Journal of Plankton Research, 2025, **47**, fbaf050
https://doi.org/10.1093/plankt/fbaf050
Original Article

Bet hedging via intermediate hatching fractions: demographic evidence from rotifer diapausing egg banks

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Corresponding editor: Lars-Anders Hansson

ABSTRACT

In this study, we investigated how hatching fractions of diapausing eggs in rotifers are related to variation in reproductive success in different habitats. It is postulated that an intermediate hatching fraction may be a bet hedging strategy, an adaptation that reduces the risk of reproductive failure by prolonging diapause, which decreases success in favorable seasons but ensures survival in unfavorable ones. Through the analysis of age-structured egg banks from eight populations of the rotifer *Brachionus plicatilis* in Spain, we were able to estimate variation across time in reproductive success. In addition, we used data obtained in previous studies, in which diapausing eggs were isolated from the most recent sediment layer of the ponds inhabited by the studied rotifer populations and these eggs were subjected to hatching experiments under controlled laboratory conditions. We found that hatching fractions were lower in populations with greater variation in reproductive success, confirming the predictions of bet hedging. However, no significant relationship was found between variation in reproductive success and hydrological conditions, such as the predictability of alternating dry and wet periods. Therefore, it is still necessary to identify the specific environmental factors that generate time uncertainty in the reproductive success of these rotifers.

KEYWORDS: hatching; diapause; diversification of risk; variation in reproductive success; rotifers

INTRODUCTION

Many zooplankton can produce resistance forms, typically in the form of diapausing eggs, to overcome periods of adverse conditions in the water column. The accumulation of these resistant forms in the sediments can lead to the formation of very dense egg banks, which serve as both demographic and genetic reservoirs for the populations (Hairston, 1996). The dynamics of these egg banks—particularly the hatching strategy of diapausing eggs, understood here as the conditions and patterns under which they resume development and hatch in response to environmental cues—are relevant to a variety of ecological and evolutionary processes in zooplankton, including population persistence in temporary habitats, colonization dynamics, local adaptation to environmental unpredictability and the evolution of bet hedging strategies (e.g. Hairston and Kearns, 2002; Brendonck and De Meester, 2003). The hatching fraction of diapausing eggs is informative on the duration of diapause and has been considered as a model trait for studying adaptations to environmental uncertainty (Brendonck and De Meester, 2003; García-Roger *et al.*, 2017; Pinceel *et al.*, 2021). Lower hatching fractions indicate that a larger proportion of eggs remain dormant, extending diapause across seasons and thereby reducing the risk of complete failure in unpredictable environments. Drawing inspiration from theoretical studies on the optimal duration of seed dormancy in variable environments (Cohen, 1966; MacArthur, 1972; Venable and Brown, 1988; Venable, 1989), it has been advocated that intermediate hatching fractions of diapausing eggs—i.e. when only a subset of diapausing eggs hatch under favorable conditions while others remain dormant—may represent a bet hedging strategy (Brendonck and De Meester, 2003; García-Roger *et al.*, 2014, 2017; Franch-Gras *et al.*, 2017a): an adaptive reduction of temporal variation in a genotype's fitness across generations in unpredictable environments, at the expense of reducing its expected fitness in any given season (Philippi and Seger, 1989). Among zooplankton, cyclical parthenogens have been a recurring subject of study in relation to bet hedging (e.g. Serra *et al.*, 2019). In these organisms, each diapausing egg is the product of sexual reproduction and, upon hatching, gives rise to a clonal lineage through asexual proliferation. All individuals within the lineage share the same genotype, so the population can be conceptualized as an assemblage of clones (genotypes) during the asexual phase (Gómez and Carvalho, 2000). When environmental cues trigger the onset of sexual reproduction, each genotype contributes to the production of a new cohort of diapausing eggs. These eventually settle in the sediment, where they can persist through adverse conditions, enabling the recolonization of the water column when suitable conditions return, thereby starting a new growing season (Brendonck and De Meester, 2003; Serra *et al.*, 2019). Delayed hatching of a fraction of a genotype's diapausing egg progeny buffers it from the risk of a complete reproductive failure

Received: March 30, 2025. **Revised:** August 22, 2025. **Editorial decision:** August 26, 2025. **Accepted:** August 27, 2025

during an unexpectedly unfavorable growing season, although it simultaneously reduces the potential to fully exploit the habitat—i.e. to take advantage of suitable conditions in the water column for rapid population growth and maximum reproductive success—when growing seasons are favorable (García-Roger *et al.*, 2014). Compared to theoretical development, empirical evidence of bet hedging in diapausing egg hatching is still scarce to date. This is true even for the main qualitative prediction of bet hedging models, namely that the higher the risk in the growing season, the lower the hatching fraction.

