Modes and mechanisms of a Daphnia invasion

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Whether exotic species invade new habitats successfully depends on (i) a change in the invaded habitat that makes it suitable for the invader and (ii) a genetic change in the invading taxon that enhances its fitness in the new habitat, or both. We dissect the causes of invasions of Swiss lakes, north of the Alps, by Daphnia galeata (a zooplankter typical of eutrophic lakes, e.g. those south of the Alps, which are also warmer) by comparing the fitness performance of eight geographically distributed clones that were fed algal-food typical of oligotrophic versus eutrophic conditions at two temperatures. Daphnia longispina, native to oligotrophic Swiss lakes, served as a reference. Daphnia galeata requires eutrophic food to persist, whereas D. longispina survives and grows on oligotrophic food but does even better on eutrophic food. Invasion by D. galeata is further explained because invading clones from the north perform better on eutrophic food and at cooler temperatures than native clones from the south, suggesting a local response to countergradient selection. Our data support the hypothesis that populations of the invader in northern lakes are dominated by well-adapted genotypes. Our results illustrate how environmental change (i.e. eutrophication) and local adaptation can act together to drive a successful invasion.

Keywords: Daphnia galeata; eutrophication; invasive species; genetic mechanisms of invasion success

1. INTRODUCTION

Biological invasions often drive important changes in community composition and dynamics as the arriving taxon inserts itself into an existing food web [1,2]; so it is important to define the characteristics of the environment or of the invading species that determine why a particular taxon invades in a particular place at a particular time [3,4]. Invasions succeed when either the receiving environment becomes more suitable (i.e. more inhabitable) for the non-indigenous taxon [1,4], or when establishing individuals differ genetically from their populations of origin in a way that makes them particularly suited to the new environment [5,6], or both. Studies of invasions following environmental change provide insight into how fundamental biological mechanisms are altered by human activities as well as into management options and priorities [7].

Among the most pervasive perturbations of lakes is nutrient enrichment [8], and eutrophication has been invoked as a cause of successful invasion [9,10]. Among the ecologically most important freshwater taxa responding to environmental change are members of the genus Daphnia [11,12], which in many lakes are critical species in the pelagic food chain [13]. Recently, Brede et al. [14] and Rellstab et al. [15] proposed that the changes during the past half century in the geographical distribution of Daphnia galeata in central Europe represent invasion success as Lake Constance and other unproductive lakes in Switzerland became eutrophied by nutrient pollution that accompanied population and economic growth following World War II [16]. Using molecular genetic analyses of diapausing eggs isolated from dated lake sediments, these authors showed that genes diagnostic of D. galeata first appeared in the 1950s when phosphorus (P) concentrations began to increase. Over the course of several decades, the relative abundance of D. galeata genotypes increased as that of its close relative Daphnia longispina (formerly Daphnia hyalina [17]), native to Swiss lakes, declined. Furthermore, as P-loading diminished as a result of successful environmental management, D. galeata again became rare relative to D. longispina. Consistent with the view that the invasion success of D. galeata is a product of its superior performance under increasingly eutrophic conditions, Keller et al. [18] showed that this species inhabits the lakes in Switzerland and northern Italy that have, on average, greater P concentrations and are warmer than those inhabited by D. longispina. Finally, for lakes where D. galeata and D. longispina coexist (i.e. where D. galeata has recently invaded), D. galeata is typically found in the warmer, more productive surface waters, whereas D. longispina migrates during the day into deeper, colder, less-productive regions [19,20]. In lakes that were invaded by D. galeata, it was shown that they hybridize with D. longispina [14,15], a process that facilitates rapid gene exchange and potentially rapid adaptation.

