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SPECIAL ISSUE-LETTER

Widespread variation in salt tolerance within freshwater zooplankton species reduces the predictability of community-level salt tolerance

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Author Contribution Statement: SEA, VF, SJM, CCS, and AMD conceived the ideas for this study, and MCA, BEB, and MPH contributed to initial discussions. SEA, CCS, and AMD compiled LC₅₀s for Cl⁻ tolerance from published laboratory studies. VF performed analyses and made figures, with help from SJM and CCS. SEA, VF, SJM, CCS, and AMD interpreted results. SEA, VF, SM, CCS, and AMD all contributed to the writing. SEA, JAB, MC-A, ALD, AMD, DKG, DG, WDH, AM, SJM, RAR, JAR, CCS, and CLS contributed to data compilation and initial conceptualization of a community-LC₅₀ metric. As part of the Global Salt Initiative led by SEA and WDH, all authors contributed to coordinated experiments in the form of experimental set-up, field or laboratory work, data preparation, or financial support, and all authors critically reviewed and edited the manuscript.

Data Availability Statement: Data are available in borealis—the Canadian Dataverse Repository at <https://doi.org/10.5683/SP3/BIDMCI>

Shelley E. Arnott, Vincent Fugère, Celia C. Symons, Stephanie J. Melles, and Alison M. Derry made equal contributions in leading the manuscript.

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

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Scientific Significance Statement

Not all populations of a species have the same sensitivity to contaminants, yet no studies have assessed this variation for multiple species across large geographic regions. Our study is the first to quantify within-species variation in salt tolerance (chloride, Cl^-) by conducting experiments at 16 environmentally diverse locations and compiling published tolerances from laboratory studies. Across our study sites, we found that Cl^- sensitivity varied up to $4.2x \pm 3.0$ SD within species. This variation was related to the species they co-existed with, suggesting that species interactions can modulate Cl^- tolerance, making it difficult to predict how individual communities respond to Cl^- increases. To adequately protect freshwater zooplankton from harm, water quality guidelines should be based on multiple populations and communities to incorporate variation in sensitivity.

Abstract

The salinization of freshwaters is a global threat to aquatic biodiversity. We quantified variation in chloride (Cl^-) tolerance of 19 freshwater zooplankton species in four countries to answer three questions: (1) How much variation in Cl^- tolerance is present among populations? (2) What factors predict intraspecific variation in Cl^- tolerance? (3) Must we account for intraspecific variation to accurately predict community Cl^- tolerance? We conducted field mesocosm experiments at 16 sites and compiled acute LC_{50} s from published laboratory studies. We found high variation in LC_{50} s for Cl^- tolerance in multiple species, which, in the experiment, was only explained by zooplankton community composition. Variation in species- LC_{50} was high enough that at 45% of lakes, community response was not predictable based on species tolerances measured at other sites. This suggests that water quality guidelines should be based on multiple populations and communities to account for large intraspecific variation in Cl^- tolerance.

Agriculture, mining, and road-deicing practices, as well as climate change, resulting in sea level rise and drought, are causing the salinization of freshwater ecosystems (Hebert et al. 2015; Dugan et al. 2017; Castillo et al. 2018; Kaushal et al. 2018; Thorslund et al. 2021), leading to severe impacts on aquatic communities and food webs (Hintz and Relyea 2017; Astorg et al. 2022; Hebert et al. 2022). Yet, there remain significant knowledge gaps about how salinization is changing the biodiversity of freshwaters. Studies of lake salinization have almost entirely focused on species- or community-level responses of phytoplankton (Ballot et al. 2009; Fay and Shi 2012; Porter-Goff et al. 2013) or zooplankton (Hintz et al. 2017; Hintz and Relyea 2019; Arnott et al. 2020; Moffett et al. 2020). However, responses to freshwater salinization may vary substantially among regions (Jeppesen et al. 2007; Moffett et al. 2020; Greco et al. 2021). Intraspecific variation is often overlooked in community ecology (Violle et al. 2012), despite evidence that trait variation within species can have equal or greater effects than between-species effects on community and ecosystem processes (Des Roches et al. 2018; Rafford et al. 2019). Intraspecific

variation at local sites can influence species-level tolerance to salinity (Weider and Hebert 1987; Loureiro et al. 2012; Venâncio et al. 2018), but studies have not examined intraspecific variation in salt tolerance across broad spatial scales.

