

Title	“Step by step”: high frequency short-distance epizoochorous dispersal of aquatic macrophytes
Authors	Coughlan, Neil E.; Kelly, Thomas C.; Jansen, Marcel A. K.
Publication date	2016-10-14
Original Citation	Coughlan, N. E., Kelly, T. C. and Jansen, M. A. K. (2016) ‘“Step by step”: high frequency short-distance epizoochorous dispersal of aquatic macrophytes’, <i>Biological Invasions</i> , 19(2), pp. 625-634. doi:10.1007/s10530-016-1293-0
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1007/s10530-016-1293-0
Rights	© 2016, Springer International Publishing. This is a post-peer-review, pre-copyedit version of an article published in <i>Biological Invasions</i> . The final authenticated version is available online at: http://dx.doi.org/10.1007/s10530-016-1293-0
Download date	2023-10-01 12:19:12
Item downloaded from	https://hdl.handle.net/10468/3267



UCC

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

1 “Step by step”: High frequency short-distance epizoochorous dispersal of
2 aquatic macrophytes.

3

4 Neil E. Coughlan*, Thomas C. Kelly & Marcel A.K. Jansen.

5 School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland.

6 *Correspondence: neil.coughlan.zoology@gmail.com

7 Author Contributions: NC proposed the study topic. TK and MJ advised on experimental design. All
8 authors (NC, TK and MJ) participated in the writing-up and editing of the manuscript.

9 We declare that all authors have contributed to and approved the final draft of the article.

10

11 1. Abstract

12 Aquatic macrophytes can successfully colonise and re-colonise areas separated by space and time. The
13 mechanisms underlying such “mobility” are not well understood, but it has often been hypothesised
14 that epizoochory (external dispersal) plays an important role. Yet, there is only limited, and mostly
15 anecdotal, evidence concerning successful epizoochorous dispersal of aquatic macrophytes,
16 particularly in the case of short-distance dispersal. Here we examine *in situ* and *ex situ* dispersal of
17 aquatic macrophytes, including three invasive alien species. A high frequency of *Lemna minor*
18 Linnaeus dispersal was observed *in situ*, and this was linked to bird-mediated epizoochory. We
19 concluded that wind had no effect on dispersal. Similarly, in an *ex situ* examination *Lemna minuta*
20 Kunth and *Azolla filiculoides* Lamarck, were found to be dispersed with a high frequency by mallard
21 ducks (*Anas platyrhynchos*). No dispersal was measured for *Elodea nuttalli* (Planchon) H. St. John. It
22 is concluded that short-distance or “stepping-stone” dispersal *via* bird-mediated epizoochory can occur
23 with high frequencies, and therefore can play an important role in facilitating colonisation, range
24 expansion and biological invasion of macrophytes.

25

26

27 2. Introduction

28 Freshwater systems can be viewed from a classical island biogeographic perspective as islands of
29 freshwater in a 'sea' of terrestrial habitats (Magnuson 1976). However, despite the isolation of aquatic
30 habitats, and the limited life span of lakes and wetlands on geological and evolutionary time scales,
31 aquatic plants tend to have broader distributions than their terrestrial counterparts (Santamaría 2002).
32 Moreover, freshwater systems have been shown to be at high risk from biological invasions (Sala et
33 al. 2000; Kelly et al. 2014). Thus, in spite of a restricted or total lack of ability to self-disperse, an
34 abundance of aquatic organisms have successfully managed to colonise and re-colonise areas separated
35 by space and time (De Meester et al. 2002; Shepherd et al. 2009). Therefore, it has often been
36 concluded that means of assisted dispersal must be readily available (Clausen et al. 2002; Santamaría
37 2002; Green and Figuerola 2005; Trakhtenbrot et al. 2005).

38

39 Dispersal can be defined as any movement of individuals or propagules with potential consequences
40 for gene flow across space (Ronce 2007). A propagule can be defined as a structure acting as an agent
41 of reproduction and/or propagation (seeds, vegetative bodies, spores, eggs, ephippia, gemmules,
42 statoblasts, cysts) (Reynolds et al. 2015). Recognised as a fundamental process in ecology, dispersal
43 is essential for colonisation, range expansion and the long term survival of species (Cain et al. 2000;
44 With 2002; Holt 2003; Ramakrishnan 2008; Thorsen et al. 2009; Viana et al. 2013).

45

46 Frequency of successful dispersal events of aquatic macrophytes remains a matter of speculation;
47 indeed, our understanding of the dispersal processes operating in wetlands remains limited (Cohen and
48 Shurin 2003; Figuerola et al. 2003; Soomers et al. 2013). While various mechanisms of dispersal are
49 essential in facilitating meta-community connectivity, research on the topic of how organisms
50 effectively surmount dispersal barriers has been largely neglected in meta-community analyses (Moritz

51 et al. 2013; Cañedo-Argüelles et al. 2015). Potential vectors of passive dispersal include water currents
52 (hydrochory), wind (anemochory), other animals (zoochory) and anthropogenic activity (Bilton et al.
53 2001; Trakhtenbrot et al. 2005; Nathan et al. 2008; Pollux 2011; van Leeuwen et al. 2012a,c).
54 Anemochorous seed dispersal of wetland plants across fragmented landscapes has been experimentally
55 investigated and modelled by many studies (Soomers et al. 2013). While wind can play an important
56 role in passive dispersal of the vegetative parts of aquatic macrophytes, water currents are considered
57 to be the dominate mechanism for many free-floating plants (Downing-Kunz and Stacey 2011). In
58 addition, research suggests zoochory to be important in surmounting dispersal limitations, thus
59 facilitating the spread of species (Cunze et al. 2013). Mammals, such as ungulates and rodents, can
60 facilitate zoochorous dispersal within aquatic and terrestrial habitats (Waterkeyn et al. 2010;
61 Vanschoenwinkel et al. 2011; Albert et al. 2015; Ginman et al. 2015; Nomura and Tsuyuzaki 2015).
62 Birds, in particular, are considered effective dispersers by both internal (endozoochory) and external
63 (epizoochory) means (Green and Elmberg 2014). In his seminal work, Landolt (1986) states that
64 animals (birds, mammals, amphibians and reptiles) are the main distributors of Lemnaceae, and this
65 hypothesis appears to be widely accepted (Les et al. 2003; Mackay and James 2016). However, in fact,
66 there is limited, and mostly anecdotal, evidence concerning bird-mediated dispersal of Lemnaceae
67 (Coughlan et al. 2015). Additionally, while several studies demonstrate bird-mediated epizoochorous
68 dispersal of plant seeds (see for example: Figuerola and Green 2002; Brochet et al. 2010; Raulings et
69 al. 2011; Aoyama et al. 2012), few studies have documented attachment or dispersal of vegetative
70 propagules. In exception, while examining shot blue-winged teal (*Anas discors*) and mallard (*Anas*
71 *platyrhynchos*) ducks, Jacobs (1947) observed viable *L. minor* within the breast plumage of one
72 individual. Similarly, Reynolds et al. (2015) observed and photographed *L. minor* attached to
73 underwing feathers of yellow-billed duck *Anas undulate*. However, much more work is required to
74 assess the extent to which bird-mediated dispersal can contribute to biological invasion (Green 2016).

