Microbial Ecology (2020) 80:366–383
https://doi.org/10.1007/s00248-020-01513-w

ENVIRONMENTAL MICROBIOLOGY

Intra-Lake Arcellinida (Testate Lobose Amoebae) Response to Winter De-icing Contamination in an Eastern Canada Road-Side "Salt Belt" Lake

Charlotte F. Cockburn[1,2] · Braden R. B. Gregory[2] · Nawaf A. Nasser[2] · R. Timothy Patterson[2]

Received: 15 March 2019 / Accepted: 7 April 2020 / Published online: 8 May 2020

Abstract

Salt contamination of lakes, due to the application of winter de-icing salts on roads, presents a significant environmental challenge in the "salt belt" region of eastern North America. The research reported here presents the first deployment of a previously published proxy tool based on Arcellinida (testate lobose amoebae) for monitoring road salt contamination. The research was conducted at Silver Lake in Eastern Ontario, a 4-km-long lake with the heavily traveled Trans-Canada Highway (HWY 7) transiting the entire southern shore. The lake showed elevated conductivity (297–310 μS/cm) and sub-brackish conditions (0.14–0.15 ppt). Sodium levels were also elevated near the roadside (median Na = 1020 ppm). Cluster analysis and nonmetric multidimensional scaling results revealed four distinct Arcellinida assemblages: "Stressed Cool Water Assemblage (SCWA)," "Deep Cold Water Assemblage (DCWA)," both from below the 8-m thermocline, and the shallower water "Shallow Water Assemblage 1 (SWA-1)" and "Shallow Water Assemblage 2 (SWA-2)". Redundancy analysis showed a minor response of Arcellinida to road salt contamination in shallower areas of the lake, with confounding variables significantly impacting assemblage distribution, particularly beneath the thermocline (e.g., water temperature, water depth, sediment runoff from catchment [Ti], sediment geochemistry [Ca, S]). The results of this study indicate that the trophic structure of the lake has to date only been modestly impacted by the cumulative nature of road salt contamination. Nonetheless, the Silver Lake results should be considered of concern and warrant continued arcellinidan biomonitoring to gauge the ongoing and long-term effects of road salt on its ecosystem.

Keywords Lakes · Arcellinida · Testate lobose amoebae · Road salt contamination · Environmental monitoring · Bioindicators

Introduction

Road salt, most commonly sodium chloride (NaCl), has been used to minimize the hazards associated with icy roads in eastern North America since the 1940s. Road salt is an inexpensive de-icing agent effective to − 18° C whose use is credited with an estimated 88% reduction in road accidents, and a 200% reduction in the severity of accidents on multi-lane highways [1]. However, the mass application of winter de-icing salts to roads has led to salt contamination of freshwater lakes and ponds near roadways [2, 3]. Salt-laden runoff enters lakes as overland flow and through the water table during and after the mass snowmelt of early spring. This has a detrimental effect on environmental systems, reducing aquatic biodiversity and populations, which can change the overall ecological health of lakes [4–6]. With thousands of tonnes of road salt being used on eastern North American roads every year, it is imperative to characterize the impact of the mass application of road salt on lacustrine biota to forestall irreparable damage to the biodiversity of water bodies.

Arcellinida (testate lobose amoebae) are a group of shelled protozoans that inhabit most lentic and lotic environments, from the tropics to the arctic regions [7–10] and can live in fresh (e.g., lakes, rivers, and ponds) to brackish (e.g.,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-020-01513-w) contains supplementary material, which is available to authorized users.

✉ Nawaf A. Nasser
Nawaf.nasser@carleton.ca

[1] Department of Earth and Planetary Science, McGill University, 845 Sherbrooke St. W, Montreal, Quebec H3A 0G4, Canada

[2] Ottawa-Carleton Geoscience Center and Department of Earth Sciences, Carleton University, 1125 Colonel By Dr, Ottawa, Ontario K1S 5B6, Canada

peatlands, and salt marshes) habitats [9, 11–16]. Their amoeboid cell is protected by a test (shell) that ranges in size from 5 to 300 μm and is typically well-preserved in Quaternary lacustrine sediments. The tests are produced by secretion or through agglutination and are highly resistant to decay. Arcellinida are useful bioindicators due to their high preservation potential, rapid reproduction rates (days to weeks), and relative ease of taxonomic identification compared to other lacustrine microfaunal groups such as diatoms [17, 18]. They have been used as bioindicators for many environmental parameters, including water table fluctuations [19], lake acidity [20, 21], land-use change [22], metal mining [23], water quality [24], ecosystem health [25], nutrient loading [26], and pH variability [18]. The spatio-temporal distribution of the group has been shown to be sensitive to climate, human settlement, sediment input, and biogeography [27–29].

Arcellinida have recently been shown to be sensitive bioindicators for monitoring and determining changes in road salt contamination [2]. By examining changes in faunal assemblages and abundance across lakes, it is possible to gauge the severity of impact of road salt contamination. Certain species are more indicative of stress than others, and several of these species have been further classified into informal infra-sub-specific strains (indicated by quotation marks in this paper), which are characterized by distinct morphotypes attributable to specific habitats or environmental stressors (e.g., road salt). A comprehensive study of road-salt-contaminated lakes and ponds in the Great Toronto Area (GTA) showed that water bodies with the highest degree of salt contamination (bottom water chloride concentrations greater than 400 mg/l and conductivities greater than 800 μS/cm) exhibited depressed Arcellinida diversity and higher abundances of stress-tolerant species and strains, such as *Arcella vulgaris* Ehrenberg 1832 (AV; [2]). Thus, by examining changes in faunal assemblages and abundance within a given lake, it is possible to gauge the severity of impact of NaCl contamination and evaluate the ecological health of low-trophic-level ecosystems.

In this study, the arcellinidan response to several lake parameters (e.g., conductivity, mineral concentrations, water depth, dissolved oxygen, and substrate composition) was analyzed in 30 sediment-water interface samples from Silver Lake, a small lake located along Highway 7 near Maberly in eastern Ontario (Fig. 1), to identify the primary controls over the arcellinidan intra-lake distribution and confirm whether road-salt contamination has any impact on the lake's Arcellinida community. Silver Lake was selected for analysis based on concerns expressed by the Silver Lake Association and Mississippi Valley Conservation Authority (MVCA) that road salt-contaminated runoff from the adjacent highway is having a cumulative negative effect on the lake ecosystem.

Study Area

Silver Lake is a relatively small lake (area = 2.46 km^2) located in rural eastern Ontario near Maberly (44° 82′ 78.11″ N, 76° 59′ 94.37″ W; Fig. 1). The lake is elongate and relatively narrow, with shallow areas on the eastern and western margins that dip steeply toward a primary basin (maximum water depth = 23 m). The elevation of the lake is 200 m above sea level. The lake is surrounded by relatively flat terrain and bordered by Highway 7 to the south, Silver Lake Provincial Park to the east and residences along the north and west shores. Silver Lake is also bounded by marshland and boreal forest and is not fed by any major stream inlets. The average winter (October to March) temperature in Maberly, Ontario, is − 2.3 °C, and the average summer (April to September) temperature is 14.7 °C. The lake receives an average of 798 mm of precipitation annually, the majority (619.4 mm) of which occurs as rainfall during the summer [30].

Silver Lake is close to four lakes located along the Highway 7 that have been previously studied for salt contamination by Roe and Patterson [2]; Kaladar Jack Pine, Blueberry Lake, Mytopo Lake, and Cox's Lake. The distances between these lakes and Silver Lake are similar. The proximity of these lakes to Silver Lake (i.e., the distance between Cox's Lake and Silver Lake is just 28.4 km). Therefore, it is useful to compare the faunal consistency of Arcellinida assemblages in Silver Lake to the four Highway 7 lakes in response to road salt contamination and other possible drivers.

Possible Environmental Drivers in Silver Lake

Road Salt

Road salt is of particular concern for lotic ecosystems in the North American salt belt because the impacts of contamination are cumulative. If the residence time of NaCl in a given waterbody is more than 1 year, progressive salinization of the fresh water system will occur due to carry-over contamination from previous years. This increase in lake water salinity is often represented by an increase in water conductivity. Previous work by Roe and Patterson [2], on lakes and ponds in the Greater Toronto Area (GTA) showed that the water bodies had been impacted, with conductivity levels ranging from 400 to 1700 μS/cm in highly road-salt-impacted lakes, corresponding to salinities of 0.2 to 0.9 ppt, with the more contaminated lakes exhibiting brackish salinity (0.5–30 ppt), similar to what might be expected in tidally influenced river systems and estuaries. In lakes from regions where the impact of road salt has been less severe, conductivities of ~ 350 μS/cm (0.17 ppt) were observed [2]. In contrast, pristine lacustrine systems in regions of northern Ontario that have been unimpacted by urbanization typically exhibit much lower conductivity. In a survey of 44 lakes in Killarney Provincial

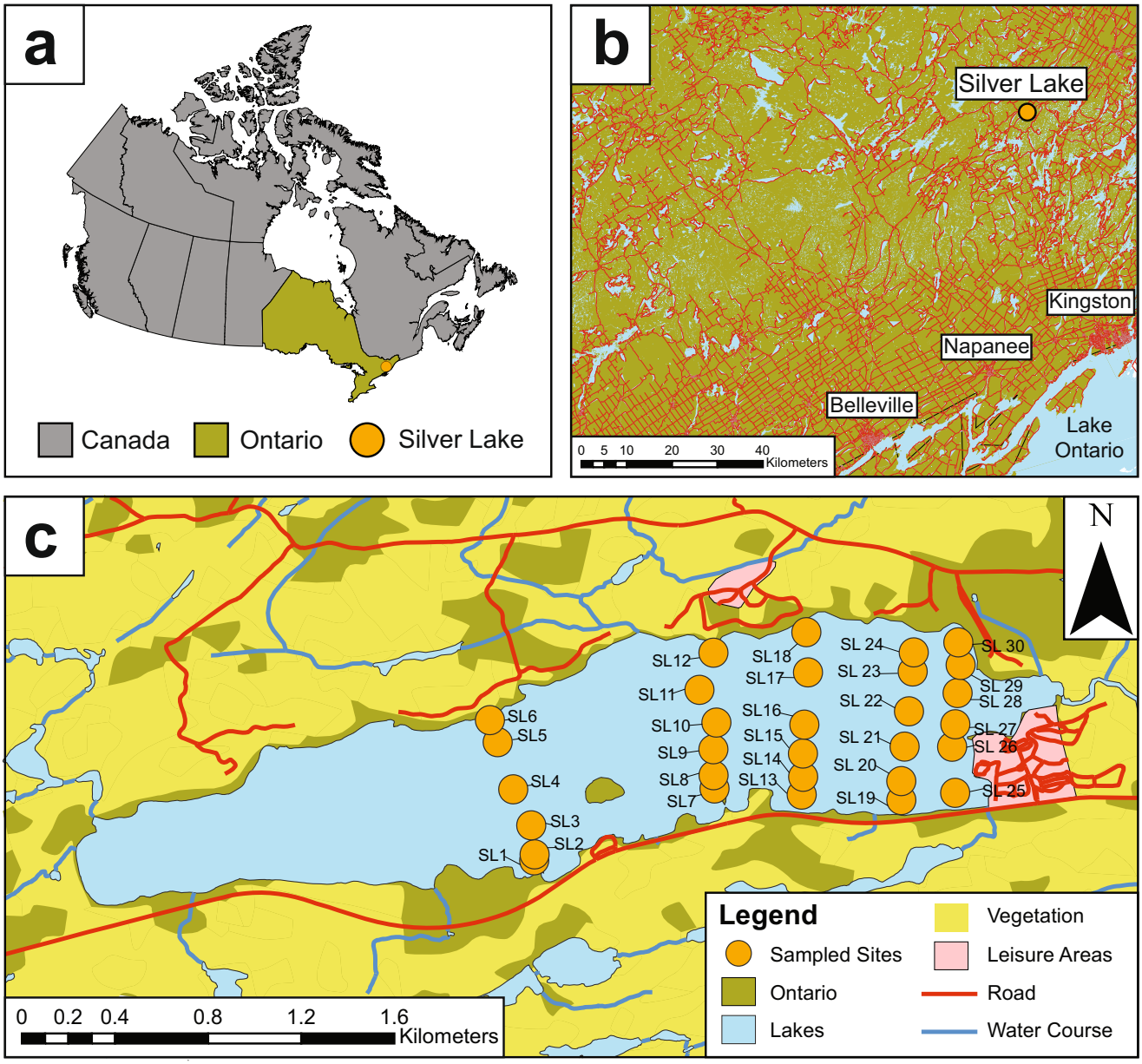

Fig. 1 Location map of Silver Lake, Maberly, Ontario, Canada. Map **a**—the location of Silver Lake in North America. Map **b**—the location of Silver Lake (black circle) in eastern Ontario. Map **c**—the intra-lake sampling stations ($n = 30$)

Park, Ontario, Suenaga [31] reported annual average conductivity values of < 30 μS/cm (0.02 ppt). Similarly, Roe and Patterson [2] reported values of < 100 μS/cm (0.05 ppt) for lakes adjacent to less-traveled roadways in central and eastern Ontario on the Canadian Shield where application of winter de-icing salt is reduced. It is assumed that the average Ontario lake unimpacted by road salt would typically exhibit conductivity values < 100 μS/cm (0.05 ppt).

