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Assessing the potential of Albufera de València Lagoon sediments for the restoration of charophyte meadows

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ABSTRACT

Albufera de València Lagoon, the largest littoral lagoon on the Iberian Peninsula, has been affected by eutrophication processes since the 1970s due to the direct dumping of sewage waters of industrial, agricultural and urban origin. Consequently, the submerged vegetation that covered the bottom of the lagoon has been lost (charophytes and *Potamogeton* spp., *Myriophyllum* spp. and *Ceratophyllum* spp.). Despite efforts to improve water quality, this vegetation has not recovered. No information about the potential of the recent sediments to host charophyte development is available. In this study, we analysed several sediment cores taken from different areas of the lagoon to determine the spatial distribution of past charophyte communities (based on the fructifications, i.e., oospores and gyrogonites, found in the sediments), to ascertain whether propagules remain in the upper sediments and to determine the potential of the surface sediments (containing pollutants, e.g., organochlorinated pesticides, heavy metals and pharmaceuticals) to host the growth and germination of charophytes if clear water is restored to the lagoon. The study was based on laboratory experiments. Generally, the same species of charophytes were found in all studied areas of the lagoon. The oospores and gyrogonites were located immediately surficially (in the first 5 cm) in certain areas, and these might be capable of germinating in this sediment stratum. The more recent sediment strata (the upper 14 cm), corresponding to the eutrophication period, can support the vegetative growth of *Chara hispida* and *Chara vulgaris* and promote the germination of *Chara aspera* and *Chara baltica*. We conclude that if the underwater light conditions are improved and nutrient concentrations are reduced in Albufera de València Lagoon, the reestablishment of charophytes might be possible both naturally and through specific management measures (namely, programmes involving seeds as planting stock or large-scale planting activity). This approach necessarily requires an improvement in the water quality of the inputs to the lagoon and an increase in the amount of water discharge to reduce the water residence time. These measures would restore the highly desirable positive feedback between submerged vegetation and water quality.

1. Introduction

The disappearance of submerged vegetation in lakes, lagoons and other types of aquatic environments as a result of anthropogenic effects is, unfortunately, a worldwide trend (Scheffer et al., 1993; Lu et al., 2012). On many occasions, lakes have undergone eutrophication due to the direct dumping of sewage waters of industrial, agricultural and urban origin. Thus, many pollutants (e.g., organochlorinated pesticides and heavy metals) are expected to accumulate in the sediments (Mateo et al., 1998; Peris et al., 2005), and pharmaceuticals may even be present as pollutants (Vázquez-Roig et al., 2011). This is the case for Albufera de València Lagoon (AV Lagoon), presently a hypertrophic Mediterranean system, which lost its submerged vegetation (primarily charophytes, with other macrophytes) more than 40 years ago (Dafauce, 1975; Rodrigo et al., 2010). The scientific literature cites many examples of the negative effects of eutrophication on charophyte meadows due to phytoplankton proliferation and the resulting decrease in light penetration (Blindow, 1992; Simons et al., 1994), as well as the harmful effects of certain herbicides and other pollutants on charophytes and submerged vegetation in general (Biernacki et al., 1997; Gao et al., 2002; Cedergreen et al., 2005, 2007).

Several types of measures, including the urgently needed implementation of sanitation systems, have been applied to improve the water quality of AV Lagoon (TYPSA, 2005). Currently, efforts to enhance the trophic status of the lagoon continue with the development of a pilot project consisting of the installation of

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several free water-surface constructed wetlands and two shallow lagoons to improve the water quality of the inputs to the lagoon (Martín et al., 2013; Rodrigo et al., 2013). Nevertheless, the lagoon still lacks submerged vegetation. The requirements for successfully re-establishing submerged macrophyte communities in eutrophicated lakes are not easy to determine. However, the restoration of these communities in lakes that have entirely lost their submerged macrophyte vegetation can be supported by studies of historical vegetation data and banks of remnant fructifications in the old sediments (Ozimek, 2006; Rodrigo et al., 2010; Lu et al., 2012). Therefore, the critical issues in developing restoration strategies are to evaluate whether submerged vegetation can develop (i) naturally from the remaining macrophyte stands (in case they still exist), (ii) from the seed banks (Ozimek, 2006; Ma et al., 2012; van Zuidam et al., 2012) and (iii) from naturally introduced propagation units or founder colonies of macrophytes to give rise to submerged vegetation meadows (Smart and Dick, 1999; Shafer and Bergstrom, 2010; Rodrigo et al., 2013). An additional issue is to determine whether polluted sediments can host the germination and/or vegetative growth of macrophytes. As indicated above, AV Lagoon has no remnant macrophyte stands, but the past composition of the charophyte communities of one part of the lagoon is known (Rodrigo et al., 2010). Thus, the only feasible restoration strategy must be based on sediment seed banks and on naturally occurring or supported propagation units.

In this study, we analysed several sediment cores taken from different parts of AV Lagoon (i) to determine the spatial distribution of past charophyte communities, (ii) to ascertain whether propagules remain in the upper sediments and (iii) to determine the potential of the surficial sediments (containing pollutants) to host the growth and germination of charophytes if clear water is restored to the lagoon. The study was based on laboratory bioassay experiments. Finally, we provide a recommendation for restoration based on our investigations of AV Lagoon relative to sediments and submerged vegetation.

