Aquatic Sciences (2019) 81:40
https://doi.org/10.1007/s00027-019-0638-z

Aquatic Sciences

RESEARCH ARTICLE

Living on the edge: reproduction, dispersal potential, maternal effects and local adaptation in aquatic, extremophilic invertebrates

Guntram Weithoff[1,2] · **Catherin Neumann**[1] · **Jacqueline Seiferth**[1] · **Thomas Weisse**[3]

Received: 7 March 2018 / Accepted: 5 April 2019

Abstract

Isolated extreme habitats are ideally suited to investigate pivotal ecological processes such as niche use, local adaptation and dispersal. Extremophilic animals living in isolated habitats face the problem that dispersal is limited through the absence of suitable dispersal corridors, which in turn facilitates local adaptation. We used five rotifer isolates from extremely acidic mining lakes with a pH of below 3 as model organisms to test whether these isolates are acidotolerant or acidophilic, whether they survive and reproduce at their niche edges (here pH 2 and circum-neutral pH) and whether local adaptation has evolved. To evaluate potential dispersal limitation, we tested whether animals and their parthenogenetic eggs survive and remain reproductive or viable at unfavourable pH-conditions. All five isolates were acidophilic with a pH-optimum in the range of 4–6, which is well above the pH (<3) of their lakes of origin. At unfavourable high pH, in four out of the five isolates parthenogenetic females produced a high number of non-viable eggs. Females and eggs produced at favourable pH (4) remained vital at an otherwise unfavourable pH of 7, indicating that for dispersal no acidic dispersal corridors are necessary. Common garden experiments revealed no clear evidence for local adaptation in any of the five isolates. Despite their acidophilic nature, all five isolates can potentially disperse via circum-neutral water bodies as long as their residence time is short, suggesting a broader "dispersal niche" than their realized niche. Local adaptation might have been hampered by the low population sizes of the rotifers in their isolated habitat and the short time span the mining lakes have existed.

Keywords Common garden experiments · Extreme habitats · Extremophiles · Rotifers · Zooplankton

Introduction

The fundamental niche of aquatic organisms is constrained by a variety of abiotic factors such as salinity, temperature, concentrations of certain chemicals, ions and protons, i.e. pH. The vast majority of natural water bodies, marine and freshwater, have a circum-neutral pH in the range of 6–8.5. Exceptions include peat bogs which are moderately acidic (pH around 5–6) and extremely acidic water bodies [pH <2.8, according to the classification by Nixdorf et al. (2005)] such as volcanic lakes (Pedrozo et al. 2001). Acidic anthropogenic lakes originate from the cessation of open cast mining activities and acid mine drainage. Furthermore, a very low pH, is often accompanied by high concentrations of metal ions (Bowers and Wiegel 2011). Under such harsh conditions, bacteria and archaea typically dominate the biota and only few eukaryotic species thrive (Amaral Zettler et al. 2002; Packroff and Woelfl 2000; Méndez-Garcia et al. 2015; Mesa et al. 2017). Most of these eukaryotes are protists (Bell et al. 2006; Aguilera et al. 2007; Aguilera 2013; Amaral-Zettler 2013; Weisse et al. 2013b) and only a few are metazoans such as rotifers and crustaceans [reviewed by Deneke (2000), Belyaeva and Deneke 2013]. Whereas the ecology and physiology of archaea, bacteria and also protists have been intensively investigated [for reviews see Bowers and Wiegel (2011), Canganella and Wiegel (2011), Dhakar and

---

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00027-019-0638-z) contains supplementary material, which is available to authorized users.

✉ Guntram Weithoff
weithoff@uni-potsdam.de

[1] Department of Ecology and Ecosystem Modelling, University of Potsdam, Potsdam, Germany

[2] Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstr. 34, 14195 Berlin, Germany

[3] Research Department for Limnology of the University of Innsbruck, Mondseestraße 9, Mondsee, Austria

Pandey (2016)], studies on their consumers, the extremophile metazoans, are very rare.

Although extremely acidic lakes (pH < 2.8) can be found on all continents, they are rare and isolated habitats (Kwong and Lawrence 1998; Pedrozo et al. 2001). This isolation might hamper the dispersal of acidophilic species if they do not tolerate circum-neutral conditions in potential stepping stones. However, zooplankton can disperse on a local (metre to kilometre) and regional (> 100 km) scale (Jenkins and Buikema 1998; Cáceres and Soluk 2002; Figuerola et al. 2005; Vanschoenwinkel et al. 2009).

In isolated habitats, where dispersal capabilities are limited, the colonizing individual(s) might locally adapt to their new environment. Such local adaptation can be manifested by improved fitness of each deme (= local population) in its home habitat (Kawecki and Ebert 2004). In this context, two scenarios are important: the "local vs. foreign" and the "home vs. away" criterion. The local vs. foreign criterion is fulfilled, when the local population has a higher fitness in its home habitat than a foreign population and the home vs. away criterion is fulfilled, when a population has a higher fitness in its home habitat than in a foreign habitat (Kawecki and Ebert 2004).

In the present study we investigated three aspects of the pH tolerance of five rotifer isolates from extremely acidic mining lakes and their potential for local adaptation. In the first step, we aimed to elucidate the pH tolerance of the five isolates covering a broad range from pH 2–8 using life-table experiments. We tested whether their occurrence in the field is in the middle of their pH niche or at the niche edge. A striking result was that all five isolates produced eggs at pH values of 6–8 that were not viable, i.e. the parthenogenetic mothers allocated resources into reproduction without reproductive success. Furthermore, egg production at pH 2 was low and eggs were also non-viable. In a second step, we further elucidated this phenomenon and tested (a) whether it is a direct pH effect on the eggs or a maternal effect and (b) whether eggs and adult females can tolerate periods of pH-conditions beyond their pH niche, which might facilitate dispersal. In the third step, we tested the hypothesis that the five isolates are locally adapted to their home environment by using "common garden" experiments. Therefore, we experimentally measured the fitness of each isolate in its home and foreign environment.

