Rapidly evolving zooplankton in a salinizing world: To what extent does microevolutionary adaptation to one salt increase tolerance to another one?

Shuai-Ying Zhao [1,2] Libin Zhou [2,3*] Guangjie Chen [1] Steven A. J. Declerck [2,4]

[1]Yunnan Key Laboratory of Plateau Geographical Processes and Environmental Change, Faculty of Geography, Yunnan Normal University, Kunming, China
[2]Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands
[3]Institute of Ecology, College of Urban and Environmental Science, Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, Beijing, China
[4]Laboratory of Aquatic Ecology, Evolution and Conservation, Department of Biology, KU Leuven, Leuven, Belgium

Abstract

Salinization is a worldwide emerging threat to freshwater systems. It can be caused by various salts with different toxicity to freshwater organisms. Populations of freshwater organisms have been shown to rapidly evolve adaptations to salinity, yet it remains unclear whether adaptation to one salt increases tolerance or, conversely, susceptibility to other salts. Using an experimental evolution approach, we subjected replicate populations of a planktonic monogonont rotifer species (*Brachionus calyciflorus* Pallas) to non-saline and NaCl-enriched environments. A subsequent multigenerational common garden (CG) transplant experiment demonstrated rapid microevolutionary adaptation to salinity. A second CG experiment provided evidence that adaptation to NaCl increases tolerance mainly to $CaCl_2$ but to a much lesser extent to Na_2SO_4. Increased tolerance to $CaCl_2$ was associated with increased tolerance to Ca^{2+} ion toxicity. In contrast, acquired tolerance to Na_2SO_4 could not be shown to involve an improved ability to cope with SO_4^{2-} ion toxicity. Our results highlight that the evolution of cross-tolerance may expand the temporal and spatial dimensions under which the ecological consequences of salt adaptation apply.

Salinization of freshwater ecosystems is an emerging issue of global concern (Kefford et al. 2016; Cañedo-Argüelles 2020; Cunillera-Montcusí et al. 2022), as it has impacted many lakes, rivers and wetlands around the world (Herbert et al. 2015; Dugan et al. 2017; Thorslund et al. 2021). Freshwater salinization is caused by various human activities, including land clearing, improper irrigation, mining, resource extraction and the application of de-icing salts (Cañedo-Argüelles et al. 2013;

Herbert et al. 2015). This issue is expected to worsen in the future because of the combination of intensified anthropogenic activities with climate change (Cañedo-Argüelles 2020). Owing to its various adverse effects on freshwater organisms, salinization can reduce the abundance and biodiversity of freshwater communities even at relatively low concentrations (Mo et al. 2021; Hintz et al. 2022; Hébert et al. 2023), posing a threat to ecosystem functioning (Castillo et al. 2018; Cañedo-Argüelles 2020).

Depending on the context such as geology and human impact, freshwater salinization can be caused by various ions (Griffith 2017; Kaushal et al. 2021). The weathering of rock beds is a natural source of ions such as Ca^{2+}, Mg^{2+}, K^+, HCO_3^-, Cl^-, and SO_4^{2-} (Kaushal et al. 2021). Human activities, such as irrigation (e.g., Na^+, Cl^-, HCO_3^-, CO_3^{2-}, SO_4^{2-}), the application of lime for agriculture (e.g., Mg^{2+}, Ca^{2+}, HCO_3^-) and the use of building materials (e.g., Ca^{2+}, Mg^{2+}, Na^+, K^+, HCO_3^-) are also known to be sources of a wide variety of ions to freshwater systems (Kinsman-Costello et al. 2023). NaCl, $CaCl_2$, and $MgCl_2$ are widely used as deicing salts (Schuler et al. 2017; Coldsnow and Relyea 2018) whereas Na_2SO_4 pollution is often the result of long-term mining activities (Kennedy et al. 2003; Leppänen et al. 2019).

*Correspondence: libin.zhou@pku.edu.cn; libzhou@hotmail.com

Additional Supporting Information may be found in the online version of this article.

Author Contribution Statement: S.-Y.Z., L.Z., and S.A.J.D. developed the idea and designed the experiments; S.-Y.Z. conducted the evolution and CG1 experiment, and L.Z. performed the CG2 experiment. L.Z. analyzed the data. S.-Y.Z. wrote the first draft of the manuscript and L.Z., S.A.J.D., and G.C. contributed substantially to the revision. All authors approved the final submitted manuscript.
Shuai-Ying Zhao and Libin Zhou should be considered joint first authors.

An important but often ignored aspect of salinization is that it simultaneously affects organisms in two ways, namely through osmotic stress and ion toxicity (Munns and Tester 2008; Latta et al. 2012). Osmotic stress depends on the concentration of osmolyte particles in the ambient environment, regardless of ion properties, whereas ion toxicity is ion-specific and depends on both the ion's concentration and chemical properties (Mount et al. 1997). Hence, the negative effects of a salt may be determined not only by its concentration but also by its ion composition (Blinn 1993; Bos et al. 1996; Cañedo-Argüelles et al. 2016). Despite the potential importance, the distinct roles of osmotic pressure and salt identity are seldom explicitly considered in ecological and ecotoxicological studies. This is remarkable, given that an effective prediction and remediation of the effects of salinization on aquatic biodiversity will necessarily require a better understanding of both aspects (Cañedo-Argüelles 2020).

Rapid evolution is increasingly recognized as a key mechanism through which populations of organisms are able to adapt to emerging environmental stressors within an ecologically relevant timescale (Hoffmann and Sgrò 2011; Dam 2013; Bitter et al. 2019). In a rapidly changing world, populations are most often exposed to multiple stressors simultaneously or sequentially (Orr 2020; Jackson et al. 2021; Orr et al. 2022*b*). Importantly, evolutionary adaptation to one stressor can affect a populations' ability to cope with other stressors (e.g., Van de Maele et al. 2021; Xu 2021; Zhou and Wang 2023). Indeed, adaptation to one stressor may enhance the ability of a population to cope with another stressor ("evolved cross-tolerance"), but the opposite may also apply, for example, due to trade-offs ("evolved cross-inhibition") (Bell 2017; Orr et al. 2022*b*).

We expect that adaptation to one salt should result in evolved cross-tolerance to osmotic stress caused by other salts. However, whether such cross-tolerance will be effective will also depend on how adaptation mediates the effects of ion toxicity. Cross-tolerance may apply if adaptations that reduce toxic effects of one ion are also effective in reducing toxicity by other ions. Conversely, trade-offs among coping strategies may also result in cross-inhibition. The question of how adaptation to one specific salt determines the abilities of populations to cope with other salts through evolutionary adaptation is relevant, given that the relative contribution of salts to salinization may vary with time. For example, some ions tend to vary stronger in time than other ions (e.g., Na^+ and Cl^- compared to Mg^{2+}, Ca^{2+}, and HCO_3^-, Kerr 2017; Cl^- compared to SO_4^{2-}, Laceby et al. 2019). Seasonal variation in the relative composition of ions is often pronounced, especially in estuaries (Cl^-, HCO_3^-, SO_4^{2-}, Na^+, Ca^{2+}, and Mg^{2+}, Crosa et al. 2006). Apart from human inputs due to agriculture (e.g., Crosa et al. 2006) or the use of road salts (Laceby et al. 2019), temporal variations in salt composition can also be influenced by extreme events like storm runoff (Kinsman-Costello et al. 2023) or chemical processes like internal ion exchange (e.g., Na^+ and Ca^{2+} exchange, Rhodes and Guswa 2016).