76 Research suggests bird-mediated dispersal may be an overlooked link between habitats, facilitating
77 connectivity and gene flow for some species (Green 2016; Reynolds et al. 2015). The literature on
78 avian-mediated dispersal has mostly focused on endo- (reviewed by Traveset, 1998) and
79 epizoochorous (reviewed by Sorensen, 1986) transport of seeds and fleshy fruits of terrestrial plant
80 species. For example, Aoyama et al. (2012) found seeds of nine terrestrial plant species, including
81 several alien plant species, adhering to seabirds. Remarkably, some of the seeds identified were
82 generally considered to be dispersed by wind or bird-mediated endozoochory. In addition, a positive
83 relationship was observed between the distributions of plants and seabirds, indicating the ecological
84 impact of bird-mediated dispersal. Much less attention has been paid to bird-mediated dispersal of
85 aquatic species (Green and Elmberg 2014; – but see, van Leeuwen et al. 2012b), many of which do
86 not produce fleshy fruits, and/or are predominantly dispersed as vegetative propagules. Recently, the
87 role of bird-mediated epizoochory in the dispersal of invasive alien species, including many aquatic
88 plant species, has been firmly established (Green 2016; Reynolds et al. 2015). Nevertheless, many
89 basic questions, such as the frequency and ecological importance of bird-mediated epizoochorous
90 dispersal, remain under-researched. Few empirical studies have focused on the epizoochorous
91 dispersal of vegetative propagules, and even fewer studies have examined the ability of birds to
92 facilitate external dispersal and population connectivity of aquatic plants over local scales (but see, for
93 example, Brochet et al. 2010 and Reynolds and Cumming 2016). Thus, although it is well known that
94 colonisation of new ponds by aquatic plants can be a rapid process (Barnes 1983; Williams et al. 2008),
95 the mechanism of their dispersal remains largely unknown. This is due, in part, to the inherent
96 difficulty in designing an experimental protocol to examine dispersal mechanisms.

97

98 Here, we tested the hypothesis that free-floating aquatic plants are frequently dispersed over short
99 distances. Moreover, we explored the roles of anemochorous and epizoochorous transport in such

100 dispersal. In a follow-up, *ex situ* experiment, we tested the hypothesis that waterbirds readily facilitate
101 external dispersal of both free-floating and submerged invasive aquatic macrophytes.

102

103

104 3. Materials and methods

105 Here, we examined *in situ* dispersal of *Lemna minor* Linnaeus (experiment no. 1) over a short distance
106 of 1 m between a central source bowl and multiple receiving bowls. Receiving bowls were designed
107 to exclude various potential vectors. In addition, (experiment no. 2) we examined the potential for
108 waterbird-mediated epizoochorous dispersal of two floating (*Azolla filiculoides* Lamarck, *Lemna*
109 *minuta* Kunth) and one submerged (*Elodea nuttallii* (Planchon) H. St. John) invasive plant species
110 over short distances.

111

112 *Species selection*

113 *Lemna minuta* is an aquatic invasive alien that is present in many parts of Eurasia (Iberite et al. 2011).
114 The species is native in temperate regions of North and South America (Lucey 2003; Iberite et al.
115 2011). The congeneric species *Lemna minor* is native to most of Europe, Asia and North America, and
116 was used for *in situ* experiments. Through asexual reproduction, both species can quickly establish
117 floating mats that alter environmental conditions, including pH, dissolved oxygen and light penetration
118 of the water column, which in turn may affect the biotic composition of water bodies (Janes et al.
119 1996).

120

121 *Azolla filiculoides* an aquatic fern native to the tropics, subtropics, and warm temperate regions of
122 Africa, Asia, and the Americas, has invaded many parts of Europe and South Africa (Hill 2003;
123 Sadeghi et al. 2013). By impeding navigation, water flow and angling, causing fish kills and

124 threatening wetland nature reserves, thick floating mats of *A. filiculoides* have become a serious
125 environmental problem (Janes 1998).

126

127 Invasive *Elodea nuttallii* is a submerged freshwater plant species which occurs in lakes and slow
128 moving rivers (Champion et al. 2010). *E. nuttallii* can significantly alter freshwater communities based
129 on its rapid spread and high abundance (Champion et al. 2010). However, recent research suggests that
130 *E. nuttallii* may be less detrimental to European wetlands than previously thought (see Kelly et al.
131 2015).

132

133 All plant species were collected locally (Co. Cork, Ireland) during spring and summer months, and
134 maintained on-site in outdoor tanks. Stocks of *Lemna minor* were kept all year-round, while stocks of
135 *Azolla filiculoides*, *Lemna minuta* and *Elodea nuttallii* were collected ~ 6 weeks before experimental
136 use. *E. nuttallii* was stored in a large freshwater tank (120 cm (L) × 100 cm (W) × 88 cm (H)), while
137 free-floating species were stored in smaller tanks 31 cm in depth with a surface area of 1753 cm².

138

139 *Experiment I: In situ dispersal of L. minor*

140 Dispersal of *L. minor* was examined in the research gardens adjacent to the School of Biological, Earth
141 and Environmental Sciences, University College Cork, Ireland. Six independent dispersal monitoring
142 “stands” were constructed. Each dispersal monitoring stand consisted of a central source-bowl
143 containing *L. minor* and four receiving-bowls. Each bowl sat on a wooden platform (320 mm (L) ×
144 150 mm (W)) on top of a 1200 mm high wooden stake. Receiving-bowls were positioned as if on the
145 corners of a square around the central source-bowl (See Fig. 1). The source-bowl was positioned in
146 the centre of the stand, 1 m from each receiving-bowl within the square shaped layout. Bowls were
147 150mm in diameter and 25mm deep. The 6 dispersal monitoring stands were positioned in a line, each
148 spaced between 10 and 21 m apart from the next, from the first to the last. At just over 1m in height,

149 the bowls were accessible to birds for bathing and as song posts, but also for the most common rodent
150 species (e.g., mice *Apodemus sylvaticus* and rat (*Rattus norvegicus*)), which are excellent climbers.

151

152 The receiving-bowls were constructed to test for specific methods of facilitated dispersal; one excluded
153 birds, one excluded rodents, one excluded both rodents and birds, while one excluded nothing.
154 Hereafter referred to as 'exclude-bird', 'exclude-rodent', 'exclude-all' and 'exclude-nothing'
155 respectively. Potential anemochorous dispersal was not excluded from any. Rodents were excluded by
156 means of an inverted funnel, while birds were excluded by a cage constructed of plastic mesh (mesh
157 size: 18 mm × 25 mm) within which the receiving-bowl was enclosed (see Fig. 1). All bowls were
158 filled with rainwater and *L. minor* was added to the source-bowl. Drainage holes near the rim prevented
159 overflowing and loss of *L. minor*.

