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Within season short-term hatching delays suggest risk-spreading behaviour in populations of the freshwater cladoceran *Daphnia*[1]

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Abstract: Many organisms produce dormant stages, building up large germ banks in the sediments. Timing of emergence from these germ banks is a key feature in the recurrent cycle of dormant and active life stages. We exposed dormant egg bank samples of *Daphnia magna* from (semi-)permanent ponds to seasonal cues mimicking temperature and photoperiod during early spring, late spring, and summer in the region of sampling and investigated hatching success and response time after incubation. Whereas no differences in hatching success were observed between seasonal treatments, within each seasonal treatment a variable fraction of eggs postponed the hatching response for several days. We link immediate *versus* short-term delayed hatching to a trade-off between the advantages of quick niche filling and the unpredictability of reproductive success under unstable environmental conditions. A scenario where hatching investment is spread over immediate and delayed hatchers as a bet-hedging strategy is corroborated by environmental data on the risk of abortive hatching in early spring and by modeling results that show that immediate *versus* short-term delayed hatching is a valid alternative to bet-hedging over years in the investigated habitats to cope with variability in the onset of favourable conditions.
Keywords: bet-hedging, dormancy, modeling, seasonality, zooplankton.

Résumé : De nombreux organismes produisent des stades dormants créant ainsi d'importantes banques de germes dans les sédiments. Le moment choisi de germination de ces banques est une caractéristique clé des cycles récurrents des stades de vie active et de dormance. Nous avons exposé des échantillons d'une banque d'oeufs dormants de *Daphnia magna* d'étangs (semi-)permanents à des indices saisonniers reproduisant la température et la photopériode du début du printemps, de la fin du printemps et de l'été dans la zone d'échantillonnage et nous avons examiné le succès d'éclosion et le temps de réponse après incubation. Aucune différence n'a été observée dans le succès d'éclosion entre les traitements saisonniers, mais, pour une fraction variable des œufs à l'intérieur d'un traitement saisonnier, l'éclosion était retardée de plusieurs jours. Nous suggérons un lien entre ces deux stratégies, une éclosion immédiate *versus* retardée à court terme, et un compromis entre l'avantage d'occuper rapidement une niche et l'imprévisibilité du succès de reproduction dans des conditions environnementales instables. Un scénario où l'investissement reproducteur est réparti dans le temps entre une éclosion immédiate et retardée comme stratégie de répartition des risques est corroboré par des données environnementales sur le risque d'éclosion avortée au début du printemps. Ce scénario est aussi corroboré par des résultats de modélisation qui montrent qu'une éclosion immédiate *versus* retardée à court terme est une alternative valable à une répartition des risques sur plusieurs années dans les habitats étudiés afin de faire face à la variabilité dans l'initiation des conditions favorables.
Mots-clés : dormance, modélisation, répartition des risques, saisonnalité, zooplancton.

Nomenclature: *Daphnia magna* Straus, 1820.

Introduction

Transition to a resistant, dormant phase is a strategy adopted by many organisms to survive temporally unfavourable conditions. Such an escape in time is an alternative to an escape in space via dispersion or migration. Besides being resistant to harsh conditions, these dormant stages can remain viable for many years and build up large germ banks buried in the sediments (Hairston, 1996; Evans & Dennehy, 2005). The timing of hatching of dormant eggs from the egg bank is a key feature in the recurrent cycle of active and dormant phases. Environmental variability, sedimentation, and mixing events can cause dormant stages produced at the same moment to hatch at different moments. In addition, a proportion of the germs may postpone hatching on acquiring appropriate stimuli due to genetic variability in the hatching response (Evans & Dennehy, 2005) or as a "bet-hedging" strategy to ensure a single genotype's successful reinvestment in the germ bank by at least part of the hatching eggs in unpredictable environments (Seger & Brockmann, 1987; Philippi & Seger, 1989). Theoretical studies indicate that the optimal proportion of propagules to hatch is a function of the variability of reinvestment to the propagule bank and of the survival probability of unhatched propagules (Cohen, 1966; Ellner, 1985).

Many aquatic organisms, including crustacean zooplankton, produce dormant stages to bridge periods of unfavourable conditions like frost or drought (Fryer, 1996; Brendonck, De Meester & Hairston, 1998). In sufficiently

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large ponds and lakes in a temperate seasonal environment, the period of favourable conditions (growing season) for growth and reproduction often greatly exceeds the generation time of planktonic organisms, reducing the risk of unsuccessful reinvestment in the egg bank due to unpredictability in the onset of unfavourable conditions (winter). Still, although sufficient time is ensured to successfully complete the reproduction cycle, one may predict strong selection against delayed hatching beyond the onset of the growing season. In organisms with multiple generations within one growing season (year) and rapid population growth, a delay of several days to weeks can entail a severe competitive disadvantage compared to earlier established hatchlings, even at relatively low densities. Indeed, a peak of hatching is often observed at the onset of the growing season (Wolf & Carvalho, 1987; Cáceres, 1998; Hairston, Hansen & Schaffner, 2000). Although the occurrence of successful growing seasons may be predictable, the onset of the growing season may still be partly unpredictable. The start of the favourable period for growth after winter, for instance, is not fully reliable, as a few warm and sunny days early in spring may be followed by a period of cold temperatures, or the time at which sufficient food becomes available may be variable between years. The strategy to hatch as soon as hatching conditions become favourable so as to have a head start in competition thus may entail a risk of failure to survive until reproduction. In a manner similar to their responses to unpredictability in the termination and productivity of the growing season, genotypes may spread this risk by spreading the hatching response over different growing seasons (years). An alternative mechanism may lie in prolonging the time between first exposure to a hatching stimulus and the actual hatching response on a smaller time scale. By delaying the hatching response by a few days or weeks, individuals may build in some security with respect to the initial instability of favourable environmental conditions. Obviously, this delayed hatching response has a cost when conditions remain suitable for rapid population growth, and different hatching strategies (genetic or bet-hedging) may co-occur within a single population on this smaller time scale.

In the present study, we experimentally investigated whether there are differences in the hatching response of the cyclical parthenogen *Daphnia magna* associated with differences in seasonal conditions. First, we tested whether a preference exists to hatch under environmental conditions mimicking the onset of the growing season (early spring) compared to conditions associated with late spring and summer. Second, we investigated the extent to which hatching is synchronized at the start of the incubation period (application of the hatching cue) within each seasonal treatment. In addition, we used meteorological data from the region of sampling to provide an estimate of the risk of abortive hatching in spring, compared it to the results from our experiments, and developed a model to further explore the conditions under which a bet-hedging strategy with immediate and delayed hatching within a season may be selected for.

