1   **Plant dispersal by teal (*Anas crecca*) in the Camargue:**

2   **duck guts are more important than their feet**

3

4   **Brochet, A.L.[a,b,1], Guillemain, M.[a], Fritz, H.[c], Gauthier-Clerc, M.[b] and Green, A.J.[d,1]**

5   [a] Office National de la Chasse et de la Faune Sauvage, CNERA Avifaune Migratrice, Le

6   Sambuc, 13200 Arles, France

7   [b] Centre de Recherche de La Tour du Valat, Le Sambuc, 13200 Arles, France

8   [c] Université de Lyon, Université Claude Bernard Lyon 1, CNRS UMR 5558 Biométrie et

9   Biologie Evolutive, Bâtiment 711, 43 bd du 11 novembre 1918, 69622 Villeurbanne

10   cedex, France

11   [d] Department of Wetland Ecology, Estación Biológica de Doñana-CSIC, C/ Américo

12   Vespucio s/n, 41092 Sevilla, Spain

13

14   Brochet, A.L.- brochet@tourduvalat.org

15   Guillemain, M. - matthieu.guillemain@oncfs.gouv.fr

16   Fritz, H. - fritz@biomserv.univ-lyon1.fr

17   Gauthier-Clerc, M. - gauthier-clerc@tourduvalat.org

18   Green, A.J. - ajgreen@ebd.csic.es

19

20   [1] These authors contributed equally to this work.

21

26 **Summary**

27 1. Migratory waterbirds are major vectors for the dispersal of aquatic plants. However,

28 quantitative field studies of the frequency of transport are scarce and the relative importance

29 of internal and external transport remains unclear.

30 2. We quantified and compared the rates of internal and external transport of aquatic plant

31 propagules by teal (*Anas crecca*) in the Camargue (southern France), inspecting the lower gut

32 contents of birds that had been shot (n = 366) and washing birds that had been live-trapped (n

33 = 68) during the winters of 2006-7 and 2007-8.

34 3. Intact propagules (n = 902) of 21 plant taxa were recorded in the rectum of teal, of which

35 16 germinated or were shown to be viable. Intact propagules were recorded in the rectum of

36 20% of teal, with up to 171 propagules per individual bird. *Chara* oogonia were most

37 abundant (60% of intact propagules), suggesting that small size favours internal transport.

38 *Eleocharis palustris*, *Juncus* spp. and *Potamogeton pusillus* (17%, 7% and 6% of intact

39 propagules, respectively) were also very abundant.

40 4. Intact propagules (n = 12) of 10 plant taxa were found on the outside of live teal, and four

41 of these taxa later germinated. Intact propagules were found on 18% of teal. No teal was

42 found to carry more than one propagule externally. There was no difference in size between

43 propagules transported internally and externally.

44 5. Teal are major dispersers of plants within the Camargue, despite being highly granivorous.

45 Contrary to widespread assumptions in the literature, endozoochory by ducks appears to be a

46 much more important mode of dispersal for aquatic plants than exozoochory. We found no

47 evidence of changes in the probability of plant propagule dispersal at a landscape scale over

48 the course of the winter, so propagule production and zoochory appear to be decoupled over

49 time in aquatic systems.

Introduction

Passive dispersal of aquatic plants by birds is assumed to be a major ecological process, with advantages over dispersal via wind or water. Wind-dispersal is considered unfavourable for aquatic plants because it does not necessarily direct diaspores (the plant reproductive body and dispersal unit, i.e. seeds and oospores) towards aquatic habitats, whilst water-dispersal does not allow dispersal between waterbodies lacking a physical connection (Barrat-Segretain, 1996). Since aquatic plants often have a very large geographic range, ducks and other migratory waterbirds have long been proposed as major vectors for their dispersal (Darwin, 1859; Ridley, 1930). As these birds repeatedly move between different waterbodies at both local and broader scales, there is a relatively high probability that any propagules they carry will be dispersed to suitable habitat patches (Green, Figuerola & Sánchez, 2002). The ability of aquatic plants to undergo long-distance dispersal via migratory birds is likely to explain why they generally have a much wider geographical distribution than animals of a similar size (Fenchel & Finlay, 2004).

Plant propagules can be carried either in waterbird guts ("endozoochory" or internal dispersal) or attached on the outside of their body ("exozoochory" or external dispersal), and the latter has been widely assumed to be the most important process, especially because of the presence of small seeds in mud stuck to birds' feet (Darwin, 1859; Ridley, 1930). However, most studies of either mechanism have been anecdotal in nature, and detailed quantitative studies in the field remain scarce (Figuerola & Green, 2002a). There have been particularly few studies of external transport (but see Vivian-Smith & Stiles, 1994; Figuerola & Green, 2002b). Several recent field studies have quantified numbers of propagules in waterbird faeces and confirmed their viability (Figuerola, Green & Santamaría, 2002, 2003; Charalambidou & Santamaría, 2005; Sánchez, Green & Castellanos, 2006; Green *et al.*, 2008). We are unaware of any previous studies comparing the importance of external and

3

75   internal transport simultaneously in a given bird population, though previous studies

76   collectively confirm that dispersal mediated via waterbirds can be frequent in the field both by

77   internal and external transport.

78   In this study, we investigated the role of teal (*Anas crecca* L.) as a dispersal vector of

79   aquatic plant diaspores in the Mediterranean wetlands of the Camargue (southern France),

80   comparing exo- and endozoochory. The Camargue area covers 140,000 ha in the Rhone

81   Delta, and hundreds of thousands of ducks spend the winter there or stop to feed during

82   migration. Teal are extremely abundant, representing 20 to 30 % of the wintering duck

83   population and the annual peak count of this species averages 36,000 birds (Tamisier &

84   Dehorter, 1999). Wintering teal in natural conditions feed mainly on aquatic plant propagules

85   (Tamisier, 1971) and make regular movements between different waterbodies, spending the

86   day on roosting sites and moving to feeding grounds at night (Tamisier, 1978). Experiments

87   in captivity confirm that seeds can survive passage through the teal gut and be retained long

88   enough to permit dispersal over hundreds of km or more during migratory flights

89   (Charalambidou, Santamaría & Langevoord, 2003; Pollux, Santamaría & Ouborg, 2005). All

90   these characteristics make teal likely vectors of plant propagules.

91   The main objective of this study was to quantify the rates of internal and external

92   transport of plant propagules by teal within the Camargue. We quantified the presence of

93   propagules in the lower gut and carried on feathers or feet, and tested their viability. We

94   compared the frequencies of endo- and exozoochory for propagules of different sizes. We

95   tested the hypothesis that smaller propagules were more likely to be dispersed externally,

96   since they would be more likely to remain attached (Cousens, Dytham & Law, 2008).

97   Although previous research has also suggested that smaller seeds are more likely to be

98   transported internally (Soons *et al.*, 2008), we hypothesized that small seed size was more

99   important in external than internal transport. We considered the importance of spatial and

100 temporal variation in rates of plant propagule transport. We tested the hypothesis that

101 propagule dispersal would be coupled to propagule production, and would therefore be more

102 frequent during the early part of the wintering period (see Clausen *et al.*, 2002). Finally, we

103 reevaluated the importance of external *versus* internal plant propagule transport by ducks in

104 the light of our findings.