160

161 The experiment was run for 20 weeks from early January until the end of May 2015. During this time
162 dispersal monitoring units were examined every 3 - 4 days for the presence of *L. minor* in any of the
163 receiving-bowls. If any *L. minor* was found in a receiving-bowl, this was recorded as a single dispersal
164 event. Moreover, the colony and frond numbers of any observed *L. minor* were also recorded. All
165 dispersal monitoring units were examined on the same sampling days. *L. minor* within the source-
166 bowls was maintained at 50 - 75% surface coverage for the duration of the experiment. *Lemna* found
167 in the receiving-bowls was removed immediately.

168

169 Two motion-sensor trail-cameras (Spypoint Digital Surveillance Camera; TINY-D model) were used
170 to film dispersal events and potential vector species. The trail-cameras were set to record 1 minute
171 long videos when triggered. The cameras were attached to dispersal monitoring units 3 and 4. All
172 replicates were visually monitored for bird and rodent activity each time replicates were checked for

173 *L. minor* dispersal, and on an incidentally basis, such as when observers were in vicinity and observed
174 birds interacting with a replicate.

175

176 Weather data was obtained from the Irish Meteorological Service collected at Cork Airport weather
177 station.

178

179 *Experiment II: Ex situ dispersal of Azolla filiculoides, Lemna minuta and Elodea nutallii*

180 Twelve game-farm reared mallard ducks (*Anas platyrhynchos*) were acquired and kept in a large,
181 outdoor, free-range enclosure (15 m (L) × 3 m (W) × 3 m (H)), which included a housing unit for
182 shelter and an artificial pond. The group of mallards was comprised of 2 males and 10 females. All
183 birds were adults and of mixed ages. Birds displayed a variety of natural behaviours and were housed
184 within the enclosure for ~ 12 months prior to commencement of experimental work. The potential for
185 waterbird-mediated epizoochorous dispersal of *Azolla filiculoides*, *Lemna minuta* and *Elodea nutallii*
186 was examined within the enclosure, over summer months.

187

188 Three plastic containers (610 mm (L) × 410 mm (W) × 220 mm (H)) were placed within the enclosure,
189 each 1 m from the next, and filled with clean tap water. The mallards were then allowed to become
190 accustomed to the presence of the containers for a two day period. In order to limit the mallards to the
191 water sources provided by the experimental containers, the artificial pond was drained for the duration
192 of the entire experiment. The containers (baths) were checked at least four times daily on non-
193 experimental days and every 30 minutes on experiment days. Baths were re-filled with clean water as
194 required.

195

196 The examination of waterbird-mediated dispersal of each species was replicated five times using a
197 distance of 1 m between the baths. Dispersal was further monitored (× 3 replicates) using a distance

198 of 3 m between the baths. The centre bath was used as the 'source-bath' on all occasions. Plant species
199 were examined separately and every replicate took place over a 24 hour period. The mallards were
200 confined to the shelter within the enclosure at 20:30 hrs. Free-floating plants were then added to the
201 middle container (source-bath) until 80% of the water surface had been covered. The total fresh weight
202 (FW) was recorded. For submerged *E. nutallii*, a mass of 500g (FW) of whole plant material was
203 added. All three containers (1 'source-bath' and 2 'receiving-baths') held clean water at 90% of their
204 total volume. The mallards remained confined to the shelter for the night and were re-released into the
205 main enclosure at 07:30 hrs. The baths could potentially have been visited by small rodents (e.g., mice
206 and rat spp.) at night. Therefore, before the birds re-entered the enclosure the receiving-baths were
207 examined for the presence of plant material. Any plant biomass found in the 'receiving-baths' was
208 removed, patted dry with paper towels to remove excess water, and weighed (FW). The number of *L.*
209 *minuta* colonies found dispersed were recorded.

210

211 The mallards were left to interact with the experimental baths. Birds were free to roam, and displayed
212 a variety of behaviours, including preening. Dispersal potential of all three plant species was monitored
213 on a 30 minute basis once the birds had entered the main enclosure. Again, any plant biomass found
214 dispersed was removed and weighed (FW), or colonies counted. Monitoring was ceased at 20:35 or
215 when the entirety of plants had been consumed by the birds, whichever came first. Any plant biomass
216 remaining within the enclosure was removed and total fresh weight was recorded. A total of 500g
217 (FW) of *A. filiculoides* and 250g (FW) of *L. minuta* were determined to be sufficient quantities to cover
218 ~80% of the water surface within the 'source-bath'. While 500g of whole *E. nutallii* plants was added
219 to the 'source-bath' for each replicate as a mass of tangled vegetation, typical of *in situ* growth
220 formation.

221

222 On completion of the experiment any remaining invasive species material was destroyed by
223 autoclaving.

224

225

226 4. Statistical analysis

227 Data were analysed using (where applicable) Correlation and Regression, Pearson's Chi-Square tests
228 and one-way ANOVAs with the post-hoc Tukey HSD in SPSS (version 22; SPSS Inc, Chicago, IL,
229 USA). Post-hoc analysis of the Chi square test was conducted *via* examination of adjusted Z-values
230 against a Bonferroni corrected *P*-value (see García-pérez 2003). A logistic analysis of dispersal events
231 (binary; No_dispersal & Yes_dispersal) was also conducted. An Odds Ratio was generated to examine
232 the likelihood of wind force as a predictor of dispersal events. The highest mean wind speed, or
233 maximum gust if ≥ 34 knots, recorded on or between sampling days was selected as a measurement of
234 wind force preceding examination of the receiving-bowls; allowing lag effects to be accounted for
235 when selecting the highest wind force.

236

237

238 5. Results

239 *Experiment I: In situ dispersal of L. minor*

240 On 27 of 42 sampling days (64.3 %) at least one dispersal event (i.e. minimum 1 colony of 1 frond)
241 was observed in a receiving-bowl. A total of 67 dispersal events were recorded. Overall, dispersal
242 events comprised of 156 colonies. Most dispersed colonies consisted of 1 – 2 fronds, and a total of 317
243 fronds was found to be dispersed.

244

245 Few dispersal events were observed within 'exclude-all' receiving-bowls ($n = 5$), while 'exclude-
246 nothing' bowls displayed the greatest number of dispersal events ($n = 29$) ($X^2_3 = 21.89$, $P \leq 0.0001$)

247 (Fig. 2A). Post-hoc examination of the Chi square test determined observed dispersal in ‘exclude-all’
248 and ‘exclude-nothing’ receiving-bowls to be significantly different from all other receiving-bowl
249 types. Similarly, dispersed colony and frond numbers were highest in ‘exclude-rodent’ bowls and
250 lowest in ‘exclude-all’ receiving-bowls (ANOVA 1: $F_{3, 164} = 3.842$; $P \leq 0.05$ and ANOVA 2: $F_{3, 164} =$
251 3.651 ; $P \leq 0.05$ respectively) (Fig. 2B & C). Frond numbers were different in ‘exclude-rodent’ and
252 ‘exclude-bird’ bowls (ANOVA 2: $F_{3, 164} = 3.651$; $P = 0.05$). No other significant difference was
253 observed for colony numbers.

