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SPECIAL ISSUE-LETTER

Effects of freshwater salinization on a salt-naïve planktonic eukaryote community

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Scientific Significance Statement

Freshwater salinization is an increasing ecological and socioeconomic concern, with widespread evidence for increased salinity of inland waters associated with anthropogenic inputs and climate change. However, there remains an incomplete understanding of how increasing salinity of freshwater will alter the diversity and abundance of planktonic communities. We found that at salt concentrations relevant to U.S. and Canada water quality guidelines, the planktonic eukaryote community was highly simplified by the loss of almost all zooplankton and the loss of taxonomic diversity across planktonic groups, with only fungi and phytoplankton remaining at > 640 mg $Cl^- L^{-1}$. Overall, our findings support that freshwater salinization can have devastating effects on the freshwater planktonic community in a lake without previous history of salt exposure, even at concentrations recommended by guidelines for water quality standards.

Abstract

Freshwater salinization is a widespread issue, but evidence of ecological effects on aquatic communities remains scarce. We experimentally exposed salt-naive plankton communities of a north-temperate, freshwater lake to a gradient of chloride (Cl^-) concentration (0.27–1400 mg Cl L^{-1}) with in situ mesocosms. Following 6 weeks, we measured changes in the diversity, composition, and abundance of eukaryotic 18S rRNA gene. Total phytoplankton biomass remained unchanged, but we observed a shift in dominant phytoplankton groups with increasing salt concentration, from Cryptophyta and Chlorophyta at lower chloride concentrations (< 185 mg $Cl^- L^{-1}$) to Ochrophyta at higher chloride concentrations (> 185 mg $Cl^- L^{-1}$). Crustacean zooplankton and rotifers were sensitive to the salinity, and disappeared at low chloride concentrations (< 40 mg $Cl^- L^{-1}$). While ciliates thrived at low chloride concentrations (< 185 mg $Cl^- L^{-1}$), fungal groups dominated at intermediate chloride concentrations (185–640 mg $Cl^- L^{-1}$), and only phytoplankton remained at the highest chloride concentrations (> 640 mg $Cl^- L^{-1}$).

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Author Contribution Statement: LA was the overall lead on the study. LA and AD designed the study. JCG and CSL conducted and supported lab analysis (DNA extraction and sequencing). LA, JCG, and AD conducted field work to implement the experiment. LA conducted the bioinformatics and statistical analyses. LA and AD wrote the paper.

Data Availability Statement: Data and metadata are available in the environmentaldatainitiative.org data repository (Astorg et al. 2021) https://doi.org/10.6073/pasta/e22781fd9df659ce107e58d200f1a6e9. Sequences are available at http://www.ncbi.nlm.nih.gov/bioproject/733220.

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

Salt pollution and accelerated weathering from human activities are drastically changing freshwater ion composition (Kaushal et al. 2018). Such freshwater salinization syndrome (Kaushal et al. 2018) is a widespread yet underappreciated environmental problem that profoundly affecting freshwater community composition, diversity, and stability (Hintz et al. 2017; Hintz and Relyea 2019; Mo et al. 2021; Moffett et al. 2021), which threatens freshwater ecosystems globally (Cañedo-Argüelles et al. 2013; Herbert et al. 2015; Castillo et al. 2018; Hintz and Relyea 2019). Plankton communities are the resource base that supports higher trophic levels, such as fisheries, and essential ecosystem functions such as primary production, decomposition, and nutrient and carbon cycling (Kalff 2001). Recent studies have addressed the effects of anthropogenic salinization on zooplankton (Hintz et al. 2017; Hintz and Relyea 2019; Arnott et al. 2020; Setubal et al. 2020; Moffett et al. 2021), but little research has addressed salinization effects on other planktonic groups, such as phytoplankton (Blinn et al. 2004; Ballot et al. 2009; Fay and Shi 2012; Porter-Goff et al. 2013; Hintz et al. 2017), fungi (Schäfer et al. 2012; Sauer et al. 2016; Gonçalves et al. 2019; da Silva et al. 2021), and to our knowledge one other study has addressed ciliates and other unicellular eukaryotes (Mo et al. 2021). We present one of the first studies to address freshwater salinization impacts using a DNA metabarcoding framework that considers all of these groups together within the same community.

Planktonic eukaryote communities are comprised of diverse organisms such as ciliates and flagellates, fungi, phytoplankton, and zooplankton (copepods, cladocerans, and rotifers) (Kalff 2001). Zooplankton is a critical food resource for higher trophic levels, and regulate water clarity, energy, and nutrients in freshwater ecosystems (Pace and Orcutt 1981; Carpenter et al. 1985). Cladocerans are usually relatively tolerant to salinization (Sarma et al. 2006; Searle et al. 2016; Lind and Jeyasingh 2018; Moffett et al. 2021). However, *Daphnia* in soft water lakes were sensitive to low chloride concentrations (between 5 and 40 mg $Cl^- L^{-1}$) (Arnott et al. 2020). Copepods can also vary in their response to salinization; while certain calanoids decrease in abundance at 200 mg $Cl^- L^{-1}$ (Evans and Frick 2001; Moffett et al. 2021), cyclopoid species seem more resistant and can maintain high abundance over 500 mg $Cl^- L^{-1}$ (Van Meter and Swan 2014; Moffett et al. 2021). Rotifers possess a wide range of tolerances to salinization, with tolerant species showing signs of reduced abundance at or beyond 1.5 g $Cl^- L^{-1}$ (Sarma et al. 2006; Van Meter and Swan 2014). Increased salinity has also been found to impact zooplankton sediment egg bank (Brock et al. 2005). Overall, tolerance thresholds to chloride concentrations are greatly variable among zooplankton taxa and seem to be influenced by local conditions (Arnott et al. 2020; Moffett et al. 2021). For phytoplankton, responses to elevated chloride levels can increase phytoplankton biomass (Fay and Shi 2012; Hintz et al. 2017) and shift community composition (Ballot et al. 2009; Porter-Goff et al. 2013; Mo et al. 2021). Freshwater salinization can reduce growth and abundance of diatoms (Porter-Goff et al. 2013), which are nutritious food sources for higher trophic levels (Taipale et al. 2013). However, overall, our understanding of the impact of elevated chloride concentration on phytoplankton is deficient (Hintz and Relyea 2017).