In salinizing landscapes, rapid adaptation to increased salt concentrations potentially has important ecological consequences as it may allow freshwater species to persist through evolutionary rescue at local and regional scales (Bell 2017), and contribute to the stabilization of food web and ecosystem functioning (Hendry 2017). Although few studies have demonstrated rapid adaptation of freshwater organisms to environments with increased salinity (Scheuerl and Stelzer 2013; Coldsnow et al. 2017; Sun and Arnott 2023; Wersebe and Weider 2023), there is hardly any information available whether adaptation to one type of salt affects the ability to cope with other salts through cross tolerance or inhibition, but see Hintz et al. (2019). With this study, using an experimental evolution approach, we aimed to test (1) whether populations of planktonic freshwater metazoans are able to adapt to salinization of their environment at an ecologically relevant time scale, (2) if evolved tolerance to one type of salt confers cross-tolerance to other types of salts, (3) whether the degree of such evolved cross-tolerance varies with the chemical identity of the second salt and (4) if evolved cross-tolerance applies to the toxicity of specific ions. For this, we used the cyclic parthenogenetic rotifer *Brachionus calyciflorus*, a common and cosmopolitan freshwater zooplankter. We first performed a selection experiment, in which we exposed replicate populations of *B. calyciflorus* to environments with contrasting salinities (i.e., non-saline vs. NaCl-enriched). Subsequently, we conducted two common garden (CG) experiments. The first common garden experiment (CG1) aimed at demonstrating rapid evolutionary adaptation to NaCl. The second common garden experiment (CG2) was specifically designed to investigate if adaptation to one salt (NaCl) results in cross tolerance against negative effects of two other common salts (i.e., $CaCl_2$ and Na_2SO_4) and, more specifically, if adaptation to toxicity of Na^+ and Cl^- ions increases the ability of populations to cope with the toxicity of Ca^{2+} and SO_4^{2-} ions.

Methods

Ancestral genotypes

We initiated our experiments with 44 genotypes (clones) of *B. calyciflorus* (Pallas). Each clone had originally been hatched from one diapausing egg isolated from the surficial sediments of one of seven freshwater ponds in the Netherlands (Supporting Information Table S1). Before use in this study, all clones had been cultured for months in the laboratory under semi-standardized conditions, and fed with chemostat grown *Chlamydomonas reinhardtii*. *B. calyciflorus* is a complex of hybridizing species for which four species have recently been described (Michaloudi et al. 2018). The genotypes used in our study were all identified as *B. calyciflorus* s.s.

Selection experiment

The selection experiment was designed with the aim to mimic the reproductive cycles of natural populations in

consecutive growing seasons, during which a phase of clonal reproduction is followed by a bout of sexual recombination and a period of dormancy after which newly produced genotypes establish a new population. We started with creating six populations with identical genotype composition (44 clones; Fig. 1). Three replicate populations were cultured in a non-saline food suspension throughout the entire experiment ("non-salt selected," further referred to as "NSS"). The other three replicate populations were reared in the same medium, but the salinity was stepwise increased by the addition of NaCl ("salt selected," further referred to as "SS"). During the selection experiment, all populations went through three consecutive cycles (Supporting Information Fig. S1). Each cycle consisted of a phase of clonal reproduction with exponential population growth, and followed by a phase of sexual reproduction resulting in the production of dormant propagules. At the end of each cycle, dormant propagules were collected and used to establish clonal lines representing newly produced, unique genotypes. Each new cycle was started by recomposing the original populations using the genotypes obtained from the previous cycle (Supporting Information Fig. S1). The salinity of the SS treatment during the three consecutive cycles was set at 2, 3, and $4 \, g \, L^{-1}$ NaCl, respectively, which lie well within the range of salinities in natural freshwaters that are subject to salinization (Kaushal et al. 2005; Mo et al. 2021). The selection experiment lasted 82 d in total and involved 3 sexual generations and < 60 clonal generations. For more detailed information, see Supporting Information.

Common garden experiment 1

We conducted a CG experiment to test for local adaptation of the populations to the salinity regimes under which they
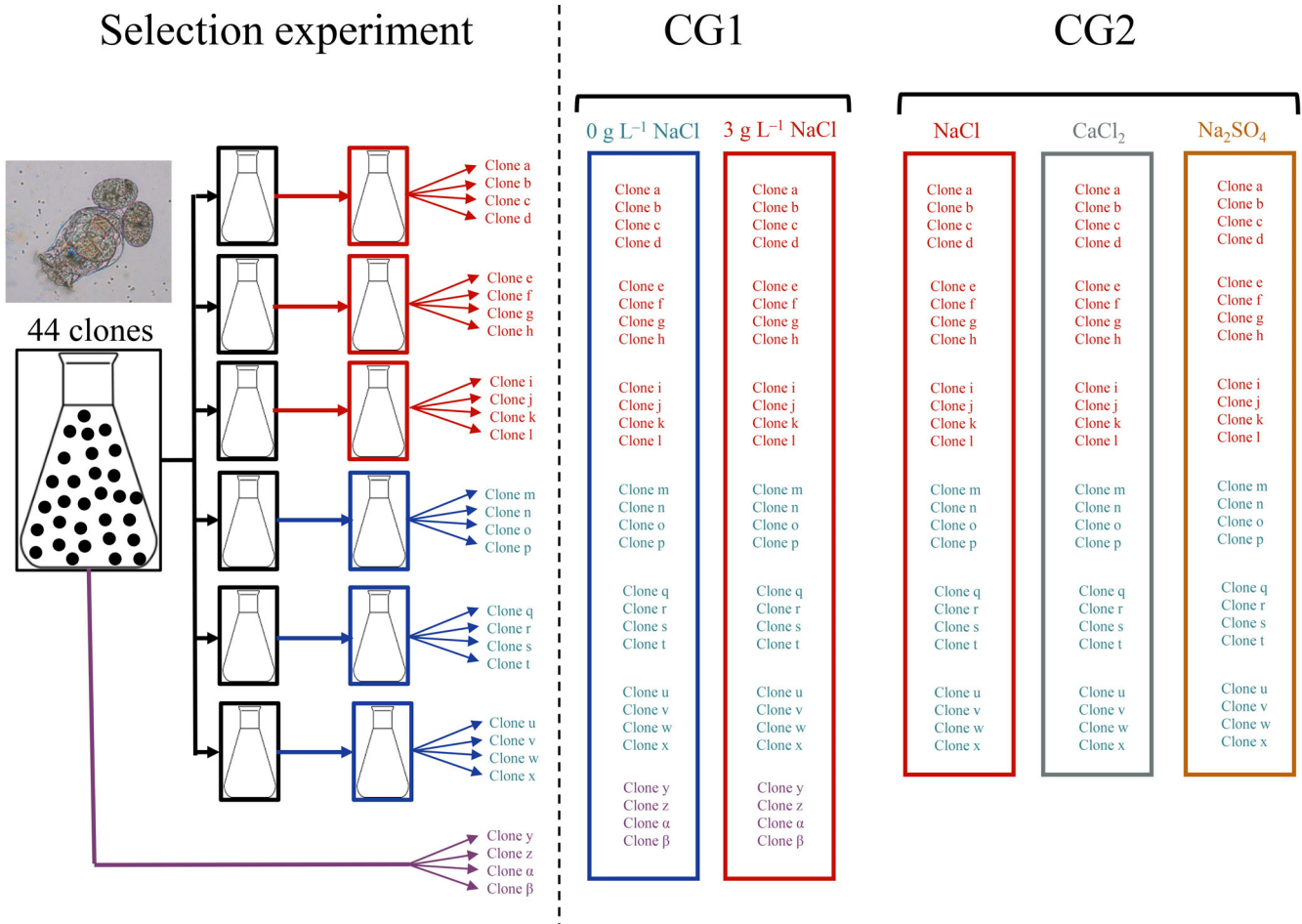