Intermediate hatching fractions of diapausing eggs have been reported in several zooplankters, in cyclical parthenogens such as branchiopods (Simovich and Hathaway, 1997; Philippi *et al.*, 2001; Brendonck and Riddoch, 2001; Cáceres and Tessier, 2003; Pinceel *et al.*, 2017; Wang and Rogers, 2018) and rotifers (García-Roger *et al.*, 2014; Tarazona *et al.*, 2017; Franch-Gras *et al.*, 2017a), but also in copepods (De Stasio, 2004). For bet hedging to be unequivocally demonstrated in this trait it is imperative that a variable response (i.e. staggered hatching over time of a cohort of diapausing eggs) occurs at the within-genotype level and in anticipation of an uncertain next growing season (García-Roger *et al.*, 2017). While these aspects have been addressed experimentally in the laboratory confirming bet hedging predictions (Tarazona *et al.*, 2017), evidence in the field regarding the anticipation of unpredictable adverse conditions remains inconclusive (Franch-Gras *et al.*, 2017a). This is partly due to the fact that field-based studies have majorly relied on rough proxies of habitat uncertainty—such as the interannual variation in rainfall at the study sites—or, at best, on metrics of the degree of environmental predictability that the target organism is supposed to perceive—like Colwell's predictability index, based on the interannual variation in the presence or absence of water (Franch-Gras *et al.*, 2017a, 2017b)—but not on direct measures of reproductive success and its variation. Obviously, there must be a causal link between habitat suitability in a particular growing season and reproductive success—i.e. variation in reproductive success constitutes the demographic expression of environmental heterogeneity (Pake and Venable, 1996; Venable, 2007)—but this is not always established accurately enough to determine the uncertainty experienced by the organisms (Pinceel *et al.*, 2021). For instance, even when abundant rainfall occurs periodically (and water is present in the habitats), conditions may be unsuitable due to factors like salinity exceeding tolerance ranges of the focal organisms, or the presence of antagonistic species. In fact, some of these factors—most notably salinity—may serve as cues for zooplankton in temporary water bodies to assess the phase of flooding-desiccation cycles. For example, high salinity may be informative of a short hydroperiod, which is likely to result in reduced reproductive success (Nielsen *et al.*, 2003; Vanschoenwinkel *et al.*, 2009). The accuracy of such a cue in predicting the actual length of the hydroperiod would determine the degree of predictability of that habitat and could influence the evolution of more or less bet hedging in the hatching of diapausing eggs. Furthermore, the adaptive value of delayed hatching relies not only on the likelihood of reproductive failure during a growing season but also on the chances to survive in the sediment egg bank. While diapausing eggs are generally assumed to be less vulnerable to environmental hazards than hatchlings, they are not invincible (García-Roger *et al.*, 2019). Egg burial, predation, disease and deterioration can reduce the benefits of delayed hatching (Ellner, 1985a, 1985b; García-Roger *et al.*, 2006a). Theoretical models of bet hedging also predict that hatching rates will increase as sediment adversity rises (García-Roger *et al.*, 2014)—that is, as abiotic or biotic conditions in the sediment become more hostile to diapausing egg survival (e.g. anoxia, toxic compounds or the presence of egg predators; García-Roger *et al.*, 2006a)—but this prediction has not been empirically verified either.

In this study, we investigated whether demographic data from egg banks can be used to reconstruct the history of variation in reproductive success of a cyclical parthenogenetic rotifer and infer a measure of habitat stability to be tested against diapausing egg hatching fractions. Rotifer diapausing egg banks provide an excellent system for such analyses, as they act as accurate demographic archives (García-Roger *et al.*, 2006b). The euryhaline rotifer *Brachionus plicatilis*—undoubtedly the rotifer species for which the most data on diapausing egg banks are available (García-Roger *et al.*, 2006a–c)—produces diapausing eggs that accumulate in the sediment of temporary lakes and ponds. These eggs consist of embryos that enter developmental arrest after several cleavage divisions and are encased in a protective multi-layered shell. Many of these eggs remain viable at varying depths within sediment, which is often vertically structured and undisturbed, reflecting historical sedimentation layers (García-Roger *et al.*, 2006b). A notable feature of rotifer egg banks is that they allow the identification of diapausing eggs at different stages of deterioration, as well as the remains of empty shells left after egg hatching (García-Roger *et al.*, 2005, 2006a–c). This enables the estimation of total diapausing egg production at a given time and the reconstruction of the growth dynamics in the egg bank, thereby reflecting the historical variation in reproductive success of the passive population over time, by using sediment cores. Further, it is also possible to quantify the incidence of diapausing egg deterioration while buried, and hence to assess sediment adversity. The present study used demographic data from diapausing egg banks of *B. plicatilis* from various natural populations—obtained in a previous study from García-Roger *et al.* (2006b) and freely available at Zenodo (10.5281/zenodo.16356262)—to derive a measure of variation in reproductive success and to test it against the hatching fraction of diapausing eggs. The underlying rationale is that populations in more environmentally stable habitats are expected to show more consistent reproductive success across years, while those in unstable habitats experience greater fluctuations in reproductive success due to irregular opportunities for growth and reproduction. Thus, the temporal variability in diapausing egg production serves as a demographic proxy for habitat stability. Based on general bet hedging theory, we expected that populations experiencing lower variation in reproductive success over time exhibit higher diapausing egg hatching fractions. Further, we explored the correlation between this measure of variation in reproductive success and other commonly used metrics of habitat predictability for *B. plicatilis* based on water permanence conditions. Finally, we tested whether populations with lower diapausing egg survival rates in the sediments showed increased