Most studies investigating intraspecific variation in freshwater zooplankton tolerance to salinity were performed in laboratories and were focused on individual- or population-level responses of cladoceran or rotifer species, often at lethal concentrations of chloride (Cl^-), a common tracer of salinity (Loureiro et al. 2012; Coldsnow et al. 2017; Venâncio et al. 2018; Adamczuk and Mieczan 2019). These studies have shown that genetic adaptation (Coldsnow et al. 2017), phenotypic plasticity (Adamczuk and Mieczan 2019), epigenetics (Jeremias et al. 2018), and maternal effects (Venâncio et al. 2018) can be important adaptive strategies for freshwater zooplankton in response to salinization. Moreover, among natural populations of zooplankton along salinity gradients, there is evidence for genetically based adaptation in salinity tolerance in Daphniidae (Weider and Hebert 1987; Teschner 1995; Loureiro et al. 2012; Liao et al. 2015). Variation in Cl^- sensitivity can also be attributed

to environmental context; laboratory studies have demonstrated that food quantity (Brown and Yan 2015), food quality (Isanta-Navarro et al. 2021), and ion composition (Elphick et al. 2011) alter individual response to Cl⁻. Thus, phenotypic variation within species could result from genetic variation, environmental effects, or their interaction (Agrawal 2001; Barrett and Schluter 2008).

Intraspecific variation can constrain our ability to predict community-level responses—especially if it occurs in multiple species—and it is unclear if information derived from single species toxicity tests can inform responses in populations, communities, or ecosystems (Forbes and Galic 2016; Galic et al. 2018, but see Venâncio et al. 2022). This has implications for management, as failure to account for intraspecific variation in the development of water quality guidelines may not protect sensitive populations and communities that are not commonly studied in toxicity tests (most toxicity tests are conducted using *Daphnia* and *Ceriodaphnia*). Despite this, no studies have assessed intraspecific variation of salt tolerance of multiple species under diverse environmental contexts that are found across continents.

Our study provides the first experimental investigation of intraspecific variation in Cl⁻ tolerance of zooplankton from regions spanning wide environmental and geographic gradients. We considered responses to NaCl additions because in the Great Lakes region of North America, and many other places that experience cold winters, salinity of freshwaters is increasing due to the use of NaCl for road deicing (Dugan et al. 2017; Kaushal et al. 2018; Sorichetti et al. 2022). We addressed three questions: (Q1) How much intraspecific variation in salt tolerance is present among freshwater zooplankton species in (a) acute laboratory tests and (b) natural communities in mesocosms? (Q2) What factors predict the magnitude of intraspecific variation in salt tolerance among species? (Q3) Do we need to account for intraspecific variation to accurately predict community-wide salt tolerance?

Methods

We quantified intraspecific variation in zooplankton Cl⁻ tolerance using two approaches: (a) a compilation of acute LC₅₀s from published laboratory studies (to answer Q1a), and (b) coordinated field mesocosm experiments among 16 sites in North America and Europe (to answer Q1b, Q2, and Q3).

Literature synthesis

In our literature review of acute toxicity to Cl⁻ for freshwater zooplankton, we considered laboratory experiments that estimated the lethal concentration of Cl⁻ that resulted in 50% mortality (LC₅₀). Our assessment of intraspecific variation is relevant for policy and management because this type of assay was used to establish Canadian and US water quality guidelines for Cl⁻ (USEPA 1988; CCME 2011). The Google Scholar database was searched between

30 November 2020 and 02 December 2020, using the terms: [taxon name] AND salinity OR sodium chloride OR NaCl OR salt OR road salt AND LC₅₀ OR toxicity OR experiment OR assay OR bioassay. Taxon search terms were: *Daphnia*, *Ceriodaphnia*, *Brachionus*, *Bosmina*, calanoid, copepod, crustacean, cyclopoid, *Cyclops*, *Eudiaptomus*, rotifer, zooplankton. To standardize information across studies, we only included laboratory experiments where NaCl was manipulated across at least five concentrations and assays were run for 24 h (*Brachionus*) or 48 h (all other species). Overall, 84 individual assays from 19 studies fit our criteria (Fig. 1; Supporting Information Table S1). We compiled LC₅₀ and 95% confidence intervals for each species, and classified whether the variation in Cl⁻ LC₅₀s occurred across studies (genetic and environmental sources of variation), across clones of a given species assayed in the same environment (genetic), across the same clone cultured in different media or environments (environmental), or across experimental replicates when the clone and the culture environment were identical (nongenetic or error).

