

To sink or swim: Variable diapause strategies among *Daphnia* species

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Abstract

Although dormancy is assumed to be fundamental to population persistence in temporary ponds, it has been suggested that diapause may be less frequent in populations that inhabit permanent lakes. We compared the seasonal timing and magnitude of investment in diapausing eggs and their subsequent storage in the sediment among *Daphnia* species from both temporary ponds (*Daphnia pulex* and *Daphnia ephemeralis*) and permanent lakes (*Daphnia pulicaria* and *Daphnia dentifera*). Species exhibited strong temporal segregation in the timing of diapausing eggs production in both temporary and permanent systems. All populations of *D. ephemeralis* produced ephippia in early April whereas *D. pulex* and *D. pulicaria* produced the majority of ephippia in May–June and *D. dentifera* did not produce males or ephippia until autumn. Maximum investment in dormant clutches (as opposed to immediately hatching eggs) was always 100% in the temporary pond species but ranged from 3% to 100% among populations living in the lakes. The number of eggs stored in the egg bank varied within and among species but was usually lower in the temporary pond species. Our results indicate that the use of dormancy in *Daphnia* varies considerably among populations and species and that whether the system is temporary or permanent is not necessarily a good predictor of a population's investment in dormancy.

Dormancy has long been recognized as a fundamental component of the life cycle of zooplankton living in temporary ponds (Sars 1885; Wiggins et al. 1980). For aquatic invertebrates living in a habitat that contains water for only a fraction of the year, the need for a desiccation-tolerant dormant stage is obvious. In contrast, it has been suggested that, in permanent lakes, the cost of entering dormancy can be so high that selection should favor a reduced dormancy investment (Lynch 1983; Korpelainen 1986). Nevertheless, many studies in permanent lakes and near-shore marine environments have documented that copepods, cladocerans, and rotifers often produce vast numbers of diapausing eggs that accumulate in sediment egg banks (see Hairston 1996 for a review). The presence of these egg banks indicates that selection has not eliminated dormant egg production in permanent systems but provides no information regarding the relative investment in dormant eggs (as compared with immediately hatching eggs) in these populations. Hence, the question remains: Is dormancy investment actually lower in populations inhabiting permanent lakes than it is in populations that live in temporary ponds?

Theoretical models developed for annual plants and dia-

pausing insects suggest that more than just the presence or absence of water needs to be addressed in considering how selection should shape dormancy strategies in zooplankton (Cohen 1966, 1970). These models illustrate that an individual's fitness is influenced by both the seasonal timing of and relative investment in diapause (in comparison with the production of nondiapausing offspring). When the habitat regularly becomes completely inhospitable, such as the dry period of a temporary pond, at some point prior to the catastrophe, 100% of the reproduction should be in the form of dormant offspring. Moreover, there is an optimal time to switch to producing these dormant offspring. Switching too early sacrifices an opportunity to make daughters who could produce their own dormant offspring, but switching too late risks being eliminated from the habitat (Hairston and Munns 1984). In general, the optimal timing of dormancy is predicted to occur when the expectation of reproduction in the active stage drops below the expectation of survival in the dormant stage (Cohen 1970). If the frequency and magnitude of risks experienced by active and dormant individuals varies among populations, then the timing and magnitude of dormancy investment is expected to vary among populations as well.

Predicting the optimal timing of dormancy in any particular population is not simple. Although pond drying is obviously a major catastrophe for zooplankton, in both temporary ponds and permanent lakes, annual or interannual fluctuation in predation, parasites or competitors can also eliminate a population from the water column (Lampert and Sommer 1997). Moreover, while dormancy does provide a temporal refuge from hardships in the water column, it is neither a risk-free endeavor nor a guarantee for survival. For many populations of zooplankton, investment in dormancy requires a switch from asexual to sexual reproduction, requiring the location of a suitable mate. When the dormant

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eggs are fertilized, they may suffer from a reduction in viability through either inbreeding or outbreeding depression (Innes 1989; DeMeester 1993). Once in the sediment, diapausing eggs may be eaten, infected with parasites, buried too deeply ever to hatch, or just fail to get an appropriate diapause termination cue (Cáceres and Hairston 1998). Hence, theory predicts that to fully understand the diapause strategies among populations, the risks associated with both remaining active and entering dormancy must be considered (e.g., Cohen 1970). To date, few studies have collected the appropriate field data to test these theoretical predictions across multiple species in multiple habitats.