Our study provides one if the first investigation to empirically address community changes in diversity and relative abundance from unicellular to zooplankton within a freshwater eukaryote community of a salt-naïve boreal lake in response to increasing salinity. Our objectives were to: (1) determine the sensitivity/tolerance of different plankton groups to freshwater salinization and (2) evaluate how planktonic eukaryote community sensitivity relates to salinity water quality guidelines for the protection of aquatic freshwater life in U.S., Canada, and European Union (EU) drinking water guidelines. We predicted that the abundance and composition of most planktonic groups would be negatively affected by salinization in a salt-naïve freshwater ecosystem. Furthermore, we predicted that phytoplankton biomass would increase as a result of the release of grazing pressure associated with a loss of sensitive zooplankton groups (Fay and Shi 2012; Hintz et al. 2017; Moffett et al. 2021). Traditional ecological monitoring of communities is labor intensive, costly, and morphological identifications can be challenging. Molecular approaches, such as DNA metabarcoding can be very useful for monitoring freshwater biodiversity (Cristescu 2014; Lim et al. 2016). In this study, we investigated the planktonic eukaryote community using DNA metabarcoding with the eukaryotic 18S rRNA gene as target sequence. This approach allowed us to detect major shifts in community composition of eukaryotic organisms in semi-natural experimental setting. Our study addresses the paucity of research on ecological effects of salinization on freshwater eukaryote communities, and our findings provide perspectives on the applicability of water quality guidelines for the protection of aquatic life in Canada and in the United States (US EPA 1988; Environment and Climate Change Canada 2011), and drinking water guidelines of the EU.

Methods

We conducted an in situ field experiment in which we employed a gradient design in sodium chloride (NaCl) treatment to increase chloride concentration ranging from 0.27 to 1400 mg $Cl^- L^{-1}$ in a salt-naïve, north-temperate lake with no prior history of salt exposure. Our experimental design concentrations representative of long-term (Canada: 120 mg $Cl^- L^{-1}$; United States: 230 mg $Cl^- L^{-1}$) and short-term (Canada: 640 mg $Cl^- L^{-1}$; United States: 860 mg $Cl^- L^{-1}$) water quality guidelines for chloride concentrations in freshwater, for the protection of aquatic life in Canada and the United States (US EPA 1988; Environment and Climate Change

Canada 2011). Drinking water guideline throughout most of the EU are 250 mg Cl^- L^{-1} (Schulz and Cañedo-Argüelles 2019); however, in Europe, salinity water quality standards for the protection of aquatic life of freshwater ecosystems are mostly absent (Schuler et al. 2019).

In situ field experiment

The mesocosm experiment was implemented with water and biological communities from Lake Croche (45°59′17.34″N/74°00′20.75″W), which is located at the Station de biologie des Laurentides de l'Université de Montréal (Québec, Canada). Lake Croche is a salt-naïve oligo-mesotrophic lake situated in the Laurentian region of Québec, which lies within the Canadian Shield, and has a low ambient chloride concentration of 0.27 mg Cl^- L^{-1}. The experimental design involved 21 mesocosms, from which we focused DNA sampling on 9 mesocosms (Fig. 1). The experiment was conducted for 6 weeks (23 June to 03 August 2018). Lake water used to fill the mesocosms was sampled and analyzed at the beginning of the experiment (hereafter referred as "start"), and each mesocosm was sampled after 3 and 6 weeks from the start of the experiment (*see* Appendix for supplementary methods; Appendix Table A1: summary of environmental conditions in mesocosms). Initial chloride concentrations were measured along with conductivity from water samples collected from the mesocosms following the NaCl addition. Conductivity was subsequently utilized as a proxy of chloride concentration because of a strong relationship between chloride and conductivity in this experiment ($R^2 = 0.986$, $p = < 2\text{e-}16$) (Fig. 2), and further analyses were performed using calculated chloride concentration from conductivity measures. Sodium cation might have been partly or jointly responsible for measured effects, but since we had no direct measures of sodium and most of the literature refers to chloride concentration, we present our results in units of chloride concentration.

Molecular analyses

To detect changes in the diversity, relative abundance, and community composition of planktonic eukaryotes, we analyzed eukaryotic 18S rRNA gene (region V7) diversity from water samples collected from the mesocosms at the start of the experiment, and at each of weeks 3 and 6 of the experiment in nine different mesocosms (Fig. 1, red circles). DNA field sampling methods and laboratory methods for filtration, DNA extraction, PCR amplification, and sequencing are provided in Appendix. For bioinformatics of 18S metabarcoding, the DADA2 (Callahan et al. 2016) workflow was used. Pseudo-pooling was used to infer amplicon sequence variants (ASVs). ASVs refer to single DNA sequences recovered from a high-throughput marker gene analysis and are used to classify groups of species based on DNA sequences. We applied a variance stabilizing transformation method to the dataset of the different samples (McMurdie and Holmes 2014). We investigated the potential positive relationship between Sequencing depth and ASV diversity in the different samples which was found to be negative (Fig. A1). We used R 4.0.3 (R Core Team 2020) phyloseq package (McMurdie and Holmes 2013) rarefaction function to analyze rarefied data. Downstream analysis results of rarefied data are reported in the Supplementary Material (Figs. A4–A6). Rarefaction did not have major impact on the results of the manuscript (Figs. A4–A6). We therefore chose to report nonrarefied data in the main sections of the manuscript. Sequences are available at http://www.ncbi.nlm.nih.gov/bioproject/733220, and other supporting data for the experiment are stored in a public data repository (Astorg et al. 2021).