Fig. 1. Experimental design of the three experiments. Selection experiment: 6 populations composed of the same set of 44 clones were initially established. Three populations were exposed to a selection regime of stepwise increased salinity (NaCl), whereas the other three were subjected to non-saline control conditions. After three cycles of clonal population growth followed by sexual reproduction (Supporting Information Fig. S1), we established clonal lines of four genotypes per population, including four ancestral genotypes, to be used in CG1 and CG2. CG1: test for local adaption by comparing the population growth rate of all clonal lines in two salinity treatments (0 and $3 \, g \, L^{-1}$ NaCl). CG2: test for evolved cross-tolerance by estimating osmotic pressure and ion concentrations at zero growth of clonal lines exposed to three salts (NaCl, CaCl$_2$, and Na$_2$SO$_4$).

had been selected for during the selection experiment. First, from each of the six populations of the selection experiment we hatched and established clonal lines of four genotypes from the dormant propagules produced during the last cycle of this experiment (Fig. 1). In addition, we randomly selected four clones to represent the initial populations in the selection experiment ("Ancestral," Fig. 1). We then subjected populations of each of these clones to a low (0 g L^{-1} NaCl) and high salinity (3 g L^{-1} NaCl) treatment, and measured their population growth rates. The CG1 experiment thus consisted of 56 experimental units, that is, 2 salinities × 7 populations × 4 clones (Fig. 1). The experiment was conducted in six-well plates filled with 8 mL of algal food at ad libitum concentrations (1200 μmol C L^{-1}).

We started each unit of the CG1 experiment with transferring 10 individuals into a food suspension with the target salinity. The culture plates were kept in a dark incubator at 24°C. After 24 h, we manually counted the total numbers of females in each unit and transferred 10 randomly selected females to a new well with fresh medium and corresponding salinity. We repeated this process for seven consecutive 24-h time intervals. For each time interval, we calculated exponential population growth rate as follows:

$$r = \frac{\ln N_t - \ln N_0}{t} \tag{1}$$

where N_0 and N_t represent the population sizes at the start and end of the time interval, respectively, and where t is the duration of the time interval (i.e., 1 d). Prior to the CG1 experiment, we removed maternal effects by acclimating all experimental units to their respective experimental treatments for two parthenogenetic generations.

Common garden experiment 2

To test the hypothesis that adaptation to one salt is able to increase tolerance of animals to other salts, we exposed genotypes generated through the selection experiment to three naturally occurring common salts, that is, NaCl, CaCl$_2$ and Na$_2$SO$_4$ (Fig. 1). The clonal lines were the same as those used in CG1, except that we did not include clones from the ancestral population. This experiment thus consisted of 72 experimental units (3 salt types × 6 populations × 4 clones). Populations were cultured and monitored for population growth rate in the same way as in CG1, except that salt concentrations were gradually increased and adjusted on a daily basis for each unit separately until net population growth rate stabilized around zero. From that moment on, we continued the experiment for 5 more days to monitor the population growth rates and recorded salt concentrations administered to keep net population growth rates equal to zero as close as possible (*see* Supporting Information for data on population growth rates and salt additions throughout the acclimation

phase and the actual experiment). This CG2 experiment lasted for 25 d in total.

Data analysis

We performed a linear mixed effects model to test for the impacts of population selection history (SS vs. NSS), salinity and their interaction on the exponential population growth rates of CG1.

To be able to compare the relative toxicity of the different salt types in CG2, we calculated the osmotic pressure of the medium at which net zero population growth occurred (further referred to as "osmotic pressure at zero growth" or "OPZG"). We assume that salts with identical toxicity should result in equal OPZG, whereas salts associated with low OPZG values should be relatively more toxic than salts with higher OPZG values. We calculated osmotic pressure (kPa) using the van 't Hoff equation (Janáček and Sigler 1996):

$$\pi = i\Phi CRT \tag{2}$$

where i represents the number of ions produced by salt dissociation, Φ is osmotic coefficient, C is molar concentration of the salt, R is the gas constant, and T is the absolute temperature (Kelvin). Given that salt concentrations in our experiment were very low, we assume Φ values to equal 1).

To evaluate the relative toxicity of Ca^{2+} vs. Na$^+$ ions for SS and NSS populations, we calculated the concentrations of dissolved Cl$^-$ ions in the NaCl and CaCl$_2$ treatments at zero population growth (*see* Fig. 4a,c, for a more detailed rationale). Similarly, to estimate the relative toxicity of the SO$_4^{2-}$ vs. Cl$^-$ ions for both population types we calculated the concentrations of dissolved Na$^+$ ions in the NaCl and Na$_2$SO$_4$ treatments (Fig. 4b,d, for a detailed rationale). We applied linear mixed effects models to test for the impacts of selection history (SS vs. NSS), salt type (NaCl, CaCl$_2$, Na$_2$SO$_4$) and their interaction on time averaged OPZG. Similarly, we applied mixed models to evaluate differences in the effects of selection history and Ca^{2+} toxicity on time averaged zero growth Cl$^-$ ion concentrations, and the effects of selection history and SO$_4^{2-}$ toxicity on time averaged zero growth Na$^+$ ion concentrations. Given that the WC medium had a very low salinity (0.087 g L^{-1}; Supporting Information Table S2) and consequently made a negligible contribution to the osmotic pressure of salt solutions, the solutes in WC medium were not taken into account in the calculation of osmotic pressure.

In all mixed model analyses, salt type and selection history were specified as fixed factors whereas populations of the selection experiment were specified as random variables. Mixed models with the lowest AIC values were selected for interpretation after their performance was also compared to competing models using Chi square difference tests. All statistical analyses were performed in R (R core Team 2016), and the mixed models were performed using the *lmer* function of the lme4 package (Bates et al. 2015).
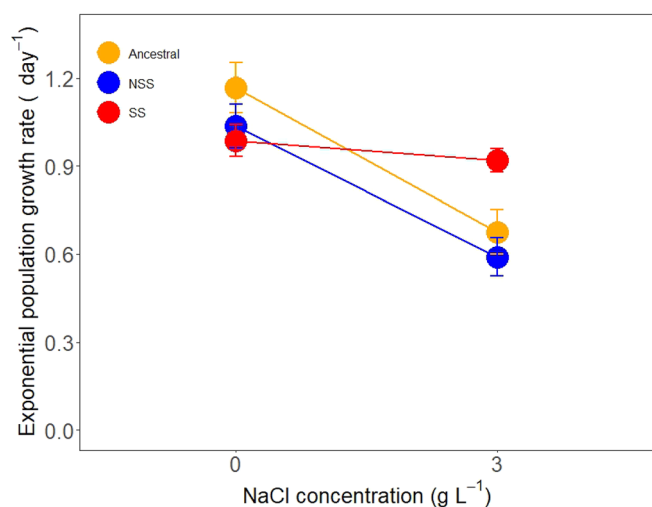
Fig. 2. Effect of salinity treatments (0 and 3 g L^{-1} NaCl additions) on exponential population growth rates of rotifer genotypes. Ancestral: original population. Symbols and error bars represent the means across populations ± 2 standard errors.

