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Road Salt Impacts Freshwater Zooplankton at Concentrations below Current Water Quality Guidelines

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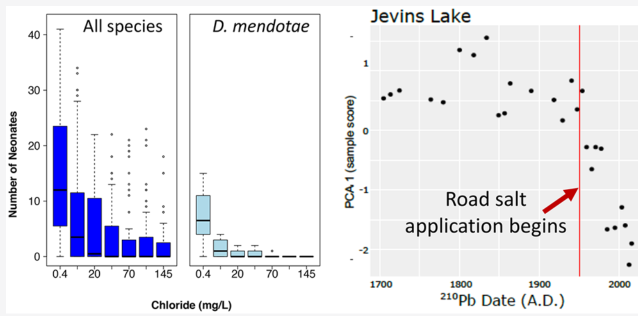
ABSTRACT: Widespread use of NaCl for road deicing has caused increased chloride concentrations in lakes near urban centers and areas of high road density. Chloride can be toxic, and water quality guidelines have been created to regulate it and protect aquatic life. However, these guidelines may not adequately protect organisms in low-nutrient, soft water lakes such as those underlain by the Precambrian Shield. We tested this hypothesis by conducting laboratory experiments on six *Daphnia* species using a soft water culture medium. We also examined temporal changes in cladoceran assemblages in the sediments of two small lakes on the Canadian Shield: one near a highway and the other >3 km from roads where salt is applied in the winter. Our results showed that *Daphnia* were sensitive to low chloride concentrations with decreased reproduction and increased mortality occurring between 5 and 40 mg Cl^-/L. Analysis of cladoceran remains in lake sediments revealed changes in assemblage composition that coincided with the initial application of road salt in this region. In contrast, there were no changes detected in the remote lake. We found that 22.7% of recreational lakes in Ontario have chloride concentrations between 5 and 40 mg/L suggesting that cladoceran zooplankton in these lakes may already be experiencing negative effects of chloride.



1. INTRODUCTION

Deicing agents (usually NaCl) are frequently applied to roads and other paved surfaces in cold regions to improve driving conditions and increase human safety during winter. In Canada, as much as 7 million tonnes of road salt is used annually,[1] while in the United States, the annual application is approximately 24.5 million tonnes.[2] In Europe, including the United Kingdom, Slovenia, Austria, and the Czech Republic, total annual road salt use ranges from ∼150,000 to 2 million tonnes.[3−5] Because salt dissolves easily in water, pulses of high salinity in streams near roads occur with winter and spring runoff events.[6] Dissolved chloride (Cl^-) also permeates soils and is retained in watersheds,[7,8] producing gradual, long-term Cl^- concentration increases in lakes near roads and urban areas.[9−11] Increasing Cl^- concentrations have the potential to impair water quality (including drinking water[12]) and exceed water quality guidelines aimed at protecting aquatic life.[6,13,14]

Increased salinity in freshwater ecosystems is often associated with lower population growth rates, reduced diversity, and changes in community composition that have implications for nutrient and energy flow in food webs (reviewed in ref 15). Zooplankton, which are a critical link between primary producers and higher trophic levels, including macroinvertebrates and fish, are particularly sensitive to increasing Cl^- concentrations. Although crustacean zooplankton are hyper-regulators that maintain higher ion concentrations in their hemolymph, increasing NaCl interferes with osmoregulation and active transport of ions across epithelial membranes[16] with implications for reproduction and survival.[17] Consequently, high concentrations of Cl^- reduce the abundance[18−21] and alter population dynamics[22,23] of filter-feeding Cladocera, including *Daphnia* spp. A reduced abundance of *Daphnia* and other zooplankton has implications for ecosystem services because reductions in the abundance of *Daphnia* and other herbivores can lead to increased algal abundance and degraded water quality,[24−26] as well as limit production available to higher trophic levels.[20]

In Canada, the current chloride water quality guideline for the protection of aquatic life is set at 120 mg/L for chronic exposure and 640 mg/L for acute exposure.[27] The United States Environmental Protection Agency has recommended a chronic water quality criterion of 230 mg/L,[28] whereas the EU Directive for surface water regulation is 250 mg/L.[29] The

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Canadian guidelines are based on the 5th percentile of the species sensitivity distribution (SSD) resulting from 24 h to 60 day toxicity tests for 28 species of aquatic plants, invertebrates, amphibians, and fish.[27] Three *Daphnia* species included in this assessment, *Daphnia ambigua*, *D. pulex*, and *D. magna*, experienced 10−25% reductions in reproduction during 10 or 21 day experiments at concentrations ranging from 250 to 421 mg Cl^-/L. This suggests that *Daphnia* in lakes with <120 mg Cl^-/L should be protected by current water quality guidelines for chronic exposure to chloride.

Several lines of evidence, however, suggest that the current water quality guidelines for chloride may not adequately protect zooplankton in thousands of low-nutrient, soft water ecosystems that typify lakes underlain by the Precambrian Shield. First, chloride is more toxic in soft water (generally defined as <60 mg/L of $CaCO_3$[30]), compared to hard water ecosystems,[31,32] which suggests that sensitivity to Cl^- may be higher in lakes with low concentrations of Ca^{2+} and other ions. This is especially a concern in Precambrian Shield lakes in Canada and Scandinavia where a widespread Ca^{2+} decline[33−35] is already impairing the growth and reproduction of *Daphnia* and other zooplankton.[36−38] Second, food availability may influence chloride toxicity. For example, *D. pulicaria*, a common inhabitant of soft water lakes, is more sensitive to Cl^- at low food concentrations typical of many soft water Shield lakes compared to high food concentrations used in standard toxicity tests.[39] Third, Cl^- sensitivity can vary among species and populations of *Daphnia*[40,41] and may depend on history of exposure.[42,43] Thus, the level of protection required may vary among species and populations in heterogeneous landscapes (e.g., ref 44).

