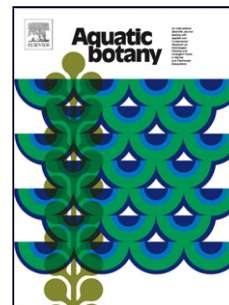


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Title: Seasonal effects of waterfowl grazing on submerged macrophytes: The role of flowers

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Highlights

- Waterfowl abundance increased from summer to winter.
- Significant herbivore effects on macrophyte biomass were only found for one macrophyte species (*Ruppia cirrhosa*) and only in summer.
- High flower abundance may have facilitated herbivory.

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1 **Seasonal effects of waterfowl grazing on submerged**
2 **macrophytes: the role of flowers**

3
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11

12 **Abstract:** Seasonal effects of waterfowl (*Fulica atra* and *Anas platyrhynchos*) grazing on
13 submerged macrophytes (*Ruppia cirrhosa* and *Potamogeton pectinatus*) and the mediating role of
14 flowers on plant consumption were evaluated by exclusion cages and tethering experiments
15 deployed in a Mediterranean lagoon throughout the annual cycle. Despite the low waterfowl
16 abundance recorded in summer, exclusion-cage experiments evidenced intense herbivory on the
17 biomass, canopy height and flowers of *R. cirrhosa* (flowers abundance was ~8 times higher inside
18 exclusion cages; $1015.7 \pm 269.8 \text{ flw} \cdot \text{m}^{-2}$). For *P. pectinatus*, exclusion cage experiments did not
19 evidence waterfowl consumption, in spite of the presence of flowers, which suggest preference
20 for reproductive tissues of *R. cirrhosa*. In addition, the higher abundance of *R. cirrhosa* flowers
21 compared to *P. pectinatus* (~10 times higher inside the exclusion cages) was likely influenced by
22 more intense herbivory on the former species. Although waterfowl abundance increased in
23 autumn and winter, experiments did not evidence herbivory effects during that period, possibly
24 because of enhanced availability of alternative resources and decreased plant biomass and canopy
25 height reducing encounter rates. Hence, our results suggest that waterfowl effects on submerged
26 macrophytes in Mediterranean aquatic ecosystems are strongly influenced by seasonal changes in
27 the availability of food resources and its flowering events. The higher herbivory on *R. cirrhosa*
28 and its flowers in summer suggest that waterfowl grazing may be driven by food preference for
29 reproductive tissues, and could have a strong effect on the community structure and abundance of
30 submerged macrophytes.

31
32 **Keywords:** Exclusion experiments, *Ruppia cirrhosa*, *Potamogeton pectinatus*,
33 Waterfowl, Herbivory.

34

35 **1. Introduction**

36 In aquatic ecosystems such as coastal lagoons and lakes, the submerged aquatic
37 vegetation (SAV) plays a vital role: influencing nutrient dynamics and water chemistry;
38 modulating the structure and dynamics of food webs; and increasing habitat diversity (see
39 Jeppesen et al., 1998). These aquatic ecosystems are habitats for many herbivorous waterfowl
40 that can also heavily use aquatic macrophyte resources during migratory stopovers and/or in
41 locations hosting permanent populations (e.g. Michot and Nault, 1993; Baldwin and Lovvorn,
42 1994 a,b). Several studies have reported long-term changes in aquatic vegetation coinciding with
43 changes in the size of waterfowl abundances (Perrow et al., 1997; Søndergaard et al., 1998;
44 Mitchell and Perrow, 1998; Blindow et al., 2000). High densities of SAV can attract waterfowl
45 (by providing food and shelter) that cause strong qualitative and quantitative effects on plant
46 communities through effects on vegetation structure, species composition and by reducing stand
47 biomass (Bortolus et al., 1998; Nolet et al., 2001).

48 Most of these studies conducted in temperate areas of North America, Europe and New
49 Zealand, suggest that major impacts of waterfowl on the SAV occur during the autumn (Perrow
50 et al., 1997; Mitchell and Perrow, 1998; Marklund et al., 2002) and winter (Kiørboe, 1980; van
51 Donk, 1998), when macrophyte productivity is low and migratory events result in increased
52 abundance of individuals (Søndergaard et al., 1996; Mitchel et al., 1994). Waterfowl herbivory is
53 also important in temperate lakes during plant colonisation stages and at very low vegetation
54 densities (Marklund et al., 2002, Körner & Dugdale, 2003, Hilt, 2006). In contrast, the few
55 studies conducted in Mediterranean aquatic ecosystems suggest that, in general, waterfowl
56 grazing does not have a strong effect on the biomass of submerged vegetation due to the high
57 level of primary production (Mitchell and Perrow, 1998; Marklund, 2002; Sandsten et al., 2002).
58 However, it has also been suggested that waterfowl in Mediterranean areas can have a strong

59 qualitative effect on the structure of plant communities by selecting the most palatable species or
60 their reproductive structures (Gayet et al., 2012; Rodriguez-Villafañe et al., 2007). A marked
61 preference of herbivores for plants bearing abundant flowers and/ or developing fruits has been
62 suggested as eventually leading to a reduction in the number of seeds produced by these plants
63 (Herrera et al., 2002) and could strongly impact the reproductive success of macrophytes.

64 *Ruppia cirrhosa*, *Potamogeton pectinatus* and *Zoostera spp* have been reported as the
65 dominant macrophyte species in Mediterranean lagoons, with a seasonal cycle characterised by
66 declining biomasses in autumn and winter, –particularly *R. cirrhosa* (Menéndez et al., 2002;
67 Rodríguez-Pérez and Green, 2006)– and flowering event in summer (Menéndez and Comín,
68 1989; Prado et al., 2013). The waterfowl community in Mediterranean wetlands is dominated by
69 the duck *Anas platyrhynchos* and the Eurasian coot *Fulica atra* whose abundances increase in
70 autumn and winter, due to migratory concentrations (Mañosa et al., 2001; Hidding et al., 2009).
71 *A. platyrhynchos* is considered to be mostly granivorous (Arzel et al., 2007) and coots (*F. atra*)
72 mainly herbivorous, with both species having long been recognised to feed on submerged
73 macrophytes such as *Potamogeton spp* and *Ruppia spp* as well as their seeds and flowers (Tubbs
74 and Tubbs, 1983; Perrow et al., 1997; Figuerola et al., 2002, 2003, Green et al., 2002). However,
75 ecological interactions between waterfowl and aquatic plant communities in Mediterranean
76 lagoons need to be further investigated for the conservation of these natural habitats and the long-
77 term sustainability of endangered and/or economically valued animal species, as well as the
78 natural diversity of ecosystems.

