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Lacustrine Arcellinida (testate lobose amoebae) as bioindicators of arsenic concentration within the Yellowknife City Gold Project, Northwest Territories, Canada

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ABSTRACT

Arcellinida (testate lobose amoebae) were examined in near-surface sediment samples from 30 lakes located within the Gold Terra Resource Corporation (GTRC) Yellowknife City Gold Project, Northwest Territories, Canada, to assess the applicability of using the group as a robust tool to assess Arsenic (As) contamination. Lake contamination by As is of concern in the Yellowknife area because it is derived from geogenic (bedrock) and anthropogenic sources (legacy pollution from former mining operations, particularly the Giant Mine [1948–2004]). Statistical multivariate analyses (cluster analysis, non-metric multidimensional scaling [NMDS], and redundancy analysis [RDA]) were performed on geochemical (inductively coupled plasma mass spectrometry [ICP-MS]) and organic (loss-on-ignition) data to identify geochemical and hydrogeological controls on the arcellinidan distribution in the lakes. Three distinct faunal assemblages were identified using Cluster analysis and NMDS: 1) Elevated Arsenic Assemblage (EAA; Approximately Unbiased (AU) *p*-value = 97 %; median As =272.4 mg/kg, range = 42–1353 mg/kg ; $n = 9$); 2) Centropyxid-Dominated Assemblage (CDA; AU *p*-value = 87 %; median As =169.7 mg/kg, range 60–233 mg/kg ; $n = 9$); and, 3) Difflugiid-Dominated Assemblage (DDA; AU *p*-value = 95 %; median As =61.3 mg/kg, range = 16–316 mg/kg ; $n = 12$). Six statistically significant controls on assemblage structure were identified using RDA. These were As, calcium, iron, strontium, water depth, and total organic carbon (Arcellinida variance explained = 36.2 %) — As is the most significant control (12.2 %; *p-value* < 0.002). Stress-tolerant centropyxids dominate in lakes characterized by elevated sedimentary As concentrations (64 % centropyxids in EAA; 40 % centropyxids in CDA), while stress-sensitive taxa thrived in samples associated with lower As concentrations (DDA). Our results corroborate the findings of previous Arcellinida studies in subarctic Canada and confirms the reliability of using Arcellinida as a reconnaissance tool for tracking As contamination in impacted lakes.

1. Introduction

For over a century, gold mining has been a substantial driver of Canada's economy, contributing $9.6 billion in 2018 alone (Natural Resources Canada, 2019). The Yellowknife area, Northwest Territories (NT) is a gold mineralization-rich area where several major gold mines have been developed within the orogenic Yellowknife Greenstone Belt (e.g., Giant Mine,1948–2004; Con Mine, 1938–1943, 1946–1998, 1999–2003; Discovery Mine, 1950–1969). Of these mines the Giant Mine went on to become the longest running and most productive mining operation in the NT mining history, producing more than 220 tonnes of gold over a 50-year period (Silke, 1999). Substantial amounts of As in the form of the highly toxic and bioavailable arsenic trioxide (As_2O_3) were released to the environments surrounding Giant Mine due to the need to roast refractory ore (Galloway et al., 2012, 2015, 2018; Hocking et al., 1978; Houben et al., 2016; Schuh et al., 2018; Nasser et al., 2016, 2020b; Palmer et al., 2019). Due to geogenic input from mineralized bedrock, pre-mining background As concentrations in lake

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sediments are ~25 mg/kg (Galloway et al., 2015, 2018), exceeding the Canadian Council of Ministers of the Environment (CCME) interim sediment quality guideline (ISQG =5.9 mg/kg) and the probable effect level (PEL =17 mg/kg) for this metalloid (Canadian Council of Ministers of the Environment (CCME, 2002). The contribution of As from anthropogenic sources has resulted in many lakes in the vicinity of Giant Mine having elevated levels of As (Galloway et al., 2015, 2018; Nasser et al., 2016) that range up to as high as 30,000 mg/kg (Thienpont et al., 2016).

