The power of numbers: dynamics of hatching and dormant egg production in two populations of the water flea *Daphnia magna*

Aurora N. Geerts · Kelle Moreau · Bram Vanschoenwinkel · Joost Vanoverbeke · Luc Brendonck · Luc De Meester

A.N. Geerts (✉) · J. Vanoverbeke · L. Brendonck · L. De Meester

KU Leuven – University of Leuven. Laboratory of Aquatic Ecology, Evolution and Conservation. Charles Deberiotstraat 32 - box 2439. 3000 Leuven, Belgium.

e-mail: aurora_geerts@yahoo.com

telephone: +32 16323948 fax: +32 16 3 24575

K. Moreau

Management Unit of the North Sea Mathematical Models (Directorate Natural Environment), KBIN, Vautierstraat 29, 1000 Brussels, Belgium.

B. Vanschoenwinkel

Biology Department, Faculty of Science and Bioengineering Sciences. Vrije Universiteit Brussel. Pleinlaan 2, 1050 Brussels, Belgium.

Acknowledgements:

AG was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT Vlaanderen) during this study. This study was partly supported by the KU Leuven Research Fund projects PF/2010/007 and C16/2017/02. We thank the three anonymous reviewers whose comments helped improve and clarify this manuscript.

Abstract

Many organisms that live in inland standing waters produce dormant life stages that can accumulate in propagule banks to survive temporarily unfavourable periods. These egg banks have important effects on the ecology of populations and communities in terms of phenology, population densities, the probability of extinction, species diversity and habitat connectivity in time and space. They also have important consequences for the evolutionary versatility of populations. Although diapause and dormant egg banks in freshwater zooplankton have been studied for several decades, little is known about the quantitative contribution of egg production and hatching to yearly egg bank budgets and their seasonality in natural ponds and lakes. Here we quantified inter- and intra-annual variation in hatching and dormant egg production in the water flea *Daphnia magna* in two natural shallow ponds in Flanders (Belgium) using high intensity sampling and *in situ* measurements. Hatching started in spring and occurred in several bouts (April-July), accumulating to a yearly average total of 3.5 $x10^3$ hatchlings m^{-2}. Dormant egg production occurred in one to three bouts mainly during late spring and summer (May-August), resulting in a total yearly production ranging from 1.2 $x10^4$ up to 17.3 $x10^4$ ephippia m^{-2}. In both years there was an average surplus of 3.14 $x10^4$ and 15.24 $x10^4$ ephippia produced m^{-2} for ponds OM2 and OM3, respectively, contributing to the accumulation of the persistent egg bank. We discuss the ecological and evolutionary consequences of both the high number of ephippia that are produced and the high number of hatchlings at the start of each growing season.

Keywords: Dormant egg-bank dynamics, propagule-bank, dormancy, zooplankton, seasonality, *Daphnia*

Introduction

To survive periods of unsuitable conditions many organisms have developed resistant life stages such as seeds, dormant eggs or spores (Harper 1977; Wiggins et al. 1980). Dormancy -a metabolic resting phase- allows species to increase their generation time: from decades to even centuries (Hairston 1996). The propagule bank can be an effective refuge against unfavourable conditions and thus facilitates population persistence (Hairston 1996), but also contributes to the genetic resilience of populations (Latta et al. 2010) and to the persistence of species and genes at the metapopulation level through facilitating dispersal (Gyllström and Hansson 2004). The presence of a dormant reservoir of genotypes and species in the sediment has important consequences for population and community dynamics (Brendonck and De Meester 2003; Gyllström and Hansson 2004; Orsini et al. 2016). It influences species and genotype diversity through the storage effect (Chesson 1994; Chesson and Warner 1981) and impacts ecological dynamics not only in terms of reducing extinction rates of local populations but also by initiating a new population and augmenting population sizes at the start of the growing season (Bozelli et al. 2008). A mixed egg bank with delayed hatching on the one hand potentially increases rates of evolution through an increased evolutionary potential but can, on the other hand, slow down evolution in response to directional selection (Hairston and De Stasio Jr 1988). Banks of resistant and dispersal prone propagules can also increase connectivity at the metapopulation and metacommunity level by increasing dispersal rates (Figuerola and Green 2002; Vanschoenwinkel et al. 2007; Waterkeyn et al. 2010).

In zooplankton populations inhabiting lakes and ponds, the dormant stages collect in the, often layered, sediment and form a dormant propagule bank (Brendonck and De Meester 2003). The size of propagule banks in lakes and ponds at a certain point in time is thought to be determined by a number of processes that together also determine the budget of the propagule bank. These include the amount of dormant eggs produced, the proportion of these that hatch in the subsequent and later seasons, and their mortality due to predation, infection or senescence (Gyllström and Hansson 2004). Most studies suggest the amount of dormant propagules in ponds and lakes can be very high, from thousands to millions per square meter (Brendonck and Riddoch 2000; Cousyn and De Meester 1998; Hairston 1996). Generally

it is expected that the amount of dormant eggs in the sediment reflects the population size during previous' reproduction periods (De Stasio 1989; Verschuren and Marnell 1997).

How do egg banks build up in the sediments of lakes and ponds? The number of dormant eggs that are transported among habitats, such as separate lakes, tends to be low compared to the number of resident individuals in an established population (Badosa et al. 2017; Green et al. 2005; Hulsmans et al. 2007; Louette et al. 2007; Vanschoenwinkel et al. 2008). Local propagule production is thus by far the dominant process that determines whether seasonal losses due to hatching and mortality are compensated (Gyllström and Hansson 2004). Little is known, however, about the phenology of hatching and dormant egg production of *Daphnia* under field conditions, likely because it is a laborious endeavour to collect such data. Relevant data should also be collected in the field, as it has been shown that there can be large differences between laboratory and field measurements of hatching rates (Cáceres and Schwalbach 2001). Thus far field estimates of egg production are only available for a limited number of species (Montero-Pau et al. 2017). A well-known example is the freshwater copepod *Diaptomus sanguineus*, where a net accumulation of dormant eggs in the sediment was found over a three year period in Bullhead Pond, a permanent shallow body of water (De Stasio 1989; De Stasio 1990). In Oneida Lake, a large shallow lake in NY, Cáceres (1998) quantified both hatching and dormant egg production of *Daphnia galeata mendotea* and *Daphnia pulicaria* over a three year period (1992-1995). She documented strong seasonality in the hatching and dormant egg production processes, showing that dormancy played a key role for the long-term persistence of the daphnid population in Oneida Lake. Similarly, Gyllström (2004) measured hatching and dormant egg production of *Ceriodaphnia*, *Daphnia* and *Bosmina* for 16 months in Lake Krankesjön, a shallow lake in southern Sweden. These studies used inverted funnel traps to quantify hatching, and zooplankton nets (75um) and sediment core samples (Cáceres 1998; De Stasio 1989) to quantify dormant egg production.

We studied the dynamics of hatching and egg production of *Daphnia magna*, a large-bodied cladoceran that holds a central position in aquatic food webs as the main grazer of unicellular algae and the preferred prey of fish and macro-invertebrate predators (Miner et al. 2012). *D. magna* has a cyclical parthenogenetic life cycle (De Meester et al. 2004; Hebert 1987). In general, this entails that at the start

of the growing season, which is early spring in the study region, female *D. magna* individuals hatch from dormant eggs. As these eggs have been produced sexually, this results in a genetically diverse population. These individuals give rise to clonal lineages that reproduce asexually as long as conditions are favourable. Sexual reproduction is induced when conditions start to deteriorate: dormant eggs are produced that are encapsulated by a protective membrane called an ephippium (Mitchell et al. 1998).