Increases in chloride ion concentration in lake water are also indicative of road salt contamination. Dugan et al. [32] reported that the mean chloride concentration of lakes in Ontario is 2.3 mg/l (0.004 ppt; $n = 8$) and for North American lakes is 8.1 mg/l (0.014 ppt; $n = 371$). Data from the Broadscale Monitoring Program, which documented chloride concentrations in hundreds of lakes across Ontario, found that the median chloride concentration in provincial lakes was 0.4 mg/l (0.0007 ppt; $n = 826$), with values ranging from 0 to 90.5 mg/l (0 to 0.16 ppt, Fig. 3, OMECP, 2012). Roe and Patterson [2] observed chloride concentrations of > 200 mg/l (0.36 ppt) in highly impacted lakes within the GTA, 100–125 mg/l (0.18–0.22 ppt) in rural Ontario lakes near roadways further north, and < 10 mg/l (0.018 ppt) in remote lakes away from roads. In Killarney Provincial Park, an area beyond the impact of anthropogenic chloride sources, chloride concentrations in 44 lakes were on average 1.1 mg/l (0.0020 ppt; [31]). These

values are much lower than the Canadian Environmental Quality Guidelines, which state that for the safety of aquatic life, chloride concentrations must remain below 120 mg/l (0.21 ppt) for long-term exposure (> 20 days) and below 640 mg/l (1.16 ppt) for short-term exposure (< 72 h; [33]). An unimpacted lake in Ontario would be expected to exhibit chloride concentrations < 10 mg/l (0.018 ppt) and may be considered at risk when chloride concentrations begin to approach 120 mg/l (0.21 ppt) monthly average chloride concentrations.

Runoff

Runoff can impact faunal assemblages because it brings minerals and nutrients to the water and increases turbidity. Titanium is frequently used as a proxy for runoff and is associated with fine sand particles [34, 35]. Silty substrates lead to higher arcellinidan biodiversity [8, 36, 37]. Aside from possible salt contamination, runoff entering Silver Lake is likely transporting silty materials into the basin, which can have an impact on the faunal assemblages identified in this study.

Methods

Field Methods

To assess whether road salt has impacted low-intermediary trophic level ecosystems within Silver Lake, 30 sediment-water interface samples were collected and analyzed for their arcellinidan assemblages, as well as geochemical and sedimentological characteristics. Only the eastern half of the lake was sampled as this region, adjacent to Highway 7, has potentially been most impacted by road salt runoff. Samples were collected from 30 stations along five N-S transects, evenly distributed throughout the eastern half of the lake (Fig. 1) on May 28, 2018. Samples were collected using an Ekman grab sampler with the upper 0.5 cm of sediment being retained for analysis—arcellinidan fauna within this interval are representative of contemporary lake conditions [38]. Conductivity (μS/cm), temperature (°C), and oxygen content (mg/l) were recorded at the surface and at 1-m intervals down the water column at each station using a YSI Professional Plus multiparameter water quality meter (Table 1). Water depth at each station was determined using a commercial "fish-finder". A handheld global positioning system (GPS) was used to record the location of each sample station.

Additional water property data was provided by the MVCA to help contextualize the observations of this study. A YSI multiparameter water quality meter was used on multiple occasions during the open water seasons of 2005, 2010, and 2018 to characterize changes in water body stratigraphy ([39, 40]; ESM Table 1; ESM Table 2). Secchi disk analysis data, used to measure the water clarity, was made available by the MVCA for 1975 to 1995, 2000, and 2005 (ESM Table 3; [39, 40]). The MVCA also provided total chlorophyll *a* concentrations for the lake for these same years plus data for 2010 (ESM Table 3; [40]). To determine chloride concentrations across Silver Lake, the MVCA used a composite sampler to collect water ~ 1 m above the lake bottom in the summer of 2018 (ESM Table 3). To contextualize chloride concentrations observed in Silver Lake, data on chloride concentrations from lakes within the Salt Belt of North America were retrieved from the Dugan et al. [32] dataset on worldwide chloride concentrations and the Ontario Ministry of the Environment, Conservation, and Parks Broadscale Monitoring Program, which provides chloride concentrations from lakes in Ontario measured from 2008 to 2012 ($n = 826$; [41]).

Laboratory Methods

Particle Size Analysis

Particle size analysis was carried out to identify variations in the sedimentological composition of sampled substrates across the lake. Subsamples (~ 5 cc) were initially treated with 10% HCl to digest organic matter [42, 43]. Carbonates were then digested using 35% H_2O_2 [42, 43]. The samples were analyzed using a Beckman Coulter LS 13 320 laser diffraction analyzer with a measurement range of 0.4 to 2000 μm. An obscuration level of 10 ± 3% was attained once the samples were loaded into the instrument. The results were compiled using GRADISTAT (Version 8; [44]). The inverse distance weighting (IDW) geospatial interpolation tool in ArcMap 10.1 was used to interpolate shifts in grain size between sampled points as a method of visualizing spatial trends.

Geochemical Analysis

Sediment subsamples were sent to ACME Analytical Laboratories Ltd. (Vancouver) for analysis of trace element concentrations using the inductively coupled plasma mass spectrometry (ICP-MS) method. Metals were extracted using aqua regia digestion protocol (ICP-MS 1F/AQ250 package), which is most suitable for determining the concentration of bioavailable elements (i.e., not contained within mineral lattices) that might potentially impact the distribution of Arcellinida [45, 46]. To visualize the spatial trends of elemental concentrations across the sampled basin, select elemental concentrations were plotted with the IDW tool.

Loss on Ignition

Loss on ignition was carried out on subsamples from all 30 stations following the methods of Heiri et al. [47] to determine the relative water, organic, carbonate, and minerogenic

Table 1 Total dissolved solids (mg/l) and chloride concentrations (mg/l) from locations across Silver Lake. Water samples were recovered 1 m from the bottom. RDL = relative detection limit in milligrams per liter. Latitude and longitude present in UTM. This data was collected by the MVCA during summer

Sample label	UTM zone	Latitude	Longitude	Site description	Analysis	Result	RDL	Date and time sampled	
BGY356	11 S	375367	4965687	Silver Lake Outlet	Total dissolved solids	158	10	17/05/2018	11:30:00
BGY357	11 S	375146	4965184	Silver Lake SE	Total dissolved solids	160	10	17/05/2018	11:50:00
BGY358	11 S	373441	4964635	Silver Lake SW	Total dissolved solids	164	10	17/05/2018	12:10:00
BGY359	11 S	373111	4965065	Silver Lake NW	Total dissolved solids	130	10	17/05/2018	13:00:00
BGY352	11 S	374791	4965730	Silver Lake NE	Total dissolved solids	98	10	17/05/2018	13:30:00
BBV512	11 S	373438	4904615	Silver Lake SW	Total dissolved solids	196	10	09/07/2018	9:20:00
BBV516	11 S	373146	4965070	Silver Lake MW	Total dissolved solids	164	10	09/07/2018	9:50:00
BBV509	11 S	375069	4965181	Silver Lake SE	Total dissolved solids	142	10	09/07/2018	10:10:00
BBV513	11 S	375294	4965640	Silver Lake Outlet	Total dissolved solids	160	10	09/07/2018	10:25:00
BBV517	11 S	374791	4965730	Silver Lake NE	Total dissolved solids	158	10	09/07/2018	10:35:00
BGY356	11 S	375367	4965687	Silver Lake Outlet	Chloride	16.2	0.1	17/05/2018	11:30:00
BGY357	11 S	375146	4965184	Silver Lake SE	Chloride	16	0.1	17/05/2018	11:50:00
BGY358	11 S	373441	4964635	Silver Lake SW	Chloride	16.3	0.1	17/05/2018	12:10:00
BGY359	11 S	373111	4965065	Silver Lake NW	Chloride	16.9	0.1	17/05/2018	13:00:00
BGY352	11 S	374791	4965730	Silver Lake NE	Chloride	16.2	0.1	17/05/2018	13:30:00
BBV512	11 S	373438	4904615	Silver Lake SW	Chloride	16.4	0.1	09/07/2018	9:20:00
BBV516	11 S	373146	4965070	Silver Lake MW	Chloride	16.9	0.1	09/07/2018	9:50:00
BBV509	11 S	375069	4965181	Silver Lake SE	Chloride	16.4	0.1	09/07/2018	10:10:00
BBV513	11 S	375294	4965640	Silver Lake Outlet	Chloride	16.6	0.1	09/07/2018	10:25:00
BBV517	11 S	374791	4965730	Silver Lake NE	Chloride	16.6	0.1	09/07/2018	10:35:00

content. For this procedure, the dry weight of clean crucibles was determined, and then reweighed after being filled with approximately 1–3 g of each sediment sample. The subsamples were then left to dry in the oven at 100 °C for 24 h. The dried subsamples were placed in a muffle furnace at 550 °C for 4 h and then at 950 °C for 2 h to remove organic matter and carbonate content, respectively. Because the 950 °C combustion only removed carbon dioxide and not all carbonates, the mass of combusted carbon dioxide was multiplied by 1.36, which is a constant derived from the molar relationship of the mineral.

Micropaleontological Analysis

To prepare for micropaleontological analysis, 3 cc of sediment was subsampled from each sample and sieved through a 297-μm sieve to remove coarse debris, then through a 37-μm sieve to separate Arcellinida from smaller particulate matter. Using a wet splitter (after [48]), samples were subdivided into six aliquots. Aliquots were analyzed wet on a gridded petri dish using a stereomicroscope (Olympus × 7.5–64 magnification). Arcellinida were identified following the strain concept [12] using key papers, illustrations, and SEM plates from previous studies (e.g., [14, 24]).

Statistical Methods

The arcellinidan content of sample aliquots were quantified until a statistically significant number of Arcellinida were obtained (greater than 150 in 18 samples and between 100 and 150 in 12 samples) after Patterson and Fishbein [49]. The determination of samples with statistically significant arcellinidan populations was achieved by calculating the probable error (*pe*) for each sample [49], such that

$$pe = 1.96\left(\frac{s}{\sqrt{X_i}}\right)$$

where s is the standard deviation of the counts at a particular sample location and X_i is the total number of counts at that station. A sample was deemed statistically insignificant if the total count of the sample did not exceed the probable error [50].

To determine the statistical significance of identified species and strains, the standard error (S_{xi}) was calculated for each sample [49], such that

$$S_{xi} = 1.96\sqrt{\frac{F_i(1-F_i)}{N_i}}$$

where F_i is the fractional abundance of a species or strain and N_i is the total species/strain count in a sample. This protocol

was based on the principal that if the standard error exceeded the total fractional abundance for that species in all samples, it was statistically insignificant and would not be included in further analyses [49].

