorophores analyzed in this study. Ultraviolet-visible absorbance and fluorescence analyses were conducted all at once with samples warmed to room temperature. Absorption spectra were obtained between 240 and 750 nm on a Beckman DU-700 spectrophotometer using a 1-cm path length cuvette and applying regular background and baseline corrections. Spectral slopes were then determined for the intervals of 275–295 nm ($S_{275\text{-}295}$) and 350-400 nm ($S_{350\text{-}400}$) by fitting the absorption spectra to a linear regression on log-transformed spectra (Helms et al. 2008). These slopes coefficients represent a measure of how the absorption decreases with respect to wavelength. The slope ratio (*Sr*) was calculated as the ratio of $S_{275\text{–}295}$ to $S_{350\text{–}400}$. Slopes $S_{275\text{-}295}$ and $S_{350\text{-}400}$ are used as surrogates of DOM molecular weight, low and high respectively. To normalize the data obtained from $S_{275\text{-}295}$ these were divided by the DOC concentration.

Fluorescence parameters were acquired from excitation-emission matrix (EEMs, Figure 2.4) performed with an F-7000 Hitachi fluorescence spectrophotometer using a 1 cm quartz fluorescence cell following Coble (1996). Three separate fluorescent components were identified and labeled as previously described in the chapter 3

Considering that the position of peaks in the matrix may change rather, values were considered as the maximum fluoresce emission within a defined analytical range of excitation and emission pairs. Accordingly, for the humic-like substances, the maximum fluorescence signal at Ex/Em wavelength of 250-260 nm/380-480 nm was defined as peak FDOM-A, at 330–350 nm/420–480 nm as peak FDOM-C, and at 310–320 nm/380–420 nm as peak FDOM-M.

Derived index from EEMs was additionally used as surrogates of DOM origin and nature (Gabor et al. 2014). The freshness index (BIX) is a proxy of DOM age, with higher values representing a higher proportion of fresh DOM and this was calculated as the ratio of fluorescence signal at em 380 nm divided by max intensity between em 420 nm and em 435 nm at ex 310 nm. The fluorescence index (FI) indicates the DOM precursor material, and this is the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm, obtained with an excitation of 370 nm.

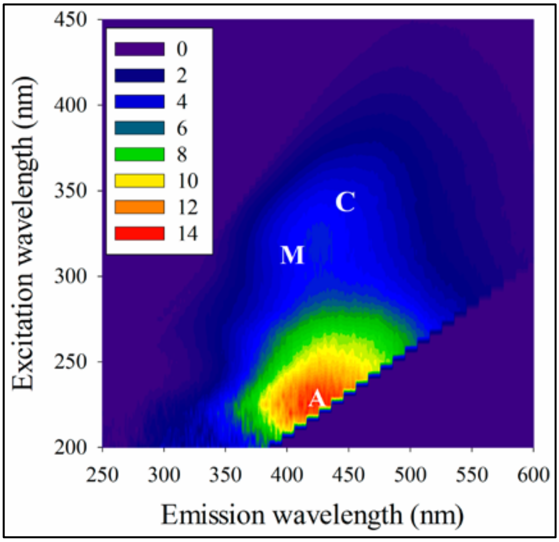


Figure 2.4. *The fluorescence excitation-emission matrix (EEM) spectrum of a water organic matter sample. Note: Image shows the three studied fluorophores at Excitation/Emission (Ex/Em) wavelength pairs. The 230/440 and 350/450 nm peaks were designated as A and C, respectively (humic-like substances). The 330/400 nm peak was designated as M peak (microbial degraded humic). Image taken from Wei et al., 2015.*

II.6. Quantification of chlorophyll-a concentrations in water

The concentration of chlorophyll *a* (Chl-*a*) was used as a proxy for phytoplankton biomass. For this, the seston retained in GF/F filters was submerged in 90% acetone overnight at -20 ºC to extract photopigments, which were subsequently determined by HPLC according to Picazo et al. (2013). The HPLC method reduced potential overestimations by discriminating native Chl-*a* from its major degradation products, which may be abundant in shallow lakes because the strong interaction between the sediment and water column.

II.7. Determination of bacterioplankton abundances and activity by flow cytometry

Flow cytometry analyses were performed with samples previously fixed with paraformaldehyde and glutaraldehyde. These samples (0.5 mL) were mixed with SYBR Green-I (Molecular Probes) diluted 1:10,000 (V/V) final concentration, and incubated in the dark for 20 min at room temperature for the cell staining. A Beckman Coulter flow cytometer (Cytomics FC 500 MPL) equipped with five fluorescent channels and 488 nm (argon laser) and 635 nm (red emitting diode) lasers was used. The bacterial cells were visualized during the acquisition by plotting the side scatter light (SSC), which is a proxy of the cell size, versus the green fluorescence (FL1) channel (Figure 2.5). In order to refine the analysis, the ratios of the green (FL1) versus red (FL4) fluorescence channels were also plotted, so as to discriminate the bacteria from cytometric noise. Fluorescent beads of 1 µm in diameter were used as size markers. This analysis allowed the separation of two main subpopulations of bacteria that were distinguished, high DNA (HDNA) and low DNA (LDNA) bacteria, depending of their green fluorescence intensity, which is proportional with their DNA content. The HDNA subpopulation was assumed as metabolically more active and constituted the dynamic fraction of the whole bacterial assemblage (Lebaron et al., 2001). The cell numbers were then obtained by considering gravimetrically the processed volume. The percentage of active cells (%HDNA) was calculated by dividing the number of HDNA bacterial cells in each sample by the total bacterial abundance (HDNA plus LDNA) (Jellett et al., 1996).

Table 2.3. *Spectral slopes in the ranges 275–295 nm ($S_{275-295}$), 350–400 nm ($S_{350-400}$), and the slope ratio of this slopes (Sr) calculated following Helms et al. (2008). Identified regions of observed fluorescence peaks (fluorophores M, C and A) sensu Coble (1996) in an excitation–emission spectrum of aquatic DOM. Fluorescence indices (BIX and FI) used in this study and their definitions.*

Parameters	Description
$S_{275-295}$	Indicates DOM of low molecular weight and/or decreasing aromaticity.
$S_{350-400}$	Indicates DOM of high molecular weight.
Sr	Ratio between $S_{275-295}$ and $S_{350-400}$. Negatively correlated to DOM molecular weight, higher *Sr* values are generally observed during CDOM photobleaching.
Peak M	Represents recently created (likely microbial) DOM, autochthonous nature
Peak C	Represents DOM of autochthonous nature; photolabile.
Peak A	Humic-like compounds of allochthonous origin; refractory.
Freshness Index (BIX)	Ratio of M and C peaks, therefore indicates proportion of recently produced DOM.
Fluorescence Index (FI)	Ratio of em 470 nm/em 520 nm at ex 370 nm. This index has a value of ~1.9 for microbially derived fulvicacids and a value of ~1.4 for terrestrially derived fulvic acids. Intermediary values suggest contributions from a combination of DOC sources.

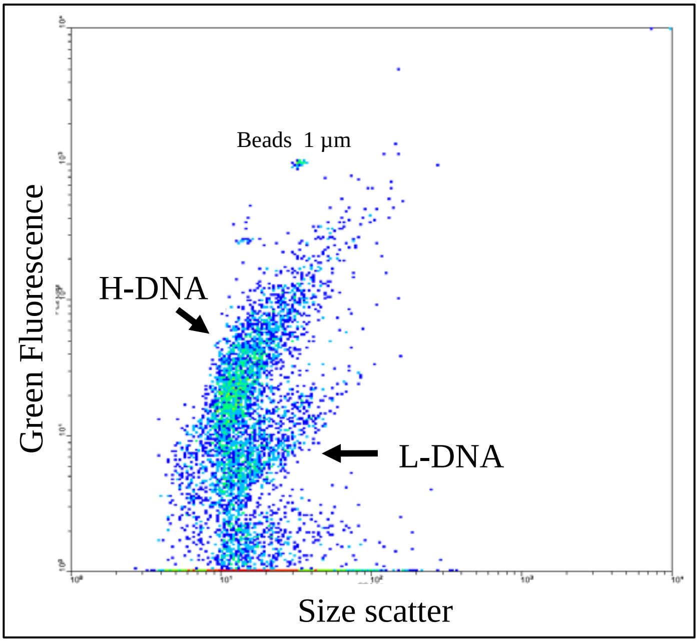


Figure 2.5. *Flow cytometer plot obtained in this study showing bacterioplankton populations HPP (Heterotrophic picoplankton). L-DNA (subpopulation with low DNA content); H-DNA (subpopulation with high DNA content).*

II.8. Measure of gross photosynthesis and respiration rates of plankton

Photosynthesis and aerobic respiration rates of plankton were estimated using the standardized method of the oxygen-exchange (Camacho et al., 2012; 2016; Morant et al., 2020). Water used for measurements was distributed into eight 250 ml Winkler bottles (Figure 2.6), four transparent replicates (net ecosystem production; NEP) and four dark replicates (respiration; R). These were incubated *in situ* for 2-4 h. Both NEP and R rates were then calculated based on the oxygen variations in the respective bottles and the incubation time. The gross primary production (GPP) was subsequently obtained by summing NEP and R, assuming equal dark and light respiration rates. Finally, Production/Respiration ratios (P/R) were calculated as the quotient between GPP and R. P/R ratios greater and lower than 1 indicate an autotrophic or heterotrophic metabolic balance, respectively.

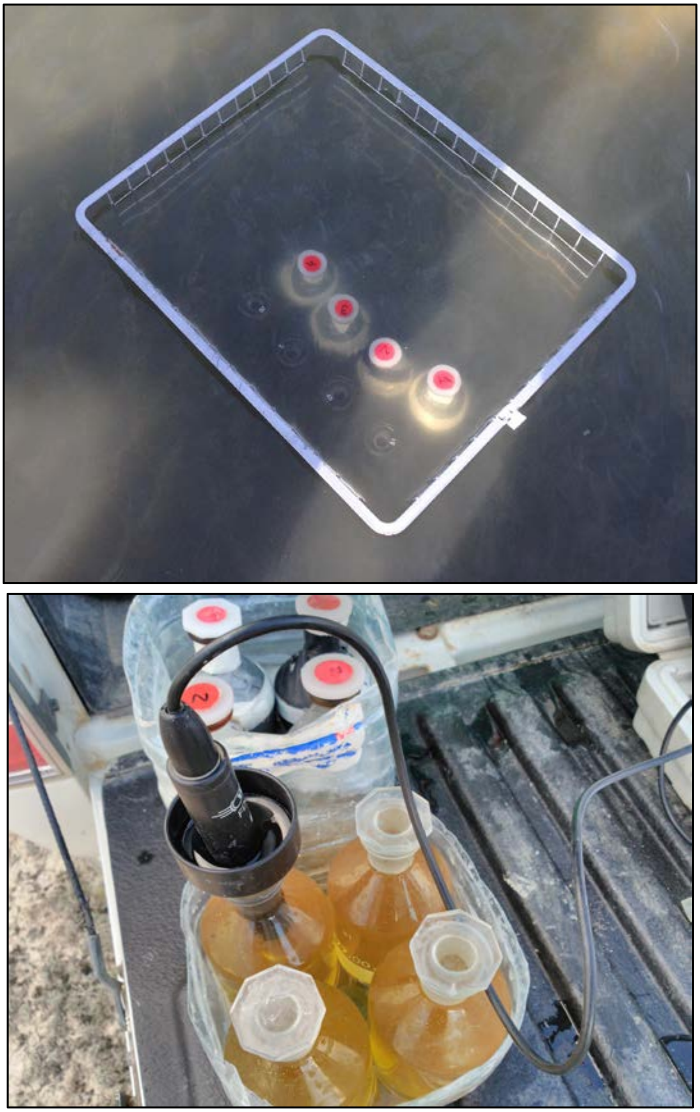


Figure 2.6. *Winkler bottles for measuring planktonic metabolic rates (dark and light bottles). The water samples were submerged to perform incubations at the in-situ light and temperature conditions.*

Chapter III: Trophic status and metabolic rates of threatened Mediterranean shallow saline lakes: Retrieving diagnostic elements to predict ecosystem functioning

III.1. Introduction

Anthropogenic nutrient enrichment of water bodies is the main cause of aquatic ecosystem pollution (Johnson et al., 2010; Seitzinger et al., 2010; Carey et al., 2013), and it is likely to increase further in the coming decades due to increased human activity (Moss, 2011; Crossman et al., 2013). Natural eutrophication might otherwise occur through the aging of the waterbodies, the extent of which depends on the catchment and lake basin characteristics (Nõges, 2009).

Shallow saline lakes in La Mancha Húmeda (Central Spain), a UNESCO Biosphere Reserve, are endorheic threatened systems, in which all the processes described previously converge (Florín and Montes, 1999; Camacho et al., 2017). Both cultural and natural eutrophication has led to an overall increase in nutrient availability in their basins, and most of them deserve urgent conservation and restoration actions. These shallow saline lakes are naturally temporary, which drives diverse process such as the patterns of nutrient transport in the catchment, the seasonal evaporative concentration of solutes and the productivity of the system (García-Ferrer et al., 2003; Camacho et al., 2017; Corrales-González et al., 2019). The hydroperiod of these lakes is the result of the transitional Mediterranean-continental climate condition of the area, which is characterized by warm and dry summers. Accordingly, seasonality plays an important role in the ecological functioning of these lakes compared to those from temperate regions (Nöges et al., 2003). However, the occurrence of wastewater inputs in some of them has increased the hydroperiod while decreasing the salts content, as well as increasing the availability of nutrients compared to pristine conditions (Florín and Montes, 1999). Nevertheless, those lakes not affected by wastewaters may be impacted by the diffusive nutrient runoff originating from the catchments (Camacho et al., 2017), which are mainly dedicated to agricultural activities. Also, natural eutrophication resulting from the catchment lithology may play a role in some of these lakes. This is the case of the volcanic lakes from the location within the reserve known as Campo de Calatrava, where the mechanisms allowing the phosphorus (P) immobilization in sediments are restrained compared to the non-volcanic lakes of the region (Cebriá and López-Ruiz, 1995; Corrales-González et al., 2019), thereby allowing a high availability of P in the water column.

The different hydrological scenarios explained above are expected to produce different trophic statuses (They et al., 2017). P is usually the limiting nutrient, but nitrogen (N) may also limit algal growth (Camacho and de Wit 2003, Camacho et al., 2003). High amounts of P in sediments may cause persistent eutrophication due to a chronic release (Søndergaard et al., 2003), which represents a problem which can remain for many years. This could be the case of the lakes in La Mancha Húmeda, in which sedimentary P is mainly occluded to calcareous and iron compounds, or immobilized in organic forms, but it can be released to the water column if the redox potential decreases as a consequence of organic matter (OM) degradation (Corrales-González et al., 2019). The water salinity might, indirectly, favor this P desorption by facilitating oxygen depletion, thus redox drops as the solubility of gases decreases with ionic strength. On the other hand, water salinity is known to slow the rates of dissolved organic matter (DOM) breakdown (Minor et al., 2006, Seo et al., 2008), thereby influencing the release of C and N. All these represent internal lake processes that, jointly with the anthropogenic pressures, are expected to alter the stochiometric balance of nutrients. This is in turn expected to impact on the metabolic function of the lakes as demonstrated by several experimental and observational studies (Elser et al., 2000; Vrede et al., 2009; Peñuelas et al., 2011; Hilt et al., 2017).

Dealing with the previous considerations, this chapter is aimed at addressing the role of the main environmental factors (i.e., water salinity, seasonality and nutrient inputs) in the nutrient availability and functioning of representative saline lakes from the Biosphere Reserve of La Mancha Húmeda (Spain). We conducted a wide-range survey attempting to establish simple and effective diagnostic parameters associated with the resource limitation patterns and C cycle functioning. The photosynthetic and the aerobic respiration rates of plankton were determined as proxies of the ecosystem functioning. These metabolic functions should provide insights into the biological turnover rates of nutrients in these lakes, which ultimately depend on availability and the stoichiometric balance. This study will help to better understand ecological concerns currently arising in these saline lakes. This should provide support for restoration measures that are in progress, this being justified by the high ecological significance of these lakes within the European context.

III.2. Methods

III.2.1. Sampling

A total of 17 lakes were studied for this chapter (Table 3.1) The lakes were selected based on a gradient of water mineralization, including both lakes with and without wastewater inputs (Table 1). This selection was made to cover the range of conditions observed in the region. They are all shallow, and range from subsaline to hypersaline (sensu Hammer, 1986). The lakes were sampled monthly over two hydrological cycles (Figure 3.1). Precipitation and temperature data were obtained from the meteorological observatory in Herencia (JCCM, 2014). The physical-chemical determinations and sampling of the relative flooding level of the lakes (%FL), the water electrical conductivity (EC), the oxygen saturation and the dissolved oxygen (DO) and the pH are explained in the Material and Analytical Methods section.

III.2.2. Statistical analyses

A multivariate analysis of variance (MANOVA) with Tukey´s Honest Significant Difference for multiple comparisons was designed to evaluate the effect of the three studied factors (i.e., *Nutrient source, Salinity and Season*) on the measured variables. The effect size of the explored factors was assessed by observing partial eta-squared (η^2) provided by MANOVA analysis, which indicates the proportion of variance accounted for a given factor (Lakens, 2013). The criteria for establishing the categories of these factors were as follow:

1. *Factor Nutrient source*: Three different categories were established (i.e., catchment runoff, wastewater and volcanic substrate), which refer to the main sources of nutrients entering the lakes. Catchment runoff (*R*) indicates a major role of diffusive inputs of nutrients, which is mainly related to the surrounding land uses. On the other

hand, lakes classified in the wastewater group (*Ww*) show point-source wastewater inflows from nearby towns. Finally, a lake was differentiated in the so-called volcanic category (*V*) because of the critical influence of this type of lithology, which provides large amounts of inorganic P (Cebriá and López-Ruiz, 1995). Lake Almodovar is representative of the volcanic area of the "Campo de Calatrava", where other similar volcanic lakes occur.

2. *Factor Salinity:* Four categories of water mineralization were established, namely, *subsaline*, *hyposaline, mesosaline* and *hypersaline*. Classification was carried out according to the ranges given by Hammer (1986), based on data of lake water conductivity (Figure 3).

3. *Factor Season*: Four groups were established for this factor: *Autumn*, when the lakes fill up; *Winter*, the period of maximum flooding; *Spring*, the most productive period associated with the onset of the drying process; *Summer*, the period when the lakes dry out, unless they remain artificially flooded by wastewater inputs.

Table 3.1. *Name, code, saline typology (sensu Hammer, 1986), size (ha), and depth (cm) of studied lakes. Gray scale in sampling schedule indicates the relative monthly flooding level of the lakes.*

Lake	Code	Surface (ha)	Max depth (cm)	Wastewater inputs	Saline typology
Alcahozo	ALC	70.00	21	No	Mesosaline
Almodovar	ALM	20.15	99	No	Hyposaline
Camino de Villafranca	CAM	142.66	78	Yes	Mesosaline
Caracuel	CAR	66.16	98	No	Subsaline
Grande de Villafranca	GRA	135.58	119	No	Hyposaline
Larga de Villacañas	LAR	88.02	71	Yes	Mesosaline
Longar	LON	93. 31	60	Yes	Hypersaline
Manjavacas	MAN	230.40	48	Yes	Mesosaline
Mermejuela	MER	9.57	68	No	Hypersaline
Nava Grande	NAV	118.80	90	No	Subsaline
Peñahueca	PEÑ	113.87	19	No	Hypersaline
Pozuelo	POZ	48.76	70	Yes	Hyposaline
Quero	QUE	79.43	30	No	Hypersaline
Salicor	SAL	58.15	20	No	Hypersaline
Tirez	TIR	92.48	19	No	Hypersaline
Veguilla	VEG	79.15	68	Yes	Subsaline
Yeguas	YEG	66.75	16	Yes (in the past)	Hypersaline

The overall variability observed in the lakes was examined by principal component analysis (PCA) using descriptive variables of the trophic status (i.e., nutrients and Chl-*a*). A Spearman correlation analysis was performed to assess relationships between all the studied variables, and between the PCA-scores and metabolic rates. The data were log transformed, when required, to ensure a normal distribution for the statistical analyses. The raw (untransformed) data are reported in the figures and tables as average values. In all cases, the level of statistical significance was set to $p < 0.05$. All the statistical analyses were performed using the University of Valencia-licensed statistical package SPSS v. 22 (SPSS Inc., Chicago, IL, USA).

III.3. Results

III.3.1. Physical-chemical characteristics of lakes

The monthly cumulative rainfall and average values of air temperature during the sampling period are shown in Figure 3.1. Average monthly temperatures ranged around 5-25ºC. Rainfall occurred mainly from autumn to early spring, and decreased when air temperatures started to rise, thereby yielding a negative water balance that explains the seasonality of lakes. However, some of the lakes remained flooded most of the year, sustained by inputs of wastewater (see the lakes affected in Table 3.1). During the studied period, the water column depth among the lakes ranged from a few centimeters to around 1 meter (Table 3.1). The water electrical conductivity (EC) varied notably among the lakes, ranged from 0.89 to 594 mS cm^{-1}. Following Hammer`s (1986) classification of saline lakes, the lakes were defined as subsaline, hyposaline, mesosaline or hypersaline (Figure 3.2). Regarding seasonal patterns, EC increased in all the groups as the hydroperiod advanced, and the depth decreased. In general, the pH of the water was slightly to moderately alkaline and averaged 8.62 ± 0.57 (Table 3.2).

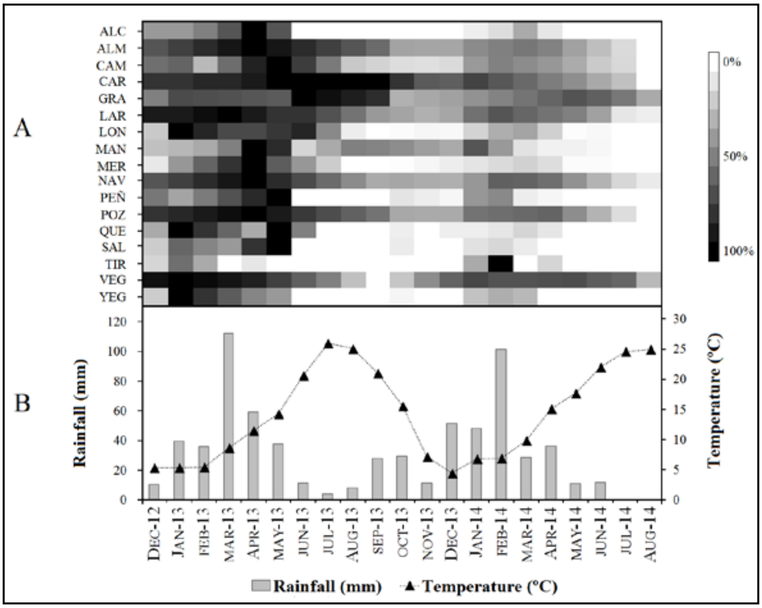


Figure 3.1. *A) Annual variation of the hydroperiod of the lakes. Gray scale indicates the relative flooding level (%FL, with respect to maximum level) of lakes based on monthly observations; the white and black colors indicate 0 and 100% of flooding respectively. B) Monthly accumulated rainfall (bars) and average air temperature (black triangles) in the study area.*

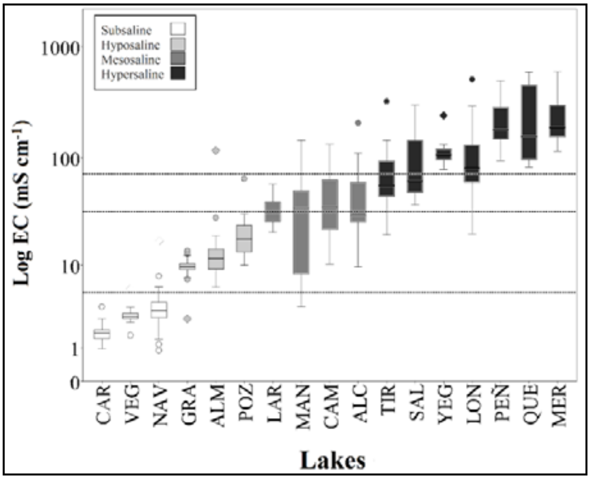


Figure 3.2*. Water electrical conductivity (EC) of the lakes during the studied period. The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). Codes of the lakes names as in Table 1.*

Oxygen saturation ranged from 81.45% to 120.91%. The lower and higher values were found in lakes Almodovar and Camino de Villafranca, which are a hyposaline and a mesosaline lake, respectively. In contrast to salinity, oxygen saturation in the lakes was highest in winter (average: 122.25 ± 42.97 %), whereas it was below saturation in the lakes that remained flooded in summer. Dissolved oxygen (DO) concentrations (range: 0.15-23.10 mg L^{-1}) showed levels of hypoxia more commonly in the hypersaline lakes such as Peñahueca, Mermejuela and Longar (Table 3.2), and the highest concentrations occurred in the subsaline lakes during winter. The turbidity produced by suspended particles or colloids in lakes averaged from values below 4 NTU, as observed in the subsaline lakes, to around one order of magnitude higher in the hypereutrophic lake Yeguas and the volcanic lake Almodovar (Table 3.2).

III.3.2. Phosphorus content in the water column and sediment of the lakes

The average concentrations of P in water and sediment indicated major differences in the trophic status of the lakes (Table 3.3). Data grouped based on MANOVA factors and the resulting post-hoc tests are shown in Figure 3, respectively. In general, soluble reactive phosphorus (SRP) increased with salinity and ranged for most of the lakes from 0.26 to 24.67 µM, representing on average around 10 to 20% of the total P content in most of the lakes. However, SRP accumulated remarkably in lakes Almodovar and Yeguas, reaching average concentrations of around 200 µM. In general, total phosphorus (TPw) concentrations in lake waters varied accordingly to the SRP; however, the SRP:TPw ratios were exceptionally high (>0.5) in lakes Almodovar and Yeguas, as well as in lake Veguilla. The total phosphorus in sediments (TPs) also varied broadly among sites (Table 3). The lowest values were measured in Lake Alcahozo, whereas the highest were observed in lakes Yeguas and Veguilla, which are both eutrophic sites affected by historical or present wastewater inputs, respectively. P also accumulated in the sediment of Lake Almodovar, but to a lesser extent compared to the sites previously mentioned.

Table 3.2. *Average values and standard deviation of pH, dissolved oxygen (DO), oxygen saturation and turbidity during the studied period. Codes of the lakes names as in Table 3.1.*

Lake name	pH	DO (ppm)	Oxygen saturation (%)	Turbidity (NTU)
Alcahozo	8.07 ± 0.48	8.46 ± 0.48	96.27 ± 15.53	19.91 ± 26.89
Almodovar	9.16 ± 0.58	7.54 ± 2.87	81.46 ± 37.30	39.69 ± 22.15
Camino de Villafranca	8.78 ± 0.37	9.98 ± 4.33	120.91 ± 68.37	16.71 ± 13.47
Caracuel	8.88 ± 0.57	8.49 ± 3.07	86.33 ± 26.31	3.84 ± 5.87
Grande de Villafranca	8.37 ± 0.40	7.87 ± 2.23	88.29 ± 19.30	2.21 ± 4.88
Larga de Villacañas	8.75 ± 0.70	9.05 ± 4.14	109.98 ± 56.21	21.84 ± 30.27
Longar	8.73 ± 0.63	8.55 ± 6.08	114.21 ± 79.88	26.37 ± 29.71
Manjavacas	8.53 ± 0.39	8.81 ± 2.79	93.35 ± 28.27	12.72 ± 13.34
Mermejuela	8.09 ± 0.63	4.86 ± 4.12	83.90 ± 74.41	32.19 ± 28.49
Nava Grande	8.09 ± 0.52	9.35 ± 3.24	98.02 ± 30.47	3.46 ± 5.30
Peñahueca	7.78 ± 0.61	5.36 ± 3.40	90.20 ± 46.41	38.37 ± 45.63
Pozuelo	8.63 ± 0.59	10.25 ± 4.07	118.75 ± 52.87	9.72 ± 11.39
Quero	8.19 ± 0.65	3.60 ± 2.65	108.14 ± 70.24	27.10 ± 37.45
Salicor	8.43 ± 0.37	7.38 ± 2.41	115.11 ± 28.32	30.95 ± 25.66
Tirez	8.33 ± 0.33	8.39 ± 1.70	101.50 ± 5.77	32.19 ± 41.29
Veguilla	8.24 ± 0.38	9.83 ± 5.25	106.73 ± 54.00	9.70 ± 13.67
Yeguas	8.29 ± 0.82	6.80 ± 4.34	108.96 ± 53.16	43.77 ± 41.20

Nutrient source was the factor explaining most of the variance observed for the different P fractions (Table 3.4). The effect size of this variance is indicated by the partial eta-squared (η2) obtained in the MANOVA analysis, which indicates the proportion of variance accounting for a given factor. The η2 values for SRP, TP_w and TP_s were 0.45, 0.64 and 0.59, respectively, which means that 45%, 64% and 59% of variance observed for these P fractions was explained by the Nutrient source factor, respectively. For SRP and TPw, the post-hoc analyses showed significant differences between the three subgroups ($p < 0.01$) (Figure 3.3). In the case of TPs, the average concentrations were significantly lower ($p < 0.01$) in the lakes where the catchment runoff constituted the most important input of nutrients (R subgroup); however, no significant differences were observed ($p > 0.05$) between the volcanic lake Almodovar (V subgroup) and those affected by wastewater inputs (Ww subgroup) (Figure 3.3). The Salinity factor explained 55% of variance observed for TPw (η2 = 0.55) (Table 3.4). Regarding the post-hoc grouping (Figure 4), the TPw was significantly lower ($p < 0.01$) in the subsaline lakes compared to the others. The hypersaline lakes showed a significantly lower ($p < 0.01$) average concentration of TPs (0.44 ± 0.54 mg g^{-1}) compared to the other subgroups. By contrast, TPs values were significantly higher in the subsaline lakes (0.96 ± 1.20 mg g^{-1}). No significant differences were observed regarding the Season factor ($p > 0.05$).

Table 3.3. *Concentration of soluble reactive phosphorus (SRP), total phosphorus in water TP_w, total phosphorus in the sediment (TP_s). Values are mean ± standard deviation of two consecutive hydrological cycle. Codes of the lakes names as in Table 1.*

Lake	SRP (µM)	TP_w (µM)	SRP:TP_w	TP_S (mg g^{-1})
ALC	0.26 ± 0.17	18.38 ± 26.44	0.03 ± 0.05	0.09 ± 0.06
ALM	188.82 ± 73.26	371.84 ± 479.85	0.75 ± 0.24	1.28 ± 0.21
CAM	9.54 ± 12.39	48.71 ± 28.82	0.18 ± 0.15	1.53 ± 0.42
CAR	1.25 ± 1.73	9.51 ± 11.90	0.35 ± 0.86	0.35 ± 0.16
GRA	0.59 ± 1.01	3.89 ± 2.76	0.17 ± 0.17	0.17 ± 0.07
LAR	5.63 ± 6.09	83.57 ± 118.71	0.09 ± 0.06	0.97 ± 0.25
LON	3.11 ± 4.18	78.32 ± 55.39	0.05 ± 0.06	0.33 ± 0.11
MAN	2.68 ± 4.23	32.22 ± 41.31	0.12 ± 0.18	0.55 ± 0.14
MER	5.47 ± 9.10	92.65 ± 68.18	0.06 ± 0.06	0.16 ± 0.04
NAV	0.90 ± 0.93	8.75 ± 15.44	0.19 ± 0.16	0.27 ± 0.15
PEÑ	24.67 ± 13.28	149.97 ± 73.66	0.20 ± 0.11	0.27 ± 0.06
POZ	3.44 ± 3.52	25.63 ± 17.21	0.23 ± 0.30	1.35 ± 0.16
QUE	10.60 ± 13.58	101.91 ± 74.48	0.11 ± 0.11	0.27 ± 0.03
SAL	4.47 ± 10.68	45.87 ± 40.53	0.06 ± 0.07	0.36 ± 0.19
TIR	4.11 ± 5.77	26.41 ± 24.18	0.18 ± 0.19	0.35 ± 0.12
VEG	17.63 ± 10.23	39.28 ± 27.83	0.56 ± 0.29	2.14 ± 1.37
YEG	203.37 ± 70.80	402.23 ± 253.97	0.61 ± 0.24	2.04 ± 0.50

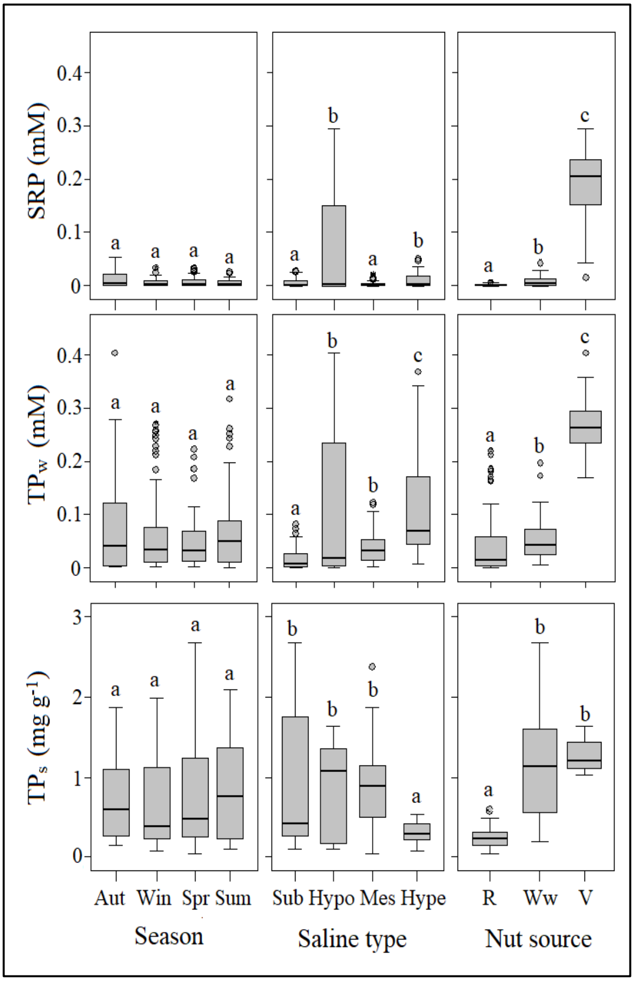


Figure 3.3. *Concentration of soluble reactive phosphorus (SRP), total phosphorus in water (TP_w) and total phosphorus in the sediments (TP_s) based on the factors explored in the MANOVA analysis: Season (Aut: autumn, Win: winter, Spr: spring, Sum: summer), Saline type of lakes (Sub. Subsaline, Hypo: hyposaline, Mes: mesosaline, Hype: hypersaline) and Nutrients source (R: run-off, Ww: wastewater, V: volcanic substrate). Letters W and S as subscript indicate water and sediment samples, respectively. The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). Letters above the boxes indicate post-hoc groups of Tukey HSD tests. Accordingly, the absence of a letter in common denotes significant differences ($p < 0.05$) between subgroups.*

Table 3.4. *MANOVA model of the effect of Season (S) (autumn, winter, spring and summer), saline typology (ST) (Subsaline, hyposaline, mesosaline and hypersaline) and Nutrients source (NS) (runoff, wastewater and volcanic sediments). Lowercase letter s, together with total phosphorus (TP) and nitrogen (TN) indicate sediment samples. Lowercase letter w, together with TP and TN indicate water samples. The statistical significance is set at the 0.05 probability level.*

		S				NS				ST			
		df	F	*p*	η^2	df	F	*p*	η^2	df	F	*p*	η^2
Water	SRP	3	1.29	0.28	0.02	2	78.16	<0.00	0.45	3	11.36	<0.00	0.15
	TP_w	3	0.74	0.53	0.01	2	169.05	<0.00	0.64	3	77.82	<0.00	0.55
	DOC	3	1.40	0.24	0.02	2	10.98	<0.00	0.10	3	112.02	<0.00	0.64
	TOC	3	2.77	0.04	0.04	2	10.81	<0.00	0.10	3	110.03	<0.00	0.63
	DTN	3	3.15	0.03	0.05	2	16.30	<0.00	0.15	3	69.30	<0.00	0.52
	TN_w	3	2.72	0.04	0.04	2	22.38	<0.00	0.19	3	87.14	<0.00	0.58
	TOC:TN_w	3	6.44	<0.00	0.09	2	1.68	0.19	0.02	3	16.95	<0.00	0.21
	TOC:TP_w	3	0.54	0.66	0.01	2	102.47	<0.00	0.52	3	5.90	<0.00	0.08
	TN_w:TP_w	3	1.02	0.38	0.02	2	94.00	<0.00	0.49	3	7.31	<0.00	0.10
	SRP:TP_w	3	2.10	0.10	0.02	2	14.12	<0.00	0.11	3	9.57	<0.00	0.11
Sediment	TN_s	3	5.86	<0.00	0.17	2	10.74	<0.00	0.20	3	3.13	0.03	0.10
	TP_s	3	0.29	0.84	0.01	2	61.38	<0.00	0.59	3	10.08	<0.00	0.26
	TN_s:TP_s	3	4.54	0.01	0.14	2	47.71	<0.00	0.53	3	4.68	<0.00	0.14
Metabolism	Chl-*a*	3	0.23	0.88	0.00	2	25.25	<0.00	0.21	3	10.51	<0.00	0.14
	GPP	3	14.10	0.00	0.10	2	72.26	<0.00	0.27	3	29.93	<0.00	0.19
	R	3	8.89	0.00	0.07	2	29.31	<0.00	0.13	3	28.08	<0.00	0.18
	P/R	3	0.47	0.71	0.00	2	20.23	<0.00	0.09	3	8.26	<0.00	0.06

Table 3.4 (continuation). *MANOVA model of the effect of interactions between the studied factors: Season (S) (autumn, winter, spring and summer), saline typology (ST) (Subsaline, hyposaline, mesosaline and hypersaline) and Nutrients source (NS) (runoff, wastewater and volcanic sediments). Lowercase letter s, together with total phosphorus (TP) and nitrogen (TN) indicate sediment samples. Lowercase letter w indicates water samples. The statistical significance is set at the 0.05 probability level.*

		S x NS				S x ST				NS x ST				S x NS x ST			
		df	**F**	***p***	**η^2**	**df**	**F**	***p***	**η^2**	**df**	**F**	***p***	**η^2**	**df**	**F**	***p***	**η^2**
Water	**SRP**	6	0.61	0.73	0.02	9	0.79	0.62	0.04	3	4.55	<0.00	0.07	8	0.58	0.79	0.02
	TP_w	6	1.70	0.12	0.05	9	1.79	0.07	0.08	3	7.48	<0.00	0.1	8	1.84	0.07	0.07
	DOC	6	1.52	0.17	0.05	9	2.83	<0.00	0.12	3	6.58	<0.00	0.09	8	2.18	0.03	0.08
	TOC	6	1.17	0.32	0.04	9	2.52	0.01	0.11	3	4.26	0.01	0.06	8	2.35	0.02	0.09
	DTN	6	1.20	0.31	0.04	9	2.22	0.02	0.09	3	0.78	0.51	0.01	8	0.96	0.47	0.04
	TN_w	6	1.80	0.10	0.05	9	3.01	<0.00	0.12	3	0.90	0.44	0.01	8	1.42	0.19	0.06
	$TOC:TN_w$	6	0.95	0.46	0.03	9	2.40	0.01	0.10	3	8.33	<0.00	0.12	8	2.49	0.01	0.09
	$TOC:TP_w$	6	0.40	0.88	0.01	9	1.79	0.07	0.08	3	13.64	<0.00	0.18	8	0.29	0.97	0.01
	$TN_w:TP_w$	6	0.87	0.52	0.03	9	3.02	<0.00	0.12	3	5.76	<0.00	0.08	8	0.58	0.80	0.02
	$SRP:TP_w$	6	2.22	0.04	0.05	9	1.74	0.08	0.06	3	1.42	0.24	0.02	8	0.35	0.94	0.01
Sediment	**TN_s**	6	1.29	0.27	0.08	9	0.49	0.88	0.05	3	1.18	0.32	0.04	6	1.03	0.41	0.07
	TP_s	6	0.58	0.75	0.04	9	0.79	0.63	0.08	3	7.42	<0.00	0.21	6	0.74	0.62	0.05
	$TN_s:TP_s$	6	0.22	0.97	0.02	9	0.57	0.82	0.06	3	7.43	<0.00	0.21	6	0.40	0.88	0.03
Metabolism	**Chl-*a***	6	0.98	0.44	0.03	9	2.03	0.04	0.09	3	1.71	0.17	0.03	8	1.07	0.38	0.04
	GPP	6	7.48	< 0.00	0.10	9	6.60	<0.00	0.13	3	14.28	<0.00	0.10	6	7.61	<0.00	0.10
	R	6	5.39	< 0.00	0.08	9	1.97	0.04	0.04	3	7.90	<0.00	0.06	6	7.40	<0.00	0.10
	P/R	6	2.49	0.02	0.04	9	4.31	<0.00	0.09	3	0.73	0.53	0.01	6	2.77	0.01	0.04

III.3.3. Nitrogen content in the water column and sediment of the lakes

The average concentrations of different nitrogen fractions in water and sediment of the studied lakes are shown in Table 3.5. Data grouped based on the MANOVA factors and the resulting *post-hoc* tests are shown in Figure 3.4. The total nitrogen in water (TN_w) of the lakes ranged from 0.05 mM to 13.79 mM, with higher values displayed by the meso and hypersaline lakes Manjavacas and Longar, respectively. Total dissolved nitrogen (TDN) represented around 50% of total nitrogen in most of the lakes (Table 3.5), and around half of this (48-66%) was dissolved organic nitrogen (DON). In sediments, seasonal average concentrations of total nitrogen (TN_s) ranged from 0.81 to 3.99 mg g^{-1}, in lakes Tirez and Veguilla, respectively, and it was double in those lakes affected by wastewater inputs (Table 3.5). The three factors of the MANOVA had a significant effect (p < 0.05) on the nitrogen concentrations both in water and sediments (Figure 3.4). The *Salinity* factor explained most of the variance ($\eta^2 > 0.5$) as shown in Table 3.4. In this case, both TDN and TN_w increased significantly (p < 0.01) with salinity.

