The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2013, ••, ••–••. With 3 figures

Flexible dispersal dimorphism in zooplankton resting eggs: an example of repeated phenotypic coin flipping?

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Received 28 May 2013; revised 9 July 2013; accepted for publication 9 July 2013

Theory predicts that spatio-temporal variation in habitat suitability will promote selection for dispersal. In addition to movement in space, dispersal gains an extra dimension in freshwater zooplankton because resting eggs can disperse in time as well, via dormancy. Potential trade-offs between both strategies, however, remain largely unexplored. Using a temporary pool fairy shrimp population as a model, we tested for consistent differences in buoyancy among resting eggs during consecutive inundations in a standardized laboratory experiment and explored a potential trade-off between dispersal (floaters versus sinkers) and dormancy (high versus low hatching fractions). Although discrete dispersal morphs were present, this trait was not fixed. Irrespective of their dispersal phenotype during previous inundations, floating eggs hatched more frequently than sinking eggs. Egg morphology did not affect buoyancy and, between inundations, approximately half of the eggs changed their dispersal phenotype. Although this mechanism has affinities with conservative bet hedging and adaptive coin flipping, it is unique because the dispersal phenotype can switch at the onset of each inundation. Despite possible selection against dispersal at the population level, such a strategy ensures variation in dispersal ability at any moment and could promote population persistence in a metapopulation context. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, ••, ••–••.

ADDITIONAL KEYWORDS: bet hedging – buoyancy – dormancy – fairy shrimp – risk spreading – temporary pool.

INTRODUCTION

Temporary pools are often unpredictable in terms of the timing, frequency, and duration of their wet phases (Brendonck & Williams, 2000; Williams, 2006; Vanschoenwinkel *et al*., 2009a). As a result, many freshwater zooplankton species in these systems have developed specific adaptations to cope with this variation in habitat suitability. These include short life-cycles, high fecundity, and the production of dormant propagules that accumulate in the sediment (Stearns, 1992; Brendonck & De Meester, 2003; Cáceres, Christoff & Boeing, 2007). Staggered hatching of this reserve of dormant genotypes over different inundations is considered to be an important risk-spreading strategy, which enables populations to buffer against demographic catastrophes (Cáceres, 1997; Brendonck *et al*., 1998; Childs, Metcalf & Rees, 2010). Alternatively, resting eggs may also differ in their dispersal propensity. In this respect, the ability to float (buoyancy) is considered to be a particularly important trait that facilitates the mobilization of resting eggs by wind, animal vectors or flowing water (Cáceres *et al*., 2007; Hulsmans *et al*., 2007; Vanschoenwinkel *et al*., 2008a).

Consequently, organisms that produce dormant propagules such as eggs or seeds can invest in dispersal in space (from here on referred to as dispersal) and/or dispersal in time (from here on referred to as dormancy) to avoid demographic catastrophes (Levin, Cohen & Hastings, 1984; Venable, 1985) as part of an evolutionary risk-spreading strategy (bet hedging;

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Cohen, 1966; Philippi & Seger, 1989; Venable, 2007). The success of both strategies, however, depends on propagule traits, habitat structure, and the scale and synchrony of environmental fluctuations (Cohen & Levin, 1987; Snyder, 2006). Dormancy is a useful strategy to survive unsuitable environmental conditions *in situ*, although it is only feasible if propagule mortality is sufficiently low for populations to recover when suitable conditions return (Evans & Dennehy, 2005). In turn, dispersal may be selected for as an alternative or complementary bet-hedging strategy when there is strong variation in habitat quality both in time and space (Levin *et al*., 2003; Siewert & Tielbörger, 2010). The spatial scale at which this environmental variation in habitat suitability occurs must, however, allow for sufficient exchange of individuals (Venable & Brown, 1988). Moreover, theory predicts that, given these assumptions, there may be trade-offs between investment in dormancy and dispersal (Venable & Lawlor, 1980; Cohen & Levin, 1987). Conversely, when relative differences in habitat suitability among patches are constant, dormancy is expected to be a more efficient risk-spreading strategy to buffer against temporal variation than dispersal (Siewert & Tielbörger, 2010). Nonetheless, in this case, dispersal may still be important to sustain populations in suboptimal habitat patches through source-sink dynamics and spatial rescue effects (Brown & Kodric-Brown, 1977; Bilton, Freeland & Okamura, 2001). Consistent with model predictions, several annual plant species produce both poorly dispersed seeds, characterized by prolonged dormancy, and seeds with a high dispersal capacity and limited dormant fractions (Venable & Lawlor, 1980; Cohen & Levin, 1987, 1991; Snyder, 2006). Despite the strong resemblance to annual plants in terms of their life cycles and survival strategies, the link between dormancy and dispersal strategies has not been investigated thoroughly in freshwater zooplankton yet.

