A comparison of egg-bank and long-term plankton dynamics of two *Daphnia* species, *D. hyalina* and *D. galeata*: Potentials and limits of reconstruction

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Abstract

Resting eggs of planktonic organisms from datable sediment cores are increasingly used to reconstruct historical information on the abundance, size, genetic composition, and microevolution of planktonic organisms. All of these studies have relied on the (up to now) mostly untested assumption that the resting egg bank in sediment will indeed allow an accurate reconstruction of past populations. Here we test the performance of the egg bank to reconstruct historical data of the *Daphnia* population of Lake Constance, which was thoroughly investigated throughout the past century. We show that it is possible to reproduce the variability in the abundance, size, and genetic composition of *Daphnia galeata* within a period of ~2 decades. Furthermore, resting egg data allowed us to reconstruct the timing of the invasion of *D. galeata* into Lake Constance. However, the egg bank did not reconstruct (1) the dynamics of the native *Daphnia* species of Lake Constance, *D. hyalina,* and (2) the relative importance of the two *Daphnia* species. We present evidence that the latter is caused by differences in the sexually reproductive activity between the two species. The failure to reconstruct the long-term dynamics of *D. hyalina* in the lake is most probably due to a change in the frequency and timing of sexual activity during the course of eutrophication.

The existence of long-lived dormant eggs in lake sediment offers aquatic ecologists powerful opportunities to reconstruct past population abundances (Yan et al. 1996), population genetics (Weider et al. 1997), food web structure (Jeppesen et al. 2001*a,b*), and microevolutionary changes (Hairston et al. 1999*a;* Kerfoot et al. 1999). The occurrence and abundance of resting stages were used to investigate past population abundance, species invasions, and the species recovery after the restoration of lakes (Verschuren and Marnell 1997). The size of daphnid ephippia was used to infer fish predation pressure in shallow lakes and to reconstruct past fish predation pressure (Verschuren and Marnell 1997; Jeppesen et al. 2002). Genetic analysis of resting stages (Duffy et al. 2000; Limburg and Weider 2002) and of hatchlings from resting stages (Weider et al. 1997; Hairston et al. 1999*b*) revealed information about the genetic composition of past populations. Because cladocerans and calanoid copepods produce resting stages that remain viable for several decades or more, the hatching of such resting stages from sediment of known age and conducting experiments with those past populations can yield important insight into the mechanisms that cause microevolutionary changes. This promising new research agenda, termed ''resurrection ecology'' (Kerfoot et al. 1999), was used to study, for instance, microevolutionary changes in the phototactic behavior of a *Daphnia magna* population in response to differences in fish predation (Cousyn et al. 2001) and microevolutionary changes in the copper sensitivity of Lake Superior daphnids in response to mining (Kerfoot et al. 1999). Using the methods of resurrection ecology, it was suggested that the *Daphnia galeata* population of Lake Constance showed microevolutionary responses to increased abundances of toxic cyanobacteria resulting from eutrophication (Hairston et al. 1999*a,* 2001).

Obviously, all of these applications rely on the assumption that sedimentary eggs banks do really reflect past populations. Up to now, there has been no study that has critically examined this assumption. Here, we use the long-term data set of *Daphnia* in Lake Constance to check the reliability of the egg bank record. The zooplankton populations of Lake Constance have been studied since the beginning of the 20th century (Straile and Geller 1998*a*). In particular, the *Daphnia* population has been studied in great detail (Stich and Lampert 1981, 1984; Geller 1986; Stich 1989; Weider and Stich 1992; Straile and Geller 1998*a,b;* Straile 2000). This enables us to compare various characteristics of the *Daphnia* population with their egg bank reconstruction. The findings from historical observations that can be used to test the possibilities of egg bank reconstructions, are as follows.

Daphnia invasion and change in *Daphnia* species composition: In the course of the eutrophication of Lake Constance, *D. galeata* invaded the lake during the 1950s and produced hybrids with the native *D. hyalina* population (Einsle 1978; Weider and Stich 1992). We expect to find this pattern in the egg bank record.

Change in *Daphnia* abundance: *Daphnia* abundance in Lake Constance increased strongly during the past century as a result of eutrophication (Straile and Geller 1998*a,b*). We expect a strong increase in the ephippia abundance.

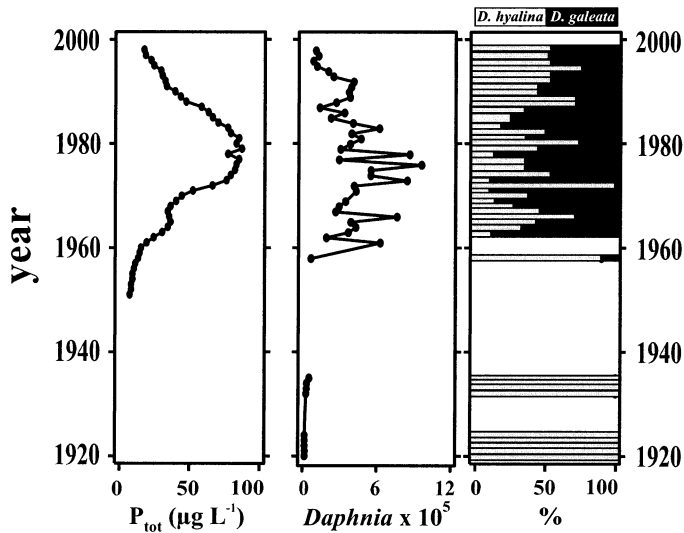*Daphnia* body size: Body size of planktonic *Daphnia* dur-