Methods

POPULATIONS AND SAMPLING

Three ponds inhabited by *Daphnia magna* were sampled: OM1, OM2, and DV. All 3 are man-made shallow and (semi-)permanent ponds (DV occasionally dries out during summer) of which the dormant egg bank has been described before (Cousyn & De Meester, 1998). OM1 and OM2 have a surface area of 1.2 and 2.5 ha, respectively, and DV has a surface area of 0.75 ha. OM1 and OM2 border each other within metres and are located at a distance of about 70 km from DV. In all 3 ponds, individuals may survive during mild winters, and populations will remain active in the water column for prolonged periods of time (several months to years). These populations engage periodically in extensive sexual reproduction, with production of dormant eggs (De Meester & Vanoverbeke, 1999), and for the OM populations there are strong indications of periodic recruitment of clones from the dormant egg bank (indicated both by analyses on neutral genetic markers [De Meester *et al*., 2006] and by direct observations in the field [K. Moreau, unpubl. data]). In *Daphnia*, the dormant eggs are encased in an ephippium, which is a protective envelope that contains a maximum of 2 eggs (Stross, 1987). During early spring 1997, when no hatching was yet observed in the field, we collected mud samples containing ephippia by scraping off the surface layer of the pond sediments at the sampling sites. The samples were stored at 4 °C in the dark. Within a week after sampling, we isolated the ephippia by sieving the samples through a 200-µm mesh to remove the mud and picking out the ephippia from the remaining debris. To avoid stimulation of the dormant eggs during manipulation, the debris was immersed in water collected from the pond at the time of sampling and cooled to about 0 to 4 °C. In addition, the material was screened within 1 h after sieving to reduce light exposure of the ephippia. The ephippia were stored in Eppendorf tubes (25 ephippia per tube) in the dark and at 4 °C for a period of 2 to 4 months before the experiments were started (see De Meester & De Jager, 1993a).

HATCHING EXPERIMENT

We performed a hatching experiment in which the isolated ephippia were incubated under 3 experimental conditions. We co-varied temperature and photoperiod to mimic seasonality, representing early spring (ES; 10 °C, 12/12 Light/Dark), late spring (LS; 16 °C, 15.5/8.5 L/D), and summer (SU; 22 °C, 17/7 L/D) in the region of sampling. The 3 environmental conditions were established in 3 different incubators. Light intensity during the light periods ranged between 30 and 80 $W \cdot m^{-2}$. The incubation medium consisted of tap water aged for 48 h at 18 °C that was filtered over a mesh of 0.45 µm and aerated for about 10 min before use. The experiment was run in 2 blocks, separated in time.

Table I gives the number of ephippia (containing 0–2 eggs) incubated for each population, seasonal treatment, and experiment. As the eggs were not decapsulated, it was impossible to know the exact number of eggs incubated for each population. Prior to the experiment, however, we estimated the average number of eggs contained per ephippium by decapsulating 140 to 160 ephippia from each population and counting the viable eggs. Based on the counts of decapsulated ephippia with 0, 1, or 2 healthy eggs, we performed a bootstrapping procedure of sampling with replacement of ephippia to obtain a better estimate of the

TABLE I. Number of ephippia incubated (Inc Eph) per population, seasonal treatment, and experimental round (exp1 – exp2); number of individuals hatched from the resting eggs (Hatch; pooled over experimental rounds); and number of incubated healthy eggs (Est Eggs; pooled over experimental rounds) as estimated by the bootstrapping procedure.

Population	Season	Inc Eph (exp1 – exp2)	Hatch	Est Eggs (SD)
DV		184 – 132		
	Early spring		182	199 (± 12)
	Late spring		171	195 (± 13)
	Summer		151	193 (± 16)
OM1		152 – 148		
	Early spring		175	251 (± 16)
	Late spring		183	250 (± 16)
	Summer		170	251 (± 16)
OM2		72 – 72		
	Early spring		172	227 (± 9)
	Late spring		169	228 (± 9)
	Summer		173	228 (± 9)

number and variability of eggs incubated per population and treatment. As a correction, bootstrap samples with a number of eggs smaller than the observed amount of hatching in a given population and treatment were omitted.

For each treatment, population, and experimental block, the incubated ephippia were equally divided over 4 Petri dishes as an additional blocking factor. Each experimental block lasted for 60 d. In the first block, the medium was changed and the Petri dishes were screened for new hatchlings daily during the whole period. In the second block, daily screening was only done during the first 30 d. After that, the Petri dishes were screened and the medium changed every other day.

TOTAL AMOUNT OF HATCHING

Since the exact number of dormant eggs incubated per population was not known and the total hatching proportions were only estimated by approximation, we tested for an interaction between population and seasonal treatment only on the total amount of hatching. This was done in R (R Development Core Team, 2007) by means of GLMM (generalized linear mixed models; library lme4) using quasi-Poisson error distribution (quasi-Poisson instead of Poisson to account for overdispersion) and log link function. We included population and seasonal treatment as fixed explanatory factors in the model and experimental block (fixed) and Petri dish (random) as blocking factors. The interaction between population and seasonal treatment was tested against a model including all other two-way interactions of fixed effects (population × experiment + season × experiment).

TIMING OF THE HATCHING RESPONSE WITHIN EACH SEASONAL TREATMENT

To analyze whether a significant proportion of the hatchlings delayed the hatching response beyond the start of the incubation period within each seasonal treatment, we performed a finite mixture model fitting analysis and compared the results for fitting unimodal *versus* bimodal models for each seasonal treatment and population. In both the unimodal and bimodal analyses, we used gamma distributions to fit the observations to take into account a strong skewness to the right. Indeed, in some treatments (see Figure 1), a unimodal distribution with a long tail to the right may be more likely than a bimodal distribution. In order to have enough counts in each data cell, we pooled counts over experimental blocks and over a number of days depending on the season (results of individual experiments are shown in Figure 2b, where it can be seen that the proportions of immediate and delayed hatchers are similar in both blocks of the experiment). As a slight difference in the onset of hatching was observed between both blocks, counts for the second experiment were shifted to the left before pooling to match the onset of hatching of the first experiment. This shift involved 2 d at 10 °C and 1 d at 16 °C. The 22 °C counts were not shifted before pooling. In addition, days were pooled in groups of 4 at 10 °C and in groups of 2 at 16 °C. Finite mixture model fitting was performed using Mixdist (J. Du, unpubl. data), an extension of the statistical package R (R Development Core Team, 2007). Mixdist uses a maximum likelihood estimation procedure based on a combination of the Expectation-Maximization algorithm and a Newton-type method. It provides a likelihood ratio (LR) test statistic. This LR test statistic was used to calculate the Akaike Information Criterion for small samples (AIC_c) (Burnham & Anderson, 2002) and Akaike weights to evaluate unimodal *versus* bimodal distributions within each seasonal treatment and population. We incorporated the shift of the data in the second experiment and the pooling of days as extra parameters in the AIC_c calculations.