The Shannon Diversity Index (SDI; [51]) was calculated to characterize species diversity and to obtain a general idea of the lake health. The SDI is defined as:

$$\mathrm{SDI} = -\sum_{i=1}^{S}\left(\frac{X_i}{N_i}\right) \times \ln\left(\frac{X_i}{N_i}\right)$$

where X_i is the sample abundance of a species or strain, N_i is the total abundance of all species and strains in a sample, and S is the species richness of the sample. If the SDI is < 1.5, the environment is considered to be stressed. SDI values between 1.5 and 2.5 indicate an environment in transition. A healthy environment generally has an SDI > 2.5 [52–54].

Data Screening

Prior to statistical analysis, the micropaleontological and geochemical datasets were screened to remove potential outliers and statistically insignificant data. Following the protocol of Reimann et al. [55], variables showing issues with more than 25% of contained data were removed (i.e., data values below or above instrumental detection limit, or missing values). Following established protocols for situations where less than 25% of the cases were below the lower detection limit, the value was given as half of the lower detection limit [55]. When the values were above the upper detection limit, values were reported as equal to the upper detection limit. Following this screening, all concentration data were converted to parts per million (ppm).

Reducing Variables

In order to reduce redundancy and enable a smoother analysis, several variables were screened out if they were determined to have no influence on the species distribution (after [56]). The variance inflation factor (VIF), which is a component of the "usdm package" in the RStudio statistical programming environment (R version 3.5.1;[57]), was used to remove select variables with VIF > 0.8 that were deemed to be highly co-linear and were eliminated from subsequent analysis.

Cluster Analysis, Detrended Correspondence Analysis, and Nonmetric Multidimensional Scaling

Q- and R-mode cluster analyses were carried out using the "hclust" function of the "stats" package in RStudio (R version 3.5.1; [58]) to group samples and species into assemblages. A Hellinger transformation was performed on the species dataset to reduce the influence of rare species. Ward's minimum variance method [59] was used to group samples containing similar populations of Arcellinida. The cluster distances in this method are defined as the squared Euclidean distance between points. This approach has been demonstrated to best simulate ecological relationships [50]. R-mode cluster analysis was carried out using the same approach to group species and strains of Arcellinida most often found together. These groupings of species were then used to characterize particular assemblages. The resulting dendrograms were merged into a two-way hierarchical dendrogram using the "cim" function in the "mixOmics" R package [60].

Detrended correspondence analysis (DCA; [61]) was performed on the Hellinger-transformed data to calculate the gradient length, which is a necessary criterion for selecting a properly constrained multivariate methodology when analyzing a dataset (e.g., redundancy analysis [RDA] vs. canonical correspondence analysis [CCA]). A gradient length of 1.44 was determined for the transformed data, which implied a unimodal response (gradient length < 2) for these samples, thus favoring the use of RDA over CCA. Nonmetric multidimensional scaling (NMDS) was performed to examine the spatial variability of Arcellininda in multidimensional space to improve the interpretation, and provide validation of the results obtained from the cluster analysis.

The Arcellinida data from Silver Lake and the Roe and Patterson [2] study were combined and analyzed using cluster analysis, NMDS, and DCA analyses in order to determine the position of the Arcellinida assemblages identified in Silver Lake in the context of the Roe and Patterson [2] assemblages.

Redundancy Analysis

RDA [62] was used to determine the relationship between the environmental parameters and the arcellinidan assemblages identified using cluster analysis and NMDS. This analysis allows for the identification of statistically significant environmental variables that contribute toward explaining the variance in the Arcellinida distribution. A series of partial RDAs along with a variance proportioning test were also performed to identify the number of statistically significant axes to be retained, and to determine the proportion of variance explained by the selected variables.

Salinity Calculations

Water salinity was calculated using water conductivity measurements from Silver Lake based on the formula provided by the United States Geological Survey [63] for conversion of conductivity to salinity based on formula:

$$S = 0.0120 + \left(-0.2174\, R^{1/2}\right) + (25.3283\, R) + \left(13.7714\, R^{\frac{3}{2}}\right) + \left(-6.4788\, R^{2}\right) + \left(2.5842\, R^{5/2}\right)$$

where S is the salinity of a given sample in practical salinity units (psu), and R is the ratio of measured conductivity at 25 °C and atmospheric pressure ($C(S, 25, 0)$) to the conductivity of seawater at 25 °C and atmospheric pressure ($C(35,25,0)$). This equation, based on the equation derived by Lewis [64], assumes conductivity which has been converted to specific conductivity at 25 °C and atmospheric pressure. No temperature correction has been applied to our data as the Handheld YSI device used in measuring conductivity applied temperature correction automatically in the measuring of conductivity.

Salinity was calculated from chloride concentrations using the simplified formula presented by Lewis and Perkin [65], for converting chloride concentrations to salinity:

$$S = 0.0018066 \times Cl^{-}$$

where S is salinity in parts per thousand and Cl^{-} is the chloride concentration of water in milligrams per liter.

Results

Lake Water Parameters

Lake water parameters measured during sampling in May, 2018, showed a weak thermocline established from 4- to 8-m depth and a well-oxygenated water column (Fig. 2a). Conductivity measurements, only recorded in 2018 as part of the field work for this study, exhibited a small decrease from 297 μS/cm (0.14 ppt) to a minimum of 293 μS/cm (0.14 ppt) from the surface to 5-m depth. This was followed by a steady increase to 310 μS/cm (0.15 ppt) at the maximum lake water depth of 23 m (Fig. 2).

Mississippi Valley Conservation Authority (MVCA) Data

Monitoring data from the MVCA suggest that temperature and oxygen stratification in Silver Lake varied considerably between spring and fall (Fig. 2b; ESM Table 1). A weak thermocline was present in May in all years that water stratigraphy was measured, generally at a shallow depth around 5 m. The thermocline strengthened and deepened throughout the summer to a maximum depth in October of ~ 12–15 m. Surficial waters remained well oxygenated through the open water season, but oxygen levels below the thermocline declined progressively, generally reaching dysoxic or anoxic conditions below 20 m by October (Fig. 2b). Secchi disk depths measured by the MVCA yearly from 1975 to 2015, showed a gradual increase in Secchi depth from 1975 through to the present, reaching a maximum of 7.9 m in 2010 (mean = 4.3, $n = 23$; Fig. 2c, ESM Table 3) and remaining > 7-m depth until 2015. Chlorophyll *a* concentrations collected alongside Secchi disk measurements showed values ranging from 1.2–3.6 (mean = 2.4 μg/L, $n = 23$; Fig. 2c, ESM Table 3), trending toward progressively lower values. The years with the lowest chlorophyll *a* values were 2000, 2005 and 2010 (< 1.3 μg/L). The oxygen concentrations and temperature measured through the water column in May, 2018 most closely resembled conditions that did not develop until late summer in 2005 and 2010.

During May and July 2018, water samples collected 1 m above the bottom at several locations across Silver Lake were tested for total dissolved solids (TDS) and chloride concentrations. The TDS concentrations in Silver Lake varied from 98 to 159 mg/l (mean = 153 mg/l, SD = 23 mg/l, $n = 10$; Fig. 3; ESM Table 4). Chloride occurred in much lower concentrations, varying from 16 to 16.9 mg/l (mean = 16.45 mg/l, SD = 0.28 mg/l, $n = 10$; Fig. 3a). Chloride and TDS concentrations were slightly higher in July than May. On average, chloride values comprised approximately 11% of TDS in Silver Lake. Additional data on chloride levels in lakes collected by Dugan et al. [32] and the BSM indicated that levels of chloride in North American Lakes show median concentrations that range from < 1 mg/l up to ~90 mg/l (Fig. 3b).

Silver Lake Water Quality

Silver Lake is categorized as oligotrophic based on water clarity and chlorophyll *a* levels, (ESM Table 3) which are linked to variations in the trophic status [66–68]. The faunal structure of the arcellinidan community of Silver lake similarly suggests oligotrophism; low to moderate diversity SDI values (SDI range = 1—2.4) coupled with the presence of significant populations of opportunistic and ecological stress-indicating centropyxid taxa that are typical of oligotrophic lakes (i.e., higher proportions of members of the genus *Centropyxis*; e.g., [12, 28, 69]).

Ongoing MVCA monitoring since the mid-1970s indicates a trend toward reduced nutrient concentrations and oxygen and pycnocline temperature stratification being established earlier in the season (ESM Table 1). Data pertaining to the dates of ice-melt on Silver Lake were not available, but shifts in lake water column temperature stratification from 2005 to 2018 suggested a trend toward earlier open water conditions in 2018 compared to 2005 and 2010, permitting earlier solar warming of lake waters ([70, 71]; Fig. 2a, b).

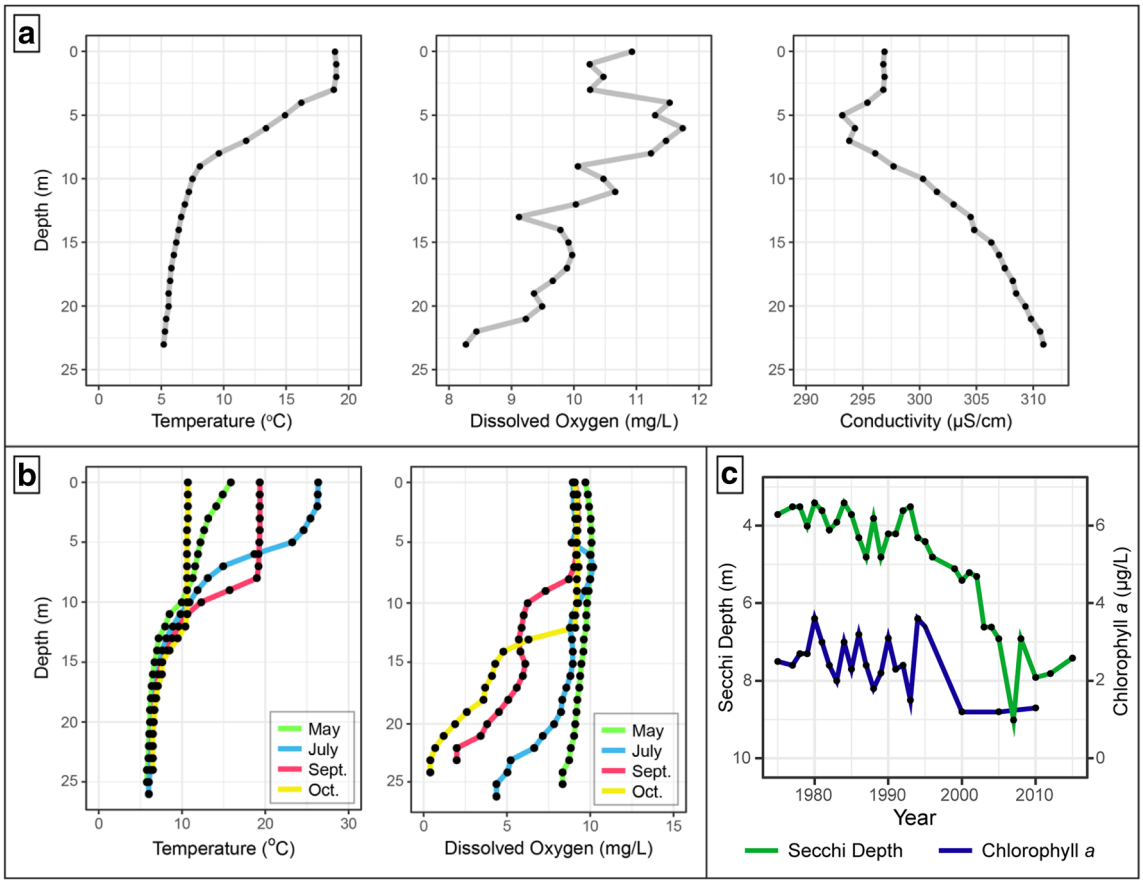

Fig. 2 Water stratigraphy in the main basin of Silver Lake. **a** Temperature, conductivity, and dissolved oxygen data measured at 1-m intervals at Station 4 during sample collection in May, 2018. **b** Temperature and oxygen for May (green), July (blue), September (red), and October (yellow); values represent the average of temperature/DO values measured by the Mississippi Valley Conservation Authority in 2005 and 2010. Individual values are provided in ESM Table 1. **c** Secchi disk depth (blue) and chlorophyll a concentrations (green) measured by the MVCA from 1975 to 2010. These profiles were averaged between 2005 and 2010

