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Salt variation induces oxidative stress response in aquatic macrophytes: The case of the Eurasian water-milfoil *Myriophyllum spicatum* L. (Saxifragales: Haloragaceae)

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ABSTRACT

Wetlands are very fragile systems and susceptible to being affected by human activity. *S'Albufera de Mallorca* Natural Park is the main wetland in Mallorca Island (Spain), and undergoes a salinization process derived from the overexploitation of adjacent aquifers, which favors marine intrusion. The objective was to evaluate the effects of salinity changes in a channel in *s'Albufera de Mallorca* through the analysis of biomarkers of oxidative stress in the submerged macrophyte *Myriophyllum spicatum*. Six different points were analysed along a channel characterized by different salinity values, ranging from ~2 to ~11. The % of macrophyte coverage in the studied stations followed an inverse salinity pattern with greater coverage in areas of low salinity and minimal coverage in those with higher saline concentration. The photosynthetic pigments index (D430/D665), determined in *M. spicatum* leaves, was significantly higher at the stations most affected by salinity. All antioxidant enzyme activities – catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase – followed a similar pattern of response with an increase in activity as salinity increases. Similarly, the levels of malondialdehyde, as marker of lipid peroxidation, were also increased the stations with the highest salinity. In conclusion, marine water intrusion causes an increase in the salinity of inland waters in *s'Albufera de Mallorca*, which is evidenced by the increase in the antioxidant enzyme activities and oxidative damage.

1. Introduction

Historically, wetlands have been considered as a waste of valuable land that could only be 'improved' through drainage and subsequently taken advantage of by humans (Mitsch and Gosselink, 2000). Today, it is well recognized that wetlands provide valuable ecological services for humans and wildlife such as protecting and improving water quality, providing fish and wildlife habitats or storing floodwaters (Bhowmik, 2020; Woodward and Wui, 2001). This is particularly important in coastal flood plains (Neri-Flores et al., 2019). Paradoxically, their functions can easily be overwhelmed in areas of intense human development, thus lessening those ecological values (Mitsch and Gosselink, 2000).

S'Albufera de Mallorca is the largest wetland in Mallorca island (Balearic Islands, Spain) comprising an area of 1,646.48 ha (1,450 ha of which correspond to wetlands, and the rest to dune ecosystem), surrounded by an area with high urban and agricultural development. However, it is considered one of the most important biodiversity hotspots of the Balearic Islands, mainly due to its condition of wetland (Pinya et al., 2008). For that reason, it was declared Natural Park in 1988, a RAMSAR wetland a year later (Riddiford and Mayol, 1996), and it is included at Natura 2000 European network as a Special Protection

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Area for Birds ES0000038 - *s'Albufera de Mallorca*. The protected area consists of a system of artificial channels that collect water from an extensive hydro geographical basin (Taberner et al., 1995). Predominant aquatic habitats contain fresh or brackish waters, according to the proximity of the sea. The wetland is mainly composed of reeds, with *Phragmites australis* (Cav.) Steud. and *Arundo donax* L. as dominant species (Ebejer, 2006).

S'Albufera de Mallorca is an area in a state of constant flux mainly due to continuous change of anthropogenic factors, creating ecotonal instabilities in the fresh and salt water coastal ecocline (Slater, 2016). In the last years, an increase in salinity has been detected in the southwest of this Natural Park derived from human activities causing overexploitation of aquifers and favouring marine intrusion (Galimont et al., 2003; Rebassa, 2015; Veraart et al., 2004). The main human activities responsible for the overexploitation of aquifers in S'*Albufera de Mallorca* derive from the great coastal urban and tourist development in the 1970s, just in front of the wetland, together with a greater and progressive demand for fresh water to supply agricultural tasks surrounding the natural park (Custodio, 2018; Slater, 2016). High salinity means an increase in ion concentration which represents an important abiotic stress factor. At cellular level, the excess of ions in the medium causes toxicity in the cells, due to an excessive uptake mainly Cl^- and Na^+, which induces a decrease in nutrient absorption leading to nutrient imbalance (Parihar et al., 2015). On the other hand, ions increase causes osmotic stress resulting in water deficit (Uddin and Juraimi, 2013). These effects induced by the high salinity affect aquatic plants at different levels such as germination, growth, photosynthesis, and photosynthetic pigments (Parihar et al., 2015). If the ion concentration reaches cytotoxic levels, metabolic processes are slowed down, premature senescence appears and, ultimately, cell death occurs (Isayenkov and Maathuis, 2019; Läuchli and Grattan, 2007).