Materials and methods

Origin of animals and algae, stock cultures

All five rotifer isolates were obtained from acidic mining lakes: *Cephalodella* sp. was isolated from mining lake 111 (L111, ca. 51°30′N, 13°38′E) in Lusatia, Germany, and was used in previous studies (Weithoff 2005, 2007; Weithoff and Wacker 2007; Wacker and Weithoff 2009). The exact species delimitation is still under way. Two strains of the recently described *Cephalodella acidophila* (Jersabek et al. 2011) were isolated from mining lake 130 (L130, 51°33′N, 13°43′E) in Lusatia and from an acidic mining lake in Langau (LLG, ca. 48°51′N, 15°44′E) in North–East Austria. Two strains of *Elosa woralli* Lord, 1891 were isolated from L111 and L130; *E. woralli* has never been recorded in LLG (Weithoff et al. 2010; Moser and Weisse 2011). All rotifer strains occurred in the upper water layers of their respective lakes, only *Cephalodella* sp. exhibited a population maximum at a depth of ca. 8 m in L111 (Weithoff 2004). The two Lusatian lakes are ca. 8 km apart, and they are about 330 km north of Lake Langau. All three lakes originated from lignite open-cast mining activities with subsequent filling with rising groundwater. L 111 is about 70 years old, L130 10 years and LLG 50 years. The low pH was generally uniform with depth in lakes 111 and 130, but increased to pH 5 from 5 to 8.5 m in LLG (Moser and Weisse 2011). The food web in the lakes is similar (Kamjunke et al. 2004; Moser and Weisse 2011): besides heterotrophic bacteria, mixotrophic *Chlamydomonas acidophila* (Chlorophyceae) and *Ochromonas* sp. (Chrysophyceae) dominate the plankton community (Tittel et al. 2005; Schmidtke et al. 2006) and only few other algae were found e.g., diatoms and chlorophytes in the shallow, wind-exposed L130 (Weithoff et al. 2010). The only planktonic metazoans are rotifers; in L111 and L130 the potential top predator is the heliozoon *Actinophrys sol* (Bell et al. 2006).

Stock cultures of animals and algae were grown in a medium adjusted to field conditions (Bissinger et al. 2000) with the key characteristics of pH = 2.65 and high iron and sulfate concentrations of 145 mg L^{-1} and 440 mg L^{-1}, respectively. For all experiments, WC media with pH ranging from 2 to 8 were prepared. At pH 2 and 3 no buffer was used, citric acid was used at pH 4, MES was used at pH 5, TES was used at pH 6 and 7, and HEPES was used at pH 8 [modified after Spijkerman (2005)].

Life-table experiments to determine the pH niche

The life-table experiments followed the procedure as in Weithoff (2004, 2007). Prior to the experiments, animals were acclimated to the target pH. When pH conditions were too harsh for reproduction (e.g., pH 2, see Results), the closest pH where animals successfully reproduced was used for adaptation. From stock cultures, eggs were collected and supplied with an algal suspension of the target pH and an algal density of 50,000 cells mL^{-1} *Chlamydomonas acidophila* (~ 1.5 µg C mL^{-1}). The neonates that hatched were then transferred randomly into separate microtitre well plates with 200 µL algal suspension of the same density and pH.

From then onwards, animals were transferred daily into new wells with fresh algal suspensions. The survival, reproduction and viability of eggs were recorded until all animals had died, thus there were no censored data. Each treatment (i.e. each isolate at each pH) comprised 24 animals. In none of the total 35 life table runs (five isolates × 7 pH) the mothers became mictic, i.e. they reproduced exclusively parthenogenetically. The experiments were performed at 20 °C in the dark to prevent algal growth.

To test for differences in the mean number of eggs between species and pH a general linear model with a tuckey post hoc test was applied using strain and pH as fixed factors and number of eggs as the response variable. Hatching success was calculated as the percentage of viable eggs to the total number of eggs pooled for all 24 females per treatment. Since percentage is an aggregated value without variance, hatching success in response to pH was compared using a test for homogeneity for each strain separately. To calculate population growth rates (r days^{-1}) from the results of the life table experiments, the equation by Lotka was used: $\sum e^{-rx} \times l_x m_x = 1$, where x is the time in days, m_x is the number of viable eggs per surviving female at time x and l_x is the proportion of surviving females at time x. It is assumed that all births in an interval between two consecutive observations occurred in the middle of each time interval. The growth rate r was estimated via an iterative procedure to an accuracy of r to the nearest 0.001 and the 95% confidence interval was calculated using the bootstrap procedure with 199 iterations with the software package R (Weithoff and Wacker 2007).

Egg-transfer experiment

To test whether the pH has a direct effect on the viability of eggs, the hatching success of eggs that were produced at pH 4 (typically viable) and 7 (typically non-viable) was tested at pH 2, 4 and 7. On day one, eggs (without any mothers) from stock cultures were collected in a Petri dish separately at pH 4 and 7 and provided with a satiating food algal suspension of 50,000 cells mL^{-1}. The next day (day 2) ca. 300 hatched neonates at each of the two pH values were transferred individually into wells of a 96-well microtiter plate with 200 µl algal suspension. Since the neonates start reaching maturity within ca. 1 day, they were inspected on day 3whether or not they had reproduced; animals that had already reproduced were discarded. Then, every hour, newly produced eggs were recorded, the mothers of these eggs and the surrounding algal suspension removed and the eggs were washed with the target algal suspension of pH of 2, 4 or 7. On the following days the hatching success was recorded. For each separate treatment between 27 and 54 eggs were used. Since egg production at pH 2 was either very low or absent (see "Results") these experiments were only performed at an almost optimal pH and at a pH higher than optimal. All five rotifer isolates were tested.

Mother-transfer experiments

Since mothers produced non-viable eggs at pH 7, it was tested whether this effect is reversible, i.e. if mothers regain their ability to produce viable eggs when transferred back from pH 7 to 4. To test whether mothers lose their ability for successful reproduction when transferred from pH 4 to 7, this direction was also tested. Therefore, experiments were run similar to the egg-transfer experiments until day three. Mothers that had reproduced at the starting pH were then transferred to the target pH (from pH 4 to 7 or from pH 7 to 4). The following 3 days the hatching success of the eggs was recorded allowing for recovery in case females got stressed by the transfer to the new environment. No quantification was done, i.e. we monitored whether or not mothers regain production of viable eggs when transferred to pH 4 after they had produced non-viable eggs at pH 7 and vice versa.

