


SPECIAL ISSUE-LETTER

Effects of chloride and nutrients on freshwater plankton communities

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Scientific Significance Statement

Current water quality guidelines for chloride may not protect aquatic life against rising lake salinity in cold regions where de-icing salts are applied to paved surfaces. Although chloride tolerance of *Daphnia pulex* × *Daphnia pulicaria* can increase with food availability, it is unclear how nutrient- and salt-driven changes in phytoplankton and protist composition will impact zooplankton sensitivity to chloride. We exposed freshwater zooplankton communities to a chloride gradient under either ambient nutrients (mesotrophic) or high nutrients (meso-eutrophic) and found that while additional nutrients increased available prey, this did not increase zooplankton tolerance, resulting in large declines in zooplankton abundance and biomass at the current Canadian Water Quality Guideline limit for chloride.

Abstract

Increasing chloride concentrations threaten freshwater zooplankton. We questioned the protectiveness of the Canadian Water Quality Guideline for chloride because it is based on individual species studied under laboratory conditions and does not account for potential interactive factors, such as nutrient concentration. We exposed plankton communities to 30 chloride concentration increments for 6 weeks, crossed with either ambient or high nutrient treatments. Total zooplankton abundance, biomass, and richness declined with increasing chloride, with losses observed below the Canadian Water Quality Guideline. Nutrients did not affect the impact of chloride on zooplankton. Phytoplankton and protist responses varied by nutrient level. Under low nutrients, phytoplankton and protist abundance, biomass, and richness increased with chloride. Under high nutrients, phytoplankton and protist abundance and biomass were unaffected while richness decreased with chloride. These results indicate that current water quality guidelines do not sufficiently protect plankton and that nutrient context may alter phytoplankton and protist response.

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Associate editor: Alison Margaret Derry

Author Contribution Statement: D.A.G. and S.E.A. led the conception and design of the experiment. B.S.S. contributed to the design of the experiment. D.A.G. and S.E.A. conducted the experiment. D.A.G. and I.B.F. processed lab samples, conducted the data analyses and led the writing of the manuscript. All authors provided feedback on the manuscript.

Data Availability Statement: Data and metadata are available in the Dryad repository at <https://doi.org/10.5061/dryad.b8gtht7c8>.

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

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About half of long-term monitored North American lakes show evidence of salinization, driven by decades of road salt use (Dugan et al. 2017). This steady chloride increase threatens many organisms, including amphibians (Karraker et al. 2008) and fish (Corsi et al. 2010). Zooplankton have particularly low chloride tolerances (CCME 2011).

The freshwater chronic chloride limit proposed by the Canadian Water Quality Guidelines (CWQG) for the Protection of Aquatic Life is 120 mg L^{-1} (CCME 2011). These guidelines were determined from a series of laboratory toxicity tests that focused on single species' responses (inhibition of growth, reproduction, development, juvenile survival, and mortality) to elevated chloride concentrations. Species of fish, invertebrates, aquatic plants, and algae, were ranked by sensitivity and a value was chosen that fell below the sensitivity of 95% of tested species, $120 \text{ mg Cl}^{-1} \text{ L}^{-1}$. For several reasons, these guidelines are unlikely to be applicable to all lakes. Only four cladoceran (*Daphnia ambigua*, *Daphnia magna*, *Daphnia pulex*, and *Ceriodaphnia dubia*) and one rotifer species (*Brachionus calyciflorus*) were included, which do not represent existing zooplankton diversity. Large interspecific differences in response exist. For example, LC_{50} values vary between *Daphnia carianata* ($667 \text{ mg Cl}^{-1} \text{ L}^{-1}$; Hall and Burns 2002) and *D. magna* ($4732 \text{ mg Cl}^{-1} \text{ L}^{-1}$; Schuytema et al. 1997). Additionally, these guidelines rely on laboratory-based studies that focus on individual species, precluding indirect, community-level effects (Rohr et al. 2006). Chloride elevation could induce sensitive species loss and species dominance changes, resulting in changes to food web dynamics (Hintz et al. 2017). Lastly, the CWQG does not consider the presence of other factors that can modify response to chloride. Documented factors that can modify species responses to chloride include water hardness (Elphick et al. 2011) and other stressors such as insecticide runoff (Stoler et al. 2017a), predator presence (*Notropis bifrenatus*; Hintz et al. 2017), and increased water temperature (Thompson and Shurin 2012).

