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The influence of pool volume and summer desiccation on the production of the resting and dispersal stage in a *Daphnia* metapopulation

Florian Altermatt · Dieter Ebert

Abstract Dispersal is a key process in metapopulations, as migrants genetically connect populations and enable the colonization of empty habitat patches. Sub-populations may differ in their numerical contribution of migrants within a metapopulation. This has strong implications on evolutionary and ecological dynamics and has led to two different hypotheses about the *Daphnia* metapopulation studied here: the assessment by some authors is that sub-populations contribute equally to the production of migrants, while others have postulated long-lived core populations in large "mainland" habitat patches as the dominant source of migrants. We have studied the resting and dispersal stage (ephippium) in a natural *Daphnia* metapopulation and in mesocosm experiments, and tested for effects of habitat size and summer desiccation. We found that a 1000-fold increase in rock pool volume resulted on average in only in a 2.8-fold increase in ephippium production. Mesocosm experiments confirmed these results: a 1000-fold increase of the mesocosms' volume resulted in a 7.2-fold increase in ephippium production. Additionally, we showed that ephippium production did not depend on the initial population size. Thus, populations in small pools may contribute only marginal fewer potential migrants in the whole metapopulation than populations in large pools. In a second mesocosm experiment we found that summer desiccation, which is a typical occurrence in small pools, is not detrimental for the populations. *Daphnia* hatched out of ephippia that were produced earlier within the same season and built up viable populations again. The substantial production of ephippia by populations in small pools suggests that these populations might be important for both the dynamics and global stability of metapopulations.

Keywords Desiccation · Dispersal propagule · Ephippium production · Migrant · Migration · Resting egg · Rock pool

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Introduction

Many animal and plant species occur in metapopulations that are assemblages of spatially delimited local populations coupled by some degree of dispersal (Hanski and Gaggiotti 2004; Levins 1970). Dispersal is the process that genetically connects separated populations and enables the colonization of empty habitats (Clobert et al. 2001; Townsend et al. 2000), counteracting local extinction. Ultimately, the long-term survival of a metapopulation depends on the balance between local extinction and colonization and thus on the ability to produce migrants (Ovaskainen and Hanski 2004). An understanding of dispersal and the ability to identify key populations for species survival is also important in the light of habitat fragmentation. Due to the increased isolation of populations, there will be fewer successful migrants. In order to set priorities for conservation, one would like to know the number of migrants and their populations of origin (Hanski and Gaggiotti 2004; Watts et al. 2005). To obtain realistic estimates and to incorporate potential feedbacks between different factors that influence the production of migrants, migrant production must be studied in the field.

Theoretical studies indicate the significance of dispersal within metapopulations (Brown and Kodrick-Brown 1977; Clobert et al. 2001), but empirical data on the various aspects of dispersal are still scarce (Bullock et al. 2002). In the original metapopulation model (Levins 1970) and in subsequent extensions of it, dispersal and the production of migrants were seen as a fixed trait of any individual in the metapopulation. Later models (Hanski 1999) considered dispersal as a function of the population density in the patch of departure, the patch size and the distance between patches. A more specific model is the mainland-island metapopulation (Harrison 1991), with populations in one or more very large patches—the mainland—with negligible risk of extinction. The remaining populations in small habitat patches run a high risk of extinction. Although the mainland-island model is strongly based on the extinction risk of individual populations, it also implicitly makes a statement about the origin of migrants: migrants predominantly or exclusively originate from the permanent "mainland" populations in large patches, while populations in small "island" patches only receive immigrants (Hanski 1999; Kawecki 2004). Population size is assumed to be proportional to patch size. Thus, these models not only make statements about the extinction risk of populations, but also assume a positive relationship between population size and the contribution of migrants. In contrast, a different type of model focuses on condition-dependent dispersal that includes ecological, genetic and social factors (Clobert et al. 2001). Empirical studies are starting to incorporate factors such as, for example, the genetic predisposition of becoming a migrant (Haag et al. 2005), increased dispersal from heavily disturbed patches (Bates et al. 2006), changes in dispersal dynamics due to climate change (Altermatt et al. 2008) and a higher success of immigrants due to local parasites (Altermatt et al. 2007) or hybrid vigor (Ebert et al. 2002). These studies illustrate that it is important to know which factors influence dispersal and migrant production to better understand the dynamics of metapopulations. Data on the origin of migrants are especially needed to make predictions about gene flow, local adaptation and parasite dispersal (Clobert et al. 2001).

We studied the influence of habitat size and summer desiccation on the production of the resting and dispersal stage (ephippium) in a natural metapopulation of the crustacean *Daphnia magna*. *Daphnia magna* commonly occurs in freshwater rock pools along the coast of the Baltic Sea and is an ideal model system to study metapopulation processes (Bengtsson and Ebert 1998; Ebert et al. 2001; Green 1957; Hanski and Ranta 1983; Pajunen 1986; Pajunen and Pajunen 2003). Metapopulations in southwest Finland and east Sweden consist of many thousands of populations that occur in rock pool habitats (Bengtsson and Ebert 1998; Ebert et al. 2001; Pajunen and Pajunen 2003). *Daphnia*

reproduce both asexually as well as sexually. An ephippium is produced by the female *D. magna* as a result of sexual reproduction. It consists of a part of the maternal carapace that forms a protective shell around up to two eggs, comparable with a plant seed capsule containing two seeds. The ephippium is released during the next moult. The enclosed eggs are capable of surviving desiccation and/or freezing and can be passively dispersed.

While the ephippium production of some *Daphnia* species has been quantified in single lakes (Cáceres 1998; Cáceres and Tessier 2004; Kerfoot et al. 2004), nothing is known about the ephippium production of *Daphnia* inhabiting small rock pools (water volume <10 m^3) and how it relates to pool size. Ephippia are essential for survival in such metapopulations for two reasons (Hanski 1999; Hanski and Ranta 1983). First, they are the dispersal stage (Maguire 1963), and dispersal is mainly dependent on the production of ephippia. Ephippia are passively dispersed by wind or birds (Maguire 1963) and allow for long-distance dispersal. Functionally, ephippia are very similar to plant seeds, and the same dispersal mechanisms may operate (Bullock et al. 2006; Figuerola and Green 2002). In the *Daphnia* metapopulation studied here, extinction and colonization in local patches occurs at a high annual rate (Pajunen 1986; Pajunen and Pajunen 2003), stressing the importance of dispersal. Second, ephippia are an essential life-history stage that enables the population to endure freezing in winter and droughts in summer. Due to the obligate freezing of the rock pools, only populations that produce ephippia can survive during winter (Pajunen 1986; Pajunen and Pajunen 2003). Thus, ephippium production can be used both as a surrogate of a population's migrant production and a population's long-term survival ability. *Daphnia* populations in lakes tend to produce ephippia towards the end of the season (Cáceres 1998; Cáceres and Tessier 2004). In unpredictable habitats such as rock pools, however, the seasonal timing of ephippium production may be different, as ephippia must be produced before possible droughts during the summer. We thus monitored the phenology of ephippium production in natural populations.