We hypothesized that *Daphnia* and other zooplankton in lakes on the Precambrian Shield may be sensitive to Cl^- concentrations below current chronic water quality guidelines, perhaps even at current Cl^- concentrations. Although individual studies infrequently test multiple species, we expected sensitivity to Cl^- to vary among *Daphnia* species that are common in Shield lakes. To test these hypotheses, we implemented a three-pronged approach: first, we evaluated the chronic toxicity of NaCl to six native species of *Daphnia* in culture media typical of Shield lakes using 21 day toxicity tests; second, we used paleolimnological studies to assess the response of zooplankton communities to the application of salt on a nearby twinned highway; and finally, we estimated the percentage of recreational lakes in Ontario, Canada, with potentially harmful Cl^- levels, based on our laboratory results.

2. METHODS

2.1. Life History Experiments. We conducted 21 day life history experiments on *Daphnia* species that are commonly found in soft water Canadian Shield lakes. Five of these species (*D. catawba*, *D. mendotae*, *D. minnehaha*, *D. pulicaria*, and the hybrid *D. pulicaria***D. pulex*) were collected from soft water lakes in Muskoka, Ontario, Canada, and were maintained in stable culture for two years prior to our experiments. The sixth *Daphnia* species, *D. pulex*, was originally obtained from a pond in Oregon (USA) and has been in culture for more than a decade.[45] For long-term culturing and our life history experiments, we used FLAMES medium,[30] a soft water medium that mirrors the chemical characteristics of two lakes in Muskoka with stable, species-rich zooplankton assemblages;[46] medium [Na] = 0.76 mg/L, [sulfate] = 9.04, [P] = 0.01 mg/L, and [Nitrate-Nitrite-N] = 0.06 mg/L (see Table 3 in ref 30 for a comparison with Blue Chalk and Red Chalk lakes). The medium calcium concentration is 2.54 mg/L, and hardness is 9.41 mg/L as $CaCO_3$, reflecting low Ca concentrations in Shield lakes; in the Muskoka River Watershed, more than 65% of the lakes currently have [Ca] below 2.5 mg/L.[47]

We grew genetically identical, <24 h-old neonates from the third brood of each *Daphnia* species individually in glass tubes containing 30 mL of FLAMES medium that we amended with reagent-grade NaCl (Fisher brand, Certified ACS) to achieve seven nominal Cl^- concentrations: 0.4 (FLAMES medium, the control) and 10, 25, 50, 75, 100, and 150 mg Cl^-/L. Each treatment had 10 replicates. Samples from the test media were taken every second day. Cl^- was analyzed at the Dorset Environmental Science Centre's chemistry laboratory in Dorset, Ontario, Canada, using established standard operating procedures for ion chromatography, method E3147. The samples were analyzed in triplicate, with distilled water blanks and quality control solutions of chloride within and outside the range of determination, with the following acceptance limits: ±0.43 mg/L for samples ranging from 5.01 to 50.0 Cl^-/L and ±2.60 mg/L for samples 50.01 to 200 Cl^-/L.

Life history experiments were conducted in Conviron CMP4030 culture chambers at 20 °C under a light regime of 16:8 h light:darkness. The test medium was renewed every 48 h, and daphniids were fed 1.0 mg C/L/day of *Pseudokirchneriella subcapitata* that was cultured in Bold's Basal Medium at 20 °C, 100 μmol m^{-2} s^{-1} light regime × 24 h. To prevent evaporation and fluctuations in Cl^- content, the experimental tubes were covered with Parafilm moisture-resistant sealing films. Treatments were randomly distributed in the culture chambers.

Survival and neonate production per individual were recorded every day during the 21 day bioassay. We counted and then discarded neonates that were produced each day. Day of first reproduction, number of clutches per individual, number of neonates per brood, and total number of neonates were recorded.

The analytical determinations of Cl^- were generally 5 mg/L lower than the nominal concentrations (Table S1); therefore, actual values were used in statistical analysis and figures. All cations, anions, and dissolved oxygen concentrations were within the expected limits, pH was consistently above 6.4 in all treatments, and oxygen was always above 8.0 mg/L. Mortality in the control treatment (0.4 mg Cl^-/L) was low; on average, 95 ± 5% standard deviation of individuals, across all species, remained alive at the end of the experiment.

2.1.1. Statistical Analyses. We assessed Cl^- toxicity for each *Daphnia* species using several life history response variables: total neonate production over the 21 day bioassay, mean clutch size, day of first reproduction, and modeled daily mortality rate. For each life history variable, we evaluated the response to increasing Cl^- concentration as a continuous predictor variable and calculated the effect size of the predicted response at 120 mg Cl^-/L, the Canadian water quality guideline for chloride, compared to control conditions.