Fig. 1. Long-term data set from Lake Constance. Shown are the average winter total phosphorus concentration during mixes (left, data from Güde et al. 1998), mean yearly *Daphnia* abundance (middle), and taxa composition (right) of *Daphnia hyalina* (gray) and *D. galeata* (black). *Daphnia* data were taken from several studies.

ing 1979–1998 exhibited interannual variability that was possibly associated with interannual variability in the predation pressure of juvenile fish (D.S. unpubl. data). Because large *Daphnia* produce large ephippia (Boersma et al. 2000; Jeppesen et al. 2002), we expect to find interannual variability in ephippia size in the sediment core to be associated with *Daphnia* size.

Daphnia clonal composition: Allozyme studies of Lake Constance *Daphnia* were conducted during 1989–1990 (Weider and Stich 1992) and during 1999–2001 (authors' unpubl. data) and revealed distinct allozyme frequencies. We expect to find these allozyme frequencies in the respective sediment layers.

Hence, our test covers most aspects of *Daphnia* egg bank reconstructions: *Daphnia* invasion, species composition, clonal composition, abundance, and size. We will discuss our results, to improve our understanding of the factors which control the production and deposition of ephippia.

Methods

Lake Constance is located on the northern fringe of the Alps (9°18′E, 47°39′N). It is a large (476 km²) and deep (z_{max} = 254 m, z_{mean} = 100 m) warm-monomictic lake. The trophic state changed dramatically during the past century, as was indicated by total phosphorus (P_{tot}) concentrations during winter mixes (Fig. 1): originally an oligotrophic lake, it underwent massive eutrophication beginning in the 1950s, with a maximum at the end of the 1970s, followed by reoligotrophication (Güde et al. 1998).

Palaeodata of ephippia—Ephippia were isolated from 15 laminated and dateable cores (acrylglass tube; 1 m long and 5.8 cm inner cross-section) sampled in 2000 (seven cores)

and 2001 (eight cores) from 220 m water depth (between Friedrichshafen and Romanshorn) in the main basin of Upper Lake Constance. Layers were dated, as in previous studies (Weider et al. 1997; Hairston et al. 1999*a*), by the counting of laminae (Wessels 1995). Each core was cut in half lengthwise and photographed, and the layers were dated. Sediment samples were taken from different layers representing 1–5 yr in the 2000 cores and 1–4 yr in the 2001 cores, from each half of the core. The 1-yr samples represent prominent high water markings. To avoid the transfer of ephippia between different layers, the outer edge (~0.5 cm) of each section was not sampled. Ephippia were isolated either by sugar-centrifugal-flotation (Onbe 1978; Cromar and Williams 1991) or by sieving through a 250-μm sieve. The ephippia of the 2000 cores were cleaned with filtered lake water (0.45 μm) and then subdivided into two parts. One part was transferred into clean 6 × 6 microtiter plates and incubated in a walk-in environmental chamber at constant light (24 h) and constant temperature (15°C) for the subsequent allozyme analysis of the hatchlings. Ephippia were monitored daily to every other day for a period of 35 d. Newly hatched daphnids were removed and carefully transferred to culture jars to grow up for 3–5 d. The hatchlings were frozen and stored at −80°C for subsequent allozyme analysis. The second part was counted and measured (without the spine) with a dissecting microscope (magnification, 2 × 10). The ephippia of the 2001 cores were measured (without spine) with a dissecting microscope (magnification, 2–5 × 10). *Daphnia* ephippia were distinguished by the occurrence (*D. hyalina*) or absence (*D. galeata*) of small spines at the dorsal edge of the ephippia (Flössner 2000). In addition to these two categories, we found mixed-type ephippia with only few dorsal spines and classified them as potential hybrids.

The genetic architecture of planktonic daphnids and hatchlings was determined, using cellulose acetate electrophoresis, with allelic variation at allozyme loci. Three enzyme loci were screened for polymorphism: aspartate amino transferase (AAT; E.C. 2.6.1.1.), phosphoglucomutase (PGM; E.C. 5.4.2.2.), and phosphoglucose isomerase (PGI; E.C. 5.3.1.9.). Protocols for electrophoresis were as described by others (Hebert and Beaton 1993), except that electrophoresis was run at 300 V.

