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Anthropogenic eutrophication shapes the past and present taxonomic composition of hybridizing *Daphnia* in unproductive lakes

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Abstract

It has been proposed that anthropogenic eutrophication of lakes facilitated the establishment of populations of the cladoceran *Daphnia galeata* into the originally oligotrophic lakes north of the European Alps in the 1960s. This hypothesis lacks the support of studies on unproductive lakes, in which the past eutrophication is assumed to have never been on the level necessary for *D. galeata* to reach high abundances and to establish permanently. In order to investigate if such species shifts also happened in unproductive systems, we studied the past and present taxonomic composition of three ultra-oligotrophic Swiss lakes that were only marginally affected by eutrophication using molecular methods on diapausing eggs sampled from sediment cores. *D. galeata* temporarily established in unproductive lakes, but its colonization success seemed to depend on the general trophic state of the lake and the magnitude of eutrophication. In two of the studied lakes, *D. galeata* could establish a significant population size, whereas it was not successful in the most unproductive lake with the weakest eutrophication. Even in unproductive lakes, eutrophication led to partly irreversible species changes, providing evidence that this anthropogenic disturbance is responsible for species shifts in many pre-alpine lakes in Central Europe.

As a consequence of increasing human population size and ongoing industrialization in the past 100 yr, most freshwater systems and their food webs have been directly or indirectly altered and disturbed (Wetzel 2001). A well-known example of habitat disturbance is the anthropogenic eutrophication of lakes, with an increased input of phosphorus and nitrogen, the key elements determining productivity in lakes (Correll 1998; Schindler 2006). In general, productivity of lakes increased with eutrophication and led in many cases to algal and cyanobacterial blooms, fish kills, and species shifts at all trophic levels (Correll 1998). After the 1970s, successful measures to reduce the anthropogenic phosphorus input led to subsequent re-oligotrophication in many lakes, resulting in a decrease in primary production (Jeppesen et al. 2005), zooplankton biomass (Manca and Ruggiu 1998), and fish yield (Gerdeaux et al. 2006). Today most lakes are again close to their natural trophic states.

The cyclical parthenogen *Daphnia* (Crustacea, Cladocera) is one of the most important grazers in lakes and a major food source for many planktivorous fish. *Daphnia* usually reproduce clonally but can switch to sexual reproduction and produce diapausing eggs covered by an ephippium when conditions start to deteriorate (Cáceres 1998). Because ephippia and other cladoceran remains sink to the lake bottom, sediment cores are a useful source of information for the investigation of past *Daphnia* populations (Korhola and Rautio 2001). Since the diapausing eggs can stay viable for several decades or longer, genetic analysis of the egg banks can be performed either on the hatchlings (Weider et al. 1997; Hairston et al. 1999; Jankowski and Straile 2003) or directly on the diapausing eggs (Duffy et al. 2000; Brede et al. 2009). Moreover, ephippia are important for dispersal and colonization of *Daphnia* into new habitats (Havel and Shurin 2004).

Colonization by a new *Daphnia* species or changes in the genetic composition of native *Daphnia* populations during the course of eutrophication have been shown in several studies conducted using sediment cores (Hairston et al. 1999; Jankowski and Straile 2003; Brede et al. 2009). *Daphnia galeata* invaded the formerly oligotrophic to mesotrophic Lakes Constance and Greifensee in the period of eutrophication and produced hybrids with the native *Daphnia longispina* population (Jankowski and Straile 2003; Brede et al. 2009). Today both parental species and their inter-specific hybrids are present in these lakes; as a matter of fact, the hybrids are the most frequent taxon in Lake Greifensee (Keller and Spaak 2004). An analysis of the present *Daphnia* populations in 43 lakes north and south of the Swiss Alps (including the three lakes of the present study) revealed that *D. longispina* (formerly known as *Daphnia hyalina*; Petrusek et al. 2008) is the dominant taxon in large oligotrophic lakes, *D. galeata* is present in warm and productive systems, and the hybrids are most frequent in lakes that have experienced a history of high anthropogenic phosphorus load (Keller et al. 2008).

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The reason for the success of *D. galeata* in the northern pre-alpine lakes of Europe is thought to be linked to the process of eutrophication (Weider et al. 1997; Jankowski and Straile 2003; Brede et al. 2009), as it altered, among others, food conditions and food web interactions, providing the basis for establishing a significant population of this species. Until now this hypothesis lacked the support of studies on reference systems (i.e., unproductive lakes, in which the past eutrophication is assumed to have never been on the level necessary for *D. galeata* to reach high abundances and to establish permanently). If evidence for a *D. galeata* invasion in such reference lakes exists, alternative processes, such as a general invasion during the eutrophication period or human-induced changes in predation pressure, could have been responsible for its geographically widespread colonization. To fill that knowledge gap we investigated the present and past taxonomic structures of the *Daphnia* populations from three ultra-oligotrophic lakes combining allozyme and microsatellite markers. The following questions were addressed: Is there any evidence for an invasion of *D. galeata* and for a change in the taxonomic composition in the course of eutrophication, and if so, could *D. galeata* establish itself in all three lakes or does the trophic difference between the lakes result in different patterns? We present evidence that *D. galeata* could temporarily establish in unproductive lakes, but its establishment success seemed to depend on the general trophic state of the lake and the magnitude of its eutrophication.

Methods

Study systems—Lake Brienz, Lake Thun, and Lake Walensee represent the three most unproductive large lakes of Switzerland (Keller et al. 2008) and are all situated just north of the Swiss Alps (Fig. 1). Lake Brienz (564 m above sea level [asl]) has a volume of 5.1 km^3, a surface area of 30 km^2, and a maximum depth of 259 m. Annually it receives over 3×10^8 kg (59 kg m^{-3}) of detrital particles that are mostly of glacial origin (Finger et al. 2006; Anselmetti et al. 2007). Connected to Lake Brienz, downstream Lake Thun (558 m asl) is larger (volume, 17.5 km^3; surface area, 48 km^2) but not as deep (217 m) or as rich in suspended particles (5×10^8 kg yr^{-1}, 29 kg m^{-3} yr^{-1}) as Lake Brienz (Sturm and Matter 1972). Lake Walensee (419 m asl) has a volume of 2.5 km^3, a surface of 24.1 km^2, and a maximum depth of 145 m. Annually it receives over 9×10^7 kg (36 kg m^{-3}) of suspended particles (Lambert 1978).