Environment alterations, including salinity changes, can induce an increase in the production of reactive oxygen species (ROS) in affected organisms. An increase in ROS production, if not effectively deactivated, can damage proteins, lipids and DNA, inducing oxidative stress (Gil et al., 2019; Zhu, 2001). Organisms are provided with a complex protective mechanism consisting of antioxidant enzymes and other non-enzymatic defences to cope with the increase in ROS in order to avoid oxidative damage (Capo et al., 2015; Gil et al., 2019; Sureda et al., 2017). Antioxidant enzymes include catalase (CAT) which catalyses the detoxification of hydrogen peroxide, superoxide dismutase (SOD) which suppresses superoxide anion, and glutathione reductase (GRd) and glutathione peroxidase (GPx) which play a central role in glutathione regeneration (Espinosa-Diez et al., 2015). Non-enzymatic defences such as polyphenols, antioxidant vitamins, or glutathione can rapidly inactivate radicals and oxidants reducing the oxidative damage. Glutathione s-transferase (GST) is a phase II detoxifying enzyme that catalyses the conjugation of glutathione to a wide variety of electrophilic substrates (Cunha et al., 2005).

Since the induction of oxidative stress is an important way through which salinity exerts its effect on aquatic plants (Rout and Shaw, 2001), we hypothesize that in the areas with higher salinity of *s'Albufera de Mallorca* there is an activation of antioxidant defence mechanisms. The aim of the present study was to assess the impact of salinity changes in a channel of *s'Albufera de Mallorca* due to anthropogenic factors in oxidative stress biomarkers in the submerged macrophyte *Myriophyllum spicatum* L.

2. Material and methods

2.1. Study species

The Eurasian water-milfoil, *M. spicatum* is an aquatic submersed plant native to Europe, Asia, and north Africa, that usually grows in less than 5 m depth (Sivaci et al., 2007). It is a monoic hydrophyte with a cosmopolitan distribution comprising the European, Asian and North African regions (GBIF, 2019). Although some authors consider that *M. spicatum* is a freshwater species (Cirujano, 1997), others reported its occurrence into oligohaline waters (Kaijser et al., 2019) or even into brackish waters with variable levels of salinity values (Hillmann and La Peyre, 2019). *M. spicatum* forms together with *Potamogeton* L., *Zannichellia* L., *Ceratophyllum demersum* L. or *Najas marina* L. the habitat 3140 Hard oligo-mesotrophic waters with benthic vegetation (Llorens et al., 2007). This habitat is listed at the Council Directive 92/43/EEC as those with community interest whose conservation requires the designation of special areas of conservation.

2.2. Study site and samples collection

Samples were collected in six different points of the Canal des Sol at the *s'Albufera de Mallorca* Natural Park (Alcúdia, Mallorca Island, Balearic Islands, Spain) in August 2017 (Fig. 1). The selected channel is located in the central area of the Natural Park to minimize the possible effects of other stressors derived from urban/tourist and agricultural activities. Because the study area is located within a protected natural area, a premise was requested from the Natural Park management to collect the samples. In each point salinity and temperature were determined by using a multiparametric probe (HANNA model HI 9828). An assessment of the abundance of aquatic plants was also measured by the percentage of occupancy, in each of the six study points. Other species found in the study site were *C. demersum* and *Zannichellia pedunculata* Rchb.

One composed group of leaves were taken from ten different specimens of *M. spicatum* in each point. Samples were rapidly introduced in hermetic recipients containing water from each sampling area and were transported to the laboratory in less than 30 min. Once in the laboratory, samples were dried with filter paper to remove excess water and immediately frozen at −80 °C until biochemically analysis. Prior to biochemical analysis, all samples were carefully checked avoiding specimens colonized by epiphytes and discarding those evidencing any sign of damage by grazing activity. Macrophyte samples were rinsed with distilled water and homogenized in five volumes (w/v) of 50 mM Tris–HCl buffer, 1 mM EDTA, pH 7.5 using a small sample dispersing system (Ultra-Turrax® Disperser, IKA). Homogenates were centrifuged at 9000×*g*, 10 min, 4 °C and supernatants were used for biochemical assays. All biochemical analyses were referred per mg protein measured with a commercial kit (Biorad®) using bovine serum albumin as a standard.

2.3. Antioxidant enzymes activities

Catalase, SOD, GPX, Grd and GST enzyme activities were determined in *M. spicatum* homogenates (Capo et al., 2015; Sureda et al., 2017). Catalase activity (mK(s^{-1})/mg of protein) was determined using the method described by Aebi (1984), based on the decomposition of H_2O_2, monitoring the decrease in absorbance at 240 nm. SOD activity (pKat/mg of protein) was determined by the degree of inhibition of the reduction of cytochrome C by the superoxide anion generated by the xanthine oxidase/hypoxanthine system (Flohe and Otting, 1984). GPx activity was determined (nKat/mg protein) following an adaptation of the spectrophotometric method described by Flohe and Gunzler (1984). GRd activity was determined (nKat/mg of protein) by a modification of the Goldberg and Spooner's procedure (Goldberg and Spooner, 1984). GST activity (nKat/mg protein) was determined at 314 nm using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates (Sureda et al., 2017). All enzymatic activities were determined in the homogenates with a Shimadzu UV-2100 spectrophotometer at 25 °C.