Results

Common garden experiment 1

We observed a significant interaction effect between selection history and salinity on the exponential population growth rate ($p < 0.001$; Fig. 2). Growth rates of SS and NSS populations were very similar under non-saline conditions. In the salinized treatment, growth rates of the NSS populations were strongly reduced, while increased salinity had no effect on the SS populations. Growth rates of ancestral clones were
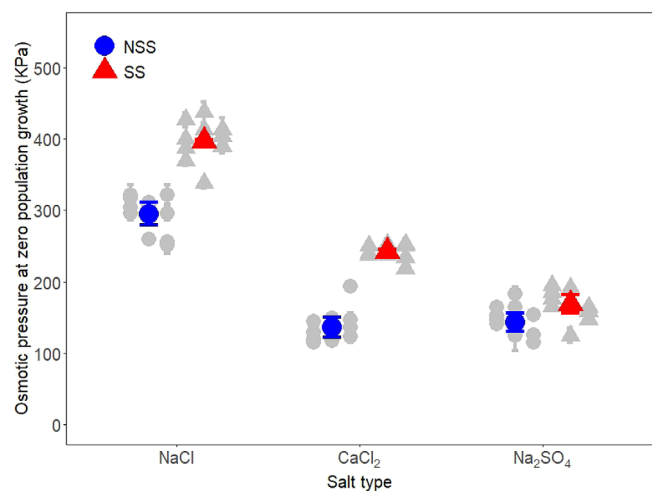

Fig. 3. The osmotic pressure at which rotifer populations showed zero population growth for each of the salt type treatments. Gray symbols refer to populations of individual clones. Blue circles and red triangles, respectively, represent means of the NSS and SS populations. Error bars represent 2× standard errors. Error bars of gray symbols represent variation of clonal populations through times. Blue and red error bars represent variation across the means of NSS and SS populations, respectively.

very similar to those of the NSS populations in both salinity treatments (Fig. 2).

Common garden experiment 2

OPZG was determined by salt type ($p < 0.001$), selection history ($p < 0.001$), and the interaction of these two factors ($p < 0.001$; Fig. 3; Supporting Information Tables S3–S6). For NSS populations, OPZG of NaCl was, respectively, 2.16 and 2.05 times higher than that of CaCl$_2$ and Na$_2$SO$_4$ (Fig. 3; Supporting Information Table S3). Given that OPZG of NSS populations is expected to be equal for salts with the same toxicity, this indicates that CaCl$_2$ and Na$_2$SO$_4$ are relatively more toxic to the rotifers than NaCl. OPZG of CaCl$_2$ tended to be slightly lower than that of Na$_2$SO$_4$, suggesting that CaCl$_2$ is somewhat more toxic to rotifers than Na$_2$SO$_4$. In the NaCl and CaCl$_2$ treatments, OPZG of SS populations appeared to be 1.35 and 1.77 times higher than that of NSS populations, respectively ($p < 0.001$ for both treatments; Fig. 3). In contrast, in the Na$_2$SO$_4$ treatment, OPZG of SS populations was on average only 1.17 times higher than that of NSS populations ($p = 0.02$; Fig. 3; Supporting Information Table S3).

To evaluate whether the increased ability of SS populations to cope with CaCl$_2$ was due to a higher ability to cope with toxicity of Ca^{2+} ions, we compared the Cl$^-$ concentrations at zero population growth in the CaCl$_2$ with that of the NaCl treatments for both population types (Fig. 4a,c,e; Supporting Information Tables S3–S6). At zero population growth, the Cl$^-$ concentration in the CaCl$_2$ treatment was significantly lower than that in the NaCl treatment for both NSS ($p < 0.001$) and SS populations ($p = 0.002$; Fig. 4e), indicating that Ca^{2+} ions are more toxic than Na$^+$ ions. At zero population growth, the Cl$^-$ concentration in the SS populations was always higher than in the NSS populations ($p < 0.001$ for both treatments; Fig. 4e), indicating that the SS populations were able to better cope with the negative effect of Ca^{2+} than the NSS populations. The reduction of Cl$^-$ at zero population growth in the CaCl$_2$ compared to the NaCl treatment was less pronounced in the SS (12.7%) than in the NSS populations (31.2%, Fig. 4e), indicating that SS populations have lowered relative sensitivity to CaCl$_2$ comparing to NSS populations (Fig. 4c).

Similarly, to evaluate relative toxicity of Cl$^-$ and SO$_4^{2-}$, and whether the slightly increased ability of SS populations to cope with Na$_2$SO$_4$ was due to a higher tolerance to the toxicity of SO$_4^{2-}$ ions, we compared the Na$^+$ concentrations at zero population growth in the Na$_2$SO$_4$ with that in the NaCl treatment (Fig. 4b,d,f; Supporting Information Tables S3–S6). The Na$^+$ concentration at which animals showed zero growth was significantly lower in the Na$_2$SO$_4$ than in the NaCl treatment for both NSS populations ($p = 0.017$) and SS populations ($p < 0.001$; Fig. 4f), suggesting that SO$_4^{2-}$ ions are more toxic than Cl$^-$ ions. In the NaCl treatment, the Na$^+$ concentration at zero population growth was considerably higher in the SS than in the NSS populations. In contrast, we observed no differences in Na$^+$ concentrations at zero growth between SS and

Theoretical Predictions   **Empirical Results**
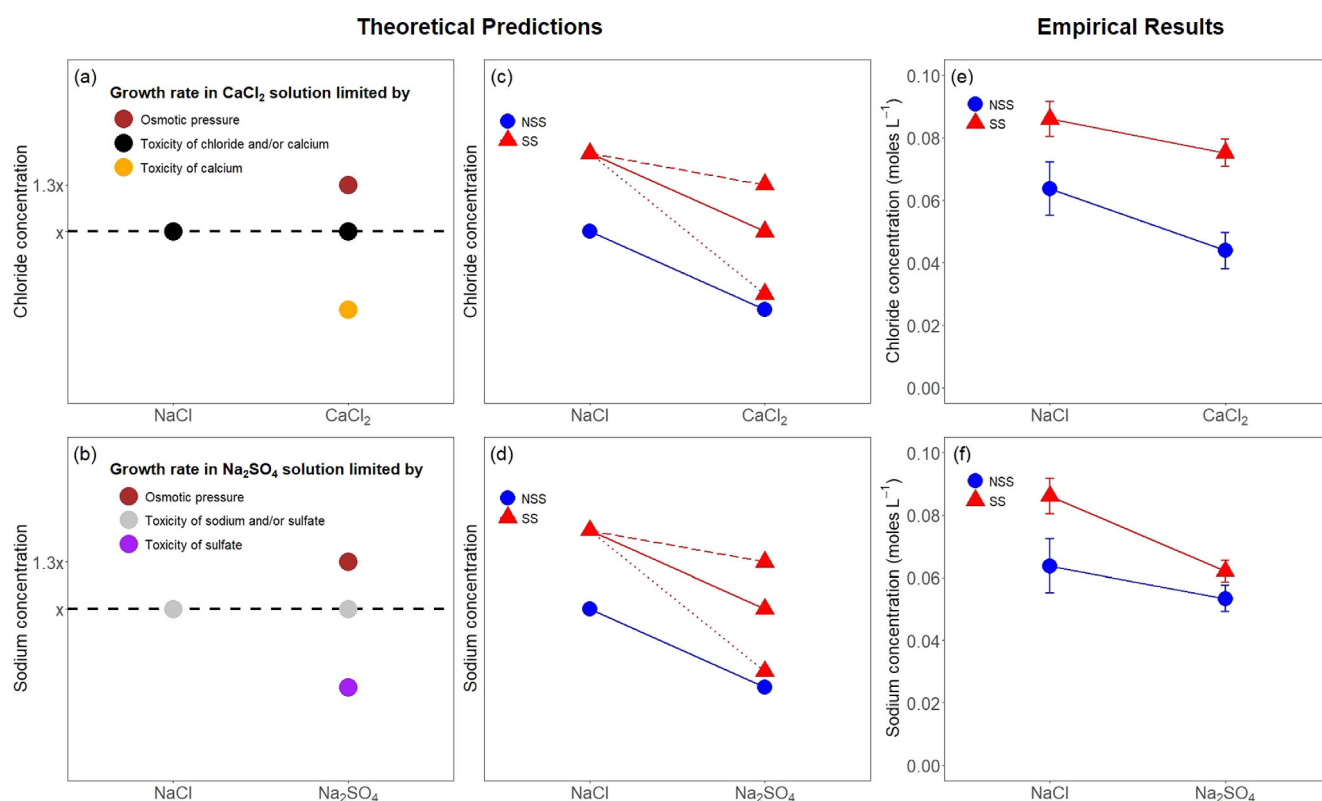