The environmental legacy left in the wake of the closure of the Giant Mine is not unique, as several mines across the NT have previously been left abandoned, with sites in the care and receivership of the federal government of Canada. Today, remediation programs are underway at former gold mines in the NT including the Tundra Mine (1962–1968, 1982–1987), Discovery Mine (1950–1969), and Colomac Mine (1990–1997), to restore these sites (AANDC, 2014; INAC, 2002; Silke, 1999). To eliminate reclamation debt and environmental liability issues associated with gold mining in the NT, the Mine Site Reclamation Policy (MSRP) was issued in 2002 by the federal government Department of Crown Indigenous Relations and Northern Affairs Canada (INAC, 2002). The policy has three primary objectives: 1) to ensure that all mines have a framework for closure; 2) to remediate any areas and environments within a mine's property that are affected by mining activities; and 3) monitor environmental change in remediated areas and environment within a mine's property after the cessation of mining activities (INAC, 2002).

Despite the closure of the old mines, the Yellowknife Greenstone Belt (YKGB) continues to have a high potential for gold. Mineral exploration is currently being carried out by several companies to identify new economic gold deposits. Amongst them is Gold Terra Resources Corporation (GTRC), which is conducting work approximately 6 km north of the former Giant Mine site. For future mines to be permitted, mine developers must have a good understanding of geochemical baseline As concentrations in the NT to comply with the requirements of the MSRP. For current and prospective mines operating within the YKGB compliance with the MSRP is complicated by a legacy of past contamination and the propensity of the post-depositional mobility of As in lake sediments. Research to address the issue in Yellowknife area lakes has linked As remobilization to a combination of seasonally influenced physical and biogeochemical processes (Galloway et al., 2018; Miller et al., 2019; Palmer et al., 2019; Van Den Berghe et al., 2018). The mobility of As is attributed to the metalloid's redox sensitivity and is influenced by either natural (e.g., changes in environmental parameters and climate change) and/or anthropogenic factors (e.g., land-use) that can affect the redox conditions of surface waters and shallow sediment pore waters (e.g., Palmer et al., 2019). For example, prolonged duration of ice cover results in near complete oxygen consumption during winter months in Yellowknife area lakes, resulting in reduction of pentavalent species (As^{+5}) to trivalent (As^{+3}) until seasonal open water conditions oxygenate the lake once again (Palmer et al., 2019). This seasonal cycling creates conditions for the lake sediment to act as sinks of As (under oxic conditions) or as sources of As (under reducing conditions; Palmer et al., 2019). However, in shallow reducing sediments, As may also be sequestered in As-bearing sulfides such as pyrite (Miller et al., 2019; Schuh et al., 2018), and this process may be biologically mediated (Galloway et al., 2018). Thus, climate conditions that determine the length of seasonal open water and ice cover, as well as organic matter loading, affect the mobility and fate of As in lake sediments. The cycling of As between shallow sediments and overlying lake waters may explain why many Yellowknife area lakes show little sign of recovery from roaster stack contamination despite cessation of the majority of emissions 60 years ago (Van Den Berghe et al., 2018; Palmer et al., 2019). The post-depositional remobilization of As also hinders determination of pre-mining background and baseline As concentrations in lake sediments and surface waters. In order to ensure the environmental sustainability of exploration and mining and compliance with the stipulations of the MSRP, mineral exploration and new mine developers as well as regulators require reliable tools for tracking the impact of As contamination on the ecological health of lakes and differentiating sources of As.

Arcellinida (testate lobose amoebae) are benthic protists in the size range of 5−300 μm. These organisms, which have a long record of being used as bioindicators of lacustrine environmental variability (Patterson and Kumar, 2002), play a key role in aquatic ecosystems where they exert considerable predatory pressure on bacteria and smaller eukaryotic microbes and thus, represent an important intermediary food web component (Anderson, 2012; Beyens and Meisterfeld, 2002; Patterson and Kumar, 2002). As such the distribution of arcellinidan assemblages is closely linked to lake productivity, which is in turn related to the availability of their preferred food; bacteria, algae and fungi.

