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Changes in submerged macrophyte diversity, coverage and biomass in a biosphere reserve site after 20 years: A plea for conservation efforts

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ABSTRACT

The conservation of submerged vegetation is crucial in the functioning of aquatic ecosystems. We have assessed, by means of subaquatic quantitative sampling transects, the current status of the hydrophytic flora in 14 lakes in the *Lagunas de Ruidera* Natural Park, a Biosphere Reserve site in central Spain, surrounded by a semi-arid landscape. The conforming lakes have endured several anthropic pressures for decades (e.g., eutrophication, land-use changes, etc.). By comparing the current with the former status (20 years ago) of hydrophytes in the natural park, substantial negative (species richness loss, decrease in submerged meadow coverage, particularly in the upstream lakes) and positive (natural recovery of meadows after the cut-off of sudden disturbances caused by point pollution sources, improving of trophic state in some lakes, no presence of exotic hydrophytes) changes in the lakes were noticed. We have analyzed that the disappearance of all the hydrophyte (mainly charophytes) stands would represent the loss of ecosystem services. For example, up to 70 t CH_4 might be released from the charophyte detritus, an internal pool of phosphorus (as high as 1400 mgP/m^2) stored in the charophytes stands could be liberated. Some lakes require special measures, but overall, to preserve this lake complex, and other similar ones, we propose to study, in detail, the causes of submerged vegetation deterioration (focusing on the presence of pesticides –in water and sediments-, and other reasons) to assess the ecological risks, to protect the groundwater resources, preventing the lakes from receiving surficial point and non-point sources of pollution.

1. Introduction

The existence of submerged vegetation (particularly hydrophytes) is a key element in the long-term conservation of aquatic communities, and it plays important roles in the functioning of aquatic ecosystems (Scheffer, 2004; Jeppesen et al., 2007). A wide representation of macrophytes, in terms of diversity and coverage, is generally indicative of a good health status in waterbodies (Brisson et al., 2020), and aquatic plants are critical to avoid regime shifts from clear water states to unhealthy turbid states with the problematic and frequent occurrence of toxic algal blooms (Asaeda and Van Bon, 1997; Zerrifi et al., 2021). Moreover, macrophytes have been considered ecosystem engineers because they not only build up their own habitat architecture (Jeppesen et al., 2012) but also provide additional structure for the settlement of other organisms (Teubner et al., 2022).

Regarding benefits for humans, the macrophyte beds in lakes are responsible for many of the ecosystem services (Millennium Ecosystem Assessment, 2005) these waterbodies provide (Madsen et al., 2001; recently reviewed in Thomaz, 2023): nature-based solutions to reduce eutrophication, climate regulation (gas sequestration), esthetic values, etc. Furthermore, submerged macrophytes can be highly sensitive to environmental changes, hence, they are powerful and effective sentinels of global change (Calero and Rodrigo, 2019). Thus, as Choudhury et al. (2019) stated, one of the main challenges for both the scientific community and water managers, currently, is to reveal patterns of change along gradients of multiple environmental disturbances. This will allow the identification of abrupt biological responses of ecosystem functioning and biodiversity. For these reasons, a baseline of the status of conservation of macrophyte beds is essential in order to be aware of the effects of the possible environmental changes the Earth is facing.

The *Lagunas de Ruidera* Natural Park constitutes a unique wetland area of high conservation value in the inner part of the arid Castilla-La Mancha plateau (Spain), and forms part of a World Biosphere Reserve located in the provinces of Albacete and Ciudad Real (De la Hera and

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Villarroya, 2013). The area is formed by a lacustrine system made up of 15 lakes, and it is renowned for its rich biodiversity in the form of magnificent freshwater flora and fauna. It is a Special Bird Protection Area and a Wetland of International Significance under the Ramsar Convention. Moreover, the geological and geomorphological features which meet in this karstic system confer extraordinary value in the European continent (Ordóñez et al., 2005). The Natural Park contains well-preserved examples of current fluvial and lacustrine carbonates (tufas), in which the primary organic structure is made up mainly of macrophytes as well as some microphytes. The lacustrine area stands out due to its beautiful landscape, the color of the clear waters, as well as the traditional folklore related to its subterranean rivers; mentioned in the famous book Don Quixote by the Spanish writer Cervantes (De la Hera and Villarroya, 2013). Therefore, the value, in cultural and recreational terms, of the *Lagunas de Ruidera* Natural Park is high (e.g., increasing the number of visitors from 405,000 in 2007 to 570,000 in 2022, according to the Spanish Ministry for Ecological Transition and Demographic Challenge).

The Ruidera lake complex belongs to an ecosystem type which receives protection in the European Union Habitats Directive (European Commission DG Environment, 2007) which is habitat 3140: "hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.". The macrophyte biodiversity of this lake complex was studied by Cirujano (1980, 1981a, 1981b) and Velayos et al. (1989) many years ago. Several years later, this information was unified in a book about the aquatic flora of the Castilla-La Mancha wetlands (Cirujano et al., 2002). However, the Ruidera lakes, like other wetlands in the center of Spain (i.e., *Tablas de Daimiel* National Park, also a Ramsar site), have endured several anthropic pressures for decades, affecting their conservation status (e.g., land-use changes, aquifer abstractions, eutrophication, high-pressure tourism, etc.; Álvarez-Cobelas et al., 2001; Rodríguez-Murillo et al., 2011; Fidalgo et al., 2018). Moreover, the area is closely linked to stressing climatic conditions and enters into cyclical 'crisis' periods during recurrent drought episodes which clearly affect the macrophytes. A decade ago, De la Hera and Villarroya (2013) assessed the wetland status and changing trends in the services provided by this lake complex. However, since then, no studies concerning the diversity and conservation status of the important macrophyte beds the lakes harbor have been carried out, despite the submerged macrophytes being very good witnesses of environmental changes.

Within this framework, we have assessed, by means of qualitative and quantitative sampling transects, the current status of the hydrophytic flora in the lakes from the *Lagunas de Ruidera* Natural Park and have quantified, for the first time in almost all of the lakes (the exception being lake Colgada), the coverage and biomass of the macrophyte beds. Additionally, we have made an effort to relate this information to the ecosystem services that submerged vegetation provides to this valuable ecosystem. Hence, this is a pioneering study that aim to lay the foundations to determine possible future changes experienced by the diversity, biomass and coverage of hydrophytes of all the Ruidera lakes. Furthermore, we have assessed the current and future threats for the conservation of this important element in the Ruidera lakes while proposing measures to counteract them, which could be applied in other areas for the conservation of submerged vegetation stands in lakes.

