

SPECIAL ISSUE-LETTER

Lake salinization drives consistent losses of zooplankton abundance and diversity across coordinated mesocosm experiments

Marie-Pier Hébert^{1,2,3a*}, Celia C. Symons^{4a}, Miguel Cañedo-Argüelles,^{5a} Shelley E. Arnott⁶, Alison M. Derry^{1,3}, Vincent Fugère,^{1,7} William D. Hintz⁸, Stephanie J. Melles⁹, Louis Astorg,^{1,3} Henry K. Baker¹⁰, Jennifer A. Brentrup¹¹, Amy L. Downing,¹² Zeynep Ersoy¹³, Carmen Espinosa,¹⁴ Jaclyn M. Franceschini,¹⁵ Angelina T. Giorgio,¹⁶ Norman Göbeler,¹⁷ Derek K. Gray¹⁵, Danielle Greco,⁶ Emily Hassal,¹⁸ Mercedes Huynh,¹⁵ Samuel Hylander,¹⁹ Kacie L. Jonassen,²⁰ Andrea Kirkwood,¹⁸ Silke Langenheder²¹, Ola Langvall²², Hjalmar Laudon,²³ Lovisa Lind,²⁴ Maria Lundgren,¹⁹ Alexandra McClymont,⁶ Lorenzo Proia,¹⁴ Rick A. Relyea,²⁵ James A. Rusak^{15,26}, Matthew S. Schuler,²⁷ Catherine L. Searle^{15,20}, Jonathan B. Shurin,¹⁰ Christopher F. Steiner,²⁸ Maren Striebel,²⁹ Simon Thibodeau,^{1,3} Pablo Urrutia Cordero^{15,30}, Lidia Vendrell-Puigmitja^{15,14}, Gesa A. Weyhenmeyer^{15,21}, Beatrix E. Beisner^{1,3*}

¹Interuniversity Group in Limnology (GRIL), Montréal, Québec, Canada; ²Department of Biology, McGill University, Montréal, Québec, Canada; ³Department of Biological Sciences, Université du Québec à Montréal, Montréal, Québec, Canada; ⁴Department of Ecology and Evolutionary Biology, University of California, Irvine, California; ⁵Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals (FEHM-Lab), Institut de Recerca de l'Aigua, Universitat de Barcelona, Barcelona, Spain; ⁶Department of Biology, Queen's University, Kingston, Ontario, Canada; ⁷Département des sciences de l'environnement, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada; ⁸Department of Environmental Sciences and Lake Erie Center, The University of Toledo, Toledo, Ohio; ⁹Department of Chemistry and Biology, Urban Water Research Centre, Ryerson University, Toronto, Ontario, Canada; ¹⁰Section of Ecology, Behavior, and Evolution, University of California San Diego, La Jolla, California; ¹¹Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire; ¹²Department of Biology, Ohio Wesleyan University, Delaware, Ohio; ¹³Rui Nabeiro' Biodiversity Chair, MED-Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Évora, Portugal; ¹⁴BETA Technological Center, TECNIO Network, University of Vic-Central-University of Catalonia (Uvic-UCC), Barcelona, Spain; ¹⁵Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada; ¹⁶Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, New York; ¹⁷Tvärminne Zoological Station, University of Helsinki, Hanko, Finland; ¹⁸Biological Science, Ontario Tech University, Oshawa, Ontario, Canada; ¹⁹Centre for ecology and evolution in microbial model systems, Department of Biology and Environmental Science, Linnaeus University, Kalmar, Sweden; ²⁰Department of Biological Sciences, Purdue University, West Lafayette, Indiana; ²¹Department of Ecology and Genetics/Limnology, Uppsala University, Uppsala, Sweden; ²²Unit for Field-based Forest Research, Siljansfors Research Station, Swedish University of Agricultural Science, Mora, Sweden; ²³Department of Forest Ecology, Swedish University of

*Correspondence: mphebert4@gmail.com, beisner.beatrix@uqam.ca

^aCo-first authors.

Associate editor: Meryem Beklioglu

Author Contribution Statement: MPH, MCA, and BEB conceived the initial ideas for this study. MPH compiled trait information and curated taxonomic data. CCS and MCA performed analyses, with help from MPH and VF. MPH, CCS, MCA, and BEB interpreted results. MPH led the writing, with help from BEB, MCA, and CCS. CCS led the revisions, with help from MPH, MCA, and BEB. AD, AMD, CFS, CS, DG, GAW, JAR, JB, LL, NG, PUC, SEA, SH, SL, SJM, VF, WDH, and ZE critically reviewed the manuscript; all authors approved the manuscript. As part of the Global Salt Initiative led by SEA and WDH, all authors contributed to coordinated experiments in the form of field work, laboratory assistance, or financial support.

Data Availability Statement: The data reported in this paper have been deposited on Dataverse (environmental, abundance, and taxonomic data) and Figshare (trait data): Dataverse: <https://doi.org/10.5683/SP3/BIDMCI>; Figshare: <https://doi.org/10.6084/m9.figshare.17109143>.

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

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Agricultural Science, Umeå, Sweden; ²⁴Department of Environmental and Life Sciences, Karlstad University, Karlstad, Sweden; ²⁵Department of Biological Sciences, Darrin Fresh Water Institute, Rensselaer Polytechnic Institute, Troy, New York; ²⁶Dorset Environmental Science Centre, Ontario Ministry of the Environment, Conservation and Parks, Dorset, Ontario, Canada; ²⁷Department of Biology, Montclair State University, Montclair, New Jersey; ²⁸Department of Biological Sciences, Wayne State University, Detroit, Michigan; ²⁹Institute for Chemistry and Biology of Marine Environment (ICBM), Carl-von-Ossietzky University Oldenburg, Wilhelmshaven, Germany; ³⁰Department of Biology/Aquatic Ecology, Lund University, Lund, Sweden

Scientific Significance Statement

Freshwater salinization from anthropogenic activities—including the application of road deicing salts—is a global environmental concern, harming aquatic biota and drinking water quality. However, the relative sensitivity of zooplankton communities to salinization across large scales remains largely unknown. Performing experiments in parallel across North America and Europe, we show that there is substantial variation in the sensitivity of different zooplankton taxa to salinization, but that chloride sensitivity is more common and pronounced in crustaceans. This study demonstrates that the abundance and diversity of the communities can be reduced at chloride levels below common water quality guidelines (120–250 mg Cl[−] L^{−1}, depending on the study country). Our results suggest that freshwater biodiversity might be reduced by chloride contamination at a global scale.

Abstract

Human-induced salinization increasingly threatens inland waters; yet we know little about the multifaceted response of lake communities to salt contamination. By conducting a coordinated mesocosm experiment of lake salinization across 16 sites in North America and Europe, we quantified the response of zooplankton abundance and (taxonomic and functional) community structure to a broad gradient of environmentally relevant chloride concentrations, ranging from 4 to ca. 1400 mg Cl[−] L^{−1}. We found that crustaceans were distinctly more sensitive to elevated chloride than rotifers; yet, rotifers did not show compensatory abundance increases in response to crustacean declines. For crustaceans, our among-site comparisons indicate: (1) highly consistent decreases in abundance and taxon richness with salinity; (2) widespread chloride sensitivity across major taxonomic groups (Cladocera, Cyclopoida, and Calanoida); and (3) weaker loss of functional than taxonomic diversity. Overall, our study demonstrates that aggregate properties of zooplankton communities can be adversely affected at chloride concentrations relevant to anthropogenic salinization in lakes.