Data analyses

Multivariate regression tree

The multivariate regression tree (MRT) technique splits the data into clusters of samples similar in their species composition based on environmental value thresholds. We included temperature, chloride concentration, DO, pH, and TP to construct the regression tree. Prior to MRT, community abundance data based on read counts was Hellinger transformed to down-weight the influence of rare species (Legendre and Gallagher 2001). MRT analyses were performed in R 4.0.3 (R Core Team 2020).

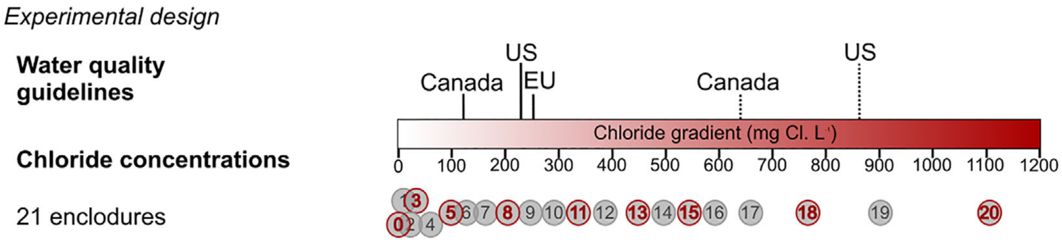

Fig. 1. Experimental gradient design with chloride (Cl^-) concentrations of the 21 mesocosm enclosures (gray and red circles), 9 mesocosms enclosures sampled for 18S eukaryotic rRNA gene (red circle), and how mescocosm Cl^- concentrations relate to the short-term application (dashed line) and long-term (solid line) water quality guidelines recommended for Cl^- concentrations in freshwater environments by Canada and the United States (US EPA 1988; Environment and Climate Change Canada 2011) for the protection of aquatic life, and the EU drinking water guidelines (Schuler et al. 2019).