Total phosphorus (TP) concentration in all three lakes decreased from the beginning of the 1980s until today (Fig. 1) as a result of a reduction of anthropogenic phosphorus input (Müller et al. 2007*a*). Unfortunately, no data are available from the time period prior to the 1970s, but based on fish yield (Müller et al. 2007*b*) and data from other lakes (Liechti 1994), it can be assumed that TP values were very low (< 5 μg L^{-1}) before the 1950s. Because the TP content of all three lakes today rarely exceeds 5 μg L^{-1}, they can be considered as ultra-oligotrophic lakes (Lampert and Sommer 2007). Lake Brienz is the most unproductive (for a comparison with Lake Thun, *see* Finger et al. [2007*a*,*b*]), as a great part of its phosphorus is bound to inorganic glacial particles and therefore is not available for biological production (Müller et al. 2007*a*). Moreover, the turbidity increases light attenuation and consequently decreases primary production (Finger et al. 2007*b*).

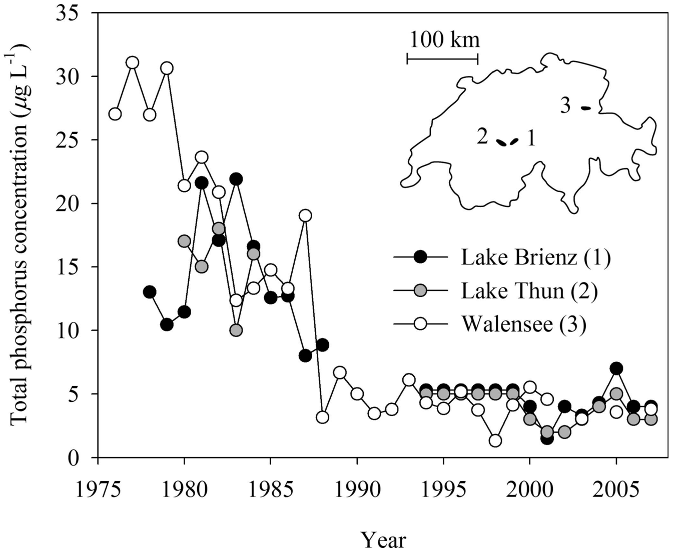

Fig. 1. Total phosphorus (TP) concentration in Lakes Brienz, Thun, and Walensee (Switzerland) from 1976 to 2007 (spring circulation values, deepest location of each lake). Data: Gewässer- und Bodenschutzlabor Bern and Wasserversorgung Zürich. Detection limit of TP in Lakes Brienz and Thun was 5 μg L^{-1} until 1999 and 1 μg L^{-1} thereafter. Numbers in parentheses indicate the location of the lakes on the Swiss map.

Present pelagic Daphnia *populations*—The pelagic *Daphnia* populations of all three lakes were sampled between October 2006 and April 2008 using a single net with a mesh size of 250 μm at the depth range of 70–0 m. Whereas Lake Brienz and Lake Thun were sampled three times between autumn and summer 2006–2007, Lake Walensee was only sampled in spring 2008. Samples were cooled during transport to the laboratory and were processed within 24 h to avoid selective mortality. For each sampling date, 40–80 asexual females with a minimum body size of 1 mm were randomly selected and frozen for future genetic analyses.

Past nutrient levels and Daphnia *populations*—In total, 10 sediment cores were taken with a gravity corer (diameter, 63 mm) at different dates and locations in all three lakes (five from Lake Brienz, two from Lake Thun, and three from Lake Walensee). To collect diapausing eggs we chose suitable coring locations with constant sedimentation rates and great depths so that ephippia had not been exposed previously to hatching stimuli. Cores were maintained at 4°C in the dark until further processing. All cores were cut into slices and used for dating, nutrient measurements, counting of ephippia, and taxonomic classification of the diapausing eggs. Data from two of the Lake Brienz cores (ephippial density from 1930 to present) had already been used in a previous study (Rellstab et al. 2007). Dating of the cores was done by determining the ^{137}Cs concentration of the sediment layers (Appleby 2001) and, additionally, by manually counting varves in some Lake Brienz cores. In one sediment core from each lake, TP and total nitrogen (TN) concentrations in freeze-dried sediment subsamples (average of three subsamples per sediment layer) were determined by employing peroxodisulfate oxidation (Ebina et al. 1983; Müller et al. 2007*a*) using a Bran+Luebbe Autoanalyzer 3. Counting of ephippia was performed after sieving the wet sediment (mesh size, 250 μm). Ephippia containing diapausing eggs were opened and individual eggs removed for further microsatellite analyses.

Genetic methods—We used two different genetic markers: allozymes (for pelagic females) and microsatellites (for pelagic females and diapausing eggs). Allozyme electrophoresis can be applied to fresh or frozen animals, but not to diapausing eggs. In contrast, microsatellite analysis can be applied to both, but the knowledge about species-specific alleles is limited (Brede et al. 2006; Dlouhá et al. 2010). Allozyme electrophoresis was performed, as described by Keller and Spaak (2004), using the two species-specific loci aspartate amino transferase (enzyme commission number [EC] 2.6.1.1) and aldehyde oxidase (EC 1.2.3.1). Results were compared to two reference clones that originated from Tjeukemeer, the Netherlands (*D. galeata*) and Lake Constance (*D. longispina*). Specimens were assigned to six possible genotype classes (parental species *D. longispina* and *D. galeata*, first and second generations of hybrids, and first-generation backcrosses to *D. longispina* and *D. galeata*) using the classification method of Nason and Ellstrand (1993).

For deoxyribonucleic acid (DNA) isolation of adult females we put 2 μL (out of 8 μL) of the *Daphnia* homogenate used for allozyme electrophoresis into 50 μL of H3 buffer (10 mmol L^{-1} Tris-HCl [pH 8.3] at 25°C; 0.05 mol L^{-1} potassium chloride; 0.005% Tween-20; and 0.005% NP-40) and added 1 μL Proteinase K (Roche, 18.2 mg mL^{-1}). Samples were incubated at 50°C for 14 h. Proteinase K was deactivated by heating the sample for 13 min to 95°C. For DNA isolation of diapausing eggs we used only 40 μL of H3 buffer.