The salinization of freshwater ecosystems from human activities is a growing issue globally. Anthropogenic drivers of freshwater salinization include agricultural practices, resource extraction, land clearing, and climate change (Williams 2001; Cañedo-Argüelles 2020); in colder regions, however, one of the most pervasive sources of salts is road deicing (Dugan et al. 2017a). Long-term increases in chloride—the anion of most road salts—have occurred in waterbodies across North America and Northern Europe (Dugan et al. 2017b; Kaushal et al. 2018), sparking toxicity concerns for biota and drinking water quality. As expanding urbanization increases the risk of chloride contamination (e.g., Dugan et al. 2020), it is critical to understand the ecological consequences of salinization to develop effective water quality management.

In most inland waters, chloride naturally occurs at low concentrations (< ca. 20 mg Cl[−] L^{−1}) but can increase to 1000–10,000 mg Cl[−] L^{−1} in lakes, rivers, and wetlands contaminated with road salt (Hintz and Relyea 2019). Current water quality guidelines for aquatic life chronic exposure in Canada and the U.S.A. are 120 and 230 mg Cl[−] L^{−1} (US-EPA 1988; CCME 2011), respectively; although variable within the European Union,

250 mg Cl[−] L^{−1} is a common recommendation. Most benchmarks for the protection of freshwater biota rely on laboratory toxicity tests of individual species. For zooplankton, which play a critical functional role in lake food webs, most toxicity studies have focused on large cladocerans, especially *Daphnia* (e.g., *Daphnia longispina* and *Daphnia galeata*; Gonçalves et al. 2007; Loureiro et al. 2015). Arnott et al. (2020) demonstrated reductions in *Daphnia* (*Daphnia catawba*, *Daphnia mendotae*, and *Daphnia minnehaha*) survival and reproduction at concentrations as low as 5–40 mg Cl[−] L^{−1}. However, knowledge remains scarce about copepod and rotifer species, and relatively few studies have thus far investigated lake salinization effects at the community level (Hintz and Relyea 2019). Recent work addressed effects on zooplankton assemblages within single lakes (Hintz et al. 2017) or along naturally-occurring salinity gradients (Gutierrez et al. 2018); but experimentally testing how community structure responds to salinization under different environmental conditions could help reveal the mechanisms by which lake zooplankton may cope with salinity stress. Yet, large-scale experimental examinations across lakes and regions are, to our knowledge, nonexistent.

Variation in chloride sensitivity across taxa can mediate community-wide responses to salinization, altering taxonomic and functional community structure following contamination. Diversity loss or compositional shifts in favor of salt-tolerant taxa may have implications for ecosystem functions and services, given the strong influence of zooplankton on both lower and upper trophic levels in lakes. For example, large-bodied filter-feeding cladocerans could be replaced with smaller, less efficient grazers such as rotifers (via compensatory increases; Jeppesen et al. 2007), thereby promoting algal blooms (Korosi et al. 2012) and reduced food quality for predators of zooplankton and upper trophic levels (Hintz et al. 2017). While zooplankton communities tend to be less diverse with increased salinity (Nielsen et al. 2003a), certain biotic factors may mitigate abundance or species losses. For instance, communities with high diversity may be buffered from the adverse effects of salinization given their broad range of life-history traits (including salt tolerance) and functional redundancy (Setubal et al. 2020).

Local environmental conditions may also modulate zooplankton responses to salinization. Notably, historic chloride levels at which communities have evolved may influence tolerance to contamination events (Coldsnow et al. 2017). Naturally-occurring calcium (Elphick et al. 2011) and phytoplankton availability (Brown and Yan 2015) may also mitigate chloride toxicity. Thus, irrespective of composition, communities may exhibit a range of chloride sensitivity across lake types and regions. Although biotic and abiotic drivers of community responses have been investigated in riverine macroinvertebrates (Kefford et al. 2012), there is a need to expand such assessments to other freshwater ecosystems and communities experiencing salinization.

We performed a cross-continental experimental study of lake salinization to investigate the effects of chloride contamination on zooplankton communities. Through 16 coordinated mesocosm experiments across North America and Europe, we exposed naturally-occurring zooplankton assemblages (spanning ca. 130 taxa) to a range of environmentally relevant chloride concentrations, along opportunistic gradients of baseline conditions (chloride, calcium, algal availability) owing to regional differences among lakes. We compared zooplankton abundance, taxonomic, and trait-based responses to salinization and tested four main hypotheses:

H1: Zooplankton communities will decline in abundance, owing to local species extinctions, concomitant with declines in taxonomic and functional evenness resulting from greater dominance by salt-tolerant taxa. Functional diversity will decline less markedly than taxonomic diversity owing to functional redundancy.

H2: Large-bodied cladoceran grazers will be the most salinity-sensitive (functional) group, while rotifers and smaller omnivorous crustaceans will be less sensitive. Rotifers will show compensatory increases in abundance.

H3: Originally more diverse communities (taxonomically and functionally) will show weaker declines in abundance and diversity with increasing chloride.

H4: Communities originating from lakes with higher calcium, chloride, or chlorophyll *a* (Chl *a*) concentrations will show weaker declines in abundance and diversity with increasing chloride.

Methods

Coordinated experiments

We conducted 16 coordinated mesocosm experiments across lakes in Canada, U.S.A., Sweden, and Spain. The experiments were based on a standardized protocol to examine how a wide range of Cl^- concentrations may affect zooplankton assemblages. All experiments ran between June and August 2018; except for sites Purdue (September) and Tavernoles (November). Site characteristics and field protocols are provided in the Appendix (Section I), Arnott et al. (2021), and Hintz et al. (In press).

Briefly, at each location, we filled 20–32 mesocosms (volume range: 80–2500 L) with local lake or well water, filtered using a 40- to 100- μm mesh to remove zooplankton, and inoculated them with zooplankton and phytoplankton from a local lake. We then applied treatments in a regression design by amending mesocosms with NaCl to obtain an unreplicated nominal gradient from ambient Cl^- (ranging from <2 to $18 \text{ mg Cl}^- \text{ L}^{-1}$) to $1400 \text{ mg Cl}^- \text{ L}^{-1}$. NaCl treatments were only applied once, at the beginning of the experiment. Two sites did not reach the target gradient due to rain (Dartmouth, maximum concentration reached = $800 \text{ mg Cl}^- \text{ L}^{-1}$) and evaporation (Convict, maximum concentration reached = $3000 \text{ mg Cl}^- \text{ L}^{-1}$). Given that Cl^- concentrations varied with weather conditions over time, we averaged Cl^- concentrations throughout the duration of the experiment at each site in analyses. Dissolved nitrogen and phosphorus were added fortnightly to preclude resource limitation except for sites Dartmouth, Feresjön, Sturgeon, and Stortjärn.

Each mesocosm experiment ran for 6 weeks (44 ± 3.3 d of exposure). We measured chloride concentrations directly or indirectly (conductivity) on weeks 0, 3, and 6 of each experiment. We sampled Chl *a* at the beginning and end of each experiment via fluorometry or spectrophotometry. We also sampled the zooplankton community at the beginning and end of the experiment (10–20% of the mesocosm volume). Zooplankton were preserved with ethanol (70–95%) or Lugol's solution. We identified and enumerated all week-6 zooplankton samples and processed week-0 samples for a subset of the sites (12/16). Week-0 zooplankton data were discarded from the main results; however, a separate analysis indicated that communities sampled within 2 d of salt addition were not associated with the chloride treatment, except for Stortjärn, which showed positive relationships between crustacean

diversity and evenness and nominal chloride (Appendix Section III).