79 In this context, the general objective of this study was to investigate whether seasonal
80 differences in the two main populations of waterfowl (*A. platyrhynchos* and *F. atra*) and in the
81 abundance of the two main submerged macrophytes (*R. cirrhosa* and *P. pectinatus*) can explain
82 patterns of plant consumption within Mediterranean lagoons. In addition, we investigated the

83 potential role of macrophytes' flowers in mediating waterfowl feeding preferences and overall
84 impacts on macrophytes' biomass. With these aims, three specific objectives were assessed
85 during three seasons: (1) waterfowl abundances of *A. platyrhynchos* and *F. atra*; (2) grazing
86 impacts on both macrophyte species and their flowers (only in summer) by deploying exclusion
87 cage experiments; and (3) plant consumption rates by tethering experiments.

88

89 **2. Material and Methods**

90 *2.1. Study site*

91 The study was conducted at the Encanyissada coastal lagoon located within the Ebro
92 Delta Natural Park (Spain, NW Mediterranean), a Natura 2000 wetland area of recognised
93 international importance for waterbird conservation by the Ramsar Convention and by BirdLife
94 International (Viada, 1998) where ca. 70 % of the total surface is devoted to rice cultivation. The
95 submerged vegetation in the lagoons is dominated by *R. cirrhosa* in high salinity areas (12–27
96 ‰) and by *P. pectinatus* in low salinity areas (3–12‰). Seasonal variation in macrophytes'
97 biomasses within the lagoon have been reported values from $151.3 \pm 16.6 \text{ gDWm}^{-2}$ in August to
98 $21.6 \pm 2.7 \text{ gDWm}^{-2}$ in February for *R. cirrhosa* and values from $162.6 \pm 24.4 \text{ gDWm}^{-2}$ in August to
99 $54.8 \pm 13 \text{ gDWm}^{-2}$ in February for *P. pectinatus*. Flowering of *R. cirrhosa* has been reported in
100 August in the lagoon, although flowers can start in June (personal observation). For *P. pectinatus*,
101 flowering occurs in June to July (personal observation) ending by August, when only fruits
102 (achenes) were observed (Prado et al., 2013). In summer, water conditions (mainly increased
103 water temperature and nutrient supply from rice agriculture) also contribute to the proliferation of
104 fast-growing species such as floating macroalgae or epiphytic loads (Valiela et al., 1997;
105 Menéndez, 2005). Waterfowl abundances in this area are especially notorious during autumn and
106 winter, due to the migratory events and the abundance of wintering grounds, when ducks and

107 coots become the most important species (Martínez-Vilalta, 1989, 1994, 1996). In this study, we
108 focused on the herbivory of *A. platyrhynchos* and *F. atra* as both have been reported to feed on
109 macrophytes as well as their seeds and flowers (Tubbs and Tubbs, 1983; Perrow et al., 1997;
110 Figuerola et al., 2002).

111

112 2.2. Waterfowl abundance and behavioural observations

113 Monitoring the waterfowl community was conducted on a previously delimited area of
114 the lagoon which included the two experimental areas of plots deployment. Waterfowl abundance
115 was counted (using binoculars) from a fixed point located approximately 100 m from each area.
116 At each study season (summer, autumn and winter) and during the 30-day experimental period,
117 waterfowl were counted at the same time of the day on 4 random days. The number of
118 individuals of *F. atra* and *A. platyrhynchos* in each study area was added to estimate total
119 waterfowl abundance.

120 Feeding on the submerged vegetation and the possible disturbance of the experimental
121 area by other bird species was also monitored by deploying a game camera (Day 6 Plotwatcher)
122 facing the tethering and exclusion cages experiments at different days throughout the study
123 period.

124

125 2.3. Exclusion cages experiment

126 To evaluate the grazing effect by waterfowl on macrophytes' biomass, six bird exclusion
127 and six open cages were deployed randomly in two shallow areas of the lagoon (80-100 cm
128 depth; separated ~1.5 km); one monospecific area with *R. cirrhosa* and another with *P. pectinatus*.
129 Each plot (exclusion and control) covered an area of 1.5 m² and contained plant biomass (either
130 *Ruppia* or *Potamogeton*) that was representative of the lagoon (Prado et al., 2013). Exclusion

131 plots consisted of a rigid, plastic net above the canopy top (1 cm² mesh size) tied to four poles
132 (1.5 m long, 10 mm diameter) inserted into the sediment, preventing the entrance of birds and
133 enabling water circulation on the sides during occasional storms (total experimental area
134 covered: ~300 m², see Fig. 1).

135 Cage experiments were deployed for a 30-day period in three different seasons: summer
136 2010 (from mid-June to mid-July: when flowering started and waterfowl abundances are the
137 lowest); autumn 2010 (from mid-September to mid-October: when flowers are no longer
138 available and waterfowl abundance increases); and winter 2011 (from mid-February to mid-
139 March: when macrophytes abundance is the lowest and waterfowl abundance is the highest).
140 After this period, 3 corers of 16 cm (Ø) were collected from central areas (defined by a minimum
141 security margin of 0.3 m from each side) of each plot. To assess a possible shading effect by the
142 cage mesh net, 3 additional corers were collected from the edges of the exclusion cages for
143 further comparison (within the 0.3 m margin area). In each season, exclusion cages were removed
144 after the 30-day experiment period to avoid the destruction of the plots or overlapping by
145 repeated sampling. In autumn, green macroalgal blooms occurred in the experimental area and, as
146 they were attached to macrophytes, their biomass was also quantified. At each sampling event,
147 plants were placed into bags and carefully washed in the laboratory to remove attached
148 sediments. We measured the canopy height, counted the number of flowers (in summer) and
149 separated the attached macroalgae (in autumn). All samples were dried at 60 °C to constant
150 weight and weighted to the nearest 0.1 g. The following macrophytes' variables were assessed:
151 canopy height (cm); biomass (g DW·m⁻²); number of flowers per·m²; and attached biomass of
152 macroalgae (g DW·m⁻²).