Microsatellite analysis was performed using seven potentially species-specific polymorphic loci (Brede et al. 2006), as follows: SWI D1 (annealing temperature 53°C), SWI D5 (59°C), SWI D7 (59°C), SWI D8 (54°C), SWI D10 (59°C), SWI D12 (55°C), and SWI D15 (53°C). For Lake Brienz and Lake Thun, single polymerase chain reactions (PCRs) were performed, each in a reaction volume of 10 μL. Four microliters of DNA were mixed with 6 μL PCR mastermix containing 1X PCR buffer, 1.5 mmol L^{-1} $MgCl_2$ (Qiagen), 150 μmol L^{-1} deoxynucleoside triphosphate (Qiagen), 0.3 μmol L^{-1} labeled forward primer (Life Technologies), 0.3 μmol L^{-1} unlabeled backward primer (Microsynth), and 0.5 units HotStart Taq DNA Polymerase (Qiagen). The following temperature cycling profile was used: a 15-min initial denaturing and HotStart activation step at 95°C, followed by 30 cycles of 1 min each at 95°C, 1 min at the specific annealing temperature of the primer, and 1 min at 72°C. Final extension was at 72°C for 15 min.

For Lake Walensee and the reference clones, we ran the samples in two multiplex PCRs. Multiplex set 1 consisted of SWI D1, SWI D10, SWI D12, and SWI D15. Multiplex set 2 included SWI D5, SWI D7, and SWI D8. We used a reaction volume of 12 μL. One microliter of DNA was mixed with 11 μL PCR mastermix containing 0.05–0.5 μmol L^{-1} unlabeled backward primers (Microsynth), 0.05–0.5 μmol L^{-1} labeled forward primers (Life Technologies), and multiplex mastermix (Qiagen). The following temperature cycling profile was used: a 15-min initial denaturing and HotStart activation step at 95°C followed by 30 cycles (diapausing eggs 35 cycles) of 30 s each at 95°C, 1.5 min at the annealing temperature (set 1: 54°C; set 2: 56°C) and 1 min at 72°C. Final extension was at 60°C for 30 min. The Walensee data set originally included seven more loci in the two multiplex sets. However, for better comparison to the Lake Brienz and Thun populations, only the seven common loci mentioned above were used. We tested the taxa determination of Lake Walensee with seven and 14 loci, and the results differed only marginally.

PCR products were analyzed on an Applied Biosystems Prism® 3130xl Genetic Analyzer (Life Technologies), used according to the manufacturer's instructions, and alleles were identified using the GeneMapper software version 4.0 (Life Technologies). To test if the two reference clones represented their species, an additional 13 *D. longispina* and 11 *D. galeata* laboratory clones originating from various Swiss lakes were analyzed using both allozyme and microsatellite markers.

Population genetic analysis and taxonomic classification—To assess the present population structure between all lakes (by microsatellite markers) we computed the pairwise fixation index (F_{ST}) and determined the significance of the population differentiation using the log-likelihood statistic G, after Bonferroni correction with Fstat 2.9.3.2 (Goudet 2002). Microsatellite data of diapausing eggs from each lake were pooled into decades. For multi-dimensional illustration of population structure over time we performed a factorial correspondence analysis (FCA) including the reference clones, the pelagic females, and the diapausing eggs from all lakes with Genetix 4.05.2 (Belkhir et al. 1996–2004).

For the reconstruction of past taxonomic compositions, we combined the use of allozyme and microsatellite markers. We pooled microsatellite data from the past (diapausing eggs) and present (pelagic females) into one data set and analyzed it with the genealogical classification software NewHybrids 1.1β (Anderson and Thompson 2002). We determined the taxon of the resulting taxonomic classes by comparing the two different classification methods (NewHybrids, based on microsatellites, vs. that of Nason and Ellstrand [1993], based on allozymes) of those individuals that were subjected to both allozyme and microsatellite genotyping (pelagic females). To increase the number of taxonomic classes from the present pelagic sample, we combined the data sets of Lake Brienz and Lake Thun. The two lakes are only 5 km apart and therefore offer the same probability that a new taxon will arrive. Moreover, the results of this study showed that gene flow between the two populations is high and that the same taxa do not greatly differ between lakes. The data set of Lake Walensee was analyzed separately.

NewHybrids uses Bayesian statistical methods to calculate the probability that an individual belongs to various hybrid categories. These categories included the following: two parental taxa (P1, P2), first- and second-generation hybrids (F1, F2), and backcrosses to both parental species. All analyses are based on at least 10^6 Markov Chain Monte Carlo simulation sweeps following a burn-in period of at least 10^6 sweeps, six genotype frequency classes, and no prior information. Data sets were analyzed three times with different priors, lengths of burn-in period, and numbers of sweeps, as recommended by the authors. Individuals were assigned to a taxonomic class if their average classification probability was $\geq$ 95%. The remaining individuals that could not be classified most likely represent later-generation backcrosses and hybrids.

Correlation analyses—We calculated Pearson's r to test for correlation between the following parameters in each lake: TP and TN content in the sediment and ephippial density; TP and TN content (average per decade); and the proportion of *D. longispina* in the egg bank (only in decades with at least five genotyped diapausing eggs).

Results

Present pelagic Daphnia *populations*—In Lake Brienz, *D. longispina* was the dominant taxon on all three sampling dates (Fig. 2). The taxonomic composition did not substantially change during the year. In Lake Thun, the taxonomic composition was more diverse and more variable through time. *D. longispina* reached frequencies of up to 50%, but first-generation hybrids (with *D. galeata*) and backcrosses to *D. longispina* were also present in significant abundances. In Lake Walensee, the taxonomic composition was similar to that of Lake Thun. *D. galeata* could not be found in any of the three lakes. Microsatellite analyses show that the two populations from Lake Brienz and Lake Thun were only slightly differentiated from each other (pairwise F_{ST} = 0.029). The Lake Walensee population differed reasonably from those of Lake Brienz (F_{ST} = 0.105) and Lake Thun (F_{ST} = 0.097). All F_{ST} values were significantly different from zero.

Past nutrient levels and Daphnia *populations*—In all three lakes, both TP and TN concentrations started to increase in the 1950s and peaked in the 1970s to 1980s (Fig. 3). Whereas the decrease in nutrients in the sediment of Lake Walensee is visible starting from the 1970s, TP values in Lake Brienz and Lake Thun do not show a decrease until the 1990s. The increase of nutrients during eutrophication is highest in Lake Walensee, followed by Lake Thun and Lake Brienz. In all tested lakes, ephippial density significantly correlated with nutrient concentrations (n = 27–65; Pearson's r = 0.28–0.68; $p < 0.05$), except in Lake Walensee, where the correlation between ephippial density and TP concentration was not significant (n = 34; r = 0.24; p = 0.17). In Lake Brienz no ephippia could be found in the sediment from 1730 until 1955 (Fig. 3 shows only data from 1860). In contrast, in Lakes Thun and Walensee, ephippia could also be found in layers before eutrophication. The proportion of ephippia containing eggs was normally low in all three lakes. The oldest egg in Lake Walensee dated from the 1890s, in Lake Thun from the 1940s, and in Lake Brienz from the 1950s.