Long-term data set of daphnids—*Daphnia* plankton data were taken from the long-term data set of the Limnological Institute of the University of Konstanz and the Institut fuer Seenforschung in Langenargen (partly published in Elster and Schwoerbel 1970; Muckle and Dillmann-Vogel 1976; Muckle and Muckle-Rottengatter 1976; Einsle 1978, 1983; Straile and Geller 1998*a*). From 1979 to 1995, *Daphnia* were collected at weekly or biweekly intervals by vertical net hauls from 140 m depth at the sampling station of the Limnological Institute in the fjordlike branch of the Upper Lake Constance (Überlingersee); data on the abundance, frequency of *D. hyalina* and *D. galeata,* and size are available. Data from 1920–1924 (Auerbach et al. 1924, 1926), 1932–1935 (Elster and Schwoerbel 1970), 1952–1962 (Muckle and Dillmann-Vogel 1976; Muckle and Muckle-Rottengatter 1976), and 1962–1993 (partly published in Einsle 1978, 1983) were
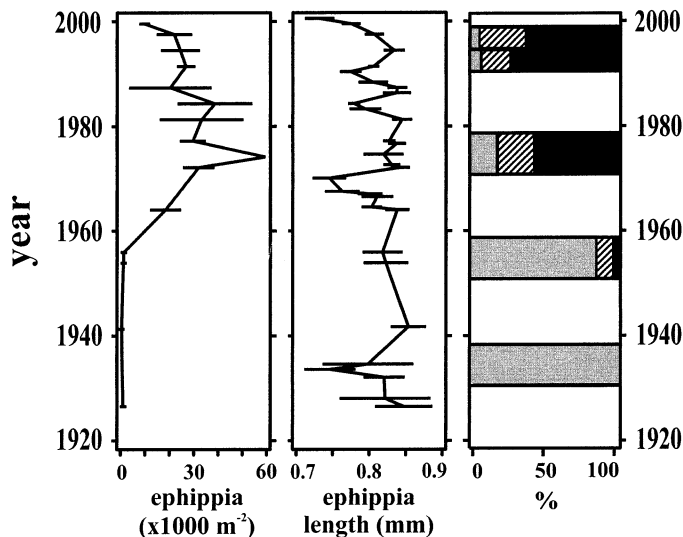
Fig. 2.   Data obtained from the ephippial record. Shown are the mean (±SE) ephippia densities (left), the mean (±SE) ephippia size of replicated cores (middle), and the composition of the ephippial record using the presence or absence of small dorsal spines (right). The peak density in 1974 was based on one core. Ephippia with dorsal spines indicate *D. hyalina* (gray), and ephippia without spines indicate *D. galeata* (black). Additionally, we found ephippia with few spines, probably indicating hybrids (hatched).
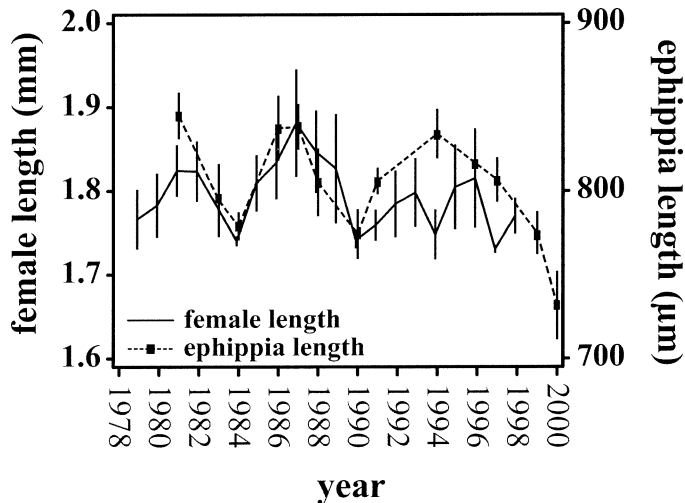

Fig. 3.   Size of ephippia isolated from the sediment record and size of parthenogenetic adult females obtained from the historical plankton record from the beginning of the 1980s to the end of the 1990s. Shown are the mean and standard error for the selected years of the ephippia record ($n = 5$ cores) and the mean and standard error from May to July for each year of the historical record.

taken at different sampling stations in the main basin of the Upper Lake Constance. Data on the abundance, percentage of males, and frequencies of *D. hyalina* and *D. galeata* for 1999, 2000, and 2001 were taken from our unpublished data. Plankton allozyme data of daphnids for 1989–1990 were taken from Weider and Stich (1992), for 1999 from unpublished data from one of us (J.T.), and for 2000 from our unpublished data.

For statistical comparison of the ephippia data from the sediment cores and the long-term data sets, we calculated the mean value for each time period sampled in the cores and (for the same time periods) in the plankton samples. We used correlation analysis to relate the egg bank reconstruction with the historical data. Data were plotted using the midpoint of the sampled intervals. Data analysis was performed using SAS software (SAS Institute 1988).

Because electrophoretical data of the ephippial hatchlings suggested that most ephippia produced belonged to *D. galeata,* we used only the long-term data sets for the adult size of *D. galeata* for the statistical analysis of size differences. Additionally, because *D. galeata* produced ephippia between May and July (authors' unpubl. data), only size data from that time of the year were analyzed.