Environmental changes can have multiple effects on the habitat suitability for an invading species. While strong circumstantial temporal and spatial evidence suggests that the invasion of eutrophied lakes by D. galeata is related to its ability to take advantage of
the change in nutritional conditions, an alternative explanation could be that lake enrichment leads to increased fish predation intensity to which *D. galeata* is adapted. There is evidence, for example, that *D. galeata* has high birth rates and early age at first reproduction [19,21] that permit it to survive planktivory. In addition, lake surveys in high-latitude Swedish lakes [22] and in high-elevation Polish lakes [23] indicate that *D. galeata* tends to occur in lakes with planktivorous fishes, whereas *D. longispina* does not. Furthermore, an indirect link to eutrophication exists because—in at least one Swiss lake where invasion by *D. galeata* coincided with eutrophication [15]—the intensity of fish predation increased along with rising P concentrations through bottom-up forcing [24]. There are, however, reasons why this mechanism is unlikely to explain the success of *D. galeata* invasion. A recent study of whitefish populations in eutrophied Swiss lakes [25] concludes that nutrient enrichment has, in fact, resulted in decreased or elimination of zooplanktivorous species (and morphs) by causing declines in the oxygen concentration in the cool hypolimnetic waters where they live. Finally, Müller et al. [24] found that despite both higher *Daphnia* densities and greater growth of whitefish in a eutrophied Swiss lake, planktivory by whitefish actually imposed negligible mortality on the *Daphnia*.

The accumulating evidence from field-survey and palaeogenetic studies thus suggests that *D. galeata* has been able to invade lakes originally dominated by *D. longispina* as a result of a change in the nutritional environment. As strong as these correlations are in time and space, they do not provide a direct test of a mechanism. Critical questions remain: (i) is *D. galeata* excluded from oligotrophic lakes by limited food availability? (ii) does enrichment make these lakes habitable? (iii) does eutrophication simultaneously alter the ability of *D. longispina* to persist? and (iv) because eutrophic lakes are generally warmer than oligotrophic ones, does temperature interact with food availability to determine *Daphnia* performance? Finally, (v) is *D. galeata* intrinsically better suited to eutrophic than oligotrophic environments or are the particular genotypes that have invaded enriched lakes different in some way that has enhanced their likelihood of success in a eutrophied environment? To answer these questions requires experiments, and although some relevant studies exist, they were not designed to address our questions; so their results are equivocal. In particular, previous studies show that *D. galeata* and *D. longispina* are effectively identical in growth rate at either low-food concentrations [26] or food-rich conditions [27] and, in a field-enclosure experiment, neither species had an apparent competitive advantage [28]. Previous studies used only single clones of each species [19,28,29], making it impossible to distinguish interspecific differences from among-lineage differences. To test directly the hypothesis that *D. galeata* invaded enriched lakes because it thrives in eutrophic conditions (either in general or for specific invading genotypes), we measured its performance (as clutch size, body size at first reproduction, and juvenile somatic growth rate) when reared in food conditions typical of both moderately eutrophic lakes and highly oligotrophic lakes. For comparison, we made the same measurements on the native *D. longispina*. We crossed these food-resource differences with water temperature to test the importance of an environmental driver correlated with the trophic state in Swiss and northern Italian lakes [30]. Using multiple clones of each species taken both from their respective native ranges and from lakes invaded by *D. galeata*, we compare the within-species performance (crossed with geographical region) with that between species, something not possible in previous studies with single clones.

If *D. galeata* responds to eutrophic conditions to a greater extent than *D. longispina*, this would support the proposal that *D. galeata* was able to invade Swiss lakes and displace *D. longispina* as the lake environments became enriched. Alternatively, if *D. galeata* does not respond any differently from *D. longispina*, then this would suggest that lake eutrophication by itself was not adequate to explain the observed range expansion. Our design further allows us to explore the genetically determined phenotypic basis for the invasion success of *D. galeata* by comparing the performance of clones of this species from both a part of its native range (northern Italy) and its invaded (Swiss) range. A similar comparison of the performance of *D. longispina* clones from Swiss and Italian lakes then depicts the general geographical distribution of fitness traits in relation to food conditions of these two closely related taxa. This experiment allows us to explore explicitly the mechanisms underlying an ecologically important species invasion that appears to have been driven by anthropogenetic environmental changes.

