biology
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Biol. Lett. 2008 **4**, 494-496
doi: 10.1098/rsbl.2008.0323

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doi:10.1098/rsbl.2008.0323
Published online 15 July 2008
Community ecology

Size-selective dispersal of *Daphnia* resting eggs by backswimmers (*Notonecta maculata*)

Frank van de Meutter*, Robby Stoks and Luc de Meester

Laboratory of Aquatic Ecology and Evolutionary Biology, Katholieke Universiteit Leuven, Charles Debériotstraat 32, 3000 Leuven, Belgium
**Author for correspondence (frank.vandemeutter@bio.kuleuven.be).*

Freshwater zooplankton is increasingly used to study effects of dispersal on community and metacommunity structure. Yet, it remains unclear how zooplankton disperses. Clearly, birds and wind play a significant role as zooplankton dispersal agents, but they may not always be the main vectors. This experimental study shows that a cosmopolitan aquatic insect, *Notonecta*, can be an important vector of cladoceran resting eggs (ephippia). Dispersing *Notonecta* frequently transported ephippia during flight, with a bias towards smaller ephippia in two species. A similar trend was present at the species level: *Daphnia* species with smaller ephippia were more often dispersed, suggesting that *Notonecta* could generate specific colonist communities. In addition, buoyancy appeared a critical trait, as non-floating ephippia of *Daphnia magna* were never dispersed. Our data suggest that *Notonecta* could be important dispersers of *Daphnia*, and that knowledge of dispersal dynamics of *Notonecta* may be used to predict *Daphnia* dispersal, colonization and resilience to disturbance.

Keywords: dispersal; *Notonecta*; zooplankton; *Daphnia*; size-selective; aquatic insects

1. INTRODUCTION

Dispersal has long fascinated biologists, because it has important ecological effects ranging from the individual level to metacommunity dynamics. Currently, freshwater zooplankton is increasingly used to study the impact of dispersal on communities and metacommunities (e.g. Shurin 2001; Cottenie & de Meester 2004); however, which are its prime modes and vectors of dispersal is open to debate. Understanding zooplankton dispersal will enable us to improve predictive models of how colonization of passively dispersed freshwater zooplankton occurs and how their communities may recover from natural or anthropogenic disturbance.

In freshwater zooplankton, no dispersive stage exists that can actively cross land boundaries, yet the colonization of new, isolated habitats may occur surprisingly rapidly (Louette & de Meester 2005). This is generally explained by the passive transport of desiccation-resistant resting stages by water flow, wind or animal vectors. Whereas dispersal by water flow is mainly restricted to floodplains and connected systems (Michels *et al.* 2001), dispersal by wind may be a more general mechanism for zooplankton dispersal. Cáceres & Soluk (2002) and Cohen & Shurin (2003) both found that restricting access to potential vectors by covering mesocosms with nettings did not restrict colonization by zooplankton, which they interpreted as evidence for a dominant role of wind dispersal. However, most studies that quantified wind dispersal yielded no dispersal of cladoceran ephippia (Jenkins & Underwood 1998; Allen 2007), except for Vanschoenwinkel *et al.* (2007) where wind dispersal of ephippia seemed to be relatively unimportant compared with smaller propagules such as rotifers.

Recent research on phoresy has mainly focused on the potential role of mammals (Allen 2007) and birds, especially waterbirds (Frisch *et al.* 2007). Allen (2007), and showed that mammals can disperse adult zooplankton, but this cannot explain colonization in regions where large mammals are rare, as is the case in the study of Louette & de Meester (2005) (G. Louette 2007, personal communication). There is good evidence that waterbirds can contribute to intermediate- and long-distance dispersal of zooplankton in bird-rich wetlands, but it remains unclear whether waterbirds can account for the rapid colonization of small ponds where they are much less common (Maguire 1963). Other supposed zooplankton vectors have been seldom studied and evidence is scarce or anecdotal. Insects, for example, have been shown to carry small zooplankton (e.g. ostracods attached to *Notonecta* and *Sigara*; Lansbury 1955, F. van de Meutter 2007, personal observation) and transport micro-organisms during flight (flagellates, protozoa; Maguire 1959, 1963; Schlichting & Sides 1969). Whether insects are also capable of dispersing larger zooplankton is not known.