Dataset, functional traits, and diversity metrics

The community dataset used for this study consisted of zooplankton abundances at the end of experiments (spanning 60 crustacean and 69 rotifer taxa) by chloride treatment level by source lake. We validated taxonomy and curated data to ensure consistency across sites. Details and data sets are provided in Arnott et al. (2021).

To assess functional responses to salinization, we assembled a trait matrix for crustaceans. We collated taxon-specific information on trophic group, body length, body mass, and feeding type. In addition to being well-documented, these traits reflect ecological strategies and roles (niche space) occupied in food webs (Hébert and Beisner 2020). We first extracted trait information from existing syntheses and known trait relationships (McCauley 1984; Barnett et al. 2007; Hébert et al. 2016a,b); then compiled remaining data as described in the Appendix (Section II). The assembled trait matrix can be found in Hébert et al. (2021). Data were not compiled for rotifers, as traits were either known to be strongly phylogenetically constrained or redundant across species.

We calculated functional diversity indices using a Gower dissimilarity matrix, with the function *dbFD* in the R package *FD* (Laliberté et al. 2014). We performed a principal coordinate analysis and used the axes to estimate functional richness and evenness, as per Villéger et al. (2008). We calculated functional dispersion as an extension of the average distance from the centroid (Laliberté and Legendre 2010). For taxonomic diversity, we quantified richness, diversity (Shannon), and evenness (Pielou) separately for crustaceans and rotifers. We performed diversity calculations and all subsequent data analyses in R (R Core Team 2020).

Statistical analyses

For each set of analyses, we included experimental sites (referred to as sites hereafter) with either ≥ 3 functionally distinct crustacean groups or different rotifer taxa; we analyzed the two groups separately. We used mean chloride concentrations for each mesocosm as the predictor variable. We applied a \log_{10} -transformation to abundances but left diversity indices untransformed. We quantified the effect of chloride using linear mixed-effects models (LMMs), with the *lmer* function in the R package *lme4*, setting site as a random effect. To model taxon richness, we used generalized LMMs to accommodate the discrete nature of the data (Poisson distribution) with a log link and the *glmer* function.

To identify which taxa and traits were most sensitive to chloride, we used linear models adapted to multivariate abundance data with the R package *mvabund* (Wang et al. 2012). We built site-specific models using the *manyam* function, fitting a model for each taxon (Appendix Section III). To assess crustacean functional trait responses, we performed a

fourth corner analysis for each site using the *traitglm* function available with *manyam* (Brown et al. 2014).

To determine whether zooplankton responses could be explained by lake abiotic or biotic characteristics, we tested for the effect of chloride, calcium, temperature, and Chl *a* concentrations, as well as taxonomic and functional diversity. We used lake chloride, calcium, and Chl *a* concentrations measured prior to the start of experiments; additionally, we tested for the effect of Chl *a* concentrations in control mesocosm at the end of experiments. Although not part of our hypotheses, we tested for the effect of temperature on chloride sensitivity and included these results in Appendix Section III. Our community diversity predictor variables were calculated using compositional data at the end of each experiment to account for both the “mesocosm effect” and the ecological drift that may have affected communities during the previous 6 weeks. To estimate the taxonomic and functional diversity of control communities without relying exclusively on the one untreated mesocosm per site, we used data from all mesocosms to fit site-specific multivariate abundance models, using the *manyam* function of *mvabund* with a Tweedie distribution, and predicted taxonomic and functional community diversity at the lowest (control) chloride concentration. Then, we performed a two-step regression analysis: we (1) quantified the effect of chloride on community response metrics for each site and extracted significant slopes as a measure of effect size; and (2) regressed site-specific slopes against predictors (chloride, calcium, Chl *a*, temperature, and taxonomic and functional diversity of modeled control communities; tested individually), and weighted relationships by model r^2 values obtained in (1).

Finally, to compare chloride sensitivity among community metrics and across sites, we extracted three indicator values from our models: (1) chloride concentrations corresponding to a 50% reduction in each metric relative to modeled control communities, and proportions (%) reduction in each metric observed at (2) Canadian and (3) U.S. water quality guideline thresholds.

Results

Increasing chloride reduced crustacean abundance, taxon richness, and Shannon diversity across sites (Fig. 1a–c; H1). Declines in abundance and richness were most pronounced, with highly similar rates of decrease along the chloride gradient (slopes declining in parallel; Tables S3 and S4). Evenness did not respond significantly (Fig. S2). For rotifers, only abundance and richness decreased significantly, although weakly (Fig. 1d,e).

Taxon-specific sensitivity to chloride was more common (proportions of sensitive taxa) and pronounced (magnitude of response) in crustaceans of all sizes than in rotifers (Figs. 2a,b, 3; H2). Among-site analyses identified 35 sensitive crustaceans, spanning all major taxonomic units (Fig. 2a). Most