2. METHODS

Our experiment was designed: (i) to compare the performance of invaded *D. galeata* (Switzerland) with *D. galeata* clones from part of its native range (south of the Alps [18]); (ii) to make, as a control, a similar comparison for *D. longispina*; and (iii) to compare performance between species from the two regions. Because we did not want to confound our comparisons between biogeographical regions with comparisons among lakes, we collected *Daphnia* from multiple lakes north and south of the Alps and then selected a single clone from each lake. Thus, our measure of the performance of each species from each region is based on replication within regions rather than within lakes.

(a) Origin of the clones

In April–May 2008, we collected live *Daphnia* from seven Swiss lakes north of the Alps and eight Italian lakes south of the Alps (figure 1). Lakes were selected using the survey by Keller et al. [18], with the goal of finding environments on both sides of the Alps with either *D. galeata* or *D. longispina*. We obtained *D. galeata* and *D. longispina* from eight Italian lakes and six Swiss lakes from Lake Constance (Bodensee) at the Swiss, German and Austrian border. Apart from Lake Constance, every clone was selected from a different lake. For more details, see the electronic supplementary material.

To investigate the distribution of genetic variation, all clones were screened for 12 polymorphic microsatellite loci (Dgm112, SwiD5, SwiD7, Dgm105, SwiD15, Dp281, SwiD1, Dp196, SwiD12, Dp512, SwiD14, Dgm109) [32]. These were visualized with a factorial correspondence analysis (FCA; GENETIX [33]) of multi-locus genotypes, allowing us to assess the clustering patterns of clones according to their geographical origin and taxon background.
We tested clonal performance in a two-by-two design of food typical of eutrophic or oligotrophic conditions at temperatures typical for these lakes. Prior to the experiment, all clones were raised for at least four generations in the laboratory under standardized conditions to minimize maternal and environmental (e.g. crowding) effects. These conditions included a 16 L: 8 D regime at 19°C and daily feeding with chemostat-grown *Scenedesmus obliquus* (1 mg carbon l⁻¹).

Figure 1. The locations of lakes (squares) from which we obtained *Daphnia* clones in 2008 illustrated on a map of the taxon composition of *Daphnia* asexual females across 44 lakes north and south of the Alps in 2003 and 2004 (as described by Keller et al. [18]). Numbers refer to lakes: 1 and 9 Constance, Obersee; 2 Pfäffikersee; 3 Zürichsee; 4 Zugsee; 5 Lago d’Endine; 6 Lago d’Iseo; 7 Lago del Segrino; 8 Lago di Varese; 10 Neuenburgersee; 11 Sarnersee; 12 Walensee; 13 Lago di Como; 14 Lago Maggiore; 15 Lago di Monate; 16 Lago di Orta (see also table 1 in the electronic supplementary material). Pie charts represent relative frequencies of *Daphnia galeata* and *Daphnia longispina*. *Daphnia cucullata* and a variety of hybrid classes were also present in these lakes and are grouped here in an ‘other taxa’ category. Black squares around the pie diagrams indicate lakes from which we sampled *D. galeata* and dashed squares indicate lakes from which *D. longispina* originated. Lake Constance taxon (pie chart) data originate from Jankowski & Straile (figure 1 average May and September observations [31]). Asterisks indicate that in Lago Maggiore (14) and Lago di Orta (16), we did not find *D. longispina* during our survey in 2003–2004.

Table 1. Results from a linear mixed-effects model examining the clutch size (number of eggs produced in the first clutch), size at maturity and juvenile growth rate using clone as a random variable. (No terms were dropped from any of the three models during stepwise backward selection. Values given are residual maximum-likelihood estimates (for parameter estimates, see the electronic supplementary material, table S2). *p < 0.05; **p < 0.01; ***p < 0.001.)