Results

Continuous measurement of total phosphorus concentrations during winter mixes started during the 1950s (Fig. 1). These measurements showed an exponential increase until the 1970s and a fast decrease of P_{tot} starting in the early 1980s. The abundance of daphnids showed a similar development, with abundances $<0.4 \times 10^5$ individuals m^{-2} during

the 1920s and 1930s. After eutrophication, abundances increased toward $>1 \times 10^6$ individuals m^{-2} during the 1970s and then decreased markedly again, especially during the late 1990s. During the course of eutrophication, *D. galeata* invaded the lake during the 1950s and soon reached a relative abundance of $\geq 50\%$. During more recent years, the relative contribution of both *Daphnia* species to overall *Daphnia* abundance was roughly similar (Fig. 1).

The ephippia abundance in the egg bank ranged between 1,000–60,000 m^{-2} yr^{-1} (Fig. 2). Before the 1950s, the ephippia abundance was very low (350–2,800 m^{-2} yr^{-1}), but it increased from 1960s onward, with a maximum from the 1970s to the early 1980s (mean, 32,000 m^{-2} yr^{-1}). From the late 1980s onward, ephippia abundance decreased to the level of the late 1960s (Fig. 2). The mean ephippia abundance was positively associated with P_{tot} ($r = 0.737$, $P = 0.010$, $n = 11$) and with mean *Daphnia* abundance ($r = 0.739$, $P = 0.006$, $n = 12$) throughout the study period. The size of the egg bank ephippia (range, 720–880 μm) was highly variable within the past century. From the beginning of the 1990s, the mean ephippia size decreased to levels of 700–750 μm. A similar decrease was also observed during the 1930s and the 1970s. Ephippia size was correlated with neither *Daphnia* abundance ($r = 0.105$, $P = 0.589$, $n = 29$) nor with P_{tot} concentration during winter mixes ($r = 0.165$, $P = 0.402$, $n = 28$). Ephippia size was, however, strongly correlated with the size of *D. galeata* within the period 1979–1998 ($r = 0.702$, $P = 0.007$, $n = 13$; Fig. 3)—that is, when size measurements of planktonic animals were available.

Using the absence or presence of small spines at the dorsal margin of the ephippia, we distinguished among three types of ephippia: with spines, without spines, and with few spines. The proportion of ephippia with dorsal spines (native *D. hyalina*) changed dramatically from the 1930s ($\sim$100%)

to the 1990s (<10%). In contrast, the proportion of ephippia without spines (*D. galeata*–like ephippia) increased from 0% to 80% during the same time period. Additionally, we found ephippia with few spines, which may represent hybrids. This type of ephippia also occurred for the first time during the 1950s, and their relative frequency increased from the 1950s onward, from 10% to 30% (Fig. 2).

Mean hatching success was 50%, but it showed high variability between different years, ranging between 10% during the 1960s and 78% during late 1970s. In general, we found the same picture as that obtained by Weider et al. (1997). A total of 304 animals hatched from ephippia were electrophoretically screened and sample size per layer varied between 10 and 60 animals; for the years of particular interest, the beginning of the 1990s and 1999–2000, we screened at least 30 individuals per year. At the AAT locus, all but two individuals belonged to the genotype "ff"—that is, *D. galeata*—whereas the two individuals showed an "sf" genotype—the *D. hyalina × galeata* hybrid. The latter were found in the sample of the beginning in the 1970s. No "ss" genotype was found. At the PGI locus, we found two alleles ("m" and "f"). The m allele decreased in frequency from 90% to 100% during the 1960s and 1970s to ~80% during 1999–2000, whereas the f allele increased in frequency during this period (Fig. 4). At the PGM locus, we found four different alleles. The f allele reached the highest frequencies, ranging between 40% and 80%. From the 1970s onward, the amount of this allele decreased from >75% to <60%. The alleles with the lowest contribution ("s" and "f+") at the PGM locus never reached >20% (Fig. 4).

A comparison of the allele frequencies of the hatchlings from the early 1990s and 1999–2000 with the plankton data of similar periods using a pairwise G-test showed no significant differences, neither between the parthenogenetic females of the plankton and the ephippial hatchlings nor between the ephippial females and the ephippial hatchlings (Table 1, Fig. 5).

Discussion

The detailed comparison between sediment ephippia characteristics and long-term data revealed striking possibilities but also potential limitations of egg bank reconstruction that have to be taken into account when reconstructing paleolimnological and historical trajectories. Ephippial data allowed the successful reconstruction of the invasion of *D. galeata* into Lake Constance, the abundance increase of *Daphnia* after the eutrophication of Lake Constance, the allozyme composition of *D. galeata* for selected years, and interannual variability of *Daphnia* body size during 1979–1998. In contrast, morphological and allozyme data did not allow a reconstruction of the relative abundance of *Daphnia* taxa, especially during more recent years, which also indicates limitations in reconstructing species-specific *Daphnia* abundance.