The group can be found worldwide in a variety of freshwater and brackish habitats (Charman, 2001; Dalby et al., 2000; Patterson and Kumar, 2002) ranging from the Arctic to the tropics (Collins et al., 1990; Dalby et al., 2000; Medioli and Scott, 1988; Patterson et al., 2015; Roe and Patterson, 2006). The potential of using Arcellinida as bioindicators of anthropogenic contamination has received much attention over the past two decades (Asioli et al., 1996; Kihlman and Kauppila, 2012; Kumar and Patterson, 2000; Nasser et al., 2020b, 2020a, 2016; Neville et al., 2011; Patterson et al., 2019; Roe and Patterson, 2014). The successful use of the group as a tool for tracking industrial contamination can be attributed to their fast reproduction rate, high abundance in aquatic systems, excellent preservation potential with decay-resistant tests, combined with a sensitivity to environmental change, particularly geochemical change (Nasser et al., 2020b; Patterson et al., 2012; Steele et al., 2018).

In the NT, Nasser et al. (2016) quantified the relationship between the inter-lake spatial distribution of Arcellinida and As concentration in sediment samples from lakes in the region surrounding Giant Mine. Similarly, Gavel et al. (2018) demonstrated a clear response of Arcellinida assemblages to temporal changes in As concentrations in a freeze core from Frame Lake within the City of Yellowknife. Most recently, Nasser et al. (2020b) determined the As tolerance limits of 25 arcellinidan taxa, thus permitting this group to be used to infer As levels in impacted lakes in subarctic Canada. Using Arcellinida as a bioindicator of As contamination is proving to be a very useful area of research as conventional ICP-MS techniques cannot distinguish between As species (e.g., As^{+3} and As^{+5}), and commercially available As speciation techniques are expensive and time consuming (e.g., Miller et al., 2019). Arcellinida are a prospective biomonitoring tool for measuring modern concentrations and bioavailability of As in the bottom waters and shallow sediment in which they live and were seasonal changes in inorganic As species have been previously observed (Palmer et al., 2019). Further, the immobility of arcellinidan tests in lake sediments offers a mean to reconstruct As at the time of sediment deposition regardless of the post-depositional mobility of As (e.g., Gavel et al., 2018).

This work in the GTRC was designed to specifically address what the pre-mining background concentrations of As are in 30 lakes in this geologically unique and economically prospective area (covers ~ 102 km^2) by evaluating the site-specific responses of Arcellinida to As concentrations in the sediments of these lakes. The results of this study will allow the establishment of a baseline for current As concentrations in the area being investigated by GTRC. This sets the stage for future high-resolution Arcellinida paleolimnological analysis to determine historic As concentrations in lakes of the area. Paleo-arsenic concentrations as determined using arcellinidan proxy data, which are uninfluenced by post depositional As remobilization, will allow for the determination of pre-mining background As concentration, and baseline levels for MSRP compliance purposes.

2. Background

2.1. Yellowknife mining history

One of the first gold deposit discoveries in the NT occurred in 1935 on the shore of Back Bay, on Great Slave Lake. Later that same year, gold was discovered at the future Giant Mine site. There were many gold discoveries in the region through the following years. For example, the Con Mine went into production in 1938 and continued until 2003. Gold was discovered 84 km northeast of Yellowknife in 1944 and the Discovery Mine that was established there was in production between 1950 and 1969. The Giant Mine went into production in 1948 and went on to be the longest running and one of the most productive mines (~198,446.6 kg) in the NT history, eventually closing in 2004 (Silke, 1999; Royle, 2007). During the refining process gold was liberated from refractory ore by roasting the ore at 500 ℃. This procedure converted gold-bearing arsenopyrite to porous Fe-oxides (hematite and maghemite), which are more amenable to the process of cyanidation (Walker et al., 2005). The residues of roasting included a fine particulate As-bearing solid phase As_2O_3 that was released into the atmosphere from the roaster stack and deposited downwind across the landscape and within area lakes (Galloway et al., 2012, 2015, 2018; Palmer et al., 2015; Houben et al., 2016; Palmer et al., 2019). The Giant Mine emitted approximately 2.6 million kg/year of As, primarily As_2O_3, and SO_x vapours into the atmosphere through the roaster stack during the first decade of gold production (MacDonald, 1997). Efforts to decrease As_2O_3 emissions began in 1951 with the installation of a Cottrell precipitator and further reductions occurred with the installation of another Cotrell precipitator in 1955 (Hocking et al., 1978). These new clean technologies reduced the release of arsenic from 2.6 million kg/year to approximately 5700 kg/year (MacDonald, 1997; SRK Consulting, 2002). Diverted emissions were collected and eventually resulted in the storage of 237,176 tonnes of As, backfilled into mine stopes, but it is estimated though that a total of 20,000 tonnes of $As_2O_3$was released into the atmosphere (Hocking et al., 1978).