Food availability is another factor that may alter how zooplankton respond to chloride. Salt regulation necessitates increased osmoprotectant and ion transporter production, both energetically costly processes (Latta et al. 2012). Research shows that hybrid *D. pulex* \times *D. pulicaria* is more tolerant to chloride when given more food (Brown and Yan 2015). In natural settings, food availability depends on phytoplankton and protist composition and abundance, partly driven by lake nutrient concentration (Barnett and Beisner 2007). If food availability can modify chloride sensitivity, zooplankton in higher nutrient lakes are likely more chloride tolerant. However, food quality is also important. Cyanobacteria, for example, are difficult to consume (Paerl and Otten 2013) and lack sterols, which increases chloride sensitivity of zooplankton consumers by increasing membrane permeability (Isanta-Navarro et al. 2021). For these reasons, cyanobacteria are poor food sources for zooplankton during periods of salt stress. Effects of chloride on phytoplankton and protist composition

are currently unknown. Only three species of algae (*Chlorella emersonii*, *Chlorella minutissima*, *Chlorella zofingiensis*) were studied to determine the CWQG, and field studies rarely include detailed taxonomic composition, the majority presenting chlorophyll *a* concentrations to represent phytoplankton response (Van Meter et al. 2011).

To assess the impact of salinization on freshwater plankton communities, we investigated the response of zooplankton, phytoplankton, and protists to increasing chloride concentrations under the potential modifying influence of nutrients. Additionally, we quantified the effects of chloride at 120 mg L^{-1} to assess the broad applicability of the CWQG for chloride.

Methods

Experimental design

We performed a mesocosm experiment at Long Lake (Ontario, Canada, $44^{\circ}31'51.4''\text{N}$ $76^{\circ}23'58.8''\text{W}$) from 08 June to 16 August 2018 (Table S1). Long Lake is 1.5 km from roadways and minimally impacted by road salt. On 08 June, we installed five wooden frames in the lake, attaching twelve $1 \times 2.5 \text{ m}$ polyethylene bags (Filmtech Plastics) to each frame, totaling sixty 1570 L mesocosms. We added $80 \text{ }\mu\text{m}$ -filtered water from Long Lake, containing smaller phytoplankton and protists, and covered each mesocosm with 1 mm mesh to minimize insect colonization. On 10 June, we added phosphorus (as KH_2PO_4 , Fisher Scientific) to 30 mesocosms to establish the high nutrient treatment (meso-eutrophic, $31.4 \text{ }\mu\text{g P L}^{-1}$). Nitrogen (as NH_4NO_3 , Sigma-Aldrich, $1297 \text{ }\mu\text{g N L}^{-1}$) was added to maintain the N : P ratio based on data from previous years. The remaining 30 mesocosms comprised the ambient nutrient treatment (mesotrophic, $13.6 \text{ }\mu\text{g P L}^{-1}$, $330 \text{ }\mu\text{g N L}^{-1}$). We added nutrients weekly to account for $\sim 35\%$ losses to periphyton and sedimentation (Downing 2005). On 19–20 June, we added zooplankton, larger phytoplankton and protists at ambient densities from Long Lake, sampled from a 0 to 4 m depth using $80 \text{ }\mu\text{m}$ nets. We added sodium chloride (>99% pure, Fisher Scientific) to each enclosure to establish a chloride gradient from 0.41 to 1500 mg L^{-1} (Table S2) within each nutrient treatment on 22 June.

Sampling protocol

We collected water chemistry samples prior to the start of the experiment, on 10 June. On 22 June and 31 July, we collected water samples to quantify particulate organic carbon (POC) concentrations. We estimated POC using the Flash 2000 Organic Elemental Analyzer (Thermo Fischer Scientific), after filtration on GF/F glass fiber circles (precombusted at 400°C for 4 h, $0.7 \text{ }\mu\text{m}$ pore size; Whatman plc). We sampled zooplankton on 22 June (week 0) and 02 August (week 6) by filtering 176.7 L of water through a $50 \text{ }\mu\text{m}$ zooplankton net and preserved them in 70% ethanol. We enumerated and

measured zooplankton using the Plankton Counting Tool software (Wong 2018) and estimated biomass using published biomass-length associations (Table S3). On 22 June and 02 August, we sampled phytoplankton and protists by collecting 250 mL of water. We preserved samples in brown plastic bottles using 1 mL of acid Lugol's solution, kept at 4°C until they were analyzed with the Utermöhl protocol (Karlson et al. 2010). Additionally, we enumerated microbial plankton with an inverted Zeiss Axiovert 100 microscope at 400X or 1000X until we reached 400 cells per colonies at each magnification. We estimated biovolumes from simple geometric forms (Hillebrand et al. 1999) based on photographs taken through a calibrated micrometric ocular, using the MB Ruler free software (MB-Softwaresolutions). Finally, we estimated biomass from biovolume according to relationships presented in Menden-Deuer and Lessard (2000). On 24 June (after chloride addition) and 30 July, we collected chloride samples from each mesocosm.