Studies from the first half of the past century reported only one *Daphnia* species in Upper Lake Constance, *D. hyalina* (Auerbach et al. 1924, 1926; Elster and Schwoerbel 1970). This is supported by ephippia morphology, by the exclusive
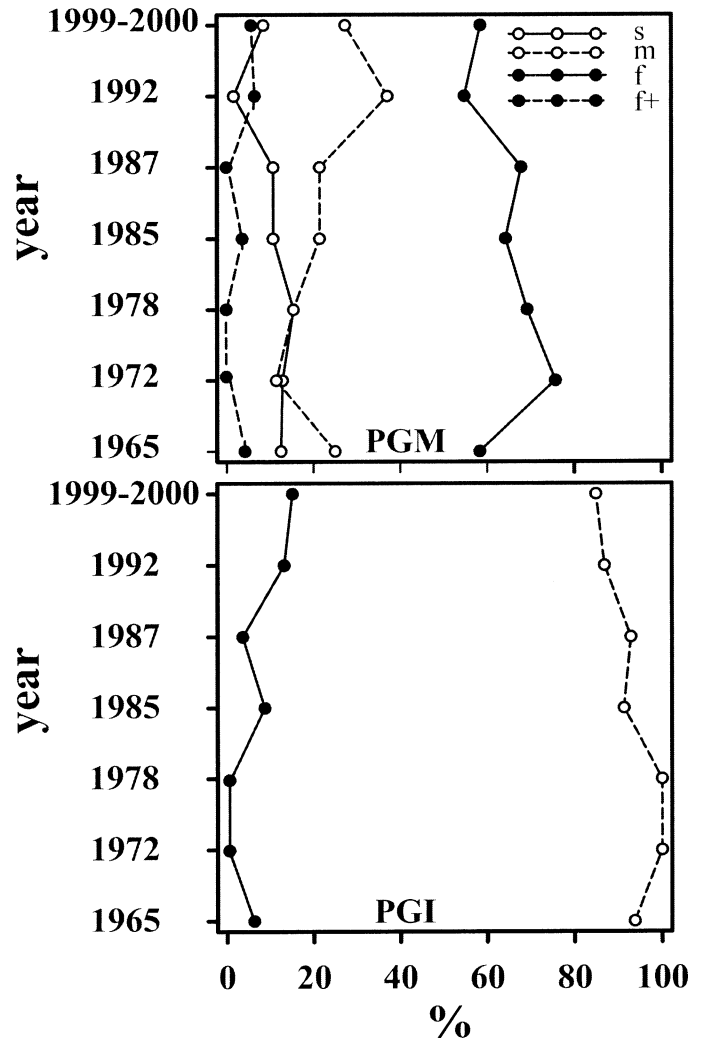

Fig. 4. Changes in the allele frequencies at the phosphoglucomutase (PGM; upper panel) and phosphoglucose isomerase (PGI; lower panel) loci from the 1960s to the end of the century obtained by the ephippial hatchlings from the sediment record. Shown are mean value for each time period sampled in the cores.

occurrence of ephippia with spines (Fig. 2). Because it is not possible to investigate the allozyme composition of ephippia, and because we did not observe the hatching of ephippia prior to the 1950s, we were not able to check species identity prior to the 1950s with allozymes. However, ephippial genetics can be studied directly using DNA analysis, and the first results from Lake Constance ephippia from the 1920s supports our morphology data and shows that these ephippia were indeed *D. hyalina* (K. Schwenk unpubl. data). Morphological data also allowed a reconstruction of the *D. galeata* invasion into Lake Constance: the first occurrence of ephippia without spines during the 1950s corresponds to historical observations (Figs. 1, 2; Muckle and Dillmann-Vogel 1976; Muckle and Muckle-Rottengatter 1976).

The genetic composition of the *D. galeata* population seems to be well represented by the ephippial record. As was shown by a previous study (Weider et al. 1997), our

Table 1. Comparison of the allele frequencies at the PGM and PGI loci for the two periods (1989–1990 and 1999–2000) when allele frequencies were available for the plankton parthenogenetical and ephippial females and ephippial hatchlings isolated from respective sediment depths (pairwise G-tests). Allele frequency data of parthenogenetical females from 1989–1990 were taken from Weider and Stich (1992). Significance level ($\alpha = 0.05$) after Bonferroni correction is $p = 0.00625$.

Period	Loci	df	Value	p
1999–2000				
Ephippial females vs. ephippial hatchlings	PGM	3	0.958	0.812
Ephippial females vs. ephippial hatchlings	PGI	2	2.946	0.229
Parthenogenetical females vs. ephippial hatchlings	PGM	4	9.134	0.058
Parthenogenetical females vs. ephippial hatchlings	PGI	2	7.725	0.021
Ephippial females vs. parthenogenetical females	PGM	4	6.315	0.177
Ephippial females vs. parthenogenetical females	PGI	2	5.505	0.064
1989–1990				
Ephippial hatchlings (early 1990) vs. parthenogenetical females	PGM	3	3.211	0.360
Ephippial hatchlings (early 1990) vs. parthenogenetical females	PGI	2	0.431	0.806