105

106 **Methods**

107 *Collection and analysis of samples*

108   To study internal transport, the terminal part of the digestive tract (rectum) was extracted

109 from teal shot at seven different hunting estates (Fig. 1), between September and January of

110 the wintering seasons 2006-7 (n = 183 teal, hereafter the 2007 winter) and 2007-8 (n = 183

111 teal, hereafter the 2008 winter) (Table 1). We focussed on the rectum because any intact

112 propagules present in this part of the digestive tract can be considered to have survived gut

113 passage (Charalambidou & Santamaría, 2002). Almost all birds were shot when flying out of

114 feeding sites towards roosting sites, so that viable propagules in their rectum would very

115 likely have been dispersed from one wetland to another. Ducks were sexed and aged using

116 plumage criteria (Carney, 1992), although 2.2% of birds had an undetermined age. The

117 rectum was removed by dissection, and then stored in a plastic bag in the fridge until analysis

118 over the next few days. For statistical analyses, we pooled the few birds shot at sites 4 to 7.

119 The upper gut contents of these same birds were used in a diet study (Brochet *et al.*,

120 unpublished). Birds with a full oesophagus were preferentially selected for dissection in the

121 2008 winter, which may explain why more plant propagules were found in the rectum in the

122 second year (see Discussion).

123   To study external transport, samples were collected from 68 live teal captured for ringing.

124 Teal were caught using standard baited funnel traps concealed in the vegetation (Bub, 1991),

125　at the Research Centre of Tour du Valat (Fig. 1), between October and March of 2007 (n = 10

126　teal) and 2008 (n = 58 teal) (Table 1). Again, ducks were sexed and aged using plumage

127　criteria, and the age of 1.5% of the birds was undetermined. Two samples were taken from

128　each bird. First, samples from the feet were obtained by rinsing both feet into a plastic

129　container. Then, samples from feathers were collected by brushing the whole plumage over a

130　tray with a nylon bristle brush, then rinsing this material into a second plastic container. Using

131　these techniques, we may have retrieved most or all propagules from the feet, but perhaps

132　only the more easily detachable propagules from the plumage. It is possible that propagules

133　deeply embedded in the plumage were missed (Vivian-Smith & Stiles, 1994). Samples were

134　stored in the fridge before analysis during the next few days. We excluded from our dataset

135　those seeds found on the outside of teal that may have come from bait used in the trap (*Oryza*

136　*sativa* L., *Polygonum persicaria* L. and *Scirpus maritimus* L., but see Table 2 for details). Our

137　bait was waste rice collected after the commercial harvest, hence the presence of rice weeds.

138　　　In the laboratory, samples were rinsed with mineral water through a 63-μm sieve, and the

139　residue was examined under a binocular microscope. Intact plant seeds and algal oogonia

140　were separated from the residue. They were counted and identified using Campredon *et al.*

141　(1982), Cappers, Bekker & Jans (2006) and a reference collection from the Camargue. Taxa

142　whose intact seeds could not be identified were listed as unknown. All intact oogonia

143　belonged to the genus *Chara* (*C. vulgaris* L., *C. globularis* Thuill. and *C. aspera* Deth. ex

144　Willd. are the most frequent species in the Camargue, J.B. Mouronval pers. comm).

145　　　Propagule size (maximum length) was measured to the nearest 0.02 mm on graph paper

146　under a binocular microscope for all taxa recorded. Ten propagules per species were

147　measured, these being taken from the oesophagus of some of the same birds collected for the

148　rectum sample. Oesophagus propagules provided a better estimate of size because they had

149　not been subjected to mechanical erosion in the gizzard. Median values were used in

150   statistical analyses. The size of *Papaver* sp. and *Tamarix* sp. were taken from Cappers,

151   Bekker & Jans (2006), because these species were absent from the oesophagus of teal

152   analysed.

153

154   *Germination and viability tests*

155   Intact propagules collected in the 2008 winter were germinated in microtitre trays with up

156   to 10 propagules per cell, which was filled with mineral water. The *Chara* oogonia from a

157   given individual duck were germinated in one or two small containers (diameter 6 cm, height

158   5 cm), because oogonia tended to develop fungi when placed in microtitre trays. Trays were

159   positioned in the laboratory at room temperature (20.6 ± 0.3°C [$\bar{x} \pm$ s.e.], ranging from 12° to

160   32°C over the whole germination test period) and natural light condition. Germination was

161   checked every 7 days for 56 days. At the same time, water was replenished and germinated

162   propagules were removed. The few propagules that became infected by bacteria and/or fungi

163   were immediately removed and the water of the cell refreshed. We did not apply antibiotics

164   because increased infection risk is one of the processes affecting natural seed viability

165   following gut passage (Figuerola, Green & Santamaría, 2002). After 56 days, non-germinated

166   propagules were placed in the fridge for two months. Their germinability was then retested

167   with the above protocol. After another 56 days, non-germinated propagules were dried at

168   room temperature during one month and their germinability was then retested with the same

169   protocol. Cold and dry periods were applied in order to break the dormancy of non-

170   germinated seeds (Baskin & Baskin, 1998). After a final period of 56 days, propagules were

171   tested for viability using a standard tetrazolium method (De Vlaming & Proctor, 1968; Holt-

172   Mueller & van der Valk, 2002). By cutting propagules, embryos were exposed, and then were

173   incubated on filter paper saturated with 1% tetrazolium solution in a Petri dish, for 24h in the

174   dark at room temperature. Red staining indicated reduction of 2,3,5-triphenyltetrazolium

175 chloride by respiratory activity in the cells (Nachlas, Margulies & Seligman, 1960). Hence,

176 propagules showing a positive tetrazolium response (*i.e.* with respiring embryos) were

177 assumed to be viable.

178

179 *Statistical analyses*

180   We used a logistic regression with a binomial error distribution to model the probability

181 that a bird had at least one intact plant propagule in its rectum as a function of the year of

182 collection (2007 or 2008 winters), the site of collection (factor of four levels: site 1, 2, 3 and

183 others), the day within the year at which it was examined (Julian date), sex (female or male),

184 and age (juvenile, adult or undetermined). We analysed presence or absence rather than the

185 number of propagules present owing to the high proportion of zeros (80 % of teal had no

186 propagule in the rectum). The interaction Sex * Age was also included. Day was included as a

187 second order polynomial (i.e. adding Day² to the model) in order to take into account potential

188 non-linear patterns across the wintering season. To reduce colinearity in polynomial models

189 (see Legendre & Legendre, 1998), the 15 November was considered as day 0 (to divide the

190 hunting season into two equal parts), earlier dates being considered as negative values. We

191 carried out models for all plant species pooled together, then for *Chara* spp. and *Potamogeton*

192 *pusillus* L. (the two most frequent species) separately. For each of the three cases, we tested

193 all possible models (64 possible subsets of the six predictor variables). Then we used the

194 Akaike Information Criterion (AIC) to compare different models. The best one has the

195 smallest AIC value. However, if the difference of AIC (ΔAIC) between two models was less

196 than two, they were considered as equivalent (McCullagh & Nelder, 1989). As several best

197 models were found, a model-averaging approach was carried out with all the best models

198 (Burnham & Anderson, 2002). Final estimates and standard-errors of the parameters

199 associated with each variable were thus obtained by weighting them across models with their

200 respective weight of AIC (ωAIC). Higher ωAIC resulted in a better explanation of the data

201 variation by models. This last parameter was thus considered as evidence that the candidate

202 model with higher ωAIC was the best out of the set of models considered (Burnham &

203 Anderson, 2002). Similar logistic regressions of the presence/absence of viable propagules

204 were conducted for the 2008 winter for all plant species pooled, for *Chara* spp. and for

205 *Potamogeton pusillus*, but no predictor variables had significant effects (results not shown).