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(b) Clonal performance experiment

We tested clonal performance in a two-by-two design of food typical of eutrophic or oligotrophic conditions at temperatures typical for these lakes. Prior to the experiment, all clones were raised for at least four generations in the laboratory under standardized conditions to minimize maternal and environmental (e.g. crowding) effects. These conditions included a 16 L: 8 D regime at 19°C and daily feeding with chemostat-grown *Scenedesmus obliquus* (1 mg carbon l⁻¹).

All clonal rearing and the experimental treatments were carried out using 0.45 μm filtered lake water from Greifensee as medium. Both species live in Greifensee [14,18], but no clone from Greifensee was used in the experiment to minimize the chance of any local environment by the clone effect.

The experiment comprised a full factorial design with two temperatures (16°C and 22°C) and two food conditions. All clone × treatment combinations were replicated four times. This resulted in an experimental design with
2 taxa (D. galeata and D. longispina) × 2 regions (north and south) × 4 clones × 2 food levels (high and low) × 2 temperatures (16°C and 22°C) × 4 replicates = 256 experimental units. Mean temperatures differ north and south of the Alps, but we were not able to obtain sufficiently extensive water temperature data for our study lakes to define mean summer values. Instead, electronic supplementary material, table S1 gives, for the 15 selected lakes, the mean summer air temperatures over the 1961–1990 period. Livingstone et al. [34] showed that lake water temperatures are well correlated with local air temperatures. Although mean summer air temperature differs by 3°C between the north and south lakes, we chose a 6°C difference between our temperature treatments (16°C versus 22°C) to provide a greater discrimination of any temperature (and temperature interaction) effects.

Because D. galeata occurs, on average, in more eutrophic conditions, and D. longispina in oligotrophic conditions (cf. \( P_{\text{max}} \) data in the electronic supplementary material, table S1), our food treatments consisted of ‘high food’ (high density and high P-content algae, representative of moderately eutrophic conditions) or ‘low food’ (low density and low P-content algae representative of oligotrophic conditions) [35]. All algae used were S. obliquus, grown in the laboratory, with high-food algae cultured in a high-P culture medium, centrifuged and resuspended in 0.45 μm filtered Greifensee lake water and were fed at a concentration of 0.9 mg carbon l⁻¹, and low-food algae cultured in a low-P culture medium and were fed at 0.3 mg carbon l⁻¹. Subsequent measurements showed that the high-food algae contained a C:P molar ratio of 137 and that the low-food algae has a C:P molar ratio of 954 (for more details, see Spaak & Keller [36] and the electronic supplementary material).

Each experimental unit was a 100 ml glass jar. The experiment was initiated by placing a maximum of three second or third clutch neonate Daphnia (less than 24 h old) of the appropriate clone in each jar. The animals were moved into fresh water, and daily food and survival were determined. The experiment was run until one day after the first eggs appeared in an experimental unit, i.e. until the Daphnia matured but before any young were released. On the day the experiment started, a sample of 10 neonates from each clone was dried for 24 h at 50°C and weighed on a Mettler Ultra Micro Balance to get the starting weight for calculating juvenile growth rate.

(c) Data analysis
At the end of the experiment, for daphnids reaching maturity, we measured the time to maturity, the number of eggs per clutch and the total body length. When there was more than one animal in a jar, the values of the individual daphnids were averaged to get the value for that experimental unit. This average was used in all further statistics. On the basis of size of the first clutch and the age of the Daphnia when it was produced, we calculated the intrinsic rate of population increase: \( r \) (per day). We also measured the performance of each daphnid in each experimental unit as somatic juvenile growth rate (day⁻¹)—the increase in the specific rate of mass during the period from neonate to maturity (for details, see [36,37]). Note that the juvenile growth rate has been found to be highly correlated with \( r \) [37], as was also the case in this study (see later text). Because our calculated \( r \) was based on only the first clutch, we used the juvenile growth rate rather than \( r \) as our summary performance measure, although we discuss the latter as well. The performance of clones in the different treatment conditions was compared using a linear mixed-effects model, including taxon, source (north/south), temperature, food and all possible two-, three- and four-way interactions as fixed effects and clone as a random variable. Model simplification was then carried out by backward selection, using Akaike information criterion (AIC) and likelihood ratio tests [38], after which a traditional maximum likelihood was used for model simplification. The selected models were then refit using a residual maximum likelihood. All statistics were conducted using R [39]: the mixed effects models were fit using the lme function in the nlme package [40] and model simplification was conducted using the stepAIC function in the MASS package [41].