2.2. Arsenic mobility and toxicity

Arsenic (As) is a trace metalloid, which can be of environmental concern because even at low concentrations it can be dangerous to plants, animals, and as a carcinogen in humans (Haffert and Craw, 2010). This metalloid is often associated with potentially economic gold deposits in the forms of arsenopyrite and arsenian pyrite. Arsenic is naturally mobilized from these deposits but ore extraction processing can significantly increase mobility (Haffert and Craw, 2010). Arsenic is present in the environment in many different compounds with the toxicity levels and biological effects being dependent on its molecular form and oxidation state. Arsenic can be either inorganic (arsenite [As^{+3}] and arsenate [As^{+5}]), or organic (monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB), arsenocholine (AsC), arsenolipids and arsenosugars). Inorganic As is found to be more mobile and toxic compared to organic forms (de Rosemond et al., 2008). Many factors influence the bioavailability of As to organisms, including mineralogy and speciation, pH, and redox conditions of the environment. The bioavailability of As can change when the metal is transformed from a solid phase to an aqueous phase in water, making it more accessible to aquatic life (Basta and Juhasz, 2014). Arsenic trioxide, as was produced at the Giant Mine, is one of the most bioaccessible and toxic forms of arsenic (Plumlee and Morman, 2011).

3. Study Area

The study area in Yellowknife, NT is characterized by a continental subarctic climate with short and dry summers. The mean annual air temperature is -4.3 ℃ and the mean annual precipitation of 170.7 mm, of which more than half falls as rain (Environment Canada, 2011). Average wind speeds range from 4.8 m/s to 5.5 m/s with the dominant wind direction flow being from the east and south (Pinard et al., 2008).

Lakes investigated for this study are underlain by Archean metavolcanic and metasedimentary rocks as well as younger granitoid rocks of the Yellowknife Supergroup and granodioritic plutons of the Defeat Suite, in the Slave Province portion of the Canadian Shield (Fig. 1; Cousens, 2000). The local terrain is a mixture of 25 % bare outcrop with the balance of the landscape covered by various glacial and glaciolacustrine sediment cover (Jolliffe, 1942; Kerr and Wilson, 2000). This entire area was once covered by postglacial Lake McConnell. The lakebed sediments of coarse and fine sand, silt, and clay can be as thick as 20 m in some regions (Kerr and Wilson, 2000). Till deposition in the Yellowknife area tends to be loosely compacted stony till with diamicton as the matrix and is generally less than 2 m thick, forming a discontinuous veneer (Kerr and Wilson, 2000).

In the area of Yellowknife, the terrain elevation ranges from <200 m above mean sea level (amsl) on the shores of Great Slave Lake to 400 m amsl north of Thistlethwaite Lake. Drainage catchments in the Yellowknife area are influenced by bedrock, where many small elongated lakes have been formed along fault boundaries. Streams and rivers are relatively shallow without significant incision into the bedrock (Kerr and Wilson, 2000). The main drainage system in the area is within the catchment of the Yellowknife River, which flows into the Yellowknife Bay, Great Slave Lake.