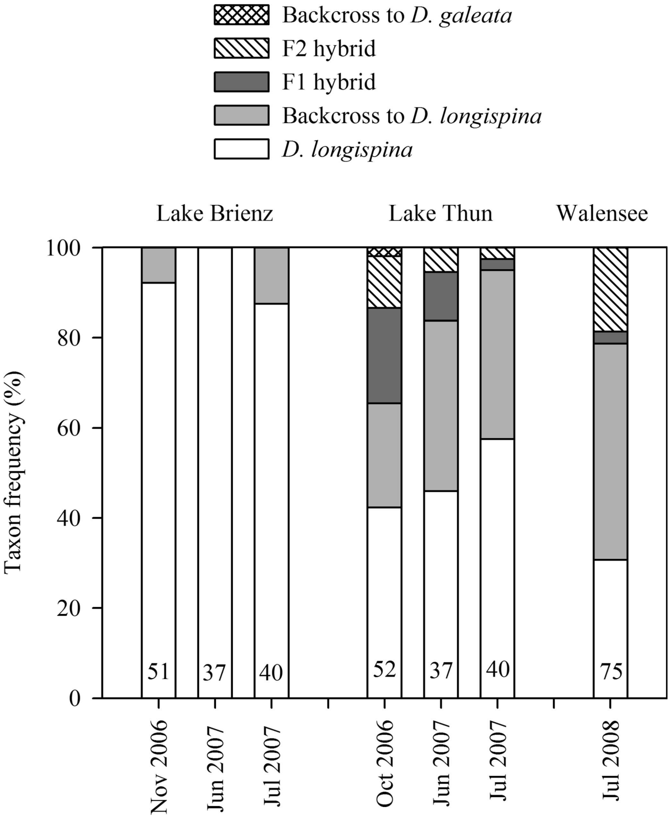

Fig. 2. Taxonomic composition of pelagic *Daphnia* females in Lakes Brienz, Thun, and Walensee at different sampling dates between 2006 and 2008, based on species-specific allozyme markers. Total sample size per sampling date is also given.

Past genetic and taxonomic architecture—As a result of ultra-oligotrophic conditions, sample sizes are low before the 1960s in Lakes Thun and Walensee and after the 1980s in Lakes Brienz and Thun. Figure 4 shows the results of the FCA including 330 pelagic females, 572 diapausing eggs, and 2 reference clones. Axis 1 represents a gradient of genotypes from *D. longispina* (right) to *D. galeata* (left). The taxonomic composition of the egg bank of Lake Brienz experiences only a minor shift in the direction of *D. galeata* during eutrophication. Today, as in the 1950s with the first appearance of diapausing eggs, almost all genotypes resemble the *D. longispina* reference clone. In Lake Thun, *D. galeata*–like genotypes appear in the 1960s and are frequently present in the 1970s but are absent nowadays. Intermediate genotypes appear in the 1970s and are still