2.4. Malondialdehyde (MDA) assay

Malondialdehyde (MDA) levels in homogenates, as lipid

Fig. 1. Sampling locations of *Myriophyllum spicatum* in the *s'Albufera de Mallorca* Natural Park (Mallorca, Spain).

peroxidation marker, were analysed by a colorimetric assay for MDA determination based on the reaction of MDA with a chromogenic reagent to yield a stable chromophore with maximal absorbance at 586 nm (Sigma-Merk, Spain, ref. MAK085). Briefly, samples or standards were placed in glass tubes containing n-methyl-2phenyl-indole (10.3 mM) in acetonitrile:methanol (3:1). HCl 12 N was added, and the samples were incubated for 1 h at 45 °C. Absorbance was measured at 586 nm.

2.5. Photosynthetic pigments (D430/D665) index

Photosynthetic pigments index was determined following a method previously described (Vázquez et al., 2013). This index relates the amount of chlorophylls and carotenoids versus only chlorophylls, and since carotenoids are more resistant to degradation, the ratio will increase in stressful situations (Margalef, 1983). To proceed with the extraction of photosynthetic pigments, leaves were incubated in acetone during 48 h at 4 °C in dark conditions. Extracts were centrifugated and supernatants were used to determine photosynthetic pigments. Absorbance at 430 nm and 635 nm were measured and the D430/D665 index was calculated dividing absorbance at 430 nm between absorbance 665 nm.

2.6. Statistical analysis

Statistical analysis was carried out using a statistical package (SPSS 25.0 for Windows®). The normal distribution of the data was assessed by applying the Kolmogorov–Smirnov test. When these assumptions were fulfilled, the statistical significance of the differences in all the determinations carried out was evaluated by one-way analysis of variance (ANOVA). Post hoc LSD paired comparisons were further made to recognize deviant groups. Results were expressed as mean ± S.D and $P < 0.05$ was considered statistically significant.

3. Results

Table 1 represents temperature, salinity levels and percentage of macrophyte coverage on the 6 different stations. Significant differences in the salinity levels were observed between the investigated areas. No significant differences were observed between areas 1, 2 and 6, increasing significantly in 3, 4 and 5. The increase in salinity in areas 4 and 5 was significantly higher than in area 3. The % of macrophyte coverage followed a pattern similar to salinity but inverse, with greater coverage in areas 1, 2 and 6 (>90%) and minimal coverage in zones 4 and 5 (<5%). Other macrophyte species such as *C. demersum* occurred at all the stations, except for the zones 4 and 5 where they were absent because of their lower tolerance to higher salinity values.

Table 1
Temperature, salinity and % macrophyte coverage on sampling zones.

Station	Temperature (°C)	Salinity	% macrophyte coverage
1	27.1 ± 0.6	2.30 ± 0.08^{a}	95–100
2	26.9 ± 0.5	2.31 ± 0.07^{a}	90–95
3	27.1 ± 0.6	4.01 ± 0.28^{b}	40–45
4	27.0 ± 0.2	8.58 ± 0.15^{c}	<5
5	26.9 ± 0.5	11.13 ± 0.13^{d}	<5
6	27.0 ± 0.2	2.76 ± 0.16^{a}	90–95

Temperature and salinity values and % macrophyte coverage in the different sampling stations. One-way ANOVA, $p < 0.05$. Different letters indicate significant differences with respect other groups.

Fig. 2 represents the photosynthetic pigments index determined in *M. spicatum* leaves. No differences in the D430/D665 index were observed between the stations 1, 2, 3 and 6, whereas the index was significantly higher in stations 4 and 5.

Antioxidant enzymes activities on *M. spicatum* recollected in the six stations are represented in Table 2. No differences in GST activity between different stations were observed. All antioxidant enzyme activities followed a similar pattern of response with an increase in their activities as salinity increases, although with slight differences. In all enzymes, the stations 1, 2, 3 and 6 presented similar activities. The activities of CAT and SOD from the stations 4 and 5 were significantly higher respect to the stations 1, 2 and 6 but similar to the station 3 for SOD. No significant differences were found in CAT between stations 3 and 4. GPx evidenced similar activities in all the investigated stations except for the station 6 which has a lower activity when compared with stations 4 and 5. The activity of GRd was significantly increased in the stations 4 and 5 respect of other sites.

MDA levels in homogenates of *M. spicatum* were represented on Fig. 3. Macrophytes sampled in stations 4 and 5 presented higher MDA levels respect to the other stations. Furthermore, no differences in MDA levels were observed between homogenates from stations 1, 2 and 6. Finally, macrophytes from station 3 present high MDA levels than station 1.