3. RESULTS
FCA of the 16 clones used in this study shows that the two taxa we used are distinct species (electronic supplementary material, figure S1). In addition, we found little or no differentiation by this method within the species between clones that originated either north or south of the Alps (except for the D. longispina clone from Lago di Orta, although excluding this clone from the analysis did not change the results and therefore remained in the dataset), but documented that all clones were genetically distinct and therefore are valid replicates for measures of species performance.

We used body size at first reproduction, clutch size at first reproduction and juvenile somatic growth rate as our measures of Daphnia performance under different temperature and food treatments, intended to mimic eutrophic or oligotrophic conditions at northern and southern temperatures. We call these ‘performance measures’ rather than ‘fitness’ because the latter term implies more general integration life-history parameters; however, we expect that a species, or a clone within a species, will have a greater value of one or more of these metrics if it is well adapted to the treatment conditions, and will have lower values if it is not. The survival of our clones was not significantly influenced by the experimental factors (data not shown).

For all three response variables, the AIC-based model simplification did not remove any terms, and the full model (with the four-way interaction and thus all lower order terms) was the best statistical description for each. We observed significant main effects of temperature and food for clutch size and juvenile growth rate, confirming that these treatments were adequate to detect differences in basic physiological processes: as a group, both Daphnia species grew faster (greater juvenile growth rate) and made larger clutches at a higher temperature and when given more and better food (\( p < 0.001 \) in all cases; table 1). In addition, the size at maturity was greater for animals reared in the high-food treatment than in the low-food (\( p < 0.01 \), table 1) one, although there was no temperature main effect on size at maturity.

Our primary hypothesis is that D. galeata requires more eutrophic conditions than D. longispina; so D. galeata could only invade D. longispina dominated lakes when they became more eutrophic. Second, we propose that D. longispina dominates in oligotrophic lakes because its performance is better in these conditions than that of D. galeata. A test of this hypothesis is the comparison

of the performance of both taxa on high-food (enough food of good quality) and low-food (limited food of low quality) conditions. We found a significant food × taxon interaction for all three performance measures (table 1 and figure 2). For juvenile growth rate, the interaction temperature × food × taxon was also significant.

For juvenile growth rate, we found a significant temperature × food interaction indicating, not surprisingly, that both species grew disproportionately quickly at high-temperature and high-food (p < 0.05; p < 0.001; table 1) conditions. However, when both species are considered together, there were no significant two-way interactions for any of our performance measures between either temperature or food and the region (north/south) of origin of the Daphnia: that is, Daphnia from lakes north of the Alps did not respond differently from those collected from the south. We found a significant temperature × taxon interaction for size at maturity (table 1 and figure 3), meaning that D. galeata is smaller at 16°C. For other performance measures, no evidence of an interaction of temperature × taxon was found (table 1).

The most direct test of our hypothesis that D. galeata from its hypothesized native range was able to invade lakes north of the Alps when those lakes became enriched with nutrients lies in the interaction terms between the region of origin of the clones (north/south) and taxon (D. galeata or D. longispina) and temperature, for our three performance measures. These interaction terms also provide a reciprocal view of the performance of D. longispina, which occurs mainly north of the Alps, but which is sometimes found south of the Alps. We observed a significant three-way temperature × north/south × taxon interaction for clutch size (p < 0.05; table 1 and figure 3a). The crossing reaction norms for this treatment combination show that D. galeata collected from lakes that it had invaded north of the Alps in Switzerland outperformed the clones of the same species collected from its native range south of the Alps in Italy—at least at temperatures typical of northern lakes where it invaded.