In this study we aimed to reconstruct the pattern of hatching, population densities and dormant egg production of *D. magna*. To this end, we engaged in a detailed sampling of two shallow ponds over a period of three years. We tested three hypotheses: (1) that hatching is characterized by a pronounced hatching peak in early spring, perhaps followed by several smaller hatching peaks later in the season (as observed by De Stasio 1989; De Stasio and Hairston 1992); (2) that dormant egg production is high just after the population peak in summer as resources are depleted (Alekseev and Lampert 2001; Gyllström 2004) as well as in autumn in response to cues indicating the onset of the unfavourable winter conditions (Gyllström and Hansson 2004); and (3) that the egg budget shows a positive balance that would lead to a gradual accumulation of dormant eggs in the mixed egg bank.

Material & Methods

- Study sites

Both study systems (OM2 and OM3) are small (OM2: 2.53 ha; OM3: 1.84 ha) shallow (average depth 0.71 m) ponds located in Abdij van 't Park (Heverlee, Belgium; 50°51′48″N, 4°43′16″E). They are part of a group of four neighbouring eutrophic ponds that were created in the 18th century for fish production, which continued until 1993. Overall, the ponds have a simple morphometry, with littoral zones that are partly covered by reed and quite rapidly reach a depth of 0.5 to 1 m. At the time of the sampling, a fish community was still present, consisting mainly of carp (*Cyprinus carpio*), perch (*Perca fluviatilis*), Prussian carp (*Carassius gibelio*), and tench (*Tinca tinca*) (Decaestecker et al. 2005). The *D. magna* populations and dormant egg banks in these ponds have been used in earlier studies, including a resurrection ecology study (Decaestecker et al. 2007) and population genetic analyses (Vanoverbeke et al. 2007; Vanoverbeke and De Meester 2009). From this background information, we know that the ponds have a well-developed dormant egg bank and that there are yearly hatching bouts replenishing genetic diversity at the start of the growing season (De Meester et al. 2006). These studies have also shown that individuals may survive during mild winters and that populations can remain active in the water column for prolonged periods of time, from several months to years (Vanoverbeke and De Meester 2009).

- Quantifying hatching

We quantified hatching throughout the year in both 2002 and 2003. Starting in February, the ponds were monitored daily for hatchlings using a Schindler-Patalas zooplankton trap of 12 L. Each day, four 12-L samples were taken at eight sampling points at random locations in the pond (total: 384L). Starting late February, eight sediment cores of each 21.24 cm^2 (total surface area: 169.92 cm^2, total volume: 339.8 cm^3) were taken every week at different, randomly chosen locations per pond but avoiding sites that had been sampled before to avoid disturbance. Sediment sampling was intensified to once every four days after first hatching was observed. The total volume of mud sampled for each pond was 6,117.1 cm^3 in 2002 and 10,195.2 cm^3 in 2003. To quantify hatching as it would occur in the pond, the top 2 cm of each

core sample was mixed and exposed in the laboratory to optimal hatching conditions of 20°C and a light regime of 14:10 L:D. Given that resting egg development time at 20°C is 2.69 days (Bottrell et al. 1976), all animals that hatched within 48 hours after isolation were considered to have also been ready to hatch in the field. Hatching for each time period was calculated based on the sum of the hatchlings for all eight sediment cores. Average hatching per 24h was calculated by summing the number of hatchling over the two days and then dividing by two. In this way, every four days we obtained an estimate of the number of animals that would have hatched during a period of 24 h in the field from a surface area of 169.92 cm^2. By multiplying the resulting number by 58.89 we calculated the amount of hatchlings per m^2 of surface sediment per 24h (Fig.1). Calculations for total hatchlings (per pond and per day) and for hatchlings per litre (per pond and per day) were calculated in the following way:

$$Total\ hatchlings = \left(\frac{hatchlings}{m^2}\right) \times pond\ area\ (m^2)$$

$$Hatchlings\ per\ litre = \frac{Total\ hatchlings}{pond\ volume\ (litre)}$$

To explore the relationship between pond temperature and hatching, we obtained average daily temperature over the study period from the Uccle weather station of the Royal Meteorological Institute of Belgium (KMI).

- Quantifying ephippial production

During three consecutive years (2001, 2002 and 2003), we sampled the active zooplankton community of both ponds weekly from February to October using a 12-L Schindler-Patalas trap at each of eight different locations, i.e. 96 L per week in each pond. These samples were filtered over 250 μm and visually screened for the presence of ephippia-bearing *Daphnia* and subsequently preserved in EtOH for population density counts of *Daphnia magna*. Whenever ephippial females were discovered, a 384-L sample was taken from each pond (4 x 12 L Schindler-Patalas samples at each of eight sampling points) each week. The animals caught per pond were kept in a container and after 24 h the number of

deposited ephippia was counted. As ephippia take 2-3 days to develop, all ephippia deposited during the first 24 h after isolation were considered to be the result of natural induction in the pond. This method allowed us to quantify ephippia production at weekly intervals during the growth season, with the number of ephippia produced during a 24 h period every week. At three separate moments during the sampling seasons of 2001 and 2002, 100 newly produced ephippia were decapsulated to check for empty ephippia or damaged dormant eggs. On average the percentage of damaged resting eggs or empty ephippia was 15% for OM2 and 23.5% for OM3. This percentage was used to correct the collected data: the number of ephippia produced was multiplied by the percentage of viable ephippia. The numbers of ephippia production presented here always refer to the corrected numbers. Ephippia production was next quantified as ephippia per m^2 (the total amount of ephippia were calculated for the total volume and divided by the area of each pond). Both monitoring of hatching and ephippia production were stopped when in late summer or autumn the populations disappeared from the water column.

- Dormant egg budget calculation

Assuming that all eggs that hatched were deposited in the preceding season, we can calculate the percentage of eggs produced in a given season (2001 and 2002) that hatched in the subsequent growing seasons (2002 and 2003, respectively). In this way we also calculated an approximated dormant egg budget for the two studied growth seasons. In the overall balance, we neglected unmeasured factors such as egg mortality in the egg bank. Our egg budget calculations are based on detailed data on both the number of hatchlings and ephippia production, but need to be interpreted by taking into account that we did not quantify egg mortality in the sediments. For our calculations we assumed a 1:1 relation between ephippia and dormant eggs. This is a conservative assumption since we know that *D. magna* usually deposits two dormant eggs in one ephippium. Using the measured production and hatching data, interpolations were done in order to have data points for each calendar date.

Results

- Hatching

In 2002 a first, quite pronounced hatching peak of 118 hatchlings m^{-2} per 24 h (reflecting a total of 2 hatchlings per day when summed over eight sediment cores) was observed in the first half of April for OM2. During the second half of April a peak of 88 hatchlings m^{-2} (i.e. reflecting a total of 1.5 hatchlings per day over eight sediment cores) was observed for OM3. The second and third hatching peaks occurred simultaneously in May and July (Fig. 1A). The peak hatching rate observed was 206 hatchlings m^{-2} per 24h (i.e. reflecting a total of 3.5 hatchlings per day over eight sediment cores) in OM2 and 177 hatchlings m^{-2} per 24h (i.e. based on average hatching *per 24h* of 3 hatchlings over eight sediment cores) in OM3. The total amount of hatchlings during April, May and July were 1266, 1118, and 1595 hatchlings m^{-2}, respectively, in OM2 and 442, 1680, and 880 hatchlings m^{-2}, respectively, in OM3.

In 2003, hatching started earlier, with a small hatching burst at the end of March and a pronounced peak in both ponds in April, with a maximum number of hatchlings of 265 m^{-2} per day (i.e. 4.5 hatchlings on a total of eight sediment cores) in OM2 and 177 m^{-2} per day (i.e. 3 hatchlings on a total of eight sediment cores) for OM3 (Fig. 1B). For OM3 this hatching bout lasted almost the entire month of April. In the beginning of May both ponds showed simultaneous hatching peaks, but these were smaller compared with the early spring peak. The total amount of hatchlings during March, April, May, and June were 74, 2186, 524, and 132 hatchlings m^{-2}, respectively, in OM2 and 191, 2657, 213, and 795 hatchlings m^{-2}, respectively, in OM3.