2. Methods

2.1. Study site

AV Lagoon (39°20′00″ N, 0°21′22″ W, Fig. 1) has a surface area of 23 km^2 (it is the largest littoral lagoon on the Iberian Peninsula) and a mean depth of approximately 1 m. It originated from a closed gulf and is currently connected to the sea by three sluice gates. It is the main component of a Natural Park established in 1986 by the Regional Government. The Park has been included in the Ramsar list of important wetlands since 1990, has been considered a relevant habitat under the EU Bird Directive (79/409/CEE) since 1991 and possesses species and habitats considered under the EU Habitats Directive (92/43/CEE). The lagoon is surrounded by 140 km^2 of fields devoted to rice crops. It is fed with freshwater from a large number of channels and gullies associated with agricultural uses and from springs located either within the lagoon (in the past, Roselló, 1976) or in the surrounding marshland. The freshwater inflows are related to the development of rice growing in the area. High-intensity rice culture developed around the 18th century and caused a progressive decrease of the salinity levels of the lagoon from their past high levels (24–16 g l^{-1} in XVII-XVIII centuries, Alonso-Guillén, 2011) to the current value of 1–2 g l^{-1}. Presently, the lagoon is hypertrophic (67 ± 58 μg Chl *a* l^{-1}, 0.26 ± 0.10 mg TP l^{-1} and 3.0 ± 1.8 mg TN l^{-1}, annual means ± standard deviations from monthly measurements for 2009–2012; unpublished results), with low water transparency (Secchi depth = 0.27 ± 0.15 m; Soria, 2006), mainly due to the high chlorophyll *a* levels. Its submerged vegetation (charophytes and other macrophytes) were lost many years ago. During the past decades, sewage waters from industrial sources (in response to the industrialisation of the neighbouring areas), agricultural sources (extensive agricultural land uses) and urban sources (due primarily to the demographic expansion of the neighbouring villages) were discharged directly into the lagoon without any type of treatment, causing massive eutrophication. The Chl *a* concentrations shifted from mean values of 15 μg l^{-1} in 1972 (Dafauce, 1975) to maxima of 700–800 μg l^{-1} in the early eighties (Vicente and Miracle, 1992). Currently, the Chl concentrations are half of the time over 50 μg l^{-1} (Rodrigo et al., 2010), and are over 100 μg l^{-1} in 23% of the occasions in the period 2009–2012. The exception are some clear-water phases during which the phytoplankton populations drastically decrease to values below 5 μg l^{-1}, events primarily related to the flushing of the lagoon (Sahuquillo et al., 2007).

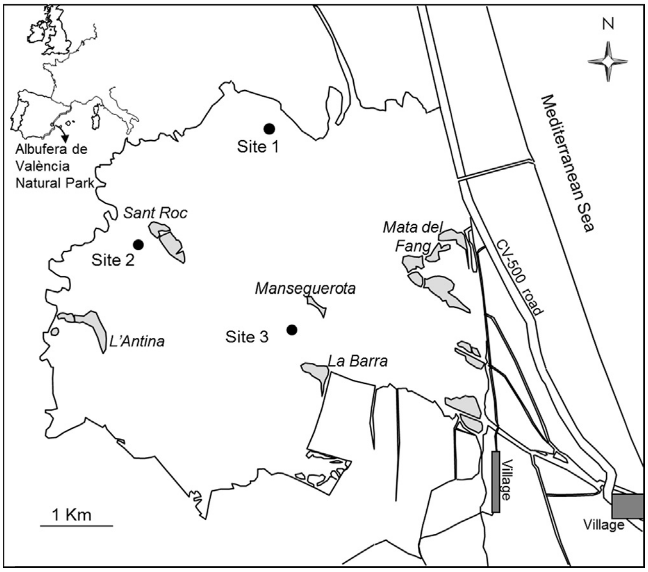

Fig. 1. Location of AV lagoon and sediment core sampling sites.

2.2. Sediment core collection

Six sediment cores were extracted from three sites (i.e., two cores from each site) in the lagoon in January 2008 (Fig. 1): site 1, locally called El Brossar, northern portion of the lagoon; site 2 to the west of Mata de Sant Roc, one of the vegetation (reed-rhizome created) islands; and site 3 to the west of another island, Manseguerota. These cores were used to isolate, identify and quantify the fructifications produced by the charophytes. The sediment cores were taken from a boat with a Beeker-type sampler (Eijkelkamp, Giesbeek, The Netherlands). The cores were 80–88 cm in length and 5 cm in diameter. Based on information about sedimentation rates, these strata represent approximately 340 years, reaching approximately the year 1675 at the 86–88 cm sediment stratum (Alonso-Guillén, 2011). Immediately after sampling, the sediment cores were divided into equal lengths of 2 cm with a hydraulic sediment sampler accessory. The sediment samples were stored in a refrigerator in darkness until the process of isolation and identification of fructifications was complete. In March 2009, three more cores were extracted from site 1. The cores were divided equally, as described above, and the upper 14 cm was used for the vegetative growth and germination experiments. Site 1 was chosen because the northern part of the AV Lagoon drainage area has the largest human population and, accordingly, the most severe pollution problems and because, according to Peris et al. (2005), the concentrations of persistent organochlorinated pesticides at site 1 were the highest at any of the three sites, ranging between 5.1 and 10 ng g^{-1} sediment.