Statistical analyses

To assess the effect of chloride, nutrients, and their interaction on response variables, we fit linear models (LMs) and generalized additive models (GAMs) with chloride (mean chloride concentration between week 0 and 6; Table S2), nutrient level, and chloride \times nutrient level as predictors. In our GAMs, we specified chloride and chloride \times nutrient level as smooth terms, using cubic regression splines. We used R.3.6.0 (R Core Team 2019) for analyses, using the packages “car” v.3.0-4 (Fox and Weisberg 2019), “pscl” v.1.5.2 (Jackman 2017), and “mgcv” v.1.8-30 (Wood 2011). Zooplankton-centric response variables included: total abundance, biomass, richness, group abundances, and abundance of common crustacean species and rotifer genera. Phytoplankton and protists were assessed together, with response variables: total abundance, biomass, richness, and group abundances (Greco et al. 2021). We included chloride in our week 0 models to confirm that there were no pre-existing trends among mesocosms prior to salt addition. We refer to changes in response variables with increasing chloride, indicating increases in mean chloride concentrations across the gradient in mesocosms, not increases through time. Additionally, we calculated the percent difference in response variables between ambient chloride concentration and the CWQG chronic limit (120 mg Cl⁻ L⁻¹) using model-predicted values. To check statistical assumptions for LMs, we used residual vs. fitted plots, normal quantile–quantile plots, scale location plots, and constant leverage in the “autoplot” function from the ggfortify v.0.4.7 package (Tang et al. 2019). We checked statistical assumptions for GAMs using enhanced residual quantile–quantile plots, residual vs. linear predictor plots, response vs. fitted plots, and the k-index from the “gam.check” function in the “pscl” package (Fox and Weisberg 2019). When necessary, we used log₁₀ and square root transformations. We fit models with all predictor combinations (chloride, nutrient level, chloride \times nutrient level) and used Akaike information

criterion, corrected for small sample sizes, for model selection. Some taxa were not found in all samples; however, we could not determine if these taxa were absent from the mesocosm or occurred at levels below detection. To address this, we added the equivalent of one individual per sample to all mesocosms for analyzing abundances.

To assess the zooplankton and the phytoplankton and protist communities' response to chloride and nutrient level, we performed a distance-based redundancy analysis (dbRDA) using log₁₀-transformed taxa abundances and Bray–Curtis dissimilarities. We conducted RDA with all possible combinations of chloride, nutrient level, and chloride \times nutrient level as predictors using the “capscale” function from “vegan” v.2.5-6 (Oksanen et al. 2019). To confirm linearity of responses, we used the “decorana” function from “vegan” v.2.5-6 (Oksanen et al. 2019).

Results

POC concentrations

We observed higher POC in the high nutrient treatment 11 days after nutrient addition (Fig. S1). By week 6, mean POC was 0.81 ± 0.46 mg L⁻¹ in the ambient and 2.83 ± 1.4 mg L⁻¹ in the high nutrient treatment. Chloride and nutrients were both significant predictors of POC concentration in mesocosms (GAM, $p < 0.001$, $r^2 = 0.65$; Fig. S1).

Week 0 abundance

We collected week 0 samples 11 days after establishing nutrient treatments, 2–3 d after stocking and a few hours before salt addition. Nutrient addition increased the initial total zooplankton abundance (LM, $p < 0.001$, $r^2 = 0.26$; Fig. S2A), rotifer abundance (LM, $p < 0.001$, $r^2 = 0.26$; Fig. S2B) and rotifer *Keratella spp.* abundance (LM, $p < 0.001$, $r^2 = 0.26$; Fig. S2C). There were no significant relationships between richness or abundance (group or species) and our proposed chloride gradient for zooplankton or phytoplankton and protists.