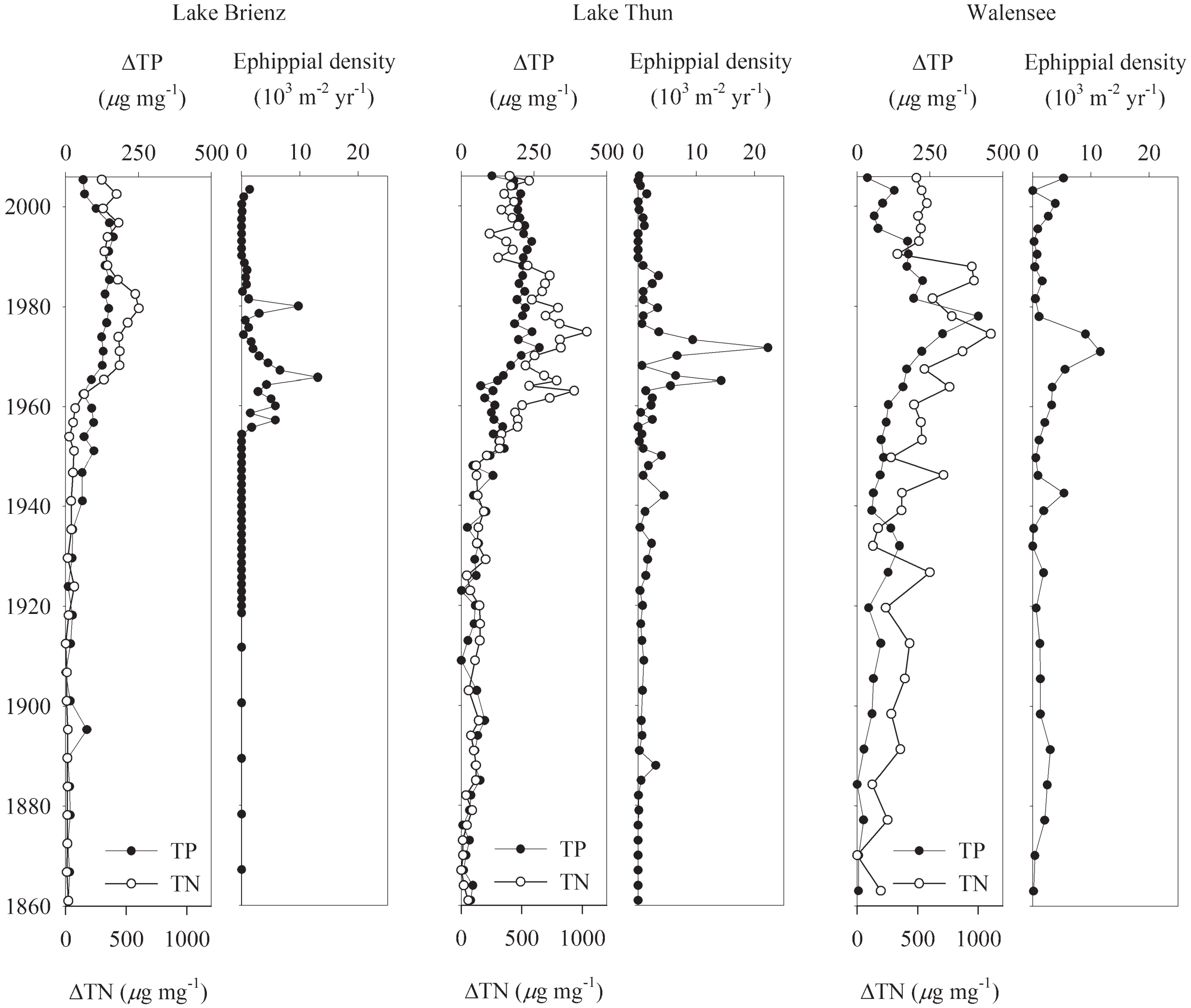

Fig. 3. Change in nutrient concentrations and historical ephippial densities in Lakes Brienz, Thun, and Walensee, derived from sediment cores. ΔTP: change in total phosphorus; ΔTN: change in total nitrogen (in μg per mg freeze-dried sediment). Change of nutrients represents the difference compared to the pre-eutrophication level in each lake (minimum nutrient content: 576 $\mu g\ mg^{-1}$ TP and 199 $\mu g\ mg^{-1}$ TN in Lake Brienz, 356 $\mu g\ mg^{-1}$ TP and 370 $\mu g\ mg^{-1}$ TN in Lake Thun, and 506 $\mu g\ mg^{-1}$ TP and 270 $\mu g\ mg^{-1}$ TN in Lake Walensee). Ephippial density includes ephippia with and without eggs. Ephippial density (1930–2004) of Lake Brienz from Rellstab et al. (2007).

present. Lake Walensee shows a similar appearance of *D. galeata*–like genotypes in the 1970s that cannot be found anymore. Compared to Lake Thun, these new genotypes are low in relative frequencies. Considering Axis 2, the *D. galeata*–like genotypes from Lake Walensee differ from those of Lake Thun.

In both data sets used in NewHybrids (Lake Brienz and Lake Thun combined and Lake Walensee), two parental species (P1, P2) and one hybrid (F2) were classifiable when reconstructing the past taxonomic composition of the egg bank. Depending on the decade and lake, 0–36% of the individuals could not be classified and most likely represent later-generation backcrosses and hybrids. In the combined Lakes Brienz and Thun data set, P1 consisted of 75% *D. longispina,* 21% backcrosses, and some first- and second-generation hybrids. F2 represented 80% first-generation hybrids and some *D. longispina*, second-generation hybrids, and backcrosses. P2 could only be found in the sediment cores of Lake Thun; therefore, no allozyme taxon could be assigned to it. It represents a second parental species that appeared in Lake Thun in the 1960s and then disappeared again after 2000. Therefore, we assigned P1 to *D. longispina*, P2 to *D. galeata,* and F2 to its hybrid. In Lake Walensee—most likely as a result of the lower taxonomic variation in the pelagic sample—the classification was not so straightforward. P1 consisted of 32% *D. longispina*, 48% backcrosses to *D. longispina*, and 1% first-generation and 19% second-generation hybrids. However, we assigned P1 to parental *D. longispina*, knowing that this taxonomic class is not a 'pure' species and that it also includes backcrosses and later-generation hybrids. P2 and F2 could only be found in the sediment. P2 was assigned to *D. galeata* and F2 to the hybrid.

A FCA supports this classification: Fig. 5 illustrates the taxonomic classes of all individuals used in this study,

