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"Step by step": High frequency short-distance epizoochorous dispersal of aquatic macrophytes.

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

1. Abstract

Aquatic macrophytes can successfully colonise and re-colonise areas separated by space and time. The 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 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 aquatic macrophytes, including three invasive alien species. A high frequency of *Lemna minor* Linnaeus dispersal was observed *in situ*, and this was linked to bird-mediated epizoochory. We concluded that wind had no effect on dispersal. Similarly, in an *ex situ* examination *Lemna minuta* Kunth and *Azolla filiculoides* Lamarck, were found to be dispersed with a high frequency by mallard ducks (*Anas platyrhynchos*). No dispersal was measured for *Elodea nuttalli* (Planchon) H. St. John. It is concluded that short-distance or "stepping-stone" dispersal *via* bird-mediated epizoochory can occur with high frequencies, and therefore can play an important role in facilitating colonisation, range expansion and biological invasion of macrophytes.

2. Introduction

Freshwater systems can be viewed from a classical island biogeographic perspective as islands of 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, aquatic plants tend to have broader distributions than their terrestrial counterparts (Santamaría 2002). Moreover, freshwater systems have been shown to be at high risk from biological invasions (Sala et al. 2000; Kelly et al. 2014). Thus, in spite of a restricted or total lack of ability to self-disperse, an 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 concluded that means of assisted dispersal must be readily available (Clausen et al. 2002; Santamaría 2002; Green and Figuerola 2005; Trakhtenbrot et al. 2005).

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).

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

et al. 2013; Cañedo-Argüelles et al. 2015). Potential vectors of passive dispersal include water currents (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). Anemochorous seed dispersal of wetland plants across fragmented landscapes has been experimentally investigated and modelled by many studies (Soomers et al. 2013). While wind can play an important role in passive dispersal of the vegetative parts of aquatic macrophytes, water currents are considered 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 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; Vanschoenwinkel et al. 2011; Albert et al. 2015; Ginman et al. 2015; Nomura and Tsuyuzaki 2015). 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 animals (birds, mammals, amphibians and reptiles) are the main distributors of Lemnaceae, and this hypothesis appears to be widely accepted (Les et al. 2003; Mackay and James 2016). However, in fact, there is limited, and mostly anecdotal, evidence concerning bird-mediated dispersal of Lemnaceae (Coughlan et al. 2015). Additionally, while several studies demonstrate bird-mediated epizoochorous dispersal of plant seeds (see for example: Figuerola and Green 2002; Brochet et al. 2010; Raulings et al. 2011; Aoyama et al. 2012), few studies have documented attachment or dispersal of vegetative 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 individual. Similarly, Reynolds et al. (2015) observed and photographed *L. minor* attached to 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).

Research suggests bird-mediated dispersal may be an overlooked link between habitats, facilitating 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 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 several alien plant species, adhering to seabirds. Remarkably, some of the seeds identified were generally considered to be dispersed by wind or bird-mediated endozoochory. In addition, a positive relationship was observed between the distributions of plants and seabirds, indicating the ecological 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 not produce fleshy fruits, and/or are predominantly dispersed as vegetative propagules. Recently, the 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 basic questions, such as the frequency and ecological importance of bird-mediated epizoochorous dispersal, remain under-researched. Few empirical studies have focused on the epizoochorous dispersal of vegetative propagules, and even fewer studies have examined the ability of birds to facilitate external dispersal and population connectivity of aquatic plants over local scales (but see, for example, Brochet et al. 2010 and Reynolds and Cumming 2016). Thus, although it is well known that colonisation of new ponds by aquatic plants can be a rapid process (Barnes 1983; Williams et al. 2008), 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.

Here, we tested the hypothesis that free-floating aquatic plants are frequently dispersed over short distances. 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.

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.

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).

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 serious environmental problem (Janes 1998).

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).

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 cm^2 .

Experiment I: In situ dispersal of L. minor

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 "stands" were constructed. Each dispersal monitoring stand consisted of a central source-bowl containing *L. minor* and four receiving-bowls. Each bowl sat on a wooden platform (320 mm (L) × 150 mm (W)) on top of a 1200 mm high wooden stake. Receiving-bowls were positioned as if on the corners of a square around the central source-bowl (See Fig. 1). The source-bowl was positioned in 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 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

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 confined to the shelter within the enclosure at 20:30 hrs. Free-floating plants were then added to the 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 added. All three containers (1 'source-bath' and 2 'receiving-baths') held clean water at 90% of their 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 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 removed, patted dry with paper towels to remove excess water, and weighed (FW). The number of *L. minuta* colonies found dispersed were recorded.