For lakes and ponds surrounding Kellogg Biological Station in southwest Michigan, U.S.A., decades of limnological research indicate that the relative risks for populations of *Daphnia* vary from lake to lake (Threlkeld 1979; Mittelbach 1981; González and Tessier 1997; Wetzel 2001), which in theory should influence a population's investment in dormancy. In this study, our primary goal was to compare diapause strategies in the field among four species of *Daphnia*, two of which inhabit temporary ponds and two of which inhabit permanent lakes. We first focus on the water column and explore the seasonal timing and relative investment in dormant eggs. We then explore the subsequent storage of eggs in the dormant egg bank and consider possible differences in hatching strategies among the populations. Our results indicate that dormancy strategies in *Daphnia* vary considerably among species, suggesting that species perceive the risks associated with a particular habitat in markedly different ways.

Methods

Study systems—We sampled 14 habitats in southern Michigan (Barry and Kalamazoo Counties), each of which have two to five coexisting *Daphnia* species (Table 1). Three are temporary ponds (Roughwood Pond, West Gull Pond, Woodfrog Pond) that contain populations of *Daphnia pulex* and *Daphnia ephemeralis*. The ponds fill in spring and usually dry by June, but in a wet year can contain water until August. The other 11 are lakes that vary in surface area (4.8–67.6 ha) and maximum depth (9–15 m). All of these lakes contain *Daphnia pulicaria* and *Daphnia dentifera*. Ten of these lakes stratify thermally during the summer (all except Little Long Lake), creating a deep-water refuge in which the large-bodied *D. pulicaria* can avoid competition and predation (Wright and Shapiro 1990). In some of these stratified lakes, however, this refuge always becomes inhospitable by midsummer due to anoxia (Cloverdale Lake and Baker Lake; Tessier and Welser 1991; Cáceres and Tessier unpubl. data).

This variation in basin morphometry, in combination with lake-specific interspecific interactions, creates a natural gradient of risk for the large daphniids in the water column. We collected field data for all four daphniid species, but the focus of our experimental work has been *D. pulicaria* (Cáceres and Tessier 2003). As a result, our study lakes were chosen to create a gradient of habitat permanence for the *D. pulicaria*. We refer to this gradient in risk as habitat permanence because some lakes do not always provide suitable condi-

Table 1. Characteristics of the lakes and ponds sampled in this study. The ponds are temporary and dry by midsummer whereas all lakes contain water year round. All of the lakes contain *D. pulicaria* and *D. dentifera* and all of the ponds contain *D. pulex* and *D. ephemeralis*. For the ponds, reported surface areas and depths are maximum seasonal values. Values for total phosphorus (TP) are from spring turnover averaged for 2–5 yr (Tessier and Woodruff 2002; Tessier and Cáceres, unpubl. data). A Yes for summer refuge indicates an oxygenated hypolimnion throughout thermal stratification.

Name	Surface area (ha)	Maximum depth (m)	Spring TP ($\mu\text{g L}^{-1}$)	Summer refuge
Baker Lake	23.8	9	25	No
Bassett Lake	17.0	11.5	15	Yes
Bristol Lake	56.8	15	11	Yes
Cloverdale Lake	44.1	15	15	No
Lawrence Lake	5.0	11.5	9	Yes
Little Long Lake	67.6	9.5	8	No
Little Mill Lake	4.8	10	23	Yes
Pine Lake	24.1	10	5	Yes
Three Lakes 2	21.2	10	12	Yes
Warner Lake	23.5	14	10	Yes
Whitford Lake	8.1	8.5	9	Yes
Roughwood Pond	0.28	0.85	36	No
West Gull Pond	0.93	0.95	60	No
Woodfrog Pond	0.08	0.82	191	No

tions for this *Daphnia* species. In some of the lakes (Baker Lake, Cloverdale Lake, Little Long Lake), the *D. pulicaria* populations are annual, meaning they are abundant in spring but become very rare every summer. At the other extreme, in lakes with a well-oxygenated hypolimnion, populations are perennial (Little Mill Lake, Pine Lake, Three Lakes 2), meaning the population is always in the water column, and finally, in some of the lakes, the population survives in some years but is eliminated in others (Cáceres and Tessier unpubl. manuscript). In all of our study lakes, *D. dentifera* are annual populations that are found in the water column between May and December. In addition, differences among lakes in the rates of sedimentation and resuspension as well as in the abundance of benthic predators and availability of hatching cues likely influences the relative costs and benefits experienced by populations of both species in the sediment egg bank (Cáceres and Hairston 1998; Cáceres and Tessier 2003). Hence, although we do not have quantitative data on the relative risks experienced by the *Daphnia* in the water column and in the sediment, basic limnology makes it clear that these risks must vary among lakes and among species.