2.1.2. Total Neonate Production. To test the hypothesis that reproduction decreases with increasing Cl^- concentration, we fit a zero-inflated negative binomial model to the total number of neonates produced in 21 days (using the package pscl and function zeroinfl in R version 3.6.1[48]) because the reproduction data had many zeros. The zero-inflated model statistically separates the reproductive outcome into two

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components. The first is the distribution of reproduction that comes from a count process and could include counts (i.e., number of neonates) that are zero and greater. The second component, the zero-inflated process, represents additional zero counts (i.e., no neonates produced). The count process was modeled using a negative binomial distribution with a log link. The zero-inflated component was modeled using a binomial distribution with a logit link. The full model included an interaction between species and Cl^- in both the count and the zero-inflated components. The best model was identified as the model with the lowest AIC. If top models were within 2 AIC units, the simplest model was chosen.

2.1.3. Mean Clutch Size and Day of First Reproduction. Changes in reproduction could result from differences in the numbers of neonates produced in each brood or changes in the timing of brood release. To test the hypotheses that mean clutch size decreased and/or day of first reproduction increased as Cl^- concentration increased, we compared both mean clutch size and day of first reproduction across Cl^- treatments and among species for individuals that successfully reproduced using linear models. We assessed the overall model fit using residual versus fitted plots, normal quantile-quantile plots, and scale-location plots. Clutch size was log-transformed to improve fit. We compared models with different complexity using AIC. If top models were within 2 AIC, the simplest model was chosen.

2.1.4. Daily Mortality Rate. To test the hypothesis that increasing Cl^- is associated with higher mortality, we calculated the daily mortality rate for each Cl^- treatment and each species using a parametric regression model for interval censored data (pc_par in R package icenReg) fitting an exponential decay with increasing Cl^-. We used linear models to compare daily mortality rates among Cl^- treatments and species. We evaluated the fit of models of different complexity using AIC and found that the inclusion of an interaction between Cl^- and species was supported. The relationship between the Cl^- concentration and daily mortality rate was compared among species using the package CompSlopes in R package "FAS".[49]

2.2. Temporal Trends in Cladoceran Community Composition. Our second objective was to determine if there is evidence of community change in salt-impacted lakes that are currently below water quality guidelines for Cl^-. To achieve this, we used paleolimnological methods to compare temporal changes in cladoceran zooplankton assemblage between two lakes that differed in salt-impact owing to their distance from major roads.

We examined cladoceran remains preserved in a lake sediment core from Jevins Lake (latitude, longitude = 44.3873, −79.2681), a moderately sized (34 ha), shallow (maximum depth = 3 m) lake located within the Muskoka River Watershed (MRW) of south-central Ontario, Canada. The catchment of Jevins Lake is traversed by a major twinned highway, passing approximately 70 m from the lakeshore, and includes urban development within the Town of Gravenhurst, Ontario. Measured Cl^- and Na^+ concentrations from a water sample taken in May 2016 were 90.9 (2.56 meq/L) and 59.8 mg/L (2.60 meq/L), respectively, and represent the highest values of any monitored lake in the MRW (District of Muskoka 2016; mean Cl^- = 8.0 ± 12.85 mg/L (0.22 ± 0.362 meq/L), mean Na^+ = 5.4 ± 8.39 mg/L (0.23 ± 0.365 meq/L)).

Heney Lake (latitude, longitude = 45.1278, −79.1031) is a small (21.7 ha), shallow (maximum depth = 6 m) lake located in the MRW with similar physical and chemical characteristics to Jevins Lake. However, Heney Lake is located >3 km from a winter-maintained highway and thus has a much lower Cl^- concentration (0.94 mg/L or 0.026 meq/L).

Sediment cores were collected in May 2016 (Jevins Lake) and July 2011 (Heney Lake) using standard high-resolution paleolimnological methods.[50] The cores were collected from the deepest basin of each lake using the Glew[51] gravity corer and were sectioned at 0.5 cm intervals throughout using the Glew[52] extruder (with the top 2 cm sectioned in 0.25 cm intervals in Heney Lake).

2.2.1. Laboratory Methods. The preparation of samples for ^{210}Pb dating followed standard methods.[53] Briefly, a germanium crystal detector was used to measure the gamma activity of radioisotopes, ^{210}Pb, ^{214}Bi, and ^{137}Cs. The unsupported ^{210}Pb concentration in sediments was used to estimate ages based on the constant rate of supply (CRS) model.[54]

Cladocera slides were prepared following the methods described by Frey[55] and Korhola and Rautio.[56] Approximately 0.1 g of freeze-dried sediment was used for each interval. Sediments were deflocculated by mixing a 10% KOH solution with the sediment and heating to 80 °C for 30 min. The sediment/KOH mixture was rinsed through a 37 μm sieve with deionized water. The sediment retained on the filter was mixed with deionized water, ethanol, and safranin to preserve and stain the sample. Fifty μL of the preserved sediment solution was pipetted onto a microscope slide, mounted using glycerin, and examined under a compound microscope using bright-field illumination at 200× magnification. The identification of cladoceran subfossils, which were abundant and well preserved, principally followed Korosi and Smol[57,58] with additional identification references used as general guides.[59−61] *Eubosmina* and *Bosmina* relative abundance data were combined into a single taxon. A minimum of 75 microfossils were identified at each sediment interval, a sum shown to provide reliable estimates of the cladoceran community structure.[62]

2.2.2. Statistical Analysis. Principal component analyses (PCA) were used to examine variation in assemblage structure in Cladocera taxa over time in each lake. Rare species that did not meet a relative abundance of >2% in at least one interval were removed prior to analyses. Statistical analyses were performed in R 3.1.3[48] using the vegan[63] and rioja[64] packages.

