REVIEW ARTICLE

Bet-hedging in diapausing egg hatching of temporary rotifer populations – A review of models and new insights

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Habitat unpredictability is a local adaptation factor shaping life-history traits in rotifer populations. It may select for the evolution of bet-hedging through risk-spreading strategies in diapausing egg hatching. This means that a fraction of diapausing eggs in wild populations do not hatch even when the conditions are favorable for population growth. Thus, there is a remaining fraction of viable diapausing eggs standing in the sediments for longer periods. According to theory, it is expected that the incidence of bet-hedging strategies for diapausing egg hatching will be higher in more uncertain habitats. Here, we review the major predictions derived from theoretical models applied to the case of monogonont rotifers. In the simplest "bad versus good season" models, the highest environmental uncertainty occurs when the probability of a good season is 0.5, and then the optimal hatching fraction is 0.5 too, implying maximum variance in hatching (i.e., maximum bet-hedging). However, there is still little evidence to support this prediction. This is most likely due to the lack of long-term data of habitat fluctuations and the difficulties in identifying and analyzing bet-hedging strategies, as well as the potential to confound genetic variation and phenotypic plasticity. Moreover, we provide new evidence supporting the occurrence of bet-hedging strategies associated with diapausing egg hatching in the *Brachionus plicatilis* species complex. Our analyses suggest a gradient of predictability in the habitats of these rotifers, and the existence of a significant positive correlation between the hatching fraction of diapausing eggs and an index of habitat predictability.

1 Introduction

At temperate latitudes, monogonont rotifer populations are typically temporary, so that they periodically re-colonize the water column in what is known as the planktonic growing season. Such a re-colonization starts with the hatching of diapausing eggs from pond and lake sediments [1, 2]. Diapausing egg hatching is risky because these sexually produced eggs are the only link between one growing season and the next, and there may be season-to-season fluctuations in the length of the growing season. These fluctuations may be related to the physical environment (e.g., presence/absence of water) or to biotic factors (e.g., presence/absence of competitors or predators), and impose habitat regimes for rotifers, which vary from highly predictable (when the length of the growing season extends regularly among seasons) to highly unpredictable (when there is great among-season variance in the length of the growing season). As a result of this uncertainty, diapausing egg hatching could be followed by a failure to complete the monogonont life cycle and produce a new cohort of diapausing eggs. A good strategy to deal with this risk would be that not all diapausing eggs

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produced by a single genotype hatch at once but over a series of growing seasons. If so, rotifers may bet-hedge in response to habitat unpredictability as proposed for annual plant seeds [3–9].

Bet-hedging is a concept first introduced by Bernouilli in 1738, which is now recognized in many research areas from economics to evolutionary biology [7]. It describes those mixed strategies that tend to minimize the global risk by spreading it across a set of independent events, hence trading-off some potential short-term benefit for a long-term benefit. Bet-hedging is thus expected to be adaptive in unpredictably changing habitats. Through bet-hedging, a genotype would randomly produce different phenotypes in advance of unknown future conditions. Such a variable offspring would reduce temporal fitness variance at the expense of lowered arithmetic mean fitness [10]. However, decreased variance is advantageous because it increases the expected geometric mean of fitness, which is the appropriate fitness measure in time-varying habitats [7, 11–13]. Unlike phenotypic plasticity, bet-hedging is conceived as a maternal strategy expressed in the offspring [14, 15].

Bet-hedging has been reported in biological traits from a broad range of organisms including micro-organisms [16–18], plants [19], invertebrates [20–24], birds [25], amphibians [26], and humans [27]. However, despite the well-developed theory for bet-hedging evolution – see Childs et al. [15] and references therein, but also Starrfelt and Kokko [28] and Olofsson et al. [29] –, few empirical studies have quantified the extent to which traits fit theoretical predictions [7]. One of the life-history traits in which bet-hedging has much theoretical development is the timing for leaving dormancy [3, 30, 31], although empirical evidence supporting it is still scarce [32].

In monogonont rotifers, not all diapausing eggs hatch in the season following to their production, so that eggs accumulate in the sediments of lakes and ponds forming diapausing egg banks, where they can remain viable for decades or even centuries [33–35]. This plays a crucial role for the persistence of populations in randomly varying habitats, and thus the proportion of dormant stages that hatch when favorable conditions resume (i.e., the hatching fraction) becomes a key life-history trait for the evolution of rotifer populations. Schröder [36, 37] suggested that this might be a case of bet-hedging if "early" (i.e., ready to hatch) and "delayed" (i.e., remaining in the egg bank) hatchlings are derived from the same clone. However, variability in the timing of diapausing egg hatching may be also the consequence of either adaptive phenotypic plasticity and/or genetic variability. Several mechanisms have been suggested in order to explain delayed hatching of diapausing eggs in rotifers, such as the presence/absence of appropriate cues promoting hatching in the water column – e.g., salinity, temperature, and light [38, 39] – conditions in

the sediment – including sediment mixing by bioturbation, and summarized as the degree of sediment adversity [40] or maternal effects [41, 42]. Notwithstanding, only the latter fits well with a bet-hedging strategy if rotifer mothers can control the offspring phenotype [36, 37]. To the date, adaptive strategies and mechanisms of rotifer diapausing egg hatching are still poorly understood.

In this contribution, our aim is to explore whether leaving dormancy is a life-history trait susceptible to bet-hedge in rotifers. To this end, the contribution is organized into two sections. First, we revisit the main bet-hedging models as applicable to rotifer diapausing egg hatching. Second, we report preliminary empirical data suggesting bet-hedging in this trait.

2 Models of bet-hedging for the hatching of diapausing eggs

Without exception, the many studies modeling bet-hedging for leaving dormancy [3, 30–32, 43–48] are inspired in the seminal approach developed by Cohen [3] to explain the optimal germination fraction of plant seeds under different degrees of environmental predictability. However, these model variants have features that have not been illustrated in detail (see below) and can be further developed to account for specific organisms – e.g., copepods and anostracans [31, 47, 48]. Here, we revisit and explore several bet-hedging models under Cohen's approach, being either parameterized or further developed to be applicable to rotifer diapausing egg hatching (Table 1). Note that in our conception, a rotifer clone is equivalent to an individual plant in the original model by Cohen [3].