2. Material and methods

2.1. Study site

The *Lagunas de Ruidera* Natural Park (declared in 1979) is part of the World Biosphere Reserve (*Mancha Húmeda*; central Spain). The lakes' water is basically groundwater; it resurges forming springs and pools and constitutes a chain of 15 lakes (Fig. 1). These lakes are distributed along a 35-km-long series of steps, with a difference in height of

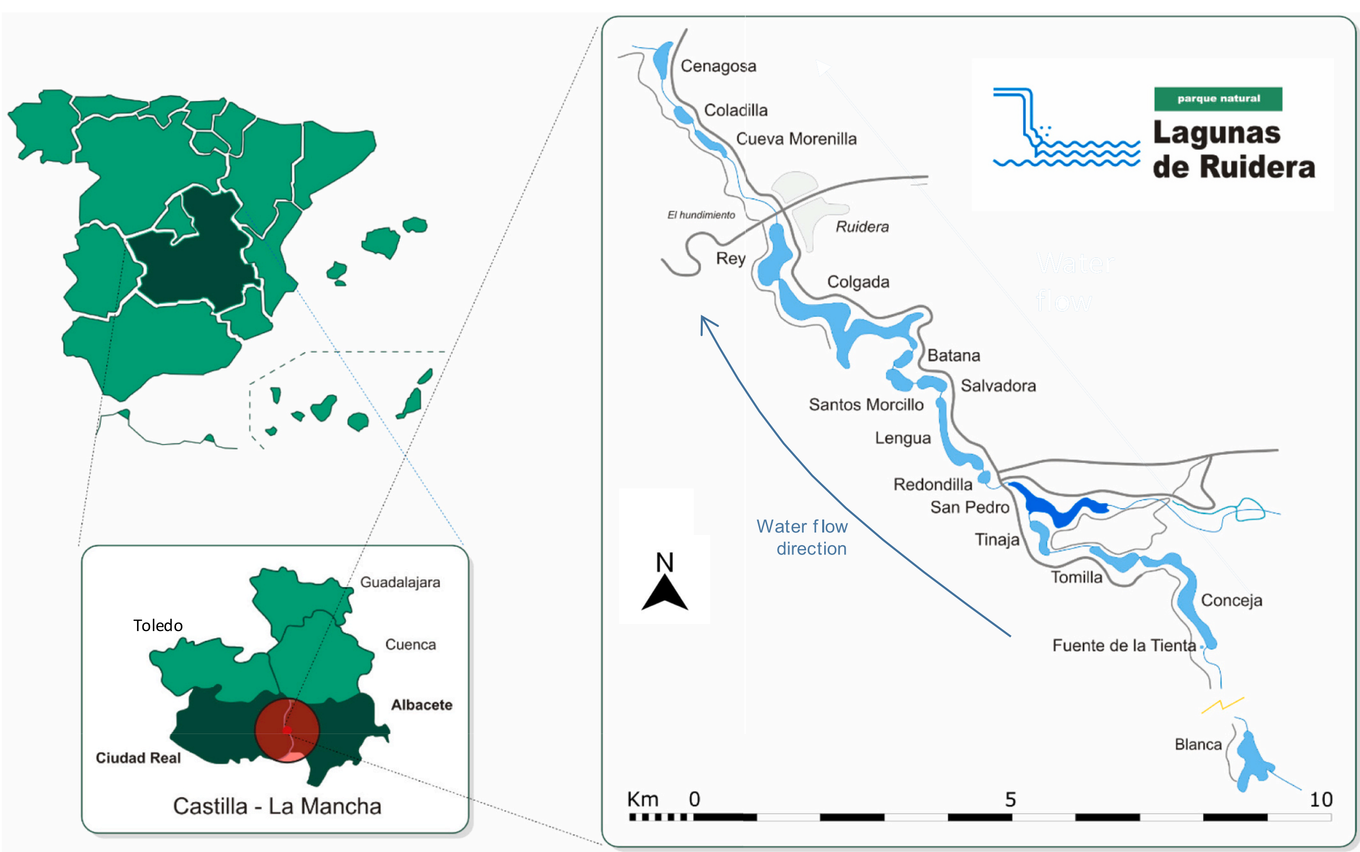

Fig. 1. Location map of the *Lagunas de Ruidera*. The arrow indicates the main water flow.

120–130 m between the highest and the lowest ones (De la Hera and Villarroya, 2013). The lakes are connected by waterfalls, torrents or channels and are dammed by a series of natural dikes, resulting from carbonate precipitation (travertine). The system ends in the Peñarroya reservoir, built in 1959, whose waters cover, since its construction, an old wetland area. The total surface area of the lake system is approximately 300 ha. The dammed volume has been calculated to be as large as 23 Mm^3 (De la Hera and Villarroya, 2013).

Regarding geology, the *Lagunas de Ruidera* Natural Park is located on a carbonated upland on the northern edge of the central peninsular plateau (De la Hera and Villarroya, 2013), with the local geology composed of a Palaeozoic metamorphic basement, with a heterogeneous deposit of Mesozoic sediments (Triassic and Jurassic). This Triassic-Jurassic complex varies in depth and is affected by fractures and folds. There is compartmentalization of the aquifer materials which affects the hydrological function. The upper Triassic layer is formed by marls and gypsum-rich clays, forming an impermeable bedrock under the Jurassic carbonate materials of the *Campo de Montiel* aquifer. There are several meter-thick Quaternary travertine deposits, on which the series of lakes are located (De la Hera and Villarroya, 2013). All the lakes are bicarbonate and calcium-rich environments and have alkalinities ranging between 2.2 and 4.4 meq/L (Álvarez-Cobelas et al., 2019).

Maximum water depth in all lakes may range between 2 m and 21 m (Table 1). The water level in the basically groundwater-fed lakes depends considerably on the lake basin geometry, the geological structure and the level of abstractions from the aquifer and its function (Montero, 1994). This means that some lakes are closely linked to the Jurassic aquifer, others are mainly fed by surface water, by and large from the Pinilla river and also by the channels connecting them to the lake immediately above, and a third group of lakes (i.e., lakes Colgada and Rey) are supplied from lateral surface water and from the lakes upstream (Montero, 1994). Moreover, during the first three decades of the 20th century, several channels were excavated in almost all the lake barriers, in addition to a tunnel from lake Tinaja to lake San Pedro. These interventions were associated with small hydroelectric power stations built on the banks of the Concejo (or Conceja), Tinaja, Batana and Rey lakes (Moya et al., 2018). Recent efforts (Delgado et al., 2022) have detected 24 subterranean flow sites in Conceja, San Pedro, Salvadora, Santos Morcillo, Batana and Colgada lakes.

The water level can change dramatically depending on the dry and wet periods (Álvarez-Cobelas et al., 2006). There was a dramatic fall in the water levels (1986–1995) within several lakes in the upper areas of the lake system as a consequence of several successive years of aridity. This resulted in enormous drops in the local water table, associated with partial to total desiccation of several lakes. After a prolonged wet period (1996–2000), the lake levels rose to regain their former water levels. A new, and short, dry period (2001) dried up some lakes. Since May 2002–September 2003 all the lakes have risen to their former level in the Ruidera system (Ordóñez et al., 2005). In 2017 some lakes dried out again during the summer season (e.g., lake Redondilla, pers. observ.); however, in 2021, this lake had a maximum depth of 9 m. Furthermore, the lake water levels are highly sensitive to aquifer pumping, since the aquifer has very little regulatory power due to its high permeability, resulting from the karstification of the aquifer (meaning it empties quickly) and also due to the shallow saturated layer (Montero and Rincón, 2004; Eugercios, 2013).

The vegetation in the surrounding catchment area consists, mainly, of open woodland with evergreen oak (*Quercus ilex* subsp. *ballota*) and incense Juniper (*Juniperus thurifera*) (Ordóñez et al., 2005). Agricultural areas can also be found, which produced nitrate inputs into the lakes through groundwater to cause total nitrogen concentrations of 7–17 mg N/L, of which 75–90 % is nitrate (Álvarez-Cobelas et al., 2019).

