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Arcellacea (Testate Amoebae) as Bio-indicators of Road Salt Contamination in Lakes

Helen M. Roe · R. Timothy Patterson

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Abstract Winter deicing operations occur extensively in mid- to high-latitude metropolitan regions around the world and result in a significant reduction in road accidents. Deicing salts can, however, pose a major threat to water quality and aquatic organisms. In this paper, we examine the utility of Arcellacea (testate amoebae) for monitoring lakes that have become contaminated by winter deicing salts, particularly sodium chloride. We analysed 50 sediment samples and salt-related water property variables (chloride concentrations; conductivity) from 15 lakes in the Greater Toronto Area and adjacent areas of southern Ontario, Canada. The sampled lakes included lakes in proximity to major highways and suburban roads and control lakes in forested settings away from road influences. Samples from the most contaminated lakes, with chloride concentrations in excess of 400 mg/l and conductivities of >800 μS/cm, were dominated by species typically found in brackish and/or inhospitable lake environments and by lower faunal diversities (lowest Shannon diversity index values) than samples with lower readings. Q-R-mode cluster analysis and detrended correspondence analysis (DCA) resulted in the recognition of four assemblage groupings. These reflect varying levels of salt contamination in the study lakes, along with other local influences, including nutrient loading. The response to nutrients can, however, be isolated if the planktic eutrophic indicator species *Cucurbitella tricuspis* is removed from the counts. The findings show that the group has considerable potential for bio-monitoring in salt-contaminated lakes, and their presence in lake sediment cores may provide significant insights into long-term benthic community health, which is integral for remedial efforts.

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H. M. Roe (✉)
School of Geography, Archaeology and Palaeoecology, Queen's University Belfast, Belfast BT7 1NN, UK
e-mail: h.roe@qub.ac.uk

R. T. Patterson
Ottawa-Carleton Geoscience Centre and Department of Earth Sciences, Carleton University, Ottawa, Ontario K1S 5B6, Canada

Introduction

Arcellacea, also informally known as testate lobose amoebae [1] or thecamoebians [2], are a group of unicellular protozoans that occur widely in fresh to brackish water environments including lakes and in other moist terrestrial habitats such as peatlands, damp soils and saltmarshes [3–10]. They form small tests (~10–500 μm), either by secretion or by agglutination of xenogenous mineral grains or other materials (e.g. diatom frustules) within an autogenous cement. Highly resistant to decay, these tests fossilize well and often occur in abundance in lacustrine sediments, a characteristic that makes arcellaceans particularly valuable as palaeoenvironmental indicators [3]. Common from the tropics to the poles, arcellaceans are also sensitive to a wide range of environmental variables [11, 12]. In lakes, for example, individual species as well as faunal assemblages have been correlated with many parameters, including substrate changes associated with land clearance [13–15], pH [16], water temperature [17], eutrophication [13, 18–20], salinity [9, 21, 22] and metal and organic pollutant contamination [23–26]. Reproduction rates are rapid (days to weeks), making the group especially useful for monitoring lakes and other environments vulnerable to contaminant loading [27, 28].

In this paper we examine the utility of arcellaceans for monitoring lakes that have become contaminated by winter deicing salts, especially rock salt (NaCl). In particular, we build on a reconnaissance study by Roe et al. [29] in the

Toronto region, where deicing operations occur on a large scale, and where there is great concern about the impact of salt contamination on aquatic ecosystems and groundwater [30–33]. This study examined arcellacean faunas from 21 lakes to provide new insights into the ecology and distribution of arcellaceans in urbanizing settings and to quantify relationships between faunal assemblages and 24 water quality and substrate-related parameters. Statistical analyses revealed that there was a significant relationship with conductivity in this dataset, which indicated that road salts are having a strong imprint on the faunas [29]. Nutrient enrichment was also found to have an important local influence [29]. In this paper, we analyse additional salt-impacted lakes from the Greater Toronto Area (GTA) and adjacent areas of southern Ontario (i) to test whether these relationships are observed in other lakes, particularly lakes in proximity to major highways and other vulnerable salt-impacted road settings; and (ii) to examine in more detail the faunal response to salt-related water quality parameters. These in turn will form an important basis for assessing the suitability of arcellaceans for biomonitoring in salt-degraded environments.

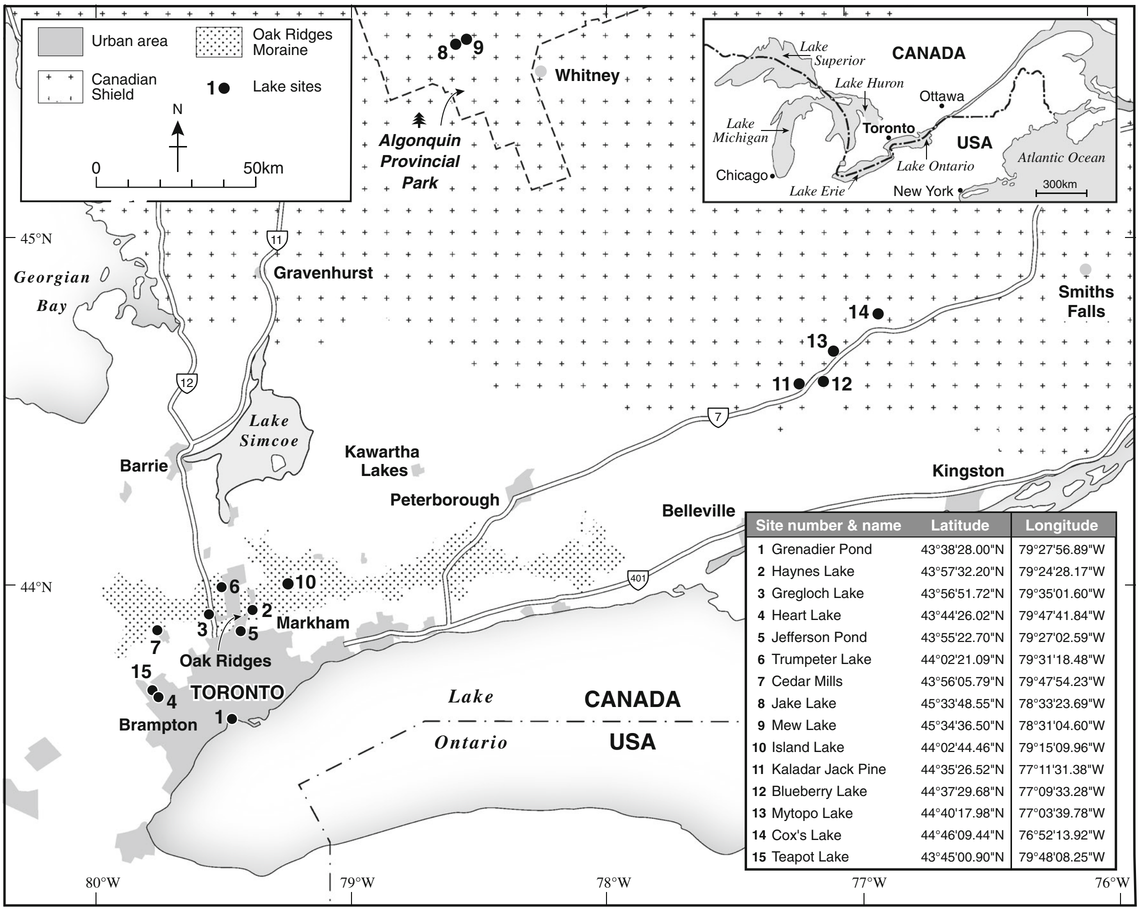
Biomonitoring approaches utilise the sensitivity of organisms to contaminants and complement the use of physicochemical methods in the study of water quality [34, 35]. Such methods form an important component of integrative monitoring of surface waters and are being increasingly adopted by regulation agencies [31, 34]. A variety of biological groups have been employed, including macroinvertebrates [31, 36], cladocera [37] and diatoms [38] amongst others. Testate amoebae have also been used in monitoring, for example, to study airborne deposition of heavy metals in urban environments [27] and the impacts of mining operations on lakes (e.g. [24, 25, 28, 39]). Biomonitoring approaches can have significant advantages over physicochemical measurements in that they can integrate the level of the contaminant over longer timescales, providing information on average pollution levels at a given location [27]. Biomonitoring can also permit the detection of extreme events that may not be recorded by non-continuous instrumental observations [27]. As well as their excellent preservation and rapid turnover, arcellaceans have considerable potential for monitoring salt degradation in lakes because they are largely confined to benthic habitats; these environments are especially vulnerable to deicing salt damage as salt loading can increase the density of bottom waters and significantly alter bottom water and sediment chemistry [40].