Several studies have reported that the resting eggs of a number of zooplankton species float or that this trait can be induced (Mura & Nagorskaja, 1995; Fryer, 1996; Cáceres *et al*., 2007). For example, initially sinking ephippia of *Daphnia* floated after air was allowed to penetrate the ephippial capsule (Pietrzak & Slusarczyk, 2006) and there are indications that most zooplankton resting eggs will float if they are stuck in the surface water film (Davison, 1969). Nevertheless, Cáceres *et al*. (2007) demonstrated that, although individual *Daphnia* clones produced both buoyant and sinking eggs, there were consistent differences in the fraction of buoyant eggs produced by different genotypes. This is suggestive of some genetic variation. Field observations attest that buoyancy can be an important trait that promotes passive dispersal, particularly in habitats connected by flowing water (Michels *et al*., 2001; Hulsmans *et al*., 2007; Vanschoenwinkel *et al*., 2008a). Additionally, floating eggs were also shown to be dispersed more readily by animal vectors such as water bugs (Van de Meutter, Stoks & De Meester, 2008) and birds (Figuerola & Green, 2002). In deep permanent waters, sinking eggs will probably never leave the habitat in which they were produced (Brendonck & De Meester, 2003), whereas, in temporary ponds, eggs can be picked up by vectors when they are exposed during dry periods (Vanschoenwinkel *et al*., 2008b). Additionally, because sinking eggs are more readily covered by sediment and hence deprived of essential hatching stimuli such as light (Murugan & Dumont, 1995; Pinceel *et al*., 2013), it is probable that prolonged dormancy is more prominent in sinking than in floating eggs.

Zooplankton populations from temporary pools on top of rocky outcrops (inselbergs) (Fig. 1A) are suitable model systems for studying the evolution of risk-spreading strategies. Rock pools are generally small (diameter < 4 m), shallow (depth < 25 cm) depressions that are solely dependent on rainfall for filling and they typically exhibit spatial and temporal variation in habitat suitability in terms of hydroperiod (i.e. the length of wet periods) (Vanschoenwinkel *et al*., 2009a; Brendonck *et al*., 2010; Vanschoenwinkel, Seaman & Brendonck, 2010). What is more, nearby pools are often connected by overflows after heavy rains, facilitating the dispersal of floating resting eggs (Brendonck & Riddoch, 1999; Hulsmans *et al*., 2007). For example, resting eggs produced by the African rock pool fairy shrimp *Branchipodopsis wolfi* Daday 1910 (Crustacea, Branchiopoda, Anostraca) (Fig. 1B) include both floating and sinking morphs, indicative of differences in dispersal capacity (Brendonck & Riddoch, 2000). Field observations consistently confirm that floating eggs of this species frequently disperse via overflows during and immediately after rains (Hulsmans *et al*., 2007). Besides water, wind was shown to be the dominant dispersal vector within rock pool clusters (Vanschoenwinkel *et al*., 2008a), fuelling the redistribution of large numbers of eggs over short time scales. The superficial floating egg fraction is most likely to be picked up by wind when floating eggs accumulate near the water's edge and when they are exposed after the last water has evaporated (Vanschoenwinkel *et al*., 2008b). Consequently, both dispersal promoted by buoyancy and dormancy could contribute to persistence of populations, illustrating the potential of dispersal in contributing to persistence at the metapopulation level via spatial rescue effects (Gotelli, 1991). Potential links between the dispersal phenotype and dormancy strategy of individual resting eggs, however, have thus far not been investigated.