Crustaceans

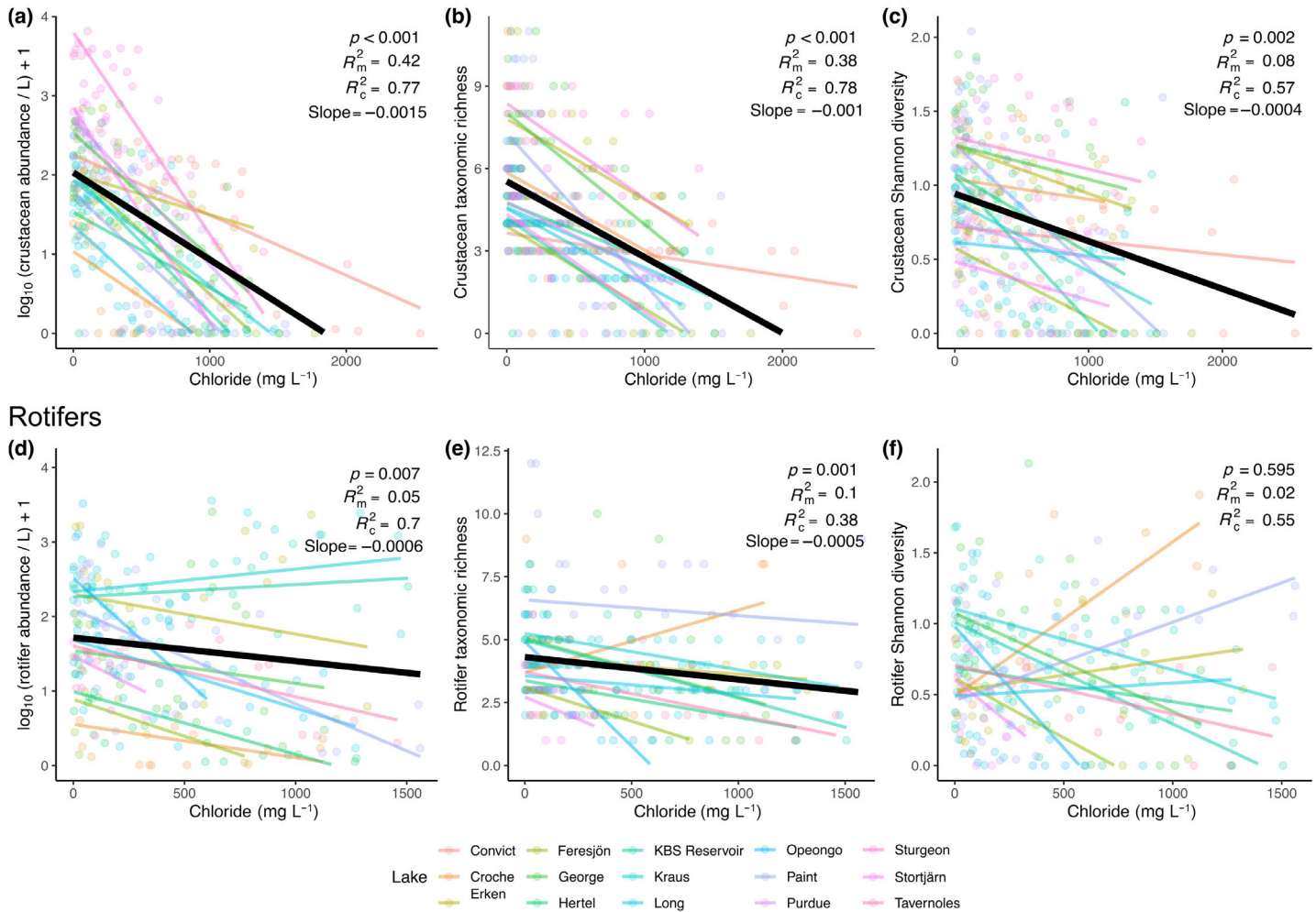


Fig. 1. Variation in (a–c) crustacean and (d–f) rotifer community abundances and diversity metrics in response to chloride additions across lake experiments. Each colored line distinctly represents one of the source lakes (individual lake linear regression models), while thicker black lines represent the general trend across lakes (LMM; significant models only, slope coefficients provided). For among-lake LMMs, both marginal and conditional R^2 are provided (R_m^2 and R_c^2 , respectively). See Table S3 for parameter estimates.

sensitive genera (highest quartile; Table S5) were also found across major groups: Calanoida (*Eudiaptomus*), Cyclopoida (*Microcyclops*, *Acanthocyclops*, *Diacyclops*), and Cladocera (*Chydorus*, *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*). *Daphnia pulex* x *pulicaria* was the sole member of *Daphnia* that declined significantly. Most rotifers were unresponsive to chloride (Fig. 2b), with only five taxa declining. No zooplankton taxon responded positively to chloride, indicating that no compensatory increases occurred.

We found no detectable pattern with chloride in crustacean trait assemblages. Feeding type and body size responded idiosyncratically to chloride across experiments (Figs. 3a, S4; H1–H2). Despite substantial among-site variation in trait-based responses, chloride weakly reduced crustacean functional richness and dispersion (Fig. 3b,c). Crustacean

functional evenness did not respond to salinity (Fig. S2). Overall, chloride caused weaker loss of functional than taxonomic diversity in crustaceans (Figs. 1b,c vs. 2b,c; Table S4). Furthermore, none of the tested biotic and abiotic drivers (H3–H4) could explain zooplankton community responses to salinization (Fig. S4; Table S7).

Across sites, we found that many metrics of crustacean community structure showed 50% reductions at concentrations slightly exceeding 500 mg $\text{Cl}^- \text{L}^{-1}$ (Fig. 4a). Furthermore, most crustacean metrics showed $\sim 10\%$ and $\sim 20\%$ decreases when chloride levels reached Canadian (120 mg $\text{Cl}^- \text{L}^{-1}$) and U.S. (230 mg $\text{Cl}^- \text{L}^{-1}$) water quality guidelines, respectively (Fig. 4b,c). Rotifer community responses showed greater variation across sites, with no apparent pattern (Fig. 4d–f).

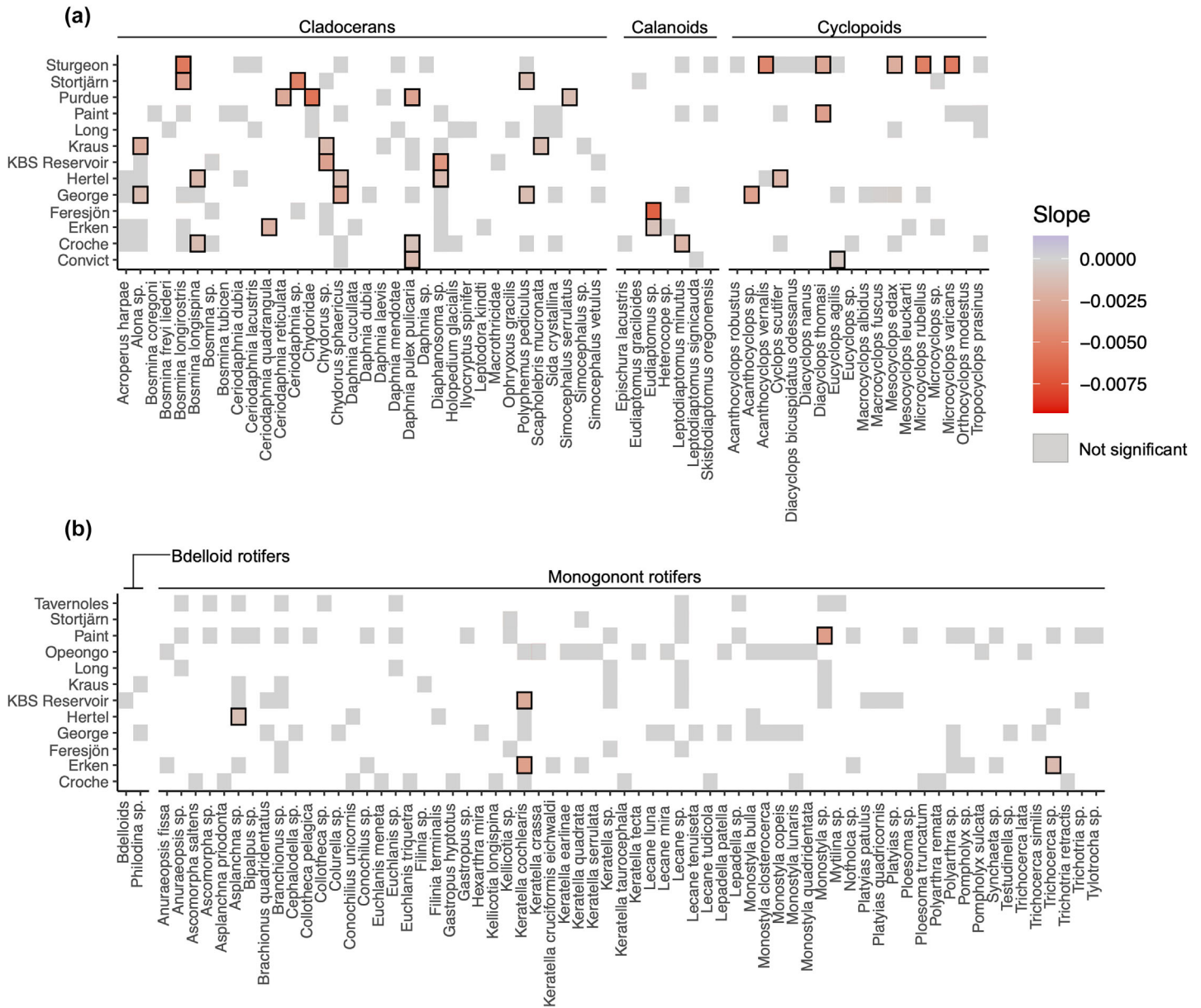


Fig. 2. Taxon-level abundance responses of (a) crustaceans and (b) rotifers to chloride additions across lake experiments. Color saturation represents lake-specific slopes of models, reflecting taxon-level sensitivity to chloride. Unframed, gray squares represent nonsignificant relationships whereas white areas indicate the absence of taxa in each lake. Abundance data are \log_{10} -transformed. See Table S5 for parameter estimates.