Table 3.5. *Concentration of total dissolved nitrogen (TDN), total nitrogen in water (TN_w), total nitrogen in sediments (TN_s), dissolved organic carbon (DOC) and total organic carbon (TOC). Values are mean ± standard deviation of two consecutive hydrological cycle. Codes of the lakes names as in Table 3.1.*

Lake	TDN (mM)	TN_w (mM)	TN_S (mg g^{-1})	DOC (mM)	TOC (mM)
ALC	0.29 ± 0.29	0.63 ± 0.98	1.20 ± 0.26	5.70 ± 8.05	7.21 ± 12.78
ALM	0.65 ± 1.36	0.94 ± 1.68	1.52 ± 0.77	10.42 ± 26.22	12.29 ± 30.05
CAM	0.53 ± 0.35	0.87 ± 0.52	3.92 ± 1.91	8.37 ± 6.16	10.54 ± 6.75
CAR	0.15 ± 0.09	0.22 ± 0.10	2.11 ± 1.10	2.19 ± 1.36	2.59 ± 1.59
GRA	0.14 ± 0.09	0.19 ± 0.09	2.03 ± 0.81	1.65 ± 0.63	2.07 ± 0.59
LAR	0.72 ± 0.44	1.56 ± 1.62	3.86 ± 1.37	9.34 ± 4.32	13.01 ± 10.48
LON	1.72 ± 1.51	3.35 ± 3.66	2.41 ± 0.70	32.32 ± 24.72	55.35 ± 59.96
MAN	0.51 ± 0.41	0.72 ± 0.56	2.97 ± 1.43	5.98 ± 4.84	8.14 ± 7.45
MER	1.95 ± 1.51	3.31 ± 2.31	1.72 ± 0.48	35.73 ± 34.78	62.03 ± 65.77
NAV	0.13 ± 0.11	0.17 ± 0.11	2.45 ± 1.25	1.82 ± 1.21	2.00 ± 1.37
PEÑ	0.60 ± 0.24	1.25 ± 0.49	0.91 ± 0.35	15.77 ± 9.60	20.10 ± 11.69
POZ	0.34 ± 0.26	0.52 ± 0.43	2.72 ± 1.54	3.68 ± 3.35	5.25 ± 6.71
QUE	0.94 ± 0.58	1.68 ± 1.35	1.52 ± 0.32	22.78 ± 21.47	26.84 ± 21.32
SAL	0.35 ± 0.12	0.62 ± 0.22	1.42 ± 0.28	5.01 ± 1.82	5.89 ± 2.03
TIR	0.28 ± 0.18	0.50 ± 0.55	0.81 ± 0.26	4.04 ± 4.70	5.25 ± 6.70
VEG	0.19 ± 0.12	0.29 ± 0.18	3.99 ± 1.70	1.10 ± 0.35	1.55 ± 0.83
YEG	1.24 ± 0.77	2.46 ± 1.08	2.99 ± 1.19	22.54 ± 16.25	30.15 ± 18.40

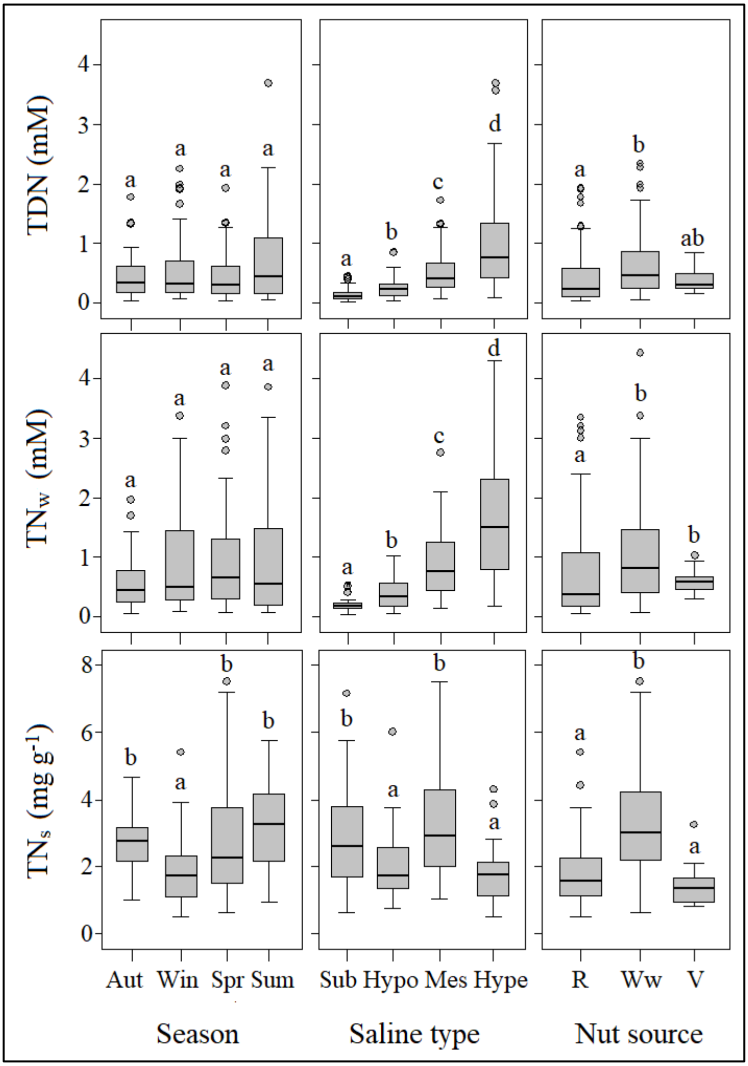


Figure 3.4. *Concentration of total dissolved nitrogen (TDN), total nitrogen in water (TNw) and total nitrogen in the sediments (TNs) based on the factors explored in the MANOVA analysis: Season (Aut: autumn, Win: winter, Spr: spring, Sum: summer), Saline type of lakes (Sub. Subsaline, Hypo: hyposaline, Mes: mesosaline, Hype: hypersaline) and Nutrient source (R: run-off, Ww: wastewater, V: volcanic substrate). Letters W and S as subscript indicate water and sediment samples, respectively. The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). Letters above the boxes indicate post-hoc groups of Tukey HSD tests. Accordingly, the absence of a letter in common denotes significant differences (p < 0.05) between subgroups.*

III.3.4. Organic carbon (OC) content in the water column of the lakes

Total organic carbon (TOC) in lake waters occurred mainly in dissolved form (~80%) and ranged from 2.00 ± 1.37 mM to 62.03 ± 65.77 mM, in lakes Nava Grande and Mermejuela, respectively (Table 3.5). The Salinity factor explained most of the variance observed for both the TOC ($\eta^2 = 0.63$) and dissolved organic carbon (DOC) ($\eta^2 = 0.64$). The post-hoc comparisons showed significant differences between all salinity subgroups ($p < 0.01$), in such way that TOC and DOC increased gradually with salinity (Figure 3.5). As for the Nutrient source factor, TOC and DOC concentrations were significantly higher ($p < 0.01$) in lakes with wastewater inputs compared to the others. On a seasonal basis, TOC concentrations were slightly, though significantly, higher during summer ($p = 0.04$), coinciding with the higher temperatures and the drying out of the lakes (Figure 3.5).

III.3.5. Nutrient molar ratios in the water column and sediment of the lakes

The stoichiometric ratios of TOC, P and N in both water and sediments of the lakes are shown in Figure 3.6. The TOC:TP_w ratio ranged from 12.50 to 3112.63, while the TN_w:TP_w ratio ranged from 0.83 to 213.03. The values of these ratios were higher than those expected by Redfield ratios (i.e., 106 and 16, respectively), except for the highly polluted lakes Veguilla and Yeguas, as well as for the volcanic lake Almodovar. In lakes Almodovar and Yeguas these molar ratios were low because of their high concentrations of TP_w, whereas in Lake Veguilla this was due to the low concentrations of TN_w and TOC (Table 3.5). This contrasted with the P-deficiency observed in unpolluted lakes such as Grande de Villafranca, Alcahozo or Caracuel.

TOC:TP_w and TN_w:TP_w ratios were significantly affected by the factors *Nutrient source* and *Salinity* ($p < 0.01$) (Figure 3.7), particularly by the former, which explained around 50% of the variance (Table 3.4). The interaction between these two factors was also significant ($p < 0.01$). On the other hand, no clear pattern was observed for the TOC:TN_w ratios (Figures 3.6 and 3.7), and no segregation between polluted and unpolluted lakes was detected in this case. However, *Salinity* significantly explained ($p < 0.01$) 21% of variance observed for the TOC:TN$_w$ molar ratio (Table 3.4), and the interaction between salinity and the *Season* factor explained nearly 10% of variance (p = 0.01). In this case, the lowest TOC:TN$_w$ ratios were found in summer for subsaline lakes, whereas the highest occurred in autumn, during the flooding period, in the hypersaline lakes (Figure 3.7).

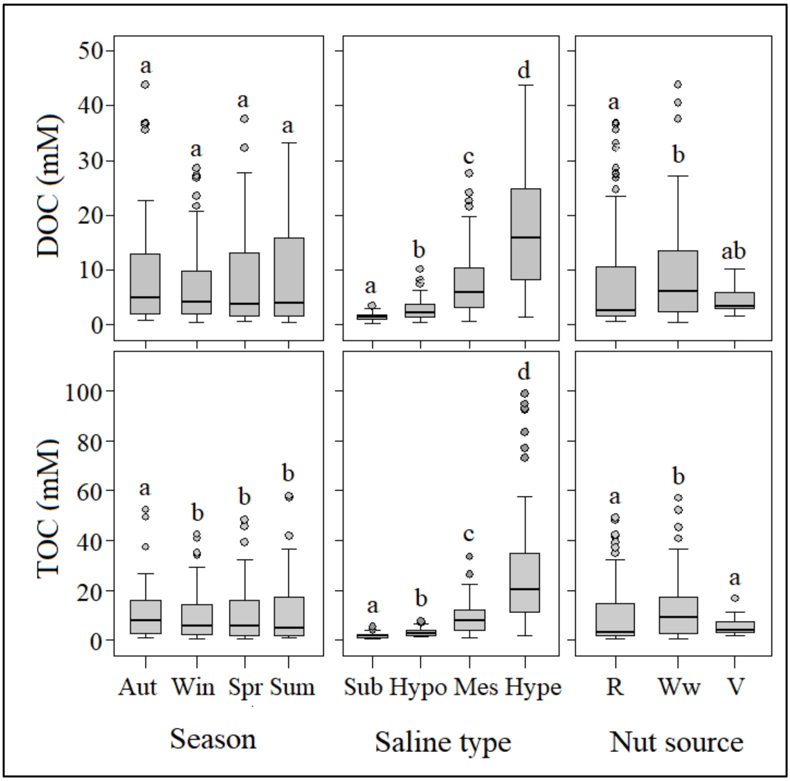


Figure 3.5. *Concentration of dissolved organic carbon (DOC) and total organic carbon based on the factors explored in the MANOVA analysis: Season (Aut: autumn, Win: winter, Spr: spring, Sum: summer), Saline type of lakes (Sub. Subsaline, Hypo: hyposaline, Mes: mesosaline, Hype: hypersaline) and Nutrient source (R: run-off, Ww: wastewater, V: volcanic substrate). Letters W and S as subscript indicate water and sediment samples respectively. The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). Letters above the boxes indicate post-hoc groups of Tukey HSD tests. Accordingly, the absence of a letter in common denotes significant differences (p < 0.05) between subgroups.*

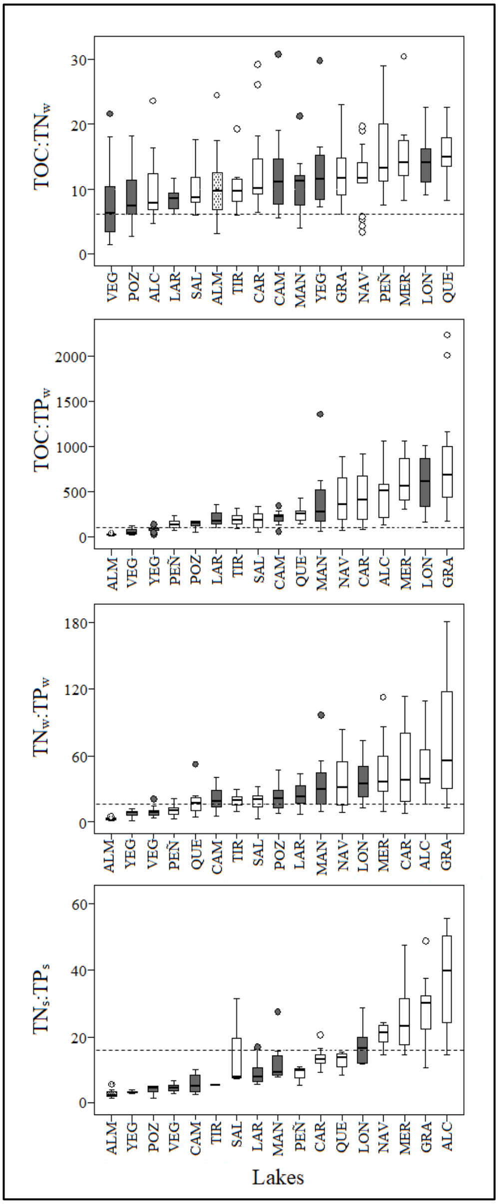


Figure 3.6. *Stoichiometric ratios of nutrients in the water (TOC:TN$_w$, TOC:TP$_w$ and TN$_w$:TP$_w$) and sediment (TN$_s$:TP$_s$) of the studied lakes. Dotted lines indicate the molar Redfield Ratio (Redfield, 1958). White boxes represent unpolluted lakes, gray boxes represent lakes with wastewater inputs and the box filled with black dots represents the volcanic lake Almodovar (ALM). The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots).*

As expected, the TN_s:TP_s molar ratios in sediments indicated a relative P enrichment compared to the water. As observed for water, the groups based on both *Nutrient source* and *Salinity* factors showed significant differences ($p < 0.01$) (Figure 3.7), but only *Nutrient source* explained an important percentage of the variance ($\eta^2 = 0.53$). In this case, the P enrichment of sediments was greater (i.e., TN_s:TP_s molar ratio <16) in polluted lakes, particularly in the volcanic lake Almodovar (Figure 3.7). On the other hand, in lakes where nutrients mainly come from runoff (*R* subgroup), the average TN_s:TP_s molar ratios were around 20.

III.3.6. Chlorophyll-a concentrations, gross photosynthesis, and aerobic respiration rates of plankton

The Chlorophyll-*a* (Chl-*a*) concentrations in the water column varied greatly among the lakes (Figure 3.8), displaying average values that ranged from oligo-mesotrophic (2.45 ± 2.76 µg L^{-1}) to hypertrophic (185.14 ± 201.15 µg L^{-1}) conditions. Higher concentrations occurred in the lakes still receiving wastewater inputs such as Larga de Villacañas or Longar, but also in those where these inputs have ceased at present, but are still affecting the internal load (i.e., Lake Yeguas). As observed in Table 3.4, the variance displayed by Chl-*a* concentrations was mainly explained by the *Nutrient source* ($\eta^2 = 0.21$; $p < 0.01$). *Salinity* significantly ($p < 0.01$) explained only around 10% of the variance. Thus, concentrations were lower in the sub- and hyposaline lakes compared to the meso- and hypersaline lakes (Figure 3.9). On the other hand, no significant differences depending on the season ($p > 0.05$) were observed (Figure 3.9).

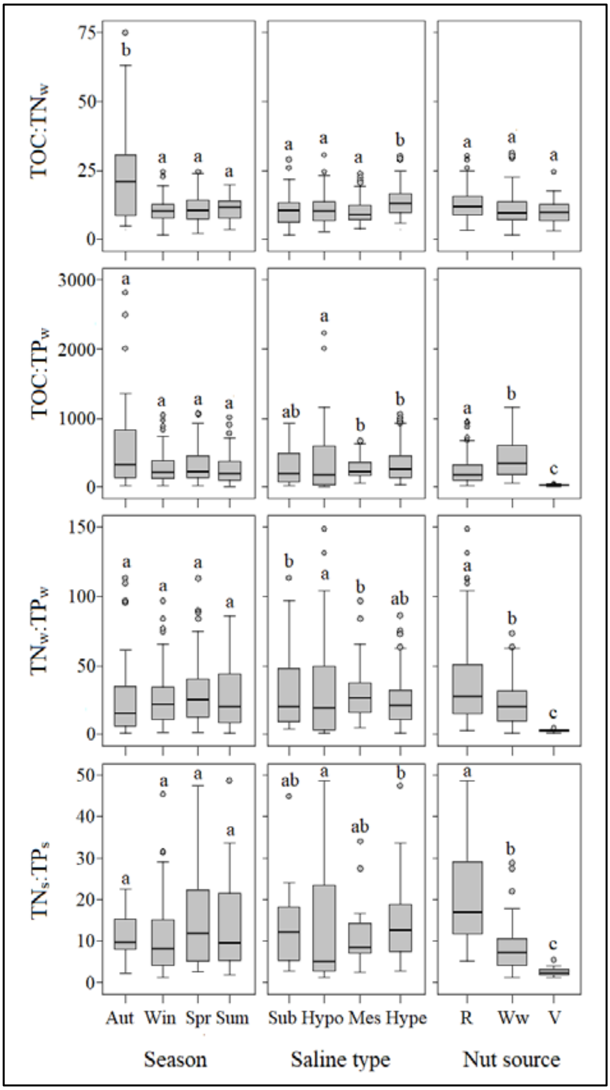


Figure 3.7. *Stoichiometric relationships of nutrients in the water (TOC:TN$_w$, TOC:TP$_w$ and TN$_w$:TP$_w$) and sediment (TN$_s$:TP$_s$) of the studied lakes based on the factors explored in the MANOVA analysis: Season (Aut: autumn, Win: winter, Spr: spring, Sum: summer), Saline type of lakes (Sub: Subsaline, Hypo: hyposaline, Mes: mesosaline, Hype: hypersaline) and Nutrient source (R: run-off, Ww: wastewater, V: volcanic). The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). Letters above the boxes indicate post-hoc groups of Tukey HSD tests. Accordingly, the absence of a letter in common denotes significant differences ($p < 0.05$) between subgroups.*

The gross primary production (GPP) of plankton varied according to the trophic status of the lakes. Extremely high values of GPP were measured in Lake Longar (>300 µmoles O_2 L^{-1} h^{-1}), while lower values occurred in Lake Nava Grande (Figure 3.8). Also, the highest average aerobic respiration (R) rates of plankton were measured in Lake Longar, whereas the lowest rates were measured in oligotrophic and mesotrophic lakes such as Alcahozo, Grande de Villafranca and Quero (Figure 3.8). The three MANOVA factors showed significant differences for the GPP rates ($p < 0.01$) (Figure 3.9), and the higher variance was explained by the *Nutrient source* ($\eta^2 = 0.27$) and *Salinity* ($\eta^2 = 0.19$) factors. Aerobic respiration rates displayed the same pattern (Figure 3.9) but with slightly lower values of η^2. Among these factors, significantly higher rates ($p < 0.01$) of both GPP and R were measured in lakes affected by wastewaters and in hypersaline lakes (Figure 3.9).

The Production/Respiration ratio (P/R) increased roughly with the trophic status, and it was consistently higher than 1 (Figure 3.8). This was higher in the eutrophic lakes such as Almodovar and Veguilla and lower in oligotrophic ones such as lakes Tirez and Alcahozo. The increase in the metabolic rates with salinity was proportionally higher in the case of respiration, and P/R ratios decreased accordingly (Figure 3.9). Regarding *Season*, both GPP and R rates were significantly higher in summer ($p < 0.01$), showing similar dynamics during the studied period (Figure 3.9). Consequently, the P/R ratios were rather stable through the hydroperiod (Figure 3.9).

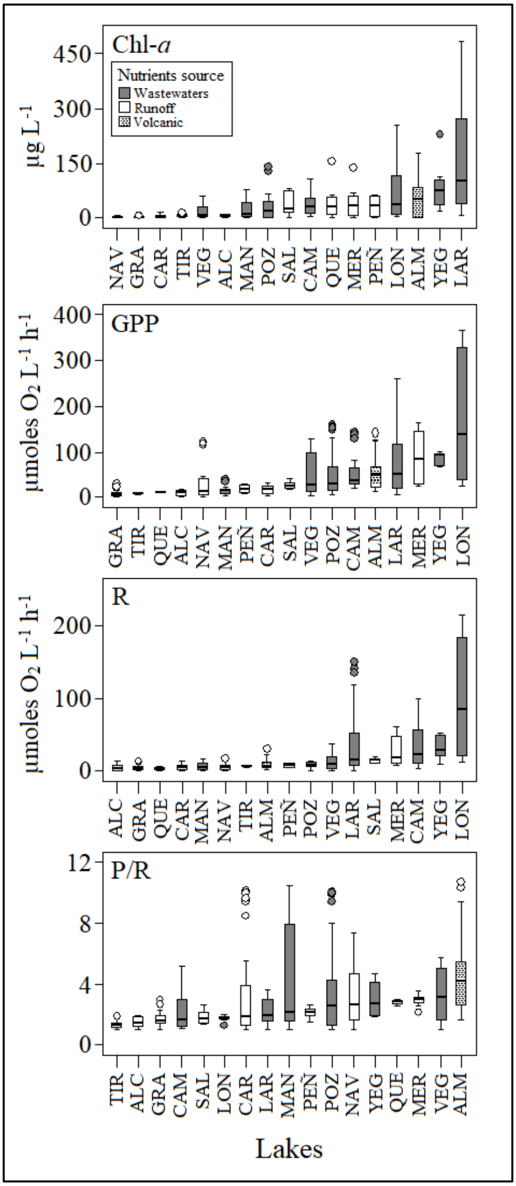


Figure 3.8. *Algal biomass and metabolism in the plankton of lakes: Chlorophyll a concentration (Chl-a), gross primary production (GPP), respiration rates (R) and production/respiration ratio (P/R). White boxes represent unpolluted lakes, gray boxes represent lakes with wastewater inputs and the box filled with black dots represents the volcanic Lake Almodovar (ALM). The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots).*

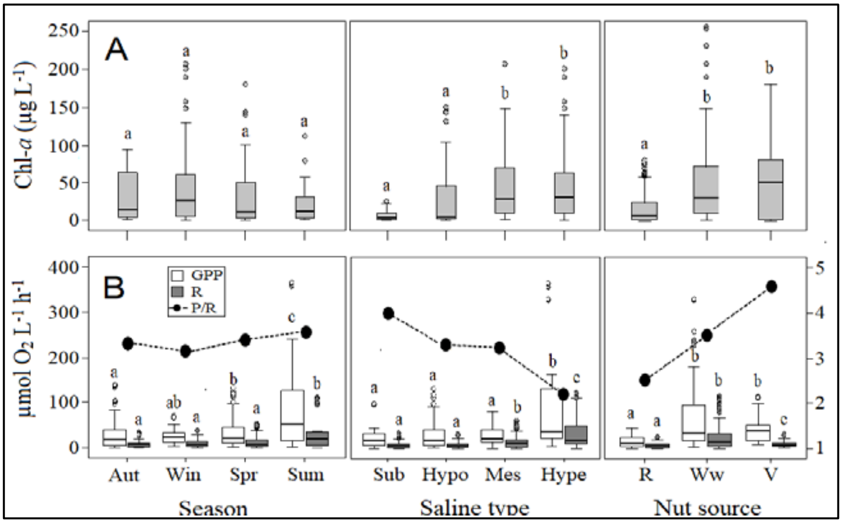


Figure 3.9. *A) Chlorophyll a concentration (Chl-a), B) gross primary production (GPP), respiration rates (R) and production/respiration ratio (P/R) of the plankton of studied lakes. Data are grouped based on the factors explored in the MANOVA analysis: Season (Aut: autumn, Win: winter, Spr: spring, Sum: summer), Saline type of lakes (Sub. Subsaline, Hypo: hyposaline, Mes: mesosaline, Hype: hypersaline) and Nutrient source (R: run-off, Ww: wastewater, V: volcanic substrate). The box plot shows the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). Letters above the boxes indicate post-hoc groups of Tukey HSD tests. Accordingly, the absence of a letter in common denotes significant differences (p < 0.05) between subgroups.*

III.3.7. <u>Principal components (PCA) analysis</u>

Sorting of samples resulting from the principal component analysis (PCA) is shown in Figure 3.10. The first (PC1) and second (PC2) components explained 34% and 29% of the total variance, respectively. PC1 showed the largest positive loadings for all P stocks and Chl-*a* concentrations, thereby arranging samples principally based on the trophic status shown by these variables. On the positive side of this axis, the samples most affected by point-source pollution were positioned, whereas the negative side displayed samples associated with runoff. Despite being unaffected by wastewater inputs, samples from Lake Almodovar are located in the positive part of this axis due to a high release of P related to volcanic lithology. On the other hand, PC2 showed the highest positive loadings for TOC and TN_w. Samples arranged on the positive side of this axis were related to longer water residence times and evaporative concentration processes. The loadings for TN_s were lower, thus indicating that this variable played a minor role in the clustering of the lakes. The PCA-scores of the samples for the first and second components increased with salinity in both wastewater and runoff subgroups (Figure 10). As proof of the bias from the general pattern, samples from the volcanic Lake Almodovar displayed the highest and lowest average scores for the first and second components, respectively.

III.3.8. Correlation analysis

The Spearman correlations obtained between the studied variables and the statistical confidence are shown in Table 3.6. In general, both nutrient concentrations in water and EC correlated negatively with the depth of the water column, indicating the occurrence of an overall process of evapo-concentration of solutes. Phosphorus measured in water correlated significantly with the P content in sediments; however, no N fraction from water showed a statistically significant correlation with the sedimentary N. On the other hand, TN_w positively correlated with P and organic C measured in water. Likewise, the Chl-*a* concentration positively correlated with the concentration of nutrients in water, as well as with TP_s.

Correlations between GPP and R were assayed to evaluate if carbon supporting secondary production in plankton derives mainly from the planktonic photosynthetic rates (i.e., stronger correlations) or it is also fueled by external sources (i.e., weaker correlations). The complete data set showed a strong correlation ($r = 0.84$; p-value < 0.001) between both rates. Correlation sorted by season was also significant in all cases, although visibly lower during winter ($r = 0.70$: p-value < 0.001) and higher in summer ($r = 0.91$; p-value < 0.001). With regard to the saline type, the lowest and highest, though significant, correlations were observed for the mesosaline ($r = 0.79$; p-value < 0.001) and hypersaline ($r = 0.95$; p-value < 0.001) subgroups, respectively. By sorting the data based on nutrient source, both wastewater and runoff subgroups showed significant (p-values < 0.001) correlations (r > 0.80), whereas this was lower, though significant (r = 0.71; p-value = 0.047), for Lake Almodovar, the only lake in the volcanic subgroup.

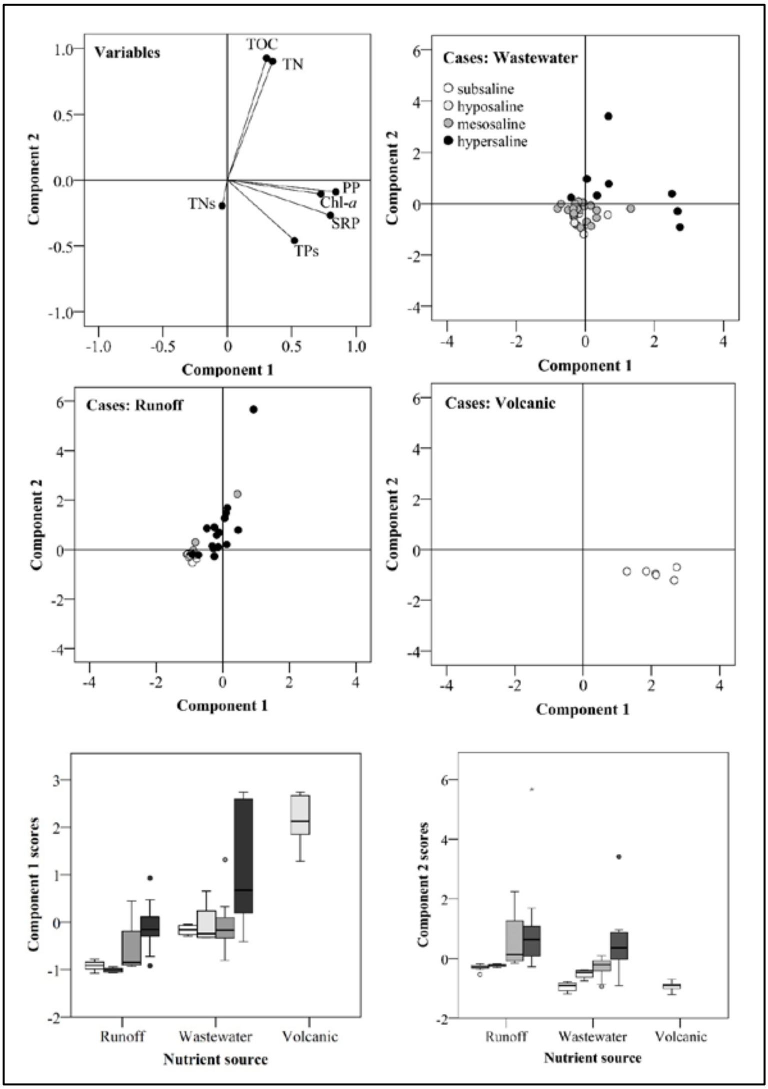


Figure 3.10. *Principal components analysis based on the trophic variables of the studied lakes. Biplots for the first and second components of the PCA are represented separately based on the Nutrient factor (Runoff, Wastewater and Volcanic). Colors of the circle symbols and boxplots are based on the Salinity factor (subsaline, hyposaline, mesosaline and hypersaline) as indicated in the legend.*

The correlation between the PCA-scores of the samples (i.e., cause variables) and the metabolic rates (i.e., effect variables) were also explored. In this case, the scores of first component of the PCA, which represented the trophic status of the lakes, correlated positively with GPP (r = 0.68; p<0.001) and, to a lesser extent, with R (r = 0.46; p < 0.001). However, no statistically significant correlation was observed between these metabolic rates and the scores of the second component of the PCA, which corresponds to higher water residence times and the enhancement of evapo-concentration processes.

Table 3.6. *Spearman correlation coefficients for the relationships between the studied variables in the lakes. Bold type indicates significant relationships (* p < 0.05; ** p < 0.01). Letters W and S as subscript indicate water and sediment samples, respectively.*

	EC	SRP	TP_w	DOC	TOC	TDN	TN_w	TP_s	TN_s	Chl-*a*
Depth	-0,48**	-0,10	-0,32**	-0,49**	-0,49**	-0,33**	-0,33**	0,27**	0,09	-0,22**
EC		0,21**	0,58**	0,83**	0,85**	0,74**	0,79**	-0,13	-0,12	0,45**
SRP			0,70**	0,29**	0,31**	0,33**	0,34**	0,42**	-0,00	0,31**
TP_w				0,70**	0,71**	0,69**	0,74**	0,30**	-0,09	0,53**
DOC					0,98**	0,85**	0,88**	-0,05	-0,01	0,49**
TOC						0,87**	0,89**	-0,01	0,00	0,50**
DTN							0,94**	0,09	0,04	0,45**
TN_w								0,11	0,06	0,52**
TP_s									0,53**	0,40**
TN_s										-0,09

III.4. Discussion

The environmental factors explored in this chapter that are affecting lakes functioning are diverse on their own as they are related to anthropic pressures, biogeochemical characteristics of the catchments, or seasonality. However, they are interconnected and influence each other. Accordingly, wastewater discharges have modified the water regime of some of these lakes, which has altered their natural hydroperiod to the point that some which were previously temporary are currently semi-permanent waterbodies. Seasonality makes a distinction between the functioning of these Mediterranean shallow lakes compared to cold-temperate lakes from central Europe (Nöges et al., 2003), and should be preserved as a particular ecological pattern.

Urban wastewaters, whether treated or not, are also a direct driver for desalination, as observed in this study. Extensive agriculture also promotes eutrophication (Bayley et al., 2013), but in this study it is quantitatively less important compared to pollution associated with wastewaters.

The PCA analysis shows the progression of the different eutrophication process occurring in the lakes. The arrangement of samples in the first component is related to an increasing influence of the previously mentioned point-source pollution, whereas the second component illustrates the present endorheic nature of these lakes that also imposes a natural and more gradual accumulation of nutrients. Thus, in the absence of wastewaters, the progressive increase in concentrations of nitrogen and organic carbon along with greater water retention times is evident.

The metabolism of the planktonic compartment behaves according to the previously described trophic scenarios. This is expressed by the correlations between the cause (i.e., PCA components) and response (i.e., metabolic rates) variables. In the present study, the anthropic eutrophication, which is more clearly reflected in the first component of the PCA, better explains primary (GPP) rather than secondary production (R). Although both metabolisms are highly coupled, indicating that it is the autochthonous photosynthetic activity which fuels respiration by the release of fresh and labile dissolved organic matter (DOM), a certain mismatch between them is still observed, particularly in winter. This is probably caused by contributions of allochthonous inputs of organic matter (OM) associated with the recharge of the lakes that occurs during this period. This has been observed in other endorheic basins (Zagarese et al., 2017; Huang et al., 2018), and it has been associated with inputs of detritic materials with a low nutritional quality (Kritzberg et al., 2006; Berggren et al., 2015). The higher TOC:TN$_w$ and TOC:TP$_w$ ratios also measured in the lakes during this period support the latter idea. These ratios decrease as the hydroperiod advances and the water residence time increases. The GPP and R show, in general, a high correlation for the different saline types, but the significant reduction in the P/R ratio observed for the hypersaline lakes compared to the others is noteworthy. The proportional increases of oxygen consumption that produce this drop could be caused by the solubilization of organic material accumulated in sediments, which may occur due to the enhancement of clay dispersion and OM mobilization caused by the Na-organic linkages (Sumner et al., 1998; Rengasamy et al., 2016).

The second component of the PCA denotes both an intensified deficiency of P and a weaker influence on the metabolic activities. This is particularly evident in the unpolluted lakes arranged based on this component (e.g., Alcahozo, Tirez, Peñahueca, Salicor). In them, the concentrations of TOC (~80% as dissolved organic carbon; DOC) are notably elevated, yet within the range described for saline lakes (Osburn et al., 2011; Boros et al., 2017; Butturini et al., 2022). TOC increases proportionally more than the other nutrients as the salinity and the water retention time increase. A buildup of uncolored DOC, likely recalcitrant, apparently occurs in these unimpaired lakes. This agrees with what has been observed in other steppe lakes (Waiser and Robarts, 2000; Osburn et al., 2011; Torremorell et al., 2015) and likely explains the lower respiration rates measured in them. Elevated solar UV radiation, intensified by prolonged water retention times, has been argued to explain the accumulation of colorless DOM (Osburn et al., 2011; Torremorell et al., 2015). However, it should be noted that photobleaching may decay, in part, if water ionic strength is high (Minor et al., 2006; Song et al., 2017; Zhu et al., 2018) as is the case of the studied lakes. In any event, this accumulation of low biodegradable DOM has major implications in C cycling, as it involves a high stock of organic matter left aside from mineralization. This is expected to promote the C sink role of these lakes when they are unthreatened, as also identified in other studies (Camacho et al., 2017).

A major deviation from the patterns described previously is observed in Lake Almodovar, which is representative of the volcanic area Campo de Calatrava. It shows a catchment substrate containing alkali basalts (Cebriá and López-Ruiz, 1995), which have a high content of P (Porder and Ramachandran, 2013) and a limited capacity to adsorb the excess of this P. This causes the high release of soluble reactive phosphorus (SRP) observed in this lake and underscores the sensitivity of volcanic lakes to suffer eutrophication if N is concurrently supplied.

However, the elevated turbidity, which is typical of volcanic lakes (Fernández et al., 2009), produces a high attenuation of light that limits the primary production. This would explain the moderate phytoplankton biomass and photosynthetic activity measured in Lake Almodovar, despite the exceeded availability of nutrients.

In summary, this study provides knowledge related to nutrient dynamics and function in shallow saline lakes; a subject which has still not been fully explored. They are very sensitive systems because of their closed, sinking nature, hence both environmental and human-produced perturbations may dramatically alter the stoichiometric relationships of nutrients and the C-related metabolisms. A relevant point here is that further inputs of nutrients, whether N or P, may add to the surplus of other nutrients which already exists. The implications of this in C cycling are important. In this sense, our results indicate that the secondary production of plankton, which represents an important part of the potential C export from the system, is mainly fueled by autochthonous primary production, which is ultimately regulated by non-natural inputs of nutrients. This is likely relevant in lakes from the site where the benthos plays a minor role compared to the plankton (Camacho et al., 2017). Our findings must serve as a background to assess the progression of the ecological status of shallow saline lakes under different scenarios. These include lakes subjected to pollution caused by humans as well as those naturally eutrophicated by either the existence of outsized evaporative and endorheic processes or specific catchment lithologies. These are concerns that should be appraised by management strategies. Therefore, different actions should consider the role of salinity (and artificial desalting) in the cycling of nutrients, as well as the vulnerability of volcanic lakes to suffer eutrophication. More particularly, restoration measures should focus on reducing the point-source inputs of nutrients rather than modifying agricultural practices. This is based on the idea that wastewaters play a major role in deteriorating the ecological status of these lakes as demonstrated in this study. Furthermore, due to diverse economic and social reasons, it would represent a more affordable task compared to the modification of land uses.

Chapter IV: Effect of wastewater management on phosphorus content and sedimentary fractionation in Mediterranean saline lakes

IV.1. Introduction

Phosphorus (P) loads are often the sustaining productivity factor in aquatic ecosystems (Schindler, 1978). Phosphorus cycling depends on several lake-internal factors, both abiotic and biotic, which can be interrelated. Among them are: the ionic strength of the water (Odum, 1988; Bailey et al., 2006; Van Diggelen et al., 2014; Herbert et al., 2015), the nature of deposited organic P (Ishiwatari, 1985; Oluyedun et al., 1991; Lü et al., 2016; Resmi et al., 2016), andmicrobial activities related to the iron and sulfur cycles (Gächter et al., 1988; Hupfer and Lewandowski, 2008; Wang et al., 2019). Sequential P fractionation is a frequently used approach to understand P transformations and dynamics in aquatic sediments (Psenner et al., 1984; Jensen and Thamdrup, 1993; Lukkari et al., 2007; Lü et al., 2016). This approach has been extensively used in freshwater ecosystems, although, so far, its application has been very limited in hypersaline lakes.

Saline endorheic lakes from the La Mancha Húmeda Biosphere Reserve (Central Spain) represent the main steppe wetland district of Western Europe (Alonso, 1998; Florín and Montes, 1999) showing high physiographical and ecological diversity (Camacho et al., 2003, 2017). This region exhibits a transitional Mediterranean-Continental climate, characterized by hot and dry summers, with wetter periods during the rest of the year. The lakes are naturally temporary and saline, with shallow waters where a gradual accumulation of nutrients occurs as a consequence of catchment inputs and high evaporation rates. The water quality of some lakes is affected by the input of sewage, or semi-treated wastewater effluents (mainly urban wastewater), which increase the trophic status, produce desalinization, and lengthen the hydroperiod (Florín andMontes, 1999; Camacho et al., 2003; García-Ferrer et al., 2003). We hypothesize that, despite general similarities among lakes, P-mobilization varies due to differences in the trophic status and water mineralization. This was partly investigated in a study performed by Gilbert et al. (2014) on two Mediterranean lakes (a subsaline and a hypersaline lake), which demonstrated how the P sorption capacity of sediments varied widely depending on water salinity. These authors highlighted the effect of seasonal drying on the availability of P in the water column, as well the critical role of organic matter in sequestering

P in this type of lake. Our study delves deeper into these concerns, and provides valuable information and conclusions that have a direct application in the design of restoration measures.