Based on the bimodal mixture-model fits on the pooled data (see results), we calculated the day (averaged over populations) until which the proportion of individuals belonging to the first mixture distribution was larger than the proportion of individuals belonging to the second mixture distribution within each seasonal treatment. This threshold day was used to divide hatched individuals into a group of "immediate" and a group of "delayed" hatchers. The relative contribution of immediate hatchers *versus* delayed hatchers was subsequently analyzed using GLMM with a binomial error distribution (no overdispersion was observed) and logit link function (library lme4 in R; R Development Core Team, 2007). We used population and season as explanatory fixed factors and experimental block (fixed) and Petri dish (random) as blocking factors. The interaction between population and seasonal treatment was tested against a model including all other two-way interactions of fixed effects (population × experiment + season × experiment). Main effects of population and season were tested against a model including all other main effects (season + experiment and population + experiment, respectively).

RISK OF ABORTIVE HATCHING: METEOROLOGICAL DATA

In early spring, a few warm days triggering hatching may be followed by a period of temperatures too cold for survival, entailing a risk of abortive hatching when hatching immediately. To calculate an estimate of this risk of abortive hatching over the year, we used meteorological data from 1833 to 1999 (Klein Tank *et al*., 2002; data from Ukkel,

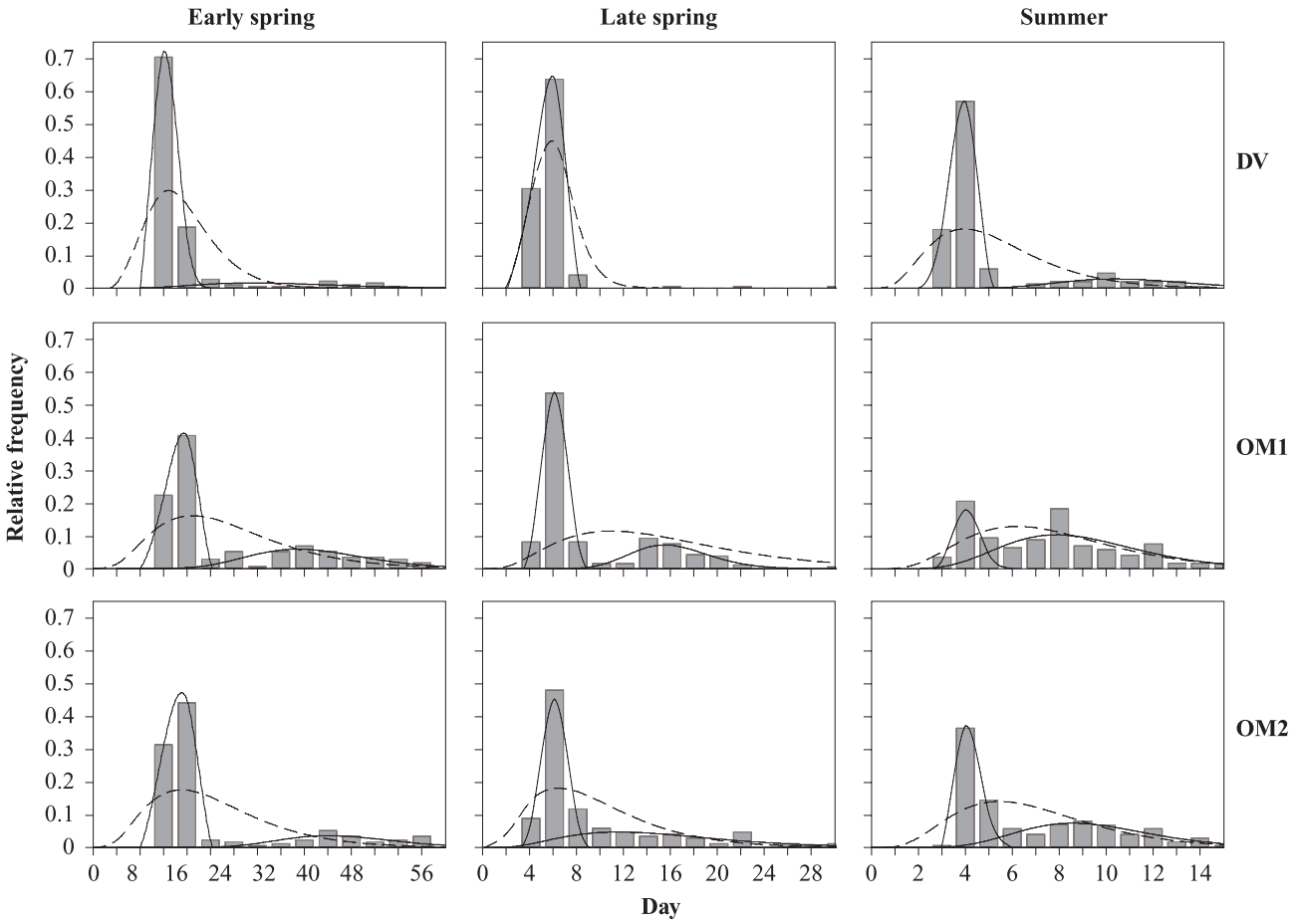

FIGURE 1. Relative amount of hatching over incubation time; data are pooled over both experimental blocks. For the early spring treatment, data are pooled over 4 consecutive days, and for the late spring treatment, data are pooled over 2 consecutive days. Dashed lines represent unimodal fitted gamma distributions; solid lines represent bimodal fitted gamma distributions.

Belgium; 25 km from OM1 and OM2, 45 km from DV) on 5-d mean air temperatures.