153

154 *2.4. Tethering experiments*

155 Tethering experiments were conducted simultaneously to the deployment of exclusion
156 cages to quantify consumption rates of waterfowl in the lagoon. For each macrophyte species a
157 tethering line was deployed within each monospecific area during the three different seasons
158 studied (summer; autumn and winter). Each tethering line consisted of macrophytes' shoots
159 previously weighted in the laboratory (ca.3 gWW; n = 6). Shoots were attached to pickets using
160 cable ties, secured between them using a thin rope and randomly deployed within each
161 monospecific experimental area during 30 days. Tethering controls (n = 6), consisting of
162 equivalent plant biomasses covered with a protective mesh, were also placed in the submerged
163 macrophyte areas in order to assess possible growth and/or decomposition of tethered plants
164 during the experimental period . After the 30-day period, all replicates were collected and
165 reweighted for variations in wet weight, and biomass changes in control tethers were used to
166 correct consumption estimates, expressed in terms of $\text{g WW} \cdot \text{lost d}^{-1}$.

167

168 2.5. Data analyses

169 Seasonal variation in the total waterfowl abundance (*A. platyrhynchos* and *F. atra*) was
170 first evaluated using a one-way ANOVA with season as fixed factor (three levels). Then, we
171 investigated seasonal differences in the abundance of each waterfowl species using a two-way
172 ANOVA with season (three levels) and waterfowl specie (two levels) as fixed factors.

173 The effects of waterfowl grazing on each macrophyte over the study period (i.e., cage
174 experiments) were investigated with a two-way ANOVA with season (three levels) and treatment
175 (two levels) as fixed factors. The possible “shading effect” by exclusion cages was first
176 investigated for each macrophyte species using a two-way ANOVA with season (three levels)
177 and shade (two levels) as fixed factors. Waterfowl effects on abundance of flowers and on the
178 biomass of attached macroalgae was also evaluated using a two-way ANOVA with macrophyte

179 (two levels) and treatment (two levels) as fixed factors. Seasonal variation in macrophytes
180 consumption (i.e., tethering experiments) was investigated using a two-way ANOVA design with
181 season (three levels) and macrophyte species (two levels) as fixed factors.

182 For all ANOVAs, assumptions of normality and homogeneity of variance were assessed
183 with the Kolmogorov-Smirnov and Cochran's C-test, respectively. When assumptions could not
184 be met by variable transformation, the significance level was set at 0.01 to reduce the possibility
185 of a Type I error (Underwood, 1997). The Student Newman-Keuls post-hoc test (Zar, 1984) was
186 then used to investigate the presence of significant groupings.

187

188 **3. Results**

189 *3.1. Waterfowl abundance and behavioural observations*

190 The total waterfowl abundance in the lagoon was significantly different among seasons
191 with increasing values from summer to winter (One-way ANOVA, $P < 0.01$; Table 4a). For
192 *F. atra* and *A. platyrhynchos* abundances, analyses showed significant effects of season and
193 waterfowl species. The abundance of *Fulica atra* was significantly higher than that of *A.*
194 *platyrhynchos*, with higher values in autumn and winter than in summer (Two-way ANOVA, Fig.
195 3b; see SNK in Table 4b). Feeding observations recorded by the camera proved that both species
196 were grazing on *R. cirrhosa* and *P. pectinatus* in the experimental area.

197

198 *3.2. Exclusion cage experiments*

199 During the seasonal study, analyses did not detect significant "shading effects" inside
200 exclusion cages on the biomass and canopy height of *R. cirrhosa* and *P. pectinatus* (Two-way
201 ANOVA; $P > 0.5$).

202 *Ruppia cirrhosa* biomass displayed significant differences between seasons, with higher
203 values in autumn than in summer and winter, with no effects for treatment (Fig. 2a, see SNK in
204 Table 1). Yet, a significant season x treatment interaction was observed, caused by significantly
205 higher biomasses inside exclusion cages during the summer period (Fig. 2a, see SNK in Table 1).
206 The highest biomass was recorded in autumn control cages ($284.4 \pm 19.8 \text{ g DW}\cdot\text{m}^{-2}$) and the
207 lowest in winter exclusion cages ($69.3 \pm 7.2 \text{ g DW}\cdot\text{m}^{-2}$). For canopy height, similar patterns were
208 observed (i.e., season and season x treatment effects), but there was also a significant effect of
209 treatment, with higher heights within exclusion cages (Fig. 2a, see SNK in Table 1). The highest
210 values were recorded in summer exclusion cages ($42.1 \pm 5.4 \text{ cm}$) and the lowest in winter control
211 cages ($13.1 \pm 0.7 \text{ cm}$).

212 For *P. pectinatus*, analyses showed that biomass and canopy height were only
213 significantly different between seasons (Fig. 2c and d, Table 1). The highest biomass was
214 recorded in autumn ($538.9 \pm 88.2 \text{ g DW}\cdot\text{m}^{-2}$) and the lowest in winter ($76.8 \pm 7.4 \text{ g DW}\cdot\text{m}^{-2}$).
215 The highest canopy height was recorded in summer ($76.2 \pm 4.7 \text{ cm}$) and the lowest in winter
216 ($27.8 \pm 1.2 \text{ cm}$).

217 The abundance of flowers in summer and macroalgal biomass in autumn showed a
218 significant macrophyte x treatment interaction (see SNK in Table 2). The highest flower
219 abundance was recorded inside *R. cirrhosa* exclusion cages ($1015.7 \pm 269.8 \text{ flw}\cdot\text{m}^{-2}$) and the
220 lowest inside *P. pectinatus* exclusion cages ($93.9 \pm 33.8 \text{ flw}\cdot\text{m}^{-2}$; see SNK in Table 2). For
221 attached macroalgae, the highest biomass was recorded in control cages of *P. pectinatus* ($405.7 \pm$
222 $91.5 \text{ g DW}\cdot\text{m}^{-2}$) and the lowest in control cages of *R. cirrhosa* ($8.8 \pm 3.4 \text{ g DW}\cdot\text{m}^{-2}$; see SNK in
223 Table 2).