In our study, P speciation was studied in the sediments of a set of 11 lakes from the La Mancha Húmeda Biosphere Reserve, displaying contrasting degrees of eutrophication, hydroperiod length, and salinity. We evaluated the relationship between P bioavailability and the productivity of the lakes based on P-chemical fractionation. Despite their particular biogeochemical features, there are almost no studies on these types of Western-European saline lakes addressing aspects such as the cycling of nutrients. The studied lakes offer an observational setting with wide ranges of salinities and trophic status, thus making them appropriate models to assess P burial and mobilization processes. Furthermore, our results would contribute to focus restoration efforts and management plans in the region, aiming to recover the natural features of these lakes, specifically those facing eutrophication problems.

IV.2. Methods

IV.2.1. Sampling

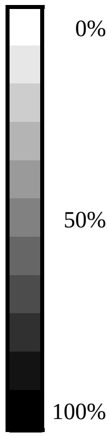
A total of 11 lakes were selected (Table 4.1) based on a gradient of water mineralization, and whether there was wastewater disposal in the lakes or not, in order to try to cover the entire range of conditions found in the region. The selected lakes range from subsaline to hypersaline (sensu Hammer, 1986) (Table 4.1). The lakes were sampled monthly over a complete hydrological cycle following the schedule set out in Table 4.2. Weather conditions (i.e., precipitation and temperature) were obtained from the SIAR (Irrigator Integral Advice Service) meteorological observatory located in the nearby town of Herencia (JCCM, 2014). In addition to the P fractionation analysis, a thorough analysis of water mineralization was carried out with samples obtained in January 2014, to assess concentrations of the main elements also associated with the P cycle.

Table 4.1. *Name, code, saline typology (sensu Hammer, 1986), size (ha), and depth (cm) of studied lakes. Gray scale in sampling schedule indicates the relative monthly flooding level of the lakes.*

Lake	Code	Saline Typology	Surface (ha)	Max depth (cm)
Alcahozo	ALC	Mesosaline	70	21
Almodóvar	ALM	Hiposaline	20.15	99
Caracuel	CAR	Subsaline	66.16	98
Grande de Villafranca	GRA	Hiposaline	135.58	119
Larga de Villacañas	LAR	Mesosaline	88.02	71
Longar	LON	Hypersaline	93. 31	60
Manjavacas	MAN	Mesosaline	230.4	48
Salicor	SAL	Hypersaline	58.15	20
Tirez	TIR	Hypersaline	92.48	19
Veguilla	VEG	Subsaline	79.15	68
Yeguas	YEG	Hypersaline	66.75	16

Table 4.2. *Name and occurrence of wastewater inputs (i.e. Yes or No). Gray scale in sampling schedule indicates the relative monthly flooding level of the lakes. Black indicates 100% and white 0%. Codes of the lakes names as in Table 1.*

Lake	Wastewater Inputs	Dec.	Jan.	Feb.	Mar.	Apr.	May
ALC	No						
ALM	No						
CAR	No						
GRA	No						
LAR	Yes						
LON	Yes						
MAN	Yes						
SAL	No						
TIR	No						
VEG	Yes						
YEG	Yes (in the past)						



IV.2.2. P fractionation analysis

The extraction of different P fractions was performed on the homogenized sediment samples, following the sequential procedure described by Lukkari et al. (2007). By doing this, P fractions in the sediments are characterized based on their differential solubility in various chemical extractants. This method slightly modifies the fractionation procedure first introduced by Psenner et al. (1984), and later modified by Jensen and Thamdrup (1993).

First, the sediment sample was extracted with 0.5 M NaCl for 1 h to extract loosely adsorbed and pore water P (Loosely-P). Subsequently, the sediment residue from the previous extraction was extracted with a buffered (pH 7.0) mixture of 0.11 M sodiumdithionite ($Na_2S_2O_4$) and 0.11 M sodium bicarbonate ($NaHCO_3$). This step allows for the extraction of the redox-sensitive P fraction (occluded-P fraction) bound to hydrated oxides (Fe/Al-P), particularly to iron. At this point it is worth mentioning some methodological issues. The presence of dithionite interferes with the spectrophotometric readings when the molybdenum blue method is used, as in our case. So, to efficiently quantify the Occluded-P fraction extracted in this step, samples were first aerated overnight to oxidize sodium dithionite following Jensen and Thamdrup (1993). The next extraction step was performed to obtain polyphosphates (Poly-P), and consisted of an extraction of sediments with NaOH 0.1 M for 18 h. This concentration of NaOH is expected to extract the easily hydrolysable organic P compounds. In the next extraction step, the remaining sediment was extracted for 1 h with 0.5 M HCl to bind P to the apatite minerals (Ca/Mg-P). Finally, to determine residual or recalcitrant organic P (Resid-P), the sediment residue from the previous extraction was boiled in 1 M HCl for 1 h. This fraction mainly includes refractory organic forms of non-labile P; however, it was simply referred to as residual P since it might contain some occluded inorganic P, which is more likely to occur in Fe-rich sediments (Lukkari et al., 2007). All the extractions were performed under moderate but continuous shaking, and were separated by centrifugation at 4000 rpm for 15 min. Supernatant filtered through GF/F was then subjected to persulphatic-acid hydrolysis, and later determination of the resulting SRP as described for the P analyses in water. The results of P fraction content in sediments are expressed on a dry weight basis. By summing up all the fractions, we attained the total-P (TP_S) extracted from the sediment.

IV.2.3. Statistical analyses

Two different types of statistical analyses were conducted to assess the variability observed for the phosphorus fractions and the main limnological variables in the lakes. First, a multivariate analysis of variance (MANOVA) with Tukey's honest significant difference (HSD) was performed for multiple comparisons in order to evaluate the effects of three different factors (i.e., fixed variables) namely, season (i.e., winter or spring), the lake's saline typology (i.e. subsaline, hyposaline, mesosaline or hypersaline; sensu Hammer, 1986), and the occurrence of wastewater inputs (i.e. presence or absence). The MANOVA was conducted with all phosphorus fractions obtained from water and sediment and 4 selected limnological variables (i.e., Chl-*a*, Fe, LOI_{460} and Ca). With regard to the factor season, winter was the period of maximum flooding, whereas a progressive decrease in the flooded area occurred during spring, before drying out. Secondly, a correlation analysis was carried out to assess the relationships between all the studied variables. Data were rank transformed to meet assumptions of equal variance and normality. In all cases, the level of significance was established at $p < 0.05$. All the statistical analyses were performed using the University of Valencia-licensed statistical package IBM SPSS v.24 (SPSS Inc., Chicago, IL, USA).

IV.3. Results

IV.3.1. Physical and chemical characteristics of water

The daily mean air temperature during the study period was 12.05 ± 6.11 °C, and the accumulated rainfall was 259 mm. The depth of water column among the lakes fluctuated from a few centimeters to around 1 m throughout the study (Table 4.1). The maximum flooding level of the lakes was measured in winter, while drying started in spring. The EC mean values in the studied lakes ranged broadly from 1.75 to 136.73 mS cm−1 (Table 4.3). The EC for each saline typology was (median ±

interquartile range): subsaline (1.85 ± 1.13 mS cm^{-1}), hyposaline (10.69 ± 2.62 mS cm^{-1}), mesosaline (35.57 ± 31.16 mS cm^{-1}) and hypersaline (80.10 ± 92.70 mS cm^{-1}). Extreme values of EC were found in the drying season (spring). In general, the pH of the lake water was moderately alkaline (8.62 ± 0.57) (Table 4.3). The oxygen saturation in the studied lakes ranged from 47.10% to 196%. Lower or higher values were respectively found in lakes Veguilla and Yeguas, a subsaline and a hypersaline lake, respectively. Regardless of salinity, winter was the season when the lakes showed higher values of oxygen saturation (108.35 ± 36.80%). The DO concentrations varied broadly among the lakes (Table 4.3), from0.86 to 16.50mg L^{-1}. The levels of hypoxia were more commonly measured in lakes such as Veguilla and Longar during the spring. On the contrary, higher concentrations of DO occurred during winter in undisturbed lakes (Table 4.1 and 4.2) with lower salinity.

The Chl-a concentrations varied three orders of magnitude among the lakes (Table 4.4), ranging from oligotrophic (1.84 ± 0.19 μg L−1) to hypertrophic (134.36 ± 88.51 μg L−1) conditions. The concentration of TPw ranged from 1.92 to 105.92 μM, except in lakes Almodovar and Yeguas, where the highest TPw concentrations were consistently measured, ranging from 222.92 to 341.28 μM. Chl-a and TPw indicate major differences in the trophic status of the lakes (Table 4.4). Particulate phosphorus (PP) was usually the most abundant fraction of P, except in lakes Almodovar, Veguilla and Yeguas (Figure 4.1A), which proportionally showed higher amounts of the inorganic soluble fraction (SRP). In general, the concentration of TPw was higher in spring (Figure 4.1A), except in permanently flooded lakes such as Grande de Villafranca and Larga de Villacañas (Figure 4.1A). In these lakes, this trend was the contrary because the flooding level was higher in the spring (Table 4.1), when our study had finished.

Table 4.3. *Mean ± standard deviation of main limnological variables measured in the lakes during the study period. Lowercase letters, s and w, together with total phosphorus (TP) indicate sediment and water samples, respectively. See the text for abbreviations. Codes of the lakes names as in Table 4.1.*

Lake	EC ($mS\ cm^{-1}$)	pH	DO ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Fe ($mg\ L^{-1}$)
ALC	102.97 ± 75.23	8.13 ± 0.81	7.03 ± 0.26	98.78 ± 6.82	0.22 ± 0.33
ALM	12.96 ± 3.59	9.50 ± 0.12	10.20 ± 2.32	2.78 ± 0.23	2.90 ± 1.67
CAR	1.75 ± 0.10	8.83 ± 0.48	9.71 ± 2.04	4.80 ± 1.35	0.19 ± 0.10
GRA	9.10 ± 0.75	8.57 ± 0.41	8.02 ± 2.17	95.15 ± 2.02	0.15 ± 0.00
LAR	26.88 ± 2.71	9.05 ± 0.24	11.30 ± 3.28	86.10 ± 0.76	0.26 ± 0.20
LON	107.70 ± 59.75	8.60 ± 0.36	7.51 ± 4.11	59.60 ± 11.02	0.49 ± 0.14
MAN	39.60 ± 12.44	8.69 ± 0.29	8.63 ± 2.38	83.40 ± 5.54	0.15 ± 0.17
SAL	74.65 ± 60.98	8.37 ± 0.06	8.40 ± 0.00	64.50 ± 4.85	0.57 ± 0.75
TIR	131.37 ± 165.91	8.27 ± 0.58	8.83 ± 0.58	69.20 ± 33.88	34.28 ± 59.25
VEG	2.63 ± 0.60	8.37 ± 0.25	8.31 ± 5.23	21.68 ± 3.86	1.23 ± 2.27
YEG	136.73 ± 90.09	7.93 ± 0.68	8.59 ± 6.87	50.07 ± 11.88	0.57 ± 0.19

Table 4.4. *Mean ± standard deviation of main limnological variables measured in the lakes during the study period. Lowercase letters, s and w, together with total phosphorus (TP) indicate sediment and water samples, respectively. See the text for abbreviations. Codes of the lakes names as in Table 4.1.*

Lake	Chl-*a* (µg L^{-1})	LOI_{460} (% dw)	LOI_{950} (% dw)	TPw (µM)	TPs (µg g^{-1} dw)
ALC	15.41 ± 16.17	22.99 ± 0.82	14.25 ± 20.82	36.56 ± 46.34	67.44 ± 18.33
ALM	113.32 ± 48.74	4.80 ± 1.72	4.53 ± 1.55	272.00 ± 29.76	857.17 ± 127.92
CAR	6.88 ± 10.27	6.75 ± 5.46	7.11 ± 13.79	8.59 ± 10.60	323.15 ± 178.90
GRA	1.84 ± 0.19	15.08 ± 1.47	6.55 ± 1.12	4.64 ± 3.86	376.45 ± 317.93
LAR	53.42 ± 32.67	19.53 ± 1.29	10.15 ± 1.25	48.47 ± 23.40	853.31 ± 292.31
LON	94.27 ± 70.03	26.95 ± 3.71	2.19 ± 1.79	66.70 ± 17.45	334.92 ± 93.90
MAN	32.49 ± 30.38	21.10 ± 2.64	7.52 ± 2.32	45.13 ± 29.48	527.92 ± 133.77
SAL	76.82 ± 3.71	18.56 ± 2.33	7.88 ± 1.79	32.70 ± 13.55	579.86 ± 105.81
TIR	5.68 ± 0.93	18.18 ± 1.85	6.32 ± 0.98	41.50 ± 39.78	470.48 ± 85.79
VEG	60.14 ± 109.11	12.21 ± 2.79	7.47 ± 6.82	38.58 ± 12.59	1463.32 ± 196.60
YEG	134.36 ± 88.51	25.98 ± 3.76	4.85 ± 1.32	277.97 ± 54.91	1163.43 ± 303.24

The Ca concentration in the water column of the lakes differed greatly (Table 4.3), ranging from 2.40 to 107.30 mg L^{-1}. The lowest concentrations were found, particularly, in the volcanic Lake Almodovar. This probably explains the exceptionally high concentrations of SRP found in the water column of this lake, given that the co-precipitation of Ca is a main mechanism to remove P from the water column in saline lakes. However, lakes with higher Ca concentrations (Table 4.3) showed, conversely, the lowest SRP concentrations (Figure 4.1A). In addition, the Fe concentration in the water column of the lakes ranged from 0.01 (Lake Alcahozo) to 102.69 mg L^{-1} (Lake Tirez) (Table 4.3).

The survey conducted in January to assess the main dissolved salts in lake waters reflected great differences, both in terms of salt concentration and ionic composition (Table 4.5). SO_4^{-2} was notably abundant (>1 g L^{-1}) in all the lakes except lakes Almodovar and Veguilla, nevertheless, it was the dominant anion measured in the water of these lakes. Likewise, lower concentrations of Mg were observed in both lakes, particularly in the former, as well as in Lake Caracuel. However, in the rest of the studied lakes, Mg concentrations were notably high, ranging from 1,093 mg L^{-1} (Lake Grande de Villafranca) to 16,362 mg L^{-1} (Lake Yeguas). On the other hand, the highest Na concentrations were measured in the hypersaline lakes. Seasonal determinations showed that Ca and Fe reached the lowest and the highest concentrations, respectively, in Lake Almodovar. The highest concentrations of Mn (~100 mg L^{-1}) were measured in lakes Manjavacas and Larga de Villacañas, whereas in lakes Yeguas and Almodovar they were around half of these concentrations, but still higher than those observed in the other sites. Total inorganic carbon (IC) in lake waters ranged from 24.87 to 364.17mg L^{-1}, the lowest and highest concentrations being measured for lakes Grande de Villafranca and Almodovar, respectively. This IC was mainly found as bicarbonate (>80%) in all the lakes. However, in Lake Almodovar bicarbonate represented only 68% of total IC as this lake had the highest pH.

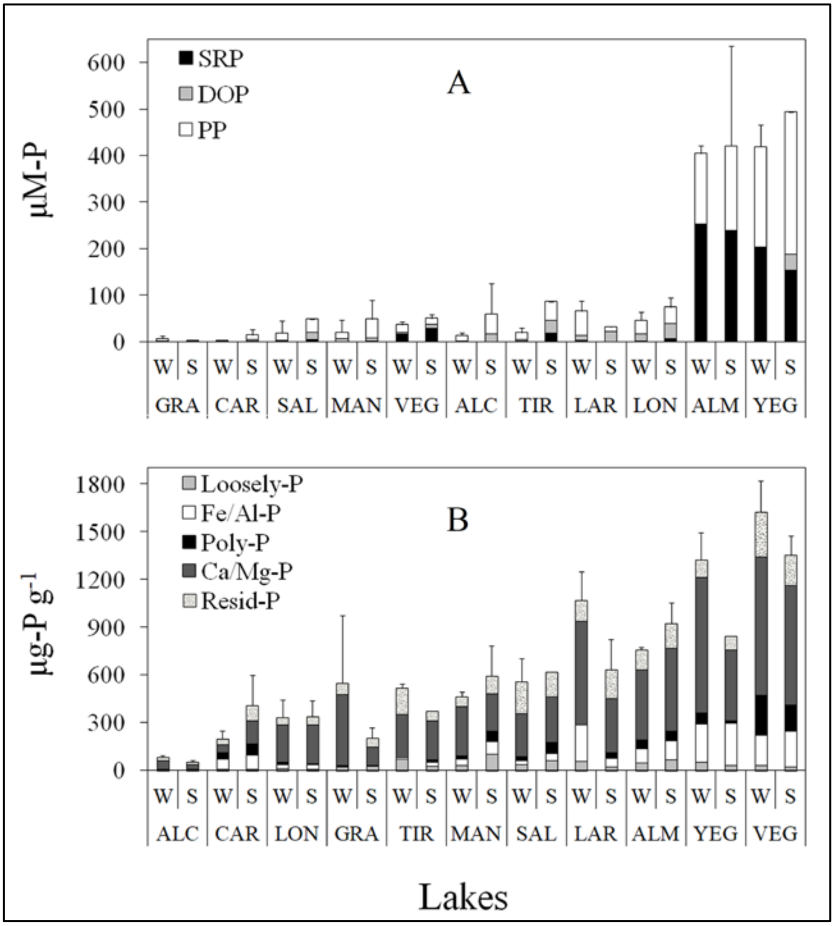


Figure 4.1. *Phosphorus fractionation in the studied lakes. A) Average concentration inwater of soluble reactive phosphorus (SRP), dissolved organic phosphorus (DOP) and particulate phosphorus (PP) during winter (W) and spring (S). B) Phosphorus fractions attained from sediments during winter (W) and spring (S). The error bars indicate the standard deviation for the addition of fractions. Abbreviations for the names of the lakes as in Table 4.1.*

Table 4.5. *Water mineralization (main dissolved ions; mg L-1) in the studied lakes in January 2014. Concentrations of inorganic carbon compounds are shown as carbon units (mg C L^{-1}).*

Lake	Fe	SO_4^{-2}	Cl	Na	Mg	K	Ca	Si	Mn	CO_3^{-2}	CO_3H^-	CO_2
ALC	0.01	6927.00	6705.00	2135.00	2670.00	165.00	90.60	<0.10	6.83	1.55	60.67	0.73
ALM	2.52	771.00	12816.00	2388.00	26.60	34.10	2.95	6.94	57.10	115.50	248.51	0.16
CAR	0.06	1094.00	1290.00	1975.00	72.70	6.38	5.30	1.83	2.75	4.00	66.74	0.34
GRA	0.08	5265.00	1341.00	4295.00	1093.00	59.40	96.90	7.07	2.83	1.10	23.61	0.16
LAR	0.08	9279.00	10864.00	2451.00	2447.00	555.00	85.00	<0.10	111.00	11.63	56.01	0.08
LON	0.43	10423.00	42098.00	8527.00	8725.00	1316.00	61.80	3.66	16.40	4.10	111.10	0.92
MAN	0.04	10074.00	20455.00	4394.00	4699.00	680.00	78.60	<0.10	117.00	3.66	78.68	0.52
SAL	1.44	14366.00	15787.00	47544.00	7202.00	662.00	63.70	<0.10	6.52	1.35	46.96	0.50
TIR	0.10	8318.00	16126.00	3516.00	2221.00	498.00	87.70	<0.10	3.94	2.87	42.72	0.20
VEG	0.07	630.00	1697.00	235.00	73.10	93.40	19.00	6.47	3.68	1.85	77.77	1.00
YEG	0.38	34387.00	40061.00	16832.00	16362.00	1709.00	59.40	<0.10	51.10	3.93	68.74	0.37

IV.3.2. Chemical characteristics of lake sediments

The TP_s content showed a great variation between the studied lakes (Table 4.4). This ranged from 46.81 to 1763.91 µgP g^{-1} dw in the whole set of samples, with lakes Alcahozo and Veguilla showing the lowest and highest average values, respectively. The general trend in the studied lakes showed higher amounts of TP_s in winter compared to spring (Figure 4.1B), Ca/Mg-P being the most abundant and dynamic fraction. The sum of fractions tentatively representing the inorganic sedimentary P (Ca/Mg-P, Fe/Al-P and the exchangeable or Loosely-P), dominated in sediments compared to the Poly-P and residual fractions, which are supposed to be mainly composed of P bound to organic matter. This overall pattern responded to the highest occurrence in all the sediments of the Ca/Mg-P fraction (Figure 4.2), which usually made up to 60% of total P content and exhibited concentrations ranging from 27.44 to 919.10 µg g^{-1} dw. The Fe/Al-P fraction ranged in the lakes from 2.42 to 247.88 µg g^{-1} dw, representing, on average, around 12% of total P content in the sediments (Figure 4.2). Neither consistent seasonal depletion, nor accumulation was observed for this fraction. On average, Resid-P constituted around 19% of P content in sediments, with amounts ranging from 11.33 to 396.56 µg g^{-1} dw (Figure 4.2). These range ends were measured in lakes Alcahozo and Veguilla, respectively. Poly-P and Loosely-P were the less abundant fractions, representing on average 5.75% and 7.04% of total P content in sediments, respectively (Figure 4.2), and ranging from 2.50 to 197.51 and 1.54 to 68.27 µg g^{-1} dw, respectively. LOI_{460} displayed concentrations ranging from 1.59% to 34.16% dw (Table 4.4). The lakes with high values of LOI_{460} also showed high DOP concentrations in the water column, which suggests that this organic matter is also an important a source of P. The mean values of LOI_{950} are shown in Table 4.4, ranging from <1% (Lakes Caracuel, Longar and Veguilla) to 45.44% dw (Lake Alcahozo).

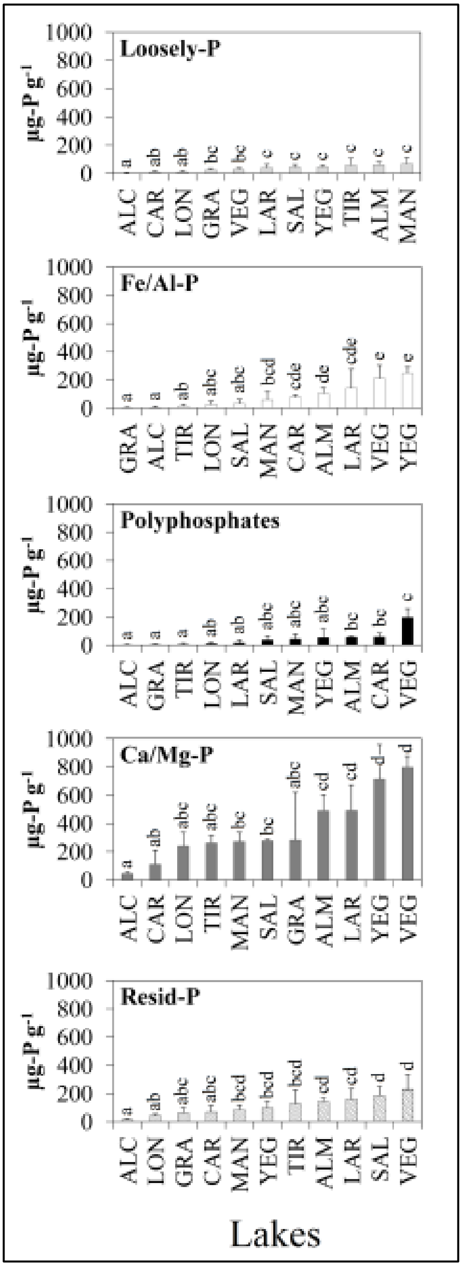


Figure 4.2. *Average seasonal values of different P fractions measured in the sediments of the studied lakes. Letters on top of the bars indicate pair-wise significant differences observed in the post-hoc ANOVA Tukey HSD test. Lakes that share the same letters did not show statistically significant differences among them.*

IV.3.3. Influence of environmental factors on the measured variables in the water column

The results of the multivariate analysis of variance (MANOVA model) are shown in Table 4.6. In general, the average concentration of the different P fractions and Fe measured in water was higher in the drying season (spring), compared to the flooding season (winter). However, the season factor only significantly affected TDP and DOP (Table 4.6). On the other hand, the salinity factor had the greatest effect on P fractions, as well as Ca and Fe concentrations in water, showing that salinity is a main driver of P cycling. The partial eta-squared (η^2) of the MANOVA analysis (Table 4.6), which is a measure of the effect size indicating the proportion of variance accounted for some factor, was 0.54 and 0.23 for Ca and Fe, respectively, when considering salinity. Meaning that the variance of 54% and 23% observed for these two variables, respectively, was explained by this factor. With regard to the post-hoc grouping of saline types (Table 4.7), DOP, Ca and Fe were significantly higher in the hypersaline lakes compared to the others. On the other hand, the hyposaline lakes showed the highest average concentrations of SRP, mainly due to Lake Almodovar, although differences were not statistically significant due to the high dispersion of data. Considering P fractions in relative terms, PP dominated in mesosaline and hypersaline lakes (73% and 56% respectively), whereas SRP was proportionally the highest in both subsaline and hyposaline lakes (45% and 59% respectively). The lowest proportion of DOP in the water column was found in hyposaline lakes (b1% of total P), mainly due to the volcanic Lake Almodovar, while in the other saline type lakes it represented between 12% to 25% of total P in the water column.

The effect of wastewater inputs on P concentrations in water was somewhat lower compared to salinity, but still significant for most of the fractions. Related to this, wastewater inputs explained 23% of the variance observed for TDP, and this was significantly higher in lakes with these inputs (Table 4.6). In these lakes, DOP was three times higher compared to the other lakes (Table 4.7), although these differences were only marginally significant (Table 4.6). Considering the P fraction in relative terms, polluted lakes showed a proportion of PP 10% higher than unaltered ones, while a higher proportion of SRP was found in lakes without wastewater inputs (50% of total P). In addition, the average Chl-*a* concentrations in the lakes almost doubled in winter compared to spring (Table 4.7), and a similar trend was also found in lakes with wastewater inputs (Table 4.7). However, differences slightly missed the significance level because of the high data dispersion.

Table 4.6. *Two-way MANOVA model of the effect of season (S) (winter and spring), saline typology (ST) (subsaline, hyposaline, mesosaline and hypersaline) and wastewater inputs (W) (presence or absence), and their interactions, on the phosphorus fractions and on some measured limnological variables both in water and sediments. Lowercase letters, w and s, together with total phosphorus (TP) indicate water and sediment samples, respectively. The statistical significance is set at the 0.05 probability level. See the text for abbreviations.*

		S				ST				W			
		df	F	*p*	η^2	df	F	*p*	η^2	df	F	*p*	η^2
Water	SRP	1	0.03	0.87	0.00	3	3.30	0.03	0.25	1	4.00	0.05	0.12
	TDP	1	4.43	0.04	0.13	3	2.61	0.07	0.21	1	9.13	0.01	0.23
	DOP	1	16.17	0.00	0.35	3	6.77	0.00	0.40	1	4.02	0.05	0.12
	PP	1	0.09	0.76	0.00	3	3.39	0.03	0.25	1	3.42	0.07	0.10
	TP_w	1	1.41	0.24	0.04	3	3.92	0.02	0.28	1	7.40	0.01	0.20
	Ca	1	0.15	0.70	0.00	3	11.68	0.00	0.54	1	0.44	0.51	0.01
	Fe	1	2.89	0.10	0.09	3	3.03	0.04	0.23	1	2.05	0.16	0.06
	Chl-*a*	1	2.63	0.12	0.08	3	2.35	0.09	0.19	1	2.88	0.10	0.09
Sediment	Loosely-P	1	0.67	0.42	0.02	3	5.91	0.00	0.37	1	8.93	0.01	0.23
	Fe/Al-P	1	0.06	0.80	0.00	3	4.01	0.02	0.29	1	13.83	0.00	0.32
	Poly-P	1	4.92	0.03	0.14	3	13.12	0.00	0.57	1	3.46	0.07	0.10
	Ca/Mg-P	1	2.51	0.12	0.08	3	7.12	0.00	0.42	1	44.62	0.00	0.60
	Resid-P	1	0.00	0.95	0.00	3	3.95	0.02	0.28	1	12.89	0.00	0.30
	TP_s	1	1.16	0.29	0.04	3	5.70	0.00	0.36	1	32.08	0.00	0.52
	LOI_{460}	1	6.71	0.01	0.18	3	69.97	0.00	0.87	1	12.24	0.00	0.29

Table 4.6 *(continuation). Two-way MANOVA model of the effect of interactions between the studied factors: season (S) (winter and spring), saline typology (ST) (subsaline, hyposaline, mesosaline and hypersaline) and wastewater inputs (W) (presence or absence), and their interactions, on the phosphorus fractions and on some measured limnological variables both in water and sediments. Lowercase letters, w and s, together with total phosphorus (TP) indicate water and sediment samples, respectively. The statistical significance is set at the 0.05 probability level. See the text for abbreviations.*

		S x ST				S x W				ST x W				S x ST x W			
		df	F	*p*	η^2	df	F	*p*	η^2	df	F	*p*	η^2	df	F	*p*	η^2
Water	SRP	3	0.32	0.81	0.03	1	0.56	0.46	0.02	2	1.07	0.36	0.07	2	0.61	0.55	0.04
	TDP	3	0.45	0.72	0.04	1	2.08	0.16	0.06	2	0.64	0.54	0.04	2	0.30	0.74	0.02
	DOP	3	3.44	0.03	0.26	1	1.59	0.22	0.05	2	1.17	0.32	0.07	2	1.68	0.20	0.10
	PP	3	0.89	0.46	0.08	1	3.11	0.09	0.09	2	0.49	0.62	0.03	2	0.17	0.84	0.01
	TP_w	3	0.27	0.85	0.03	1	1.41	0.24	0.04	2	0.23	0.80	0.01	2	0.69	0.51	0.04
	Ca	3	0.41	0.75	0.04	1	0.44	0.51	0.01	2	1.45	0.25	0.09	2	0.29	0.75	0.02
	Fe	3	0.79	0.51	0.07	1	2.54	0.12	0.08	2	0.08	0.93	0.01	2	0.00	1.00	0.00
	Chl-*a*	3	1.26	0.31	0.11	1	1.52	0.23	0.05	2	0.06	0.94	0.00	2	0.27	0.76	0.02
Sediment	Loosely-P	3	0.28	0.84	0.03	1	0.34	0.56	0.01	2	13.58	0.00	0.48	2	0.31	0.73	0.02
	Fe/Al-P	3	0.14	0.93	0.01	1	0.71	0.41	0.02	2	0.79	0.46	0.05	2	0.10	0.90	0.01
	Poly-P	3	1.34	0.28	0.12	1	1.01	0.32	0.03	2	1.74	0.19	0.10	2	5.47	0.01	0.27
	Ca/Mg-P	3	0.71	0.55	0.07	1	2.36	0.14	0.07	2	6.54	0.00	0.30	2	0.01	0.99	0.00
	Resid-P	3	0.72	0.55	0.07	1	0.42	0.52	0.01	2	16.39	0.00	0.52	2	1.38	0.27	0.08
	TP_s	3	0.59	0.63	0.06	1	1.37	0.25	0.04	2	7.79	0.00	0.34	2	0.14	0.87	0.01
	LOI_{460}	3	1.04	0.39	0.09	1	0.24	0.63	0.01	2	15.58	0.00	0.51	2	0.36	0.70	0.02

IV.3.4. Influence of environmental factors on the measured variables in sediment

The general trend found in most of the studied lakes showed smaller amounts of sedimentary P at the end of the hydroperiod. However, the season factor only significantly affected the Poly-P concentration, showing significantly higher values in the spring (Tables 4.6 and 4.7). Differences in both absolute and relative terms of P sedimentary fractions were found depending on salinity (Table 4.6). Whatever the P fraction, a higher accumulation occurred significantly in subsaline lakes compared to the rest, except for Loosely-P which was significantly higher in the hyposaline lakes. The latter included both polluted and/or volcanic lakes, such as Manjavacas or Almodovar, respectively. The most remarkable difference was observed for Poly-P, which was up to three times higher in subsaline lakes, particularly in Lake Veguilla. In relative terms, Loosely-P and Ca/Mg-P were also lower in subsaline lakes compared to the other saline lake types. With regard to the high proportion of variance explained by this factor (η^2 in Table 4.6), salinity explained 57% and 42% of the variance observed for the Poly-P and Ca/Mg-P fractions, respectively (Table 4.6).

Compared to salinity as a determinant factor, the occurrence or absence of wastewater inputs had a stronger effect on some of the dominant P fractions, particularly on Ca/Mg-P and Fe/Al-P (Table 4.6), explaining 60% and 32% of the variance observed for these factors according to η2. In all cases, the concentrations of all P sedimentary fractions were significantly higher in lakes with wastewater inputs (Tables 4.6 and 4.7). Concerning the changes in the distribution of P among the different fractions, polluted lakes showed the highest and the lower proportions of Fe/Al-P and Resid-P, respectively, whereas the proportions of the other measured fractions were very similar, for lakes with or without wastewater inputs. Finally, the effect of the interaction between saline typology and wastewater inputs was found to be more important than their separate effect on the variance of Loosely-P and Resid-P, which were 48% and 52%, respectively (Table 4.6). All three studied factors significantly affected the LOI_{460} (Table 4.6).

The greatest effect was observed, in all cases, for salinity, which jointly with the other two determinant factors (wastewater inputs and season) explained 87% of the variance observed for the organic matter content (Table 4.6). Higher LOI_{460} values were found in spring compared to winter (Table 4.7) and, whatever the season, LOI_{460} was also higher in hypersaline lakes compared to the less saline types (Table 4.7), as well as when wastewater inputs were present (Table 4.7).

Table 4.7. *Concentrations of phosphorus fractions in the sediments (µg g^{-1} dw) and water (µM), total P in the sediments (TPs) and water (TPw), total iron in water (Fe, mg L^{-1}), organic matter content in the sediments (LOI460, %dw), calcium in water (Ca, mg L^{-1}) and chlorophyll a (Chl-a, µg L^{-1}). Average values ± standard deviations of sampled lake parameters are shown, according to season (winter and spring), saline typology (subsaline, hyposaline, mesosaline and hypersaline) and wastewater inputs (presence or absence). Superscript letters indicate post-hoc groups of Tukey HSD tests. Accordingly, the absence of a letter in common denotes significant differences ($p < 0.05$) between groups.*

		Season		Saline typology				Wastewater	
		Winter	Spring	subsaline	hyposaline	mesosaline	hypersaline	presence	absence
Water	SRP	45.71 ± 92.06	43.54 ± 83.80	12.32 ± 14.69 ^{a}	135.97 ± 129.21 ^{a}	0.78 ± 1.30 ^{a}	40.36 ± 77.28 ^{a}	32.16 ± 64.88	52.26 ± 101.04
	TDP	28.72 ± 46.71	49.29 ± 67.22	16.98 ± 16.98 ^{a}	88.24 ± 90.86 ^{a}	10.99 ± 9.64 ^{a}	44.58 ± 55.42 ^{a}	40.29 ± 48.13	38.8 ± 67.13
	DOP	3.67 ± 5.30	13.27 ± 14.38	4.65 ± 3.61 ^{b}	0.45 ± 0.68 ^{a}	10.20 ± 9.80 ^{b}	15.76 ± 16.22 ^{b}	13.58 ± 13.72	4.61 ± 8.17
	PP	52.02 ± 72.36	55.89 ± 101.21	10.35 ± 10.65 ^{a}	95.89 ± 135.65 ^{ab}	29.13 ± 29.03 ^{ab}	70.79 ± 97.12 ^{b}	56.48 ± 83.89	47.3 ± 90.18
	TP_w	72.47 ± 95.84	85.14 ± 101.28	23.59 ± 19.24 ^{a}	153.17 ± 142.49 ^{ab}	43.23 ± 31.81 ^{ab}	99.29 ± 101.98 ^{b}	84.48 ± 87.52	74.6 ± 107.25
	Ca	58.94 ± 33.79	50.14 ± 37.89	13.24 ± 9.30 ^{a}	43.83 ± 48.70 ^{ab}	89.43 ± 8.38 ^{c}	60.59 ± 16.56 ^{b}	58.83 ± 25.42	50.61 ± 43.37
	Fe	0.64 ± 1.22	5.21 ± 21.29	0.71 ± 1.61 ^{a}	1.68 ± 1.87 ^{a}	0.21 ± 0.23 ^{a}	7.28 ± 26.40 ^{a}	0.56 ± 1.08	5.06 ± 20.84
	Chl-*a*	65.60 ± 74.13	39.24 ± 49.30	33.51 ± 78.27 ^{a}	63.78 ± 68.12 ^{ab}	33.77 ± 29.63 ^{ab}	81.08 ± 69.12 ^{b}	73.32 ± 74.55	38.23 ± 50.59
Sediment	Loosely-P	34.31 ± 25.84	33.18 ± 32.66	18.42 ± 12.72 ^{a}	45.74 ± 22.28 ^{b}	37.19 ± 39.05 ^{ab}	34.00 ± 29.50 ^{ab}	35.29 ± 29.15	32.32 ± 29.65
	Fe/Al-P	81.96 ± 96.39	86.07 ± 89.50	144.79 ± 93.43 ^{b}	59.92 ± 60.26 ^{a}	68.39 ± 95.40 ^{a}	70.45 ± 94.59 ^{ab}	126.05 ± 110.8	45.49 ± 45.94
	Poly-P	42.54 ± 73.37	53.59 ± 52.04	128.24 ± 85.90 ^{b}	33.70 ± 28.83 ^{a}	21.73 ± 27.79 ^{a}	24.31 ± 32.66 ^{a}	66.87 ± 82.78	30.83 ± 29.93
	Ca/Mg-P	396.42 ± 293.83	322.71 ± 231.6	453.48 ± 372.66 ^{a}	397.34 ± 248.80 ^{a}	268.95 ± 216.15 ^{a}	346.78 ± 220.53 ^{a}	483.25 ± 266.22	246.19 ± 209.41
	Resid-P	110.40 ± 91.72	107.06 ± 70.85	148.3 ± 114.78 ^{a}	106.82 ± 50.48 ^{a}	86.64 ± 74.78 ^{a}	101.18 ± 72.11 ^{a}	122.24 ± 90.99	96.35 ± 70.43
	TP_s	665.63 ± 477.13	602.60 ± 404.39	893.23 ± 626.51 ^{a}	643.51 ± 332.08 ^{a}	482.89 ± 376.39 ^{a}	576.72 ± 346.67 ^{a}	833.7 ± 481.52	451.17 ± 302.39
	LOI_{460}	16.52 ± 7.88	17.54 ± 8.03	9.48 ± 5.00 ^{a}	9.37 ± 5.63 ^{a}	21.21 ± 2.17 ^{b}	23.32 ± 5.09 ^{b}	21.06 ± 6.31	13.34 ± 7.45

IV.3.5. Correlation analysis

Pearson correlations, and their statistical significances, between the studied variables are compiled in Table 4.8. A significant positive correlation was found for EC and TP_w. However, considering the P forms separately, EC only correlated significantly with DOP and PP. On the other hand, EC was negatively correlated with %FL, something which is to be expected since an increase in EC is associated with an increase in evaporation processes. The SRP concentration in water correlated positively with all P fractions in sediments, the correlation being higher for the inorganic fractions (Loosely- P, Ca/Mg- P and Fe/Al- P). Chl-*a* concentrations and Loosely-P correlated significantly with all P fractions in water except with DOP. Additionally, Loosely-P correlated positively with Ca/Mg-P and Fe/Al-P fractions from sediment. LOI460 correlated negatively with DO and positively with DOP. On the other hand, the concentration of Ca in water was inversely correlated with SRP and TP_s. Significant inverse correlations were also observed between Ca and Fe/Al-P and the amount of Poly-P in sediments. Finally, the Fe concentration in water, which correlated negatively with Ca, showed significant positive correlations with the amount of Fe/Al-P sedimentary fraction, as well as with TDP and SRP concentrations in water.

Table 4.8. Pearson's correlation coefficients among the studied variables. (n=46; * $p < 0.05$; ** $p < 0.01$). Flooding level (%FL), water electrical conductivity (EC, mS cm^{-1}), dissolved oxygen (DO, mg L^{-1}), organic matter content in sediments (LOI460, %dw), calcium (Ca, mg L^{-1}), concentrations of P fractions in sediments (μg g^{-1} dw), concentrations of P fractions in water (μM), iron (Fe, mg L^{-1}) and chlorophyll-a (Chl-a, μg L^{-1}).