The mallards were left to interact with the experimental baths. Birds were free to roam, and displayed 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 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 remaining within the enclosure was removed and total fresh weight was recorded. A total of 500g (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 to the 'source-bath' for each replicate as a mass of tangled vegetation, typical of *in situ* growth formation.

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

4. Statistical analysis

Data were analysed using (where applicable) Correlation and Regression, Pearson's Chi-Square tests and one-way ANOVAs with the post-hoc Tukey HSD in SPSS (version 22; SPSS Inc, Chicago, IL, USA). Post-hoc analysis of the Chi square test was conducted *via* examination of adjusted *Z*-values 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 the likelihood of wind force as a predictor of dispersal events. The highest mean wind speed, or maximum gust if $\geq$ 34 knots, recorded on or between sampling days was selected as a measurement of wind force preceding examination of the receiving-bowls; allowing lag effects to be accounted for when selecting the highest wind force.

5. Results

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.

Few dispersal events were observed within 'exclude-all' receiving-bowls (n = 5), while 'exclude-nothing' bowls displayed the greatest number of dispersal events (n = 29) (X_3^2 = 21.89, $P \leq 0.0001$)

(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: $F_{3, 164} = 3.842$; $P \leq 0.05$ and ANOVA 2: $F_{3, 164} = 3.651$; $P \leq 0.05$ 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.

Experiment I: Digital and visual surveillance

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

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.

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_1^2 = 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*.

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 was observed during the night periods when birds were inside the shelter. The mallards consumed all 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 mallards often displaced large amounts of *A. filiculoides* from the source onto the surrounding area, 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* occurred during the night periods. The mallards did not disperse any *E. nuttallii* plant material. Once again, no dispersal was observed during night periods. Birds consumed most of the *E. nuttallii* and fragmented whole plants during feeding. It is not known if mallards dispersed and then subsequently removed plant material from a receiving-bath for any of the examined species.

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; $P \leq$ 0.05 respectively) (Fig. 3B).

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

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*

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 this *in situ* experiment. Exclusion of birds led to a drop in dispersal events, although not a cessation of 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 contributes to the dispersal of *L. minor*. However, the importance of anemochory might be underestimated as *Lemna* dispersed by wind could have by-passed the receiving bowls and therefore would not have been recorded. Unlike avian assisted epizoochory where birds could be expected to 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 rodent activity in this experiment. Moreover, exclusion of rodents did not affect the dispersal frequency. While rodent species have previously been observed to disperse aquatic organisms (see 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 (e.g. placement of bowl directly on the ground), rodents may well facilitate epizoochorous dispersal.

- *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 comparison, no dispersal was found for *E. nuttallii*. Coughlan et al. (submitted) has argued that 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 in our experimental set-up would have been in close contact with all 3 species, it is suggested that 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 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. nuttallii* 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

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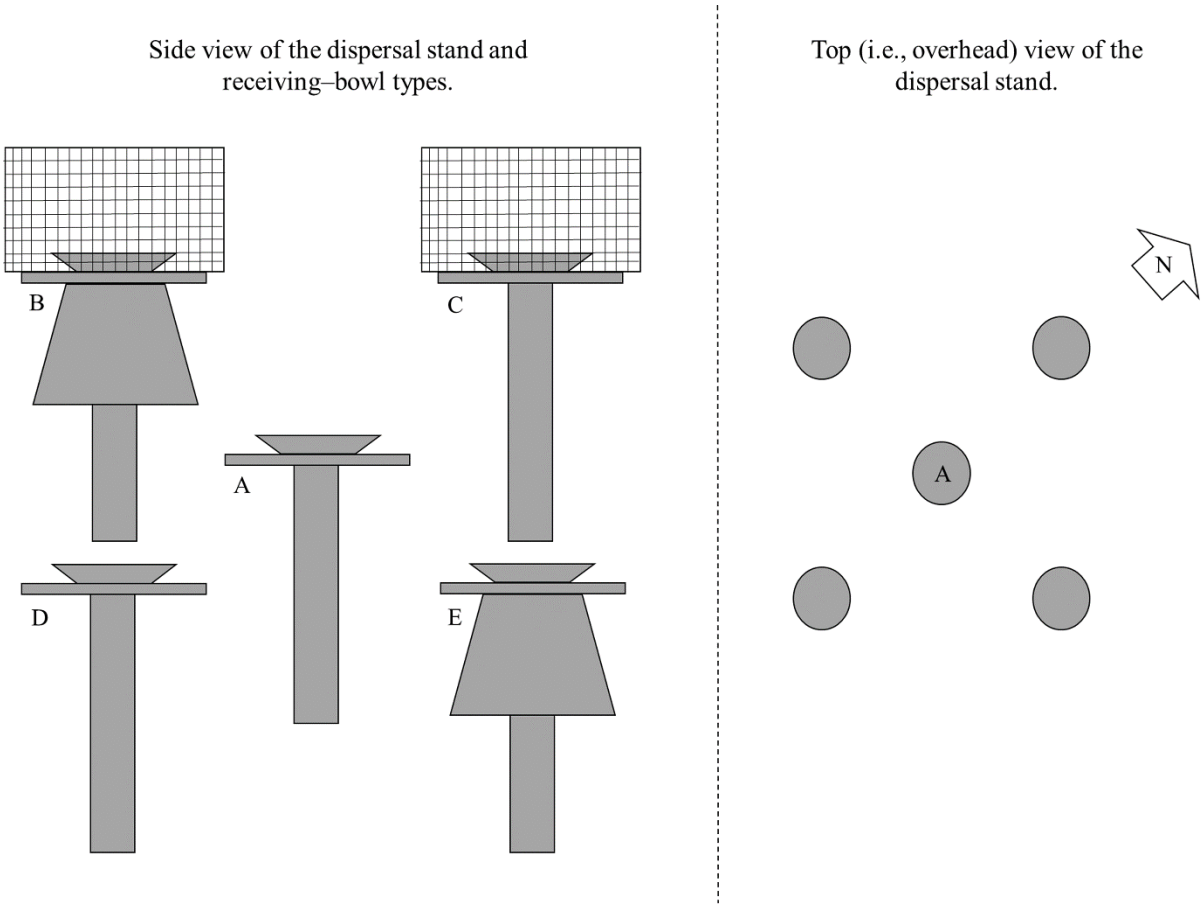
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Figure 1: Dispersal monitoring stand constructed to test for specific methods of facilitated dispersal. Each stand (n = 6) consisted of a central source-bowl (A) containing *L. minor* and four receiving-bowl types that excluded particular vectors of 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.