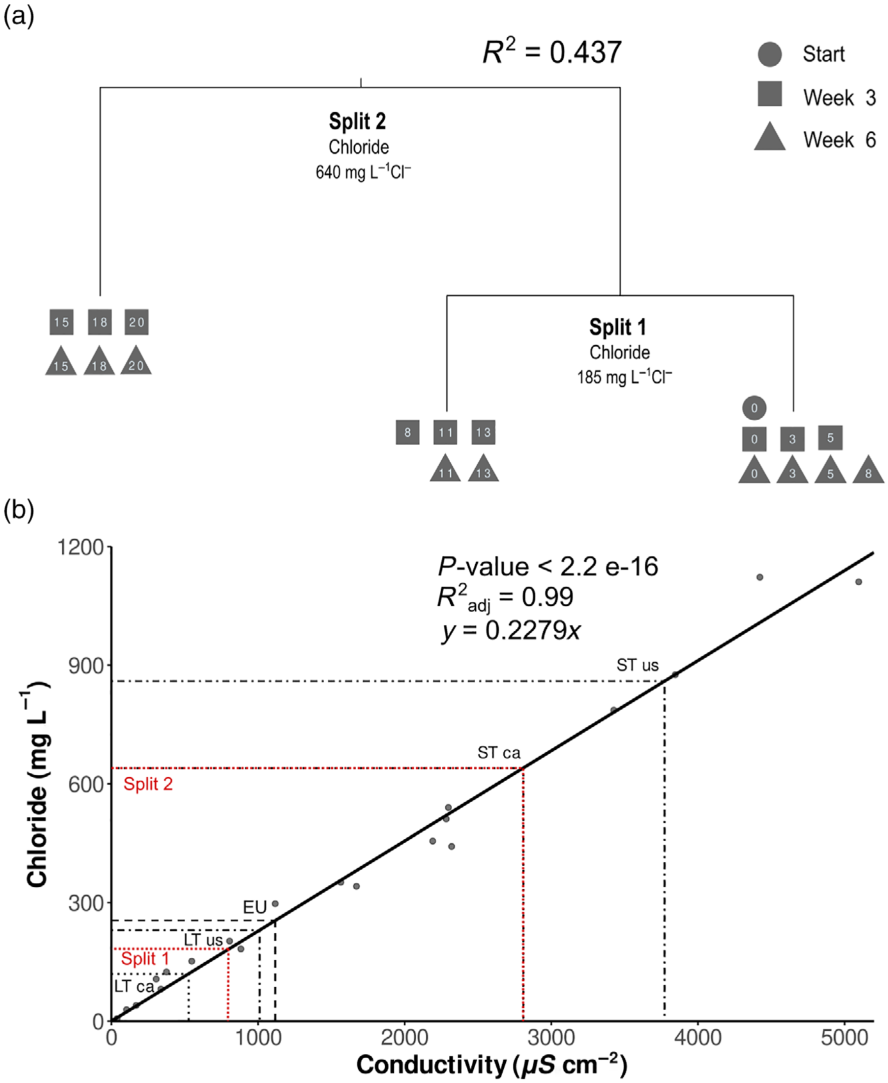

Fig. 2. MRT of plankton community clustering in relation to chloride concentration and freshwater salinity water quality guidelines: (**a**) MRT obtained from multiple regression analysis with environmental variables on the Hellinger-transformed ASV matrix. Each branch represents mesocosms with similar plankton communities, and splits are explained by chloride concentration. Enclosure shapes represent different sampling dates (circles refers to lake water at the start of the experiment, squares refer to week 3, and triangles refer to week 6) and numbers refer to the identity of mesocosms that correspond with different levels of the NaCl treatment (Fig. 1). (**b**) Linear regression between measured conductivity and chloride concentration in the larger set of mesocosms (21 mesocosms; Fig. 1). Black dashed lines are showing short-term (ST) and long-term (LT) water quality guidelines that are recommended for chloride concentrations in freshwater environments in Canada and the United States (US EPA 1988; Environment and Climate Change Canada 2011) for the protection of aquatic life. The EU dashed line represents drinking water guideline across much of the EU (Schulz and Cañedo-Argüelles 2019). Red dashed lines indicate splits obtained with MRT analysis displayed above in panel (**a**).

Indicator species

To highlight indicator species in the different clusters generated by the MRT analysis, we used the indispecies package (De Cáceres and Legendre 2009) in R 4.0.3 (R Core Team 2020). Indicator species are determined by using species occurrences, species abundances, and sites classification representing habitats type or disturbance states.

Diversity indices and generalized linear models

Community diversity indices were calculated using 18S metabarcoding read counts. We calculated taxon richness, Shannon diversity (Shannon 1948), and Pielou's evenness (Pielou 1966). These diversity indices were included as response variables in different generalized linear models that tested the influence chloride concentration (Appendix).

Principal coordinate analysis

To visualize differences in mesocosms ASV composition and abundance, we performed principal coordinate analysis (PCoA) on ASVs. We used Bray–Curtis dissimilarity distance on squared ASV community matrix. Each analysis produced a two-dimensional plot based on the first two PCoA scores. We used PERMANOVA using 9999 permutations to test the influence of salinity on the multivariate community data. All analyses were performed in R 4.0.3 (R Core Team 2020).

Results

Community shift with elevated chloride

Three plankton community clusters of ASVs were identified and splits were explained by chloride concentration corresponding to community splits at 185 and 640 mg Cl^- L^{-1} (Fig. 2; MRT). The first cluster spanned low chloride concentrations between 0.27 and 185 mg Cl^- L^{-1}, the second cluster spanned intermediate chloride concentrations between 185 and 640 mg Cl^- L^{-1}, and the third cluster spanned high chloride concentrations greater than 640 mg Cl^- L^{-1}. The low chloride mesocosms (identified as 0–5 at week 3 and as mesocosm 8 at week 6) (Fig. 2) were comprised of diversified planktonic communities. These included phytoplankton (indicator phylums: Ochrophyta, Cryptophyta, Diatomea, Phragmoplastophyta) (details on indicator taxa Table A2), fungi (indicator phylums: Peronosporomycetes), as well as protozoans (indicator phylums: Ciliophora, MAST, and Cercozoa) and multicellular eukaryotes, including zooplankton and rotifers, but with an increasing dominance of unicellular eukaryotes (mainly Ciliophora) as chloride increased within the group of low chloride mesocosms (Fig. 3). The intermediate chloride mesocosms (identified as 8 at weeks 3–13; Fig. 2) showed a sharp increase in fungal taxa (indicator phylums: Cryptomycota and Basidiomycota) in the planktonic community, but most zooplankton and other unicellular eukaryotes were lost (Fig. 3). The high chloride mesocosms (identified as 15–20; Fig. 2) were characterized by the dominance of phytoplankton ASVs (indicator phylums: Ochrophyta and Diatomea), which represented almost the totality of the planktonic community at such water chloride (Fig. 3). Overall, chloride concentration had a significant effect on planktonic community composition (PERMANOVA p-value $= 0.0001$). The first planktonic community split as a response to increased chloride occurred at 185 mg Cl^- L^{-1} and is between the long-term freshwater chloride concentration recommended by Canada (120 mg Cl^- L^{-1}) and the United States (230 mg Cl^- L^{-1}) (US EPA 1988; Environment and Climate Change Canada 2011; Fig. 2). The second planktonic community split occurred at 640 mg Cl^- L^{-1}, and represents the short-term freshwater chloride concentration recommended by Canada for the protection of aquatic life (Environment and Climate Change Canada 2011; Fig. 2).