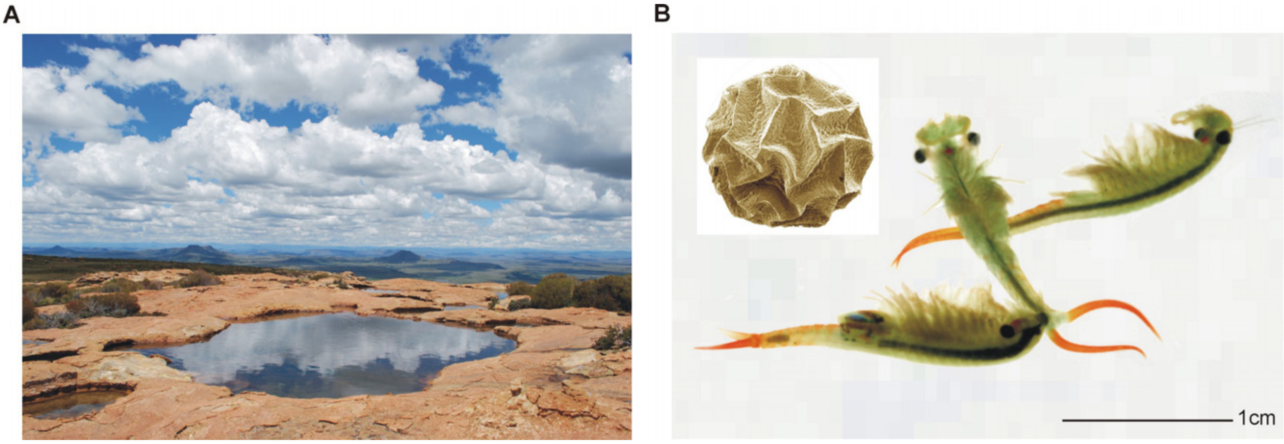

Figure 1. A, example of a typical rock pool fairy shrimp habitat in southern Africa. B, the fairy shrimp *Branchipodopsis wolfi* showing two males and a female (bottom left corner), as well as an enlarged scanning electron microscopic picture of a dormant resting egg (actual egg size: 200 μm) (photographs courtesy of Bram Vanschoenwinkel and Dirk Ercken).

In the present study, we investigated intrapopulation variation in dispersal phenotype and associated diapause strategy in resting eggs of the fairy shrimp *B. wolfi* to test the general hypothesis that individuals investing in dispersal would invest less in dormancy and vice versa. First, by separating floating and sinking eggs during subsequent experimental inundations, we tested whether there are consistent differences in the dispersal phenotype of resting eggs and investigated the potential contribution of egg size and egg shell morphometry with respect to explaining this variation. Second, by hatching batches of floating and sinking eggs under controlled conditions, we explored a potential trade-off between dispersal and dormancy.

MATERIAL AND METHODS

Study site and model organism

Branchipodopsis wolfi is a dominant pelagic filter feeder in temporary rock pool habitats in southern Africa. Resting eggs typically hatch during the first 3 days after rain and larvae require approximately 7–14 days to mature and produce a next generation of dormant eggs (Vanschoenwinkel *et al.*, 2010). By contrast to many other zooplankton species, *B. wolfi* is characterized by a strictly sexual reproduction and exclusively produces dormant eggs that need a dry period before they can hatch. Because not all eggs hatch during a single inundation (Brendonck *et al.*, 1998), persistent egg banks are generated, which may contain between 200 and 500 000 eggs per pool in the studied rock pool systems (1000 and 220 000 eggs m^{-2}, respectively) (Brendonck & Riddoch, 2000). The outer layer of the egg shell shows external sculpturing, consisting of ribs surrounding polygonal depressions (from here on referred to as valleys) (Fig. 1B).The studied population was originally collected from one temporary rock pool that is part of a pool cluster on a granite outcrop near the city of Gaborone in Botswana (Kgale Hill; S24°41′45″, E25°52′03″). Dry sediment containing resting eggs was collected from different quadrates (10 × 10 cm) in the middle and at the edges of the pool using a spoon and brush so that approximately 30% of the total sediment bank of the pool was sampled.