Fig. 4. (**a**) Schematic representation guiding the interpretation of Cl^- concentrations at zero net population growth in the NaCl and $CaCl_2$ treatments. The brown symbol represents the case where all ions have equal toxicity and zero net population growth will be reached at a critical osmotic stress that is equal for both NaCl and $CaCl_2$ treatments. In this scenario, the $CaCl_2$ solution will then contain 1.33 times the Cl^- ion concentration of the NaCl solution given that $CaCl_2$ dissociates into three parts (with two Cl^- ions) and NaCl dissociates into two parts (with one Cl^- ion). As the relative importance of Cl^- and/or calcium toxicity increases, Cl^- concentrations at zero net population growth will be reduced in the $CaCl_2$ compared to the NaCl solution (cf. range between black and brown symbols). Only if the toxicity of Ca^{2+} is stronger than the toxicity of Na^+ will the Cl^- concentrations at zero net population growth in the $CaCl_2$ solution be lower than in the NaCl treatment. (**b**) Inferences regarding the relative toxicity of SO_4^{2-} and Cl^- can be made through a comparison of Na^+ concentrations in the Na_2SO_4 and NaCl treatments following the same reasoning as in (**a**). (**c**) Schematic representation of the possible responses of Cl^- concentration at zero net population growth in a solution of $CaCl_2$ compared to NaCl for rotifer populations with different selection history ("SS" vs. "NSS"), for the case where Ca^{2+} ions are more toxic than Na^+-ions. Identical Cl^- reductions (equal slopes, full lines) indicate equal sensitivity of populations to the relative toxicity of Ca^{2+} and Na^+ ions. A stronger Cl^- reduction for SS (dotted lines) compared to NSS populations indicates a relatively lower tolerance of SS populations to Ca^{2+} toxicity. A weaker Cl^- reduction for SS (dashed lines) compared to NSS populations indicates a relatively higher tolerance of SS populations to Ca^{2+} toxicity. Similar deductions regarding differences in sensitivities between population types regarding the relative toxicity of SO_4^{2-} and Cl^- ions can be made using (**d**). (**e**) Observed Cl^- concentrations in the NaCl and $CaCl_2$ treatments at zero net population growth. (**f**) Observed Na^+ concentrations in the NaCl and Na_2SO_4 treatments at zero net population growth. In (**e**) and (**f**), blue circles and red triangles represent means across replicates of NSS and SS adapted populations, respectively. Error bars represent ± 2 standard errors based on variation across replicates of the different population types.

NSS populations in the Na_2SO_4 treatment ($p > 0.05$; Fig. 4f), indicating that adaptation to NaCl does not allow populations to better cope with toxicity from SO_4^{2-} ions. The reduction of Na^+ at zero population growth in the Na_2SO_4 compared to the NaCl treatment was more pronounced in the SS (27.9%) than in the NSS populations (16.3%; Fig. 4f), indicating that SS populations have higher relative sensitivity to Na_2SO_4 comparing to NSS populations.

Discussion

Our results show that populations of freshwater rotifers are able to rapidly evolve tolerance to considerably enhanced levels of salinization in a very short time span (i.e., three sexual generations and < 60 clonal generations). In CG1, exposure of naïve populations (NSS) to NaCl concentrations of $3\ g\ L^{-1}$ resulted in a fitness reduction of approximately 43% compared to freshwater conditions. In contrast, for populations with a selection history of gradually increased exposure to this salt (SS) we were unable to demonstrate any significant performance reductions. Similarly, in CG2, when exposed to NaCl, OPZG of SS-populations was 34.7% higher than that of NSS populations. These results indicate that within a limited number of sexual generations, SS populations acquired a higher ability to cope with stresses (osmotic stress and ion toxicity) associated with salinization. Our results are

well in line with other studies that have shown rapid evolutionary adaptation of microscopic freshwater metazoans to increased salinity both in experimental evolution approaches (Scheuerl and Stelzer 2013; Coldsnow et al. 2017; Sun and Arnott 2023) and field populations (Wersebe and Weider 2023). Although all these studies reveal a great adaptive potential of these organisms when exposed to one specific salt (mostly NaCl), the question how such adaptation affects the ability of organisms to cope with toxic stress from other salts has largely remained unaddressed.

The results of CG2 demonstrate that the ion composition of a salt is clearly an important determinant of its toxicity (Mount et al. 1997; Mount 2016). $CaCl_2$ and Na_2SO_4 were more toxic to the investigated rotifers than NaCl given the lower values for OPZG observed in these treatments. The higher toxicity of $CaCl_2$ can only be explained by the presence of Ca^{2+}, given that the concentration of Cl^- ions at zero population growth was larger in the NaCl compared to the $CaCl_2$ treatment (0.064 and 0.044 mol L^{-1} for NSS populations, respectively). Similarly, the higher toxicity of Na_2SO_4 compared to NaCl was due to the SO_4^{2-}, given that the concentration of Na^+ ions at zero population growth was larger in the NaCl than Na_2SO_4 treatment (0.064 and 0.053 mol L^{-1} for NSS populations, respectively). Furthermore, we found strong evidence for evolved cross tolerance given that populations adapted to NaCl had a higher OPZG when exposed to $CaCl_2$ or Na_2SO_4 than populations without a history of exposure to NaCl. Remarkably, the degree of cross-tolerance depended strongly on the chemical nature of the salt. Whereas adaptation to NaCl resulted in a strong degree of cross tolerance to $CaCl_2$ (i.e., almost a doubling of the OPZG of SS compared to NSS populations in $CaCl_2$), the relative increase in the OPZG upon exposure to Na_2SO_4 was much smaller. Consequently, the impact of evolved cross tolerance was found to be largely dependent on the chemical identity of a salt's ions.

Our study is unique because it also demonstrates that rapid adaptation to the ionic composition of one salt results in cross-tolerance to the toxicity of other ions. More specifically, adaptation to NaCl resulted in cross-tolerance to $CaCl_2$ not only due to an increased tolerance to osmotic stress or Cl^- ion toxicity but also to Ca^{2+} ion toxicity. This can be derived from a closer inspection of the Cl^- concentrations observed at zero population growth in CG2 (Fig. 4a,c,e). Compared to Na^+, Ca^{2+} toxicity strongly reduced population performance given that $CaCl_2$ resulted in OPZG-values and Cl^- concentrations lower than in the NaCl treatment, both for adapted as well as non-adapted populations. Similar to what was observed in the NaCl treatment, adaptation to NaCl allowed populations to realize zero growth at higher osmotic pressure and Cl^- concentrations in the $CaCl_2$ treatment than populations without a history of exposure to NaCl. Although this increased coping ability may partly reflect a higher tolerance to osmotic stress and Cl^- toxicity, it must have involved a higher ability to

cope with Ca^{2+} toxicity as well, given the weaker response slope of Cl^- at zero growth for SS compared to NSS populations.