224

225 *3.3. Tethering experiments*

226 Analyses showed that macrophyte consumption was not significantly different across
227 seasons, but was significantly higher in *P. pectinatus* than in *R. cirrhosa* (Fig. 3a; Table 3).

228

229 **4. Discussion**

230 Contrary to previous findings in Northern Europe, our study shows that major herbivory
231 impacts of waterbirds in Mediterranean regions are neither restricted to periods of early growth,
232 or to autumn when macrophyte productivity is low and wildfowl form migratory concentrations.
233 Our results show that waterfowl grazing effects on submerged macrophytes in Mediterranean
234 aquatic lagoons were influenced by the seasonal changes in the availability of food resources and
235 flowering events rather than by waterfowl abundances. The higher abundance of flowers recorded
236 in *R. cirrhosa* (~10 times higher than *P. pectinatus* inside exclusion cages) concurred with higher
237 waterfowl consumption on this specie, and appears to be a key factor controlling herbivory
238 pressure.

239

240 *4.1. Waterfowl abundance*

241 Total waterfowl abundance (*F. atra* and *A. Platyrhynchos*), in the lagoon increased from
242 summer to winter (from 0.51 ind·ha⁻¹ to 3.14 ind·ha⁻¹) with *F. atra* being the most abundant
243 species in the entire lagoon throughout the study. This seasonal pattern has been previously
244 reported for coots and ducks in other Mediterranean wetlands, with abundances peaking in
245 October-November during the post-breeding period, and with a mean density of 2.9 ind·ha⁻¹
246 (Rodríguez-Pérez and Green, 2006). Although the grazing activity in Central and Northern
247 Europe takes place in late autumn and winter due to populations' increase (Van Donk and Otte,
248 1996; Søndergaard et al., 1996; Froelich and Lodge, 2000; Santamaría and Rodríguez-Girone's,

249 2002), our study suggests that major effects of waterbirds on submerged macrophytes in
250 Mediterranean lagoons are not restricted to periods of high waterfowl concentrations (Rodríguez-
251 Pérez and Green 2006).

252

253 4.2. Seasonal herbivory impacts on macrophytes

254 Experiments with exclusion cage showed that waterfowl grazing effects on *R. cirrhosa*
255 and *P. pectinatus* were not driven by seasonal variations in waterfowl abundance. In summer,
256 although waterfowl abundance was lower, grazing effects were evident in *R. cirrhosa* biomass,
257 canopy height and flowers which suffered the most intense herbivory in open cages (flowers
258 abundance were ~8 times higher inside exclusion cages). The higher abundance of flowers
259 recorded in *R. cirrhosa* (ca. 10 times higher than *P. pectinatus* inside exclusion cages) concurred
260 with higher waterfowl consumption on this specie, which suggest that this may be a key factor
261 controlling herbivory pressure. In fact, preferential consumption of flowers has been reported in
262 previous exclusion experiments with coots in Mediterranean lagoons featuring a diverse
263 community of macrophytes (Rodríguez-Villafañe et al. 2007). Yet, we did not observe higher
264 herbivory pressure on *P. pectinatus* despite the presence of flowers and the higher canopy height
265 of this species, which can also influence waterfowl grazing (Hurter, 1972). Hence, our results
266 suggest that waterfowl have an important impact on *R. cirrhosa* in summer, which is likely
267 influenced by preference for flowers that are locally very abundant during the summer period (as
268 previously described for this lagoon, see Prado et al., 2013).

269 A marked preference of herbivores for plants bearing abundant flowers and/or developing
270 fruits has been suggested as eventually leading to a reduction in the number of seeds produced by
271 these plants (Herrera et al., 2002) and could strongly impact the reproductive success of
272 macrophytes. Rodríguez-Villafañe et al., (2007) conducted a bird-exclusion experiment in Lake

273 Sentiz (Spain) and found that *Potamogeton gramineus* only developed leaves and flowers under
274 waterfowl exclusion, thus decreasing in abundance until becoming codominant with
275 *Myriophyllum alterniflorum* which also suffered higher consumption of flowers outside the
276 cages. This suggests that by selecting the most palatable species or their reproductive structures
277 waterfowl can have a strong qualitative effect on the structure of plant communities and become
278 the central force driving species' composition in some aquatic ecosystems (Bonser and Reader,
279 1995; Rachich and Reader, 1999). In addition, it is possible that waterfowl selectivity for *R.*
280 *cirrrosa* influence vegetative regrowth during the following year, as has been reported to occur
281 with other macrophytes species (Van Dijk et al., 1992; Fishman and Orth, 1996; Piazzini et al.,
282 2000). Yet, some studies also suggest that plants may have mechanisms to compensate herbivory
283 pressure such as increasing the proportion of female flowers (Howe and Westley 1988), or the
284 amount of belowground structures, which may enhance substrate fixation and facilitate lateral
285 expansion (Orth 1977). These mechanisms of compensatory growth are the main drivers of
286 evolutionary responses for plant- animal coexistence (McNahugton 1983). Although we did not
287 measure how waterfowl grazing affected macrophytes' grow rates, a previous study in
288 Mediterranean wetlands suggested that a strong grazing effect on macrophytes' biomass and the
289 reproductive structures in one year are likely to influence *Ruppia* growth the following year
290 (Rodriguez-Pérez and Green, 2006).