Common garden experiments to test for local adaptation

To test for local adaptation, the population development of all five isolates was investigated in lake water of the three lakes of origin: Water samples of the three lakes were taken and transported to the laboratory. The ambient pH of the three lake waters at the sampling day were 2.70 for L111, 2.83 for L130 and 2.84 for LLG. These samples were filtered through 0.2 µm filters to remove all organisms including bacteria. Then, 50,000 cells mL^{-1} of the alga *Chlamydomonas acidophila* (Chlorophyta) were added to the three water samples. For each lake water, a *Chlamydomonas* isolate of the same lake was used (Weisse et al. 2011). From these algal suspensions, 4 mL were pipetted into the wells of a 6-well microtiter plate and 10 neonates were added to each well as founder population. In the following, the algal suspension was renewed daily and the number of animals and eggs was recorded. All treatments were run in four replicates resulting in 60 separate wells (3 lakes × 5 isolates × 4 replicates). To test for differences in fitness response (measured as final abundance) among strains, a general linear model with strain and source water as fixed factors and ln final abundance of animals as response variable was applied. To further evaluate potential local adaptation a modified approach from Hereford (2009) was used. Local adaptation was calculated as

$$\frac{W_{local\ population\ i} - \overline{W}_{foreign\ populations}}{\overline{W}_{all\ populations\ in\ habitat\ i}},$$

where W is the fitness of an isolate (ln of final abundance). Positive values, when the 95% confidence interval does not include zero, indicate that local populations are better adapted to their habitat than foreign populations. Analogous, the home vs. away criterion was measured as

$$\frac{W_{population\ i\ at\ home} - \overline{W}_{population\ i\ away}}{\overline{W}_{populations\ i\ in\ all\ habitats}}.$$

Here, positive values, when the 95% confidence interval does not include zero, indicate that populations have a higher fitness in their home habitat than in other habitats.

Results

The pH niche (life-table experiments)

The five rotifer isolates differed in their reproduction (number of viable eggs, F = 171, df = 4, p < 0.001) and this was strongly driven by the pH (F = 379, df = 6, p < 0.001). All isolates had their highest reproduction at acidic conditions: *C. acidophila* from both lakes had the highest reproduction at pH 4, *Cephalodella* sp. (L111) at pH 4–6 and *E. woralli* from L111 at pH 4–6 and from L130 at 5–6 (post hoc comparisons) (Fig. 1). The hatching success varied among species and was strongly dependent on the pH (separate tests for homogeneity, χ^2 ranged from 143 to 239, df = 6, p < 0.001). At the lower end of the investigated pH range (pH 2) either no eggs were produced as in *C. acidophila* and *E. woralli* from L130 or very few with low hatching success as in *Cephalodella* sp. from L111. Only *C. acidophila* from LLG and *E. woralli* from L111 produced a number of eggs theoretically sufficient to maintain a population i.e. more than one egg per female, but none of these eggs was viable. At the upper end of the pH range (pH 6–8), the three species exhibited a different pattern. *Cephalodella* sp. reproduced at a similarly high level as at pH 4 and 5, however the hatching success drastically declined to almost zero at pH 8. A slightly different pattern was found for *C. acidophila* from both lakes, L130 and LLG. From optimal reproduction i.e. a high number of eggs along with a high hatching success at pH 4, the number of eggs decreased with increasing pH up to pH 6 and 7. At pH 8, the total number of produced eggs was relatively high and comparable to pH 3 and 4. Interestingly, hatching success was high only at pH 3–5 and zero at all other tested pH values. Thus, positive growth is restricted to a narrow pH range from slightly below pH 2.65 (pH of the stock cultures) to a pH of 6. *E. woralli* tolerated a broader pH range than *C. acidophila* with a broader maximum and high reproduction and hatching success at pH 6. However, as for the other isolates, reproduction at pH 8 was still high but eggs were completely non-viable.

From the life table data population growth rates were calculated assuming all eggs were viable (maximum potential growth rate) as well as taking only the viable eggs into account (realized growth rate). At optimal conditions (i.e. acidic pH), all five isolates exhibited high realized population growth rates in the range of 0.8–1.2 day^{-1}, which are comparable to rotifers from neutral habitats, demonstrating that all isolates are well adapted to the low pH conditions. If all eggs were assumed viable at pH 8 the estimated growth rates ranged between 0.41 and 1.08 day^{-1} indicating a high, though worthless, reproductive investment given the lack of successful hatching (Table 1 in Supplementary Material).

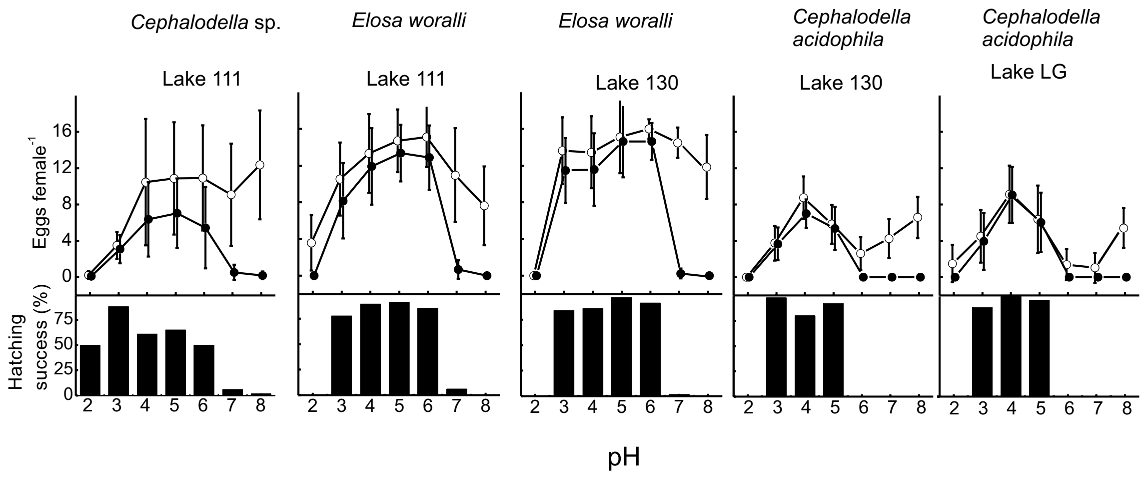

Fig. 1 Results from life-table experiments. Top row, number of eggs per individual female, filled circles, viable eggs, open circles, total number of eggs. Bottom row, hatching success in percentage of viable eggs to total number of eggs, pooled for all 24 animals in each experimental trial