2.2. Underwater and superficial samplings

Underwater samplings were performed by specialist SCUBA divers in 13 waterbodies in 2022 (in different sampling campaigns from April to October) (Supplementary Material, Fig. A.1). Surface samplings were carried out in one lake (lake Blanca) from a boat, using a hook in the shallower lakes. In each lake, between one and four transects, depending on the size of the waterbody, were established. Lake Colgada was the only exception, since nine transects were performed in order to compare it with previous studies (2003–2004; Álvarez-Cobelas et al., 2007). In each transect, four vegetation samples were taken (mainly at depths of 4 and 8 m, from each shore of the lake, unless, in some cases, the lakes' depth did not allow sampling at these depths; see details of sampled depths for each lake in Supplementary Material, Table A.1). These depths were chosen as a representative of more littoral environment (4 m) and deeper environment where the growth of macrophytes could be light limited (8 m). Coordinates in each sampling site were taken by the SCUBA divers equipped with GPS devices. The sampling unit was a 0.25 m^2 metal frame placed in each sampling site. All the aboveground hydrophyte biomass was collected with the help of a manual saw, and all the material was kept in labeled plastic bags (see photographs in the Supplementary Material section). The bags were brought to the surface, where the SCUBA divers were assisted by researchers piloting a kayak, and the samples were kept in cool, dark conditions until they were studied and analyzed.

2.3. Species identification, biomass and coverage determination

From each sample, some specimens were taken for taxonomic identification. In order to compare the results with previous ones, the taxonomic keys of Spanish aquatic flora were used both for aquatic submerged plants and for charophytes (Cirujano et al., 2007a, 2007b, 2014). Submerged vegetation samples were washed in the lab with tap water to remove any remaining sediment or other materials (detritus, living organisms such as crayfish or insect larvae) and separated into species. Each species was put on trays in desiccation heaters at 70 °C until they were completely dry. Later, the dry weight was determined using a Mettler PJ3000 balance. Biomass results (Dry Weight, DW) are expressed as $KgDW/m^2$. The macrophyte coverage in each lake (in ha) was estimated by combining the information given by updated orthophotos (by means of Google Earth) and that of the transects performed by the SCUBA divers. Then, the total biomass of each lake was estimated by multiplying the biomass per unit surface and the total coverage (expressed in tons). Two groups of lakes were considered based on their altitude (classifying those lakes above 820 m of altitude as "higher lakes" and those below this limit as "lower lakes"). Moreover, the biomass of charophyte stands was considered separately between the

Table 1
Main morphometric features of the Ruidera lakes studied in 2022 (most data from Álvarez-Cobelas et al., 2007; the data from Fuente Tienta are from this study).

	Blanca	Fuente Tienta	Conceja	Tomilla	Tinaja	San Pedro	Redondilla	Lengua	Salvadora	Santos Morcillo	Batana	Colgada	Rey	Cueva Morenilla
Surface (ha)	21.6	0.2	29.4	8.8	8.0	28.6	3.5	20.1	8.7	11.7	6.5	103.0	37.9	7.1
Maximum depth (m)	2	2	14	14	17	21	9	14	12	14	8	18	20	8
Mean depth (m)	0.5	1.1	8.4	7.9	8.1	10.4	5.7	6.8	7.6	7.8	4.5	8.4	9.8	5.5

right and the left sides of the lakes due to the existence of much more human constructions (houses, campings, hotels, restaurants, etc.) in the right side.

2.4. Determination of carbonate encrustations in the charophytes

Some charophyte specimens were used to determine the percentage of carbonate encrustations in the dry weight. In order to do this, clean, dry specimens (70 °C, 24 h) were weighed and treated with 0.5 M HCl to remove the carbonate according to the protocol described by Rojo et al. (2015). After removing the acid, the charophytes were rinsed, dried and weighed again. The difference in weight was used to calculate the percentage of carbonate on the surface of the charophytes in relation to the dry biomass.

2.5. Statistical analyses

Mean submerged vegetation biomass among groups of lakes, sampling depths and the margin of the lakes was compared by means of one-way analysis of variance (ANOVA) after checking that the normality and homoscedasticity criteria were met. When necessary, *post-hoc* comparisons were performed by Tukey tests. Statistical analyses were performed using the PAST 4.11 program (Hammer et al., 2001) considering statistically significant differences at $p < 0.05$.

3. Results

Fifteen taxa of hydrophytes were identified in the Ruidera lakes, out of which six were charophyte species (Table 2). Moreover, several amphibious plants were detected living totally submerged. This is the case of *Lythrium salicaria* L., *Veronica anagallis-aquatica* L., *Samolus valerandi* L. and *Teucrium scordium* L. In particular, the latter developed dense submerged meadows mainly in lake Redondilla. The most abundant macrophytes were three taxa of charophytes (*Chara hispida* var. *major* (Hartm.) R.D. Wood, *C. hispida* var. *hispida* f. *polyacantha* (A. Braun) R.D. Wood and *C. hispida* L. var. *hispida*), which developed dense meadows in most of the lakes (see Supplementary Material, Fig. A.2). Most of the lakes showed a decrease in the species richness of hydrophytes in comparison with former data (Table 2), ranging from 38 % to 75 %. The exception was lake Conceja, whose richness increased by 175 %, from 4 species detected previously to 7 species currently. The richest lake was lake Lengua (according to the data before −15 species– and now −7 species–).

Overall, the mean submerged vegetation biomass was 1.0 ± 0.9 kg DW/m^2. In this average, the value from the Fuente de la Tienta was excluded since this small waterbody had an eleven-fold greater biomass (11.5 kg DW/m^2) in comparison to the mean (Fig. 2A). The mean biomass was significantly lower in the lakes located upstream (from lake Conceja to lake Lengua) compared to the lakes located downstream (from lake Salvadora to lake Cueva Morenilla) (Fig. 2B). The mean biomass was also statistically higher in the shallower areas (1.4 ± 0.9 kg DW/m^2 at 4 m deep and 0.9 ± 0.7 kg DW/m^2 at 8 m deep; Fig. 2C).

Analyzing each lake individually (Fig. 3), only lake Batana showed statistically significant differences in the mean biomass of each studied transect; this biomass being greater in the transect located northeast of the lake. For the rest of the lakes, the mean biomass was statistically similar when comparing the two, three or four transects (or nine in the case of lake Colgada). There were not statistically significant differences in the mean macrophyte biomass in any of the lakes when comparing the right vs left sides of each basin.

Taking into account the surface occupied by the macrophyte beds in each lake, and the biomass per square meter, the total macrophyte biomass ranged from 1 t (lake Salvadora) to 542 t DW (lake Conceja) (Fig. 4); the mean biomass (± standard deviation) per lake was 118 ± 158 t DW, and the total biomass considering the lake complex was 1537 t DW.

