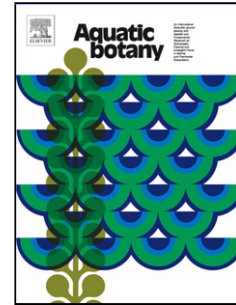# Accepted Manuscript

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.

1   **Seasonal effects of waterfowl grazing on submerged**

2   **macrophytes: the role of flowers**

3

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

2

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

3

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

4

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 ± 16.6 gDWm^{-2} in August to

98 21.6 ± 2.7 gDWm^{-2} in February for *R. cirrhosa* and values from 162.6 ± 24.4 gDWm^{-2} in August to

99 54.8 ± 13 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

5

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 delimitated 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 m^2 and contained plant biomass (either

130 Ruppia or Potamogeton) that was representative of the lagoon (Prado et al., 2013). Exclusion

6

131   plots consisted of a rigid, plastic net above the canopy top (1 cm^2 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 m^2, 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 ℃ 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·m^2; and attached biomass of

152   macroalgae (g DW·m^{-2}).

153

154   *2.4. Tethering experiments*

7

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 g WW· 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

8

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; $P > 0.5$).

9

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 ± 19.8 g DW·m^{-2}) and the

207   lowest in winter exclusion cages (69.3 ± 7.2 g DW·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 ± 5.4 cm) and the lowest in winter control

211   cages (13.1 ± 0.7 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 ± 88.2 g DW·m^{-2}) and the lowest in winter (76.8 ± 7.4 g DW·m^{-2}).

215   The highest canopy height was recorded in summer (76.2 ± 4.7 cm) and the lowest in winter

216   (27.8 ± 1.2 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 ± 269.8 flw·m^{-2}) and the

220   lowest inside *P. pectinatus* exclusion cages (93.9 ± 33.8 flw·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 g DW·m^{-2}) and the lowest in control cages of *R. cirrhosa* (8.8 ± 3.4 g DW·m^{-2}; see SNK in

223   Table 2).

224

225   *3.3. Tethering experiments*

10

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 (Rodriguez-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,

11

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 (Rodriguez-

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 thismay 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. Rodriguez-Villafañe et al., (2007) conducted a bird-exclusion experiment in Lake

12

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　*cirrhosa* 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; Piazzi 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 (McNauhgton 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,

13

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

14

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   Villafañe 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.