2.3. Chloride Concentrations in Recreational Lakes in Ontario. To examine Cl^- concentrations across the province of Ontario, Cl^- data were drawn from two provincial water quality data sets: 447 lakes from the Ontario Ministry of the Environment, Conservation, and Parks (MECP)'s Lake Partner Program (LPP) and 375 lakes from the Broadscale Monitoring Program (BSM). The LPP is a citizen-based lake monitoring program that provides lake water quality and trophic status information for cottage- and recreational-use lakes. The BSM is a collaboration between the MECP and the Ontario Ministry of Natural Resources and Forestry, with the purpose of providing detailed fisheries and water quality information on hundreds of inland lakes that support a recreational fishery. Together, they represent recreational lakes in Ontario that provide important ecosystem services to residents and visitors.

For both the LPP and BSM programs, water samples for Cl^- analyses were collected in the spring (April or May) of 2016−

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17 from the deepest location of each lake using an integrated water sampler lowered and raised through a depth equivalent to the Secchi transparency depth. The water samples were filtered through an 80 μm mesh and stored at 4 °C in an 80 mL clear polypropylene jar (LPP) or a 500 mL clear polyethylene terephthalate jar (BSM). Water samples from both programs were analyzed in the water chemistry laboratory at the MECP's Dorset Environmental Science Centre using ion chromatography with an analytical range of 0.05−110 mg Cl^-/L.[65]

To examine Cl^- concentrations in recreational lakes in Ontario, water quality data from the LPP and BSM programs were pooled (822 lakes). Cl^- concentrations from multiple stations and for the spring of 2016 and 2017 were averaged when more than one sample was taken. To characterize the distribution of Cl^- across the province, a cumulative frequency plot was created using R version 3.6.1.[48]

3. RESULTS

3.1. Life History Experiments. *3.1.1. Total Daphnia Reproduction.* We found that neonate reproduction decreased at Cl^- concentrations well below the current Canadian Water Quality Guideline of 120 mg Cl^-/L. Increasing chloride concentration decreased the mean total number of neonates produced in 21 days (Figure 1; Table S1, Supporting Information) resulting in a 64% decrease in the predicted total number of neonates produced at 120 mg Cl^-/L, compared to 0.4 mg Cl^-/L, the control. The strength of the chloride effect was species-dependent (i.e., the best model included an interaction between species and Cl^-, Table S2, Supporting Information) and was strongest for *D. mendotae*, which is a widespread species that suffered a 99.8% reduction in total reproduction at 120 mg Cl^-/L compared to 0.4 mg Cl^-/L (Figure 1). In addition, as Cl^- increased, the proportion of failed reproduction in the zero-inflated component of the model increased, which might represent animals dying before maturation (Table S3, Supporting Information). The probability of not reproducing increased by 188% at 120 mg Cl^-/L compared to the control, although this effect was not as strong for *D. pulex*.

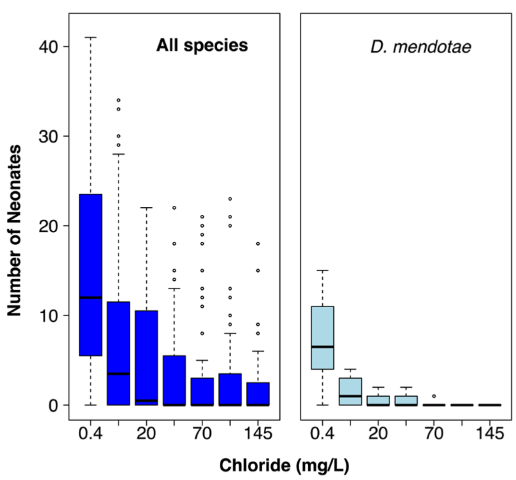

Figure 1. Number of neonates produced during the 21 day bioassay for each chloride treatment, including zeros for individuals that failed to reproduce. "All species" represents the pooled data from six *Daphnia* species, whereas "*D. mendotae*" represents the data from bioassays for only *D. mendotae*. The box represents the 1st and 3rd quartiles, whereas the center line represents the median. Upper whiskers represent the interquartile range times the smaller of the maximum number of neonates and the 3rd quartile. Lower whiskers represent the interquartile range times the larger of the minimum number of neonates and the 1st quartile.

3.1.2. Clutch Size. To assess the effect of Cl^- on reproduction that is independent of survival, we examined the effect of Cl^- on mean clutch size for all reproducing daphniids. There was no interaction between species and Cl^- on mean clutch size of species that reproduced (Table S4, Supporting Information), although clutch size varied among species with *D. mendotae* having the smallest clutch size and *D. pulicaria* having the largest (Table S5, Supporting Information). Across all species, increasing Cl^- was associated with a reduction in mean clutch size. At 120 mg Cl^-/L, the predicted mean clutch size was 29.2% smaller than mean clutch size in our control media (Figure S2, Supporting Information).

3.1.3. Day of First Reproduction. There was no effect of Cl^- on the timing of first reproduction (Table S6, Supporting Information) although we detected significant variation among species, with *D. pulex/pulicaria* and *D. pulex* reaching primaparity later than *D. catawba* and *D. mendotae*, which reached primiparity later than *D. minnehaha* and *D. pulicaria* (post hoc tests, $P < 0.05$; Figure S3, Supporting Information).