Field sampling—To estimate annual timing and magnitude of ephippial production in each species of *Daphnia*, we sampled each lake with an 80- μm mesh Wisconsin-style bucket net. On each sampling date, we collected three vertical hauls from the deepest section of each lake. In 1999–2001, we sampled in early April after ice-out, weekly from May until July, once in August, and two or three times from September to November. Winter samples were also collected through the ice in early 2000 and 2001. The three samples were combined and preserved in 70% ethyl alcohol. In the ponds, two quantitative zooplankton samples (12–21 liter)

were taken using a 3-liter pitcher. Each sample was individually preserved in 70% EtOH. The ponds were sampled weekly in 1999 and 2001, although in 1999, we did not sample immediately after the ponds had filled. In 2000, low precipitation prevented sampling of the ponds for much of the season. Samples from the lakes were subsampled for population abundance estimates, but the entire sample was searched for males and females carrying ephippia. Pond samples were most often processed without subsampling.

To obtain an estimate of the size, distribution, and turnover rate of the dormant egg bank, we collected multiple sediment cores from each lake. In June and July 2000, we used SCUBA to collect six sediment cores (7.3-cm inner diameter) from each lake, i.e., three from near shore and three from the sediments in the deepest portion of the lake. In each core, all *Daphnia* eggs and ephippia in the top 2 cm of sediment were removed and identified to species based on ephippial morphometry (Hebert 1995; Cáceres 1998a). The top 2 cm were used to include multiple years of deposition and based on the depth of bioturbation in freshwater (Kearns et al. 1996).

In the temporary ponds, we used plastic tubes (5-cm inner diameter) to core dry pond sediments in August 1999 (Roughwood and Woodfrog Ponds) and August 2001 (West Gull Pond). Three transects were established radiating from the center of each pond, and one core was taken at three sites along each transect. One site was at the deepest spot in the pond, one site was the edge of the pond in high-water years, and the third site was halfway between the other two sites. Cores were sliced at 1-cm intervals, and the three samples from each pond depth were combined for processing. For comparison to the lakes, only the ephippia found in the top 2 cm of sediment are reported here.

Statistical analyses—We used analysis of variance (ANOVA) to test for differences in the annual timing of diapause among the four species. For each population, the Julian date on which the first ephippium was found was used as an indicator of the annual timing of the onset of diapausing egg production. Prior to analysis, the data were $\ln(X + 1)$ transformed to normalize the residuals. Bonferroni post hoc tests were used to contrast differences in diapause timing between *D. pulex* and *D. ephemeralis*. Because the data on magnitude of diapause investment did not conform to the assumptions of ANOVA, among-species differences were investigated with a multiresponse permutation procedure available in BLOSSOM (Cade and Richards 2001).

For the egg bank data, the residuals from most models indicated that variance tended to increase with treatment mean; hence, all egg counts were $\ln(X + 1)$ transformed. The relative size of the egg banks found in lakes versus ponds was tested with an ANOVA in which species was nested within habitat type (lake or pond), and one average value was used for each population. A paired *t*-test was used to compare the number of eggs in the sediment egg bank of *D. pulicaria* versus *D. dentifera* across the 11 different lakes. Again, one estimate of egg density was used for each population. Two separate two-way ANOVA models were used to investigate the effects of lake and location (nearshore vs. offshore) on the size of egg bank for each of the two lake