data from 1989–1990 are very similar and showed no bias between parthenogenetic plankton females and the ephippial record at either the PGI or the PGM locus. Also, the allozyme composition of the ephippial hatchlings from 1999–2000 did not differ between sexual females or parthenogenetic females. The temporal shifts in genetic composition through time at the PGI and PGM loci (Fig. 4) were similar to those reported by Weider et al. (1997).
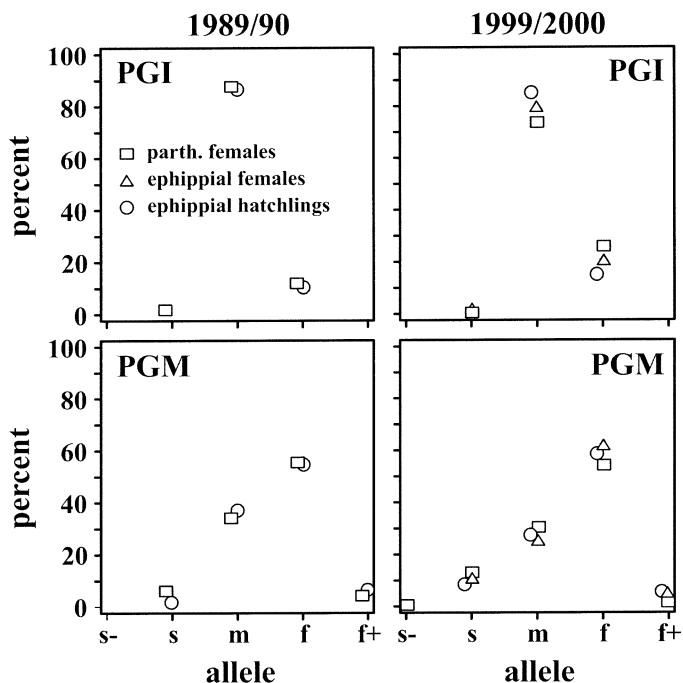

Fig. 5. Allele frequencies at the phosphoglucose isomerase (PGI; upper panels) and phosphoglucomutase (PGM; lower panels) loci of ephippial hatchlings and parthenogenetic and ephippial plankton females from 1989–1990 (left panels) and 1999–2000 (right panels). Data on parthenogenetic females from 1989–1990 were taken from Weider and Stich (1992).

The population size of the two planktonic daphnid populations combined seems to be well reflected by the abundance of ephippia in the respective sediment layers. This is not only true regarding the large differences in population size, which occurred during a century of eutrophication, but also for more subtle differences during the period of reoligotrophication from 1979 to the present ($r = 0.866$, $P = 0.026$, $n = 6$). The ephippial record seems also to be a good archive to reconstruct the mean female size. Both female and ephippial size showed cyclic fluctuations during the 1980s and 1990s, with periods of larger and smaller ephippia/females (Fig. 3). Hence, the variability in size was not related to the trophic status of the lake, as indicated by P_{tot} concentrations, which showed no fluctuations but decreased monotonically during this period. Variability in ephippia size between lakes (Jeppesen et al. 2002) and between years within a single lake (Cousyn et al. 2001; Jeppesen et al. 2001a), has been suggested to result from variability in fish predation pressure. This suggests that ephippia size variability in Lake Constance is also related to fish predation pressure, which in Lake Constance seems to be associated with climatic variability (D.S. unpubl. data).

Hence, significant variability in the egg bank is not only associated with processes that result from that eutrophication and oligotrophication of Lake Constance. As a consequence, studies of "resurrection ecology" (Kerfoot et al. 1999) in Lake Constance should not only consider eutrophication and oligotrophication as the driving forces behind, for instance, microevolutionary changes (e.g., Hairston et al. 1999a, 2001), but have also to consider alternative explanations.

Our study also revealed important differences between egg bank reconstructions and historical data. These differences suggest that egg bank reconstructions might be complicated by species-specific differences in *Daphnia* life history, such as the timing and frequency of sexual reproduction, the hydrodynamic conditions during the time of sexual reproduction, and changes in response to changes in the trophic status of a lake.

Although the morphological analysis of the ephippial record seems to reflect very well the taxa composition at the beginning of the 20th century and also the time of invasion by *D. galeata* around the 1950s, there seems to be a bias not only between ephippial record and plankton data but also between the allozyme and morphological analysis of the ephippial record from the middle of the 20th century onward. From morphology-based historical records, we would expect to find a ratio of *D. hyalina* to *D. galeata* ~50:50 (Fig. 1) or, if hybrids are included, ~40 (*D. hyalina*):40 (*D. galeata*):20 (hybrid) for the 1990s (Weider and Stich 1992; T.J. unpubl. data). In fact, the morphological analysis of ephippia suggested a proportion of ~5% *D. hyalina* and ~15% hybrids. This bias is even more pronounced when looking at the allozyme composition of hatched individuals. From 237 individuals successfully screened at the AAT locus, 235 *D. galeata*, no *D. hyalina*, and only 2 hybrids (~0.8%) were identified. The same proportion (0.8%) of hybrid genotypes was found by Weider et al. (1997), and they also found no *D. hyalina*. Given the high number of hatchlings, 237 in our investigation and 1,200 in the investigation of Weider et al. (1997), it is surprising that not a single *D. hyalina* was detected. Several factors might contribute to this discrepancy.

First, species-specific differences in embryo mortality and hatching success may bias the genetic analysis of ephippial hatchlings. However, the high number of screened individuals in Weider et al. (1997) and our study and the high hatching success, at least during some years, suggests that this is an unlikely explanation. Furthermore, the first results of molecular genetic analysis using DNA isolated directly from ephippia also suggested that all ephippia in recent years were *D. galeata* (K. Schwenk unpubl. data). Hence, the lack of *D. hyalina* in the more recent layers of the egg bank seems to be real and not a methodological artifact.