Resting egg production and buoyancy

After 3 months of dry storage, the field collected sediment was inundated in the laboratory using demineralized water and incubated at 27 °C under a 12 : 12 h light/dark cycle. Hatched larvae were transferred to 2-L aquaria filled with diluted tap water (conductivity: 20 μS cm^{-1}) and fed *ad libitum* (*Selenastrum capricornutum*, *Chlamydomonas reinhardii* and micronized Tetramin®; Tetra Europe). At day 4, juveniles were transferred to 9-L aquaria at a density of 20 individuals L^{-1} and fed *ad libitum*. After 1 month, resting eggs were harvested from the laboratory population and stored on small sieves under constant conditions (40 °C, 0 : 24 h light/dark cycle, 30% relative humidity) for 4 weeks before use in the laboratory experiments.

To separate floaters from sinkers, the eggs were inundated in a 200-mL vial with demineralized water and the vial was shaken for 1 min. After 30 min of stabilization, both fractions were separated using a micropipette. Only a subset of these two egg fractions was used in a first hatching experiment, whereas the remaining eggs of both floating and sinking fractions were dried on small sieves for 2 weeks (40 °C, 0 : 24 h light/dark cycle, 30% relative humidity). Afterwards, these dried eggs, which were derived from the sinking and floating egg fractions in the first experiment, were once more separated into floaters and sinkers using the same protocol, resulting in a total of four egg fractions corresponding to four buoyancy phenotypes at the start of the second hatching experiment. These included eggs that sank or floated during both inundations (S-S, F-F), eggs that sank during the first and floated during the second inundation (S-F), and those that floated during the first and sank during the second inundation (F-S).

HATCHING PROCEDURE AND EXPERIMENTAL SET-UP

Branchipodopsis wolfi resting eggs were incubated under optimal hatching conditions (demineralized water, 15 °C, conductivity: 1 μS cm^{-1}, pH: 6.1, 24 h light) as identified in a previous study (Brendonck *et al*., 1998). A first 36-replicate hatching experiment with batches of either 20 sinking or 20 floating eggs per replicate was set up in a total of 12 polystyrene multiwell plates, each consisting of six individual wells filled with 10 mL of medium. This tested for differences in hatching fractions of eggs previously isolated from sinking and floating egg fractions. Wells with floating or sinking egg batches were randomized among and within multiwell plates. Using the same experimental protocol as in the first experiment, batches of 20 eggs of each of the four egg fractions (S-S, F-F, S-F, and F-S) were hatched to assess whether the dispersal phenotype is preserved during subsequent inundations and whether differences in hatching fractions of eggs are associated with differences in dispersal phenotype.

During each hatching experiment, the number of hatchlings was checked on a daily basis until no additional hatching was observed for 48 h, after which the experiment was terminated. Emerging nauplius larvae were counted and removed. Eggs that did not hatch by the end of the experiment were decapsulated with commercial hypochlorite 2.12% (Sorgeloos *et al*., 1977) to check for the presence of viable embryos. This corrected for the number of dead eggs in each well when calculating hatching fractions.