Study Area and Context

The GTA is a rapidly expanding urban area on the northern shore of Lake Ontario (Fig. 1). Currently, this region supports a population of over six million [41], although this is projected to increase to over 8.9 million by 2036 [42]. Like other large metropolitan areas in North America that experience prolonged sub-zero winter temperatures, thousands of tonnes of deicing salt are applied to the region's roads and highways each year. The City of Toronto is the largest municipal user of road salt in Canada, using an average of 135,000 tonnes of road salts per year on 5,300 km of roads and 7,100 km of sidewalks [43]. Ontario similarly has the highest rates of salt application of all Canadian provinces [43]. Several deicing chemicals are used for winter maintenance, although rock salt ($NaCl$) is the most common because of its cost effectiveness, ease of application and suitability for the regional climate ($NaCl$ is an effective deicing compound for temperatures down to −9 °C) [43, 44]. Sodium chloride has been applied in the region as an ice disbonding and melting agent since the 1940s [45].

Whilst the application of winter deicing salts is tolerated and indeed popular as a result of the significant (88 %) ensuing reduction in road accidents and fatalities [46], the impacts of $NaCl$ deicing salt application are a subject of major environmental concern in this region. This not only reflects the large volumes of salt that are applied, but the geography of the transport networks, which, with continued urban growth, will inevitably expand into areas where groundwater is regularly used for potable supply [47]. The regional groundwater aquifers are relatively shallow and occur within unconsolidated Quaternary sediments (up to 200 m thick) above bedrock [48, 49]. These are charged every winter with deicing salts from melting snow, which enter the sub-surface and eventually discharge into urban streams via baseflow [30]. Salt contamination of groundwater has already occurred in several watersheds, with many registering elevated chloride levels [50]. Areas in proximity to major highways are of particular concern [33, 44, 47]. Chloride concentrations in many monitored streams in the Toronto area periodically exceed chronic and acute aquatic freshwater thresholds established by the United States Environmental Protection Agency [51] and also similar thresholds recently set for chloride in aquatic environments in Canada [52]. These salt-impacted areas also often show ecological damage, with a variety of aquatic organisms being affected, including macroinvertebrates [31], amphibians [53, 54] and fish populations [32]. Winter deicing salts can also pose a risk to plants, and many salt-tolerant plants such as the invasive common cattail (*Typha latifolia*), common reed (*Phragmites australis*) and other halophytes locally proliferate in salt-damaged wetlands [32, 55].

One area that is particularly vulnerable to salt contamination in this region is the Oak Ridges Moraine (ORM), a prominent morainic ridge located to the north of the GTA (Fig. 1). This has been the subject of intense public and scientific debate regarding proposed urbanization [49]. Land use is largely agricultural, with areas of woodland and small urban centres, but the area is under threat as permitted 'development corridors' encroach progressively northwards from



Site number & name	Latitude	Longitude
1 Grenadier Pond	43°38'28.00"N	79°27'56.89"W
2 Haynes Lake	43°57'32.20"N	79°24'28.17"W
3 Gregloch Lake	43°56'51.72"N	79°35'01.60"W
4 Heart Lake	43°44'26.02"N	79°47'41.84"W
5 Jefferson Pond	43°55'22.70"N	79°27'02.59"W
6 Trumpeter Lake	44°02'21.09"N	79°31'18.48"W
7 Cedar Mills	43°56'05.79"N	79°47'54.23"W
8 Jake Lake	45°33'48.55"N	78°33'23.69"W
9 Mew Lake	45°34'36.50"N	78°31'04.60"W
10 Island Lake	44°02'44.46"N	79°15'09.96"W
11 Kaladar Jack Pine	44°35'26.52"N	77°11'31.38"W
12 Blueberry Lake	44°37'29.68"N	77°09'33.28"W
13 Mytopo Lake	44°40'17.98"N	77°03'39.78"W
14 Cox's Lake	44°46'09.44"N	76°52'13.92"W
15 Teapot Lake	43°45'00.90"N	79°48'08.25"W

Fig. 1 Map showing location of sampling sites and major highways

the GTA. The area not only provides an important source of clean drinking water for the growing population, but includes hundreds of small lakes, including many kettle lakes, which are particularly sensitive to contaminant loading [32]. It also provides habitat for many endangered and threatened species. Salt degradation of water bodies has already been reported in this area near highways, suburban roads and in urban stormwater management ponds [32].

In this study we examine the arcellacean faunas of salt-impacted lakes in the GTA and western areas of the adjacent ORM region. We also examine roadside lakes in forested settings in the Algonquin Provincial Park near Gravenhurst and along Highway 7 northwest of Belleville (Fig. 1). In contrast to the GTA-ORM region where many lakes have been influenced by nutrient enrichment from suburban and agricultural sources [20, 29], these lakes are less impacted by anthropogenic activities within the lake catchments and are typically mesotrophic to oligotrophic. These lakes were also selected to examine the impacts of salt application in areas where lower volumes of salt are applied, but where salt degradation has nevertheless been reported [56].

Materials and Methods

Sampling Design

To examine the arcellacean assemblages of lakes in different road salt-impacted settings, samples were collected from two broad types of lakes: (i) lakes in proximity (<50 m) to major highways; and (ii) lakes adjacent to suburban roads, where the topography of the land around the lake promotes runoff of road-borne contaminants into the lake, or where roads border a significant part (ca. >30 %) of the shoreline. Grenadier Pond in downtown Toronto is an example of a lake in the first category. This 0.8 km long natural lake lies to the immediate north of Lake Ontario (Fig. 1) and receives runoff from a large urban catchment. Two six-lane highways the southern end of the lake, whilst the other areas of the lake are surrounded by parkland (Supplementary Fig. 1). Haynes Lake near Oak Ridges (Fig. 1) falls into the second category. This small (0.22 km^2) lake is skirted by a road that bends around the eastern shoreline (Fig. 2). Deicing salt is routinely applied to the bend, but salt-laden runoff flows downslope into the lake. Indeed, conductivity measurements taken from ponded up water and snowmelt on this bend in February 2003 averaged

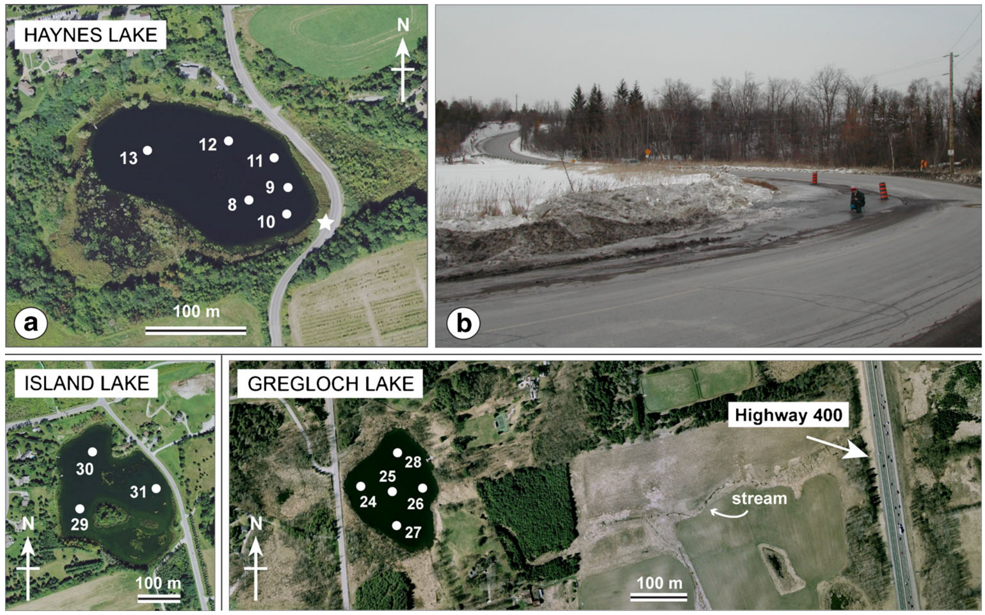


Fig. 2 Aerial photographs of Haynes Lake, Island Lake and Gregloch Lake in the GTA-ORM study region (source: Google Earth 2013). Sampling stations are indicated. *Insets* **a** and **b** show Haynes Lake in summer and winter. *Inset* **b** shows a large area of ponded water and melting snow on a bend in the road on the eastern shore of the lake in February 2006 (the location is indicated by a *star* in **a**). Conductivity readings taken from this puddle averaged 3,450 μS/cm. The *white colour* of the road reflects dried road salt. The lake is surrounded by thick stands of *Typha latifolia* and *Phragmites australis*, which are both salt tolerant

3,450 μS/cm (Roe and Patterson, unpublished data) (Fig. 2b). The eastern side of the lake is fringed by a thick stand of common reed (*P. australis*) and cattails (*Typha angustifolia*). Island Lake, Gregloch Lake and Cedar Mills (informal name) Lake in the GTA-ORM region are similarly bordered by roads (Fig. 1) and have fringing *Phragmites* and *Typha*-dominated bankside vegetation.