Discussion

Through coordinated experiments of lake salinization, we compared the response of zooplankton density and community structure along a gradient of chloride concentrations (4 to ca. 1400 mg $\text{Cl}^- \text{L}^{-1}$), while accounting for differences in regional assemblages and environmental conditions. We found that crustaceans were more sensitive to elevated chloride than rotifers, with no evidence of compensatory increases by the latter. Based

on the multifaceted response of crustaceans (i.e., community vs. individual taxa, taxonomic vs. functional), our results indicate that salinization: (1) leads to marked, highly consistent declines in abundance and taxon richness; (2) impairs multiple taxa across Cladocera, Cyclopoida, and Calanoida; and (3) causes greater losses of taxonomic than functional diversity, with substantial among-site variation in trait-based responses. Despite among-site variation in community structural responses, many crustacean metrics showed 50% reductions at ca. 500 mg $\text{Cl}^- \text{L}^{-1}$; although

adverse effects of salinity already occurred at lower concentrations. Our study demonstrates that key community metrics can be affected at chloride levels relevant to anthropogenic salinization, pointing to broad implications for lake ecosystems.

Salinization altered crustacean abundance and taxonomic structure, in agreement with previous studies (H1; Schallenberg et al. 2003; Jensen et al. 2010; Hintz et al. 2017). Elevated chloride strongly reduced richness and Shannon diversity but had no effects on evenness. While richness declines could result from an inability to detect taxa reduced to very low abundances, this observation suggests that diversity loss was potentially driven by local extinctions. The striking consistency in the loss of abundance and taxa for each chloride level increment across sites highlights a general response pattern to salinization; that is, independent of initial abundance and richness. Salinity can induce mortality and diversity loss in freshwater crustaceans in different ways, interfering with osmoregulation, ionoregulation, and egg hatching (Nielsen et al. 2003b; Griffith 2017). Irrespective of the causal mechanisms, the trends uncovered in our study indicate that increased chloride may impair crustacean biomass stocks, biodiversity, and associated functions (e.g., grazing control, dietary supply for upper trophic levels), which may in turn reduce food chain length and energy transfer in lakes (Golubkov et al. 2018).

Chloride additions had weak to no effect on rotifer communities and individual taxa (H1–H2). This result is consistent with studies reporting relatively high salt tolerance in rotifers (Schallenberg et al. 2003). However, in contrast to our expectation, the decrease in crustacean abundance and, therefore, competition for phytoplankton, was not accompanied by an increase in rotifer abundance, as observed elsewhere (Jeppesen et al. 2007; Van Meter and Swan 2014; Lin et al. 2017). Furthermore, the parallel study by Hintz et al. (In

press) showed that Chl *a* increased as a result of cascading effects in most experiments (as observed by Moffett et al. 2020), suggesting that rotifers were not limited by phytoplankton availability. Although we found no evidence of compensatory increases, the concomitant decrease in crustaceans and maintenance of rotifer abundance support the general suggestion that lake salinization is likely to induce weaker top-down control by zooplankton, with implications for algal proliferation and food web stability (Jeppesen et al. 2015).

Crustacean community-wide responses were attributable to declines in multiple species, rather than a few distinct (“sentinel”) sensitive taxa (H2). Surprisingly, chloride sensitivity was common across all major crustacean groups, with 25% of taxa showing declines in all of Cladocera (23/89), Calanoida (3/12), and Cyclopoida (9/34). While salt sensitivity has been observed in copepods (Van Meter et al. 2011), toxicological effects are mainly documented for large cladocerans, especially *Daphnia*. Such toxicity tests form the basis of most water quality guidelines, representing the criteria upon which governmental agencies base monitoring programs. Yet, our study reports only one sensitive *Daphnia* taxon, and suggests that copepods and various other cladocerans may be equally or even more sensitive to chloride. Furthermore, our results indicate that a given species may exhibit variable chloride sensitivity across lakes, highlighting the role of environmental forcing and intraspecific variation in crustacean responses to increased salinity. Current knowledge based on toxicity tests may, thus, be insufficient to properly predict and protect lake communities from the effects of anthropogenic salinization.

Relative to effects on taxonomic structure, salinization weakly affected crustacean functional structure (H1–H2). This result is consistent with our a priori hypothesis, whereby functional diversity may be buffered from salinity effects

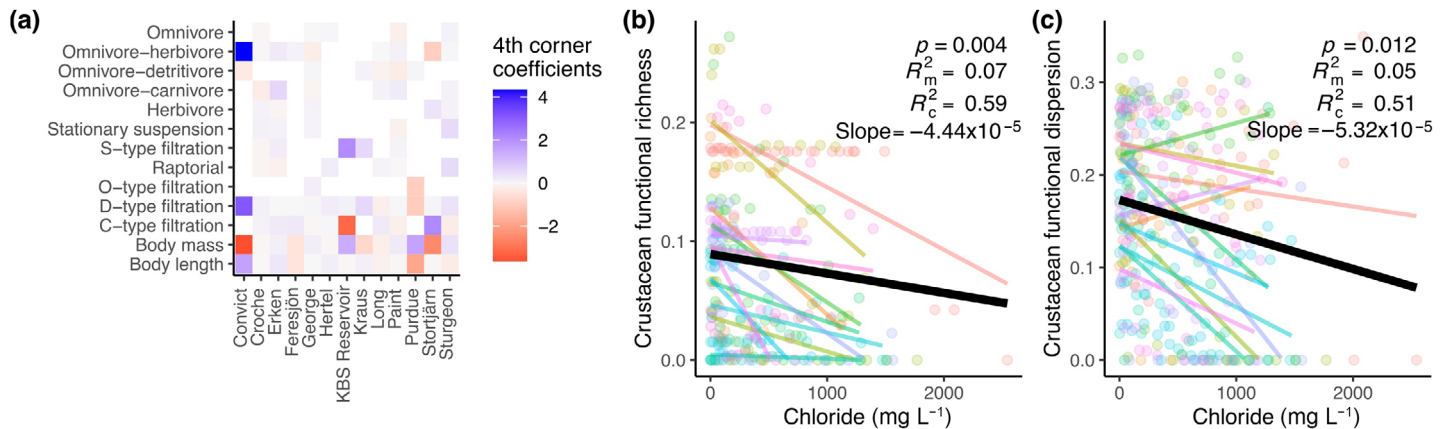


Fig. 3. Variation in crustacean (a) trait assemblages and (b, c) functional diversity in response to chloride additions across lake experiments. (a) For each lake, fourth corner coefficients show the strength of association between traits and chloride concentrations, from positive (blue) to negative (red). See Table S5 for parameter estimates. (b, c) Each colored line distinctly represents one of the source lakes (individual lake linear regression model; colors as in Fig. 1), while thicker black lines represent the general trend across lakes (LMMs, slope coefficients provided). For among-lake LMMs, both marginal and conditional R^2 are provided (R_m^2 and R_c^2 , respectively). See Tables S3 and S4 for parameter estimates.