To modify Cohen's approach for rotifers, we simplify the life-cycle of monogonont rotifers to two stages: active (i.e., females in the water column) and dormant (i.e., diapausing eggs in the sediments). Of these two stages, we focus on diapausing eggs because their dynamics meet all rotifer life-cycle processes and are related to the long-term measure of fitness in rotifers [49, 50]. Hence, in a given pond or lake, at the beginning of a new planktonic growing season a fraction h of the total number of viable diapausing eggs present in the sediments hatches to restore the active population. Then, rotifer clones proliferate, and, if the population reaches high density, sexual reproduction takes place and a new cohort of diapausing eggs is produced [51, 52]. Under Cohen's approach (Table 1, upper panel), during the planktonic growing season, a fraction d of the eggs that did not hatch deteriorate in the sediments (Fig. 1a). Unlike Cohen, we assumed that diapausing egg deterioration mostly occurs during the period between growing seasons (Table 1, lower panel). This assumption was first considered for anostracans by

Table 1. Models, as named in this study, within Cohen's framework used or referred to in the text

Dynamic equation	Model name	Description	Author
$\lambda_i = (1-h)(1-d) + hP_i$ (deterioration of diapausing stages occurs only during the growing season)	Binomial-Cohen	Two possible outcomes for diapausing stage production (P_i) in a growing season: (i) bad seasons with null production; (ii) good growing seasons with production overcompensating deterioration. The type of season cannot be anticipated. It is a "good vs. bad season" model	Cohen [3]
	Trinomial-Cohen	Three possible outcomes in a season: (i) and (ii) as in the Binomial-Cohen model; (iii) good seasons with diapausing stage production undercompensating deterioration	This study
	Normal-Cohen	Reproductive outcome of growing seasons distributed normally	Cohen [3]
	Clauss and Venable	Three possible types of seasons: (i) and (ii) as in the Binomial-Cohen model; (iii) seasons in which the cue promoting hatching is absent. Note that deterioration still operates in the sediments. Thus, there are cues informing that a growing season certainly starts, while there are good and bad seasons still uncertain	Clauss and Venable [32]
$\lambda_i = (1-h)(1-d) + hP_i(1-d)$ (deterioration of diapausing stages occurs within and between growing seasons)	Binomial-D[a)]	As in the binomial-Cohen, except for deterioration of diapausing stages	This study
	Trinomial-D	As in Trinomial-Cohen, except for deterioration of diapausing stages	This study
	Spencer et al.	Multiple possible outcomes for diapausing stage production in a growing season; density-dependent dynamics is explicitly considered	Spencer et al. [31]

Depending on the organisms, the original model might not refer to diapausing stages but to seeds, diapausing eggs, etc. The Spencer et al. model was not evaluated in this study; the other models were explored and or simulated using parameter values for rotifers (λ_i: finite growth rate; P_i: per capita production of diapausing eggs; h: hatching rate; d: deterioration rate of diapausing eggs).
a) D for deterioration.

Spencer et al. [31], and we further explore their dynamic equation here. Hence, we assume that the same yearly deterioration rate affects all diapausing eggs (i.e., unhatched and newly produced eggs) regardless of the timing of their production (Fig. 1b). This assumption is plausible if the growing season is short compared to the time period between growing seasons [53, 54]. Hence, in our approach the finite growth rate λ_i of the viable egg bank from the growing season $i-1$ to the growing season i is

$$\lambda_i = (1-h)(1-d) + hP_i(1-d) \qquad (1)$$

where P_i is the per capita diapausing egg production (i.e., the number of new diapausing eggs produced per

hatchling) within the ith growing season. For a sequence of t seasons, the long-term finite growth rate $\bar{\lambda}$ (i.e., the measure of fitness) of the diapausing egg bank is the geometric mean of the growth rates,

$$\bar{\lambda} = \left[\prod_{i=1}^{i=t} \lambda_i \right]^{1/t} \qquad (2)$$

Given a distribution of P_i for successive growing seasons, using computer simulation it is possible to find the optimum hatching fraction h_{opt} that maximizes the long-term finite growth rate $\bar{\lambda}$.

In the simplest instance of the dynamics described in Eq. (1), a binomial distribution of P_i values can be assumed

a

b
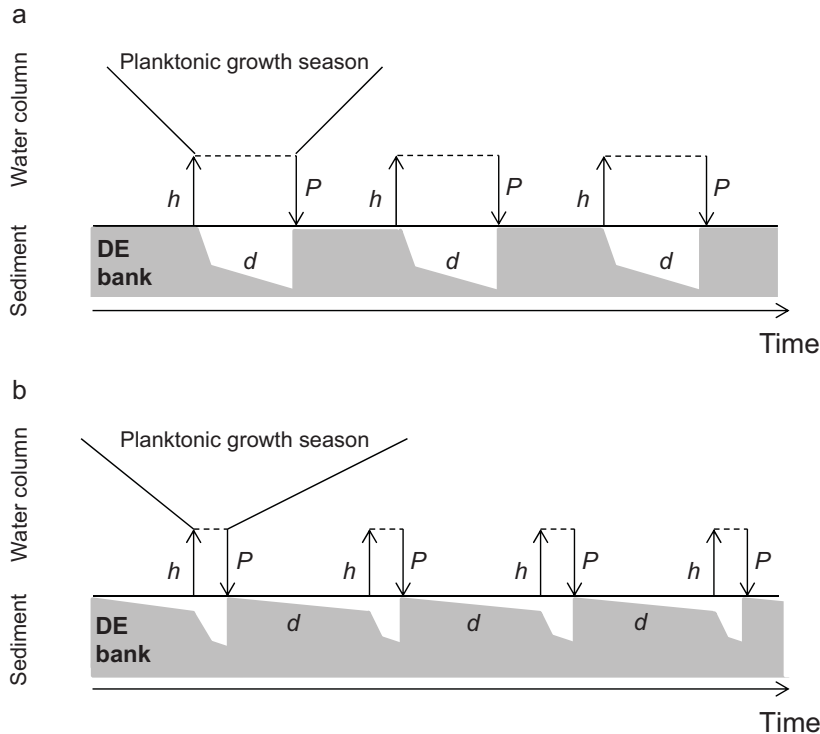
Figure 1. Outline of the dynamics of a diapausing egg bank during several planktonic growing seasons. (a) Deterioration of the diapausing eggs occurring only during the growing season (e.g., Binomial-Cohen model). (b) Deterioration of the diapausing eggs occurring continuously (e.g., Binomial-D model). Dashed lines indicate variable length of the planktonic growing season. Gray area height is the amount of diapausing eggs in the bank: *h*, hatching; *P*, production of new diapausing eggs; *d*, deterioration of diapausing eggs.

(Binomial-D model; Table 1). Such a distribution of P_i describes a "good versus bad season" scenario, which was originally analyzed by Cohen (here named Binomial-Cohen model; Table 1). In the good growing seasons, a high, constant per capita diapausing egg production ($P_g \gg 1$) occurs, while no production occurs in the bad growing seasons ($P_b = 0$). Being *p* and *q* ($=1-p$), respectively, the frequencies of good and bad planktonic growing seasons, the optimal fraction of diapausing egg hatching, h_{opt}, is

$$h_{opt} = \frac{pP_g - 1}{P_g - 1} \qquad (3)$$

(see the details for the analytical computation in Appendix 1).