Monitoring of hatching in OM2 in 2002 and 2003 showed 2 distinct hatching peaks in spring, 1 in April and 1 in May (K. Moreau, unpubl. data). This monitoring was done by collecting on an (approximately) weekly basis a standardized area of surface sediments and observing hatching within the first 2 d after transportation to the laboratory. At 18–20 °C (= conditions in the laboratory), development of dormant eggs from induction to hatching takes at least 3 d (De Meester & De Jager, 1993a). Thus, individuals that hatched in the first 2 d after collection of the sediments had already been induced to hatch in the field before the sediment was collected. This procedure thus yields reliable data on hatching under field conditions. Based on the meteorological data, the average temperature at the onset of April is 7–8 °C and at the onset of May is 10–11 °C. Although these are air temperatures, averaging over 5 d may give a good estimate of the average water temperature in the investigated shallow lakes (maximum 1–2 m depth). A life-table experiment by Carvalho (1987) with clones of *D. magna* collected from the field in spring showed that such clones survive at temperatures around 10 °C but exhibit high mortality rates at temperatures around 5 °C. The temperature at the onset of April (7–8 °C) may give us an indication of the minimum temperature cue required for hatching, and the data of Carvalho (1987) may give us a minimum temperature limit for survival after hatching (4–5 °C). Using these data, we calculated the probability of a 5-d mean equal or above this minimum temperature for hatching followed within 30 d by a 5-d period with temperature below the mortality threshold. This may give us an indication of the risk of abortive hatching associated with low temperatures at any given moment of the year.

IMMEDIATE *VERSUS* DELAYED HATCHING AS A BET-HEDGING STRATEGY: SIMULATION MODELING

To explore the possibility that immediate and delayed hatching result from a bet-hedging strategy to cope simultaneously with the advantages of quick niche filling and the unpredictability of reproductive success when hatching immediately, we developed a discrete time simulation model. In this model, immediate and delayed hatching as a bet-hedging strategy is compared with the classic bet-hedging over growing seasons (years; Cohen, 1966). The presented model is a discrete model written in C^{++}. The model consists of iterations of the equations given in Table II, which describe cycles (years) of hatching and production of new eggs. The model starts with constructing a

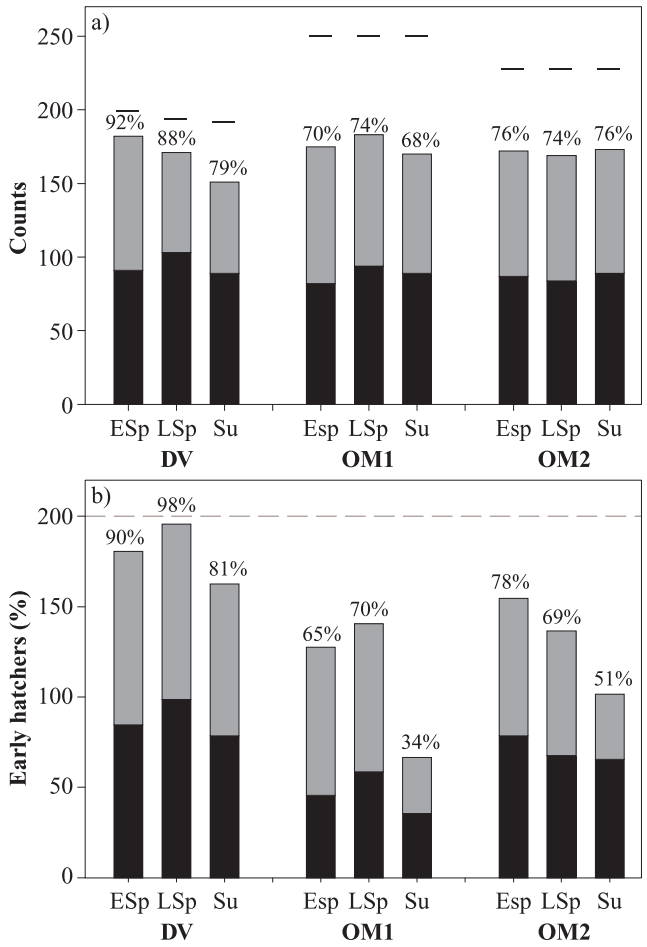

FIGURE 2. a) Total number of hatched eggs (absolute counts) in each experimental block; horizontal lines indicate the total number of eggs incubated (bootstrap estimates of pooled data over both experimental blocks); percentage values indicate the percentage of hatched eggs (bootstrap estimates of pooled data over both experimental blocks). b) Relative contribution of immediate hatchers in each experimental block; percentage values indicate the relative contribution of early hatchers averaged over the 2 experimental blocks. Black bars = results of first experimental block; grey bars = results of second experimental block; ESp = Early spring, LSp = Late spring, Su = Summer.

number of genotypes, where each genotype is characterized by the proportion of hatching each cycle (h_c; bet-hedging between years) and by the proportion of immediate *versus* delayed hatching within each cycle (h_{im}; bet-hedging within years) (equation [1] and [2]), Table II). Each genotype thus has 2 independent dimensions: 1 dimension determining the between-year bet-hedging and 1 dimension determining the within-year bet-hedging. For each cycle in the model, there is a total amount of hatching that is dependent on the remaining number of eggs per genotype (equation [3] and [4]), Table II) and there is a total yield of new eggs, drawn from a random normal distribution with given average α_1 and standard deviation σ_1, to be divided among the hatched individuals (equation [5]), Table II).

We define *a* as a coefficient that determines the strength of the advantage of early hatching. Small values of *a* reflect a clear advantage of immediate over delayed hatching with respect to reproductive output, as in the case of exponential growth conditions. Large values of *a* reflect only a slight to no advantage of immediate over delayed hatching, as in the case where competition strength is more important than exponential growth. There is, however, a normally distributed ($\alpha_2 = 1$, σ_2) random error *e* among cycles in the advantage of immediate over delayed hatchers (equations [6] and [8]), Table II). σ_2 thus determines the risk of hatching early and may result in a smaller yield of immediate compared to delayed hatchers in a given year (equation [8], Table II). We also performed simulations with a cut-off on *e*, where values of *e* lower than the cut-off *t* result in zero yield for immediate hatchers (equation [7], Table II) to simulate an increasing risk of abortive hatching in immediate hatchers (without changing the variability when the yield of immediate hatchers is larger than zero). Within the groups of immediate and delayed hatchers, the yield per genotype is proportional to the number of hatched individuals of that genotype (equation [10], Table II). Finally, between cycles, only a given fraction ($1 - m$) of the eggs survives (equation [11], Table II).

