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HOW LONG TO REST: THE ECOLOGY OF OPTIMAL DORMANCY AND ENVIRONMENTAL CONSTRAINT

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Abstract. Dormancy is a common mechanism employed by short-lived organisms for persistence in a variable environment. Theory suggests that the fraction of propagules that terminate dormancy each year should be <100% when recruitment success varies temporally. Moreover, the fraction of propagules that resumes development should vary across habitats that differ in the probability of successful recruitment or the probability of survival during dormancy. We tested these predictions by using dormant eggs from five populations of the freshwater cladoceran *Daphnia pulicaria* that differ in their ability to recruit to and persist in the water column. In two separate experiments, newly produced dormant eggs were incubated in situ for one year at various sites on the bottom of the lakes. A series of reciprocal transplants among four of these populations separated the effects of lake-specific environmental cues from the genetic and maternal effects of the different populations. Additional eggs were incubated in the laboratory under photoperiod–temperature combinations representative of those in the field. We found that the annual hatching fraction ranged from 6% to 50% among lakes, and that hatching fraction was primarily driven by environmental cues rather than being a result of the source of the eggs. However, laboratory incubations demonstrated significant differences among populations in the trajectories of the hatching curves, and a much higher rate of hatching than the field incubations. Our results suggest that variation in dormancy strategies within these systems is likely influenced both by the seasonal risk experienced by the active individuals and by risks associated with entering the dormant egg bank.

Key words: *bet hedging;* Daphnia pulicaria; *diapause; dormancy, optimal; dormancy termination and environmental cues; resting eggs; zooplankton.*

INTRODUCTION

Most organisms live in variable environments and have evolved life-history traits that facilitate survival and reproduction in habitats of fluctuating quality. For example, many species produce long-lived dormant propagules such as seeds, eggs, or cysts that can not only survive conditions that are lethal to active individuals, but can also create overlapping generations with the formation of persistent seed or egg banks (Leck et al. 1989, Hairston et al. 1995, Cáceres 1997). When only a fraction of propagules resume development at the first available opportunity (e.g., first rainfall after seed drop), short-term fitness gains are sacrificed, but this bet-hedging strategy of variable germination pays off in habitats that are occasionally so bad that recruitment back to the dormant stage fails entirely (Cohen 1966, Seger and Brockmann 1987). Just what fraction of propagules should forgo resuming development is predicted by theory to be related to the frequency of favorable habitat conditions and the probability of survival in the dormant stage (Cohen 1966, Ellner 1985*a*).

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If this theory is correct and dormancy termination schedules can be shaped by selection, habitats that differ dramatically in either the frequency of ''bad'' conditions or the mortality rate of propagules should select for populations that differ in their dormancy strategies. Annual plants have served as the primary model system for testing this theory, and variation in germination fractions at the level of species, population, and genotype is well established (Went 1949, Grime et al. 1981, Phillipi 1993*a*). The studies that have rigorously addressed the theory indicate that delayed germination is consistent with a bet-hedging strategy, and some suggest the occurrence of ''predictive germination'' in which phenotypic plasticity in response to the hatching cues results in the highest germination fractions in years that are favorable for recruitment (Rice 1985, Phillipi 1993*b*, Evans and Cabin 1995, Pake and Venable 1995, Clauss and Venable 2000).

Much like annual plants, many species of freshwater and nearshore marine zooplankton produce long-lived dormant eggs that accumulate in vast numbers in sediment egg banks (De Stasio 1989, Marcus et al. 1994, Hairston 1996, Cáceres 1998). The existence of these egg banks indicates that not all eggs resume development soon after they were produced, raising the question of whether or not this accumulation of viable

offspring represents a bet-hedging strategy parallel to that seen in annual plants. Laboratory studies have indicated substantial variation in response to hatching cues across species, populations, and genotypes of zooplankton (Pancella and Stross 1963, Schwartz and Hebert 1987, Van Dooren and Brendonck 1998) and have documented significant genetic effects on the hatching fraction (De Meester and De Jager 1993*a*). A few field studies have directly estimated hatching rates from the sediment egg bank, and all conclude that a very small fraction of the viable eggs terminate dormancy within a given year (De Stasio 1989, Cáceres 1998, Hairston et al. 2000).

If dormancy schedules in zooplankton populations are shaped by selection, then we would expect the hatching fraction of eggs to vary among lakes, depending on the probability of successful recruitment back to the egg bank (sensu Cohen 1966, Ellner 1985*a*, *b*). Successful recruitment is likely influenced by a number of ecological factors (e.g., resource levels, abundance of competitors, and predation risks) known to vary widely among lakes in the same geographic region. Alternatively, rather than genotypes bet hedging by producing some fraction of offspring that will delay hatching, diapausing eggs may accumulate in the sediments simply because some fail to get the ''right'' cue soon enough and are subsequently buried. Since eggs may need to be within the top few millimeters of sediment to receive the hatching cue and terminate diapause, the majority of the eggs produced can quickly become buried too deeply to contribute to the active population (Kasahara et al. 1975, Cáceres and Hairston 1998). If this is the case, mother and offspring may have little control over hatching schedules and the presence or absence of egg banks in a particular system may be driven to a large extent by physical processes such as the degree to which sediments accumulate and are subsequently mixed. Hence, if diapause strategies are shaped by selection in these populations, the risks experienced in the water column balanced against the risks in the sediment will determine the optimal strategy.