Lakes next to major highways were also targeted in the northern study areas, including a set of lakes along Highway 7, north of Belleville (Kaladar Jack Pine, Blueberry and Mytopo Lakes—informal names) and Jake Lake, which is located in the corridor of a smaller provincial highway, Highway 60, in the Algonquin Provincial Park (Fig. 1).

In addition to the salt-influenced lakes, samples were collected from four control lakes, which at the time of sampling were located well away from major roads. These include Heart Lake and Teapot Lake near Brampton in the GTA, which are located in conservation areas and are surrounded by woodland, and Mew Lake and Cox's Lake in the northern study areas (Fig. 1).

Field and Laboratory Methods

Surface sediment samples were collected from the lakes with an Ekman grab sampler suspended from a small boat. The upper 0.5 cm of the sediment was retained for analysis. Multiple samples were collected from each lake at varying distances from roads (and inferred point-source salt-contaminant inputs) on the margins. Sampling was undertaken in late Spring (May–June), well after snow had melted and deicing operations had ceased. Fifty samples were collected in total from 15 lakes, 37 from the GTA-ORM (with 10 controls) and 13 from the northern study areas (five controls).

To assess salt contamination, chloride and conductivity readings were taken from each sampling station with a YSI Professional Plus water quality sensor, which was suspended into the water to a depth of ~0.2 m above the sediment-water interface. These bottom water readings provided optimal estimates of conditions near the benthic habitat of the arcellaceans. Water depths for each sampling station were determined using a Knudsen BP-320 sub-bottom profiler.

Sediment subsamples of 3 cc were used for arcellacean analysis. Samples were agitated for 1 h using a Burrell wrist shaker, screened with a 250-μm sieve to remove coarse organic debris and then with a 37-μm sieve to remove fine organic and mineral detritus. The residues on the 37-μm sieves were retained for arcellacean enumeration. The 37–250 μm size fraction was selected to allow comparability with other recent lake-based arcellecean studies. The 37–250-μm fraction samples were subdivided into aliquots for quantitative analysis using a wet splitter [57]. Wet aliquots were examined under a dissecting binocular microscope (×40–80 magnification) until a statistically significant number of specimens were quantified [58]. In most cases, >150 and often >250 arcellaceans were counted per sample.

Identification of arcellaceans was undertaken with reference to standard systematic keys (e.g. [3, 59]). Lacustrine species can display a significant amount of environmentally controlled morphological variation [3, 60, 61]. The accepted practice by lacustrine researchers has been to designate informal infrasubspecific strain names for these ecophenotypes [11, 23]. Although these infraspefic level designations have no status under the International Zoological Code of Nomenclature, they are useful for delineating environmentally significant populations within lake environments [2, 16, 22, 25, 26] so these terms were employed during counting. Scanning electron micrograph images of common species and strains were obtained using a Tescan Vega-II XMU VP scanning electron microscope (Fig. 3).

Statistical Analysis

Twenty-six arcellacean species and strains were identified in the 50 collected samples. The standard error (S_{xi}) associated with each taxon was calculated using the following formula:

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

where F_i is the relative fractional abundance of each taxon and N_i is the total of all the species counts in that sample. The methodology employed requires that if the calculated standard error exceeds the fractional abundance for a particular species in all samples, then that species should not be included in successive statistical analyses [58]. As all species were found in statistically significant numbers in at least one sample, none were removed from ensuing data analyses.

The 50 samples were also assessed to determine which were statistically significant. The probable error (pe) for each of the total sample counts was calculated using the following formula:

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

where s is the standard deviation of the population counts and X_i is the number of counts at the station being investigated. A sample is judged to have a statistically significant population (SSP) if the total counts obtained for each taxon were greater than the pe [62]. All 50 sediment samples were deemed to have SSP counts.

The Shannon diversity index (SDI) was used to examine the faunal diversity of the species found in each sample and provide an indication of the relative health of the lakes and ponds [63]. The SDI is defined as:

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

where X_i is the abundance of each taxon in a sample, N_i is the total abundance of the sample and S is equal to the species richness of the sample. Environments are considered to be stable if the SDI falls between 2.5 and 3.5, in transition between 1.5 and 2.5, and stressed between 0.1 and 1.5 [2, 64]. Low SDI values characterise environments where harsh conditions severely limit species numbers.

R-mode cluster analysis was used to determine which species were most closely associated with others and thus best characterised a particular assemblage [62]. Q-mode cluster analysis was used to group statistically similar populations using Ward's minimum variance method and recorded as squared-Euclidean distances.

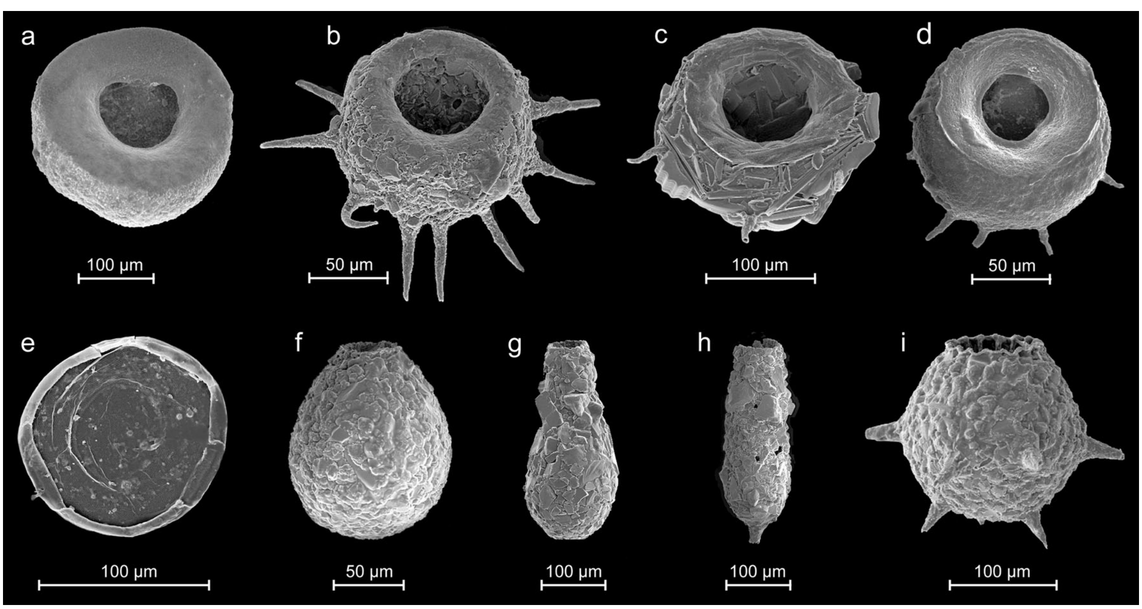


Fig. 3 Scanning electron micrographs (SEM) of selected specimens of Arcellacea from the study lakes. **a** *Centropyxis aculeata* (Ehrenberg 1832) strain 'discoides'; **b** *Centropyxis constricta* (Ehrenberg 1843) 'spinosa'; **c** *Centropyxis constricta* (Ehrenberg 1843) strain 'constricta' from Mew Lake. Note the test is largely composed of diatom frustules. **d** *Centropyxis aculeata* (Ehrenberg 1832) strain 'aculeata'; **e** *Arcella vulgaris* Ehrenberg 1832; **f** *Cucurbitella tricuspis* Carter 1856; **g** *Difflugia oblonga* Ehrenberg 1832 strain 'oblonga'; **h** *Difflugia protaeiformis* Lamark 1816 strain 'acuminata'; **i** *Difflugia corona* Wallich 1864

This clustering methodology is widely used in quantitative ecology and has been demonstrated to be a valuable data exploration tool [62]. Q-mode and R-mode cluster analyses were carried out on the 26 arcellacean species and strains and organized into a hierarchical diagram of the combined dataset. Detrended correspondence analysis (DCA) was carried out to explore the inter-site characteristics of the arcellacean communities and to examine associations between species.