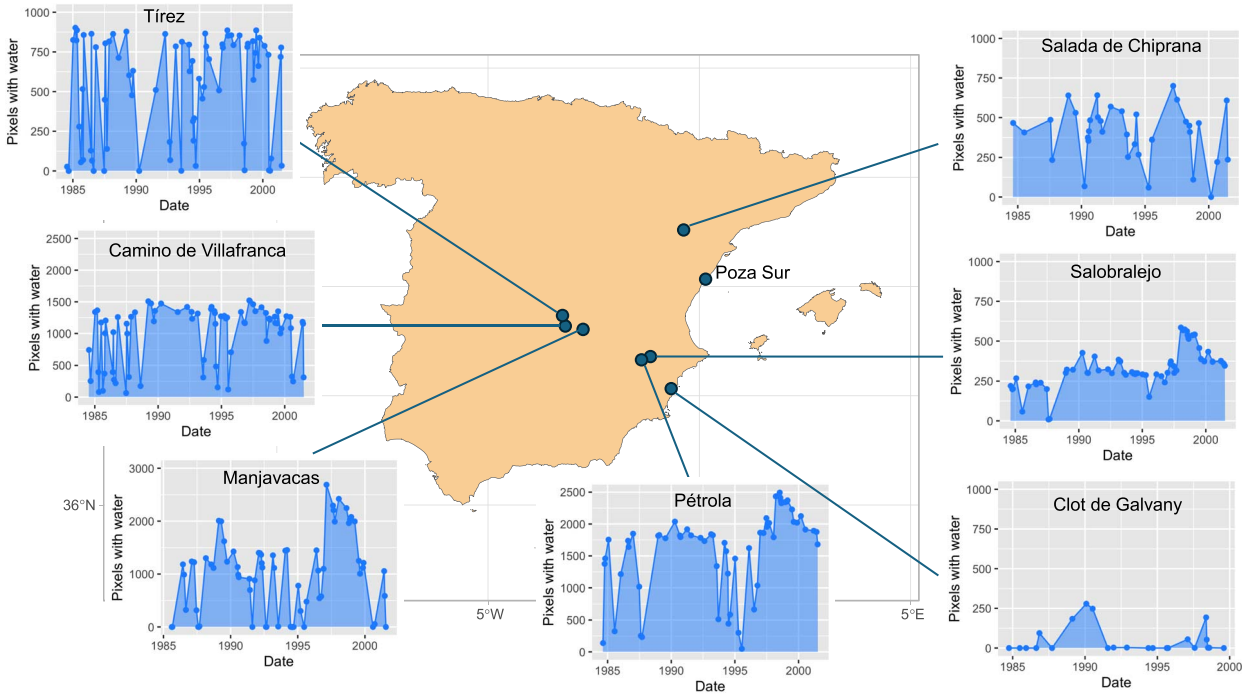

Figure 1. Map of Spain showing the location of the studied habitats. Hydrographs for the study period (1984–2000) are shown for each lake and pond, derived from satellite data gathered by LANDSAT's 5 and 7 missions (see main text for details). The hydrograph could not be determined for Poza Sur because of its size being smaller than the maximum spatial resolution of satellite imagery. One pixel corresponds to an area of 30 × 30 m.

hatching fractions as a strategy to escape from adverse sediment conditions.

METHOD

Studied populations and habitat characterization

This study focused on eight natural populations of *B. plicatilis* from Eastern Spain (Fig. 1). These populations inhabit a set of salt and brackish ponds and lakes where diapausing egg banks of this species had been previously detected and explored (García-Roger *et al.*, 2005, 2006a–c). The species belongs to a complex of cryptic species occasionally occurring in sympatry. However, from previous studies we know that the selected habitats predominantly harbor diapausing eggs of *B. plicatilis.* Based on PCR-RFLP analysis of the mitochondrial COI gene in clonal lines hatched from diapausing eggs, 80–100% of *Brachionus*-type diapausing eggs were identified as belonging to this species (unpublished results); for methodological details on species identification see Campillo *et al.* (2005).