There are two hypotheses that describe these *Daphnia* metapopulations, and these differ with respect to the persistence of individual populations and the origin of migrants. Hanski and Ranta (1983) suggested a Levins-type metapopulation in an extinction–colonization equilibrium where all populations contribute migrants equally. Pajunen (1986) and Pajunen and Paujnen (2007) favor a mainland-island model, with the long-lived populations in large rock pools being the dominant sources of migrants. Populations in small pools have a higher risk of extinction due to, for example, frequent desiccation, and they are generally more short-lived (Altermatt et al. in preparation; Bengtsson 1989). Pajunen and Pajunen (2003) classified short-lived populations in

small pools as being less important in terms of metapopulation dynamics (mainland-island model). The two hypotheses are based on different assumptions about the production of migrants in individual populations. Hanski and Ranta (1983) did not assume that dispersal is related to any pool variable other than the presence of other *Daphnia* species. Pajunen and Pajunen (1986, 2003) assumed that the origin of migrants is associated with population persistency. Long-lived populations in large pools should produce more migrants, while populations in small pools are usually short-lived and should not contribute migrants due to the unpredictability of their habitat with respect to desiccation. None of the earlier studies quantified ephippium production, and it is not yet understood which populations produce how many ephippia (Pajunen and Pajunen 2003). However, the origin and number of ephippia is a relevant aspect for distinguishing between these two hypotheses.

To fill this gap, we measured the seasonal production of ephippia in natural populations in experimental populations in mesocosms. Both the rock pools and containers in the respective experiments varied in size over five orders of magnitude. We further included initial population size as a factor. Populations in unstable habitats in particular may more often go through population bottlenecks and be recruited out of a few individuals. We especially focused on the ephippium production of populations in pools that may frequently dry up. We tested whether populations can produce ephippia before a drought and then build up viable populations from hatchlings afterwards within the same season, which has not yet been shown. A population is classified as "viable" when the planktonic animals are able to produce ephippia and, thereby, guarantee long-term survival. We intentionally did not relate densities of adult *Daphnia* throughout the season with pool size, as densities vary strongly on short timescales (personal observation). By using the number of ephippia, we have a measurement that integrates over the whole time-span and all densities—and provides the number of potential migrants, which is relevant in an evolutionary and ecological context. It was not our intention to make mechanistic statements on ephippium production of adult *Daphnia*. Our findings will improve our understanding of the origin and number of migrants in a metapopulation and focus on the significance of the numerous populations in habitat patches that are small or where the environment is less predictable.

Material and methods

The natural system

The freshwater crustacean *D. magna* Straus, 1820 (Crustacea: Cladocera) is widely distributed along the coast of the Baltic Sea. It inhabits rock pools ranging from 10 to 30,000 l in volume on the skerry islands of southwest Finland (Pajunen and Pajunen 2003). *Daphnia* hatch from ephippia at the beginning of May, and the populations are usually in their planktonic phase until autumn (September/October). During this time, *D. magna* reproduces predominantly asexually, with intermittent periods of sexual reproduction when ephippia are produced. Various stress-linked factors, such as high population density (Banta and Brown 1929; Carvalho and Hughes 1983), food limitation (Kleiven et al. 1992), increased salinity and short-day photoperiod, have been suggested as triggers for sexual reproduction and the production of ephippia. Only ephippia can survive unfavorable conditions, such as the obligate freezing during winter or the occasional desiccation of pools during summer (Ebert 2005). They also serve as wind-drifted dispersal stages (Maguire 1963; Ranta 1979). The ephippia rest on the bottom of the pools until a hatching stimulus occurs. *Daphnia* females can produce ephippia that do not contain eggs and only consist of a shell. In all our studies and experiments, only ephippia containing eggs were counted, and those without eggs were excluded. Empty ephippia, however, were rare and their proportion negligible.

Most rock pools contain little to no sediments and are washed out frequently during autumn storms. Thus, contrary to lakes, rock pools do not have yearly strata of sediments. A *Daphnia* population consists of both planktonic animals and ephippia. The local extinction of a population only occurs when all of the individuals in both of these two life-stages are dying. Several diapause termination cues occur per year (Pajunen and Pajunen 2003). In accordance to other publications, we define local extinction to have occurred when no *Daphnia* have been observed within an 18-month period (Altermatt et al. 2008; Pajunen and Pajunen 2003). An average population persists for about 3–5 years (Pajunen and Pajunen 2003). Populations that persist for less than about 3 years are called short-lived, while populations that persist for 10 to up to 25 years are called long-lived (Pajunen and Pajunen 2003).

Ephippium production of natural populations

We quantified the production of ephippia by populations in natural rock pools of various volumes throughout the season. We chose populations in 34 rock pools on 14 different islands near the Tvärminne Zoological Station, Finland (59°50′N, 23°15′E). All of these rock pools had contained a *D. magna* population the previous year. We measured the longest axis of each pool, the greatest width perpendicular to this and the maximal water depth in order to estimate the volume of the rock pool as an inverted pyramid (width × length × depth/3, following the method of Ebert et al. 2001). The measurements were carried out in 2005

and 2006 when the pools were filled with water up to their maximal level. The volume estimates of the chosen rock pools (between 24 and 24,200 l) spanned almost the entire spectrum of pools in that metapopulation. We also estimated the surface (width × length).