3.1.4. Daily Mortality Rate. The daily mortality rate increased with increasing Cl^- concentrations (Table S7, Supporting Information), but the slope of the relationship differed among species (Table S8, Supporting Information). On average, the daily mortality rate increased to 0.108 individuals/day ± 0.064 SD at 120 mg Cl^-/L. This resulted in a mean effect size of 800% ± 494 SD at 120 mg Cl^-/L. *D. mendotae* and *D. minnehaha* were the most sensitive to Cl^- followed by *D. catawba*. In contrast, *D. pulex*, *D. pulicaria*, and the hybrid were more tolerant to increasing Cl^- (Figure S4, Supporting Information).

3.2. Temporal Trends in Cladoceran Community Composition. Thirty-six cladoceran taxa were identified within the sediment cores (28 taxa in Jevins Lake, 31 taxa in Heney Lake, with 24 taxa in common between the two lakes). Note that *Daphnia*, the genus we used in our experiments, was rare in Jevins Lake. After removing the rare species that did not meet a relative abundance of >2% in at least one interval, 12 species remained in Jevins Lake and 22 in Heney Lake for statistical analyses.

Our paleolimnological analyses provide the first evidence, in a natural system, of cladoceran assemblage change associated with the 1950s onset of road salt use in Muskoka. In Jevins Lake, we observed a clear division between cladoceran species assemblages, as represented by the first PCA axis (41% variation explained), in the older sediment intervals (ca. 1740−1954) compared to the more recent intervals (ca. 1954−2016; Figure 2). The more recent sediment intervals coincide with the time period where road salts have been applied during winter (1950s to present).

Detailed descriptions of taxonomic changes in the paleolimnogical record for Jevins and Heney lakes are presented in Valleau et al.[66] In summary, *Bosmina* spp. was the dominant cladoceran taxon throughout the Jevins Lake core, with a mean abundance of 77%. However, *Bosmina* spp. began to decrease in relative abundance beginning around 11 cm (ca. 1950), reaching a minimum relative abundance of 56% at 1 cm (ca. 2012). An increasing trend in the relative abundance of *Chydorus brevilabris* commenced at a core depth

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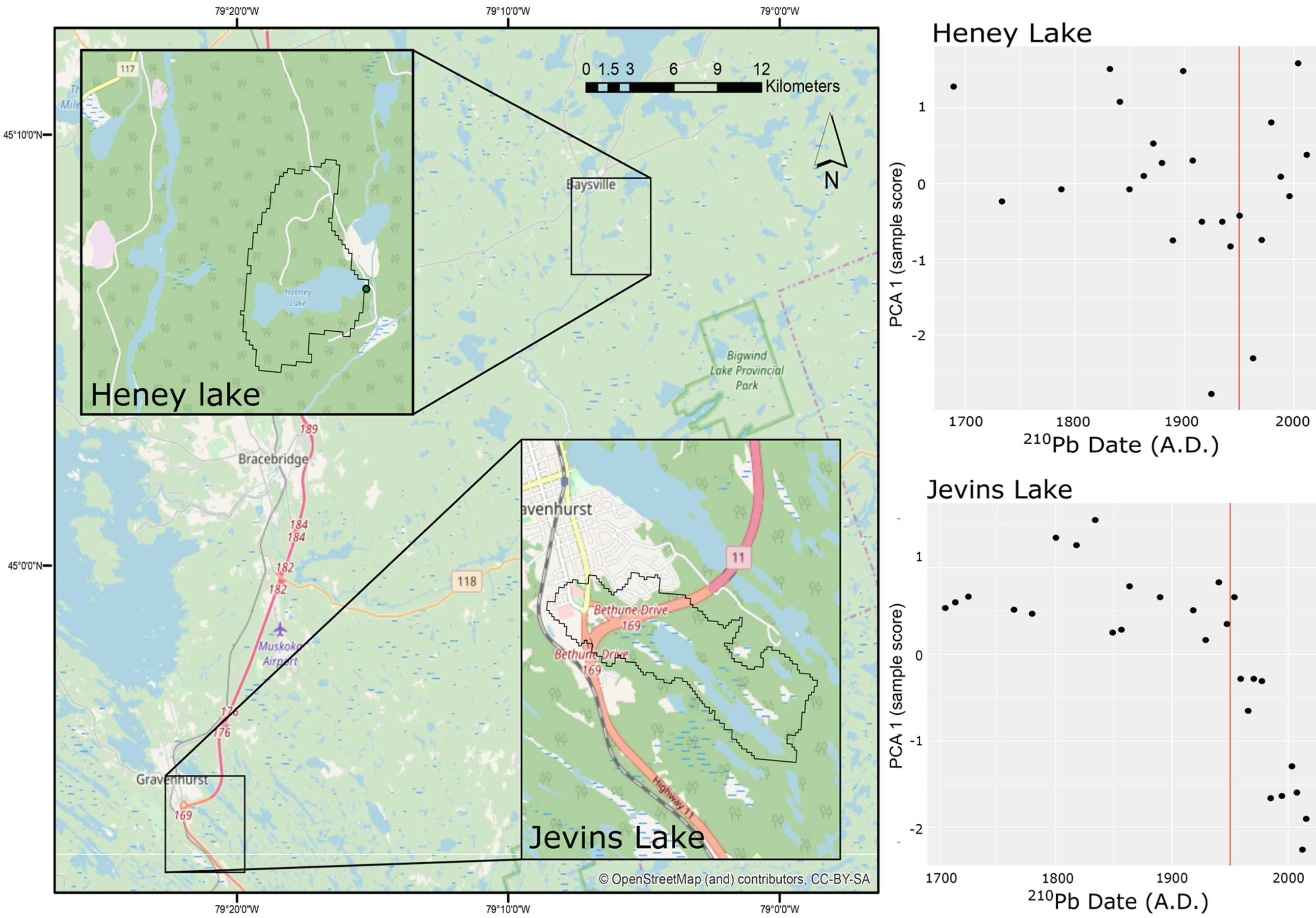

