

Starvation tolerance of rotifers produced from parthenogenetic eggs and from diapausing eggs: a life table approach

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*Monogonont rotifers have to cope with environmental variation by producing diapausing stages. Cyclically, parthenogenetic rotifers produce females by (i) parthenogenetic eggs and (ii) sexually produced eggs. Parthenogenetic eggs hatch shortly after ovoposition, frequently while they are carried by their mothers. Sexual eggs go into dormancy and hatch in the sediment, in response to specific environmental cues. Therefore, it is expected that post-diapause and parthenogenetic offspring would face different environments. Moreover, resource allocation is higher in diapausing eggs than in parthenogenetic eggs. In this study, the response to starvation of females obtained from parthenogenetic eggs and from diapausing eggs of the rotifer *Brachionus plicatilis* was compared. Starved individuals showed unexpectedly long maximum longevity (~12 days). The average lifespan and the lifespan variance of individuals hatched from parthenogenetic eggs were higher than those of individuals hatched from diapausing eggs. Contrasting with a previous experimental finding, our results do not support the hypothesis that high resource allocation in diapausing eggs causes high starvation tolerance. Lower survival of individuals hatched from diapausing eggs could be caused by the diversion of energy to other functions (i.e. maintenance during diapause or the hatching event), or the result of the variance in the allocation of resources in parthenogenetic eggs. We hypothesize that resource allocation in diapausing eggs follows a conservative pattern, while it is more opportunistic in parthenogenetic eggs.*

INTRODUCTION

Many zooplankters inhabit isolated and temporarily suitable habitats and are expected to invest significant amount of resources and to evolve specific adaptations for time and spatial dispersal. Monogonont rotifers are cyclical parthenogenetic zooplankters, whose life-cycle combines parthenogenesis with episodic bouts of sexual reproduction (Gilbert, 1977). This cycle is matched to dormancy and dispersal. During the parthenogenetic phase, the most frequent mode of reproduction, females produce asexually parthenogenetic diploid eggs that develop into daughters genetically identical to their mothers. After a usually high number of parthenogenetic generations, sex is induced by some environmental factor which may predict rotifer habitat deterioration (Serra *et al.*, 2004). In the case of the monogonont rotifer

Brachionus plicatilis, the environmental signal that triggers sex initiation has been found to be the population density (Snell and Boyer, 1988; Carmona *et al.*, 1995; Stelzer and Snell, 2003). Sexual reproduction starts with the parthenogenetic production of sexual females. These sexual females produce parthenogenetic haploid eggs. If a sexual female is not inseminated while young, her haploid, unfertilized eggs will develop into dwarf males (Snell and Childress, 1987). If inseminated, her fertilized eggs will result in a diploid embryo, the so-called diapausing egg. These eggs may remain in an arrested state of development (i.e. diapause) for long periods. They can resist desiccation and can disperse spatially. Diapausing eggs hatch in response to environmental cues thought to be correlated to favourable conditions. Hatchlings are parthenogenetic females, and so the cycle is completed.

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Parthenogenetic diploid eggs (hereafter, simply parthenogenetic eggs) hatch shortly after ovoposition, frequently when they are carried by the mother. Thus, offspring would experience similar environmental conditions to their mothers. By contrast, sexually produced diapausing eggs do not hatch immediately after their deposition, even in good environmental conditions. Diapausing eggs are released by their mothers into the water column and fall to the bottom of ponds and lakes where many of them will hatch. Therefore, it is expected that post-diapausing and post-parthenogenetic egg offspring would face different environments what should be reflected in differences in their respective life histories (Stearns, 1992). Studies by Gilbert and coworkers on females produced from diapausing eggs have shown several of these differences (Gilbert, 2002, 2004; Gilbert and Schröder, 2004).