	EC	pH	DO	LOI_{460}	Ca	Loosely-P	Fe/Al-P	Poly-P	Ca/Mg-P	Resid-P	TP_s	SRP	TDP	DOP	TP_w	PP	Chl-*a*	Fe
%FL	-0.76**	0.16	0.28	-0.67**	-0.25	0.06	0.32*	0.18	0.31*	0.36*	0.35*	0.11	-0.05	-0.14	-0.29	-0.26	-0.28	-0.24
EC		-0.27	-0.22	0.85**	0.38**	-0.03	-0.27	-0.38**	-0.17	-0.28	-0.25	0.01	0.23	0.39**	0.45**	0.40**	0.41**	0.21
pH			0.34*	-0.31*	-0.23	0.23	0.13	0.09	0.2	0.20	0.18	0.1	0.06	-0.34*	0.09	0.05	0.13	0.09
DO				-0.34*	-0.20	0.24	0.21	0.02	0.27	0.10	0.24	0.19	-0.01	-0.39**	0.04	0.05	0.40**	0.03
LOI_{460}					0.50**	-0.2	-0.24	-0.41**	-0.13	-0.36*	-0.24	-0.21	0.07	0.53**	0.25	0.27	0.21	0.03
Ca						-0.14	-0.63**	-0.67**	-0.35*	-0.34*	-0.41**	-0.60**	-0.50**	0.20	-0.37*	-0.14	-0.27	-0.49**
Loosely-P							0.42**	0.23	0.56**	0.64**	0.65**	0.46**	0.31*	-0.29	0.40**	0.28	0.28	0.26
Fe/Al-P								0.62**	0.63**	0.57**	0.75**	0.66**	0.56**	-0.02	0.43**	0.25	0.18	0.33*
Poly-P									0.38**	0.49**	0.51**	0.41**	0.36*	-0.13	0.17	-0.05	0.15	0.27
Ca/Mg-P										0.64**	0.95**	0.61**	0.55**	-0.09	0.47**	0.32*	0.36*	0.28
Resid-P											0.78**	0.46**	0.34*	-0.09	0.26	0.10	0.19	0.23
TP_s												0.65**	0.52**	-0.13	0.42**	0.27	0.27	0.27
SRP													0.72**	-0.17	0.73**	0.56**	0.35*	0.48**
TDP														0.22	0.84**	0.51**	0.48**	0.68**
DOP															0.08	0.03	-0.10	0.05
TP_w																0.82**	0.62**	0.67**
PP																	0.40**	0.37*
Chl-*a*																		0.46**

IV.4. Discussion

The studied lakes are representative of both natural and altered systems in UNESCO's La Mancha Húmeda Biosphere Reserve. Our survey reveals different types of lakes, both in terms of mineralization and trophic status. Human impacts, such as wastewater discharges, modify the water regime and trigger differences in the hydroperiod and the pollution level. The contributions derived from urban waters, regardless of whether these are treated or not, are a source of freshwater, nutrients and materials, causing major differences in the trophic status and other ecological features of the lakes.

The most abundant fraction in water was usually PP, particularly in the most polluted lakes. This indicates that an important part of inorganic P entering the lakes is transformed into biomass, which is also suggested by the significant correlation observed between PP and Chl-*a* concentrations. In addition, in polluted lakes DOP concentrations were higher in relative terms compared to the undisturbed lakes. This DOP can be transformed into SRP by extracellular enzymatic hydrolysis, thus favoring the uptake of the resulting PO_4 (Labry et al., 2002). This would increase the availability of P for phytoplankton production even more.

In this study, we have assessed the influence of three main environmental factors (i.e., season, salinity and the presence of wastewater inputs) in the accumulation and fractionation of phosphorus in the sediment of some saline lakes. Our findings indicate that the main drivers of P cycling were the occurrence of wastewater inputs, as well as lake salt composition and abundance. However, the effect linked to the season had less of an influence. P accumulates naturally in the sediments of the lakes; however, this process is likely enhanced in endorheic lakes such as those in La Mancha Húmeda, since they show long residence times and experience marked evaporative processes (i.e., nutrient evapo-concentration). This implies the major role of local climatic conditions on nutrient dynamics, as observed in other sites (Anderson and Stedmon, 2007; Bayley et al., 2013). Also, the marked endorheic character of these lakes would explain the higher concentrations observed for some metals, such as Mn. This was particularly high in some of the polluted lakes (i.e., Manjavacas and

Larga de Villacañas), which receive wastewaters enriched with this metal in particular (Mañas et al., 2009).

The MANOVA results showed that the relative importance of some P fractions in the water column were in part influenced by the season, with a marked increase in TDP and DOP in the studied lakes from winter to spring. This suggests a potential fertilization of the water column from sediments, which has been already described in both eutrophic shallow freshwater (Søndergaard et al., 2003) and saline lakes (Pilati et al., 2016), and responds to the increase in decomposition rates of the organic matter in sediments when temperature rises. Furthermore, we observed a positive correlation between LOI_{460} and DOP concentrations, indicating that this mechanism of P mobilization is enhanced in polluted lakes. Similar results were found by Wang et al. (2008), who concluded that DOP was mainly released directly from the organic matter that was stored in sediments. It should be noted that this problem is getting worse in those lakes affected by wastewater inputs (e.g., Larga de Villacañas, Longar, Manjavacas and Veguilla), which remained flooded during the warmest months. This artificial increasing in the flooding period further alters the redox conditions, whereby promoting the mobilization of P from sediments, as will be explained later. The increase in P concentrations in the water column at the end of the hydroperiod comes from the interplay of the phosphorus release from sediments and the evapo-concentration processes, the latter promoted by the reduction in the water volume of the studied lakes.

As a general trend, higher amounts of P were in the sediment of the polluted lakes, Lake Veguilla being an extreme case. In general, P fractions in sediments of the studied lakes ranked, in terms of abundance, as follows: Ca/Mg-P > Resid-P > Fe/Al-P > Poly-P > Loosely-P. This trend did not change depending on the season, the lake salinity type or wastewater inputs; however, the Poly-P fraction was three times higher in subsaline lakes compared to the others. Our results showed that Ca/Mg-P was the most dynamic among all sedimentary fractions, showing greater reductions from winter to spring. This indicates that the release of sedimentary P comes from this fraction, thereby explaining the close relationship observed between Ca/Mg-P, Loosely-P and SRP. The occurrence of Ca/Mg-P and Resid-P as dominant fractions indicates that the settling and immobilization of P occur mainly via precipitation with divalent cations (either Ca or Mg), or by the burial of organic matter, respectively. Both mechanisms represent an accumulation of relatively stable P-pools. Heavy precipitation with divalent cations agrees with the significant negative correlation observed between Ca and P dissolved fractions (SRP and TDP). This is a pattern largely observed in hard waters (House et al., 1986; Driscoll et al., 1993). In calcareous lakes, apatite ($Ca[PO_4]_3OH$) has been identified as the dominant phosphorus mineral (Golterman, 2004). In our case, the higher proportion of this P fraction could be explained by the high calcium content coming from the dissolution of calcareous rocks in the catchment, which in turn favors the accumulation of calcium bound P.

The formation of apatite requires both chemical and biogenic reactions. However, even though Ca concentrations are also high, lake waters in the region have high alkalinity and very low Ca/Mg, due to the dominance of gypsum-rich marls in the catchment (Florín et al., 1993). This is expected to leave large amounts of Mg available for the adsorption of P. Interestingly, some authors suggested that, as a result of the high magnesium availability, the carbonates associated with Mg would play a more prominent role than that typically expected for P immobilization in wetlands (e.g., Pant and Reddy, 2003). As mentioned before, Ca/Mg bound P is thought to be a chemically stable pool; however, a slow dissolution of this P might be maintaining a steady flux into the water column, thus sustaining the P supply to lake waters. This probably explains the close relationship observed between Ca/Mg-P, Loosely-P and SRP. In turn, a strong relationship is also observed between SRP and phytoplankton production (i.e., Chl-*a* concentration), thus sustaining the idea that productivity of the studied lakes largely relies on the P internal loading. This causes changes not only in phytoplankton composition and abundance (Ferriol et al., 2017), but also in the benthic phototrophic communities usually developing in saline lakes (Camacho and de Wit, 2003).

Our fractionation scheme provides a distinction of P into labile, moderately labile, and non-labile fractions of the total P pool, in such a way that the weakest extractant used (NaCl) extracts P that can be readily transformed into soluble and/or available forms for a rapid algae uptake (Loosely-P). There are other methods to extract the P loosely adsorbed to sediments, such as that carried out with NH4F. However, we discarded this because it strongly reacts with $CaCO_3$ in calcareous sediments to form CaF_2, which will then precipitate soluble P and produce underestimates. Loosely-P regularly shows a low concentration in sediments, due to its fast assimilation and/or diffusion into the water column. Accordingly, and as mentioned above, we observed a positively significant relationship between the amount of Loosely-P in sediments and the P concentration in the water column, this correlation being higher with the SRP fraction.

The higher content of Loosely-P in sediments of the studied lakes occurs at the beginning of the flooding period (hydroperiod), when the temporary lakes flood again after the summer drying. An experimental study performed in two Mediterranean wetlands with contrasting salinities evidenced a significant increase in the P released from the sediments of the saline lake studied, which had been dry for a long period (up to 6 months), compared to a freshwater one (Gilbert et al., 2014). This phenomenon was observed in other lacustrine systems (Dieter et al., 2015; Kinsman-Costello et al., 2016) and was explained due to different causes (Qiu and McComb, 1994; Steinman et al., 2014 and articles cited therein). On the one hand, it can be attributable to the dissociation of P-bound Fe in sediments as a result of anoxia induced by flooding (Boström et al., 1982). On the other hand, it may also be due to sulfide oxidation during seasonal drying, which reduces the pH in sediment and enhances the dissolution of calcareous minerals and the associated P (Pant and Reddy, 2003; Kinsman-Costello et al., 2016). The latter would better fit with our case, especially when considering the abundance of gypsum materials within the catchment, which provide large amounts of sulfate. In any case, both mechanisms could explain the positive correlation observed between Loosely-P and P bound to Fe and Ca. This temporary supply of P, at any rate, would justify the overgrowth of phytoplankton perceived at the beginning of hydroperiod, thus demonstrating its transitory significance on the productivity of the studied lakes. Unfortunately, our monthly samplings do not have sufficient temporal resolution to properly detect the highly variable Chl-*a* concentrations associated with rapid phytoplankton growth episodes. For this reason, it is likely that Chl-*a* did not reveal any dependence on the factors explored in the MANOVA analysis. In addition, cyanobacterial growth occurring in these lakes (Camacho et al., 2003) could also facilitate the release of Fe bound P and organic P as observed in reported cyanobacterial blooms (Zhu et al., 2013; Wang et al., 2019).

Other extractants, such as dithionite-bicarbonate, extract the P bound to reducible forms of metals that requires reducing conditions to become bioavailable. This implies a higher P mobilization from sediments if the concentration of dissolved oxygen drops. Related to this, a high biological oxygen demand may occur in these lakes which may lead to anoxia or hypoxia states in the surface layer of sediments. In other aquatic systems this is supposedly a more inert fraction, hence it does not contribute as much to P cycling compared to the other fractions (Golterman, 2004). However, sediments might release this adsorbed P when their retention capacity is exceeded, or when particular environmental conditions, such as pH and redox, are altered (Maine et al., 1992; Dieter et al., 2015). Due to the high oxygen demand, it is expected that alternating aerobic/anaerobic cycles occur in the sediment surface layer in contact with the water during day/night periods. High salinities also decrease the solubility of gases in water, further favoring oxygen depletion. Although weak, there is a significant negative relationship between the organic matter content in surface sediments and the dissolved oxygen, suggesting that there is a certain oxygen demand promoting oxygen consumption in this surface layer. At night, when photosynthesis ceases, a rapid decrease in oxygen would likely occur, thus severely hypoxic, or even anoxic, conditions can be established. These conditions favor the desorption of phosphate bound to Fe (Perkins and Underwood, 2001; Søndergaard et al., 2003) and Mn (Moore et al., 1992; Hongve, 1997) oxy-hydroxides, which probably occurs in our studied lakes. Generally, the waters of the studied lakes present high concentrations of SO_4, allowing the reduction of sulfate, which actively occurs during anoxic periods, to produce the iron-sulfur complexation (FeS_2 formation). This insoluble iron sulphide formation, and its subsequent precipitation, reduces, in turn, the binding of phosphorus iron oxides (Holmer and Storkholm, 2001; Herbert et al., 2015). As a direct consequence, P is released to the overlaying water and increases the eutrophication of the water (Rozan et al., 2002; van derWelle et al., 2007). Another important fact is the positive correlation between Fe and dissolved forms of P and Chl-*a*, which would indicate that fluxes in P, and the consequent phytoplankton overgrowth, must have been, at least in part, controlled by the reductive dissolution of FeOOH~P complexes.

A good example of the major role of ferric complexes on P release in the studied lakes is that of Lake Almodovar, which shows the highest concentrations of dissolved Fe and SRP. The reduced iron (Fe^{2+}), which likely prevails during anoxic events, has a lower affinity for P than the oxidized form (Fe^{3+}) (Lamers et al., 1998); however, our analytical method is not able to distinguish the proportions of both iron forms in our samples. In addition, Lake Almodovar is also characterized by presenting lower concentrations of Ca, as well as higher Fe concentrations, compared to the rest of the studied lakes, despite its high mineralization. This evidenced that the formation of the $CaCO_3$~P complex would play, in relative terms, a minor role compared to the other lakes. It appears then that the P-calcite co-precipitation mechanism is saturated in this lake, thus favoring a high proportion of P to remain dissolved in the water column. Lake Almodovar is representative of the volcanic area of the “Campo de Calatrava”, where other similar volcanic lakes occur. The P behavior in this lake represents an example of the sensitivity of this particular type of volcanic lake to the eutrophication process, given that the main precipitation mechanisms of P described for the other lakes do not work in this lake. The high P release, which produces SRP concentrations >200 μM, would provide almost unlimited P-availability for phytoplankton growth in Lake Almodovar, which should result in the highest Chl-*a* concentrations among the studied lakes. However, this does not occur because of the high turbidity that characterizes volcanic lakes (Fernández et al., 2009) which limits the availability of light and, thus, phytoplankton productivity.

An important fraction (~20%) of total P in our lakes settles in sediments as organic P. This is progressively buried and may remain sequestered over long periods (Søndergaard et al., 2001). However, its origin would also determine the way it is stored and hence its suitability for microbial degradation. Among the studied lakes, only those with wastewater inflows have surrounding helophytic vegetation, allowed by desalinization (Gosálvez et al., 2012; Camacho et al., 2017). Although part of the organic P comes from the algal biomass, a significant accumulation of residual P (Resid-P), mainly organic in these lakes, might come from the decaying of this helophytic plant material; which otherwise is supposed to be particularly recalcitrant.

The Poly-P, which were extracted using a weak solution of NaOH, involve condensed phosphate which can still be regarded as an available source of P for the biota (Hupfer et al., 2007; Rier et al., 2016). According to Nesmeyanova (2000), the formation of polyphosphates is enhanced with increasing concentrations of exogenous orthophosphate, constituting a type of P stored as intracellular granules which are extensively accumulated by the microorganisms inhabiting sediments. Our results show that Poly-P concentrations were higher in most of the lakes with wastewater inputs; however, they were very low in unpolluted lakes such as lakes Alcahozo, Grande de Villafranca and Tirez. Regarding Lake Alcahozo, which has the lowest TP_S values measured, this may be due to its small catchment and the absence of regular surface water inflows, except those coming from rainfall (Doña et al., 2016: Camacho et al., 2017).

We also found a close relationship between Poly-P, SRP and TDP, suggesting the occurrence of biological P removal carried out by polyphosphate-accumulating organisms, as described by Davelaar (1993). The sediments of Lake Veguilla, where the amounts of polyphosphates were significantly the highest, can be seen as a paradigmatic case to illustrate this phenomenon. This is a land depression, artificially flooded with wastewaters from a nearby wastewater treatment plant (WWTP). It is worth noting how, through this process, Lake Veguilla would be acting as a continuous green filter, operating as a tertiary treatment of the outflow from the previously mentioned WWTP. This is useful, because the nutrient concentration in the WWTP effluent remains too high for the receiving waters (Sánchez-Ramos et al., 2016). However, in other polluted lakes such as

Manjavacas or Larga de Villacañas, the wastewaters effluents circulate, prior to reaching the lake, through slow flow channels which facilitate water selfpurification. However, they are not long enough, and have also accumulated large amounts of organic matter and nutrients over time, thus decreasing their effectiveness (García-Ferrer et al., 2003; Sánchez-Ramos et al., 2016). It appears then that these facilities may be a reliable countermeasure to reduce the impact of wastewater effluents in other lakes from La Mancha Húmeda, as they can promote biogeochemical transformations of nutrients, some of which should involve P binding to sediments. Nevertheless, increasing the length of the channel linking the point of effluent discharges to the receiving lakes, and improving its maintenance, should be management priorities in order to maintain the good ecological status of these unique lakes when they act as end points of wastewater disposal in this endorheic landscape.

In conclusion this chapter shows that P-partitioning in the saline lakes from La Mancha Húmeda is linked to its internal loads and water mineralization. The settling and immobilization of P occur mainly via precipitation with Ca and/or Mg, which involves a relatively stable pool of total P in sediments. The internal P loading of these lakes increases, in an expected way, when external wastewater inputs occur, but the forthcoming release of P from sediments will depend on the particular physical and chemical characteristics of each lake. Phosphorus appears in these sediments in several chemical forms, whose cycling has different timescales. Short-term dynamics involves the release of immediately available P as a nutrient source. Our results suggest a higher mobilization of this labile P upon sediments re-wetting, which could be induced, as previously mentioned, in part by the dissolution of calcareous minerals during seasonal drying. These seasonal fertilization pulses are, in this case, closely related to the fluctuating nature of these types of lakes and should also be considered when planning management strategies. Nevertheless, most of the P accumulated in sediments follows longer-term dynamics, which involves the transformation of inorganic phosphorus into highly occluded, or stable, organic P forms. However, the release of these hypothetically inert forms into labile P may still occur in these lakes if environmental conditions, such as pH and redox, are altered, causing the long-term fertility of the water column. This fact could be even more relevant in those lakes that remain artificially flooded during summer, when the temperature and the oxygen demand increase, altering the pH and the redox conditions of the lakes. Moreover, we cannot exclude other reasons to explain phosphorus mobilization which were not studied here, such as wind-induced sediment resuspension, or the bioturbation produced by benthic invertebrates and waterfowl (Søndergaard et al., 2003 and references therein). Regarding the latter, the current management of some wetlands in the region aims to extend the lakes' hydroperiod by increasing the water supply with treated wastewaters as a measure to favor the permanence of waterfowl and increase the diversity. However, as we demonstrated in this study, this may lead to negative effects on the trophic status of the lakes. Moreover, the long-term consequences far from benefiting waterfowl, could end up putting them at serious risk (Anza et al., 2014).

Chapter V: Environmental drivers of the optical characteristics of dissolved organic matter in saline lakes from Central Spain

V.1. Introduction

Dissolved organic matter (DOM) is a complex mixture of organic compounds that plays a pivotal role in the functioning of lake ecosystems (Creed et al., 2018). DOM in lakes originates from both allochthonous and autochthonous sources. Allochthonous DOM leaches from the catchment area into the lake and can be partially or highly refractory to the immediate bacterial mineralization. Autochthonous DOM includes both the in situ biologically produced and transformed DOM and that modified by photoreactions. Regarding this, natural sunlight degradation offers labile substrates that may stimulate bacterial production (De Lange et al., 2003).

The occurrence of chromophoric dissolved organic matter (CDOM), which is the light-absorbing fraction of dissolved organic carbon (DOC), can be assessed through the examination of DOM optical properties. Both absorption coefficients and spectral slope parameters obtained at different wavelengths can be employed to address the origin, molecular weight, or biogeochemical processing of CDOM (Helms et al., 2008; Fichot and Benner 2012). Likewise, high-resolution fluorescence spectroscopy is also used for the diagnosis of DOM characteristics (McKnight et al., 2001; Fellman et al., 2010; Osburn et al., 2011; Coble et al., 2014; Gabor et al., 2014) by providing fluorescence markers (FDOM). Thus, three-dimensional fluorescence scans, originally referred to as excitation-emission matrices (EEMs) by Coble (1996), have become widely used for this purpose and this has represented a notable advance in the field of aquatic ecology and biochemistry. However, the use of both spectral and fluorescence metrics simultaneously in environmental studies is less common (Osburn et al., 2011), which demonstrates that it is possible to infer the origin of DOM even when both biological and photochemical alterations occur at the same time (Hansen et al., 2016).

Saline lakes deprived of surface outlets are characterized by long hydraulic residence times. DOM transformations under these circumstances are expected to be strengthened and significantly impact C cycling. Saline lakes from UNESCO's Biosphere Reserve, La Mancha Húmeda (Central Spain Plateau) show a priori these characteristics (Florín and Montes, 1999; Camacho et al., 2017), displaying different degrees of salts and organic matter accumulation, an absence of surface outlets, and a negative hydrologic balance (i.e., evaporation and infiltration exceed precipitation, runoff and groundwater discharge). On the other hand, some of these lakes have been reported as being altered by the occurrence of sewage discharges, which enhances nutrient accumulation and produces water desalination (Florín and Montes, 1999; Camacho et al., 2017), producing an even broader environmental variability that should be considered when conducting environmental studies.

The scientific background in lakes from La Mancha Húmeda is diverse (Prieto-Ballesteros et al., 2003; Montoya et al., 2013; Castillo-Escrivà et al., 2015; Camacho et al., 2017), but it does not reflect its overall limnological significance. Thus, current knowledge on the DOM features of these lakes is virtually absent. In this study, we adopted both spectral and fluorometric metrics of DOM as chemical expressions of its biotic and abiotic processing. The occurrence of lakes varying greatly in their salinity and disturbance, but subjected to equal climatic conditions, represents an ideal research frame to assess how changeable local factors may produce different DOM environments. Here, we determine DOM optical metrics of different saline shallow lakes from La Mancha Húmeda and assess their relationships with main environmental variables.

V.2. Methods

V.2.1. Sampling and determination of environmental variables

The lakes in this study (*n* =17) were selected (Table 5.1) based on a gradient of water mineralization, and whether there was wastewater disposal in the lakes or not, to try to cover the entire range of conditions found in the region. The selected lakes range from subsaline to hypersaline (sensu Hammer, 1986) (Table 5.1), some of them being permanent and other ephemerals (Table 1).

Table 5.1. *Location and main limnological characteristics. The flooding period refers only to the period covered in this study, which could vary for other years.*

Lake	Code	Flooding period (months)	Wastewater discharges	Conductivity (mS cm^{-1})	Chl-*a* (μg L^{-1})
Caracuel	CAR	12	No	1.73 ± 0.62	6.01 ± 6.16
Alcahozo	ALC	5 - 6	No	53.01 ± 53.29	10.93 ± 12.22
Almodovar	ALM	12	No	12.11 ± 5.29	50.69 ± 52.85
Camino de Villafranca	CAM	10 - 11	Yes	45.02 ± 31.19	46.05 ± 50.89
Grande de Villafranca	GRA	12	No	9.61 ± 2.22	2.45 ± 2.76
Longar	LON	10 - 11	Yes	124.67 ± 119.12	66.53 ± 72.82
Manjavacas	MAN	9 - 11	Yes	35.34 ± 32.31	24.10 ± 23.81
Mermejuela	MER	7 -8	No	267.50 ± 157.50	38.26 ± 36.98
Nava Grande	NAV	12	No	4.02 ± 3.39	3.35 ± 5.57
Peñahueca	PEÑ	6	No	228.71 ± 132.86	32.47 ± 25.63
Pozuelo	POZ	11 - 12	Yes	20.63 ± 11.87	35.12 ± 44.55
Quero	QUE	5 - 6	No	252.31 ± 189.99	50.33 ± 63.94
Salicor	SAL	4 - 5	No	90.89 ± 79.00	38.46 ± 30.13
Tirez	TIR	4 - 5	No	93.31 ± 99.23	6.85 ± 4.02
Veguilla	VEG	11 - 12	Yes	3.05 ± 1.03	32.29 ± 64.16
Yeguas	YEG	4 - 5	Yes (in the past)	120.34 ± 47.75	85.39 ± 68.06

Water sampling and physical and chemical determinations were carried out monthly, during two hydrological cycles, in open waters in fixed stations of 17 lakes in the region. Conductivity was measured with a WTW LF-191 conductivity meter. Water samples were collected for the quantification of dissolved organic carbon (DOC) and the optical parametrizing of dissolved organic matter (DOM). All the containers used for sampling were rinsed several times with the sample before filling. For DOC analysis, a fraction of the sample was immediately filtered at the lake shore through combusted Whatman GF/F fibre-glass filters (0.7 µm nominal size cut-off) and kept in acid-washed glass bottles. The samples were preserved at 4ºC until determination, within 48 hours, and acidified with 0.2 ml of 1 N HCl. Samples for the optical analyses of DOM were processed similarly and stored without acidification. For chlorophyll-*a* determination, the samples were filtered through Whatman GF/F filters and kept frozen until proceeding with extraction and analysis.

V.2.2. Spectrofluorometric analysis of DOM

All fluorescence parameters were acquired from an excitation-emission matrix (EEMs) performed with an F-7000 Hitachi fluorescence spectrophotometer using a 1 cm quartz fluorescence cell following Coble (1996). EEMs consisted of a series of emission scans (240–600 nm) collected over excitation wavelengths ranging from 240 to 450 nm in 5-nm increments. The bandwidth was set to 5 nm for both excitation and emission. The water Raman scattering effects from samples were removed by subtracting the signal of the EEMs obtained from Milli-Q water.

To avoid nonconformity when comparing our data with other studies, spectra correction was performed on both the excitation and emission side using, respectively, an excitation correction spectrum derived from a concentrated solution of rhodamine-B, and an emission correction spectrum derived using a ground quartz diffuser. These corrections were carried out following the specifications of the spectrophotometer manufacturer. Samples with high a(320) coefficients were diluted to avoid inner filtering effects, which otherwise only affect, in particular, substances absorbing at low excitation wavelengths (Guo et al., 2010). Five separate fluorescent components were identified and labeled as previously described by Coble et al. (1996) to establish better comparisons with other studies. Assuming that the position of peaks in the matrix may change slightly, values were considered as the maximum fluoresce emission within a defined analytical range of excitation and emission pairs. Accordingly, for the humic-like substances, the maximum fluorescence signal at Ex/Em wavelength of 250-260 nm/380-480 nm was defined as peak FDOM-A, at 330–350 nm/420–480 nm as peak FDOM-C, and at 310–320 nm/380–420 nm as peak FDOM-M. For protein-like substances, the maximum fluorescence signals at Ex/Em wavelengths of 270-280 nm/300-320 nm and 270–280 nm/320–350 nm were defined as peaks FDOM-B and FDOM-T, respectively. Standards for humic-type and protein-like FDOM were prepared with quinine sulfate and bovine serum albumin, respectively (Coble et al., 2014). Fluorescence units were expressed in ppt equivalents of QS (QS-ppt) for FDOM types A, M and C, and in ppt equivalents of BSA (BSA-ppt) for types B and T.

Two derived indexes from the EEMs were used, in addition, as surrogates of DOM origin and nature (Gabor et al., 2014). The fluorescence index (FI) was obtained as the ratio of the fluorescence signal at emission wavelengths of 450 and 500 nm after excitation at 370 nm. This ratio varies usually between 1.2 and 1.9, higher and lower values being indicative of more terrestrially (allochthonous) or microbially (autochthonous) derived FDOM (Gabor et al., 2014). On the other hand, the freshness index (BIX) was calculated as the ratio of a fluorescence signal at Em 380 nm divided by max intensity between Em 420 nm and Em 435 nm at Ex 310 nm. This index is a proxy of DOM age, with higher values representing a higher proportion of fresh DOM (Gabor et al., 2014).

V.2.3. Statistical analysis

Non-linear regressions were performed with DOM parameters and environmental variables to identify relationships among them. ANOVA tests were used to determine whether the Pearson correlation coefficients were statistically significant, establishing confidence at $p \leq 0.05$. A principal component analyses (PCA) was conducted with DOM metrics and selected environmental variables to summarize, in two dimensions, most of the variability present in the samples. The descriptive environmental variables used in PCA covered changes in the seasonal hydrological period (% of flood level of water column) and the trophic status of the lakes (Chl-*a*). All statistical analyses were performed using the University of Valencia-licensed statistical package IBM SPSS 24.0 Statistics.

V.3. Results

V.3.1. Environmental conditions and trophic status of lakes

The main environmental characteristics of the 17 studied lakes and their main anthropic stressors are listed in Table 5.1. They are ordered in the table according to their water electrical conductivity. The length of the flooding period varied among the lakes from 4 to 12 months per year. The most ephemeral lakes remained flooded from mid-autumn to early spring. The seasonal flooding patterns did not show notable differences during the 2012-2013 and 2013-2014 hydrological periods, although the second period was a bit drier. Water temperature showed a clear seasonally dependent pattern, ranging from 1.6 to 31.1 ºC, being highest in the lakes that remained flooded during the summer months. Slight, although not significant differences were observed in relation to the lakes' latitudes.

The studied lakes spanned a large range of salinity and trophic statuses (Table 5.1). Average conductivities ranged from subsaline (1.73 mS cm^{-1}) to hypersaline (267.5 mS cm^{-1}). Extreme values (> 400 mS cm^{-1}) and higher seasonal variations were observed in lakes more prone to suffer the evaporative accumulation of salts. In contrast, the seasonal variation of conductivity was narrow in subsaline lakes. The lakes were classified as subsaline (n=3), hyposaline (n=3), mesosaline (n=5), and hypersaline (n=7) based on descriptive statistics of complete conductivity data (Figure 2, *sensu* Hammer, 1986). As a general pattern, conductivity increased seasonally in all these subtypes while the flooding level of the lakes' basins decreased.

The trophic status of the lakes based on the chlorophyll-a (Chl-*a*) concentrations varied broadly from oligotrophic to hypertrophic (Table 5.1). Seasonal averages for each lake ranged from 2.45 to 185.14 µg L^{-1}, with lower averages observed in subsaline lakes, such as Nava Grande de Malagón, Caracuel and Grande de Villafranca, all of which are not affected by wastewater inflows. In contrast, lakes with point-source inputs of nutrients (see Table 1) usually showed higher average concentrations. The artificial increase of the flooding period in these lakes worsens this situation, in such a way that the highest Chl-*a* concentrations usually occurred during summer, when these lakes should be dry if undisturbed. Another hypersaline lake, i.e., Yeguas, had also been affected by a wastewater discharge, although it was not currently active, nonetheless this lake showed equally elevated mean Chl-*a* concentrations (85.39 µg L^{-1}), likely supported by the internal nutrient load from sediments. Currently, this lake belongs to the Alcázar de San Juan Lake Complex, where the mesosaline Camino de Villafranca and the subsaline La Veguilla lakes are also located and receive wastewater inflows at present, although they show mid-level Chl-*a* concentrations (Table 5.1). Although there were no signs of contamination, other mesosaline and hypersaline lakes (e.g., Salicor, Mermejuela, Peñahueca) showed moderate Chl-*a* average concentrations of around 30 µg L^{-1}, exemplifying a process of a developing natural eutrophication, typical of lakes that are deprived of surface water outlets and of the evaporative concentration of seston in them. However, lower Chl-*a* concentrations likewise occurred concurrently with high salinities and ephemerality as in the case of lakes Tirez and Alcahozo, where concentrations averaged 6.85 and 10.93 µg L^{-1} , respectively.

V.3.2. DOC concentration in lakes

Dissolved organic carbon (DOC) showed a significant power-law dependence with water conductivity, although with a moderate coefficient of determination (R^2 = 0.67) as shown in Figure 5.1A. In-lake average DOC concentrations ranged from 1.10 to 32.32 mM. The lakes displaying the highest values (> 20 mM) were, in decreasing order, Longar, Mermejuela, Yeguas and Quero. Furthermore, these lakes showed the highest seasonal increments of DOC. Among highly mineralized lakes, the most ephemeral and unimpaired (e.g., Tirez, Salicor, Alcahozo) showed significantly lower concentrations compared to the former, displaying average seasonal concentrations in the range of 4.04 - 5.69 mM. Seasonal evaporative DOC concentration in these lakes never produced concentrations over 30 mM. On the other hand, DOC concentrations in subsaline lakes (i.e., Veguilla, Nava Grande, Caracuel) were usually below 5 mM, thereby representing the lower extreme of the range.

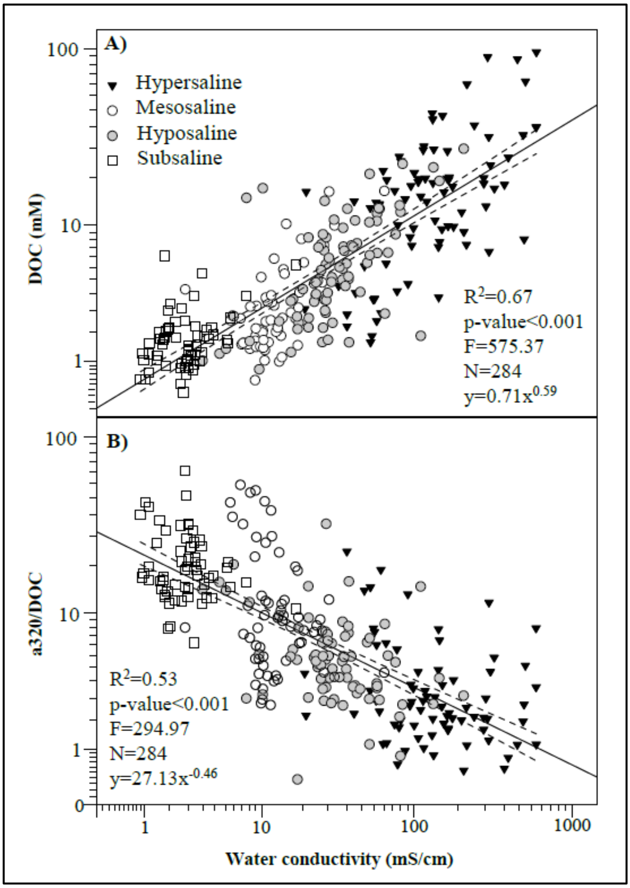


Figure 5.1. *Log-to-log relationships for water conductivity versus A) DOC concentrations and B) Napierian coefficient a320 normalized to DOC concentrations. Dashed lines indicated 95% confidence interval for the regression line.*

V.3.3. *Optical characteristics of DOM in lakes*

The mean values and standard deviation of optical parameters in the lakes derived from spectroscopic and fluorescence analyses of DOM are listed in Table 5.2. The average Napierian absorption coefficients $a(320)$ ranged in the lakes from 12.22 to 80.31 m^{-1}. The values were usually higher in hypersaline lakes, with the only exception being lake Peñahueca, whose average range was similar to the meso and subsaline lakes. The hyposaline turbid lake Almodovar displayed the highest average among all the lakes. These coefficients varied by more than an order of magnitude within salinity subtypes, still, the entire data range of these coefficients normalized to DOC concentrations showed a decreasing power relationship with water conductivity (Figure 5.1B). The spectral slope coefficient $S_{275–295}$ exhibited a positive nonlinear dependence with DOC concentrations (Figure 5.2A). In-lake average values ranged from 0.0081 to 0.0171 and increased slightly with salinity. On the contrary, the slope coefficient $S_{350-400}$ was highly unresponsiveness to variations in DOC (Figure 5.2B) concentrations and averaged from 0.00502 to 0.00966. Consequently, the variability observed in the ratio between slopes (*Sr*), which also showed a weak correlation with DOC concentrations (Figure 5.2C), was modulated overall by the slope coefficient $S_{275–295}$.

Table 5.2. *Average (± standard deviation) values for the DOC concentrations and DOM optical parameters of dissolved organic matter in the studied lakes during the entire survey period. Codes of the lakes' names as in Table 1.*

Lake	DOC	a320	$S_{275-295}$	$S_{350-400}$	*Sr*
ALC	5.70 ± 8.05	18.49 ± 20.09	0.013 ± 0.003	0.009 ± 0.005	1.83 ± 0.93
ALM	4.74 ± 3.4	80.31 ± 47.74	0.009 ± 0.002	0.005 ± 0.001	1.79 ± 0.51
CAM	8.37 ± 6.16	30.12 ± 13.87	0.014 ± 0.002	0.007 ± 0.002	2.17 ± 0.82
CAR	2.19 ± 1.36	28.76 ± 11.94	0.011 ± 0.002	0.007 ± 0.001	1.68 ± 0.46
GRA	1.65 ± 0.63	12.22 ± 11.49	0.013 ± 0.002	0.007 ± 0.004	2.10 ± 0.64
LAR	9.34 ± 4.32	34.22 ± 11.80	0.014 ± 0.001	0.007 ± 0.002	2.08 ± 0.34
LON	32.32 ± 24.72	66.89 ± 71.43	0.016 ± 0.004	0.007 ± 0.002	2.31 ± 0.60
MAN	5.98 ± 4.84	32.31 ± 13.71	0.014 ± 0.002	0.008 ± 0.002	1.83 ± 0.52
MER	28.91 ± 21.15	71.54 ± 71.64	0.017 ± 0.002	0.007 ± 0.002	2.82 ± 0.91
NAV	1.82 ± 1.21	30.35 ± 10.01	0.009 ± 0.002	0.007 ± 0.002	1.49 ± 0.64
PEÑ	15.77 ± 9.60	19.92 ± 8.66	0.017 ± 0.005	0.006 ± 0.003	3.52 ± 1.74
POZ	4.00 ± 3.55	30.13 ± 12.11	0.012 ± 0.001	0.007 ± 0.002	1.79 ± 0.46
QUE	22.78 ± 21.47	41.97 ± 33.20	0.016 ± 0.003	0.007 ± 0.002	2.54 ± 0.76
SAL	4.94 ± 1.76	44.2 ± 21.71	0.013 ± 0.002	0.007 ± 0.002	1.88 ± 0.44
TIR	4.04 ± 4.70	30.39 ± 25.80	0.012 ± 0.002	0.008 ± 0.002	1.62 ± 0.31
VEG	1.10 ± 0.35	24.77 ± 9.01	0.008 ± 0.001	0.006 ± 0.002	1.37 ± 0.38
YEG	22.54 ± 16.25	52.14 ± 24.87	0.015 ± 0.003	0.008 ± 0.002	2.02 ± 0.42

*Units: DOC, (mM); a320 (m^{-1}).