Figure 2. Map of a portion of the Muskoka River Watershed, showing the locations of Heney and Jevins lakes. Graphs represent the PCA score for axis 1 plotted against the ^{210}Pb-inferred dates for sediment intervals. The vertical red line indicates the approximate date when winter road salt application began in Muskoka.

of 10 cm (ca. 1950), coincident with a subtle increase in the relative abundance of *Alona circumfimbriata* (*rectangula*) after ca. 1960.

In contrast, there was no directional change over time in cladoceran community structure in our reference Heney Lake, based on PCA axis 1 scores of the cladoceran species assemblage (Figure 2). *Bosmina* spp. was the dominant cladoceran taxon throughout the core (53−71%), and PCA axis 1 scores of the species data were variable across time.

3.3. Chloride Concentrations in Recreational Lakes in Ontario. Most recreational lakes (76.2%) in the Broadscale Monitoring and Lake Partner programs had Cl^- concentrations below 5 mg/L (Figure 3) and, based on our *Daphnia* life history bioassays, likely have zooplankton communities that are not impacted by Cl^-. However, 22.7% of the lakes had concentrations of 5−40 mg/L, which corresponds to Cl^- concentrations where *Daphnia* reproduction declined and mortality increased. Only 1.1% of the lakes had concentrations above 40 mg/L where sensitive species, such as *D. mendotae*, *D. minnehaha*, and *D. catawba*, failed to reproduce in most replicates (Figure S1, Supporting Information).

3.4. Discussion. Using three very different approaches (lab, field, and paleolimnological assessments), we provide strong evidence that zooplankton in Canadian Shield lakes are sensitive to Cl^- concentrations well below current Canadian water quality guidelines for long-term exposure (120 mg Cl^-/L[25]). Approximately 23% of Ontario's recreational lakes currently have Cl^- concentrations between 5 and 40 mg/L, a chloride range where we detected dramatic reductions in reproduction, suggesting that aquatic communities in these lakes may be at risk from winter salt application to nearby

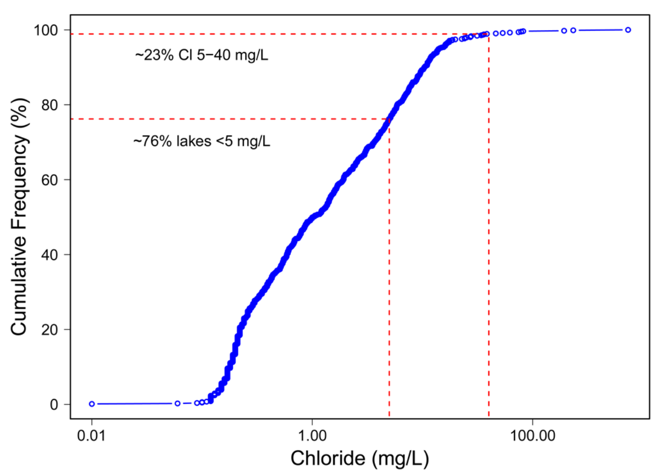

Figure 3. Cumulative frequency plot of chloride in n = 822 of Ontario's inland lakes for the years 2016−17. Dashed lines show that 76.2% of the lakes in the data set have chloride concentrations at or less than 5 mg/L, while 22.7% of the lakes have chloride concentrations between 5 and 40 mg/L Cl. The remaining 1.1% have Cl concentrations above 40 mg/L.

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roads. Indeed, the cladoceran assemblage structure has already been altered by road salt application in Jevins Lake, a lake situated near a twinned highway. Changes in the Jevins Lake cladoceran assemblage began almost immediately with the commencement of winter road salt application in the 1940s (Figure 2), a result that is consistent with declines in reproduction and increased mortality at Cl^- concentrations as low as 5−40 mg/L in our *Daphnia* life history experiments.

Our 21 day life history experiments, conducted using soft water media typical of thousands of Precambrian Shield lakes, revealed high sensitivity of six common *Daphnia* species at low Cl^- concentrations. Total reproductive output decreased with increasing Cl^- concentrations, and this effect was particularly strong for *D. mendotae*, a species with a widespread distribution in Shield lakes (e.g., 38% occurrence in MRW, Canadian Aquatic Invasive Species Network (CAISN) 360-lake database). This effect was coupled with reduced survival and clutch sizes, indicating likely decreases in population growth rates. Increased sensitivity to Cl^- under low food quantity[39] suggests that zooplankton in oligotrophic Shield lakes will be particularly vulnerable to road salt as nutrient concentrations decline.[67,68]

