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The contribution of differential hatching success to the fitness of species and interspecific hybrids

N. Brede · D. Straile · B. Streit · K. Schwenk

Abstract Resting egg banks of microcrustaceans have been used to reconstruct the evolutionary and ecological history of species. However, recent studies provided evidence for a discrepancy between dormant propagules in the sediment and the planktonic population. This pattern raises two questions: First, what is the value of data on resting egg banks for population dynamics over time and second, which component of the reproductive cycle causes the observed inconsistency? In our study we focussed on the second question by comparing the taxon composition of a resting egg bank with the reproductive success of ex-ephippial hatchlings. Species and interspecific hybrid identification of dormant and hatched stages was achieved through the application of restriction fragment length polymorphism analysis of an internal transcribed spacer region. We found no significant deviation between the proportion of hatched *Daphnia galeata*, *D. galeata* × *hyalina* and *D. hyalina* individuals and the observed taxon composition of the resting egg bank. However, species and hybrids differed in their mode and relative success of reproduction. We conclude that the components of reproductive success in *Daphnia* contribute differentially to the fitness of species and interspecific hybrids. The discrepancy between resting egg banks and "active" planktonic populations results not from differential hatching of species but from the reproductive success of ex-ephippial females and the timing and frequency of sexual reproduction of the different taxa.

Keywords Ephippia · Hatching experiments · Cladocera · Ecology · Genetics · Lake Constance

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Introduction

Dormant egg banks of microcrustaceans have been generally recognized as biological archives that allow to reconstruct microevolutionary and ecological changes (reviewed in: Brendonck & De Meester, 2003). Paleogenetic data of resting eggs have been used to understand changes of species assemblages due to invasive species (Kerfoot et al., 2004), morphological differentiation associated with variation in predation levels (Kerfoot & Weider, 2004) and natural selection for grazer resistance to toxic cyanobacteria (Hairston et al., 1999, 2001). Recent studies, however, revealed notable discrepancies

between species assemblages derived from dormant eggs and species assemblages of "active" pelagic populations (Jankowski & Straile, 2003; Keller & Spaak, 2004). This discrepancy was attributed to differential levels (and timing) of sexual reproduction among taxa.

In general, species assemblages of "active" pelagic populations will only reflect the taxon composition of resting egg banks if the following assumptions are met: No differential (species specific) rates of (1) hatching, (2) survival of hatchlings and (3) reproductive output of ex-ephippial adults. In addition, successfully hatched individuals should not differ in their (4) level of clonal propagation, (5) induction of males and sexual females and (6) mating success (numbers correspond to those in Fig. 1).

Although we have ample information on the differential levels of selection which directly affect clonal propagation (4; Pfrender & Lynch, 2000), taxon specific levels of sexual reproduction (5; Spaak et al., 2004) and mating success (6; Keller & Spaak, 2004), we lack information on the initial steps of the life cycle (1–3; Fig. 1). In order to bridge this gap, we compared the taxon composition of "non developing" resting eggs and hatchlings isolated from sediments of Lake Constance. In addition, we measured the reproductive success of ex-ephippial adults

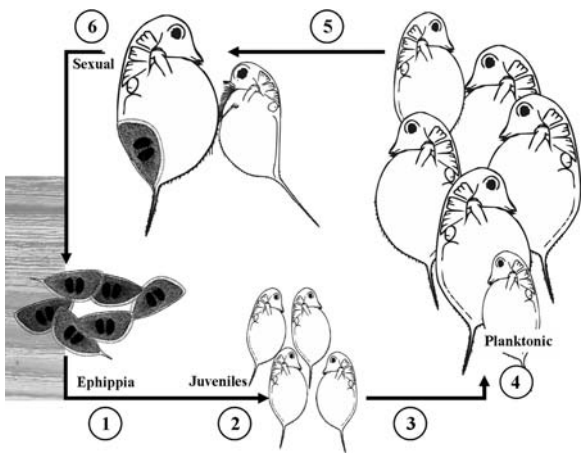

Fig. 1 Reproductive cycle of natural and laboratory populations of *Daphnia* illustrating the different components of fitness. 1: Hatching from ephippia, 2: survival of juveniles, 3: reproductive mode of ex-ephippial adults, 4: level of clonal propagation, 5: induction of males and sexual females, 6: mating success

for each taxon (*D. galeata*, *D. hyalina* and the interspecific hybrid *D. galeata* × *hyalina*).