Our research focuses on the variation in diapause strategies of several populations of *Daphnia pulicaria* inhabiting small lakes in southwest Michigan (USA). More than six years of field sampling indicate that the annual phenology of the *Daphnia pulicaria* in the water column varies considerably across these populations, reflecting a natural gradient of persistence ability in the active stage (Tessier and Welser 1991, Geedey et al. 1996, Tessier and Leibold 1997, A. J. Tessier and C. E. Cáceres, *unpublished data*). We combine field and laboratory incubations of newly-produced diapausing eggs to assess variation in dormancy termination in these populations. Specifically, we address the following questions: Do dormant eggs from different populations of *D. pulicaria* exhibit variation in annual hatching rates? Is hatching in a particular system primarily the result of genetic or environmental effects? Is there any evidence that egg banks represent an optimal strategy for these populations? Our results suggest that despite differential responses to environmental cues both within and between populations, the optimal hatching strategies predicted by theory are constrained by among-lake variation in availability of environmental cues.

Methods

Study systems

Our study lakes are small glacial kettles in southwest Michigan (Barry and Kalamazoo Counties) that vary in surface area (4.8 ha–67.6 ha) and maximum depth (9 m–14 m). The zooplankton assemblage of each lake is dominated by *Daphnia pulicaria* during spring. Four of the lakes thermally stratify in summer, creating a deep-water refuge in which the *D. pulicaria* can avoid competition and predation (Wright and Shapiro 1990) and therefore persist year-round (perennial populations: Bristol Lake, Lawrence Lake, Warner Lake, 3 Lakes 2). Among these perennial populations, however, there is considerable variation in the annual water-column abundance and seasonal dynamics of *D. pulicaria*. This variation is in part associated with the fact that in some lakes, deep-water anoxia reduces the size of available refuge, leading to a decline of summer densities (Tessier and Welser 1991, C. E. Cáceres and A. J. Tessier, *unpublished data*). The fifth lake, Little Long, has a large surface area and therefore does not stratify thermally during the summer. The water-column abundance of *D. pulicaria* in this lake typically falls below detection by mid-summer due to a lack of deep-water refuge (annual population). These differences in water-column dynamics likely create annual variation within and among lakes in a population's ability to hatch from the egg bank and successfully recruit back to the dormant stage, thereby creating variation in the frequency of ''good'' and ''bad'' years among populations. Moreover, differences in basin shape and productivity among the lakes likely influence the rates of sedimentation and re-suspension in these lakes, two key processes in determining the risk of eggs being buried too deeply to ever receive a hatching cue (Cáceres and Hairston 1998).

For most of the year, female *D. pulicaria* produce daughters parthenogenetically. During May and June, males and diapausing (dormant) eggs are also produced. One or two dormant eggs are encased in a protective modification of the female's carapace known as an ''ephippium,'' which closes around the eggs after the female molts. The timing and intensity of the production of diapausing eggs varies among populations and is correlated with persistence ability in the water column (C. E. Cáceres and A. J. Tessier, *unpublished manuscript*).

Hatching fraction of newly produced eggs

To investigate hatching rates in newly produced diapausing eggs, we collected ephippia-bearing *Daphnia* from the water column of Warner and Little Long Lakes during May–June 1999. The *Daphnia* were incubated at room temperature in the laboratory for up to 48 hours, and ephippia released during that time were transferred to six-well tissue-culture trays (50 two-egg ephippia per tray = 100 eggs). Six holes drilled in the lids of each tray were covered with a 200-μm mesh to allow water exchange. During the second week of June, ephippia were returned to the lake from which they had been isolated. When diapausing eggs are released by a female in the field, they can either settle to the sediment in the mixing zone (epilimnion) or into sediment in deeper water below the thermocline (hypolimnion). Eggs that settle in the mixing zone receive higher light levels, lower sediment load, and warmer temperatures than those that settle into deeper water. Hence, when we returned our trays to the field we established two transects in each lake, with two sites per transect. Each transect had one nearshore site located in the mixing zone and another site in the offshore region. In a thermally stratified lake (all lakes but Little Long), the offshore site was not only deeper, colder, and darker than the nearshore site, but also experienced periods of summer anoxia. Each tray, which served as the experimental unit, was secured inside a frame constructed of plastic garden fencing and rebar (two trays per frame). We used scuba to firmly attach the frames to the bottom of the lakes (two frames per site). One frame at each site was removed in early November 1999, while the other remained in place until May 2000. All trays were successfully recovered at the end of their incubation.

At the end of the 6-mo or 1-yr incubation, all ephippia were removed from each tray and examined individually. Based on visual inspection, each ephippium was scored as containing no eggs (open and empty), one, or two eggs. A small number of eggs were clearly no longer viable (Cáceres 1998) and were recorded as such. Since all ephippia contained two eggs at the beginning of the experiment, and the open and empty ephippia remain in the tray after the *Daphnia* have hatched, ''missing'' eggs from the recovered ephippia were considered to have hatched. A small fraction of the initial ephippia ($<3\%$ in experiment 1 and $<5\%$ in experiment 2) were not found at the end of the experiment, hence we define ''hatching fraction'' as the proportion of recovered eggs (i.e., two times the number of recovered ephippia) that had hatched.