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) (± 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.

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.*

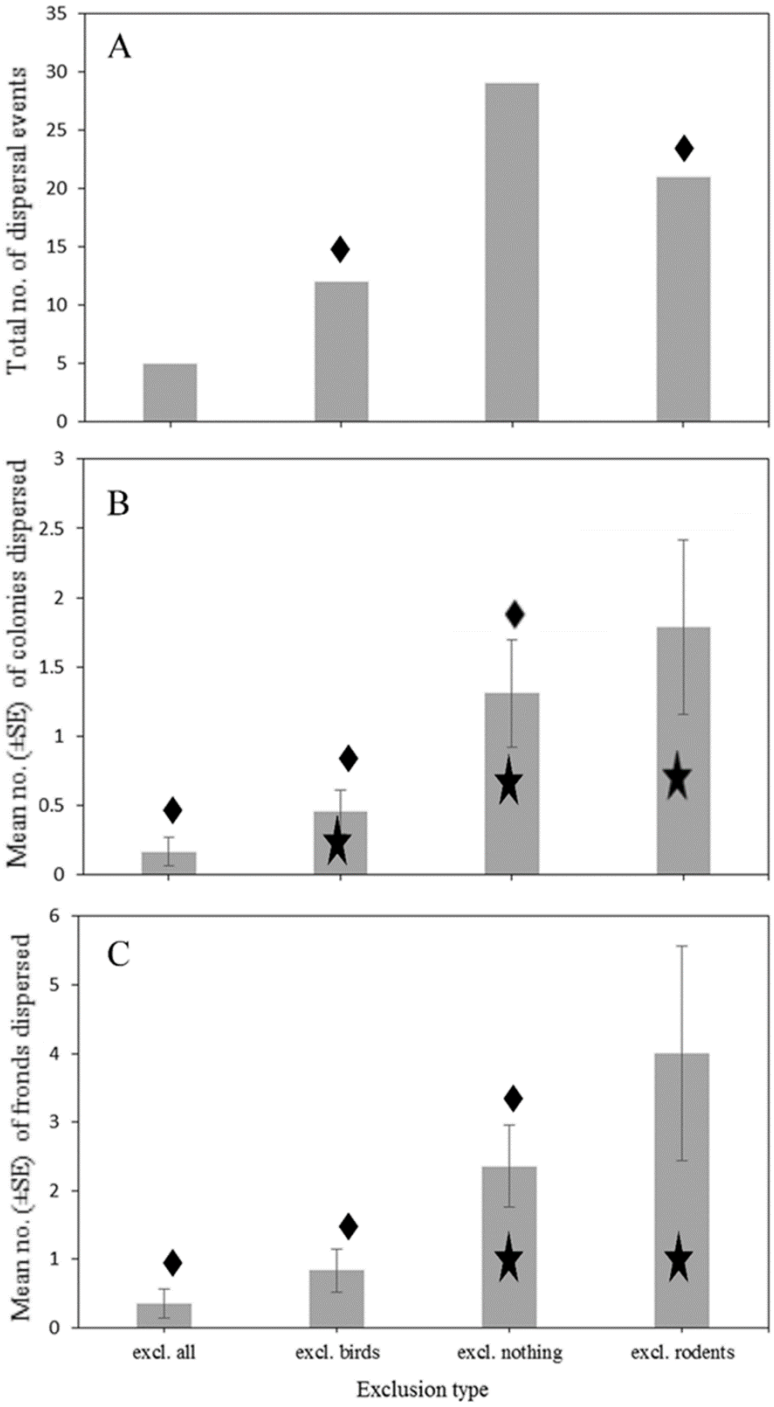
Figure 1:


Side view of the dispersal stand and receiving–bowl types.
B
C
A
D
E
Top (i.e., overhead) view of the dispersal stand.
N
A

643 Figure 2:

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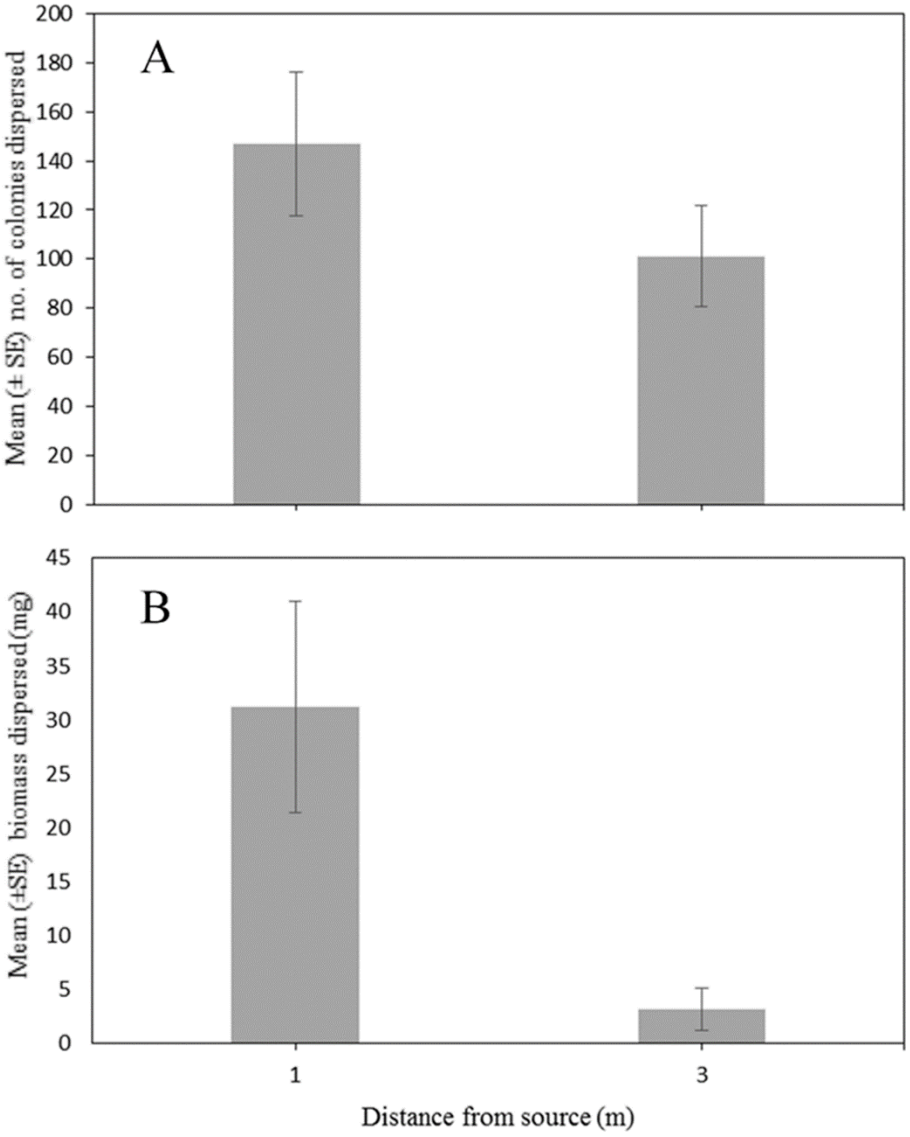
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Figure 3:


A
Mean (± SE) no. of colonies dispersed
200
180
160
140
120
100
80
60
40
20
0
B
Mean (±SE) biomass dispersed (mg)
45
40
35
30
25
20
15
10
5
0
1
3
Distance from source (m)