It is well known that diapausing eggs in some freshwater monogononts living in temperate climates have more lipid reserves than parthenogenetic eggs (Wurdak *et al.*, 1978; Gilbert, 1983). This finding, besides the fact that diapausing-egg producing females have a lower fecundity than parthenogenetic females, suggests that resource allocation per egg is higher in diapausing eggs than in parthenogenetic eggs. In a recent paper, Gilbert (Gilbert, 2004) has shown that *Brachionus calyciflorus* females emerging from diapausing eggs have higher tolerance to starvation than females emerging from parthenogenetic eggs. This result suggests that the observed higher tolerance is the result of a higher resource allocation, and that survival is the selective pressure for the evolution of resource allocation. Differences in hatching environments between diapausing and parthenogenetic eggs would ultimately account for the evolution of the differences in resource allocation.

However, hatchling starvation is not the only differential condition that may have shaped resource allocation into diapausing eggs. For instance, these hatchlings might need to have an increased swimming effort to migrate from the sediment to the upper water layers, and diapausing eggs might consume resources during diapause due to low respiration rates. The relative importance of these and other factors on demanding resources is expected to be dependent on the ecological conditions prevalent in the rotifer environment (i) during diapausing egg formation, (ii) during the diapause stage and (iii) in the habitat of the diapausing egg hatchling. For instance, shallow lakes would impose different demands on diapausing egg hatchlings, if compared to deep lakes. Therefore, it is interesting to know if enhanced starvation tolerance of hatchlings from diapausing eggs is found in rotifer species other than *B. calyciflorus*.

Our study compares the response in survival and reproduction under food deprivation (i.e. starvation) between two cohorts of females of the rotifer *B. plicatilis* developed from diapausing (hereafter, post-diapausing egg cohort) and from parthenogenetic diploid eggs (hereafter, post-parthenogenetic egg cohort) and produced under identical environmental conditions.

METHOD

Experimental stock population

A total amount of 4000 females of the rotifer *B. plicatilis* were isolated from a plankton sample collected in January 2004 in Hondo Sur, a shallow pond located in a wetland area in Eastern Spain (38°10' N; 0°44' W). These females, most likely representing a variety of genotypes, were used to found our experimental stock population. Rotifers were mass cultured in 2 L of f/2 enriched medium (Guillard and Ryther, 1962) at 22.5°C, 12 g L⁻¹ artificial seawater (Instant Ocean; Aquarium Systems), constant illumination (~35 µmol quanta m⁻² s⁻¹) and fed with the algae *Tetraselmis suecica* (7–12 µm length, 5–7 µm width; Ciro-Pérez *et al.*, 2004) up to a density of 2 × 10⁵ cells mL⁻¹. Weekly, a fraction of the rotifer culture was replaced with fresh medium (dilution rate: 0.7 week⁻¹). *Tetraselmis suecica* provided as food was grown under the same culture conditions as rotifers.

Parthenogenetic and diapausing egg production

Six hundred females were isolated from the experimental stock culture and transferred to 6 L of fresh culture medium. The culture was kept at the same conditions that the experimental stock population, but algae density was controlled daily, and adjusted to 26 mg C L⁻¹ (2 × 10⁵ cells mL⁻¹) *T. suecica* if a lower density was measured. Algal density was estimated by taking samples of the rotifer culture without animals and measuring light extinction at 750 nm (Shimadzu UV-1603 spectrophotometer). Light extinction at 750 nm was transformed to algae density and carbon concentration as in Ciro-Pérez *et al.* (Ciro-Pérez *et al.*, 2004). When needed, a high-concentrated algae suspension was added to the culture. We kept sampling and algae addition volumes low in order to minimize changes in culture volume. Under these culture conditions, rotifers were able to grow up to high female density (typically above 100 females mL⁻¹) and produced high numbers of both parthenogenetic and diapausing eggs. Sexual reproduction was therefore induced by crowding.

The mictic ratio (i.e. the proportion of sexual females over total females) was monitored by counting egg-carrying females and classifying them as asexual or sexual according to the type of eggs carried (i.e. parthenogenetic diploid eggs, if asexual, or parthenogenetic haploid eggs or diapausing eggs, if sexual). Females were counted from daily samples (2–200 mL). At the 10th day, high numbers of both parthenogenetic and diapausing eggs occurred in the culture. Females carrying both types of eggs were collected from the culture and placed separately in 45 mL plastic tubes (one for each type of females) containing 12 g L⁻¹ artificial seawater. Both types of eggs were detached from their mothers by shaking. Then, eggs and females were sieved using a 5 cm diameter, 30- μ m Nylal mesh. The mesh was rinsed on a Petri dish in order to collect the eggs in a small volume (<5 mL) of 12 g L⁻¹ artificial seawater. Therefore, 430 parthenogenetic eggs and 640 diapausing eggs were collected from the same environmental conditions.