In spring 2000 we repeated this experiment but revised the design to include additional lakes and to perform a series of reciprocal transplant experiments to address the relative importance of population and environmental effects on hatching fraction. In May–June 2000 we isolated newly produced ephippia from five lake populations (Bristol, Lawrence, Little Long, Warner, 3 Lakes 2 [3L2]). Thirty ephippia (60 eggs) from each population were transferred to tissue-culture trays and returned to the bottom of their own lake along three transects (one tray per site). In Little Long, Warner, and 3L2, each transect had a nearshore and offshore site. In Lawrence Lake, steep banks prohibited the establishment of nearshore sites, hence we only established three offshore sites. In Bristol, we could collect only enough eggs for three nearshore sites. In addition to the eggs being incubated in their own lake, eggs from Little Long were incubated at all established sites in all lakes. Eggs from 3L2 and Bristol were also incubated at the nearshore sites in Little Long, and eggs from Warner Lake were incubated in both nearshore and offshore locations in Little Long. All eggs were incubated until May 2001, and hatching fraction was determined as in the first experiment. Once again, all trays were successfully recovered.

In addition to the field experiments, we incubated newly produced diapausing eggs from our populations in two Percival environmental chambers (Percival Scientific, Perry, Iowa, USA). One chamber was set up with the photoperiod–temperature combination representative of our nearshore sites, and the other chamber was set up with the photoperiod–temperature conditions typical of our offshore sites in a thermally stratified lake. Prior research indicates that photoperiod and temperature are proximate cues for terminating dormancy in *Daphnia* (Stross 1966). Each week, settings on the chambers were set to reflect field conditions typically found in stratified lakes in southwest Michigan (Wetzel 1983). Both chambers were always set to the same photoperiod, but temperatures differed in the two chambers June–October to reflect the average temperature at the sediment in the epilimnion and the hypolimnion. Conditions in the two chambers were identical (ambient photoperiod, temperature $\leq 10^{\circ}$C) during November–April, which represented the periods of complete mixing and ice cover in the lakes. Treatments were periodically rotated between the chambers to reduce the possibility of chamber effects.

These laboratory experiments were conducted in both 1999 and 2000. In 1999, three trays (100 eggs each) of both Little Long and Warner were incubated in both the nearshore and offshore treatments. In 2000, we were limited by the number of eggs collected, hence only Little Long had three trays (50 eggs each) in each treatment, all other populations had two trays (50 eggs each), except for Warner which had one tray. In addition to the five lakes used in the field experiment, we also examined the hatching fraction of a sixth population (Big Long, two trays each treatment). Hatchlings were counted weekly for 48 weeks in 1999–2000 and 45 weeks in 2000–2001, at which point no viable eggs remained. The cumulative hatching fractions as well as the hatching trajectories for Little Long and Warner in

the 1999–2000 experiment were combined with those from the 2000–2001 experiment for analysis.

Statistical analysis

For the first field experiment, we used three-way ANOVA to analyze the effect of source population (Warner vs. Little Long), duration of incubation (six months vs. one year), and incubation depth (nearshore vs. offshore) on the hatching fraction of newly produced eggs. In the second field experiment (2000–2001), basin morphometry and egg limitation precluded the establishment of a fully factorial design in the reciprocal transplants (see above). Since the experimental design did not allow the use of a single model, we used a series of one-way and two-way ANOVAs to address different questions. Specifically, since two of the five lakes (Bristol and Lawrence) were placed at a single incubation depth, we exclude incubation depth as a factor in the first three analyses. First we used one-way ANOVA to examine if hatching fraction varied among the five populations when eggs were incubated in their own lake. We then used two one-way ANOVA models to address the question of source vs. environment by examining hatching fraction of Little Long eggs in the five different lakes and the hatching fraction in the four populations incubated in the common garden of Little Long. The potential effect of incubation depth was considered for the three lakes (Little Long, Warner, and 3L2) that had both nearshore and offshore sites, first by using two-way ANOVA to compare the effects of lake environment and incubation depth on the hatching fraction of eggs incubated in their own lake, and then by using two-way ANOVA to compare the environmental effects (host lake and incubation depth) on hatching fraction of Little Long eggs. Finally, we used three separate two-way ANOVA models, with a sequential Bonferroni correction, to address the effects of source population vs. host lake in the three reciprocal transplant experiments (Little Long × Warner, Little Long × 3L2, Little Long × Bristol).

The five lakes, two seasons, and two incubation depths were chosen specifically to define the range of population phenology and environmental variation of interest, and so were treated as fixed effects. Each tissue-culture tray served as the experimental unit. Because the residuals from most models indicated that variance tended to increase with treatment mean, hatching fractions from the field and laboratory hatching experiments were $\log(X + 1)$ transformed. Unless otherwise noted, interactions were not significant. Analyses were performed in SYSTAT 10.0 (Wilkinson 2000).