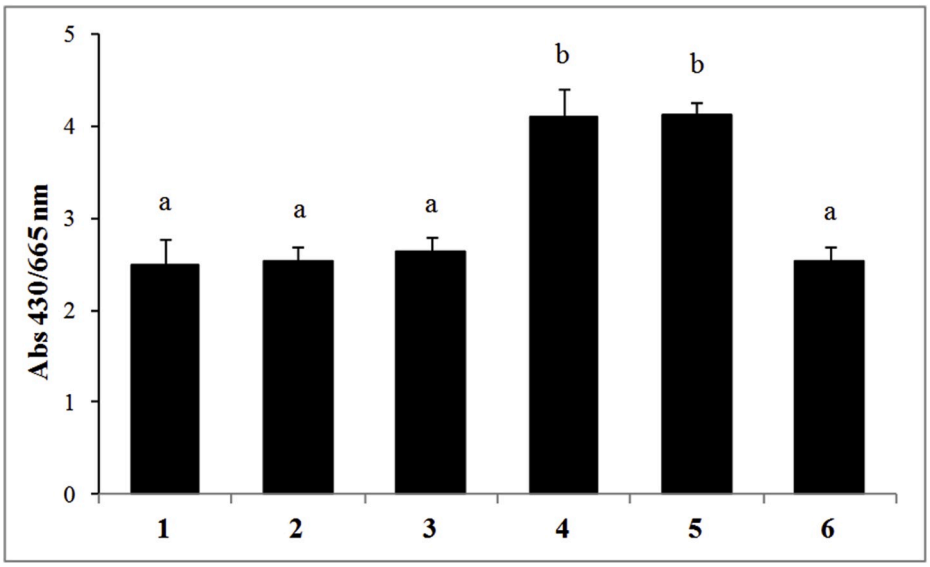

Fig. 2. Photosynthetic pigments (D430/D665) index in *Myriophyllum spicatum* homogenates from the different sampling stations. One-way ANOVA, $p < 0.05$. Different letters indicate significant differences with respect other groups.

Table 2
Enzyme activities.

Station	Catalase	SOD	GPx	GRd	GST
1	473 ± 42.7[a]	14.8 ± 1.3[a]	38.1 ± 4.8[a,b]	17.8 ± 1.8[a]	19.1 ± 2.2
2	476 ± 42.4[a]	15.3 ± 1.0[a]	39.7 ± 2.8[a,b]	18.9 ± 0.7[a]	17.1 ± 2.3
3	573 ± 93.1[a,c]	20.4 ± 3.1[a,c]	46.3 ± 6.3[a,b]	22.4 ± 3.5[a]	19.8 ± 3.4
4	721 ± 81.8[b,c]	26.2 ± 3.8[b,c]	48.3 ± 4.9[a]	31.4 ± 3.7[b]	19.8 ± 3.5
5	752 ± 61.1[b]	25.1 ± 0.5[b,c]	48.7 ± 3.0[a]	32.9 ± 6.9[b]	18.4 ± 1.4
6	456 ± 23.8[a]	15.9 ± 0.5[a]	35.1 ± 2.8[b]	17.1 ± 1.8[a]	16.5 ± 1.2

Activities of antioxidant enzymes – catalase (mK(s^{-1})/mg of protein), superoxide dismutase (SOD, pKat/mg of protein), glutathione peroxidase (GPx, nKat/mg protein) and glutathione reductase (GRd, nKat/mg protein) – and detoxifying enzyme glutathione *s*-transferase (GST, nKat/mg protein) in *Myriophyllum spicatum* homogenates from the different sampling stations. One-way ANOVA, $p<0.05$. Different letters indicate significant differences with respect other groups.

4. Discussion

In the last decade an increase of salinity values at *s'Albufera de Mallorca* Natural Park inland waters occurred, because of different anthropic pressures. This increase in the degree of salinity derives mainly from seawater intrusion due to overexploitation of aquifers (Candela et al., 2009; Galimont et al., 2003). Water hypersalinization can cause stress in affected aquatic plants. It has been demonstrated that saline stress is related to an increase in hydric stress, salts toxicity, and oxidative stress (Parihar et al., 2015). Plant populations that inhabit the channels of *s'Albufera de Mallorca* are exposed to different salinity degree. Higher values of salinity at waters close to the sea coast (sea water), followed at inland waters with medium values of salinity into brackish waters and the lower values of salinity at the most inland waters thus classifying this last type of water into fresh water. However, in the present study we have observed a significant increase in salinity in three of the intermediate stations, and far away from the sea. The most likely cause of the increase of salinity in these stations is the intrusion of seawater through a salty water upwelling that reaches the area from the aquifer.

M. spicatum is a hygrophyte species that can tolerate both fresh and brackish waters (Hillmann and La Peyre, 2019; Kaijser et al., 2019; Taberner et al., 1995). This tolerance range allows *M. spicatum* to live under a wide range of salinity and under different oxidative stress conditions. On the contrary, other species less tolerant salinity such as *C. demersum* or *Z. pedunculata* were only present in the areas with low salt levels (zones 1, 2, 6 and in low amounts in 3). The current results are in agreement with those observed in the Modern Yellow River Delta (China) where a higher resistance to salinity of *M. spicatum* was evidenced with respect to other macrophytes such as *C. demersum* and *Potamogeton perfoliatus* L. (Li et al., 2011). In addition, it has been observed that an increase of salinity can reduce germination and, consequently, the abundance and distribution of macrophytes such as *Z. pedunculata* and *C. demersum* (Short and Neckles, 1999; Stoler et al., 2018). However, and although it has been reported that *M. spicatum* is more salt tolerant than many other freshwater macrophyte species, it also exhibits reduced growth when exposed to high salinity (Stoler et al., 2018).