Egg transfer

To investigate the effect of high reproductive effort despite zero percent hatching success, egg-transfer experiments were conducted. Eggs that were produced at pH 4, i.e. at or close to optimum conditions, were transferred to unfavourable pH conditions at pH 2 and pH 7 (Fig. 2a); the control group remained at pH 4. These eggs exhibited a hatching success comparable to that determined in the life table experiments (Figs. 1 and 2) and served as controls. The eggs that were transferred to pH 2 within 1 h after they had been released from their mothers were all not viable for all five isolates of rotifers, suggesting a direct negative effect of the low pH on egg/embryonic development (Fig. 2a). In contrast, eggs remained viable when they were transferred from pH 4 to pH 7. Thus, a pH of 7 alone does not explain why the hatching success at pH 7 was very low or even zero when the eggs were also produced at pH 7. In accordance to that, eggs that were produced at pH 7 were almost completely non-viable at pH 4 and 2 (Fig. 2b). Only some neonates hatched at pH 7 from eggs from *E. worallii* L111, which is in agreement with the results from the life table experiments when also some neonates hatched at pH 7 (Fig. 1g). To summarize, at pH 2 it is a direct pH effect that prevents neonates from hatching whereas at pH 7 it is a maternal effect.

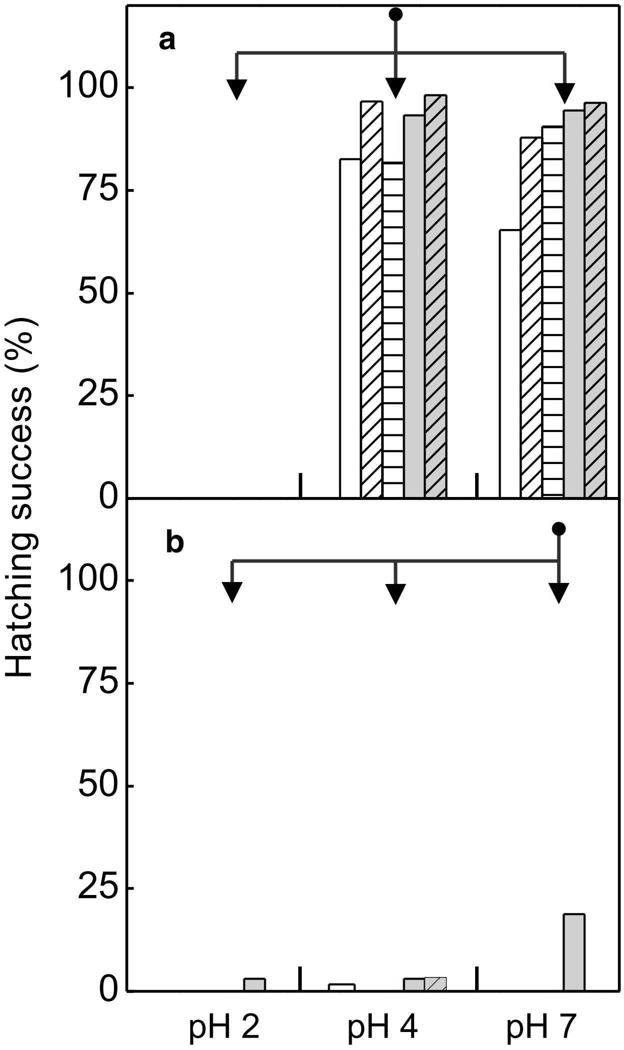

Fig. 2 Results from the egg transfer experiment. Hatching success of eggs produced at pH 4 (**a**) and pH 7 (**b**), which were then transferred to pH 2 and 7 and to pH 2 and 4 respectively, a control group remained at the hatching pH. Open, white bars, *Cephalodella* sp. (from Lake 111), hatched, white bars *Cephalodella acidophila* from Lake 130 (diagonal) and Lake LG (horizontal), open, grey bars, *Elosa worallii* from Lake 111 and hatched grey bars *Elosa worallii* from Lake 130. When no bars are visible, the hatching success was 0%

Mother transfer

To further elucidate the maternal effect on hatching success and the relevance for dispersal, mothers were reared at pH 4 until they reproduced successfully and then transferred to pH 7. After the transfer to pH 7, almost all animals of the three isolates tested ceased successful reproduction, i.e. they produced eggs at pH 7 but the eggs were non-viable (Fig. 3). When mothers were transferred from pH 7 (after unsuccessful reproduction) to pH 4, then mothers from *E. worallii* and *C. acidophila* produced viable eggs within the following days (Fig. 3d, f, h). *Cephalodella* sp. behaved differently in that only about half of the animals that were transferred to pH 4 continued to reproduce (Fig. 3a) and from these mothers only very few produced viable eggs (Fig. 3b). These results demonstrate that at least *E. worallii* and *C. acidophila* are not affected during maturation at pH 7 since they reproduce successfully when transferred to pH 4.