Daphnia hyalina represents the indigenous *Daphnia* taxon of Lake Constance; Lake Constance is the type locality for *D. hyalina* (Flößner, 2000). *D. galeata* invaded Lake Constance successfully in the early 1950s associated with a continuous shift in habitat quality through the long-term process of increasing eutrophication. During the following decades multiple hybridization events and introgression altered the genetic structure of the species complex (Jankowski & Straile, 2004). During peak eutrophication (1970s) *D. galeata* was the most abundant taxon found in the resting egg bank whereas in the 1980s *D. hyalina* was present only in the plankton population and could not be found in the resting egg bank of that time (Jankowski & Straile 2003; N. Brede, unpublished data). Due to effective pollution control of Lake Constance inflows, the lake recovered in the subsequent years and regained its characteristic oligotrophic conditions.

The aim of our study was to determine the level of differential hatching among *Daphnia* taxa and to identify the life history stages explaining the discrepancy between "active" pelagic and the dormant populations. Specifically, we addressed the question whether the relative frequencies of taxa found in the resting egg bank differ from the relative proportions of successfully reproducing individuals. To do so, we measured the (i) hatching rate, (ii) proportion of individuals reaching maturity and (iii) reproductive fitness of ex-ephippial females of the three *Daphnia* taxa inhabiting Lake Constance.

Materials and methods

Sampling

Sediment cores were sampled in Lake Constance in Germany. Cores were recovered in December 2002 from 220 m depth close to the deepest point of the lake between Konstanz and Langenargen (47°34′46″ N, 9°27′54″ E) and in September 2004 from 180 m depth close to the Langenargener Bucht (47°37′21″ N, 9°26′24″ E). Sediments were dated by lamination counting (Wessels et al., 1995) and prepared as in previous studies (Weider et al., 1997). In general, the sediments are well laminated and reference cores

have been dated by ^{137}Cs dating before (Wessels et al., 1995). Ephippia were isolated by washing the sediments through a 220 µm mesh sieve.

Hatching experiments

Four hatching experiments were conducted in which ephippia from three different time periods were exposed to hatching stimuli (1970s, 1990s and 2000s; Table 1). All experiments were carried out in a 16:8 h light dark cycle at 18°C (pers. comm. T. Jankowski; Vandekerkhove et al., 2005). Two different media were used for the experiments, pond water and Lake Constance water in order to obtain maximum hatching success. Both, the Lake Constance (drawn in winter right before use) and the pond water were filtered (0.45 µm Whatman filters) and autoclaved. Pond water originates from small artificial (concrete) pools filled with rainwater which are cleaned once a year. All ephippia isolated from the sediments were subjected to the hatching experiments. To avoid any damage of viable resting eggs we did not open ephippia to determine the presence or absence of eggs. The experiments "1970/1" and "1990" as well as the experiments "1970/2" and "2000" were conducted simultaneously.

Each plate was checked in the morning and if necessary also in the afternoon in search of neonates. Hatching started after 2–6 days and each hatchling was transferred to a 10 ml vessel and fed with *Scenedesmus obliquus* suspension containing ~1 mg C l^{-1} to guarantee a food supply above the incipient limiting level. Animals were controlled by eye until reaching maturity to monitor developmental differences between individuals. Experiment "1970/1" was carried out with two times 96 ephippia extracted from sediments of 1971–74 and placed individually in 96 well plates with filtered Lake Constance water. After transferring hatchlings to 100 ml jars animals were controlled by eye until reaching maturity in this experiment to monitor developmental differences between individuals: All hatched individuals were observed daily and categorized in three groups. "Asexually reproducing" categorizes ex-ephippial adults establishing a clonal lineage, "not reproducing" represents animals that did not reproduce at all (after max. 43 days) and "ephippium producing" accounts for the observed fraction of hatchlings that built up an ephippial shell (without depositing eggs) right after molting to maturity. The definition of sexual females is imprecise and usually connected to the visibility of promoted ovary activity. The generation of an ephippial structure on the carapace is an indication, but not the ultimate proof of the status of a female. Experiment "1970/2" (same time period as in "1970/1") was replicated four times; each replicate was carried out by exposing 96 ephippia in well plates with filtered pond water to hatching stimuli. For experiment "1990" 192 ephippia (two replicates) from the sediments of 1994–98 were exposed to hatching stimuli in filtered Lake Constance water. Experiment "2000" was carried out with ephippia extracted from sediments of 1999–2004 in filtered pond water in six replicates (one replicate = 96 ephippia).