206 We did not carry out a similar logistic regression with plant propagules recorded externally

207 because of the small sample size.

208   Given the high number of zeros, we used non-parametric Mann-Whitney U tests to

209 compare the total number of intact or viable propagules in the rectum of teal from different

210 years and/or for early and later parts of the winter season (comparing teal collected before and

211 after 15 November). Similarly, Kruskal-Wallis tests were used to compare the number of

212 propagules between collection sites, using pairwise *post-hoc* tests with sequential Bonferroni

213 correction (Rice, 1989). Differences between years and early and late winter in the numbers

214 of propagules recorded externally on teal were also tested with Mann-Whitney U tests.

215   To analyse the propagule viability in rectal samples (for data from the 2008 winter), we

216 used a logistic regression with a binomial error distribution to model the proportion of viable

217 propagules (summing germinated and non-germinated viable propagules) of *Chara* spp. (n =

218 31 teal) and *P. pusillus* (n = 12 teal), as a function of the Day (Julian date) and Day². The total

219 number of intact propagules of each species found in the rectum was included in the logistic

220 regression model as a binomial denominator. We applied the same method as for the previous

221 logistic regression analysis.

222   To test the effect of propagule size on the kind of transport, we carried out a Fisher exact

223 test to compare the proportion of propagules < 1, 1-2, and > 2 mm in length in rectal and

224 external samples. A χ^2 test was not applied because some categories had low expected values.

225   R software (version 2.8.0) was used for all statistical analyses (R Development Core

226   Team, 2008).

227

228   **Results**

229   *External transport*

230   Intact diaspores were not found externally on any of the 10 teal studied in 2007. In 2008,

231   intact propagules of 10 taxa were found on a total of 12 of 58 teal, and four of these taxa later

232   germinated (Table 2). No teal was found to carry more than one plant propagule. There was

233   no evidence of a linear seasonal trend in the probability of carrying propagules (Mann-

234   Whitney U test comparing the number of propagules present on teal before and after 15

235   November for 2008: n = 27 and 31 teal respectively, U = 493, P = 0.121). Although only teal

236   in the second winter carried some propagules, the difference between winters was not

237   statistically significant (Mann-Whitney U test, n = 10 and 58 teal respectively, U = 220, P =

238   0.086).

239

240   *Internal transport*

241   Intact diaspores of a total of 21 plant taxa were recorded in the rectum of teal, of which 16

242   were later germinated or shown to be viable. Overall, intact propagules were recorded in

243   19.9% of teal studied. *Chara* spp. was the most abundant taxon, representing 60.2% of all

244   intact propagules and being present in 9.8% of teal (Table 3). *Potamogeton pusillus* was the

245   most frequent of the remaining taxa and was recorded in 4.4% of birds, although *Eleocharis*

246   *palustris* and *Juncus* spp. were more abundant in terms of total number of seeds (Table 3). Of

247   propagules recorded in the 2008 winter, 38 % of *Chara* oogonia and 75% of seeds of other

248   taxa germinated or were shown to be viable (Table 3).

249   We found 7, 7 and 12 best models of logistic regression for all propagule species

250   combined, *Chara* spp. and *P. pusillus* respectively (see Appendix). A significant positive year

251   effect was found for each dependent variable (Table 4), because the probability of the

252   presence of plant propagules was consistently higher during the 2008 winter (27.9% of teal

253   with intact propagules, compared to 12.0% in 2007). A significant site effect was also found

254   for all plant species pooled (Table 4), owing to differences between sites in the frequency

255   with which teal carried propagules in the rectum (Fig. 2). Tukey *post-hoc* tests suggested only

256   one pairwise difference between sites 1 and 2 (n = 98 and 110 respectively; P = 0.052), with

257   greater propagule presence in the former, though this was only marginally significant (Fig. 2).

258   Mann-Whitney U tests showed that the number of intact propagules in rectal samples in

259   2008 was significantly greater than in 2007, for all propagules combined (U = 13810, P <

260   0.001) as well as for *Chara* spp. (U = 14529, P < 0.001) and *P. pusillus* (U = 16013, P =

261   0.04). Similar tests found no evidence of a seasonal effect, with no difference for either winter

262   between the number of intact or viable propagules recorded in teal shot before or after 15

263   November (all U-values < 4336, all P-values > 0.171).

264   When comparing differences in total number of propagules between sites with Kruskal-

265   Wallis tests, there was a statistically significant effect in 2007 (2007: $H_{3,183}$ = 12.794, P =

266   0.005; 2008: $H_{3,183}$ = 4.828, P = 0.074). This site effect was mainly due to the different

267   proportions of teal carrying at least one intact propagule in the rectum (Fig. 2). *Post-hoc* tests

268   showed that the only significant pairwise difference was between sites 1 and 3, (n = 55, 40

269   respectively, U = 1360, P = 0.006) with more propagules in the former ($\bar{x}$ ± s.e. of number of

270   propagules per bird: 0.62 ± 0.03 and 0.00 ± 0.00, respectively).

271   When analysing with logistic regression the proportion of intact propagules in rectal

272   samples that were viable, we found no significant effect of the Day or Day² for *Chara* spp. or

273   *P. pusillus* (all P-values > 0.1).

274

275 *Propagule size effect*

276   The size range of propagules transported internally and externally was very similar (Fig.

277 3) with no significant difference in the proportion of propagules of different size classes

278 between transport modes (Fisher exact test: P = 0.350).

279

280 **Discussion**

281   To our knowledge, this study is the first to make such a detailed comparison of rates of

282 internal and external transport of plants by waterbirds. Since birds were shot when flying

283 from feeding sites towards day-roosts, viable propagules in their rectum were very likely to be

284 dispersed from one wetland to another. Teal at the site for live-trapping are also known to

285 move between different wetlands on a daily basis. Moreover, teal undergo long-distance

286 movements. There are approximately 2 million teal wintering in Europe (Wetlands

287 International, 2006), many of which undergo long-distance migrations to the Camargue and

288 other areas. Long-distance dispersal of propagules between the Camargue and other areas is

289 favoured by the important fraction of propagules retained in the gut for 12 h or more

290 (Charalambidou, Santamaría & Langevoord, 2003; Pollux, Santamaría & Ouborg, 2005;

291 Brochet *et al.*, unpublished). Long-distance dispersal can occur throughout the winter, as there

292 is a high turnover rate within the teal wintering population (Pradel *et al.*, 1997) and teal often

293 move away from the Camargue in response to weather (Ridgill & Fox, 1990).