4. Materials and methods

An inter-lake survey of 115 lakes within the GTRC property was conducted in July 2016 and resulted in the collection of 115 sediment-water interface samples. Each sample was analyzed for ICP-MS and loss-on-ignition (LOI). A subset of 30 sediment-water interface samples was used in this study. The selection of the 30 samples is based on the As levels derived from the ICP-MS results (low [<100 mg/kg; $n = 12$], moderate [100–300 mg/kg; $n = 12$], high levels were selected for this study [>300 mg/kg; $n = 6$]). All lakes were accessed by air using a pontoon-equipped Bell Long Ranger helicopter. Samples were collected using an Eckman grab sampler deployed from the helicopter pontoons, with the top ~0.5 cm sediment-water interface retained from each grab for various analyses. Location of samples were recorded using the helicopter's GPS. Water depth at each sampling station was determined using a Vexilar hand-held sonar.

4.1. Laboratory

4.1.1. Geochemical analysis

The selected 30 sediment-water interface samples were analyzed for their geochemical composition using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following the *aqua regia* digestion protocol (ICP-MS 1 F/AQ250 package) at Bureau Veritas, Vancouver, British Columbia (Supplementary Table 1). *Aqua regia* digestion was employed instead of a 4-acid digestion protocol because the latter protocol can volatilize As (Parsons et al., 2012), which would generate results inappropriate for comparison with Arcellinida data.

Analytical precision was assessed using duplicate samples. Relative Percent Difference (RPD) was less than 7 % for As (median RPD = 6.1; RPD range = 1.129 %–6.896 %, $n = 4$). Analytical accuracy was assessed using standard reference materials STD DS10 ($n = 4$) and STD OREAS45EA ($n = 4$). Median As concentration measured in STD DS10 was 44.7 mg/kg (range = 43.5–44.7 mg/kg; $n = 4$) compared to an expected concentration of 43.7 mg/kg (median RPD = 2.3 %; RPD range = 0.5–4.0 %). Median measured As concentration for STD OREAS45EA was 12.7 mg/kg (range = 11.8–12.9 mg/kg; $n = 4$) compared to an expected As concentration of

10.3 mg/kg (median RPD = 20.9 %; RPD range = 13.6–25.4 %). Arsenic was not detected in any of the four laboratory methods blanks.

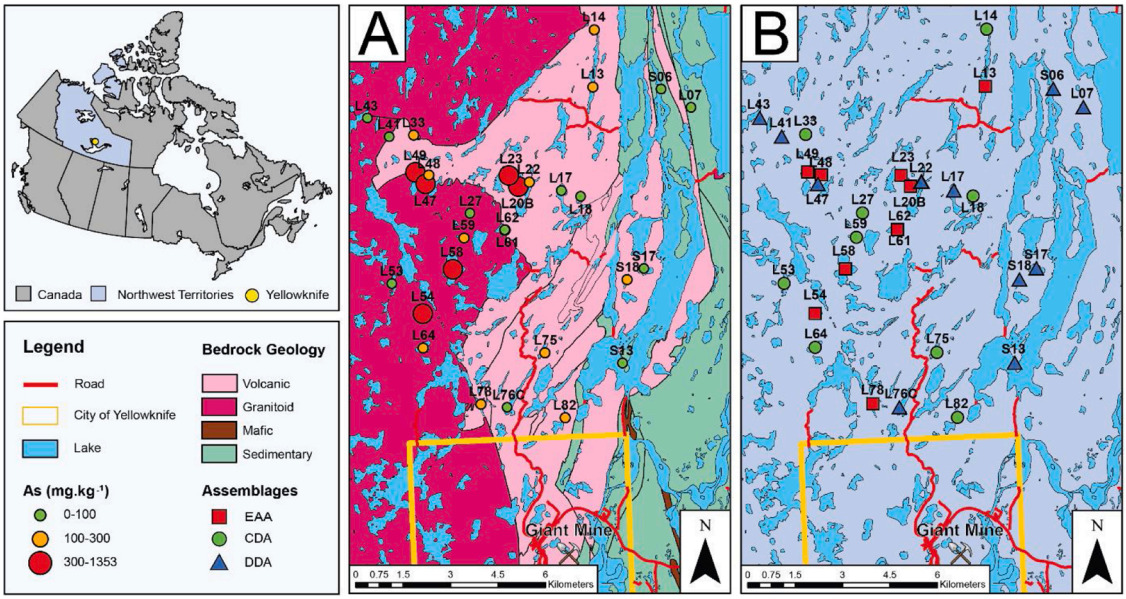


Fig. 1. Map of the study region. A. Location of the 30 sampled lakes in relation to the City of Yellowknife along with the bedrock geology. The graduated symbology and the color-coding represent the As concentration. B. A map showing the distribution of the three identified Arcellinida assemblages (represented by square, circle, and triangle symbols).