Similar to the Binomial-Cohen model, the main prediction derived from the Binomial-D variant is that, if P_g is large, the hatching fraction that maximizes fitness (h_{opt}) equals the frequency of good seasons (*p*).

P_g can be roughly estimated for the well-studied case of *Brachionus plicatilis* using: (i) emergence rates of stem females from the uppermost 4 cm incubated sediments of several Spanish ponds – i.e., number of emerging females cm^{-2} [39], and (ii) the estimated egg bank sizes from the same depth and ponds as a measure of total diapausing egg production – i.e., number of diapausing eggs cm^{-2} [35]. By dividing total diapausing egg production by emergence rates for each pond we obtained a set of

values for the per capita diapausing egg production, P_g, which ranged from 50 to 15 000 diapausing eggs per hatchling depending on the pond.

Equation (3) can be illustrated by plotting h_{opt} against the variance of the binomial distribution of the planktonic growing seasons ($s^2 = tpq$, where *t* is the total number of seasons) (Fig. 2a). As intuitively expected, for large values of P_g (>50), h_{opt} was 1 when growing seasons were constantly good (i.e., massive hatching events in predictably good habitats). When good and bad seasons were equally frequent (i.e., maximum variance: completely unpredictable habitats), h_{opt} was 0.5 (i.e., maximum bet-hedging: 50% of "early hatchers" and 50% of "delayed hatchers"). As the frequency of bad seasons exceeded 50%, h_{opt} decreased (i.e., high dormant fraction in predictably bad habitats). For low P_g (e.g., $P_g \leq 10$) the h_{opt} curve shifted to low values (Fig. 2a).
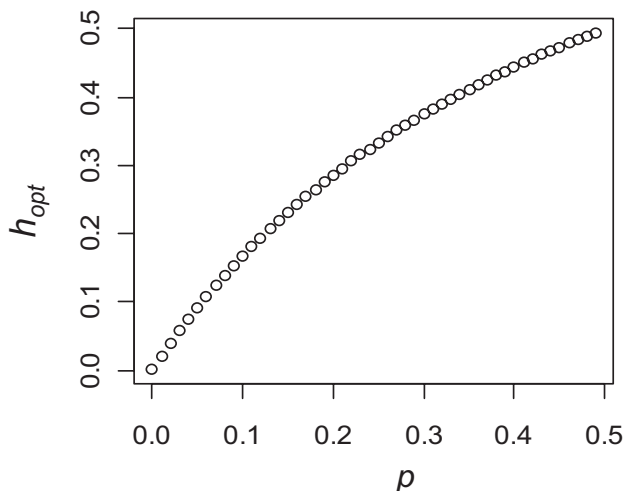
Most theoretical developments from Cohen's approach have typically omitted deterioration of diapausing stages in order to simplify the exploration of other parameters [43, 44]. However, recent studies have demonstrated that egg deterioration is very important in the dynamics of rotifer diapausing egg banks [35, 40, 55, 56]. In Fig. 2b, we show how egg deterioration in the sediment reduces the range of conditions in the water column in which the egg bank, and thus the population, can persist.

The predictions from the "good versus bad season" variants in both the Binomial-Cohen and Binomial-D model are generally robust when complexities are included, as for
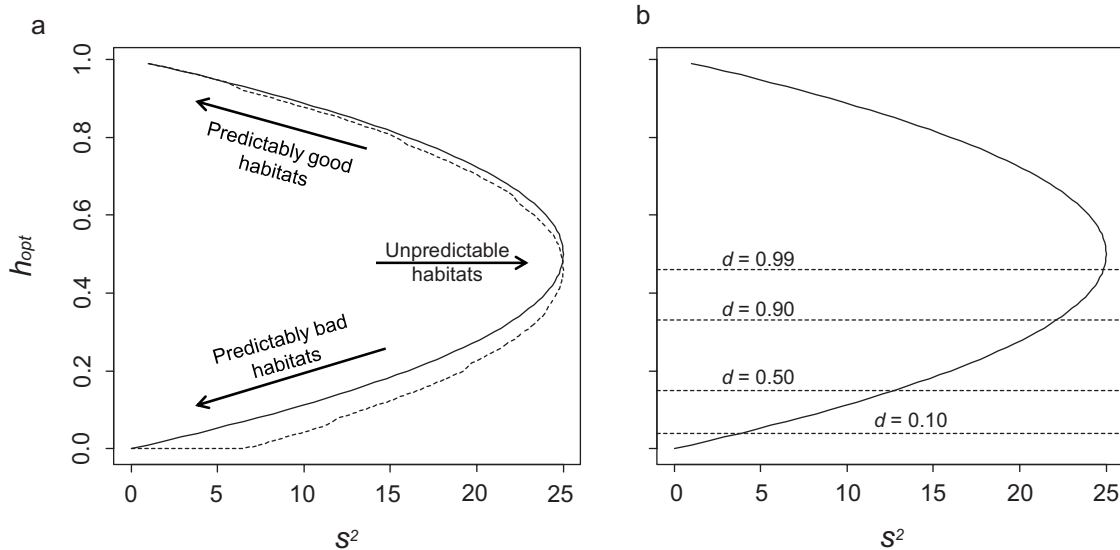
Figure 2. (a) Relationship between the optimal hatching rate (h_{opt}) and the environmental variance (s^2; variance over seasons of diapausing eggs produced per hatchling) according to the Binomial-D model. Results obtained after simulating rows of 100 growing seasons. (a) Solid line: 50 diapausing eggs per hatchling were produced in the good seasons (for diapausing egg production higher than 50, the corresponding curves are indistinguishable) Dashed line: 10 diapausing eggs per hatchling were produced in the good seasons. (b) Curve h_{opt} versus s^2 for a production of 1000 diapausing eggs per hatchling in the good seasons. Each horizontal dashed line correspond to a different deterioration rate (d) and splits the curve in two segments: viable populations (upper; long-term growth rate, $\bar{\lambda}$, >1) and non-viable populations.