The D430/D665 index has been interpreted as a marker of the degree of maturity of the population and the levels of stress to which plants are subjected (Margalef, 1983). Values close to 2 in the D430/D665 index indicate productive, young and non-stressed populations, while higher values, close to 5 represent mature, low productive and stressed populations. Macrophytes from zones 4 and 5, zones where the salinity is higher than in the other studied areas, present values between 4 and 5, suggesting that salinity causes a stressful situation in *M. spicatum* population. In addition, high salinity decreases water potential and induces disturbances in ion homeostasis and toxicity leading to growth reduction and limited productivity in affected plants (Parihar et al., 2015). In this sense, a significant reduction in the levels of chlorophyll and, consequently, in photosynthetic rates has been reported in different plant species under salt stress (Amirjani, 2011; Mane et al., 2010) and also in aquatic macrophytes (Rout and Shaw, 2001; Upadhyay and Panda, 2005).

Antioxidant enzyme activities, as well as oxidative stress products such as MDA, are often used as biomarkers of stress levels to which a plant population is exposed (Gil et al., 2019; Sureda et al., 2017). Accordingly, it is widely described that exposure to high salinity conditions results in the induction of an excessive accumulation of ROS that establishes a situation of oxidative stress (Gil et al., 2019; Parihar et al., 2015). Specifically, saline stress can lead to stomatal closure by reducing the availability of carbon dioxide in the leaves and inhibiting carbon

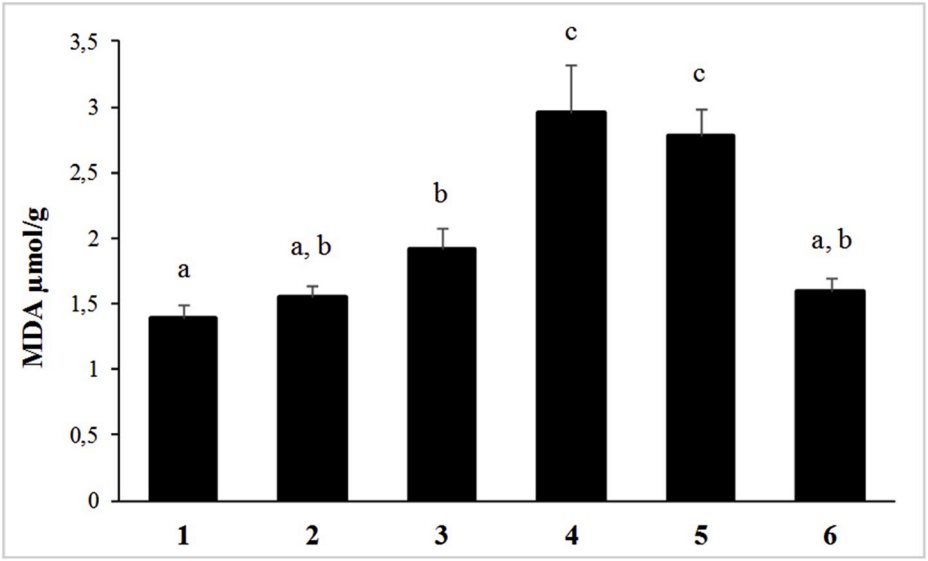

Fig. 3. Malondialdehyde (MDA) levels in *Myriophyllum spicatum* homogenates from the different sampling stations. One-way ANOVA, $p < 0.05$. Different letters indicate significant differences with respect other groups.

fixation, which exposes chloroplasts to excess excitation energy and, in turn increases ROS generation (Ahmad et al., 2010). In the present study it has been observed that an increase in water salinity causes a significant increase in the activity of antioxidant enzymes CAT, SOD, GPx and GRd activities. These results suggest that high salinity causes an increase in antioxidant defences in *M. spicatum* to cope with saline stress. In an *in vitro* study, it was observed that *M. spicatum* leaves responded to increased levels of salinity with a progressive increase in CAT, SOD, GPx and ascorbate peroxidase activities (Li et al., 2011). Moreover, the results obtained in the present study are in agreement with previous works, which documented an increase in ROS production, in the activities of antioxidant enzymes and a disturbance in the uptake and translocation of macro and micronutrients when submerged aquatic macrophytes *C. demersum*, *Najas graminea* Delile, *Najas indica* (Willd.) Cham., and *Hydrilla verticillata* (L.f.) Royle were exposed to high salinity (Dogan and Demirors Saygideger, 2018; Mallik et al., 2011; Rout and Shaw, 2001). However, despite the increased antioxidant enzymes activities ROS production was not completely deactivated, since a significant increase in MDA, a marker of lipids peroxidation, was observed in areas with higher salinity. Similar results were found in the submerged macrophytes *Spirodela polyrhiza* (L.) Schleid., *Pistia stratiotes* L., *Salvinia molesta* D.Mitch. exposed to salinity stress, evidenced by an increased H_2O_2 accumulation and lipid peroxidation (Cheng, 2011; Upadhyay and Panda, 2005).