2.3. Isolation, identification and quantification of charophyte fructifications

Each 2-cm sediment slice was homogenised with a spatula, and an aliquot was used to determine the water content of the sediment immediately subsequent to sampling. Another aliquot was used for sediment dating in one of the cores from site 1 (Rodrigo et al., 2010). The remaining sediment (approximately 25 g dry weight (DW) per layer) was sequentially washed with tap water and sieved through 1000, 500 and 250 μm mesh. After sieving, the >250 and >500 mm fractions were observed with a stereomicroscope to search for charophyte remains. These remains consisted of (i) oospores formed by the organic cell wall produced around the fertilised egg cell and (ii) gyrogonites, the calcified oospores. The gyrogonites and oospores were removed with a brush and/or forceps and separated by species. Four types of fructifications were distinguished: "apparently viable" and non-viable oospores and gyrogonites. Apparently viable structures were defined as intact and hard oospores that did not deform if they were pressed with the forceps and had "healthy" starch granules inside (de Winton et al., 2004) and gyrogonites with an intact calcium carbonate covering. The taxonomic characteristics used to identify the oospores and/or gyrogonites were as follows: theapical zone (the presence or absence of a "shoulder"), the basal zone (the presence of a basal column on the gyrogonites, the presence of a basal cage on the oospores), the shape of the basal plate of the gyrogonites and oospore wall ornamentation as well as other features such as the size, overall outline and number of striae (Soulié-Märsche, 1989; Rodrigo et al., 2010). The observations and measurements were made with a stereomicroscope at 160× and 400×, respectively. All the oospores and gyrogonites were then counted. The number of fructifications was originally based on an analysed area of 20 cm^2. However, because we observed that the sediment layers differed in water content, the number of fructifications was expressed per gram DW of sediment. Depth profiles of the total oospore and gyrogonite densities, as well as detailed distributions of each species, were obtained with the Psimpol 4 program.

2.4. Vegetative growth and germination experiments on recent AV Lagoon sediments

The upper 14 cm of the sediment cores from site 1 corresponded to the eutrophication period of the lagoon, when the submerged macrophytes disappeared. According to a previous sediment-dating study (Rodrigo et al., 2010), the cores covered approximately 35 years. The first 8 cm corresponded to the period between 2008 and 1984, and the portion from 8 to 14 cm corresponded to the period from 1984 to 1973. Each of the 2-cm sediment slices from the last three sediment cores taken from site 1 were pooled together, homogenised and used as "substrate treatments" in the charophyte vegetative growth and oospore germination experiments.

The growth of *Chara hispida* L. and *Chara vulgaris* L., two species that could have covered and covered, respectively (*C. hispida* has appeared spontaneously in restored habitats in the boundary of AV Lagoon; Rodrigo et al., 2013), the lagoon bottom in the past (Rodrigo et al., 2010), was tested in the different AV sediment layers. A total of 59 small plastic containers were filled with the homogenised sediment (2 species × 4 replicates × 7 treatments: 0–2 cm, 2–4, 4–6, 6–8, 8–10, 10–12, 12–14, and 3 "control" pots: a mixture of sediment and commercial sand used for maintenance of charophyte cultures in the lab). Two 1.5-cm apical portions of charophyte individuals cultured from specimens brought from waterbodies within the Natural Park were planted in the small pots containing the homogenised sediment. The pots were submerged in 8 containers (one for each treatment plus the "control") filled with dechlorinated tap water. One container was used for each treatment to avoid interference with charophyte growth by pollutants that possibly could have been released from a particular sediment layer. The experiment was performed in a culture room at 20 °C with a 12/12 L:D photoperiod and a light level of 20 μmol m^{-2} s^{-1} at the water surface. The experiment began on 27 March 2009 and ended on 27 June 2009. During this period, the maximum length of each charophyte was measured weekly. At the end of the experiment, the charophytes were pulled from the sediment and washed, and the DW was determined.

For the germination experiments, viable charophyte oospores of *Chara aspera* Dethard. ex Willd. and *Chara baltica* A. Bruzelius were collected from the sediment of a site near AV Lagoon (mallada Nova del Fang) where these species coexist (Rodrigo and Alonso-Guillén, 2008). The sediment was sieved and the oospores picked by means of a forceps. Groups of 40 fructifications were located inside double layers of 200 μm Nytal nets, which were further sealed with silicone. The netting allows the protonema to emerge after the oospores have germinated and facilitates further counting. Each net was buried 0.5–1 cm deep in Petri dishes containing duplicates of the same experimental sediment used for the vegetative growth trials. As "controls", cleaned sand and sediment from the original collection site were used as germination substrates. Three replicates per treatment were used for *C. aspera*; for *C. baltica*, due to the low number of oospores available, only the 0–2, 6–8 and 10–12 cm sediment layers were assayed. All Petri dishes containing the sediment and the buried fructifications were submerged in separate containers filled with dechlorinated tap water, one for each sediment depth treatment to avoid the possible release of pollutants from the sediment to the water, which could affect oospore germination in the different sediment layers. The containers were incubated in the culture room under the same conditions described above. The experiment began on 26 May 2009 and ended on 26 August 2009. The germlings emerging from the sediment were counted weekly. At the end of the experiment, the bags were extracted from the sediments, opened and observed under a stereomicroscope to check for recent germinations. The germination rates were calculated based on the data collected at the end of the experiment.