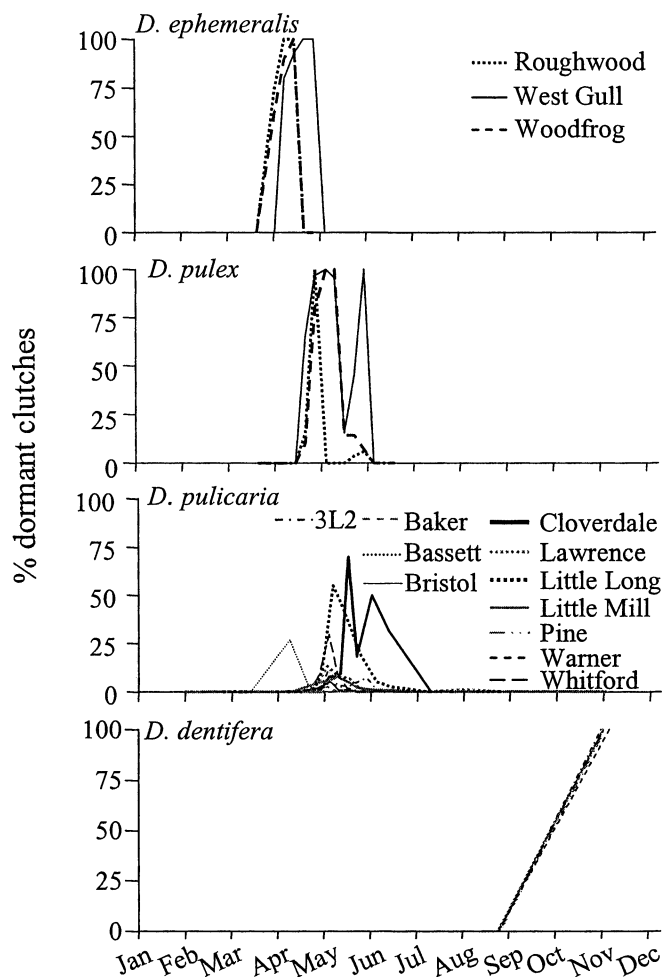


Fig. 1. The seasonal phenology of diapausing egg production in four species of *Daphnia*. The top two panels are for the pond species and the bottom two panels are for the lake species.

species (one for *D. pulicaria* and the second for *D. dentifera*). For the pond species, a paired *t*-test was used to compare the number of eggs in the sediment egg bank of *D. pulex* versus *D. ephemeralis* across the three ponds, with one estimate of egg density for each population. Finally, for each of the two pond species, we used an additional ANOVA model to compare the sizes of each species' egg bank in the three ponds.

To explore the potential hatching fraction of diapausing eggs among the species, we used ANCOVA to investigate the relative number of eggs per ephippia in the two lake species after confirming that there was no interaction between the treatment (species) and the covariate (number of ephippia) in the model ($F_{1,40} = 1.02$, $P = 0.32$). Except for the randomization test, all analyses were performed in SYSTAT 10.0 (Wilkinson 2000).

Results

We observed pronounced differences in the seasonal onset of diapause in the four species of *Daphnia* (Fig. 1; $F_{3,24} = 487.1$, $P < 0.0001$). Three of the four species produced their

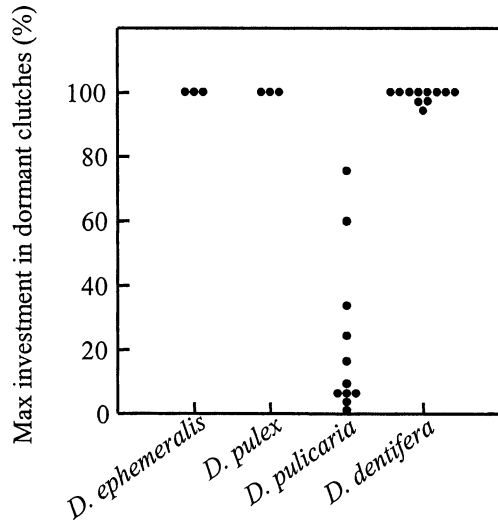


Fig. 2. Maximum investment in ephippial clutches in four species of *Daphnia* (measured as number of ephippia per total number of clutches). For each population of *D. pulicaria*, we first calculated the average maximum investment in dormancy for the 3 yr 1999–2001, then plotted a single value for each population. For the two pond species, only data from 2001 were available and each population is plotted as an individual symbol. The symbol for each *D. dentifera* population is the averaged values for 1999 and 2000 except for Cloverdale Lake, which was sampled with higher resolution in fall 2002.

dormant eggs in the spring and the fourth only made dormant eggs in the fall. This pattern was in part a result of the differences in the annual phenology of each species in the water column. The two pond species cannot persist in the water column for more than the few months that the pond holds water and therefore must produce their eggs during the spring or early summer. Even within this window, however, we found interspecific variation in phenology and diapause timing (Bonferroni post hoc comparison; $P = 0.03$). *D. ephemeralis* emerged from the egg bank and were present in the water within 1 week of the pond filling. Males and females carrying ephippia were found in all three ponds by early April, and all *D. ephemeralis* were absent from the water by early May. Although a few *D. pulex* were found during our first sampling in all three ponds, we did not find any females with ephippia in any of the ponds until after the *D. ephemeralis* population had crashed the first week in May. *D. pulex* often remained in the water column until the pond dried in June or July. In all of our study lakes, *D. pulicaria* was most abundant in spring (Cáceres and Tessier unpubl. manuscript) and most populations began producing their dormant eggs in May, at approximately the same time as the closely related *D. pulex*. In *D. dentifera*, ephippial females began to appear in most lakes in October and the majority of ephippia were produced in November. In all of our lakes, *D. dentifera* was absent in the early spring but common during summer and fall.