Lake Colgada is the only one that can be compared with previous data (Fig. 5). Overall, considering the whole lake, there were no statistically significant differences in the mean biomass of the macrophyte meadows between 2003 (0.97 ± 0.40 kg DW/m^2; Sosnovsky et al., 2005) and 2022 (1.14 ± 0.39 kg DW/m^2) (Fig. 5A). However, the mean biomass in transects 1–4 (closer to buildings) was almost two-fold greater in 2022 compared to 2003 (Fig. 5B), and, again, no statistical differences in mean macrophyte meadow biomass between 2003 and 2022 in transects 5–9 (far from buildings) were found. However, a total absence of charophyte stands was observed just close to the shore in transect 6, something that did not occur in the other transects (Supplementary Material, Fig. A.1). The relationship between macrophyte biomass and depth (less biomass at greater depths) is significant in lake Colgada, considering, independently, the two groups of transects (1 to 4 vs 5 to 9) (Biomass = −0.3*Depth + 3; Fig. 5C), and it is similar to that found in 2003 for transects 5 to 9.

Carbonate in the encrustations of the charophytes represented 50 % ± 10 % of dry weight, with a maximum of 63 % ± 2 % in lake Redondilla and a minimum of 40 % ± 5 % in lake Colgada (Fig. 6A). Charophytes from lake Cueva Morenilla only showed 29 % ± 6 %, but this was the only lake sampled in October. The mean carbonate content was statistically higher (55 % ± 10 %) in the lakes located upstream compared to the lower ones (44 % ± 11 %) (Fig. 6B), even excluding the lowest values measured in lake Cueva Morenilla from the lower lake group. There were no significant statistical differences in the content of carbonates in the charophyte biomass when comparing the shallower and deeper sites (a depth of 4 m versus 8 m) (Fig. 6C).

4. Discussion

4.1. Temporal and spatial changes in richness, biomass and coverage of hydrophytes and the main threats the lakes face

Specific richness comparisons among different studies are not always possible, since the sampling effort can substantially differ (ter Steege et al., 2011). Taking into account this circumstance, we can state that the hydrophyte richness observed during this study represents a reduction of 30 % in comparison with the data reported by Álvarez-Cobelas et al. (2007), due to the reduction observed in most lakes (the only exception being lake Conceja). These authors described 20 hydrophyte taxa, nine of which were charophytes. We found all charophyte taxa except for *Chara vulgaris* (cited previously with three subspecies). We have followed the same taxonomical nomenclature for Characeae as in the previous studies (Álvarez-Cobelas et al., 2007; Cirujano et al., 2007a) for comparison. However, it should be remarked that the taxonomic classification of charophytes has been recently unified for Europe (Schubert et al., 2024) and some of the varieties considered in this study have nowadays the category of species. On the other hand, we detected other hydrophyte species not previously described in the Ruidera lakes (see Table 2). However, in some lakes, we observed an eventual shift towards eutrophication-related species. Nevertheless, we stress the importance of amphibian plants such as *Teucrium scordium* (Šraj Kržič and Gaberščik, 2005), in lakes subjected to drastic fluctuations in the water level, such as lake Redondilla, in maintaining a wide coverage of submerged vegetation for quite long periods until the most appropriate or typical submerged species replace it.

Most lakes exhibited a very dense coverage of charophytes, with mean biomasses above 1 kgDW/m^2, a value similar (even higher) to other hard-water lakes in Europe (Rodrigo et al., 2015; Pukacz et al., 2016a); the high biomass (11.7 KgDW/m^2) found in the small lake Fuente de La Tienta is remarkable, an amount that has never been reported for charophytes in the literature. Overall, the mean charophyte biomass was higher in the shallower parts of the lakes compared to the deeper ones. This result is expected due to the light limitation these photosynthetic organisms are exposed to in deeper habitats (Middelboe and Markager, 1997; Pełechaty et al., 2013; Pukacz et al., 2016b).

Table 2
Hydrophyte taxa identified in the Ruidera lakes in this study (white circles). Black circles correspond to taxa identified in previous studies, summarized in Álvarez-Cobelas et al. (2007).

Hydrophytes Lakes	Blanca	Fuente Tienta	Conceja	Tomilla	Tinaja	San Pedro	Redondilla[a]	Lengua	Salvadora	Santos Morcillo	Batana	Colgada	Rey	Cueva Morenilla	Occurrence
Chara hispida var. major (Hartm.) R.D. Wood	●○		●○	●○	●○	●○	●○	●○	●○	●○	●	●○	●○	●	13/11
Chara hispida var. *hispida* f. polyacantha (A. Braun) R.D. Wood	●	○	●○	●	●○		○	●	○		●○	●○	●○	●○	9/9
Chara hispida L. var. hispida	●						○	●○	●	●○		●			5/3
Chara vulgaris L. var. vulgaris	●								●	●					3/0
Chara vulgaris var. *contraria* (A. Braun ex Kütz.) J.A. Moore										●					1/0
Chara vulgaris var. *crassicaulis* (Schleicher ex A. Braun) Kütz.									●						1/0
Chara aspera Deth. Ex Willd. var. *aspera*	●								○	○					1/2
Nitella hyalina (DC.) C. Agardh	●○		○	●		●○	●	●○	●○	●○	●○				8/7
Nitella confervacea (Bréb.) A. Braun ex Leonhardi							●	●○	○	○					2/3
Myriophyllum spicatum L.			○		●	●○	●	●	●	●			●	○	7/3
Myriophyllum verticillatum L.			○			●	●	●			●				4/1
Stuckenia pectinata (L.) Börner (*Potamogeton pectinatus* L.)	●		●○	●	●	●○	●	●○	●	●○			●	●	11/4
Potamogeton coloratus Hornem.					●			●							2/0
Potamogeton lucens L.								○							0/1
Ranunculus peltatus Schrank subsp. peltatus					●		●	●							3/0
Ranunculus peltatus Schrank subsp. *saniculifolius* (Viv.) C.D.K. Cook								●							1/0
Ranunculus trichophyllus Chaix								●○							1/1
Ranunculus sp.									○						0/1
Najas marina L.			○	○		●		●	●	●					4/2
Polygonum amphibium L.						●○		●							2/1
Utricularia australis R. Br.	●○		●	●○									●		4/2
Zannichellia palustris L.						●	●	●							3/0
Number of taxa	8/3	/1	4/7	5/3	6/2	8/5	8/3	15/7	8/6	8/6	4/2	3/2	5/2	3/2	

[a] In lake Redondilla, the amphibious plant *Teucrium scordium* was found, totally submerged and having lived there for a long period of time.