Based on the raw data, we counted an average of 11.75 hatchlings per year and per pond hatching from the sediment samples, i.e. a total of 47 hatchlings over all sediment samples in the study. Per unit of volume water in the ponds, we estimated that in 2002 a total of 1.2 animals hatched per litre water in OM2 and a total of 0.6 animals hatched per litre water in OM3 (assuming an average depth of 0.55 m for OM2 and 0.87 m for OM3). In 2003, a total of 1.3 and 1.0 animals hatched per litre water of OM2 and OM3, respectively.

In figure S1 we plotted the hatching peaks per year (hatching m^{-2}) for each pond and the corresponding ambient air temperatures (see Supplementary materials). There was no relationship between spring temperatures and hatching rates. It is important to note that we found active populations in both ponds and all studied years at the start of every sampling season (March). This means we missed overwintering populations and possibly early hatching events.

- Ephippia production

At the end of May 2001 both study ponds had a peak production of ephippia (3835 ephippia m^{-2} for OM2, 1544 ephippia m^{-2} for OM3; see Fig. 2A). OM2 also had relatively smaller ephippia production peaks in mid-July (855 ephippia m^{-2}) and mid to end of August (30 ephippia m^{-2}), while OM3 had a small production peak at the end of June (309 ephippia m^{-2}). The raw data for 2001 reveal that we counted a total of 5,388 and 987 ephippia in our samples from OM2 and OM3, respectively. In 2002 both ponds had a peak ephippia production in mid-June. The *D. magna* population of OM3 showed a peak production of over 11 000 ephippia m^{-2} per day, while for OM2 this was less than 1000 ephippia m^{-2} (Fig. 2B). The latter pond, however, showed a second peak of ephippia production in mid-July, reaching 1975 ephippia m^{-2} per day. Based on the raw data for 2002, a total number of 3,371 and 5,752 ephippia were counted for OM2 and OM3, respectively. In 2003, in both OM3 and OM2, an ephippia production peak occurred during mid-June, with a production of up to 20 000 ephippia m^{-2} per day in OM3 (Fig. 2C). There was no peak in ephippium production associated with the end of the growth season. Based on the raw data for 2003, a total number of 2,221 and 10,118 ephippia were counted for OM2 and OM3, respectively. In 2001 and 2002 the active population gradually disappeared during the month of August, but without a peak of dormant egg production. Rather, ephippium production peaked earlier in the season. The total amount of ephippia produced per pond and per year ranged from 6.3 x 10^8 to 10.6 x 10^8 for OM2 and from 2.1 x 10^8 to 32.0 x 10^8 for OM3 (see table 1).

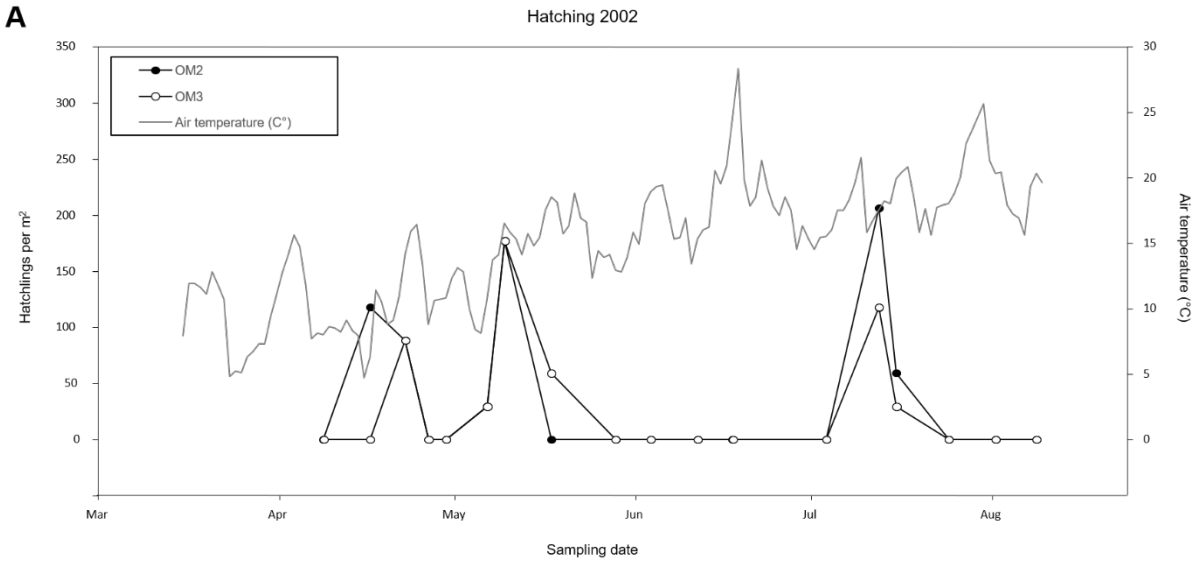
- Hatching fractions and egg budget

Assuming that all eggs that hatched were deposited in the preceding season, we can calculate the percentage of eggs produced in a given season (2001 and 2002) that hatched in the subsequent growing

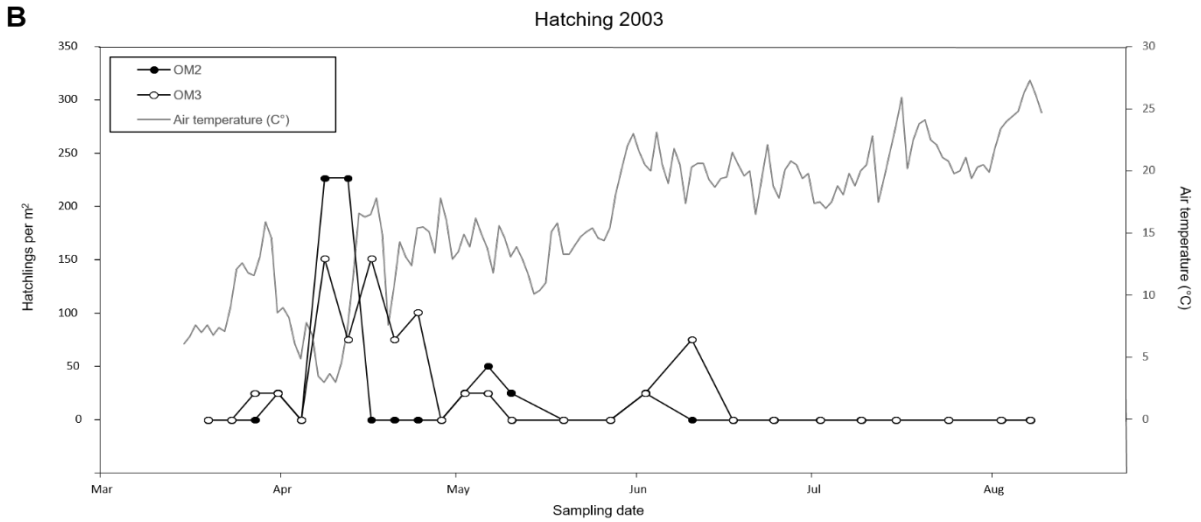
237 season (2002 and 2003, respectively). Reconstructed hatching percentages in 2002 and 2003 were
238 comparable for both ponds and averaged 11.7% (Table 1).

239 In the spring-summer growth season of 2002, the total reconstructed dormant egg budget provides an
240 assessment of the degree to which the dormant egg bank of the local populations increased in size
241 (assuming on average only one dormant egg per ephippium). For 2001-2002, OM2 showed a positive
242 balance of 4.1 $x10^4$ dormant eggs m^{-2} while this was 13.5 $x10^4$ for OM3; for 2002-2003 these numbers
243 were 2.2 $x10^4$ and 17.0 $x10^4$, respectively. Given the surface area of the ponds, this translates into a large
244 number of dormant eggs that were added to the ponds: 10.3 $x10^8$ and 24.9 $x10^8$ for OM2 and OM3,
245 respectively, in 2001-2002 and 5.6 $x10^8$ and 31.2 $x10^8$ for OM2 and OM3, respectively, in 2002-2003.