Local adaptation (common garden experiments)

Overall, all five isolates exhibited a similar response to the transfer to the different lake waters (F = 2.23, df = 4, p = 0.081) however, final abundance differed in response to the lake water (F = 4.99, df = 2, p = 0.011) and was consistently high in L 111 water. This indicates that this environment is favourable for all isolates independent of their lake of origin. Consequently, when testing for the home vs away criterion (Fig. 4, shaded areas), only the isolates from L111 exhibited positive values for local adaptation, whereas the other strains had negative values (*E. woralli* and *C. acidophila* from L130) or values around zero (C. *acidophila* from LLG). The local vs foreign criterion could only be tested for *C. acidophila* and *E. woralli* because these two species were found in more than one lake. No consistent result was found, but *E. woralli* from L111 and *C. acidophila* from LG performed slightly better in their home environment as their conspecifics from the other lake. To summarize, lake water from L111 together with

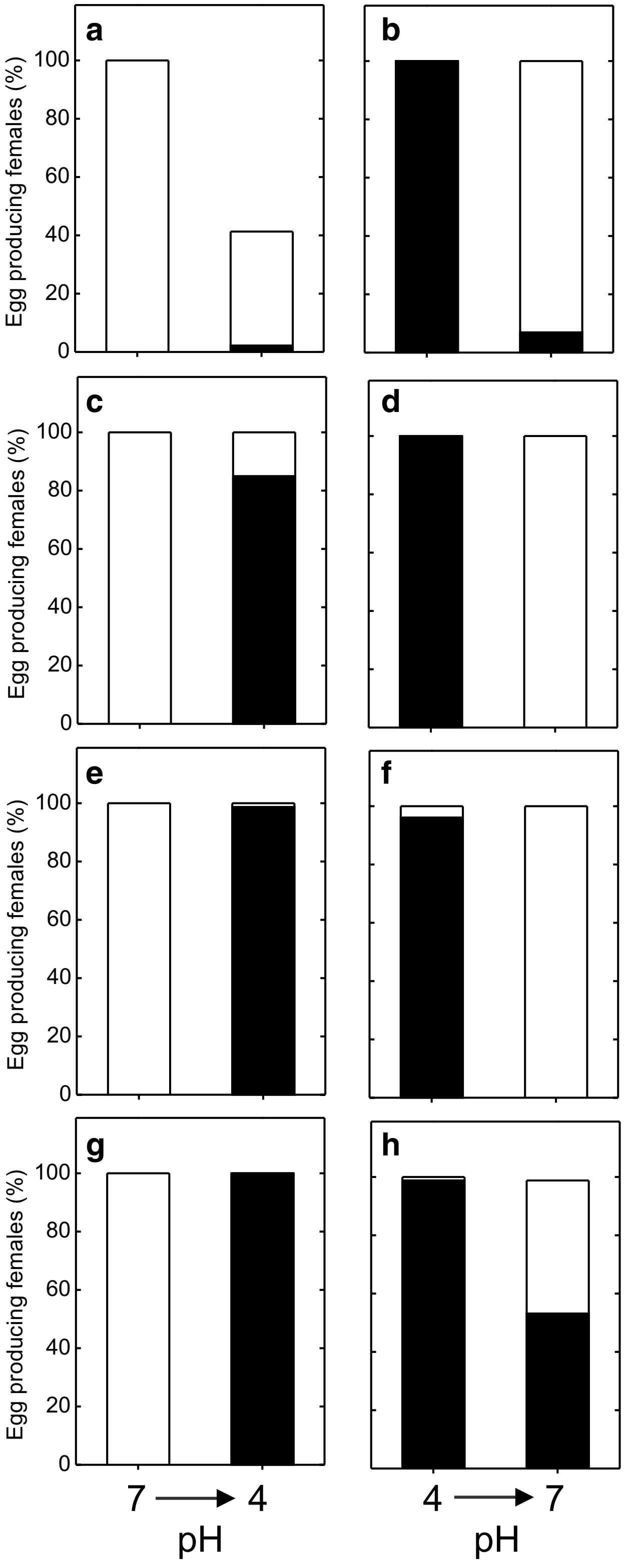

Fig. 3 Results from the mother transfer experiment. Percentage of females that reproduced at pH 4 after their first reproduction at pH 7 (left, **a**, **c**, **e**, **g**) and vice versa (right, **b**, **d**, **f**, **h**). *Cephalodella* sp., (**a**, **b**); *Cephalodella acidophila* from Lake 130, (**c**, **d**); *Cephalodella acidophila* from Lake LG, (**e**, **f**); *Elosa worallii* from Lake 111, (**g**, **h**). Open bars, production of non-viable eggs, solid bars, production of viable eggs

the food source from L111 provided the best environment for all five isolates. No home vs. away effect was found and only weak evidence (in two out of four cases) was found for a local vs. foreign effect demonstrating no clear sign for local adaptation.

Discussion

pH niche

All five isolates are extremophiles i.e. their maximal reproduction was found at acidic conditions and population growth rates were negative at neutral pH. This finding is new for *Cephalodella* sp. from L111 and *C. acidophila* from L130 as well as for the *Elosa* isolates and confirms previous findings for *C. acidophila* from LLG (Weisse et al. 2013a). However, the pH in the lakes of their origin was well below the pH optimum for all five isolates demonstrating that the environmental conditions in their habitat are suboptimal. It was hypothesized earlier that their success in the extremely acidic environment is mainly due to low predation pressure and competitor release, because in L111 mortality by heliozoan is low and food has been proven a limiting factor (Weithoff 2004). This suggests that their occurrence in the extremely acidic mining lakes indicates rather a refuge from otherwise adverse conditions than their preferred habitat (Weisse et al. 2013a).

A striking feature found for all five rotifer isolates was that they reproduce at circum-neutral pH conditions, but eggs were not viable. A similar pattern has been found for five different *Brachionus* species, however at their lower pH-range (Yin and Niu 2008). In that study, egg mortality was exceptionally high at pH 5 and/or 6 compared to circum-neutral or alkaline pH conditions. From an evolutionary point of view, it is extremely disadvantageous to allocate resources into egg production without reproductive success. Although the ultimate mechanism behind this pattern is not yet clear, two potential factors can be excluded by our results: (1) we demonstrate that the high (here: circum-neutral) pH itself does not cause the high egg mortality since eggs that were produced at pH 4 remained viable at pH 7. (2) It is not an aberration during the maturation of the females, since mothers that produced non-viable eggs at pH 7 were able to produce viable eggs at pH 4 when transferred after their first reproduction, except for *Cephalodella* sp. which appears to be irreversibly adversely affected by pH 7. Thus, it is most likely that processes during egg production lead to the high egg mortality. This is underlined by the fact that eggs produced at pH 7 remained non-viable when transferred to pH 4, the optimal pH for population growth for all five isolates in this study.