The characterization of the habitats of the eight populations aimed to estimate pond and lake sizes as well as water permanence conditions (Table 1). These features were derived from a 16-year dataset (1984–2000) of satellite imagery collected from the Landsat Thematic Mapper (Landsat 5) and Enhanced Thematic Mapper Plus (Landsat 7) missions. This timeframe includes the earliest available data from the Landsat missions and the period immediately preceding the field sampling conducted to characterize the diapausing egg banks of the studied populations (see below). Raw satellite scenes were downloaded as Landsat Collection 2 L1 products (https://earthexplorer.usgs.gov/) and then radiometrically corrected to obtain top-of-atmosphere (TOA) reflectance values for each spectral band. These were used to compute the Modified Normalized Difference Water Index (MNDWI; Xu, 2006) with a pixel spatial resolution of 30 × 30 m. Pixels identified as potentially water-covered (MNDWI > 1) were further evaluated using near-infrared (NIR) reflectance to assess the presence of salt crust left by water evaporation (NIR > 0.4), which could generate false positives for water presence pixels (see Franch-Gras *et al.*, 2017b). Cloud-covered scenes were excluded from the analyses. Satellite data handling and analyses were performed using functions from the "raster" package (Hijmans, 2023) and customized scripts in R 4.2.2 (R Core Team, 2023). These data were used to construct time series of water presence (which can be consulted at 10.5281/zenodo.16356262) and, following the methodology described by Franch-Gras *et al.* (2017a, 2017b), to calculate the maximum inundation area, annual probability of desiccation events, and Colwell's (1974) predictability of desiccation and flooding patterns of the studied ponds and lakes. Dry periods were defined as reductions in inundation area exceeding 90% of the maximum, conditions under which the little water that remains forms small, scattered pools of brine with salinities beyond the tolerance range of rotifers (>250 g L^{-1}; *in situ* personal observation). Briefly, Colwell's metric quantifies unpredictability as the deviation from the product of marginal probabilities in a contingency table of state (with counts of either water presence or absence) versus months of the year. Therefore, in this metric the main source of environmental predictability is the inter-annual repetition of the within-year water presence pattern. Previous studies have proposed that this metric would

Table 1: *Location and hydro-morphological features of the studied ponds and lakes. Water body area, desiccation probability and Colwell's predictability were estimated from satellite data gathered by the LANDSAT's 5 and 7 missions over a 16-year period (1984–2000). Area is estimated from the maximum number of pixels covered by water in the whole series for each water body, and desiccation probability and Colwell's predictability are derived from water presence/absence data. These features could not be determined in the case of Poza Sur because of its small size (note that the minimum pixel size is 30 × 30 m).*

Pond/population	Acronym	Location	Area (km^2)	Desiccation probability (y^{-1})	Colwell's predictability
Laguna de Manjavacas	MAN	39° 24.615′ N, 2° 51.899′ W	1.37	0.53	0.40
Clot de Galvany	CDG	38° 14.999′ N, 0° 32.228′ W	0.25	0.40	0.06
Poza Sur (Torreblanca marsh)	TOS	40° 08.715′ N, 0° 10.059′ E	0.01	–	–
Laguna de Pétrola	PET	38° 50.555′ N, 1° 34.448′ W	2.24	0.06	0.92
Laguna Salada de Chiprana	CHI	41° 14.417′ N, 0° 10.874′ W	0.63	0.12	0.86
Laguna de Tírez	TIR	39° 32.631′ N, 3° 21.114′ W	0.82	0.18	0.87
Laguna del Salobralejo	SAL	38° 54.765′ N, 1° 28.275′ W	0.53	0.47	0.41
Laguna del camino de Villafranca	CVF	39° 25.009′ N, 3° 16.324′ W	2.42	0.24	0.93

match the unpredictability perceived by generalist zooplankton species like *B. plicatilis* (Franch-Gras *et al.*, 2017b).

Demographics of diapausing egg banks

Demographic data of diapausing egg banks of the studied populations were obtained from previous research (García-Roger *et al.*, 2005, 2006a–c). In short, three sediment cores were collected from randomly selected sampling points in each pond or lake studied during the summer of 2001 (Fig. 2). Each core was sliced into 2-cm layers, up to a maximum depth of 10 cm. In each layer, the numbers of diapausing eggs were classified based on their appearance and separately counted as viable (healthy looking and intact), hatched (empty shells with an operculum) and deteriorated (showing signs of structural damage or embryo degradation) (for details, see García-Roger *et al.*, 2006b). Following García-Roger *et al.* (2006a), the total egg production in a sediment layer i (E_i) was calculated as the sum of viable, hatched and deteriorated eggs, while the ratio of deteriorated to viable eggs was used as a measure of the sediment adversity in each habitat. Total egg abundance data were averaged from the three cores of each pond or lake at each depth layer and used to compute the finite growth rate of the diapausing egg bank between successive sediment layers ($\lambda_i = E_{i+1}/E_i$). As sedimentation rates, for the cases in which they were available, were relatively consistent across habitats (0.08 ± 0.01 cm y^{-1} after averaging available ^{137}Cs dating for a subset of three of the ponds studied see García-Roger *et al.*, 2006b), sediment layers were assumed to represent comparable periods of time, and λ_i was considered a measure of reproductive success in each period. For a sequence of periods, the long-term finite growth rate of the diapausing egg bank for each population was calculated as the geometric mean of λ_i's (hereafter $GM(\lambda_i)$), while the temporal variation in reproductive success was calculated as the standard deviation of the log-transformed λ_i values (hereafter, $SD(log(\lambda_i))$). Note that for a log-transformed variable, the standard deviation is a scale-invariant measure of variability and more robust to outliers than the coefficient of variation (Pake and Venable, 1996; Venable and Pake, 1999). These calculations were performed using data from all sediment layers containing eggs across the entire core for each population. As shown in the results (Fig. 3), in three populations no eggs were found in the deepest layer, so only the upper layers with egg presence were used in those cases.