Interestingly, and unexpectedly, D. longispina showed a pattern reciprocal to that of D. galeata: clones from Italian lakes south of the Alps had larger clutch sizes than did clones collected from Swiss lakes north of the Alps (p < 0.05; table 1 and figure 3a), even though it is certainly native to the northern lakes and possibly a recent invader in lakes south of the Alps. There was no significant temperature × north/south × taxon interaction for the size at maturity and the juvenile growth rate. Finally, there is a significant four-way interaction (figure 4) for clutch size that we discuss below in the context of a broader interpretation of how adaption facilitated invasion.

4. DISCUSSION

Our results are fully consistent with the hypothesis that D. galeata was only able to invade previously oligotrophic Swiss lakes after they had become eutrophied, but they reveal a more complex picture in that not all D. galeata are alike, and they show that D. longispina is not placed at a disadvantage by the high (‘eutrophic’) food conditions. Not surprisingly, all D. galeata clones performed much better for all performance metrics at high-food conditions than those at low (‘oligotrophic’) food (figure 2). Performance in our low-food treatment was so poor that the D. galeata clones are probably simply unable to sustain populations under these conditions. For example, in our study, juvenile growth rate (G_j) was significantly positively correlated with intrinsic rate of population increase, r, where we calculated r based only on data for the first clutch (for D. galeata: r = −0.161 + 0.92 × G_j, r^2 = 0.62; p < 0.0001). Using this regression, the expected r
for *D. galeata* under low-food conditions is near zero \((r = 0.029)\). By contrast, *D. longispina*, which is native to oligotrophic Swiss lakes, also has a juvenile growth rate correlated with \(r\) (for *D. longispina*: \(r = -0.048 + 0.50 \times G_3\); \(r^2 = 0.36\); \(p < 0.0001\)), but with an expected \(r\) under low-food conditions that is three times greater than that for *D. galeata* \((r = 0.090)\). As a result, not only is *D. galeata* able to invade Swiss lakes after they are eutrophied, this taxon is unable to persist after a lake has experienced eutroigotrophication, whereas *D. longispina* survives under both conditions. This pattern provides a mechanistic underpinning for the palaogenetic patterns observed in several lakes north of the Alps: the appearance of either *D. galeata* clones or its genes in *D. galeata \times D. longispina* hybrids as primary productivity increased and their subsequent decline following nutrient reduction owing to governmental regulation of wastewater inputs [14,15]. Interestingly, under high–food conditions, *D. galeata* outperformed *D. longispina* clones in terms of only clutch size (figure 2a; \(F_{1,243} = 3.948, p = 0.048\), and did not differ in either size at maturity (figure 2b; \(F_{1,243} = 0.305, p = 0.581\)) or juvenile growth rate (figure 2c; \(F_{1,244} = 2.610, p = 0.107\). *Daphnia longispina* by contrast outperformed *D. galeata* at low food in both the size at maturity (figure 2b; \(F_{1,243} = 5.907, p = 0.016\) and juvenile growth rate (figure 2c; \(F_{1,244} = 9.970, p = 0.002\). The resulting significant food \(\times\) taxon interactions (table 1) for all three performance measures summarize how *D. galeata* clones are more sensitive to the change in the trophic state as represented by food environment (i.e. they have a steeper reaction norm in figure 2) than *D. longispina* clones. These results are consistent with those of others showing that *D. galeata* is more sensitive to low-food conditions than *D. longispina* [29], has little or no advantage over *D. longispina* under food-rich conditions [27] and does not dominate *D. longispina* in competition experiments [28].

Eutrophic and oligotrophic lakes differ not only in algal quantity and quality, but also in temperature [18]; this pattern is driven entirely by the fact that the southern lakes are both warmer and more productive (table 1). It is only in the northern Swiss lakes, which are consistently cool whether oligotrophic or eutrophic, that temperature and trophic state can be disarticulated. In our experiment, there was a highly significant temperature effect for all performance metrics (table 1), but only for size at first reproduction was there a significant temperature \(\times\) taxon interaction, and that effect was relatively small.