Coordinated regional mesocosm experiments

Sixteen coordinated mesocosm experiments were conducted for ~ 6 weeks between 22 June 2018 and 20 December 2018 at sites in Canada, the United States, Sweden, and Spain (Supporting Information Fig. S1) following standardized experimental methodology. Detailed descriptions of study sites and field and laboratory methodology are provided in Hintz et al.

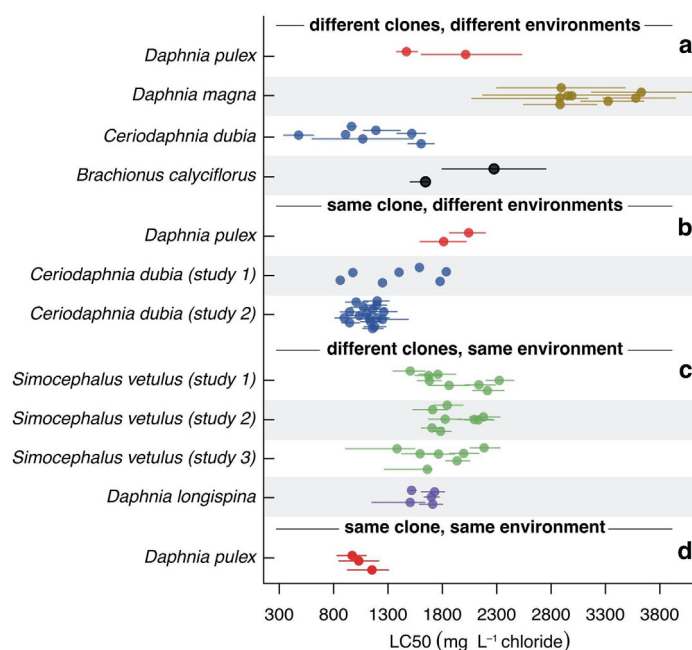


Fig. 1. Literature synthesis of freshwater zooplankton LC₅₀ to Cl⁻ as NaCl. Horizontal lines indicate 95% confidence intervals of reported Cl⁻ LC₅₀ estimates.

(2022) and Arnott et al. (2021). Briefly, at each of the 16 sites, in-lake (4) or on-land mesocosms (12) with volumes that ranged from 80 to 2500 liters (mean 643.6 liters) were filled with lake, stream, or well water and inoculated with local zooplankton and phytoplankton communities without a history of salinization. We applied 20–32 Cl^- treatments using a regression design where nominal concentrations ranged from ambient to 1500 $\text{mg Cl}^- \text{L}^{-1}$ (actual concentrations ranged from 0.21 to 2.543 $\text{mg Cl}^- \text{L}^{-1}$), focusing on low concentrations near water quality guidelines, but also including realistic high values (Supporting Information Fig. S1). To compensate for losses of nutrients resulting from sedimentation and periphyton growth (Downing et al. 2008), at 13 sites we added phosphorus (KH_2PO_4) and nitrogen (NH_4NO_3) every 2 weeks (70% of initial concentrations). Following 41–48 d (43.4 ± 2.8 d) of exposure, we sampled zooplankton communities (1.2–15% of mesocosm volume) and analyzed abundances. See Supporting Information Table S2 for additional information.

Data analyses

Nineteen species were found at more than one site across our mesocosm experiment. For each species and site combination (“populations”), we calculated a site- and species-specific population- LC_{50} . Then, for each of the 19 species, we calculated a species-level LC_{50} based on a weighted mean of the population- LC_{50} s (Fig. 2). The site-specific salt tolerance (population- LC_{50} s; Fig. 2a) and R^2 were calculated by modeling log (density + 1) estimates using generalized additive models (GAMs) with Cl^- as the predictor. Cl^- was the mean of Weeks 0 and 6 concentrations to account for changes associated with rainfall or evaporation. The LC_{50} was the Cl^- concentration where a 50% reduction in abundance occurred relative to the modeled abundance at the lowest Cl^- concentration. A population- LC_{50} was only calculated if the species was detected in at least five mesocosms at that site. To account for uncertainty associated with population- LC_{50} s at each site, species-level LC_{50} was estimated as the mean of all