We measured ephippium production in representative rock pools in 2005 (18 rock pools) and 2006 (20 rock pools, four of which were also used in 2005). We did not include the very small proportion of pools with a thick layer of soft sediments in our study. In May 2005 and 2006, we placed large glass petri dishes (diameter either 182 or 193 mm) in each of these rock pools at depths that were representative of the rock pool. This is the time of year that *Daphnia* populations start growing. The petri dishes passively collect all particles that sink from the water column above them, including the ephippia produced by the *Daphnia*. Ephippia of *D. magna* do not float but only sink to the bottom of the pool. Before the study, we confirmed that the petri dishes did not collect resuspended ephippia from the bottom of the pool. In 2005, we focused on temporal changes in ephippium production throughout the season. All rock pools were equipped with one trap, and the content of each trap was collected at four different time points (after about 21, 50, 97 and 127 days). In 2006, we focused on differences in the total ephippium production throughout the season between populations in pools of different sizes. Thus, ephippia were sampled only twice in 2006 (after about 54 and 105 days). In 2006, two petri dishes were used per rock pool whenever the pool was sufficiently large (17 out of 20 rock pools) to obtain a better estimate for each population's ephippium production. The arithmetic mean of the number of ephippium in the two petri dishes was then used in the analysis. By using two traps per pool we could also affirm that our method gave representative catches (thus little variation between the petri dishes within a rock pool). The depth of the traps in the rock pools was recorded at each sampling to calculate the water volume above the traps. The last sampling was carried out on 3 and 6 September in 2005 and 2006, respectively; the monitored time thus spanned the entire season when *Daphnia* produced significant numbers of ephippia. Some *Daphnia* may remain in September and October, but water temperatures are so low then that ephippium production becomes insignificant. All ephippia in the collected sediments were counted with a stereomicroscope at tenfold magnification. Ephippium production per day per trap (adjusted for the two slightly different sizes of the petri dishes) as well as per day and water volume was calculated.

Habitat size experiment

We measured the influence of habitat size (water volume) and of the initial number of *D. magna* on the populations'

seasonal ephippium production in an experiment. We used four different, parasite-free *D. magna* genotypes that were collected in rock pools on four different islands near Tvärminne Zoological Station in spring 2003 and 2004 and kept in their asexual phase since then. At the end of April 2005, mass cultures of each genotype, starting from one single female, were established. The *D. magna* were kept in their exponential growth phase in artificial medium (Klüttgen et al. 1994) at room temperature and fed ad libitum with the green alga *Scenedesmus obliquus*. On 22 May 2005, the experiment was started with females of the same age class (about 10-day-old animals, i.e. most of them had their first asexual egg clutch in their brood chamber). The animals were released into plastic containers (=mesocosms) filled with water from a natural rock pool filtered with a 20-μm filter. Per 10 l of filtered pool water, 30 ml of a horse manure suspension (10 kg horse manure suspended in 60 l of seawater) and 0.5 l of seawater were added to increase the nutrient content and the salinity of the water. Containers of seven different sizes were used (1, 2, 5, 8, 30, 75 and 320 l). We had four (sizes 1–30 l), two (size 75 l) and one replicate (size 320 l) for each container size class and treatment respectively, giving in total 46 container replicates. The containers were arranged within 32 m^2 in a Latin square outdoors on an island next to natural rock pools. Each container received all four *D. magna* genotypes. We had two different treatments where we introduced the genotypes: either at the same density (one animal per clone and liter = "same density" treatment) or at the same number per container (one animal per clone = "same number" treatment). The water volume was kept constant during the whole season, and losses due to evaporation were replaced with deionized water. After 100 days, which is close to the length of the natural season and comparable to 10–12 asexual generations, the sediments in all replicates were sampled and frozen at −20°C. The numbers of ephippia were counted with a stereomicroscope at tenfold magnification.

Sunlight is the energy source for the primary production of algae, which are the food of the *Daphnia*. Sunlight irradiation is proportional to the surface and not to the volume of the water body. We therefore analyzed the production of ephippia using both water volume as well as water surface area as the explanatory variable.

Desiccation-experiment

As rock pools commonly experience dessication during summer droughts (Pajunen and Pajunen 2003), we tested the influence of desiccation intermitting the ongoing asexual reproduction of a *D. magna* population. We performed an experiment in plastic containers where we could standardize both desiccation and the genetic composition of the *Daphnia* populations. It was impossible to exclude variability

in the dessication/refilling rates in natural pools (due to different catchment areas, evaporation rates, among others; personal observations). We were interested if a forthcoming drought can stimulate ephippium production. It is known that *Daphnia* hatch after droughts in rock pools with persisting populations (Lass and Ebert 2006), but it is unclear if these animals hatch from ephippia of the previous year or from ephippia that did not pass a winter diapause. We thus tested if—after refilling with water—*D. magna* can hatch out of ephippia that were produced immediately before the drought without any additional winter diapause. Although this had never been tested prior to our experiment, it has important implications for dynamics in pools that dry up within the first year of a population's presence.

For this experiment, *D. magna* populations were kept under outdoor conditions in containers containing 5 l of water originating from a rock pool and subsequently filtered through a 20-μm filter and charged with a horse manure suspension and seawater (analogous to the previous experiment). We measured absolute ephippium production before (A; see Fig. 5 for definition of letters), during (B_1) and after desiccation (B_2, desiccation treatment) as well as ephippium production in control populations without desiccation C_1, C_2). At the end of April 2005, a mass-culture of one of the *D. magna* genotypes used in the habitat size experiment was established (analogous to the previous experiment). The experiment was started on 31 May 2005, and 20 adult females were released into each of 75 plastic containers. The containers were placed outdoors next to natural rock pools on an island. After 33 days, 11 randomly chosen replicates were destructively sampled and all sediments, including the ephippia, collected and stored at −20°C (A, ephippium production before evaporation). To study the effect of desiccation, we added wicks to increase evaporation in 32 randomly chosen containers of the remaining 64 replicates. The wicks were made out of three layers of Whatman filter paper (size 20 × 40 cm) clamped between two plastic grids with a mesh size of 1 cm. One wick with cable ties was fixed in an upright position to each container. The lower half of the wick reached the bottom of the container, while the upper half exceeded the container and was exposed to wind. In the 32 control replicates, the same wicks were installed, but the filter paper was interrupted at the water surface level, which prevented an increased evaporation rate. During a natural period of dry weather in July 2005, the water in all replicates of the desiccation treatment evaporated completely within 16 days. In the controls, the water volume decreased only slightly and never fell below 4 l. There was in total 2.4 mm of rainfall during these 16 days (weather data from the Tvärminne Zoological Station's weather station). On 19 July, all wicks were removed, and the desiccated containers were covered with a lid to keep out rainwater. Containers remained