We used JMP 4.0 (SAS Institute 2000) to examine the effects of source population and treatment on hatching rates in the laboratory. Product-limit (Kaplan-Meier) hatching estimates for each population were calculated as:

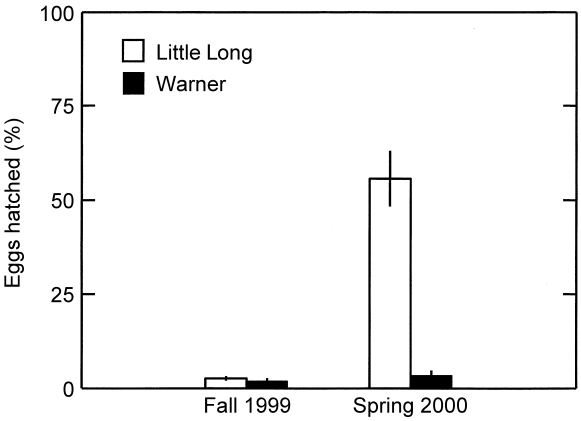

FIG. 1. Hatching fraction in the first field experiment (1999–2000) for eggs produced by *Daphnia pulicaria* in Little Long and Warner lakes (southwest Michigan, USA). Eggs were incubated in the lake in which they were produced for either 6 mo (fall) or 1 yr (spring). The effect of incubation depth (nearshore vs. offshore) was not significant but contributes to the variance. Data are means ± 1 SE.

$$S(t) = 1 - (h_i/n_i)$$

where $S(t)$ is the fraction of eggs remaining dormant at time t, h_i is the number hatching during interval i and n_i is the number that were dormant at the beginning of the interval. Eggs that degraded during the laboratory incubation were right censored. Differences among treatments and among populations within each treatment were determined with log-rank tests. Cumulative hatching fraction was examined by two-way ANOVA, with each tray serving as the experimental unit.

RESULTS

Hatching in the lakes

In our first hatching-fraction experiment (1999–2000) we found a significant effect of season retrieved (fall vs. spring) and population (Little Long vs. Warner), but not of incubation depth (nearshore vs. offshore), on the fraction of *Daphnia pulicaria* eggs that terminated dormancy (Fig. 1; season $F_{1,24} = 38.39$, $P < 0.0001$; population $F_{1,24} = 37.32$, $P < 0.0001$; incubation depth $F_{1,24} = 0.83$, $P = 0.37$). In both lakes, <3% of the eggs had terminated dormancy by the November 1999 sampling, but the following spring >55% of the eggs hatched in the annual population (Little Long) and <4% hatched in the perennial population. The large increase in the Little Long population compared to consistently low hatching in Warner resulted in a significant season × lake interaction as well ($F_{1,24} = 22.85$, $P = 0.0001$). Incubation depth was not significant because so few eggs hatched at any site in Warner and hatching at the nearshore and offshore sites was similar in Little Long.

In the second experiment (2000–2001) we again observed a higher hatching fraction in Little Long eggs

(49%) as compared to Warner eggs (7%), suggesting that hatching fraction within a particular system may be relatively constant from year to year. By including three additional populations and a series of reciprocal transplants in the 2000–2001 experiment we found that hatching fraction varied substantially across a range of lake types and that this difference was primarily the result of lake-specific environmental cues. When incubated in their own lake, the average hatching fraction of the five populations of *D. pulicaria* ranged from 6 to 50% ($F_{4,19} = 3.78$, $P = 0.02$). Although this variation could result from differences in either the environmental cues associated with each lake or the genetic and maternal effects of each population's eggs, the hatching fraction of Little Long eggs incubated in each of the five systems showed a similar range of variation ($F_{4,19} = 4.40$, $P = 0.011$). Further, no significant difference was observed across the four populations incubated in the common-garden environment provided by Little Long ($F_{3,13} = 0.09$, $P = 0.96$). These results suggest that most of the variation in hatching fraction among populations can be explained by environmental differences among the lakes.

In addition to comparing environmental differences among the lakes, we also examined the environmental effects of nearshore and offshore sites. In the second experiment three of the five lakes (Little Long, Warner, and 3L2) had their own eggs incubated at both nearshore and offshore sites. We found a significant effect of lake, incubation depth, and the lake × depth interaction on the hatching fraction in these three populations (lake $F_{2,12} = 12.83$, $P = 0.001$; depth $F_{1,12} = 9.00$, $P = 0.01$; lake × depth $F_{2,12} = 4.75$, $P = 0.03$; Fig. 2). This analysis indicates clearly the effects of incubation depth, but because we only considered eggs of each of the three populations incubated in their own lake, it is unclear whether the lake effect results from a genetic or maternal effect, or from the lake-specific environmental cues of each incubation lake. We therefore examined the Little Long eggs incubated at nearshore and offshore sites in the three lakes (Little Long, Warner, 3L2). The hatching fraction of Little Long eggs was significantly influenced by both the host lake and incubation depth (Fig. 2; host $F_{2,12} = 12.44$, $P = 0.001$; depth $F_{1,12} = 5.07$, $P = 0.04$). The depth effect seemed primarily to be driven by the differences between nearshore and offshore sites in 3L2 (Fig. 2, open bars nearshore vs. offshore), but the host × depth effect was not significant ($F_{2,12} = 3.17$, $P = 0.08$).