The cosmopolitan waterbug, genus *Notonecta* is a common inhabitant of ponds and pools (Nieser 1982). The ventral surface of the abdomen in *Notonecta* has a hairy keel and hair-fringed lateral margins. Small aquatic organisms such as ostracods may attach to these hairs (Lansbury 1955) and could then be dispersed. Ephippia of cladocerans are often produced *en masse* and may aggregate in the pond littoral, from where *Notonecta* typically disperses. This experimental study aims at answering the following two questions: (i) could *Notonecta* be a vector of *Daphnia* ephippia? (ii) do differences in ephippium size, and buoyancy among and within *Daphnia* species affect dispersal propensity?

2. MATERIAL AND METHODS

(a) *Outdoor experiment*

First, we investigated whether dispersing *Notonecta maculata* could transport *Daphnia* ephippia. The experiment was run on 11 October 2006 in a 15 m×5.5 m×3 m (L×W×H) insectary (mesh 1.35 mm). Ten *Notonecta* and 1000 *Daphnia pulex* ephippia were introduced into a 280 cm^2 Petri dish filled with 3 cm of water and with four 3.5 cm high cobbles. Densities of ephippia mimicked field densities (see the electronic supplementary material). The dish was placed at the northern end of the insectary, because previous trials showed that flying *Notonecta* oriented towards the sun (which was on average in the south, experiments were run between 11.00 and 16.00 hours).

Flying *Notonecta* were caught with a bucket. We quantified the distance travelled with a tape measure to the nearest 0.5 m and checked the *Notonecta*'s body and the bucket for ephippia after each catch.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2008.0323 or via http://journals.royalsociety.org.

Received 13 June 2008
Accepted 25 June 2008

We used a different set of 10 *Notonecta* per trial in 10 subsequent flight trials. *Daphnia pulex* ephippia and *N. maculata* were collected on 5 October 2006 from ponds in Leuven and Engsbergen, respectively. *Notonecta maculata* were washed under a water flow prior to the experiments to remove any ephippia already present.

(b) *Comparison of different* Daphnia *species*

In a second experiment, we compared the propensity for ephippia phoresy by *N. maculata* of three *Daphnia* species (*Daphnia longispina*, *Daphnia magna* and *D. pulex*) that differ in ephippium size and buoyancy. *Daphnia longispina* has the smallest and *D. magna* the largest ephippia. *Daphnia longispina* and *D. pulex* usually have a large fraction of floating ephippia, ephippia of *D. magna* generally sink (Ślusarczyk & Pietrzak 2008). Because sinking ephippia may become floating ones after a drought, we additionally tested *D. magna* ephippia that had become floating ones after drying. We used the same 280 cm^2 Petri dish as in the outdoor experiment, placed in a cubic 90 cm^3 insectary (mesh 1.35 mm) in a room at 18°C. Flying *Notonecta* fell into white trays and were checked for ephippia. We measured the length of the comb (without the spine) and the maximal width for all dispersed ephippia and 50 non-dispersed ephippia for each *Daphnia* species using an OLYMPUS SZX-ILLB-200 stereomicroscope. Ephippia of *D. magna* were collected from a multiclonal laboratory culture, *D. pulex* from a pond in Leuven and *D. longispina* from a pond in Engsbergen on 5 October 2006. We inoculated 400 ephippia for each species, which is below observed field densities (see the electronic supplementary material). Ephippia of the different species behaved similarly in the Petri dish, and more than 95% aggregated near edges and cobbles. For each *Daphnia* species, three trials with 10 different *Notonecta* each time were run on 26–27 October 2006.