With the proposed scheme, the variability in total yield (variability between years) is equal for immediate and

TABLE II. Model equations.

	E_i	number of eggs for genotype *i*
	$h_{c,\,i}$	between-cycle hatching proportion for genotype *i*
	$h_{im,\,i}$	proportion of immediate hatchers for genotype *i*
[1]	$T_{im,\,i} = E_i * h_{c,\,i} * h_{im,\,i}$	immediate hatchers for genotype *i*
[2]	$T_{del,\,i} = E_i * h_{c,\,i} * (1 - h_{im,\,i})$	delayed hatchers for genotype *i*
[3]	$T_{im} = \Sigma_i\, T_{im,\,i}$	total immediate hatchers
[4]	$T_{del} = \Sigma_i\, T_{del,\,i}$	total delayed hatchers
[5]	$Y = \max(0, \text{normal}(\alpha_1, \sigma_1))$	total yield
	a	advantage of immediate hatchers over delayed hatchers
[6]	$e = \max(0, \text{normal}(1, \sigma_2))$	error on advantage of immediate over delayed hatchers
	t	threshold for abortive hatching of immediate hatchers
[7]	if $e < t$ then set $e = 0$	
[8]	$y_{im} = [e * T_{im} / (T_{del} + e * T_{im})]^a$	proportion of total yield attributed to immediate hatchers
[9]	$y_{del} = 1 - y_{im}$	proportion of total yield attributed to delayed hatchers
[10]	$Y_i = y_{im} * Y * (T_{im,\,i} / T_{im}) + y_{del} * Y * (T_{del,\,i} / T_{del})$	yield for genotype *i*
	m	mortality of eggs
[11]	$E_i = (E_i - T_{im,\,i} - T_{del,\,i} + Y_i) * (1 - m)$	number of eggs for genotype *i* at start of next cycle

delayed hatchers, but the distribution of the total yield over immediate and delayed hatchers (variability within years) is independent of the variability in total yield. This mimics situations in which the events early in the season, when individuals hatch, are independent from the events later in the season, when hatched individuals (both immediate and delayed) produce the next generation of dormant eggs.

We performed simulations for a range of values of σ_1, σ_2, a, t, and m. We started each simulation run with genotypic values h_c (= the proportion of hatching each cycle) and h_{im} (= the proportion of immediate hatching) ranging from 0 to 1 in steps of 0.02 and with an equal number of eggs per genotype (10^3 eggs). The average total yield α_1 was set equal to the total initial number of eggs. For each parameter setting we performed 5 runs of 10^6 cycles.

Results

TOTAL AMOUNT OF HATCHING

Based on 1000 bootstrap samples for each seasonal treatment and population, the number of eggs incubated (pooled over experiments) was between 193 and 251, with a standard deviation of 9 to 16 eggs (Table I). Hatching percentages were fairly high in all treatments, ranging from 68 to 92% (pooled over both experiments; Figure 2a), with a bootstrap standard deviation of 4 to 9%. The GLMM analysis on total amount of hatching showed no significant interaction between population and seasonal treatment (Table III).

TIMING OF THE HATCHING RESPONSE WITHIN EACH SEASONAL TREATMENT

Figure 1 shows the relative amount of hatching over time for each population and seasonal treatment (data pooled over both experiments). Overall, the first hatchlings were observed 10 to 13 d after the start of the incubation period under early spring conditions. Under late spring conditions, the first hatchlings occurred on the fourth or fifth day after the start of the incubation period, and under summer conditions, they occurred on the third or fourth day. Figure 1 also shows that hatching was not stringently synchronized at the beginning of the incubation period but occurred in 2 bouts over time ("immediate" *versus* "delayed" hatchers). The bimodal fitted distributions (Figure 1, solid lines) accord much better with the data than the unimodal fitted distributions (Figure 1, dashed lines): whereas all unimodal fitted distributions deviated highly significantly from the observed counts (Table IV; all 9 unimodal distribution fits: $P < 0.001$) even after sequential Bonferroni correction (Rice, 1989), only 3 of the 9 bimodal fitted distributions deviated significantly from the observed counts (Table IV; lowest $P = 0.014$), and none remained significant after sequential Bonferroni correction. Table IV also gives the AIC_c values and Akaike weights (w) for the comparison of unimodal *versus* bimodal distributions. As can be seen from Table IV, there is 100% support for bimodal over unimodal distributions in every seasonal treatment and population. In DV, a bimodal pattern with very low proportions of delayed hatchlings in early spring and late spring indeed is still 100% supported by AIC_c compared to a unimodal distribution. Apparently a unimodal gamma distribution cannot account simultaneously for the high initial peak of hatching and following long tail. These results are not changed when right-tailed distributions other than a gamma distribution are considered (results not shown). Given the distribution patterns in the other populations and treatments, with 100% support of bimodal distributions over unimodal in all cases, a bimodal pattern with a very low contribution of delayed hatchers does not seem illogical in DV. Table III gives the results of the GLMM analysis on the contribution of immediate hatchers *versus* delayed hatchers to the total amount of hatching. Both the effect of population and the effect of season were highly significant. The interaction between population and season was also highly significant. Figure 2b shows that, on average, the highest contribution of immediate hatchers was found in DV, whereas the lowest contribution of immediate hatchers was observed in OM1. Moreover, in all 3 populations the contribution of immediate hatchers was reduced under summer conditions, with the strongest reduction observed in OM1 and the weakest reduction in DV.

TABLE III. Results of GLMM analysis on total amount of hatching (quasi-Poisson with log link) and on the relative proportion of immediate *versus* delayed hatching (binomial with logit link); *P* values in bold = significant at the 0.05 level.

	Total hatching			Immediate hatching		
Effect	df	χ^2	*P*	df	χ^2	*P*
Population (1)	2	0.563	0.75	2	63.196	**< 0.001**
Season (2)	2	1.368	0.50	2	38.511	**< 0.001**
1 × 2	4	2.376	0.67	4	18.877	**< 0.001**

RISK OF ABORTIVE HATCHING: METEOROLOGICAL DATA

Figure 3 shows the risk of abortive hatching over the year, as calculated from the meteorological data. The graph represents a running average (over 3 consecutive 5-d periods) of the risk of abortive hatching, expressed as the probability of experiencing a 5-d period inducing mortality (M) within 30 d after experiencing a 5-d period with favourable temperature for hatching (H). Both an upper limit and a lower limit of the risk are given. The risk of abortive hatching when initiating hatching in early April is between 10% and 25%, while this risk is nonexistent when initiating hatching in early May or later.