To compare investment in dormancy across populations, we calculated the fraction of clutches in each sample that were dormant eggs relative to the total number of clutches (diapausing and immediately hatching eggs). This number

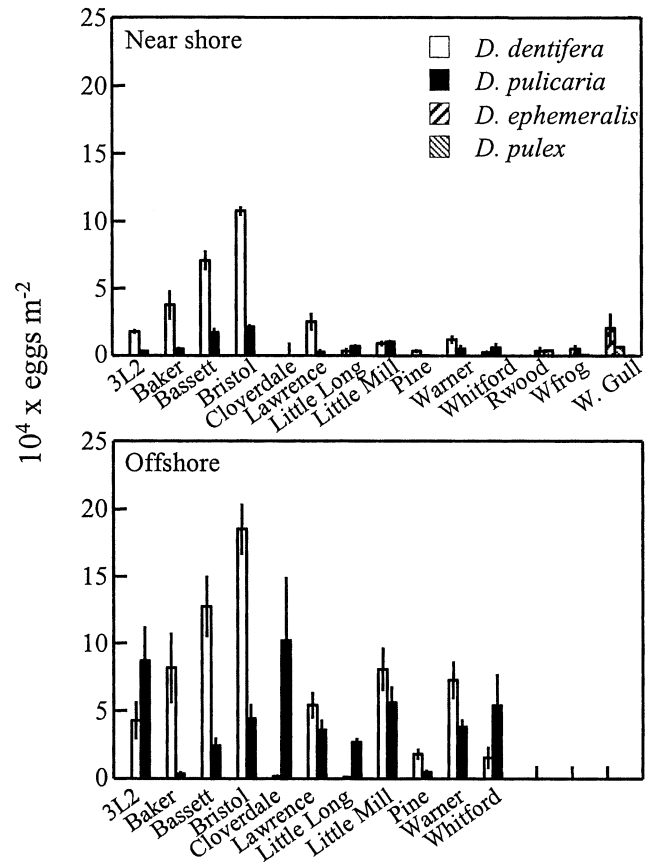


Fig. 3. Number of diapausing eggs found in the top two centimeters of sediment. Only eggs that appeared to be viable on visual inspection were included in these counts. For the lake species, data are the average ± 1 SE from three cores taken in either shallow water (near shore) or from the deepest area of the lake (offshore). Data from the ponds are averages ± 1 SE from multiple sites at each of three locations that radiated from the center of the pond, and are plotted only with the near-shore lake data.

allows for a comparison of the relative investment in dormancy by each population but does not allow for a comparison of the absolute number of eggs produced by each population. That calculation depends on the water temperature experienced by each adult, and this information was not collected as part of this study. In all four of these species, the maximum investment in dormancy can be quite high (Fig. 2), but we found significant among-species differences in this investment (randomization test, $\delta = -8.6$, $P < 0.0001$). In *D. pulex*, *D. ephemeralis*, and *D. dentifera*, maximum investment in dormancy reached 100% and showed little or no variation among populations (Fig. 2). In contrast, maximum investment in dormancy in the 11 populations of *D. pulicaria* varied from 3% to 75%.

In the sediment egg bank for each species, we found considerable variation both within and among species (Fig. 3). In general, sediment egg banks found in lakes were larger than those found in ponds ($F_{1,12} = 8.0$, $P = 0.01$). Across the 11 lakes, we found no difference in the average size of the egg banks of *D. dentifera* and *D. pulicaria* ($t_{10} = 0.18$, $P = 0.85$). Within each of these two lake species, we found

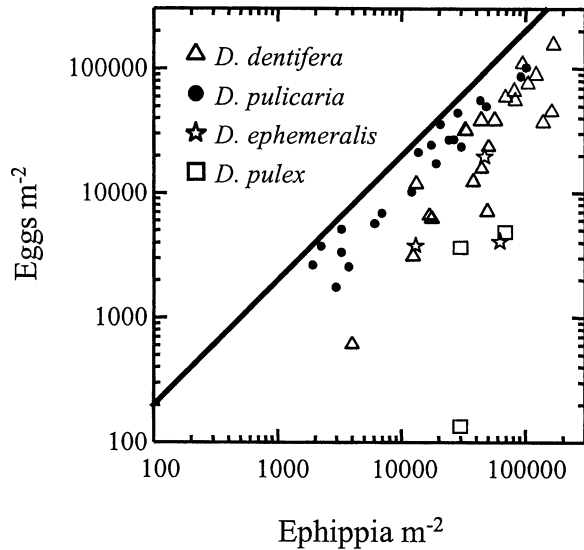


Fig. 4. Average number of eggs m^{-2} (from Fig. 3) plotted as a function of the average number of ephippia m^{-2} in which those eggs were found. The diagonal line is the 2:1 line. The further away a point lies from the line provides an indication of the relative loss of eggs from the sediment egg bank.