246



247

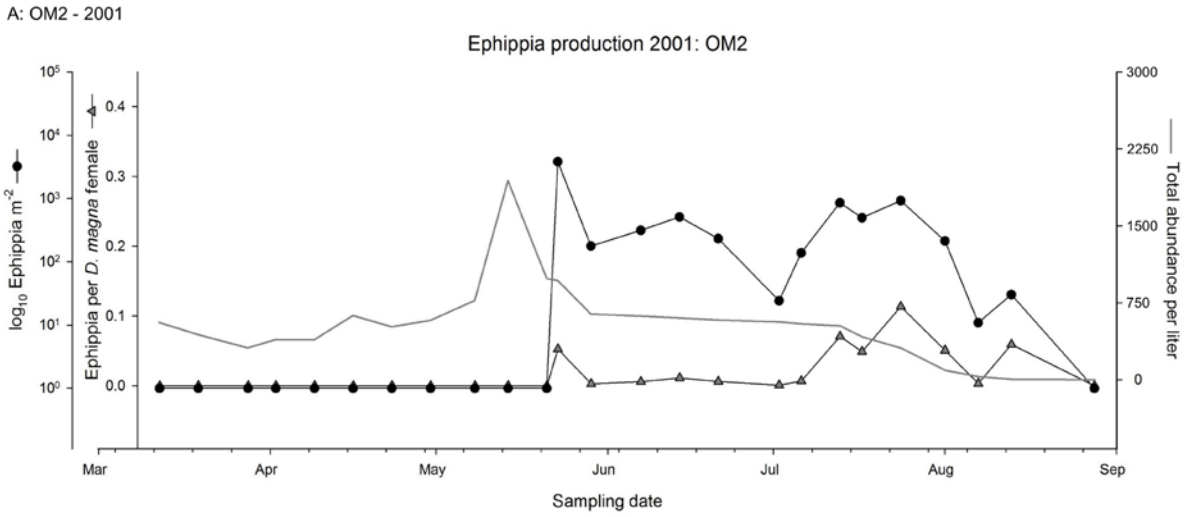


248

249 **Figure 1**. Number of hatchlings m^{-2} in OM2 (filled circles, full line) and OM3 (open circles, full line)

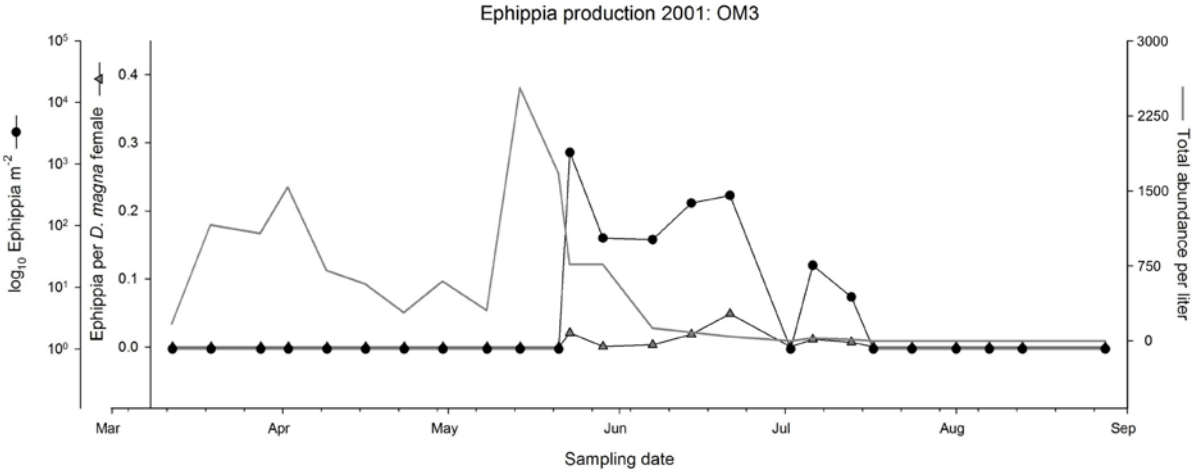
250 plotted together with ambient temperatures (grey line) for A. 2002 and B. 2003.