Hatching fractions were calculated in a previous study García-Roger *et al.* (2006c; raw data available at 10.5281/zenodo.16356262) as the percentage of hatchlings from viable diapausing eggs isolated from the uppermost 2-cm of sediment from three cores per pond or lake, each taken at a different sampling point. Eggs were incubated under standard hatching conditions for *B. plicatilis* (details in García-Roger *et al.*, 2006c). These conditions—generally, a combination of moderate to high temperatures, light exposure and relatively low to intermediate salinities is required—simulate those at the beginning of the growing season and ensure rotifers to emerge in a favorable environment conducive to their development and survival.

Data analysis

Generalized linear models (GLMs) were used to test for differences in diapausing egg hatching fraction among populations. Hatching data were analyzed on the counts of hatchlings relative to viable diapausing eggs using a binomial distribution of errors and the logit link function. Pearson's correlation analysis was used to evaluate the relationships between the hatching fraction, the ratio of deteriorated to viable *B. plicatilis* diapausing eggs, and $SD(log(\lambda_i))$ of the egg bank of each population. We used this same approach to explore the association of $SD(log(\lambda_i))$ and the hatching fraction with the hydrological conditions of the set of ponds and lakes studied (i.e. the probability of desiccation events per year and Colwell's predictability metric based on water presence/absence). All statistical analyses were performed in R v 4.2.2 (R Core Team, 2023) using the "stats" package. GLM on hatching data was performed using the *glm* and *anova* functions, while correlation analyses were performed using the *cor.test* function.

RESULTS

The studied populations of *B. plicatilis* exhibited distinct growth dynamics in their diapausing egg banks, as evidenced by the varying depth profiles of total diapausing egg abundance and the differences in $GM(\lambda_i)$ values (Fig. 3). Some populations displayed relatively constant growth of their diapausing egg banks,

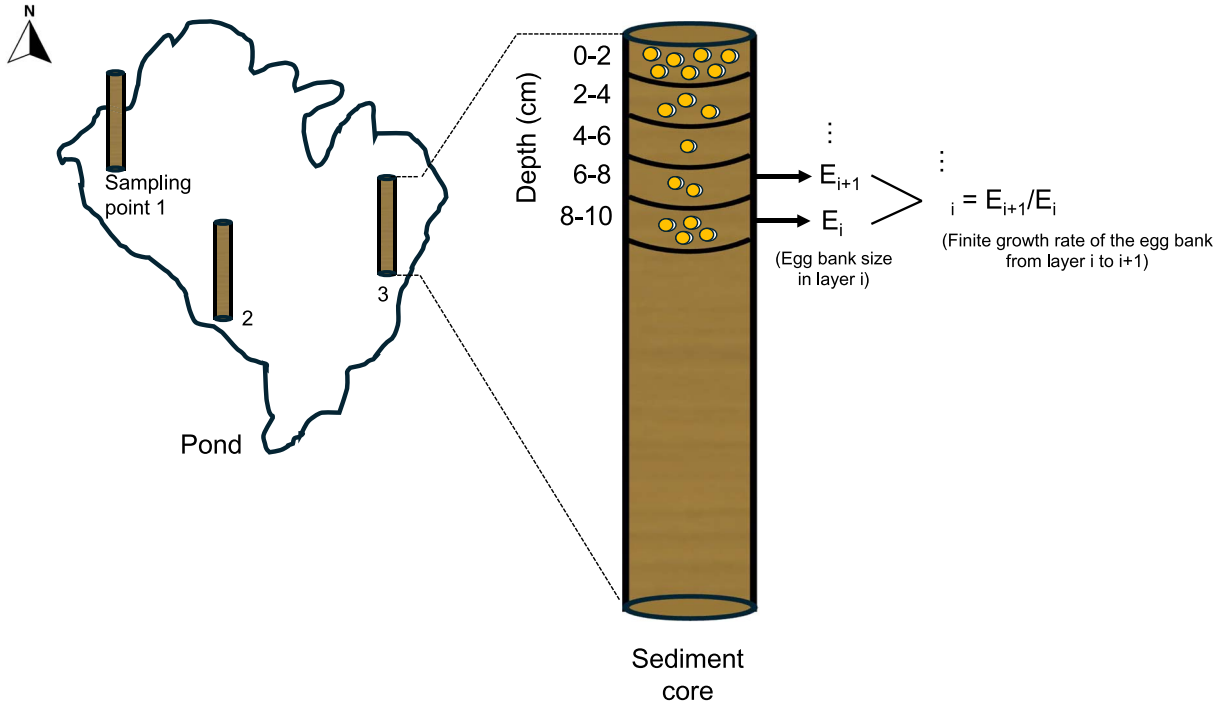

Figure 2. Schematic representation of the sampling design and procedure used to estimate the demographic properties of the diapausing egg bank, namely the population egg bank size at different sediment depth layers (E_i, for layer i) and the finite growth rate between two successive depth layers (λ_i).