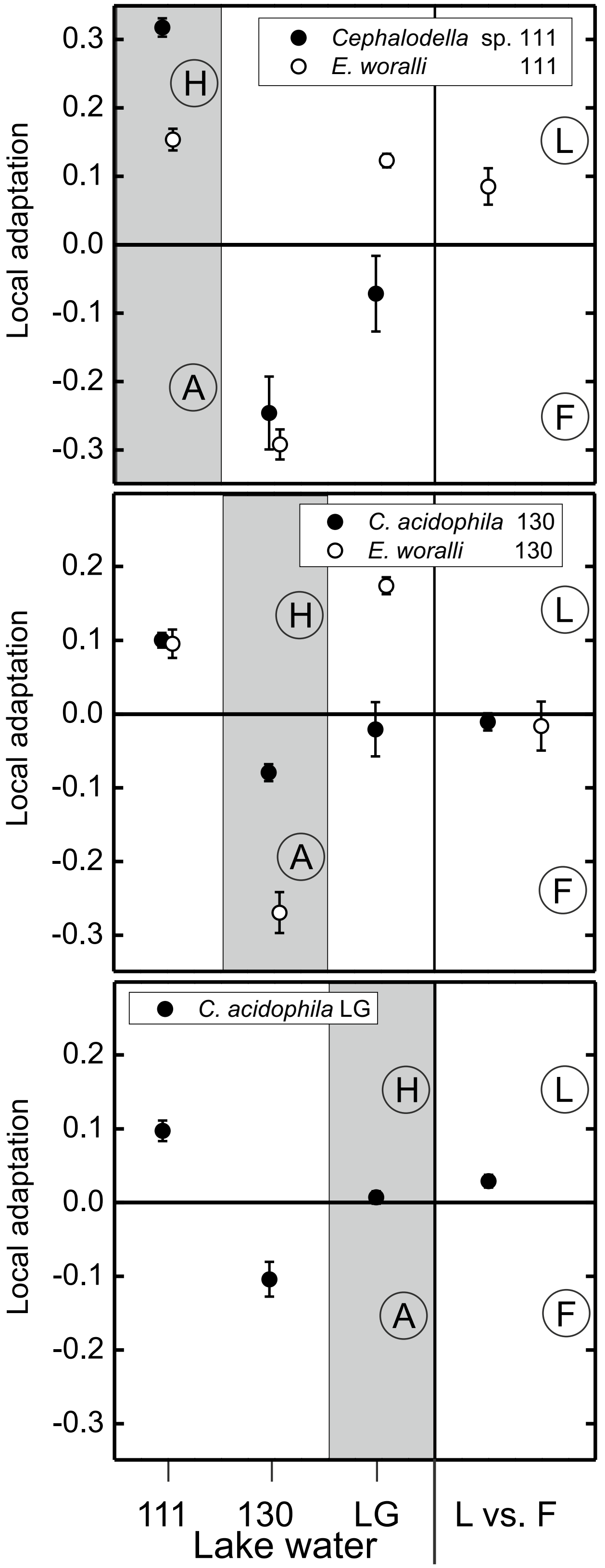

Fig. 4 Results from the common garden experiments on local adaptation, error bars indicate the 95 % confidence interval. Left, Shaded areas display the home (H) versus away (A) criterion. When data are positive and the error bars do not include zero, the strain had a higher fitness in its own habitat than foreign habitats (away); right, local (L) versus foreign (F) criterion. When data are positive and the error bars do not include zero, the local strain had a higher fitness than the foreign strain. Each isolate was reared in lake water from the three study lakes with their respective isolate of the chlorophyte *Chlamydomonas acidophila*

Implications for dispersal–dispersal niche

Despite the acidophilic nature of all five investigated isolates, dispersal via circum-neutral dispersal corridors is possible, because animals and eggs do survive pH-conditions beyond their realized ecological niche in their respective habitat. In directly connected water bodies, e.g. ponds that are connected by running waters, zooplankton dispersal can be measured relatively easily and leads to homogenized species richness between ponds, whereas local factors, both biotic and abiotic, structure the communities (Cottenie et al. 2003). Dispersal between unconnected water bodies is much more difficult to measure and thus less well understood. Using experimental mesocosms, Cáceres and Soluk (2002) showed that dispersal by wind can be an effective pathway for the colonization. In that study, rotifers were the fastest colonizers with *Cephalodella* sp. being the fastest among rotifers. Crustaceans needed much more time to colonize the mesocosms than rotifers, a pattern also found for newly created ponds in Belgium (Frisch et al. 2012) or experimental ponds in the US (Jenkins and Buikema 1998). Another dispersal pathway is via animal vectors such as water birds, either internally in the digestive system or externally attached to birds´ feet or plumage (Figuerola and Green 2002; Figuerola et al. 2005; Frisch et al. 2007). These vectors move fast enough to disperse animals with life spans as short as the studied rotifers. The dispersal ways and the colonization of the lakes from where the isolates of this study originate are unclear. Water birds appear to avoid extremely acidic lakes (pers. observation), however, un-experienced birds might visit these lakes from time to time. Since in our laboratory cultures males or resting eggs have never been found we might speculate that live animals or subitaneous eggs are relevant for the dispersal. It was shown, for example for bacteria and archaea, that even pH-neutral lake water harbours extremophilic species (Low-Décarie et al. 2016). In conclusion, although the pH niche of all five isolates is restricted to acidic waters, the “dispersal niche” might include circum-neutral water bodies as stepping stones, at least when the residence time of the animals or eggs is short. This might be true for many other passively dispersing animals that do not require suitable dispersal corridors.