outdoors during the entire study period. On 31 July, these desiccated replicates were refilled with 4 l of deionized water, and the dried sediments were resuspended. From then on we visually checked all replicates daily and recorded the occurrence of the first hatchlings. We also recorded when these animals became adult and produced their first clutch of asexual eggs. At the same time as we refilled the containers, all sediments including ephippia were destructively sampled in half of the remaining control treatment replicates and the density of the planktonic *D. magna* population was reduced to 5% (about 50 animals) to mimic the bottleneck of the populations after hatching in the desiccation treatment (D). The other 16 controls were not manipulated. In 16 randomly chosen replicates of the refilled desiccation treatment, the number of ephippia and the number of hatchlings were counted on 9 August. By then the *Daphnia* had already hatched again, but not yet started to produce ephippia. The shells of empty resting eggs are long-lasting and can be studied even after hatching of the *Daphnia*. Therefore, the number of ephippia produced before the desiccation event could be determined retrospectively (B_1, all ephippia counted. We then subtracted the number of ephippia that were open due to hatching, which gave the decrease indicated by the dotted line). The ephippia in the remaining replicates [16 of the desiccation treatment (B_2), 16 of the continuous control treatment C_2) and 16 of the density reduction treatment (D)] were collected and stored at −20°C on 10 September. All ephippia were counted with a stereomicroscope at tenfold magnification to determine ephippium production during the different phases and in the different treatments.

Analysis

All variables were log-transformed prior to the analysis to fulfil the requirements of the analysis of variances (ANOVAs) and covariances (ANCOVAs). If transformed data still did not fulfil the requirements, nonparametric tests were used. Statistical analyses were performed with R (R Development Core Team 2007). Statistical comparison of estimated and theoretical slopes was performed according to Scherrer (1984). The number of ephippia per day per trap was used when comparing ephippium production relative to pool volume. By doing so, differences in the volume of the water column above the different ephippium traps were not taken into account. The range of the water column volume above the trap was more than three orders of magnitude smaller than the range of the numbers of ephippia collected per trap. Also, the potential bias is conservative with respect to our interpretation of the data that small pools have an over-proportional ephippium production. Pool surface and not pool volume was used when comparing the depths of the ephippium traps, and pool

size was used to avoid any potential autocorrelations between depths and size. To avoid pseudoreplication, only the data from 2006 were used from the four rock pools sampled in both years. In that year, we had two traps in each of these rock pools instead of one, as in 2005; thus, our estimates of ephippium production are more precise. For comparing the results of the habitat size experiment with the natural pool data, we calculated a population's total ephippium production per rock pool by extrapolating the ephippium production per liter of water above the trap to the total volume of the rock pool (ephippia $\times \, l^{-1} \times$ volume of the rock pool).

Results

Ephippium production of natural populations

Ephippia were produced throughout the summer, but the production per day per trap varied significantly between the four measurement periods in 2005 (Kruskal–Wallis test, $C^2 = 12.91$, $df = 3$, $P = 0.005$; Fig. 1a). As expected, the production of ephippia was low early in the season (May), increased to a maximum in July and decreased afterwards. The same pattern was also seen when ephippium production per day per liter was used as response variable (Kruskal–Wallis test, $C^2 = 12.27$, $df = 3$, $P = 0.007$; Fig. 1b). There was also a significant difference in the total number of ephippia produced between the different populations (Kruskal–Wallis test, $C^2 = 35.51$, $df = 17$, $P = 0.005$), ranging from 1 to 2266 ephippia (mean 220; median 41). There was little variation between the two traps within one pool, indicating that the method gave representative estimates.

The number of ephippia produced per day per trap by populations in 34 natural rock pools (data from 2005 and 2006 combined) correlated negatively with the volume of the rock pools (ANCOVA, $F_{1,31} = 14.6$, $P = 0.0006$; Fig. 2), indicating that the productivity (ephippia produced per trap or volume) was higher by populations in small pools. There was no significant difference between the 2 years (ANCOVA, $F_{1,31} = 1.8$, $P = 0.18$; Fig. 2). The time × volume interaction was not significant ($P = 0.87$) and taken out during model-simplification (Crawley 2002). The depths of the ephippium traps were slightly deeper in larger pools and, consequently, the water column above the trap was larger, though the correlation was not significant {linear model between pool surface [log(m^2)] and depth of ephippium trap [log(cm)], $F_{1,32} = 1.75$, $R^2 = 0.088$, $P = 0.09$}. We extrapolated the ephippium counts per trap to the whole pool to estimate the total number of ephippia produced by populations in pools of various size (see below and Fig. 4).
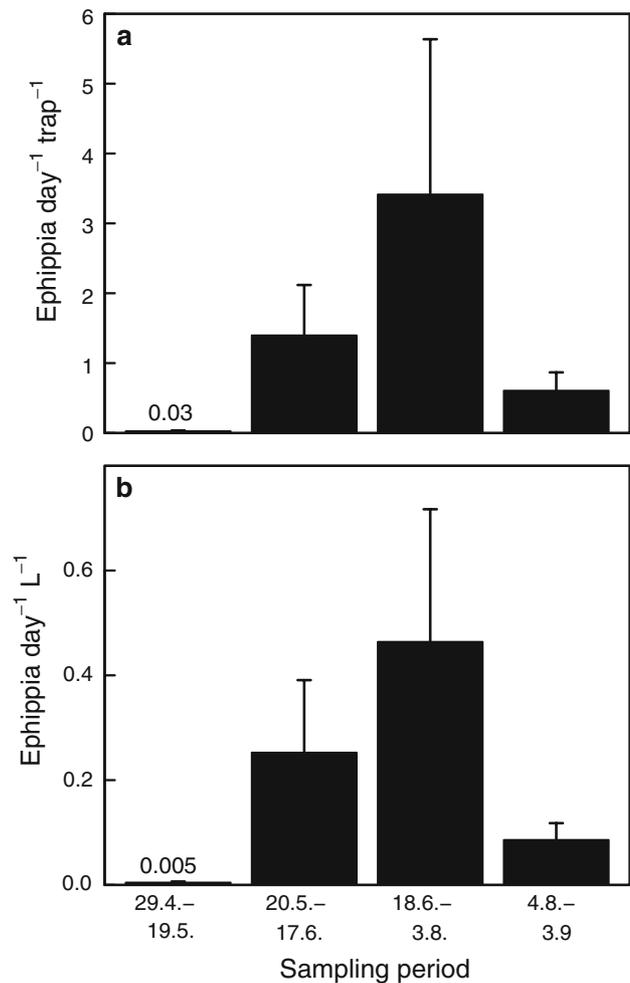