254

255 *Experiment I: Digital and visual surveillance*

256 Cameras recorded a total of five instances of black-billed magpie (*Pica pica*) visits to the monitored
257 replicates. Magpies were also visually observed to interact with all dispersal units replicates over the
258 duration of the experiment. Moreover, magpies were videoed moving between source- and receiving-
259 bowls. European robin *Erithacus rubecula* was recorded twice and likewise visually observed on all
260 dispersal unit replicates. Blackbirds (*Turdus merula*) were also frequently observed on replicates. The
261 droppings of passerine species were regularly found on all replicates, on the platforms and within the
262 bowls. Replicate 1 and 2 also appear to have been used consistently as song posts and for bathing
263 purposes. No rodent activity was observed, i.e. faeces or chew marks, at any replicate.

264

265 Visual observation of the bowls indicated that ‘Exclude-bird’ bowls may not have excluded the
266 possibility of bird-mediated dispersal. Birds appear to have perched on the cage structures as droppings
267 were often observed both on the platforms and within the bowls.

268

269 *Experiment I: Assessment of potential anemochorous dispersal*

270 Assessed wind speeds did not correlate with dispersed colony ($R^2 = -0.007$; $F_{1, 40} = 0.28$; $P > 0.05$) or
271 frond ($R^2 = -0.00007$; $F_{1, 40} = 0.003$; $P > 0.05$) numbers. A logistic analysis of dispersal events also

272 indicated that wind speed does not contribute to *L. minor* dispersal ($b = -0.54$, Wald $X_1^2 = 4.214$; $P <$
273 0.05 : OR = 0.947:1). The Odds Ratio (OR) is < 1 , which indicates that an increase in the predictor (i.e.
274 wind force) leads to a decrease in the odds of the outcome occurring (i.e., dispersal). Wind directional
275 data was not examined as wind force appears not to have facilitated dispersal of *L. minor*.

276

277 *Experiment II: Ex situ dispersal of A. filiculoides, L. minuta and E. nuttallii by mallard ducks.*

278 *Lemna minuta* was dispersed by the mallards on all occasions at both 1 and 3 m distances. No dispersal
279 was observed during the night periods when birds were inside the shelter. The mallards consumed all
280 non-collected duckweed in its entirety. *Azolla filiculoides* was dispersed by the birds from the source
281 to a receiving-bath on four occasions at a distance of 1m and twice at the 3m distance. However, the
282 mallards often displaced large amounts of *A. filiculoides* from the source onto the surrounding area,
283 mostly within a 50 cm radius from the source. All *A. filiculoides* was consumed by the birds, except
284 for the amounts found within and removed from the receiving-baths. No dispersal of *A. filiculoides*
285 occurred during the night periods. The mallards did not disperse any *E. nuttallii* plant material. Once
286 again, no dispersal was observed during night periods. Birds consumed most of the *E. nuttallii* and
287 fragmented whole plants during feeding. It is not known if mallards dispersed and then subsequently
288 removed plant material from a receiving-bath for any of the examined species.

289

290 Dispersal of *L. minuta* colonies by mallards was not significantly different between the examined
291 distances (ANOVA 3: $F_{1,8} = 1.614$; $P > 0.05$)(Fig. 3A). A significant difference was observed in the
292 dispersal of *A. filiculoides*, in relation to the distance from the source (ANOVA 4: $F_{1,8} = 7.881$; $P \leq$
293 0.05 respectively) (Fig. 3B).

294

295 Anemochorous dispersal was not considered as a viable method of dispersal for experiment no. II
296 given the extreme sheltered nature of the experimental site. In addition, while rodents were observed

297 in the vicinity of the experimental site, it is unlikely any visited the baths while the mallards were
298 present.

299

300

301 6. Discussion

302 - *Local dispersal occurs with a high frequency*

303 A high frequency of Lemnaceae dispersal was observed *in situ* (Fig. 2). On 27 out of 42 sampling days
304 at least one dispersal event was noted. We conclude that *Lemna minor* is a highly mobile species *via*
305 passive dispersal. Similarly, in an *ex situ* event *Lemna minuta* and *Azolla filiculoides* were found to be
306 dispersed with a high frequency (Fig. 3). High frequency of dispersal can contribute to an increased
307 rate of colonisation of barren water bodies, but also facilitate biological invasions through sustained
308 propagule pressure (see De Meester et al. 2002). For example, in a newly established mosaic of pond
309 and semi-aquatic habitats monitored over a 7 year period, Williams et al. (2008) observed rapid
310 colonisation, resulting in a rich assembly of macrophyte and aquatic macroinvertebrates. Similarly,
311 Barnes (1983) noted that the initial colonization of ponds was a rapid process, with a predictable
312 sequence of species arrival. Yet, typically it is not known how vegetatively-distributed, sessile plant
313 species colonise new ponds. In this study, it is demonstrated that bird-mediated short-distance or
314 “stepping-stone” dispersal is a frequent and rapid process, which may well underpin reported rapid
315 colonisation of water bodies and long distance dispersal (LDD) between ecosystems. Indeed, repeated
316 transport over short-distances may be a more advantageous method of dispersal for some aquatic
317 macrophytes. For example, *L. minuta* can suffer reduced viability due to desiccation when removed
318 from the aquatic medium for extended periods of time (Coughlan et al. 2015).

319

320 - *Local dispersal of Lemnaceae is associated with bird movements*

321 The highest incidence of dispersal events was observed when either rodents or nothing was excluded
322 from the dispersal set-up (Fig. 2). Therefore, it is concluded that birds played a key role in dispersal in
323 this *in situ* experiment. Exclusion of birds led to a drop in dispersal events, although not a cessation of
324 dispersal. It is highly likely that “exclude-bird” bowls may have contained bird-dispersed *Lemna*, as
325 birds tended to perch on the cage structures. From the present study it does not appear that anemochory
326 contributes to the dispersal of *L. minor*. However, the importance of anemochory might be
327 underestimated as *Lemna* dispersed by wind could have by-passed the receiving bowls and therefore
328 would not have been recorded. Unlike avian assisted epizoochory where birds could be expected to
329 move from one suitable habitat to another, anemochory would be a much more random process and
330 therefore will involve a much higher “hit or miss” outcome. There was no documented evidence of
331 rodent activity in this experiment. Moreover, exclusion of rodents did not affect the dispersal
332 frequency. While rodent species have previously been observed to disperse aquatic organisms (see
333 Waterkeyn et al. 2010), the experiments detailed here yielded no evidence for this process. However,
334 rodent species were not encouraged to interact with the bowls, therefore, under different circumstances
335 (e.g. placement of bowl directly on the ground), rodents may well facilitate epizoochorous dispersal.