Second, *D. hyalina* might have a lower sexual reproductive activity (e.g., lower ephippial production) than *D. galeata*. This is supported by a 3-yr study of the sexual reproductive activities of daphnids in Lake Constance. We observed all three taxa to produce ephippia, but overall *D. galeata* produced >100 times more ephippia than *D. hyalina* and 30 times more than the hybrid (Fig. 6).

Third, ephippia might differ in their buoyancy because of, for example, the presence of spines, gas chambers, or lipid content (Wesenberg-Lund 1939; Hebert 1995; Weider et al. 1997; Flössner 2000) or different hydrodynamic conditions (see below). With increasing buoyancy, the chances for ephippia to be transported to shallow-water regions should increase. The presence of spines on *D. hyalina* resting eggs might indicate increased buoyancy. Indeed, ephippial hatchlings caught in emergence traps put on the sediment in the littoral zone of Lake Constance revealed a different taxonomic composition of the littoral hatchlings than ephippia from our deep-water cores (T.J. unpubl. data). We found considerably higher hatchling proportions of *D. hyalina* (25%) and hybrids (7%) from the littoral sediments, which suggests higher floating capabilities of *D. hyalina* ephippia. In sum, a 100-fold lower ephippial production and, additionally, a higher floating capability of *D. hyalina* ephippia, compared with *D. galeata*, might explain why none of the hatched
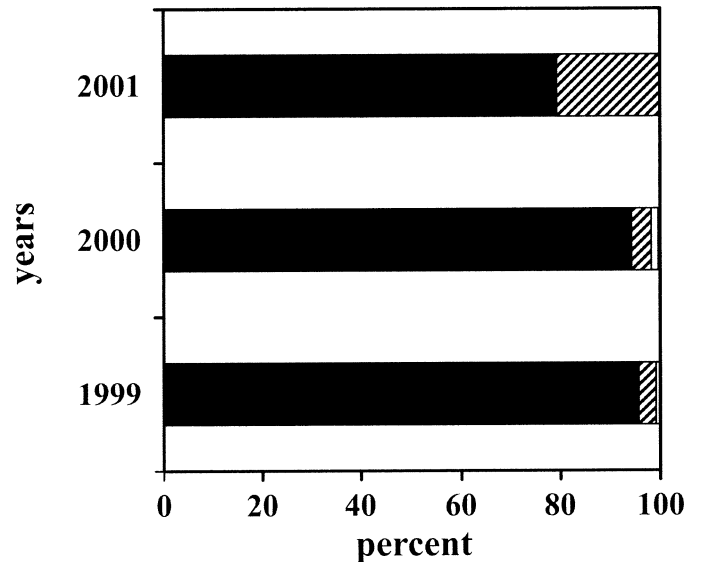

Fig. 6. Relative contribution of *D. galeata* (black), *D. hyalina* (gray), and *D. galeata* × *D. hyalina* hybrids (hatched) to total occurrence of ephippial females during 3 study years. Taxa were identified using the aspartate amino transferase (AAT) locus as marker (Wolf 1987). Data from authors' unpublished data.

individuals in Weider et al.'s (1997) and in our study were *D. hyalina*.

However, these observations do not explain why there are *D. hyalina* ephippia in the deep sediment prior to the 1950s. The ephippia found in these sediment layers have to be *D. hyalina*, because during that time only *D. hyalina* occurred in the lake, as supported by the first DNA analysis (K. Schwenk unpubl. data). Furthermore, because the abundance of *D. hyalina* was much lower prior to the 1950s, we would expect even fewer ephippia in these sediment layers. This suggests a difference in the sexual reproductive activity of *D. hyalina* and/or a change in the buoyancy of its ephippia during the course of eutrophication.

Increasing food levels during eutrophication changed the seasonal pattern of *D. hyalina* occurrence in Lake Constance (Straile and Geller 1998a). In contrast to the more eutrophic years, during which *D. hyalina* occurred in all plankton samples, *D. hyalina* was a typical summer species and thus was not found in the plankton during winter months during the 1920s (Auerbach et al. 1924, 1926) and only rarely during the 1930s (Elster and Schwoerbel 1970). This suggests that *D. hyalina* in oligotrophic Lake Constance was more dependent on the production of resting eggs than in eutrophic/mesotrophic Lake Constance. Data on male occurrence as a proxy for sexual reproductive activity supports this hypothesis. During the 1930s, the frequency of male production was 5–10-fold higher than it was between 1999 and 2001 (Fig. 7). Hence, the *D. hyalina* population seems to have responded with reduced sexual activity to an increased possibility to overwinter in the plankton after eutrophication.