a significant effect of the lake from which the eggs had been collected (*D. pulicaria*: $F_{10,44} = 99.2$, $P < 0.0001$; *D. dentifera*: $F_{10,44} = 34.7$, $P < 0.0001$), spatial location within the lake (near shore vs. offshore, *D. pulicaria*: $F_{1,44} = 678.8$, $P < 0.0001$; *D. dentifera*: $F_{1,44} = 33.2$, $P < 0.0001$), as well as an interaction among lake and location (*D. pulicaria*: $F_{10,44} = 82.4$, $P < 0.0001$; *D. dentifera*: $F_{10,44} = 8.35$, $P < 0.0001$) on the number of eggs stored in the sediment. In general, more eggs are found in the offshore areas of each lake for both species, but the magnitude of this difference depends on the specific lake. In the ponds we found no difference in the average size of the egg banks of *D. ephemeralis* and *D. pulex* ($t_2 = 1.48$, $P = 0.28$). For *D. ephemeralis* we found no effect of the pond from which the eggs had been collected on the size of the egg bank ($F_{2,6} = 1.59$, $P = 0.27$). However, for *D. pulex*, significantly fewer eggs were found in Woodfrog as compared to the other two ponds ($F_{2,6} = 63.9$, $P < 0.0001$; Bonferroni post-hoc comparisons of Woodfrog vs. W. Gull and Woodfrog vs. Roughwood, $P < 0.001$).

The size of the dormant egg bank is the result of variable annual contributions to the egg bank combined with differential survival and hatching. We do not have estimates of the loss rates for our populations, but the fact that ephippia remain open and empty on the bottom of the lake after the *Daphnia* have hatched provides an opportunity to investigate relative hatching among species. If all ephippia were produced with two eggs, and eggs in the egg bank never hatched or died, a plot of the number of eggs in the egg bank against the number of ephippia would have all points falling exactly on the 2 to 1 line (two eggs per ephippia). For our lakes, this is clearly not the case (Fig. 4). Not surprisingly, the number of eggs in the sediment egg bank of the two lake species was positively related to the number of ephippia found at that site ($F_{1,41} = 209.6$, $P < 0.0001$). We also found a significant effect of species ($F_{1,41} = 13.1$, $P = 0.0008$).

Across this gradient of ephippial densities, *D. dentifera* tends to have fewer eggs per ephippia than does *D. pulicaria*, indicating a higher loss rate of *D. dentifera* eggs from the sediment egg bank. This pattern does not result from *D. dentifera* producing a large number of empty ephippia. Across our study lakes, on average, $96.4 \pm 1.2\%$ of *D. dentifera* ephippia are produced with two eggs, but only $30.7 \pm 2.7\%$ of the ephippia in the egg bank were found to contain two eggs. All estimates for the pond species fall well below the 2:1 line (Fig. 4), indicating that a large proportion of the ephippia in the temporary pond were empty.

Discussion

These field surveys indicate that there is considerable among-species variation in both the timing and magnitude of diapausing egg production as well as in the dynamics of eggs in the egg bank. Both of our pond species (*D. ephemeralis*, *D. pulex*) and one of the lake species (*D. dentifera*) showed little among-population variation in the timing and magnitude of ephippia production, whereas *D. pulicaria* showed considerable among-population variation in the magnitude of investment. The two pond species have temporally segregated life cycles, even during the brief time that the pond holds water. Both *D. pulicaria* and *D. dentifera* are lake species but show considerable differences in their life histories. Clearly, the diapause strategy exhibited by a population cannot be predicted simply by whether or not the habitat in which the zooplankton live is temporary or permanent.

Theory predicts that diapause strategies will evolve based on the risk experienced in both the active stage as well as in the dormant stage (Cohen 1970). In general, females should continue to produce active offspring until such a time when those offspring are more likely to survive in dormancy. For *Daphnia*, decades of research have provided numerous examples of the relative risks experienced by females in the water column, primarily as a result of either competition or predation (Sommer 1989; Lampert and Sommer 1997; Wetzel 2001). For our study populations, the timing of diapausing egg production seems to be a trait associated with a particular species. Our results suggest that, at least among the lakes surrounding Kellogg Biological Station, each population of a particular species experiences the greatest risk in the water column at approximately the same time of year. In ponds, *D. ephemeralis* always began producing diapausing eggs before *D. pulex* did, and in lakes, the water-column risks that most influence the life history of *D. pulicaria* occurred much earlier than did those for *D. dentifera*.