Results

Interpretation of the Q-mode cluster analysis resulted in the recognition of four distinct arcellacean assemblages (1–4) (Fig. 4). The first three comprised samples from the GTA-ORM region, which we name based on the water property characteristics (Fig. 5) and other environmental inferences: (1) 'lakes with spatial salt gradients'; (2) 'control lakes (GTA-ORM region)'; and (3) 'salt-influenced lakes with nutrient

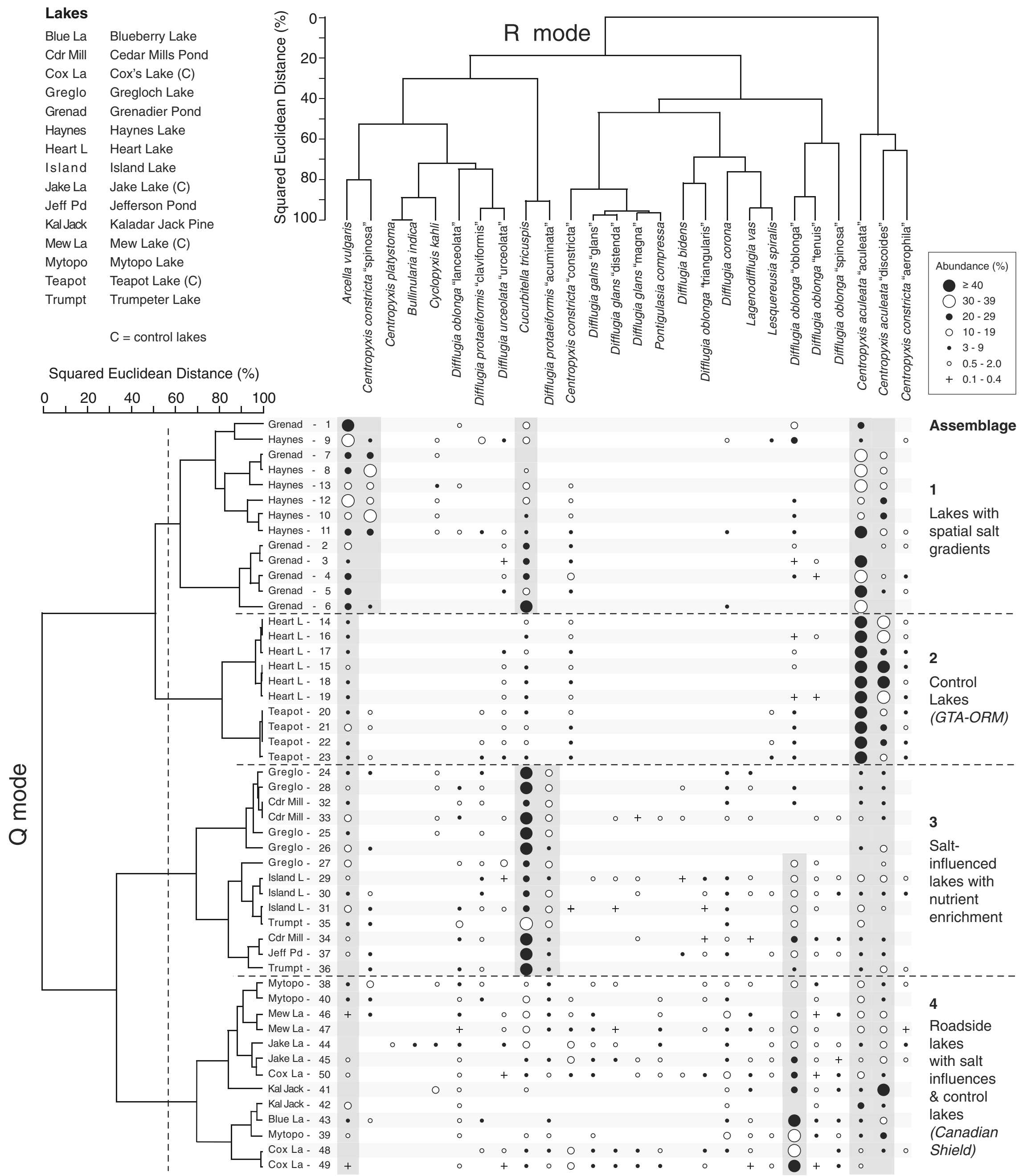


Fig. 4 R-mode vs Q-mode cluster diagram for the 50 arcellacean samples. Four faunal assemblages (*1–4*) are indicated. The *dashed vertical line* discriminates clusters of samples with correlation coefficients greater than the selected level of significance

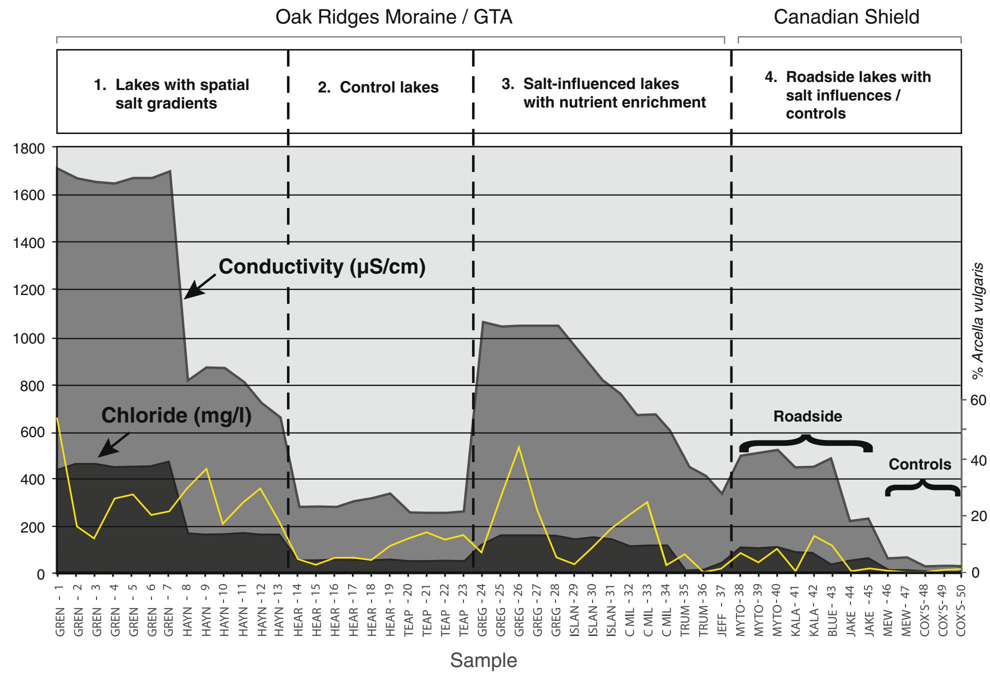


Fig. 5 Conductivity and chloride readings associated with the 50 arcellacean samples. The samples are grouped into four faunal assemblages as identified by Q-mode cluster analysis (Fig. 4). The *yellow line* shows the re-calculated abundance (%) of a notable indicator taxon, *A. vulgaris*, following the removal of the planktic *C. tricuspis* from the total counts

enrichment'. The fourth comprised samples from the northern set of lakes near Highway 7 and from Algonquin Provincial Park. Since these lakes share a common bedrock, metamorphic and igneous rocks of the Canadian Shield [65], we label this assemblage (4) 'roadside lakes with spatial salt influences (Canadian Shield)'.