Life table experiment

Immediately after collection, parthenogenetic eggs were individually placed into 96-multiwell sterile dishes (Nunc) containing 200 μ L of 12 g L⁻¹ artificial seawater and incubated at 25°C under constant illumination (150–170 μ mol quanta m⁻² s⁻¹) for hatching. By contrast, diapausing eggs were stored dry at 4°C for 2 months in order to ensure the completion of the obligate period of diapause and thus optimize hatching success (Hagiwara and Hino, 1989). After the storage period, hatching conditions for diapausing eggs were the same as those used for the parthenogenetic ones. The majority of eggs showed a normal appearance (García-Roger *et al.*, 2005) when rehydrated in 12 g L⁻¹ artificial seawater.

After hatching, 72 neonates from each group (i.e. post-parthenogenetic and post-diapausing egg offspring) were used to find the experimental cohorts. The neonates were individually isolated in sterilized, polystyrene tissue culture plates (Iwaki brand, Asahi Techno Glass) with 1 mL of 12 g L⁻¹ artificial seawater and incubated at 22.5°C under constant illumination in the absence of food. In order to produce the experimental neonates from the experimental stock population, a filtration with rinsing and two transfers to new medium culture (to isolate the egg and then to isolate the neonate) were involved, which remove algae. Algae were not observed during the life table experiment. Neonates were observed from birth to death every 12 h. Two neonates died before the first observation (12 h), and they were discarded for the life table computations, since it was assumed that they were unable to complete successful emergence. Survival time (defined as the midpoint of the

time interval in which death happened) and number and type (sexual or asexual) of offspring produced in each time interval were recorded for each individual in the life table experiment. As three animals were lost during handling, a censored data analysis approach was adopted (see Data analysis).

Data analysis

Only females of experimental cohorts that reproduced parthenogenetically were used for life table computations; sexual females (i.e. females producing males or diapausing eggs, neither of which contributing to population growth) were excluded. Only neonates observed alive at the monitoring time were counted for fecundity; eggs that did not hatch were also neglected. Pearson's correlation coefficient was computed in order to estimate the association between survival times of females and number of descendants produced. A *t*-test was applied on this coefficient.

From the life table data, the following parameters were estimated: net reproductive rate (R_0), mean generation time (G_c , days), survival function (l_x , x being age), hazard function (or instantaneous mortality rate, h_x) and age-specific fecundity (m_x). The survival function (l_x) and the associated hazard function (h_x) were estimated for each cohort by using the Berkson-Gage method corrected for censored data (Lee, 1992). The median survival (S_{50}) times (age, x , at which $l_x = 0.5$) were estimated by linear interpolation.

Non-parametric Gehan-Wilcoxon and log-rank tests were used to compare survival functions of the cohorts. These statistical tests use different assumptions. Wilcoxon-Gehan test gives more weight to early cohort observations, as they are based on more individuals than late observations. Hence, it is more sensitive to early survival function differences. On the other hand, log-rank test gives equal weight to all observations. All these statistics were performed using STATISTICA 6.0 (StatSoft).

We fitted four theoretical hazard functions (exponential, Weibull, Gompertz and linear exponential) to the experimental hazard functions, previously estimated by the Berkson-Gage method (see above). Fitting was performed by the method of weighted least squares (Gehan and Siddiqui, 1973; see also Lee, 1992). Following Gehan and Siddiqui (Gehan and Siddiqui, 1973), three different weights (w_x) were considered: (i) $w_x = 1$ (no weight), (ii) $w_x = 1/\text{VAR}(h_x)$, the inverse of the estimated variance of hazard function at x age, and (iii) $w_x = b_x n_x$, the result of the product between the timespan between two consecutive cohort observations (b_x) and number of animals exposed to risk at age x (n_x , this number is related to the number of animals observed alive at age x but modified accordingly to procedures for censored data). The best fitted theoretical hazard function was

selected based on log-likelihood criterion. This analysis was performed using STATISTICA 6.0 and the additional criteria implemented there.