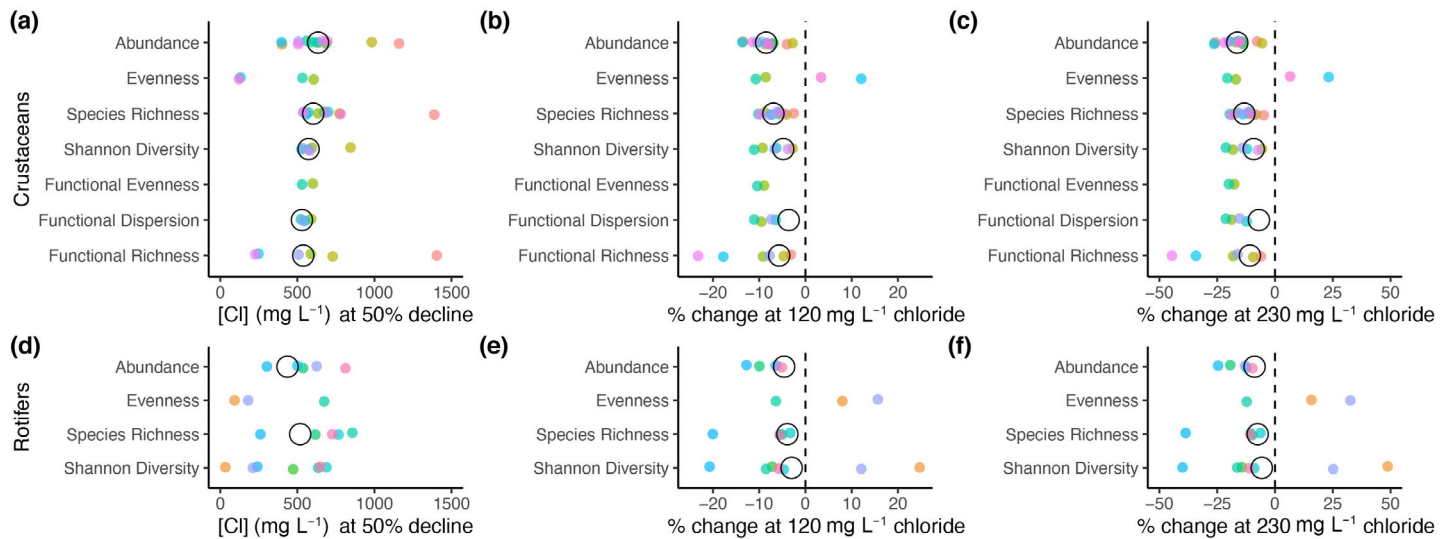


Fig. 4. Comparisons of chloride sensitivity among (a–c) crustacean and (d–f) rotifer community-level measures (abundance and diversity metrics) and across source lakes. (a, d) Chloride concentrations correspond to 50% reductions in community-level measures (analogous to half maximal effective concentration EC_{50}), and (b, c, e, f) specific proportions (%) of reduction are shown at Canadian ($120 \text{ mg Cl}^{-1} \text{ L}^{-1}$, middle column) and U.S. ($230 \text{ mg Cl}^{-1} \text{ L}^{-1}$, right column) water quality guidelines. Each colored point distinctly represents one of the source lakes (individual lake linear model; colors as in Figs. 1 and 3); large transparent circles illustrate the overall value across lakes (among-lake LMMs). Only values from significant models are shown.

owing to functional redundancy among taxa. Losses of functional diversity were overall minor in our experiments, especially when compared to effects reported in studies using similar size- and feeding-related traits (Gutierrez et al. 2018; Setubal et al. 2020). Divergence in initial trait composition could be a possible explanation for this discrepancy between studies; although generally, salinity is a weak driver of zooplankton size structure (Brucet et al. 2010). For a deeper understanding of the mechanisms underlying functional responses to salinization, future studies could incorporate direct measurements of traits more closely related to stress coping strategies.

Local water chemistry, algal availability, and (taxon and trait) diversity could not explain among-site variation in community responses (H3–H4). History of exposure to chloride had no detectable effect, likely owing to the narrow range of ambient chloride concentrations across source lakes ($\leq 18 \text{ mg Cl}^{-1} \text{ L}^{-1}$). Our sites covered relatively wider ranges of calcium ($2.5\text{--}77 \text{ mg L}^{-1}$) and Chl *a* concentrations (up to 14.7 and $22.6 \mu\text{g L}^{-1}$ across source lakes and control mesocosms, respectively); yet, we found no indication that neither calcium nor algal availability mitigated chloride toxicity, unlike previous studies (Soucek et al. 2011; Brown and Yan 2015). Perhaps more surprising was that neither the taxonomic nor the functional diversity of communities influenced responses to salinization, despite our diverse arrays of species and traits. Other unmeasured factors could have mediated chloride toxicity in our experiments, such as the dietary quality of algae (Isanta-Navarro et al. 2021). Overall, our inability to predict

community responses using drivers of known relevance highlights the need to further investigate how abiotic and biotic factors may operate at large scales to modulate salinization effects.

Each of our 16 experiments includes limitations that are generally associated with mesocosm-based studies. For example, the diversity of species in the mesocosms was likely lower than that of natural lakes, and we did not allow dispersal from the regional species pool or allot sufficient time for adaptation, reducing natural pathways by which salt-tolerant species or genotypes may buffer communities over longer timescales. Furthermore, our set of experiments also includes site-to-site variation that must be considered. Notably, mesocosm volume varied among experimental sites and plankton dynamics may differ based on mesocosm volume; however, volume differences were rarely associated with the strength of community responses to Cl^{-} (Appendix Section III). Nevertheless, despite variation in mesocosm volume and local site characteristics, our among-site comparisons revealed similar effects of Cl^{-} on some community properties, highlighting a general response to salinization.

The salinization of inland waters threatens water quality and biota globally. Yet current management practices rely on benchmarks that may be too permissive to protect the structure of freshwater communities. Our study shows that aggregate properties of crustacean communities can be adversely affected at chloride concentrations relevant to salt contamination (Dugan et al. 2017a), even below common water quality guidelines. Although we estimated that the 50% decline in many crustacean community metrics occurs at ca. 500 mg

$\text{Cl}^- \text{ L}^{-1}$, a 50% loss of abundance or species have serious implications for ecosystems and declines of smaller magnitude at lower chloride concentrations can already have far-reaching ecological consequences (e.g., cascading effects on lake food webs). Given the global trends in anthropogenic freshwater salinization, we believe that national water quality criteria should be revised downwards whenever a “one-size-fits-all” approach is adopted.