Week 6 abundance

Community response: Zooplankton

Total zooplankton abundance decreased as chloride concentration increased across mesocosms, and was unaffected by nutrient addition (LM, $p < 0.001$, $r^2 = 0.53$; Fig. 1A). At the CWQG of 120 mg Cl⁻ L⁻¹, total abundance was reduced by 35% compared to the ambient chloride concentration. Similarly, total zooplankton biomass decreased with increasing chloride (GAM, $p < 0.001$, $r^2 = 0.53$; Fig. 1B), and there was no difference between ambient and high nutrient levels. At 120 mg Cl⁻ L⁻¹, total biomass was reduced by 69%. Taxonomic richness significantly decreased with increasing chloride, with no effect of nutrient addition (GAM, $p < 0.001$, $r^2 = 0.35$; Fig. 1C). Richness decreased by 8% by 120 mg Cl⁻ L⁻¹.

Zooplankton community composition changed along the chloride gradient but was unaffected by nutrients; only chloride was retained as an axis after model selection in the

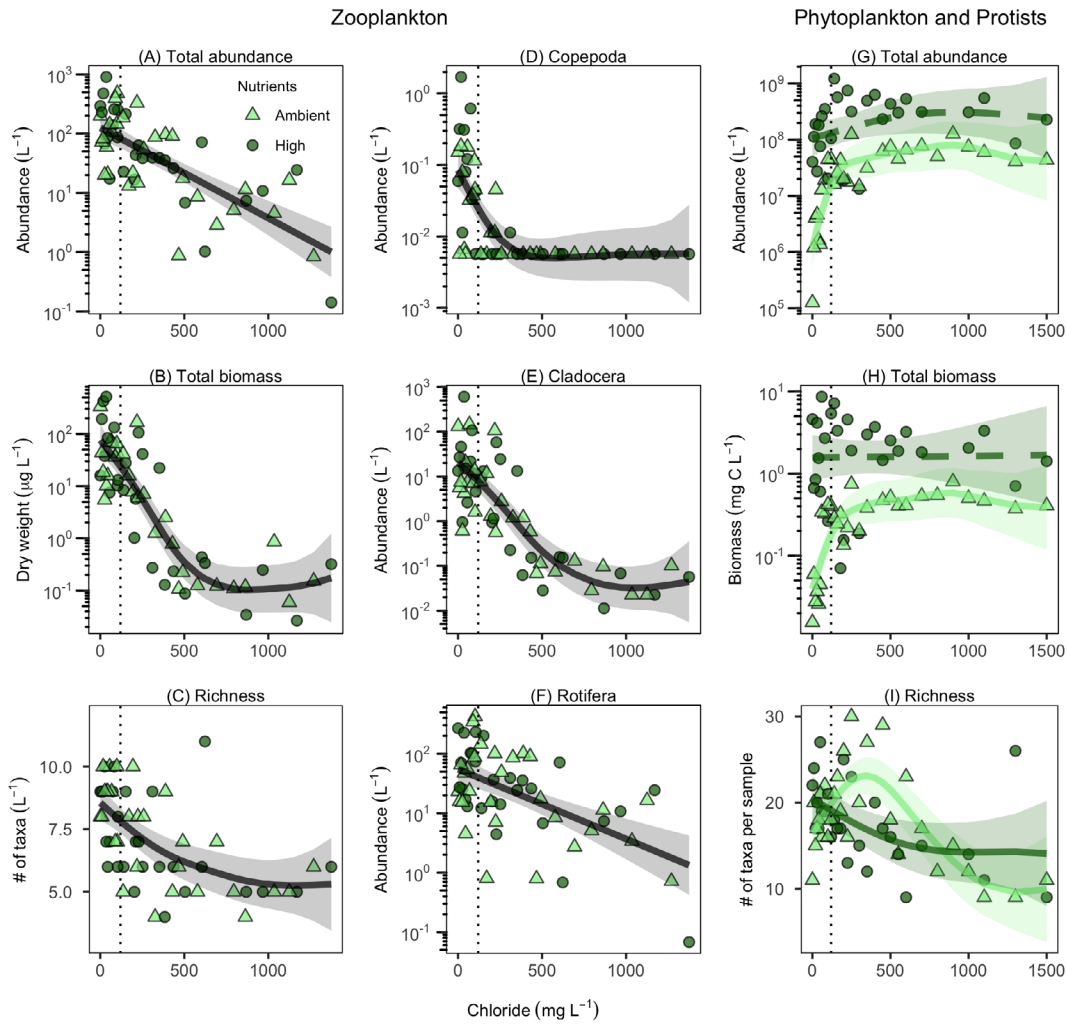


Fig 1. Week 6 (final) total abundance (A), total biomass (B), and richness (C; as the sum of crustacean species and rotifer genera) of zooplankton, total cladocerans (D), copepods (E) and rotifers (F), and phytoplankton and protist total abundance (G), total biomass (H), and richness (I, as the sum of taxa) under increasing chloride concentrations (0.45–1375 mg L⁻¹; n = 57). Shaded areas represent 2× the standard error. For zooplankton, we log₁₀ transformed total abundance and total biomass to meet normality requirements. We fit total abundance with a linear model and both total biomass and richness with generalized additive models. Zooplankton total abundance (A, $p < 0.001$, $r^2 = 0.53$), biomass (B, $p < 0.001$, $r^2 = 0.77$), and richness (C, $p < 0.001$, $r^2 = 0.38$) as well as total copepod (D, $p < 0.001$, $r^2 = 0.5$), cladoceran (E, $p < 0.001$, $r^2 = 0.69$), and rotifer abundance (F, $p < 0.001$, $r^2 = 0.31$) declined significantly with increasing chloride. As there was no effect of nutrient level on abundance, biomass, or richness, both treatments were combined into one line of best fit. For phytoplankton and protists, we log₁₀ transformed abundance and biomass, and they were both fit with generalized additive models. The nutrient level had a significant effect, and ambient and high responses were modeled separately. For the ambient nutrient level (represented by light green triangles), there was a relationship between chloride and abundance (G, $p < 0.001$, $r^2 = 0.73$), biomass (H, $p < 0.001$, $r^2 = 0.66$), and richness (I, $p = 0.001$, $r^2 = 0.52$). For the high nutrient treatment (dark green circles), there was no relationship between abundance (G, $p = 0.2$) or biomass (H, $p = 0.9$) and chloride, but richness decreased with increasing chloride (I, $p = 0.04$, $r^2 = 0.2$).

dbRDA ordination (dbRDA1, $p < 0.001$, pseudo- $F_{1,55} = 12.2$; Fig. 2A). Chloride explained 18.2% of the total variation. Most species and genera had higher abundances with low chloride, based on loadings opposite to chloride on the dbRDA1 axis (Table S4).

Community response: Phytoplankton and protists

Community response of phytoplankton and protists to chloride was dependent on nutrient level. Under ambient

nutrients, total abundance ($p < 0.001$, $r^2 = 0.73$; Fig. 1G) and total biomass significantly increased with increasing chloride ($p < 0.001$, $r^2 = 0.66$; Fig. 1H), while richness peaked at ≈ 350 mg L⁻¹ ($p = 0.001$, $r^2 = 0.52$; Fig. 1I). At 120 mg Cl⁻ L⁻¹, total abundance was 1734% greater, total biomass was 450% greater, and richness was 22% greater. Under high nutrients, there was no relationship between total abundance ($p = 0.2$; Fig. 1G) or biomass ($p = 0.9$; Fig. 1H) and chloride, but richness declined with increasing chloride ($p = 0.04$,