Our results also show that the scope of evolved cross-tolerance to ion toxicity is limited and may only apply to one or a confined subset of salt ions. Indeed, adaptation to NaCl incurred cross tolerance to Ca^{2+} toxicity, but it provided no tolerance against toxicity by SO_4^{2-}. Interestingly, the strong difference in the degree to which adaptation to NaCl enabled populations to cope with different ions resulted in a reversal of the relative sensitivity of populations to exposure to different salts. For populations with no history of exposure to NaCl, $CaCl_2$ was more toxic than Na_2SO_4 (Fig. 3). In contrast, populations adapted to NaCl were more sensitive to Na_2SO_4 than to $CaCl_2$. An important implication of this finding is that differences in the recent evolutionary history of populations of a given species can determine their capacity to tolerate different salts. Although monitoring programs and legislation still focus mainly on the total concentration of salts or Cl^- ions, the need for the consideration of specific ion toxicities is increasingly recognized (Cañedo-Argüelles et al. 2016) and research efforts directed at evaluating the toxicity of different salts are increasing (Evans and Frick 2001; Mount 2016). Our results indicate that such efforts would strongly benefit from also considering the evolutionary history of the populations under study and more explicitly the identity of the ions populations have been exposed to in the past (Brady et al. 2017).

Our observation of cross tolerance of NaCl adapted populations to $CaCl_2$ confirms the findings of Hintz et al. (2019), who showed that outdoor mesocosm populations of the water flea *Daphnia pulex* adapted to NaCl have increased tolerance to $CaCl_2$. Our laboratory study, however, also shows that cross tolerance strongly depends on the chemical composition of the secondary salt, as we found much lower increase in the tolerance of NaCl adapted populations to Na_2SO_4. Furthermore, the unique design of our second garden experiment (CG2) provides deeper mechanistic insights because it allows evaluating the role of the toxicity of specific ions (i.e., Ca^{2+} and SO_4^{2-}) that were not part of the salt to which populations had originally adapted to (i.e., Na^+ and Cl^-).

Although the physiological or molecular basis of cross tolerance mechanisms is beyond the scope of this study, the question why adaptation to NaCl incurs a better tolerance to Ca^{2+} toxicity and not SO_4^{2-} is nevertheless interesting. Mechanisms underlying the evolution of tolerance to osmotic and ionic stress in freshwater organisms have been shown to involve genes associated with osmoregulation and ATPase activity (Kefford et al. 2016). For example, Latta et al. (2012) observed that adaptation to NaCl in the zooplankton species *Daphnia pulex* resulted in a constitutive upregulation of genes involved with osmoregulation and Na^+, K^+-ATPase activity (responsible for Na^+ transport). The evolution of cross-tolerance to Ca^{2+} toxicity as observed in our study suggests that adaptation to

NaCl also involves the Ca^{2+} metabolism. Indeed, acclimation and adaptation to salinity are known to involve Ca^{2+} regulation (Rengmark et al. 2007; Munns and Tester 2008). In eukaryotes, including aquatic animals, Ca^{2+}-ATPase activity has been found to increase upon exposure to high salinity (e.g., Shephard 1981; Rengmark et al. 2007; Wang et al. 2013). Because Ca^{2+} serves as an intracellular signaler, exposure to salinity stress typically results in a rapid accumulation of cytosolic Ca (Knight et al. 1997). To maintain intracellular Ca^{2+} homeostasis (Bose et al. 2011; Larsen et al. 2014), excess Ca^{2+}-ions need to be removed via Ca^{2+} transport proteins, such as Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger (aquatic animals). Possibly, evolutionary responses to selection by NaCl-induced salt stress also involved changes in the expression of genes responsible for intracellular Ca^{2+} transport. In contrast, in our experiment adaptation to NaCl failed to provide rotifers with cross-tolerance to Na_2SO_4. Although SO_4^{2-} is an important ion for normal cellular function (Markovich and Aronson 2007), it becomes toxic at high concentrations (Griffith 2017). In freshwater animals, SO_4^{2-} uptake and efflux are both mediated by SO_4^{2-} transporters (Griffith 2017). SO_4^{2-} regulation is generally accompanied with transport of Na^+ or Cl^- (Markovich and Aronson 2007; Markovich et al. 2008; Kato et al. 2009) but there are no indications that Na^+ and Cl^- stress regulation shares the same pathways with SO_4^{2-} regulation. This may explain why we observed no increased ability of NaCl-adapted populations to cope with increased SO_4^{2-} concentrations.

We found no evidence for costs associated with adaptation to NaCl, given that the fitness of salt adapted populations in the non-salinity treatment did not differ significantly from the fitness of the ancestral populations and the populations that evolved in the non-salinity treatment. Similar observations have been made for salinity exposed *Daphnia* (Weider and Hebert 1987; Latta et al. 2012; Sun and Arnott 2023), salinity exposed salamanders (Brady 2012), and rotifer populations subjected to poor food quality in an experimental evolution study (Declerck et al. 2015). The absence of such trade-offs suggests that populations may be able to maintain the acquired ability to cope with salt stress in the long run. It should be noted, however, that we only tested for such trade off under relatively benign conditions (e.g., plenty of good quality food, absence of other stressors) and that potential tradeoffs could still become apparent when populations would be compared under the influence of one or more other stressors (Tikhonova et al. 2020; Orr et al. 2022*a*).

The ability of rotifer populations to rapidly adapt to salinization and associated cross-tolerance may have various ecological implications. Through rapid adaptation populations may prevent local extirpation in the face of increased salt stress through evolutionary rescue (Gonzalez and Bell 2013). Adaptation may also result in a reduction of sublethal effects, allowing a higher population performance than in the absence of micro-evolution. In systems isolated from saline systems where dispersal limitation hampers colonization by halophile species, adaption may secure important ecosystem functions and as such stabilize trophic interactions and food web structure (Hendry 2017). Local adaptation may also contribute to an increased resistance of freshwater communities to invasion by more halophile species, reducing the rate at which freshwater species are replaced by brackish water species (for an example of adaptation to elevated temperatures, *see* Van Doorslaer 2009).

Evolved cross-tolerance may play an important role in contributing to species persistence and community stability especially in situations where the identity of dominant salts is altered through time due to changes in land use, pollution from mining and industry or deicing practices, and so on. For example, although Ca^{2+} and Mg^{2+} ions are generally considered more toxic to aquatic organisms than Na^+ ions (Evans and Frick 2001; Coldsnow and Relyea 2018), $CaCl_2$ and $MgCl_2$ become increasingly popular alternatives to NaCl as deicing salts (Schuler et al. 2017; Coldsnow and Relyea 2018). Our observation that adaptation to NaCl results in a strong evolved cross-tolerance to Ca^{2+} toxicity suggests the potential ability of natural populations to evolve better abilities to cope with alternative deicing salts after having been exposed to NaCl before. Evolution of cross tolerance may also contribute to the persistence of metapopulations (Urban 2020). Irrespective of its cause, most types of salinization affect multiple populations across landscapes (Herbert et al. 2015; Cunillera-Montcusí et al. 2022). The evolution of increased tolerance to salts in local populations may foster colonization of vacant salinized patches by pre-adapted genotypes, increasing the regional occupancy of a species and hence reduce its risk of becoming regionally extinct especially when salinization gradually progresses across the entire landscape. If the chemical composition of salts shows spatial variation, cross-tolerance may contribute to the colonization of patches salinized by a wider range of salts than genotypes have locally adapted to. To summarize, the identity of dominant salts may vary through space and time. Evolved cross-tolerance may expand the temporal and spatial dimensions under which the ecological consequences of salt adaptation apply.