Although 26 arcellacean taxa were included in the analysed dataset, R-mode cluster analysis indicated that only seven key species and strains significantly influence assemblage composition: *Arcella vulgaris* Ehrenberg 1832; *Centropyxis constricta* (Ehrenberg 1843) 'spinosa'; *Cucurbitella tricuspis* Carter 1856; *Difflugia protaeiformis* Lamark 1816 strain 'acuminata'; *Difflugia oblonga* Ehrenberg 1832 strain 'oblonga'; *Centropyxis aculeata* (Ehrenberg 1832) strain 'aculeata'; and *Centropyxis aculeata* (Ehrenberg 1832) strain 'discoides' (Figs. 3 and 4; Supplementary Appendix 1). The DCA results (Fig. 6) show similar trends to the Q-mode cluster analysis and are discussed below in the context of the four identified faunal assemblages.

Assemblage 1—'Lakes with Spatial Salt Gradients'

A. vulgaris and *C. aculeata* 'aculeata' dominate this assemblage, although other centropyxids, principally *C. constricta* 'spinosa' are abundant in several samples. The samples came from two lakes, Grenadier Pond and Haynes Lake, and are associated with some of the highest conductivity and chloride readings observed in the study (Fig. 5). The conductivity values were particularly high for Grenadier Pond (>1,600 µS/cm), confirming the strong impact of winter deicing salts, and potentially other road-borne metal contaminants on the entire lake. The chloride concentrations are also consistently high (430–470 mg/l) and far exceed background levels for the Great Lakes region (10–30 mg/l) [43]. These values are comparable to those recorded in the winter months (1990–1993) in salt-contaminated streams in the Highland Creek watershed, a major river that drains the eastern area of the GTA [31]. They are also well in excess of the Ontario drinking water standard (250 mg/l) [66] and the chronic aquatic freshwater toxicity threshold set by the United States Environmental Protection Agency (230 mg/l) for stream flow peaks resulting from snowmelt runoff [67]. (For comparison, the chronic effect water quality thresholds established in 2011 for chloride in Canada are even lower, at 120 mg/l [52]). Interestingly, the faunas of Sample 1, collected from the southern end of the lake near two major highways (Supplementary Fig. 1), and associated with the highest conductivity reading of 1,705 µS/cm, show some differences to those from the centre of the lake (e.g. samples 3 and 4). *A. vulgaris* dominates in this sample (48 %), whereas in the centre lake samples, it declines (to 7–11 %) and *C. aculeata* 'aculeata' dominates (46–58 %). It is unlikely that these differences are driven by variations in water depth, as samples were taken from comparable depths (~2–4 m) in open water locations. *A. vulgaris* is a common arcellacean species of brackish water environments (<5 ppt) and has been observed in slightly saline lakes [11, 68], lakes influenced by sea spray [22] and coastal cenotes [9, 69]. *C. aculeata* 'aculeata' often accompanies *A. vulgaris* in these settings. Both taxa have also been reported in a range of other hostile lake environments, including mine-acidified lakes [25, 28, 70] and lakes with elevated metal concentrations (including Al, Fe and As) [24] and low pH [71]. Low pH can

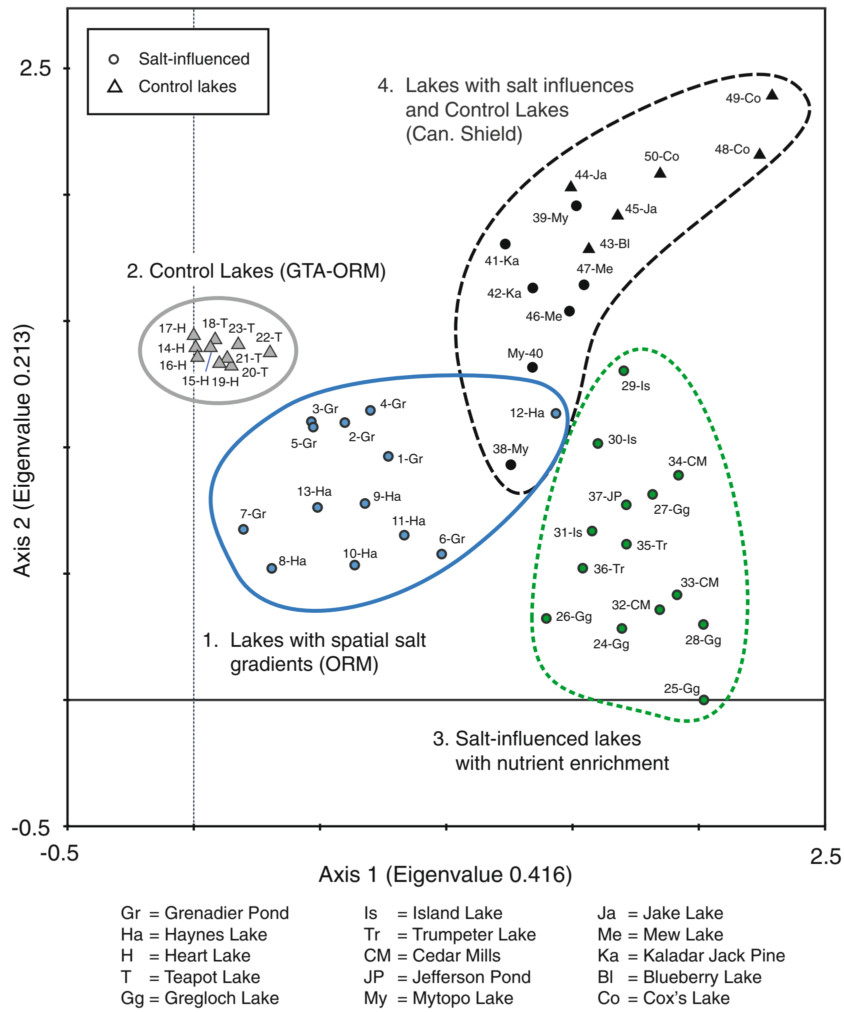


Fig. 6 Detrended correspondence analysis (DCA) results showing principal patterns of variation in the arcellacean populations for the analysed samples. Clusters are *encircled* following the assemblage groupings defined by Q-mode cluster analysis (Fig. 4)

be ruled out as a factor in this case, however, as all the lakes included in the study had a circumneutral to slightly alkaline pH.

The faunas from Haynes Lake also show a number of intra-lake differences that are consistent with water quality changes across the lake. The samples from the eastern side of the lake near the road, for example, yielded significant abundances of *A. vulgaris* (21–31 %), although *C. constricta* 'spinosa' co-dominated in some samples (25–30 %). *D. oblonga* 'oblonga' attained frequencies of 20 % in one roadside sample (11). The conductivity readings were correspondingly higher in the eastern lake stations (812–862 μS/cm), than in station 13 in the western part of the lake (667 μS/cm) (Figs. 2 and 5). Chloride concentrations were relatively even across the lake (160–167 mg/l). Like other centropyxids, strains of *C. constricta* are able to withstand a variety of stressed conditions better than most other arcellacean taxa and have been reported previously in brackish coastal lakes [21, 68] and lakes influenced by mine tailings [70]. The SDI values for Assemblage 1 are generally quite low (1.1 to 1.7). Two samples from Haynes Lake (samples 11 and 12) gave values of 2.0 (Supplementary Appendix 1).

Assemblage 2—'Control Lakes (GTA-ORM Region)'

This assemblage is dominated by *C. aculeata* 'aculeata' (>40 %) and *C. aculeata* 'discoides' (12–44 %). The samples were collected from two control lakes, Teapot Lake and Heart Lake, which are both located in a wooded conservation area north of Brampton (Fig. 1). The DCA analysis shows that the samples are closely grouped at both the intra- and inter-lake levels (Fig. 6). The SDI values are low to moderate (SDI= 1.1–1.9), whilst the chloride values (50–55 mg/l), and conductivity readings (266–340 μS/cm) are much lower than in the roadside and highway-influenced lakes of assemblage 1.

Whilst these two lakes do not directly receive contaminants from adjacent highways, the assemblage points to sub-optimal conditions. A previous study [29] reported that the faunas in these two lakes could be impacted by other stressors, including low oxygen conditions and/or sandy substrates. As noted above, centropyxids, particularly the strains *C. aculeata* 'discoides' and *C. aculeata* 'aculeata', are able to withstand a variety of inhospitable conditions. These include low salinities [72], lakes contaminated by metals and other pollutants

[25, 73, 74] as well as low oxygen levels [11, 75, 76]. Unlike other lakes included in the study which were less than 6 m deep, Heart Lake and Teapot Lake are >8 m and have well-developed thermoclines. They are also characterised by sandy substrates, which could have impacted the faunas. It is interesting to note that whilst a significant buffer of woodland surrounds the two lakes, the chloride and conductivity values are significantly higher than in control lakes in the northern study region (Fig. 5). This suggests that salt-contaminated groundwater is impacting the lakes. Hydrogeological modelling studies [47] have estimated that chloride contamination of groundwater may extend for some distances, potentially 500 m or more, away from major, multiple lane highways in the GTA-ORM region, due in part to the local permeability of the underlying glacigenic sediments, coupled with strong hydraulic gradients. Roe et al. [29] previously hypothesised that elevated levels of metals in these two lakes, including copper, lead and zinc, might be attributed to pollutant inputs from residential areas to the south and east of the lakes. However, distal highway-borne contaminants carried in via groundwater may also have impacted the lake water geochemistry.