REFERENCES

- Arnott, S. E., M. P. Celis-Salgado, R. E. Valleau, A. M. DeSellas, A. M. Paterson, N. D. Yan, J. P. Smol, and J. A. Rusak. 2020. Road salt impacts freshwater zooplankton at concentrations below current water quality guidelines. *Environ. Sci. Technol.* **54**: 9398–9407. doi:[10.1021/acs.est.0c02396](https://doi.org/10.1021/acs.est.0c02396)
- Arnott, S. E., and others. 2021. Global Salt Experiment. Dataverse, data sets and protocols. doi:[10.5683/SP3/BIDMCI](https://doi.org/10.5683/SP3/BIDMCI)
- Barnett, A. J., K. Finlay, and B. E. Beisner. 2007. Functional diversity of crustacean zooplankton communities: Towards a trait-based classification. *Freshw. Biol.* **52**: 796–813. doi:[10.1111/j.1365-2427.2007.01733.x](https://doi.org/10.1111/j.1365-2427.2007.01733.x)
- Brown, A. H., and N. D. Yan. 2015. Food quantity affects the sensitivity of *Daphnia* to road salt. *Environ. Sci. Technol.* **49**: 4673–4680. doi:[10.1021/es5061534](https://doi.org/10.1021/es5061534)
- Brown, A. M., D. I. Warton, N. R. Andrew, M. Binns, G. Cassis, and H. Gibb. 2014. The fourth-corner solution – using predictive models to understand how species traits interact with the environment. *Methods Ecol. Evol.* **5**: 344–352. doi:[10.1111/2041-210X.12163](https://doi.org/10.1111/2041-210X.12163)
- Brucet, S., and others. 2010. Factors influencing zooplankton size structure at contrasting temperatures in coastal shallow lakes: Implications for effects of climate change. *Limnol. Oceanogr.* **55**: 1697–1711. doi:[10.4319/lo.2010.55.4.1697](https://doi.org/10.4319/lo.2010.55.4.1697)
- Canadian Council of Ministers of the Environment (CCME). 2011. *Canadian water quality guidelines for the protection of aquatic life: Chloride*. Environment Canada.
- Cañedo-Argüelles, M. 2020. A review of recent advances and future challenges in freshwater salinization. *Limnetica* **39**: 185–211. doi:[10.23818/limn.39.13](https://doi.org/10.23818/limn.39.13)
- 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. doi:[10.1016/j.envpol.2016.12.024](https://doi.org/10.1016/j.envpol.2016.12.024)
- Dugan, H. A., and others. 2017a. Salting our freshwater lakes. *Proc. Natl. Acad. Sci.* **114**: 4453–4458. doi:[10.1073/pnas.1620211114](https://doi.org/10.1073/pnas.1620211114)
- Dugan, H. A., and others. 2020. Lakes at risk of chloride contamination. *Environ. Sci. Technol.* **54**: 6639–6650. doi:[10.1021/acs.est.9b07718](https://doi.org/10.1021/acs.est.9b07718)
- Dugan, H. A., and others. 2017b. Long-term chloride concentrations in North American and European freshwater lakes. *Sci. Data* **4**: 1–11. doi:[10.1038/sdata.2017.101](https://doi.org/10.1038/sdata.2017.101)
- Elphick, J. R., M. Davies, G. Gilron, E. C. Canaria, B. Lo, and H. C. Bailey. 2011. An aquatic toxicological evaluation of sulfate: The case for considering hardness as a modifying factor in setting water quality guidelines. *Environ. Toxicol. Chem.* **30**: 247–253. doi:[10.1002/etc.363](https://doi.org/10.1002/etc.363)
- Golubkov, S. M., N. V. Shadrin, M. S. Golubkov, E. V. Balushkina, and L. F. Litvinchuk. 2018. Food chains and their dynamics in ecosystems of shallow lakes with different water salinities. *Russ. J. Ecol.* **49**: 442–448.
- Gonçalves, A. M. M., B. B. Castro, M. A. Pardal, and F. Gonçalves. 2007. Salinity effects on survival and life history of two freshwater cladocerans (*Daphnia magna* and *Daphnia longispina*). *Ann. Limnol. - Int. J. Lim.* **43**: 13–20. doi:[10.1051/limn/2007022](https://doi.org/10.1051/limn/2007022)
- 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. doi:[10.1002/etc.3676](https://doi.org/10.1002/etc.3676)
- Gutierrez, M. F., and others. 2018. Salinity shapes zooplankton communities and functional diversity and has complex effects on size structure in lakes. *Hydrobiologia* **813**: 237–255. doi:[10.1007/s10750-018-3529-8](https://doi.org/10.1007/s10750-018-3529-8)
- Hébert, M.-P., and B. E. Beisner. 2020. Functional trait approaches for the study of metazooplankton ecology, p. 3–27. In M. A. Teodósio and A. B. Barbosa [eds.], *Zooplankton Ecology*. CRC Press, Taylor & Francis Group. doi:[10.1201/9781351021821-2](https://doi.org/10.1201/9781351021821-2)
- Hébert, M., B. E. Beisner, and R. Maranger. 2016a. A compilation of quantitative functional traits for marine and freshwater crustacean zooplankton. *Ecology* **97**: 1081–1081. doi:[10.1890/15-1275.1](https://doi.org/10.1890/15-1275.1)
- Hébert, M., B. E. Beisner, and R. Maranger. 2016b. A meta-analysis of zooplankton functional traits influencing ecosystem function. *Ecology* **97**: 1069–1080. doi:[10.1890/15-1084.1](https://doi.org/10.1890/15-1084.1)
- Hébert, M.-P., C. C. Symons, M. Cañedo-Argüelles, and B. E. Beisner. 2021. Trait data for “Lake salinization drives consistent losses of zooplankton abundance and diversity across coordinated mesocosm experiments”. Figshare, Data Set. doi:[10.6084/m9.figshare.17109143](https://doi.org/10.6084/m9.figshare.17109143)
- Hintz, W. D., and others. In press. Current water quality guidelines across North America and Europe do not protect lakes from salinization. *Proc. Natl. Acad. Sci.* doi:[10.1073/pnas.2115033119](https://doi.org/10.1073/pnas.2115033119)
- Hintz, W. D., B. M. Mattes, M. S. Schuler, D. K. Jones, A. B. Stoler, L. Lind, and R. A. Relyea. 2017. Salinization triggers a trophic cascade in experimental freshwater communities with varying food-chain length. *Ecol. Appl.* **27**: 833–844. doi:[10.1002/eap.1487](https://doi.org/10.1002/eap.1487)
- Hintz, W. D., and R. A. Relyea. 2019. A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. *Freshw. Biol.* **64**: 1081–1097. doi:[10.1111/fwbi.13286](https://doi.org/10.1111/fwbi.13286)
- Isanta-Navarro, J., S. E. Arnott, T. Klauschies, and D. Martin-Creuzburg. 2021. Dietary lipid quality mediates salt