Local adaptation

In principal, it is assumed that natural selection is the key driving force for genetic differentiation and local adaptation (Linhart and Grant 1996). This mechanism is reinforced, when gene flow is hampered by geographic isolation (Slatkin 1985; Savolainen et al. 2007). For all five isolates, a clear habitat effect was found i.e. the animals performed differently in different lake waters. However, no clear indication of local adaptation was found. These findings differ in some respect from previous results for protists: the chlorophyte *Chlamydomonas acidophila*, the chrysophyte *Ochromonas* sp. and the ciliates *Oxytricha acidotolerans* and *Urosomoida* sp. [originally named *Oytricha* sp. in Weisse et al. (2011), taxonomic affiliation corrected by Weisse et al. (2013b)]. *Chlamydomonas acidophila* from L 111, for example, performed equally well in all lake waters but in its home environment the L 111 isolate exhibited higher growth rates than isolates from the other two lakes, thus fulfilling the ‘local vs. foreign’ criterion of local adaptation (Kawecki and Ebert 2004). This effect levelled off in lake water from L LG where *Chlamydomonas acidophila* isolates from all three lakes exhibited the same growth rate (Weisse et al. 2011). Taking into account that local adaptation takes time (= many generations), one might speculate that protists with high abundances and high (potential) growth rates adapt faster to local conditions than multicellular organisms such as rotifers with their lower growth rates, low abundances and food limitation (Weithoff 2004). Leimuu and Fischer (2008) found in a meta-analysis of plant studies that local adaptation was much more common in large plant populations compared to small populations. Since the population size of the rotifers are much lower than that of the flagellate *Chlamydomonas acidophila* (e.g. by a factor of ca. 10^5 in Lake 111, Kamjunke et al. 2004), this might explain that local adaptation has not (yet) evolved.

Acknowledgements We greatly acknowledge the excellent support of Christina Schirmer and Michael Moser in the laboratory. Financial support was provided by the Austrian Science Fund (FWF), project P20118 (TW).

Author contributions GW and TW conceived the study. GW, TW and CN designed the experiments. GW, CN and JS performed the experiments. GW analyzed the data. GW wrote and TW and CN commented on the manuscript.

References

Aguilera A (2013) Eukaryotic organisms in extreme environments, the Río Tinto case. Life 3:363–374. https://doi.org/10.3390/life3030363

Aguilera A, Souza-Egipsy V, Gómez F, Amils R (2007) Development and structure of eukaryotic biofilms in an extreme acidic environment, Río Tinto (SW Spain). Microb Ecol 53:294–305. https://doi.org/10.1007/s00248-006-9092-2

Amaral Zettler LA, Gómez F, Zettler E, Keenan BG, Amils R, Sogin ML (2002) Eukaryotic diversity in Spain’s River of fire. Nature 417:137. https://doi.org/10.1038/417137a

Amaral-Zettler LA (2013) Eukaryotic diversity at pH extremes. Front Microbiol 3:441. https://doi.org/10.3389/fmicb.2012.00441

Bell EM, Weithoff G, Gaedke U (2006) Temporal dynamics and growth of *Actinophrys sol* (Sarcodina: Heliozoa), the top predator in an extremely acidic lake. Freshw Biol 51:1149–1161. https://doi.org/10.1111/j.1365-2427.2006.01561.x

Belyaeva M, Deneke R (2013) Biology and ecosystem of acidic pit lakes: Zooplankton. In: Geller W, Schultze M, Kleinmann R, Wolkersdorfer C (eds) acidic pit lakes. Springer, Berlin, pp 117–126. https://doi.org/10.1007/978-3-642-29384-9

Bissinger V, Jander J, Tittel J (2000) A new medium free of organic carbon to cultivate organisms from extremely acidic mining lakes (pH 2.7). Acta Hydroch Hydrob 28:310–312. https://doi.org/10.1002/1521-401X(200012)28:6%3c310:AID-AHEH310%3e3.0.CO;2-H

Bowers KJ, Wiegel J (2011) Temperature and pH optima of extremely halophilic archaea: a mini-review. Extremophiles 15:119–128. https://doi.org/10.1007/s00792-010-0347-y

Cáceres CE, Soluk DA (2002) Blowing in the wind: a field test of overland dispersal and colonization by aquatic invertebrates. Oecologia 131:402–408. https://doi.org/10.1007/s00442-002-0897-5

Canganella F, Wiegel J (2011) Extremophiles: from abyssal to terrestrial ecosystems and possibly beyond. Naturwissenschaften 98:253–279. https://doi.org/10.1007/s00114-011-0775-2

Cottenie K, Michels E, Nuytten N, de Meester L (2003) Zooplankton metacommunity structure: regional vs. local processes in highly interconnected ponds. Ecology 84:991–1000. https://doi.org/10.1890/0012-9658(2003)084%5b0991:ZMSRVL%5d2.0.CO;2

Deneke R (2000) Review of rotifers and crustaceans in highly acidic environments of pH values ≤ 3. Hydrobiologia 433:167–172. https://doi.org/10.1023/A:1004047510602

Dhakar K, Pandey A (2016) Wide pH range tolerance in extremophiles: towards understanding an important phenomenon for future biotechnology. Appl Microbiol Biotechnol 100:2499–2510. https://doi.org/10.1007/s00253-016-7285-2

Figuerola J, Green AJ (2002) Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. Freshw Biol 47:483–494. https://doi.org/10.1046/j.1365-2427.2002.00829.x

Figuerola J, Green AJ, Michot TC (2005) Invertebrate eggs can fly: evidence of waterfowl-mediated gene flow in aquatic invertebrates. Am Nat 165:274–280. https://doi.org/10.1086/427092

Frisch D, Green A, Figuerola J (2007) High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. Aquat Sci 69:568–574. https://doi.org/10.1007/s00027-007-0915-0

Frisch D, Cottenie K, Badosa A, Green AJ (2012) Strong spatial influence on colonization rates in a pioneer zooplankton metacommunity. PLoS One 7:e40205. https://doi.org/10.1371/journal.pone.0040205

Hereford J (2009) A quantitative survey of local adaptation and fitness trade-offs. Am Nat 173:579–588. https://doi.org/10.1086/597611

Jenkins DG, Buikema AL (1998) Do similar communities develop in similar sites? A test with zooplankton structure and function. Ecol Monogr 68:421–443. https://doi.org/10.1890/0012-9615(1998)068%5b0421:DSCDIS%5d2.0.CO;2

Jersabek CD, Weithoff G, Weisse T (2011) *Cephalodella acidophila* n. sp. (Monogononta: Notommatidae), a new rotifer species from highly acidic mining lakes. Zootaxa 2939:50–58

Kamjunke N, Gaedke U, Tittel J, Weithoff G, Bell EM (2004) Strong vertical differences in the plankton composition of an extremely acidic lake. Arch Hydrobiol 161:289–306. https://doi.org/10.1127/0003-9136/2004/0161-0289