In the two replicated experiments "1970/2" and "2000" a minor fraction of eggs and individuals was

Table 1 Absolute numbers of analyzed individuals and eggs of all experiments

	1970/1 (2, L)			1970/2 (4, P)			1990 (2, L)			2000 (6, P)			Total[a]
	G	GH	H	G	GH	H	G	GH	H	G	GH	H	
"Non developing"	25	4	1	117.8	21.4	4.8	51	0	0	125.5	11.7	3.8	299
Hatched	106	5	0	167.8	13.2	0	242	0	0	64	7.1	0	501
Sum	131	9	1	285.6	34.5	4.8	293	0	0	189.5	18.7	3.8	
Sum all	141			325			293			211.6			800

Numbers refer to the amount of resting eggs or individuals subjected to a RFLP analysis of G: *D. galeata*, GH: *D. galeata* × *hyalina* H: *D. hyalina*. Hatching experiments were conducted either in filtered lake water from Lake Constance (1970/1 and 1990; L) or in filtered pond water (1970/2 and 2000; P) experiments. The experiment's name is followed by the number of replicates with every replicate containing 96 ephippia (in brackets). The number of eggs or individuals was corrected with the number of genetically not analyzed data. First row: "Non developed": Eggs that did not hatch, Hatched: number of hatchlings, Sum: number of eggs or individuals per taxon and per experiment, Sum all: Total amount of experimental eggs per experiment. The total number of "non developed" eggs and hatchlings (uncorrected values, see text) are provided in the last column (Total[a])

not identified genetically ("1970/2" total $N = 384$, "2000" total $N = 576$; Table 1). In order to correct the observed frequencies of taxa we multiplied the number of unidentified eggs or individuals with the observed proportion of each taxon. Hatching success per taxon was calculated by dividing the total number of eggs (per taxon) over the whole experiment or per replicate by the number of hatched individuals. The total number of eggs was calculated by summing the number of genetically identified hatchlings and "non developing" eggs. In order to test whether the observed hatching frequencies of taxa are explained by the initial taxon composition of the exposed resting eggs we conducted a goodness-of-fit *G*-test with a Williams correction for small sample sizes (Sokal & Rohlf, 1995).

Genetic analysis

Resting eggs were isolated from their ephippial shells and DNA was prepared separately in 35 µl H3 buffer (1×: 10 mM Tris–HCl; pH 8.3 at 25°C, 0.05 M potassium chloride, 0.005% Tween 20 and 0.005% NP-40) and 1.2 µl proteinase K (Sigma; 10 mg/ml). Adults were directly transferred to 70 µl H3 buffer and 2 µl proteinase K. After an incubation time of 12 h proteinase K was deactivated by heating the sample for 12 min at 95°C. An ITS fragment was amplified using a total reaction volume of 14 µl. About 2 µl of template and 3 mM $MgCl_2$, 1× PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer (ITS2-5.8S: 5′-GGA AGT AAA AGT CGT AAC AAG G-3′; 10 µM; ITS1-18S: 5′-CGG TGG TCG ACG ACA CTT CGA CAC GC-3′; 10 µM) and 1 U *Taq* DNA polymerase (all chemicals and primers: Invitrogen) in 94°C for 3 min, five cycles at 94°C for 1 min; 52°C for 1 min; 72°C for 1.5 min; 35 cycles: 94°C for 1 min; 50°C for 30 s; 72°C for 1 min; final synthesis step at 72°C for 5 min.