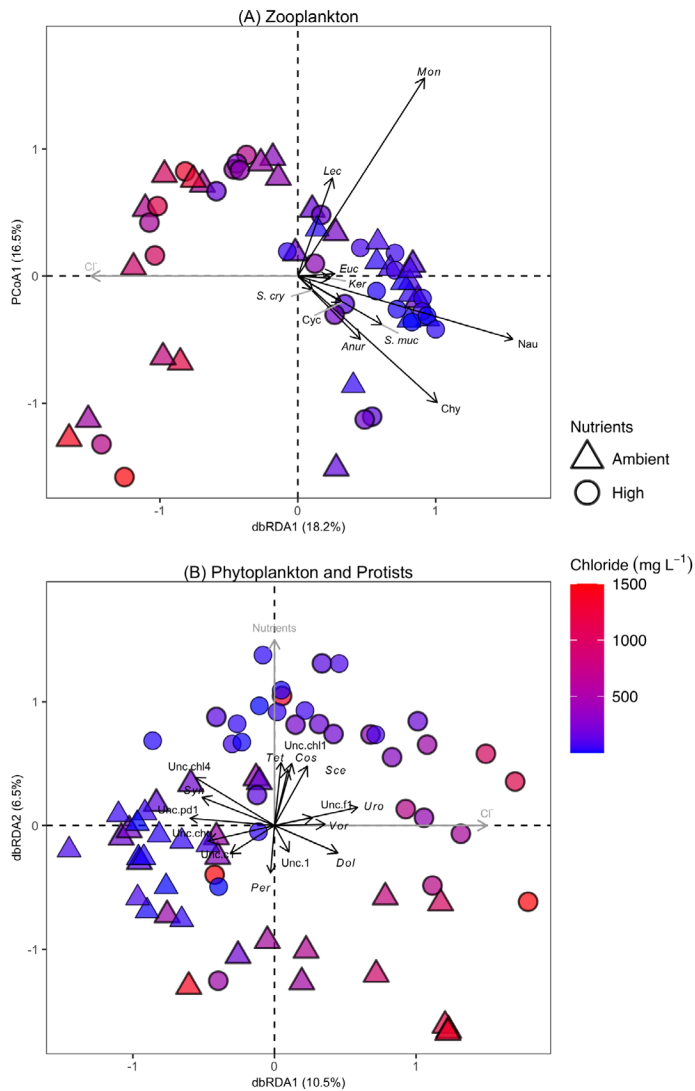


Fig 2. Distance-based redundancy analysis (dbRDA) ordination using Bray–Curtis dissimilarities among zooplankton communities **(A)** with chloride concentration as a constrained axis and phytoplankton and protists communities **(B)** with chloride concentration and nutrients as constrained axes. Chloride level (gray arrow) explained 18.2% of the total variation in zooplankton communities (A, dbRDA1, pseudo- $F_{1,55} = 12.2$, $p < 0.001$), while nutrient level was not a significant source of variation. Chloride level (horizontal gray arrow) explained 10.5% of the total variation in phytoplankton and protists community (B, dbRDA1, pseudo- $F_{1,55} = 6.55$, $p = 0.005$), while nutrients (vertical gray arrow) explained 6.5% of the total variation (B, dbRDA2, pseudo- $F_{1,55} = 6.09$, $p = 0.005$). Each point represents a mesocosm community. Thickened margins are for mesocosms with chloride concentration $> 120 \text{ mg L}^{-1}$. In **(A)**, *S. cry*, *Sida crystallina*; *Cyc*, cyclopoid copepodids; *Anur*, *Anuraeopsis spp.*; *Chy*, chydoridae; *S. muc*, *Scapholeberis mucronata*; *Nau*, nauplii; *Ker*, *Keratella spp.*; *Euc*, *Euchlanis spp.*; *Mon*, *Monostyla spp.*; *Lec*, *Lecane spp.* In **(B)**, *Unc. chl4*, unclassified chlorophytes 4; *Syn*, *Synedra*; *Unc. pd1*, unclassified pennate diatom 1; *Unc. cho*, unclassified choanoflagellate; *Unc. c1*, unclassified ciliate 1; *Per*, *Peridinium*; *Unc. 1*, unclassified 1; *Dol*, *Dolichospermum*; *Vor*, *Vorticella*; *Uro*, *Uronema*; *Unc. f1*, unclassified flagellate 1; *Sce*, *Scenedesmus sp.1*; *Cos*, *Cosmarium*; *Unc. chl1*, unclassified chlorophyte 1; *Tet*, *Tetraedron minimum*.

$r^2 = 0.2$; Fig. 1I). At $120 \text{ mg Cl}^{-1} \text{ L}^{-1}$, the richness decreased by 6.4%.

Phytoplankton and protist community composition varied with chloride and nutrients; both these terms and their interaction were selected for the dbRDA (chloride $F = 6.55$, $p = 0.005$; nutrients $F = 6.09$, $p = 0.005$; chloride \times nutrients $F = 1.71$, $p = 0.03$; Fig. 2B). Chloride explained 10.5% of the variation, nutrients explained 6.5%, and the interaction between chloride and nutrients explained 1.6%.

Taxonomic response: Zooplankton

Total adult cyclopoid copepod abundance significantly decreased with elevated chloride (GAM, $p < 0.001$, $r^2 = 0.48$; Fig. 1D). Nutrient level did not affect abundance or response to chloride in any copepod species. At the CWQG limit, cyclopoid copepods declined by 71%. No calanoid copepods were found in mesocosms by week 6.

Cladoceran abundance significantly decreased with increasing chloride (GAM, $p < 0.001$, $r^2 = 0.69$; Fig. 1E). Changes in nutrient level did not affect abundance or response to chloride, except for *Sida crystallina* which was only significantly affected by chloride under high nutrients (Table S6). Cladoceran abundance decreased by 62% at $120 \text{ mg Cl}^{-1} \text{ L}^{-1}$.