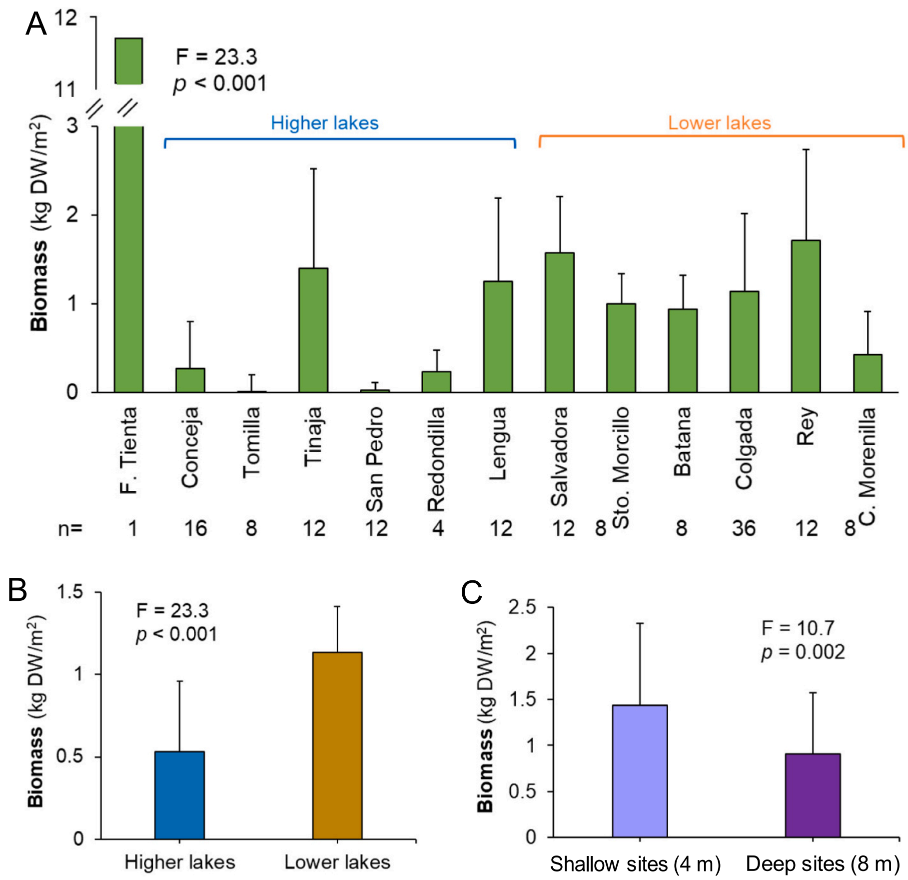

Fig. 2. A: Means of submerged vegetation biomass (kg dry weight (DW)/m^2) in the Ruidera lakes calculated from the 4 samples taken in each transect ("n" indicates the number of total samples collected in the lake, considering the performed transects and the sampling depths). The lakes are ordered from left to right following the water flow. B: Comparison of submerged vegetation biomass means between higher and lower lakes. C: Comparison of submerged vegetation biomass means between samples taken at shallower and higher depths. Variability bars are standard deviations. F and p values for ANOVA analyses are also shown.

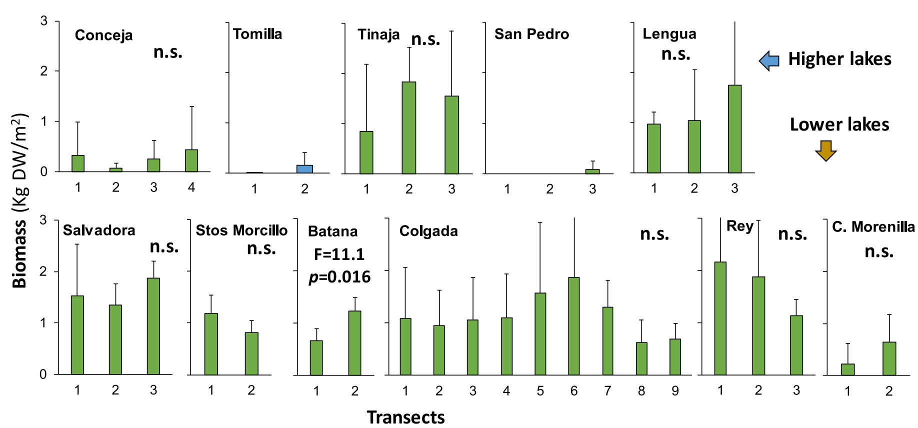

Fig. 3. Means (n = 4, i.e. considering the samples from 4 m and 8 m depth) of submerged vegetation (mainly charophytes) biomass (kg dry weight (DW)/m^2) in each transect of the Ruidera lakes (in lake Tomilla the biomass belongs to submerged *Schoenoplectus lacustris* = blue bar). Variability bars are standard deviations. F and p values for ANOVA analyses are shown; n.s.: non-significant differences in the ANOVA analyses among transects. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Overall, no other spatial differences were found, either horizontally or between the right and left sides of the lake basins, despite the right margin being where more human presence can be found (houses, hotels, restaurants, etc.). The total absence of charophyte stands in the south shore of transect 6 in lake Colgada is related to an underwater point source of pollution visually observed by the SCUBA divers during the samplings, since as Montero (1994) described, some lakes such as Colgada and Rey are supplied from both lateral subsurface water and from the lakes upstream. Most of the wastewater discharge that occurred several years ago has almost been eliminated as a result of the functioning of the wastewater treatment plant in 2013, located in the town of Ruidera; however, this kind of uncontrolled point source of pollution should be contained because it represents a clear threat to the survival of the macrophyte meadows. The reduction of 63 % in the charophyte