294   Our study suggests that teal are dispersers of many different aquatic plants within and

295 beyond the Camargue wetlands. We detected intact propagules in the rectum or on the outside

296 of 20% and 18% of teal, respectively, although more propagules were found in the rectum and

297 these figures underestimate the numbers transported internally (see below). Previous field

298 studies have used different methods that are not readily comparable, but propagules have been

299   found in 12% to 50% of teal faecal samples, varying according to sites and seasons

300   (Figuerola, Green & Santamaría, 2003; Charalambidou & Santamaría, 2005). We are not

301   aware of previous quantitative studies of external transport in teal, but small numbers of seeds

302   were recorded on most waterbirds studied by Vivian-Smith & Stiles (1994) and Figuerola &

303   Green (2002b).

304   We identified 21 plant taxa in the teal rectum, including the exotic species *Ludwigia*

305   *peploides*, *Paspalum distichum*, *Heteranthera reniformis* and *H. limosa*. These species are not

306   native to Europe and were introduced in the Camargue as ricefield weeds (Marnotte *et al.*,

307   2006). Our results thus suggest that teal are important vectors of exotic species (see also

308   Brochet *et al.* 2009). Many other plants are likely to be dispersed by the Camargue teal

309   population, and we have detected propagules of an additional 57 plant taxa when analysing

310   the foregut contents of teal included in this study (Brochet *et al.*, unpublished; see also

311   Brochet *et al*. 2009). Most or all of these additional taxa can potentially undergo

312   endozoochory by teal, and their absence from our rectal samples is explained by their relative

313   rarity and the low probability of them being in the rectum at the particular time of our

314   sampling. Diaspores are much more abundant in the oesophagus (1310 ± 827 [$\bar{x} \pm$ s.e.] (n =

315   183 teal) and 4857 ± 1192 propagules (n = 183 teal) in 2007 and 2008, respectively) and,

316   unlike propagule size or hardness, abundance of a given taxon in the oesophagus is a

317   significant predictor of its presence in the hindgut (Brochet *et al.*, 2009). Furthermore, in

318   captive experiments 41% of diaspores fed to teal were recovered intact in faeces (Brochet *et*

319   *al.*, unpublished).

320

321   *Relative importance of endo- versus exozoochory*

322   Our results suggest that, in general, internal transport is much more important than

323   external transport for aquatic plants dispersed by ducks. We found both abundance (means of

324   2.5 *versus* 0.2 propagules per sample) and diversity (means of 0.3 *versus* 0.2 taxa per sample)

325   of propagules to be higher in internal samples than in external samples. In addition, rates of

326   internal dispersal were severely underestimated, as teal held many propagules further along

327   the digestive tract that were likely to have been egested if they had not been hunted (see

328   above).

329   Some idea of the actual extent of transport can be given as follows. At the time of

330   collection, the gizzard and oesophagus of a teal contained only part of the daily intake of

331   diaspores, since most are egested within 8 h (Charalambidou, Santamaría & Langevoord,

332   2003; Pollux, Santamaría & Ouborg, 2005; Brochet *et al.*, unpublished). The teal we studied

333   contained a mean of 49 ± 4 intact *P. pusillus* seeds in the oesophagus and gizzard combined,

334   and during an experiment in captivity 46% of *P. pusillus* seeds survived gut passage (of which

335   78% were viable, Brochet *et al.*, unpublished). These figures are probably extreme values,

336   because teal used in the feeding experiment were not used to feeding on seeds. However this

337   would suggest that each teal could egest 17 viable *P. pusillus* seeds on a daily basis,

338   equivalent to over 300,000 a day for the whole Camargue teal population.

339   Rates of exozoochoric dispersal are also likely to be underestimated to some extent in our

340   study. We cannot rule out the possibility that some propagules became detached between

341   entering the trap and being examined (birds were transported in mesh cages from the trap

342   within the marsh to the ringing site on the shore). However, teal spend more than one hour per

343   day preening their feathers (Tamisier & Dehorter, 1999) and are likely to detect and remove

344   seeds quickly from their plumage (Sorensen, 1986). Our data, together with a review of

345   literature, strongly suggest that the relative importance of exozoochory has been

346   overemphasised in the past. For example, Ridley (1930) proposed that *Chara*, *Juncus* and

347   *Myriophyllum spicatum* L. reach new wetlands on birds' feet, whereas we found all these taxa

348   to be transported internally. Similarly, Carlquist (1967) proposed that *Eleocharis* and

349 *Ranunculus* are only transported externally by birds, but we found them to be transported

350 internally. In contrast, our findings agree with those of Proctor (1962), who found *Chara*

351 oogonia to be abundant in the lower guts of six waterbird species in North America, and

352 argued that internal transport of oogonia is more important than external transport.

353   We found no evidence that exozoochory is relatively more important for smaller

354 propagules than endozoochory. We may possibly have found a different result with a larger

355 duck species such as mallard (*A. platyrhynchos* L.), since teal are the smallest European duck

356 and tend to ingest relatively small propagules (Pirot, Chessel & Tamisier, 1984; Guillemain &

357 Fritz 2002). However, small size is considered to improve the dispersal potential of seeds in

358 both aquatic (De Vlaming & Proctor, 1968; Holt-Mueller & van der Valk, 2002, Soons *et al.*,

359 2008) and terrestrial systems (Traveset, 1998), not only due to their large numbers (that

360 increases overall dispersal probability; Jakobsson & Eriksson, 2000), but also because small

361 propagules are more likely to survive passage through the gizzard (Soons *et al.*, 2008). The

362 dominance of *Chara* oogonia amongst the propagules we recovered supports the view that, as

363 a rule in macroecology, smaller organisms are more readily dispersed (Fenchel & Finlay,

364 2004). It is possible that, the smaller the propagule of an aquatic plant in the Camargue, the

365 more likely that species is to be dispersed by teal both externally and internally.

366   Most of the propagules that were transported externally lacked hooks or other

367 morphological adaptations for external dispersal (see Sorensen, 1986), and five of the eight

368 identified taxa found externally were also found internally, suggesting that exozoochory is

369 largely a secondary mechanism for taxa dispersed more often by endozoochory. *Papaver* sp.

370 was one of three taxa only found externally, but we found and germinated one *Papaver* seed

371 in a dropping collected from one of the live-trapped teal (results not shown), confirming the

372 potential for internal transport of this species also. Of the other two, the ability of *Phragmites*

373 *australis* seeds to survive gut passage has been shown experimentally (Soons *et al.*, 2008).