Although rotifers were the most chloride tolerant, overall total rotifer abundance declined as chloride increased (LM, $p < 0.001$, $r^2 = 0.31$; Fig. 1F). Nutrient addition did not affect abundance or response to chloride. Two rotifer genera did not follow this pattern; *Lecane spp.* peaked at $\approx 400 \text{ mg Cl}^{-1} \text{ L}^{-1}$ but declined at higher chloride concentrations and *Keratella spp.* was unaffected by chloride or nutrients (Table S6). At $120 \text{ mg Cl}^{-1} \text{ L}^{-1}$, total rotifer abundance decreased by 28%.

Taxonomic response: Phytoplankton and protists

Phytoplankton and protist responses to increased chloride were taxa specific and nutrient level dependent. We observed four types of responses: type I, a decrease (e.g., the pennate diatom *Synedra*, Fig. S3A; Table S8), type II, increase followed by decrease (e.g., unclassified ciliate 2, Fig. S3B; Table S8), type III, rapid increase followed by stability (e.g., the green alga cf. *Coelastrrella*, Fig. S3C; Table S8), and type IV, increase (e.g., the cyanobacterium *Dolichospermum*, Fig. S3D; Table S8). Some taxa only responded to chloride increase under one nutrient treatment, and some did not respond at all (Table S8). These response differences led to changes in taxonomic community structure, both between extreme ends of the chloride gradient and between the two nutrients levels (Fig. 2B).

Discussion

Community and taxa-level response to chloride

In our mesocosm experiment, increasing chloride led to significant changes in zooplankton community composition (as indicated by dbRDA), as well as reduced biomass and group abundance. Chloride generally had a more negative

impact on aquatic taxa than observed in previous work. In many studies, zooplankton declines (if any) were not detected until $727 \text{ mg Cl}^- \text{ L}^{-1}$ (Hintz et al. 2017; Jones et al. 2017; Stoler et al. 2017a,b; Lind et al. 2018). Likewise, Sinclair and Arnott (2018) found an increase in Long Lake cladocerans after chloride was elevated to $250 \text{ mg Cl}^- \text{ L}^{-1}$, driven by littoral species, and no differences in rotifers or copepods. In contrast, Loughborough Lake ($19 \text{ mg Cl}^- \text{ L}^{-1}$) copepods declined at $250 \text{ mg Cl}^- \text{ L}^{-1}$ while cladocerans and rotifers were unaffected (Sinclair and Arnott 2018). We detected a 69% decrease in total zooplankton biomass and a 62% decrease in cladoceran abundance at $120 \text{ mg Cl}^- \text{ L}^{-1}$. These results align with those of Thompson and Shurin (2012) who detected a zooplankton biomass decline at $162 \text{ mg Cl}^- \text{ L}^{-1}$, and Petranka and Francis (2013) who detected few cladocerans above $255 \text{ mg Cl}^- \text{ L}^{-1}$. However, Petranka and Francis (2013) found a weak chloride effect on copepods, whereas we observed a strong effect with no copepods observed above $350 \text{ mg Cl}^- \text{ L}^{-1}$.

Drivers of variation in chloride sensitivity among studies are unclear. Differences in water chemistry (Elphick et al. 2011) and food quantity and quality (Brown and Yan 2015; Isanta-Navarro et al. 2021) could be contributors. Previous chloride exposure is also a potential driver, as zooplankton have demonstrated some ability to adapt to chloride (Hintz et al. 2019). Our study lake had little previous exposure (mean $[\text{Cl}^-] = 0.43$ compared to $15\text{--}60 \text{ mg L}^{-1}$ for source lakes in other published studies). Additionally, species composition differences could drive variation in chloride sensitivity. As most studies only report total or taxa-level responses, we do not know the degree to which our communities differ from previous studies. Furthermore, we lack information on many species' chloride sensitivity in published literature. Toxicity studies generally focus on daphniids and *Brachionus spp.* (CCME 2011), which were uncommon in our mesocosms.

In our experiment, zooplankton groups differed in the relative severity of their chloride response; copepods experienced the greatest declines, followed by cladocerans and then rotifers. In previous studies, cladocerans tended to be the most sensitive (Van Meter et al. 2011; Petranka and Francis 2013; Stoler et al. 2017b), except in Sinclair and Arnott (2018) where increases in littoral cladoceran abundance at high chloride ($250 \text{ mg Cl}^- \text{ L}^{-1}$) drove the overall response. Rotifers are generally the most tolerant, only showing declines in one study (Stoler et al. 2017b). Copepods are more variable in their response to chloride, sometimes declining (Van Meter et al. 2011; Petranka and Francis 2013; Lind et al. 2018) or not responding to chloride (Stoler et al. 2017a,b, Sinclair and Arnott 2018).