instance when using multinomial distributions of P_i values (i.e., not all good seasons are equally good; Table 1), normal-like distributions of P_i values differing in average and variance (Normal-Cohen model; Table 1) or assuming density-dependent effects on P_i in addition to stochastic length of the planktonic growing season (Spencer et al. model; Table 1). However, predictions based on multino-

mial distributions of P_i may differ from the predictions of the binomial ones, especially if P_i is low. We showed this by developing the Trinomial-D model, where three possible types of growing season were considered: (i) over-compensating good seasons – with frequency p – in which diapausing egg production takes high values that overcompensate the loss of eggs due to deterioration [$P_o > 1 + d/(h(1-d))$]; (ii) bad seasons – with frequency q – in which no diapausing egg production occurs ($P_b = 0$), and (iii) undercompensating good seasons – with frequency $r[=1-(p+q)]$ – in which diapausing egg production does not compensate the loss of eggs due to deterioration [$P_u < 1 + d/(h(1-d))$]. In this model, h_{opt} does not equal the frequency of good planktonic growing seasons (p) but becomes

$$h_{opt} = \frac{pP_o - (p+q)}{(p+q)P_o - (p+q)} \tag{4}$$

An instance showing the relationship between h_{opt} and the frequency of overcompensating seasons (p) is depicted in Fig. 3. On the other hand, when considering these three possible types of growing season under the Trinomial-Cohen model [note that the condition for $\lambda_i > 1$ is $P_u > 1 + d(1-h)/h$], h_{opt} was found to converge to $p+r$ as deterioration rate (d) increased (Fig. 4). This result is very interesting as it describes a tendency of diapausing eggs to escape from the sediments when they are adverse.

Figure 3. Relationship between h_{opt} and the frequency of overcompensating seasons (p) according to the Trinomial-D model. A fixed $q = 0.5$ was assumed so that $r = 0.5 - p$.
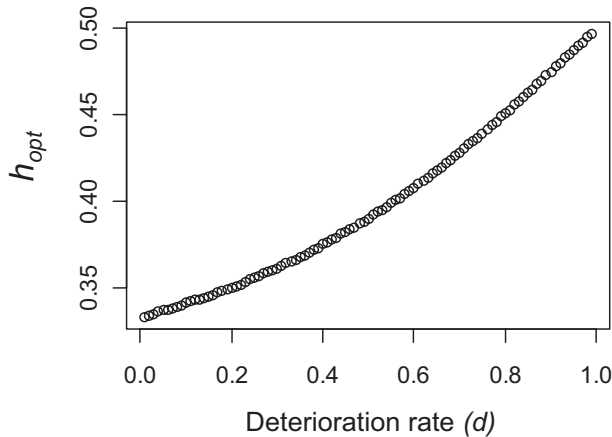
Figure 4. Relationship between h_{opt} and the deterioration rate (d) obtained after simulating rows of 1000 growing seasons for the Trinomial-Cohen model ($p = 0.25$, $q = 0.5$, $r = 0.5$, $P_o = 1000$, $P_u = 1$).

Clauss and Venable [32] developed a three-growing season variant (Table 1) from the original dynamic equation by Cohen [3]. They assumed that, besides unpredictable good and bad seasons, diapausing stages may also face seasons in which the cue promoting hatching is absent, what in the case of rotifers might be applicable to ponds that undergo desiccation and do not refill every year. We parameterized this model for rotifers and considered the three different kinds of seasons described by Clauss and Venable [32]: (i) seasons without any cue promoting hatching in which the absence of cue is an indicator that the season will be certainly bad ($\lambda_i = 1 - d$; i.e., the diapausing egg bank is depleted by deterioration); (ii) seasons in which the hatching cue occurs and is followed by favorable conditions in the water column [$\lambda_i = (1 - h)(1 - d) + hP_i$; i.e., suitable length of the growing season to produce a new cohort of diapausing eggs]; and (iii) seasons in which the hatching cue occurs but then is followed by unfavorable conditions resulting in the failure to produce new diapausing eggs [$\lambda_i = (1 - h)(1 - d)$; i.e., the diapausing egg bank is depleted by hatching and deterioration]. Hence, after a cue occurs, the season can be either good or bad with some uncertainty. Figure 5a–c shows the values of the long-term finite growth rate $\bar{\lambda}$ for different hatching strategies (h values) after running the model by Clauss and Venable [32] in three different simulated habitats: (i) a habitat in which the cue promoting hatching occurs in 90% of the seasons and then it is highly probable to be a good season for rotifer population growth and diapausing egg production (predictably good habitat, Fig. 5a), (ii) a habitat in which the cue promoting hatching only occurs in 10% of the seasons and it is likely that the growing season is bad (predictably bad habitat, Fig. 5b), and (iii) a habitat in which the cue
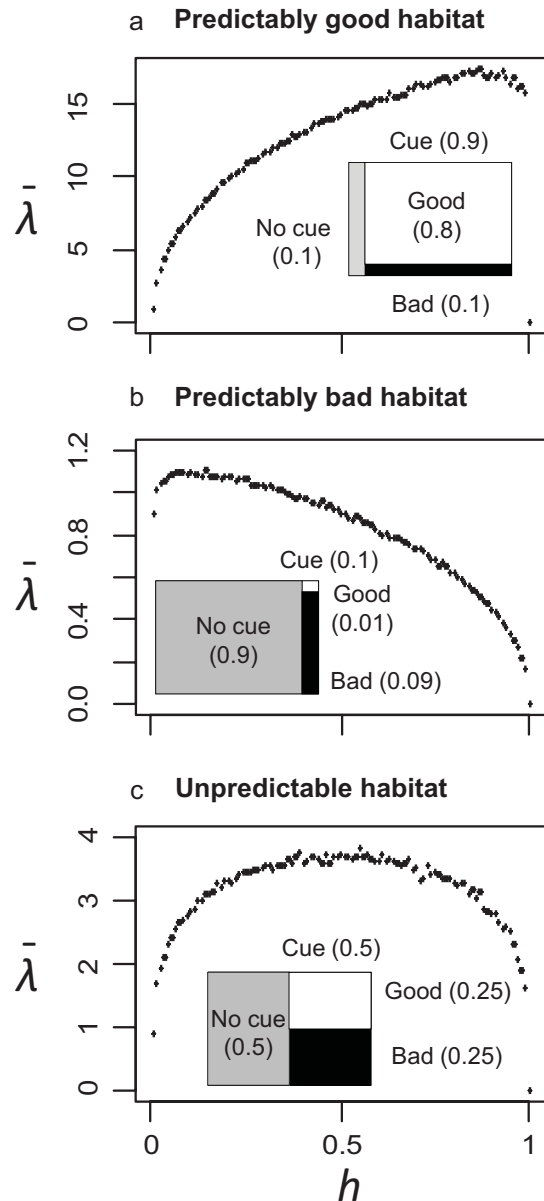
Figure 5. Relationships between the long-term growth rate ($\bar{\lambda}$) and the hatching rate (h) according to the Clauss and Venable model [32] in three different habitats: (a) a rather predictably good habitat, (b) a rather predictably bad habitat, and (c) an unpredictable habitat. Results obtained after simulating rows of 1000 growing seasons with $P = 1000$ in the good seasons and deterioration rate (d) = 0.1. Boxes and numbers within plots show the frequency of good (white) and bad (black) seasons, and seasons in which the hatching cue is absent (gray). h_{opt} can be obtained without computer simulation as the probability of a good season conditional to the hatching cue occurred [i.e., $h_{opt} = p(\text{Good} \mid \text{Cue}) = p(\text{Good} \cap \text{Cue}) / p(\text{Cue})$, therefore for a predictably good habitat as in (a), $h_{opt} = 0.8/0.9 \approx 0.9$].

promoting hatching occurs in 50% of the seasons and then there is an additional 50% probability of the growing season being good (unpredictable habitat, Fig. 5c). Considering that this model assumes that the absence of the cue is a constraint rather than an optimization strategy, results were similar to the Binomial-Cohen model as h_{opt} was found to converge to the probability of a season being good given that the cue promoting hatching has occurred [32].