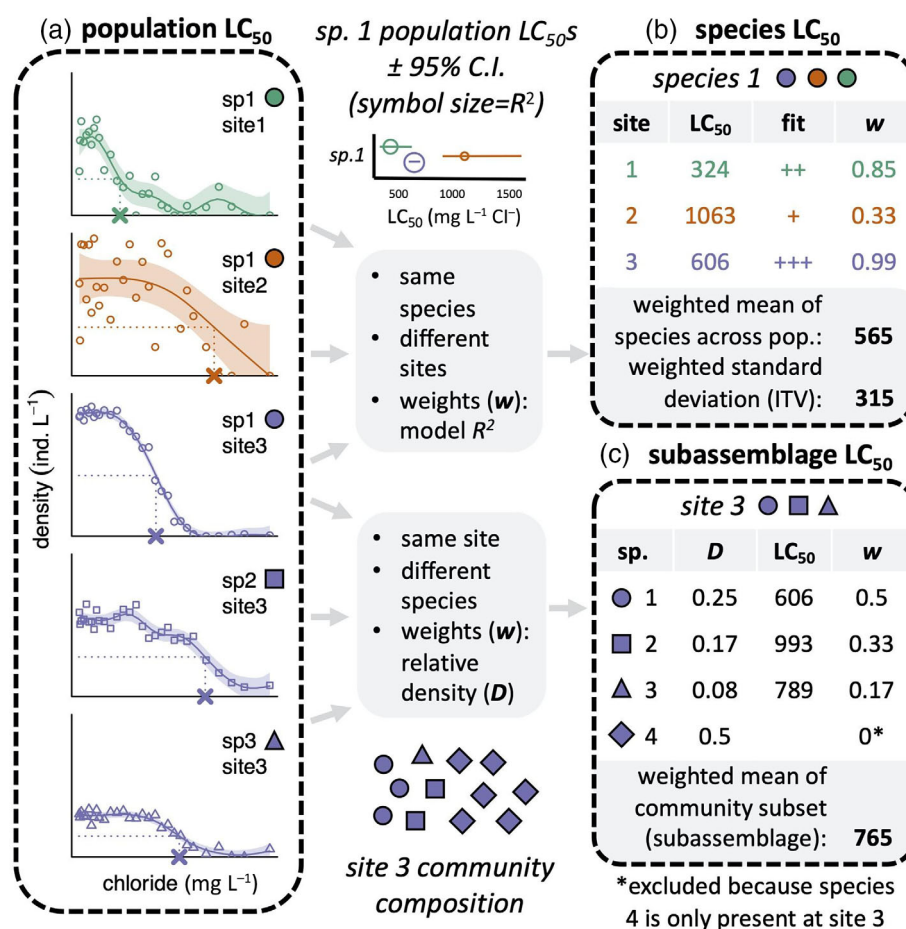


Fig. 2. Conceptual figure with simulated data showing how population (a), species (b), and subassemblage (c) LC_{50} s were calculated. In all panels, colors denote sites while symbols represent species. In (a), crosses on the x-axis indicate LC_{50} s, calculated as the Cl^- concentration where the population density is predicted to be 50% of the value with no Cl^- . In (b), species LC_{50} s were calculated as the mean of population estimates, weighted based on model R^2 . The predicted subassemblage LC_{50} in (c) was calculated for individual sites using a subset of the 19 species found at that site. Note that numbers are provided for illustrative purposes only and do not correspond to observed densities or LC_{50} s. C.I., confidence interval; ind., individual; pop., populations; sp., species.

population- LC_{50} s, weighted by the R^2 of site-specific population GAMs (Fig. 2b).

To identify explanatory factors of intraspecific variation (Q2), we quantified variation as the weighted standard deviation among population- LC_{50} s (again, using GAM R^2 as weights; Fig. 2b). We used the standard deviation because this allowed us to express variation in relation to Cl^- concentration, which is relevant to management. Species intraspecific variation estimates were then modeled as a function of several predictors, estimated for each species based on the sites where it was found: (1) the species mean LC_{50} , to account for a potential association between the mean and standard deviation; (2) the number of sites at which a species was found; (3) the mean geographic distance among sites (average great-circle distance); (4) the mean community dissimilarity among sites (mean Euclidean distance of Hellinger-transformed zooplankton communities among sites in the absence of Cl^- pollution); (5) the standard deviation (s) in calcium (Ca^{2+}) among sites as an indication of variation in water hardness; (6) the variation in food quantity, using the standard deviation of chlorophyll *a* (Chl *a*) as a proxy for algal biomass; and (7) the overall dissimilarity (mean Gower dissimilarity) in environmental conditions and water chemistry across sites when considering nine variables (listed in the Supporting Information Appendix S1). We also included taxonomic category (rotifer, cladoceran, copepod) and species body size as predictors, as phylogeny and differences in life history could influence intraspecific variation. After standardizing all continuous predictors, stepwise model selection by AIC was used to identify significant drivers of intraspecific variation in our coordinated experiment (an alternative model averaging approach led to the same inferences). Linear models were fitted with the function `stepAIC` from the “MASS” package and GAMs with the “mgcv” package. Additional information on independent variables and statistical analyses can be found in the Supporting Information Appendix S1.