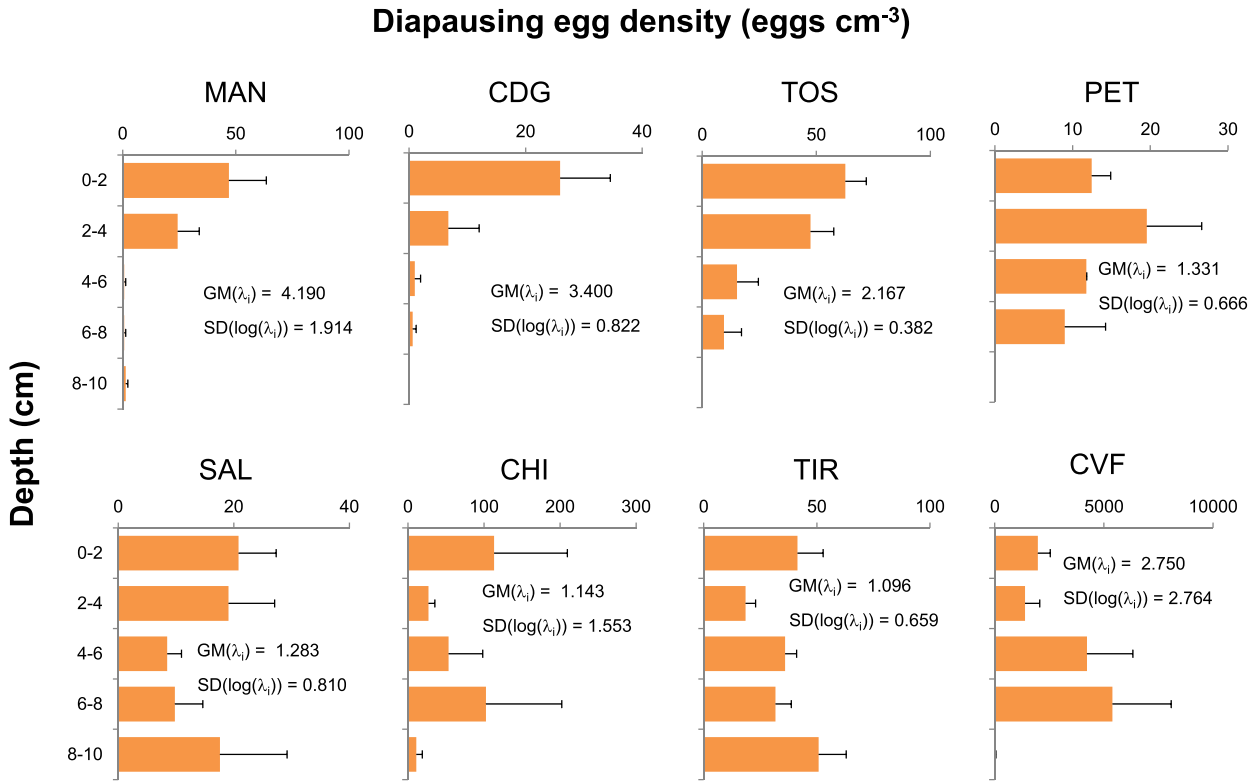

Figure 3. Depth profiles of the density of total diapausing eggs of *B. plicatilis* in the sediments of the studied ponds and lakes (acronyms are provided in Table 1). Data for each sediment layer are means from three sampling stations ±1 S.E. Note the different scales on the *x*-axes. The average of the logarithm of the finite growth rate of the egg bank between successive depth layers (λ_i) in a population ($M(log(\lambda_i))$) and the standard deviation of $log(\lambda_i)$ values ($SD(log(\lambda_i))$) are enclosed in each plot. Figure modified from García-Roger *et al.* (2006b)

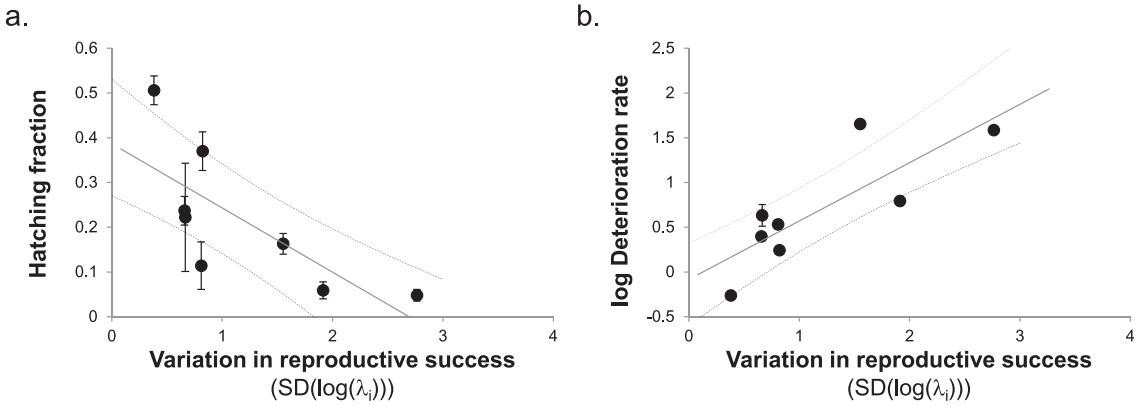

Figure 4. (a) Mean hatching fraction of diapausing eggs from eight natural populations of the rotifer *B. plicatilis*, calculated from eggs isolated from the upper 2 cm of three sediment cores per pond or lake, plotted against variation in reproductive success. (b) Mean values of the logarithm of the ratio of deteriorated to viable diapausing eggs in the sediments of the same eight populations plotted against variation in reproductive success. In both plots, demographic variation was measured as the standard deviation of the logarithm of the finite growth rate in the egg bank ($SD(log(\lambda_i))$) and error bars represent the standard errors of the mean values.

while others showed a pronounced variation in diapausing egg recruitment over time. These differences were effectively captured by the $SD(log(\lambda_i))$ values, which revealed that the populations under study covered a gradient from greater to lesser stability in growth conditions.