IMMEDIATE *VERSUS* DELAYED HATCHING AS A BET-HEDGING STRATEGY: SIMULATION MODELING

All simulations converged to a single optimal genotype or occasionally 2 genotypes with adjacent proportions of bet-hedging (*cf.* due to discrete steps in changing the proportion of bet-hedging in the simulations, sometimes the optimal proportion is in between 2 steps), with the proportion of bet-hedging within and between years depending on the parameter settings. The modeling results show that immediate *versus* delayed hatching as a bet-hedging strategy within years (cycles) is indeed possible, and that the amount of bet-hedging within years is largely independent of the amount of bet-hedging between years. Whereas the optimal proportion of bet-hedging between years is dependent on the variability of total yield σ_1 and on mortality of

TABLE IV. Results of mixture-model fitting for unimodal and bimodal gamma distributions. pr = relative contribution to total distribution; μ = average of distribution; σ = standard deviation; df = degrees of freedom; *P* values in bold = significant at the 0.05 level; * = significant at the 0.05 level after sequential Bonferroni correction; k = number of parameters; AIC_c = Akaike Information Criterion for small samples; w = Akaike weight.

	Early spring			Late spring			Summer		
	Unimodal	Bimodal		Unimodal	Bimodal		Unimodal	Bimodal	
DV									
n		182			171			151	
pr		0.88	0.12		0.98	0.02		0.81	0.19
μ	13.18	11.45	30.42	5.02	4.45	23.38	4.74	3.34	10.56
σ	5.70	0.67	12.75	1.70	0.83	8.54	2.49	0.44	2.72
df	11	9		6	9		12	8	
χ^2	251.06	14.39		141.70	5.92		205.44	4.50	
P	**< 0.001 ***	0.109		**< 0.001 ***	0.748		**< 0.001 ***	0.809	
k	4	7		4	7		2	5	
AIC_c	259.29	29.03		149.94	20.61		209.52	14.91	
w	0	1		0	1		0	1	
OM1									
n		175			183			170	
pr		0.64	0.36		0.70	0.30		0.25	0.75
μ	20.89	12.70	35.67	8.04	4.98	15.09	7.23	3.55	8.45
σ	11.30	1.62	10.02	4.53	0.84	3.31	3.39	0.46	3.03
df	12	8		12	8		12	9	
χ^2	167.93	19.15		176.46	13.62		48.88	18.11	
P	**< 0.001 ***	**0.014**		**< 0.001 ***	0.092		**< 0.001 ***	**0.034**	
k	4	7		4	7		2	5	
AIC_c	176.17	33.82		184.68	28.26		52.95	28.48	
w	0	1		0	1		0	1	
OM2									
n		172			169			173	
pr		0.78	0.22		0.61	0.39		0.48	0.52
μ	18.70	12.51	40.51	8.55	5.00	14.21	6.46	3.78	8.99
σ	10.42	1.71	9.82	5.27	0.89	7.35	3.15	0.35	2.89
df	12	8		12	9		12	8	
χ^2	230.13	18.03		120.82	12.87		119.06	6.07	
P	**< 0.001***	**0.021**		**< 0.001***	0.169		**< 0.001***	0.639	
k	4	7		4	7		2	5	
AIC_c	238.37	32.71		129.06	27.57		123.13	16.43	
w	0	1		0	1		0	1	

the eggs *m* (results not shown), the optimal proportion of within-year bet-hedging is dependent on the variability of the advantage of immediate over delayed hatching σ_2 and especially on the risk of abortive hatching *t* and the relative advantage of immediate over delayed hatching *a* (Figure 4). The optimal proportion of delayed hatching decreases with decreasing risk of abortive hatching of the immediate hatchers, and it increases with a decreasing advantage of immediate over delayed hatching. Changes in the variability of the advantage of immediate over delayed hatching σ_2 mainly affect the results at low risk of abortive hatching *t*. At high values of σ_2 (σ_2 = 0.5), the proportion of immediate hatching is reduced with decreasing relative advantage of immediate over delayed hatching *a*.

Discussion

VARIATION AMONG SEASONS

The total estimated hatching percentages ranged from around 70 to 90%, with no significant differences among seasons. Total hatching percentages in our experiments were thus fairly high. Other experimental studies on hatching behaviour of *Daphnia* species have obtained widely varying hatching success (*e.g.*, Stross, 1966; Stross & Hill, 1968; Schwartz & Hebert, 1987; Carvalho & Wolf, 1989; De Meester, 1993; De Meester & De Jager, 1993a; Cáceres & Schwalbach, 2001; Cáceres & Tessier, 2003). Nevertheless, although laboratory conditions often invoke excessively strong hatching stimuli, such as decapsulation from the protective ephippium and exposure to high light availability (see also Cáceres & Schwalbach, 2001), hatching responses close to 100% are rarely obtained. The observed hatching success in our experiments indicates that a large proportion of the eggs (40% or more) must have a generalistic hatching behaviour, hatching in all 3 seasonal treatments. If the proportion of generalists were less than 40%, the total amount of hatching over all seasonal treatments would add up to more than 100%. The lack of preferential hatching under early spring conditions is in contrast with observations from field surveys (Wolf & Carvalho, 1989;

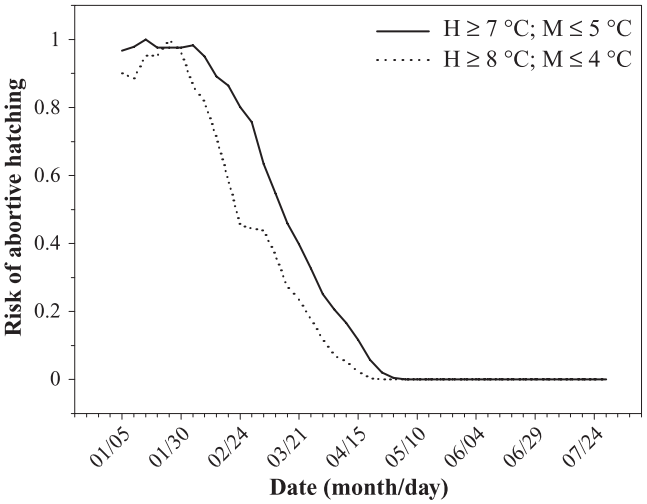

FIGURE 3. Running average (over 3 consecutive 5-d periods) of the risk of abortive hatching, expressed as the probability of experiencing a 5-d period inducing mortality (M) within 30 d after experiencing a 5-d period with favourable temperature for hatching (H). For validation of value settings, see Methods. Solid line: upper limit (H ≥ 7 °C and M ≤ 5 °C); dashed line: lower limit (H ≥ 8 °C and M ≤ 4 °C).