251



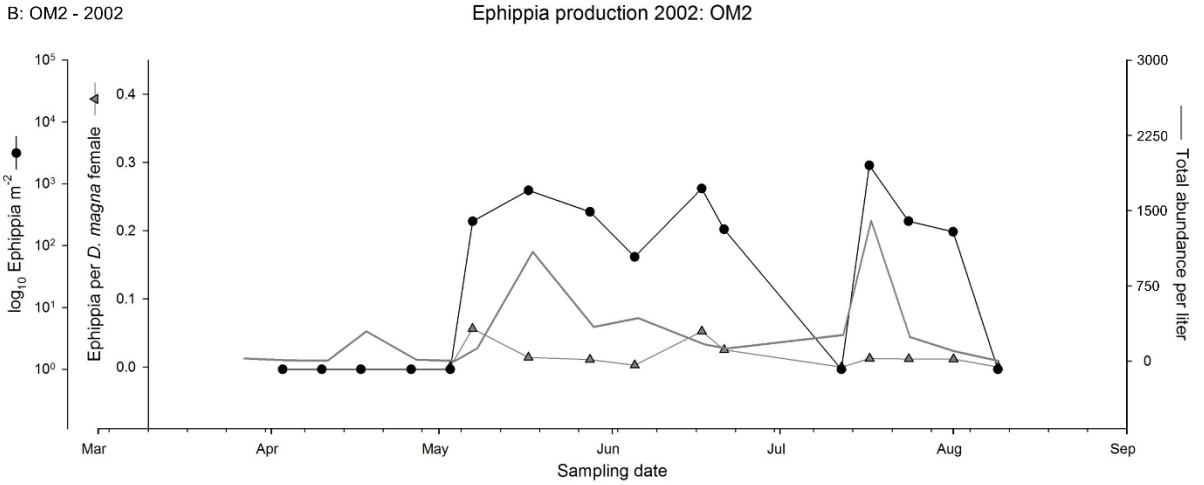
252

A: OM3 - 2001



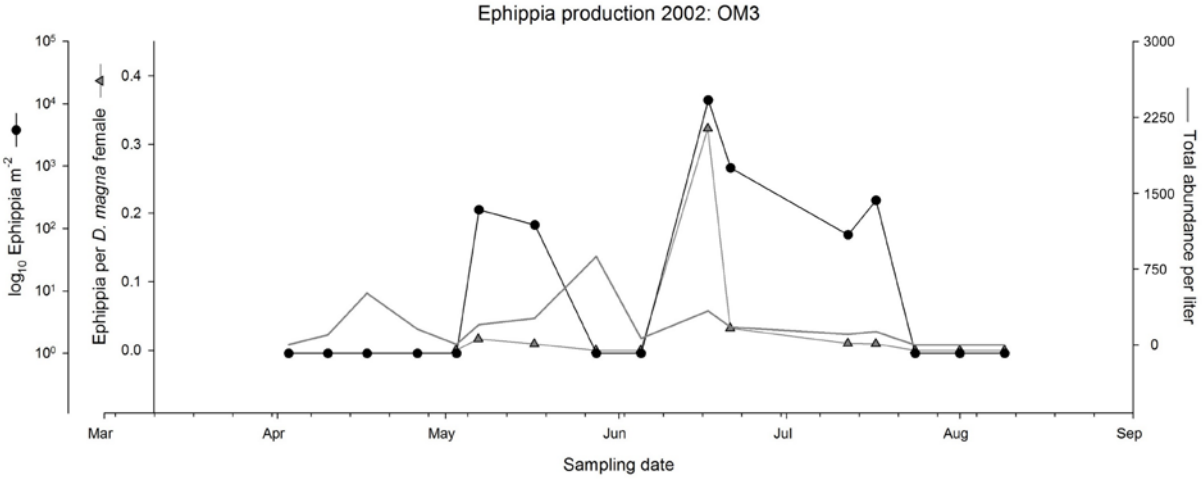
253

B: OM2 - 2002

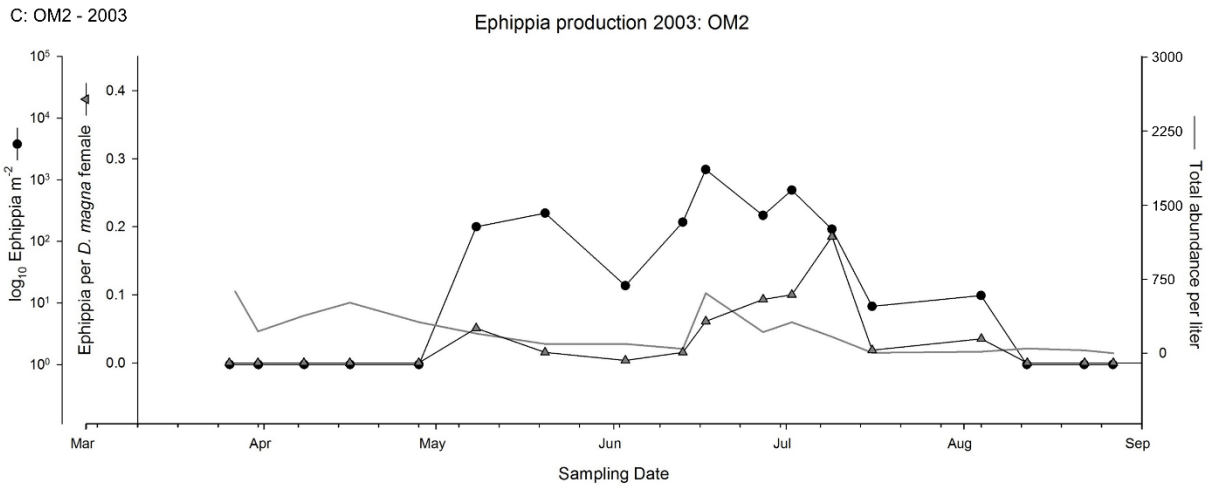


254

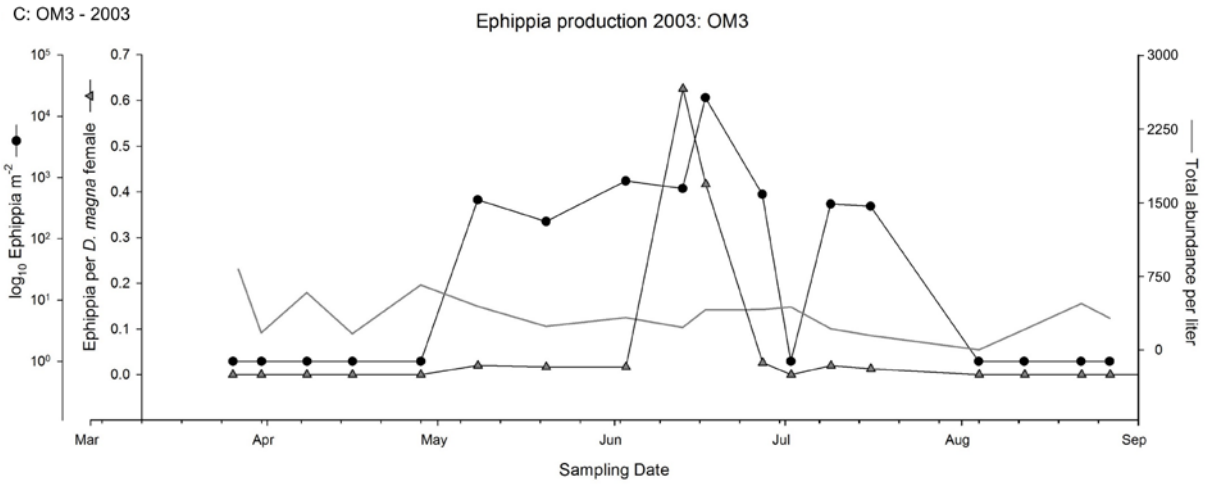
B: OM3 - 2002



255



256



257

258

259 **Figure 2**. Log_{10} of ephippia production m^{-2} plotted per year (A. 2001, B. 2002, and C. 2003) and per
260 pond (OM2 and OM3). Ephippia production (black circle, full line) plotted against amount of ephippia
261 per female (gray triangle, full gray line) and total abundance of *D. magna* per liter (thick gray line).
262 Note that due to a high percentage of ephippia per females in OM3 in 2003, the range of the y-axis
263 (Ephippia per *D. magna* female) is different in this plot (C: OM3 2003).

Discussion (1300 words)

We quantified ephippia production, hatching and their phenology in two natural *D. magna* populations during two (three for ephippia production) consecutive years. We discovered large numbers of hatchlings and a very high number of ephippia produced in these populations from moderately sized habitats. Both egg production and hatching showed marked seasonality. The surplus of dormant eggs being produced compared to the number of hatchlings resulted in the production of a persistent dormant egg bank.

- Hatching rate

We used a reliable method to quantify hatching of induced eggs in the laboratory as it would occur in the field for two ponds and two consecutive years, and collected detailed data for the whole period starting late winter until late summer, when the active populations disappeared. While earlier studies used hatching traps to monitor seasonal patterns in hatching, this method did not work properly in the shallow ponds we studied, as the hatchlings did not immediately swim upwards but rather built up populations in the traps (KM, pers. obs.). As the method we used reliably quantifies hatchlings whose development was induced in the field, we could assess the amount of animals that would hatch on a given day per unit surface area. Our approach might, however, have resulted in an overestimation of daily hatching rates especially in early spring. This is because we incubated the eggs at 20°C. Whereas part of the development of induced eggs will have taken place in the field at ambient temperatures, final development until hatching took place at an accelerated rate in the lab. Assuming that on average half of the development of the eggs took place in the field and half in the lab and taking into account the typical development time of *Daphnia* resting eggs (see Bottrell 1976; Vanoverbeke and De Meester 2009), this overestimation is probably marginal for ambient temperatures above 15°C. For ambient temperatures around 10°C or lower (April 2002 and 2003), hatching rates may have been increased considerably. We note, however, that in all cases all eggs that we observe to hatch were initiated in the field and would have hatched anyway. As such, our assessment of hatching peaks in terms of the amount

289 of eggs that hatched in total based on one set of sediment cores, is correct. Yet, our estimates of daily
290 hatching rates might be overestimated for early spring samples.