To determine whether intraspecific variation could influence our ability to predict community-level salt tolerance from existing data (Q3), we calculated community-weighted LC_{50} s (weighted by the species' relative abundance) using either site-specific population- LC_{50} s or inferred community LC_{50} s based on values derived from other sites in the absence of site-specific information. We first estimated the observed subassemblage- LC_{50} for each site using the subset (“subassemblage”) of 19 species that were found at individual sites (Fig. 2c). Nine sites, which had subassemblages with at least three species, were considered in this analysis. The observed subassemblage- LC_{50} of a site was estimated as the mean of all population- LC_{50} s for that site, weighted by the species' relative abundance. Next, we asked if we could predict the observed subassemblage- LC_{50} s at a given site from species LC_{50} s using information from other sites. We simulated subassemblage LC_{50} s 1000 times, drawing values from normal distributions parameterized from the species- LC_{50} mean and standard deviation. Instead of using

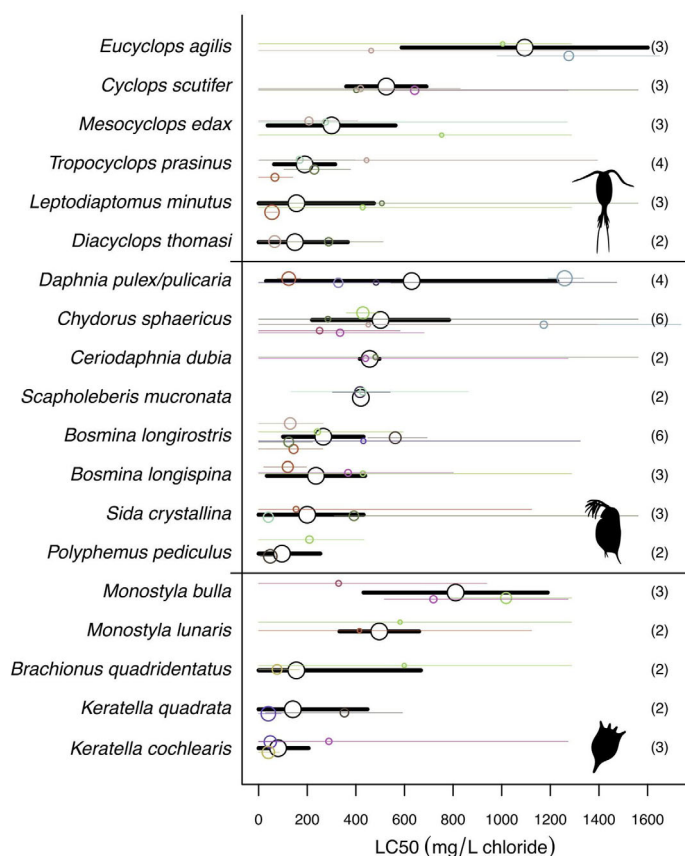


Fig. 3. Cl^- tolerance (LC_{50}) of freshwater zooplankton species occurring at more than one site in the coordinated mesocosm experiment. Species are grouped by major taxon (top = copepods; middle = cladocerans; bottom = rotifers). Colored circles and lines represent site-specific population $LC_{50} \pm 95\%$ confidence intervals. Colors correspond to sites in Fig. 5. The size of the circles represents the confidence in the model predicting the population LC_{50} (GAM R^2). The number of sites at which each species is found is shown in parentheses. The bolded black line and black circle are the weighted mean \pm standard error (95% confidence intervals) representing species- LC_{50} s when averaging population- LC_{50} s across sites.

species- LC_{50} s reported in Fig. 3, values were recalculated after excluding the focal population, to determine whether the salt tolerance of an assemblage can be predicted using LC_{50} from other sites. For each site, we then compared the observed vs. predicted LC_{50} , calculating a z-score and a *p*-value for the observed LC_{50} (function `pnorm` in R) to quantify its deviation from the distribution of simulated LC_{50} s.

Results

Intraspecific variation in salt tolerance among freshwater zooplankton species

High intraspecific variation in freshwater zooplankton Cl^- LC_{50} s was evident from published laboratory studies (Fig. 1). Using these data, we characterized intraspecific variation for

five cladoceran and one rotifer species. We found that intraspecific variation attributable to genetics (Fig. 1c) and environmental variation (Fig. 1b) was high. However, the greatest intraspecific variation occurred when comparing bioassays that included both genetic and environmental variation (Fig. 1a). Experimental variation (Fig. 1d) was small but detectable.