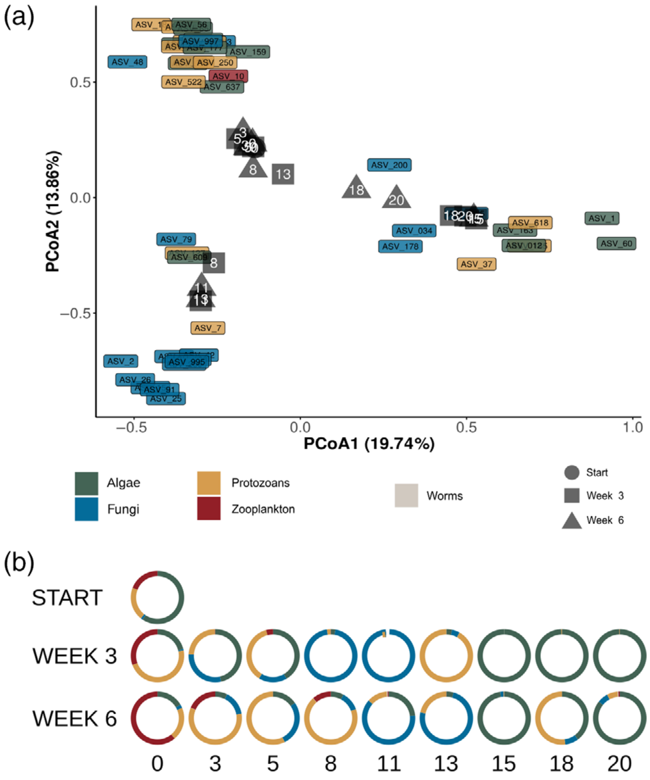

Fig. 3. The response of the plankton community to elevated chloride concentration. (**a**) PCoA with Bray–Curtis dissimilarity matrix of squared ASV matrix. Labels are unique ASVs with colors showing their taxonomic group. Enclosure shapes represent different sampling dates (circles refers to lake water at the start of the experiment, squares refer to week 3, and triangles refer to week 6) and numbers are different levels of the NaCl treatment. Only significant ASVs from the indispecies analysis are displayed. (**b**) Relative abundance of the different plankton groups. Each circle is an enclosure and the colors within the circle are the proportions of ASVs from the different taxonomic groups displayed above. The different rows represent different sampling dates and numbers are different levels of the NaCl treatments in the experiment.

Phytoplankton community

The 18S rRNA gene showed that certain freshwater phytoplankton groups were sensitive to elevated chloride concentrations (e.g., Cryptophyta, Dinoflagellata, Bacillariophyta, Charophyta, Protalveolata, and Streptophyta), while others were more salt-tolerant because they had large increases in relative abundance in medium and high chloride mesocosms (e.g., Chlorophyta and Ochrophyta) (Fig. 3). Chlorophyta persisted at low and increased at intermediate water chloride. High water chloride concentrations, however, could only be withstood by Ochrophyta (of the Chrysophyceae class), and all other phytoplankton groups disappeared completely or were maintained at very low relative abundances. We also noted that Ochrophyta relative abundance increased in control mesocosms compared to the lake community over the duration of the experiment, and so may also partially reflect a positive response to mesocosm conditions. Chloride concentration had a significant effect on the phytoplankton community composition (PERMANOVA p-value = 0.0001). Overall, phytoplankton community diversity decreased with elevated chloride concentrations (Appendix Table A3; Fig. A2). However, chlorophyll (Chl) a, an estimate of total phytoplankton biomass, had no detectable aggregate response to the salt addition across the experimental gradient. (Appendix Fig. A3; Table A4).

Discussion

Our experiment supports that freshwater salinization may cause the simplification and potential collapse of aquatic eukaryote communities, especially in salt-naïve lakes with no prior exposure to salt. First, we found that major compositional shifts in the plankton community occurred at chloride concentrations that corresponded with long-term and short-term recommendations for water quality guidelines in Canada, the EU, and the United States (Fig. 2). Second, we observed that compositional shifts in the plankton community were associated with important shifts toward by fungal taxa at intermediate chloride concentrations (185–640 mg Cl^- L^{-1}), and toward phytoplankton at elevated chloride concentrations (above 640 mg Cl^- L^{-1}; Fig. 3). Third, we highlight that, with increasing chloride concentrations, the compositional shifts in the phytoplankton community were not associated with a strong decline in biomass (Fig. 4). Lastly, we found that the overall diversity of planktonic organisms was greatly reduced with elevated chloride concentration (Fig. A2; Table A3). Our findings highlight the profound effects of freshwater salinization on all compartments of the planktonic eukaryote community, and that chloride concentrations recommended by water quality guidelines might not be sufficient to prevent major changes in salt-naïve aquatic eukaryote communities.

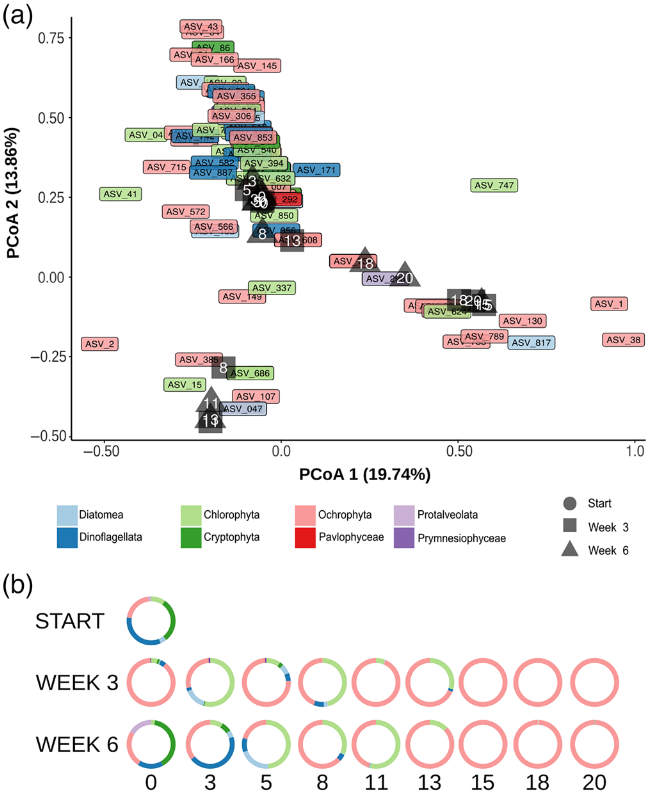

Fig. 4. The response of the phytoplankton community to elevated chloride (Cl^-) concentration. (**a**) PCoA with Bray–Curtis dissimilarity matrix of squared phytoplankton ASV matrix. Labels are unique ASVs with colors showing the phytoplankton phylum. Enclosure shapes represent different sampling dates (circles refers to lake water at the start of the experiment, squares refer to week 3, and triangles refer to week 6) and numbers are different levels of the NaCl treatment. (**b**) Relative abundance of the different phytoplankton phyla. Each circle is an enclosure and the colors within the circle are the proportions of ASVs from the different phytoplankton phyla. The different rows represent different sampling dates and numbers are different levels of the NaCl treatment in the experiment.