Taxon differences in sensitivity carry important implications as chloride in freshwater systems continue to rise. Zooplankton are essential to ecosystem function, contributing to respiration, moderation of predator and algal biomass and composition, and nutrient recycling (Hébert et al. 2017). With compositional changes, species replacement will not

necessarily maintain ecosystem function if they are incapable of similar functioning. Growth, feeding, excretion, and respiration rate increase with body size, in turn increasing ecosystem respiration, predator biomass, and nutrient recycling, and decreasing algal biomass (Hébert et al. 2017). As relative abundances shift from large crustaceans to small rotifers, total biomass will drop (as observed in this study; Fig. 1B), and these processes will decline.

Impact of nutrients on response to chloride

Added nutrients increased food availability, as observed by POC and phytoplankton and protist biomass within the high nutrient treatment. In high nutrient mesocosms, increasing chloride shifted phytoplankton and protist taxonomic composition to a higher proportion of less palatable taxa (as described by Porter 1973) such as desmids, filamentous cyanobacteria, and filamentous green algae (Fig. 2B). However, palatable small green alga cf. *Coelastrrella* was dominant above $120 \text{ mg Cl}^- \text{ L}^{-1}$ under both nutrient treatments. Despite this availability of palatable prey, nutrient addition did not increase chloride tolerance in any zooplankton group or species. Lind et al. (2018) found a similar result with phosphorus treatments of 4.36 and $100 \mu\text{g L}^{-1}$; nutrient addition did not affect response to chloride, although they did find that zooplankton abundance was higher with increased nutrients. Our observed lack of response may be due to high POC concentrations in our ambient treatment (0.81 mg L^{-1} under ambient nutrients vs. $0.2\text{--}1 \text{ mg L}^{-1}$ for Brown and Yan 2015), close to the *D. pulex* food threshold, above which, growth rates saturate (1 mg L^{-1} , Lampert 1977 in Brown and Yan 2015). Despite previous evidence that food availability influences zooplankton chloride tolerance, our results suggest that a lake's trophic status and total phytoplankton and protist biomass cannot predict its zooplankton chloride tolerance.

Applicability of the CWQG for the Protection of Aquatic Life

Zooplankton biomass and abundance across taxa were significantly lower in mesocosms receiving even relatively low concentration chloride additions. Total biomass, copepod, and cladoceran abundance declined by 62–71% in mesocosms where chloride concentrations reached this supposedly safe limit. This level of decline would likely lead to a decline in ecosystem services. While phytoplankton and protist total biomass and abundance were higher with increasing chloride, likely driven by a greater proportion of tolerant taxa, specific taxa abundance was 50% lower in mesocosms at the CWQG. The CWQG should be re-evaluated to address these shortcomings. Our results are in agreement with recent laboratory- and field-based studies that demonstrate zooplankton sensitivity to chloride at concentrations below CWQG (Arnott et al. 2020; Valteau et al. 2020; Isanta-Navarro et al. 2021). Together, these results suggest that interactive stressors should be considered. Although we did not find evidence that nutrient level influences chloride tolerance, studies are needed

under conditions of lower food quantity and quality. Finally, in our study, copepods were highly sensitive, yet none were included by CCME (2011) when the chronic chloride limit was calculated. A new guideline should consider a greater diversity of species, their interactions, and environmental context to ensure that plankton are fully protected across all regions.

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Acknowledgments

This project is part of the Global Salt Initiative, a global collection of mesocosm experiments which aim to understand the impact of salinization on plankton communities. This initiative was led by SEA and Bill Hintz and was the product of a Global Lakes Ecological Observatory Network (GLEON) working group formed in 2017. We thank Brooke Rathie, Alex McClymont, and Haley Richardson for their assistance in the field, QUBS staff for logistical support, Nathaniel Wong for technical support, and our anonymous reviewers for their constructive feedback that greatly improved this manuscript. Funding was provided by a Craigie Fellowship to D.A.G., NSERC Discovery Grants to S.E.A. and B.S.S., the Alexander and Cora Munn Summer Research Award to Brooke Rathie, and the Queen's Summer Work Experience Program.

Submitted 16 April 2021

Revised 25 June 2021

Accepted 27 June 2021