RESULTS

Egg production and rotifer culture dynamics

Daily changes in total female density and mictic ratio as well as the dynamics of diapausing and parthenogenetic eggs are shown in Fig. 1. Parthenogenetic and diapausing eggs were produced at high numbers after a 10-day culture of the experimental population of the rotifer *B. plicatilis*. Exponential growth began on day 2 and lasted until day 9. Mictic ratio rose sharply by day 5 and 7 and kept at ~0.6 from day 7 until the end of the culture. As expected, density of both parthenogenetic and diapausing eggs increased with time but peaked at different times. Parthenogenetic eggs peaked at day 8, whereas the density of diapausing eggs peaked by day 9. At day 10, numbers of both types of eggs were large enough (estimation: 6000 diapausing eggs and 36000 parthenogenetic eggs) to allow an efficient harvest in order to perform subsequent experiments.

Hatching

Hatching of parthenogenetic and diapausing eggs followed different patterns. Dynamics for the first 72

hatchlings of each type of eggs are shown in Fig. 2. Notice that a lower number of parthenogenetic eggs, if compared to diapausing ones, were needed to be incubated in order to reach 72 hatchlings. Hatching of parthenogenetic eggs was rather synchronous, happening in a 4-h time window. Contrasting with this, hatchlings from diapausing eggs after 2 months of storage conditions started to emerge after 48 h of incubation, and emergence had to be monitored until 288 h in order to get 72 individuals.

Survival under starvation

Survival curves of starved neonates of *B. plicatilis* from parthenogenetic and diapausing eggs are shown in Fig. 3. Curves crossed at day 4, since some neonates from the post-parthenogenetic egg cohort died earlier than neonates emerged from diapausing eggs, and the survival of post-diapausing egg cohort dropped sharply after around day 2. Median survival time under starvation was higher for the post-diapausing egg cohort than for the post-parthenogenetic egg cohort, whereas expectancy of life at birth followed the opposite pattern (Table I). No non-parametric test (i.e. Wilcoxon-Gehan's test and log-rank test) revealed significant statistical differences between survival functions of both cohorts, regardless the different weight assigned to the early survival records.

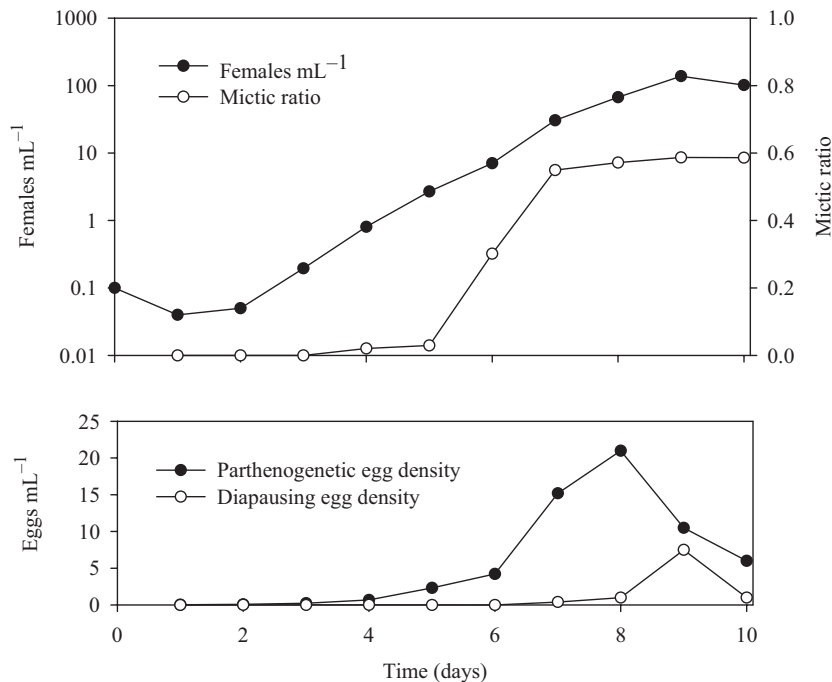


Fig. 1. Population dynamics in the 10-day culture of *Brachionus plicatilis* from which the experimental diapausing and parthenogenetic eggs were collected.