Fig. 1 Mean (±SE) ephippium production of *Daphnia magna* populations in 18 natural rock pools during four time periods in summer 2005. The number of ephippia produced differed significantly between the four periods and the different rock pools. **a** Mean number of ephippia produced per day per trap, **b** mean number of ephippia produced per day and water volume above the trap

Habitat size experiment (mesocosms)

There was a significant positive correlation between the total number of ephippia produced during one season and the water volume of the mesocosm containers inhabited by the *Daphnia* populations (Fig. 3). However, the increase of ephippium counts with volume was significantly smaller than an increase with a slope of one (on a log–log scale; same density treatment $t_{21} = 11.06$, $P < 0.0001$; same number treatment $t_{21} = 12.38$, $P < 0.0001$). An increase with a slope of one (1:1 line) would indicate a proportional increase and thus a constant productivity per volume. As the increase was lower than one, the populations' productivity significantly decreased with increasing container size. A 1000-fold increase in habitat volume resulted only in a 5- to 11-fold increase in the populations' ephippium production [same density treatment:
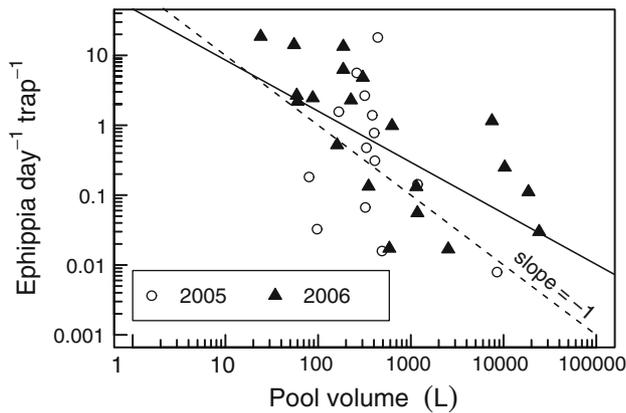
Fig. 2 Correlation between pool volume and the ephippium production of *Daphnia magna* populations in 34 natural rock pools in 2005 and 2006. The rock pool water volume ranged from 24 to 24,200 l. *Solid line* Overall linear regression $\log_{10} (y) = 1.66 - 0.731 \times \log_{10} (x)$, *dashed line* 1:1 line with slope -1 for visualization. Both axes are on a logarithmic scale

Table 1 ANCOVA for the effects of water volume of the containers (l), treatment (same density or same number) and their interaction on a populations' ephippium production per season and replicate

Source of variation	df	MS	F value	P value
Volume	1	1.731	44.03	<0.0001
Treatment	1	0.045	1.15	0.29
Volume × treatment	1	0.079	2.00	0.16
Error	42	0.039		

The results were very similar when water surface area was used instead of water volume as the explanatory variable (Fig. 3c, d; Table 2): the total number of ephippia produced by a population increased with the water surface area of the container in both treatments [same density treatment: $\log_{10}$ (ephippia day^{-1}) $= -0.55 + 0.54 \times \log_{10}$ (surface); same number treatment: $\log_{10}$ (ephippia day^{-1}) $= -0.14 + 0.37 \times \log_{10}$ (surface)]. Again, this increase was significantly smaller than an increase with a slope of one (on a log–log scale; same density treatment: $t_{21} = 4.57$, $P = 0.0002$; same number treatment: $t_{21} = 5.99$, $P < 0.0001$).

We then combined the data from the habitat size experiment with the extrapolated data from the natural pools (Fig. 4). The numbers of ephippia produced relative to inhabited

$\log_{10}$ (ephippia day^{-1}) $= 2.64 + 0.35 \times \log_{10}$ (volume); same number treatment: $\log_{10}$ (ephippia day^{-1}) $= 2.61 + 0.22 \times \log_{10}$ (volume)]. The initial number of *D. magna* females in spring did not influence the overall production of ephippia per season (Fig. 3a, b; Table 1).

Fig. 3 Total number of ephippia produced by *D. magna* populations in mesocosms under semi-natural outdoor conditions as a function of water volume (**a, b**) or water surface (**c, d**). The initial population in the two treatments "same density" and "same number" was manipulated. Populations in the same density treatment had an initial population density of 4 individuals l^{-1} while populations in the same number treatment had a total initial population size of 4 individuals irrespective of the container's volume. The populations' ephippium production correlated significantly with the water volume and the water surface of the containers (*solid lines*), but there was no significant difference in the number of ephippia produced between the two different treatments. The slopes were significantly smaller than the slope of 1 (*dashed*). Both axes are on logarithmic scale
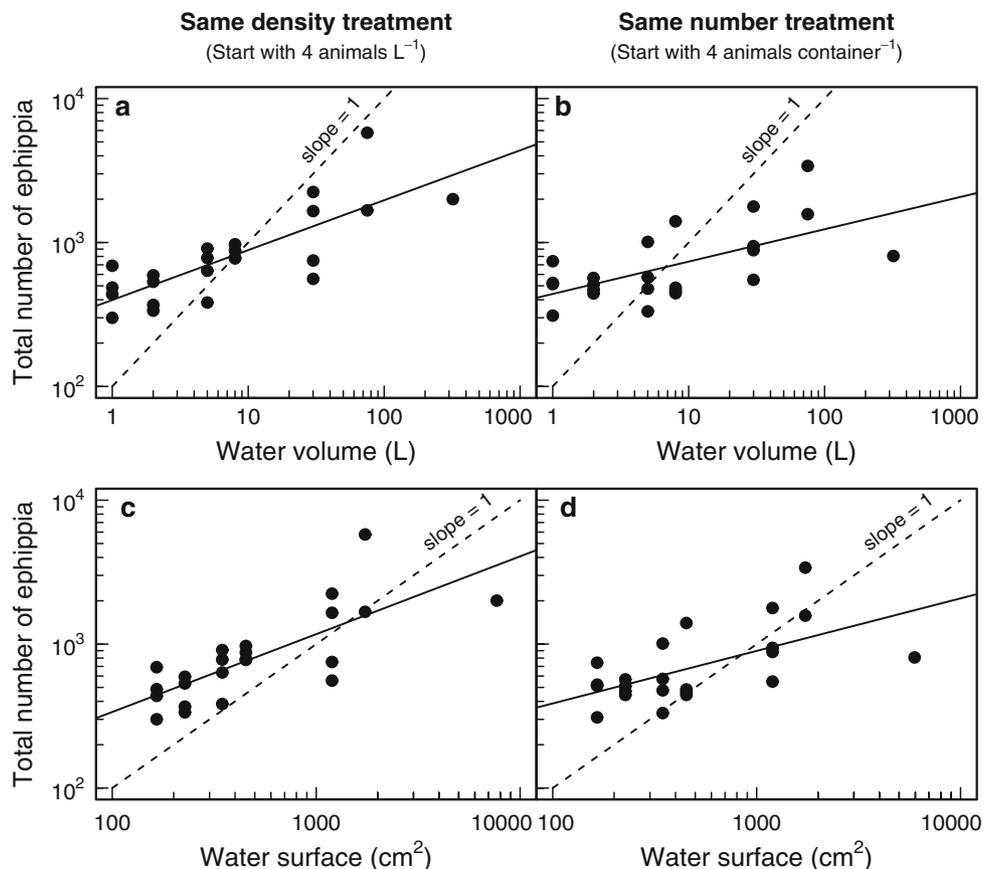
Table 2 ANCOVA for the effects of water surface of the containers (cm^2), treatment (same density or same number) and their interaction on a populations' ephippium production per season and replicate