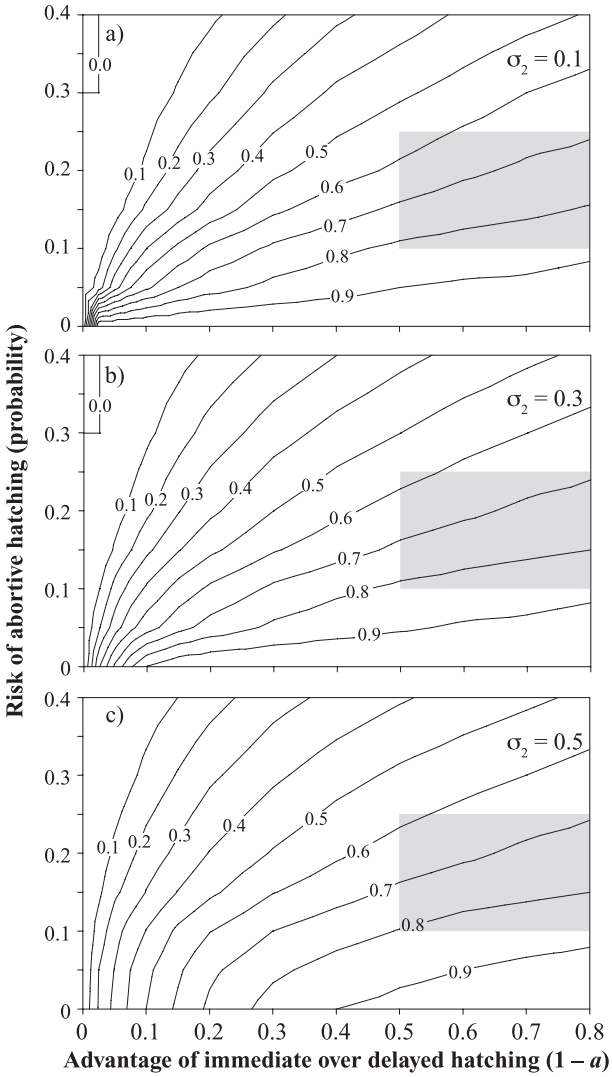

FIGURE 4. Modeling results for the optimal proportion of immediate hatching. The x-axis represents the average advantage of immediate over delayed hatching (determined by parameter *a*). The higher the value on the x-axis (*i.e.*, 1 – *a*) the higher the advantage of immediate hatching. The y-axis represents the risk of abortive hatching (probability of *e* being lower than *t*, resulting in zero reproductive output) when hatching is immediate. Figures (a), (b), and (c) give the results for different levels of variability (σ_2 = 0.1, 0.3, and 0.5, respectively) of the advantage of immediate over delayed hatching. The grey squares within each figure represent the area of expectation of immediate hatching in early spring corresponding to the risk of abortive hatching as calculated from the meteorological data (Figure 3).

Cáceres, 1998; Hairston, Hansen & Schaffner, 2000, but see De Stasio, 1990, for synchronized hatching later in the growing season). Our data, however, are in agreement with other observations from laboratory experiments (Cáceres & Schwalbach, 2001). Although differences between laboratory results and field observations may suggest that the stimuli offered in the laboratory are not comparable to the field conditions (Cáceres & Tessier, 2003), detailed monitoring of hatching in pond OM2 and a neighbouring pond (OM3) indicates that in these ponds hatching in the field is not restricted to the onset of the growing season (early spring) but rather shows distinct peaks throughout the growing season (K. Moreau, unpubl. data). Moreover, hatching response under a wide range of conditions may still translate into peaked hatching at the beginning of the growing season under field conditions if eggs sensitive to hatching cues are rapidly depleted at the sediment surface. Eggs that subsequently get exposed due to bioturbation of the sediments or by resuspension by wind will also hatch but are more difficult to detect in the field, as their numbers will be low compared to total population size. In spite of the competitive disadvantage of hatching late in the season (under late spring or summer conditions), a generalistic hatching behaviour may still be adaptive if sedimentation rates are fairly high and the probability of getting exposed again in a later year is low. For the OM ponds, sedimentation rates are indeed estimated to be high (about 2 $cm \cdot y^{-1}$; Cousyn & De Meester, 1998).

IMMEDIATE *VERSUS* DELAYED HATCHING

Within the different seasonal treatments, we observed that not all eggs hatched immediately upon exposure to hatching conditions. Our observations are better explained by a bimodal hatching pattern than with 1 hatching event concentrated at the onset of the incubation period. Even if the pattern were not bimodal, it is clear that hatching is not synchronized at the start of the incubation period but spread over a relatively long period in all experimental conditions. The differences between seasonal treatments in the time of appearance of the first hatchlings and in the time between the peak of the first and second hatching bout can be mainly attributed to the effect of temperature on the duration of egg development. Based on Bottrell *et al*. (1976), the time needed for the development of (parthenogenetically produced) eggs in *Daphnia* is about 8 d at 10 °C, 4 d at 16 °C, and 2 d at 22 °C. The bimodal hatching pattern itself and the interaction between the proportion of delayed hatching and the seasonal conditions, however, cannot be attributed to such temperature effects. A bimodal hatching pattern has also been observed by Stross (1966), but the start of the second