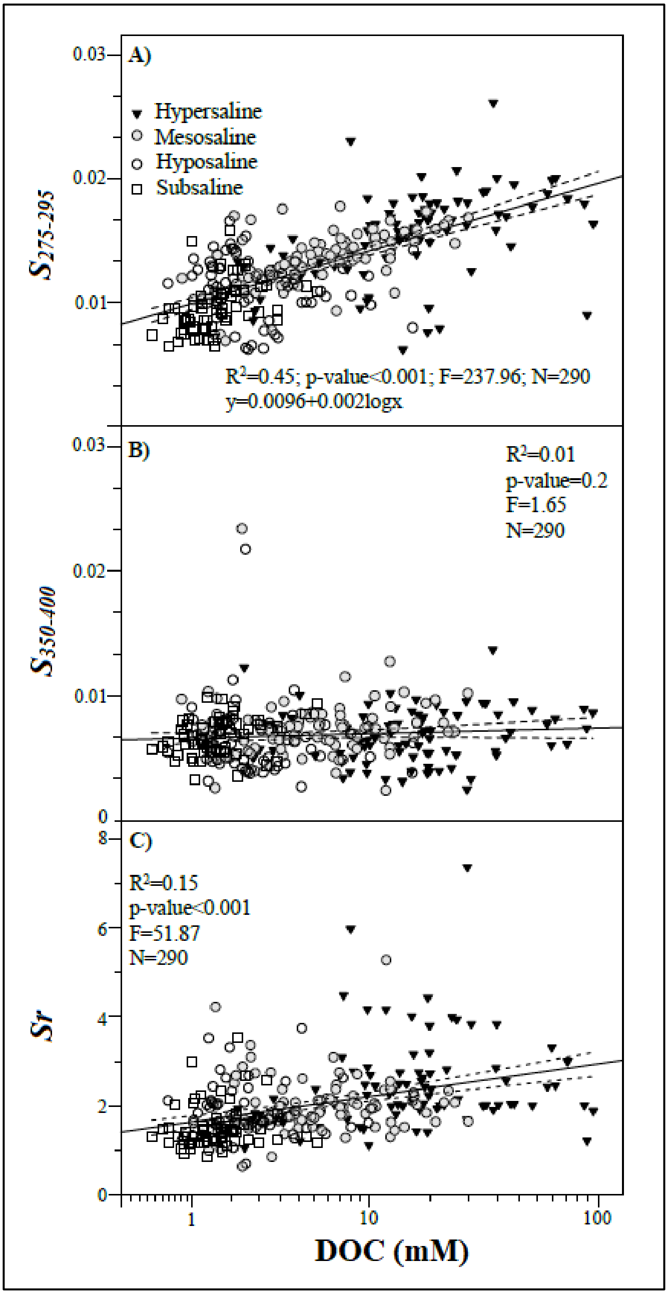


Figure 5.2. *Semi-log relationships for DOC concentrations with A) Slope $S_{275-295}$, B) Slope $S_{350-400}$ and C) Slope ratio Sr. Dashed lines indicated 95% confidence interval for the regression line.*

Table 5.3. *Average (±standard deviation) values for FDOM in the studied lakes during the entire survey period. Codes of the lakes' names as in Table 1. Units: FDOM types A, C and M (SQ-ppt); FDOM types B and T (BSA-ppt). The other parameters are dimensionless.*

Lake	FDOM-A	FDOM-B	FDOM-C	FDOM-M	FDOM-T	FI	BIX
ALC	53.33 ± 17.29	3.54 ± 1.32	38.03 ± 35.93	46.62 ± 37.46	6.11 ± 2.01	1.62 ± 0.05	0.96 ± 0.05
ALM	81.60 ± 24.73	3.31 ± 1.04	52.85 ± 15.49	62.18 ± 19.12	6.04 ± 2.03	1.34 ± 0.06	0.74 ± 0.02
CAM	63.91 ± 15.77	8.72 ± 2.33	56.58 ± 25.87	70.94 ± 29.41	12.59 ± 3.37	1.61 ± 0.06	1.00 ± 0.04
CAR	80.28 ± 14.27	4.05 ± 1.28	35.90 ± 13.29	46.47 ± 11.38	6.61 ± 1.80	1.47 ± 0.07	0.81 ± 0.06
GRA	37.10 ± 10.44	4.52 ± 1.13	12.47 ± 2.68	17.81 ± 3.60	5.66 ± 1.39	1.56 ± 0.13	0.95 ± 0.08
LAR	79.89 ± 15.38	12.4 ± 2.97	64.55 ± 20.08	77.59 ± 23.09	16.67 ± 3.49	1.64 ± 0.06	0.98 ± 0.04
LON	35.95 ± 20.64	7.79 ± 3.47	85.89 ± 40.38	97.79 ± 32.89	11.02 ± 4.58	1.61 ± 0.07	1.01 ± 0.06
MAN	61.75 ± 13.27	5.68 ± 2.20	51.62 ± 25.23	65.68 ± 30.43	9.00 ± 3.30	1.56 ± 0.07	0.96 ± 0.12
MER	26.56 ± 12.50	6.74 ± 3.44	74.40 ± 25.26	85.36 ± 20.73	9.86 ± 4.37	1.61 ± 0.05	0.99 ± 0.07
NAV	97.38 ± 25.72	2.80 ± 0.87	45.40 ± 19.69	56.04 ± 23.11	4.96 ± 1.30	1.42 ± 0.06	0.67 ± 0.04
PEÑ	40.63 ± 10.19	7.07 ± 2.35	32.15 ± 8.72	45.29 ± 12.30	10.42 ± 3.52	1.61 ± 0.08	1.06 ± 0.05
POZ	87.86 ± 12.87	5.43 ± 1.44	56.22 ± 21.32	70.15 ± 26.80	9.82 ± 2.92	1.61 ± 0.09	0.92 ± 0.04
QUE	38.12 ± 17.22	7.09 ± 2.92	52.72 ± 22.51	63.99 ± 24.27	9.91 ± 3.62	1.54 ± 0.06	0.97 ± 0.06
SAL	78.08 ± 16.29	4.27 ± 0.83	63.24 ± 22.02	72.90 ± 20.87	8.49 ± 1.10	1.50 ± 0.06	0.92 ± 0.03
TIR	70.74 ± 15.18	2.76 ± 0.72	61.64 ± 59.55	68.28 ± 58.06	6.15 ± 1.26	1.47 ± 0.05	0.89 ± 0.01
VEG	82.22 ± 29.23	7.78 ± 3.09	36.09 ± 10.45	43.83 ± 12.83	11.48 ± 4.45	1.61 ± 0.14	0.88 ± 0.11
YEG	40.73 ± 19.75	7.69 ± 3.71	101.39 ± 25.72	112.89 ± 23.97	12.61 ± 5.20	1.60 ± 0.04	0.97 ± 0.03

Five fluorophores attributed to different DOM sources were commonly found in the samples. They consisted of three humic-like (FDOM-A, FDOM-M, and FDOM-C) and two protein-like (FDOM-B and FDOM-T) fluorophores (Table 5.3). The average concentrations of allochthonous DOM, represented by the fluorophore FDOM-A, varied in the lakes from 26.56 to 97.38 SQ-ppt, and the entire data set showed a negative power-law relationship with DOC concentrations (Figure 5.3A) similar to that observed for the absorption coefficient *a*(320). On the contrary, the more degraded humics of autochthonous nature, types FDOM-C and FDOM-M, showed a significant positive correlation with DOC concentrations (Figures 5.3B and 5.3C), and displayed ranges of 12.47-101.39 and 17.81-112.89 SQ-ppt, respectively. The amino acid-like fluorophores FDOM-B and FDOM-T were, in general, less abundant compared to humic acids, and correlated poorly with DOC concentrations (Figure 5.4). The derived parameters from the fluorescence analysis were the florescence (FI) and freshness (BIX) index (Table 5.3). The average values of FI in the lakes, which is considered to increase with the autochthonous nature of DOM, ranged narrowly from 1.34 to 1.64, and was usually lower in subsaline lakes. The average values of the BIX index, which indicates the relative proportion of FDOM-M relative to the FDOM-C peak, ranged from 0.67 to 1.06. Among the lakes, this index roughly increased with salinity, thereby showing a similar pattern of variation to the FI index.

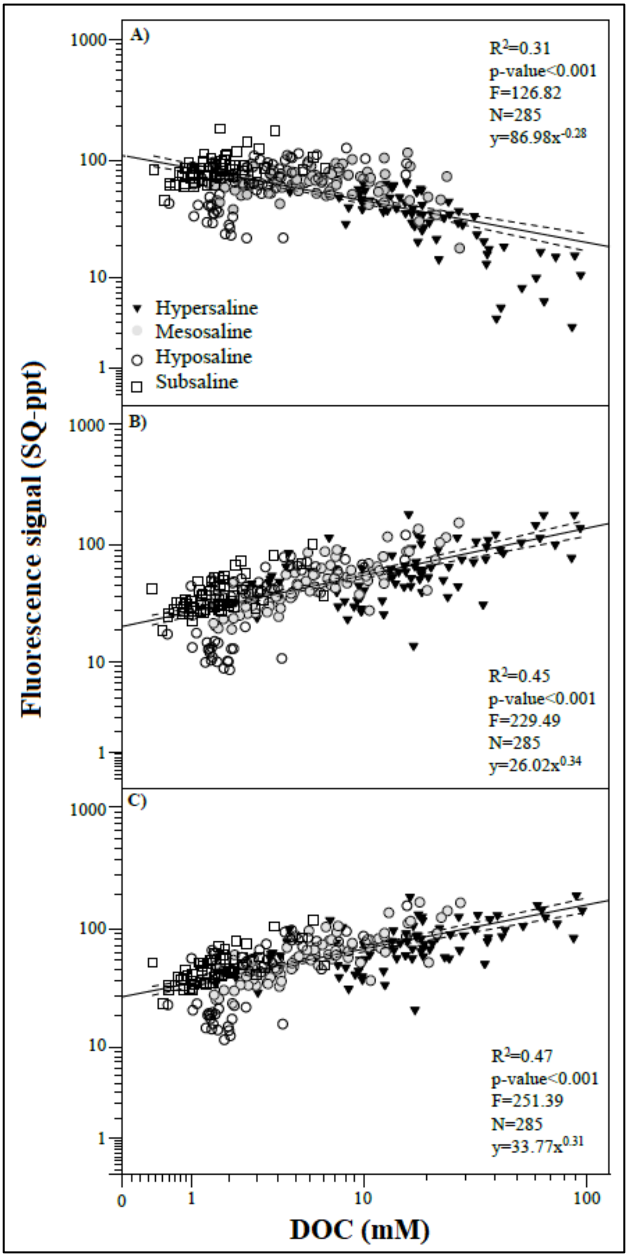


Figure 5.3. *Log-to-log relationships between fluorescence peaks acquired from the EEMs and DOC concentrations in the water of the studied lakes. The humic-like substances correspond to peaks A) FDOM-A, B) FDOM-C and C) FDOM-M. Dashed lines indicated a 95% confidence interval for the regression line.*

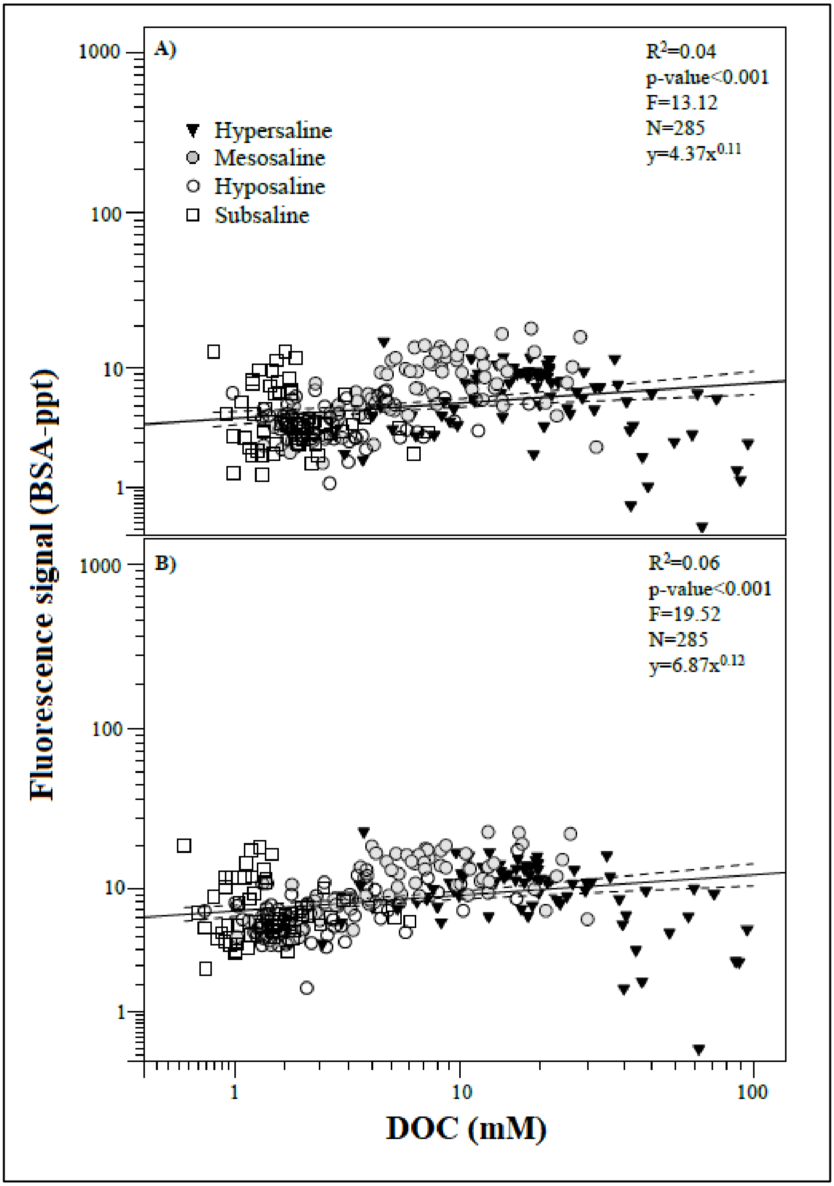


Figure 5.4. *Log-to-log relationships between fluorescence peaks acquired from the EEMs and DOC concentrations in the water of the studied lakes. Protein-like substances correspond to peaks A) FDOM-B and B) FDOM-T. Dashed lines indicated a 95% confidence interval for the regression line.*

V.3.4. Multivariate analysis of different DOM environments

The sorting of samples resulting from the principal components analysis (PCA) performed with the DOM metrics and main environmental variables (i.e., % of flood level of water column and Chl-*a* concentrations) is shown in Figure 5.5. The first component (PC1) accounted for 33.7% of the total variance and showed the largest positive loadings of spectral slope coefficient $S_{275\text{-}295}$, DOC, FDOM-C, FDOM-M, whereas FDOM-A, and the relative flooding level of the lakes, showed the opposite trend. In general, this component arranged samples based on their salinity, positioning the subsaline lakes in the negative extreme, and samples with higher salinity and ephemerality in the positive extreme of the axis, whereas intermediate salinities were positioned in-between. The variance explained for the second component (PC2) was 22.2% and showed the highest positive loadings for protein-like FDOM-B and FDOM-T, as well as the FI and BIX indexes. The highest loadings at the negative extreme were, in this case, shown by the Napierian absorption coefficient $a(320)$.

A graphical representation of the seasonal variation of the sample scores for the two first components of PCA is shown in Figure 5.6. In the case of the first component (PC1), the seasonal variation was greater in the meso and hypersaline lakes compared to the lakes with lower salinity. However, all of them showed the same pattern, consisting of a progressive increase in scores as the hydroperiod advanced, with lower and higher values during autumn-winter and spring-summer, respectively. This pattern was less obvious in the lakes ranked as hypersaline. However, the occurrence of a seasonal pattern in the scores of the second component (PC2) was more obvious in the case of the hypersaline lakes (Figure 5.6). The subset of mesosaline lakes, which was mainly composed of artificially eutrophicated lakes (i.e., Camino, Larga de Villacañas, Manjavacas), showed consistently higher average scores for this second component compared to the other subtypes.

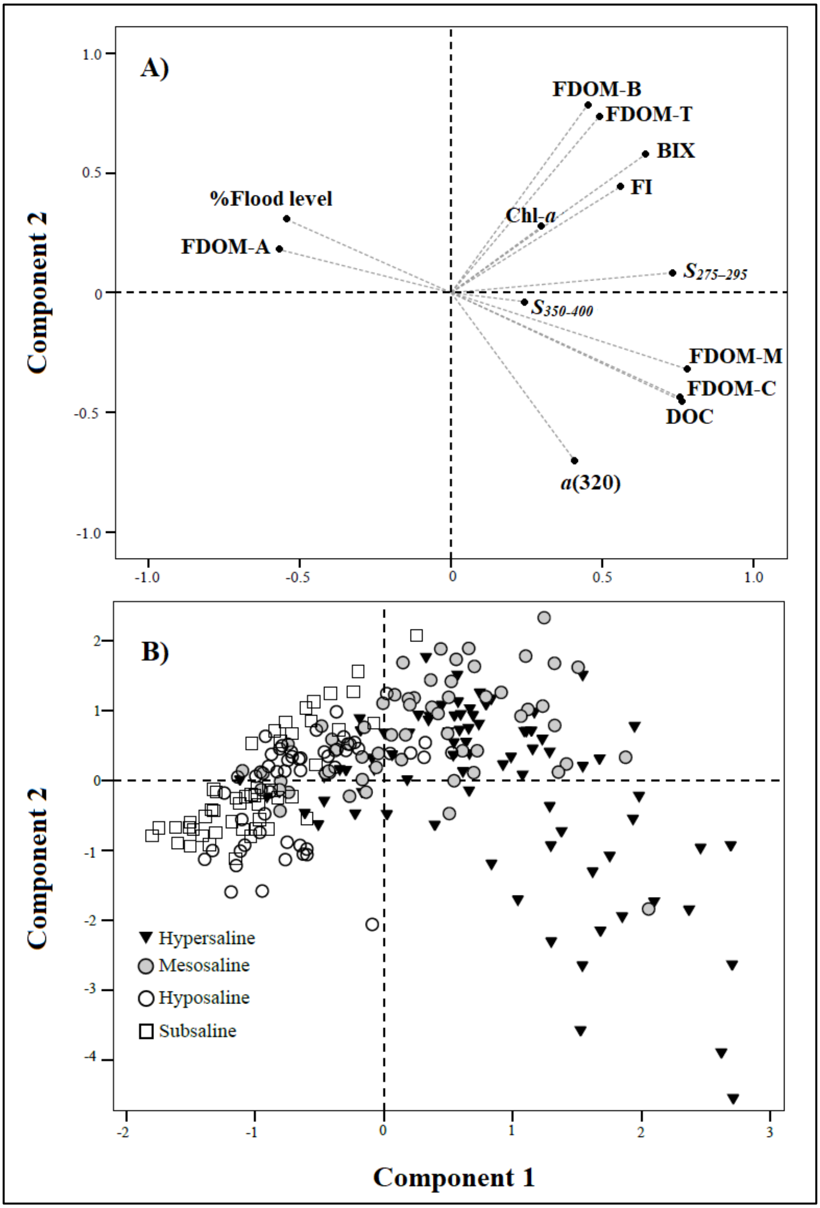


Figure 5.5. *Principal component analysis of dissolved organic matter (DOM) and environmental parameters for the studied lakes. A) Loading plot showing correlations between the measured variables. B) Score plots displaying variation among lake subtypes during the entire studied period.*

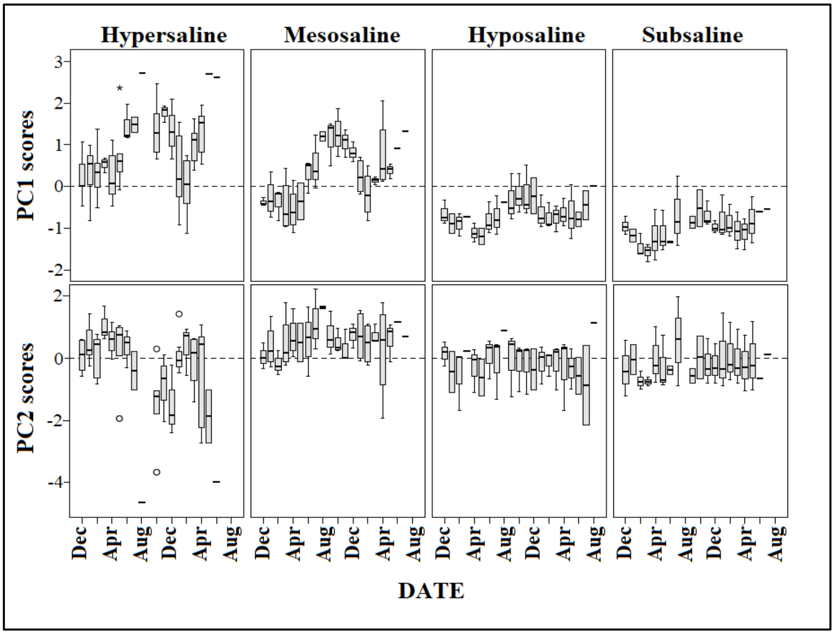


Figure 5.6. *Seasonal variation of scores of the two first components obtained in the principal component analysis (PCA) for the different typologies of lakes.*

V.4. Discussion

The lakes studied here represent the range of limnological conditions encountered in UNESCO's Mancha Húmeda Biosphere Reserve. They range from freshwater to hypersaline, and show trophic states varying from oligotrophic to hyper-eutrophic. Most of the DOM metrics measured here vary greatly among the lakes, and over time. Evaporative processes mainly control seasonal variations of DOC concentrations, as the overall chemistry of the lakes, following a pattern characterized by marked spring-summer maxima. The highest average DOC concentrations measured in the lakes are comparable, or even exceed, those observed in other saline lakes (Mariot et al., 2007; Osburn et al., 2011; Boros et al., 2017; Butturini et al., 2022)). However, some data can be partially skewed by the extreme DOC concentrations marginally measured in some of the lakes at the beginning and end of the hydrological cycle, just when the flood in the basins is minimal.

Both allochthonous and autochthonous sources account for these DOC-rich environments as deduced from the fluorescence metrics.

Among the peaks indicating the presence of humic-like compounds, FDOM-A clearly denotes the occurrence of allochthonous inputs (Coble et al., 1996). This peak and the absorptivity of DOM (i.e., DOC-normalized coefficients *a*(320)) display a negative dependency on DOC and water conductivity, respectively, hence showing a wide-ranging pattern consisting of a reduction in DOM aromaticity, and its relative content of chromophores, as the salinity and the water residence time of the lakes increase.

The displacement of the DOM fluorescence emission to longer wavelengths, which reduces the values of the FI index, indicates the occurrence of more conjugated fluorescent molecules, and thereby a DOM which is more aromatic in nature (McKnight et al., 2001). The average values of this index in the studied lakes vary narrowly; however, and in general terms, the lakes showing lower values had correspondingly both higher fluorescence signals of peak FDOM-A and DOC-normalized *a*(320) coefficients. This reinforces the idea that the less saline lakes have, in relative terms, a highly aromatic DOM, likely deriving from recent terrestrial humic matter. On the other hand, in-lake transformations lead to the build-up of FDOM-C and FDOM-M fluorophores. Both fluorophores highly correlate with DOC concentrations, thus indicating that they are major components of the DOM pool.

The BIX index represents the quotient between the M and C peaks, and its increases can be interpreted as an increase in the proportion of recently produced DOM (Huguet et al., 2009; Wilson and Xenopoulos, 2009). Based on this, the increases that we observe likely respond to the activity of primary producers. The detection of peak M in its ‘‘pure’’ form has been related with biological processes, either deduced from the correlations observed with chlorophyll-a concentrations (Coble et al., 1998) or the bacterial activity (Stedmon and Markager, 2005). On the contrary, relative increases in peak C occur when DOM is aged and degraded. The M and C humic peaks may overlap when they are in large amounts. Accordingly, fluorescence signals in samples more skewed to the positive side of PC1 component (Figure 5.5) cannot unequivocally be attributed to one of them in particular. This would explain why they correlate greatly and display high loading coefficients for the first component of the PCA, when on the contrary the peak M should be more associated with the positive side of the second component. However, the results become more convincing if we rely on the use of the BIX index. In our case, this index is more informative regarding the primary production of the lakes than chlorophyll-a concentrations, something which has also been noted by other authors (Chari et al., 2013). This may respond to the fact that chlorophyll-*a* only reflects planktonic production, and it does not consider benthic primary production, which otherwise can be significant in shallow ecosystems.

The fluorescence signals of protein-like compounds (i.e., FDOM-B and FDOM-T), which have a high loading coefficient for the PC2 component (Figure 5.5), denote the presence of the aromatic amino acids tyrosine and tryptophan. The occurrence of these fluorophores has also been attributed to phytoplankton primary production (Coble et al., 1998). In our case, this algal origin is very conceivable considering that these proteins are freshly formed. This is supported by the strong correlation observed between both protein-like fluorophores, which differs with the enrichment observed in the fluorophore B when proteins are highly degraded (Fellman et al., 2010; Yu et al., 2015; He et al., 2021). On the other hand, there are recent studies showing the occurrences of compounds other than these amino acids that have a fluorescence emission overlapping in the same low-UV region (Hansen et al., 2016 and articles cited therein). Nonetheless, they are also related with the occurrence of freshly formed DOM and therefore represent an analogous proxy. Accordingly, samples showing positive scores for both components of PCA can be inferred as containing recently formed autochthonous DOM from primary production. Most of these samples belong to lakes suffering greater eutrophication (i.e., Camino, Larga de Villacañas, Manjavacas). Also, the higher and lower values of FI and *a*(320) measured, respectively, in samples positioned on the positive side of PC2 indicate, as previously mentioned, DOM of a more microbial rather than terrestrial nature. Exceptionally, the lowest average FI values observed in the turbid Lake Almodovar may respond to its volcanic nature, which causes very fine particles to remain suspended in water even in the GF/F filtered samples.

The absorbance spectra of DOM should not necessarily behave in the same way, given that not all the CDOM emits fluorescence, likely resulting in a complementary proxy for the DOM analysis. We rely on the size-fractionation study performed by Helms et al. (2008) to establish the slope $S_{275–295}$ as a proxy of DOM with low molecular weight, opposed to the slope $S_{350-400}$, that otherwise indicates a higher molecular weight. Our findings show, in general, steep $S_{275–295}$, shallow $S_{350-400}$, and then relatively high values of *Sr*, which suggests waters with a DOM significantly altered by photo-degradative reactions (Fichot and Benner, 2012). Other processes such as microbial degradation have been experimentally shown to produce hardly any variation in the steepness of the $S_{275–295}$ slope (Yamashita et al., 2013), principally when DOM is mainly protein-rich plankton-derived, as in our case (Fichot and Benner, 2012). In our study, $S_{275–295}$ slopes significantly correlate with DOC concentrations, thereby indicating the major role of photobleaching in these lakes. In agreement with Helms et al. (2008), we consider this slope as an appropriate retrieval method of the DOC concentration, particularly when the DOM is highly altered photochemically, as in our case.

The principal component analysis (PCA) conducted with samples covers, simultaneously, the long-term and seasonal variance of DOM metrics. The PCA segregates the lakes based on different DOM environments, the order of the samples being inherently related to the water residence time. The lakes which are more skewed to the positive side of the first component (PC1) are those showing longer residence times and aged DOM of a more refractory nature, likely suffering more photochemical alterations. The slope $S_{275–295}$ shows a high loading coefficient for the PC1 component, but opposed to the fluorescence metrics, it displays the lowest coefficient for the PC2 component. This reinforces this idea that the photochemical alteration of DOM explains the distribution of samples throughout this first component of PCA. This underlying cause, which produces a variance in this component, evolves seasonally as observed in Figure 5.6. Thus, the lakes are, in general, displaced unto more positive scores in the first component as the season advances, reaching higher scores in the warmer months.

Furthermore, this seasonal increase in the PCA scores occurs whatever the lakes' trophic status or salinity.

In summary, we have assessed the optical characteristics of dissolved organic matter of a group of saline lakes representing organic-rich environments. Our survey shows how contrasting water residence times lead to different degrees of accumulation of aged DOM. Both photolytic and biotic degradation lead to the biochemical conversion of this DOM, thus regulating, to a great degree, carbon cycling within these lakes. Our findings show how an intense accumulation of chromophoric organic matter occurs in the more saline lakes with higher water residence times, which likely represents a non-labile carbon pool with a very low potential for microbial mineralization. Since these lakes lack water outflows, this notable accumulation over time, combined with the expected high chemical precipitation of carbonates because of the elevated alkalinity of the waters, leads these lakes to act as carbon sinks and thereby contributes to their sequestration.

Chapter VI: Time course of dissolved organic matter and bacterial populations in two close saline shallow lakes showing a contrasting trophic status

VI.1. Introduction

An important part of nutrients in aquatic ecosystems are supplied by the dissolved organic matter (DOM), which originates from natural and anthropogenic liquid sources, atmospheric deposition, and autochthonous production. The analysis of the optical properties of the dissolved organic matter (DOM) carried out in the previous chapter provides valuable information regarding its origin, composition, and reactivity. The chemical and biological transformation of DOM may occur at different temporal scales (Bertilsson and Tranvik, 2000; Zhang et al., 2009; Guillemette and del Giorgio, 2011; Soto-Cardenas et al., 2017). The more reactive the DOM coming from the catchment is, the faster it will be processed by the bacterial heterotrophic community. Furthermore, the natural sunlight degradation of DOM may produce labile substrates over time that are able to stimulate bacterial production (De Lange et al., 2003). This is because the absorption of UV radiation causes CDOM photobleaching and a subsequent release of carbon and nitrogen photoproducts (Moran et al., 2000, Stedmon et al., 2007). Accordingly, the fluxes of materials and energy towards the bacterioplankton (i.e., bacterial component of the water column) could be controlled not only by DOM inputs but also by its physical-chemical transformations. Flow cytometry applied to the analysis of bacterioplankton may provide information about its physiological condition, which can be related with its DOM environment. Among other approaches, this can be made by distinguishing bacterial subpopulations with a high apparent content of nucleic acid (DNA), which can be referred as the active growing fraction of the community (Lebaron et al., 2001).

In previous chapters, the dynamics of nutrient concentrations and functional aspects of the studied lakes have been described on a seasonal basis. However, faster and low predictable changes may occur because the susceptibility of these lakes to the weather conditions and catchment process. Both the shallowness and a low ratio of lake to catchment area would explain it. Hence, changing conditions related with alterations of the water level (flooding or drying), rain events, or wastewater inputs, may occur in short time periods and be unnoticed following other sampling regularity. These dynamics should be considered because they can force the biological productivity of lakes and have an impact in their management (de Vicente et al., 2006).

In this last chapter of the thesis, the dynamics of nutrients and DOM optical properties were surveyed in lakes Manjavaccas and Alcahozo, which are near each other. They show different management modes and trophic status but experience the same weather conditions because its proximity. A cytometric analysis of bacterioplankton populations was conducted in parallel to assess their abundances and growth condition. Differently to previous chapters, the data collection frequency in this case was notably increased to obtain more accurate information with respect to rapid lacustrine dynamics. The knowlege generated is expected to improve our ability to manage these saline lakes by complementing the information gained in previous chapters when following regular monitoring.

VI.2. Methods

VI.2.1. Study site and sampling plan

The study was conducted in lakes Manjavacacs and Alcahozo (Figure 6.1), which contrast in terms of management and trophic status. Lake Alcahozo is an unpolluted and temporary shallow water body with a flooding area of around 70 ha and a small catchment (~7 km^2). The water balance of this lake is basically controlled by climatic conditions. Accordingly, water usually enters the lake through groundwater inflows (62%), from direct precipitation (~37%) and surface runoff (~1%). Major water losses result from evapotranspiration (~ 80%), while infiltration remains smaller (Ballesteros et al., 2018). On the other hand, Lake Manjavacas is a polluted and originally temporary shallow lake. This lake shows an artificially prolongated hydroperiod, as it is currently supplied with treated (from 2003, previously untreated) wastewater inflows. Lake Manjavacas has a flooding area of around 230 ha and a catchment basin close to 57 km^2. Water inputs in this lake come from direct precipitation (56%), groundwater (13%), diffuse surface runoff (0.07%) and human management via wastewater inflow (31%) (Ballesteros et al., 2018). Similarly to Lake Alcahozo, water losses in Manjavacas are mostly due to evapotranspiration. The wastewater inputs to this lake, which are previously subjected to a secondary treatment, are justified as a management measure to promote habitat for aquatic birds (Navarro et al. 2011). According to Hammer (1986) and using conductivity as a proxy of salinity, these lakes range from mesosaline (at maximum flooding) to hypersaline conditions, with the highest conductivities found at the beginning of the seasonal filling and before drying. However, wastewater inputs in Lake Manjavacas may still produce desalinization. The surrounding landscape mostly shows agricultural uses. Weather conditions (precipitation and temperature data) during the study period were obtained from the SIAR (Irrigation Integral Advice Service) nearest meteorological observatory (JCCM, 2015).

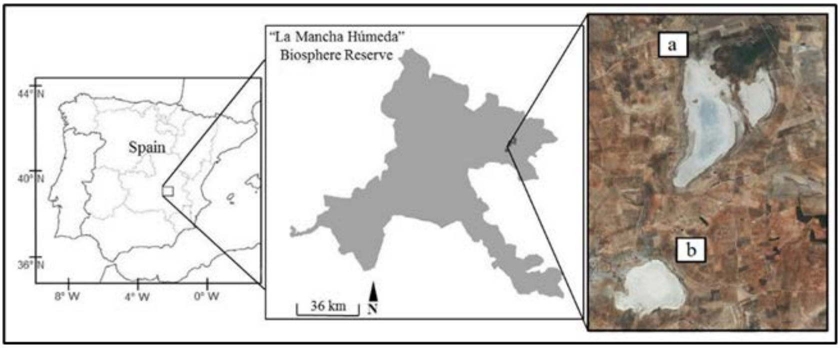


Figure 6.1. *Location of study area. a) Lake Manjavacas; b) Lake Alcahozo.*

Sampling was conducted weekly from November 2014 to April 2015 in the open waters of both lakes. The relative flooding level of the basins (%FL), the water electrical conductivity (EC) and the pH were measured as explained in the chapter II of the thesis. Water samples were obtained during each sampling event for the quantification of major nutrients, Chl-*a* concentrations, the cytometric analysis of bacterioplankton and the optical characterization of DOM. See chapter II for all the analytical details.

VI.2.2. Statistical analyses

Statistical analyses were performed using the University of Valencia-licensed statistical package SPSS v. 22 (SPSS Inc., Chicago, IL, USA). Because ANOVA assumptions were violated, a nonparametric Kruskal-Wallis test was used to examine overall differences between lakes and periods (using the season as a factor). Correlation analyses were done to determine the association between the studied variables. In both analyses, values of $p<0.05$ were considered as statistically significant.

VI.3. Results

VI.3.1. Environmental characteristics

The rainfall occurring during the study period is shown in figure 6.2. The total accumulated precipitation was 188 mm, it was lower in spring (53 mm) and a bit higher in autumn (73 mm). The daily mean air temperature was 10.63±7.08 ºC. In autumn, when the lake basins started to fill up, this ranged between -4.1 and 18.4 ºC (mean 7.76 ± 3.00 ºC), whereas in winter (when flooding is higher) it ranged between -8.3 and 22.8 ºC (mean 4.88 ± 3.25 ºC). During spring (when the drying of lakes started), air temperature increased and ranged from -0.9 and 26.5 ºC (mean 11.50 ± 2.54 ºC). The depth of the water column fluctuated from few centimeters to around 50 cm along study, being these usually higher in Lake Manjavacas.

The mean electrical conductivity (EC) averaged 39.15±28.69 mS cm^{-1} and 31.35±12.26 mS cm^{-1} in Lake Alcahozo and Manjavacas respectively, and non-significant differences were observed among them. This EC varied significantly along the hydroperiod in both lakes, being this higher at the beginning of the flooding period (38.9 and 42.2 mS cm^{-1} respectively) and lower in January-February during the maximum flooding (24.6 and 23.9 mS cm^{-1} respectively). The pH in both lakes was moderately alkaline, averaging 8.69±0.47 and 8.94±0.34 in Lake Alcahozo and Lake Manjavacas respectively.

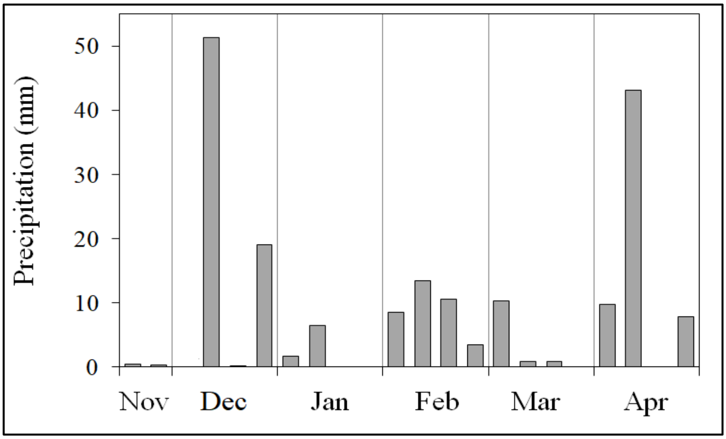


Figure 6.2. *Weekly accumulated precipitation as registered in the meteorological observatory of "El Pedernoso" town during the study period.*

VI.3.2. Nutrient dynamics

The average concentrations of the main nutrients for both lakes are shown in table 6.1. In general, nutrient concentrations were significantly higher in Lake Manjavacas, which receives wastewaters, compared to the well-preserved Lake Alcahozo (Figure 6.3). Only NH_4 concentration were higher but not significantly in Lake Alcahozo. In both lakes, the highest concentrations of nutrients were measured during the initial filling and the drying of the basins (Figure 6.3), although different trends were observed for each lake. Thus, higher concentrations of SRP, NO_3, DOC and DON were found in Lake Manjavacas at the beginning of hydroperiod (Figure 6.3), being these differences significant ($p < 0.05$) for SRP and DON. By contrast, higher concentrations of these nutrients in Lake Alcahozo occurred when the drying started at spring (Figure 6.3); being in this case significant ($p <$ 0.05) for NO_3^-, DON and DOC. The NH_4 evolved similarly in both lakes (Figure 6.3), being higher at the beginning of the hydroperiod and decreasing towards the end, except for a particular increase detected in Lake Alcahozo during drying.

VI.3.3. Optical characteristics of DOM

The spectral slope coefficient $S_{275–295}$ exhibited a nonlinear dependence with DOC concentrations in both lakes (Lake Alcahozo: R^2=0.64; Lake

Manjavacas: R^2=0.63). This contrasted with the lack of dependence exhibited by the coefficient $S_{350–400}$ (Lake Alcahozo: R^2=0.05; Lake Manjavacas: R^2=0.08). In general, results indicated a higher CDOM photobleaching in Lake Alcahozo compared to Manjavacas. Accordingly, when coefficients were normalized to DOC concentrations ($S_{275–295}$:DOC and $S_{350-400}$:DOC), these showed significantly (p<0.001) higher average values in Lake Alcahozo (0.0064±0.0025 and 0.0036±0.0021 respectively) compared to those of Lake Manjavacas (0.0029±0.0008 and 0.0017±0.0006 respectively). Likewise, the *Sr* ratio was higher but not significantly in Lake Alcahozo (1.92 ± 0.54) compared to Lake Manjavacas (1.68±1.78).

The temporal dynamics of these spectral coefficients also differed between both lakes (Figure 6.4). In Lake Alcahozo, they changed significantly along time (p < 0.05), particularly the $S_{275–295}$:DOC values, that were significantly higher at the beginning of hydroperiod and decreased towards the end. Also, in Alcahozo, variations of these spectral slopes and its ratio indicated important qualitative changes of DOM (Figure 6.4). This coincided in time with the main rain events occurring in the area (Figure 6.2), which could indicate DOM input pulses via runoff. The low values observed for these coefficients in Lake Manjavacas did not allow to stablish clear relationships with rain events, although a moderate increasing trend with time was observed (Figure 6.4).

Table 6.1. *Average and range of the nutrient and Chl-a concentrations measured in the water of lakes Alcahozo and Manjavacas during the studied period.*

Lake		**SRP (µM)**	**NO_3 (µM)**	**NH_4 (µM)**	**DON (mM)**	**DOC (mM)**	**Chl-*a* (µg L^{-1})**
Alcahozo	Average	0.51	79.14	20.19	0.17	2.83	5.45
	Min	0.23	19.87	0.35	0.08	0.99	0.33
	Max	0.74	322.74	69.64	0.51	8.26	16.18
Manjavacas	Average	4.24	137.90	14.89	0.35	5.06	17.88
	Min	0.34	43.23	1.05	0.15	3.02	2.88
	Max	23.07	323.81	70.55	1.02	12.26	61.28

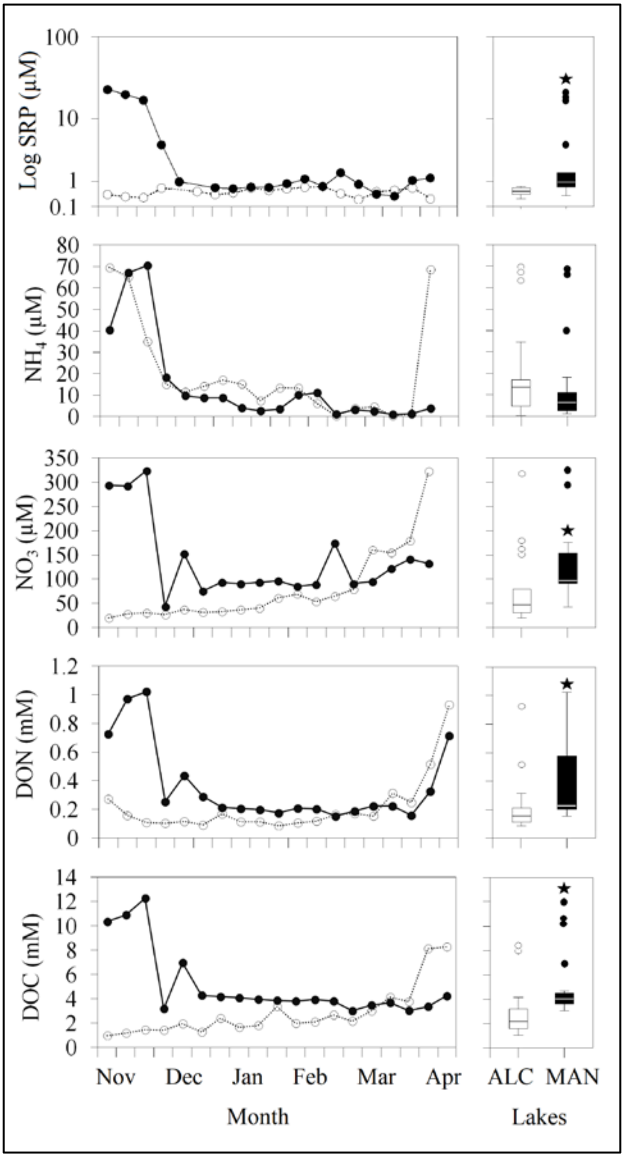


Figure 6.3. *Dynamics of the nutrient concentrations in the water of lakes Alcahozo (ALC) and Manjavacas (MAN) during the studied period. SRP data are shown in a logarithmic scale. The dotted line plot shows the temporal evolution of the nutrient, whereas boxplots show data for the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). The asterisks in the boxplot charts indicate significant difference between lakes (* p<0.05, ** p<0.01).*

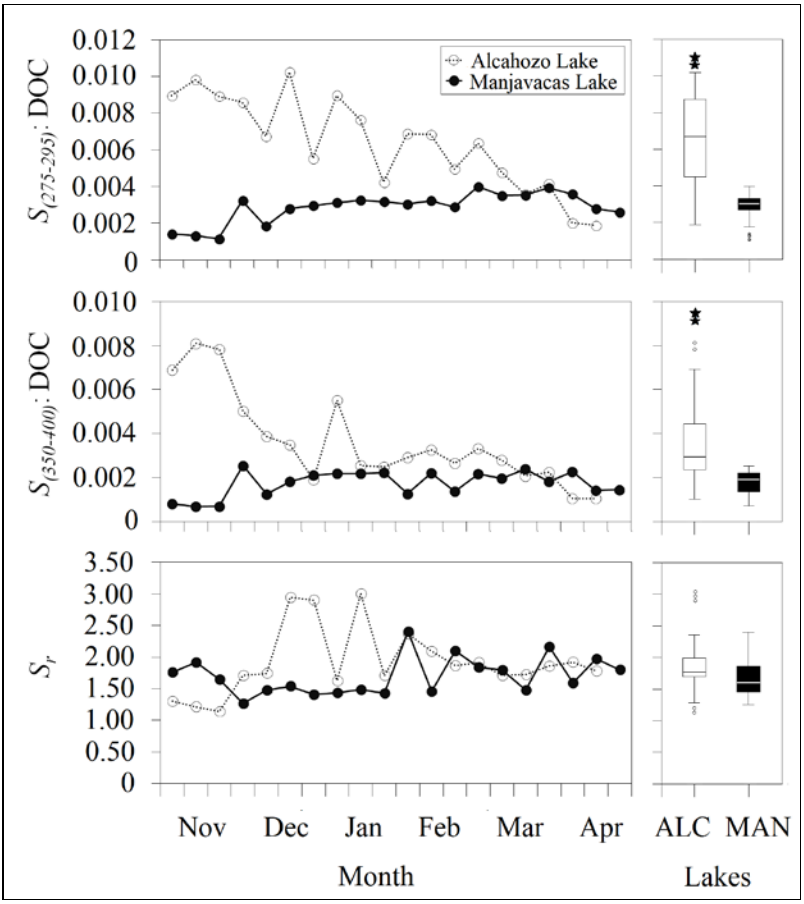


Figure 6.4. *Changes in DOC normalized $S_{275–295}$ and $S_{350–400}$ coefficients, and the slope ratio (Sr), in the water of lakes Alcahozo (ALC) and Manjavacas (MAN) during the study period. The dotted line plot shows the temporal evolution of the nutrient, whereas boxplots show data for the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). The asterisks in the boxplot charts indicate significant difference between lakes (* $p<0.05$, ** $p<0.01$).*

The fluorophores detected by the excitation/emission fluorescence matrices (EEMs) were related with different DOM sources and ages (see Table 2.3 from Chapter II for further details). The humic-like FDOM-M, FDOM-C and FDOM-A peaks were in average significantly higher ($p < 0.001$) in Lake Manjavacas (2279.89, 1899.29 and 1816.57 SQ-ppt, respectively) compared to Lake Alcahozo (1163.16, 872.60

and 1353.84 SQ-ppt, respectively). The fluorescence signal of these compounds varied significantly along time ($p < 0.05$), and as observed for the spectral slopes, its temporal dynamics differed between lakes (Figure 6.5). In Lake Manjavacas, the FDOM-M and FDOM-C peaks were higher at the beginning of the hydroperiod, in autumn, and decreased near the end in spring (Figure 6.5). An opposite trend was observed in Lake Alcahozo, being the signal of these peaks lower at the beginning of the hydroperiod and increasing at the end (Figure 6.5). Otherwise, the FDOM-A showed a similar trend in both lakes (Figure 6.5), that is, lower and higher values were found during the fill up and drying of the basins, respectively.