Simplified planktonic eukaryote community at elevated chloride concentrations

Increasing freshwater salinity was associated with decreased eukaryotic community diversity in a salt-naïve freshwater lake (Fig. A2). The eukaryotic community was greatly simplified and held few dominant ASVs in high chloride mesocosms compared to the hundreds of ASVs detected in low chloride mesocosms (Fig. 3). In the intermediate and high chloride mesocosms, the eukaryote community was almost entirely reduced to phytoplankton or fungal species, and zooplankton were almost entirely lost. While other studies have found that certain zooplankton persist higher chloride levels up to 1300 mg Cl^- L^{-1} (Coldsnow et al. 2017; Hintz et al. 2017; Moffett et al. 2021), this was not the case in our study where cladocerans, copepods, and rotifers were all sensitive to increasing freshwater salinity. The simplification of the planktonic eukaryote community detected in our experiment has the potential to modify ecosystem function and productivity in salt-naïve freshwater lakes, if the responses that we measured in experimental mesocosms extend to the whole ecosystem scale in lakes (energy pathways: Sherwood et al. 2011; O'Gorman et al. 2019; productivity: Liess et al. 2009; Higgins et al. 2014; and food web stability: Fussmann et al. 2014; Binzer et al. 2016; Mo et al. 2021).

Phytoplankton

The phytoplankton community shifted toward Ochrophyta (Chrysophyceae) with the addition of NaCl in mesocosms. This shift also occurred over time in the control mesocosms, suggesting that this response may also be a response to mesocosm conditions, but it was most strongly pronounced at high salinity. Moreover, there was no change in total phytoplankton biomass, indicated by Chl a concentration, over the course of the experiment. This suggests that as certain phytoplankton groups were eliminated when their ecological tolerances were exceeded with increasing salinity, phytoplankton biomass was compensated by the increased abundance of salinity-tolerant taxa such as Ochrophyta. Only a handful of studies, including our study, have addressed community compositional shifts in phytoplankton as a response to elevated chloride concentrations (Ballot et al. 2009; Porter-Goff et al. 2013; Mo et al. 2021). Studies that analyzed phytoplankton composition changes have found that small changes in salinity can alter dominance of phytoplankton phylums (Mo et al. 2021), benthic diatom were sensitive to relatively low chloride concentration (35 mg $Cl^- L^{-1}$) (Porter-Goff et al. 2013), and very high chloride concentration (2000–5000 mg $Cl^- L^{-1}$) favor cyanobacteria in eutrophic conditions with high nutrient loadings (Ballot et al. 2009). We also found that highly nutritious phytoplankton (cryptophytes and diatoms) were sensitive to increased chloride concentration, and were replaced by less nutritious groups (chlorophytes and chrysophytes) at relatively low chloride concentration (< 185 mg $Cl^- L^{-1}$). Elevated chloride can result in an increase in the total biomass of phytoplankton under certain conditions (Ballot et al. 2009; Hintz et al. 2017; Moffett et al. 2021), but this response is not consistent across studies (Hintz et al. 2021, unpubl.). For our experiment, the oligo-mesotrophic nutrient status of the lake and nutrient additions to experimental mesocosms (Appendix) may explain the stability of phytoplankton biomass despite the reduction and near total loss of zooplankton grazers at elevated chloride concentrations.

Different phytoplankton groups have different nutritional value in terms of the essential fatty acids that they provide to higher consumers in pelagic food webs of lakes (Taipale et al. 2013). The shift in phytoplankton community composition as a response to increasing salinity represented a change toward a lower quality resource base. Cryptophyta and Bacillariophyta (diatoms) are highly nutritious phytoplankton groups containing a high proportion of essential highly unsaturated fatty acids (Taipale et al. 2013) that are critical for organismal reproduction at higher trophic levels such as zooplankton (Brett et al. 2009; Trommer et al. 2019). Chlorophyta and Chrysophyceae provide an intermediate level of dietary quality for zooplankton (Taipale et al. 2013). While the diverse phytoplankton assemblage at low chloride concentrations included highly nutritious groups of phytoplankton, such as diatoms and cryptophytes, the high salinity mesocosms contained less nutritious chrysophytes (Ochrophyta). Phytoplankton nutritional quality is important for somatic growth, reproduction, disease resistance, tissue development, and pigmentation at higher trophic levels in aquatic food webs (Twining et al. 2016). Moreover, the susceptibility of freshwater zooplankton to salinization can be highly dependent on the dietary quality of lipid supply available in the phytoplankton community (Isanta-Navarro et al. 2021). Therefore, increasing freshwater salinization on landscapes may provide challenges to aquatic food webs not only through direct toxicity, but also through reduced nutritional provisioning to higher consumers such as zooplankton, fish, and other vertebrates.