High intraspecific variation in freshwater zooplankton Cl⁻ LC₅₀s was also evident from the mesocosm experiments (Fig. 3) where both environments and genotypes varied among populations. In our experiments, 19 of 129 species were found in >2 sites, including 6 copepods, 8 cladocerans, and 5 rotifers. Intraspecific variation for species-LC₅₀s (among populations at different sites) varied among species with some exhibiting high variation (e.g., *Eucyclops agilis*, *Daphnia pulex*, *Monostyla bulba*, and *Brachionus quadridentatus*), and others exhibiting lower variation (e.g., *Ceriodaphnia dubia* and *Scapholeberis mucronata*; Fig. 3). Some populations of a given species were, on average, 67% ± 27 SD more sensitive to chloride than populations of species from other lakes.

Factors predicting intraspecific variation in salt tolerance among species

Stepwise model selection indicated that community dissimilarity among sites containing a particular species was the only variable predicting variation in species-LC₅₀s for Cl⁻ from the coordinated mesocosm experiments, with higher intraspecific variation found across highly dissimilar communities (regression $\beta \pm SE = 73.1 \pm 24.5$, $p = 0.009$; Fig. 4). Intraspecific variation was not predicted by any environmental variables (environmental dissimilarity, Ca²⁺ variation, Chl *a* variation) nor number of sites in which species occurred, mean geographic distance, taxonomic group, body size or species mean LC₅₀s (all $p > 0.05$; Fig. 4).

Predicting community-wide salt tolerance

The predicted subassemblage-LC₅₀s differed from the observed LC₅₀s in four of our nine sites (Fig. 5). Zooplankton subassemblages were more sensitive to Cl⁻ in two sites (Croche and Sturgeon) and less sensitive in two sites (Convict and Stortjärn) than predicted based on species-LC₅₀s estimated from other sites (Fig. 5). Our ability to accurately

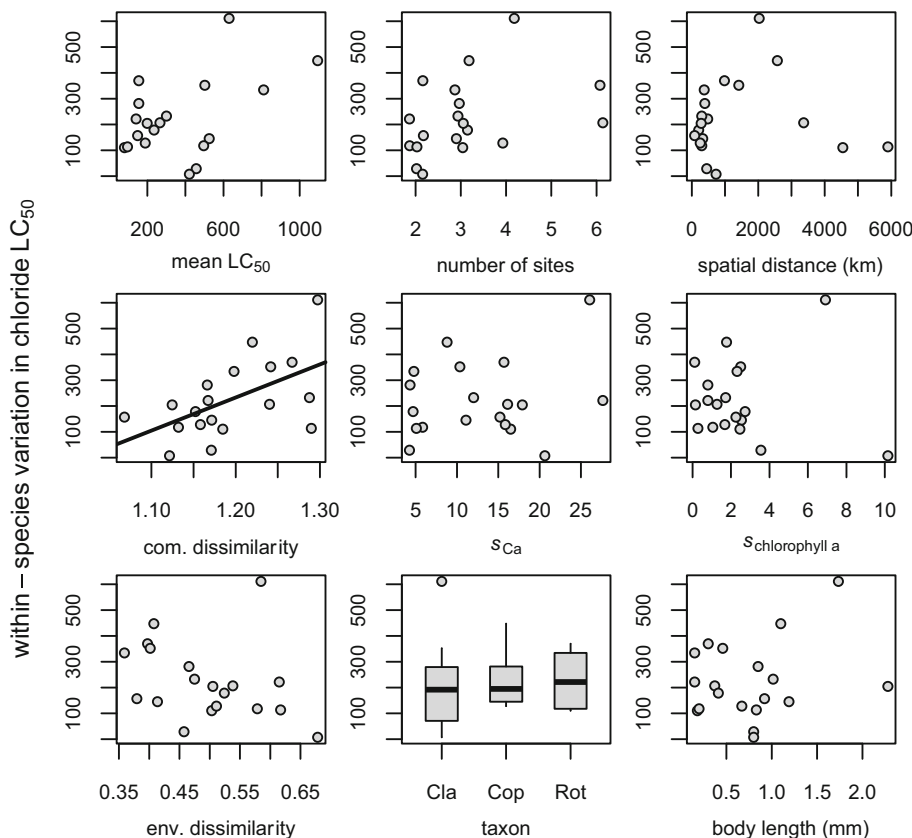


Fig. 4. Predictors of intraspecific variation in Cl⁻ tolerance. Each circle represents a species listed in Fig. 3. A regression line indicates the relationship between intraspecific variation and the one significant predictor of intraspecific variation (community dissimilarity). Cla., cladocerans; com., community; Cop., copepods; env., environmental; Rot., rotifers; s, standard deviation.