3 Empirical evidence for bet-hedging in rotifer diapausing egg hatching

Despite the potential usefulness of a theory of optimal diapausing egg hatching, it has not been empirically evaluated in rotifers. An option for its evaluation would require the acquisition of long-term demographic data on hatching rates, deterioration in the sediments, and water column production of diapausing eggs, parameters generally not available. Moreover, it should be confirmed that non-genetic phenotypic variability for this trait exists in the populations.

Preliminary evidence for bet-hedging in rotifer diapausing egg hatching reduces to the observation that not all eggs derived from single clones in *Epiphanes senta* [36], and *Rhinoglena frontalis*, and *B. calyciflorus* [37] hatched after the exposure to a first hatching stimulus, while they did it in subsequent hatching events. The same applies for other zooplankters, such as anostracans [57, 58]. This observation of "early" and "delayed hatcher" phenotypes within a clone suggests bet-hedging. However, as predicted by theory [19], stronger evidence needs (i) to relate hatching fraction to predictability in the habitat, and (ii) to test for constant hatching fractions in subsequent events of hatching induction.

In this paper, in order to collect empirical evidence suggesting bet-hedging in rotifers, we tested the predicted relationship between habitat uncertainty and the hatching fraction of diapausing eggs in Spanish populations of the *B. plicatilis* species complex.

East Spain harbors a huge variety of ponds and small lakes, varying from small ephemeral to almost permanent ones, which are characterized by variable salinity. Strong seasonality and temporal unpredictability are also common [59]. Hence, these ponds and lakes likely embrace a gradient in habitat predictability. Information on the geographical distribution and population ecology of the *B. plicatilis* species complex in Eastern Spain is available after a series of studies in the last decade [35, 53, 55, 56, 60, 61]. We used these data for the multivariate ordination (PCA; Fig. 6) of 25 Spanish saline lakes and ponds inhabited by the *B. plicatilis* species complex, including variables thought to be related to water
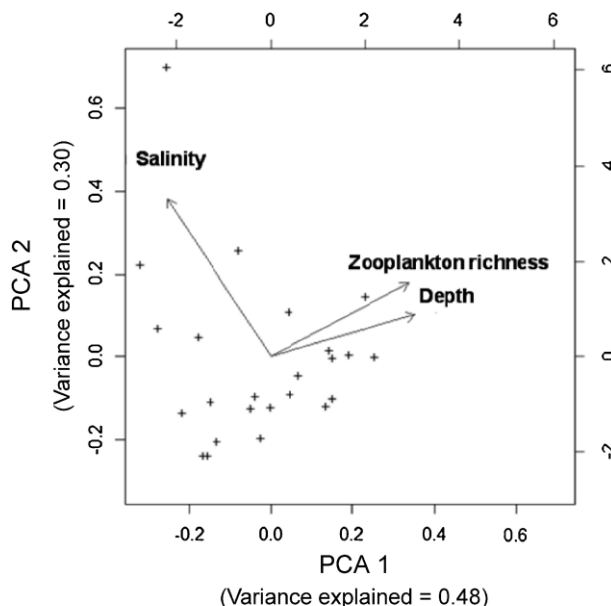
Figure 6. Ordination of the Spanish ponds and lakes where rotifers belonging to the *B. plicatilis* species complex have been observed in the space defined by the first two factors of the principal component analysis. Correlation coefficients: PCA 1 versus Zooplankton species richness = 0.65, PCA 1 versus depth = 0.61, PCA 2 versus salinity = 0.86. Limnological variables obtained from Ortells [60], Lapesa [53], García-Roger et al. [35, 56], and Campillo et al. [61].

permanence (e.g., salinity, depth, and area) or habitat predictability. For the latter, we followed Slobodkin and Sanders [62], who claimed that biological communities tend to be poorer in species in habitats with irregular fluctuations of environmental factors than in habitats characterized by regular and predictable fluctuations of the same magnitude. Therefore, zooplankton species richness was used as a proxy of habitat predictability and computed as the total number of zooplankton species observed during the growing season in each pond – data obtained from Ortells [60], Lapesa [53], and García-Roger et al. [39]. The first PCA axis (48% total variance) was positively related to the log transformations of pond depth and zooplankton richness, and so interpreted as a gradient from unpredictable to predictably good habitat conditions. The second PCA axis (30% total variance) ranked ponds along a gradient of increased salinity.

According to the expectations from a bet-hedging strategy, optimal hatching fraction in a habitat is expected to be independent on diapausing egg age, because every time viable eggs in the bank are exposed to hatching conditions, the same fraction should hatch. This provides a rationale for using here the hatching fraction estimated from a sediment layer likely integrating several bouts of
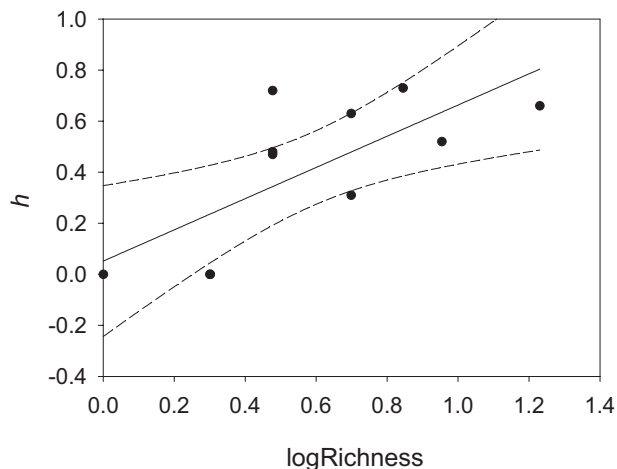
Figure 7. Relationship between (i) hatching fraction (*h*) of *B. plicatilis* species complex diapausing eggs induced in the laboratory and (ii) log-zooplankton species richness from the sites where the eggs were collected. Hatching data were originally published in García-Roger et al. [56].

diapausing egg production [19]. Hatching data of diapausing eggs of the *B. plicatilis* species complex were available for a subset of the ponds ordered in the PCA from a previous study by García-Roger et al. [56]. In that study, a combination of high temperature (25°C), low salinity ($6\,g\,L^{-1}$) and constant light was used to induce diapausing egg hatching. These conditions have been observed to successfully induce egg hatching in clones belonging to the *B. plicatilis* species complex from a wide variety of habitats [35, 41, 56, 60, 61]. Our assumption is that hatching fraction estimated in these optimized laboratory conditions is correlated to the hatching ratio in the wild. Furthermore, since most of the studied ponds undergo periods of desiccation, these conditions likely mimic water refilling and the start of a new growing season for the rotifer populations.