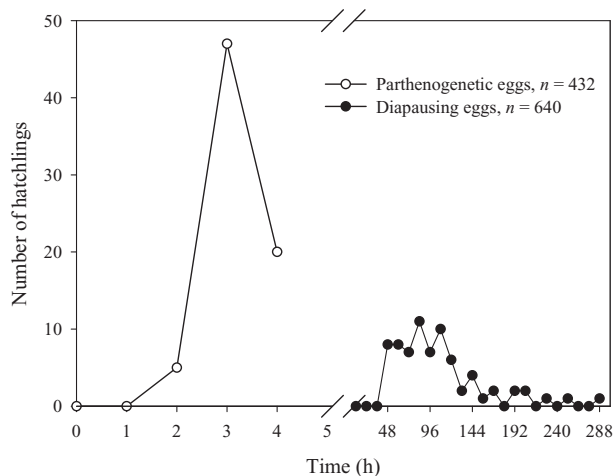


Fig. 2. Hatchling dynamics of the experimental parthenogenetic and diapausing eggs. Initial numbers of eggs exposed to hatching conditions are shown.

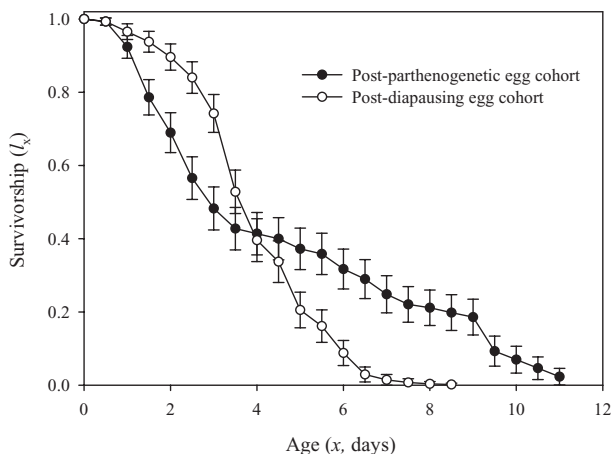


Fig. 3. Age-specific survival (l_x) of starved females. Vertical bars are ± 1 SE.

A Levene’s test for homogeneity of variances revealed that the variance of the death distribution for the post-parthenogenetic egg cohort was significantly higher than that for the post-diapausing egg cohort (Levene’s $W = 63.73$, $df = 1, 142$, $P < 0.001$), confirming that the post-diapausing egg cohort died more synchronously than the post-parthenogenetic one. Survival variation in post-diapausing egg cohort was not correlated with the time needed for diapausing egg hatching (linear determination coefficient: 0.0448).

When parametric survival analysis methods were applied, we found that the post-parthenogenetic egg cohort did not fit to any of the four theoretical distributions tested (exponential, Weibull, Gompertz and linear exponential; $P < 0.001$, in all cases). For the post-diapausing egg cohort, the fitting of three theoretical

Table I: Survival parameters of starved females, as estimated from Berkson-Gage method for censored data

Cohort	N	S_{50} (days)	e_0 (days)
Post-parthenogenetic egg	72	2.89	4.40
Post-diapausing egg	72	3.61	3.80
Test	Statistic	P	
Wilcoxon-Gehan ^a	-0.823	0.410	
Log-rank ^b	-1.762	0.078	

S_{50} , age at 50% cohort survival; e_0 , expectancy of life at birth (average lifespan). Statistical significance for differences in survival distributions are shown, after non-parametric Wilcoxon-Gehan test and log-rank test for censored data.