A restriction fragment length polymorphism analysis (RFLP) was used for taxon identification (Billiones et al., 2004). Amplicons of the ITS region were digested with the restriction enzyme *Mwo* I (5′-GVNNNNN↓NNGC-3′; NEB) for 2.5 h at 60°C in a total reaction volume of 9.6 µl containing 8 µl PCR product and 10× NEBuffer for *Mwo* I, 5 U of the restriction enzyme and autoclaved dH_2O.

The digestion products were transferred to a 2% agarose gel and bands were separated by applying 115 volts. Specific banding patterns allow identification of the taxa: *D. galeata* (100, 320, 380 and 490 bp), *D. hyalina* (100, 520 and 680 bp) and their hybrid who displays an additive banding pattern in the RFLP analysis. ITS RFLP analyses were compared with microsatellite analyses (six loci: DaB 10/15, DaB 17/17, DaB 17/16, DaB 10/14, Dp512 and Dp519; see Brede et al., 2005) and resulted in similar taxon classifications.

Results

Our genetic analyses of the resting egg bank revealed a very low frequency of *D. hyalina* and the interspecific

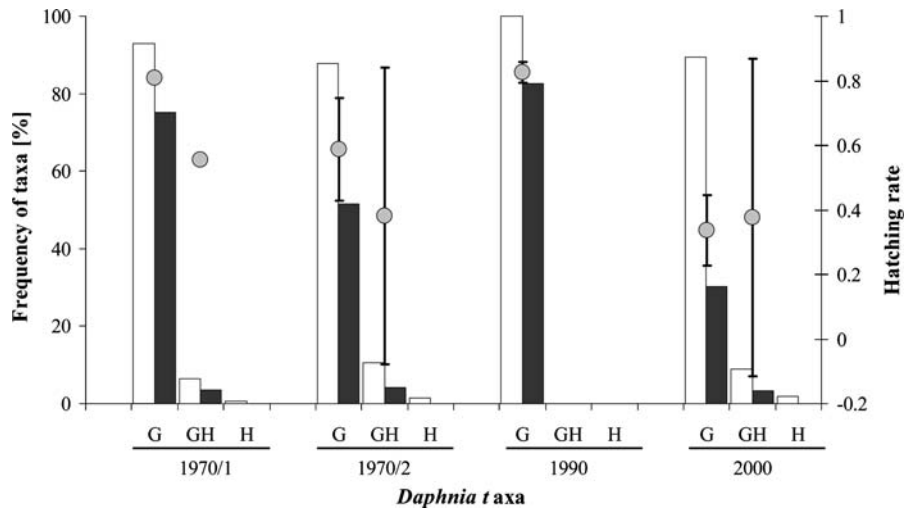

Fig. 2 Comparison of hatching rate, taxon composition of resting eggs present in the experiment (white bars) and those that hatched (black bars) in percent (left y axis). G: *D. galeata*, GH: *D. galeata* × *hyalina*, H: *D. hyalina*. Dots represent the hatching rate (right y axis) and error bars standard deviation among replicates

hybrid compared to *D. galeata* ephippia (1:6.5:88; s.d. 0.8:4.6:4.4; Fig. 2).

In all cases, hatching peaked within 2 days and slowly decreased over the next 1 or 2 weeks. In the experiments "1970/1" and "1970/2" with ephippia exposed to different water characteristics (filtered Lake Constance and pond water respectively) the hatching success for the lake water experiment was 78.7% whereas for the pond water experiment the average hatching success was 55.7%. Hatching success between lake and replicated, pond water experiments could not be tested because of large variation within treatments among replicates (hatching success differed over four replicates between 37.6 and 77.6%). Eggs from the sediments of the 1990s hatched most successfully with 82.3%. The hatching success for recent ephippia (experiment "2000") was lower at 33.5%. We found no differences in hatching success among ephippia isolated from different sediment layers.