EGG MORPHOMETRY

To assess potential links between egg morphometry and dispersal phenotypes and to evaluate the impact of hydration on egg size, the diameter of floating and sinking egg fractions was measured and contrasted both in a hydrated and a dry state using a calibrated microscope eye piece (accuracy of 0.1 μm). A first measurement of 100 floating and 100 sinking eggs was performed 2 h after the start of the inundation when eggs were fully hydrated. The same eggs were measured a second time after they were dried for 7 days at 60 °C. Additionally, to detect differences in the thickness of the egg shell of floating and sinking eggs, 50 eggs of each dispersal phenotype were prepared for scanning electron microscopic (SEM) analyses under a Philips SEM 515. Eggs were first cut in half using a razor blade when immersed in liquid nitrogen and subjected to critical point drying. Subsequently, eggs were transferred to stubs and coated in 30 nm of gold prior to analysis. Because of the presence of ridges on the egg shell of this species (Fig. 1B), thickness was measured both at these ridges and in valleys. Estimates of the thickness of the egg shell were calculated as the mean thickness measured at five randomly chosen ridges and the thickness measured in five valleys on the egg surface for each egg using the internal scale of the SEM.

STATISTICAL ANALYSIS

All analyses were performed in R, version 2.15.2 (R Core Development Team). Differences in hatching fractions between dispersal phenotypes (F and S in Experiment 1; S-S, F-F, S-F, and F-S in Experiment 2) were tested using generalized linear models specifying a binomial error distribution and a corresponding logit link function. We used a sequential Bonferroni correction to correct *P*-values for pairwise contrasts. Size differences between dispersal phenotypes and between hydrated and dried eggs were evaluated using nonparametric Kruskal–Wallis and Wilcoxon matched pairs tests, respectively. Differences in egg shell thickness between floating and sinking eggs were assessed at ridges and valleys using a nonparametric Mann–Whitney *U*-test.

RESULTS

HATCHING EXPERIMENTS

Overall, 28.6% of *B. wolfi* resting eggs floated during the first experimental inundation. The dispersal phenotype of individual eggs, however, was not consistent over different inundations. Over two experimental hydrations alternated by a dry period, 55.1% of initially floating eggs sank during a second inundation. In turn, 43.8% of eggs that sank during the first inundation floated during the second one.

Hatching fractions of floating eggs were significantly higher than those of sinking eggs in both experimental inundations. In the first experiment, 39 ± 14% of the floating eggs hatched compared to 15 ± 10% of the sinking ones (d.f. = 70, $P < 0.0001$; Fig. 2A). In the second experiment, eggs that floated during both inundations (F-F: 31.6 ± 8.3%) or started to float in the second inundation (S-F: 28.0 ± 11.1%)

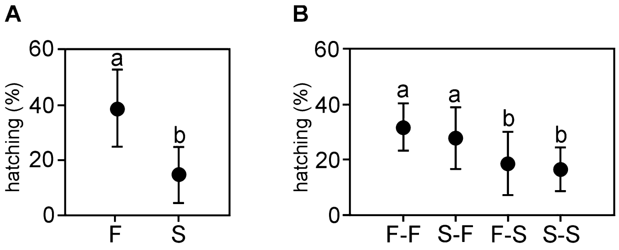

Figure 2. Relationships between dispersal phenotypes (F, floating; S, sinking) and hatching strategies of fairy shrimp resting eggs. A, comparison of the hatching fraction of floating and sinking eggs during inundation one. B, comparison of the hatching fraction of the four dispersal phenotypes (F-F, floating-floating; S-F, sinking-floating; F-S, floating-sinking; S-S, sinking-sinking) during inundation two. Significant differences in hatching between dispersal phenotypes are indicated by the lowercase letters 'a' and 'b' within both panels.

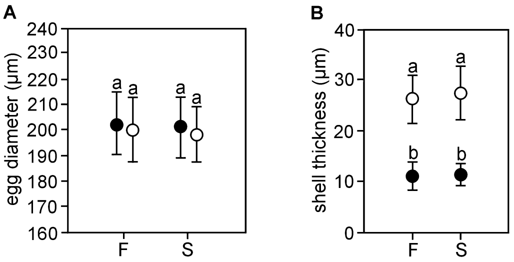

Figure 3. Relationships between dispersal phenotypes (F, floating; S, sinking) and resting egg morphology. A, comparison of egg diameter of floating and sinking eggs. The same eggs were measured in dehydrated (empty circle) and hydrated (solid circle) states. B, comparison of variation in the thickness of the egg shell of floating and sinking resting eggs measured at a ridge (empty circle) or in the valleys between two consecutive ridges (solid circle), respectively. Significant differences in size or shell thickness between floating and sinking eggs are indicated by the lowercase letters 'a' and 'b' within both panels.