The hatching fractions of diapausing eggs differed significantly among rotifer populations (GLM, % deviance = 20.5; $df = 7$, 1 216; P-value < 0.001), ranging from 4.8% in CVF to 50.6% in TOS. Populations with lower $SD(log(\lambda_i))$ values, indicative of a lower variance in reproductive success through time, generally exhibited higher hatching fractions (Fig. 4a). The correlation between hatching fraction and $SD(log(\lambda_i))$ was negative and statistically significant (Pearson's $r = -0.739$, $t = -2.690$, $df = 6$, P-value = 0.036). No significant relationship was found between $SD(log(\lambda_i))$ and Colwell's predictability (Pearson's $r = 0.288$, $t = 0.673$, $df = 5$, P-value = 0.531) or the probability of desiccation events per year (Pearson's $r = 0.122$, $t = 0.275$, $df = 5$, P-value = 0.795). The hatching fraction of diapausing eggs did not correlate with either Colwell's predictability index (Pearson's $r = -0.355$, $t = -0.849$, $df = 5$, P-value = 0.435) or the probability of desiccation events (Pearson's $r = -0.215$, $t = -0.492$, $df = 5$, P-value = 0.643).

Sediment adversity was higher in those populations with higher $SD(log(\lambda_i))$ values (Fig. 4b), as evidenced by the positive and significant relationship found between the logarithm of the ratio of deteriorated to viable diapausing eggs and $SD(log(\lambda_i))$ (Pearson's $r = 0.730$, $t = 2.613$, $df = 6$, P-value = 0.04). Contrary to predictions, a negative correlation was found between the logarithm of the ratio of deteriorated to viable diapausing eggs and the hatching fraction (Pearson's $r = -0.770$, $t = -2.953$, $df = 6$, P-value = 0.026).

DISCUSSION

This study provides evidence that the hatching fractions of diapausing eggs are related to the variation in reproductive success experienced by rotifer populations in different habitats. The finding that lower hatching fractions occurred in populations experiencing higher temporal variation in reproductive success fits with the predictions of bet hedging theory, whereby risk is avoided in uncertain environments. According to recent theoretical research (Starrfelt and Kokko, 2012; Haaland *et al.*, 2019), bet hedging strategies are considered part of a continuum of responses to environmental variance, including the so-called variance-sensitive strategies. Within this framework, bet hedging is expected to be favored in the case of high environment correlation among offspring—that is, in coarse-grained environments where entire cohorts are affected by the same environmental conditions, that are distinct to those experienced by the mother—and when there is a significant risk of experiencing zero-fitness events in the offspring generation. Thus, the delayed hatching of a fraction of diapausing eggs and the formation of long-lived egg banks appear to be key adaptive bet hedging traits in the dynamics of rotifer genotypes and populations inhabiting water bodies where reproductive success of a whole generation may be occasionally threatened. Note that the demographic consequence of a prolonged diapause—mediated by delayed hatching—is to lengthen the generation time of the organism, which enhances the possibility of bridging different environmental conditions in its life. This in turn implies a refinement of the environmental grain experienced.

The finding that the hatching fraction differed between populations when subjected to a similar stimulus (i.e. under a common garden setting) suggests the existence of genetic differences in the trait, with genotypes exhibiting varying degrees of bet hedging. The negative relationship between hatching fractions and the variability in reproductive success within each population further suggests that rotifers may be adapted to local conditions of environmental predictability.

Our study shows how long-term data on reproductive success can be derived from previously collected demographic information from egg banks. This approach leverages the capacity of rotifer egg banks to function as historical records for reconstructing population dynamics and so inferring environmental properties, such as habitat stability (García-Roger *et al.*, 2006a). The demographic data utilized in this study came from natural

populations spanning a gradient in the variation of their reproductive success over time. This enabled a direct quantification of the association between bet hedging in the hatching of diapausing eggs and the potential effects on reproductive success under conditions of environmental uncertainty in the wild. In contrast to previous studies on bet hedging in zooplankton (Philippi *et al.*, 2001; Pinceel *et al.*, 2017; Franch-Gras *et al.*, 2017b), our study links variation in the hatching fraction to a direct measure of variation in reproductive success, rather than to proxies of the environmental uncertainty supposedly perceived by the organisms. How, or whether, the variation in these putative risk factors translates into actual reproductive success outcomes is rarely addressed (but see Venable, 2007, for seed germination).