2.5. Data analyses

Differences in growth between *C. hispida* and *C. vulgaris* in the mean final length, weight, growth rate of the charophytes and oospore germination rates in the different sediment layers (treatments = age ∼ degree of pollution) were analysed with a one-way analysis of variance. Bonferroni tests were used as a post hoc test if necessary. Homeoscedasticity was checked with a Levene test. All analyses were performed with the statistical package SPSS (v. 11).

3. Results

3.1. Charophyte fructifications identified in AV Lagoon sediments

Oospores and gyrogonites of *C. aspera* (Fig. 2) were distributed differently in each sediment core. At sites 1 and 2, they appeared from 52 and 28 cm, respectively, and reached the end of the cores, with a maximum density of 2 oospores-gyrogonites (O-G)

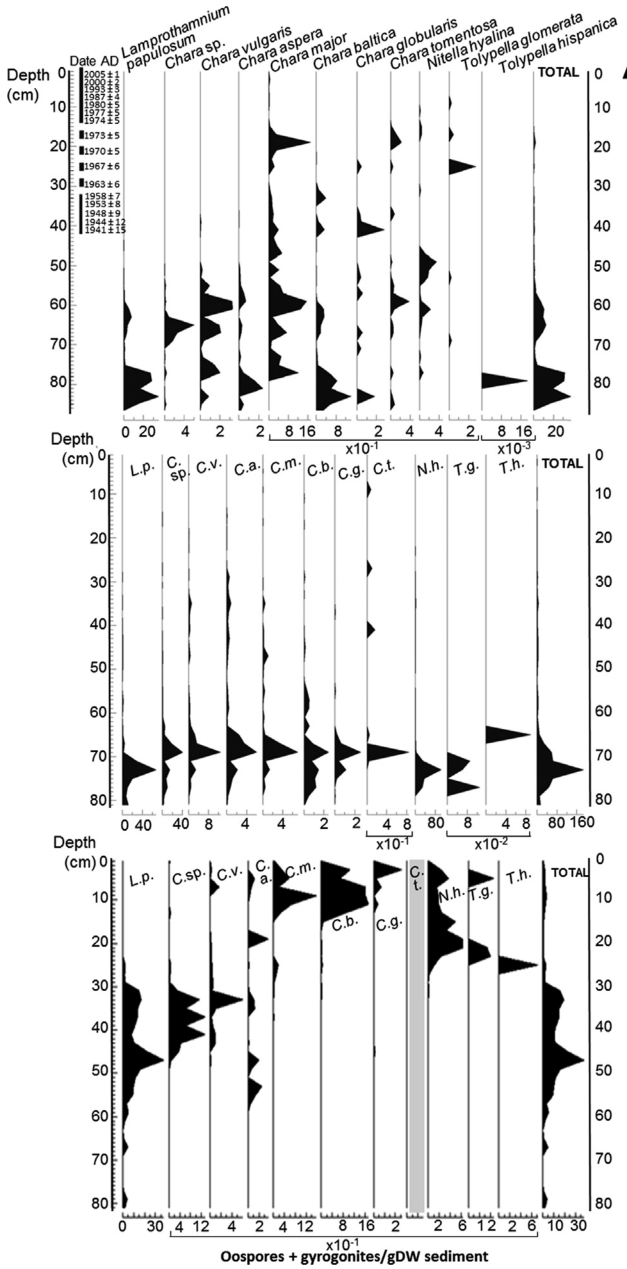

Fig. 2. Age-depth distribution and densities of the fructifications for each charophyte species in the sediments of AV lagoon. Cores from sites 1 (A), 2 (B) and 3 (C). Values are mean densities calculated from two cores in each site. Notice the different scale in fructificacitions density for each taxon. Chronological frame based on ^{210}Pb dating for the sediment core from site 1 (see Rodrigo et al., 2010).

g DW sed.$^{-1}$. Most of the fructifications were apparently viable oospores. At site 3, they occurred from the upper part of the core to 56 cm and at lower densities. *Chara globularis* Thuillier fructifications were more abundant at site 2. Their maximum density was 2.6 O-G g DW sed.$^{-1}$. In most cases, they were apparently viable gyrogonites. *C. baltica* fructifications occurred at maximum densities at site 2 (2.5 O-G g DW sed.$^{-1}$). At site 3, they were found in the first 32 cm, and most were apparently viable gyrogonites. *Chara major* Valliant ex Hy fructifications were found at maximum densities of 7 O-G g DW sed.$^{-1}$ (site 2), and most were also apparently viable gyrogonites. Fructifications were more scarce at site 3, but most were apparently viable oospores. *Chara tomentosa* L. fructifications were found only at sites 1 and 2 in certain sediment layers, with maximum densities of 0.9 O-G g DW sed.$^{-1}$. *C. vulgaris* fructifications were more abundant at site 2 (maximum of 13 O-G g DW sed.$^{-1}$), but with a narrower depth distribution. Most of them were apparently viable gyrogonites. *Chara* sp. fructifications, a morphotype that could not be assigned to any known species (Rodrigo et al., 2010), were found from 50 cm to the end of the core at site 1 with a maximum density of 6 O-G g DW sed.$^{-1}$. Most were apparently viable gyrogonites. At site 2, these fructifications appeared with a maximum density of 20 O-G g DW sed.$^{-1}$ at 68–70 cm. They were much less common at site 3 (1.3 O-G g DW sed.$^{-1}$). At all sites, most were apparently viable gyrogonites. Fructifications with a tuberculated morphology represented approximately 20% of the total at sites 1 and 2 and up to 80% at site 3. *Lamprothamnium papulosum* (K. Wallroth) J. Groves fructifications appeared from 56 cm and from 48 cm at sites 1 and 2 respectively. At site 3, they were concentrated at approximately 26–62 cm. *Nitella hyalina* (DC.) C. Agardh oospores were scarce at sites 1 and 3. However, they were very abundant at site 2, particularly at 72–74 cm (104 oospores g DW sed.$^{-1}$) and were apparently viable. *Tolypella glomerata* (Desv.) Leonh. fructifications were very scarce (0.02–0.2 O-G g DW sed.$^{-1}$) and appeared scattered in the sediment profile. Only a few *Tolypella hispanica* Nordstedt oospores were found (0.2–0.09 O-G g DW sed.$^{-1}$).