Our observed sensitivity of *Daphnia* and other cladocerans to Cl^- concentrations within the tested range of 5 to 145 mg/L contrasts with other toxicity studies that have demonstrated threshold tolerances that range from 285 to 516 mg Cl^-/L for several *Daphnia* species. *D. magna*, which is not found in lakes on the Canadian Shield, has high tolerance, with half the population surviving (i.e., LC50) short-term experiments in salinity up to 6−7 g/L,[69,17] although total reproduction over 21 days was reduced by 25% at 421 mg Cl^-/L.[31] *D. longispina*, a species that occurs in <3% of lakes in MRW, experienced reduced reproduction and population growth rates at 1040 mg Cl^-/L in a 21 day test.[70] Population dynamics of *D. pulex* were altered at 2.0 g of NaCl (1210 mg Cl^-/L),[23] and 21 day tests demonstrated a 10% loss of reproduction at 368 mg Cl^-/L (71 in ref 31). For *D. ambigua*, found in 24% of MRW lakes, the lowest effect concentration (LOEC) for reproduction over 3 broods was 516 mg Cl^-/L.[72] Overall, the effect of Cl^- was greater in our study; total reproduction at 21 days was reduced by 65% and the daily mortality rate increased by 800% at 120 mg Cl^-/L compared to control conditions, averaged across all six *Daphnia* species.

There are two logical explanations for greater sensitivity to Cl^- in our study compared to previously published studies. First, we employed species that were relevant to Shield lakes (and originated from Shield lakes − with the exception of *D. pulex*). Second, we conducted our studies in a soft water medium that is more representative of the conditions in Shield lakes. An important aim of our study was to compare chloride toxicity among multiple species that are common in soft water lakes. We examined six *Daphnia* species: *D. catawba*, 51% occurrence; *D. mendotae*, 38% occurrence; *D. minnehaha*, 1% occurrence, and members of the *D. pulex* complex, 14% occurrence in MRW lakes (CAISN 360-lake database). Some species commonly used in standard toxicity tests, such as *D. magna* (e.g., refs 31 and 73), do not inhabit soft water lakes and have limited value for assessing regional sensitivity. While other species used in toxicity tests, including *D. pulex* and *Ceriodaphnia dubia* (19% occurrence in MRW),[17,31,69,73] are common in Shield lakes, our results reveal considerably higher sensitivity to chloride than published studies. The use of natural populations of *Daphnia* and other test organisms may provide a more accurate assessment of sensitivity to Cl^- and other pollutants (e.g., ref 74).

An important difference between our test medium and that used in published chloride toxicity tests is water hardness. With some exceptions,[31] most tests have used standard hard water media or local water with higher hardness than Shield lakes in Ontario. Ions, including Ca^{+2}, decrease toxicity of chloride.[31,32,75] Elphick et al.[31] quantified the relationship between chronic chloride sensitivity (IC25) for *C. dubia* reproduction and water hardness ($CaCO_3$). Based on these results, they suggested that water quality guidelines for *C. dubia* should range from 388 mg Cl^-/L at 160 mg $CaCO_3$/L (64 mg Ca^{2+}/L) to 64 mg Cl^-/L at 10 mg $CaCO_3$/L (4 mg Ca^{2+}/L). Our results that *Daphnia* species suffer losses in reproduction and increased mortality at low Cl^- concentrations ranging from 5 to 40 mg/L support these findings and indicate that water quality guidelines should be tailored to protect organisms in soft water lakes. Our test medium (FLAMES) was designed to mirror the water chemistry of the two lakes in Muskoka, ON,[30] and contains only 2.5 mg Ca^{2+}/L, compared to 10 mg Ca^{2+}/L in COMBO[76] and 13.5 mg Ca^{2+}/L in EPA synthetic water,[77] which are commonly used in toxicity tests. Approximately two-thirds of the lakes in the Muskoka River Watershed, Ontario, have Ca^{2+} concentrations less than 2.5 mg Ca^{2+}/L.[47] Despite evidence that water hardness ($CaCO_3$) can modify sensitivity to Cl^- for several organisms, including daphniids, a hardness-based Canadian Water Quality Guideline was not developed owing to insufficient data.[27] Our study provides important evidence that failure to develop water hardness-based guidelines may leave organisms in thousands of lakes vulnerable to increasing Cl^- associated with winter road deicing. The combination of increasing Cl^- and declining Ca^{2+}[33] and total phosphorus[67,68,71] in Canadian Shield lakes may lead to a "perfect storm" that could result in altered energy flow and ecosystem services as large grazers, such as *Daphnia*, are reduced or lost from aquatic food webs.

As expected, we detected differences in sensitivity among species with *D. pulex*, *D. pulicaria*, and their hybrid was generally being more tolerant to Cl^- than *D. catawba*, *D. minnehaha*, and *D. mendotae*. Although it remains unclear why some species were more sensitive than others, one explanation could be that smaller-bodied individuals are more sensitive to ionoregulatory toxins.[78] This is, at least in part, because of the negative relationship between body mass and whole-body Na^+ uptake, resulting in higher Na^+ influxes in small-bodied individuals. Ionoregulation is energetically expensive with Na-K-2Cl cotransport being fueled by a Na-K-ATPase driven sodium pump,[79] and therefore increased osmoregulatory actions associated with increases in external NaCl concentrations could limit energy available for other functions including reproduction, particularly in small individuals.