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22

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}.
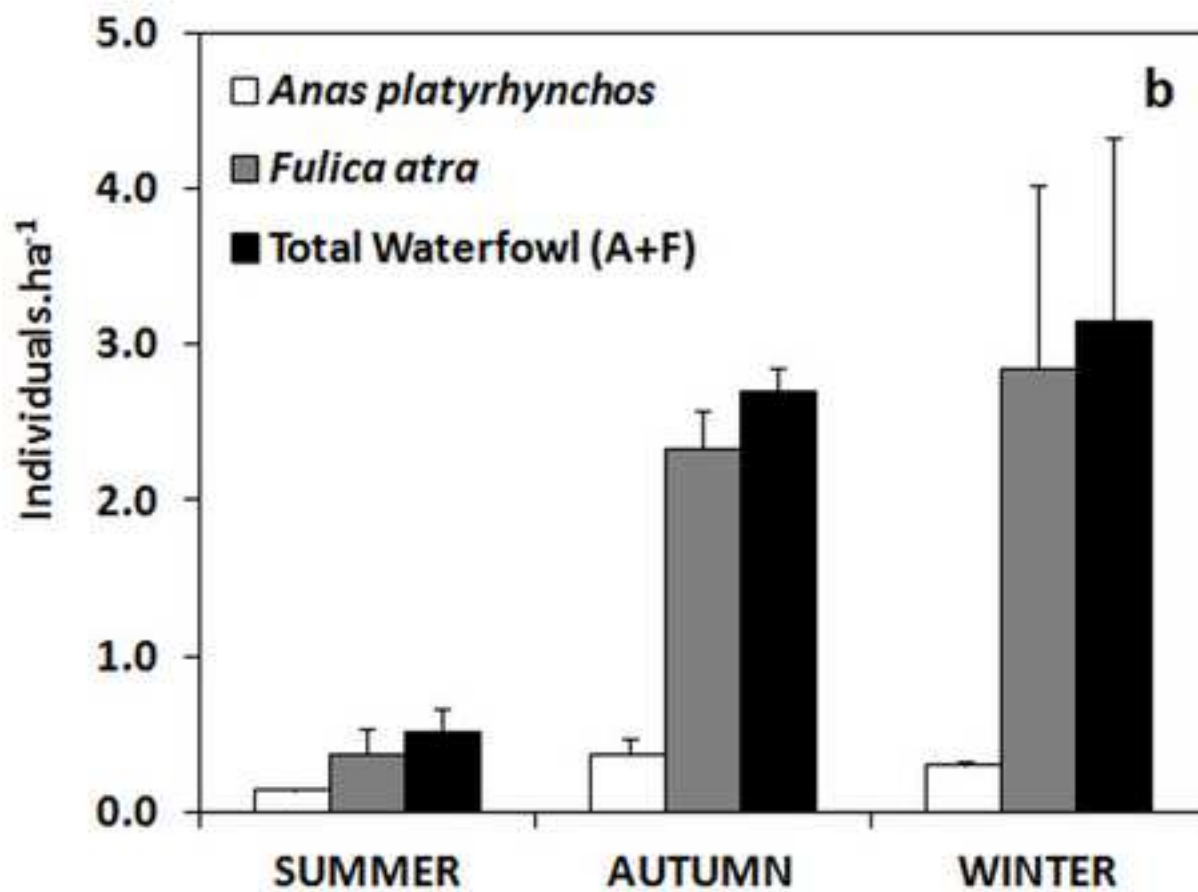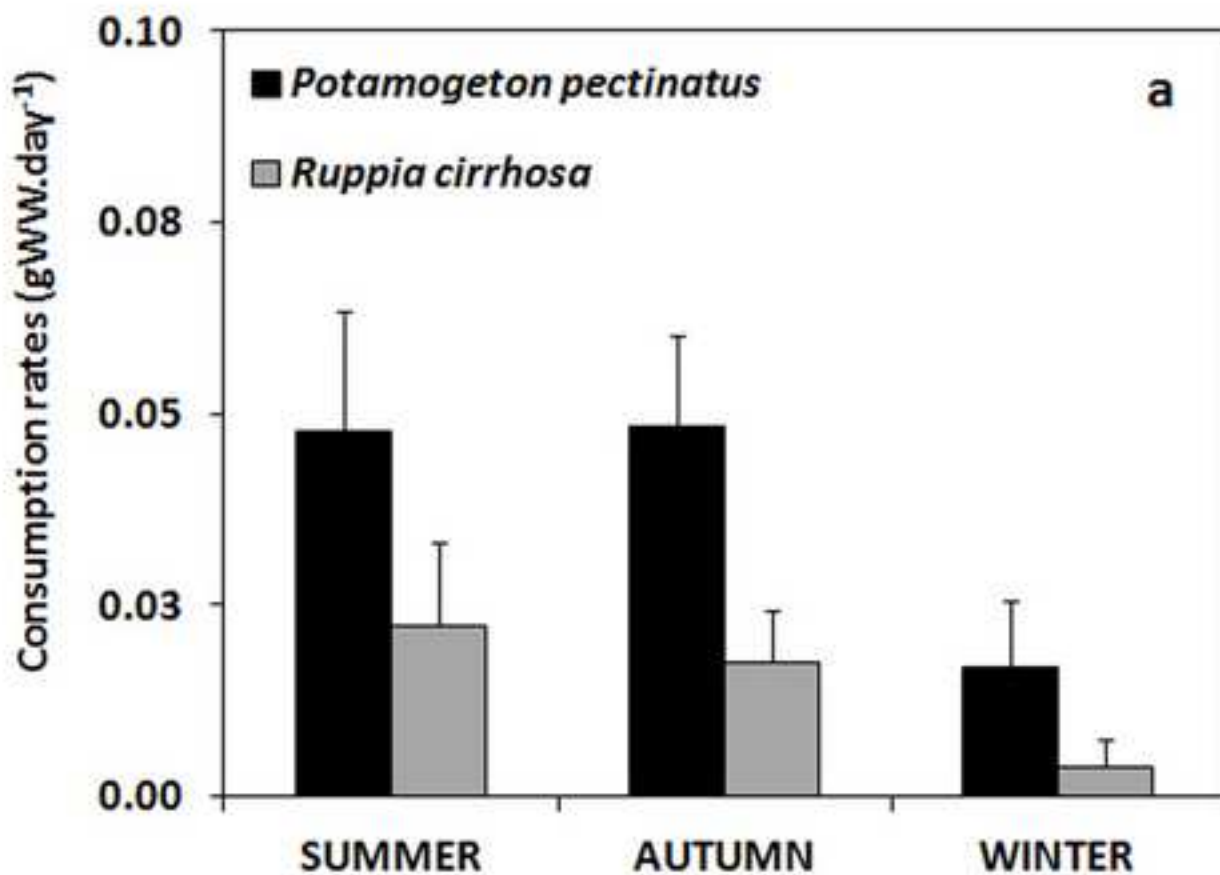
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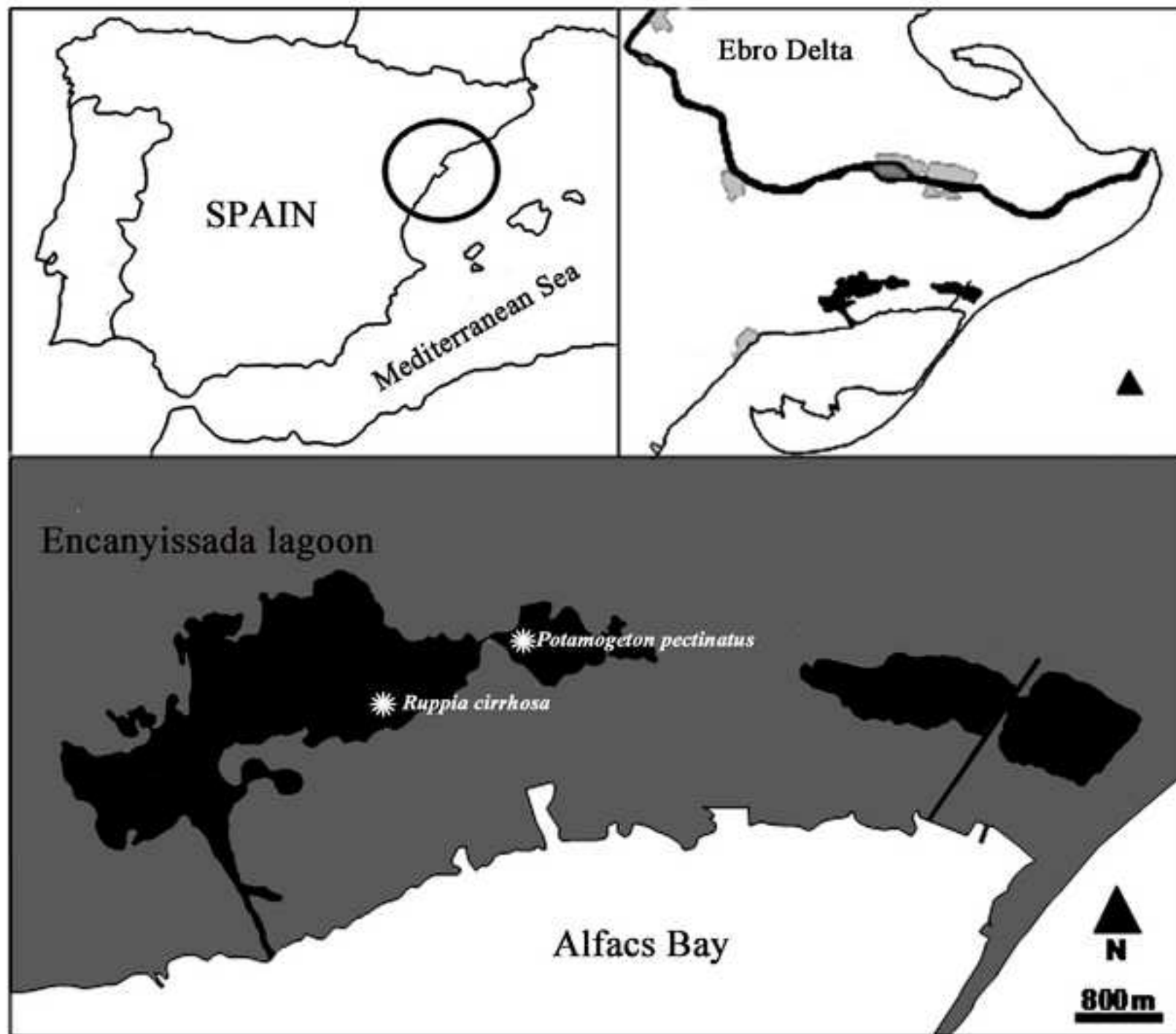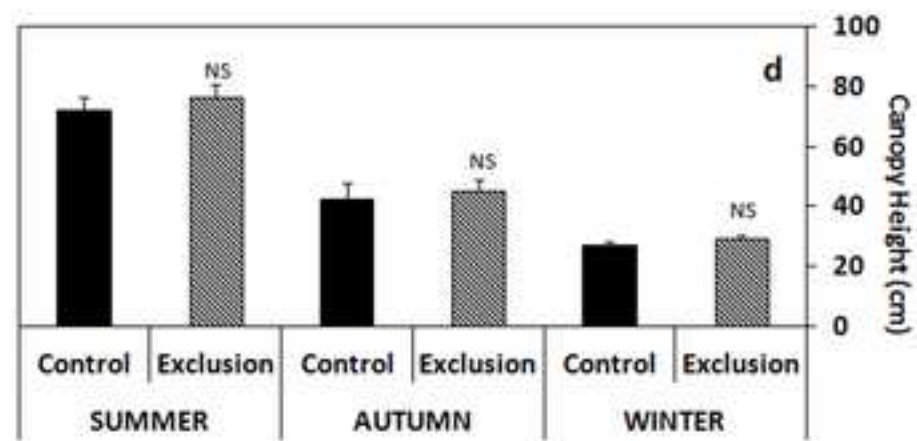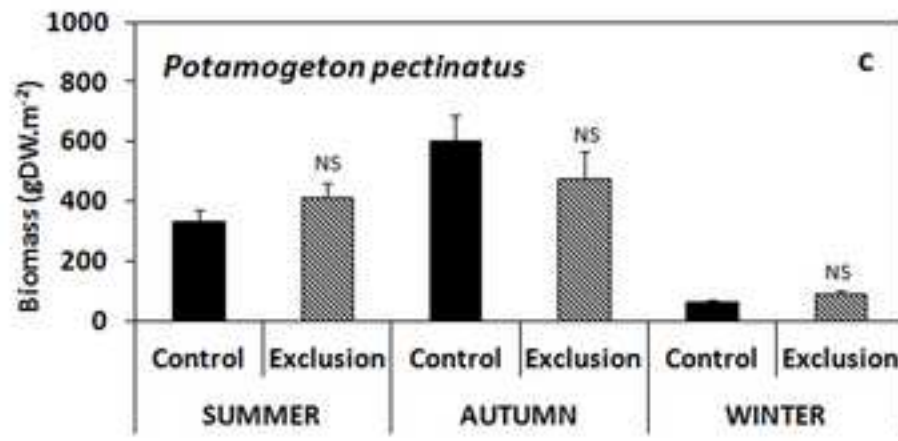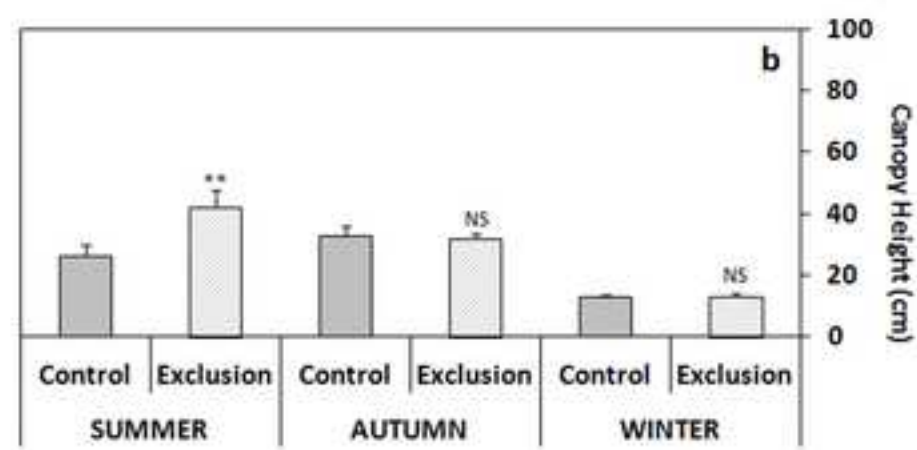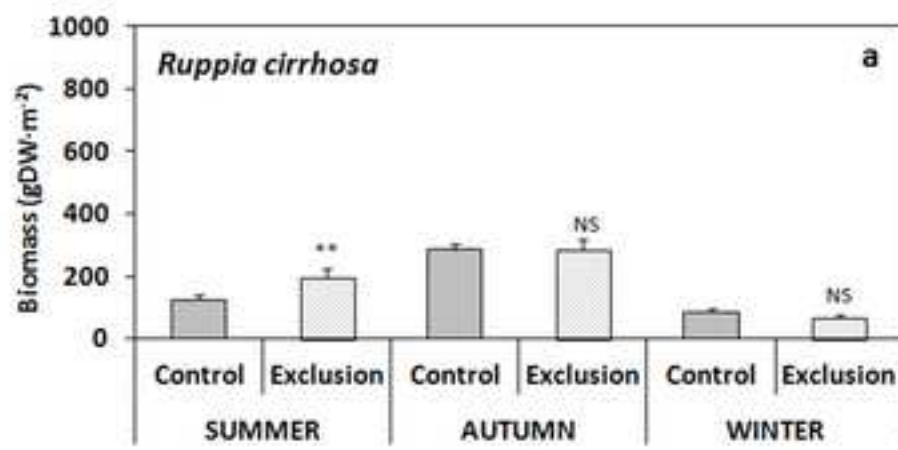
Table 1. Two-way ANOVA testing for differences on biomass (g DW·m^{-2}) and canopy height (cm) among seasons (S: summer; A: autumn; W: winter) and treatments (C: control; E: exclusion) in *Ruppia cirrhosa* and *Potamogeton pectinatus*. Significant differences are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, 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	*Rupppia cirrhosa*								*Potamogeton pectinatus*							
	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

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			

2

Table 3. Two-way ANOVA testing for differences on consumption (g WW·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 *Ruppia cirrhosa* and *Potamogeton pectinatus* 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			

3

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 (AAn) andwinter (WAn), *F. atra* population in summer (SF), autumn in (AF), and winter (WF) are indicated.

	a.Total waterfowl Census (A+F)			
Source of variation	**df**	**MS**	**F**	**p**
Season (S;A;W)	2.00	4.35	16.24	**
Residual	9.00	0.27		
Transformation		Ln x		
	b.Waterfowl Census (An and F)			
Source of variation	**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 = AAn = WAn			
Transformation		Ln x		

2

4