Conclusions

Our study demonstrates a remarkable ability of planktonic freshwater rotifers to show rapid evolutionary adaptation to increased salt stress. Our study provides the novel insight that adaptation to one salt (i.e., NaCl) may induce cross tolerance to toxicity of other ions (i.e., Ca^{2+}, but not SO_4^{2-}). As a result, adaptation to one salt (e.g., NaCl) may reduce the negative impact of stress caused by other salts (i.e., $CaCl_2$ and Na_2SO_4). Our insights have important implications regarding the interpretation of responses of natural populations to salinization and highlight the importance of salt ion composition and prior selection regime.

Data availability statement

The data that support the findings of this study are openly available in Figshare at http://doi.org/10.6084/m9.figshare.24197490.

References

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**: 1–48.

Bell, G. 2017. Evolutionary rescue. Annu. Rev. Ecol. Evol. Syst. **48**: 605–627.

Bitter, M. C., L. Kapsenberg, J.-P. Gattuso, and C. A. Pfister. 2019. Standing genetic variation fuels rapid adaptation to ocean acidification. Nat. Commun. **10**: 5821.

Blinn, D. W. 1993. Diatom community structure along physicochemical gradients in saline lakes. Ecology **74**: 1246–1263.

Bos, D. G., B. F. Cumming, C. E. Watters, and J. P. Smol. 1996. The relationship between zooplankton, conductivity and lake-water ionic composition in 111 lakes from the Interior Plateau of British Columbia, Canada. Int. J. Salt Lake Res. **5**: 1–15.

Bose, J., I. I. Pottosin, S. S. Shabala, M. G. Palmgren, and S. Shabala. 2011. Calcium efflux systems in stress signaling and adaptation in plants. Front. Plant Sci. **2**: 85.

Brady, S. P. 2012. Road to evolution? Local adaptation to road adjacency in an amphibian (*Ambystoma maculatum*). Sci. Rep. **2**: 235.

Brady, S. P., J. L. Richardson, and B. K. Kunz. 2017. Incorporating evolutionary insights to improve ecotoxicology for freshwater species. Evol. Appl. **10**: 829–838.

Cañedo-Argüelles, M. 2020. A review of recent advances and future challenges in freshwater salinization. Limnetica **39**: 185–211.

Cañedo-Argüelles, M., B. J. Kefford, C. Piscart, N. Prat, R. B. Schäfer, and C.-J. Schulz. 2013. Salinisation of rivers: An urgent ecological issue. Environ. Pollut. **173**: 157–167.

Cañedo-Argüelles, M., and others. 2016. Saving freshwater from salts. Science **351**: 914–916.

Castillo, A. M., D. M. T. Sharpe, C. K. Ghalambor, and L. F. De León. 2018. Exploring the effects of salinization on trophic diversity in freshwater ecosystems: A quantitative review. Hydrobiologia **807**: 1–17.

Coldsnow, K. D., B. M. Mattes, W. D. Hintz, and R. A. Relyea. 2017. Rapid evolution of tolerance to road salt in zooplankton. Environ. Pollut. **222**: 367–373.

Coldsnow, K. D., and R. A. Relyea. 2018. Toxicity of various road-deicing salts to Asian clams (*Corbicula fluminea*). Environ. Toxicol. Chem. **37**: 1839–1845.

Crosa, G., J. Froebrich, V. Nikolayenko, F. Stefani, P. Galli, and D. Calamari. 2006. Spatial and seasonal variations in the water quality of the Amu Darya River (Central Asia). Water Res. **40**: 2237–2245.

Cunillera-Montcusí, D., and others. 2022. Freshwater salinisation: A research agenda for a saltier world. Trends Ecol. Evol. **37**: 440–453.

Dam, H. G. 2013. Evolutionary adaptation of marine zooplankton to global change. Ann. Rev. Mar. Sci. **5**: 349–370.

Declerck, S. A. J., A. R. Malo, S. Diehl, D. Waasdorp, K. D. Lemmen, K. Proios, and S. Papakostas. 2015. Rapid adaptation of herbivore consumers to nutrient limitation: Eco-evolutionary feedbacks to population demography and resource control. Ecol. Lett. **18**: 553–562.

Dugan, H. A., and others. 2017. Salting our freshwater lakes. Proc. Natl. Acad. Sci. U.S.A. **114**: 4453–4458.

Evans, M., and C. Frick. 2001. The effects of road salts on aquatic ecosystems. Environment Canada-Water Science and Technology Directorate.

Gonzalez, A., and G. Bell. 2013. Evolutionary rescue and adaptation to abrupt environmental change depends upon the history of stress. Philos. Trans. R. Soc. B **368**: 20120079.

Griffith, M. B. 2017. Toxicological perspective on the osmoregulation and ionoregulation physiology of major ions by freshwater animals: Teleost fish, crustacea, aquatic insects, and mollusca. Environ. Toxicol. Chem. **36**: 576–600.

Hébert, M. P., and others. 2023. Lake salinization drives consistent losses of zooplankton abundance and diversity across coordinated mesocosm experiments. Limnol. Oceanogr. Lett. **8**: 19–29.

Hendry, A. P. 2017. Eco-evolutionary dynamics. Princeton Univ. Press.

Herbert, E. R., and others. 2015. A global perspective on wetland salinization: Ecological consequences of a growing threat to freshwater wetlands. Ecosphere **6**: art206.

Hintz, W. D., D. K. Jones, and R. A. Relyea. 2019. Evolved tolerance to freshwater salinization in zooplankton: Life-history trade-offs, cross-tolerance and reducing cascading effects. Philos. Trans. R. Soc. B **374**: 20180012.

Hintz, W. D., and others. 2022. Current water quality guidelines across North America and Europe do not protect lakes from salinization. Proc. Natl. Acad. Sci. U.S.A. **119**: e2115033119.

Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. Nature **470**: 479–485.

Jackson, M. C., S. Pawar, and G. Woodward. 2021. The temporal dynamics of multiple stressor effects: From individuals to ecosystems. Trends Ecol. Evol. **36**: 402–410.

Janáček, K., and K. Sigler. 1996. Osmotic pressure: Thermodynamic basis and units of measurement. Folia Microbiol. **41**: 2–9.

Kato, A., and others. 2009. Identification of renal transporters involved in sulfate excretion in marine teleost fish. Am. J. Physiol. Regul. Integr. Comp. Physiol. **297**: R1647–R1659.

Kaushal, S. S., P. M. Groffman, G. E. Likens, K. T. Belt, W. P. Stack, V. R. Kelly, E. B. Lawrence, and G. T. Fisher. 2005. Increased salinization of fresh water in the northeastern

United States. Proc. Natl. Acad. Sci. U.S.A. **102**: 13517–13520.

Kaushal, S. S., and others. 2021. Freshwater salinization syndrome: From emerging global problem to managing risks. Biogeochemistry **154**: 255–292.

Kefford, B. J., D. Buchwalter, M. Cañedo-Argüelles, J. Davis, R. P. Duncan, A. Hoffmann, and R. Thompson. 2016. Salinized rivers: Degraded systems or new habitats for salt-tolerant faunas? Biol. Lett. **12**: 20151072.