3.2. Vegetative growth of charophytes and oospore germination on recent AV Lagoon sediments

C. vulgaris and *C. hispida* reached the same final length under the “control” conditions. However, the growth of *C. vulgaris* individuals was almost double (26 ± 9 cm final length; 0.13 ± 0.09 g final DW) than that of *C. hispida* (15 ± 6 cm; 0.08 ± 0.05 g) (ANOVA$_{\text{length}}$: $F_{d.f.:1;102} = 49.8$; $p < 0.0001$) (ANOVA$_{\text{weight}}$: $F_{d.f.:1;104} = 12.7$; $p = 0.001$) under the treatments (2-cm sediment layers from different depths). The growth rates based on total length for the first 25 days of the experiment were 0.4 cm d^{-1} for *C. vulgaris* and 0.2 cm d^{-1} for *C. hispida* (Table 1). The ANOVA results showed no statistically significant differences among treatments in the final length of *C. hispida* and *C. vulgaris* specimens. However, when the data were pooled for the first 8 cm (sediments accumulated between 1984 and 2008) and compared to the pooled data for the 6 cm below (from 8 to 14 cm, sediments accumulated between 1973 and 1984), the final length was significantly greater and the dry weight significantly higher for the charophytes grown on deeper and older sediment layers for both species (Fig. 3) (*C. vulgaris*: ANOVA$_{\text{length}}$: $F_{d.f.:1;51} = 9.6$; $p = 0.030$; ANOVA$_{\text{weight}}$: $F_{d.f.:1;51} = 26.5$; $p < 0.0001$; *C. hispida*: ANOVA$_{\text{length}}$: $F_{d.f.:1;49} = 15.7$; $p = 0.001$; ANOVA$_{\text{weight}}$: $F_{d.f.:1;49} = 22.7$; $p < 0.0001$).

In the germination trials, the first germlings were observed at 15 days after the beginning of the experiment and continued to appear throughout the experiment. The germination rates of *C. aspera* oospores were significantly lower in both “controls” than in the different sediment layers (ANOVA: $F_{d.f:1;26} = 5.8$; $p = 0.024$) (Fig. 4). The highest rates were found in the shallowest and deepest sediment layers (mean approximately 20%). The germination rates of *C. baltica* oospores were greater and showed a depth germination pattern similar to that of *C. aspera*, with rates close to 50% in the shallowest sediment layer, approximately 70% in the deepest layer assayed and less than 10% in the intermediate layers (Fig. 4). Pooling the *C. aspera* germination data with the same criteria as for vegetative growth, no statistically significant differences were found among the shallower and deeper layers (ANOVA: $F_{d.f.:1;20} = 2.7$; $p = 0.114$). However, based on pooled data for 4–8 cm, the germination rates were significantly lower than for deeper and older layers (ANOVA: $F_{d.f.:2;19} = 3.8$; $p = 0.042$).

Table 1
Growth rates of the two charophytes species in each AV lagoon sediment layer.

Sediment depth	*Chara hispida*		*Chara vulgaris*	
	cm d^{-1}	(gDW d^{-1}) × 10^{-4}	cm d^{-1}	(gDW d^{-1}) × 10^{-4}
0–2	0.18	9	0.3	9
2–4	0.15	6	0.2	6
4–6	0.14	7	0.3	13
6–8	0.16	9	0.3	13
8–10	0.22	14	0.4	29
10–12	0.27	16	0.4	24
12–14	0.18	12	0.3	20
0–8 (~2008–1984)	0.16	7	0.26	10
8–14 (~1984–1973)	0.22	14	0.36	24