We tested for differences in the propensity for dispersal (yes/no) between the different *Daphnia* species with a mixed model ANOVA with species as a fixed factor and run nested in species as a random factor. We used a binomial error structure. We tested for differences between *Daphnia* species in the average number of ephippia transported per successful dispersal event with a similar mixed model ANOVA with a Poisson error structure. Species differences were tested using the estimate function in SAS. Analyses were done in proc MIXED using the GLIMMIX macro of SAS 9.1. Differences in morphology between dispersed and non-dispersed ephippia were analysed with a two-way MANOVA with dispersal (yes/no) and species as categorical and the logarithmically transformed comb length and ephippium width as dependent variables. Significant interactions were further explored with separate MANOVAs per species.

3. RESULTS

(a) *Outdoor flight cage experiment*

Of the 45 dispersing *Notonecta* that were caught in flight, 30 were carrying ephippia. The mean number of transported ephippia per *Notonecta* was 2.6 (maximum: 15). The number of ephippia on a *Notonecta* was not correlated with the distance travelled ($r=0.012$, $p=0.45$; range: 1–13 m, median: 6 m). Most ephippia fell off when catching the *Notonecta*, but in nine *Notonecta* we found ephippia still present on the body: 12 ephippia were attached to the hair fringes on the abdomen and two to the hair fringes on the hind legs.

(b) *Comparison of different* Daphnia *species*

Thirty *Notonecta* flights resulted in 58 transported ephippia in *D. longispina*, 26 in *D. pulex*, 8 in floating *D. magna* and none in sinking *D. magna* ephippia. The propensity for ephippia phoresy by *Notonecta* differed between floating and non-floating ephippia in *D. magna* (Fisher exact, $p=0.012$). For floating ephippia, dispersal propensity decreased from *D. longispina* over *D. pulex* to *D. magna* ($F_{2,84}=8.95$, $p=0.0003$, table 1, figure 1, all comparisons significant after sequential Bonferroni). The average number of transported ephippia per *Notonecta* (excluding flights where no ephippia were transported) did not differ between the three species ($F_{2,38}=0.78$, $p=0.47$). Dispersal of ephippia was size selective, depending on the species (species × dispersal: $F_{4,468}=4.84$, $p<0.001$, figure 1). Dispersal by *Notonecta* was not selective in *D. longispina* ($F_{2,105}=0.69$, $p=0.50$), but favoured smaller *D. pulex* ($F_{2,73}=3.41$, $p=0.039$) and *D. magna* ephippia ($F_{2,54}=3.96$, $p=0.029$).

Table 1. Pairwise comparisons of dispersal propensity among *Daphnia longispina*, *Daphnia pulex* and *Daphnia magna* (floating ephippia only). (Values in the upper right corner are the t_{84} values of a contrast analysis in a mixed model ANOVA; values in the lower left corner are p-values. p-values remained significant after sequential Bonferroni correction.)

species	*D. longispina*	*D. pulex*	*D. magna*
D. longispina		−2.55	−4.22
D. pulex	0.012		2.11
D. magna	<0.0001	0.038	

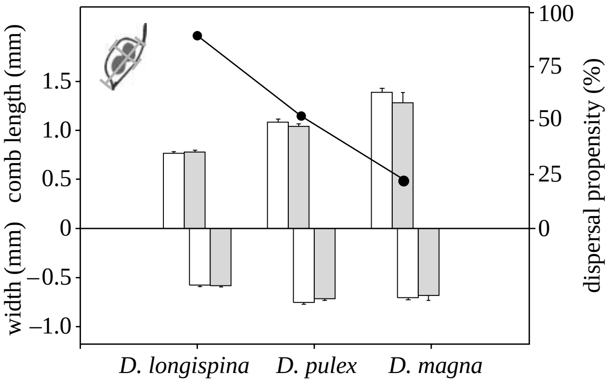

Figure 1. Ephippium comb length (upper bars) and width (lower bars) for non-dispersed (white bars) and dispersed (grey bars) ephippia of *Daphnia longispina*, floating *Daphnia magna* and *Daphnia pulex*. Dots indicate dispersal propensity (percentage of successful dispersal events).