374   Overall, apart from size, seed morphology seems to be an unreliable predictor of which taxa

375   are passively dispersed by birds or other means (see also Calviño-Cancela *et al.*, 2006;

376   Brochet *et al.*, 2009).

377

378   *Spatial and temporal variation in dispersal rates*

379   We found no change in the probability of passive dispersal by teal during the course of a

380   given winter. This strongly suggests there is a decoupling in the timing of plant propagule

381   production and their dispersal by birds in this system. This is largely because propagules can

382   be ingested by ducks from the sediments or become attached to bird feet or feathers long after

383   they have dropped from the mother plant (Green, Figuerola & Sánchez, 2002). Even for

384   fleshy-fruited terrestrial plants dispersed by passerines, there is extensive decoupling between

385   fruit production and rates of avian dispersal (Herrera, 1998). Thus, we found no evidence to

386   support previous suggestions (Clausen *et al.*, 2002) that submerged macrophyte seed dispersal

387   period is probably limited to the seed production period in later summer and autumn. Previous

388   studies have also shown viable seeds to be abundant in duck faeces in late winter (Figuerola,

389   Green & Santamaría, 2002, 2003).

390   There is annual and spatial variation in the availability of different plant foods and their

391   presence in the diet of teal in the Camargue, so it is no surprise that we found differences

392   between years and sites in the numbers of intact propagules present in the rectum. However,

393   the difference between years is likely to be a consequence of our change in methodology.

394   During the second year of collection, teal were partly selected on the basis of whether or not

395   they had a full crop, as judged externally by palpation of the neck (so as to study their diet).

396   Approximately 87% of teal were considered to have a full crop. Plant propagule abundance in

397   the upper gut is a significant predictor of their presence in the lower gut (Holt-Mueller & van

398   der Valk, 2002; Brochet *et al.*, 2009), and the number of propagules found in the teal

399 oesophagus was significantly higher during the second winter (see above). Hence, we were

400 probably more likely to study teal holding propagules in their rectum during the second

401 winter.

402

403   In conclusion, our data demonstrate that teal are major dispersers of plant diaspores,

404 despite being highly granivorous. They can disperse propagules both internally and

405 externally, but the former is quantitatively much more important, contrary to previous

406 assumptions. Roughly 10 times as many diaspores were found in a teal rectum (mean of 2.5)

407 as carried externally (mean of 0.2), and taking into account the propagules found in the higher

408 gut that survive gut passage, as many as a thousand may be dispersed daily by each teal,

409 equivalent to tens of millions at the scale of the teal population wintering in the Camargue.

410 Propagule morphology may not affect the importance of internal versus external transport,

411 and may not a predictor of which propagules are dispersed by teal, although teal

412 predominantly disperse small seeds. There is significant spatial variability in transport rates

413 between sites, but propagule dispersal occurs throughout the entire winter season and is

414 decoupled from propagule production.

415

426

References

428 Barrat-Segretain M.H. (1996) Strategies of reproduction, dispersion, and competition in river

429   plants: a review. *Vegetatio*, **123**, 13-37.

430 Baskin C.C. & Baskin J.M. (1998) *Seeds - Ecology, Biogeography, and Evolution of*

431   *Dormancy and Germination*. Academic Press, San Diego.

432 Brochet A.L., Guillemain M., Fritz H., Gauthier-Clerc M. & Green A.J. (2009) The role of

433   migratory ducks in the long-distance dispersal of native plants and the spread of exotic

434   plants in Europe. *Ecography*, doi: 10.1111/j.0906-7590.2009.05757.x

435 Bub H. (1991) *Bird trapping and bird banding. A handbook for trapping methods all over the*

436   *world*. Cornell University Press, New-York.

437 Burnham K.P. & Anderson D.R. (2002) *Model selection and multimodel inference: a*

438   *practical information – theoretic approach, 2nd Edition*. Springer, New York.

439 Calviño-Cancela M., Dunn R.R., Van Etten E.J.B. & Lamont B.B. (2006) Emus as non-

440   standard seed dispersers and their potential for long-distance dispersal. *Ecography*, **29**,

441   632-640.

442 Campredon S., Campredon P., Pirot J.Y. & Tamisier A. (1982) *Manuel d'analyse des*

443   *contenus stomacaux de canards et de foulques*. ONC, Paris.

444 Cappers R.T.J., Bekker R.M. & Jans J.E.A. (2006) *Digital seed atlas of the Netherlands*.

445   Barkhuis Publishing, Eelde.

446 Carlquist S. (1967) The Biota of Long-Distance Dispersal. V. Plant Dispersal to Pacific

447   Islands. *Bulletin of the Torrey Botanical Club*, **94**, 129-162.

448   Carney S.M. (1992) *Species, age and sex identification of ducks using wing plumage*. U.S.

449   Department of the interior, U.S. Fish and Wildlife Service, Washington.

450   Charalambidou I. & Santamaría L. (2002) Waterbirds as endozoochorous dispersers of

451   aquatic organisms: a review of experimental evidence. *Acta Oecologica*, **23**, 165-176.

452   Charalambidou I. & Santamaría L. (2005) Field evidence for the potential of waterbirds as

453   dispersers of aquatic organisms. *Wetlands*, **25**, 252–258.

454   Charalambidou I., Santamaría L. & Langevoord O. (2003) Effect of ingestion by five avian

455   dispersers on the retention time, retrieval and germination of *Ruppia maritima* seeds.

456   *Functional Ecology*, **17**, 747-753.

457   Clausen P., Nolet B.A., Fox A.D. & Klaassen M. (2002) Long-distance endozoochorous

458   dispersal of submerged macrophyte seeds by migratory waterbirds in northern Europe

459   - a critical review of possibilities and limitations. *Acta Oecologica*, **23**, 191-203.

460   Cousens R., Dytham C. & Law R. (2008) *Dispersal in plants, a population perspective,*

461   Oxford University Press, Oxford.

462   Darwin C. (1859) *On the Origin of Species by Means of Natural Selection*. Murray, London.

463   De Vlaming V. & Proctor V.W. (1968) Dispersal of aquatic organisms: viability of seeds

464   recovered from the droppings of captive killdeer and mallard ducks. *American Journal*

465   *of Botany*, **55**, 20-26.

466   Fenchel T. & Finlay B.J. (2004) The ubiquity of small species: patterns of local and global

467   diversity. *Bioscience*, **54**, 777-784.

468   Figuerola J. & Green A.J. (2002a) Dispersal of aquatic organisms by waterbirds: a review of

469   past research and priorities for future studies. *Freshwater Biology*, **47**, 483-494.

470   Figuerola J. & Green A.J. (2002b) How frequent is external transport of seeds and

471   invertebrate eggs by waterbirds? A study in Donana, SW Spain. *Archiv für*

472   *Hydrobiologie*, **155**, 557-565.

473 Figuerola J., Green A.J & Santamaría L. (2002) Comparative dispersal effectiveness of
474   wigeongrass seeds by waterfowl wintering in South-West Spain: quantitative and
475   qualitative aspects. *Journal of Ecology*, **90**, 989-1001.