291 Despite the increased waterfowl abundance in autumn and winter, grazing effects on the
292 submerged macrophytes were negligible in both seasons. In autumn, flowers disappeared, and the
293 lack of effects on biomass and canopy height of both *R. cirrhosa* and *P. pectinatus* may be due to
294 the enhanced availability of other resources such as rice seeds (due to the harvest season) or the
295 proliferation of floating macroalgal mats, which have been commonly reported to proliferate in
296 spring and summer due to higher water temperature and irradiance (Menéndez and Sánchez,

297 1998; Menéndez and Comín, 2000). During our experiment, floating macroalgae ended up
298 attached to macrophytes' leaves, particularly in *P. Pectinatus*, possibly due to differences in
299 water salinity and/or nutrient availability within the lagoon (Prado et al., 2013). Yet, conversely
300 to enhanced palatability effects commonly reported for epiphytes and macroalgae (Gayet et al.,
301 2012; Marco-Méndez et al., 2012), increased algal biomass did not result on preferential
302 waterfowl grazing on *P. Pectinatus*. Conversely, given the large accumulation of macroalgae, it is
303 possible that the availability of this alternative resource decreased waterfowl effects on both
304 macrophytes species. Later in winter, the lower biomass and canopy height recorded for both
305 macrophytes could have made them a less accessible resource and therefore, harder to be found
306 by waterfowl, particularly due to the enhanced water turbidity during this period. We hypothesize
307 that in this season, the reported ability of ducks and coots switching to feeding on invertebrates
308 and seeds may help them to persist within the lagoon area, in spite of the scarcity of submerged
309 vegetation (Rodríguez-Pérez and Green, 2006).

310 Despite exclusion-cage experiments not evidencing grazing effect on macrophytes in
311 some seasons, results from tethering experiments suggests that there is some consumption
312 through the year. However, the low consumption rates recorded suggest low encounter of tethers
313 by waterfowl, possibly resulting from the high mobility of waterfowl in the lagoon or the high
314 abundance of other resources. This could explain the low consumption of *R. cirrhosa* tethers
315 during the summer period despite strong waterfowl impacts in cage experiments. It is likely that
316 tethering experiments underestimate waterfowl consumption and these results need to be
317 interpreted with caution. Yet, tethering results were supported by the video camera feeding
318 observations, evidencing that observed differences in plant biomass during the study were due to
319 waterfowl.

320

321 *Conclusion*

322 Our findings contrast with the seasonality of herbivory impacts described i for coots and
323 ducks in Northern Europe (Van Donk and Otte, 1996; Søndergaard et al., 1996), but are in
324 concordance with other Mediterranean studies (Rodriguez-Pérez and Green, 2006; Rodriguez-
325 Villafaña et al., 2007) reporting major effects of waterbirds during the summer period, when
326 plant and flowers' availability is higher. Overall, this suggests that seasonal impacts of waterfowl
327 are not a general rule, but depend on a regional combination of animal numbers and
328 compositional abundance of food resources.

329 To conclude, the strongest waterfowl impacts on the submerged vegetation within
330 brackish Mediterranean lagoons do not occur when abundance of individuals is higher, but in
331 summer when plants and flowers are largely available. In the long term, higher herbivory
332 pressure on *R. cirrhosa* and its flowers could reduce the reproductive success of this species and
333 alter the overall community structure of submerged macrophytes. This study contributes to a
334 better understanding of the interactions between waterfowl herbivory and the SAV in aquatic
335 Mediterranean ecosystems along the seasonal cycle, and may allow a better conservation of
336 natural habitats and the long-term sustainability of the natural diversity of ecosystems.