Assemblage 3—'Salt-Influenced Lakes with Nutrient Enrichment'

In contrast to assemblages 1 and 2, this assemblage is dominated by *C. tricuspis* (24–75 %), which is widely regarded as an indicator of eutrophication [17, 20, 21, 60, 68, 77]. Moderate (10–28 %) frequencies of *A. vulgaris*, *D. protaeiformis* 'acuminata' and *D. oblonga* 'oblonga' also occur, along with low frequencies of several other difflugids and centropyxids and *Lagenodifflugia vas* Leidy 1874. The samples came from six salt-impacted, shallow (<6 m) lakes in the GTA-ORM region: Gregloch Lake, Island Lake, Jefferson Pond, Cedar Mills Lake and Trumpeter Lake (Fig. 1). The conductivity and chloride readings confirm varying levels of salt contamination, with conductivity readings ranging from 330 μS/cm (Jefferson Pond, sample 37) to 1,055 μS/cm (Gregloch Lake, sample 24). Chloride concentrations follow similar trends (Jefferson Pond, sample 37=43 mg/l; Gregloch Lake, sample 25=157 mg/l). The elevated readings for Gregloch Lake are surprising given that this lake is located in a rural (agricultural) area and is bordered only by a minor road (Supplementary Fig. 1). The high values, however, probably reflect its proximity (~0.8 km) to one of the region's '400 Series' highways (Highway 400), major multi-lane highways that receive over 200,000 kg of NaCl road salt per kilometre each year [47, 78]. The introduction of contaminants from an ephemeral stream that drains from the highway into the lake (Fig. 2) seems likely.

The significant presence of *C. tricuspis* in assemblage 3 and the implication that the faunas are responding to nutrient enrichment make the task of identifying the faunal response to salt contamination less straightforward than for the other assemblages. Unlike other taxa recorded in the study, this species has a planktic phase to its life cycle and enters the water column following the ingestion of nutrients from filamentous green algae such as *Spirogyra*, upon which it adheres and feeds [24, 60, 79]. Schönborn [75, 79] hypothesised that the nutrients have a high lipid content, which aids buoyancy. Once in the water column, the tests can be carried across lakes by winds and currents and only settle to the bottom upon decay [73, 75]. *Cucurbitella tricuspis* can thus occur in high numbers even in lakes with contaminated bottom waters [24]. Torigai et al. [77], for example, found that *C. tricuspsis* was prolific in heavy metal-contaminated sediment samples from Lake Winnipeg, Manitoba, and attributed its presence to allochthonous inputs from floating algae. Similar interpretations were drawn by Patterson et al. [28] for *C. tricuspis*-enriched assemblages from a pyrite mine-contaminated lake (James Lake) in northeastern Ontario.

In spite of this trend, it is interesting that *A. vulgaris*, which, as noted above, is prevalent in many of the salt-contaminated samples from Grenadier Pond and Haynes Lake, occurs in low to moderate abundances (8–12 %) in many of the assemblage 3 samples and attains peak frequencies in samples from stations closest to roads. In a road-proximal station (sample 31) from Island Lake, for example, which is bounded on two sides by roads (Fig. 2), *A. vulgaris* was present at 12 %, whereas in Sample 29 located furthest from the roads, it attained 2 %. Only in sample 35 from Trumpeter Lake was the species absent. This lake is surrounded by gardens and woodland, which may have buffered the lake from contaminant inputs from a nearby highway. This sample was one of several to include moderate frequencies (13–23 %) of *D. oblonga* 'oblonga', which often thrives in shallow lakes with organic-rich substrates [17]. This sample was also associated with much lower chloride readings (13 mg/l) than others in the assemblage.

Alongside *A. vulgaris*, some of the Assemblage 3 samples also included *C. constricta* 'spinosa' (3–9 %), which was prevalent in some of the roadside samples from Haynes Lake. The occurrence of this taxon is quite erratic though, with no consistent relationship with the conductivity and chloride data. *D. proteaiformis* 'acuminata' is another strain that might be indicative of elevated conductivities [61]. Like *C. aculeata*, this is an opportunistic taxon that has been reported in a variety of stressed lake environments. Patterson and Kumar [11], for example, noted its presence in a mine-contaminated lake (Crossfire Lake) in northeastern Ontario, where it was able to thrive when high levels of metal pollutants (Hg, As, Cd, Cr, Cu and Pb) precluded most other species. Like *C. tricuspis*, it is also tolerant of nutrient loading, including phosphorus enrichment [20].

The SDI values for Assemblage 3 are, in general, low to moderate (1.1 to 2.4), with the highest values from Island

Lake Sample 29. One anomalously low value (0.8) was obtained from Gregloch Lake Sample 25, which was associated with conductivity and chloride readings of 1,043 μS/cm and 156 mg/l respectively. Not surprisingly, there is no overlap between the Assemblage 3 samples and those of Assemblages 1 and 2 in the DCA plot (Fig. 6); the strong imprint of *C. tricuspis* and other faunal differences has resulted in the samples plotting out separately.

Assemblage 4—'Roadside Lakes with Spatial Salt Influences (Canadian Shield)'

This assemblage includes the most diverse faunas observed in the study (SDI=1.4—2.6, with an average of 2.1). The highest diversities (SDI >2.4) occurred in samples from Jake and Mew lakes in the Algonquin Provincial Park. Community composition was generally quite even between samples and included many difflugids, centropyxids (principally *C. aculeata* strains), *Pontigulasia compressa* Carter 1864 and *C. tricuspis*, whilst *L. vas*, *Lesquereusia spiralis* (Ehrenberg 1840) and *Cylclopyxis kahli* Deflandre 1912 occurred sporadically. A deviation from this trend was noted in six samples where *D. oblonga* 'oblonga' dominated (15–40 %). These generally well-balanced faunas are indicative of healthy, mesotrophic conditions. Patterson and Kumar [11] note that in most stable climax arcellacean communities, usually associated with higher SDI values, a balanced distribution of species is typically encountered, with no single species overwhelmingly dominating the assemblages. Faunas dominated by species of *Difflugia*, particularly strains of *D. oblonga*, are also often characterised by high SDI values and large populations. Most typical of sapropelitic environments, *Difflugia* taxa typically rely on plentiful sources of organics to maintain their habitat and a high carrying capacity is possible [2, 17].

Evidence of salt contamination of the roadside lakes in Assemblage 4 is nevertheless indicated by the water property readings. Chloride concentrations, for example, ranged from 39 mg/l (Blueberry Lake) to 108 mg/l (Mytopo Lake). These are appreciably higher than those from the two local control lakes, Mew Lake (~1 mg/l) and Cox's Lake (~15 mg/l) (Fig. 5), which are within or just above background levels for surface waters in the Canadian Shield (1–5 mg/l) [43]. Conductivity values follow similar trends, peaking at 525 μS/cm in Mytopo Lake and ranging from 38 to 72 μS/cm in the controls.

Whilst the faunas of Assemblage 4 do not show any significant evidence of inhospitable conditions, there are subtle fluctuations that require explanation. The most contaminated roadside stations, for example, with higher chloride and conductivity readings, yielded lower SDI values than the other samples. The two samples from Kaladar Jack Pine Lake (41 and 42) near Highway 7 provide a case in point (Fig. 1), with scores of 1.4 and 1.5. *A. vulgaris* was also typically present at greater frequencies (3–13 %) in the roadside samples and was only absent in Kaladar Jack Pine 41. This sample, which was collected from only 5 m from the edge of the highway, is also noteworthy for its high levels (44 %) of *C. aculeata* 'discoides'. As indicated above, this is another taxon that has been reported in sub-optimal environments influenced by pollutants [2, 25, 74]. *Cyclopyxis kahli* also peaked (11 %) in this sample. This species is most common in soils and forest litter [80, 81] but has also been observed in lakes, where it has been attributed to inwashing [22]. Its occurrence again points to erosion around the lake margins.