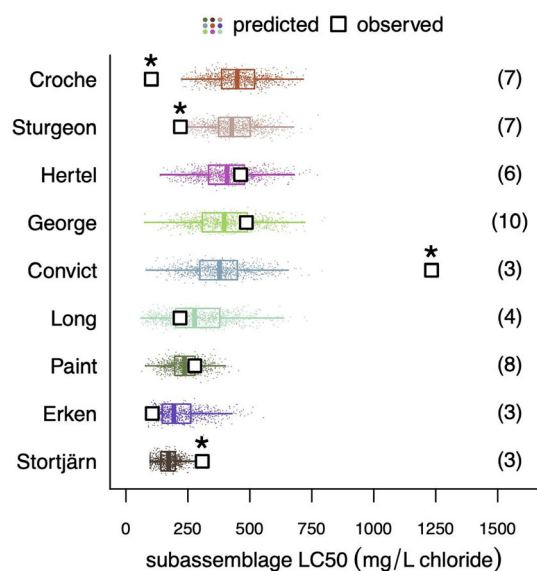


Fig. 5. Community-weighted mean Cl⁻ tolerance (subassemblage-LC₅₀) of freshwater zooplankton from each site (squares) where at least 3 of the 19 species were detected. Simulated-LC₅₀s were drawn from species' LC₅₀ mean and standard deviation excluding the focal population to test prediction accuracy when intraspecific variation was not considered. Simulated LC₅₀s are shown using colored dots and boxplots. Asterisks indicate subassemblage-LC₅₀s that deviate significantly from simulated-LC₅₀s at $p < 0.05$ (note that p -values were adjusted for multiple comparisons using the false discovery rate method). The numbers in parentheses represent the number of species used to calculate the subassemblage-LC₅₀s.

predict the observed subassemblage-LC₅₀ was not related to lake environmental parameters, community composition or experimental parameters such as study duration or mesocosm type (Supporting Information Fig. S2).

Discussion

We found substantial intraspecific variation in freshwater zooplankton salt tolerance for multiple species in both the literature and our mesocosm studies, reducing our ability to predict community-wide tolerance. There were, however, key differences in methodology between laboratory and field studies. Estimates for acute LC₅₀ from our literature review are based solely on mortality after short-term exposure to NaCl, whereas species LC₅₀s from the mesocosm study represent a combination of lethal and indirect community effects. Species interactions likely modulated salt tolerance, as community context was the only factor that explained intraspecific variation in LC₅₀s in the mesocosm experiments. Our results support a growing recognition that species interactions can modulate species responses to contaminants (Relyea and Hoverman 2006; Fischer et al. 2013; Venâncio et al. 2017), and global environmental change (Gilman et al. 2010). Intraspecific variation was high enough that, without accounting

for population differences in LC₅₀, we could only accurately predict the community response to Cl⁻ in approximately half of the sites (5/9 sites, subassemblage-LC₅₀s). High variation in salt tolerance indicates that environmental risk assessments on individual populations may not accurately reflect salt tolerance; policy makers should incorporate intraspecific variation into water quality guidelines by including community-based experiments and studies with organisms that originate from multiple populations.

Given the recognition that Cl⁻ is an important determinant of water quality (Dugan et al. 2017; Kaushal et al. 2018; Schuler et al. 2019), we found surprisingly few studies that estimated 48-h acute toxicity of NaCl (Supporting Information Table S1). A subset of primarily cladoceran species have been used in laboratory tests of Cl⁻ tolerance in zooplankton, leaving important information gaps about salt toxicity for rotifers and copepods, which dominate some plankton communities (Gannon and Stemberger 1978). Filling this gap is important because our mesocosm results indicated that many zooplankton species were more sensitive to salt than the species commonly used in toxicity tests that support environmental policy.