From these field data, however, it is not clear exactly what the major risks are for each species. While water loss is obviously a major catastrophe for the pond species, in *D. ephemeralis*, it is likely that something in addition to hydroperiod influences the timing of dormancy because this species disappears from the water column several weeks before the earliest possible date of pond drying. Hebert (1995) classifies *D. ephemeralis* as a cold stenotherm and indicates that this species always disappears from the water column in early spring. It is the only species of *Daphnia* that we have

been unable to maintain in laboratory culture above 15°C. Whether fitness in this species declines during spring as a result of predation, competition, or some other factor (e.g., increasing temperature) is not well understood. Experimental data documenting the relative competitive abilities of these two pond species at different times of the year would be useful in understanding their seasonal phenologies and why these two species are temporally segregated even during the brief time each year that the pond holds water.

In our lakes, *D. pulicaria* dominates the water column in early spring and produces its dormant eggs in late May while the majority of the *D. dentifera* population is still dormant. The exact nature of the late-spring risks for *D. pulicaria* cannot be determined from the field data. Evidence from other species of zooplankton from permanent lakes suggests that diapause is used to avoid both competition (Santer and Lampert 1995; Hansen and Hairston 1998) and predation (Hairston 1987; Ślusarczyk 2001), and both types of interspecific interactions become more intense for *D. pulicaria* as the summer progresses. Field competition experiments for *D. pulicaria* and *D. dentifera* indicate that the rank order of competitive ability changes during the season (Hu and Tessier 1995; Cáceres 1998b), and in the Michigan lakes, *D. pulicaria* is a superior competitor in the spring but *D. dentifera* is the better competitor in late summer. Although interspecific competition alone is likely not the only factor shaping the timing of dormant egg production in these species, it is clear that the production of dormant eggs allows for temporal partitioning of the water column, a strategy that may foster coexistence of competitors (Warner and Chesson 1985; Cáceres 1997). For annual populations of *D. pulicaria*, the switch to dormancy may contribute to spring population decline, but it is not the ultimate cause of the annual populations' disappearance from the water column. After the spring bottleneck, the remaining females return to parthenogenetic reproduction and continue producing small clutches until they become extremely rare in late summer (Cáceres and Tessier unpubl. manuscript).

Although populations of the same species living in different lakes may experience an increase in water-column risks at roughly the same time of year, among the permanent lakes, the magnitude of this risk can vary widely both among lakes and years. For example, it has been suggested that declining food levels can induce the switch to dormancy in some species of zooplankton (D'Abramo 1980) and the magnitude of the annual clear-water phase varies both within and among lakes (Lampert et al. 1986). In addition, basin shape and productivity determine the size and availability of a summer hypolimnetic refuge against competition and predation for large daphniid species (Wright and Shapiro 1990; Tessier and Welser 1991). This variation in risk in the water column should influence the magnitude of investment in dormancy across populations, which was exactly the case for our 11 populations of *D. pulicaria*. The annual populations in Cloverdale and Little Long Lakes that disappear from the water column each summer had over 50% of the reproduction going into dormant eggs each spring, while many of the perennial populations produced as few as 10% dormant clutches. The fact that our *D. dentifera* populations showed very little variation in their investment in dormancy may be

a consequence of the fact that we choose all annual populations. In other lakes in the region, where *D. pulicaria* is not abundant in spring, *D. dentifera* can be found in the plankton year round. An investigation of diapause strategies in these perennial *D. dentifera* populations would provide an interesting comparison with the variation we observed in *D. pulicaria*. In ponds, although variation in interspecific interactions may influence the timing of dormancy, because active individuals cannot survive the dry stage, it is not surprising that at some point all females switch to producing dormant eggs.

Because our field observations provide no information regarding potential clonal variation in diapause investment, it is possible that some individuals of all clones in the population switch to producing ephippia even though observed investment in diapause at the population level is <100%. However, we have conducted common garden experiments that quantify dormancy investment by clones from nine of these 11 *D. pulicaria* populations. The results indicate substantial genetic variation among clones and differentiation of lake populations in dormancy investment (Tessier and Cáceres unpubl. manuscript).