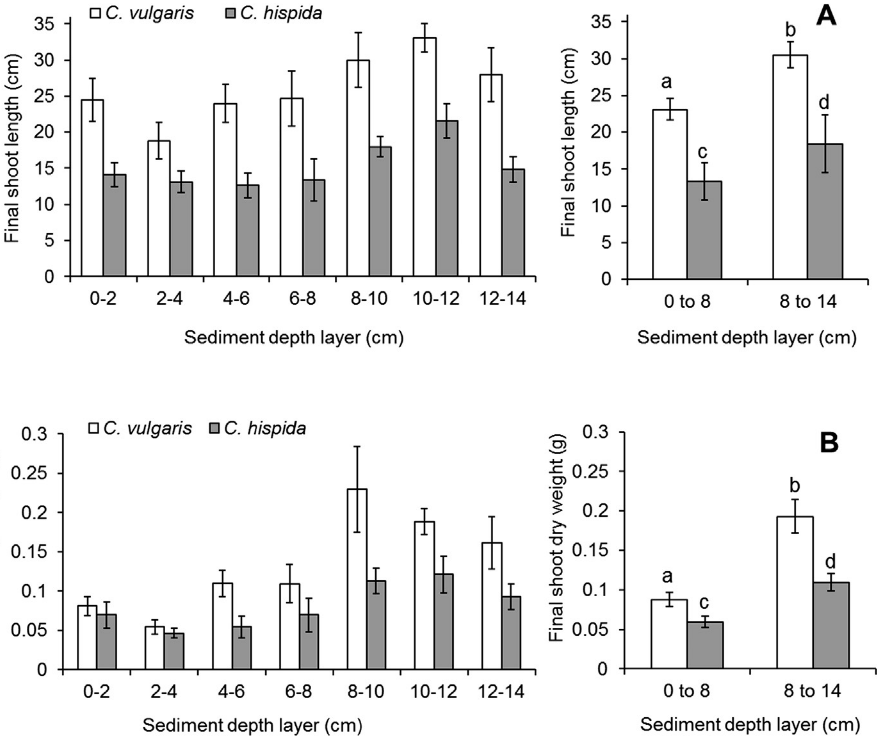

Fig. 3. Final length (A) and final weight (B) reached by *C. vulgaris* and *C. hispida* in each sediment layer (left), and integrating the eight first centimetres (ca. from 2008 to 1984) and the six deeper cm (ca. from 1984 to 1973) (right). Vertical bars show standard error of replicates. Significant differences are indicated with different lower-case letters ($p < 0.05$).

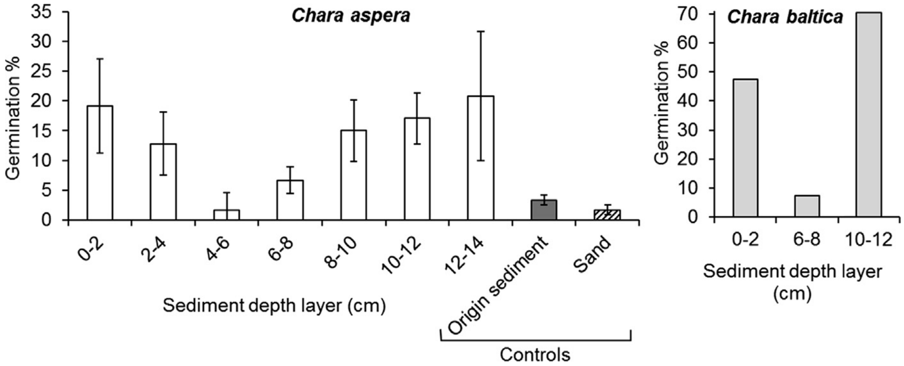

Fig. 4. Germination percentage of oospores of *C. aspera* and *C. baltica* in the AV lagoon sediment layers and in the "controls". Verticals bars show standard errors.

4. Discussion

4.1. Spatial and sediment strata distribution of charophyte propagules

Historical bibliographic data on submerged vegetation in AV Lagoon are scarce, but several references reported the following charophyte species from the lagoon: *C. hispida* (*Chara aculeolata*) (Prósper, 1910; Corillion, 1962; Guerlesquin, 1963), *Chara polyacantha* A. Braun, *C. baltica* (Corillion, 1962), *C. major*, *C. tomentosa* (*Chara ceratophylla*) (Prósper, 1910), *C. globularis* (*Chara fragilis*) (Dafauce, 1975), *C. aspera* (Corillion, 1962; Guerlesquin, 1963; Boira and Carretero, 1985), *N. hyalina* (Prósper, 1910; Boira and Carretero, 1985), *T. glomerata* (Prósper, 1910; Comelles, 1982) and *L. papulosum* (only oospores cited in the study of Dafauce, 1975). Oospores of all these species were found in the sediments of AV Lagoon during our study. Moreover, we identified fructifications of *C. vulgaris* and *T. hispanica*; thus, the charophyte communities developed in the lagoon in the past consisted of at least 11 taxa. The diversity of the charophyte flora obtained with the used sediment cores is characteristic of a long time period, extending to periods when the waters of AV Lagoon were saline (Marco-Barba, 2010; Rodrigo et al., 2010), as corroborated by the presence in the deep and old sediment layers of fructifications of charophyte taxa typical of saline water, such as *L. papulosum* (Soulié-Märsche, 1998) and *T. hispanica* (Cirujano et al., 2008; García and Chivas, 2004). Other taxa found in shallower sediment layers, such as *C. globularis* and *N. hyalina*, are characteristic of freshwater (Cirujano et al., 2008). Taxa with a broader ecological niche are represented by *C. vulgaris* and the "*C. hispida*" group. A detailed interpretation of the succession of the charophyte flora over time in AV Lagoon based on the sediment fructifications has previously been presented by Rodrigo et al. (2010) in terms of the information obtained from a single sediment core. Here, we discuss the heterogeneity of the spatial distribution of charophytes in the lagoon and we focus on the charophyte species with current re-vegetation potential.