Finally, we used our three reciprocal transplants (Little Long × Warner, Little Long × 3L2, Little Long × Bristol) to analyze the effects of source population and host environment. In each of the three transplant experiments, source population had no effect on hatching fraction (LL × W $F_{1,19} = 0.02$, $P = 0.88$; LL × B $F_{1,11} = 0.18$, $P = 0.67$; LL × 3L2 $F_{1,17} = 2.8$, $P = 0.11$). Similarly, in no case, did we observe a significant effect of host × source interaction (LL × W $F_{1,19} = 0.02$, $P = 0.89$; LL × B $F_{1,11} = 0.55$, $P = 0.47$; LL × 3L2 $F_{1,17} = 0.08$, $P = 0.78$). However, host environment was significant in one experiment, and marginally significant in a second even after sequential Bonferroni correction (LL × W $F_{1,19} = 37.77$, $P < 0.0001$; LL × B $F_{1,11} = 5.97$, $P = 0.03$; LL × 3L2 $F_{1,17} = 0.02$, $P = 0.90$). These results confirm the large effect of environmental cues relative to source-population effects in terminating dormancy in these *Daphnia* populations.

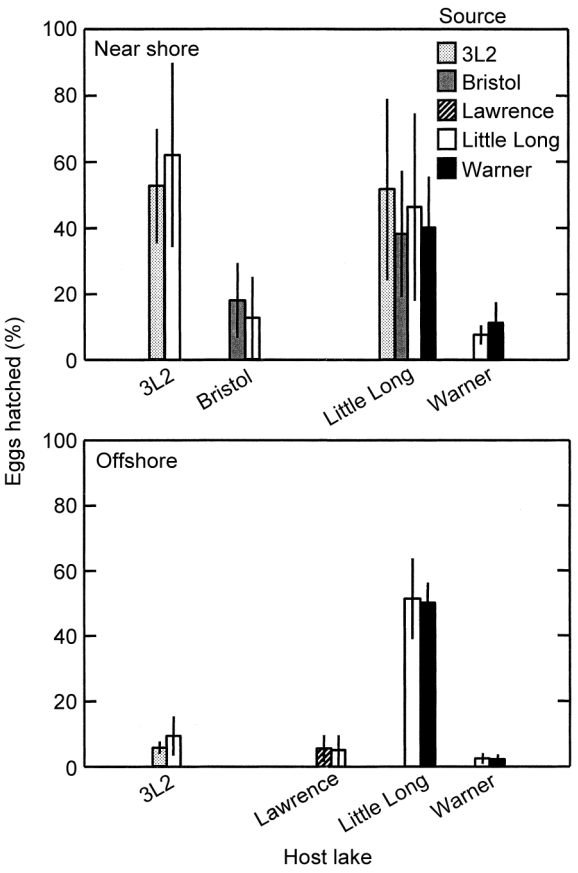

FIG. 2. Hatching fraction in the second field experiment for eggs produced by five populations of *Daphnia pulicaria*. Each population had a set of eggs incubated in the lake in which they were produced; all but Lawrence were also incubated in Little Long. Little Long was incubated in all five systems. Top panel shows hatching from nearshore sites. The bottom panel represents hatching from the offshore site in each lake, which was in the hypolimnion for 3L2, Lawrence, and Warner, but not for Little Long. Data are means ± 1 SE.

Our comparison of hatching fraction among populations assumes that eggs from the five lakes did not differ in initial viability or their ability to survive for the duration of the experiment. To test this assumption we compared the number of visibly nonviable eggs recovered after the field incubations in both years. Among the five populations, the percentage of nonviable eggs at the end of one year ranged between a low of 0 ± 0.0% (mean ± 1 SE) in Lawrence (i.e., all remaining eggs appeared to be viable) to a high of 15

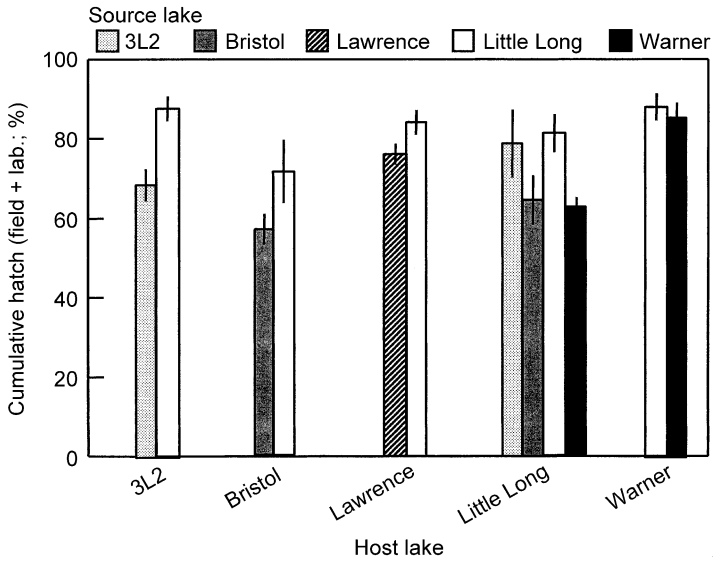

FIG. 3. Viability estimates of *Daphnia pulicaria* eggs used in the 2000–2001 experiment (% hatched). Following removal from the lakes, all seemingly viable eggs were transferred to new tissue-culture wells and incubated in the laboratory at 10°C until no viable eggs remained. The total numbers of eggs hatched (lake + laboratory) are plotted; data are means ± 1 SE.