476 Figuerola J., Green A.J. & Santamaría L. (2003) Passive internal transport of aquatic
477   organisms by waterfowl in Doñana, south-west Spain. *Global Ecology &*
478   *Biogeography*, **12**, 427-436.

479 Green A.J., Figuerola J. & Sánchez M.I. (2002) Implications of waterbird ecology for the
480   dispersal of aquatic organisms. *Acta Oecologica*, **23**, 177-189.

481 Green A.J., Jenkins K.M., Bell D., Morris P.J. & Kingsford R.T. (2008) The potential role of
482   waterbirds in dispersing invertebrates and plants in arid Australia. *Freshwater*
483   *Biology*, **53**, 380-392.

484 Guillemain M. & Fritz H. (2002) Temporal variation in feeding tactics: exploring the role of
485   competition and predators in wintering dabbling ducks *Wildlife Biology,* **8**, 81-90.

486 Herrera C.M. 1998. Long-term dynamics of mediterranean frugivorous birds and fleshy fruits:
487   a 12-year study. *Ecological Monographs*, **68**, 511-538.

488 Holt Mueller M. & Van Der Valk A.G. (2002) The potential role of ducks in wetland seed
489   dispersal. *Wetlands*, **22**, 170-178.

490 Jakobsson A. & Eriksson O. (2000) A comparative study of seed number, seed size, seedling
491   size and recruitment in grassland plants. *Oikos*, **88**, 494–502.

492 Legendre P. & Legendre L. (1998) *Numerical ecology*. Elsevier, Amsterdam.

493 Marnotte P., Carrara A., Dominati E. & Girardot F. (2006) *Plantes des rizières de Camargue*.
494   Cirad, CFR, PNRC.

495 Mc Cullagh P. & Nelder J.A. (1989) *Generalized Linear Models, 2nd Edition*. Chapman &
496   Hall/CRC, Boca Raton.

497 Nachlas M.M., Margulies S.I. & Seligman A.M. (1960) Sites of electron transfer to

498   tetrazolium salts in the succinoxidase system. *Journal of Biological Chemistry*, **235,**

499   2739-2743.

500 Pirot J.Y., Chessel D. & Tamisier A. (1984) Exploitation alimentaire des zones humides de

501   Camargue par cinq espèces de canards de surface en hivernage et en transit :

502   modélisation spatio-temporelle. *Revue d'Ecologie (Terre Vie)*, **39**, 167-192.

503 Pollux B.J.A., Santamaría L. & Ouborg N.J. (2005) Differences in endozoochorous dispersal

504   between aquatic plant species, with reference to plant population persistence in rivers.

505   *Freshwater Biology*, **50**, 232-242.

506 Pradel R., Rioux N., Tamisier A. & Lebreton J.D. (1997) Individual turnover among

507   wintering teal in Camargue: A mark-recapture study. *Journal of Wildlife Management*,

508   **61**, 816-821.

509 Proctor V.W. (1962) Viability of *Chara* oospores taken from migratory waterbirds. *Ecology*,

510   **43**, 528-529.

511 R Development Core Team (2008) R: a language and environment for statistical computing.

512   R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL

513   http://www.R-project.org.

514 Rice W.R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

515 Ridgill S.C. & Fox A.D. (1990) *Cold weather movements of waterfowl in western Europe*.

516   IWRB Publication 13, Slimbridge.

517 Ridley H.N. (1930) *The Dispersal of Plants Throughout the World*. Reeve & Co., Ltd,

518   Ashford.

519 Sánchez M.I., Green A.J. & Castellanos E.M. (2006) Internal transport of seeds by migratory

520   waders in the Odiel marshes, south-west Spain: consequences for long-distance

521   dispersal. *Journal of Avian Biology*, **37**, 201-206.

522   Soons M.B., Van Der Vlugt C., Van Lith B., Heil G.W. & Klaassen M. (2008) Small seed

523   size increases the potential for dispersal of wetland plants by ducks. *Journal of*

524   *Ecology*, **96**, 619-627.

525   Sorensen A.E. (1986) Seed dispersal by adhesion. *Annual Review of Ecology and Systematics*,

526   **17**, 443-463.

527   Tamisier A. (1971) Régime alimentaire des sarcelles d'hiver *Anas crecca* L. en Camargue (In

528   French with an English summary). *Alauda*, **39**, 261-311.

529   Tamisier A. (1978) The functional units of wintering ducks: a spatial integration of their

530   comfort and feeding requirements. *Verhandlungen der Ornithologischen Gesellshaft*

531   *in Bayern*, **23**, 229-238.

532   Tamisier A. & Dehorter O. (1999) *Camargue, canards et foulques. Fonctionnement et devenir*

533   *d'un prestigieux quartier d'hiver.* Centre Ornithologique du Gard, Nîmes.

534   Traveset A. (1998) Effect of seed passage through vertebrate frugivores' gut on germination: a

535   review. *Perspectives in Plant Ecology, Systematics and Evolution*, **1**, 151-190.

536   Vivian-Smith G. & Stiles E.W. (1994) Dispersal of salt marsh seeds on the feet and feathers

537   of waterfowl. *Wetlands*, **14**, 316-319.

538   Wetlands International (2006) *Waterbird population estimates, 4th edition*. Wetlands

539   International, Wageningen.

540

Tables

Table 1. Number of teal samples for internal and external transport collected each month in both study winters.

Month	Rectum				Washing			
	2007[1] (n = 183)		2008 (n = 183)		2007 (n = 10)		2008 (n = 68)	
	Female	Male	Female	Male	Female	Male	Female	Male
September[2]	5	12	11	35	-	-	-	-
October	15	31	9	22	-	-	11	9
November	22	22	5	12	0	2	10	9
December	15	15	25	19	-	-	1	2
January	19	27	20	25	-	-	5	9
February[3]	-	-	-	-	2	3	2	0
March[3]	-	-	-	-	2	1	-	-

[1] September 2006 to March 2007.

[2] No washing samples were collected in September, because water levels were too low to use the trap.

[3] No rectal samples were collected in February and March, because the hunting season closed at the end of January.

549 **Table 2.** List of plant taxa of which propagules were found intact in teal washing samples (P: plumage and F: feet) for winter 2008 (n = 58 teal)

550 (WP: number of birds with intact propagules; TP: total number of intact propagules; N germ: number of propagules that germinated). None of the

551 propagules that failed to germinate were found to be viable with a tetrazolium test.

Plant family	Plant taxa	Location	Length (in mm)	WP	TP	N germ
Characeae	*Chara* sp.	F	0.58	1	1	0
Chenopodiaceae	*Salicornia* sp.	P	1.13	1	1	1
Cyperaceae	*Cyperus difformis* L.	F	0.64	1	1	1
	Scirpus maritimus[1]	P	2.48	1	1	0
Juncaceae	*Juncus* sp.	F	0.52	2	2	1
	Juncus sp.	P	0.52	1	1	0
Papaveraceae	*Papaver* sp.	P	0.95	1	1	0
Polygonaceae	*Polygonum persicaria*[1]	P	2.32	1	1	0
Gramineae	*Paspalum distichum* L.	P	2.72	1	1	0
	Phragmites australis (Cav.) Trin. ex Steud.	F	1.82	1	1	0
Tamaricaceae	*Tamarix* sp.	F	0.81	1	1	0
Unknown	Unidentified (2 taxa)	P	-	2	2	1
	Total			12	12	4

552 [1] These seeds were excluded from the totals and from further analyses as they may have come from bait used in the trap.