^aWilcoxon-Gehan test gives more weight to initial observations. Hence, it is more sensitive to detect earlier life differences between groups.

^bLog-rank test gives equal weight to all observations.

distributions (exponential, linear exponential and Gompertz) did not match the data ($P < 0.05$). However, no significant differences were found between the observed post-diapausing egg cohort and a Weibull distribution ($P = 0.532$). The log-likelihood values obtained under this model for three weighting methods were very close, the highest log-likelihood value being when no weight was used to the hazard rate ($w_x = 1$). According to the Weibull model so fitted ($h_x = \lambda \gamma x^{(\gamma-1)}$, λ and γ being respectively the scale and shape parameters of the Weibull distribution), the hazard function for the post-diapausing egg cohort is $0.028 \cdot 2.47 x^{(2.47-1)}$. Given that a standard model did not fit survival data of both cohorts, a parametric comparison was not possible.

Reproduction under starvation

No reproduction was observed in the post-diapausing egg cohort, whereas 40% of starved neonates developed from parthenogenetic eggs produced offspring during their lifespan. Figure 4 shows the distributions of the lifetime reproductive success for asexual females in the latter cohort. Of the reproductive females, 82.7% were asexual, the rest (13.3%) females produced male progeny. Almost all reproductive females (91%) laid one or two eggs. No female produced more than three eggs. All eggs developed into neonates. Net reproduction rate (R_0) for the post-parthenogenetic egg cohort was 0.722, and mean generation time (G_c) was 6.23 days. These two parameters allowed us to estimate a growth rate (r) as ~ -0.05 , which implies that, despite reproduction, the post-parthenogenetic egg cohort was doomed to extinction under complete food deprivation conditions.

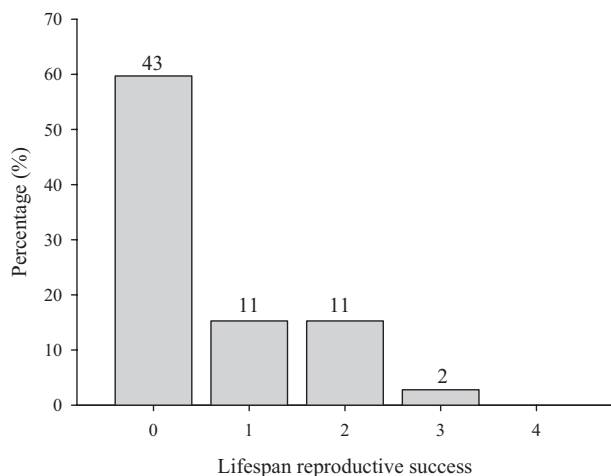


Fig. 4. Percentage of post-parthenogenetic egg females showing different lifetime reproductive successes. Numbers above bars are number of females. No offspring was observed for post-diapausing egg females under starvation.

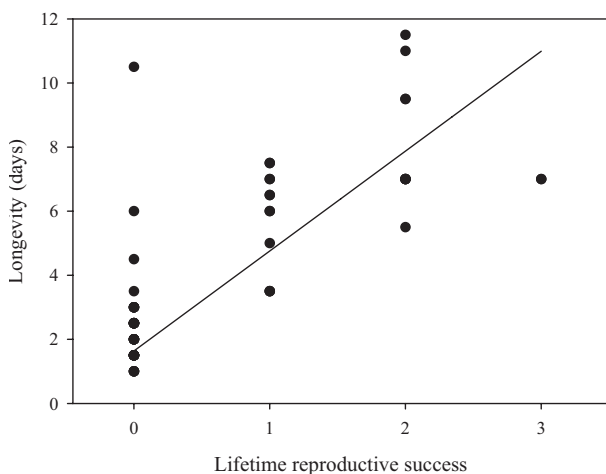


Fig. 5. Relationship between longevity and lifetime reproductive success of starved post-parthenogenetic egg females.

Figure 5 shows the association between survival time and investment in reproduction (number of descendants) in the post-parthenogenetic egg cohort. Correlation between these two variables resulted significant ($r = 0.795$, $P < 0.001$).