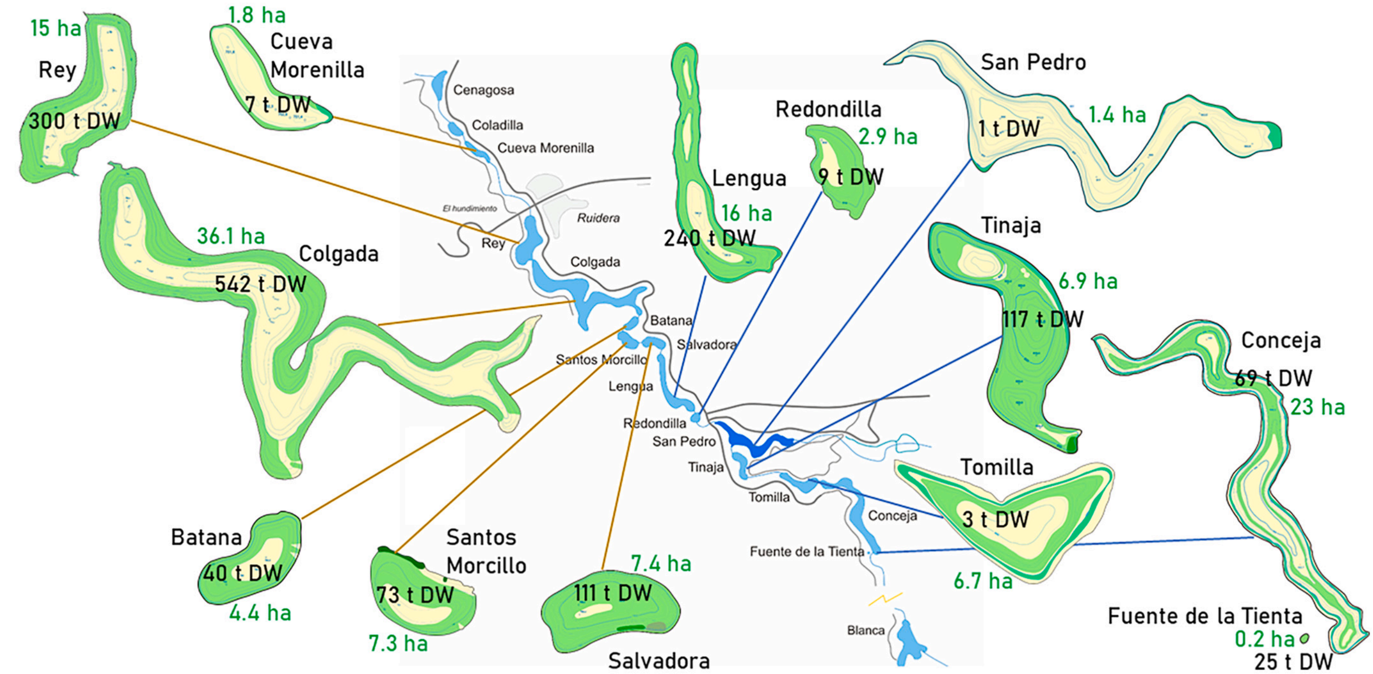

Fig. 4. Submerged vegetation maps of the Ruidera lakes (the most abundant green color represent the extension of charophyte beds; coverage (in ha) is indicated in green numbers for each lake). Blue lines indicate higher lakes and orange lines indicate lower lakes. Estimated total biomass of macrophyte beds in each lake is also indicated on each lake map (in tons of dry weight). For lake San Pedro, the indicated (different green color) area corresponds mainly to *Polygonum amphibium* coverage; for lake Santos Morcillo, the dark green area represents *Myriophyllum spicatum* coverage; for lake Salvadora, the greyish area corresponds to *Nitella* spp. coverage, and the bright green area represents *Chara aspera* coverage. For lake Tomilla, the bright green color indicates *Najas marina-Utricularia australis* coverage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

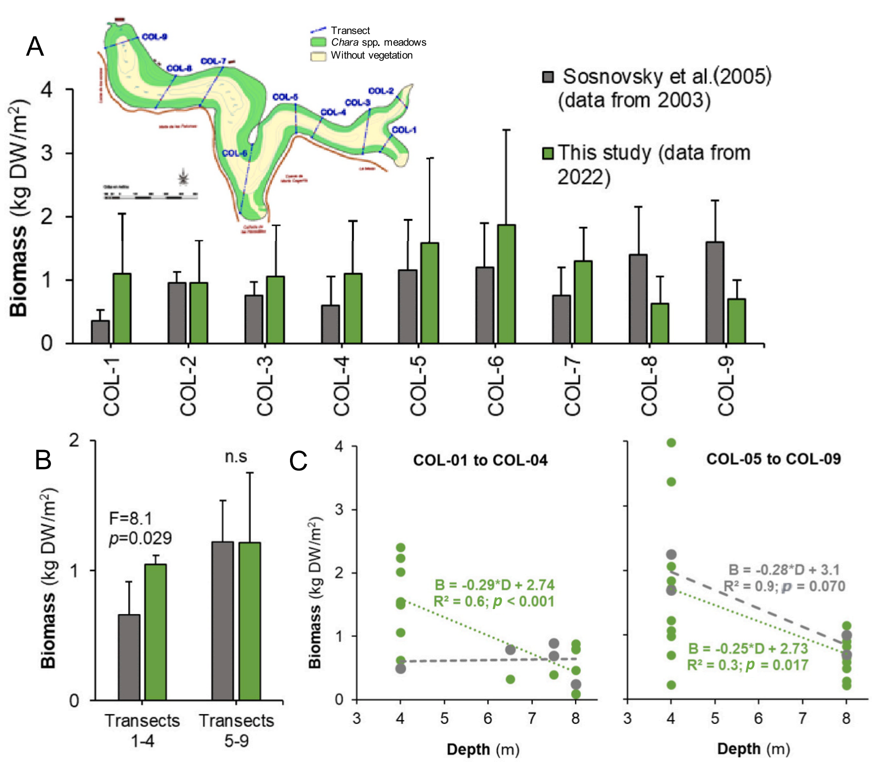

Fig. 5. A: Mean charophyte biomass in each transect studied in lake Colgada in 2003 from the previous study by Sosnovsky et al. (2005) and in 2022 (this study). The 2022 vegetation map with the location of the nine transect is also shown. Variability bars are standard deviations. B: Mean charophyte biomass (and standard deviation) in two groups of transects depending on their proximity to buildings (COL-01 to 4: closer to buildings; COL-05 to 9: far from buildings). F and p values for ANOVA analyses comparing both dates in each group of transects are shown; n.s.: non-significant differences in the ANOVA analysis. C: Relationship between the depth at which charophyte are located and their biomass in both studies in the groups of transects considered in B. Equations of the regression analyses are shown.

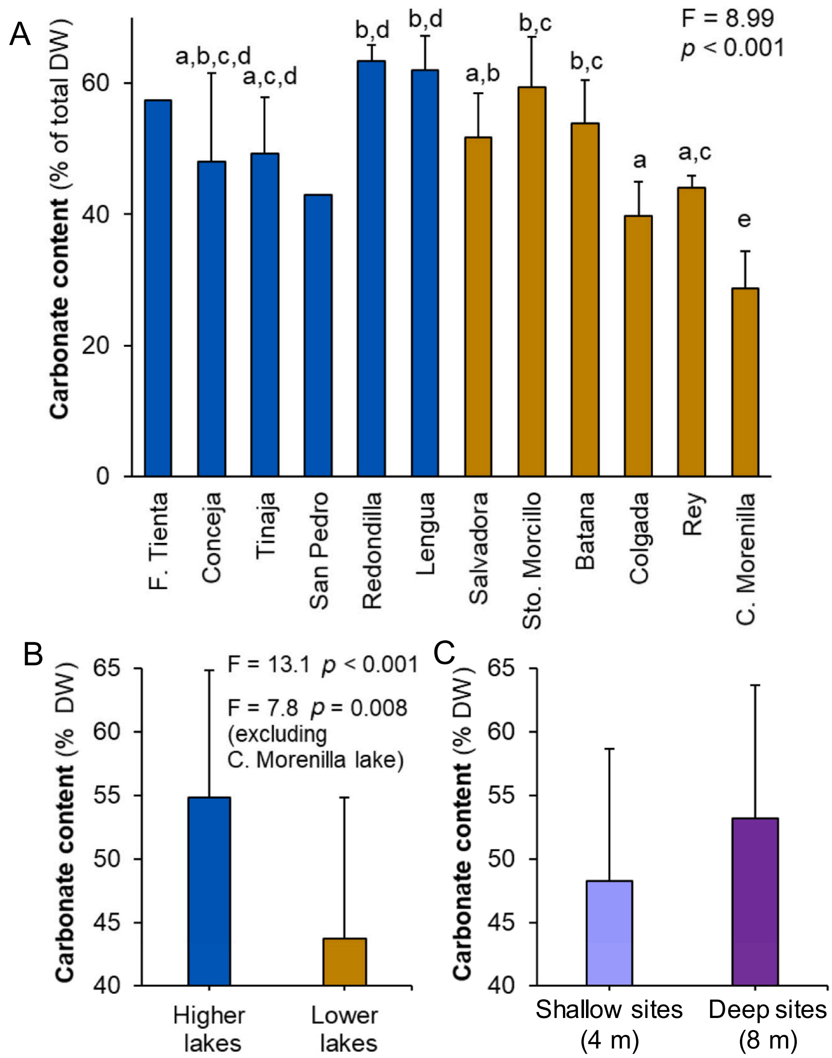

Fig. 6. A: Means of carbonate percentage in the dry weight of charophyte biomass in the Ruidera lakes. Variability bars are standard deviations. The lakes are ordered from left to right following the water flow. F values and *p*-values for ANOVA analysis are shown. Lower-cap letters above the bars indicate the results of *post-hoc* Tukey analyses. B: Comparison of carbonate percentage means in the charophytes of the higher and lower lakes. F values and *p*-values for ANOVA analysis are shown. C: Comparison of carbonate percentage means between samples taken at shallower (4 m) and higher (8 m) depths. Variability bars are standard deviations.

