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University College Cork, Ireland Coláiste na hOllscoile Corcaigh "Step by step": High frequency short-distance epizoochorous dispersal of
aquatic macrophytes.

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- 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

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

27 2. Introduction

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

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

45

Frequency of successful dispersal events of aquatic macrophytes remains a matter of speculation; indeed, our understanding of the dispersal processes operating in wetlands remains limited (Cohen and Shurin 2003; Figuerola et al. 2003; Soomers et al. 2013). While various mechanisms of dispersal are essential in facilitating meta-community connectivity, research on the topic of how organisms 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. 2001; Trakhtenbrot et al. 2005; Nathan et al. 2008; Pollux 2011; van Leeuwen et al. 2012a,c). 53 Anemochorous seed dispersal of wetland plants across fragmented landscapes has been experimentally 54 investigated and modelled by many studies (Soomers et al. 2013). While wind can play an important 55 role in passive dispersal of the vegetative parts of aquatic macrophytes, water currents are considered 56 57 to be the dominate mechanism for many free-floating plants (Downing-Kunz and Stacey 2011). In addition, research suggests zoochory to be important in surmounting dispersal limitations, thus 58 59 facilitating the spread of species (Cunze et al. 2013). Mammals, such as ungulates and rodents, can facilitate zoochorous dispersal within aquatic and terrestrial habitats (Waterkeyn et al. 2010; 60 Vanschoenwinkel et al. 2011; Albert et al. 2015; Ginman et al. 2015; Nomura and Tsuyuzaki 2015). 61 62 Birds, in particular, are considered effective dispersers by both internal (endozoochory) and external (epizoochory) means (Green and Elmberg 2014). In his seminal work, Landolt (1986) states that 63 animals (birds, mammals, amphibians and reptiles) are the main distributors of Lemnaceae, and this 64 hypothesis appears to be widely accepted (Les et al. 2003; Mackay and James 2016). However, in fact, 65 there is limited, and mostly anecdotal, evidence concerning bird-mediated dispersal of Lemnaceae 66 (Coughlan et al. 2015). Additionally, while several studies demonstrate bird-mediated epizoochorous 67 dispersal of plant seeds (see for example: Figuerola and Green 2002; Brochet et al. 2010; Raulings et 68 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 platyrhynchos) ducks, Jacobs (1947) observed viable L. minor within the breast plumage of one 71 individual. Similarly, Reynolds et al. (2015) observed and photographed L. minor attached to 72 73 underwing feathers of yellow-billed duck Anas undulate. However, much more work is required to assess the extent to which bird-mediated dispersal can contribute to biological invasion (Green 2016). 74

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

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

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

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104 3. Materials and methods

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

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112 Species selection

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

120

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

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

126

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

132

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

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

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

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

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

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Weather data was obtained from the Irish Meteorological Service collected at Cork Airport weatherstation.

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

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

187

Three plastic containers (610 mm (L) \times 410 mm (W) \times 220 mm (H)) were placed within the enclosure, each 1 m from the next, and filled with clean tap water. The mallards were then allowed to become accustomed to the presence of the containers for a two day period. In order to limit the mallards to the water sources provided by the experimental containers, the artificial pond was drained for the duration of the entire experiment. The containers (baths) were checked at least four times daily on nonexperimental days and every 30 minutes on experiment days. Baths were re-filled with clean water as 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 were examined separately and every replicate took place over a 24 hour period. The mallards were 199 confined to the shelter within the enclosure at 20:30 hrs. Free-floating plants were then added to the 200 201 middle container (source-bath) until 80% of the water surface had been covered. The total fresh weight (FW) was recorded. For submerged E. nutallii, a mass of 500g (FW) of whole plant material was 202 added. All three containers (1 'source-bath' and 2 'receiving-baths') held clean water at 90% of their 203 204 total volume. The mallards remained confined to the shelter for the night and were re-released into the main enclosure at 07:30 hrs. The baths could potentially have been visited by small rodents (e.g., mice 205 206 and rat spp.) at night. Therefore, before the birds re-entered the enclosure the receiving-baths were examined for the presence of plant material. Any plant biomass found in the 'receiving-baths' was 207 208 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

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

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

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4. Statistical analysis

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

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237

238 5. Results

239 Experiment I: In situ dispersal of L. minor

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

244

Few dispersal events were observed within 'exclude-all' receiving-bowls (n = 5), while 'excludenothing' bowls displayed the greatest number of dispersal events (n = 29) ($X_3^2 = 21.89$, $P \le 0.0001$) (Fig. 2A). Post-hoc examination of the Chi square test determined observed dispersal in 'exclude-all' and 'exclude-nothing' receiving-bowls to be significantly different from all other receiving-bowl types. Similarly, dispersed colony and frond numbers were highest in 'exclude-rodent' bowls and lowest in 'exclude-all' receiving-bowls (ANOVA 1: $F_{3, 164} = 3.842$; $P \le 0.05$ and ANOVA 2: $F_{3, 164} =$ 3.651; $P \le 0.05$ respectively) (Fig. 2B & C). Frond numbers were different in 'exclude-rodent' and 'exclude-bird' bowls (ANOVA 2: $F_{3, 164} = 3.651$; P = 0.05). No other significant difference was observed for colony numbers.

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255 *Experiment I: Digital and visual surveillance*

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

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

268

269 Experiment I: Assessment of potential anemochorous dispersal

Assessed wind speeds did not correlate with dispersed colony ($R^2 = -0.007$; F_{1,40} = 0.28; P > 0.05) or

frond ($R^2 = -0.00007$; F_{1, 40} = 0.003; P > 0.05) numbers. A logistic analysis of dispersal events also

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

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

289

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

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Anemochorous dispersal was not considered as a viable method of dispersal for experiment no. II given the extreme sheltered nature of the experimental site. In addition, while rodents were observed in the vicinity of the experimental site, it is unlikely any visited the baths while the mallards werepresent.

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301 6. Discussion

302 - Local dispersal occurs with a high frequency

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

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 from the dispersal set-up (Fig. 2). Therefore, it is concluded that birds played a key role in dispersal in 322 this in situ experiment. Exclusion of birds led to a drop in dispersal events, although not a cessation of 323 324 dispersal. It is highly likely that "exclude-bird" bowls may have contained bird-dispersed Lemna, as birds tended to perch on the cage structures. From the present study it does not appear that anemochory 325 contributes to the dispersal of L. minor. However, the importance of anemochory might be 326 underestimated as Lemna dispersed by wind could have by-passed the receiving bowls and therefore 327 would not have been recorded. Unlike avian assisted epizoochory where birds could be expected to 328 329 move from one suitable habitat to another, anemochory would be a much more random process and therefore will involve a much higher "hit or miss" outcome. There was no documented evidence of 330 331 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, rodent species were not encouraged to interact with the bowls, therefore, under different circumstances 334 335 (e.g. placement of bowl directly on the ground), rodents may well facilitate epizoochorous dispersal.

336

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

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

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

351

352 - Potential impact of frequent dispersal of aquatic plants

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

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

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

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

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















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