Source of variation	df	MS	F value	P value
Surface	1	1.639	39.007	<0.0001
Treatment	1	0.042	1.005	0.32
Surface × treatment	1	0.061	1.444	0.24
Error	42	0.042		

Fig. 5 Cumulative number of ephippia produced by *D. magna* populations in the desiccation experiment (mean ± SE) at different moments in time. Ephippium production was measured after an initial pre-desiccation phase (*A*), after a desiccation phase (*B$_1$* and in parallel in the control *C$_1$*) and at the end of the season (*C$_2$*, *D*, *B$_2$*). All measurements are from independent replicates, each of which was only once destructively sampled. The *light-grey area* schematically depicts the water level change in the desiccation treatment. In the desiccation treatment there was a reduction in ephippia due to hatching of *Daphnia* few days after refilling the water (subtracted from the total, *dotted line*); in the control, the water level stayed constant. In the density reduction treatment (*D*), *Daphnia* population density was reduced to a density equivalent to the hatchling density in the desiccation treatment

Fig. 4 Comparison of the correlation between water volume of the habitat (in liters) and number of ephippia produced by *D. magna* populations in natural rock pools pooled over 2 years (*solid line*, data pooled over 2 years) and in mesocosms in containers (*dotted line*, same data used as in Figs. 2, 3). The *dashed line* depicts the 1:1 line for visualization. Both axes are on logarithmic scale

water volume were in agreement between these two independent studies. In both studies, the increase in habitat volume resulted in a much smaller increase in the populations' total ephippium production: a 1000-fold increase in habitat volume resulted only in a 2.8- to 7.2-fold increase in a population's absolute ephippium production (extrapolated data from natural pools and data from the habitat size experiment, respectively). The linear regression for the ephippium traps in natural rock pools was $\log_{10}$ (ephippia day^{-1}) = 1.57 + 0.15 × $\log_{10}$ (volume) and that for the habitat size experiment irrespective of the treatment was $\log_{10}$ (ephippia day^{-1}) = 0.62 + 0.29 × $\log_{10}$ (volume) (Fig. 4). As one may expect, the data from natural rock pools were more variable than those from the experimental mesocosms.

Desiccation experiment

The *D. magna* in the containers produced ephippia prior to (A in Fig. 5) and during desiccation (B$_1$) as well as after post-drought reestablishment (B$_2$). Only ephippia survived the drought in the desiccation treatment. At the end of the drought, there were less ephippia in the desiccation treat-
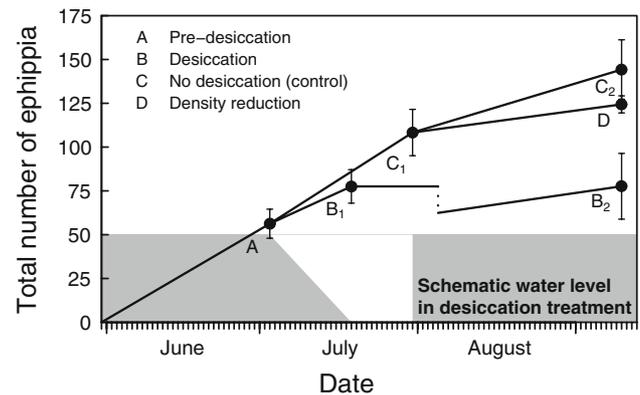
ment than in the control treatment (Fig. 5, mean number of ephippia produced per population: B$_1$ = 77.6 vs. C$_1$ = 108.3, measured just before refilling the container on 31 July; Wilcoxon rank test $W_{16,16}$ = 74.5, P = 0.046). This difference was likely to be due to the difference in the production time. The difference was no longer significant when the daily ephippium production was used (mean number of ephippia produced per day with desiccation = 1.33 and control = 1.86; Wilcoxon rank test, $W_{16,16}$ = 110, P = 0.51). Thus, the lower ephippium production in the desiccation treatment can be ascribed at least in part to the 12 days of complete drought (19–31 July), during which no ephippia were produced. The length of complete drought was arbitrarily chosen and, therefore, it is better to use ephippia produced per day rather than the total number.