Then, by using zooplankton richness as a proxy of increasingly predictably good habitat conditions in these ponds we explored the relationship between hatching fraction and habitat predictability. A positive, significant correlation between hatching fraction and the log transformation of zooplankton species richness was found (Pearson's coefficient $r = 0.724$, $t = 3.148$, df $= 9$, *p*-value $= 0.006$; see Fig. 7).

4 Discussion

Theory predicts selection for a genotype producing offspring with randomly variable phenotypes in unpredictable habitats. Such a bet-hedging strategy can enhance long-term fitness by increasing the probability that at least a subset of individuals in the offspring will have an advantageous phenotype in a future environment [13, 14, 18, 63, 64]. In the case of rotifers inhabiting unpredictable habitats, genotypes may spread risk by producing offspring that vary in their emergence time from diapausing eggs.

In this paper, we have revisited the main models of bet-hedging for the optimal timing of leaving dormancy, expressed in terms of rotifer diapausing egg hatching. The choice of a specific model depends on the available information or reasonable assumptions for the population on focus (e.g., the extent of egg deterioration or how informative environmental cues are on future conditions). In our opinion, the adaptation of Cohen's approach to rotifers needs to consider that deterioration occurring outside the planktonic growing season in rotifer diapausing egg banks is probably high [35, 40, 56], while previous modeling assumed it does not occur or it was low [31]. Notwithstanding, by assuming high deterioration rates in the Binomial-D model, we found that the optimal hatching fraction is similar to that predicted originally in the Binomial-Cohen model (i.e., the frequency of good planktonic growing seasons), but only if the production of diapausing eggs in the good growing seasons is large in both cases [3].

Regardless explicitly incorporating deterioration outside of the growing season or not, results are generally consistent between both approaches. However, in our simulations we found an instance where differences arose in relation to the role of deterioration, showing a departure from the original prediction that the optimal hatching fraction equals the frequency of good growing seasons. We illustrated this difference through the trinomial variants of both models with three types of seasons (bad, undercompensating production, and overcompensating production). While the optimal hatching fraction in the Trinomial-D model was rather insensitive to change in deterioration rates, we found that the optimal hatching fraction in the Trinomial-Cohen model increased with increased deterioration rates in the sediments during the growing season. Then, despite the probability of a neonate of being unable to exploit the water column if the growing season is not long enough, hatching may still be advantageous because the most likely fate of a diapausing egg remaining in the sediment would be death [56].

Most likely, zooplankton populations do not only face unpredictability regarding quality of growing seasons. For instance, Vanoverbeke and De Meester [48] have modeled how unpredictability in the onset of the growing season may also promote delayed hatching as a bet-hedging strategy within a growing season. Nevertheless, in this study we focused on among-season variation due to the occurrence of long-lasting diapausing egg banks in rotifers [35], which cannot be explained by the timing of

hatching within a growing season. Moreover, our analysis lies on the notion that large variations in the quality of the growing seasons occur in many geographic areas, as it is the case of the Mediterranean region.

Information on habitat fluctuations is important to test bet-hedging predictions. Maffei et al. [47] have demonstrated that the hatching fraction of anostracan populations fits Cohen's predictions. They compared the hatching fraction against specific measures of habitat predictability based on Colwell's [65] metrics for the presence/absence of water (i.e., constancy and contingency). Because estimations of environmental fluctuation require long-term monitoring and are usually scarce, alternative approaches based on proxies have been used. For instance, Clauss and Venable [32] used the amount of rainfall needed for germination of seed from weather stations in South Western Arizona and Southern California to predict the probabilities of good seasons and optimal germination fractions. Other organisms may respond to changes in temperature, photoperiod, or any other stimuli that could act in this way [1, 2, 38]. Unfortunately, to the date the data required to quantify the strength of these associations is not available for rotifers. Instead, we used zooplankton richness as an indicator of habitat predictability [54, 62, 66, 67]. Our empirical survey showed the expected relationship between hatching fraction and predictability, suggesting that those populations inhabiting more unpredictable habitats hedge their bets by spreading hatching over several occasions.

Nevertheless, testing bet-hedging in diapausing egg hatching still requires investigating additional conditions [68]: (i) low heritability in the trait due to maternal control in the offspring phenotype, and (ii) population differentiation associated to habitat unpredictability. Monogonont rotifers and their habitats have features that make them suitable for testing these requirements. First, clonal proliferation makes feasible the creation of isogenic lines to test for maternal effects in the offspring phenotype. Second, we have showed that rotifer habitats in Eastern Spain embrace a gradient of predictability conditions, so constitute an ideal study system to quantify the relationships between the extent of diversification in the timing of diapausing egg hatching and the degree of environmental unpredictability. Third, rotifer populations show signatures for local adaptation in relation to diapause patterns [50]. Therefore, rotifer populations are a promising model to test the evolutionary theory of bet-hedging in an appropriate ecological context.

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5 References

[1] Gilbert, J. J., Dormancy in rotifers. *Trans. Am. Microsc. Soc.* 1974, *93*, 490–513.

[2] Pourriot, R., Snell, T. W., Resting eggs in rotifers. *Hydrobiologia* 1983, *104*, 213–224.

[3] Cohen, D., Optimizing reproduction in a randomly varying environment. *J. Theor. Biol.* 1966, *12*, 119–129.

[4] De Witt, T. J., Langerhans, R. B., Integrated: Solutions to environmental heterogeneity: Theory of multimoment reaction norms, in: De Witt, T. J., Scheiner, S. M. (Eds.), *Phenotypic Plasticity. Functional and Conceptual Approaches*, Oxford University Press, New York 2004, 98–111.

[5] Leimar, O., The evolution of phenotypic polymorphism: Randomized strategies versus evolutionary branching. *Am. Nat.* 2005, *165*, 669–681.

[6] Leimar, O., Multidimensional convergence stability. *Evol. Ecol. Res.* 2009, *11*, 191–208.

[7] Simons, A. M., Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proc. R. Soc. B: Biol. Sci.* 2011, *278*, 1601–1609.