291 Each sampling day we quantified hatching for a total surface area of 169.92 cm^{-2}, and summing the
292 surface area of all cores from a given year, we sampled on average 0.31 m^2 in 2002 and 0.51 m^2 in 2003.
293 Extrapolating our results to the total surface area of each pond resulted in a very high total number of
294 hatchlings in the ponds, ranging from 5.5 $x10^7$ up to 10.8 $x10^7$ depending on the pond and year (see
295 table 1). While the error on these estimates is obviously high given that we need to extrapolate over a
296 much larger surface area than sampled, our results at least indicate that, in total, the number of hatchlings
297 that initiate a population is very high. Even if we consider only the individuals that hatch during the first
298 month of the growing season, the numbers range from 1.9 $x10^6$ to 3.2 $x10^7$ (total hatchlings per pond).
299 This very high number has important consequences. First, given that all dormant eggs are the product
300 of sexual recombination, all hatchlings are genetically unique. This implies that clonal variation at the
301 start of the growing season, even if only the first month of hatching is taken into consideration, is very
302 high. This clonal variation provides the raw material for clonal selection to act upon (Pfrender and Lynch
303 2000; Vanoverbeke and De Meester 2010). Even if clonal erosion is severe (Pfrender and Lynch 2000;
304 Vanoverbeke and De Meester 2010), it is likely that there will be hundreds or thousands of clones
305 remaining at the end of the season. So while clonal erosion is very strong and clonal structure of the
306 population is high, with many clones represented by thousands or even millions of individuals, it is
307 unlikely that clonal diversity in such habitats would be reduced to the extent that only one or a few
308 clones would remain. Moreover, while clonal selection is very effective as across generations both
309 additive and interactive gene effects are inherited (Lynch and Walsh 1998), it acts on the expressed
310 phenotype, and to the extent that the same phenotypes can be coded by different gene combinations, this
311 leads to the expression of “hidden” genetic variation upon recombination (Lynch 1983). This can be the
312 case when different pathways or combinations of genes lead to the same phenotype, or when the clones
313 that are selected for are heterozygous for specific loci. Several studies have reported an increase in
314 heterozygosity during the course of a growth season or following a selection experiment (Hochmuth et
315 al. 2015), suggesting that clonal selection may even favour heterozygotes, leading to the release of

316 genetic variation upon subsequent recombination. The high number of hatchlings at the start of the
317 growth season in effect then results in a very broad sampling of the evolutionary potential offered by
318 the allelic diversity present in the population (Orsini et al. 2016). The huge clonal diversity at the
319 beginning of the growth season thus likely has important consequences for evolutionary potential,
320 population genetic structure, genetic drift and among-population genetic differentiation. In addition, the
321 high number of hatchlings also has ecological consequences, as it results in a relatively high starting
322 population, thus buffering for chance extinctions and reducing the length of the period where one would
323 expect exponential growth in the absence of competition.

324 Hatching rates as quantified in the present study (on average 39.0 individuals m^{-2} day^{-1} for OM2 and
325 38.4 m^{-2} day^{-1} for OM3 across the growth season) were much higher than those measured in most other
326 studies that monitored hatching in detail in the field. In the studies by Wolf & Carvalho (1989), Hairston
327 et al. (2000) and Rother et al. (2010), the number of hatchlings ranged from 0.1 to 1 individuals m^{-2} day^{-1}. Similarly, Cáceres (1998) reported emergence rates for *D. pulicaria* of 0.1 to 0.75 individuals m^{-2} day^{-1}. The low hatching rates observed in some of these studies may be related to the fact that most of them involved relatively deep lakes that may provide a permanent habitat for the studied zooplankton species, so that populations invest less in the production of dormant eggs (Gilbert 2017). Moreover, the ponds studied by us are very productive and harbour a dense *Daphnia* population (often >50 and sometimes >200 individuals L^{-1}; see figure 2). De Stasio (1989) observed hatching rates for *Diaptomus sanguineus* up to 200 individuals m^{-2} day^{-1} in Bullhead Pond (NY). This population is eliminated each year between July and October due to intense sunfish predation. Another example of a population that relies heavily on emergence to start up in the spring is *Epischura nordenskioldi* in the same pond, which shows hatching rates of 20 individuals m^{-2} day^{-1} (De Stasio 1990).

338 - Hatching phenology

339 While hatching rates at the start of the growth season were high, hatching was not strongly synchronized
340 and occurred, more than expected, in hatching bouts spread over the growth season. In 2002, for
341 example, there were three approximately equally important hatching bouts in spring and summer, while

342 in 2003 the hatching peak early in the growth season was the most pronounced, and the three hatching
343 peaks occurred earlier in the season than in 2002. It is striking from figure 1 that the two ponds show
344 similar seasonality in hatching responses within a given year. This synchronicity among ponds suggests
345 that hatching might amongst others be triggered by weather or other environmental conditions (e.g. food
346 levels). The observation of several hatching peaks is in line with results on the patterns of genetic
347 diversity observed in these populations. Multilocus genotype diversity of both OM2 and OM3 has been
348 reported by De Meester et al. (2006; their figure 5) and their results based on a survey carried out in
349 1996-1997 suggests that clonal diversity in these populations increased at different instances during the
350 period from May to July. In addition, our observations are also consistent with the results of a hatching
351 experiment starting from dormant eggs sampled in OM2, where high hatching rates were observed under
352 both early spring and summer (Vanoverbeke and De Meester 2009).

353 Hatching early in the season has important advantages as it gives a head start during the exponential
354 growth phase (Vanoverbeke and De Meester 2009). In addition, hatchlings from dormant eggs differ in
355 life history traits from offspring that hatch from parthenogenetic eggs, as they are better adapted to the
356 high food conditions that prevail during the spring algal bloom (Arbačiauskas and Lampert 2003). On
357 the other hand, there are also advantages to a more delayed hatching response that also allows hatching
358 to occur later in the season. First, uncertainties on what is the optimal moment for early hatching, e.g.
359 in the case of erratic fluctuations of the weather, may select for bet-hedging or the coexistence of
360 alternative strategies (De Stasio and Hairston 1992; Vanoverbeke and De Meester 2009). Second, if
361 sedimentation rates are relatively high (which is the case in the OM ponds, with 1-2 cm per year Cousyn
362 and De Meester 1998), then the reduced probability of being exposed to hatching conditions may select
363 for a more generalistic response to hatching stimuli.

364 - Ephippia production

365 Ephippia production of the *D. magna* populations in the two study ponds was very high, with the total
366 number of ephippia produced yearly ranging from 1.2 $x10^4$ to more than 17.3 $x10^4$ m^{-2}. As each
367 ephippium can harbour two eggs, the total number of dormant eggs produced yearly can mount up to

34.6 x10^4 m^{-2}. Total dormant egg production in the copepod *Diaptomus sanguineus* in Bullhead Pond was similarly high, with total numbers varying between 8 x10^4 and 4 x10^5 individuals m^{-2} per year (De Stasio 1989). Cáceres (1998) found lower numbers in her study of dormant egg production by *Daphnia* species in Lake Oneida, with yearly totals ranging from 2.2 to 13.7 x10^3 diapausing eggs m^{-2} for *D. pulicaria* and from 0 to 1.2 x10^3 diapausing eggs m^{-2} for *D. galeata mendotae*.

- Phenology of ephippium production

The phenology of ephippia production is striking for both ponds, with the major peak at the end of May in 2001, in June and July in 2002, and in June in 2003. The peak in ephippium production in 2001 followed a population density peak (see figure 2.A) and is therefore consistent with the expectation of sexual reproduction of dormant eggs induced by crowding (Hobæk and Larsson 1990; Kleiven et al. 1992). This observation also corresponds with Alekseev and Lampert (2001) who revealed an association of ephippium production with maternal effects; *Daphnia* females switch to sexual reproduction if they are less well fed than their mothers. This in practice would often result in ephippium production after population density peaks, and ensures proper timing of ephippium at the onset of less optimal conditions and at a moment that there are still many individuals in the population. The peaks in ephippium production in July and August are less clearly related to density peaks of the active population, and may also be induced by the abiotic conditions in the ponds such as high water temperatures, or the occurrence of toxic algae or parasites (see also Gyllström 2004). The active *D. magna* populations inhabiting these ponds tend to disappear in late summer. The peaks in ephippium production in June and July may thus be seen as an insurance for survival. After this peak in production of sexual, dormant eggs, the females apparently go on reproducing parthenogenetically and try to survive, which sometimes results in overwintering populations (in practice, the studied populations are semi-permanent, see also De Meester et al. 2006). We also noted that the peaks in ephippium production were determined by two factors: increases in the percentage of females that carry ephippia and increases in population size (see figure 2).