- tolerance of a freshwater keystone herbivore. *Sci. Total Environ.* **769**: 144657. doi:[10.1016/j.scitotenv.2020.144657](https://doi.org/10.1016/j.scitotenv.2020.144657)
- Jensen, E., S. Brucet, M. Meerhoff, L. Nathansen, and E. Jeppesen. 2010. Community structure and diel migration of zooplankton in shallow brackish lakes: Role of salinity and predators. *Hydrobiologia* **646**: 215–229. doi:[10.1007/s10750-010-0172-4](https://doi.org/10.1007/s10750-010-0172-4)
- Jeppesen, E., and others. 2015. Ecological impacts of global warming and water abstraction on lakes and reservoirs due to changes in water level and related changes in salinity. *Hydrobiologia* **750**: 201–227. doi:[10.1007/s10750-014-2169-x](https://doi.org/10.1007/s10750-014-2169-x)
- Jeppesen, E., M. Søndergaard, A. R. Pedersen, K. Jürgens, A. Strzelczak, T. L. Lauridsen, and L. S. Johansson. 2007. Salinity induced regime shift in shallow brackish lagoons. *Ecosystems* **10**: 47–57. doi:[10.1007/s10021-006-9007-6](https://doi.org/10.1007/s10021-006-9007-6)
- Kaushal, S. S., G. E. Likens, M. L. Pace, R. M. Utz, S. Haq, J. Gorman, and M. Grese. 2018. Freshwater salinization syndrome on a continental scale. *Proc. Natl. Acad. Sci.* **115**: E574–E583. doi:[10.1073/pnas.1711234115](https://doi.org/10.1073/pnas.1711234115)
- Kefford, B. J., and others. 2012. Global scale variation in the salinity sensitivity of riverine macroinvertebrates: Eastern Australia, France, Israel and South Africa. *PLoS One* **7**: e35224. doi:[10.1371/journal.pone.0035224](https://doi.org/10.1371/journal.pone.0035224)
- Korosi, J. B., S. M. Burke, J. R. Thienpont, and J. P. Smol. 2012. Anomalous rise in algal production linked to lakewater calcium decline through food web interactions. *Proc. R. Soc. B Biol. Sci.* **279**: 1210–1217. doi:[10.1098/rspb.2011.1411](https://doi.org/10.1098/rspb.2011.1411)
- Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. *Ecology* **91**: 299–305. doi:[10.1890/08-2244.1](https://doi.org/10.1890/08-2244.1)
- Laliberté, E., P. Legendre, and B. Shipley. 2014. FD: Measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-12.
- Lin, Q., L. Xu, J. Hou, Z. Liu, E. Jeppesen, and B.-P. Han. 2017. Responses of trophic structure and zooplankton community to salinity and temperature in Tibetan lakes: Implication for the effect of climate warming. *Water Res.* **124**: 618–629. doi:[10.1016/j.watres.2017.07.078](https://doi.org/10.1016/j.watres.2017.07.078)
- Loureiro, C., A. P. Cuco, M. T. Claro, J. I. Santos, M. A. Pedrosa, F. Gonçalves, and B. B. Castro. 2015. Progressive acclimation alters interaction between salinity and temperature in experimental *Daphnia* populations. *Chemosphere* **139**: 126–132. doi:[10.1016/j.chemosphere.2015.05.081](https://doi.org/10.1016/j.chemosphere.2015.05.081)
- McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples, p. 228–265. In J. A. Downing and F. H. Rigler [eds.], *A manual on methods for the assessment of secondary productivity in fresh waters*. Blackwell Scientific Publications.
- Moffett, E. R., H. K. Baker, C. C. Bonadonna, J. B. Shurin, and C. C. Symons. 2020. Cascading effects of freshwater salinization on plankton communities in the Sierra Nevada. *Limnol. Oceanogr. Lett.* doi:[10.1002/lol2.10177](https://doi.org/10.1002/lol2.10177)
- Nielsen, D. L., M. A. Brock, K. Crossle, K. Harris, M. Healey, and I. Jarosinski. 2003a. The effects of salinity on aquatic plant germination and zooplankton hatching from two wetlands sediments. *Freshw. Biol.* **48**: 2214–2223. doi:[10.1046/j.1365-2427.2003.01146.x](https://doi.org/10.1046/j.1365-2427.2003.01146.x)
- Nielsen, D. L., M. A. Brock, G. N. Rees, and D. S. Baldwin. 2003b. Effects of increasing salinity on freshwater ecosystems in Australia. *Aust. J. Bot.* **51**: 655–665. doi:[10.1071/BT02115](https://doi.org/10.1071/BT02115)
- R Core Team. 2020. R: A language and environment for statistical computing.
- Schallenberg, M., C. J. Hall, and C. W. Burns. 2003. Consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes. *Mar. Ecol. Prog. Ser.* **251**: 181–189. doi:[10.3354/meps251181](https://doi.org/10.3354/meps251181)
- Setubal, R. B., E. O. de Sodr , T. Martins, and R. L. Bozelli. 2020. Effects of functional diversity and salinization on zooplankton productivity: An experimental approach. *Hydrobiologia* **847**: 2845–2862. doi:[10.1007/s10750-020-04276-0](https://doi.org/10.1007/s10750-020-04276-0)
- Soucek, D. J., T. K. Linton, C. D. Tarr, A. Dickinson, N. Wickramanayake, C. G. Delos, and L. A. Cruz. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. *Environ. Toxicol. Chem.* **30**: 930–938. doi:[10.1002/etc.454](https://doi.org/10.1002/etc.454)
- United States Environmental Protection Agency (US-EPA). 1988. Ambient water quality criteria for chloride.
- Van Meter, R. J., and C. M. Swan. 2014. Road salts as environmental constraints in urban pond food webs. *PLoS One* **9**: e90168. doi:[10.1371/journal.pone.0090168](https://doi.org/10.1371/journal.pone.0090168)
- Van Meter, R. J., C. M. Swan, J. Leips, and J. W. Snodgrass. 2011. Road salt stress induces novel food web structure and interactions. *Wetlands* **31**: 843–851. doi:[10.1007/s13157-011-0199-y](https://doi.org/10.1007/s13157-011-0199-y)
- Villéger, S., N. W. H. Mason, and D. Mouillot. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* **89**: 2290–2301. doi:[10.1890/07-1206.1](https://doi.org/10.1890/07-1206.1)
- Wang, Y., U. Naumann, S. T. Wright, and D. I. Warton. 2012. mvabund – an R package for model-based analysis of multivariate abundance data. *Methods Ecol. Evol.* **3**: 471–474. doi:[10.1111/j.2041-210X.2012.00190.x](https://doi.org/10.1111/j.2041-210X.2012.00190.x)
- Williams, W. D. 2001. Anthropogenic salinisation of inland waters. *Hydrobiologia* **466**: 329–337. doi:[10.1023/a:1014598509028](https://doi.org/10.1023/a:1014598509028)

Acknowledgments

This study is part of the Global Salt Initiative, a working group formed during the Global Lake Ecological Observatory Network (GLEON) meeting held in 2017. Our working group acknowledges funding from the Canadian Institute of Ecology and Evolution (CIEE) awarded to SEA and WDH, and the Québec Interuniversity Research Group in Limnology (Groupe de recherche interuniversitaire en limnologie; GRIL) awarded to AMD and BEB. We thank Kathryn L. Cottingham for her assistance with the Dartmouth site experiment. MPH was supported by the Natural Sciences

and Engineering Research Council (NSERC) and the Fonds de Recherche du Québec Nature-Technologies (FRQNT); MCA was supported by the Serra Húnter Programme. LP was supported by the Programme Beatriu de Pinós (Secretary of Universities and Research, Government of Catalonia) and the Horizon Programme of Research and Innovation of the European Union under the Marie Skłodowska-Curie Grant Agreement No. 801370. We acknowledge contributions from individual research grants awarded by the NSERC Discovery Grant program (AMD, BEB, DG, SEA, SM, and Gregor F. Fussmann) and the National Science Foundation (CFS). We are also grateful for various forms of support from the Ontario Ministry of Environment, Conservation, and Parks (Best-in-Science; SEA), Queen's University Biological Station (SEA), Dorset Environmental Science Centre (SEA, JR), Ohio Wesleyan Summer Science Research Program (AD), Jefferson Project at Lake George (WDH, RAR, MSS; a collaboration of the Rensselaer Polytechnic Institute, IBM Research, and The FUND for Lake

George), the University of Toledo (WDH) the European Commission LIFE program via the LIFE DEMINE project (LP), the Swedish Research Council (GAW, HL, PU), Linnaeus University (SH), Umeå University (LL), and Uppsala University (SL). We also acknowledge the Swedish Infrastructure for Ecosystem Science (SITES) for provisioning of the SITES AquaNet mesocosm facility at three sites (Asa, Erken and Svartberget; grant 2017-00635 funded by the Swedish Research Council).

Submitted 10 June 2021

Revised 13 December 2021

Accepted 22 January 2022