337
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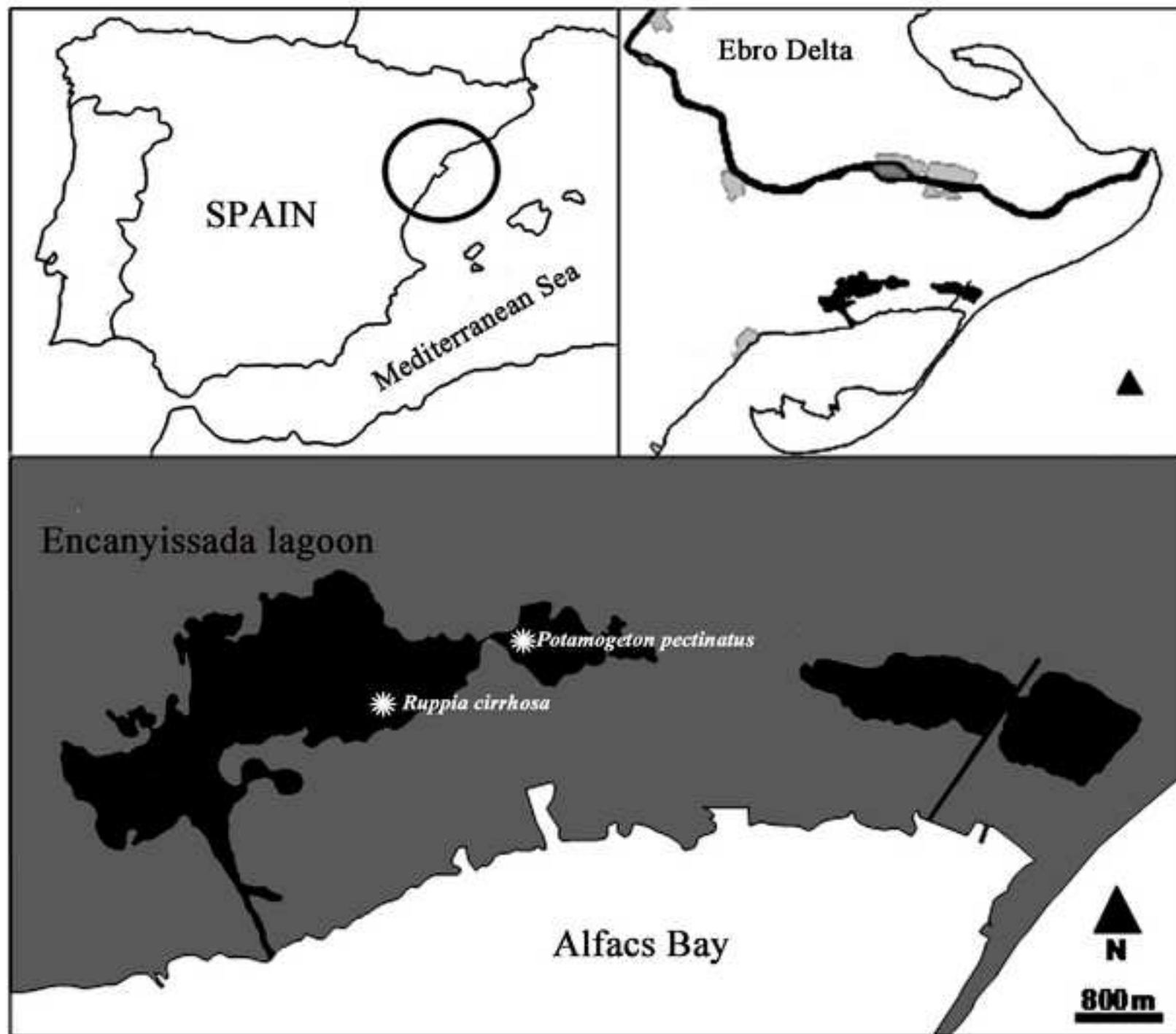
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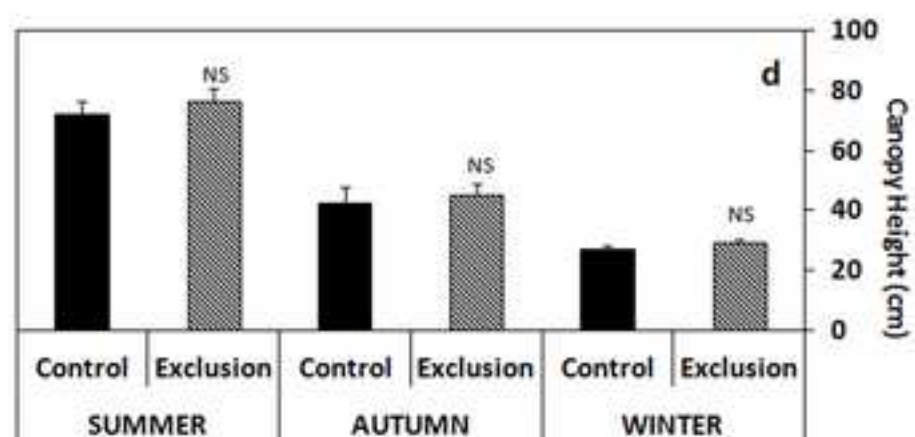
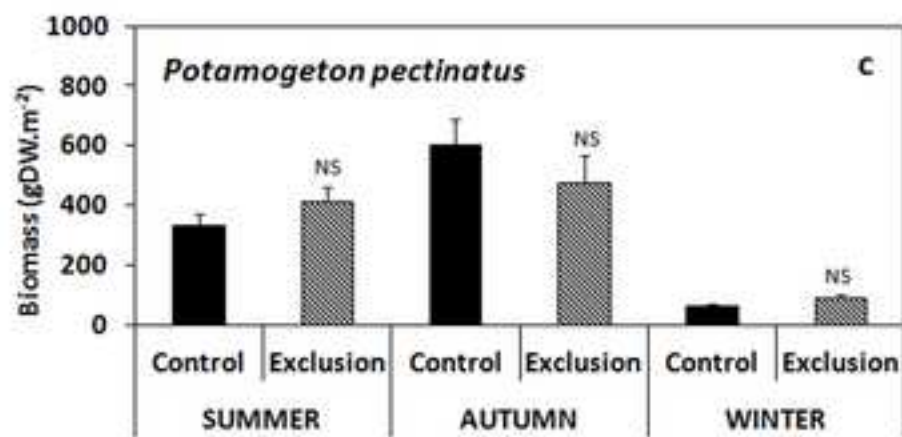
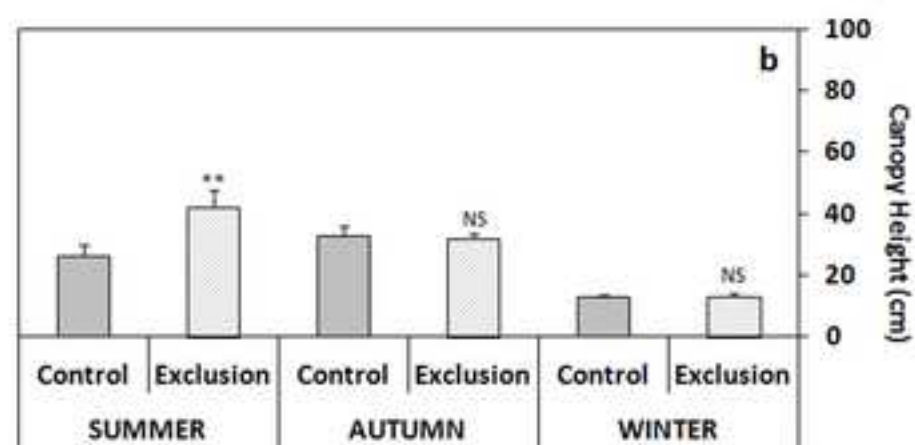
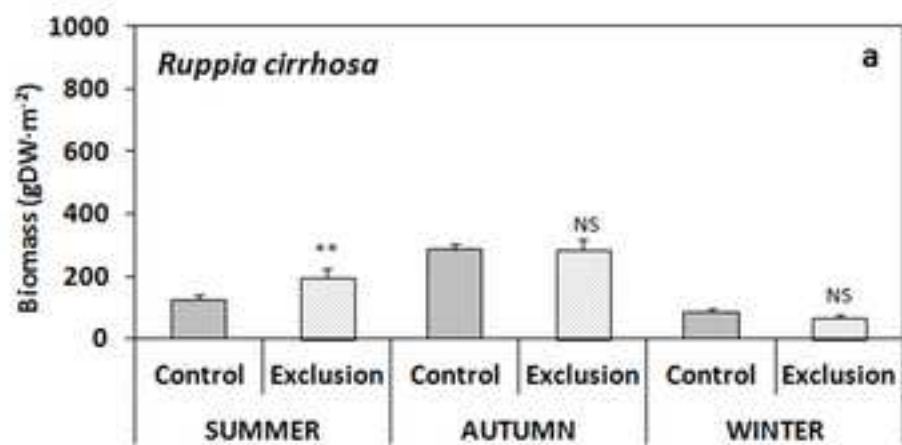
1 **Fig 1.** Map of the Encanyissada lagoon showing the position of the three sampling sites were
2 experiment were deployed: *R. cirrhosa* and *P. pectinatus* sites (adapted from Prado et al. 2013).