[8] Svardal, H., Rueffler, C., Hermisson, J., Comparing environmental and genetic variance as adaptive response to fluctuating selection. *Evolution* 2011, *65*, 2492–2513.

[9] Volis, S., Demographic consequences of delayed germination in two annual grasses from two locations of contrasting aridity. *Perspect. Plant Ecol. Evol. Syst.* 2012, *14*, 335–340.

[10] Ripa, J., Olofsson, H., Jonzen, N., What is bet-hedging, really? – Invited reply. *Proc. R. Soc. B: Biol. Sci.* 2010, *277*, 1153–1154.

[11] Dempster, E. R., Maintenance of genetic heterogeneity. *Cold Spring Harbour Symp.* 1955, *20*, 25–32.

[12] Gillespie, J. H., Natural selection for within-generation variance in offspring number. *Genetics* 1974, *76*, 601–606.

[13] Seger, J., Brockmann, H. J., What is bet-hedging?, in: Harvey, P. H., Partridge, L. (Eds.), *Oxford Surveys in Evolutionary Biology*, Oxford University Press, Oxford 1987, 182–211.

[14] Crean, A. J., Marshall, D. J., Coping with environmental uncertainty: Dynamic bet hedging as a maternal effect. *Proc. R. Soc. B: Biol. Sci.* 2009, *364*, 1087–1096.

[15] Childs, D. Z., Metcalf, C. J. E., Rees, M., Evolutionary bet-hedging in the real world: Empirical evidence and challenges revealed by plants. *Proc. R. Soc. B: Biol. Sci.* 2010, *277*, 3055–3064.

[16] Tonegawa, S., Somatic generation of antibody diversity. *Nature* 1983, *302*, 575–581.

[17] Moxon, E. R., Rainey, P. B., Nowak, M. A., Lenski, R. E., Adaptive evolution of highly mutable loci in pathogenic bacteria. *Curr. Biol.* 1984, *4*, 24–33.

[18] Beaumont, H. J., Gallie, J., Kost, C., Ferguson, G. C., Rainey, P. B., Experimental evolution of bet hedging. *Nature* 2009, *462*, 90–93.

[19] Venable, D. L., Bet hedging in a guild of desert annuals. *Ecology* 2007, *88*, 1086–1090.

[20] Hairston, N. G., Munns, W. R., The timing of copepod diapause as an evolutionary stable strategy. *Am. Nat.* 1984, *123*, 733–751.

[21] Danforth, B. N., Emergence dynamics and bet hedging in a desert bee, *Perdita portalis*. *Proc. Natl. Acad. Sci. USA* 1999, *266*, 1985–1994.

[22] Friberg, M., Dahlerus, J., Wiklund, C., Strategic larval decision-making in a bivoltine butterfly. *Oecologia* 2012, *169*, 623–635.

[23] Pélisson, P.-F., Bernstein, C., François, D., Menu, F., Venner, S., Dispersal and dormancy strategies among insect species competing for a pulsed resource. *Ecol. Entomol.* 2013, *38*, 470–477.

[24] Rossi, V., Martorella, A., Menozzi, P., Hatching phenology and voltinism of *Heterocypris barbara* (Crustacea: Ostracoda) from Lampedusa (Sicily, Italy). *J. Limnol.* 2013, *72*, 227–237.

[25] Laaksonen, T., Hatching asynchrony as a bet-hedging strategy – An offspring diversity hypothesis. *Oikos* 2004, *104*, 616–620.

[26] Lips, K. R., Reproductive trade-offs and bet-hedging in *Hyla calypsa*, a neotropical frog. *Oecologia* 2001, *128*, 509–518.

[27] Wunderlich, K., Symmonds, M., Bossaerts, P., Dolan, R. J., Hedging your bets by learning reward correlations in the human brain. *Neuron* 2011, *71*, 1141–1152.

[28] Starrfelt, J., Kokko, H., Bet-hedging: A triple trade-off between means, variances and correlations. *Biol. Rev.* 2012, *87*, 742–755.

[29] Olofsson, H., Ripa, J., Jonzen, N., Bet-hedging as an evolutionary game: The trade-off between egg size and number. *Proc. R. Soc. B: Biol. Sci.* 2009, *276*, 2963–2969.

[30] Venable, D. L., Modeling the evolutionary ecology of seed banks, in: Leck, M. A., Parker, V. T., Simpson, R. L. (Eds.), *The Ecology of Soil Seed Banks*, Academic Press, San Diego 1989, 67–87.

[31] Spencer, M., Colegrave, N., Schwartz, S. S., Hatching fraction and timing of resting stage production in seasonal environments: Effects of density dependence and uncertain season length. *J. Evol. Biol.* 2001, *14*, 357–367.

[32] Clauss, M. J., Venable, D. L., Seed germination in desert annuals: An empirical test of adaptive bet hedging. *Am. Nat.* 2000, *155*, 168–186.

[33] Marcus, N. H., Lutz, R., Burnett, W., Cable, P., Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: Evidence of an egg bank. *Limnol. Oceanogr.* 1994, *39*, 154–158.

[34] Kotani, T., Ozaki, M., Matsuoka, K., Snell, T. W., Hagiwara, A., Reproductive isolation among geographically and temporally isolated marine Brachionus strains. *Hydrobiologia* 2001, *446/447*, 283–290.

[35] García-Roger, E. M., Carmona, M. J., Serra, M., Patterns in rotifer diapausing egg banks: Density and viability. *J. Exp. Mar. Biol. Ecol.* 2006, *336*, 198–210.

[36] Schröder, T., *Lebenszyklusstrategien planktischer Rotatorien (Monogononta, Rotifera) im Zusammenhang mit den saisonalen Überflutungen in der Flussaue des Unteren Odertals*, Thesis Dissertation, Freie Universität Berlin, Berlin 1999.

[37] Schröder, T., Diapause in monogonont rotifers. *Hydrobiologia* 2005, *546*, 291–306.

[38] Minkoff, G., Lubzens, E., Kahan, D., Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. *Hydrobiologia* 1983, *104*, 61–69.

[39] García-Roger, E. M., Armengol-Díaz, X., Carmona, M. J., Serra, M., Assessing rotifer diapausing egg bank diversity and abundance in brackish temporary environments: An ex situ sediment incubation approach. *Fundam. Appl. Limnol.* 2008, *173*, 79–88.

[40] García-Roger, E. M., Carmona, M. J., Serra, M., A simple model relating habitat features to a diapause egg bank. *Limnol. Oceanogr.* 2006, *51*, 1542–1547.

[41] Hagiwara, A., Hino, A., Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. *Hydrobiologia* 1989, *186/187*, 415–421.