553 **Table 3.** List of plant taxa of which propagules were found intact in teal rectum samples for winters 2007 and 2008 (WP: number of birds with

554 intact propagules; TP: total number of intact propagules; Max: maximum number of intact propagules in one bird; N germ: number of propagules

555 collected in the 2008 winter that germinated; N viable: number of propagules collected in the 2008 winter that did not germinate but were shown

556 to be viable by a tetrazolium test).

Plant family	Plant taxa	Length (in mm)	2007 (n = 183)			2008 (n = 183)			N germ	N viable
			WP	TP	Max	WP	TP	Max		
Characeae	*Chara* spp.	0.58	5	34	21	31	525	144	182	15
Chenopodiaceae	*Salicornia* spp.	1.13	-	-	-	1	1	1	1	0
	Suaeda maritima (L.) Dumort.	0.65	-	-	-	3	7	5	5	1
Cyperaceae	*Cyperus difformis* L.	0.64	3	6	4	2	20	19	19	0
	Eleocharis palustris (L.) Roem. & Schult.	1.33	3	23	21	5	130	34	112	11
	Schoenoplectus mucronatus (L.) Palla	1.90	-	-	-	1	2	2	2	0
	Scirpus maritimus L.	2.48	-	-	-	5	6	2	1	5
Gramineae	*Echinochloa crus-galli* (L.) P.Beauv.	3.92	4	7	3	2	2	1	1	0
	Paspalum distichum L.	2.72	1	4	4	2	2	1	0	0
Haloragaceae	*Myriophyllum spicatum* L.	1.84	1	1	1	2	6	4	3	1
Juncaceae	*Juncus* spp.	0.52	3	3	1	3	61	39	16	0
Leguminosae	*Trifolium repens* L.	1.28	-	-	-	1	1	1	1	0
Onagraceae	*Ludwigia peploides* (Kunth) P.H.Raven	1.23	-	-	-	1	1	1	0	0
Polygonaceae	*Polygonum lapathifolium* L.	2.10	2	2 [1]	1	-	-	-	-	-
	Polygonum persicaria L.	2.32	1	2	2	-	-	-	-	-
Pontederiaceae	*Heteranthera limosa* (Sw.) Willd.	0.75	1	2	2	-	-	-	-	-
	Heteranthera reniformis Ruiz & Pav.	0.64	-	-	-	3	4	2	4	0
Potamogetonaceae	*Potamogeton pusillus* L.	1.55	4	16	13	12	30	12	21	1
Ranunculaceae	*Ranunculus* spp.	1.12	1	1	1	1	1	1	0	1
Unknown	Unidentified (2 taxa)	-	1	1	1	1	1	1	1	0
	Total		22	102	22	51	800	171	369	35

557 [1]One of these *P. lapathifolium* seeds germinated after removal from the rectum.

558 **Table 4.** Results of model-averaging based on alternative models (see Appendix), presenting

559 final estimate (β), standard-error (se) and p-value (s: significant, ns: non significant) for each

560 variable and for all plant species pooled together, then for *Chara* spp. and for *Potamogeton*

561 *pusillus*.

Variables	All plant species pooled			*Chara* spp.			*P. pusillus*		
	β	se	p	β	se	p	β	se	p
Year	1.334	0.478	s	2.570	0.824	s	1.354	0.460	s
Site	-0.311	0.152	s	-0.180	0.208	ns	-0.015	0.103	ns
Day	-0.0003	0.001	ns	-0.0002	0.001	ns	-0.004	0.004	ns
Day²	-0.00004	0.00006	ns	-0.0001	0.0001	ns	-0.00007	0.0001	ns
Age	-0.123	0.204	ns	-	-	-	0.813	1.333	ns
Sex	0.013	0.100	ns	0.046	0.194	ns	-0.029	1.318	ns
Age*Sex	-	-	-	-	-	-	-0.659	0.87	ns

562

Figure legend

564 **Figure 1.** Map of the Camargue showing the seven collection sites for rectum samples (dark

565 circles) and the collection site for external transport (hatched square).

566

567 **Figure 2.** Proportion of teal with intact plant propagules in the rectum according to collection

568 sites (Others: site 4 to 7, pooled together as relatively small numbers of teal were collected in

569 these sites), for study winters 2007 (in grey) and 2008 (in white). Error bars indicate 95%

570 Confidence Intervals. Sample sizes for 2007 and 2008, respectively, were: site 1, n = 55, 43;

571 site 2, n = 41, 69; site 3, n = 40, 42; others, n = 47, 29.

572

573 **Figure 3.** Percentage of intact plant propagules found in teal rectums (grey) and external