Higher values of salinity alter the density of *M. spicatum*, and of all macrophytes in general, causing severe effects throughout the ecosystem. In these lotic or running waters, where phytoplankton is poorly represented, the primary production is carried out by macrophyte species, such as *M. spicatum* (Margalef, 1983). Thus, a decrease in plant density and, consequently, a decrease in primary production, would trigger a serious threat to the entire aquatic ecosystem, which would affect trophic networks. The impact is even worse, considering that it is concomitant with the occurrence of invasive species at the natural park, specifically the common carp *Cyprinus carpio* L., 1758 (Riddiford et al., 2014). This fish species feeds on the macrophytes but also is a bottom detri-omnivore feeder that resuspends the muddy sediments and produces water turbidity decreasing light penetration. This feeding activity is detrimental to the macrophyte species (Lougheed et al., 1998; Miller and Crowl, 2006; Parkos III et al., 2003), and further worsens the survival of the macrophyte species in this highly impacted habitat. Therefore, the increase in water salinity, together with the appearance of invasive species, would act synergistically against macrophyte species, reducing their abundance and increasing their stress, affecting primary production.

In conclusion, marine water intrusion into aquifers causes an increase in the salinity of inland waters in *s'Albufera de Mallorca* Natural Park. This increased salinity induces oxidative stress in the submerged macrophyte *M. spicatum*, which is evidenced by the increase in the antioxidant enzyme activities. However, this increase in antioxidant defences damage is not able to prevent the occurrence of oxidative damage in the areas studied with the highest salinity. Major attention should be addressed to improve the management of the aquifer and to reduce the impact of the intrusion of saline waters into the Natural Park. Environmental government should focus on the conservation of aquatic macrophytes according to their relevance with the primary production, and therefore the livelihood of the entire ecosystem.

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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.

CRediT authorship contribution statement

Lorenzo Gil: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing. **Xavier Capó:** Methodology, Investigation, Writing - review & editing. **Silvia Tejada:** Methodology, Investigation, Formal analysis, Writing - review & editing. **Guillem Mateu-Vicens:** Methodology, Investigation, Writing - review & editing. **Pere Ferriol:** Methodology, Investigation, Writing - review & editing. **Samuel Pinya:** Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing, Funding acquisition. **Antoni Sureda:** Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Project

administration, Writing - review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2020.106756.

References

Aebi, H.E., 1984. Catalase. In: Bergmeyer, H.U. (Ed.), Methods in Enzymatic Analysis. Verlag Chemie, Basel, pp. 273–286.

Ahmad, P.J., Jaleel, C.A., Sharma, S., 2010. Antioxidative defence system, lipid peroxidation, proline-metabolizing enzymes and biochemical activity in two genotypes of *Morus alba* L. subjected to NaCl stress. Russ. J. Plant Physiol. 57, 509–517.

Amirjani, M., 2011. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. Int. J. Bot. 7.

Bhowmik, S., 2020. Ecological and economic importance of wetlands and their vulnerability: a review. In: Rathoure, A., Chauhan, P. (Eds.), Current State and Future Impacts of Climate Change on Biodiversity. IGI Global, Hershey, PA, pp. 95–112.

Candela, L., Igel, W., Elorza, F.J., Aronica, G., 2009. Impact assessment of combined climate and management scenarios on groundwater resources and associated wetland (Majorca, Spain). J. Hydrol. 376, 510–527.

Capo, X., Tejada, S., Box, A., Deudero, S., Sureda, A., 2015. Oxidative status assessment of the endemic bivalve *Pinna nobilis* affected by the oil spill from the sinking of the Don Pedro. Mar. Environ. Res. 110, 19–24.

Cheng, T.S., 2011. NaCl-induced responses in giant duckweed (*Spirodela polyrhiza*). Can. J. Bot. 59, 104–105.

Cirujano, S., 1997. *Myriophyllum* L. In: Castroviejo, S., Aedo, C., Laínz, M., Muñoz Garmendia, F., Nieto Feliner, G., Paiva, J., Benedí, C. (Eds.), Flora Iberica, vol. 8. Real Jardín Botánico, CSIC, Madrid, pp. 3–7.

Cunha, I., Garcia, L.M., Guilhermino, L., 2005. Sea-urchin (*Paracentrotus lividus*) glutathione S-transferases and cholinesterase activities as biomarkers of environmental contamination. J. Environ. Monit. 7, 288–294.