DISCUSSION

It has been proposed that diapausing eggs produce rotifer females more tolerant to starvation than parthenogenetic eggs (Gilbert, 2004). The suggested mechanisms for this tolerance would be that diapausing eggs would have more lipid reserves than parthenogenetic eggs, as observed in *B. calyciflorus* and *Asplanchna sieboldi* (Wurdak *et al.*, 1978;

Gilbert, 1983). This higher investment would cause the cost to produce one diapausing egg to be higher than that for a parthenogenetic one, as implied by the differences in the corresponding lifetime egg productions (Gilbert, 1983; Snell and Garman, 1986). Lipid reserves have been found to be abundant in neonates hatched from diapausing eggs in *B. calyciflorus* (Gilbert, 2004). A study by Tessier *et al.* (Tessier *et al.*, 1983) also demonstrated that lipid content is positively correlated to total energy reserves and resistance to starvation in several cladoceran *Daphnia* species. Increased starvation tolerance would have adaptive implications, since diapausing eggs hatch likely in the sediments, at some distance to the feeding sites. On the other hand, the females of many rotifer species carry their parthenogenetic eggs until they hatch, which likely implies energetic costs. This suggests that hatching in proximity to the mother (e.g. where the mother found food) is advantageous (Gilbert, 1983).

However, our results for *B. plicatilis* do not support the hypothesis of a higher starvation tolerance of females hatched from diapausing eggs than those hatched from parthenogenetic eggs. Females hatched from parthenogenetic eggs in *B. plicatilis* exhibited longer maximum survival times under starvation than those females developed from diapausing eggs; however, no significant differences were found between survival times. Moreover, a few of the former females exhibited fecundity, although it was low. By contrast, no offspring was produced by the post-diapausing egg cohort, which is also in agreement with the hypothesis that females hatched from diapausing eggs may have lower levels of energy reserves if compared to females from parthenogenetic eggs. Nevertheless, our data suggests that starvation tolerance is quite uniform in hatchlings from diapausing eggs. As a result, a high proportion (60%) of hatchlings from diapausing eggs with lowest survival, survived longer than the corresponding proportion in the post-parthenogenetic cohort.

Overall, our results contrast with the only previous report comparing starvation survival times of parthenogenetic and diapausing eggs in rotifers (Gilbert, 2004). Gilbert found that females hatched from diapausing eggs in two strains of the rotifer *B. calyciflorus* survived starvation significantly longer (1.2 times, 12 h) than those hatched from parthenogenetic eggs. Despite, the design of our experiment is similar to that used by Gilbert (Gilbert, 2004), some differences might account for the contrasting results. First, model organisms, although being co-generic, are not the same, and they are likely adapted to different habitats (e.g. salinity, temporality). Second, our experimental design tried to mimic natural conditions, and genetic variation was present in our

experimental populations. Note however that in our experiment, as in Gilbert (Gilbert, 2004), care was taken in order to collect diapausing and parthenogenetic eggs produced under the same environmental conditions, and food levels were not limiting rotifer growth. Third, we stored diapausing eggs for 2 months at low temperature and in the dark in order to obtain higher hatching rates (Hagiwara and Hino, 1989).

Regardless of the causes for our contrasting results, they show that the causal path '(i) diapausing egg, (ii) then increased allocation of reserves and (iii) then increased tolerance for starvation' is not always acting. First, high levels of energy reserves might be allocated to some parthenogenetic eggs. This is expected if resource allocation in parthenogenetic eggs would follow an opportunistic pattern, some of these eggs getting much more resources than others. Those parthenogenetic eggs with more resources could result in a longer average starvation tolerance of parthenogenetic offspring when compared to females hatched from diapausing eggs. It is worthwhile noting that if the function relating survival time under starvation to resource allocation is concave, variance in the latter would cause an increase in the average of the former (e.g. Horn and Rubenstein, 1984; Real and Ellner, 1992).