Regarding the fluorescence index, the Freshness index (BIX), that indicates recently produced DOM, exhibited high average values in Lake Alcahozo (1.43 ± 0.68) compared to Lake Manjavacas 1.22±0.05), though non-significant differences were observed among them. This index varied significantly between seasons ($p < 0.05$) in both lakes, although showing a somewhat different pattern. Thus, higher values in Lake Alcahozo were observed at the beginning of the hydroperiod and decreased towards the end in spring, whereas it evolved inversely in Lake Manjavacas (Figure 6.5). On the other hand, the FI index, which increases when the microbial contribution to the DOM pool is higher, differed significantly between lakes ($p < 0.001$), being in average higher in Lake Alcahozo (2.30±0.07) than in Lake Manjavacas (2.20±0.08). Despite this quantitative difference, this index evolved similarly in both lakes during the studied period (Figure 6.5).

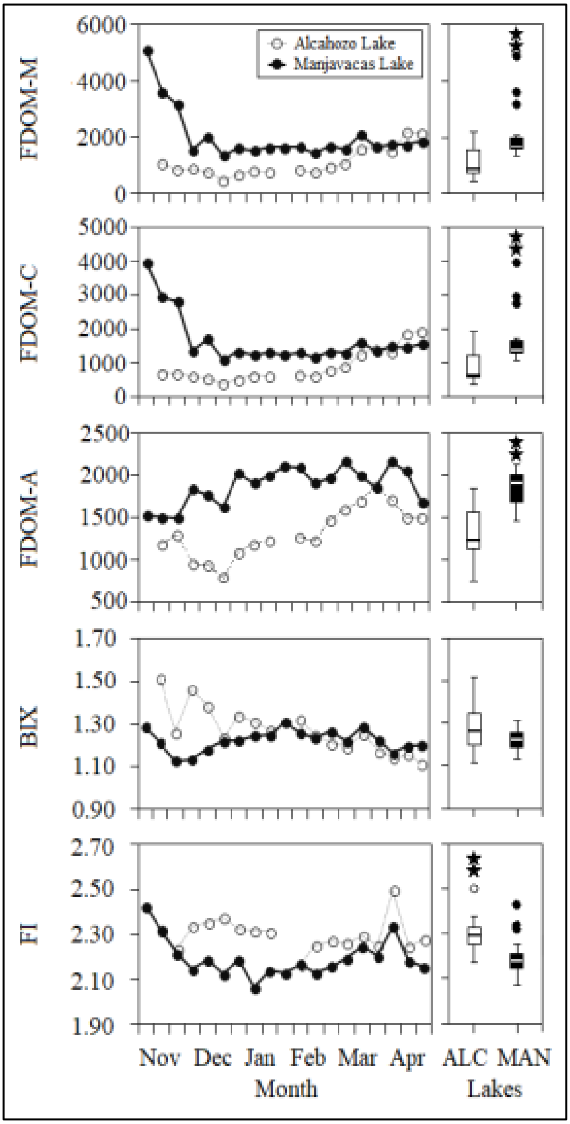


Figure 6.5. *Changes during the study period in the fluorescence peaks of three fluorophores (M, C and A) and the indices (FI and BIX) in the water of lakes Alcahozo (ALC) and Manjavacas (MAN). The dotted line plot shows the temporal evolution of the nutrient, whereas boxplots show data for the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). The asterisks in the boxplot charts indicate significant difference between lakes (* p<0.05, ** p<0.01).*

VI.3.4. Phytoplankton dynamics

The chlorophyll-a concentrations (Chl-*a*) were used as a bulk measure of total phytoplankton biomass. Mean values of Chl-*a* (Table 6.2) were significantly higher in the more polluted Lake Manjavacas ($p < 0.05$). According to these Chl-*a* concentrations, Lake Alcahozo was cataloged as mesotrophic while Lake Manjavacas evolved as eutrophic (sensu OCDE, 1982) (Table 6.1). Temporal dynamics of Chl-*a* in both lakes showed increases that nearly coincided with rain events (Figure 6.6), although Lake Manjavacas showed a higher responsiveness to these events. Despite these occasional peaks of primary production, in Lake Manjavacas, significant ($p<0.05$) higher Chl-*a* values were observed in autumn-winter period, during the fill up of the basin, whereas in Lake Alcahozo concentrations slightly increased at the dried-up period (Figure 6.6), though not significantly ($p = 0.07$).

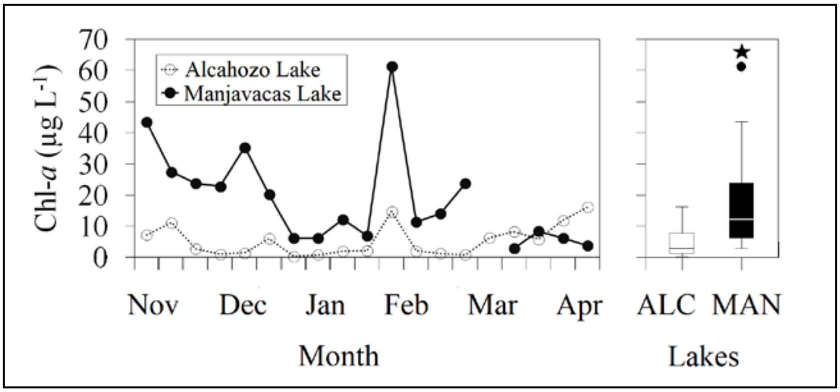


Figure 6.6. *Dynamics of the chlorophyll-a concentrations (Chl-a) in the water of lakes Alcahozo (ALC) and Manjavacas (MAN) during the studied period. The dotted line plot shows the temporal evolution of the nutrient, whereas boxplots show data for the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). The asterisks in the boxplot charts indicate significant difference between lakes (* p<0.05, ** p<0.01).*

VI.3.5. Bacterioplankton dynamics

Total abundance of bacterioplankton was also significantly influenced by the condition of the lakes ($p < 0.001$). Lake Manjavacas, which is more affected by wastewater inputs and hydrological alterations, showed higher total average abundances (4.49 x 10^7), up to four times greater than the observed in Lake Alcahozo (1.11 x 10^7). However, the %HDNA was significantly higher in Lake Alcahozo (59%) than in Lake Manjavacas (36%). Different temporal trends were also observed between both lakes (Figure 6.7). Total bacterial abundances in Lake Manjavacas showed higher values during the initial fill up but started to decrease notably in December (Figure 6.7). The values of %HDNA in Lake Manjavacas were higher during the maximum flooding, between January and February, indicating a period of increased cellular activity. This coincided with the highest phytoplankton peak detected (Figure 6.7). A different trend was found in Lake Alcahozo, which was apparently more dependent on dilution effects. Thus, bacterial abundances peaked at the extremes of hydroperiod, when water level was the lowest, whereas they were consistently lower when the basin was totally flooded (Figure 6.7). The %HDNA values in Lake Alcahozo were in general higher compared to Lake Manjavacas (Figure 6.7), but still, they showed a slight decrease during hydroperiod (Figure 6.7).

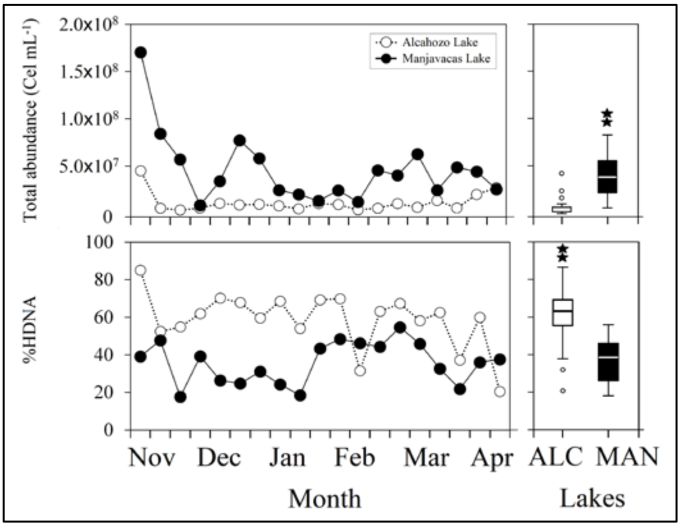


Figure 6.7. *Bacterial abundances and percentages of cells with high DNA content (%HDNA) in the water of lakes Alcahozo (ALC) and Manjavacas (MAN) during the studied period. The dotted line plot shows the temporal evolution of the nutrient, whereas boxplots show data for the median (bold horizontal line), 25-75th interquartile range (box), 10-90th interquartile range (whiskers) and outlier points (dots). The asterisks in the boxplot charts indicate significant difference between lakes (* p<0.05, ** p<0.01).*

VI.3.6. Spearman correlation analyses: Lake Alcahozo

The Spearman correlations observed between all measured variables and parameters in Lake Alcahozo are compiled in Table 6.2. As expected, the EC was highly and negatively correlated with depth of the water column. Regarding inorganic nutrients, the NO_3^- was positively correlated with DOC and $S_{275-295}$, as well as with all the measured FDOM fluorophores and the BIX index. On the other hand, NH_4 was negatively related with DOC and NO_3. However, no significant relationship was observed for SRP.

The dissolved organic fractions of nutrients showed significant relationships with several optical parameters of DOM. Hence, as observed in chapter V, DOC was highly and positively correlated with $S_{275-295}$, as well as with all measured FDOM fluorophores. By contrast, it was negatively related with the BIX index. Similarly, DON was positively related with FDOM-C and negatively with the BIX index. The correlation between DOC and DON was also significant and positive, although the relationship of DOC with NO_3 was stronger in this case. Other relations observed were the general positive correlations displayed between the $S_{275-295}$ coefficient and most of the fluorophores. On the other hand, the FI index, that denotes microbially derived DOM, was negatively related with the FDOM-A fluorophore, which is a proxy for allochthonous DOM.

Regarding the biological components, the Chl-*a* concentrations were positively correlated with the autochthonous fluorophores FDOM-M and FDOM-C, being FDOM-M associated to algal-derived DOM. On the other hand, the bacterioplankton showed significant positive correlations with DOC and DON, but only for the LDNA subpopulation.

VI.3.7. Spearman correlation analyses: Lake Manjavacas

The Spearman correlations observed between all measured variables and parameters in Lake Manjavacas are compiled in Table 6.3. As previously reported for Lake Alcahozo, the EC was strongly negatively correlated with the depth of the water column. This EC was positively related with several inorganic nutrients, DOM fluorophores and with bacterial populations, denoting in this case the evapo-concentration processes typical of these temporary lakes. By contrast, pH correlated negatively with the inorganic nitrogen and also with DON. Concerning the main nutrients, NH_4 correlated positively with both DOC and DON, whereas NO_3 correlated with some of the DOM optical parameters, more particularly with those indicating internal production. Differently to Alcahozo, SRP was correlated positively with inorganic nitrogen forms, Chl-*a* concentrations, and the autochthonous DOM (FDOM-M and FDOM-C).

Compared to Lake Alcahozo, the biological components in Lake Manjavacas showed a somewhat higher number of significant correlations with the measured variables, thereby indicating more sensitivity to the changing environmental conditions. Thus, the Chl-*a* correlated positively with the SRP as indicated previously, and with NH_4. The abundance of LDNA cells of bacterioplankton correlated with NO_3, whereas total bacterial, including HDNA cells, correlated positively with those optical parameters denoting autochthonous production of DOM (i.e., FDOM-M and FI) and its higher photobleaching (Sr).

Table 6.2. *Spearman´s correlation coefficients and statistical significance between the studied variables (n=17; * p < 0.05; ** p < 0.01) in the Lake Alcahozo. The green and red colour intensity indicate the degree of the positive or negative correlations, respectively.*

	EC	Depth	SRP	NO_3	NH_4	DTN	DOC	Chl-*a*	$S_{275-295}$	$S_{350-400}$	*Sr*	FDOM-M	FDOM-C	FDOM-A	BIX	FI	LDNA	HDNA	TOTAL
pH	0.18	-0.18	0.04	0.55*	-0.46	0.18	0.64**	-0.22	0.57*	0.06	0.31	0.04	0.12	0.21	-0.47	0.41	0.31	0.08	0.2
EC		-1.00**	-0.38	-0.13	0.08	0.37	-0.06	0.26	-0.29	0.32	-0.22	0.07	0.26	-0.08	-0.29	0.2	0.3	0	0.11
Depth			0.38	0.13	-0.08	-0.37	0.06	-0.26	0.29	-0.32	0.22	-0.07	-0.26	0.08	0.29	-0.2	-0.3	0	-0.19
SRP				0.1	-0.34	-0.31	0.11	-0.09	0.34	-0.18	0.15	-0.16	-0.25	-0.01	0.23	0.11	-0.21	-0.28	-0.25
NO_3					-0.62**	0.54*	0.90**	0.23	0.82**	0.19	0.35	0.57*	0.68**	0.77**	-0.67**	-0.35	0.35	0.15	0.22
NH_4						-0.13	-0.53*	0.17	-0.43	0.11	-0.31	-0.15	-0.28	-0.47	0.47	0	0.09	0.17	0.15
DTN							0.57*	0.51*	0.45	0.39	-0.1	0.60**	0.88**	0.59*	-0.67**	-0.32	0.64**	0.24	0.47*
DOC								0.21	0.81**	0.24	0.26	0.66**	0.61**	0.77**	-0.60**	-0.3	0.50*	0.18	0.35
Chl-*a*									0.18	0.29	-0.11	0.48*	0.52*	0.31	-0.32	-0.35	0.41	0.22	0.35
$S_{275-295}$										0.31	0.28	0.46	0.55*	0.62**	-0.63**	-0.2	0.37	0.02	0.2
$S_{350-400}$											-0.69**	0.60**	0.60**	0.37	-0.14	-0.21	0.02	-0.12	0.03
Sr												-0.43	-0.23	-0.18	-0.29	0.04	0.16	0.02	-0.01
FDOM-M													0.75**	0.81**	-0.29	-0.34	0.41	0.28	0.42
FDOM-C														0.68**	-0.69**	-0.38	0.34	0.07	0.26
FDOM-A															-0.45	-0.48*	0.37	0.19	0.28
BIX																0.18	-0.43	-0.05	-0.25
FI																	-0.01	-0.18	-0.09
LDNA																		0.69**	0.88**
HDNA																			0.91**

Table 6.3. *Spearman´s correlation coefficients and statistical significance between the studied variables (n=17; * $p < 0.05$; ** $p < 0.01$) in the Lake Alcahozo. The green and red colour intensity indicate the degree of the positive or negative correlations, respectively.*

	EC	Depth	SRP	NO_3	NH_4	DTN	DOC	Chl-*a*	$S_{275-295}$	$S_{350-400}$	*Sr*	FDOM-M	FDOM-C	FDOM-A	BIX	FI	LDNA	HDNA	TOTAL
pH	-0.37	0.09	-0.60*	-0.18	-0.84**	-0.44	-0.44	-0.59*	-0.3	0.14	-0.06	-0.38	-0.46	0.49*	0.21	-0.32	-0.13	-0.06	-0.13
EC		-0.99**	0.12	0.52*	0.41	0.80**	0,59*	0.29	0.64**	0.1	0.15	0.56*	0.60*	-0.66**	-0.3	0.59*	0.72**	0.57*	0.73**
Depth			-0.12	-0.52*	-0.42	-0.80**	-0.60*	-0.28	-0.65**	-0.11	-0.14	-0.56*	-0.60*	0.67**	0.26	-0.59*	-0.71**	-0.56*	-0.79**
SRP				0.48*	0.57*	0.43	0.21	0.58*	0.03	-0.43	0.36	0.48*	0.52*	-0.31	-0.3	0.32	0.21	0.21	0.17
NO_3					0.04	0.59*	0.39	0.13	0.51*	-0.1	0.37	0.88**	0.79**	-0.29	-0.1	0.68**	0.57*	0.41	0.53*
NH_4						0.47	0.61**	0.60*	0.31	0.08	-0.13	0.11	0.22	-0.61**	-0.3	0.06	0.2	0.03	0.14
DTN							0.66**	0.11	0.28	-0.15	0.25	0.69**	0.77**	-0.69**	-0.4	0.42	0.56*	0.41	0.57*
DOC								0.18	0.57**	0.27	-0.05	0.42	0.4	-0.71**	-0.1	0.02	0.46*	0.13	0.39
Chl-*a*									0.09	-0.25	0.24	0.16	0.11	-0.27	0.12	0.34	0.22	0.38	0.25
$S_{275-295}$										0.66**	-0.3	0.38	0.33	-0.45*	0.14	0.3	0.38	0.06	0.27
$S_{350-400}$											-0.88**	-0.29	-0.23	-0.28	0.11	-0.27	-0.08	-0.50*	-0.25
Sr												0.50*	0.35	0.16	-0	0.45*	0.27	0.57**	0.4
FDOM-M													0.94**	-0.28	-0.1	0.74**	0.49*	0.47*	0.54*
FDOM-C														-0.37	-0.4	0.74**	0.46*	0.38	0.50*
FDOM-A															0.22	-0.09	-0.34	-0.06	-0.3
BIX																-0.12	-0.14	0.16	-0.06
FI																	0.55*	0.60**	0.61**
LDNA																		0.74**	0.96**
HDNA																			0.87**

VI.4. Discussion

The methodological approach adopted in this study allowed to characterize dynamics of DOM according to its main sources, showing differences between two nearby lakes differing in the catchment size and anthropic impact. Furthermore, a weekly sampling scheme allowed us to capture rapid environmental events affecting the dissolved organic matter (DOM) dynamics, as well as its impact on the biological components. Along the hydroperiod, in both lakes, major changes in the characteristics of DOM and the availability of major nutrients were generally observed during the fill up of the basins and, to a lesser extent, during the drying period. This is in contrast with the observed during the period of maximum flooding, when conditions are more homeostatic in lakes, and were only disrupted by strong rainfall events.

The differences observed in nutrient dynamics between the studied lakes can be attributed to the anthropic pressures but also to catchment characteristics. During the studied period, the concentrations of nutrients in Lake Manjavacas, which has been historically affected by wastewater inputs, were usually higher compared to Lake Alcahozo. The progressive increase of nutrients observed in Lake Alcahozo may otherwise respond to the natural evapo-concentration process that is intensified near the end of the hydroperiod, which denotes the normal functioning of these endorheic systems. These differences are notably marked at the beginning of hydroperiod, when the water recharge of the basin likely produce a massive solubilization of the nutrients and salts stored in the dried sediments. On the other hand, the Lake Manjavacas has a bigger catchment basin (~57 km^2) compared to the Lake Alcahozo (~7 km^2), which could mean a greater allochthonous DOM input in Lake Manjavacas when significant rainfall occurs. This is supported by the progressive increase of the FDOM-A peak in both lakes, which indicates the occurrence of allochthonous DOM (Coble et al., 1996), although in Lake Manjavacas the FDOM-A peak was significantly higher. Additionally, the contribution from treated wastewater in Lake Manjavacas (~264.000 m^3 of water per year) (Ballesteros et al., 2018) may proportionally represent an important DOM source during certain periods. Thus, the significant positive correlations of NH_4 with the DOC and DON, which are only observed in Lake Manjavacas, could indicate the occurrence of wastewater discharges containing nitrogen-rich DOM that will be mineralized later. The increase of the DOC/DON ratio along the hydroperiod agrees with this hypothesis. However, during some periods, NH_4 concentrations are higher in Lake Alcahozo compared to Lake Manjavacas. This is a concomitant trend also observed in other studies comparing polluted and unpolluted sites (Wiegner and Seitzinger, 2004; Andersen et al., 2020), and could be explained by the fact that NH_4 is the preferred N-form for phytoplankton uptake. Accordingly, it is not uncommon to find this nutrient in low amounts during periods of a high development of phytoplankton (Bronk et al., 1998; Howarth et al., 2014; Andersen et al., 2020), as observed in Lake Manjavacas.

The optical study of DOM indicates, for Lake Alcahozo, a high input/solubilization of photo-oxidized and smaller DOM. The sunlight exposure has been shown to decrease the average molecular weight of soil organic matter under different environmental conditions, as it breaks down larger compounds into smaller ones (Feng et al., 2011; Cory et al., 2013; Ward and Cory, 2016). This can promote the productivity at different trophic levels, both by providing labile organic matter for bacteria but also inorganic nutrients for primary producers (Van Veen and Kuikman, 1990; Yanni et al., 2015). In Lake Alcahozo, both higher $S_{(275–295)}$:DOC values and bacterial numbers and activity matches in time during the first months of hydroperiod, suggesting that is the labile photo-degraded DOM which is mainly fueling the microbial production at this period as also observed in previous studies (Waiser and Robarts, 2000; Su et al., 2017; Kragh et al., 2022). In Lake Alcahozo, these simple organic compounds generated photolytically may originate from the solubilization of the internal loads of DOM stored in sediments.

Our results show higher fluorescence intensities of FDOM peaks (both M and C) in Lake Manjavacas, as expected by its higher trophic status compared to Lake Alcahozo. However, the high correlations observed between these two fluorescence peaks indicate an overlapping that difficults distinguishing among them. In this case, it would be more convenient to focus on the BIX index, which roughly represents a ratio between peaks M and C. A displacement in the emission wavelength due to a change in the proportion of each fluorophore is then detected by this index. Accordingly, higher values of BIX index can be interpreted as a higher proportion of recently produced DOM (i.e., increase of peak M relative to C), which seems to be an important trait in Lake Alcahozo at the beginning of hydroperiod, just when the highest values (<1.3) of this index were measured. By contrast, in Lake Manjavacas, this index increases moderately though consistently during December and January.

Other fluorescence metric reported in this study is the FI index, whose values decrease when the aromaticity of DOM increases (McKnight et al., 2001). As reported in chapter V for the entire range of the studied lakes, this index was positively related with the productivity of the lakes and the relative dominance of a more microbial derived DOM (see PCA in chapter V). The same can be inferred here, however, it is interesting to note how this index shows only significant correlations with bacterial numbers in Lake Manjavacas. Similarly, other optical parameters such as *Sr* and the FDOM-M peak correlate with the active fraction of bacterioplankton (i.e., HDNA subpopulation) but only in this lake. Also limited to Lake Manjavacas, lagged increases in the bacterial numbers are mimicked by increases in the Chl-*a* concentrations. This may respond to a rapid turnover of algal-derived DOM producing more fluctuating dynamics of the heterotrophic bacterioplankton (see figure 6.7), thus suggesting a faster cycling of carbon. By contrast, this dependence of the bacterioplankton on contemporaneous phytoplankton production was not observed in Lake Alcahozo. It should not be excluded that bacterioplankton in Alcahozo could be fueled partially by the DOM released by benthic microalgae. The occurrence of top-down mechanisms controlling bacterial dynamics should also not be ignored, although this was not addressed in this study.

In summary, the contrasting response of the biological components of the studied lakes to the environmental variation described here agrees with the partial decoupling in the metabolic balances reported in chapter III. The higher temporal resolution of sampling used in this chapter, as well the inclusion of the DOM survey, has deepened our understanding on the relationship between these two lakes and their-catchments, and the impact that this has on their ecological functioning. One case is that of Lake Alcahozo, which represents ephemeral and relatively well-preserved shallow temporary lakes from the site. Previous chapters describe an accumulation of recalcitrant DOM occurring in these lakes over long periods. However, it appears also that the seasonal recharge of the basins provides allochthonous (or internal solubilized) DOM, likely photobleached and with a low molecular weight, that can subsidize a low-to-moderate bacterial production along the season. It is conceivable in this case a coordination between the bacterioplankton physiology and this oligotrophic environment. On the other hand, Lake Manjavacas represents sites from the region suffering nutrient enrichment, which show unpredictable algal blooms that cause a deterioration of the water quality (other examples in this thesis are Camino de Villafranca and Larga de Villafranca). In these cases, the allochthonous inputs of organic matter are accompanied, and even exceeded, by the release of DOM internally produced by photosynthesis. This involves a regular occurrence of pulses of labile organic material sustaining bacterial production. The present survey provides information on the uncertainty and the non-linearity of dynamic processes that can occur in these saline lakes. This knowledge might be complementary to studies relating long-term ecological trends and processes such as climate change or maintained human disturbances.

Final remarks

The Biosphere Reserve of “La Mancha Húmeda” shows a high abundance and diversity of shallow lakes, which contrasting water salinity, management modes, and trophic status. The UNESCO Man and Biosphere Program intends to conciliate nature conservation and human development, which is not exempt from potential conflicts. Factors driving the ecological status and productivity of these lakes operate based on salinity, flooding period and the threatening level. Accordingly, the site offers a good scenario to contrast different ecological hypotheses. This thesis explores the effect of different environmental factors, both natural and anthropic, in the biogeochemistry and the cycling of major nutrients (i.e., carbon, nitrogen and phosphorus) of 17 representative lakes from the site. Moreover, the dynamics and metabolic activity of phytoplankton and bacterioplankton populations, which are highly dynamic components of the community, were studied and assessed in comparison with the condition of the lakes. This set of lakes represents an example of the main pressures and alterations that shallow lakes suffer in the Mediterranean region. This is relevant as they are part of one of the most important lake districts in southern Europe.

The main survey conducted in this study extended over two hydrological cycles, which is supposed to be time enough to show nutrient and functional dynamics in the lakes. As described in chapter III, selected sites properly represented the lake diversity in the area, both in terms of mineralization and ecological status. The studied lakes vary from subsaline to hypersaline (sensu Hammer, 1986). Based on chlorophyll-a and phosphorus concentrations, they range from oligotrophic to hypertrophic.

The regional climate, catchment processes and anthropic factors determine the hydrological patterns of the studied lakes. The length of hydroperiod is regulated by rainfall and evaporation, together with the lithological characteristics of the surrounding basin and its relationship with groundwater (Ballesteros et al., 2018). Long-term thermo-pluviometric data indicates that mid-April is the period when evaporation starts to exceed rainfall (Jerez, 2010). Then lake depths progressively decrease, and they naturally remain dry between 2 to 5 months per year. However, anthropic factors such as the input of wastewater, whether treated or not, have a significant impact in the natural functioning of the lakes. These inputs not only represent an excess of nutrients but also of freshwaters that dilutes the natural salinity and extend the hydroperiod. In these cases the lakes experience, both trophic and hydrological alterations as in lakes such as in Camino de Villafranca, Veguilla, and Larga de Villacañas.

The occurrence of permanent lakes, flooded, even in the warmest periods, can lead to additional problems related with an excessive presence of waterfowl. For instance, flamingos have been observed to exert a control in the availability dissolved nutrients by both providing guano inputs as well as enhancing sedimentary phosphorus mobilization by bioturbation (Batanero et al., 2017). This release of the internal P-loads is expected to be significative in the polluted lakes studied here as deduced from the P-fractionation study performed in chapter IV. The occurrence of waterfowl during the warmest periods in flooded lakes may also cause public health concerns. This is related to the occurrence of Clostridium perfringens and Clostridium botulinum when wastewater inputs persist during warmer season (Anza et al., 2014). These authors reported the presence of C. botulinum in bird feces from Lake Veguilla, indicating the role of waterfowl as carriers and highlighting the risk of botulinum toxin production that may cause avian botulism outbreaks. Fortunately, however, the forcing of a permanent hydroperiod is not so common. Instead, other lakes also affected by wastewater discharges (e.g., Longar or Manjavacas) still dry out completely during summer, thus allowing for a hydrological regime, although extended, being at least comparable to the natural pattern. This helps these lakes to preserve many of their ecosystem services with lower alteration.

Long-term accumulation of solutes occurs in the studied lakes. Such way, the highest concentrations of dissolved organic carbon (DOC) reported in this thesis are comparable, or even exceed, those observed for other saline lakes (Wetzel, 2001; Mariot et al., 2007; Osburn et al., 2011; Boros et al., 2017; Butturini et al., 2022). When performing DOC analyses of saline lakes, it is noteworthy that an overestimation of DOC concentrations may occur in strongly alkaline waters. This is because the dissolved inorganic carbon (DIC), which is also at high concentrations in these lake waters, may have not be totally purged during the analysis, as noted by Osborn et al. (2011). Anyhow, the DIC concentrations in the lakes studied here are not as extreme as those reported by Osborn and co-workers (< 400 mg L^{-1}). Anyhow, in order to avoid such potential error, we adopted the conservative approach of diluting the samples with the highest DIC concentrations.

Assuming these DOC measurements to be reliable, they show, as well as for other nutrient fractions, a positive correlation with the water electrical conductivity, and they also covary with the decrease of the water column. This reveals the key role of evaporative processes controlling the overall chemistry of these lakes, as also causing nutrient concentration (Waiser and Robarts, 2000; Li et al., 2016; Howard and Noble, 2018). On the other hand, the increases in DOC observed during the autumn-winter period come mainly from the inputs of allochthonous organic matter associated to the seasonal enhancement of catchment runoff, which is a common trend in endorheic systems (Guo et al., 2017).

The main chemical forms of the of sedimentary phosphorus pool have also been determined in this thesis. The potential mechanisms for the release of sedimentary phosphorus have been assessed as well. Our results stress the importance of considering the internal phosphorus loads for the design of future restoration measures, in addition to undertake the reduction of external inputs. The general pattern in the studied lakes is a P-enrichment by the accumulation due to the high endorheic nature. This trend is observed in lakes with similar characteristics (Søndergaard et al., 2001; Cobbaert et al., 2014; Cobbaert et al., 2015), where a long-term accumulation of P has also been described, which is regulated in part by physical-chemical changes occurring during the evapo-concentration cycles.

Several mechanisms can regulate the precipitation and solubilization of phosphorus. In highly mineralized lakes the phosphorus accumulates in sediments, forming either highly labile or very insoluble phosphorus forms depending on the chemical bounds (Granéli, 1999; Søndergaard et al., 2003; Horppila et al., 2017). This will control the mobility and diffusion rates from the sediment to the water. This is illustrated in figure 7.1, which shows a scheme of the main trends of sedimentary phosphorus in the saline lakes studied in this thesis. This scheme shows phosphorus as being mainly retained in insoluble forms such as calcium phosphates or apatite due to the high calcium content of these waters. To this way, the P-immobilization occurs mainly via precipitation with Ca and/or Mg, which involves a relatively stable pool. Our results demonstrate a stronger accumulation in lakes affected by wastewater discharges. On the other hand, the decaying of emergent macrophytes, that growth mainly in the desalinized lakes, may contribute to increase of other P fractions. Accordingly, the accumulation of this plant material would explain the higher amounts of the residual-P fraction observed in some of the lake's sediments. Whatever the case, the amount of P-released later to the water column would vary depending on the physical-chemical condition of each lake.

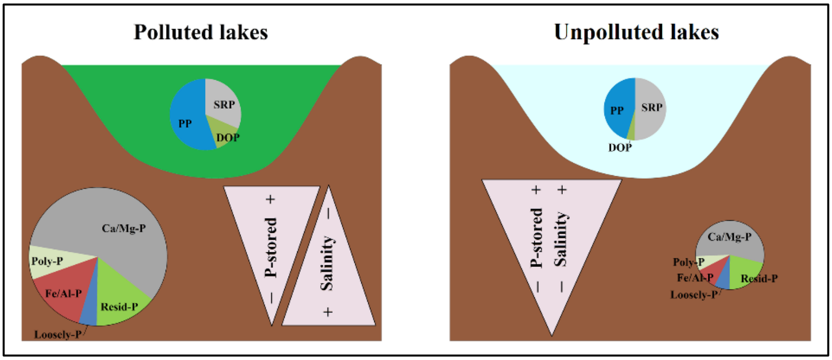


Figure 7.1. *Scheme of phosphorus retention in the sediment of the lakes where the factors studied interact. Soluble reactive phosphorus (SRP), dissolved organic phosphorus (DOP) and particulate phosphorus (PP), loosely adsorbed and pore water P (Loosely-P), P-bound to hydrated oxides (Fe/Al-P), polyphosphates (Poly-P), P-bound to apatite minerals (Ca/Mg-P), residual or recalcitrant organic P (Resid-P).*

A singular case in terms of P-availability and mobilization is that of the volcanic lake Almodovar, located in the "Campo de Calatrava" Lake District, where the concentrations of soluble reactive phosphorus (SRP) were consistently high. This responds to a high internal P-load linked to volcanism in this area, which is associated with the presence of bases rich in phosphorus (de la Horra et al., 2008), which acts as source for the P-dissolution in the water column. As a result of this lithology, Lake Almodovar has significantly lower concentrations of Ca compared to the other studied lakes. This implies a faster saturation of the main mechanism for P precipitation occurring these lakes, that is the formation of the $CaCO_3$~P complex, thus allowing in this case a higher availability of phosphorus in the water column.

Whatever the causes for the accumulation of phosphorus, solubilization of internal P loads can increase under be enhanced with anoxic conditions and at low redox potentials. The negative correlation observed between the content of organic matter in sediments and the dissolved oxygen concentration in the water column indicate a high oxygen demand in some of the studied lakes. In these cases, the onset of anoxic conditions may promote a release of phosphorus from sediments by altering the redox potential and pH. For instance, such processes are known to reduce the P-sorption capacity of Fe and Al minerals (Sundareshwar and Morris, 1999; Loh et al., 2013; Li et al., 2021), which are abundant in the sediments of the lakes studied in this thesis (chapter IV).

An important fraction of the phosphorus accumulated in sediments follows longer-term dynamics, however, a partial dissolution may occur during the seasonal drying. Accordingly, our results show how the seasonal sediment re-wetting and subsequent flooding produces pulses of rapidly available phosphorus. An experiment performed in river-floodplain systems demonstrated that phosphorus release from sediments into the water increases with increasing length of dry periods prior to re-wetting (Schönbrunner et al., 2012), which can be related with an enhancement of the mineralization rates. On that sense, it should be considered that all the studied lakes, including those well preserved that are not experiencing anthropic eutrophication, are exposed to this seasonal fertilization following the initial yearly flooding, although this fertilization will occur based on the level of the internal P-load of each lake. On the other hand, the desalination associated to wastewater inputs is expected to reduce the capability for phosphorus adsorption by reducing the Ca^{2+} availability, thereby modifying the calcium phosphate precipitation equilibrium, which as commented previously is the main mechanism of phosphorus retention in these sediments. Regarding other aspects, these anoxic cycles are supposed to facilitate the anaerobic decomposition of DOM and hence the production of methane (Zhou et al., 2019). Particularly in this type of lakes, these methane emissions can greatly alter its carbon sink capacity (Morant et al., 2020).

In relation to the buildup of DOM in these lakes, the high DOC concentrations measured respond to a gradual accumulation over the years because of the marked endorheic character of these lakes, added to the low degradation rates of this organic matter. The high residence time facilitates the aging of the DOC (Waiser and Robarts, 2000; Torremorell et al., 2015). Both photolytic and biotic degradation lead to the biochemical processing of this DOM, that is then converted into a recalcitrant carbon pool excluded from the biochemical cycling. The nutrients stoichiometry, the DOM optical analysis and the metabolic measurements performed in the thesis provide evidences on: (1) the higher TOC:TN and TOC:TP ratios measured in the water of unimpaired hypersaline lakes (see Chapter III), illustrating an enrichment in organic carbon in relation to other nutrients, (2) the lower FI (fluorescence index) and higher values of Sr observed in the DOM of unpolluted lakes (see Chapter V), denoting organic carbon significantly altered by photo-degradative reactions, and (3) the lower gross primary production rates of plankton also observed in these lakes, which results in lower amounts of freshly formed autochthonous DOM (See Chapters III and IV).

The seasonal recharge of lakes can still provide allochthonous (or internal solubilized) DOM to fuel a low-to-moderate secondary production. Regarding this, the flow cytometry analyses demonstrate how distinctively the bacterioplankton populations may response to DOM pulses. For example, in the well-preserved Lake Alcahozo, this bacterioplankton shows a high temporal stability but still high activity, indicating a capacity to use the available DOM source. Conversely, in the eutrophic Lake Manjavacas, pulses of labile materials released by phytoplankton and other biotic products sustain more fluctuating bacterioplankton dynamics, which show abundances up to four times higher than those observed in Alcahozo.

This thesis highlights the distinctive biogeochemical characteristics of the saline lakes from La Macha Húmeda compared to other wetland systems within the Mediterranean region. There are few studies on the cycling of nutrients in Western European saline lakes, and particularly scarce are those addressing aspects such as DOM characteristics or the burial and mobilization of sedimentary phosphorus, which are both important for functioning of these lakes as we demonstrated. Our observational setting covers wide ranges of salinities and trophic status, thus making these lakes appropriate models to assess the biochemical aspects here afforded. The findings of the thesis may help to better design restoration and management plans in the region, aiming to recover the natural features of these lakes, specifically those facing eutrophication.

The current status of these shallow saline lakes of La Mancha stresses the necessity to couple the current models of management with the laws and guidelines that the authorities have currently drawn up. Assuming polluted lakes from the site to still retain a certain resilience capacity, applying specific restoration measures might be successful in maintaining the capacity of these lakes to provide ecosystem services and improve its ecological status. The usefulness of management strategies to improve the ecological condition of these lakes can be assessed based on the outcomes of the thesis. In this sense, our study indicates point source pollution as the major threat to be addressed, being its impact higher than for instance the land use of the catchment promoting nutrient runoff, as demonstrated in chapter III. However, the specific characteristics of these lakes highlighted in this thesis such as natural eutrophication by either evaporative process, endorheic nature, specific catchment lithologies (e.g., volcanic), the long residence time of nutrients, and the enhanced photochemical processing of nutrients, should be also considered for management strategies. Besides, this management should also be aligned with the proposal of different authors (Olsson et al., 2004; Boyd and Folke, 2011; Berkes, 2017) that encourage governments (1) to support projects focused on understanding the dynamics, resources, and services that ecosystems provide, (2) promoting practices aimed at ecological feedback, and (3) developing adaptive management processes. Therefore, if these strategies were put into practice, the management of these peculiar lakes could be successful, provided that the biogeochemical particularities outlined in this thesis were also considered.