Custodio, E., 2018. Consequences of seawater intrusion in Mediterranean Spain. Project SASMIE. In: Calvache, M., Duque, C., Pulido-Velazquez, D. (Eds.), Groundwater and Global Change in the Western Mediterranean Area. Environmental Earth Sciences. Springer, Cham, pp. 93–100.

Dogan, M., Demirors Saygideger, S., 2018. Pysiological effects of NaCl on *Ceratophyllum demersum* L., a submerged rootless aquatic macrophyte. Iran. J. Fish. Sci. 17, 346–356.

Ebejer, M., 2006. Some Chloropidae (Diptera) from the Balearic Islands (Spain) with particular reference to Parc Natural de s'Albufera de Mallorca. Alguns Chloropidae (Diptera) de les Illes Balears (Espanya) amb especial referència al Parc Natural de s'Albufera de Mallorca. Bolleti Soc. Historia Nat.s Balears 49, 173–184.

Espinosa-Diez, C., Miguel, V., Mennerich, D., Kietzmann, T., Sanchez-Perez, P., Cadenas, S., Lamas, S., 2015. Antioxidant responses and cellular adjustments to oxidative stress. Redox Biology 6, 183–197.

Flohe, L., Gunzler, W.A., 1984. Assays of glutathione peroxidase. Methods Enzymol. 105, 114–121.

Flohe, L., Otting, F., 1984. Superoxide dismutase assays. Methods Enzymol. 105, 93–104.

Galimont, A., Candela, L., Barón, A., González, C., 2003. Evolución de la intrusión salina en el llano de Inca-Sa Pobla, Mallorca. In: López-Geta, J.A., de la Orden, J.A., Gómez, J.D., Ramos, G., Mejías, M., Rodríguez, L. (Eds.), Tecnología de la intrusión de aguas de mar en acuíferos costeros: Países mediterráneos. IGME, Madrid, pp. 533–541.

GBIF, 2019. *Myriophyllum Spicatum* L. GBIF Backbone Taxonomy. https://doi.org/10.15468/39omei. Checklist dataset accessed via GBIF.org on 2020-01-13.

Gil, L., Pinya, S., Tejada, S., Capo, X., Sureda, A., 2019. Antioxidant defenses in wild growing halophyte *Crithmum maritimum* from inland and coastline populations. Chem. Biodivers. 16, e1800448.

Goldberg, D.M., Spooner, R.J., 1984. Glutathione reductase. In: Bergmeyer, H.U. (Ed.), Methods in Enzymatic Analysis. Verlag Chemie, Basel, pp. 258–265.

Hillmann, E., La Peyre, M., 2019. Effects of salinity and light on growth and interspecific interactions between *Myriophyllum spicatum* L. and *Ruppia maritima* L. Aquat. Bot. 155.

Isayenkov, S.V., Maathuis, F.J.M., 2019. Plant salinity stress: many unanswered questions remain. Front. Plant Sci. 10.

Kaijser, W., Kosten, S., Hering, D., 2019. Salinity tolerance of aquatic plants indicated by monitoring data from The Netherlands. Aquat. Bot. 158, 103129.

Läuchli, A., Grattan, S.R., 2007. Plant growth and development under salinity stress. In: Jenks, M.A., Hasegawa, P.M., Jain, S.M. (Eds.), Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer Netherlands, Dordrecht, pp. 1–32.

Li, F., Xie, Y., Chen, X., Pan, Y., Deng, Z., Li, X., 2011. Plant distribution can be reflected by physiological responses to salinity of three submerged macrophytes from the Modern Yellow River Delta. Fund. Appl. Limnol. Arch. Hydrobiol. 179, 159–167.

Llorens, L., Gil, L., Tébar, F.J., 2007. La vegetació de l'illa de Mallorca: bases per a la interpretació i gestió d'hàbitats. Associació Jardí Botànic de Palma. Palma de Mallorca-Spain 262.

Lougheed, V., Crosbie, B., Chow-Fraser, P., 1998. Predictions on the effect of common carp (*Cyprinus carpio*) exclusion on water quality, zooplankton, and submergent macrophytes in a Great Lakes wetland. Can. J. Fish. Aquat. Sci. 55, 1189–1197.

Mallik, S.N., Nayak, M., Sahu, B., Panigrahis, A.K., Sahw, B.P., 2011. Response of antioxidant enzymes to high NaCl concentration in different salt-tolerant plants. Biol. Plantarum 55, 191–195.

Mane, A., Karadge, B., S, S., 2010. Salinity induced changes in photosynthetic pigments and polyphenols of *Cymbopogon Nardus* (L.) Rendle. J. Chem. Pharmaceut. Res. 2, 338–347.

Margalef, R., 1983. Limnologia. Ediciones Omega, S.A., Barcelona, p. 1010.