336

337 - *Can waterbirds facilitate external dispersal of both floating and submerged macrophytes?*

338 In this study we show considerable dispersal of *Lemna sp.* under *in situ* and *ex situ* conditions. In
339 comparison, no dispersal was found for *E. nuttallii*. Coughlan et al. (submitted) has argued that
340 dispersal depends on 1) contact between a viable propagule and the vector, 2) attachment to vector, 3)
341 survival of transport, 4) detachment within a suitable receiving environment. Given that the mallards
342 in our experimental set-up would have been in close contact with all 3 species, it is suggested that
343 attachment to the vector, and subsequent detachment are key processes that determine dispersal
344 frequency. Mallards likely facilitated the dispersal of *L. minuta* more readily than the other studied
345 plants as this species easily adheres to the external surfaces of the birds. Moreover, individual *L. minuta*

346 were less clumped together, compared to *A. filiculoides* and *E. nuttalli* plants (personal observation),
347 resulting in smaller units which may have facilitated dispersal. Interestingly, the birds caused *A.*
348 *filiculoides* and whole *E. nuttalli* to break apart, which may potentially aid dispersal by hydrochory.
349 Thus, even where epizoochory does not occur, birds may still facilitate aquatic plant dispersal in a
350 different way.

351

352 - *Potential impact of frequent dispersal of aquatic plants*

353 Understanding how organisms, particularly invasive species, spread is of particular concern in the
354 current era of globalisation and rapid environmental change (Kelly et al. 2014). However, very little
355 attention has been given to the role of dispersal in species invasiveness and management (Westcott
356 and Fletcher 2011; Reynolds et al. 2015). In addition, birds tend to be overlooked as vectors of
357 dispersal and are frequently omitted from risk assessments and horizon scanning initiatives (Green
358 2016). Best management practices of aquatic species and ecosystems will need to give greater
359 consideration to the potential of epizoochory. High frequencies of dispersal will contribute to
360 biological invasions, sustained propagule pressure, and potentially increase the rate of plant species
361 primary succession. Conversely, habitat fragmentation can result in high population differentiation and
362 without further gene flow remnant populations are prone to further genetic erosion and perhaps
363 extinction (Vanden Broeck et al. 2015). Retention of genetic connectivity between populations will
364 mitigate these effects. High dispersal frequencies will facilitate a greater degree of genetic connectivity
365 between populations. Quantification and modelling of dispersal between fragmented habitats,
366 particularly newly emerged aquatic habitats, such as man-made water-bodies and wetland restoration,
367 will give an insight into how many species will adapt to threats to biodiversity.

368

369

370 7. Acknowledgments

371 We are grateful to John Coughlan and Geraldine Coughlan for technical support. M.A.K.J. appreciates
372 support by WoB. We also would like to thank two anonymous reviewers for helpful comments on an
373 earlier draft.

374

375

376

377 8. References

378 Albert A, Auffret AG, Cosyns E, Cousins SAO, D'Hondt B, Eichberg C, Eycott AE, Heinken T,
379 Hoffmann M, Jaroszewicz B, Malo JE, Mårell A, Mouissie M, Pakeman RJ, Picard M, Plue J, Poschlod
380 P, Provoost S, Schulze KA, Baltzinger C (2015) Seed dispersal by ungulates as an ecological filter: a
381 trait-based meta-analysis. *Oikos* 124: 1109-1120.

382

383 Aoyama Y, Kawakami K, Chiba S (2012) Seabirds as adhesive seed dispersers of alien and native
384 plants in the oceanic Ogasawara Islands, Japan. *Biodiversity and Conservation* 21: 2787–2801.

385

386 Barnes L. (1983) The colonization of ball-clay ponds by macroinvertebrates and macrophytes.
387 *Freshwater Biology* 13: 561-578.

388

389 Bilton DT, Freeland JR, Okamura B (2001) Dispersal in freshwater invertebrates. *Annual Review of*
390 *Ecology and Systematics* 32: 159–181.

391

392 Brochet AL, Guillemain M, Fritz H, Gauthier-Clerc M, Green AJ (2010) Plant dispersal by teal (*Anas*
393 *crecca*) in the Camargue: duck guts are more important than their feet. *Freshwater Biology* 55: 1262–
394 1273.

395

396 Cain ML, Milligan BG, Strand AE (2000) Long-distance seed dispersal in plant populations. *American*
397 *Journal of Botany* 87: 1217–1227.

398

399 Cañedo-Argüelles M, Boersma KS, Bogan MT, Olden JD, Phillipsen I, Schriever TA, Lytle DA (2015)
400 Dispersal strength determines meta-community structure in a dendritic riverine network. *Journal of*
401 *Biogeography* 42: 778–790.

402

403 Champion PD, Clayton JS, Hofstra DE (2010) Nipping aquatic plant invasions in the bud: weed risk
404 assessment and the trade. *Hydrobiologia* 656: 167–172.

405

406 Clausen P, Nolet BA, Fox AD, Klaassen M (2002). Long-distance endozoochorous dispersal of
407 submerged macrophyte seeds by migratory waterbirds in northern Europe - a critical review of
408 possibilities and limitations. *Acta Oecologica* 23: 191–203.

409

410 Cohen GM, Shurin JB (2003) Scale-dependence and mechanisms of dispersal in freshwater
411 zooplankton. *Oikos* 103: 603–617.

412

413 Coughlan NE, Kelly TC, Jansen MAK (2015) Mallard duck (*Anas platyrhynchos*)-mediated dispersal
414 of Lemnaceae: a contributing factor in the spread of invasive *Lemna minuta*? *Plant Biology* 17 (Suppl.
415 1): 108-114.

416

417 Cunze S, Heydel F, Tackenberg O (2013) Are plant species able to keep pace with the rapidly changing
418 climate? *PLoS ONE* 8: e67909. doi:10.1371/ journal.pone.0067909e, Ltd.