Implications and conclusions

Short-term (640 mg $Cl^- L^{-1}$ in Canada and 840 mg $Cl^- L^{-1}$ in the United States), long-term (120 mg $Cl^- L^{-1}$ in Canada and 230 mg $Cl^- L^{-1}$ in the United States), and the EU drinking water guidelines (250 mg $Cl^- L^{-1}$) recommended chloride concentrations (US EPA 1988; Environment and Climate Change Canada 2011; Schuler et al. 2019) had profound effects on the planktonic eukaryote community of a salt-naïve lake ecosystem. Short-term salinity pulses in stream and rivers, and longer-term salinity exposure in lakes generally exceed 3 weeks of exposure (Kaushal et al. 2018; Laceby et al. 2019; Niedrist et al. 2021). Therefore, the responses that we observed in the mesocosms over a short time scale have the potential to occur in freshwater ecosystems. However, other complex and interacting factors occur in natural ecosystems that were excluded from our semi-natural mesocosm experiment, such as metacommunity dynamics through habitat connectivity and organismal dispersal, evolutionary responses, and the effects of multiple stressors interacting with the effects of salinity. Nonetheless, our experiment demonstrates that water quality guidelines for chloride concentrations in freshwater ecosystems provided by Canada, the EU, and the United States are not adequate to ensure the conservation of planktonic communities in salt-naïve freshwater ecosystems with low ambient chloride concentrations.

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

Astorg, L., J. Gagnon, C. Lazar & A. Derry 2021. Effects of freshwater salinization on a salt-naïve planktonic eukaryote community ver 1. Environmental Data Initiative. doi: 10.6073/pasta/e22781fd9df659ce107e58d200f1a6e9

Ballot, A., K. Kotut, E. Novelo, and L. Krienitz. 2009. Changes of phytoplankton communities in Lakes Naivasha and Oloidien, examples of degradation and salinization of lakes in the Kenyan Rift Valley. Hydrobiologia **632**: 359–363. doi:10.1007/s10750-009-9847-0

Binzer, A., C. Guill, B. C. Rall, and U. Brose. 2016. Interactive effects of warming, eutrophication and size structure: Impacts on biodiversity and food-web structure. Glob. Chang. Biol. **22**: 220–227. doi:10.1111/gcb.13086

Blinn, D., S. Halse, A. Pinder, and R. Shiel. 2004. Diatom and micro-invertebrate communities and environmental determinants in the western Australian wheatbelt: A response to salinization. Hydrobiologia **528**: 229–248. doi:10.1007/s10750-004-2350-8

Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. PNAS. **106**: 21197–21201. doi:10.1073/pnas.0904129106

Brock, M. A., D. L. Nielsen, and K. Crosslé. 2005. Changes in biotic communities developing from freshwater wetland sediments under experimental salinity and water regimes. Freshw. Biol. **50**: 1376–1390. doi:10.1111/j.1365-2427.2005.01408.x

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods **13**: 581–583. doi:10.1038/nmeth.3869

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. doi:10.1016/j.envpol.2012.10.011

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. Bioscience **35**: 634–639. doi:10.2307/1309989

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. doi:10.1007/s10750-017-3403-0

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

Cristescu, M. E. 2014. From barcoding single individuals to metabarcoding biological communities: Towards an integrative approach to the study of global biodiversity. Trends Ecol. Evol. **29**: 566–571. doi:10.1016/j.tree.2014.08.001

De Cáceres, M., and P. Legendre. 2009. Associations between species and groups of sites: Indices and statistical inference. Ecology **90**: 3566–3574. doi:10.1890/08-1823.1

Environment and Climate Change Canada. 2011. Canadian water quality guidelines for the protection of aquatic life - chloride. Canadian Council of Ministers of the Environment. https://ccme.ca/en/res/chloride-en-canadian-water-quality-guidelines-for-the-protection-of-aquatic-life.pdf

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

Fay, L., and X. Shi. 2012. Environmental impacts of chemicals for snow and ice control: State of the knowledge. Water Air Soil Pollut. **223**: 2751–2770. doi:10.1007/s11270-011-1064-6

Fussmann, K. E., F. Schwarzmüller, U. Brose, A. Jousset, and B. C. Rall. 2014. Ecological stability in response to warming. Nat. Clim. Change **4**: 206–210. doi:10.1038/nclimate2134

Gonçalves, A. L., A. Carvalho, F. Bärlocher, and C. Canhoto. 2019. Are fungal strains from salinized streams adapted to salt-rich conditions? Philos. Trans. R. Soc. B: Biol. Sci. **374**: 20180018. doi:10.1098/rstb.2018.0018

Herbert, E. R., et al. 2015. A global perspective on wetland salinization: Ecological consequences of a growing threat to freshwater wetlands. Ecosphere **6**: 1–43. doi:10.1890/ES14-00534.1

Higgins, S. N., B. Althouse, S. P. Devlin, Y. Vadeboncoeur, and M. J. V. Zanden. 2014. Potential for large-bodied zooplankton and dreissenids to alter the productivity and autotrophic structure of lakes. Ecology **95**: 2257–2267. doi:10.1890/13-2333.1

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

Hintz, W. D., and R. A. Relyea. 2017. A salty landscape of fear: Responses of fish and zooplankton to freshwater salinization and predatory stress. Oecologia **185**: 147–156. doi:10.1007/s00442-017-3925-1

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/fwb.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

Kalff, J. 2001. *Limnology: Inland water ecosystems*. Prentice Hall.