Miller, S., Crowl, T., 2006. Effects of common carp (*Cyprinus carpio*) on macrophytes and invertebrate communities in a shallow lake. Freshw. Biol. 51, 85–94.

Mitsch, W., Gosselink, J., 2000. The value of wetlands: importance of scale and landscape setting. Ecol. Econ. 35, 25–33.

Neri-Flores, I., Moreno-Casasola, P., Peralta-Peláez, L.A., Monroy, R., 2019. Groundwater and river flooding: the importance of wetlands in coastal zones. J. Coast Res. 92, 44–54.

Parihar, P., Singh, S., Singh, R., Singh, V.P., Prasad, S.M., 2015. Effect of salinity stress on plants and its tolerance strategies: a review. Environ. Sci. Pollut. Control Ser. 22, 4056–4075.

Parkos III, J.J., Santucci V.J., Jr, Wahl, D.H., 2003. Effects of adult common carp (*Cyprinus carpio*) on multiple trophic levels in shallow mesocosms. Can. J. Fish. Aquat. Sci. 60, 182–192.

Pinya, S., Cuadrado, E., Trenado, S., 2008. Presencia de *Mauremys leprosa* (Schweizer, 1812) en el Parque Natural de s'Albufera de Mallorca. Bol. Asoc. Herpetol. Espanola 19, 83–84.

Rebassa, M., 2015. S'Albufera, efectes de 27 anys de gestió i canvi. L'exemple de les aus aquàtiques. In: Servei de Protecció d'Espècies (Ed.), Llibre verd de la protecció d'Espècies a les Balears. Monografia de la Societat d'Història Natural, 20. Conselleria d'Agricultura, Medi Ambient i Territori & Societat d'Història Natural de les Balears, Palma, pp. 345–354.

Riddiford, N., Mayol, J., 1996. 7.2 S'Albufera de Mallorca, Spain. In: Tomas Vives, P. (Ed.), Monitoring Mediterranean Wetlands: A Methodological Guide. MedWet Publication, Wetlands International, Slimbridge, UK and ICN, Lisbon, pp. 103–118.

Riddiford, N., Veraart, J.A., Férriz, I., Owens, N., Royo, L., Honey, M., 2014. The Albufera Initiative for Biodiversity: a cost effective model for integrating science and volunteer participation in coastal protected area management. Revista de Gestão Costeira Integrada 14, 267–288.

Rout, N.P., Shaw, B.P., 2001. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci. 160, 415–423.

Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. Aquat. Bot. 63, 169–196.

Sivaci, A., Sivaci, E., Sökmen, M., 2007. Changes in antioxidant activity, total phenolic and abscisic acid constituents in the aquatic plants *Myriophyllum spicatum* L. and *Myriophyllum triphyllum* Orchard exposed to cadmium. Ecotoxicology (London, England) 16, 423–428.

Slater, F., 2016. Ecotones, ecoclines and eco-perturbations: the aquatic flora and fauna of S'Albufera Natural Park, Mallorca: a contribution and review. Mediterr. J. Biosci. 1, 120–127.

Stoler, A., Sudol, K., Mruzek, J., Relyea, R., 2018. Interactive effects of road salt and sediment disturbance on the productivity of seven common aquatic macrophytes. Freshw. Biol. 63, 709–720.

Sureda, A., Tejada, S., Capo, X., Melia, C., Ferriol, P., Pinya, S., Mateu-Vicens, G., 2017. Oxidative stress response in the seagrass *Posidonia oceanica* and the seaweed *Dasycladus vermicularis* associated to the invasive tropical green seaweed *Halimeda incrassata*. Sci. Total Environ. 602, 918–925.

Taberner, A.M., Niell, G.M., Forteza, V., Ramon, G., 1995. El medi lòtic de S'Albufera de Mallorca. In: Taberner, A.M., Mayol, J. (Eds.), S'Albufera de Mallorca. Societat d'Història Natural de les Balears, Editorial Moll, Palma, pp. 175–186.

Uddin, M.K., Juraimi, A.S., 2013. Salinity tolerance turfgrass: history and prospects. Sci. World J. 3.

Upadhyay, R., Panda, S., 2005. Salt tolerance of two aquatic macrophytes, *Pistia stratiotes* and *Salvinia molesta*. Biol. Plantarum 49, 157–159.

Vázquez, M.D., Villares, R., Carballeira, A., 2013. Biomonitoring urban fluvial contamination on the basis of physiological stress induced in transplants of the aquatic moss *Fontinalis antipyretica* Hedw. Hydrobiologia 707, 97–108.

Veraart, J.A., Groot, Perelló, G., Riddiford, N.J., Roijackers, R.M.M., 2004. Selection of (bio) indicators to assess effects of freshwater use in wetlands: a case study of s'Albufera de Mallorca, Spain. Reg. Environ. Change 4 (2004) 2-3 4.

Woodward, R., Wui, Y.-S., 2001. The economic value of wetland services: a meta-analysis. Ecol. Econ. 37, 257–270.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.