574 transport samples (white) according to size classes (< 1, 1 - 2, and > 2 mm in length).
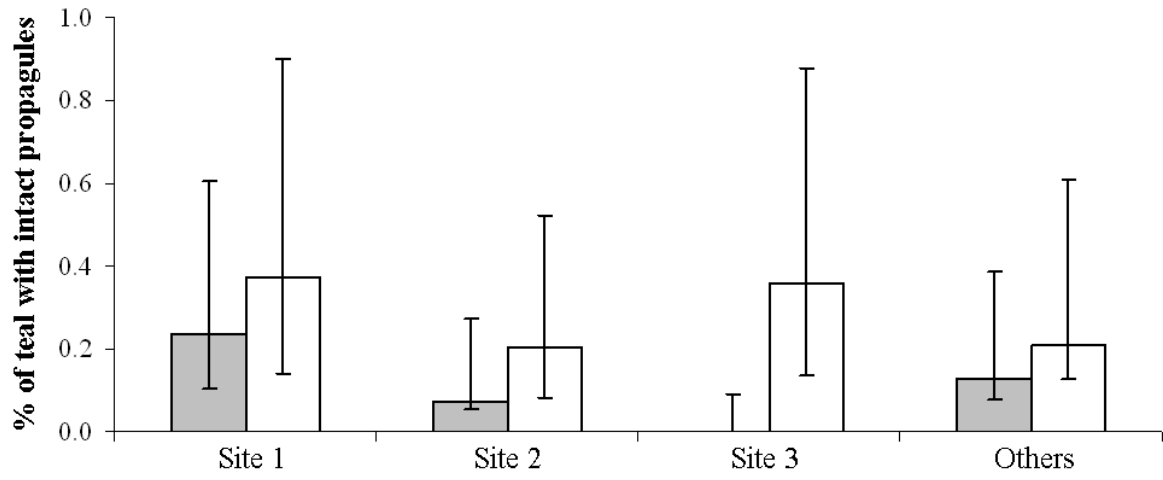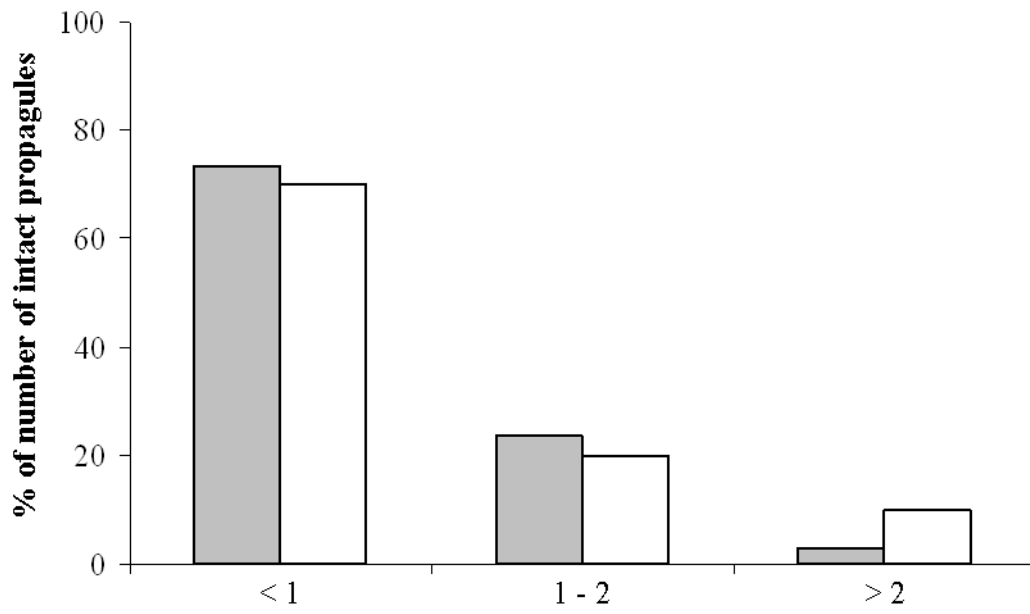
575

576

577

578
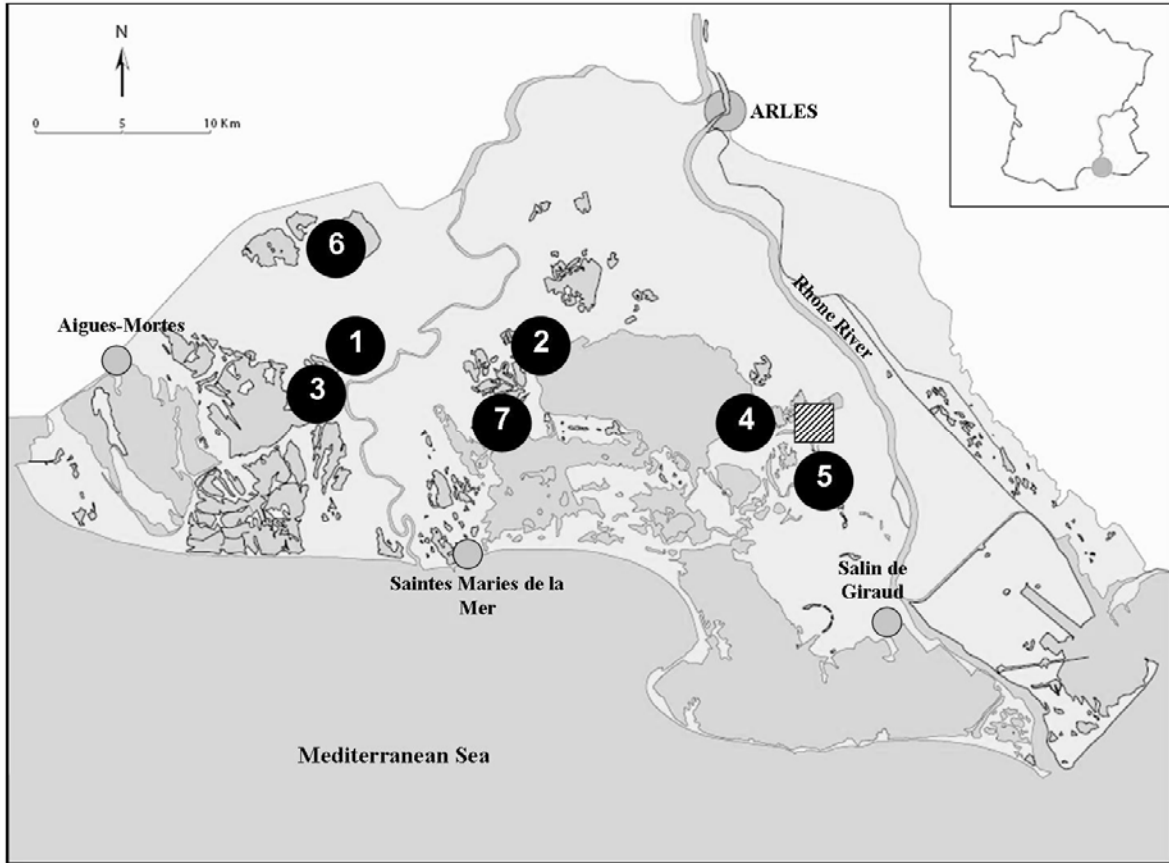
585   **Appendix.** Null and best models (ΔAIC < 2) of logistic regression for the presence/absence of

586   plant diaspores in teal rectums, for all plant species combined, then for *Chara* spp. and for

587   *Potamogeton pusillus*, ranked in decreasing order of fit.

Dependent variable	Models	AIC	ΔAIC
All plant species pooled	Year + Site	353.22	0.00
	Year + Site + Day²	353.84	0.62
	Year + Site + Age	353.90	0.68
	Year + Site + Day² + Age	354.25	1.03
	Year + Site + Day	354.64	1.42
	Year	355.02	1.80
	Year + Site + Sex	355.06	1.84
	Null model	367.74	14.52
Chara spp.	Year + Site +Day²	215.91	0.00
	Year + Day²	215.97	0.06
	Year	216.37	0.46
	Year + Site	216.44	0.53
	Year + Site + Day² + Sex	217.54	1.63
	Year + Site + Day +Day²	217.67	1.76
	Year + Day² + Sex	217.72	1.81
	Null model	237.31	21.40
P. pusillus	Year + Sex	129.95	0.00
	Year +Day + Sex	129.97	0.02
	Year + Day² + Sex	130.71	0.76
	Year + Age +Sex	130.8	0.85
	Year	131.08	1.13
	Year + Day + Age + Sex	131.09	1.14
	Year + Day² + Age + Sex	131.10	1.15
	Year + Day +Day² + Sex	131.14	1.19
	Year + Day²	131.34	1.39
	Year + Site + Day + Sex	131.55	1.60
	Year + Day + Day² + Age + Sex	131.87	1.92
	Year + Site + Sex	131.90	1.95
	Null model	133.45	3.50

588