We found that parthenogenetic offspring of *B. plicatilis* showed higher variance in survival times than neonates from diapausing eggs. Interestingly, a simple survival theoretical model (i.e. Weibull model) describes the post-diapausing egg cohort, which contrasts with the post-parthenogenetic cohort. This higher homogeneity in the post-diapause offspring was found, even though diapausing eggs are the end product of sexual reproduction, and they are expected to be more diverse due to recombination in a genetically diverse population. If the variance in starvation tolerance of females hatched from parthenogenetic eggs is the result of a variance of egg resource levels, the latter could reflect a variance in the maternal food environment and the maternal stage (e.g. age). Females of different age, because of overlapping generations in the culture, might have been involved in parthenogenetic egg production which might influence progeny aging, as observed in bdelloid rotifers (Lansing, 1954). A large variance in resource allocation into parthenogenetic eggs is also suggested by our finding of a positive correlation, and not a trade-off, between fecundity and longevity, as expected from differences in female condition. However, although not measured, we did not observe noticeable differences in the size of parthenogenetic eggs, a characteristic correlated with longevity of hatched rotifers (Kirk, 1997). The effect of variation in the maternal status and environment on egg resource allocation is expected to be lower for

diapausing eggs, since they last a longer time in being produced, and the intrinsic (e.g. age) and extrinsic (e.g. food supply) variation would be averaged.

It is still unclear whether the high costs (i.e. low rates) of diapausing egg production in *B. plicatilis* are associated to high resource allocation (i.e. lipid content), as they are in other rotifer species. Other explanations based on the cost of producing resistant shells are possible. However, even if diapausing eggs would have more resources than any of the parthenogenetic egg of the same population, diapausing egg resources could be used for processes other than starvation tolerance. Several works suggest that reserves may become partially depleted during diapause (Perona and Vallejo, 1989; Williams-Howze *et al.*, 1998; García-Roger *et al.*, 2005), although respiration rates of developing diapausing eggs have not been measured, and they are assumed to be low at least under specific conditions (e.g. anoxia; Clegg, 1997). It is also possible that neonates emerging from diapausing eggs waste great amounts of energy in the hatching event. In fact, Santer and Boldt (Santer and Boldt, 1998) have observed a sudden decrease in total lipid content after emergence from diapausing eggs in copepods. Interestingly, we observed some diapausing eggs where the animal was not able to exit from the envelope and resulted in abortive hatchlings, although embryo development seemed to have been completed (data not shown). Furthermore, post-diapausing egg rotifer neonates could have higher metabolic rates. Arbačiauskas and Lampert (Arbačiauskas and Lampert, 2003) found that post-diapause females of the cladoceran species *Daphnia magna* had higher metabolic rates than females derived from parthenogenetic eggs, which may lead to shorter survival times under starvation in the former. Higher metabolic activity in ex-diapausing egg females implies that more food resources are required for development and growth.

It is expected that rotifer diapausing eggs, as actually observed in *Daphnia* species (Arbačiauskas, 1998), will hatch in response to stimuli correlated to favourable conditions. *Brachionus plicatilis* populations usually inhabit shallow temporary ponds in which light exposure, temperature and oxygenation at the beginning of a growth season may promote algal growth and also diapausing egg hatching. For such populations, hatching stimuli might particularly be well correlated to the food availability in the near environment of the eggs. This would contrast with the common assumption for deep-lake dwelling rotifers, which should migrate long distances under food deprivation conditions after the breaking of diapause and egg hatching. In this case, starvation tolerance, and so lipid reserves, would be a critical adaptation.

In summary, as a result of our study, we hypothesize that resource allocation in diapausing egg production follows a conservative pattern, while it is more opportunistic in parthenogenetic eggs. We conclude that the resources allocated in diapausing eggs do not always result in an average starvation tolerance higher than in parthenogenetic eggs. This could be caused by the diversion of energy to other functions or the result of the variance in the allocation of resources in parthenogenetic eggs. Despite its important evolutionary, adaptive and demographic consequences, zooplankton diapause is still a poorly characterized phenomenon. Our work shows that we need to increase the number of case studies in order to find general patterns of traits associated with diapause, namely starvation tolerance of rotifer neonates from diapausing eggs. As in other life history phenomena, regularities will likely arise as an association of traits to specific type of habitats.

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