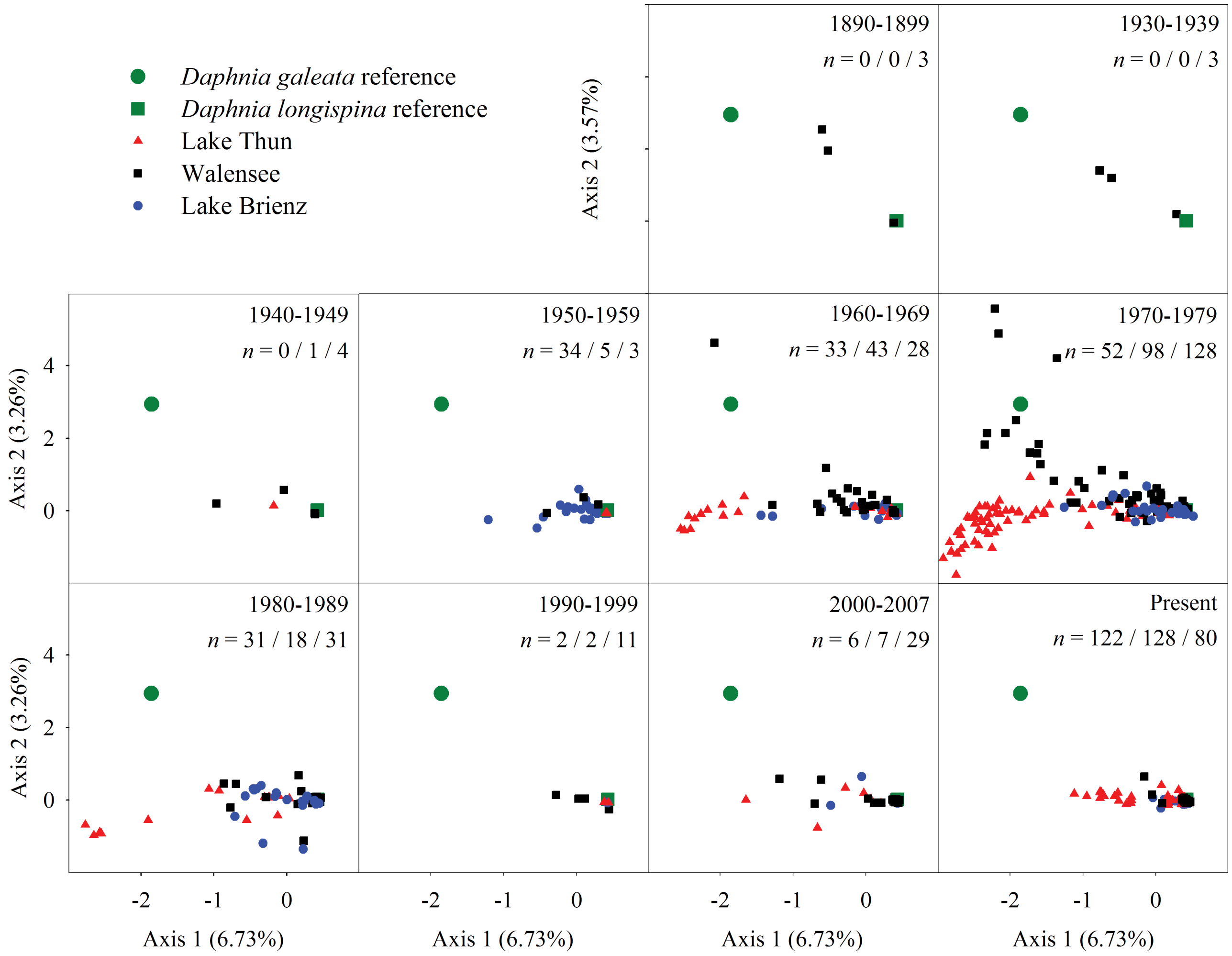

Fig. 4. Factorial correspondence analysis showing the past (diapausing eggs) and present (pelagic females) population structure of the three studied lakes during different decades. Allozyme reference clones for *D. galeata* and *D. longispina* are also included in the analysis. Each dot represents a genotype. Actual number of samples is also given (order: Lake Brienz/Lake Thun/Lake Walensee).

including additional laboratory clones for *D. longispina* and *D. galeata*. The FCA shows that the reference clones chosen for the allozyme electrophoresis are representative *D. longispina* and *D. galeata* clones. Moreover, it also supports that the linking of the two classification methods, allozyme electrophoresis with classification after Nason and Ellstrand (1993) and microsatellite genotyping with classification by NewHybrids (Anderson and Thompson 2002), is justified.

Over time, the taxonomic composition of Lake Brienz stayed mostly constant (Fig. 6): *D. longispina* represented 65–85% of the past populations and *D. galeata* × *longispina* hybrids represented 5–15% of the past populations, and *D. galeata* was never present. The present lake population consists of 98% parental *D. longispina*. In Lake Thun, the taxonomic composition experienced major shifts over time. *D. longispina* was dominant in the early years, then decreased in frequency until the 1980s and has again become the predominant taxon in more recent times. In the 1960s *D. galeata* appeared and it reached its frequency peak in the 1970s, but it disappeared later on. The hybrid appeared in the 1970s and is still present. In Lake Walensee, *D. longispina* was dominating the egg bank over the whole time period (65–80%), except for during the 1940s and 1960s, when the hybrid was the most frequent taxon. *D. galeata* could be found in the egg bank in low frequencies from the 1960s to the 1990s, but it was undetected in the recent egg bank and pelagic population.

There was a significant and strong negative correlation between the proportion of *D. longispina* diapausing eggs and TP content in the sediment in Lake Brienz ($n = 5$; $r = -0.94$; $p < 0.05$) and Lake Thun ($n = 5$; $r = -0.91$; $p < 0.05$), but not in Lake Walensee ($n = 5$; $r = -0.01$; $p = 0.99$). This correlation was not found between the proportion of *D. longispina* diapausing eggs and TN content in the sediment of all lakes.

Discussion

The disturbance of habitats can favor the establishment of new species (Elton 1958; Alpert et al. 2000). However, if most habitats in a region experience the same kind of disturbance, it is difficult to differentiate between a general invasion and an establishment due to altered conditions (as

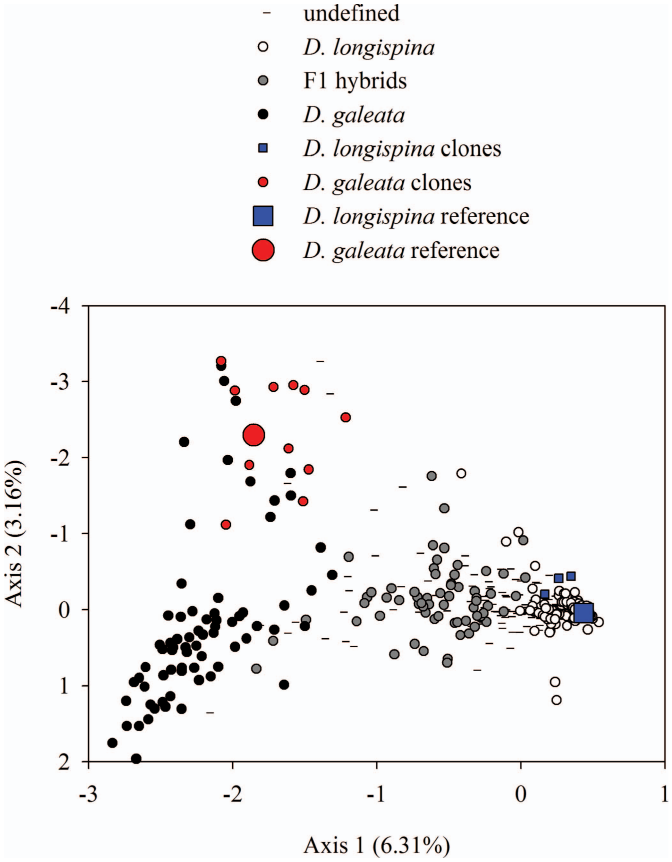

Fig. 5. Factorial correspondence analysis showing all genotypes from Fig. 4, but labeled by taxa determined by NewHybrids. Besides the two allozyme reference clones, additional *D. galeata* and *D. longispina* laboratory clones are shown. Undefined represents not significantly classifiable individuals.

discussed in Brede et al. [2009]). It is therefore necessary to test if such changes in species composition can also be found in reference habitats that were not (or were only marginally) affected by the disturbance. In the *Daphnia* species complex described in the present study, anthropogenic eutrophication was suggested to be responsible for the permanent establishment of *D. galeata* in lakes formerly dominated by *D. longispina* and its hybridization with the native species (Weider et al. 1997; Jankowski and Straile 2003; Brede et al. 2009). In this study, we present evidence that even in unproductive lakes only mildly affected by eutrophication, the process of eutrophication led to shifts in the taxonomic composition of *Daphnia* populations. However, these patterns differed between lakes: in the most unproductive lake, *D. galeata* never established a population. The success of the new species seemed to be depending on the general trophic state of the lake and the magnitude of its disturbance.