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7:1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Kwong YTJ, Lawrence JR (1998) Acid generation and metal immobilization in the vicinity of a naturally acidic lake in Central Yukon Territory, Canada. In: Geller W, Klapper H, Salomons W (eds) Acidic mine drainage. Springer, Berlin, pp 65–86

Leimuu R, Fischer M (2008) A meta-analysis of local adaptation in plants. PLoS One 3:e4010. https://doi.org/10.1371/journal.pone.0004010

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annu Rev Ecol Syst 27:237–277. https://doi.org/10.1146/annurev.ecolsys.27.1.237

Low-Décarie E, Fussmann GF, Dumbrell AJ, Bell G (2016) Communities that thrive in extreme conditions captured from a freshwater lake. Biol Lett 12:20160562

Méndez-Garcia C, Peláez AI, Mesa V, Sánchez J, Golyshina OV, Ferrer M (2015) Microbial diversity and metabolic networks in acid mine drainage habitats. Front Microbiol 6:475. https://doi.org/10.3389/fmicb.2015.00475

Mesa V, Gallego JLR, González-Gil R, Lauga B, Sánchez J, Méndez-Garcia C, Peláez AI (2017) Bacterial, archaeal, and eukaryotic diversity across microhabitats in an acid mine drainage. Front Microbiol 8:1756. https://doi.org/10.3389/fmicb.2017.01756

Moser M, Weisse T (2011) The most acidified Austrian lake in comparison to a neutralized mining lake. Limnologica 41:303–315. https://doi.org/10.1016/j.limno.2011.01.002

Nixdorf B, Lessmann D, Deneke R (2005) Mining lakes in a disturbed landscape: application of the EC Water Framework Directive and future management strategies. Ecol Eng 24:67–73. https://doi.org/10.1016/j.ecoleng.2004.12.008

Packroff G, Woelfl S (2000) A review on the occurrence and taxonomy of heterotrophic protists in extreme acidic environments of pH values ≤ 3. Hydrobiologia 433:153–156. https://doi.org/10.1023/A:1004039309694

Pedrozo F, Kelly L, Diaz M, Temporetti P, Baffico G, Kringel R, Friese K, Mages M, Geller W, Woelfl S (2001) First results on the water chemistry, algae and trophic status of an Andean acidic lake system of volcanic origin in Patagonia (Lake Caviahue). Hydrobiologia 452:129–137. https://doi.org/10.1023/A:1011984212798

Savolainen O, Pyhäjärvi T, Knürr T (2007) Gene flow and local adaptation in trees. Annu Rev Ecol Evol Syst 38:595–619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

Schmidtke A, Bell EM, Weithoff G (2006) Potential grazing impact of the mixotrophic flagellate *Ochromonas* sp. (Chrysophyceae) on bacteria in an extremely acidic lake. J Plankton Res 28:991–1001. https://doi.org/10.1093/plankt/fbl034

Slatkin M (1985) Gene flow in natural populations. Annu Rev Ecol Syst 16:393–430. https://doi.org/10.1146/annurev.es.16.110185.002141

Spijkerman E (2005) Inorganic carbon acquisition by *Chlamydomonas acidophila* across a pH range. Can J Botany 83:872–878. https://doi.org/10.1139/b05-073

Tittel J, Bissinger V, Gaedke U, Kamjunke N (2005) Inorganic carbon limitation and mixotrophic growth in *Chlamydomonas* from an acidic mining lake. Protist 156:63–75. https://doi.org/10.1016/j.protis.2004.09.001

Vanschoenwinkel B, Gielen S, Seaman M, Brendonck L (2009) Wind mediated dispersal of freshwater invertebrates in a rock pool metacommunity: differences in dispersal capacities and modes. Hydrobiologia 635:363–372. https://doi.org/10.1007/s10750-009-9929-z

Wacker A, Weithoff G (2009) Carbon assimilation mode in mixotrophs and the fatty acid composition of their consumers. Freshw Biol 54:2189–2199. https://doi.org/10.1111/j.1365-2427.2009.02251.x

Weisse T, Berendonk T, Kamjunke N, Moser M, Scheffel U, Stadler P, Weithoff G (2011) Significant habitat effects influence protist fitness: evidence for local adaptation from acidic mining lakes. Ecosphere 2:134. https://doi.org/10.1890/es11-00157.1

Weisse T, Laufenstein N, Weithoff G (2013a) Multiple environmental stressors confine the ecological niche of the rotifer *Cephalodella acidophila*. Freshw Biol 58:1008–1015. https://doi.org/10.1111/fwb.12104

Weisse T, Moser M, Scheffel U, Stadler P, Berendonk T, Weithoff G, Berger H (2013b) Systematics and species-specific response to pH of *Oxytricha acidotolerans* sp. nov. and *Urosomoida* sp. (Ciliophora, Hypotricha) from acid mining lakes. Eur J Protistol 49:255–271. https://doi.org/10.1016/j.ejop.2012.08.001

Weithoff G (2004) Vertical niche separation of two consumers (Rotatoria) in an extreme habitat. Oecologia 139:594–603. https://doi.org/10.1007/s00442-004-1545-z

Weithoff G (2005) On the ecology of the rotifer *Cephalodella hoodi* from an extremely acidic lake. Freshw Biol 50:1464–1473. https://doi.org/10.1111/j.1365-2427.2005.01423.x

Weithoff G (2007) Dietary restriction in two rotifer species: the effect of the length of food deprivation on life span and reproduction. Oecologia 153:303–308. https://doi.org/10.1007/s00442-007-0739-6

Weithoff G, Wacker A (2007) The mode of nutrition of mixotrophic flagellates determines the food quality for their consumers. Funct Ecol 21:1092–1098. https://doi.org/10.1111/j.1365-2435.2007.01333.x

Weithoff G, Moser M, Kamjunke N, Gaedke U, Weisse T (2010) Lake morphometry and wind exposure may shape the plankton community structure in acidic mining lakes. Limnologica 40:161–166

Yin X, Niu C (2008) Effect of pH on survival, reproduction, egg viability and growth rate of five closely related rotifer species. Aquat Ecol 42:607–616. https://doi.org/10.1007/s10452-007-9136-9