Conclusions

The main conclusions drawn from this thesis are listed, as follows:

1. The studied lakes represented the typical range of limnological conditions for the shallow lakes of La Mancha Húmeda Biosphere Reserve. When undisturbed, they show a temporary hydroperiod regulated by rainfall and evaporation, and a low to moderate content of nutrients. However, most of them are affected by impacts related to the alteration of the annual hydroperiod length, desalinization, and nutrient-rich wastewater inputs.

2. Accordingly, the surveyed lakes (n=17) ranged from freshwater to hypersaline and displayed trophic status ranging from oligo-mesotrophic to hyper-eutrophic. These conditions are kept throughout the hydroperiod in each lake, however, the seasonal evaporative concentration of solutes also controlled nutrient concentrations and salinity.

3. Addressing the external factors affecting the lake's trophic status, an analysis of variance was conducted by classifying lakes based on their main source of nutrients inputs (i.e., catchment runoff, wastewater spills, or volcanic lithology). Significant differences were observed among these groups on their availability of nutrients, internal load, stoichiometric relationships, as well the metabolic activity of plankton.

4. The lakes affected by ongoing and/or past wastewater inputs, as well those settled over volcanic lithology, showed a significant phosphorus enrichment both in water and surface sediments. This explained the enhancement of the photosynthetic rates for the polluted lakes, though this was not so evident for the volcanic lakes because its typical high natural turbidity, that potentially enhances light limitation.

5. By contrast, lakes not affected by watewaters, mainly subsidized by inputs from the catchment runoff, were characterized by a high accumulation of uncoloured DOC, likely recalcitrant. A natural accumulation of nutrients still occurs in these lakes because its marked endorheic nature, although with more unbalanced ratios. They are characterized by low to moderate plankton metabolic rates, and lower production/respiration ratios compared to eutrophic lakes, which suggests a higher role of allochthonous inputs of organic carbon sustaining respiration.

6. The study of sedimentary phosphorus showed that Ca/Mg-bound and organic fractions were the largest P-reservoirs in the sediments of these lakes, which, in both cases, represent relatively occluded phosphorus forms. However, the artificial flooding and water desalinization favours phosphorus mobilization into more labile forms capable to fertilize the water column. Additionally, our study demonstrated the highly suitability of volcanic lakes from the region to experience eutrophication.

7. The optical study conducted on the dissolved organic matter (DOM) using absorbance and fluorescence spectroscopy was successful in the identification of the origin, general composition, and reactivity of this main carbon source, thereby demonstrating its pivotal role in the functioning of these saline lakes and complementing the metabolic survey performed with the plankton.

8. Aged and greatly photobleached DOM accumulates in these lakes. However, partially labile carbon might originate from seasonal photolytic and biotic transformation processes occurring in the catchments and in the dry lake beds, which can fuel, after the re-flooding of lakes, the secondary production.

9. The eventual and rapid proliferation of phytoplankton, which is more pronounced and frequent in the polluted lakes, promotes the production of autochthonous algal-derived DOM, including highly labile protein substances. The occurrence of this freshly formed DOM may explain the strong coupling observed between the photosynthetic and respiration rates measured under these circumstances.

10. Short-term dynamics involving rapid mobilization of sediment nutrients, mainly phosphorus, or the availability of labile DOM, either allochthonous or autochthonous, underlay the fluctuating dynamics observed for the phytoplankton and bacterioplankton populations.

11. The flow cytometry study of the bacterioplankton suggests a complex response depending on the DOM environment. Such way, diverse physiological adaptations might exist depending on the trophic scenarios described previously. A deeper study of the functional aspects of these bacterial communities would be necessary to discover the occurrence of distinctive physiological skills considering the extreme conditions usually displayed by these lakes.

12. The main findings derived from this thesis, such as the sensitivity of the ecological functioning of these lakes to pollution, the weight of internal loads, the uncertainty of processes occurring within the lake-catchment, and the pivotal role of the transformation and mobilization of DOM, should be adopted as relevant information for management strategies, both in the Biosphere Reserve lake district, but also, as far as possible, in other sites where this type of shallow saline lakes appear.

Resumen extendido

Las lagunas salinas someras ofrecen diversos servicios ecosistémicos, siendo particularmente valiosas en términos de conservación de la biodiversidad, estando esto último relacionado con su naturaleza en muchos casos extrema. Este tipo de sistemas acuáticos se encuentran asociados a depresiones terrestres sometidas generalmente a un régimen de inundación estacional. Son masas de agua que pueden llegar a presentar una profundidad máxima de unos 5-7 metros. No obstante, en la región climática Mediterránea, no suelen exceder los 3 metros de profundidad y su temporalidad es además más marcada. Debido a esta reducida profundidad, estas zonas húmedas presentan un patrón de mezcla polimíctica, sin estratificación térmica, lo que conlleva que exista una alta interrelación entre el sedimento y la columna de agua. Por otra parte, su marcado carácter endorreico lleva a que sean importantes sumideros de materia proveniente de la cuenca circundante. La extensión y características de esta cuenca resultan por tanto determinantes a efectos de su funcionamiento, de tal modo que su efecto no será el mismo si esta la constituyen terrenos forestales o agrícolas, por ejemplo. Esta circunstancia genera que la disponibilidad de nutrientes en estas zonas húmedas sea mayor que en otras tipologías, lo que, sumado a la contribución del sedimento mencionada anteriormente, conlleva una mayor productividad en condiciones normales en comparación con lagos más profundos.

Las características climáticas y topográficas de la Península Ibérica facilitan la formación de lagunas salinas someras, cuyo balance hidrológico es característicamente negativo de primavera a verano. Estas lagunas presentan una gran relevancia ecológica, ya que son los principales reservorios de especies microbianas, animales y vegetales en la zona. La convergencia de flujos de la cuenca antes mencionada y la geología de la zona conducen a una elevada acumulación de sales y materia orgánica en estas masas de agua. Su salinidad cambia también estacionalmente, aumentando durante el período más cálido y seco debido a la evaporación, mientras que desciende por dilución durante el período más lluvioso y frío.

Entre los sistemas lacustres de estas características que existen en la Península Ibérica, cabe destacar el de La Reserva de la Biosfera de La Mancha Húmeda. Esta zona forma parte de la cuenca alta del río Guadiana, extendiéndose por las provincias de Toledo, Cuenca, Albacete y Ciudad Real (Castilla-La Mancha, España central), ocupando principalmente áreas de carácter estepario. Este sistema lacustre se caracteriza por su gran superficie, densidad y diversidad de sistemas salinos que alberga. Las Reservas de la Biosfera son áreas reconocidas internacionalmente por el Programa El Hombre y la Biosfera de la UNESCO desde 1980. Este programa de la UNESCO tiene como objetivo conciliar la conservación de la naturaleza con el desarrollo económico.

Desde un punto de vista funcional, estas lagunas de La Mancha Húmeda resultan fundamentales para el mantenimiento de diversos procesos ecológicos dentro de la región. Constituyen además ecosistemas únicos en el contexto europeo, lo que no obvia que se encuentren sujetos a diferentes niveles de degradación y presión antrópica. Las principales amenazas a las que están sometidos son la pérdida y/o fragmentación del hábitat, la alteración de su ciclo hidrológico natural o la contaminación por exceso de nutrientes. Otros aspectos como el sobrepastoreo intensivo y drenaje para promover el cultivo han transformado su funcionalidad. Otra amenaza a la que se enfrentan estos ecosistemas es su cercanía a los centros urbanos, un claro ejemplo de esto es el complejo lagunar de Alcázar de San Juan, cuyo estudio también se contempla en esta tesis. Muchas de estas perturbaciones que se señalan están asociadas a un aporte excesivo de nutrientes, así como a un proceso de desalinización en algunos casos debido a la dilución que generan los aportes de aguas residuales. Esta situación hace necesaria la adopción de planes de manejo y conservación que mejoren el estado ecológico de estas zonas húmedas, lo que exige incrementar el conocimiento acerca de las particularidades que muestran con respecto al ciclo biogeoquímico de nutrientes.

Existen diversos estudios científicos que abordan aspectos generales sobre la geoquímica de estos lagos, así como de las comunidades de flora y fauna que albergan. No obstante, el conocimiento acerca de aspectos más concretos de la biogeoquímica y ciclo de nutrientes, como puedan ser el secuestro y movilización del fósforo o la transformación de la materia orgánica disuelta, es todavía muy escaso. Esto contrasta con el interés que estos aspectos sí tienen en sistemas de características similares en otras regiones geográficas. En cualquier caso, la información científica existente sobre estas lagunas representa un punto de partida para definir nuevos objetivos y lugares de estudio, alguno de los cuales han inspirado lo trabajos de esta tesis.

El principal objetivo de esta tesis es revelar los patrones de flujos de nutrientes (carbono, nitrógeno y fósforo) y funcionamiento metabólico de lagos salinos representativos de la Reserva de la Biosfera La Mancha Húmeda, describiendo los principales procesos ambientales involucrados en el ciclo biogeoquímico y la transformación de los nutrientes. Con ello se pretende sentar las bases científicas para una adecuada gestión de estos ecosistemas acuáticos, únicos en el contexto europeo. En base a estos, los objetivos específicos de la tesis son los siguientes:

1. Caracterizar los patrones hidrológicos, fisicoquímicos y funcionales de los lagos salinos de La Mancha Húmeda, relacionándolos con su estado de conservación, así como con parámetros de diagnóstico asociados a su estado trófico y al metabolismo del carbono de las comunidades planctónicas. Este objetivo específico se aborda en el Capítulo III.

2. Estudiar la relación existente entre la biodisponibilidad del fósforo y la productividad de los lagos a partir del fraccionamiento químico del fósforo en los sedimentos, evaluando con ello los procesos de secuestro y movilización de este nutriente. Este objetivo específico se aborda en el Capítulo IV.

3. Llevar a cabo una caracterización de la materia orgánica disuelta (MOD) presente en estas lagunas en base a sus características ópticas, lo cual permitirá obtener información sobre su origen, capacidad para ser degradada y su influencia en la producción secundaria asociada al bacterioplancton. Este objetivo específico se aborda en el Capítulo V y VI.

Para alcanzar dichos objetivos en esta tesis se seleccionaron 17 lagos representativos de la Reserva de la Biosfera La Mancha Húmeda, los cuales representan la gama de condiciones limnológicas que actualmente tienen lugar en la reserva. El clima de la zona es mediterráneo de interior, con precipitaciones medias cercanas a los 400 mm anuales, los valores máximos se registran en otoño y los mínimos en invierno, siendo el verano la estación más seca. Las temperaturas oscilan de forma notable entre -15 y 43 ºC, dependiendo de la estación, siendo de media 15 ºC. La altitud media del área de estudio es de unos 600 metros sobre el nivel del mar. Las lagunas estudiadas presentan un marcado carácter endorreico, encontrándose enclavadas en un paisaje dominado por cultivos agrícolas.

De forma general, en cada una de las lagunas se obtuvieron muestras de agua y sedimento superficial con una cadencia mensual (durante dos hidroperiodos consecutivos), siguiendo procedimientos estandarizados de muestreo y procesado de muestras. Para abordar parte del objetivo específico número 3, en dos de las lagunas (Alcahozo y Manjavacas) se tomaron además muestras de agua con una cadencia aproximadamente semanal, con la finalidad de tener una mayor resolución que permitiera observar cambios rápidos en la disponibilidad de algunos nutrientes y en la respuesta de las comunidades del plancton a dichos cambios.

Para la caracterización de los patrones hidrológicos, fisicoquímicos y funcionales de los lagos (objetivo específico 1) se midieron las concentraciones tanto disueltas como totales del fósforo, nitrógeno y carbono orgánico presentes en el agua y sedimento superficial. Las diferencias observadas se evaluaron en base a factores ambientales concretos, esto es: 1) principal fuente de aporte de nutrientes (i.e., escorrentía desde la cuenca, entradas localizadas de aguas residuales y presencia de sustrato volcánico), 2) nivel de salinidad de la laguna (subsalina, hiposalina, mesosalina o hipersalina), periodo estacional (otoño, cuando se llenan los lagos; invierno, el período de máxima inundación; primavera, el período más productivo y asociado al inicio del proceso de secado de los lagos; verano, el período en que los lagos se secan de forma natural, a excepción de los alterados hidrológicamente por entradas de aguas residuales).

Para evaluar la relación existente entre la biodisponibilidad del fósforo y la productividad de los lagos (objetivo específico 2) se realizó un fraccionamiento químico del fósforo presente en los sedimentos (en este caso sólo se usaron muestras de 11 lagos). El fraccionamiento secuencial propuesto en la tesis permitió obtener cinco formas químicas de fósforo distintas: (1) fósforo fácilmente disponible y asimilable por la biota (*Lossely-P*), (2) fósforo ligado a los oxihidroxidos principalmente de hierro (Fe) y aluminio (Al) (*Fe/Al-P*), (3) los polifosfatos (*Poly-P*), (4) el fósforo unido a minerales de apatita (*Ca/Mg-P*) y, finalmente, (5) la fracción residual o recalcitrante (*Resid-P*). Con este procedimiento fue posible hacer una evaluación de los procesos de secuestro y movilización de este nutriente que potencialmente pueden tener estas lagunas.

La caracterización óptica de la materia orgánica disuelta (MOD) (objetivo específico 3) se llevó a cabo a partir de los análisis de absorbancia y fluorescencia ultravioleta-visible de muestras de agua filtrada. Los espectros de absorción se obtuvieron entre las longitudes de onda de 240 y 750 nm, con los que se determinaron las pendientes espectrales para los intervalos de 275 a 295 nm ($S_{275\text{-}295}$) y de 350 a 400 nm ($S_{350\text{-}400}$). Estas pendientes representan una medida de cómo disminuye la absorción con respecto a la longitud de onda, dando así una idea del peso molecular de la MOD presente en la muestra, por ejemplo, la pendiente $S_{275\text{-}295}$ representaría la MOD de bajo peso molecular y $S_{350\text{-}400}$ representaría la MOD de alto peso molecular. Por otro lado, los parámetros de fluorescencia se adquirieron de la matriz de emisión de excitación, en la cual se identificaron tres componentes fluorescentes separados: *sustancias de tipo húmico* con señal de fluorescencia máxima en la longitud de onda Ex/Em de 250-260 nm/380-480 nm se definió como pico *FDOM-A*, a 330–350 nm/420–480 nm como pico *FDOM-C*, y a 310–320 nm/380–420 nm como pico *FDOM-M*.

El estudio limnológico, que abarcó dos ciclos hidrológicos, mostró un rango de salinidades entre las 17 lagunas que varió desde agua dulce hasta la hípersalinidad (rango promedio: 1,75-136,73 mS cm^{-1}) y presentaban un estado trófico de oligo-mesotrófico (2,45±2,76 µg Chl-*a* L^{-1}) a hipereutrófico (185,14 ± 201,15 µg Chl-*a* L^{-1}). Los aportes por escorrentías y aguas residuales, así como los procesos de evapo-concentración de solutos, provocan una acumulación variable de nutrientes en estas lagunas, lo cual se traslada también en una distinta carga interna. El análisis multivariado de la varianza (MANOVA) realizado con datos limnológicos mostró la principal fuente de procedencia de los nutrientes (que varió de natural a antrópica) como el factor con mayor influencia sobre la disponibilidad y relación estequiométrica de los nutrientes, lo que a su vez tuvo un impacto en el metabolismo de las comunidades del plancton. Los lagos que reciben aportes de aguas residuales, así como los asentados sobre litología volcánica, muestran un notable enriquecimiento de fósforo tanto en el agua como en los sedimentos superficiales. El análisis de componentes principales (PCA), de acuerdo con lo anterior, reveló la existencia de dos escenarios tróficos diferenciados, que se refleja en un funcionamiento ecológico del compartimento planctónico diferente. Este aspecto funcional se evaluó midiendo la fotosíntesis y las tasas de respiración aeróbica del plancton, basándose en la variación de la concentración de oxígeno en botellas que contenían agua de la laguna. Estas incubaciones se llevaron a cabo en presencia y ausencia de luz, para poder distinguir los procesos autótrofos y heterótrofos. De este modo, se observaron tasas de fotosíntesis y respiración que variaron de acuerdo con el estado trófico de los lagos, con promedios en el rango de 8,12-158,41 y 3,58-93,87 µmoles O_2 L^{-1} h^{-1} de oxígeno producido o consumido respectivamente. Estas tasas mostraron un notable dinamismo y sensibilidad a la variación de las condiciones ambientales.

Uno de los escenarios tróficos apreciado en la PCA se corresponde con un conjunto de lagunas muy afectadas por contaminación a través de vertidos, tanto contemporáneos como pasados (i.e., carga interna). Ejemplos de ello son las lagunas de Longar, Larga de Villacañas y Camino de Villafranca, así como otras que presentan un estado meso-eutrófico como la Laguna de Manjavacas. Todos estos casos muestran un claro enriquecimiento de fósforo, que explica el aumento de las tasas fotosintéticas en el compartimento planctónico. En estos casos, se observa un elevado acoplamiento entre las tasas de respiración y las fotosintéticas. Esto denota un papel preponderante de la producción autóctona en el sostenimiento de la producción secundaria. No obstante, en períodos en los que los aportes difusos provenientes de la cuenca aumentan, durante la recarga estacional, proveen de material orgánico autóctono al sistema, se observa un relativo desacoplamiento entre estas actividades metabólicas.

Lo anterior contrasta con el otro escenario trófico distinguible en la PCA, el englobado por aquellas lagunas con un buen estado de conservación, las cuales mantienen una salinidad y hidroperíodo naturales (p.ej., Alcahozo, Tirez y Salicor). Las tasas metabólicas en el plancton de estas lagunas son entre bajas y moderadas. El cociente entre las tasas fotosintéticas y de respiración en este caso es además significativamente menor que el observado en los lagos con peor estado trófico, prueba de que los aportes alóctonos tendrían mayor relevancia en este caso.

En los lagos estudiados en esta tesis se produce de forma natural una acumulación gradual de nutrientes debido a su marcado carácter endorreico, lo que se traduce en la presencia de elevadas concentraciones de carbono orgánico disuelto (COD) recalcitrante. A lo largo del hidroperíodo, en los lagos oligotróficos este carbono orgánico aumentó proporcionalmente más que los otros nutrientes, incrementado con ello el desequilibrio estequiométrico. Por otra parte, la ratio producción-respiración (P:R) obtenida a partir de las mediciones del intercambio de oxígeno en los todos los lagos en general mostró un metabolismo planctónico autótrofo (P:R>1), con valores en un rango de 1,35 a 5,64. No obstante, considerando que la fotosíntesis cesa durante la noche, pero no así la respiración en la misma medida, cabe pensar que se pueda producir un balance heterótrofo neto en alguno de los casos.

Otro aspecto abordado en esta tesis ha sido el de la medición de las cargas internas de nutrientes en los sedimentos, lo que pone de manifiesto el papel histórico que han jugado en algunos casos los aportes de aguas residuales en la cronificación del deterioro ecológico de estos lagos. Para profundizar en las consecuencias de esta carga interna, se seleccionaron 11 de los lagos para evaluar el secuestro, fraccionamiento químico y movilización de fósforo en sus sedimentos. Los resultados muestran como los aportes de aguas residuales favorecieron la acumulación de todas las fracciones sedimentarias de fósforo detectadas, sobre las que dominó la fracción asociada a la precipitación con cationes divalentes como el calcio (Ca) o magnesio el (Mg). Otra fracción igualmente abundante fue la ligada orgánica. La elevada presencia de estas fracciones indica una cierta inmovilización del fósforo, pero que no obstante puede ser liberado por cambios en la físico-química del agua. Otra fracción menos abundante que las anteriores, pero también importante en el sedimento, es la de los polifosfatos. Esta fracción la constituyen polímeros lineales del ortofosfato, la cual representa un modo estable de almacenar un exceso de fosfato. Por otro lado, los resultados de fraccionamiento muestran cómo, a diferencia del resto de lagos estudiados, la retención de fósforo estaría muy limitada en los lagos volcánicos de la región. Esta circunstancia hace a estos últimos ser más propensos a la eutrofización en caso de recibir aportes externos de nutrientes, lo que guarda relación con las particulares características químicas de sus aguas, que limitan notablemente la retención del fósforo en el sedimento.

La dinámica de este fósforo sedimentario también se estudió a lo largo del ciclo hidrológico. En base a ello, se observa un aumento de la disponibilidad de fósforo asimilable por la biota, asociado a los ciclos de inundación de la cubeta, lo que a menudo contribuye a amentar la productividad de los lagos. No obstante, en base a la composición química del fosforo sedimentario, una liberación más importante de fósforo podría estar asociada al mantenimiento de la inundación durante el estiaje, lo que puede alterar las condiciones redox y promover la disolución reductora de este fósforo. En base a esto, es importante considerar que este aporte desde el sedimento al agua no disminuiría en aquellos lagos altamente contaminados, si mantuviesen un régimen excesivo de inundación, aún a pesar de cesar las nuevas entradas de aguas residuales, lo que contrarrestaría cualquier medida de restauración aplicada.

Otro factor que, junto con el del fraccionamiento químico del fósforo, juega un papel importante en el funcionamiento de estos lagos es el de la materia orgánica disuelta (MOD). En base a ello, en la presente tesis se ha realizado un estudio óptico de esta MOD mediante la aplicación de técnicas de espectrofotometría ultravioleta-visible (UV/VIS) y de fluorescencia, con la finalidad de identificar el origen, naturaleza y nivel de degradabilidad de esta MOD. Se ha llevado a cabo un estudio de la variación de diferentes parámetros ópticos a lo largo del ciclo hidrológico, obtenidos a partir de los espectros de absorbancia y de las matrices de emisión/extinción de fluorescencia. En los resultados obtenidos se observa una correlación entre la pendiente del espectro de absorbancia en el rango de longitud de onda de 275–295 nm ($S_{275–295}$) y las concentraciones de carbono COD, lo que indica que este último estaría principalmente compuesto por MOD más hidrolizada y expuesta a la fotodegradación. Los lagos muestran a su vez un patrón en base al cual se produce una disminución de la aromaticidad de la MOD conforme aumenta el tiempo de retención del agua y la evapo-concentración de solutos. Este proceso se intensifica además conforme avanza el ciclo hidrológico, lo que supone una mayor cantidad de MOD de tipo húmico alóctono durante los eventos de inundación de otoño-invierno, cuando la escorrentía en la cuenca aumenta. Los análisis de regresión llevados a cabo indican como la MOD alóctona proveniente de la cuenca no se acumula en los lagos, lo que sugiere que este presenta una cierta reactividad y potencialidad a ser degradado.

El análisis óptico muestra también como en los lagos no contaminados y que mantienen los niveles de salinidad natural, presentan una mayor cantidad de esta MOD fotoblanqueada y de bajo peso molecular, capaz todavía de sostener todavía cierta producción secundaria, a la vista de las tasas de respiración entre bajas y moderadas que muestran estos lagos. Este carbono, parcialmente lábil, podría provenir de la fotooxidación de la materia orgánica presente en la cuenca durante el periodo de estiaje, que entraría posteriormente durante la inundación. Estos aportes alóctonos de materia orgánica también se dan en los lagos eutróficos ya que presentan cuencas con características similares. No obstante, en estos últimos lagos, es principalmente la MOD autóctona, derivado de la actividad de las algas, quien sustentaría la producción secundaria del plancton.

De forma adicional a lo descrito anteriormente, en los lagos Alcahozo y Manjavacas se llevó a cabo un seguimiento también de las características ópticas de la MOD, junto con las poblaciones del fitoplancton (como concentración de Chl-a) y el bacterioplancton, pero con una mayor frecuencia de muestreo. Se trata de dos lagos próximos entre sí que pertenecen al mismo complejo lacustre, por lo que experimentan condiciones climáticas similares. Esta mayor cadencia en el muestreo (aproximadamente semanal) ofreció una visión más aproximada de la celeridad con la que las dinámicas en los lagos responden a los procesos que se dan en la cuenca, caracterizándose estos por pulsos en la disponibilidad de nutrientes y, más particularmente, de material orgánico lábil que sustenta la productividad, ya sea proveniente de MOD parcialmente fotodegradada o, por otra parte, MOD autóctona recién formada.

Un análisis de citometría de flujo mostró que las poblaciones de bacterioplancton respondían a ambas situaciones, aunque de manera diferente, lo que reveló una respuesta compleja a distintos escenarios en lo que a tipos de MOD se refiere. El número de bacterias fue significativamente mayor en el lago eutrófico de Manjavacas en comparación con el lago Alcahozo, regularmente oligotrófico. Sin embargo, las poblaciones bacterianas en Alcahozo se mostraron metabólicamente más activas atendiendo a la mayor proporción de células bacterianas con alto contenido de ADN, tendencia basada en la intensidad de fluorescencia detectada por el análisis de citometría de flujo. Un estudio exhaustivo de la composición taxonómica y funcional de estas comunidades bacterianas podría ayudar a determinar si muestran una adaptación fisiológica a estos entornos de MOD tan contrastantes.

En resumen, esta tesis ofrece una descripción amplia de las presiones a las que son sometidos los lagos salinos de Mancha Húmeda y como esto se traduce en algunos aspectos principales de su funcionamiento ecológico. Estas alteraciones, de forma más habitual, implican modificaciones en la duración del período de inundación, desalinización y eutrofización por aportes de aguas residuales. El estado trófico, el metabolismo del carbono, las características de la MOD y la relación estequiométrica de los nutrientes estudiados, en los lagos mejor conservados, pueden considerarse como valores de referencia, o próximos a ellos, para evaluar las desviaciones causadas por los impactos antrópicos más importantes que se dan en la región. Estos valores de referencia deberían contemplar el particular enriquecimiento en fósforo observado para las lagunas volcánicas de la zona del Campo de Calatrava. Las altas concentraciones naturales de fósforo soluble y la turbidez del agua son características distintivas de estos lagos volcánicos, lo que predetermina en gran medida su funcionamiento ecológico.

Las posibles estrategias de gestión conducentes a mejorar el estado ecológico de estos lagos podrían evaluarse en función de los resultados de la tesis. El estudio muestra la contaminación puntual como la principal fuente de aporte de nutrientes y, por tanto, la principal amenaza a la que hay que hacer frente. Su impacto es mayor que, por ejemplo, que el derivado de los usos del suelo de la cuenca, como se demuestra en el capítulo III. En cualquier caso, las características particulares de estos lagos, y que se abordan en esta tesis, como son la eutrofización natural por procesos evaporativos, su marcada naturaleza endorreica, la presencia de litologías particulares (ej., volcánicas), el largo tiempo de residencia de los nutrientes y el mayor procesamiento fotoquímico de los mismo, son todos aspectos que deberían tenerse en cuenta en el diseño e implementación de estas estrategias de gestión. Esta gestión también debería alinearse con aquellas propuestas que aconsejan a los gestores a (1) apoyar proyectos que se sustenten en la comprensión de la dinámica, recursos y servicios ecosistémicos que proporcionan estas lagunas, (2) promover prácticas orientadas a facilitar el feedback ecológico y (3) así como desarrollar procesos de gestión adaptativa. La puesta en práctica de estas estrategias en estas lagunas manchegas podría resultar así exitosa, siempre y cuando se tengan en consideración las particularidades biogeoquímicas expuestas en esta tesis.

A continuación, se enumeran las principales conclusiones derivadas de la presente tesis:

1. Los lagos estudiados representan el rango de condiciones limnológicas observable en la Reserva de la Biosfera La Mancha Húmeda. Con baja o nula perturbación, estos lagos muestran un hidroperíodo temporal, regulado por el balance de precipitaciones y evaporación, y un contenido de nutrientes entre bajo y moderado. No obstante, muchos de ellos reflejan alteraciones relacionadas con la duración del hidroperíodo, la desalinización y aportes de nutrientes mediante aguas residuales.
2. En base a esto, los sitios estudiados (n=17) oscilan entre lagos de agua dulce a hipersalinos, con una amplia variación también en el estado trófico, que va desde oligomesotrófico a hipereutrófico. Estas son condiciones que en líneas generales se mantienen durante todo el hidroperíodo en cada uno de los lagos, sin embargo, la evapo-concentración estacional de solutos es un factor que también modula los niveles de nutrientes y concentración de sales en general.
3. En relación con la exploración de los factores externos que afectan al estado trófico de estos lagos, se realizó un análisis de varianza mediante la clasificación de estos en función de su principal fuente de entrada de nutrientes (i.e., aportes difusos, entrada puntual de aguas residuales, o presencia de litología volcánica), en base al cual se observaron diferencias significativas entre ellos con respecto a la disponibilidad de estos nutrientes, sus relaciones estequiométricas, carga interna de los sedimentos, así como la actividad metabólica del plancton.

4. Los lagos afectados por aportes de aguas residuales, tanto actuales como en el pasado, así como aquellos asentados sobre litología volcánica, mostraron un enriquecimiento de fósforo, tanto en el agua como en los sedimentos superficiales. Esto explicaría sus altas tasas fotosintéticas, aunque esto no fue tan evidente en el caso del lago volcánico estudiado, lo que responde a la elevada turbidez que de manera natural este presenta, y que limita notablemente la disponibilidad de la luz.
5. Por el contrario, los lagos sometidos a menores impactos, cuya principal fuente de nutrientes serían los aportes difusos de la cuenca, se caracterizan por una acumulación de carbono orgánico disuelto (COD) previsiblemente fotodegradado y de naturaleza recalcitrante. Estos lagos también acumulan nutrientes por su marcado carácter endorreico, aunque más desequilibradamente. Presentan tasas metabólicas del plancton entre bajas y moderadas, con una ratio producción/respiración inferior al de los lagos eutróficos, lo que sugiere que la producción secundaria estaría en parte sostenida por aportes alóctonos.
6. El estudio del fraccionamiento del fósforo muestra las fracciones orgánicas y las ligadas a cationes divalentes (Ca^{2+} y Mg^{2+}) como los reservorios de fósforo más importantes en los sedimentos, los cuales son formas químicas relativamente estables. Sin embargo, la inundación artificial de las cubetas y su desalinización con agua dulce, pueden favorecer la movilización de fósforo a formas más lábiles capaces de fertilizar la columna de agua. El estudio también pone de manifiesto la elevada susceptibilidad de los lagos volcánicos de la región a la eutrofización debido a su limitada capacidad de retener fósforo en sus sedimentos.
7. El análisis óptico realizado sobre la materia orgánica disuelta (MOD) mediante espectroscopia de absorbancia y fluorescencia ha permitido caracterizarla en función de su origen, composición general y reactividad como fuente de carbono, lo que aporta conocimiento respecto a su papel en el funcionamiento de estos lagos, y complementa además el estudio metabólico llevado a cabo en el plancton.

8. Este estudio óptico muestra una general acumulación de MOD fotodegrada, como se sugiere anteriormente. Pero una fracción todavía lábil podría originarse por procesos de transformación biótica y fotolítica, que se darían anualmente en la cuenca y cubetas desecadas y que, tras su reinundación, podrían sustentar la actividad respiratoria.
9. La eventual proliferación de fitoplancton, que es más pronunciada y frecuente en los lagos contaminados, promueve por el contrario la producción de MOD autóctona derivada de su actividad, incluyendo sustancias proteicas altamente lábiles. La presencia de esta MOD fácilmente degradable explicaría el elevado acoplamiento observado entre las tasas de respiración y las fotosintéticas medidas en estos lagos con más carga de nutrientes.
10. Con relación a lo anterior, los procesos que a corto plazo implican una rápida movilización de nutrientes del sedimento, principalmente fósforo, o la disponibilidad de MOD lábil, ya sea alóctona o autóctona, subyacen a las dinámicas fluctuantes observada para las poblaciones del fitoplancton y bacterioplancton.
11. El estudio de citometría de flujo sugiere la existencia de una respuesta del bacterioplancton dependiente del tipo de MOD presente, que hace pensar en una fisiología adaptada a las condiciones tróficas del lago. Esto hace necesario abordar un estudio más profundo sobre aspectos funcionales de estas comunidades bacterianas, del que podría derivarse la existencia de adaptaciones metabólicas particulares, sobre todo considerando las condiciones extremas a las que están sometidos estos ambientes.
12. Las observaciones derivadas de esta tesis, tales como la susceptibilidad de los lagos en aspectos funcionales a la contaminación, la cantidad y propiedades químicas de la carga interna de nutrientes, la naturaleza dinámica y fluctuante de procesos que ocurren en el vaso lagunar y la cuenca, o el papel fundamental de la transformación de la MOD, deben considerarse como relevantes en las estrategias de gestión, inicialmente para el caso de la Reserva de la Biosfera de la Mancha Húmeda, pero también, en la medida de lo posible, para otras zonas en las que este tipo de lagos salinos someros también estén presentes.

References

Abell, J., Özkundakci, D., Hamilton, D., Reeves, P. (2022). Restoring shallow lakes impaired by eutrophication: Approaches, outcomes, and challenges. Crit Rev Environ Sci Technol. 52(7), 1199-1246.

Alfonso, M., Brendel, A., Vitale, A., Seitz, C., Piccolo, M., Perillo, G. (2018). Drivers of ecosystem metabolism in two managed shallow lakes with different salinity and trophic conditions: The Sauce Grande and La Salada Lakes (Argentina). Water. 10(9), 1136.

Alonso, M., 1998. Las lagunas de la España peninsular. Limnetica. 15, 1–176.

Andersen, I., Williamson, T., González, M., Vanni, M. (2020). Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir. Limnol Oceanogr. 65(5), 962-978.

Anderson, N. and Stedmon, C.A., 2007. The effect of evapoconcentration on dissolved organic carbon concentration and quality in lakes of SW Greenland. Freshw Biol. 52, 280–289.

Anuradha, V., Nair, S., Kumar.N. (2011). Humic acids from the sediments of three ecologically different estuarine systems - a comparison. Int J Environ Sci. 2,174-184.

Anza, I., Vidal, D., Laguna, C., Díaz-Sánchez, S., Sánchez, S., Chicote, Á., Florín,M., Mateo, R., (2014). Eutrophication and bacterial pathogens as risk factors for avian botulism outbreaks in wetlands receiving effluents from urban wastewater treatment plants. Appl. Environ Microb. 80, 4251–4259.

APHA-AWWA-WPCF. (2005). Standard methods for the examination of water and waste water, twenty first ed. APHA – American Public Health association, AWWA – American Water Works Association & WPCF – Water Pollution Control Federation, Washington DC.

Badr, E. (2016). Spatio-temporal variability of dissolved organic nitrogen (DON), carbon (DOC), and nutrients in the Nile River, Egypt. Environ monit assess. 188(10), 1-15.

Bailey, P., Boon, P., Blinn, D., Williams, W. (2006). Salinisation as an ecological perturbation to rivers, streams and wetlands of arid and semi-arid regions. In: Kingsford, D.R.T. (Ed.), Ecology of Desert Rivers, 1 ed. Cambridge University Press, Cambridge UK, pp. 280–314.

Ballesteros, B., Camuñas, C., Mejías, M., Camacho, A., Sánchez, D., Albacete, L., Rochera, C. (2018). Lagunas mesetarias de La Mancha: funcionamiento hidrológico, cultura y medio ambiente. Madrid, Spain: Instituto geológico y minero de España.

Bao, Y., Huang, T., Ning, C., Sun, T., Tao, P., Wang, J., Sun, Q. (2023). Changes of DOM and its correlation with internal nutrient release during cyanobacterial growth and decline in Lake Chaohu, China. J Environ Sci. 124, 769-781.

Batanero, G.L., León-Palmero, E., Li, L., Green A.J., Rendón-Martos M., Suttle, C.A., Reche, I. (2017) Flamingos and drought as drivers of nutrients and microbial dynamics in a saline lake. Sci Rep 7, 12173.

Battle, J. and Mihuc, T. (2000). Decomposition dynamics of aquatic macrophytes in the lower Atchafalaya, a large floodplain river. Hydrobiologia. 418, 123–136.

Bayley, S., Wong, A., Thompson, J. (2013). Effects of agricultural encroachment and drought on wetlands and shallow lakes in the boreal transition zone of Canada. Wetlands. 33, 17-28.

Berggren, M., Klaus, M., Selvam, B., Ström, L., Laudon, H., Jansson, M., Karlsson, J. (2018). Quality transformation of dissolved organic carbon during water transit through lakes: contrasting controls by photochemical and biological processes. Biogeosciences, 15(2), 457-470.

Berggren, M., Sponseller, R., Alves-Soares, A., Bergström, K. (2015). Toward an ecologically meaningful view of resource stoichiometry in DOM-dominated aquatic systems. J Plankton Res. 37(3), 489-499.

Berkes, F. (2017). Environmental governance for the anthropocene? Social-ecological systems, resilience, and collaborative learning. Sustainability, 9(7), 1232.

Bertilsson, S. and Tranvik, L. (2000). Photochemical transformation of dissolved organic matter in lakes. Limnol Oceanogr. 4, 458–463.

Bodaker, I., Beja, O., Sharon, I., Feingersch, R., Rosenberg, M., Oren, A., Hindiyeh, M., Malkawi, H. (2009). Archaeal diversity in the Dead Sea: Microbial survival under increasingly harsh conditions. NREI. 15, 137-143.

Boers P., Bongers J., Wisselo A., Cappenberg T. (1984). Loosdrecht Lakes Restoration Project: Sediment phosphorus distribution and release from the sediments. Verh Int Verein Limnol. 22, 842-847.

Boros, E., Jurecska, L., Tatár, E., Vörös, L., Kolpakova, M. (2017). Chemical composition and trophic state of shallow saline steppe lakes in central Asia (North Kazakhstan). Environ monit assess. 189(11), 1-12.

Boström, B., Janson, M., Forsberg, C. (1982). Phosphorus release from lake sediments. Arch. Hydrobiol Beih Ergeb Limnol. 18, 5–59.

Boyd, E. and Folke, C. (Eds.). (2011). Adapting institutions: governance, complexity and social-ecological resilience. Cambridge University Press.

Bronk, D. and Glibert, P. (1991). A 15 N tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. Mar Ecol Prog Ser. 77, 171-182.

Bronk, D., Glibert, P., Malone, T., Banahan, S., Sahlsten, E. (1998). Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. Aquatic Microbial Ecology, 15: 177–189.

Butturini, A., Herzsprung, P., Lechtenfeld, O., Alcorno, P., Benaiges-Fernandez, R., Berlanga, M., Boadella, J., Freixinos-Campillo, Z., Gomez, R., Sanchez-Montoya, M., Urmeneta, J., Romaníg, A. (2022). Oigin, accumulation and fate of dissolved organic matter in an extreme hypersaline shallow lake. Water Res. 118727.

Cabestrero, Ó., Sanz-Montero, M., Arregui, L., Serrano, S., Visscher, P. (2018). Seasonal variability of mineral formation in microbial mats subjected to drying and wetting cycles in alkaline and hypersaline sedimentary environments. Aquat geochem. 24(1), 79-105.

Cáceres, C. and Soluk, D. (2002). Blowing in the wind: a field test of overland dispersal and colonization by aquatic invertebrates. Oecologia. 131, 402–408.

Camacho A., Picazo, A., Rochera, C., Santamans, A., Morant, D., Miralles-Lorenzo J., Castillo-Escrivà A. (2017) Methane emissions in Spanish saline lakes: Current rates, temperature and salinity responses, and evolution under different climate change scenarios. Water. 9, 659; doi:10.3390/w9090659.

Camacho, A. and de Wit, R., 2003. Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat Microb Ecol. 32(3), 261-273.

Camacho, A., Miracle, M., Vicente, E. (2003). Which factors determine the abundance and distribution of picocyanobacteria in inland waters? A comparison among different types of lakes and ponds. Arch. Hydrobiol. 157, 321–338.

Camacho, A., Murueta, N., Blasco, E., Santamans, A., Picazo A. (2016). Hydrology-driven macrophyte dynamics determines the ecological functioning of a model Mediterranean temporary lake. Hydrobiologia. 774, 93–107.

Camacho, A., Peinado, R., Santamans, A., Picazo, A. (2012). Functional ecological patterns and the effect of anthropogenic disturbances on a recently restored Mediterranean coastal lagoon. Needs for a sustainable restoration. Est Coast Shelf Sci. 114, 105-117.

Camacho, A., Wurtsbaugh, W., Miracle, M., Armengol X., Vicente, E. (2003). Nitrogen limitation of phytoplankton in a Spanish karst lake with a deep chlorophyll maximum: a nutrient enrichment bioassay approach. J Plankton Res. 25, 397-404.

Carey, R., Hochmuth, G., Martinez, C., Boyer, T., Dukes, M., Toor, G., Cisar, J. (2013). Evaluating nutrient impacts in urban watersheds: challenges and research opportunities. Environ Pollut. 173, 138-149.