Our lab-based life history studies likely underestimate the effects that will be observed in natural populations. Searle et al.[22] detected population-level responses to NaCl at 364 mg Cl^-/L for *D. dentifera*, despite failing to detect an effect on birth, death, and population growth rates when daphniids were tested individually, in a manner similar to our life history assays. This suggests that, in populations or communities where intra- and interspecific competition occurs, the effects of increased salinity may be greater than those detected in life history bioassays. For example, at high salinity, the competitive dominance of *D. galeata* over *Simocephalus vetulus* was reversed,[80] albeit at concentrations much higher than those

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tested in our experiment (910 mg Cl^-/L). Second, increased salinity may also influence predator−prey relationships. Decreased swimming rates observed at high salinity[22,81] could alter the probability and frequency at which *Daphnia* encounter predators, making them less susceptible to sit-and-wait invertebrate predators, such as *Chaoborus*.[82−84] In contrast, there is evidence that NaCl can impair antipredator responses in *D. pulex*[85] and *D. pulicaria*[26] suggesting that increasing Cl^- in natural systems with abundant invertebrate or fish predators may result in indirect effects associated with weakened antipredator defenses, as well as direct toxicological effects. However, experiments have also indicated that when *D. pulex* is exposed to NaCl and fish kairomone (fat head minnow and bluegill), metabolic rates and acute toxicity were lower than those when it is exposed to NaCl in the absence of kairomone.[86] These contrasting results indicate that more research aimed at understanding the interactive effects of road salt and predators on zooplankton is warranted. Finally, our study considered NaCl because it is the most common deicing agent applied to roads and other paved surfaces and is responsible for the increase in Cl^- concentration in Jevins Lake. Regions where alternative chloride salts are applied may have more severe consequences for aquatic organisms because they are more toxic than NaCl (KCl > $MgCl_2$ > $CaCl_2$ > NaCl; refs 32 and 87, but see Hintz and Relyea[26] for sublethal effects on trout ($CaCl_2$ > NaCl >$MgCl_2$)). Road salt may also contain a variety of other contaminants, which could increase toxic effects on daphniids.

Our paleolimnological comparison of long-term changes in cladoceran species assemblages in Jevins and Heney lakes further supports our conclusions that organisms in soft water Shield lakes are vulnerable to road salt (NaCl) at concentrations well below current water quality guidelines. Although *Daphnia* remains were rare in Jevins Lake, we detected changes in cladoceran community structure, concurrent with the onset of road salt application in the MRW. Ordination of cladoceran species composition revealed a marked shift beginning in the mid-1950s, which strongly suggests a response to road salt additions, as similar shifts were not observed in Heney Lake, a nearby reference lake (Figure 2).

The assemblage shift found in our paleolimnological study of Jevins Lake was related to changes in a few sensitive taxa, specifically *C. brevilabris*, *Bosmina* spp. and *A. circumfimbriata* (*rectangula*). Although this shift was relatively subtle, it was persistent, and the increase in *A. circumfimbriata* is significant. This taxon is relatively saline-tolerant and has been linked in previous studies to increasing Cl^- concentrations.[88−90] The increase in *C. brevilabris* and decrease in *Bosmina* spp. are likely related to salinization as *Bosmina* spp. is known to be relatively saline-intolerant, whereas *C. brevilabris* is a more salt-tolerant species.[89]

In soft water lakes in south-central Ontario, zooplankton may have already been affected by road salt application since 22.7% of the lakes have Cl^- concentrations of 5 to 40 mg /L, concentrations where *Daphnia* life history responses to NaCl were detected in our bioassays. Monitoring programs are needed to examine *Daphnia* and other zooplankton presence and abundance in those lakes.

3.4.1. Broader Implications and Multiple Co-Occurring Stressors. The susceptibility of key aquatic herbivores in Canadian Shield lakes, at low Cl^- concentrations ranging from 5 to 40 mg/L, provides strong evidence that current water quality guidelines do not protect sensitive aquatic taxa. *Daphnia* and other cladocerans are critical components of aquatic food webs, controlling phytoplankton biomass and providing energy and nutrients for higher trophic levels; substantial reduction in their abundance is likely to result in reduced delivery of ecosystem services that include the provisioning of drinking water, fisheries, and recreation. The effects of increasing Cl^- on aquatic ecosystems are likely to be further compounded by concurrent environmental changes that include declining Ca^{2+} and total phosphorus. For example, declining Ca^{2+} has direct effects by reducing reproduction and population growth rates of *Daphnia* and other zooplankton,[36,37] as well as indirect effects by increasing Cl^- toxicity.[31] However, limited data on these interactions has thus far impeded the incorporation of water hardness into water quality guidelines, particularly at low Ca^{2+} concentrations typical of Canadian Shield lakes. Further studies on the population and community scales are needed to fully explore the interaction between increased salinity and Ca^{2+}. Declining total phosphorus concentration in Shield lakes will likely interact with Cl^- by determining food concentrations and increasing sensitivity of key herbivores;[39] further studies are needed to determine the interaction between nutrients and chloride sensitivity in field conditions. Together, these co-occurring environmental stressors are likely to magnify the risk associated with increasing Cl^- concentrations in lakes, necessitating the need to re-evaluate water quality guidelines for protecting sensitive organisms. Our lab, field, and paleolimnological studies, representing current conditions in many soft water Shield lakes, highlight the urgency of this task.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c02396.

We have included tables with results of statistical analyses and additional graphs showing individual species responses to chloride concentration as the Supporting Information (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. S.E.A. led the writing of the paper and conducted and interpreted statistical analyses for the life history experiments, M.P.C.-S. designed and conducted life history experiments, R.E.V. processed sediment samples and conducted principal component analyses, A.M.D. compiled survey data and conducted analyses, and all authors contributed to the writing, interpretation, and editing. All authors have given approval to the final version of the manuscript.

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Notes

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