Geospatial Interpolation of Key Measured Variables

The interpolation maps generated using IDW for selected environmental variables: titanium (Ti), sodium (Na), sulfur (S), calcium (Ca), % sand, % silt, bottom water temperature and bottom water conductivity are shown in Fig. 4. Sodium and conductivity were selected because of the interest in salinity, and the other variables were selected because they have been proven to be significant in previous studies (e.g., [2]). Levels of conductivity were relatively high (median = 298.4 µS/cm; n = 30) with little variation across the lake and the highest levels recorded in the deepest water areas (median = 306.7 µS/cm; n = 7 water depth range = 17–23 m; Fig. 4a). The deeper areas of the lake, along with most of the western portion of the study area, were characterized by a primarily silty substrate (median water depth = 18 m; median silt = 76%; n = 7), while the eastern and shallower section of the lake were comprised of more sand-dominated sediments (median water depth = 5 m; median sand = 88%; n = 23; Fig. 4c, d). Water temperature is the highest to the east of the sampled basin, particularly along the lake's southern and norther shorelines (median water depth = 1.5 m; median water temperature = 19.3 °C; n = 11), with colder water being characteristic of deeper water (median water depth = 12 m; median bottom water temperature = 7.8 °C; n = 19; Fig. 4b). The IDW map for Na distribution is characterized by higher concentrations in the shallower areas within the southeastern portion of the study area (median water depth = 8.5 m; median Na = 1330 ppm; n = 11), and lower concentrations elsewhere, particularly in deeper areas of the lake (median water depth = 9 m; median Na = 1020 ppm; n = 19; Fig. 4e). Sulfur concentrations were highest in the deeper basin (median water depth = 18 m; median S = 23,800 ppm; n = 7) and decreased toward the lake edges (median water depth = 5 m; median S = 3400 ppm; n = 23; Fig. 4f). Levels of Ti were higher in the shallow areas along the eastern margin (median water depth = 8.5 m; median Ti = 1300 ppm; n = 7; Fig. 4g), and lower in the deep areas of the lake (median water depth = 10 m; median Ti = 860 ppm; n = 23). Calcium concentrations were higher toward the edge of the lake (median water depth = 5 m; median Ca = 103,500 ppm; n = 23; Fig. 4h), and low in the deep basin (median water depth = 18 m; median Ca = 25,400 ppm; n = 7).

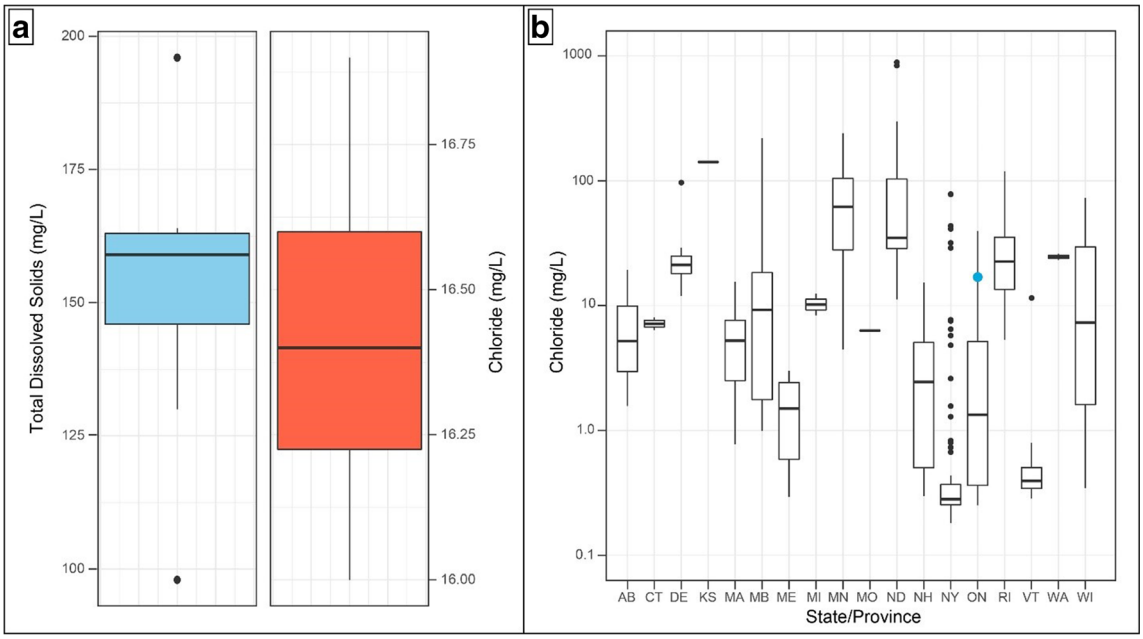

Fig. 3 **a** Boxplot of total dissolved solids (mg/l; blue) and chlorine concentrations (mg/l; red) measured across the Silver Lake basin by the MVCA during May and July, 2018. The thick black line indicates the median value. **b** Boxplots of chlorine concentrations (mg/l) of lakes throughout the Salt Belt of North America shown using a logarithmic scale. Data presented are a subset of the worldwide dataset of chlorine concentrations in waterbodies created by [32]. Thick black lines on the boxplots indicate median values. The blue dot in the ON boxplot represents the chlorine concentrations observed in Silver Lake. AB = Alberta; CT = Connecticut; KS = Kansas; Kansas; DE = Delaware; MA = Massachusetts; MB = Manitoba; ME = Maine; MO = Missouri; ND = North Dakota; NH = New Hampshire; NY = New York; ON = Ontario; RI = Rhode Island; VT = Vermont; WA = Washington; WI = Wisconsin; ON (BSM) = Chloride concentrations in Ontario lakes recorded in the Broadscale Monitoring Program

Cluster Analysis and NMDS

Q-mode cluster analysis results for bioindicator data from the 30 sample stations (Fig. 5) produced four distinct arcellinidan assemblages: 1) Stressed Cool Water Assemblage (SCWA; depth range: 7–17.5 m, Na range: 700–2140 ppm); 2) Deep Cold Water Assemblage (DCWA; depth: 17–23 m, Na: 490–820 ppm); 3) Shallow Water Assemblage 1 (SWA-1; depth: 1.5–2.5 m, Na: 510–1250 ppm); and 4) Shallow Water Assemblage 2 (SWA-2; depth: 1.5–5 m, Na: 830–1330 ppm). R-mode cluster analysis showed that, while all 25 identified Arcellinida species and strains were present in statistically significant proportions (see ESM Table 2), only six taxa contributed significantly toward shaping the faunal structure of the identified assemblages: *Difflugia oblonga* Ehrenberg 1832 strain "oblonga" (DOO); *Difflugia glans* Penard 1902 strain "glans" (DGG); *Centropyxis aculeata* (Ehrenberg 1832) strain "discoides (CAD)"; *Centropyxis aculeata* (Ehrenberg 1832) strain "aculeata" (CAA); *Centropyxis constricta* (Ehrenberg 1843) strain "constricta" (CCC); and *Centropyxis constricta* (Ehrenberg 1843) strain "aerophilia CCA)" (Fig. 5). The NMDS bi-plot further corroborated the results of cluster analysis, showing that the four assemblages grouped closely and distinct from each other (Fig. 6).

Performing cluster analysis on the combined Silver Lake and Roe and Patterson [2] Arcellinida data set revealed a strong association between samples of Silver Lake and samples from Kaladar Jack Pine, Mytopo Lake, Cox Lake, Mew Lake, and Jake Lake (ESM Fig. 1). The results of NMDS and Detrended Correspondence Analysis (DCA) corroborated the interpretation of the cluster analysis dendrograms by clustering the Silver Lake samples closely with samples of the aforementioned lakes (ESM Fig. 2; ESM Fig. 3).

Redundancy Analysis and Partial Redundancy Analysis

The RDA results were conformable with the results of cluster analysis and NMDS, indicating four distinct arcellinidan groups. Based on the variance partitioning of the RDA axes, only the first two axes were retained due to their statistical significance. Together, RDA axes one (eigenvalue = 0.0605) and two (eigenvalue = 0.02934) explained 50.6% of the total faunal variance (Fig. 7) using seven measured variables: Ti (17.9%), depth (10.1%), Na (9.7%), S (5.1%), bottom water temperature (3.1%), bottom water conductivity (2.2%), and calcium (2.21%). The six species and strains that dominated the assemblages were found to be strongly associated with these environmental parameters. DOO corresponded strongly to high Ti and Na concentrations. DGG corresponded with deeper waters and high conductivity measurements. The Centropyxids corresponded to high S, bottom water

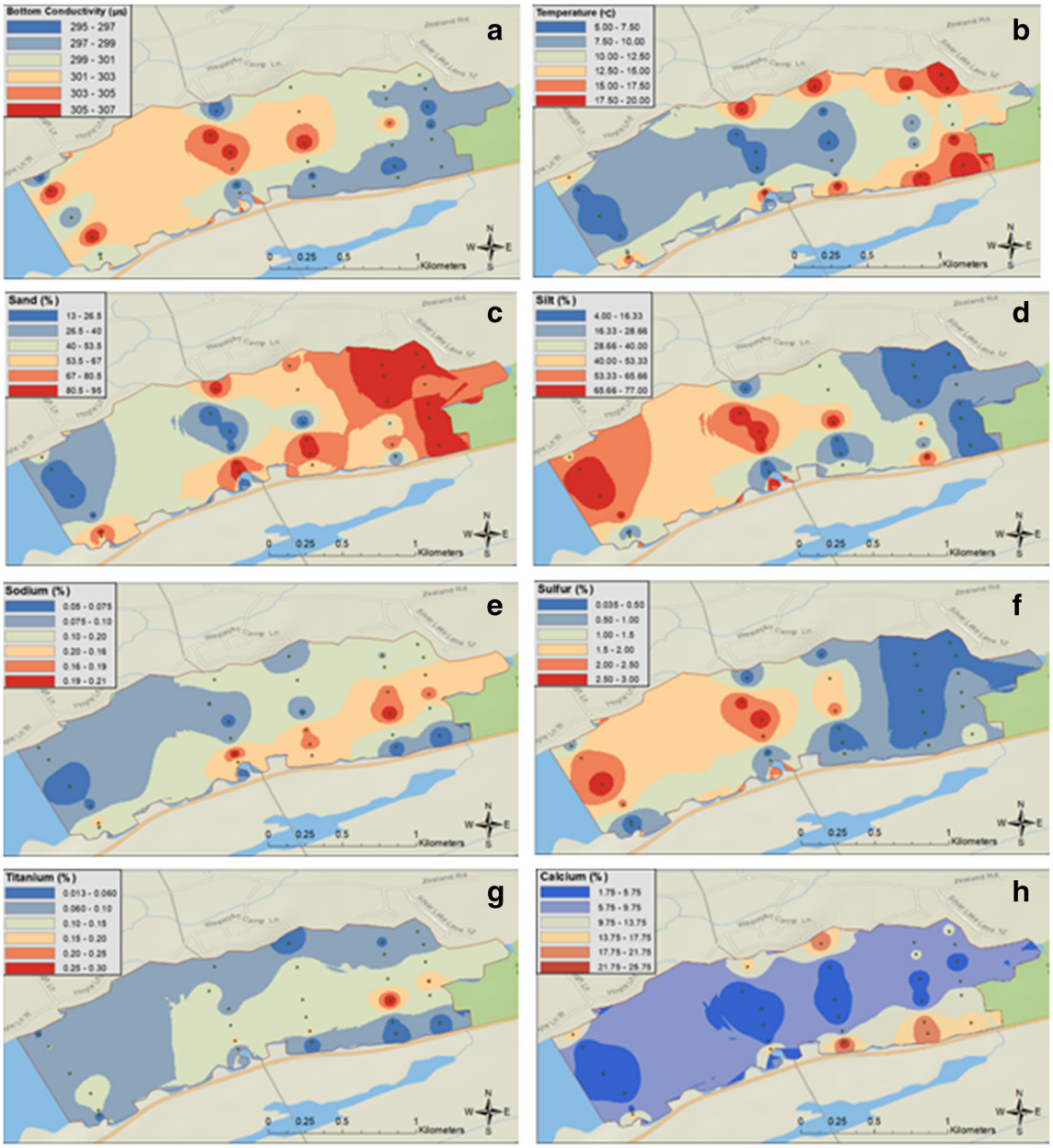

Fig. 4 Maps showing the results of Inverse distance weighting (IDW) interpolation. **a** Bottom conductivity (unit). **b** Water temperature (°C). **c** Sand (%). **d** Silt (%). **e** Sodium (%). **f** Sulfur (%). **g** Titanium (%). **h** Calcium (%)

temperature, and high Ca concentrations. CAD showed a slight preference for higher sulfur concentrations over high Ca concentrations.