The higher juvenile growth rate of *D. longispina* compared with *D. galeata* at high food raises the question of why at the peak of eutrophication *D. longispina* declined in frequency and in some cases disappeared entirely from the sediment record of Lake Constance and Greifensee, two lakes north of the Alps [14]. In Lake Constance, *D. longispina* persisted in the water column but ceased making diapausing eggs [42,43]. Because diapausing egg production in other species of *Daphnia* is induced at least in part by crowding and food scarcity [44], it may be that eutrophication eliminated the stimulus for diapause in this species. In Greifensee, where eutrophication was much more extensive than in Lake Constance (maximum water column Total Phosphorus > 450 and \(< 100 \mu g 1^{-1}\), respectively; [14]), *D. longispina* disappeared completely from the plankton. This is almost certainly because the hypolimnion of this lake became anoxic in summer [14], removing a coldwater refuge for vertically migrating *D. longispina*—a result similar to the mechanism by which deep-water morphs of zooplanktivorous whitefish have apparently been eliminated from eutrophied Swiss lakes [25].

Our temperature treatments of 16\(^\circ\)C and 22\(^\circ\)C were chosen to represent (modestly exaggerated) mean surface temperatures during the summer growing season for lakes north and south of the Alps (table 1 and [18]). As a result, a comparison of the performance of *D. galeata* clones obtained from each of these two regions, when grown in each of the two temperatures, provides an indication of how well the local *D. galeata* populations are adapted to either their possible native home habitats or the ones they invaded in the north after eutrophication. Of the resulting three-way temperature \(\times\) north/south \(\times\) taxon terms in table 1, only that for clutch size is statistically significant \((p = 0.037)\); size at maturity is marginally significant \((p = 0.064)\), and juvenile growth rate is not significant \((p = 0.300)\). The intriguing result for clutch size (figure 3a) is that the clones collected from lakes south of the Alps, where *D. galeata* is native, perform less well at 16\(^\circ\)C (a temperature typical of lakes north of the Alps) than do the clones that originated from lakes north of the Alps, where it invaded only in the past 50 years. Indeed, clutch sizes for these northern *D. galeata* clones at 16\(^\circ\)C are the same as at 22\(^\circ\)C (typical of lakes south of the Alps) and as that for clones from south of the Alps at 22\(^\circ\)C. We interpret this to mean that the *D. galeata* has adapted to the local conditions where it invaded (and did not lose performance at 22\(^\circ\)C in the process).

It is informative also to compare the performance of *D. galeata* with that of clones of the reference species, *D. longispina*. This species is native not only to cold oligotrophic lakes in Switzerland, but also occurs in a few
warm eutrophic Italian lakes (figure 1) where we do not know if it is native or a recent invader. Most striking in figure 3 is the clutch-size pattern for *D. longispina* at 16°C, which is opposite to that of *D. galeata*. Clones from warm lakes south of the Alps performed better at the cooler water temperature than did clones native to lakes north of the Alps. We do not have an explanation for this intriguing pattern because it is very unlikely that *D. longispina* was introduced from Italian lakes into Switzerland.

The significant four-way interaction for clutch size (table 1 and figure 4) represents the combined effects of food and temperature on the clones of the two species from north and south of the Alps. Food effects are much greater than temperature effects, explaining in part why the enhanced performance that *D. galeata* experiences when grown in elevated algal abundance typical of eutrophied Swiss lakes outweighs any reduction in performance because of lower temperatures, even for clones typical of Italian lakes south of the Alps. The crossing reaction norms seen in the three-way interaction for temperature × north/south × taxon (figure 3) is now seen in figure 4 to be driven almost exclusively by clutch sizes in high food, again consistent with the view that *D. galeata* succeeded in Swiss eutrophic lakes by adapting to the lower temperatures there. Clutch sizes for clones of this species originating from eutrophic warm Italian lakes are essentially identical to clutch sizes for *D. galeata* clones obtained from eutrophic cool Swiss lakes (squares in figure 4), suggesting that this species has evolved, upon invading lakes north of the Alps, so that the clutch size was ultimately unchanged. This is despite the fact that in general clutch size is lower at lower temperatures (cf. significant temperature effect on clutch size in table 1). This would appear to be an example of countergradient selection acting to maintain constant trait expression in the presence of a changed environment [45]. Interestingly, even if *D. longispina* actually did invade Italian lakes from Switzerland, it is not possible in our dataset to look for countergradient selection for tolerance by this species to warm lake temperatures because Italian lakes are generally simultaneously both warm and eutrophic (as was the case for our study lakes), so that these two environmental factors affecting clutch size covary.