4. DISCUSSION

This study demonstrates that aquatic insects can be important dispersers of zooplankton dormant eggs. Using natural *Daphnia* ephippia densities, we observed a high propensity for dispersal by the cosmopolitan waterbug *Notonecta*. Phoresy by *Notonecta* was selective among and within *Daphnia* species, favouring smaller ephippia. Also buoyancy appeared to be critical: non-floating ephippia of *D. magna* were never dispersed.

Dispersal propensity decreased from the small *D. longispina* over *D. pulex* to the large *D. magna*. For the latter two species, dispersal propensity decreased with increasing ephippium size, suggesting ephippium size itself may explain variation in dispersal propensity. Another critical trait for dispersal was buoyancy, which probably relates to the mechanism of ephippium attachment: we observed that ephippia floated towards the *Notonecta* that came to the water surface, possibly due to hydrophobic attraction (F. van de Meutter 2007, personal observation). We conclude that *Notonecta* transports mainly small, floating ephippia, and thus could generate species-specific distribution patterns in freshwater Cladocera.

Despite the artificial settings in our experiment, we believe that our results apply to natural situations. First, we noted no unnatural behaviour of the

Notonecta that could have affected attachment of ephippia. Without exception, ephippia attached to the *Notonecta* when it climbed upon a stone or floated at the water surface to prepare for flight, which is natural pre-dispersal behaviour. Second, densities of ephippia may seem high, but this reflects natural situations (see the electronic supplementary material). *Notonecta* densities were also high, but this only affected frequency of dispersal, not propensity.

Cáceres & Soluk (2002) and Cohen & Shurin (2003) both found that restricting access to potential vectors by covering experimental mesocosms with nettings did not restrict colonization by zooplankton, from which they concluded that wind dispersal was the dominant dispersal vector. However, these studies did not check for unsuccessful colonization by aquatic insects. Aquatic insects use polarized light reflected from water surfaces (Schwind 1984), which may remain visible through netting (G. Horvath 2007, personal communication), to detect water during flight. Covering the water surface prevents access to the containers, but still insects may attempt to land and possibly deposit propagules (F. van de Meutter 2007, personal observation).

Assuming that *Notonecta* is an important disperser of zooplankton can have fascinating implications for cladoceran dispersal and colonization. For example, *Notonecta* will transport ephippia from pond to pond, which is likely to be more efficient than wind dispersal. Moreover, *N. maculata* has been shown to avoid small (less than 1 m^2), often short-lived pools (Wilcox 2001), which secures transported cladocerans against abortive hatching. Interestingly, this relationship between cladocerans and *Notonecta* may be advantageous for both partners. *Daphnia* can be an important food for *Notonecta* (Arnér *et al.* 1998). Thus, by dispersing *Daphnia* ephippia, *Notonecta* may actually seed a food supply for itself and its progeny.

This study shows that aquatic insects may be important vectors of cladoceran resting eggs. *Notonecta* are frequent dispersers (Briers & Warren 2000) that can fly at least 1.6 km (Briers 1998), which can explain the rapid colonization by zooplankton observed in many small ponds (Louette & de Meester 2005). Flying *Notonecta* in our experiment frequently transported ephippia, with a bias towards smaller species, suggesting *Notonecta* could generate specific colonist communities in new habitats. Also, other aquatic Coleoptera and Hemiptera might contribute to zooplankton dispersal, but having less hairs and setae, they may be less equipped to carry resting stages than *Notonecta*. If the importance of *Notonecta* as a vector can be confirmed in the field, available knowledge on dispersal and colonization dynamics of *Notonecta* may be used to predict zooplankton dispersal, colonization and resilience to disturbance.

F.v.d.M. is postdoctoral fellow of the research foundation, Flanders (FWO-Vlaanderen).

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