biomass observed in the area close to the outlet of lake Colgada (transects 8 and 9), in comparison to 2003 data (Sosnovsky et al., 2005; Álvarez-Cobelas et al., 2007), must be related to possible inputs of non-point polluted waters. On the positive side, our data show a clear improvement in the biomass and coverage of the charophyte meadows in the area (southeast littoral) affected by the breakdown of the septic tank belonging to the hotel located on that shore of lake Colgada, described by the authors cited above, which caused a sudden and drastic disappearance of this submerged vegetation in 2004. This is evidence of the resilience capacity of charophyte stands after an important disturbance when the source of pollution is cut-off. Another proof of the reduction of wastewater in the lower lakes is the clear favorable changes observed in lake Cueva Morenilla, in terms of a reduction in the values of most of the eutrophication-related variables. For example, water transparency increased from 2 m in summer 2001 to 4.8 m in mid July 2022 and, currently, the macrophyte meadows are well developed with a biomass >1 kgDW/m^2 in some sites. Lake San Pedro deserves special mention, because, being the fourth lake in extension, its surface covered by charophyte meadows is negligible due to the elevated water turbidity. In this case, human pressure is high (several buildings are very close to the lake shores, and many recreational activities take place on the lake which produce pollution and waste –some broken chairs were found at the bottom of the lake–). However, the value of this lake is unquestionable, since it is the only one in the Ruidera lakes complex which possesses quite an extended population of the floating-leaved hydrophyte *Poligonum amphibium,* with heights of up to 8 m. Recovery measures are urgent for the, still possible, restoration of the lake and to avoid the disappearance of this unusual species.

Overall, comparing the upper and lower stream lakes, the mean biomass of charophyte meadows was significantly lower, specifically 2.4 times in the lakes located in the upper area (from lakes Conceja to Lengua). It is known that the upper lakes, although not accurately monitored, lost their submerged vegetation meadows several years ago (lake Conceja in 2014, lake Tomilla in 2016, lake San Pedro around 2004). There is widespread recognition that agriculture plays a major role in modifying, and, commonly, degrading freshwater ecosystems and the services that they provide (in Europe, for example, member states report that nutrient pollution significantly degrades 28 % of all surface water bodies classified in the Water Framework Directive; Carvalho et al., 2019). Nitrate concentrations in the *Lagunas de Ruidera*

Natural Park in surface and groundwater have increased almost threefold over the last 30 years as a result of heavy nitrate pollution (Álvarez-Cobelas et al., 2007), and the upper lakes in the area are clearly more influenced by agriculture compared to the lower ones. In fact, previous research showed how nitrate concentrations were significantly greater in the upper lakes (Álvarez-Cobelas et al., 2007; Piña-Ochoa et al., 2006). Rodrigo et al. (2017) demonstrated, in short-term laboratory experiments with high-nitrate concentrations (a range of 0.5–50.0 mg N-NO_3/L;) and with *Chara hispida* and *Chara vulgaris,* that no direct nitrate toxicity existed until 30 mg N-NO_3/L, therefore, nitrate in the water per se was not detrimental to these charophytes species below this limit, which is above the nitrogen concentration measured in the Ruidera lakes (7–17 mg N/L of TN; Álvarez-Cobelas et al., 2019). Hence, we do not consider nitrate concentrations as the exclusive cause of charophyte meadows decline in the upper lakes. The low water transparency could be another reason for the disappearance of charophyte meadows. One of the upper lakes (lake Conceja) exhibited, for example in July 2022, a lower transparency (5.3 m of Secchi depth) than the lower lake Colgada (9.0 m), despite sestonic chlorophyll *a* concentrations being much lower in the former (0.5 μgChl/L versus 2.3 μgChl/l; own data). Álvarez-Cobelas et al. (2007) described how in the upper lakes (up to lake Batana), the suspended solids (chiefly due to carbonate precipitation) contributed more than the phytoplankton to light attenuation. In any case, the water transparency has been always high enough to not consider this fact as a cause of the macrophyte stands collapse in the upper lakes, such as lakes Conceja and Tomilla.

Extensive agriculture is characterized by the use of a large number of pesticide compounds in Europe (at least 148 active pesticide substances according to Pistocchi et al., 2023) and their presence in water and sediments can be an important stress factor to aquatic ecosystems, in the end affecting both plants and animals (Wagner et al., 2015). After consulting the data from the Guadiana River Water Authorities (CHG, 2021), we saw that only terbuthylazine (0.110 μg/L), pyrene (0.120 μg/L), chlorpyrifos (0.216 μg/L) and benzo(*k*)fluoranthene (1.024 μg/L) were detected in concentrations above legal limits in three of the sampling sites where this authority regularly monitors pesticides in wells around the Ruidera lakes and over four different years (2006, 2007, 2014 and 2016). Terbuthylazine is the only herbicide among the detected compounds, and it is also frequently found in continental waters of other countries of the European Union such as Italy, Greece and Portugal (Tóth et al., 2022). It is used in corn and sunflower crops which can be found all year round. Its relevance and extensive use have increased since atrazine and simazine were prohibited in Europe (ECC, 2004a, 2004b). There is scarce information concerning the possible negative effects of terbuthylazine on charophytes. Coors et al. (2006) studied its effect on *Chara globularis* and other macrophytes, but their results are not conclusive. Although pesticides and their use are strictly regulated in the European Union, the monitoring is insufficient to characterize their actual exposure and impact, such as the cumulative toxicity (of several substances) at a given location. This is an issue that deserves meticulous attention in a natural park such as *Lagunas de Ruidera*. Moreover, high predation by animals on hydrophytes stands could cause an important reduction of their biomass. Exotic species of fish (carps) and crayfish (*Procambarus clarkii*) are present in all Ruidera lakes. Unfortunately, there is a lack of quantitative information about animal abundance which prevent to find causal relationships with vegetation regression.

On the other hand, charophyte stands in the Ruidera lakes contain around 50 % dry weight of thick calcium carbonate encrustations, a fact that has been linked to bicarbonate use in photosynthesis by these macroalgae (Sand-Jensen et al., 2018). This is a common fact found in other hard-water lakes (Pełechaty et al., 2013; Pukacz et al., 2014a, 2014b, 2016a, 2016b; Herbst et al., 2018a, 2018b). In a compilation of 79 charophyte meadows in calcium carbonate-rich lakes in Northern Europe, carbonate encrustations constituted, on average, 64 % of the total charophyte mass (see Sand-Jensen et al., 2021, and references therein). Our results are of the same order of magnitude, and this circumstance is of paramount relevance regarding phosphorus availability and ecosystem services (discussed below).