Substantial intraspecific variation in salt tolerance among species

The considerable variation in salt tolerance that we found agree with other studies that detected adaptive genetic variation in freshwater *Daphnia* populations along spatial salinity gradients (Weider and Hebert 1987; Loureiro et al. 2012; Liao et al. 2015). However, intraspecific variation in LC₅₀s that we detected in our mesocosm experiments was likely driven by genetic and unmeasured environmental differences among sites. While this is also true for some of our comparisons among laboratory studies, there were several laboratory studies where we could differentiate independent contributions of variation in genotypes and environment to intraspecific variation in LC₅₀. Regardless, intraspecific variation in Cl⁻ LC₅₀ from both the literature review and mesocosm experiments was high for multiple species.

If genetic sources contribute to intraspecific variation for Cl⁻ tolerance in our mesocosm experiment, as found for some species in laboratory studies, then species with high intraspecific variation for Cl⁻ tolerance might have higher adaptive potential through natural selection at sites facing anthropogenic salinization (Godhe and Rynearson 2017). However, genetic erosion caused by contaminants may also be associated with mal-adaptations (Brady et al. 2019) and reduced genetic diversity (Ribeiro and Lopes 2013) that could increase susceptibility to subsequent exposures or additional environmental stressors (Venâncio et al. 2017). Our mesocosm experiments were conducted at sites with low Cl⁻ concentration; studies are needed to examine the outcome of future salinization on genetic diversity and resilience to additional stressors.

Community context shapes intraspecific variation in salt tolerance among species

The magnitude of intraspecific variation across species was significantly related to differences in community composition across sites. Therefore, species interactions, including predation, intraspecific and interspecific competition, could modulate the response of individual species to Cl^- contamination. The effect of biotic interactions on stress sensitivity has been reported for other groups, including stream insects (Clements et al. 2013) and amphibians (Boone and Semlitsch 2002). There is also some evidence that increasing salinity alters *D. pulex* life-history response to invertebrate (Liu and Steiner 2017) and fish (Hintz and Relyea 2017) predators. The influence of community context on salt tolerance should be tested in future experiments by manipulating the community within which a population is embedded.

We expected environmental variables such as Ca^{2+} and Chl *a* to influence intraspecific variation in salt tolerance because ion concentrations (Mount et al. 2016) and in particular, water hardness (Elphick et al. 2011) can mediate toxicity of Cl^- . Likewise, food quantity (Brown and Yan 2015) and quality (Isanta-Navarro et al. 2021) could alter *Daphnia* response to Cl^- . However, we did not detect a relationship between intraspecific variation of LC_{50} and environmental conditions. It is possible that our measured variables (Ca^{2+} , Chl *a*) did not adequately capture differences in water hardness ($\text{Ca}^{2+} + \text{Mg}^{2+}$) and food availability or that species interactions and other unmeasured drivers overshadowed the environmental effects we measured. We recommend that relationships between intraspecific variation in Cl^- tolerance and environmental context be experimentally explored along wide environmental gradients, especially lakes with lower solute concentrations that may be more sensitive to increased salinity (Arnott et al. 2021).

We need to account for intraspecific variation to accurately predict community-wide salt tolerance

Populations of a given species can vary considerably in Cl^- tolerance such that ignoring intraspecific variation could lead to erroneous estimates of community-level sensitivity to salt pollution; in our study community LC_{50} was not predictable in four of nine sites. Similarly, incorporating intraspecific variation alters the predictions of species distribution models under climate change (Valladares et al. 2014), arguing for more conservative management approaches to account for sensitive populations (Hällfors et al. 2016). Our community-based approach allowed for indirect food web and nonlethal effects resulting in community tolerances that deviated from the predicted range. We do not know what drivers are responsible for deviations and thus suggest that the development of water quality guidelines include data from multiple regions and community-level responses to account for variation.

Conclusion

Our analyses demonstrate high intraspecific variation in salt tolerance that is driven, in part, by species interactions with the local community. Toxicity tests on individuals from cultured laboratory species often provide the foundation for environmental risk assessments which support regulations (CCME 2011 for Cl^-), yet generally do not consider how species interactions influence responses to contaminants. The occurrence of high intraspecific variation in $\text{Cl}^- \text{LC}_{50}$ in both laboratory studies and the complex environments of our mesocosms emphasizes the need to not only incorporate evolution into environmental risk assessments (Oziolor et al. 2020), but also to consider multiple community contexts where diverse species interactions occur. Water quality guidelines based on select population LC_{50} estimates might not adequately protect all populations of a given species over its entire range. This could be particularly important for communities containing species with sensitivity near water quality guidelines (e.g., *Polyphemus pediculus*). This emphasizes not only the need to test multiple populations, but also to conduct studies under multiple environmental contexts to reveal the range of community responses.

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