The DCA plot (Fig. 6) shows that there is little overlap between the faunas of this assemblage with the others. Only Mytopo Lake Sample 38 plots out close to sample 12 from Haynes Lake, probably because of the similar proportions of *C. constricta* 'spinosa' and *C. aculeata* strains.

Discussion and Conclusions

The results of this study confirm that there is clearly a link between lacustrine arcellacean faunas and the application of winter deicing salts to adjacent highways, supporting previous preliminary work from the same region [29]. Samples from the most salt-contaminated lakes, with bottom water chloride concentrations in excess of 400 mg/l and conductivities of >800 μS/cm, were typically associated with lower faunal diversities and higher frequencies of species tolerant of brackish water and/or stressed conditions than those from lakes with lower readings. *A. vulgaris*, *C. constricta* 'spinosa' and *Centropyxis aculeata* 'aculeata' were common constituents in these salt-impacted assemblages, although other centropyxids were also present in varying abundances. These trends were observed in lakes near major highways and lakes bounded by smaller roads. In both cases, elevated proportions of the 'stressed' indicator taxa were often noted in road-proximal sampling stations, suggesting that the contaminants are road-derived. This was particularly well illustrated at Haynes Lake, where poor salt management has led to seepage of salt-charged waters into one side of the lake. Island Lake, Cedar Mills Lake and some of the lakes along Highway 7 and Highway 60 showed similar trends. In other cases, the spatial patterns were less apparent, suggesting that the response to salt contamination is being obscured by other controls, for example, nutrient loading and substrate characteristics.

Of the two study areas, the GTA-ORM region was, not surprisingly, associated with higher chloride concentrations and conductivity values than lakes in the Highway 7 (Trans-Canada Highway) and Highway 60 (Algonquin Provincial Park) corridors. This is likely to reflect the fact that lower volumes of deicing salt are applied to these highways; two lane arterial highways in more rural areas of southern Ontario typically receive 10,000–20,000 kg of NaCl deicing salt per kilometre each year [78], whereas major highways in the GTA-ORM region like the Gardiner Expressway near Grenadier Pond and Highway 400 near Gregloch Lake receive >200,000 kg/km/year [47]. The Canadian Shield bedrock may have further reduced the vulnerability of the northern study lakes to deicing salt degradation. Unlike the unconsolidated glacigenic sediments that form the underlying basement geology in ORM region, the granitic and gneissic rocks of the Canadian Shield are largely impermeable [82, 83], reducing the likelihood of groundwater contamination. The more pristine character of the forest within lake catchments in the northern study areas is also likely to have contributed to the healthier faunas observed.

Nutrient enrichment was prevalent in some of the studied lakes, particularly in the ORM region. This was particularly evident for the assemblage 3 samples, which were dominated by the algal indicator taxon, *C. triscuspis.* As noted above, the tests of this seasonally planktic species can drift across lakes, achieving high totals in the thanatocoenosis. The potential inclusion of reworked tests in the counts clearly poses problems for interpreting the impact of contaminants on the lake benthos. This problem can be overcome, however, if the *C. triscuspis* counts are removed and the relative abundances

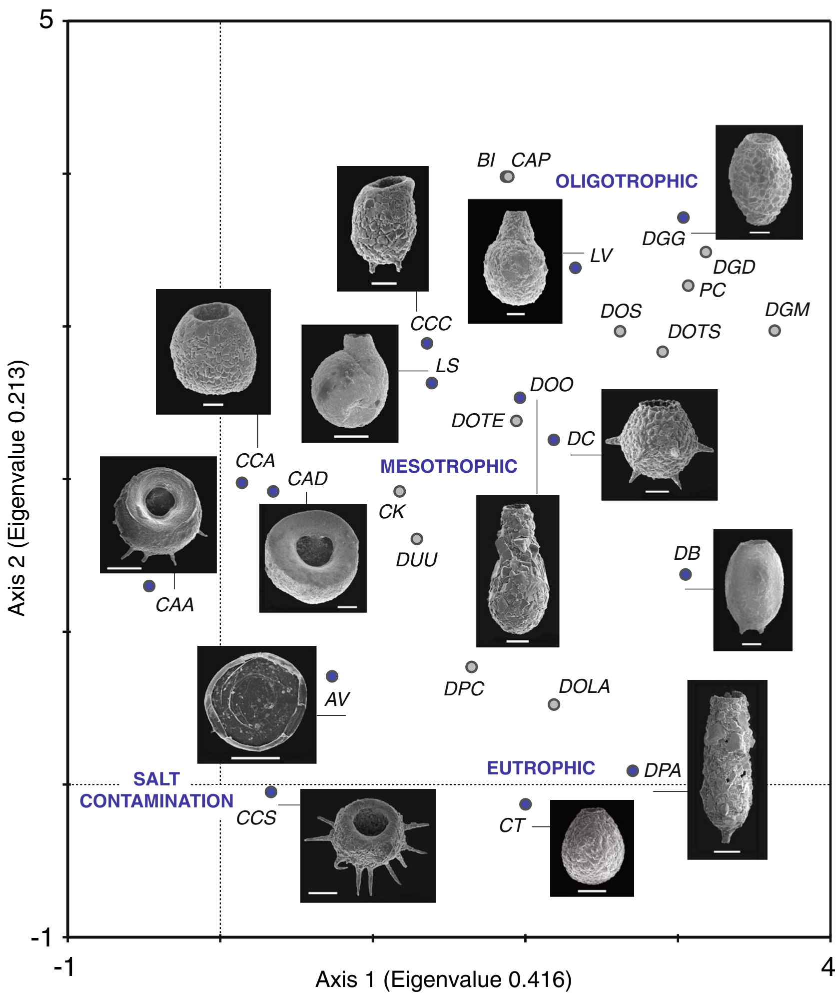


Fig. 7 Detrended correspondence analysis (DCA) results showing species scores for the 26 arcellacean taxa recorded in the study and inferred water quality preferences. *AV* = *Arcella vulgaris* Ehrenberg 1830; *BI* = *Bullinularia indica* (Penard 1907); *CAA* = *Centropyxis aculeata* (Ehrenberg 1832) strain 'aculeata'; *CAD* = *Centropyxis aculeata* (Ehrenberg 1832) strain 'discoides'; *CCA* = *C. constricta* (Ehrenberg 1843) strain 'aerophila'; *CCC* = *C. constricta* (Ehrenberg 1843) strain 'constricta'; *CCS* = *C. constricta* (Ehrenberg 1843) strain 'spinosa'; *CK* = *Cyclopyxis kahli*; *CP* = *Centropyxis platystoma* (Penard 1890); *CT* = *Cucurbitella tricuspis* Carter 1856; *DB* = *Difflugia bidens* Penard 1902; *DC* = *Difflugia corona* Wallich 1864; *DGD* = *D. glans* Penard 1902 strain 'distenda'; *DGG* = *D. glans* Penard 1902 strain 'glans'; *DGM* = *D. glans* Penard 1902 strain 'magna'; *DOLA* = *D. oblonga* Ehrenberg 1832 strain 'lanceolata'; *DOO* = *D. oblonga* Ehrenberg 1832 strain 'oblonga'; *DOS* = *D. oblonga* Ehrenberg 1832 strain 'spinosa'; *DOTE* = *D.* oblonga Ehrenberg 1832 strain 'tenuis'; *DOTS* = *D. oblonga* Ehrenberg 1832 strain 'triangularis'; *DPA* = *D. protaeiformis* Lamark 1816 strain 'acuminata'; *DPC* = *D. protaeiformis* Lamark 1816 strain 'claviformis'; *DUU* = *D. urceolata* Carter 1864 strain 'urceolata'; *LS* = *Lesquereusia spiralis* (Ehrenberg 1840); *LV* = *Lagenodifflugia vas* Leidy 1874; *PC* = *Pontigulasia compressa* Carter 1864. SEM images of the most common taxa are shown (*scale bar*=50 μm)

are re-calculated. Under this scenario, the proportions of stressed indicator taxa like *A. vulgaris* and *C. constricta* 'spinosa' in the Assemblage 3 samples become more comparable to those for Assemblage 1, where *C. tricuspis* does not feature prominently. Sample 26 from Gregloch Lake, for example, which was associated with a conductivity reading of 1,044 μS/cm and a chloride concentration of 156 mg/l, gave an adjusted *A. vulgaris* abundance of 42 % (Fig. 5), in contrast to the pre-adjusted value of 18 %. This strongly suggests that this species is responding to road salt contamination. Future studies that are concerned with monitoring impacts of contaminants on lake bottom waters should follow a similar approach.