exhibited higher hatching fractions than eggs that sank during the two inundations (S-S: 16.4 ± 8.2%; d.f. = 34, $P < 0.0001$ and d.f. = 34, $P = 0.0003$, respectively) or only sank during the last inundation (F-S: 18.9 ± 11.2%; d.f. = 34, $P = 0.0002$ and d.f. = 34, $P = 0.005$, respectively). Within floating and sinking fractions, hatching was similar during both inundations irrespective of former buoyancy (Fig. 2B).

EGG MORPHOMETRY

The diameter of sinking eggs measured on average 201.3 ± 11.6 µm when hydrated and 198.4 ± 10.9 µm when dehydrated. Floating eggs measured on average 202.6 ± 12.5 µm when hydrated and 200.0 ± 12.8 µm when dehydrated (Fig. 3A). Kruskal–Wallis tests did not reveal significant differences in diameter between eggs with a different dispersal phenotype (d.f. = 98, $P = 0.475$ for hydrated and d.f. = 93, $P = 0.213$ for dehydrated eggs). Although, for both floating and sinking eggs, the diameter was generally larger when hydrated, Wilcoxon matched pairs tests did not reveal significant differences (d.f. = 98, $P = 0.140$ and d.f. = 93, $P = 0.058$, respectively).

The thickness of the egg shell measured at ridges amounted 26.4 ± 4.8 µm for floating eggs and 27.6 ± 5.2 µm for sinking eggs, whereas the thickness in valleys was 11.1 ± 2.8 µm for floating eggs and 11.4 ± 2.2 µm for sinking eggs. Mann–Whitney *U*-tests, however, showed that the observed differences in egg shell thickness of floating and sinking eggs were not significant at ridges (d.f. = 52, $P = 0.333$), nor valleys (d.f. = 52, $P = 0.505$) (Fig. 3B).

DISCUSSION

We evaluated the relationship between dispersal phenotype and hatching probability of fairy shrimp resting eggs over two consecutive inundations separated by a dry period. Dispersal phenotypes of zooplankton resting eggs in terms of buoyancy are often assumed to be fixed (Hulsmans *et al.*, 2007; Vanschoenwinkel *et al.*, 2009b). In the brine shrimp *Artemia*, for example, all eggs float and the ability of eggs in this species to reliably utilize Archimedes' principle is typically attributed to an extensive, gas filled, alveolar layer in the egg shell (Abatzopoulos *et al.*, 2002). By contrast, our results show that buoyancy phenotype is not a fixed trait in resting eggs of the fairy shrimp *B. wolfi*. Instead, approximately half of the resting eggs showed a different dispersal phenotype during a second inundation after they were dried. A lack of consistent trends in switching of the dispersal phenotype after dry periods suggests that the buoyancy phenotype of a resting egg is not different from random expectations. Mura & Nagorskaja (1995) found that the floating eggs of another fairy shrimp species, *Thamnocephalus platyurus* Packard 1877, were consistently larger in size and had a thicker tertiary envelope than sinking eggs. The fact that a similar relationship was absent in *B. wolfi* suggests that, rather than simply being determined by the potential air volume that can be stored in the alveolar layer, buoyancy may be regulated by subtle changes in egg shell permeability during hydration and drying cycles at the level of pores and channels through which air may penetrate the egg shell. This is consistent with observations reported by Pietrzak & Slusarczyk (2006), who suggested that drought may allow air to penetrate the ephippial capsule in the water flea *Daphnia*, promoting buoyancy.