± 4.3% in Bristol. The other three populations had <8% nonviable eggs. Viability did not differ among the four populations central to the reciprocal transplant experiment presented above, either when they were incubated in their own lake ($F_{3,33} = 2.10$, $P = 0.12$) or in Little Long ($F_{3,13} = 1.64$, $P = 0.23$). On average 94.7% ± 1.3% of the eggs in 1999–2000 and 93.7 ± 1.0% of the eggs in 2000–2001 were viable throughout the experiment. The high survival rate in the field suggests that the differences we observed were strongly related to the lake-specific environmental cues rather than viability differences among populations.

The seemingly viable eggs that remained after the 2000–2001 incubation in the field were transferred to new tissue-culture wells and incubated in the laboratory at 10°C; hatching was checked until no viable eggs remained (7–15 months). By the end of this laboratory incubation the cumulative hatching (field + laboratory) was substantially greater than field hatching alone for all populations, confirming the intrinsic viability of these year-old eggs. However, under laboratory conditions a significant fraction of the eggs began to degrade, resulting in a cumulative hatch that varied among populations (Fig. 3), both for eggs that had been incubated in their own lake ($F_{4,23} = 7.1$, $P = 0.001$) and in the common-garden environment provided by the Little Long incubation ($F_{3,13} = 5.2$, $P = 0.01$). Laboratory conditions served to increase both hatching and senescence rates of the eggs.

Hatching fraction in the simulated habitats

Newly produced eggs that were incubated in the laboratory under temperature–photoperiod combinations representative of field conditions exhibited differential responses to cues (Fig. 4). For all populations the eggs placed into hypolimnion conditions began to hatch immediately, indicating that newly produced eggs have no ''latent period'' during which time they will not hatch. In contrast, in the nearshore treatment four of the six populations exhibited little or no hatching for the first three months of incubation. The other two populations had an initial burst of hatching, but then exhibited similar trajectories as the other populations. In the nearshore treatment, all populations increased hatching rate during the simulated October conditions associated with temperature dropping below 10°C.

The early hatching in the hypolimnion treatment compared to the delayed hatching for most populations in the epilimnion treatment led to clear differences in the hatching-time functions between habitat treatments (log-rank test; $\chi^2 = 215.2$, df = 1, $P < 0.0001$), a result that holds even when the hatching that occurred during the first week is excluded from the analysis (log-rank test; $\chi^2 = 291.8$, df = 1, $P < 0.0001$). Moreover, hatching clearly varied among populations in each treatment. When we excluded the first week of hatching from the analysis we still found differences among populations in both the epilimnion (log-rank test; $\chi^2 = 84.1$, df = 5, $P < 0.0001$) and hypolimnion treatments (log-rank test; $\chi^2 = 98.4$, df = 5, $P < 0.0001$). The qualitative results are also unaffected by excluding the right-censored eggs from the analysis. In addition to variation in the hatching trajectories, cumulative hatch varied both among populations and habitat (two-way ANOVA: source $F_{5,26} = 12.34$, $P < 0.0001$; habitat $F_{1,26} = 8.39$, $P = 0.008$). However, all eggs used in the laboratory experiment either completed development or degraded within 48 weeks. This contrasted greatly with the continued viability of the eggs that had been incubated in the field for a year, and confirms that laboratory conditions accelerated both hatching and senescence of both newly produced and year-old eggs.

DISCUSSION

Our field experiments document considerable variation in the hatching fraction of *Daphnia pulicaria* eggs