[42] Hagiwara, A., Kadota, Y., Hino, A., Maternal effect by stem females in *Brachionus plicatilis*: Effect of starvation on mixis induction in offspring. *Hydrobiologia* 2005, *546*, 275–279.

[43] MacArthur, R. H., *Geographical Ecology*, Harper and Row, New York 1972.

[44] Venable, D. L., Lawlor, L., Delayed germination and dispersal in desert annuals: Escape in space and time. *Oecologia* 1980, *46*, 272–282.

[45] Ellner, S. P., Hairston, N. G., Jr., Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* 1994, *143*, 403–417.

[46] Ellner, S. P., You bet your life: Life-history strategies in fluctuating environments, in: Othmer, H. G., Adler, F. R., Lewis, M. A., Dallon, J. C. (Eds.), *Case Studies in Mathematical Modeling: Ecology, Physiology, and Cell Biology*, Prentice-Hall, New Jersey 1997, 3–24.

[47] Maffei, C., Vagaggini, D., Zarattini, P., Mura, G., The dormancy problem for Crustacea Anostraca: A rigorous model connecting hatching strategies and environmental conditions. *Ecol. Model.* 2005, *185*, 469–481.

[48] Vanoberbeke, J., De Meester, L., Within season short-term hatching delays suggest risk-spreading behavior in populations of the freshwater cladoceran Daphnia. *Ecoscience* 2009, *16*, 441–451.

[49] Serra, M., King, C. E., Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. *J. Evol. Biol.* 1999, *12*, 263–271.

[50] Campillo, S., García-Roger, E. M., Carmona, M. J., Serra, M., Local adaptation in rotifer populations. *Evol. Ecol.* 2011, *25*, 933–947.

[51] Carmona, M. J., Serra, M., Miracle, M. R., Relationships between mixis in *Brachionus plicatilis* and preconditioning of culture medium by crowding. *Hydrobiologia* 1993, *255–256*, 145–152.

[52] Stelzer, C. P., Snell, T. W., Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. *Limnol. Oceanogr.* 2003, *48*, 939–943.

[53] Lapesa, S., *Efecto de la depredación por invertebrados sobre poblaciones simpátricas de especies crípticas de rotíferos*, PhD Thesis Dissertation, Universitat de València, Valencia 2004.

[54] Wallace, R. L., Walsh, E. J., Arroyo, M. L., Starkweather, P. L., Life on the edge: Rotifers from springs and ephemeral waters in the Chihuahuan Desert, Big Bend National Park (Texas, USA). *Hydrobiologia* 2005, *546*, 147–157.

[55] García-Roger, E. M., Carmona, M. J., Serra, M., Deterioration patterns in diapausing egg banks of Brachionus (Muller, 1786) rotifer species. *J. Exp. Mar. Biol. Ecol.* 2005, *314*, 149–161.

[56] García-Roger, E. M., Carmona, M. J., Serra, M., Hatching and viability of rotifer diapausing eggs collected from pond sediments. *Freshwater Biol.* 2006, *51*, 1351–1358.

[57] Saiah, H., Perrin, N., Autumnal vs. spring hatching in the fairy shrimp *Siphonophanes grubii* (Dybowski) (Crustacea, Anostraca) – Diversified bet-hedging strategy. *Funct. Ecol.* 1990, *4*, 769–775.

[58] Simovich, M. A., Hathaway, S. A., Diversified bet-hedging as a reproductive strategy of some ephemeral pool anostracans (Branchiopoda). *J. Crust. Biol.* 1997, *17*, 38–44.

[59] Blondel, J., Aronson, J., Boudiou, J. Y., Boeuf, G., *The Mediterranean Basin – Biological Diversity in Space and Time*, Oxford University Press, Oxford 2010.

[60] Ortells, R., *Diversidad genética y ecológica en especies crípticas de rotíferos: Patrones y procesos*, PhD Thesis Dissertation, Universitat de València, Valencia 2002.

[61] Campillo, S., García-Roger, E. M., Carmona, M. J., Gómez, A., Serra, M., Selection on life-history traits and genetic population divergence in rotifers. *J. Evol. Biol.* 2009, *22*, 2542–2553.

[62] Slobodkin, L. B., Sanders, H. L., On the contribution of environmental predictability to species diversity. *Brookhaven Symp. Biol.* 1969, *22*, 82–95.

[63] Philippi, T., Seger, J., Hedging one's evolutionary bets, revisited. *Trends Ecol. Evol.* 1989, *4*, 41–44.

[64] Simons, A. M., Johnston, M. O., Environmental and genetic sources of diversification in the timing of seed germination: Implications for the evolution of bet hedging. *Evolution* 2006, *60*, 2280–2292.

[65] Colwell, R. K., Predictability, constancy, and contingency of periodic phenomena. *Ecology* 1974, *55*, 1148–1153.

[66] Williams, D. D., *The Biology of Temporary Waters*, Oxford University Press, Oxford 2006.

[67] Antón-Pardo, M., Armengol, X., Effects of salinity and water temporality on zooplankton community in coastal Mediterranean ponds. *Est. Coast. Shelf Sci.* 2011, *114*, 93–99.

[68] Simons, A. M., Fluctuating natural selection accounts for the evolution of diversification bet hedging. *Proc. R. Soc. B: Biol. Sci.* 2009, *276*, 1987–1992.

[69] R Core Team, *R: A language and environment for statistical computing*, R Foundation for Statistical Computing, Vienna 2013. http://www.R-project.org/

Appendix 1

The optimum hatching fraction h_{opt} can be found analytically in the Binomial-D model variant of Cohen's approach with deterioration occurring also between planktonic growing seasons. Hence, the long-term finite growth rate $\bar{\lambda}$ (Eq. 2) of the diapausing egg bank can be rewritten as follows

$$\bar{\lambda} = \left[\left[(1-h)(1-d) + hP_g(1-d) \right]^{pt} \left[(1-h)(1-d) \right]^{qt} \right]^{1/t} \tag{A1}$$

By taking logarithms,

$$\log(\bar{\lambda}) = p \log\left[(1-h)(1-d) + hP_g(1-d) \right] + q \log\left[(1-h)(1-d) \right] \tag{A2}$$

Considering Eq. (A3) a function of h, $\log(\bar{\lambda}) = f(h)$, and deriving with respect to h,

$$f'(h) = \frac{\left[(h-p)P_g \right] - h + 1}{\left[(h^2 - h)P_g \right] - h^2 + 2h - 1} \tag{A3}$$

Then, setting Eq. (A3) equal to zero (i.e., $\log(\bar{\lambda}) = 0$) and solving for h_{opt} gives

$$h_{opt} = \frac{pP_g - 1}{P_g - 1} \tag{A4}$$