3
4 **Fig 2.** Seasonal trends on submerged vegetation during cage experiments: **a.** Biomass (g DW·m⁻²)
5 of *R. cirrhosa*; **b.** Canopy height (cm) of *R. cirrhosa*; **c.** Biomass (g DW·m⁻²) of *P. pectinatus*; **d.**
6 Canopy height (cm) of *P. pectinatus*; Mean ± SE. **P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; NS=
7 not significant results.

8
9 **Fig 3. a.** Seasonal consumption rates of *R. cirrhosa* and *P. pectinatus* during tethering
10 experiments (g WW·d⁻¹); **b.** Seasonal variability in the abundance of *A. platyrhynchos*, *F. atra*,
11 and in the overall number of individuals·ha⁻¹.

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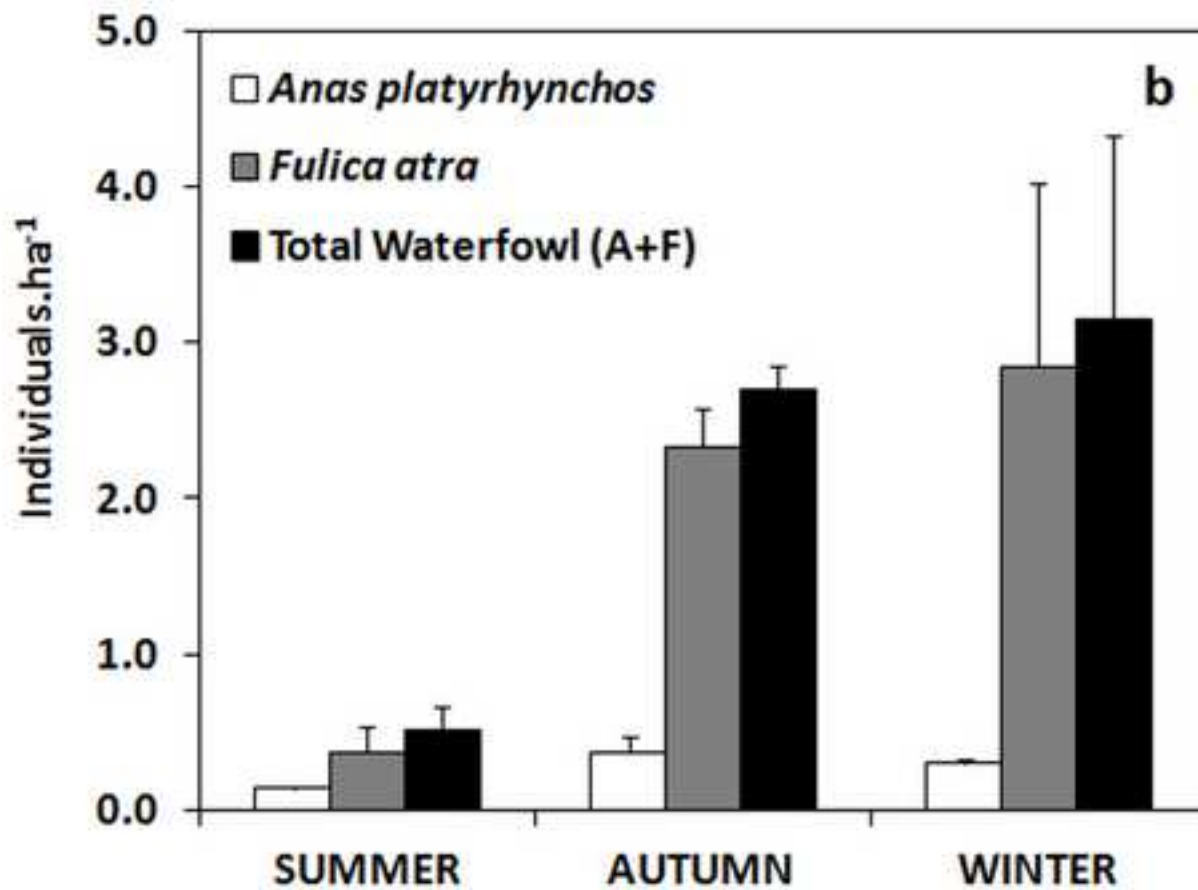
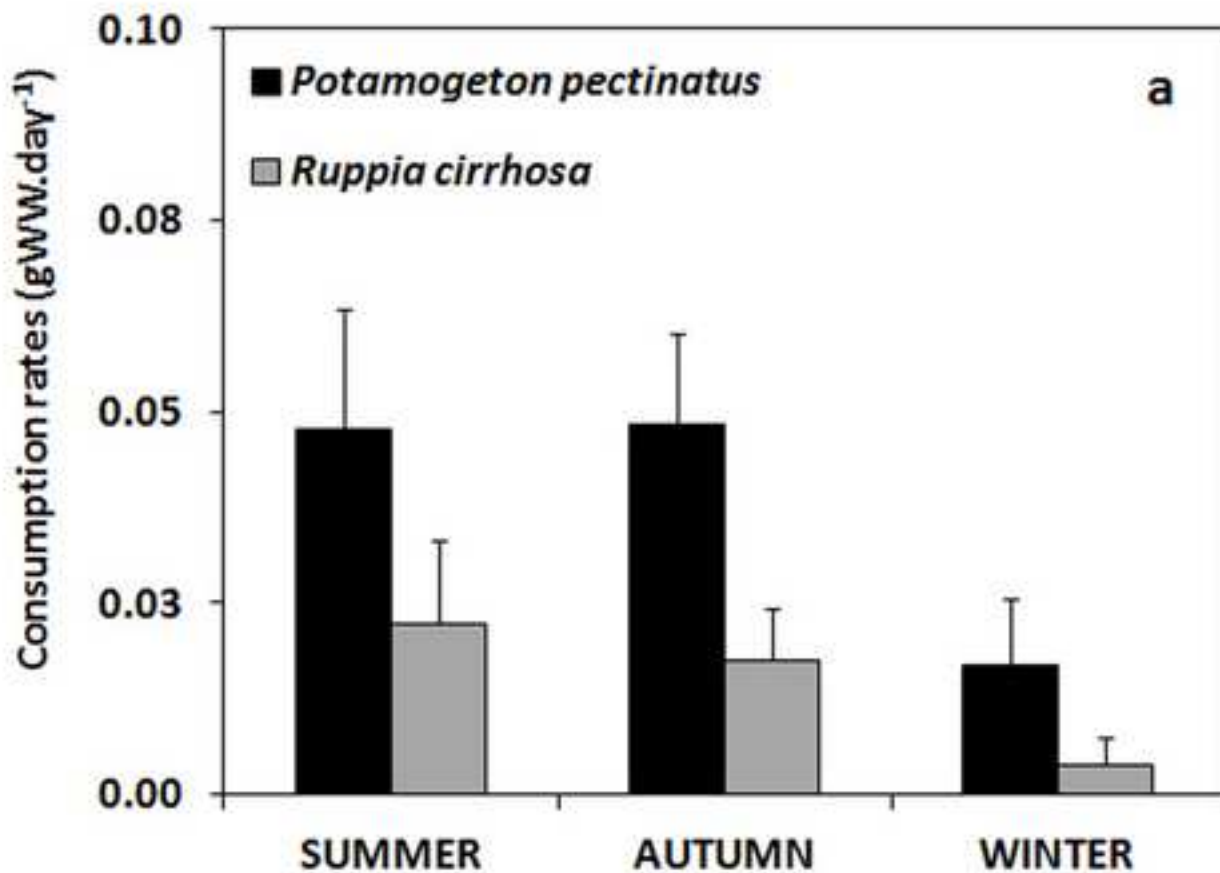