Refilling the containers with water after the drought initiated hatching, and viable populations were built up within the same season in all but one replicate. The hatching synchronously occurred 5 days after refilling in all replicates, and the animals produced the first asexual eggs within 5–8 days after hatching. The number of hatchlings was positively correlated with the number of ephippia (linear regression with the intercept forced through 0, t = 7.92, df = 15, P < 0.00001; Fig. 6). The females that hatched from the ephippia reproduced asexually and produced ephippia before the end of the experiment in the autumn. Production of ephippia per day did not differ between the desiccation treatment, the density-reduction treatment and the control
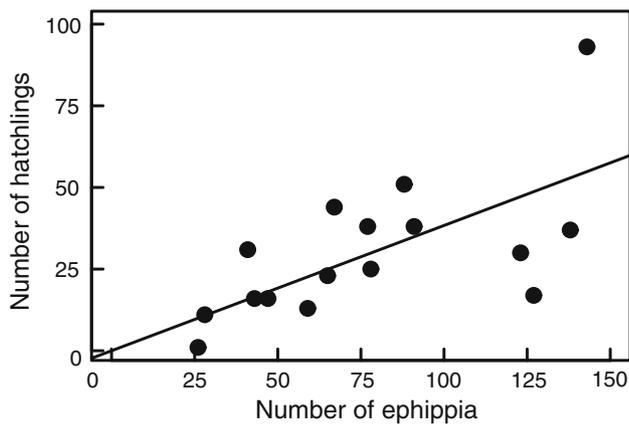
Fig. 6 Correlation between the number of ephippia and number of *D. magna* hatchlings after experimental desiccation and subsequent refilling of the containers (see desiccation treatment in Fig. 5). The linear regression line forced through the origin (slope = 0.38). As there are usually two eggs per ephippium, the estimated average hatching rate can be calculated to be about 19%

during the time when the desiccation treatment had been refilled until the end of the season (this corresponds to the slopes of the three lines in the rightmost part of Fig. 5; Kruskal–Wallis test ($\chi^2 = 4.26$, $df = 2$, $P = 0.12$). In the desiccation treatment, the absolute number of ephippia present at the end of the season was about half than in the control ($B_2 = 77.63$ vs. $C_2 = 144.25$; Wilcoxon signed rank test, $W_{16,16} = 54.5$, $P = 0.006$). Coincidently, in the desiccation treatment, the mean number of ephippia at the end of the experiment (B_2) reached about the same level as that before desiccation (B_1). Consistent with the phenology in natural pools (Fig. 1), the production of ephippia in August was much smaller than that earlier in the season and the number of ephippia produced after desiccation just substituted the "loss" due to hatching.

Discussion

We studied the production of ephippia in a natural metapopulation of *D. magna*. The number of ephippia reflects a population's contribution of potential migrants to the metapopulation, as dispersal occurs passively by wind (Maguire 1963) and possibly via waterfowl (Figuerola and Green 2002; Proctor and Malone 1965). In the metapopulation studied here, the inhabited rock pools differ in size over several orders of magnitude, and a high colonization–extinction turnover of populations has been found (Ebert et al. 2001; Pajunen and Pajunen 2003). It has been suggested that short-lived populations in small pools act as sinks that do not contribute migrants, while long-lived populations in large pools were classified as the source of migrants (Bengtsson 1989; Pajunen 1986; Pajunen and

Pajunen 2003). We were especially interested in the production of ephippium by populations in qualitatively different habitats and focused on the effects of habitat size, initial population size and summer desiccation on this process. We found that populations in small, desiccation-prone pools produced more potential migrants than previously thought and, therefore, that they may contribute substantially to the global stability of the metapopulation.

Phenology of ephippium production

Daphnia hatched in the first half of May. The *D. magna* populations then started to produce ephippia within 4 weeks (Fig. 1). They continued to produce ephippia until September, with peak production occurring during June and July. This peak occurred during the warmest month (July; data not shown), which could be due to a dependence of productivity on water temperature (Brown 1929), but it may also be a result of the typically high population densities during the summer (personal observations). The rapid initiation of ephippium production in the spring and the peak in the summer may also reflect an adaptation to avoid extinction during summer droughts. Desiccation can be rapid and unpredictable and occurs frequently in small pools in this metapopulation (personal observations). Therefore, the early and continuous production of ephippia is an essential bet-hedging trait of the present *Daphnia* populations that enables them to survive in unpredictable habitats (Cáceres and Tessier 2003; Hopper 1999). In contrast, lake *Daphnia* usually produce much fewer ephippia and usually only at distinct times, as they can survive year-round in the planktonic phase (Cáceres 1998; Cáceres and Tessier 2004). The rapid initiation and continued production of ephippia over an extended period also indicates that ephippium production is not limited to large and long-lasting "mainland" populations (Pajunen and Pajunen 2003) but that it is equal in small, desiccation-prone pools.

Influence of habitat size and initial population size on ephippium production

The fundamental difference between the Levins' type of metapopulation suggested by Hanski and Ranta (1983) and the mainland-island model suggested by Pajunen and Pajunen (2003, 2007) is the number of migrants produced by populations in different patch types, whereby patches differ both in size and risk of desiccation. We consistently found an increased ephippium production by populations in larger rock pools and larger mesocosms. However, the increase in habitat size resulted in a much smaller increase in a population's ephippium production: a 1000-fold increase in the pool volume resulted only in a 2.8- to 7.2-fold increase in the ephippium production (Fig. 4). This means that, relative

to their size, populations in small pools contributed over-proportionally to the pool of dispersal stages. The same results were found when pool surface area was used to describe the size of a habitat: a 100-fold larger surface area resulted in a 5- to 12-fold increase in ephippium production (Fig. 3c, d).

There are several possible explanations for why populations in large pool produce less ephippia than one could expect based on pool volume. In this metapopulation, many *D. magna* populations occur in small, desiccation-prone pools (Pajunen and Pajunen 2007). To survive in such an environment, it may therefore be adaptive to invest a lot of energy into the production of ephippia, especially by populations in the smallest pools. Environmental factors triggering the production of ephippia, such as shortage of food, crowding and rapid changes in temperature (Carvalho and Hughes 1983; Kleiven et al. 1992; Stross and Hill 1965), may not be buffered in small pools, and such populations are more susceptible to stochastic processes. These environmental factors may thus be the ultimate reason why populations in small pools produce many ephippia. An analogous example where populations in small habitat patches contribute most migrants in a metapopulation is given by Crone et al. (2001). They studied a vole metapopulation on skerry islands, which are small rocky, generally uninhabited islands, where the environment was unpredictable. Population densities were less stable on these small islands, and dispersal rates increased before a subpopulation's extinction. In our data from the natural metapopulation (Fig. 2), one could argue that animals from populations in small pools differ in their genetic predisposition to produce ephippia compared to animals from populations in large pools. Natural populations in small pools are often descending from recent colonizations, and colonizers may carry alleles that favor the production of dispersal stages. The case of a butterfly metapopulation is a good example: individuals of newly founded populations have a higher genetic predisposition to disperse (Haag et al. 2005; Hanski et al. 2006). However, in our experiment ,the *Daphnia* populations in the different-sized mesocosms consisted of the same genotypes (Fig. 3). Therefore, the effect was not due to a genetic difference of the populations in small versus large pools but solely due to differences in habitat size.