419

420 De Meester L, Gómez A, Okamura B, Schwenk K (2002) The Monopolization Hypothesis and the
421 dispersal–gene flow paradox in aquatic organisms. *Acta Oecologica* 23: 121-135.
422

423 Figuerola J, Green AJ (2002) How frequent is external transport of seeds and invertebrate eggs by
424 waterbirds? A study in Doñana, SW Spain. *Archiv für Hydrobiologie*, 155: 557–565.
425

426 Figuerola J, Green AJ, Santamaría L (2003) Passive internal transport of aquatic organisms by
427 waterfowl in Doñana, south-west Spain. *Global ecology and Biogeography* 12: 427-436.
428

429 García-pérez MA, Núñez-Antón V (2003) Cellwise residual analysis in two-way contingency tables.
430 *Educational and Psychological Measurement* 63: 825-839.
431

432 Ginman E, Prider J, Matthews J, Virtue J, Watling J (2015) Sheep as vectors for branched broomrape
433 (*Orobancha ramosa* subsp. *mutelii* [F.W. Schultz] Cout.) seed dispersal. *Weed Biology and*
434 *Management* 15: 61-69.
435

436 Green AJ (2016) The importance of waterbirds as an overlooked pathway of invasion for alien species.
437 *Diversity and Distributions* 22: 239–247.
438

439 Green AJ, Elmberg J (2014) Ecosystem services provided by waterbirds. *Biological reviews* 89: 105-
440 122.
441

442 Green AJ, Figuerola J (2005) Recent advances in the study of long distance dispersal of aquatic
443 invertebrates via birds. *Diversity and Distributions* 11:149–156.
444

445 Hill MP (2003) The impact and control of alien aquatic vegetation in South African aquatic
446 ecosystems. *African Journal of Aquatic Science* 28: 19-24.

447

448 Holt RD (2003) On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research* 5:
449 159-178.

450

451 Iberite M, Iamónico D, Abati S, Abbate G (2011) *Lemna valdiviana* Phil. (Araceae) as a potential
452 invasive species in Italy and Europe: taxonomic study and first observations on its ecology and
453 distribution. *Plant Biosystems* 145: 751–757.

454

455 Jacobs DL (1947) An ecological life-history of *Spirodela polyrhiza* (Greater Duckweed) with
456 emphasis on the turion phase. *Ecological Monographs* 17: 437 – 469.

457

458 Janes R (1998) Growth and survival of *Azolla filiculoides* in Britain. *New Phytologist* 138: 367 – 375.

459

460 Janes RA, Eaton JW, Hardwick K (1996) The effects of floating mats of *Azolla filiculoides* Lam. and
461 *Lemna minuta* Kunth on the growth of submerged macrophytes. *Hydrobiologia* 340: 23–26.

462

463 Kelly R, Harrod C, Maggs CA, Reid N (2015) Effects of *Elodea nuttallii* on temperate freshwater
464 plants, microalgae and invertebrates: small differences between invaded and uninvaded areas.
465 *Biological Invasions* 17: 2123–2138.

466

467 Kelly R, Lundy MG, Mineur F, Harrod C, Maggs CA, Humphries NE, Sims DW, Reid N (2014)
468 Historical data reveal powerlaw dispersal patterns of invasive aquatic species. *Ecography* 37: 001–
469 010.

470

471 Landolt E (1986) *Biosystematic investigations in the family of duckweeds (Lemnaceae) (Vol. 2), The*
472 *family of Lemnaceae – a monographic study (Vol. 1)*. Veröffentlichungen des Geobotanischen
473 Institutes Der Eidg. Technischen Hochschule, Stiftung Rubel, Zürich, Switzerland, p 566.

474

475 Les DH, Crawford DJ, Kimball RT, Moody ML, Landolt E (2003) Biogeography of discontinuously
476 distributed hydrophytes: A molecular appraisal of intercontinental disjunctions. *International Journal*
477 *of Plant Sciences* 164: 917-932.

478

479 Lucey J (2003) *Lemna minuta* Kunth (Least Duckweed) in E. Cork. *Irish Botanical News* 13: 5–8.

480

481 Mackay S, James C (2016). Aquatic vascular macrophytes of Australia's riverine systems. In: Capon
482 S, James C, Reid M (eds) *Vegetation of Australian Riverine Landscape: Biology, Ecology and*
483 *Management*. 1st edn. CSIRO, Australia, pp87-102.

484

485 Magnuson JJ (1976) Managing with exotics – a game of chance. *Transactions of the American*
486 *Fisheries Society* 105: 1–9.

487

488 Moritz C, Meynard CN, Devictor V, Guizien K, Labrune C, Guarini JM, Mouquet N (2013)
489 Disentangling the role of connectivity, environmental filtering, and spatial structure on
490 metacommunity dynamics. *Oikos* 122: 1401 – 1410.

491

492 Nathan R (2008) An emerging movement ecology paradigm. *Proceedings of the National Academy of*
493 *Sciences* 105: 19050–19051.

494

495 Nomura N, Tsuyuzaki S (2015) Hares promote seed dispersal and seedling establishment after volcanic
496 eruptions. *Acta Oecologica* 63: 22-27.

497

498 Pollux BJA (2011) The experimental study of seed dispersal by fish (ichthyochory). *Freshwater*
499 *Biology* 56: 197-212.

500

501 Ramakrishnan AP (2008) Dispersal-migration. In: Jørgensen SE, Fath BD (eds), Encyclopaedia of
502 Ecology. 1st edn. Elsevier B.V., Amsterdam, The Netherlands. pp 930-938.

503

504 Raulings E, Morris K, Thompson R, MacNally R (2011) Do birds of a feather disperse plants together?
505 *Freshwater Biology* 56: 1390–1402.

506

507 Reynolds C, Cumming GS (2016) Seed dispersal by waterbirds in southern Africa: comparing the roles
508 of ectozoochory and endozoochory. *Freshwater Biology*. doi: 10.1111/fwb.12709

509

510 Reynolds C, Miranda NAF, Cumming GS (2015) The role of waterbirds in the dispersal of aquatic
511 alien and invasive species. *Diversity and Distributions* 21: 744–754.

512

513 Ronce O (2011) How does it feel to be like a rolling stone? Ten questions about dispersal evolution.
514 *Annual Review of Ecology, Evolution, and Systematics* 38: 231–53.

515

516 Sadeghi R, Zarkami R, Sabetraftar K, Van Damme P (2013) A review of some ecological factors
517 affecting the growth of *Azolla* spp. *Caspian Journal of Environmental. Science* 11: 65-76.

518

519 Sala OE, Chapin III FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke,
520 LF, Jackson RB, Kinzig A (2000). Global biodiversity scenarios for the year 2100. *Science* 287: 1770–
521 1774.

522

523 Santamaría L (2002) Why are most aquatic plants widely distributed? Dispersal, clonal growth and
524 small-scale heterogeneity in a stressful environment *Acta Oecologica* 23: 137–154.

525

526 Shepherd LD, de Lange PJ, Perrie LR (2009) Multiple colonizations of a remote oceanic archipelago
527 by one species: how common is long-distance dispersal? *Journal of Biogeography* 36: 1972-1977.

528

529 Soomers H, Karssen D, Soons MB, Verweij PA, Verhoeven JTA, Wassen MJ (2013) Wind and
530 water dispersal of wetland plants across fragmented landscapes. *Ecosystems* 16: 434–451.