Hatching success among parental taxa did not differ significantly in any of the experiments ("1970/1": χ^2, df = 2, $P = 0.48$; "1970/2": replicated goodness-of-fit test, G (Williams) $P = 0.1168$; "1990": χ^2, df = 2, $P = 0.66$; "2000": replicated goodness-of-fit-test, G (Williams) $P = 0.6090$; Fig. 2). Apart from experiment "2000", *D. galeata* eggs developed with 80.9% hatching success in experiment "1970/1", an average success of 60.3% in experiment "1970/2" (replicated experiment) and a hatching success of 82.3% in the "1990" experiment. Further observations within this experiment showed that 28.7% of all hatchlings died before the first molt. In recent times (experiment "2000"), *D. galeata* hatched with an average success of 34.7% (ranging between 23.1 and 47.2%). *D. galeata* × *hyalina* hybrid eggs were almost as successful: In both 1970s experiments hybrid eggs hatched in lake water with 55.6%, whereas hatching success in pond water was 55.4%. No eggs of the interspecific hybrid were found in the sample from the 1990s but in recent times (experiment "2000"), the hybrid hatched with 38% success. In all three experiments in which we found *D. hyalina* eggs ($N = 7$, uncorrected value; see also Table 1) none hatched (Fig. 2).

As mentioned before, the one egg of *D. hyalina* found in experiment "1970/1" did not hatch. Within the three categories observed, only *D. galeata* and the interspecific hybrid *D. galeata* × *hyalina* were detected. Within the category "asexually reproducing" all hatchlings turned out to be *D. galeata* ($N = 74$). All interspecific hybrids ($N = 5$) divided among the two other categories with 33.3% "ephippium producing" and 22.2% "not reproducing". All categories differed significantly ($P < 0.001$) compared to the taxon composition of all hatched individuals.

Discussion

The overall observed hatching rates are comparable with those found in a previous study (Weider et al., 1997). In addition, the variation of hatching success shows a similar pattern and confirms the tendency of reduced hatching rates of eggs recovered from recent sediments (experiment 2000).

The proportion of *D. hyalina* to *D. galeata* × *hyalina* to *D. galeata* eggs is on average 1:6.5:88 (s.d. 0.8:4.6:4.4; Fig. 2). In all four experiments conducted in this study no significant frequency difference between the taxon composition within the resting eggs and those that hatched and established a clonal lineage was observed. The discrepancy between the resting egg bank and the pelagic population cannot be explained by differential hatching of taxa. In this study no *D. hyalina* resting eggs developed in three experiments representing two time periods (containing corresponding resting eggs). The most likely explanation for the lack of *D. hyalina* hatchlings is a stochastic effect due to the low number of resting eggs. Other studies showed that *D. hyalina* resting eggs hatch under natural conditions (Carvalho & Wolf, 1989; Jankowski, 2002; Wolf & Carvalho, 1989). For Lake Constance it has been shown that hatching success of *D. hyalina* may depend on lake depth; Jankowski (2002) showed that *D. hyalina* hatched in the littoral (25%) but did not hatch from the profundal. Caceres & Tessier (2003) found a similar pattern of spatial variation in hatching experiments on North-American *D. pulicaria*. Furthermore, several authors describe that some *Daphnia* species' ephippia are buoyant e.g. through spines, lipid drops or gas chambers (Flößner, 2000; Weider et al., 1997). This differential buoyancy may result in a spatial separation of *D. hyalina* resting eggs floating ashore whereas *D. galeata* ephippia sink mainly to the profundal. Recent population genetic studies of resting eggs isolated from Lake Constance

sediments show that *D. hyalina* was present as dormant stages before the 1970s (Jankowski & Straile, 2003; N. Brede, unpublished data). Based on these findings we conclude that spatial effects have to be taken into account when the resting egg bank and current populations are compared.