Arcellinida Assemblages

Assemblage 1—Stressed Cool Water Assemblage (SCWA)

The faunal composition of the SCWA ($n = 13$) was dominated by DOO (median = 41%) and DGG (median = 27%; see ESM Table 2). CAD was also common in several samples (median = 11%). The samples in this assemblage were mainly found in sites at a greater distance from the shore and closer to the central portion of the eastern section of the lake (median water depth of 10 m); the lake here is cool (median bottom temperature of 8.8 °C), well-oxygenated (median dissolved oxygen of 10.61 mg/l), and sandy (median of 83% sand; Fig. 8). These substrates were also characterized by high levels of Ti (median = 1300 ppm) and Na (median = 1630 ppm). The RDA plot shows that the SCWA corresponds positively with Ti, Na, water depth, and bottom conductivity (Fig. 7). This assemblage had a SDI range of 1.0–1.9, indicating stressed to transitional environmental conditions [53, 54].

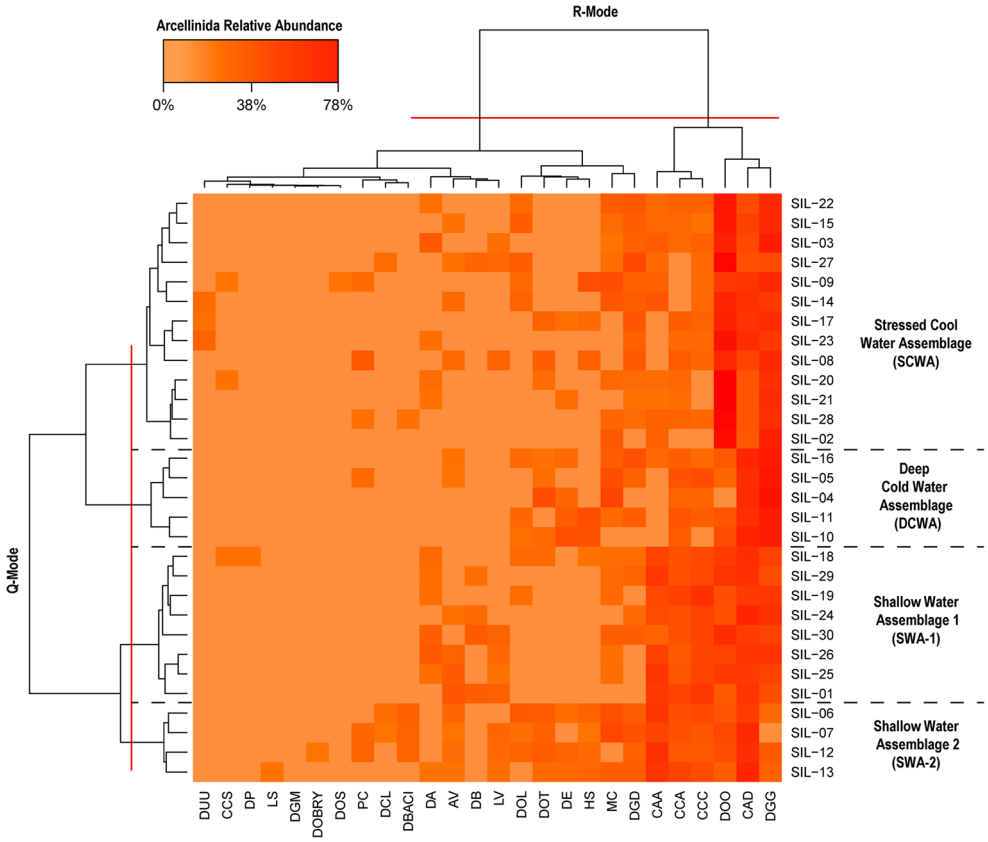

Fig. 5 A heat map (i.e., two-way cluster analysis) dendrogram for the 30 samples and (*n*) statistically significant arcellinidan species and strain. Four arcellinidan assemblages are identified. The color gradient reflects the relative abundance of arcellinidan taxa. The red line shows the distinction between assemblages

Assemblage 2—Deep Cold Water Assemblage (DCWA)

The SDI values for samples from the DCWA fell within the range of 1.4–1.8, indicating moderately stressed to transitional environmental conditions ([53, 54]; see ESM Table 2). The faunal structure of the DCWA was dominated by DGG (median = 37.5%) and CAD (median = 25.6%). The proportion of DOO was much lower in this assemblage (median = 5%) compared to the SCWA. The DCWA was mainly found in the center of the lake, and clustered within and around the deepest basin (Fig. 8). This assemblage occurred in a deep-water (median water depth of 20 m), cold (median bottom temperature of 6.1 °C), well-oxygenated (median bottom dissolved oxygen of 9.43 mg/l), and silty (median of 77% silt) area of the lake. The RDA bi-plot revealed a strong positive correlation between the DCWA samples and S (median = 2.52%) and a negative correlation with Na (Fig. 8).

Assemblage 3—Shallow Water Assemblage 1 (SWA-1)

The SWA-1 ($n = 8$) was dominated by CAD (median = 21%), DOO (median = 15%), CAA (median = 14.5%), DGG (median = 14%), CCC (median = 13.5%), and CCA (median = 8%). This assemblage occurred in shallow (median water depth of 1.5 m), warm (median temperature of 19.6 °C), well oxygenated (median bottom dissolved oxygen of 9.86 mg/l), and sandy (median of 86% sand) sediments in the eastern end of the lake (Fig. 8). Substrates associated with this assemblage were also differentiated by relatively low Ti levels (median = 410 ppm) and moderate Na levels (median = 990 ppm). On the RDA bi-plot, the SWA-1 was positively correlated with Ca (median = 14.57%) and higher bottom water temperatures, and negatively correlated with water depth and bottom conductivity. The SWA-1 was characterized by relatively high arcellinidan diversity (SDI = 1.8–2.1) indicative of a transitional environment [53, 54].

Assemblage 4—Shallow Water Assemblage 2 (SWA-2)

The SWA-2 ($n = 4$), like the SWA-1, is found in littoral areas of the lake but was characterized by a more diverse faunal structure than the SWA-1. The faunal structure of the SWA-2 was dominated by CAD (median = 26.3%) and CAA (median = 20.8%). Other common taxa included CCA (median = 8.5%), DOO (median = 8%), CCC (median = 7.8%), and *Mediolus corona* (median = 6%). The SWA-2 occurred along the northern and southern edges of the lake, which is shallow (median water depth of 2.5 m), warm (median bottom temperature of 18.9 °C), well oxygenated (median bottom dissolved oxygen of 10.46 mg/l) and sandy (median of 71% sand). The SWA-2 correlated positively with Ca (median = 17.40%) and bottom water temperature, and negatively with water depth

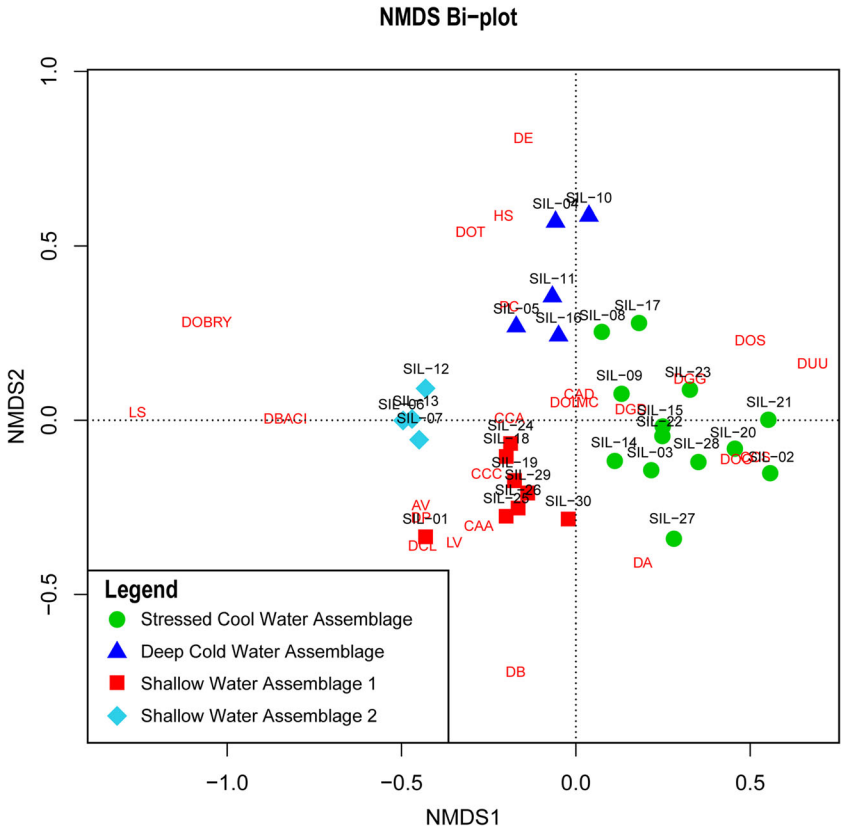

Fig. 6 Nonmetric multidimensional scaling (NMDS) plot showing the distribution of the sampled sites (colored symbols) and arcellinidan species (red acronyms) and strains in multidimensional space. Species noted: AV—*Arcella vulgaris*, CAA—*Centropyxis aculeata* "aculteata," CAD—*Centropyxis aculeata* "discoides," CCA—*Centropyxis constricta* "aerophilia," CCC—*Centropyxis constricta* "constricta," CCS—*Centropyxis constricta* "spinosa," DBACI—*Difflugia bacillarium*, DB—*Difflugia bidens*, MC—*Mediolus corona*, DGG—*Difflugia glans* "glans," DGD—*Difflugia glans* "distenda," DOBRY—*Difflugia oblonga* "bryophilia," DOL—*Difflugia oblonga* "lanceleolata," DOO—*Difflugia oblonga* "oblonga," DOS—*Difflugia oblonga* "spinosa," DOT—*Difflugia oblonga* "tenuis," DP—*Difflugia protaeiformis*, DA—*Difflugia acuminata*, DCL—*Difflugia claviformis*, DUU—*Difflugia urceolata* "urceolata," DE—*Difflugia elegans*, LV—*Lagenodifflugia vas*, LS—*Lesquereusia spiralis*, HS—*Heleopera sphagni*, PC—*Pontigulasia compressa*

and conductivity. The SDI in this assemblage ranged from 2.1 to 2.4, indicative of transitional environments bordering on healthy ecological conditions [53, 54].

Discussion

Road salt contamination from winter de-icing operations is an emerging environmental issue for many lakes adjacent to major highways throughout eastern North America [2]. As the impact of road salt contamination is cumulative, there is concern that the decades of continuous use have resulted in a degradation of lake trophic structure in lakes. Preying on bacteria and eukaryotic microbes, Arcellinida are important intermediary food web components of aquatic ecosystems, and thus are an excellent proxy of anthropogenic influences on lake health [72, 73]. Lake systems are complex and there are multiple environmental contributors that influence the distribution of organisms even within individual lake basins. For this reason, the research design for this project required assessment of samples from multiple stations, measuring multiple variables, to disentangle confounding variables (e.g., runoff, pycnocline), from parameters indicative of possible road salt contamination (e.g., conductivity, Cl, Na).