Today, the pelagic *Daphnia* population of Lake Brienz consists almost completely of *D. longispina* (Figs. 2, 6), the dominant species of deep and unproductive pre-alpine lakes in Central Europe (Keller et al. 2008). The taxonomic composition in Lake Brienz is stable and lacks *D. longispina* × *galeata* hybrids, not only over the time period presented here but also during a longer survey over the course of more than 2 yr, conducted prior to the present study (C. Rellstab unpubl.). In Lakes Thun and Walensee, which are more productive as a result of lower concentrations of inorganic particles, we find a greater variety of taxa and more temporal variation in the taxonomic composition (Fig. 2). *D. longispina*, first- and second-generation *D. longispina* × *galeata* hybrids, and their backcrosses are present in changing frequencies. Note that we only present a limited picture of the present taxonomic composition in these lakes. For example, we sampled Lake Walensee only once, but the taxonomic composition of this lake is very similar to that found by Keller et al. (2008), who sampled in different seasons in 2003 and 2004. The low F_{ST} between the two pelagic populations of the interconnected Lakes Thun and Brienz indicates that regular gene flow between the two populations takes place and justifies the use of a combined data set for all analyses.

Although the present TP content does not differ between the studied lakes within the water column (Fig. 1), Lake Brienz is the most unproductive lake of the three as a result of its high concentration of inorganic particles. Lake Brienz also experienced the weakest eutrophication of the studied lakes, as it shows the lowest increase in TP and TN measured in the sediment, compared to pre-eutrophication levels (Fig. 3). Note that at least in Lakes Brienz and Thun, changes in TP concentrations in the sediment seem to lag behind those measured in the open water column (Fig. 1), a result that could derive from ongoing chemical processes in the surface layers of the sediment (Shapiro et al. 1971). In all three lakes, the ephippial density increased during eutrophication and then decreased sharply again (Fig. 3). Ephippial density in Lake Thun was up to twice as high as in Lakes Brienz and Walensee. The differences in levels of ephippial density between lakes and decades are most likely a result of the population size and taxonomic composition, as shown for Lake Constance (Jankowski and Straile 2003). In contrast to Lakes Thun and Walensee, no ephippia were found in Lake Brienz from 1730 to 1955. This indicates that *Daphnia* was not present, or at least not permanently established, in the pre-eutrophication period (for discussion, *see* Rellstab et al. [2007]). This finding is supported by historic literature (Stingelin 1908; Flück 1926; Wuhrmann and Corti 1947).

Eutrophication not only had an effect on the population size, but it also had an effect on taxonomic composition. Whereas taxonomic composition stayed mainly constant for more than 50 yr in Lake Brienz, major shifts were found in Lake Thun during eutrophication and re-oligotrophication (Figs. 4, 6). In the 1960s, *D. galeata* appeared in the egg bank, reached its peak in the 1970s and 1980s, and disappeared again thereafter. The lagged appearance of the hybrid taxon indicates that this new species hybridized with the native *D. longispina*. The hybrids are still present in Lake Thun. In Lake Brienz, *D. galeata* was never found, and the hybrids must have recently disappeared, as they were present in all investigated sediment layers. In Lake Walensee, *D. galeata* can be found between 1960 and 1990, but in contrast to Lake Thun, it is found only in low frequencies. In the pelagic population of spring 2008, only one out of 80 individuals was classified as hybrid. However,

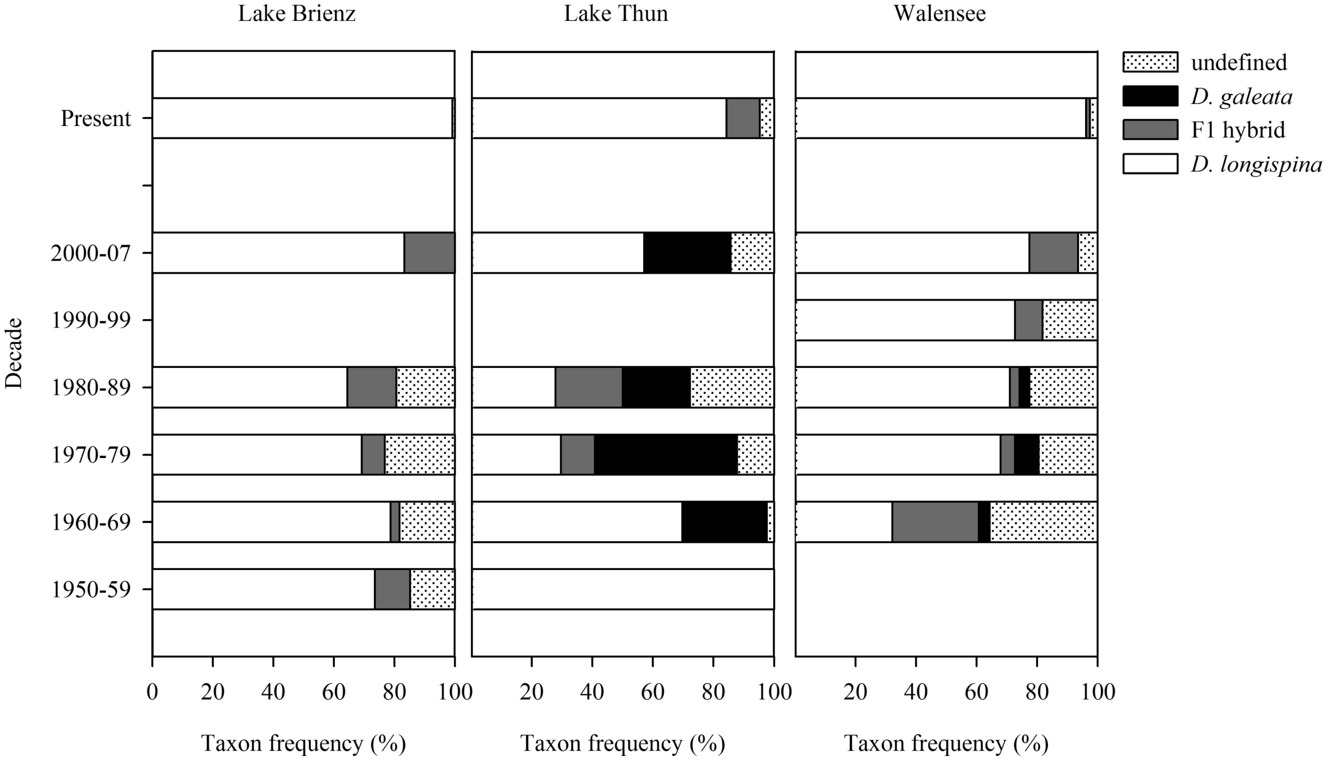

Fig. 6. Taxonomic composition of the past (diapausing eggs) and present pelagic populations of Lakes Brienz, Thun, and Walensee classified by NewHybrids. Undefined represents not significantly classifiable individuals. Decades with $n < 5$ not shown. Sample sizes are given in Fig. 4.

recent sediment layers and the work by Keller et al. (2008) indicate that hybrids might be more frequent in Lake Walensee than found in our study.