Table 1. Two-way ANOVA testing for differences on biomass (g DW·m⁻²) and canopy height (cm) among seasons (S: summer; A: autumn; W: winter) and treatments (C: control; E: exclusion) in *Ruppia cirrhosa* and *Potamogeton pectinatus*. Significant differences are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: no significant. In SNK, significant differences between groups are indicated. Summer Control: SC, Summer Exclusion: SE, Autumn Control: AC, Autumn Exclusion: AE, Winter Control: WC, Winter Exclusion: WE.

Source of variation	<i>Ruppia cirrhosa</i>								<i>Potamogeton pectinatus</i>							
	Biomass				Canopy Height				Biomass				Canopy Height			
	df	MS	F	P	df	MS	F	P	df	MS	F	p	df	MS	F	P
Season (S)	2	575.41	55.58	***	2	575.41	55.58	***	2	1962923.62	34.45	***	2	17626.45	61.09	***
Treatment (T)	1	11.17	1.08	NS	1	11.17	1.08	*	1	50.51	0	NS	1	643.38	2.23	NS
S x T	2	37.2	3.59	*	2	37.2	3.59	**	2	107705.44	1.89	NS	2	145.02	0.5	NS
Residual									102	56986.79			102	288.52		
SNK	AE=AC>SE>SC=WC=WE				SE>AC=AE=SC>WE=WC				AC=AE>SE=SC>WE=WC				SE=SC>AE=AC>WE=WC			
Transformation	Sqr (x+1)				NT				NT				NT			

1

Table 2. Two-way ANOVA testing for differences on flowering rates (No. flw·m⁻²) and on attached macroalgae biomass (g DW·m⁻²) between macrophytes (R: *R. cirrhosa*; P: *P. pectinatus*) and treatments (C: control; E: exclusion). Significant differences are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: no significant. In SNK, significant differences between investigated groups, *R. cirrhosa* control cages (RC), *R. cirrhosa* exclusion cages (RE), *P. pectinatus* control cages (PC) and *P. pectinatus* exclusion cages (PE) are indicated.

Source of variation	SUMMER				AUTUMN			
	Flowering rates				Macroalgal biomass			
	df	MS	F	P	df	MS	F	P
Macrophyte (M)	1	44.26	5.98	*	1	1555865.55	33.09	***
Treatment (T)	1	20.99	2.84	NS	1	184939.9	3.93	NS
M x T	1	44.57	6.02	*	1	190780.64	4.06	*
Residual	68	7.4			68	47018.54		
SNK		RE>RC=PC=PE				PC>PE=RE=RC		
Transformation		Ln (x+1)				NT		

Table 3. Two-way ANOVA testing for differences on consumption ($\text{g WW}\cdot\text{d}^{-1}$) between seasons (S: summer; A: Autumn; W: Winter) and macrophytes-plant type (R: *R. cirrhosa*; P: *P. pectinatus*). Significant differences are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: no significant. In SNK, significant differences between investigated groups, summer in *R. cirrhosa* (SR), autumn in *R. cirrhosa* (AR), winter in *R. cirrhosa* (WR), summer in *P. pectinatus* (SP), autumn in *P. pectinatus* (AP), winter in *P. pectinatus* (WP) are indicated.

Source of variation	Differences between <i>Ruppia cirrhosa</i> and <i>Potamogeton pectinatus</i> in all the seasons			
	Consumption rates			
	df	MS	F	p
Season (S;A;W)	2.000	0.000	3.170	NS
Macrophyte type (R;P)	1.000	0.010	8.100	**
Season X Macrophyte	2.000	0.000	0.430	NS
Residual	30.000	0.000		
SNK		AP=SP=SR=AR=WP=WR		
Transformation		NT		

Table 4. a. One-way ANOVA testing differences on Total Waterfowl population (including individuals of An: *A. platyrhynchos* and F: *F. atra*); **b.** Two-way ANOVA testing for differences on populations of waterfowls (An: *A. platyrhynchos* and F: *F. atra*) among seasons (S: summer; A: autumn; W: winter). Significant differences are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: no significant. In SNK, significant differences between investigated groups, *A. platyrhynchos* population in summer (SAn), autumn (AAAn) and winter (WAn), *F. atra* population in summer (SF), autumn in (AF), and winter (WF) are indicated.

Source of variation	a. Total waterfowl Census (A+F)			
	df	MS	F	p
Season (S;A;W)	2.00	4.35	16.24	**
Residual	9.00	0.27		
Transformation		Ln x		
Source of variation	b. Waterfowl Census (An and F)			
	df	MS	F	p
Season (S;A;W)	2.00	5.95	17.51	***
Waterfowl type (An;F)	1.00	15.09	44.37	***
Season X Waterfowl	2.00	1.12	3.30	NS
Residual	18.00	0.34		
SNK		AFu = WFu > SFu = SAn = AAAn = WAn		
Transformation		Ln x		