Impact of Road Salt on Silver Lake Arcellinida Communities

Conductivity, Salinity and Chloride

Conductivity/salinity explained only a small proportion of the observed intra-lake arcellinidan assemblage variability (2.2%) based on the results of RDA and variance partitioning (Fig. 7a, b), indicating that the ecology of Silver Lake is not overly influenced by salinity. This result was expected as, despite being elevated, conductivity/salinity was relatively homogeneous across the basin (293–297 μS/cm [0.14 ppt]) and increased only slightly with depth (315 μS/cm [0.15 ppt]). As

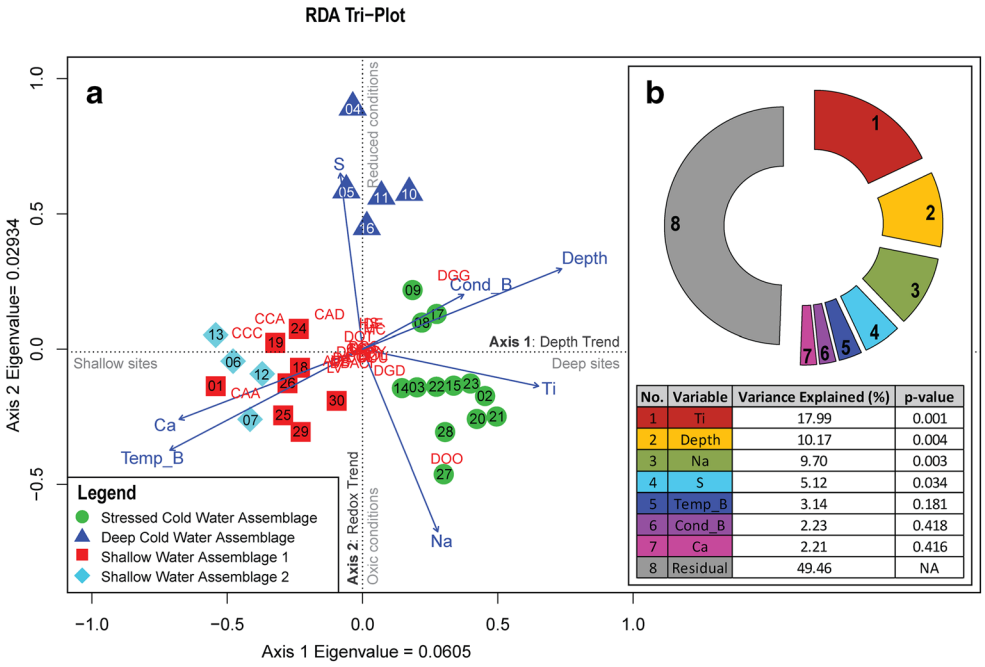

Fig. 7 **a** Redundancy analysis (RDA) bi-plot of seven significant measured variables (blue arrows) and 30 samples (black text and colored symbols) with statistically significant Arcellinida populations. The identified Arcellinidan assemblage are represented by the colored symbols. **b** Partial redundancy analysis with variance partitioning showing the percentage of Arcellininid variance explained by each of the statistically significant parameters. Parameters noted; S—sulfur, Cond_B—bottom water conductivity, Depth—water depth, Ti—titanium, Na—sodium, Temp_B—bottom water temperature, Ca—calcium. Species noted; AV—*Arcella vulgaris*, CAA—*Centropyxis aculeata* "aculteata," CAD—*Centropyxis aculeata* "discoides," CCA—*Centropyxis constricta* "aerophilia," CCC—*Centropyxis constricta* "constricta," CCS—*Centropyxis constricta* "spinosa," DBACI—*Difflugia bacillarium*, DB—*Difflugia bidens*, MC— *Netzelia corona*, DGG—*Difflugia glans* "glans," DGD—*Difflugia glans* "distenda," DOBRY—*Difflugia oblonga* "bryophilia," DOL—*Difflugia oblonga* "lanceleolata," DOO—*Difflugia oblonga* "oblonga," DOS—*Difflugia oblonga* "spinosa," DOT—*Difflugia oblonga* "tenuis," DP—*Difflugia protaeiformis*, DA—*Difflugia acuminate*, DCL—*Difflugia claviformis*, DUU—*Difflugia urceolata* "urceolata," DE—*Difflugia elegans*, LV—*Lagenodifflugia vas*, LS—*Lesquereusia spiralis*, HS—*Heleopera sphagni* and PC—*Pontigulasia compressa*

a result, the direct impact of conductivity on particular arcellinidan assemblages in the lake was difficult to discriminate. Roe and Patterson [2] found that lakes in the GTA with similar moderate-to-elevated conductivity measurements (266–340 μS/cm) had low-diversity Arcellinida assemblages and were often associated with elevated proportions of *C. aculeata* "aculeata" and *C. constricta* "spinosa," which are indicative of sub-optional conditions. The *Centropyxid* taxa are opportunistic and highly tolerant to several types of environmental stress (e.g., changes in lake pH, eutrophication, and metal(loid) contamination; [24, 25, 38]). The assemblages observed in Silver Lake were characterized by diversities generally associated with stressed to transitional arcellinidan assemblages, similar to those observed by Roe and Patterson [2]; i.e., SCWA and DCWA; SDI range = 1—1.9), but also assemblages associated with transitional to relatively healthy

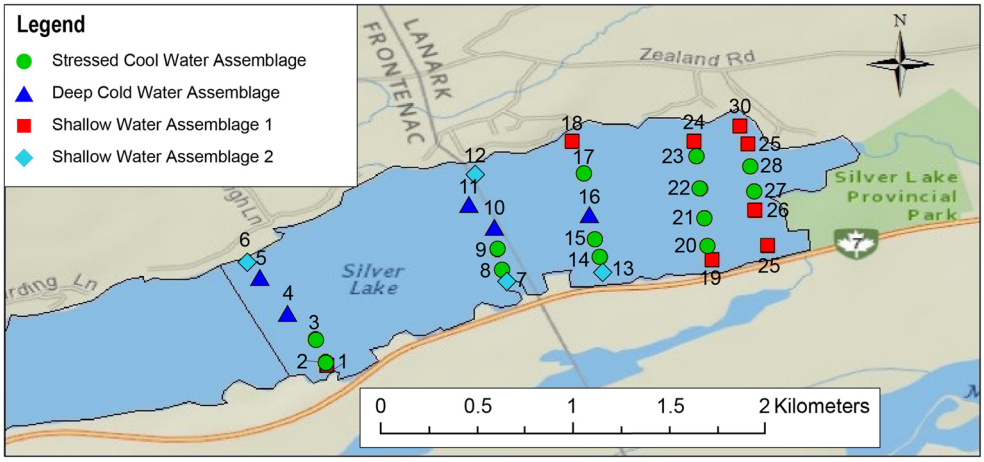

Fig. 8 Map showing the locations of stations attributed to the four identified arcellinidan assemblages within Silver Lake

environmental conditions (i.e., SWA-1 and SWA-2; SDI range = 1.8—2.4). A key road-salt contamination-indicator taxon, AV, was only present in very low proportions in the four assemblages (relative abundance range = 0.5—6.5%; n = 30).

Although chloride was not determined by the RDA analysis to explain a statistically significant amount of the observed variance in the Silver Lake system, chloride concentrations (~16 mg/l) in Silver Lake were much higher than typical for more remote typical rural lakes in the region 1.1 mg/l [31]. The lack of singular *Centropyxid*-dominance in any of the assemblages suggests that as yet road salt has had a modest influence on the arcellinidan distribution in Silver Lake. This stands in contrast to Roe et al. [24].

Sodium

Sodium, discussed separately from the proxies above as it has not been previously used as a road salt contamination proxy in lake systems, explained a moderate proportion of the variance observed in the arcellinidan community (9.7%) in Silver Lake. Previously, elevated Na values have been primarily been used as a proxy for low lake water levels resulting from high evaporation, (e.g., [74]). However, recent research has suggested elevated Na concentrations may also be caused by road salt contamination. A recent report showed that 78 of 232 United States Geological Survey freshwater monitoring sites surveyed exhibited an increasing trend in Na^+ ions present in water over the periods of monitoring, with the strongest positive trends being observed in the northeastern United States were road salt is required for road safety [75]. In a winter study of Na concentrations in river systems of southern Ontario, it was observed that Na concentrations are up to 50 times higher than baseline levels near roadways during initial periods of snow melt, reaching concentrations > 2600 mg/l before continued snowmelt diluted concentrations [76]. Sodium has also been shown to remain in soils for extended periods of time due to its ability to readily exchange with other cations in soil (e.g., Ca^+ and Mg^+; [77, 78]). Thus, the elevated Na measured in Silver Lake may be derived from the high concentrations of Na released into the environment during snow melt, and freely exchanging with other cations in compounds in sediments on the lake bottom. The elevated Na concentrations along the southern shore of Silver Lake adjacent to Highway 7 suggest that elevated salinities most likely existed along this shore earlier in the spring, which would have been associated with salt transport into the lake via overland flow, or by groundwater inputs directly following road salt application and subsequent snow melt.

The highest concentrations of Na were associated with the SCWA (median Na = 1630) and SWA-2 (median Na = 1285 ppm), while lower concentrations were associated with the DCWA (median Na = 710 ppm) and SWA-1 (median Na = 990 ppm). Surprisingly, the abundance of the road salt indicator taxa CAA was notably low in samples forming SCWA (max_{CAA} = 1.9%, $mean_{CAA}$ = 0.3%, n = 5) and DCWA (max_{CAA} = 6.5%, $mean_{CAA}$ = 2.3%, n = 13). In contrast, samples of the shallower Arcellinida assemblages (SWA-1 (max_{CAA} = 21%, $mean_{CAA}$ = 14%, n = 8) and SWA-2 (max_{CAA} = 24%, $mean_{CAA}$ = 20%, n = 4)) are characterized by higher numbers of CAA, thus suggesting a subtle influence of Na over the distribution of this centropyxid strain. Similarly, the association between the distribution of other road salt indicator taxa (e.g., AV) and Na concentrations appears to be weak. Additional research is required from additional lakes to explain the nature of the relationship between road-salt contamination species and shifts in Na observed in the Silver Lake data, as it cannot be concluded based on this study alone that salinity has a significant impact on the arcellinidan distribution in Silver Lake.

The RDA tri-plot also revealed a strong association between DOO and Na along the second RDA axis (Fig. 7). This is an unexpected result given the possibility of road-salt contamination being the primary cause of increasing Na levels in Silver Lake and the sensitivity of DOO to environmental stress. Strains of *D. oblonga* thrive in silty, organic–rich substrates and relatively healthy environmental conditions [7, 25] and have been shown to be sensitive to different types of environmental stress (e.g., [18, 24]). Therefore, the abundance of DOO in Na-rich substrates may reflect a weak influence of Na on the distribution of this arcellinidan strain, which is likely controlled by other environmental parameters (e.g., substrate composition and organic content).

Comparing Silver Lake and Roe and Patterson [2] Assemblages

Results of cluster analysis, NMDS, and DCA showed samples from Silver Lake plotting closely to Assemblage 4 ("roadside lakes with salt influences and control lakes, Canadian Shield) of Roe and Patterson ([2]; ESM Fig. 1; ESM Fig. 2; ESM Fig. 3). These results are expected because Assemblage 4 was identified in lakes that are geographically close to Silver Lake (i.e., Kaladar Jack Pine, Mytopo Lake, Cox Lake, Blueberry Lake, Mew Lake, and Jake Lake lakes). Assemblage 4 is characterized by generally high diversity (SDI range = 1.4–2.6) and well-balanced fauna, with some higher proportions of stress-indicating taxa (e.g., *Centropyxis aculeata* strains and AV). The transitional to relatively healthy nature of environmental conditions reflected by Assemblage 4 was attributed in part to the variance in conductivity (range = 38–525 μs/cm) and chloride levels (range = 39–108 mg/l), which are higher in roadside lakes (Mytopo Lake, Jake

Lake, Kaladar Jack Pine, and Blueberry Lake) and notably lower in control lakes (Cox Lake and Mew Lake). Similarly, Silver Lake has a relatively healthy and diverse faunal assemblage (SDI range = 1–2.4) and relatively elevated chloride and conductivity measurements that fall within the range measured for Assemblage 4. Therefore, the similarity between the Silver Lake assemblages and Assemblage 4 of Roe and Patterson [2] reflects a modest impact of salt-laden runoff compared to the truly salt-contaminated lakes of the salt-belt (e.g., see Roe and Patterson [2] assemblages 1 and 3).