4.2. Assessment of potential losses of ecosystem services provided by the macrophyte meadows

As stated by Kufel and Ozimek (1994) and Vermeer et al. (2003) among other researchers, dense charophyte stands may limit the growth of phytoplankton and epiphytes, because they constitute nutrient sinks in the form of organic biomass and in calcium carbonate precipitates on their surfaces. The result is the creation of suitable light conditions for their own development, but, mainly, the maintenance of clear-water status which represents a valuable ecosystem service. Regarding calcification, this process is related to phosphate dynamics by adsorption processes and the formation of calcium-carbonate-phosphate minerals. Thus, co-precipitation of phosphate in the carbonate matrix, both on charophyte and on sediment surfaces, contributes to reduce the growth of phytoplankton (Kufel and Kufel, 2002; Sand-Jensen et al., 2021). The range of phosphorus concentration in the combined organic biomass and carbonate precipitates reported by Kufel et al. (2013, 2016) in several charophyte stands in hardwater lakes in Poland was 0.85–1.93 mgP/gDW. Considering a mean value from this range of 1.4 mgP/gDW, and the mean biomass of 1000 gDW/m^2 determined in our study in the Ruidera lakes, this would amount to 1400 mgP/m^2, a large phosphorus pool contained in the charophyte stands. For comparison, this amount exceeds, >10-fold, a phosphorus pool of 50–100 mgP/m^2 in a 5–10 m water column holding an upper threshold concentration of 10 mgP/m^3 for oligotrophic lakes (Kalff, 2002). Recently, Sand-Jensen et al. (2021) also determined extensive phosphorus pools associated with charophyte meadows in Swedish ponds (200–600 mgP/m^2; about 2/3 incorporated in the macroalgae tissues and 1/3 in the carbonate encrustations). Therefore, if the charophyte stands disappear, this phosphorus would be available for other organisms such as phytoplankton, cooperating to eutrophication by internal loading.

As discussed above, perennial charophyte beds may have the potential to serve as nutrient sinks. Rodrigo et al. (2007) determined nitrogen retention estimates due to charophyte assimilation in the Ruidera lakes by the dense *Chara hispida* beds of approximately 4300 kg N/km^2 yr (net evergreen plant and detritus retention); recently, Vermaat et al. (2020) have estimated similar nitrogen retention (4275 ± 1680 kg N/km^2 yr) by the *Chara tomentosa* beds in lake Ohrid. Moreover, they may constitute greenhouse gas sinks, particularly CO_2 through its incorporation during photosynthesis (Kufel and Kufel, 2002). However, when they die, they represent a release of greenhouse gases such as CO_2, as well as CH_4 by means of decomposition from their detritus. Recently, the maximum production rate of methane from *Chara hispida* detritus (the main charophyte species in the Ruidera lakes) has been determined as 2.2 g CH_4/kgDW day (Puche et al., 2024). In the hypothetical case that all the charophyte biomass decompose in each lake, the production of CH_4 could range between 0.01 t CH_4 (lake Salvadora) and 9.2 t CH_4 (lake Colgada), and considering the biomass of all the 1537 t DW of charophyte stands in all the lakes, and a mean temperature of 20 °C, the overall emission can be estimated as high as 26 t CH_4. Furthermore, our investigations also revealed that an increase in temperature of 5 °C increased the methane production rate by three-fold in mesocosm experiments (unpublished results). Therefore, facing a global warming scenario, the overall emission directly from charophyte detritus could hypothetically reach 70 t CH_4 (which would represent 2086 t CO_2eq.; considering the methane warming potential as 1 kg CH_4 * 29.8 = 29.8 kg CO_2eq; IPCC, 2022); hence the need to preserve the macrophyte stands in the lake complex to act as C sinks and not as C sources.

The immensely large macrophyte bio-surface found in most of the Ruidera lakes, as stressed for other similar habitats (Teubner et al., 2022), represents a vast spatial dimension for additional habitats for freshwater biota, thus, macrophytic beds are essential to preserve other species. This is the case of the endangered *Salaria fluviatilis,* one of the few native fish species which still live in the Ruidera lakes, and which were able to survive the introduction of pike in 1953, which predate on them. This is a benthic fish species, endemic to circum-Mediterranean rivers and lakes (Méndez et al., 2019) which can find refuge within the submerged vegetation to avoid predators (Elvira et al., 2021). It is also the case of the freshwater shrimp (*Athyaephira desmarestii*) and the hydrides which populations has been shrunken in the last years (pers. obs.). The vulnerable species of freshwater mussel *Unio tumidiformis*, whose current distribution is limited to the south-western Iberian Peninsula, and whose populations are scarce and generally small (Reis and Araujo, 2016), has been detected in the Ruidera lakes (IUCN Red List). These lakes also harbor the Iberian pond turtle (*Mauremys leprosa*) and the Eurasian otter (*Lutra lutra*), the latter requiring a complex and stable habitat (Bueno-Enciso et al., 2014), such as that provided by submerged vegetation beds, among others. Their disappearance would largely affect these other species. The inexistence of a submerged vegetation *continuum* (lack of macrophyte beds in the upper lakes) represents habitat fragmentation for those species which need vegetation for their persistence.

5. Final remarks and conclusions

The *Lagunas de Ruidera* Natural Park represents a well-preserved example of present day fluvial and lacustrine carbonates (tufas), comparable in extent to the carbonates of the UNESCO World Heritage Site, Plitvice National Park, Croatia (Vurnek et al., 2019), but developed under more seasonally and semi-arid conditions. Therefore, it is one of the few habitats of this type throughout Europe where long-lasting surface water in arid-lands provide oases and refuge for the aquatic biota. Currently, the aquatic plant richness is lower than 20 years ago; however, on the positive side, some improvements have been observed: (i) the recovery of submerged meadows after sudden disturbances due to point pollution sources; (ii) an enhancement of the eutrophication status in some lakes; (iii) no exotic plant species were found. The mature submerged macrophyte stands of the Ruidera lakes create a unique macrophyte habitat architecture, and they represent the main component in the network between benthic (lake bottom) and pelagic (lake water) habitats. Moreover, the stands are formed by charophytes, a key group of submerged macrophytes in temperate hardwater lakes which have declined, or been replaced by angiosperms, as a result of eutrophication for >100 years in Europe (Alirangues Núñez et al., 2023). The disappearance of charophyte meadows would represent an important loss of regulating, provisioning and cultural ecosystem services and indirect/long-term benefits. Therefore, we assert the urgency of promoting management and conservation efforts regarding macrophyte meadows, mainly in the upper lakes, to restore the diverse and vast community that inhabited the natural park in the past. We envisage some measures that can be used to enable this complex of lakes to be conserved: (i) a detailed study of the causes (e.g., the presence of pesticides –in water and sediments-, or other reasons) of the disappearance of macrophyte meadows in the upper lakes to assess the relative ecological risks; (ii) the protection of the groundwater resource (aquifers): as the Ruidera lake complex has a low regulation capacity, making it vulnerable to drought, overexploitation of the aquifer must not occur, particularly over the next few years of foreseeable low rainfall due to climate change; (iii) the assurance that the lakes do not receive wastewater without tertiary treatment, and coping with point and non-point sources of pollution (primarily from the agriculture).

CRediT authorship contribution statement

Eric Puche: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Daniel Cruz:** Writing – review & editing, Methodology. **Pilar Delgado:** Writing – review & editing, Methodology. **Joanna Rosińska:** Writing – review & editing, Methodology. **Maria A. Rodrigo:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data used for this study is provided in the supplementary material

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Appendix A. Supplementary data

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