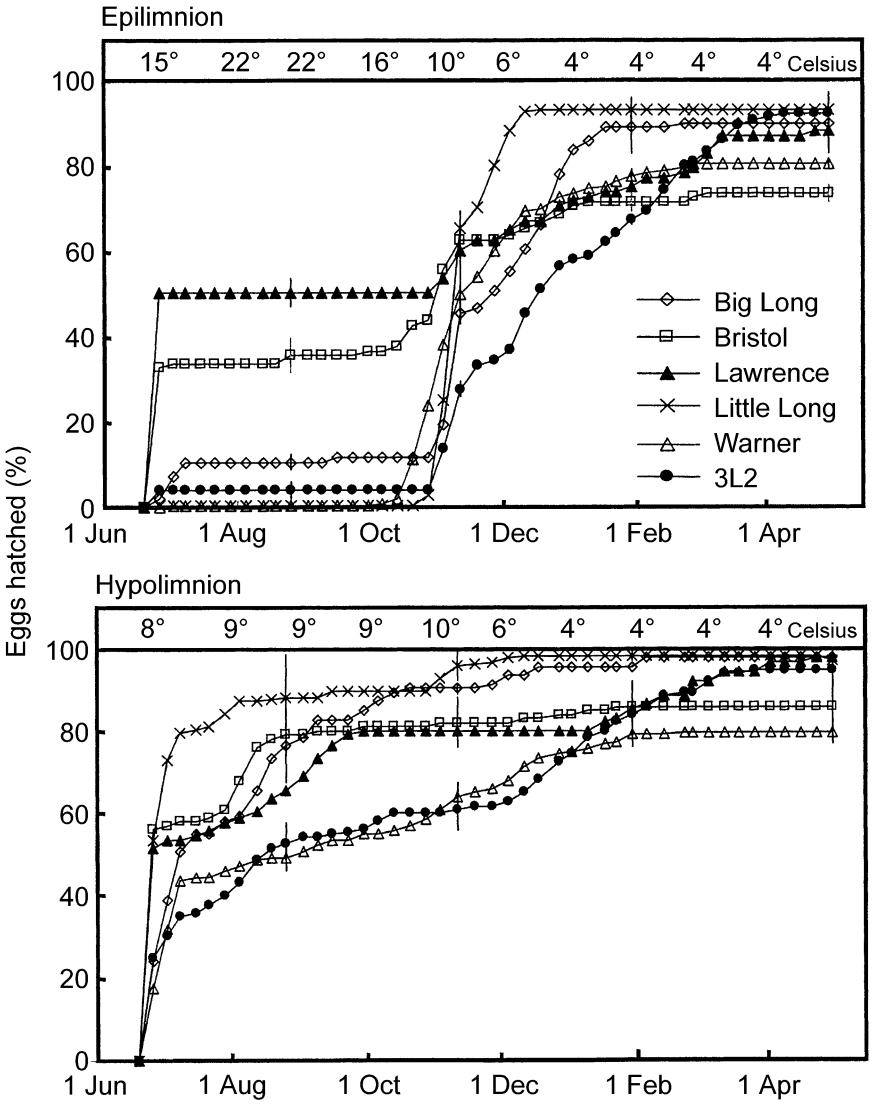

FIG. 4. Hatching schedules of newly produced, diapausing *Daphnia pulicaria* eggs in the laboratory under temperature and photoperiod conditions representative of the epilimnion (top) and hypolimnion (bottom) in lakes in southwest Michigan, USA. Big Long, Bristol, Lawrence, and 3L2 Lakes have eggs for 2000–2001 only, but Little Long and Warner were measured in both 1999–2000 and 2000–2001. For clarity, error bars (±1 SE) are shown for only four dates throughout the experiment; these error estimates are representative for the entire experiment. Numbers at the top of each panel represent the temperature on the first day of the month in each treatment; each week photoperiod was the same in both environmental chambers and representative of the photoperiod in southern Michigan (USA).

within and among lakes. Our reciprocal transplants and comparisons of offshore vs. nearshore habitats indicate that most of this variation in hatching fraction in nature is determined by the environment. Although the laboratory incubations document significant genetic or maternal variation in response of dormant eggs to the hatching cues, the resulting variation among populations in cumulative hatching is less pronounced than the effects of incubation lake and depth on the hatching of each population's eggs. The laboratory results also suggest bet hedging (variable dormancy) or predictive germination (increased hatching in response to seasonal cues). However, large differences in hatching patterns between the laboratory and lake incubations call for caution in extrapolating laboratory results as an approach to testing adaptive hypotheses. Moreover, since our field-collected ephippia were likely produced by multiple maternal genotypes, we cannot partition genetic from maternal phenotype effects, necessary in any discussion of bet hedging. It is obvious, however, that there is a general seasonal pattern to the hatch (inhibition in summer, stimulation beginning in late autumn), not all eggs respond to the environment in the same way, and there are large differences among lakes in the probability of getting an adequate seasonal cue.

In describing dormant seeds, Harper (1977:65) said that ''some seeds are born dormant, some acquire dormancy and some have dormancy thrust upon them,'' and plant ecologists often distinguish between ''innate'' vs. environmentally ''enforced'' dormancy. The range of abiotic conditions within and across lakes, coupled with the fact that the dormant eggs of zooplankton can quickly become buried and shut off from the hatching cues, makes this distinction essential to understanding the evolution of dormancy strategies in zooplankton. Despite the observed differences in response to photoperiod and temperature cues in the laboratory, much of the variation in our field results is easily explained by environmental variation in cues such as light availability and temperature. Most eggs do not hatch from sites that are perpetually cold and dark.

In the field the availability of photoperiod and temperature cues, which are known to break dormancy in zooplankton (Stross 1966, Schwartz and Hebert 1987, Arnott and Yan 2002), obviously varied both within and among lakes. In our thermally stratified lakes, where the offshore sites experienced constant cold temperatures and light levels can be as low as 3% of surface irradiance (Gerrish 2001), hatching was on average $<10\%$. Offshore sites in stratified lakes often experience higher sediment accumulation with lower levels of mixing and re-suspension (Weyhenmeyer et al. 1997). Since so few eggs are being cued to hatch early on, many are becoming permanently entombed in the egg bank where they are effectively dead unless some localized mixing action returns them to the sediment–water interface (Cáceres and Hairston 1998). Hence, rather than providing a risk-spreading strategy, in most lakes the majority of the egg bank is likely in the category of ''those that have dormancy thrust upon them.'' Diapausing eggs in Little Long Lake may be the exception to this rule. Since this lake mixes deeply and does not stratify in summer, potential environmental cues differ far less between the nearshore and offshore sites. This was the only host lake in which hatching from the offshore sites was comparable to that of the nearshore sites. Moreover, Little Long Lake in the one system in which the *Daphnia* population always falls below detection limits in the water column by midsummer and does not reestablish for several months (A. J. Tessier and C. E. Cáceres, *unpublished data*). The lack of potential competitors at times may increase the probability that a genotype that emerges from the sediment will successfully invade the water column. That, coupled with the relaxation of environmental constraint, may have contributed to the high hatching in this system.