531

532 Sorensen AE (1986) Seed dispersal by adhesion. *Annual Review of Ecology and Systematics* 17: 443–
533 463.

534

535 Thorsen MJ, Dickinson KJM, Seddon PJ (2009) Seed dispersal systems in the New Zealand flora.
536 *Perspectives in Plant Ecology, Evolution and Systematics* 11: 285–309.

537

538 Trakhtenbrot A, Nathan R, Perry G, Richardson DM (2005) The importance of long-distance dispersal
539 in biodiversity conservation. *Diversity and Distributions* 11: 173–181.

540

541 Traveset A (1998) Effect of seed passage through vertebrate frugivores' guts on germination: a review.
542 *Perspectives in Plant Ecology Evolution and Systematics* 1: 151-190.

543

544 Vanden Broeck, A., Ceulemans, T., Kathagen, G., Hoffmann, M., Honnay, O., Mergeay, J. (2015)
545 Dispersal constraints for the conservation of the grassland herb *Thymus pulegioides* L. in a highly
546 fragmented agricultural landscape. *Conservation Genetics* 16: 765-776.

547

548 van Leeuwen CHA, Tollenaar ML, Klaassen M (2012a) Vector activity and propagule size affect
549 dispersal potential by vertebrates. *Oecologia* 170: 101-109.

550

551 van Leeuwen CHA, van der Velde G, van Groenendael JM, Klaassen, M (2012b) Gut travellers:
552 internal dispersal of aquatic organisms by waterfowl. *Journal of Biogeography* 39: 2031–2040.

553

554 van Leeuwen CHA, van der Velde G, van Lith B, Klaassen M (2012c) Experimental Quantification of
555 Long Distance Dispersal Potential of Aquatic Snails in the Gut of Migratory Birds. *PLoS ONE* 7:
556 e32292. doi:10.1371/journal.pone.0032292

557

558 Vanschoenwinkel B, Waterkeyn A, Nihwatiwa T, Pinceel T, Spooren E, Geerts A, Clegg B, Brendonck
559 L (2011) Passive external transport of freshwater invertebrates by elephant and other mud-wallowing
560 mammals in an African savannah habitat. *Freshwater Biology* 5:1606–1619.

561

562 Viana DS, Santamaría L, Michot TC, Figuerola J (2013) Migratory strategies of waterbirds shape the
563 continental-scale dispersal of aquatic organisms. *Ecography* 36: 430–438.

564

565 Waterkeyn A, Vanschoenwinkel B, Elsen S, Anton-Pardo M, Grillas P, Brendonck L (2010)
566 Unintentional dispersal of aquatic invertebrates via footwear and motor vehicles in a Mediterranean
567 wetland area. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 580–587.

568

569 Westcott DA, Fletcher CS (2011) Biological invasions and the study of vertebrate dispersal of plants:
570 opportunities and integration. *Acta Oecologica* 37: 650–656.

571

572 Williams P, Whitfield M, Biggs J (2008) How can we make new ponds biodiverse? A case study
573 monitored over 7 years. *Hydrobiologia* 597: 137-148.

574

575 With KA (2002) The landscape ecology of invasive spread. *Conservation Biology* 16: 1192–1203.

576

577

578

579

580

581

582

583

584

585

586

587

588

589

590

591

592

593

594

595

596

597

598

599

600 Figure 1: Dispersal monitoring stand constructed to test for specific methods of facilitated dispersal.
601 Each stand (n = 6) consisted of a central source-bowl (A) containing *L. minor* and four receiving-bowl
602 types that excluded particular vectors of dispersal; exclude-all (birds and rodents) (B), exclude-birds
603 (C), exclude-nothing (D), exclude-rodents (E). Potential anemochorous dispersal was not excluded
604 from any. An overhead view depicts the location of receiving-bowl platforms positioned around the
605 central source-bowl. Each of the four receiving-bowl types (B – E) were randomly assigned to a
606 platform. Not drawn to scale, see main text for measurements.

607

608

609

610 Figure 2: Total number of observed dispersal events (A) (i.e. minimum 1 colony of 1 frond) and mean
611 number of colonies (B) and fronds (C) (\pm SE) found dispersed in relation to the receiving-bowl
612 exclusion type. Corresponding symbols indicate statistical similarity, otherwise each exclusion type is
613 statically different from all others.

614

615

616 Figure 3: Mean number (\pm SE) of *Lemna minuta* colonies (A) and mean biomass (\pm SE) of *Azolla*
617 *fliculoides* (B) dispersed by mallard duck over 1 and 3 m distances. No dispersal was measured for
618 *Elodea nuttalli*.

619

620

621

622

623

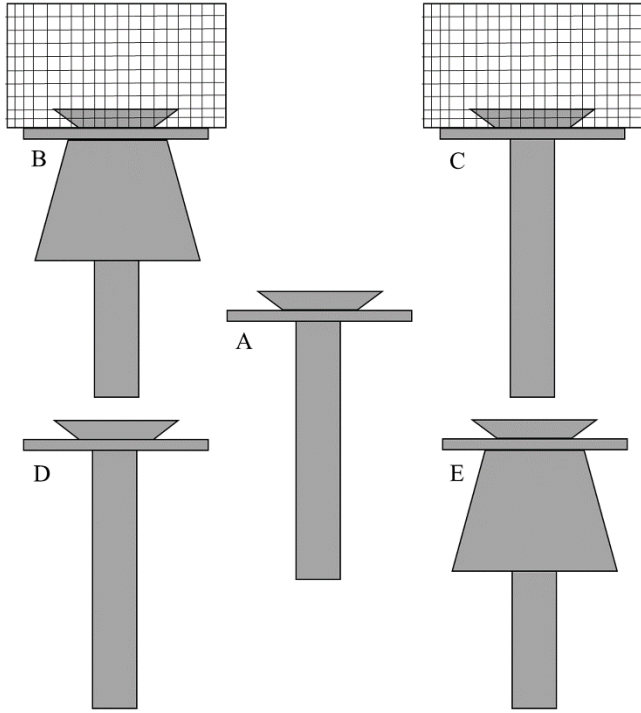
624

625

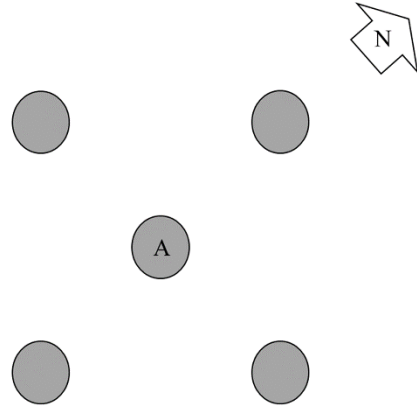
626 Figure 1:

627

Side view of the dispersal stand and receiving-bowl types.



Top (i.e., overhead) view of the dispersal stand.