The combined use of sediment cores, genetic markers, and genealogical class identification software presented a powerful tool with which to reconstruct past changes in the population structure and taxonomic composition of the three lakes. However, a few restrictions concerning our data set should be mentioned. Sample sizes are low in some sediment layers, especially before eutrophication (Fig. 4). The appearance of *D. galeata* is therefore difficult to prove. However, in Lake Thun we were able to find one diapausing egg from the 1940s and five eggs from the 1950s, all showing typical *D. longispina* alleles. In Lake Walensee we could find some eggs dating back to the 1890s showing either *D. longispina* or hybrid-like alleles. Historic literature (Stingelin 1908) using morphological taxa identification (which is not always straightforward in the studied species complex; *see* Dlouhá et al. 2010) supports that Lakes Thun and Walensee were once typical *D. longispina* lakes, as Lake Brienz is today. Moreover, results derived from egg banks of *Daphnia* should be treated with some caution, because the production of ephippia is influenced by taxon- or clone-specific investment into diapause (Jankowski and Straile 2003; Keller and Spaak 2004) and its interactions with other factors (Tessier and Cáceres 2004). The taxonomic structure of the population found in the sediment therefore does not necessarily reflect the past pelagic taxa composition (Jankowski and Straile 2003). However, we find a similar taxonomic composition of the recently produced diapausing eggs and the present pelagic populations. Finally, we find some incongruence between the classification methods of Nason and Ellstrand (1993) and NewHybrids (Anderson and Thompson 2002). Especially in Walensee, *D. longispina* (classified by NewHybrids) consists of a significant proportion of putative backcrosses and second-generation hybrids, after the classification method of Nason and Ellstrand (1993). The most likely reasons for this are (1) the fact that allozyme classification is based on only two species-specific loci (leading to misidentification of later-generation backcrosses and hybrids) and (2) the small reference population size for this lake. However, the FCA shown in Fig. 5 strongly supports our taxonomic classification.

The increase in nutrient content in the 1950s enabled the permanent establishment of a *Daphnia* population in Lake Brienz, just as it facilitated the establishment of *D. galeata* in Lakes Thun and Walensee. Because Lake Brienz and Lake Thun are situated in close proximity, we can assume that the probability of a new taxon arriving is equal for both lakes. It therefore seems that the colonization success of *D. galeata* is correlated with the general trophic state and the magnitude of eutrophication. The phosphorus curves of Lakes Brienz, Thun, Walensee, Greifensee, and Constance all have the same shape in the 20th century, but with different levels of TP (Keller et al. 2002; Jankowski and Straile 2003; Brede et al. 2009). However, it is only in Lake Brienz that *D. galeata* could not establish. In the other four previously *D. longispina*–dominated lakes the establishment of a significant *D. galeata* population most likely occurred during the same time period (1950s through 1960s), although TP levels were substantially different between lakes at that time. This indicates that the success of *D. galeata* is not only correlated with the improved food levels during eutrophication but also with changes in other factors, such as food quality and predation (for discussion, *see* Brede et al. [2009]). The successful establishment of *D. galeata* in the lakes north of the European Alps (formerly dominated by *D. longispina*) seems therefore to be related to the process of eutrophication, but only if a lake reached a trophic level enabling *D. galeata* to establish a significant population. Interestingly, in contrast to Lakes Constance and Greifensee, the three lakes presented in this study show a strong trend back toward the original taxonomic composition (Fig. 6), but introgressed *D. galeata* alleles still exist (Fig. 2).

Daphnia can reach new habitats through the transport of ephippia or living animals through vectors such as wind, rivers, birds, and humans (Havel and Shurin 2004). The colonization success of an individual or species depends on its performance under the present environmental conditions. Local genotypes are buffered against invaders by a large population size (large egg bank) and local adaptation (De Meester et al. 2002), but the disturbance of local populations (e.g., by the change in trophic state of their habitat, as proposed in this study) could result in ill-adapted populations with decreased fitness under the altered conditions. In the case of the *D. longispina* species complex, fitness differences between species could derive, for example, from differences in vertical migration behavior (Weider and Stich 1992), sensitivity to predation (Spaak et al. 2000), or food preference (Weider 1993). Because *D. longispina* × *galeata* hybrids often show intermediate responses to environmental conditions, compared to their parental species (Weider 1993), it is very likely that the hybrids we find in the egg bank of Lake Brienz could handle its low trophic conditions once they hatched. There are two possibilities for hybrids to be deposited in the sediment of Lake Brienz: Either the hybrids were locally produced (requiring a temporary presence of *D. galeata*), or diapausing eggs of hybrids were imported from elsewhere.

We are aware of the fact that we lack replication in lakes that have never been invaded by *D. galeata*. Lake Brienz, the most unproductive large pre-alpine lake of Central Europe, seems to be the only lake with an almost-pure *D. longispina* population (Keller et al. 2008) where *D. galeata* could never establish. However, in addition to the evidence that comes from our study, Keller et al. (2008) showed that the present dominance of hybrids (implying a large *D. galeata* population in the past) is correlated to the maximum phosphorus level in the past, whereas *D. longispina* is more dominant in less productive systems. Taken altogether, this supports the hypothesis that the general trophic state and the magnitude of eutrophication influenced the establishment success of *D. galeata* in pre-alpine lakes starting in the 1950s.

Our study is an example of how anthropogenic disturbance can favor the establishment of new species and change the taxonomic composition of a population. However, the outcome of these processes depends not only on the magnitude of disturbance but also on the general ecological characteristics of the habitat. Our results support the hypothesis that changes in species composition of the *Daphnia* populations in the pre-alpine lakes of Central Europe were a result of human-induced eutrophication, but depending on the general trophic state of the lake and the magnitude of nutrient increase. Although all lakes are again close to their natural ultra-oligotrophic state (and the new species is not present anymore), the introgression of genetic material into the local population as a result of hybridization with the native taxon led to a partly irreversible change in the taxonomic composition.

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