- Dormant egg budget

In both years, we observed a clear surplus of dormant eggs being produced compared to the number of hatchlings. Overall, only 3.7 to 9.5% of the eggs that are produced actually hatch at the next occasion (assuming on average only one dormant egg per ephippium). The high hatching percentage, 25%, of OM3 in 2002 was related to the low production of ephippia in 2001, especially when compared to the production in 2002 and 2003 (table 1). The egg budget that we reconstructed did not cover the entire active period, as we stopped sampling in September. This is also apparent in figure 2.C, where during our first sampling moment early spring an active population was already present, presumably consisting of individuals that had survived the winter (see also De Meester et al. 2006). In our egg budget we only considered the main growth season from spring to late summer, and did not monitor hatching and egg production in late autumn or winter. However, even considering the incomplete egg budget, our data do suggest that the dormant egg bank of the study ponds is increasing in size every year, with up to 31.2 $x10^8$ ephippia (62.4 $x10^8$ dormant eggs).

Our main conclusion is that even in moderately sized aquatic habitats, the amount of dormant eggs that are produced and hatch yearly can be very high. Moreover, the positive balance between eggs produced and eggs that hatch suggests a rapid build-up of a persistent dormant egg bank. We note that *Daphnia* densities are very high in our study systems. It might be that in ponds with lower productivity or higher predator densities, where *Daphnia* densities are lower, the ratio of egg production on the number of hatchlings is closer to one. Nevertheless, large egg banks as observed in our study systems have important consequences, amongst others because they ensure population persistence in the face of environmental change by buffering against occasional catastrophes (Rogalski et al. 2017). If, following unfavourable conditions, the habitat becomes favourable again, the population may rebound from the dormant egg bank even if the likelihood of an individual egg to hatch is very low. This is in line with the results of Mergeay et al. (2007), who showed that a *Daphnia barbata* population that was absent from Lake Naivasha for more than 50 years was re-established by hatchlings from the local dormant egg bank. The large dormant egg bank also facilitates coexistence of alleles and species through the storage effect (Chesson 1994; Chesson and Warner 1981) and reduces the impact of genetic and ecological drift within populations through time. The increased genetic and species diversity may also favour

environmental sorting and may thus lead to stronger patterns of isolation-by-environment in landscape genetic data (Orsini et al. 2013) and to stronger signals of species sorting in metacommunity data (Urban et al. 2008), although mass effects of dispersal in time may contribute to noise in the environmental signal in a metacommunity analysis (Leibold et al. 2004) or to retarded adaptation under a scenario of directional selection (Hairston and De Stasio Jr 1988). Overall, the presence of massive dormant egg banks such as reported here and in other studies (García-Roger et al. 2006; Hairston et al. 1995; Thompson and Grime 1979) may strongly contribute to a dominance of local compared to regional dynamics in determining metapopulation and metacommunity structure, because of the combined effects of large numbers and increased genetic and species diversity.

References:

Alekseev V, Lampert W (2001) Maternal control of resting-egg production in *Daphnia* Nature 414:899-901

Arbačiauskas K, Lampert W (2003) Seasonal adaptation of ex-ephippio and parthenogenetic offspring of *Daphnia magna*: differences in life history and physiology Funct Ecol 17:431-437

Badosa A, Frisch D, Green AJ, Rico C, Gómez A (2017) Isolation mediates persistent founder effects on zooplankton colonisation in new temporary ponds Scientific reports 7:43983

Bottrell HH et al. (1976) A review of some problems in zooplankton studies Norwegian Journal of Zoology 24:419-456

Bozelli RL, Tonsi M, Sandrini F, Manca M (2008) A Big Bang or small bangs? Effects of biotic environment on hatching Journal of Limnology 67:100-106

Brendonck L, De Meester L (2003) Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment Hydrobiologia 491:65-84

Brendonck L, Riddoch BJ (2000) Egg bank dynamics in anostracan desert rock pool populations (Crustacea : Branchiopoda) Archiv Fur Hydrobiologie 148:71-84

Cáceres CE (1998) Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs Ecology 79:1699-1710

Cáceres CE, Schwalbach MS (2001) How well do laboratory experiments explain field patterns of zooplankton emergence? Freshw Biol 46:1179-1189

Chesson P (1994) Multispecies Competition in Variable Environments Theoretical Population Biology 45:227-276

Chesson PL, Warner RR (1981) Environmental variability promotes coexistence in lottery competitive systems American Naturalist:923-943

Cousyn C, De Meester L (1998) The vertical profile of resting egg banks in natural populations of the pond-dwelling cladoceran *Daphnia magna* Straus Archiv für Hydrobiologie:127-139

De Meester L, Gómez A, Simon J-C (2004) Evolutionary and ecological genetics of cyclical parthenogens. Oxford University Press,

De Meester L, Vanoverbeke J, De Gelas K, Ortells R, Spaak P (2006) Genetic structure of cyclic parthenogenetic zooplankton populations–a conceptual framework Archiv für Hydrobiologie 167:217-244

De Stasio BT (1989) The seed bank of a freshwater crustacean: copepodology for the plant ecologist Ecology 70:1377-1389

De Stasio BT (1990) The role of dormancy and emergence patterns in the dynamics of a freshwater zooplankton community Limnol Oceanogr 35:1079-1090

De Stasio BT, Hairston NG, Jr (1992) Environmental variability and the persistence of multiple emergence strategies Bulletin of Mathematical Biology 54:313-334 doi:http://dx.doi.org/10.1016/S0092-8240(05)80029-1

Decaestecker E, Declerck S, De Meester L, Ebert DJO (2005) Ecological implications of parasites in natural Daphnia populations 144:382-390

Decaestecker E, Gaba S, Raeymaekers JAM, Stoks R, Van Kerckhoven L, Ebert D, De Meester L (2007) Host-parasite 'Red Queen' dynamics archived in pond sediment Nature 450:870-U816 doi:10.1038/nature06291

Figuerola J, Green AJ (2002) Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies Freshw Biol 47:483-494

García-Roger EM, Carmona MJ, Serra M (2006) Patterns in rotifer diapausing egg banks: Density and viability Journal of Experimental Marine Biology and Ecology 336:198-210 doi:http://dx.doi.org/10.1016/j.jembe.2006.05.009

Gilbert JJ (2017) Resting-egg hatching and early population development in rotifers: a review and a hypothesis for differences between shallow and deep waters Hydrobiologia 796:235-243

Green AJ, Sanchez MI, Amat F, Figuerola J, Hontoria F, Ruiz O, Hortas F (2005) Dispersal of invasive and native brine shrimps Artemia (Anostraca) via waterbirds Limnol Oceanogr 50:737-742