From an evolutionary point of view, consistently higher hatching of floating eggs, regardless of their buoyancy during a previous inundation, does not suggest strong intrinsic genetic or epigenetic variation in hatching alleles (Evans & Dennehy, 2005). Instead, hatching appears to be modulated by extra-embryonic factors associated with an egg's buoyancy phenotype. In relation to risk-spreading strategies, a negative correlation between dormancy and dispersal-promoting traits can be indicative of a trade-off between dormancy and dispersal as alternative risk-spreading strategies in variable environments (Venable & Lawlor, 1980; Levin *et al*., 1984; Kisdi, 2002). However, a lack of consistent differences in dispersal phenotypes indicates that, although a trade-off was found between dormancy and dispersal in the present study, an evolutionary origin appears to be unlikely. Instead, at the population level, all eggs have a more or less fixed probability of floating (30–40%) or sinking (60–70%) during any inundation. When they happen to float, eggs presumably hatch more readily as a result of subtle differences in hatching cues. For example, even though dissolved oxygen concentrations in the experimental hatching wells were close to saturation, more efficient gas exchange with the atmosphere may lead to higher oxygen availability and enhanced metabolic rates in the embryos of floating eggs. An alternative explanation could be that floating eggs receive additional indirect light via reflection on the water surface, which could result in slightly higher light intensities promoting hatching. Given the low reflectivity of water (Cogley, 1979), however, this appears less likely.

Overall, the maintenance of dispersal dimorphism in *B. wolfi* could be considered as a population strategy that bares resemblance to both conservative bet hedging and coin flipping risk-spreading strategies. On the one hand, the production of a single egg type, performing reasonably well under different circumstances, is consistent with a conservative bet-hedging strategy (Seger & Brockmann, 1987). However, the fact that a single egg type does not exhibit one all-purpose phenotype but is able to display one of two possible discrete dispersal phenotypes under common garden conditions (i.e. in absence of environmental modulation) is not consistent with this paradigm. Alternatively, the idea that a genotype can give rise to two different phenotypes is central to the concept of adaptive coin flipping; a diversified bet-hedging strategy (Cooper & Kaplan, 1982; Starrfelt & Kokko, 2012). The mechanism observed in the present study, however, differs from adaptive coin flipping in the fact that the phenotype may switch repeatedly during the lifetime of the individual. In the fairy shrimp *B. wolfi*, the dispersal phenotype can switch after each dry period at the onset of a new inundation. As such, this mechanism could be considered as a form of repeated phenotypic coin flipping. This concept can be defined as a risk-spreading strategy of a genotype where the phenotype may switch between different discrete phenotypic states in consecutive growing seasons independent of environmental cues.

In terms of potential adaptive value, the observed strategy ensures variation in dispersal ability at any given moment, despite possible selection against dispersal at the population level. Such a strategy could be beneficial to promote population persistence in a metapopulation context (Hamilton & May, 1977). However, to understand how the strategy works at a larger metapopulation scale, it will be necessary to explore interpopulation variation in the probability of eggs to float or sink along gradients of habitat suitability and connectivity.

Finally, the notion that dormancy strategies were confounded by the buoyancy state of resting eggs also has important consequences for egg bank and community dynamics of freshwater zooplankton. Species or genotypes with floating eggs might not only disperse more readily (Hulsmans *et al*., 2007), but also could contribute disproportionately to the recruitment of individuals to active communities compared to eggs that are embedded in the bottom sediments. As such, the cost of buoyancy related to, for example, the risk of mortality during dispersal is not only likely to be offset by the possible advantage of colonizing new patches but also by a local advantage of better hatching, which could lead to a competitive edge over other species and genotypes via priority effects (De Meester *et al*., 2002).

ACKNOWLEDGEMENTS

This research was funded by the Research Foundation Flanders (FWO-Vlaanderen) 3E090007 and 3E110799. BV currently holds a postdoctoral fellowship with the Research Foundation Flanders. We thank four anonymous reviewers for their constructive comments that helped to improve the manuscript. The authors would also like to thank Arnout Grégoir, Falko Buschke, and Jane Reniers for their valuable advice during the preparation of the manuscript.

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