Non-Road Salt Controls on Silver Lake Arcellinida Distribution

In addition to the road salt run-off proxies discussed above there were additional environmental drivers identified during the RDA analysis that significantly influenced the distribution of observed arcellinidan assemblages in Silver Lake and complicated correlation of arcellinidan assemblages with the road salt contamination signal. These confounding variables included Ti (17.9%), water depth (10.1%) and S (5.1%; Fig. 7a, b).

Titanium

Titanium is often used as a runoff indicator as it is relatively redox insensitive and can be associated with silty- to fine-sand-sized sediment [34, 35]. Substrate particle size has been shown to significantly influence arcellinidan distributions whereby silty to muddy substrates often yield healthy and abundant assemblages, while coarser sandy substrates, which contain few food resources, generally only support a meager arcellinidan presence [8, 36, 37]. In Silver Lake, samples characterized by higher Ti were associated with the low-to-moderate diversity, difflugiid-dominated SCWA (median Ti = 1300 ppm; SDI range = 1.0—1.6) and the DCWA (median Ti = 1060 ppm; SDI range = 1.5—1.9) in both sand-dominated and silt dominated substrates, respectively. Samples with lower Ti content (range = 410—590 ppm) were associated with the more diverse SWA-1 (SDI = 1.8—2.1) and SWA-2 (SDI = 2.1—2.4), which were characteristic of sand-dominated substrates. It is notable that DOO dominated the faunal structure of the SCWA (median relative abundance = 41%), which was found in sand-dominated substrates (median sand = 83%) characterized by high Ti concentrations and low organic content. The prevalence of DOO in sand-dominated substrates in Silver Lake is surprising as the taxon has generally been associated with silt-dominated, organic–rich substrates and relatively healthy environmental conditions [7, 45]. While organic content in the substrate was generally low across the study area (median TOC = 2.71%; $n = 30$), the high proportions of DOO suggest that this taxon can also thrive in sand-dominated substrates where organic content is low, so long as adequate minerogenic material required for construction of its agglutinated test is present.

Water Depth (below Thermocline)

Silver Lake is characterized by a shallow area close to the shorelines of the lake that rapidly deepens to form one central deep basin with a maximum depth of 23 m. The influence of this water depth transition on arcellinidan assemblage composition, particularly beneath the ~8 m thermocline, was associated with a notable increase in the proportions of DGG, a species that is often found in high abundance in deeper lacustrine substrates [45, 79]. This strain dominated the DCWA (median relative abundance = 37.5%; median water depth = 20 m), and was co-dominant in the SCWA (median relative abundance = 27%; median water depth = 10 m). Assemblages found on shallower substrates (SWA-1 and SWA-2) were characterized by low to moderate proportions of DGG (relative abundance range = 2.5—14%; median water depth = 1.5 m). The RDA bi-plot further corroborated these findings by showing a strong and positive association between water depth and DGG (Fig. 7).

Indirect Influence of Water Depth on Particle Size, Calcium and Sulfur

Although depth does not appear to correlate with Arcellinida assemblages in a direct manner, depth controls many other variables that do impact assemblage composition. For example, silt concentrations are higher in the deep basin, likely due to sediment focusing, a process where water turbulence moves fine grained sedimentary material from shallower to more distal and deeper basins in lacustrine systems [80, 81]. Calcium is more abundant in shallow regions, most likely due to increased authigenic precipitation in the littoral zone induced by higher productivity [82, 83].

Concentrations of S were found to be the highest in samples comprising Assemblage 2 (DCWA; median S = 25.2%; $n = 5$) compared to the rest of the assemblages (S range = 3.1–6.1%; $n = 25$). The DCWA occurred well below the thermocline (median water depth = 20 m), where borderline dysoxic/anoxic conditions typically characterize Silver Lake late in the open water season (Fig. 2b). Such conditions often result in reduced S, which becomes sequestered into the lake sediments [84]. The impact of elevated S concentrations on the identified assemblages is not clear. A study by Payne [85] showed that elevated levels of S adversely impacted the distribution of a number of peatland testate amoebae taxa. Sulfur has also been suggested to be indirectly related to arcellinidan assemblages in lacustrine systems as habitats of select species and strains co-occur with elevated S concentrations that naturally occur due to shifts in lacustrine systems (e.g., changes in depth, level

of vegetation, lake productivity; [37]). However, the impact of elevated S levels on lacustrine Arcellinida is, to our knowledge, yet to be definitively assessed.

Conclusions

At Silver Lake, road salt runoff from the adjacent busy Highway 7 has resulted in elevated conductivity, and Na concentrations that are in line with conditions found in urban and rural road side lakes, but significantly higher than found in lakes from more remote areas. The arcellinidan assemblages characterizing shallower areas of the lake, particularly near Highway 7 may be modestly impacted by road salt contamination (conductivity, 293–297 μS/cm; salinity, 0.14 ppt; Na, 1020 ppm), which is in line with observations made in similar modestly impacted lakes from the Great Toronto Area. In contrast, the arcellinidan assemblages in the deeper parts of the lake, below the thermocline, are most significantly influenced by lower temperature, reduced dissolved oxygen concentrations, sediment geochemistry (S and Ti) and particle size, and less so by conductivity and Na concentrations. A necessary follow-on study will require a high-resolution temporal assessment of changes in the arcellinidan fauna, from a core collected above the thermocline and adjacent to Highway 7. Such a study would provide details on background and baseline environmental conditions in the lake with particular emphasis on how the lake ecosystem has changed since application of road salt began in this area in the lake 1940s.

References

1. Hanbali RM, Kuemmel DA (1992) Traffic collision analysis of ice control - 47 - operation. http://www.trc.marquette.edu/publications/IceControl/ice-control-1992.pdf Accessed 30 Oct 2018
2. Roe HM, Patterson RT (2014) Arcellacea (testate amoebae) as bioindicators of road salt contamination in lakes. Microb Ecol 68(2): 299–313
3. Dugan HA, Bartlett SL, Burke SM, Doubek JP, Krivak-Tetley FE, Skaff NK, Summers JC, Farrell KJ, McCullough IM, Morales-Williams AM, Roberts DC, Ouyang Z, Scordo F, Hanson PC, Weathers KC (2017a) Salting our freshwater lakes. PNAS 114(17):4453–4458
4. Williams D, Williams N, Cao Y (2000) Road salt contamination of grounDCWAter in a major metropolitan area and development of a biological index to monitor its impact. Water Res 34(1):127–138. https://doi.org/10.1016/S0043-1354(99)00129-3
5. Rosenberry D, Bukaveckas P, Buso D, Likens G, Shapiro A, Winter T (1999) Movement of road salt to a small New Hampshire lake. Water Air Soil Pollut 109(1–4):179–206. https://doi.org/10.1023/A:1005041632056
6. Findlay S, Kelly V (2011) Emerging indirect and long-term road salt effects on ecosystems. Ann N Y Acad Sci 1223(1):58–68. https://doi.org/10.1111/j.17496632.2010.05942.x
7. Collins ES, McCarthy FM, Medioli FS, Scott DB, Honig CA (1990) Biogeographic distribution of modern thecamoebians in a transect along the eastern north American coast. In: Hemleben C, Kaminski MA, Kuhnt W, Scott DB (ed), Paleoecology, biostratigraphy, paleoceanography and taxonomy of agglutinated foraminifera. NATO Advanced Study Institute Series, Series C, Mathematical and Physical Sciences 327:783–791
8. Dalby AP, Kumar A, Moore JM, Patterson RT (2000) Utility of arcellaceans (thecamoebians) as paleolimnological indicators in tropical settings: Lake Sentani, Irian Jaya, Indonesia. J Foraminifer Res 30:135–142
9. Charman DJ, Hendon D, Woodland W (2000) The identification of peatland testate amoebae. Quaternary research association technical guide no. 9, London, 147pp
10. Patterson RT, Huckerby G, Kelly TJ, Swindles GT, Nasser NA (2015) Hydroecology of Amazonian lacustrine Arcellinida (testate amoebae): a case study from Lake Quistococha, Peru. Eur J Protistol 51:460–469. https://doi.org/10.1016/j.ejop.2015.06.009
11. Loeblich Jr AR, Tappan H (1964) Sarcodina, chiefly 'Thecamoebians' and Foraminiferida. In: Moore RC (ed) Treatise of invertebrate paleontology, part C, protista 2. Geol Soc America Univ Kansas Press, Lawrence, pp 1–900
12. Patterson RT, Kumar A (2002) A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeogr Palaeoclimatol Palaeoecol 180:225–251
13. Patterson RT, MacKinnon KD, Scott DB, Medioli FS (1985) Arcellaceans ("Thecamoebians") in small lakes of New Brunswick and Nova Scotia: modern distribution and Holocene stratigraphic changes. J Foraminifer Res 15(2):114–137
14. Scott DB, Medioli FS (1983) Testate rhizopods in Lake Erie: modern distribution and stratigraphic implications. J Paleontol 57:809–820
15. Mitchell EAD, Charman DJ, Warner BG (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodivers Conserv 17:2115–2137
16. Patterson RT, Kumar A (2000) Use of Arcellacea to gauge levels of pollution and remediation of industrially polluted lakes. In: Martin RE (ed) Environmental micropaleontology. Topics in geobiology, vol 15. Kluwer Academic/Plenum Publication, New York, pp 257–278
17. Nguyen-Viet H, Bernard N, Mitchell EAD, Cortet J, Badot P-M, Gilbert D (2007) Relationships between testate amoeba (protist) communities and atmospheric heavy metals accumulated in Barbula indica (Bryophyta) in Vietnam. Microb Ecol 53:53–65
18. Patterson RT, Lamoureux EDR, Neville LA, Macumber AL (2013) Arcellacea (testate Lobose amoebae) as pH indicators in a pyrite mine-acidified lake, northeastern Ontario, Canada. Microb Ecol 65: 541–554
19. Charman D, Roe WR, Gehrels WR (1998) The use of testate amoebae in studies of sea-level change: a case study from the Taf Estuary, South Wales, UK. The Holocene 8:209–218
20. Qin Y, Fournier B, Lara E, Gu Y, Wang H, Cui Y, Zhang X, Mitchell E (2013) Relationships between testate amoeba communities and water quality in Lake Donghu, a large alkaline lake in Wuhan, China. Front Earth Sci 7(2):182–190. https://doi.org/10.1007/s11707-013-0352-4
21. Escobar J, Brenner M, Whitmore T, Kenney W, Curtis J (2008) Ecology of testate amoebae (thecamoebians) in subtropical Florida lakes. J Paleolimnol 40(2):715–731
22. Patterson RT, Dalby A, Kumar A, Henderson L (2002) Arcellaceans (thecamoebians) as indicators of land use change: settlement history of the Swan Lake Area, Ontario as a case study. J Paleolimnol 28:297–316
23. Boudreau REA, Galloway JM, Patterson RT, Kumar A, Michel FA (2005) A paleolimnological record of Holocene climate and