Kennedy, A. J., D. S. Cherry, and R. J. Currie. 2003. Field and laboratory assessment of a coal-processing effluent in the Leading Creek Watershed, Meigs Co., Ohio. Arch. Environ. Contam. Toxicol. **44**: 324–331.

Kerr, J. G. 2017. Multiple land use activities drive riverine salinization in a large, semi-arid river basin in western Canada. Limnol. Oceanogr. **62**: 1331–1345.

Kinsman-Costello, L., and others. 2023. Mud in the city: Effects of freshwater salinization on inland urban wetland nitrogen and phosphorus availability and export. Limnol. Oceanogr. Lett. **8**: 112–130.

Knight, H., A. J. Trewavas, and M. R. Knight. 1997. Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. Plant J. **12**: 1067–1078.

Laceby, J. P., J. G. Kerr, D. Zhu, C. Chung, Q. Situ, S. Abbasi, and J. F. Orwin. 2019. Chloride inputs to the North Saskatchewan River watershed: The role of road salts as a potential driver of salinization downstream of North America's northern most major city (Edmonton, Canada). Sci. Total Environ. **688**: 1056–1068.

Larsen, E. H., L. E. Deaton, H. Onken, M. O'Donnell, M. Grosell, W. H. Dantzler, and D. Weihrauch. 2014. Osmoregulation and excretion. Compr. Physiol. **4**: 405–573.

Latta, L. C., L. J. Weider, J. K. Colbourne, and M. E. Pfrender. 2012. The evolution of salinity tolerance in *Daphnia*: A functional genomics approach. Ecol. Lett. **15**: 794–802.

Leppänen, J. J., T. P. Luoto, and J. Weckström. 2019. Spatio-temporal impact of salinated mine water on Lake Jormasjärvi, Finland. Environ. Pollut. **247**: 1078–1088.

Markovich, D., and P. S. Aronson. 2007. Specificity and regulation of renal sulfate transporters. Annu. Rev. Physiol. **69**: 361–375.

Markovich, D., A. Romano, C. Storelli, and T. Verri. 2008. Functional and structural characterization of the zebrafish Na$^+$-sulfate cotransporter 1 (NaS1) cDNA and gene (slc13a1). Physiol. Genomics **34**: 256–264.

Michaloudi, E., S. Papakostas, G. Stamou, V. Neděla, E. Tihlaříková, W. Zhang, and S. A. J. Declerck. 2018. Reverse taxonomy applied to the *Brachionus calyciflorus* cryptic species complex: Morphometric analysis confirms species delimitations revealed by molecular phylogenetic analysis and allows the (re)description of four species. PLoS One **13**: e0203168.

Mo, Y., and others. 2021. Low shifts in salinity determined assembly processes and network stability of microeukaryotic plankton communities in a subtropical urban reservoir. Microbiome **9**: 128.

Mount, D. R., D. D. Gulley, J. R. Hockett, T. D. Garrison, and J. M. Evans. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). Environ. Toxicol. Chem. **16**: 2009–2019.

Mount, D. R., and others. 2016. The acute toxicity of major ion salts to *Ceriodaphnia dubia*: I. Influence of background water chemistry. Environ. Toxicol. Chem. **35**: 3039–3057.

Munns, R., and M. Tester. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. **59**: 651–681.

Orr, J. A., and others. 2020. Towards a unified study of multiple stressors: Divisions and common goals across research disciplines. Proc. R. Soc. B Biol. Sci. **287**: 20200421.

Orr, J. A., P. Luijckx, J. Arnoldi, A. L. Jackson, and J. J. Piggott. 2022*a*. Rapid evolution generates synergism between multiple stressors: Linking theory and an evolution experiment. Glob. Chang. Biol. **28**: 1740–1752.

Orr, J. A., M. C. Rillig, and M. C. Jackson. 2022*b*. Similarity of anthropogenic stressors is multifaceted and scale dependent. Nat. Sci. **2**: e20210076.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Rengmark, A. H., A. Slettan, W. J. Lee, Ø. Lie, and F. Lingaas. 2007. Identification and mapping of genes associated with salt tolerance in tilapia. J. Fish Biol. **71**: 409–422.

Rhodes, A. L., and A. J. Guswa. 2016. Storage and release of road-salt contamination from a calcareous lake-basin fen, western Massachusetts, USA. Sci. Total Environ. **545–546**: 525–545.

Scheuerl, T., and C.-P. Stelzer. 2013. Patterns and dynamics of rapid local adaptation and sex in varying habitat types in rotifers. Ecol. Evol. **3**: 4253–4264.

Schuler, M. S., W. D. Hintz, D. K. Jones, L. A. Lind, B. M. Mattes, A. B. Stoler, K. A. Sudol, and R. A. Relyea. 2017. How common road salts and organic additives alter freshwater food webs: In search of safer alternatives. J. Appl. Ecol. **54**: 1353–1361.

Shephard, K. L. 1981. The activity and characteristics of the Ca^{2+} ATPase of fish gills in relation to environmental calcium concentrations. J. Exp. Biol. **90**: 115–121.

Sun, X., and S. E. Arnott. 2023. Evolved tolerance to NaCl does not alter *Daphnia* response to acute heat stress. Evol. Ecol. **37**: 345–361.

Thorslund, J., M. F. P. Bierkens, G. H. P. Oude Essink, E. H. Sutanudjaja, and M. T. H. van Vliet. 2021. Common irrigation drivers of freshwater salinisation in river basins worldwide. Nat. Commun. **12**: 4232.

Tikhonova, M., S. Kachru, and D. S. Fisher. 2020. A model for the interplay between plastic tradeoffs and evolution in changing environments. Proc. Natl. Acad. Sci. U.S.A. **117**: 8934–8940.

Urban, M. C., and others. 2020. Evolutionary origins for ecological patterns in space. Proc. Natl. Acad. Sci. U.S.A. **117**: 17482–17490.

Van de Maele, M., L. Janssens, and R. Stoks. 2021. Evolution of tolerance to chlorpyrifos causes cross-tolerance to another organophosphate and a carbamate, but reduces tolerance to a neonicotinoid and a pharmaceutical. Aquat. Toxicol. **240**: 105980.

Van Doorslaer, W., and others. 2009. Local adaptation to higher temperatures reduces immigration success of genotypes from a warmer region in the water flea *Daphnia*. Glob. Change Biol. **15**: 3046–3055.

Wang, Y., P. Luo, L. Zhang, C. Hu, C. Ren, and J. Xia. 2013. Cloning of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) gene from white shrimp, *Litopenaeus vannameian* and its expression level analysis under salinity stress. Mol. Biol. Rep. **40**: 6213–6221.

Weider, L. J., and P. D. N. Hebert. 1987. Ecological and physiological differentiation among low-artic clones of *Daphnia Pulex*. Ecology **68**: 188–198.

Wersebe, M. J., and L. J. Weider. 2023. Resurrection genomics provides molecular and phenotypic evidence of rapid adaptation to salinization in a keystone aquatic species. Proc. Natl. Acad. Sci. U.S.A. **120**: e2217276120.

Xu, D., and others. 2021. Acclimation and adaptation to elevated pCO_2 increase arsenic resilience in marine diatoms. ISME J. **15**: 1599–1613.

Zhou, L., and S. Wang. 2023. The bright side of ecological stressors. Trends Ecol. Evol. **38**: 568–578.

Acknowledgments

Conflict of Interest

None declared.