628

629

630

631

632

633

634

635

636

637

638

639

640

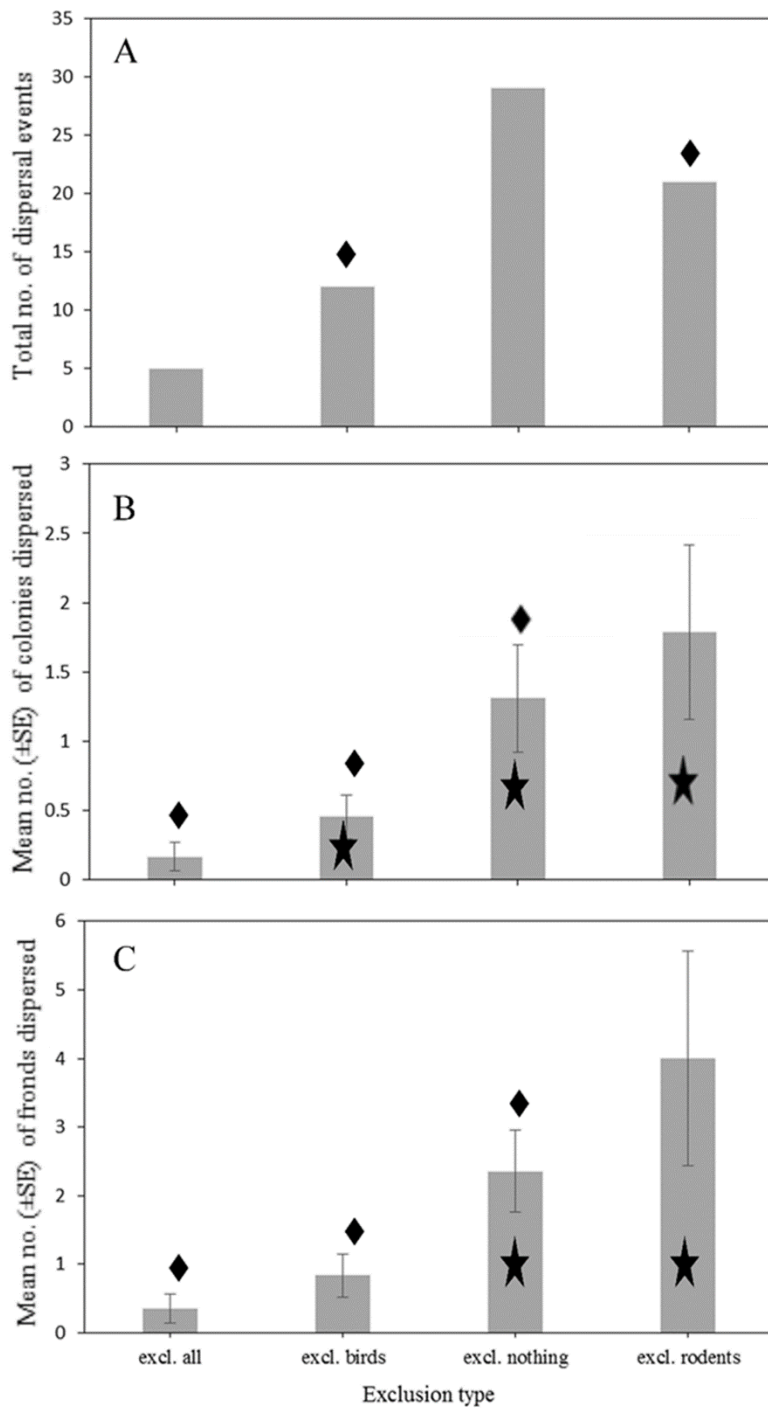
641

642

643 Figure 2:

644

645



646

647

648

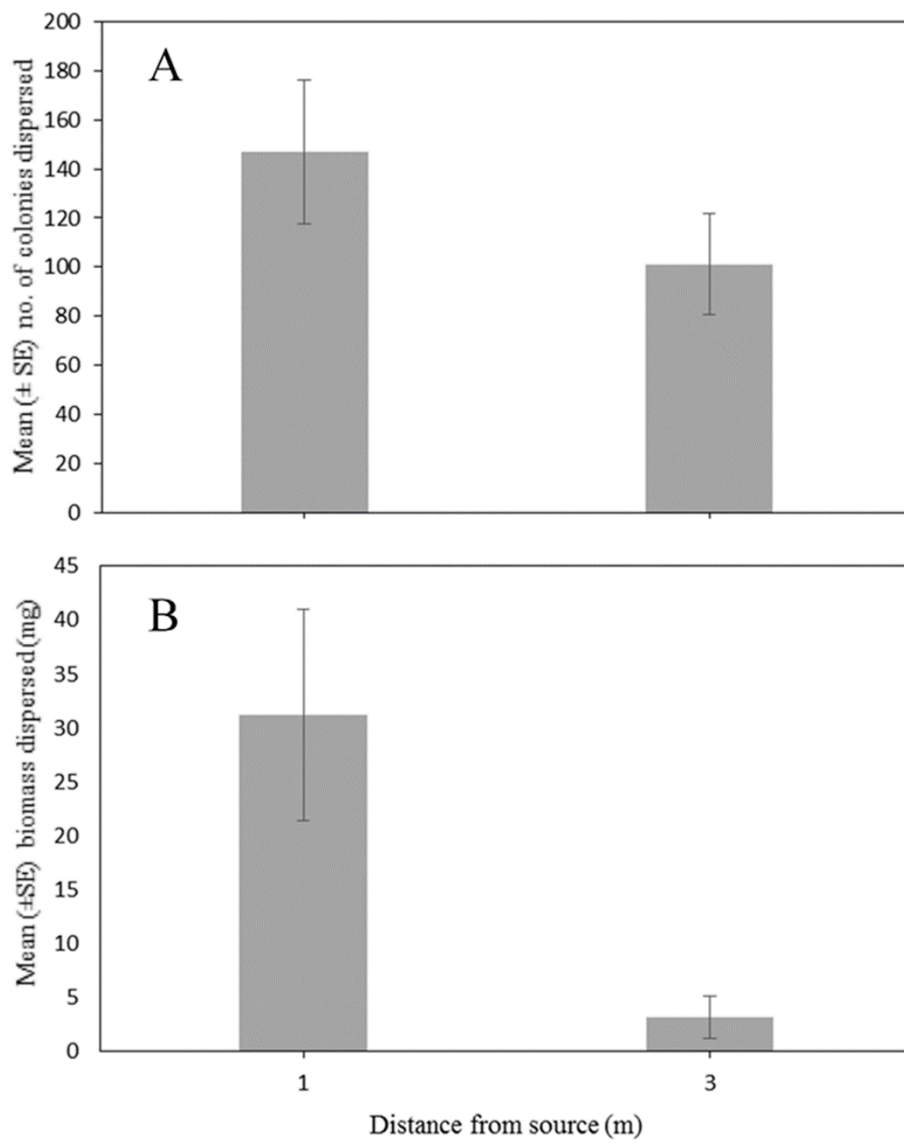
649

650

651

652 Figure 3:

653



654

655

656

657

658

659