environmental change in the Temagami region, northeastern Ontario. J Paleolimnol 33:445–461
24. Roe HM, Patterson RT, Swindles GT (2010) Controls on the contemporary distribution of lake thecamoebians (testate amoebae) within the Greater Toronto area and their potential as water quality indicators. J Paleolimnol 43:955–975
25. Neville LA, McCarthy FMG, MacKinnon MD, Swindles GT, Marlowe P (2011) Thecamoebians (testate amoebae) as proxies of ecosystem health and reclamation success in constructed wetlands in the oil sands of Alberta, Canada. J Foraminifer Res 41:230–247
26. Patterson RT, Roe HM, Swindles GT (2012) Development of an Arcellacea (testate lobose amoebae) based transfer function for sedimentary phosphorus in lakes. Palaeogeogr Palaeoclimatol Palaeoecol 348–349:32–44
27. Honig C, Scott DB (1987) Postglacial stratigraphy and sea-level change in southwestern New Brunswick. Can J Earth Sci 24:354–364
28. McCarthy FM, Collins ES, McAndrews JH, Kerr HA, Scott DB, Medioli FS (1995) A comparison of postglacial arcellacean ("thecamoebian") and pollen succession in Atlantic Canada, illustrating the potential of arcellaceans for paleoclimatic reconstruction. J Paleontol 69(5):980–993
29. Neville LA, Christie DG, McCarthy FMG, MacKinnon MD (2010) Biogeographic variation in Thecamoebian (Testate amoeba) assemblages in lakes within various vegetation zones of Alberta, Canada. Int J Biodivers Conserv 2(8):215–224
30. Environment Canada (2018) Historical climate data. Published on http://climate.weather.gc.ca/historical_data/search_historic_data_e.html
31. Suenaga EK (2018) Assessing the chemical and biological recovery of Killarney Provincial Park Lakes from historical acidification. Unpublished Master's Thesis, Department of Biology, Queen's University
32. Dugan HA, Summers JC, Skaff NK, Krivak-Tetley FE, Doubek JP, Burke SM, Bartlett SL, Arvola L, Jarjanazi H, Korponai J, Kleeberg A, Monet G, Monteith D, Moore K, Rogora M, Hanson PC, Weathers KC (2017b) Long-term chloride concentrations in north American and European freshwater lakes. Sci Data 4:170101
33. Canadian Council of Ministers of the Environment (2011) Canadian water quality guidelines for the protection of aquatic life: chloride. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment Winnipeg.C
34. Boës X, Rydberg J, Martinez-Cortizas A, Bindler R, Renberg I (2011) Evaluation of conservative lithogenic elements (Ti, Zr, Al, and Rb) to study anthropogenic element enrichments in lake sediments. J Paleolimnol 46:75–87
35. LoDico JM, Flower BP, Quinn TM (2006) Subcentennial-scale climatic and hydrologic variability in the Gulf of Mexico during the early Holocene. Paleoceanograph 21:PA3015
36. Roe HM, Patterson RT (2006) Distribution of Thecamoebians (testate amoebae) in Small Lakes and Ponds, Barbados, West Indies. J Foraminifer Res 36(2):116–134. https://doi.org/10.2113/36.2.116
37. Steele R, Nasser NA, Patterson RT, Gregory BRB, Roe HM, Reinhardt EG (2018) An assessment of sub-meter scale spatial variability of arcellinida (testate lobose amoebae) assemblages in a temperate lake: implications for limnological studies. Microb Ecol 76(3):680–694. https://doi.org/10.1007/s00248-018-1157-5
38. Kumar A, Patterson RT (2000) Arcellaceans (thecamoebians): new tools for monitoring long-term and short-term changes in lake bottom acidity. Environ Geol 39:689–697
39. Mississippi Valley Conservation Authority (2005) State of the Lake Environment Report, December 2005, Silver Lake. Published on mvc.on.ca/silver-lake/ Retrieved July 2nd, 2018
40. Mississippi Valley Conservation Authority (2010) State of the Lake Environment Report, December 2010, Silver Lake. Published on mvc.on.ca/silver-lake/ Retrieved July 2nd, 2018
41. Ontario Ministry of Environment, Conservation, and Parks (2012) Broadscale Monitoring Program data
42. van Hengstum PJ, Reinhardt EG, Medioli FS, Gröcke DR (2007) Exceptionally preserved late Albian (Cretaceous) Arcellaceans (Thecamoebians) from the Dakota formation near Lincoln, Nebraska, USA. J Foraminifer Res 37:300–3008
43. Donato E (2009) Particle-size distribution of inferred tsunami deposits in Sur lagoon, Sultanate of Oman. Mar Geol 25:54–64
44. Blott SJ, Pye K (2001) GRADISTAT: a grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surf Process Landf 26:1237–1248
45. Nasser NA, Patterson RT, Roe HM, Galloway JM, Falck H, Palmer MJ, Spence C, Sanei H, Macumber AL, Neville L (2016) Lacustrine Arcellinida (Testate amoebae) as bioindicators of arsenic contamination. Microb Ecol 72(1):130–149. https://doi.org/10.1007/s00248-016-0752-6
46. Santoro A, Held A, Linsinger TPJ, Perez A, Ricci M (2017) Comparison of total and aqua regia extractability of heavy metals in sewage sludge: the case study of a certified reference material. Trends Anal Chem 89:34–40
47. Heiri O, Lotter AF, Lemcke H (2001) Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J Paleolimnol 25:101–110
48. Scott DB, Hermelin JOR (1993) A device for precision splitting of micropaleontological samples in liquid suspension. J Paleontol 67: 151–154 83
49. Patterson RT, Fishbein E (1989) Re-examination of the statistical methods used to determine the number of point counts needed for micropaleontological quantitative research. J Paleontol 63:245–248
50. Fishbein E, Patterson RT (1993) Error-weighted maximum likelihood (EWML): a new statistically based method to cluster quantitative micropaleontological data. J Paleontol 67:475–485
51. Shannon CE (1948) A mathematical theory of communication. Bell Syst Tech J 27(379–423):623–656
52. Medioli FS, Scott DB, Collins ES, McCarthy FMG (1990) Fossil thecamoebians: present status and prospects for the future. In: Hemleben C, Kaminski MA, Kuhnt W, Scott DB (ed) Paleoecology, biostratigraphy, paleoceanography and taxonomy of agglutinated foraminifera. NATOAdvanced Study Institute Series, Series C, Math Phys Sci 327:813–884
53. Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton 185 pp
54. Patterson RT, Kumar A (2000b) Assessment of arcellacea (thecamoebian) assemblages, species and strains as contaminant indicators in variably contaminated James Lake, north eastern Ontario. J Foraminifer Res 30:310–320
55. Reimann C, Filzmoser P, Garrett RG, Dutter R (2008) Statistical data analysis explained: applied environmental statistics with R. John Wiley & Sons, Ltd ISBN: 978-0-470-98581-6
56. Birks HJB, Line JM, Juggins S, Stevenson AC, ter Braak CJF (1990) Diatoms and pH reconstructions. Philos Trans R Soc B 327:263–278
57. Naimi B, Na H, Groen TA, Skidmore AK, Toxopeus AG (2014) Where is positional uncertainty a problem for species distribution modelling. Ecography 37:191–203. https://doi.org/10.1111/j.1600-0587.2013.00205.x
58. R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07-0, URL http://www.R-project.org/
59. Ward JH (1963) Hierarchical grouping to optimize an objective function. J Am Stat Assoc 58:236–244
60. Kim-Anh Le Cao, Florian Rohart, Ignacio Gonzalez, Sebastien Dejean with key contributors Benoit Gautier, Francois Bartolo, contributions from Pierre Monget, Jeff Coquery, FangZou Yao and Benoit Liquet. (2016). mixOmics: Omics Data Integration

Project. R package version 6.1.1. https://CRAN.R-project.org/package=mixOmics
61. Hill MO (1979) Decorana—a fortran program for detrended correspondence analysis and reciprocal averaging. Ecology and systematics. Cornell University, Ithaca, New York, 14850, 52 pp
62. van denWollenberg AL (1977) Redundancy analysis. An alternative for canonical correlation analysis. Psychometrika 42:207–219
63. Wagner RJ, Boulger Jr RW, Oblinger CJ, Smith BA (2006) Guidelines and standard procedures for continuous water-quality monitors: station operation, record computation, and data reporting
64. Lewis EL (1980) The practical salinity scale 1978 and its antecedents. IEEE J Ocean Eng 5(1):3–8
65. Lewis EL, Perkin RG (1978) Salinity: its definition and calculation. J Geophys Res 83:466–478
66. Nürnberg GK (1996) Trophic state of clear and colored, soft- and harDCWAter lakes with special consideration of nutrients, anoxia, phytoplankton and fish. Lake Reserv Manage 12:432–447
67. Nürnberg G, Shaw M (1998) Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. Hydrobiologia 382:97–112
68. Henny C, Nomosatryo S (2016) Changes in water quality and trophic status associated with cage aquaculture in Lake Maninjau, Indonesia. IOP Conf Ser Earth Environ Sci 31:012027
69. Schönborn W (1984) Studies on remains of Testacea in cores of the great Woryty Lake (NE-Poland). Limnologica 16:185–190
70. Patterson RT, Swindles GT (2015) Influence of ocean–atmospheric oscillations on lake ice phenology in eastern North America. Clim Dyn 45(10):2293–2308. https://doi.org/10.1007/s00382-014-2415-y
71. Cole GA, Weihe PE (2016) Textbook of limnology. Press, Waveland, 440 p
72. Anderson OR (2012) The fate of organic sources of carbon in Moss-rich tundra soil microbial communities: a laboratory experimental study. J Eukaryot Microbiol 59:564–570
73. Patterson RT, Nasser NA, Gregory BRB, Gavel MJ, Menard E, Galloway JM, Roe HM (2019) Arcellinida (testate lobose amoebae) as sensitive bioindicators of arsenic contamination in lakes. Chapter 15, p. 71–73. H. Chenchouni et al. (eds.), Exploring the Nexus of Geoecology, Geography, Geoarcheology and Geotourism: Advances and Applications for Sustainable Development in Environmental Sciences and Agroforestry Research, Advances in Science, Technology & Innovation. https://doi.org/10.1007/978-3-030-01683-8_15
74. Ma L, Wu J, Abuduwaili J, Lio W (2016) Geochemical responses to anthropogenic and natural influences in Ebinur Lake Sediments of Arid Northwest China. PLOS one:1–12
75. Kaushal SS, Likens GE, Pace ML, Utz RM, Haq S, Gorman J, Grese M (2018) Freshwater salinization syndrome on a continental scale. PNAS 115(4):E574–E583
76. Scott WS (1976) The effect of road deicing salts on sodium concentration in an urban water-course. Environ Pollut 10:141–153
77. Ramakrishna DM, Viraraghavan T (2005) Environmental impact of chemical deicers – a review. Water Air Soil Pollut 166:49–63
78. Eimers MC, Croucher K-N, Raney SM, Morris ML (2015) Sodium accumulation in calcareous roadside soils. Urban Ecosyst 18:1213–1225
79. Reinhardt EG, Dalby A, Kumar A, Patterson RT (1998) Arcellaceans as pollution indicators in mine tailing contaminated lakes near Cobalt, Ontario, Canada. Micropaleontology 44(2):131–148
80. Hilton J (1985) A conceptual framework for predicting the occurrence of sediment focusing and sediment redistribution in small lakes. Limnol Oceanogr 30(6):1131–1143
81. Blais JM, Kalff J (2005) The influence of lake morphometry on sediment focusing. Limnol Oceanogr 40(3):582–588
82. Cangemi M, Censi P, Reimer A, D'Alessandro W, Hause-Reitner D, Madonia P, Oliveri Y, Pecoraino G, Reitner J (2016) Carbonate precipitation in the alkaline lake Specchio di venere (Pantelleria Island, Italy) and the possible role of microbial Mats. Appl Goechem 67:168–176
83. Pelechaty M, Pukacz A, Apolinarska K, Pelechata A, Siepak M (2013) The significance of Chara vegetation in the precipitation of lacustrine calcium carbonate. Sedimentology 60:1017–1035
84. Davidson W, Lishman JP, Hilton J (1985) Formation of pyrite in freshwater sediments: implications for C/S ratios. Geochim Cosmochim Acta 49:1615–1620
85. Payne R (2010) Testate amoeba response to acid deposition in a Scottish peatland. Aquat Ecol 44(2):373–385. https://doi.org/10.1007/s10452-009-9297-9