Notwithstanding, identifying the specific environmental factor that varies unpredictably and drives the risk to these rotifer populations in the wild remains an open question. In this regard, we did not detect a clear association between the variation in reproductive success and commonly used metrics of habitat stability based on hydrological conditions, namely Colwell's predictability in the interannual regime of alternation between dry and wet periods, or the probability of desiccation events in the studied water bodies. Consequently, we also found no significant correlation between the hatching fraction and Colwell's predictability index, with the direction of the relationship being negative and contrary to expectations. We did find a negative, but non-significant, relationship between hatching fraction and the probability of desiccation. This unexpected finding is consistent with results from a previous study in the same system, in which hatching fractions from diapausing eggs were also not significantly correlated to either Colwell's predictability or hydroperiod length (Franch-Gras *et al.*, 2017a). While this may suggest that reproductive success variation is, to some extent, decoupled from variation in hydrological conditions, a definitive test would require a time-consuming and costing detailed study of the year-to-year variation in reproductive success vs hydroperiod length in each growing season. Moreover, gaps in the time series (e.g. due to cloud cover in satellite scenes) can affect the hydrological characterization, so complementary variables are often necessary to confirm the degree of uncertainty experienced by the organisms. These variables may be related to water permanence conditions (e.g. salinity, depth, area) or inform about habitat stability (e.g. taxa richness, since biological communities tend to be poorer in species in habitats with irregular fluctuations of environmental conditions; Slobodkin and Sanders, 1969). The latter approach successfully demonstrated a positive relationship between the hatching fraction in diapausing eggs of different populations of *B. plicatilis* and the zooplankton taxonomic richness of their habitats, in turn related to the water permanence (García-Roger *et al.*, 2014). Beyond purely hydrological conditions, we envisage a broader suite of biotic and abiotic factors—such as temperature, ionic concentrations, food supply, competition, predation—whose variation may influence reproductive success. The more or less predictable nature of the variation in these factors and their effects on the demography of rotifer diapausing egg banks, remain important avenues for future research.

In this study, we used the deterioration rates of diapausing eggs as a measure of the demographic effect of sediment adversity. This is a habitat feature that could modulate hatching rates, as the adaptive value of delayed hatching depends not only on the likelihood of reproductive failure during a growing season in the water column, but also on the probability of surviving in the sediment. It was therefore hypothesized that populations experiencing lower diapausing egg survival rates in the sediments would compensate by increasing hatching fractions to escape from adverse conditions. Contrary to this expectation, we observed a significant negative correlation between the log transformation of the deterioration rate and hatching fractions. In this manner, our results did not show the existence of an additive effect of sediment adversity on hatching. Rather, hatching fractions appeared to be essentially determined by the greater or lesser environmental stability of the habitats of the populations studied. Interestingly, populations with higher variance in reproductive success also exhibited greater deterioration of diapausing eggs. We interpret this result in accordance with the hypothesis that environmental variability translates into a greater risk to both survival and reproductive success. Therefore, we conjecture that the mortality observed in the diapausing embryos may, at least in part, be due to abortive hatchings triggered under unfavorable conditions, rather than solely from risks during diapause in the sediment itself. Additionally, we acknowledge that differences in the intrinsic quality of diapausing eggs may influence their susceptibility to degradation. However, we argue that such quality differences can be, at least in part, shaped by variation in water column conditions during their production. Consequently, habitats characterized by more variable conditions are likely to have a higher proportion of diapausing eggs prone to deterioration.

As a final note for the improvement of future studies, we acknowledge that this study was based on pre-existing data, which limited the possibility of adapting the sampling design to certain specific features of each location, such as spatial heterogeneity in the distribution of diapausing eggs and sedimentation rates within the individual ponds or lakes. Although we consider that this limitation does not compromise the validity of our findings, addressing such heterogeneity during the initial design phase of future studies would enhance the robustness and representativeness of the analyses. To this end, it would be advisable to consider increasing the number of sediment cores per location to better capture the natural variability of the system.

CONCLUSION

In conclusion, our study highlights a direct approach for testing a key, hitherto elusive, prediction of bet hedging theory. By focusing on the demographic consequences that drive the evolution of intermediate hatching fractions of diapausing eggs in rotifer genotypes, this approach relies on egg banks with specific characteristics—notably, structural integrity or preservation of the outer shells of diapausing eggs—to enable accurate estimation of total production and deterioration. This, in turn, allows for the inference of long-term reproductive success variation across populations. According to theoretical predictions, diapausing egg hatching rates are lower in those rotifer populations subject to greater variation in their reproductive success. While the observed differences in reproductive success variation likely reflect differing levels of environmental risk experienced in the wild, identifying the ultimate environmental factors responsible

for this uncertainty remains an important challenge for future studies.

ACKNOWLEDGEMENTS

This research was supported by grant PID2020-114153GB-I00, funded by MCIN/AEI/10.13039/501100011033/and by "ESF Investing in your future". The final version of the manuscript has greatly benefited from the insightful comments of two anonymous reviewers, to whom we thank for their constructive and generous feedback.

DATA AVAILABILITY

All the data that support the findings of this study are freely available at Zenodo (10.5281/zenodo.16356262).

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Journal of Plankton Research, 2025, Vol. 47, No. 5, fbaf050
https://doi.org/10.1093/plankt/fbaf050
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