Whilst there was an interesting correspondence between the salt-related water property variables and the presence of 'stressed' elements in the arcellacean faunas, it remains unclear which aspect of salt contamination the faunas are responding to: elevated conductivities/salinities, chloride enrichment or indeed the presence of other unmeasured NaCl deicing salt constituents such as sodium or magnesium or even accompanying grit acting in combination with the above. The response could also reflect other more complex limnological changes operating in the lakes. In some small, deep lakes, for example, deicing salts can promote chemical stratification and reduced oxygen availability [84, 85]. Other than Heart Lake and Teapot Lake, two deeper lakes that are located away from major roads, all the study lakes appeared to be well mixed, however.

Notwithstanding the potentially complex nature of the chemical impacts, it is significant that for most of the analysed lakes, the conductivity and chloride data co-varied, and a high correlation was observed between the two variables (r^2= 0.911; p<0.00001) (Supplementary Fig. 2). This is reassuring, for whilst elevated chloride concentrations are unlikely to be derived from sources other than deicing salts in this region, the conductivity readings could reflect metal inputs from many other sources, particularly in urban areas [86].

It is interesting to note that low-diversity arcellacean faunas dominated by *A. vulgaris* and *C. constricta* and *C. aculeata* strains have also been reported in constructed wetlands that have been heavily impacted by oil sands processing operations in the Athabasca region of northeastern Alberta, Canada [87]. The process-impacted waters are characterised by elevated conductivity readings, high levels of Na/(Ca+Mg) and high naphthenic acid concentrations, and a strong relationship was found between the arcellacean assemblages and these constituents [87]. *A. vulgaris*- and *C. aculeata*-dominated communities have also been recorded in brackish water lakes in coastal regions as well as in other inhospitable lacustrine environments (e.g. [12, 21]). These taxa additionally occur in saltmarsh testate amoeba assemblages [7, 8, 88], although many other taxa, including idiosomic species, are also present, reflecting significant habitat differences.

Ultimately, for aquatic organisms to be useful for biomonitoring, a graded response to the contaminant is desirable, with specific indicator species or assemblage components showing predictable and sequential changes as the contaminant increases. Williams et al. [31], for example, studied the impact of chloride loading on stream invertebrates in the GTA region and developed a 'biotic index' in which faunal groupings were associated with different levels of contamination. The current study has shown that two or three lake arcellacean taxa appear to flourish in the hostile conditions induced by NaCl deicing salt application and that abundances of these taxa appear to rise with increases in salt-related water chemistry parameters. The associations between these 'stressed' indicator taxa are

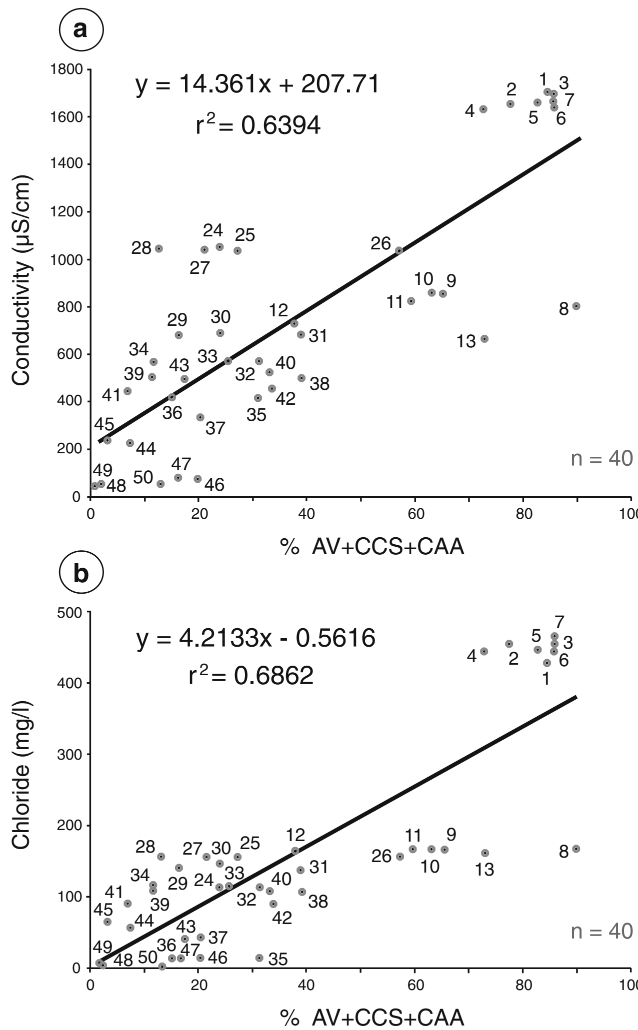


Fig. 8 Correlations between combined totals of three 'stressed', road salt indicator taxa (*A. vulgaris*, *C. constricta* 'spinosa' and *C. aculeata* 'aculeata') in 40 of the lake samples with **a** conductivity (r^2=0.6394; p<0.0001) and **b** chloride (r^2=0.6861; p<0.0001). The sample numbers follow those used in Figs. 4, 5 and 6. Planktic taxa (*C. tricuspis*) were removed prior to analysis. All samples achieved statistically significant counts [58]. Samples from Teapot Lake and Heart Lake were not included in the analyses as the arcellacean faunas in these samples are strongly influenced by other known stressors, i.e. sandy substrates and low oxygen

confirmed by the DCA analyses for species (Fig. 7), which show *A. vulgaris* plotting out near to *C. constricta* ‘discoides’ and *C. aculeata* ‘aculeata’ (Fig. 7). *A. vulgaris*, in particular, appears to be capable of withstanding the highest levels of contamination, as noted in some mine-tailing studies [28] and may have potential as an indicator species for tracking salt damage in lakes which are otherwise healthy. Moreover, if the abundances of these three taxa are combined and the planktic faunal component (*C. tricuspis*) is removed from the counts as discussed above, then strong correlations with chloride (r^2= 0.639; p<0.0001) and conductivity (r^2=0.686; p<0.0001) are observed (Fig. 8). The impact of other controls on the arcellacean faunas, particularly trophic status, is also evident from the DCA plot (Fig. 7). More quantitative distributional work is required to better discriminate salt-damaged faunas from those influenced by other stressors.

A final aspect of the DCA plot that is worthy of note is the varying environmental responses shown by different strains of the same species. *Centropyxis constricta* ‘spinosa’, for example, plots out separately to *C. constricta* ‘constricta’ (Fig. 7). The prominent basal processes that characterise the former strain (Fig. 3) may reflect an ecophenotypic response that is accentuated by road salt contamination. These differing responses underline the validity of adopting strain-based classification approaches to better understand autoecological relationships and the value of strains as environmental indicators [11, 23].

In the future, improved insights into the relationships between lake arcellaceans and salt contamination should be gained by analysing instrumental datasets that span longer timescales. Our readings only reflect salt contamination in the study lakes in early summer, yet chloride and conductivity readings of water bodies in road salt-impacted regions vary on seasonal and shorter timescales, reflecting the timing and character of deicing operations, runoff and snowmelt characteristics and temperature fluctuations [89]. Peak chloride values in streams in this region, for example, are consistently observed in the winter months [31, 50]. However, lakes in road salt-impacted regions can register high salinities and chloride concentrations in the winter and spring, but in the summer, flushing can reduce values [85]. Evaporation, associated with high summer temperatures, can also concentrate salts in lakes, particularly kettle lakes with no through flows [90], whilst inwashing of salt constituents from soils may also result in fluctuations in lake readings [85]. The study of fossil arcellacean assemblages from lakes associated with instrumental records that span several decades would be particularly useful for the analysis of long-term trends and for establishing pre-disturbance conditions. There is also considerable scope for examining the testate amoeba faunas of soils in salt-impacted regions. Preliminary analyses from this region have certainly yielded promising results (Roe and Patterson, unpublished). Comparisons with more established salinity indicators such as lake diatoms would also be useful.

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