In this study, we compared *Daphnia* clones from Swiss lakes with those from south of the Alps because the study by Keller et al. [18] provided useful survey patterns for native and non-native populations. Although invading *D. galeata* did not necessarily come from northern Italy, these lakes do represent locations where this species is native. In addition, there are circumstantial reasons to believe that Italian lakes may well have been the source. *Daphnia galeata* invaded Swiss lakes during the period immediately following World War II, a time when Swiss immigration policy led to the migration of several hundred thousand people from northern Italy to Switzerland in the first decade and a half following World War II [16], a two-order of magnitude increase over pre-war years [46]. Northern Italy is a more likely (although not definitive) source of dispersal by this mechanism than other nearby countries because post-World War II approximately 75 per cent of migrant workers to Switzerland came from Italy when compared with France, Germany and Austria [46].

If *D. galeata* invaded from lakes with warm water such as the Italian lakes, then there are three possible non-mutually exclusive mechanisms for local temperature adaptation by *D. galeata* in Swiss lakes. One is that because *D. galeata* and *D. longispina* readily hybridize [18], and did so as the former invaded Swiss lakes [14,15,18], it may be that there has been introgression of genes that confer high fitness to native *D. longispina* into the invading *D. galeata* genotypes, providing them with similar characteristics. Second, it could be that the *D. galeata* which initially established in the Swiss lakes experienced natural selection for an enhanced performance in their new habitat and rapidly evolved enhanced tolerance to the cooler temperatures. There are many examples of rapid contemporary evolution in *Daphnia* taking place on this time scale of one or a few decades [47], including for juvenile growth rate of *D. galeata* in Lake Constance in response to changes in diet [48]. Finally, it may be that a diverse set of genotypes of this species colonized Swiss lakes, but only a subset capable of producing large clutch sizes at lower temperatures (in eutrophic conditions) actually established. This explanation is the ‘favoured founder’ hypothesis [49], perhaps supplemented by the mechanism of the monopolization hypothesis in which the first really successful genotypes of a clonally reproducing species such as *Daphnia* that invade a new system can be so successful that later arriving, or more slowly growing, clones cannot succeed [50]. Indeed, it may be that *D. galeata* dispersed to the Swiss lakes from a variety of surrounding locations and the favoured founders that succeeded did not come from lakes south of the Alps but from more northern water bodies where they were already adapted to cooler water temperatures.

It has often been speculated [14] that the well-documented invasion by *D. galeata* of Swiss lakes north of the Alps was ultimately the result of eutrophication. Our data clearly support that conclusion, showing that *D. galeata* grows only very poorly in the cool oligotrophic conditions of undisturbed Swiss lakes, and requires the high algal quality and quantity of the eutrophic conditions that developed after World War II. Our data also show that although *D. longispina* has declined in some lakes following invasion by *D. galeata*, this pattern is not likely to be the result of competition or elevated algal densities: if anything, *D. longispina* has similar performance to *D. galeata* under the elevated food conditions typical of eutrophy. Its decline or disappearance from eutrophied Swiss lakes does not appear to be related to the presence of the invader. Finally, the success of *D. galeata* in eutrophied Swiss lakes has been further enhanced by its genetically based adaptation to the cooler environments of these habitats.

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REFERENCES
Invasion facilitated by eutrophication

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