Fructifications from the same charophyte species were found in the three parts of the lagoon, with the exception that *C. tomentosa* propagules were absent from site 3. The depth distributions of the density of fructifications in the cores were very similar for sites 1 and 2. However, the results from site 3 showed that the 0–30 cm sediment depth of the sediment column was absent at this location. Thus, an older age was reached (estimated as approximately 16th century from sedimentation rates, Alonso-Guillén, 2011) at the same sediment depth. The distribution of all fructifications is out of phase over these 30 cm. Site 3 was near one of the reed-rhizome formed islands in AV Lagoon in an elevated part of the lagoon basin. In the 60s, this island had a surface area of 0.12 km^2 and was surrounded by dense meadows of submerged macrophytes. The submerged vegetation disappeared during the eutrophication process, and the island and the surficial sediments were eroded by the strong waves, which were not buffered by the submerged macrophytes. The current surface area of the island is less than 0.01 km^2 (J.M. Benavent; pers. comm.), and the upper sediments found in other parts of the lagoons are missing here. As a result, buried freshwater charophyte fructifications (e.g., *C. globularis*, *C. major*, *C. tomentosa* and *N. hyalina*) are found just at the surface of the sediment in close contact with the water column. This position is important in view of the possibility that charophyte germination could result if the water transparency were improved. Charophyte oospores, despite their small size relative to the seeds of other submerged macrophytes, can germinate and emerge through 4 cm of sediment layer (Bonis and Lepart, 1994). As shown in our previous study (Rodrigo et al., 2010), charophyte oospores from AV Lagoon sediments as old as 60 years can germinate in a few days after the sediments are covered with transparent water.

The depth distributions in the sediment at sites 1 and 2 of the fructifications of certain species were quite similar (*C. vulgaris*, *Chara* sp. and *L. papulosum*), but other species (e.g., *C. aspera*, *C. globularis*, *N. hyalina*) were distributed differently at these sites. A remarkable finding was that the oospores of *N. hyalina*, a freshwater species, with the highest maximum reported for any of the species found in the AV Lagoon sediments (more than 100 oospores g DW $sed.^{-1}$) were located at a relatively great depth at site 2. Because that sediment depth corresponds to the period when the AV Lagoon was saline, one explanation of such a large accumulation of the oospores of a freshwater species might be the existence of a freshwater spring (Roselló, 1976) that could have decreased the salinity of the immediately surrounding waters. *N. hyalina* prefers to grow in freshwaters but can also grow in slightly saline water (Cirujano et al., 2008). An alternative explanation is that oospores generated at other sites had been transported to the site where they were found and had accumulated there.

In this study, we did not find oospores and gyrogonites in noteworthy amounts before a sediment depth of 14 cm at two of the three sites (depth corresponding to 1974 ± 5 according to the sediment dating; Rodrigo et al., 2010). This finding is consistent with the observation that the eutrophication of the AV Lagoon during the past 50 years (Dafauce, 1975; Soria, 2006) severely affected the development of the charophytes and caused their complete disappearance. The fructifications found in the upper sediments belonged to freshwater species that can also be found in the rice fields of the Natural Park (Alonso-Guillén, 2011). Fructifications formed in the rice fields may reach the AV Lagoon sediments when the rice fields are emptied during the crop cycle. This observation would explain why some oospores were found at sediment depths corresponding to the time when charophytes had already disappeared from those sites where the surficial sediments were intact (sites 1 and 2).

4.2. Sediment bioassays

Persistent organochlorinated pesticides (the greatest average concentrations corresponded to pp′-DDE, pp′-DDD and pp′-DDT), heavy metals, pharmaceuticals and other compounds have been reported from AV Lagoon sediments (Mateo et al., 1998; Canet et al., 2003; Peris et al., 2005; Vázquez-Roig et al., 2011). Substrate factors such as salinity, texture, organic matter content and humic acids can affect the toxicology of such pollutants. Our results indicate that the levels of these compounds in AV Lagoon sediments are not too harmful, at least for the assayed charophytes. Charophytes are resistant to herbicides such as diquat, to which vascular plants are sensitive (Tanner et al., 1990; Clayton and Matheson, 2010). However, neither the growth rates nor the germination rates were the same in sediment layers originating in different time periods. In 1992, a sanitation plan for the region promoted a series of actions in towns and tourist housing in the southern portions of AV Lagoon. As a result of the plan, sewage treatment plants and other sanitation infrastructures were installed in this area. All these actions prevented a large portion of the nutrients from reaching the lagoon (Verdú et al., 1999; TYPSA, 2005). Consequently, it is probable that the inputs of pollutants and toxic substances were also reduced. In 1995, a slight improvement of the trophic state was already evident in the lagoon (Soria, 2006). Our data show a progressive decrease in the germination rates of *C. aspera* oospores, culminating at the sediment depths corresponding approximately to 1996, in comparison with shallower and deeper sediments (although *C. baltica* oospores, with fewer data, showed a similar pattern). The shallower sediment layers (1996–2008), presumably with a lower content of toxic substances due to the sanitation measures completed (Soria, 2006), would have produced higher oospore germination rates.