Larger pools usually harbor long-lived populations with potentially many hatchlings originating out of the ephippia from the preceding year (Altermatt et al. in preparation; Bengtsson 1989; Pajunen and Pajunen 2003). In contrast, populations in newly colonized or small rock pools have a higher risk of extinction (Bengtsson 1989) and a shorter time of persistence and harbor no or small ephippium banks (Pajunen and Pajunen 2007). Due to the instability of the habitat with respect to desiccation, populations in small pools may also go more often through bottlenecks. Therefore,

we expect fewer ephippia and only few hatchlings in the spring in these pools. To incorporate differences in the number of *Daphnia* at the beginning of the season on the seasonal ephippium production, the habitat size experiment was started with different initial population sizes. The same density treatment reflected the situation in long-lived populations, while the same number treatment reflected conditions in newly colonized rock pools with only few hatchlings. The initial difference in population sizes did not have an effect on the total number of ephippia produced over the whole season (Tables 1, 2; Fig. 3). In a metapopulation context, this means that populations differing in their initial population size may still be equivalent in the production of ephippia.

The observed increase in the studied populations' ephippium production with habitat size was much more shallow than expected under a mainland-island model, in which the vast majority of migrants come from a few mainland populations and hardly or no migrant comes from populations in the small island (Harrison 1991). We showed that populations in small habitats, which are usually short-lived (Pajunen and Pajunen 2007), produced substantial amounts of ephippia, implying that populations in small habitats are more important in this metapopulation than previously thought (Pajunen and Pajunen 2003). In our *D. magna* metapopulation, most populations occur in small rock pools (Altermatt et al., in preparation). Overall, these populations may even produce in total more ephippia than those in the large pools. However, these arguments need to be worked out in a more quantitative way. Furthermore, small pools are more prone to desiccation. During droughts, ephippia are more exposed to wind and birds in the dry sediments (Maguire 1963; Proctor and Malone 1965), which may result in even more dispersal from small, frequently desiccating pools than from large and permanent pools (Vanschoenwinkel et al. 2008). It should be noted that we did not measure dispersal itself, but only the production of potential migrants. It is generally difficult to measure dispersal rate and dispersal success (Hanski et al. 2000), and the measuring of migrants usually requires either individual marking or recognition of migrants. In our system, all ephippia are potential migrants. Dispersal occurs passively either by wind or birds (Maguire 1963; Proctor and Malone 1965). We therefore assume a positive correlation between the number of ephippia and the number of effective migrants.

Desiccation of the habitat

Annual extinction rates and population turnover are high in the studied *D. magna* metapopulation (Pajunen and Pajunen 2003, 2007), and the mesocosm experiments in containers showed that populations in smaller habitats have a higher

risk of extinction (Bengtsson 1989). The instability of pools has been used as a main argument to explain why populations in small pools do not contribute migrants (Pajunen and Pajunen 2003). Desiccation of the habitat is a possible instability. While pools desiccate, the *Daphnia* population density and the salinity of the water increase. As both high population density and salinity are known to trigger ephippium production (Banta and Brown 1929; Carvalho and Hughes 1983), we speculated that these factors may boost the ephippium production in our population. Contrary to our expectations, however, desiccation did not induce a higher ephippium production in our experiment (Fig. 5). The productivity (ephippia day^{-1}) was not different between the desiccation treatment and the control, although ephippium production continued in the control populations during the complete drought period in the desiccation treatment, resulting in a higher absolute ephippium production. During the second half of the season (31 July until 10 September), the rate of ephippia produced per day was smaller than that early in the season (Fig. 6). This is consistent with the seasonal pattern of ephippium production in natural pools (Fig. 1). The rate of late season ephippium production did not differ between previously desiccated and non-desiccated treatments (Fig. 5), which is consistent with the container size experiment (Fig. 3), where the ephippium production was independent of the initial population size.

Only ephippia can survive a drought. It remains unclear if and how fast a new viable population can establish after desiccated pools have been filled with water once again. In our experiments, hatchlings emerged and formed a viable planktonic population within a few days after the containers had been refilled within the same season. Thus, *D. magna* can survive a summer drought even in the absence of an ephippium bank from previous years. Winter conditions are not obligatory for hatching after the drought, which also suggests that ephippia can be produced, disperse and colonize a new habitat patch within the very same year. Taken together, a drought seems not to boost ephippium production, but it could give the *Daphnia* a dispersal advantage as the ephippia become exposed on the dry sediments of the pool. While desiccation does not place populations in small pools at an immediate risk of extinction, it does reduce the number of end-of-season ephippia, which may reduce the likelihood of the population surviving the next winter. This may contribute to the observed high extinction rates of populations in small pools (Pajunen and Pajunen 2007).

Conclusions

We found that populations of the studied *D. magna* metapopulation that reside in small habitat patches are able to produce substantial numbers of ephippia (dispersal stage). The instability associated with these smaller patches does

not compromise their ability to produce migrants, and it would even be an evolutionary stable strategy of animals to produce more migrants in an unpredictable environment. Thus, when focusing on the production of dispersal stages, our data do not support the hypothesis of Pajunen and Pajunen (2007) who suggested that a few long-lasting populations in large pools are the key populations in this metapopulation. As droughts, which mainly affect small pools, may increase the exposure of ephippia to passive dispersal, it may even be the short-lived populations in small pools that contribute most effectively to the migrant population. If this were to be the case, it is the locally instable part of the metapopulation that contributes most to the global stability of the metapopulation. Small and instable patches may also be more important in other systems for the functioning of metapopulations than previously thought (Crone et al. 2001). Our results may change predictions about the future development of metapopulations. For example, when a protection scheme in conservation biology can only be applied to a subset of populations, the key populations with respect to the origin of migrants must be known to guarantee an ongoing of metapopulation processes.

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