The change in sexual reproductive activity might have contributed to the occurrence of *D. hyalina* resting eggs in the sediment layers prior to the 1950s. However, why did these early ephippia sink down to the deep-water sediment
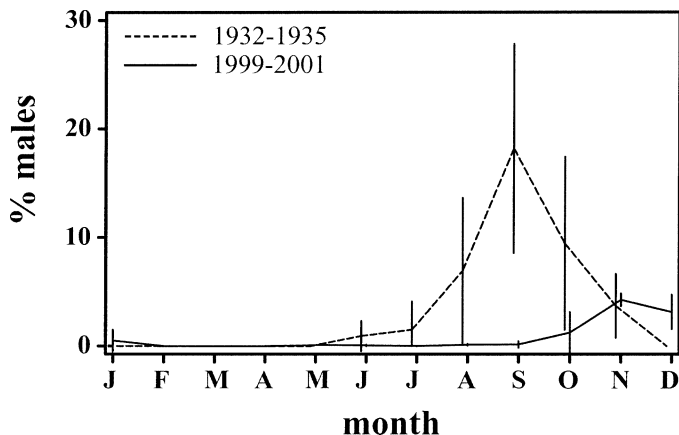
Fig. 7. Mean (±SE) monthly percentage of *D. hyalina* males prior to eutrophication (1932–1935, dashed line) and at the end of the century (1999–2001, solid line) of overall population size. Data from Elster and Schwoerbel (1970) and authors' unpublished data.

and not end up in the littoral zone, as *D. hyalina* ephippia seem to have done during recent times? The floating capabilities of ephippia are determined by the characteristics of the ephippia, such as spininess and density, and by environmental conditions, such as the depth of the mixed zone. Because the latter varies seasonally in a lake, the timing of ephippia production will influence the sinking rate of ephippia. In Lake Constance, the well-mixed epilimnion is confined to the upper 4 m during summer (i.e., June–September), whereas in November the epilimnion has extended down to a depth of 20–30 m. Hence, from June to September—the time period that encompasses the ephippial production of *D. galeata* and, presumably, the maximum ephippia production of *D. hyalina* in the oligotrophic Lake Constance—ephippia in the upper 4 m of the water column will be kept in suspension, whereas, in November—the period of maximum *D. hyalina* ephippia production during recent years—this will be the case of ephippia within the upper 20–30 m of the water column. In addition, the sinking velocity will be higher during summer than autumn and winter because of overall higher water temperatures. Obviously, ephippia of equal buoyancy will have a higher possibility of sinking down to the deep-water sediments when produced between June and September, compared with November and December. All else being equal, the shift in the timing of ephippia production from the 1930s toward recent years (Fig. 6) will reduce the probability of ephippia to sink down to deep-water sediments. The timing of ephippia production will, consequently, also contribute to our finding ephippia from *D. galeata*—that is, ephippia produced during June—in the deep sediments but no autumn- and winter-produced ephippia of *D. hyalina*. Hence, besides the frequency of ephippia production, the timing of ephippia production might be an important factor to consider when reconstructing *Daphnia* populations from the egg bank record. Another possible factor that influences the sedimentation of ephippia could be the diurnal vertical migration (DVM) of daphnids. In Lake Constance, *D. hyalina* performs a pronounced DVM, whereas *D. galeata* stays in the upper water column (Geller 1986). If there is no diurnal molt cycle, *D. hyalina*

ephippia will, on average, be released deeper in the water column, favoring their sedimentation. However, the lack of *D. hyalina* ephippia in the deep-water sediments suggests that the DVM is only of minor importance in influencing ephippia sedimentation in Lake Constance.

In conclusion, a precise reconstruction of *Daphnia* populations from sediment egg banks can be achieved if there is no clonal- or taxa-specific variability in the frequency of ephippia production and if there is no clonal or taxa specificity in ephippia buoyancy (i.e., in the probability that a produced ephippia will sink down to the sediment). Our study revealed that, for *Daphnia,* both conditions are not met in Lake Constance. *D. hyalina* and *D. galeata* differ in their allocation into ephippia production, and their ephippia also differ in spininess and probably other characters that possibly influence their buoyancy. Furthermore, the frequency of ephippia production and floating capability are most likely not taxa-specific constants but seem to be highly variable depending on environmental conditions and possibly also microevolutionary changes, even within the rather limited time period of less than a century.

The good correlation between ephippia density and *Daphnia* abundance is hence not a direct consequence of a constant allocation of daphnids into sexual reproduction but rather is the result of a variety of overlapping mechanisms. In fact, considering only *D. hyalina,* we would have found a negative relationship between planktonic females and egg bank ephippia. Hence, relying only on the sediment record—that is, without knowing the actual historical population development from the analysis of plankton samples—we might have erroneously concluded that *D. galeata* was able to almost outcompete *D. hyalina* in Lake Constance. However, this was, in fact, not the case, and both species achieved abundances of similar magnitude during spring and summer in Lake Constance after the invasion of *D. galeata* (Straile and Geller 1998*a*). As a consequence, a successful reconstruction of specific characteristics (i.e., abundance, variability in allozyme composition, and body size) of the Lake Constance *Daphnia* population was only possible for one species, *D. galeata,* within a limited period of time. Future research that uses *Daphnia* ephippia as a paleolimnological tool should note that ephippia production and deposition within a lake might change during a rather limited period of time, especially when the lake is subjected to changes in trophic status.

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