Carlson, C. and Hansell, D. (2015). DOM sources, sinks, reactivity and budgets. In Biogeochemistry of Marine Dissolved Organic Matter. New York: Academic. 2nd ed. pp. 65–126.

Castillo-Escrivà A., Valls, L., Rochera, C., Camacho, A., Mesquita-Joanes, F. (2015). Spatial and environmental analysis of an ostracod metacommunity from endorheic lakes. Aquat Sci. 78(4), 707-716.

Catalán, N., Obrador, B., Felip, M., Pretus, J. (2013). Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake. Aquat Sci. 75(4), 581-593.

Cebriá, J. and López-Ruíz, J. (1995). Alkali basalts and leucitites in an extensional intracontinental plate setting: The late Cenozoic Calatrava Volcanic Province (Central Spain). Lithos. 35, 27-46.

Champion, M. and Currie, D. (2000). Phosphorus-chlorophyll relationships in lakes, rivers, and estuaries. Verh Int Ver Theor Angew Limnol. 27, 1986–1989.

Chari N., P. Rao, P., Sarma, N. (2013). Fluorescent dissolved organic matter in the continental shelf waters of western Bay of Bengal. J Earth Syst Sci. 122, 1325–1334.

Chen, Q., Hu, W., Shen, L., Shen, W., Zhang, X. (2022). The role of nutrients, wind speed, and rainfall in determining the composition of the algal community of shallow lakes in the

Taoge water system, upstream from Lake Taihu, China. Environ Sci Pollut Res. 1-15.

Cirujano, S., Álvarez-Cobelas, M., Ruíz de la Hermosa, C. (2010). Analysis of applied environmental management strategies for wetland conservation during the last 30 years: a local history. In Sánchez Carrillo, S. & Angeler, D. G. (Eds.). Ecology of Threatened Semi-Arid Wetlands. Springer: 229-237.

Cobbaert, D., Wong, A., Bayley, S. (2014). Precipitation-induced alternative regime switches in shallow lakes of the Boreal Plains (Alberta, Canada). Ecosystems, 17(3), 535-549.

Cobbaert, D., Wong, A., Bayley, S. (2015). Resistance to drought affects persistence of alternative regimes in shallow lakes of the Boreal Plains (Alberta, Canada). Freshw biol. 60(10), 2084-2099.

Coble, P. (1996). Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. Mar Chem. 51: 325–346.

Coble, P., del Castillo, C., Avril, B. (1998). Distribution and optical properties of CDOM in the Arabian Sea during the 1995 southwest monsoon; Deep-Sea Res Part II. 45, 2195–2223.

Coble, P., Lead, J., Baker, A., Reynolds, D., Spencer, R. (Eds.). (2014). Aquatic Organic Matter Fluorescence (Cambridge Environmental Chemistry Series). Cambridge: Cambridge University Press. 375 pages.

Conley, D., Paerl, H., Howarth, R., Boesch, D., Seitzinger, S., Havens, K., Lancelot, C., Likens, G. (2009). Controlling eutrophication: nitrogen and phosphorus. Science. 323, 1014-1015.

Corrales-González, M., Rochera, C., Picazo, A., Camacho, A. (2019). Effect of wastewater management on phosphorus content and sedimentary fractionation in Mediterranean saline lakes. Sci Total Environ. 668, 350-361.

Cory, R., Crump, B., Dobkowski, J., Kling, G. (2013). Surface exposure to sunlight stimulates CO2 release from permafrost soil carbon in the Arctic. PNAS. 110(9), 3429-3434.

Cottenie, K., de Meester, L. (2004). Metacommunity structure: synergy of biotic interactions as selective agents and dispersal as fuel. Ecology. 85, 114-119.

Creed I., Bergström, A., Trick, C., Grimm, N., Hessen, D., Karlsson, J., Kidd, K., Kritzberg, E., McKnight, D., Freeman, E., Senar, O., Andersson, A., Ask, J., Berggren, M., Cherif, M., Giesler, R., Hotchkiss, E., Kortelainen, P., Palta, M., Vrede, T., Weyhenmeyer, G. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Glob Change Biol. 24(8), 3692-3714.

Crossman, J., Whitehead, M., Futter, L., Jin, M., Shahgedanova, M., Castellazzi, M., Wade, A. (2013). The interactive responses of water quality and hydrology to changes in multiple stressors, and implications for the long-term effective management of phosphorus. Sci Total Environ. 454, 230–244.

Dalsgaard, T., Canfield, D., Petersen, J., Thamdrup, B., Acuña-González, J. (2003). N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature. 422(6932), 606-608.

Dalsgaard, T., Thamdrup, B., Farías, L., Revsbech, N. (2012). Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific. Limnol Oceanogr. 57(5), 1331-1346.

Dantin, J. (1932). La población de la Mancha española en el centro de su máximo endorreísmo. Bol R Soc Geogr. 72, 25-45.

Dantin, J. (1940). La aridez y el endorreísmo en España. El endorreísmo bético. Estud Geogr. 23, 269- 312.

Davelaar, D. (1993). Ecological significance of bacterial polyphosphate metabolism in sediments. Hydrobiologia. 253, 179–192.

De La Horra, J., Serrano, F., Carlevalis, J. (2008). Estudio de suelos del Campo de Calatrava (Ciudad Real) y sus condiciones de fertilidad, vol. 32. C.S.I.C., Madrid. 415 pp. (ISBN: 978-84-00-08625-1).

De Lange H., Morris, D., Williamson, C. (2003). Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. J Plankton Res. 25, 111-117

de Vicente, I., Moreno-Ostos, E., Amores, V. Rueda, F., Cruz-Pizarro, L (2006) Low predictability in the dynamics of shallow lakes: Implications for their management and restoration. Wetlands 26, 928–938.

De Vries, P. and Ouboter, P. (1985).Water sample treatments and their effects on bioassays using Stigeoclonium helveticum Vischer. Aquat Bot. 22, 177–185.

Dieter, D., Herzog, C., Hupfer, M. (2015). Effects of drying on phosphorus uptake in reflooded lake sediments. Environ Sci Pollut Res. 22, 17065–17081.

Dodds, W. and Whiles, M. (2010) Freshwater Ecology (Second Edition). Academic Press, 829 pp.

Doña, C., Chang, N., Caselles, V., Sánchez, J., Pérez-Planells, L., Bisquert, M., García-Santos, V., Imen, S., Camacho, A. (2016). Monitoring hydrological patterns of temporary lakes using remote sensing and machine learning models: case study of laMancha Húmeda Biosphere Reserve in central Spain. Remote Sens. 8, 618.

Driscoll, C., Effler, S., Auer, M., Doerr, S., Penn, M. (1993). Supply of phosphorus to the water column of a productive hardwater lake: controlling mechanisms and management considerations. Hydrobiologia. 53, 61-72.

Elser, J., Sterner, R., Galford, A., Chrzanowski, T., Findlay, D., Mills, K., Paterson, M., Stainton, M., Schindler, D. (2000). Pelagic C:

N: P stoichiometry in a eutrophied lake: responses to a whole-lake food-web manipulation. Ecosystems. 3(3), 293-307.

Fellman, J., Hood, E., Spencer, R. (2010). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review; Limnol Oceanogr. 55 2452–2462.

Feng, X., Hills, K., Simpson, A., Whalen, J., Simpson, M. (2011). The role of biodegradation and photo-oxidation in the transformation of terrigenous organic matter. Org Geochem. 42(3), 262-274.

Fernández, A., Viedma, O., Sánchez-Carrillo, S., Alvarez-Cobelas, M., Angeler, D. (2009). Local and landscape effects on temporary pond zooplankton egg banks: conservation implications. Biodivers Conserv. 18, 2373–2386.

Ferree, M. and Shannon, R. (2001). Evaluation of a second derivative UV/visible spectroscopy technique for nitrate and total nitrogen analysis of wastewater samples. Water res. 35(1), 327-332.

Ferriol, C., Miracle, M., Vicente, E. (2017). Effects of nutrient addition, recovery thereafter and the role ofmacrophytes in nutrient dynamics of a Mediterranean shallow lake: a mesocosm experiment. Mar Freshw Res. 68, 506–518.

Fichot, C., Benner, R. (2012). The spectral slope coefficient of chromophoric dissolved organic matter ($S_{275–295}$) as a tracer of terrigenous dissolved organic carbon in river-influenced ocean margins. Limnol Oceanogr. 57:1453–1466.

Florín, M. and Montes, C. (1999). Functional analysis and restoration of Mediterranean lagunas in the Mancha Húmeda Biosphere Reserve (Central Spain). Aquat Conserv Mar Freshwater Ecosyst. 9, 97–109.

Florín, M., Montes, C., Rueda, F. (1993). Origin, hydrologic functioning, and morphometric characteristics of small, shallow, semiarid lakes (Lagunas) in La Mancha, central Spain. Wetlands.13, 247-259.

Florín, M., Montes, C., Rueda, F. (1993). Origin, hydrologic functioning, and morphometric characteristics of small, shallow, semiarid lakes (Lagunas) in La Mancha, central Spain. Wetlands. 13, 247-259.

Gabor, R., McKnight, D., Miller, M. (2014). Fluorescence Indices and Their Interpretation. In: Paula G. Coble et al. (eds.) Aquatic Organic Matter Fluorescence. pp. 303-338. [Online]. Cambridge Environmental Chemistry Series. Cambridge: Cambridge University Press. Available from: Cambridge Books Online. http://dx.doi.org/10.1017/CBO9781139045452.015

Gächter, R., Meyer, J., Mares, A. (1988). Contribution of bacteria to release and fixation of phosphorus in lake sediments. Limnol Oceanogr. 33, 1542–1558.

García, O. and de la Cruz, M. (2016). El interés didáctico de los paisajes alterados: la Reserva de la Biosfera de La Mancha húmeda (España) como ejemplo de estudio. Revista Contexto & Educação. 31(99), 52-80.

Garcia, R., Reissig, M., Queimaliños, C., Garcia, P., Dieguez, M. (2015). Climate-driven terrestrial inputs in ultraoligotrophic mountain streams of Andean Patagonia revealed through chromophoric and fluorescent dissolved organic matter. Sci Total Environ. 521, 280-292.

García-Ferrer, I., Camacho, A., Armengol, X., Miracle, M. R., Vicente, E. (2003). Seasonal and spatial heterogeneity in the water chemistry of two sewage-affected saline shallow lakes from central Spain. Hydrobiologia, 506(1), 101-110.

Gasol, J. and Del Giorgio, P. (2000). Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar. 64(2), 197-224.

Gilbert, J., Guerrero, F., de Vicente, I. (2014). Sediment desiccation as a driver of phosphate availability in the water column of Mediterranean wetlands. Sci Total Environ. 466, 965–975.

Golterman, H., (2004). The Chemistry of Phosphate and Nitrogen Compounds in Sediments. Springer, Dordrecht, 284 pp.

Gosálvez, R., Gil-Delgado, J., Vives-Ferrándiz, C., Sánchez, G., Florin, M. (2012). Seguimiento de aves acuáticas amenazadas en lagunas de la Reserva de la Biosfera de La Mancha Húmeda (España Central). Polígonos. 22, 89–122.

Granéli, W. (1999). Internal phosphorus loading in Lake Ringsjön. Hydrobiologia, 404, 19–26.

Guerrero, M. and de Wit, R. (1992). Microbial mats in the inland saline lakes of Spain. Límnetica 8, 197–204.

Guillemette, F. and del Giorgio, P. (2011). Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. Limnol Oceanogr. 56, 734–748.

Guo W, Xu1, J., Wang, J., Wen, Y., Zhuo, J., Yan, Y. (2010). Characterization of dissolved organic matter in urban sewage using excitation emission matrix fluorescence spectroscopy and parallel factor analysis. J Environ Sci. 22, 1728–1734.

Guo, W., Yang, F., Li, Y., Wang, S. (2017). New insights into the source of decadal increase in chemical oxygen demand associated with dissolved organic carbon in Dianchi Lake. Sci Total Environ. 603(604), 699-708.

Hammer, U. (1986). Saline lake ecosystems of the world, (Volume 59 of Monographiae Biologicae), Springer Science & Business Media.

Hansen, A., Kraus, T., Pellerin, B., Fleck, J., Downing, B., Bergamaschi, B. (2016). Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation. Limnol Oceanogr. 61(3), 1015-1032.

He, Q., Xiao, Q., Fan, J., Zhao, H., Cao, M., Zhang, C., Jiang, Y. (2021). Excitation-emission matrix fluorescence spectra of chromophoric dissolved organic matter reflected the composition and origination of dissolved organic carbon in Lijiang River, Southwest China. J Hydrol. 598, 126240.

He, W., Bai, Z., Li, Y., Kong, X., Liu, W., Yang, C., Yang, B., Xu, F. (2016). Advances in environmental behaviors and effects of dissolved organic matter in aquatic ecosystems. Sci China Earth Sci., 59(4), 746-759.

Heiri, O., Lotter, A.F., Lemcke, G. (2001). Loss on ignition as amethod for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J. Paleolimnol. 593, 101–110.

Helms J., Stubbins, A., Ritchie, J., Minor, E., Kieber D., Mopper K. (2008). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol Oceanogr. 53, 955–969

Herbert, E., Boon, P., Burgin, A., Neubauer, S., Franklin, R., Ardón, M., Hopfensperger, K., Lamers, L., Gell, P. (2015). A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. Ecosphere. 6, 1–43.

Hilt, S., Brothers, S., Jeppesen, E., Veraart, A. J., Kosten, S. (2017). Translating regime shifts in shallow lakes into changes in ecosystem functions and services. BioScience, 67(10), 928-936.

Holmer, M. and Storkholm, P. (2001). Sulphate reduction and sulphur cycling in lake sediments: a review. Freshw Biol. 46, 431–451.

Hongve, D. (1997). Cycling of iron, manganese, and phosphate in a meromictic lake. Limnol Oceanogr. 42, 635–647.

Horppila, J., Holmroos, H., Niemistö, J., Massa, I., Nygren, N., Schönach, P., Tapio, P., Tammeorg, O. (2017). Variations of internal phosphorus loading and water quality in a hypertrophic lake during 40 years of different management efforts. Ecol Eng. 103, 264-274.

House,W., Casey, H., Donaldson, L., Smith, S. (1986). Factors affecting the coprecipitation of inorganic phosphate with calcite in hardwaters—I laboratory studies. Water Res. 20, 917–922.

Howard, K. and Noble, P. (2018). Hydrological perturbations drive rapid shifts in phytoplankton biodiversity and population dynamics in Butte Lake (Lassen Volcanic National Park, California). *Lake Reserv Manag. 34*(1), 21-41.

Howarth, R., Hayn, M., Marino, R., Ganju, N., Foreman, K., McGlathery, K., Giblin, A., Berg, P., Walker, J. (2014). Metabolism of a nitrogen-enriched coastal marine lagoon during the summertime. Biogeochemistry, 118(1), 1-20.

Huang, C., Zhang, L., Li, Y., Lin, C., Huang, T., Zhang, M., Zhu, A.x., Yang, H., Wang, X. (2018). Carbon and nitrogen burial in a plateau lake during eutrophication and phytoplankton blooms. Sci Total Environ. 616, 296–304.

Hudson, N., Baker, A., Reynolds, D. (2007). Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters - a review. River Res Appl. 23, 631-649.

Huguet, A., Vacher, L., Relexans, S., Saubusse, S., Froidefond, J., Parlanti, E. (2009). Properties of fluorescent dissolved organic matter in the Gironde Estuary. Org Geochem. 40 706–719.

Humayoun, S., Bano, N., Hollibaugh, J. (2003). Depth Distribution of Microbial Diversity in Mono Lake, a Meromictic Soda Lake in California. Appl Environ Microbiol. 69, 1030–1042.

Hupfer, M., Gloess, S., Grossart, H.P. (2007). Polyphosphate-accumulating microorganisms in aquatic sediments. Aquat Microb Ecol. 47, 299–311.

Hupfer,M., Lewandowski, J. (2008). Oxygen controls the phosphorus release from lake sediments – a long-lasting paradigm in limnology. Internat Rev Hydrobiol. 93, 415–432.

Ionescu, D., Siebert, C., Polerecky, L., Munwes, Y., Lott, C., Häusler, S., Bižić-Ionescu, M., Quast, C., Peplies, J., Glöckner, F., Ramette, A., Rödiger, T., Dittmar, T., Oren, A., Geyer, S., Stärk, H., Sauter, M., Licha, T., Laronne, J., de Beer, D. (2012). Microbial and Chemical Characterization of Underwater Fresh Water Springs in the Dead Sea. PloS one, 7(6), e38319.

Ishiwatari, R. (1985). Geochemistry of Humic Substances in Lake Sediments. Humic Substances in Soil, Sediment and Water. John Wiley and Son Inc., New York, p. 561-582.

JCCM, 2014. Servicio Integral de Asesoramiento al Regante. Albacete, Consejería de Agricultura y Medio Ambiente (JCCM)-CREA (UCLM). On line: http://crea.uclm.es/siar/datmeteo/

Jellett, J., Li, W., Dickie, P., Boraie, A., Kepkay, P. (1996). Metabolic activity of bacterioplankton communities assessed by flow cytometry and single carbon substrate utilization. Mar Ecol Prog Ser. 136, 213–225.

Jensen, H.S., Thamdrup, B. (1993). Iron-bound phosphorus in marine sediments as measured by bicarbonate-dithionite extraction. Hydrobiologia. 253, 47–59.

Jerez, G. (2010) La Reserva de la Biosfera de La Mancha Húmeda y la Cuenca Alta del Guadiana. Guía didáctica del medio físico y de la evolución de los paisajes. Universidad de Castilla–La Mancha, 367 pp.

Jilbert, T., Couture, R. M., Huser, B. J., Salonen, K. (2020). Preface: Restoration of eutrophic lakes: current practices and future challenges. Hydrobiologia. 847(21), 4343-4357.

Johnson, P., Townsend, A., Cleveland, C., Glibert, P., Howarth, R., McKenzie, V., Rejmankova, E., Ward, M. (2010). Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecol Appl**.** 20, 16-29.

Jones, B., Naftz, D., Spencer, R., Oviatt, C. (2009). Geochemical Evolution of Great Salt Lake, Utah, USA. Aquat Geochem., 15, 95–121.

Kalinkina, N., Tekanova, E. (2022). The Dependence of Chlorophyll a Concentration on Total Phosphorus in Water Bodies with Increasing Water Color. Inland Water Biol. 15(5), 539-542.

Karayanni, H., Macingo, S., Tolis, V., Alivertis, D. (2019). Diversity of bacteria in lakes with different chlorophyll content and investigation of their respiratory activity through a long-term microcosm experiment. Water, 11(3), 467.

Kim, L., Choi, E., Stenstrom, M. (2003). Sediment characteristics, phosphorus types and phosphorus release rates between river and lake sediments. Chemosphere. 50(1), 53-61.

Kinsman-Costello, L., Hamilton, S., O'Brien, J., Lennon, J. (2016). Phosphorus release from the drying and reflooding of diverse shallow sediments. Biogeochemistry. 130, 159–176.

Kragh, T., Sand-Jensen, K., Kristensen, E., Pedersen, O., Madsen-Østerbye, M. (2022). Removal of chromophoric dissolved organic matter under combined photochemical and microbial degradation as a response to different irradiation intensities. J Environ Sci. 118, 76-86.

Kritzberg, E., Langenheder, S., Lindström, E. (2006). Influence of dissolved organic matter source on lake bacterioplankton structure and function–implications for seasonal dynamics of community composition. FEMS Microbiol. 56(3), 406-417.

Labry, C., Herbland, A., Delmas, D. (2002). The role of phosphorus on planktonic production of the Gironde plume waters in the Bay of Biscay. J Plankton Res. 24, 97–117.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 4, 863.

Lamers, L., Tomassen, H., Roelofs, J. (1998). Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environ Sci Technol. 32, 199–205.

Lanzén, A., Simachew, A., Gessesse, A., Chmolowska, D., Jonassen, I., Øvreås, L. (2013) Surprising Prokaryotic and Eukaryotic Diversity, Community Structure and Biogeography of Ethiopian Soda Lakes. PLoS ONE, 8(8), e72577.

Lebaron, P., Servais, P., Agogue, H., Courties, C., Joux, F. (2001). Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? Appl. Environ Microbiol. 67, 1775–1782.

Li, H., Song, C., Yang, L., Qin, H., Cao, X., Zhou, Y. (2021). Phosphorus supply pathways and mechanisms in shallow lakes with different regime. Water Res. 193, 116886.

Li, S., Bush, R., Mao, R., Xiong, L., Ye, C. (2017). Extreme drought causes distinct water acidification and eutrophication in the Lower Lakes (Lakes Alexandrina and Albert), Australia. J Hydrol. 544, 133-146.

Liu, H., Liu, Q., Zhao, J., Zhang, X., Ding, L., Liu, Y., Fu, G. (2022). Spatiotemporal variation of phosphorus use efficiency across 70 lakes in China: Implications for lake eutrophication management. Ecol Indic. 142, 109293.

Loh, P., Molot, L., Nowak, E., Nürnberg, G., Watson, S., Ginn, B. (2013). Evaluating relationships between sediment chemistry and anoxic phosphorus and iron release across three different water bodies. Inland Waters, 3(1), 105-118.

Lü, C., He, J., Zuo, L., Vogt, R.D., Zhu, L., Zhou, B., Mohr, C.W., Guan, R., Wang, W., Yan, D. (2016). Processes and their explanatory factors governing distribution of organic phosphorous pools in lake sediments. Chemosphere. 145, 125–134.

Lukkari, K., Hartikainen, H., Leivuori, M. (2007). Fractionation of sediment phosphorus revisited. I: fractionation steps and their biogeochemical basis. Limnol Oceanogr Methods. 5, 433-444.

Maine, M., Hammerly, J., Leguizamon, M., Pizarro, M. (1992). Influence of the pH and redox potential on phosphate activity in the Parana Medial system. Hydrobiologia. 228, 83–90.

Mañas, P., Castro, E., de las Heras, J. (2009). Irrigation with treated wastewater: effects on soil, lettuce (Lactuca sativa L.) crop and dynamics of microorganisms. J Environ Sci Health A. 44, 1261–1273.

Margalef, R. (1947). Estudios sobre la vida en las aguas continentales de la región endorreica manchega. Publ Inst Biol Apl. Barcelona, 4, 5-51.

Mariot, M., Dudal, Y., Furian, S., Sakamoto, A., Vallès, V., Fort, M., Barbiero, L. (2007). Dissolved organic matter fluorescence as a water-flow tracer in the tropical wetland of Pantanal of Nhecolândia, Brazil. Sci Total Environ. 388:184–193.

McKnight, D., Boyer, E., Westerhoff, P., Doran, P., Kulbe, T., Andersen, D., (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr. 46(1), 2001, 38-48.

Minor, E., Pothen, J., Dalzell, B., Abdulla, H., Mopper, K. (2006). Effects of salinity changes on the photodegradation and ultraviolet—visible absorbance of terrestrial dissolved organic matter. Limnol Oceanogr. 51, 2181-2186.

Montoya L., Vizioli, C., Rodríguez, N., Rastoll, M., Amils, R., Marin, I. (2013), Microbial community composition of Tirez lagoon (Spain), a highly sulfated athalassohaline environment. Aquatic Biosyst. 9, 19

Moore, P., Reddy, K., Graetz, D. (1992). Nutrient transformations in sediments as influenced by oxygen supply. J Envir Qual. 21, 387–393.

Mopper, K., Helms, J., Stubbins, A., Chen, N., Minor, E., Dalzell, B. (2006). Effect of pH and ionic strength on terrestrial DOM photoreactivity: Implications in estuarine DOM transformations. EOS Trans AGU. 87(36).

Moran, M., Sheldon Jr, W., Zepp, R. (2000). Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnol Oceanogr. 45(6), 1254-1264.

Morant, D., Picazo, A., Rochera, C., Santamans, A.C., Miralles-Lorenzo, J., Camacho-Santamans, A., Ibañez, C., Martínez-Eixarch, M., Camacho, A. (2020). Carbon metabolic rates and GHG emissions in different wetland types of the Ebro Delta. PLOS ONE 15(4): e0231713.

Moss, B. (2011). Cogs in the endless machine: lakes, climate change and nutrient cycles: A review. Sci Total Environ. 434, 130-142.

Mutlu, M., Martínez, M., Santos, F., Peña, A., Guven, K., Antón, J. (2008). Prokaryotic diversity in Tuz Lake, a hypersaline environment in Inland Turkey. FEMS Microbiol Ecol, 65, 474–483.

Myrstener, M., Jonsson, A., Bergström, A. (2016). The effects of temperature and resource availability on denitrification and relative N_2O production in boreal lake sediments. J Environ Sci, 47, 82-90.

Navarro, V., García, B., Sánchez, D., Asensio, L. (2011). An evaluation of the application of treated sewage effluents in Las Tablas de Daimiel National Park, Central Spain. J Hydrol. 401(1), 53-64.

Nesmeyanova, M. (2000). Polyphosphates and enzymes of polyphosphatemetabolism in Escherichia coli. Biochemistry (Mosc). 65, 309–314.

Nöges, P., Nõges, T., Tuvikene, L., Smal, H., Ligeza, S., Kornijów, R., Peczula, W., Becares, E., Garcia-Criado, F., Alvarez-Carrera, C., Fernandez-Alaez, C., Ferriol, C., Miracle, R. M., Vicente, E., Romo, S., Van Donk, E., van de Bund, W., Jensen, J. P., Gross, E., Hansson, L., Gyllstrom, M., Nykanen, M., de Eyto, E., Irvine, K., Stephen, D., Collins, S., Moss, B. (2003). Factors controlling hydrochemical and trophic state variables in 86 shallow lakes in Europe. Hydrobiologia. 506(1-3), 51–58.

Nõges, T. (2009). Relationships between morphometry, geographic location and water quality parameters of European lakes. Hydrobiologia. 633, 33–43.

OCDE, 1982. Eutrophisation des eaux. Métodes de surveillance, d'evaluation et de lutte. Paris. 164 pp.

Odum, W. (1988). Comparative ecology of tidal freshwater and salt marshes. Ann Rev Ecol Syst. 19, 147–176.

Olsson, P., Folke, C., Berkes, F. (2004). Adaptive comanagement for building resilience in social–ecological systems. Environ Manage. 34(1), 75-90.

Oluyedun, O., Ajayi, S., Van-Loon, G. (1991). Methods for fractionation of organic phosphorus in sediments. Sci Total Environ. 106, 243–252.

Ortega-Retuerta, E., Pulido-Villena, E., Reche, I. (2007). Effects of dissolved organic matter photoproducts and mineral nutrient supply on bacterial growth in mediterranean inland waters. Microbiol Ecol. 54, 161–169.

Osborn, S., Vengosh, A., Warner, N., Jackson, R. (2011). Methane contamination of drinking water accompanying gas-well drilling and hydraulic fracturing. PNAS. 108(20), 8172-8176.

Osburn, C., Wigdahl, C., Fritz, S., Saros, J. (2011). Dissolved organic matter composition and photoreactivity in prairie lakes of the U.S. Great Plains. Limnol Oceanogr. 56(6), 2371–2390.

Pacheco, F., Roland, F., Downing, J. (2014). Eutrophication reverses whole-lake carbon budgets, Inland Waters. 4, 41–48.

Pant, H., and Reddy, K., (2003). Potential internal loading of phosphorus in a wetland constructed in agricultural land. Water Res. 37, 965–972.

Parkhurst, D. and Appelo, C. (1999). User's guide to PHREEQC (version 2): a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations. Washington, DC: U.S. Geological Survey Water-Resources Investigations Report. 723, 312 pp.

Peñuelas, J., Sardans, J., Rivas-Ubach, A., Janssens, I. (2011). The human-induced imbalance between C, N and P in Earth's life system. Global Change Biol. 18, 3-6.

Perkins, R. and Underwood, G. (2001). The potential for phosphorus release across the sediment–water interface in a eutrophic reservoir dosed with ferric sulfate. Water Res. 35, 1399–1406.

Picazo, A., Rochera, C., Vicente, E., Miracle, M., Camacho, A. (2013). Spectrophotometric methods for the determination of photosynthetic pigments in stratified lakes: a critical analysis based on comparisons with HPLC determinations in a model lake. Limnetica. 32, 139-158.

Pilati, A., Castellino, M., Bucher, E.H., 2016. Nutrient, chlorophyll and zooplankton seasonal variations on the southern coast of a subtropical saline lake (mar Chiquita, Córdoba, Argentina). Ann Limnol - Int J Limnol. 52, 263–271.

Porder, S. and Ramachandran, S. (2013). The phosphorus concentration of common rocks—a potential driver of ecosystem P status. Plant Soil. 367, 41–55.

Prieto-Ballesteros, O., Rodríguez, N., Kargel, J., Kessler, C., Amils, R., Remolar, D. (2003) Tírez lake as a terrestrial analog of Europa. Astrobiology 3, 863-877

Psenner, R., Pijcsko, R., Sager, M. (1984). Die Fraktionierung organischer und anorganischer Phosphorverbindungen von Sedimenten-Versuch einer Definition iikologisch wichtiger Frakionen [Fractionation of organic and inorganic phosphorus compounds in lake sediments: an attempt to characterize ecologically important fractions]. Arch Hydrobiol Suppl. 70, 111–155.

Qiu, S. and McComb, A. (1994). Effects of oxygen concentration on phosphorus release from reflooded air-dried wetland sediments. Mar Freshw Res. 45, 1319–1328.

Reddy, K., Patrick, W., Broadbent, F. (1984). Nitrogen transformations and loss in flooded soils and sediments. Crit Rev Env Control. 13(4), 273–309.

Redfield, A. (1958). The biological control of chemical factors in the environment. Am Sci. 46, 205–221.

Rengasamy, P., Tavakkoli, E. and McDonald, G.K., 2016, Exchangeable cations and clay dispersion: net dispersive charge, a new concept for dispersive soil. Eur J Soil Sci, 67, 659-665.

Resmi, P., Manju, M., Gireeshkumar, T., Ratheesh-Kumar, C., Movitha, M., Shameem, K., Chandramohanakumar, N. (2016). Phosphorous fractionation in mangrove sediments of Kerala, South West coast of India: the relative importance of inorganic and organic phosphorous fractions. Environ Monit Assess. 188 (6), 1-16.

Rier, S., Kinek, K., Hay, S., Francoeur, S. (2016). Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. Freshw Sci. 35, 490–502.

Rozan, T., Taillefert, M., Trouwborst, R., Glazer, B., Ma, S., Herszage, J., Valdes, L., Price, K., Luther III, G. (2002). Iron–sulfur–phosphorus cycling in the sediments of a shallow coastal bay: implications for sediment nutrient release and benthic macroalgal blooms. Limnol Oceanogr. 47, 1346–1354.

Sánchez-Ramos, D., Sánchez-Emeterio, G., Florín, M. (2016). Changes in water quality of treated sewage effluents by their receiving environments in Tablas de Daimiel National Park. Spain. Environ Sci Pollut Res. 23, 6082–6090.

Scheffer, M. (1998). Ecology of shallow lakes (Population and Community Biology Series Vol. 22). Springer Science & Business Media. 358 pages.

Schindler, D., (1978). Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol Oceanogr. 23, 478–486.

Schindler, D., Bayley, S., Curtis, P., Parker, B., Stainton, M., Kelly, C. (1992). Natural and man-caused factors affecting the abundance and cycling of dissolved organic substances in precambrian shield lakes. In Dissolved organic matter in lacustrine ecosystems (pp. 1-21). Springer, Dordrecht.

Schönbrunner IM, Preiner S, Hein T, (2012) Impact of drying and re-flooding of sediment on phosphorus dynamics of river-floodplain systems. Sci. Total Environ. 432:329-337.

Seitzinger, S., Mayorga, E., Bouwman, A., Kroeze, C., Beusen, A., Billen, G., Van Drecht, G., Dumont, E., Fekete, B., Garnier J., Harrison, J. (2010). Global river nutrient export: A scenario analysis of past and future trends. Global Biogeochem Cy. 24(4).

Seo, D., Yu, K., Delaune, R. (2008). Influence of salinity level on sediment denitrification in a Louisiana estuary receiving diverted Mississippi River water. Arc Agron Soil Sci. 54(3), 249-257

Søndergaard, M., Jensen, J., Jeppesen, E. (2003). Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia, 506(1-3), 135-145.

Søndergaard, M., Jensen, P., Jeppesen, E. (2001). Retention and internal loading of phosphorus in shallow, eutrophic lakes. Sci World J. 1, 427–442.

Song, G., Li, Y., Hu, S., Li, G., Zhao, R., Sun, X., Xie, H. (2017). Photobleaching of chromophoric dissolved organic matter (CDOM) in the Yangtze River estuary: kinetics and effects of temperature, pH, and salinity. Environ Sci Process Impacts. 19(6), 861-873.

Soto-Cardenas, C., Gerea, M., Garcia, P., Pérez, G., Diéguez, M., Rapacioli, R., Reissig, M., Queimaliños, C. (2017). Interplay between climate and hydrogeomorphic features and their effect on the seasonal variation of dissolved organic matter in shallow temperate lakes of the Southern Andes (Patagonia, Argentina): a field study based on optical properties. Ecohydrology, 10(7), e1872.

Stedmon, C. and Markager, S. (2005). Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. Limnol Oceanogr. 50: 686–697.

Stedmon, C. and Markager, S. (2005). Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. Limnol Oceanogr. 50(5), 1415-1426.

Stedmon, C., Markager, S., Tranvik, L., Kronberg, L., Slätis, T., Martinsen, W. (2007). Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea. Mar Chem. 104(3-4), 227-240.

Steinman, A., Ogdahl, M., Weinert, M., Uzarski, D. (2014). Influence of water-level fluctuation duration and magnitude on sediment–water nutrient exchange in coastal wetlands. Aquat Ecol. 48, 143–159.

Strayer, D. and Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. J N Am Benthol Soc. 29(1), 344-358.

Su, Y., Hu, E., Feng, M., Zhang, Y., Chen, F., Liu, Z. (2017). Comparison of bacterial growth in response to photodegraded terrestrial chromophoric dissolved organic matter in two lakes. Sci. Total Environ. 579, 1203-1214.

Sudhir, P. and Murthy, S. (2004). Effects of salt stress on basic processes of photosynthesis. Photosynthetica. 42(4), 481-486.

Sumner, M., Miller, W., Kookana, R., Hazelton, P. (1998). Sodicity, dispersion and environmental quality. In: Sodic soils: distribution, properties, management and environmental quality (eds M.E. Sumner & R. Naidu), pp. 149– 172. Oxford University Press, New York.

Sundareshwar, P. and Morris, J. (1999). Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient. Limnol Oceanogr. 44: 1693–1701.

Tammeorg, O., Nürnberg, G., Nõges, P., Niemistö, J. (2022). The role of humic substances in sediment phosphorus release in northern lakes. Sci Total Environ. 833, 155257.

Thamdrup, B. and Dalsgaard, T. (2002). Production of N2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl Environ Microbiol**.** 68(3), 1312-1318.

They, N., Amado, A., Cotner, J. (2017). Redfield ratios in inland waters: Higher biological control of C:N:P ratios in tropical semi-arid high water residence time lakes. Front Microbiol. 8, 1505.

Torremorell, A., Pérez, G., Lagomarsino, L., Huber, P., Queimaliños, C., Bustingorry, J., Fermani, P., Llames, M., Unrein, F. (2015). Microbial pelagic metabolism and CDOM characterization in a phytoplankton-dominated versus a macrophyte-dominated shallow lake. Hydrobiologia, 752(1), 203-221.

Tortosa, G., Correa, D., Sánchez-Raya, A., Delgado, A., Sánchez-Monedero, M., Bedmara, E. (2011). Effects of nitrate contamination and seasonal variation on the denitrification and greenhouse gas production in La Rocina Stream (Doñana National Park, SW Spain). Ecol Eng. 37, 539-548.

Van der Welle, M., Smolders, A., Op den Camp, H., Roelofs, J., Lamers, L. (2007). Biogeochemical interactions between iron and sulphate in freshwater wetlands and their implications for interspecific competition between aquatic macrophytes. Freshw Biol. 52, 434–447.

Van Diggelen, J., Lamers, L., van Dijk, G., Schaafsma, M., Roelofs, J., Smolders, A. (2014). New insights into phosphorus mobilisation from Sulphur-rich sediments: time-dependent effects of salinisation. PLoS One 9, e111106.

Van Veen, J. and Kuikman, P. (1990). Soil structural aspects of decomposition of organic matter by micro-organisms. Biogeochemistry, 11(3), 213–233. doi:10.1007/bf00004497

Verdouw, H., Van Echteld, C., Dekkers, E. (1978). Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12(6), 399-402.

Vrede, T., Ballantyne, A., Mille-Lindblom, C., Algesten, G., Gudasz, C., Lindahl, S., Brunberg, A. (2009). Effects of N: P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshw Biol. 54(2), 331-344.

Waiser, M. and Robarts, R. (2000). Changes in composition and reactivity of allochthonous DOM in a prairie saline lake. Limnol Oceanogr. 45, 763-774.

Wang, S., Jin, X., Zhao, H., Zhou, X.,Wu, F. (2008). Effects of organic matter on phosphorus release kinetics in different trophic lake sediments and application of transition state theory. J. Environ. Manag. 88, 845–852.

Wang, Z., Huang, S., Li, D. (2019). Decomposition of cyanobacterial bloom contributes to the formation and distribution of iron-bound phosphorus (Fe-P): insight for cycling mechanism of internal phosphorus loading. Sci Total Environ. 652, 696–708.

Ward, C. and Cory, R. (2016). Complete and partial photo-oxidation of dissolved organic matter draining permafrost soils. Environ sci. 50(7), 3545-3553.

Wei, J., Han, L., Song, J., Chen, M. (2015). Evaluation of the interactions between water extractable soil organic matter and metal cations (Cu (II), Eu (III)) using excitation-emission matrix combined with parallel factor analysis. International Journal of Molecular Sciences, 16(7), 14464-14476.

Wiegner, T. and Seitzinger, S. (2004). Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. Limnol Oceanogr. 49(5), 1703-1712.

Wilson, H. and Xenopoulos, M. (2009). Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nature Geosci. 2(1), 37–41.

Wong, S. and Clark, B. (1976). Field determination of the critical nutrient concentrations for Cladophora in streams. J Fish Res. 33(1), 85-92.

Yamashita, Y., Nosaka, Y., Suzuki, K., Ogawa, H., Takahashi, K., Saito, H. (2013). Photobleaching as a factor controlling spectral characteristics of chromophoric dissolved organic matter in open ocean. Biogeosciences. 10:7207–7217.

Yanni, S., Suddick, E., Six, J. (2015). Photodegradation effects on CO_2 emissions from litter and SOM and photo-facilitation of microbial decomposition in a California grassland. Soil Biol Biochem. 91, 40-49.

Yu, H., Song, Y., Gao, H., Liu, L., Yao, L., Peng, J. (2015). Applying fluorescence spectroscopy and multivariable analysis to characterize structural composition of dissolved organic matter and its correlation with water quality in an urban river. Environ Earth Sci. 73(9), 5163-5171.

Zagarese, H.E., Ferraro, M., Queimaliños, C., del Carmen Diéguez, M., Suárez, D.A., Llames, M.E., 2017. Patterns of dissolved organic matter across the Patagonian landscape: a broad-scale survey of Chilean and Argentine lakes. Mar. Freshwater Res. 68(12), 2355-2365.

Zhang, Y., Liu, X., Wang, M., Qin, B. (2013). Compositional differences of chromophoric dissolved organic matter derived from phytoplankton and macrophytes. Org Geochem. 55, 26-37.

Zhang, Y., van Dijk, M. A., Liu, M., Zhu, G., Qin, B. (2009). The contribution of phytoplankton degradation to chromophoric dissolved organic matter (CDOM) in eutrophic shallow lakes: field and experimental evidence. Water res. 43(18), 4685-4697.

Zhang, Y., Zhou, Y., Shi, K., Qin, B., Yao, X., Zhang, Y. (2018). Optical properties and composition changes in chromophoric dissolved organic matter along trophic gradients: Implications for monitoring and assessing lake eutrophication. Water Res. 131, 255-263.

Zhang, Z. and Liu, S. (2014). Hot topics and application trends of the anammox biotechnology: a review by bibliometric analysis. SpringerPlus, 3, 220-228.

Zhou, Y., Zhou, L., Zhang, Y., de Souza, J., Podgorski, D., Spencer, R., Davidson, T. (2019). Autochthonous dissolved organic matter potentially fuels methane ebullition from experimental lakes. Water res. 115048.

Zhu, W., Zhang, J., Yang, G. (2018). Mixing behavior and photobleaching of chromophoric dissolved organic matter in the Changjiang River estuary and the adjacent East China Sea. Estuarine, coastal and shelf science, 207, 422-434.

Zhu, Y.,Wu, F., He, Z., Guo, J., Qu, X., Xie, F., Giesy, J.P., Liao, H., Guo, F. (2013). Characterization of organic phosphorus in lake sediments by sequential fractionation and enzymatic hydrolysis. Environ Sci Technol. 47, 7679–7687.