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. USA. **115**: E574–E583. doi:10.1073/pnas.1711234115

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. doi:10.1016/j.scitotenv.2019.06.208

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia **129**: 271–280. doi:10.1007/s004420100716

Liess, A., K. Lange, F. Schulz, J. J. Piggott, C. D. Matthaei, and C. R. Townsend. 2009. Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. J. Ecol. **97**: 326–336. doi:10.1111/j.1365-2745.2008.01463.x

Lim, N. K. M., Y. C. Tay, A. Srivathsan, J. W. T. Tan, J. T. B. Kwik, B. Baloğlu, R. Meier, and D. C. J. Yeo. 2016. Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. R. Soc. Open Sci. **3**: 160635. doi:10.1098/rsos.160635

Lind, P. R., and P. D. Jeyasingh. 2018. Interactive effects of dietary phosphorus and iron on Daphnia life history. Limnol. Oceanogr. **63**: 1181–1190. doi:10.1002/lno.10763

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One **8**: e61217. doi:10.1371/journal.pone.0061217

McMurdie, P. J., and S. Holmes. 2014. Waste not, want not: Why rarefying microbiome data is inadmissible. PLoS Comput. Biol. **10**: e1003531. doi:10.1371/journal.pcbi.1003531

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. doi:10.1186/s40168-021-01079-w

Moffett, E. R., H. K. Baker, C. C. Bonadonna, J. B. Shurin, and C. C. Symons. 2021. Cascading effects of freshwater salinization on plankton communities in the Sierra Nevada. Limnol. Oceanogr. Letters. doi:10.1002/lol2.10177

Niedrist, G. H., M. Cañedo-Argüelles, and S. Cauvy-Fraunié. 2021. Salinization of Alpine rivers during winter months. Environ. Sci. Pollut. Res. **28**: 7295–7306. doi:10.1007/s11356-020-11077-4

O'Gorman, E. J., and others. 2019. A simple model predicts how warming simplifies wild food webs. Nat. Clim. Change **9**: 611–616. doi:10.1038/s41558-019-0513-x

Pace, M. L., and J. D. Orcutt. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnol. Oceanogr. **26**: 822–830. doi:10.4319/lo.1981.26.5.0822

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. **13**: 131–144. doi:10.1016/0022-5193(66)90013-0

Porter-Goff, E. R., P. C. Frost, and M. A. Xenopoulos. 2013. Changes in riverine benthic diatom community structure along a chloride gradient. Ecol. Indic. **32**: 97–106. doi:10.1016/j.ecolind.2013.03.017

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Sarma, S. S. S., S. Nandini, J. Morales-Ventura, I. Delgado-Martínez, and L. González-Valverde. 2006. Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquat. Ecol. **40**: 349–360. doi:10.1007/s10452-006-9039-1

Sauer, F. G., M. Bundschuh, J. P. Zubrod, R. B. Schäfer, K. Thompson, and B. J. Kefford. 2016. Effects of salinity on leaf breakdown: Dryland salinity versus salinity from a coalmine. Aquat. Toxicol. **177**: 425–432. doi:10.1016/j.aquatox.2016.06.014

Schäfer, R. B., and others. 2012. Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. Sci. Total Environ. **415**: 69–78. doi:10.1016/j.scitotenv.2011.05.063

Schuler, M. S., M. Cañedo-Argüelles, W. D. Hintz, B. Dyack, S. Birk, and R. A. Relyea. 2019. Regulations are needed to protect freshwater ecosystems from salinization. Philos. Trans. R. Soc. B: Biol. Sci. **374**: 20180019. doi:10.1098/rstb.2018.0019

Schulz, C.-J., and M. Cañedo-Argüelles. 2019. Lost in translation: The German literature on freshwater salinization. Philos. Trans. R. Soc. B: Biol. Sci. **374**: 20180007. doi:10.1098/rstb.2018.0007

Searle, C. L., C. L. Shaw, K. K. Hunsberger, M. Prado, and M. A. Duffy. 2016. Salinization decreases population densities of the freshwater crustacean, Daphnia dentifera. Hydrobiologia **770**: 165–172. doi:10.1007/s10750-015-2579-4

Setubal, R. B., E. O. de Sodré, 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

Shannon, C. E. 1948. A mathematical theory of communication. Bell Syst. Tech. J. **27**: 379–423. doi:10.1002/j.1538-7305.1948.tb01338.x

Sherwood, G. D., J. Kovecses, A. Hontela, and J. B. Rasmussen. 2011. Simplified food webs lead to energetic bottlenecks in polluted lakes. Can. J. Fish. Aquat. Sci. **59**: 1–5. doi:10.1139/f01-213

da Silva, J. P., A. Martínez, A. L. Gonçalves, F. Bärlocher, and C. Canhoto. 2021. Fungal richness does not buffer the effects of streams salinization on litter decomposition. Ann. Limnol. Int. J. Limnol. **57**: 5. doi:10.1051/limn/2021003

Taipale, S., U. Strandberg, E. Peltomaa, A. W. E. Galloway, A. Ojala, and M. T. Brett. 2013. Fatty acid composition as biomarkers of freshwater microalgae: Analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquat. Microb. Ecol. **71**: 165–178. doi:10.3354/ame01671

Trommer, G., P. Lorenz, A. Lentz, P. Fink, and H. Stibor. 2019. Nitrogen enrichment leads to changing fatty acid composition of phytoplankton and negatively affects zooplankton in a natural lake community. Sci. Rep. **9**: 16805. doi:10.1038/s41598-019-53250-x

Twining, C. W., J. T. Brenna, N. G. Hairston, and A. S. Flecker. 2016. Highly unsaturated fatty acids in nature: What we know and what we need to learn. Oikos. **125**: 749–760. doi:10.1111/oik.02910

US EPA 1988. Aquatic Life Ambient Water Quality Criteria for Chloride. EPA 4409/5-88-001.

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

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