hatching bout (50 d at 21 °C in Stross, 1966, *versus* 6–7 d at 22 °C in our study) is not comparable. Although several hatching experiments have been carried out with dormant eggs of *D. magna* (*e.g.*, Schwartz & Hebert, 1987; De Meester, 1993; De Meester & De Jager, 1993a,b) and other *Daphnia* species (*e.g.*, Stross & Hill, 1968; Schwartz & Hebert, 1987; Cáceres & Schwalbach, 2001; Fox, 2007), a comparable bimodal pattern on a short timescale (several days to weeks) has not yet been reported. Some of the hatching cues used in these other studies, such as decapsulation, are very artificial and may have induced all eggs to hatch immediately. In other experiments (Cáceres & Schwalbach, 2001), eggs were not separated from the sediments and the observation of delayed hatching may have been blurred by stirring the sediments, exposing each time new eggs to the hatching cues. De Meester and De Jager (1993b) do report a delayed hatching response—even though they decapsulated the eggs—but only in older eggs (2.5–4.5 y old) compared to recently produced eggs (< 5 months old). The hatching patterns reported in their study for old and young eggs (20 °C, 14/10 L/D) correspond to our first and second bout of hatching under summer conditions. Similarly, Fox (2007) found delayed hatching responses in older eggs (15–20 y old) collected from the field compared to younger eggs (< 5 y old). From these observations, one may derive that the 2 hatching bouts we observe might be associated with young (produced in the previous growing season) and old (produced several years ago) eggs. Hatching experiments with eggs collected from different depths of sediment cores in DV and OM1 (decapsulated eggs), however, showed that almost all hatching occurred on the fourth or fifth day after incubation (Cousyn & De Meester, 1998). This indicates that older eggs collected from the field do not necessarily show a delayed hatching response in the studied ponds. Also, our observation that the relative importance of immediate *versus* delayed hatchers changes with the seasonal treatment cannot be explained by a difference in age structure of the eggs.

Because of the short generation time of *Daphnia*, the time between the peak of the first hatching bout and that of the second hatching bout (see Figure 1) equals about the length of 2 adult instars. Assuming logistic population growth with low starting densities, this advantage is sufficient for the immediate hatchers to dominate the population almost entirely. On the other hand, immediate hatchers will fail to mature when environmental conditions deteriorate after a few days, whereas delayed hatchers may stay dormant until favourable conditions remain more stable. Monitoring of hatching in OM2 in 2002 and 2003 (K. Moreau, unpubl. data) shows 2 distinct hatching peaks in spring, one in April and one in May, where the difference in time between both peaks (20 to 30 d) corresponds to our immediate and delayed hatching category under early spring conditions. Based on our calculations from the meteorological data, the risk of abortive hatching caused by low temperature is between 10 and 25% in early April, while this risk is nonexistent in early May or later (Figure 3). Although our data thus fit the idea of a trade-off between the advantages of immediate hatching and the variability in reproductive outcome (see De Stasio & Hairston, 1992), we cannot prove that the delayed hatching in part of the eggs is indeed an adaptive response and that our observations fulfill the criteria as outlined by Evans and Dennehy (2005) for the occurrence of bet-hedging. Our data do not enable us to directly assess whether single genotypes spread their risks by expressing 2 hatching phenotypes (Philippi & Seger, 1989; Evans & Dennehy, 2005) and producing eggs with both immediate and delayed hatching behaviour. Nevertheless, we consider it very plausible that a bet-hedging strategy is involved with respect to immediate and delayed hatchers in our data. In plants, it has been shown that a large part of the variability in emergence timing of seeds (including within season variability) can be ascribed to non-genetic effects (Simons & Johnston, 2006). It does indeed follow from our data that immediate and delayed hatching cannot be ascribed solely to genotypic differences. Given the high overall hatching success (> 70%), the change in proportion of delayed hatchers between seasons is too large to be attributed to a subset of the eggs hatching in one but not in the other season, leaving little room for genetic differences or the occurrence of differentially preconditioned eggs (*e.g.*, age, as discussed in the text) to account for our results. Still, to show beyond doubt that we are dealing with a single genotype expressing 2 hatching phenotypes and that this phenotypic variation is adaptive (see Evans & Dennehy, 2005) would require additional experiments.

Our modeling results support the idea that a bet-hedging strategy may be involved and show that whereas unpredictability in the total productivity of a given growing season results in bet-hedging over years, unpredictability in the onset of favourable conditions promotes bet-hedging within years. Within-year bet-hedging is mainly dependent on the relative advantage of immediate over delayed hatching (parameter a) and the risk of abortive hatching of immediate hatchers (parameter t). Comparing the calculated risk of abortive hatching based on meteorological data (Figure 3) with the results of our modeling under high advantage of early hatching (*i.e.*, $t = 0.1$–0.25; $1 - a > 0.5$; Figure 4) gives an optimal proportion of immediate hatchers in early spring between 0.6 and 0.9, which is what we observe in our experiments in OM1 and OM2. Our modeling results also show that in the absence of abortive hatching ($t = 0$), a large proportion of the eggs may still delay hatching if there is only a low average advantage of immediate over delayed hatching ($1 - a < 0.1$; Figure 4), especially with relatively high variability (σ_2) in the relative advantage of immediate *versus* delayed hatching. We interpret the higher proportion of delayed hatchers under summer conditions in our experiments to reflect a reduction in advantage of early hatching in the presence of an established population. As many ponds harbour an active *Daphnia* population during summer, competition will be severe and hatchlings cannot benefit much from hatching immediately. Under those circumstances, competitive strength and risk-spreading behaviour will be more important than a high intrinsic growth rate. In one of our study populations (DV), the contribution of delayed hatchers is very low, even under summer conditions. This observation, however, conforms

to the above scenario and strengthens our interpretation, as the pond occupied by this population dries out almost completely during summer, such that the active population of *D. magna* is temporally strongly reduced or even absent (J. Vanoverbeke & L. De Meester, pers. observ.). Delayed hatching is thus potentially very costly under summer conditions in this pond, especially when the risk of abortive hatching is negligible.

In summary, our data do not confirm the hypothesis of preferential hatching under early spring conditions but instead suggest a generalistic hatching behaviour of a large fraction of the dormant eggs with respect to seasonal conditions. Our results, however, also point to the occurrence of delayed hatching responses of some of the eggs within each seasonal treatment, and we argue that this reflects a bet-hedging strategy. Although our data do not enable us to test for the existence of a bet-hedging strategy at the genotype level, our hatching results, a small simulation exercise, and unpublished field observations are all in agreement with the hypothesis that the *Daphnia* populations studied exhibit an advanced bet-hedging strategy to cope simultaneously with the advantages of early hatching and the associated unpredictability in reproductive outcome and hence to optimize reproductive output within each season.

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