In addition to the relative investment in diapausing eggs in the water column, another factor to consider in examining diapause strategies is the length of time a particular egg spends in the egg bank and the relative risks it experiences while it is dormant (Ellner et al. 1998). It has been suggested that these egg banks provide a means of temporal risk spreading and that by delaying hatching for more than 1 yr, these long-dormant eggs can serve as a buffer against occasional recruitment failures (Cohen 1966). The size and availability of this temporal refuge is determined by the balance between the number of eggs added each season, the availability of appropriate termination cues, and the number of eggs lost by hatching, predation, infection, senescence, and deep burial (Cáceres and Hairston 1998). Although these gains and losses obviously must vary among systems, quantitative estimates of these rates are extremely rare. The few studies that have recorded in situ hatching rates for zooplankton all suggest that only a small fraction of the sediment egg bank terminates dormancy each year and that there is considerable variation in hatching rates among species and systems (e.g., De Stasio 1989, 1990; Wolf and Carvalho 1989; Cáceres 1998a; Hairston et al. 2000). Although we have not sampled emergence traps in any of our systems, we do have information regarding the hatching fraction of *D. pulicaria* in a subset of these lakes. By incubating newly produced ephippia on the lake bottom for 1 yr in a reciprocal transplant design, 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 as a result of the source of the eggs (Cáceres and Tessier 2003). These experimental results suggest that, for *D. pulicaria*, the majority of eggs that are deposited in the hypolimnion fail to receive a hatching cue and are subsequently buried alive. With a low hatching rate, tens of thousands of eggs m⁻² can accumulate even in lakes where the relative investment in dormancy is low. Hence, in some of these lakes, the relative costs of entering dormancy may be quite high, if only a very few of the dormant eggs ever reenter

the water column. The sediment cores from the 11 lakes seem to confirm this result because the majority of *D. pulicaria* ephippia found in deep water are full.

However, this does not appear to be the case for *D. dentifera* because the number of *D. dentifera* eggs falls considerably below the 2:1 line. There are at least three separate but not mutually exclusive explanations for the differences we observed among species. The first is that the species differ in the number of empty ephippia that are produced. For the lake species at least, this is likely not the case because well over 95% of all ephippia are produced with two eggs in these populations and males are always abundant during the time that dormant eggs are produced (Cáceres and Tessier unpubl. data). A second possibility is that the eggs of different species experience differential mortality while in the egg bank, but we have no evidence to suggest factors that would contribute to consistently higher physiological mortality of *D. dentifera* diapausing eggs in all of our lakes. Hence, the most likely explanation for the species-level differences is a difference in hatching rates. In all of our lakes, *D. dentifera* is not found in the water column during the spring, suggesting that the egg bank may serve as a source for the annual recolonization of the water column. A higher success rate of surviving the egg bank and reentering the water column may then feed back into a higher investment in the production of diapausing eggs by females in the water column.

The pond species fall even further below the 2:1 line than do the two lake species. Studies of in situ hatching indicate that emergence rates are higher nearshore than offshore (De Stasio 1989, 1990; Cáceres 1998a; Arnott and Yan 2002) and our experimental work with *D. pulicaria* suggests that the environmental conditions that a dormant egg experiences in the hypolimnion of a stratified lake constrain the hatching rates of the eggs (Cáceres and Tessier 2003). Given that the majority of dormant eggs in a temporary pond are likely to be exposed to the hatching cues and that dormant eggs are the primary contribution to the water column population each year, a higher hatching rate (i.e., a higher proportion of empty ephippia) in the shallow temporary ponds is to be expected, which likely is a major contributing factor toward the smaller sizes of egg banks in temporary ponds. However, we do not have any information regarding the number of empty ephippia that are produced in these populations and therefore cannot rule this out as a possible explanation for the observed pattern.

Finally, all of our lake populations reproduce by cyclical parthenogenesis; hence, investment in dormancy is also investment in sex. There is a considerable literature that has developed regarding the benefits of sexual reproduction (Williams 1975; Barton and Charlesworth 1998), but few studies consider the cost and benefits of dormancy and sexual reproduction in concert. It has been suggested that in a life cycle that includes several asexual generations and only one sexual generation, sexual reproduction should occur at the time where ecological differences will be the greatest between generations (Bonner 1958; Williams 1975). Because diapausing eggs rejoin the population months or years after they were produced, the coupling of diapause and sex seems appropriate. Bonner (1958) found that across a wide array

of taxa, if a dormant stage was found in the population, it was almost always produced sexually. Whether the patterns we observe in these populations are the result of selection on the timing of dormancy, the timing of sex or some combination of these factors is a question that we are continuing to investigate.

Dormancy is a key component to the life history of many invertebrates that provides an opportunity for recombination, a temporal escape from conditions that the active individuals would not survive, and a mechanism for allowing strong competitors to temporally divide the water column (Williams 1975; Warner and Chesson 1985; Cáceres 1997). Not surprisingly, we found that dormancy investment is highly variable both among species and within one species (*D. pulicaria*). Hence, future studies should treat dormancy variation as a key trait in addressing questions relating biodiversity to ecological function at both the community and population levels.

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