In the experiment "1970/1" we studied the development of *Daphnia* from the juvenile stage to maturity. Only ex-ephippial individuals of the taxon *D. galeata* reproduced parthenogenetically (Fig. 3). Among those animals which failed to reproduce and those carrying an ephippium we found *D. galeata* and all interspecific hybrids. Still, some of the hatchlings that primarily produced an ephippium later on built up a clonal lineage. Although we cannot exclude the possibility that these observations are due to a differential response of taxa to laboratory conditions, we do not expect that our standardized laboratory conditions have such detrimental effects on basic developmental processes of hybrids. The observed patterns indicate that hybrids do experience fitness deficiencies, in particular, after reaching the adult stage. This reduced reproductive success of interspecific hybrids (and recombinant genotypes) and some *D. galeata* individuals might be caused by

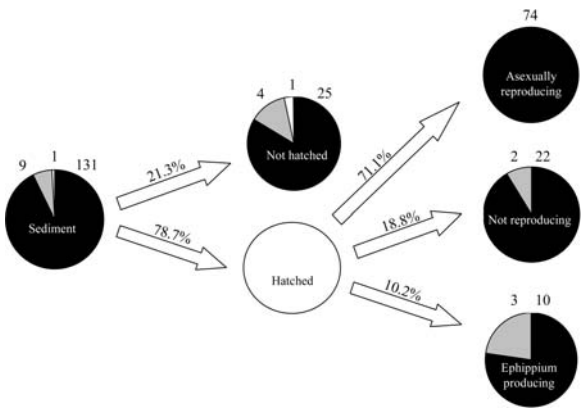

Fig. 3 Relative taxon composition of *Daphnia* species and hybrids at two life history stages, after hatching (juveniles) and after first reproduction (experiment "1970/1"). Black area = *D. galeata*, grey area = *D. galeata* × *hyalina*, white area = *D. hyalina*. The total number of identified taxa is provided above each pie in the order *D. galeata* × *hyalina*, *D. hyalina*, *D. galeata*. Numbers above arrows describe the percent of undeveloped eggs versus hatchlings (left arrows) and represent the percentage of individuals attributed to different modes of reproduction (asexual reproduction, no reproduction and production of ephippial females; right arrows)

genetic incompatibilities of recombinant hybrid genomes. Some *D. galeata* individuals within the two categories are probable to be backcrosses of the parental species. Studies have shown that backcrossing occurs in Lake Constance (Jankowski & Straile, 2004; Löffler et al., 2004).

In Jankowski's Ph.D. thesis (2002) hatching experiments in the laboratory and in the littoral zone of Lake Constance showed that *D. galeata* and *D. hyalina* hatch in different zones of the lake. After reconstructing the taxon composition over time using spininess of the ephippia, historical records and genetically (allozymes) determined hatchlings, Jankowski & Straile (2003) concluded that the resting egg bank of *Daphnia* does not represent the "active" pelagic population. Similar results were obtained by comparing egg banks and "active" populations by Keller & Spaak (2004).

Both published results and our data suggest that the components of reproductive success in *Daphnia* contribute differentially to the fitness of species and interspecific hybrids. We found no species-specific (1) hatching rates and (2) no differential survival of juveniles. We observed in one experiment ("1990") a 28.7% mortality rate among juveniles, however, since they could not be subjected to genetic analyses we were not able to determine taxon specific survival rates. Species and hybrids differed in their mode of reproduction and in their level of clonal propagation (3, 4; Fig. 1). In addition, taxa varied in their rate of sexual reproduction (Jankowski & Straile, 2003; Keller & Spaak, 2004).

The observed differences between resting egg banks and pelagic populations might also be explained by the heterogeneous spatial distribution of resting eggs (Jankowski, 2002) and the comparison of pelagic populations representing the entire population with a non-representative sample of the profundal resting egg bank. Furthermore, we have very little information on the level of random mating within and among taxa (Keller & Spaak, 2004; 6, Fig. 1). All these observations suggest that it is highly unlikely to find "active" planktonic populations that reflect dormant populations.

In general, resting egg banks represent a conglomerate of recombinants sexually produced by "successful" parental genotypes. Speaking in evolutionarily relevant terms, the resting egg bank forms a large archive of genetic variation which

results in *Daphnia* populations that can change rapidly following to ecological changes i.e. predation levels, food quality or quantity (Cousyn et al., 2001; Hairston et al., 1999). Long-term evolutionary changes, like adaptations to novel environments, the consequences of interspecific hybridization or successful invasions of species and lineages will be reflected in resting egg banks (Duffy et al., 2000; Jankowski & Straile, 2004). Since several aspects of current populations are determined by their history, future ecological studies may profit from an interdisciplinary approach using both population genetic data over time and life history studies.

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