Gyllström M (2004) Induction and termination of diapause in a freshwater zooplankton community Archiv für Hydrobiologie 161:81-97 doi:10.1127/0003-9136/2004/0161-0081
Gyllström M, Hansson LA (2004) Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling Aquat Sci 66:274-295 doi:10.1007/s00027-004-0712-y
Hairston JNG, De Stasio Jr BT (1988) Rate of evolution slowed by a dormant propagule pool Nature 336:239-242
Hairston NG, Jr, Vanbrunt RA, Kearns CM, Engstrom DR (1995) Age and survivorship of diapausing eggs in a sediment egg bank Ecology 76:1706-1711
Hairston NG, Jr. (1996) Zooplankton egg banks as biotic reservoirs in changing environments Limnol Oceanogr 41:1087-1092
Hairston NG, Jr., Hansen A-M, Schaffner WR (2000) The effect of diapause emergence on the seasonal dynamics of a zooplankton assemblage Freshw Biol 45:133-145 doi:10.1046/j.1365-2427.2000.00386.x
Harper JL (1977) Population biology of plants. Academic Press, New York, USA
Hebert PDN (1987) Genetics of Daphnia. Memorie Dell'institutio Italiano Di Idrobiolgia 45: 439±460.
Hobæk A, Larsson P (1990) Sex determination in *Daphnia magna* Ecology 71:2255-2268 doi:10.2307/1938637
Hochmuth JD, De Meester L, Pereira CMS, Janssen CR, De Schamphelaere KA (2015) Rapid adaptation of a Daphnia magna population to metal stress is associated with heterozygote excess Environmental science & technology
Hulsmans A, Moreau K, De Meester L, Riddoch BJ, Brendonck L (2007) Direct and indirect measures of dispersal in the fairy shrimp Branchipodopsis wolfi indicate a small-scale isolation-by-distance pattern Limnol Oceanogr 52:676-684
Kleiven OT, Larsson P, Hobæk A (1992) Sexual reproduction in *Daphnia magna* requires three stimuli Oikos:197-206
Latta LIV, Fisk D, Knapp R, Pfrender M (2010) Genetic resilience of *Daphnia* populations following experimental removal of introduced fish Conserv Genet 11:1737-1745 doi:10.1007/s10592-010-0067-y
Leibold MA et al. (2004) The metacommunity concept: a framework for multi-scale community ecology Ecology Letters 7:601-613 doi:10.1111/j.1461-0248.2004.00608.x
Louette G, Vanoverbeke J, Ortells R, De Meester L (2007) The founding mothers: the genetic structure of newly established Daphnia populations Oikos 116:728-741
Lynch M (1983) Ecological genetics of *Daphnia pulex* Evolution:358-374
Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Genetics and analysis of quantitative traits.
Mergeay J, Vanoverbeke J, Verschuren D, Meester LD (2007) Extinction, recolonization, and dispersal through time in a planktonic crustacean Ecology 88:3032-3043
Miner BE, De Meester L, Pfrender ME, Lampert W, Hairston NG, Jr. (2012) Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model Proceedings of the Royal Society B-Biological Sciences 279:1873-1882 doi:10.1098/rspb.2011.2404
Mitchell S, Carvalho G, Weider L Stability of genotype frequencies in an intermittent Daphnia magna population. In: Diapause in the Crustacea-with invited contributions on non-crustacean taxa, 1998. pp 185-194
Montero-Pau J, Serra M, Gómez A (2017) Diapausing egg banks, lake size, and genetic diversity in the rotifer Brachionus plicatilis Müller (Rotifera, Monogononta) Hydrobiologia 796:77-91
Orsini L et al. (2016) Temporal genetic stability in natural populations of the waterflea Daphnia magna in response to strong selection pressure Molecular ecology 25:6024-6038
Orsini L, Schwenk K, De Meester L, Colbourne JK, Pfrender ME, Weider LJ (2013) The evolutionary time machine: using dormant propagules to forecast how populations can adapt to changing environments Trends in Ecology & Evolution 28:274-282 doi:10.1016/j.tree.2013.01.009
Pfrender ME, Lynch M (2000) Quantitative genetic variation in *Daphnia*: Temporal changes in genetic architecture Evolution 54:1502-1509

539 Rogalski MA, Leavitt PR, Skelly DK (2017) Daphniid zooplankton assemblage shifts in response to
540 eutrophication and metal contamination during the Anthropocene Proc R Soc B 284:20170865
541 Rother A, Pitsch M, Hülsmann S (2010) The importance of hatching from resting eggs for population
542 dynamics and genetic composition of *Daphnia* in a deep reservoir Freshw Biol 55:2319-2331
543 doi:10.1111/j.1365-2427.2010.02441.x
544 Thompson K, Grime J (1979) Seasonal variation in the seed banks of herbaceous species in ten
545 contrasting habitats The Journal of Ecology:893-921
546 Urban MC et al. (2008) The evolutionary ecology of metacommunities Trends in Ecology &
547 Evolution 23:311-317 doi:10.1016/j.tree.2008,02.007
548 Vanoverbeke J, De Gelas K, De Meester L (2007) Habitat size and the genetic structure of a cyclical
549 parthenogen, *Daphnia magna* Heredity 98:419-426
550 Vanoverbeke J, De Meester L (2009) Within season short-term hatching delays suggest risk-spreading
551 behaviour in populations of the freshwater cladoceran *Daphnia* Ecoscience 16:441-451
552 Vanoverbeke J, De Meester L (2010) Clonal erosion and genetic drift in cyclical parthenogens–the
553 interplay between neutral and selective processes Journal of Evolutionary Biology 23:997-
554 1012
555 Vanschoenwinkel B, De Vries C, Seaman M, Brendonck L (2007) The role of metacommunity
556 processes in shaping invertebrate rock pool communities along a dispersal gradient Oikos
557 116:1255-1266 doi:doi:10.1111/j.0030-1299.2007.15860.x
558 Vanschoenwinkel B, Gielen S, Vandewaerde H, Seaman M, Brendonck L (2008) Relative importance
559 of different dispersal vectors for small aquatic invertebrates in a rock pool metacommunity
560 Ecography 31:567-577
561 Verschuren D, Marnell LF (1997) Fossil zooplankton and the historical status of westslope cutthroat
562 trout in a headwater lake of Glacier National Park, Montana Transactions of the American
563 Fisheries Society 126:21-34
564 Waterkeyn A, Pineau O, Grillas P, Brendonck L (2010) Invertebrate dispersal by aquatic mammals: a
565 case study with nutria *Myocastor coypus* (Rodentia, Mammalia) in Southern France
566 Hydrobiologia 654:267-271
567 Wiggins GB, Mackay RJ, Smith IM (1980) Evolutionary and ecological strategies of animals in
568 annual temporary pools Archiv für Hydrobiologie supplement 58:206
569 Wolf HG, Carvalho GR (1989) Resting eggs of lake *Daphnia* II. In situ observations on the hatching
570 of eggs and their contribution to population and community structure Freshw Biol 22:471-478

571

572

573 **Table 1**. Hatching and ephippia production in ponds OM2 and OM3 during 2001, 2002 and 2003: Total
574 ephippia (Eph) produced m^{-2}, total hatching m^{-2}, yearly average *Daphnia* density (individuals / liter),
575 and the hatching percentage (percentage hatchlings compared to the number of ephippia produced in the
576 previous year). The data were calculated based on our observed counts and interpolations based on these
577 counts (to estimate numbers on days on which no observations were done). For the percentage hatching
578 we make two assumptions: we assume that each ephippium contains only one egg (average of 50%
579 filling rate, the average filling rate reported by Cousyn and De Meester (1998) across multiple eutrophic
580 ponds including OM2), and we assume that only eggs that were produced in the previous growing season
581 hatched. When relaxed (higher filling rate or some hatchlings are derived from older eggs), both these
582 assumptions would result in lower hatching percentages (i.e. lower than 3.8 – 25 % reported here).

583

	2001					
			Eph m^{-2} produced	Total Eph produced	Avg *Daphnia* density (ind/L)	
OM2			4.2×10^4	10.6×10^8	571.4	
OM3			1.2×10^4	2.1×10^8	530.3	
	2002					
	Hatching m^{-2}	Total hatching	Eph m^{-2} produced	Total Eph produced	Avg *Daphnia* density (ind/L)	Hatching % from 2001
OM2	4.0×10^3	10.8×10^7	3.8×10^4	9.5×10^8	248.9	9.48
OM3	3.0×10^3	5.5×10^7	10.3×10^4	18.9×10^8	181.0	25.7
	2003					
	Hatching m^{-2}	Total hatching	Eph m^{-2} produced	Total Eph produced	Avg *Daphnia* density (ind/L)	Hatching % from 2002
OM2	3.0×10^3	7.6×10^7	2.5×10^4	6.3×10^8	113.8	7.93
OM3	3.9×10^3	7.2×10^7	17.3×10^4	32.0×10^8	320.5	3.82

584

585