Across the nearshore sites of the different lakes, where eggs experienced a full range of temperatures and the light levels were considerably higher than at the offshore sites, hatching fractions still differed dramatically across lakes and were consistently $<100\%$. Although differential rates of burial among the lakes may partially contribute to this pattern, there is still the question of why so many eggs delay development until sometime after the first year. One possible explanation for our field pattern is that genotypes are capable of ''predictive germination,'' as has been suggested for annual plants, and only resume development during times that are favorable for recruitment (Rice 1985, Evans and Cabin 1995, Pake and Venable 1995). Considerable evidence has accumulated documenting significant variation in fitness among clones coexisting in a single habitat (Weider 1985, De Meester et al. 1995, Tessier and Leibold 1997), hence eggs produced by different genotypes will be favored in the water-column under different conditions. Although we found no information indicating that the dormant eggs of *Daphnia* can assess the quality of their habitat, this has been suggested for other aquatic organisms. For example, Rengefors et al. (1998) and Hansson (2000) found that three species of algae reduced termination of dormancy when in the presence of herbivores. If *Daphnia* diapausing eggs are capable of assessing habitat quality and adjusting their hatching fraction accordingly depending on the particular environment, we would expect differential hatching among lakes.

Our results also suggest the possibility that *Daphnia* clones produce diapausing eggs with differential responses to the environment, some which hatch early and some which hatch later. In annual plants, the maternal genotype can control dormancy duration of the seeds by such mechanisms as varying seed size or the thickness of the seed coat (Harper 1977, Westoby 1981, Bradbeer 1988). The ''seed coat'' of *Daphnia* dormant eggs, the ephippium, varies considerably in levels of melanization, with some ephippia being completely transparent and some being completely opaque. Gerrish (2001) found significant variation in pigmentation of the ephippia both within and among our study lakes, and used laboratory experiments to determine that pigment levels were strongly influenced by the maternal genotype. Moreover, De Meester and De Jager (1993b) and De Meester et al. (1998) suggest both genetic differences and maternal effects on the delayed hatching schedules observed in the laboratory. However, given that many of the eggs are likely to never receive the hatching cue, the effectiveness of this bet-hedging strategy may be largely constrained by the environment.

In our laboratory experiment we did find differential responses to the photoperiod and temperature cues both within and across populations, but the level of variation differed among lakes. For example, in the epilimnion and hypolimnion treatment of Little Long, 90% of the hatching occurred in a period of about seven weeks, whereas comparable levels of hatching in 3L2 took over 40 weeks. Because Little Long does not stratify thermally, cool temperatures may be a particularly good signal for the changing seasons. This is not the

case in stratified lakes, where eggs that descend into the hypolimnion experience cold temperatures immediately after they are produced. For most populations, warm water seems to inhibit hatching, which is not surprising since *D. pulicaria* is most abundant in the water column during spring. We suspect that the short pulse of hatching during the first week in the epilimnion treatment of Lawrence, Bristol, and Big Long was a response to the experimental setup, rather than to the warm temperature since hatching in all three populations did not resume until cooler temperatures were experienced.

The accelerated hatching and senescence observed in the laboratory complicates extrapolating these results to the dynamics of the field populations. It is now well established that diapausing eggs of zooplankton can remain viable in situ for decades if not centuries (Marcus et al. 1994, Hairston 1996, Cáceres 1998), which was certainly not the case for our laboratory experiments. One major difference between the sediment egg bank and the laboratory experiment is that most of the eggs in the egg bank are surrounded by anoxic sediment, which is known to inhibit both embryonic development and metabolism in diapausing cysts of *Artemia* (Hoffmann and Hand 1990, Clegg 1997). The oxygenated conditions present in the laboratory experiment may have led to an increase in metabolism, which will eventually lead to senescence of the egg. Hatching in the laboratory could also be increased due to increased light availability and lack of any chemical inhibition that might be present in the natural water column. Although much of what is known about dormancy strategies has resulted from laboratory experiments, it is clear that the laboratory results do not adequately represent field dynamics (Cáceres and Schwalbach 2001).

As research into diapause in zooplankton increases, it is becoming clear that ephippia are more than an over-wintering stage that is produced by all individuals in the fall to hatch the following spring. These animals exhibit considerable variation in their dormancy strategies, both within and across populations. The challenge is then to understand the causes and consequences of this variation. The life-history theory developed for annual plants provides an excellent starting point, and recent theory has begun to consider optimal dormancy strategies in freshwater invertebrates (e.g., Ellner et al. 1998, Easterling and Ellner 2000, Spencer et al. 2001). However, additional information from multiple populations regarding recruitment success in the water column and survival ability in the sediment is needed before this theory can be rigorously tested in natural systems. The among-lake differences in the sizes of persistent water-column populations, coupled with differential food resources and natural enemies, likely also create a gradient of invasibility from the dormant egg bank that should strongly influence the relative costs and benefits of seeking refuge in the sediment. Finally, variable sedimentation rates and physical mixing dynamics of the sediment result in across-lake differences in the fraction of the dormant eggs lost to burial each year. The environmental constraints placed on these optimal strategies may be considerable in deep stratified lakes in which the majority of the eggs will never be exposed to a dormancy termination cue. The risks experienced in the water column and the sediment are obviously linked, and information regarding how both vary across systems is needed to fully investigate the evolution of dormancy strategies in zooplankton.

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