Variables such as sediment grain size, organic matter and nitrogen content are some of the factors affecting the breakage of dormancy in charophyte oospores (Selig et al., 2007; Kalin and Smith, 2007). Overall, we observed a much higher oospore germination percentage in the AV Lagoon sediment than in the sediment from which the assayed oospores were obtained or in commercial sand. The higher organic matter content in AV Lagoon sediments (not measured, but its appearance suggested this observation), as well as higher inorganic nutrient content, than in the sediment from the oligotrophic waterbody (Rodrigo and Alonso-Guillén, 2008) that was the source of the *C. aspera* and *C. baltica* oospores could be the reasons for the higher germination rates.

The vegetative growth of both *C. hispida* and *C. vulgaris* was more vigorous and more rapid in the deeper (8–14 cm; 1984–1973) sediment layers used as the culture substrate than in the sediment layers from 1984 to 2008 (0–8 cm). Does this finding mean that toxic compounds could have been present, with negative effects on vegetative growth, but could have lost their toxicity in the older sediments? The patterns observed for germination and growth as a function of sediment depth (age) were not the same. It is probable that toxic substances affected both processes in different ways (Kalin and Smith, 2007). It is probable that toxins from cyanobacteria also accumulated in the sediments. AV Lagoon water was found to contain microcystin concentrations that were above the recommended guidelines in several cases (Romo et al., 2013). The sediments are important sinks for cyanobacteria and, consequently, for microcystin accumulation, but the adsorption of microcystin varies significantly with texture, pH, and organic matter content of sediments (Munusamy et al., 2012). It has been recently proved that the germination of charophytes is affected by the microcystin content of the sediments, even if these microcystin levels are not extremely high. For example, in laboratory experiments performed adding 8 and 16 μg microcystin-LR/L on sediments collected in a restored small lagoon within AV Natural Park (Rodrigo et al., 2013), *Chara* germination rates were 44% and 11%, respectively, of the germination that occurred in treatments without microcystin (Rojo et al., 2013). The growth of *C. vulgaris* was greater than that of *C. hispida*. Resource competition can be excluded as the reason for this difference because both species were planted in different pots and incubated in different containers. Accordingly, this result may indicate that *C. vulgaris* has a higher tolerance to toxic substances. In fact, this species has been used in Germany in bioremediation ponds that receive mining wastes (Kalin and Smith, 2007; Marquardt and Schubert, 2009). Charophytes are increasingly considered to represent a promising group of macrophytes for use in phytoremediation efforts, e.g., the detoxification of sediments polluted with cadmium, chromium, hydrocarbons and pesticides (Marquardt and Schubert, 2009; Pattiyage and Asaeda, 2009; Triboit et al., 2009; Clabeaux et al., 2011).

4.3. *Implications for restoration*

Dredging the AV Lagoon to remove the sediments has been considered by the managers for several years. However, this process is highly impractical due to its economic cost and the problem of treating many tonnes of sediment. As an alternative, the results obtained in this study show that if the underwater light environment is improved and the nutrient concentrations are reduced, i.e., if the trophic state of the lagoon is improved, reestablishment of charophytes on the current sediments could be possible. This solution necessarily requires an improvement in the water quality of the inputs to the AV Lagoon and an increase in the water discharge to reduce the water residence time (Romo et al., 2013). Almost every year, the lagoon experiences a brief clear-water phase related to the emptying of the rice fields in mid-winter through the lagoon when the sluice gates are opened. This action produces a period of high flushing and water renewal that causes decreased phytoplankton densities and induces a shift in phytoplankton species composition, with increasing proportions of edible algae. As a result, substantial cladoceran populations develop, and this shift reinforces the process of lake clearing, by reducing phytoplankton biomass (Sahuquillo et al., 2007). If the efforts in all the areas cited above continue to reduce the eutrophication level, with effective management of winter flushing to allow longer clear-water phases, the establishment of at least the turbidity-tolerant charophyte species will potentially allow the vegetation to recover (Arthaud et al., 2012). It is already known that (1) at least some portions of the AV Lagoon bottom have surficial sediments containing old but still viable charophyte oospores that can germinate if they are covered by transparent water (Rodrigo et al., 2010); (2) the abundant waterfowl populations may act to disperse the fructifications of the submerged vegetation (Raulings et al., 2011); (3) a semi-continuous input of oospores from the rice fields may occur when the fields are emptied, and these oospores could colonise and participate in the maintenance of charophyte communities in AV Lagoon; and (4) other management decisions concerning vegetation restoration can be implemented, e.g., the use of seeds as planting stock, or even ambitious large-scale plantings, as performed elsewhere to restore submerged vegetation. For example, a surface area of 4 km^2 in the highly polluted North American Chesapeake Bay (Shafer and Bergstrom, 2010) had been planted with submerged vegetation by 2008. As stated here, the surficial sediments of AV Lagoon are able to support macrophyte development, including both germination and growth. The establishment of submerged vegetation will allow the oxygenation of the sediment (Flessa, 1994), the retention of phosphorus and iron (Jaynes and Carpenter, 1986) and the oxidation of potential toxic substances resulting from anaerobic decomposition, such as ammonium and sulphur (van Wijck et al., 1992; Azzoni et al., 2001). The formation of calcite incrustations by charophytes would result in the removal of phosphorus and thus control the phytoplankton biomass (Siong and Asaeda, 2006). With these measures, it would be possible to restore the highly desirable positive feedback between submerged vegetation and water quality.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2013.09.041. These data include Google maps of the most important areas described in this article.

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