4.1.2. Organic content

Loss-on-ignition (LOI) analysis was carried out on 30 sediment-water interface sub-samples to determine the relative percentage of moisture, organic carbon, carbonate, and minerogenic content (Heiri et al., 2001). Moisture content was determined by comparing measurements before and after samples were placed in an oven at 100 °C for 24 h. A Thermo Scientific Thermolyne Benchtop Muffle Furnace (model: F48025−60-80) was used to carry out sequential burning at 550 °C and 900 °C to determine percentages of organic carbon and carbonate, respectively (Supplementary Table 1).

4.1.3. Arcellinida bioindicator analysis

A three cm^3 aliquot of the 30 sediment-interface samples obtained from each lake was prepared for arcellinidan analysis. Each sub-sample was sieved with water through 300 μm and 38 μm sieves to respectively remove large debris matter found in the sample and retain Arcellinida tests. Once sieved, the samples were poured into a custom-built wet splitter (after Scott and Hermelin, 1993), gently agitated to fully disperse the samples in the water column, and then left to settle to the bottom of the splitter for an hour, where the samples were divided into 6 equal aliquots. Each aliquot was then collected into separate vials for subsequent analysis. Individual aliquots were poured onto a gridded Petri Dish and Arcellinida species and strains were identified and quantified under an Olympus SZH10 research stereo microscope until at least 150 tests per sample were obtained (Patterson and Fishbein, 1989). Once analysis of an aliquot began specimens were counted until the aliquot was completely analyzed to avoid any bias related to varying settling and fluid dynamic of individual arcellinidan test morphologies (Patterson and Kumar, 2002). Taxonomic identification of Arcellinida species and strains was based on several publications where the "strain" concept was utilized (e.g., Burbidge and Schröder-Adams, 1998; Dalby et al., 2000; Nasser et al., 2020a, 2020b; Patterson et al., 2013, 2012; Steele et al., 2018).

4.1.4. Statistical analysis

Thirty-one arcellinidan species and strains were identified in the 30 near-surface sediment samples. Six arcellinidan species were present in insignificant numbers and were thus removed from statistical analysis. The Shannon Diversity Index (SDI) was calculated using the vegan package in RStudio (Dixon, 2003), the integrated development environment for the R statistical language (R Team, 2020) and was used to characterize species diversity. Samples with SDI values that range between 2.5 and 3.5 are considered stable, from 2.5 to 1.5 they are considered transition and between 0.1 and 1.5 they are considered stressed (Patterson and Kumar, 2002).

4.1.4.1. Data screening. Any arcellinidan taxa, LOI, and ICP-MS variables associated with more than 25 % missing, below detection values were removed (e.g., Reimann et al., 2008). Geochemical concentrations below the method detection limit were substituted with a value ½ of the method detection limit.

4.1.4.2. Variable reduction. Fifty-one variables were considered in this study (Supplementary Table 1). Variables that were highly correlated were removed using Spearman rank correlation (Correlation coefficient (R_s) > +0.7 and <-0.7; removed variables: Ag, Al, Au, Ce, Cr, Cs, Ga, K, La, Nb, Rb, Sc, Sn, Th, Ti, V, Zr, Li, and Cd). When two variables are highly correlated, only the variable that has been shown to impact the distribution of Arcellinida communities were retained. Any variables with high or low correlation coefficients that have no known direct or indirect effects on the disreibution of Arcellinida have also been removed (U, Sb, Bi, Ba, W, Tl, Se, and Y). The collinearity of the remaining variables (n = 24) was determined using the variance inflation factor (VIF), part of the USDM package (Naimi, 2015) within RStudio. Variables with a VIF value above 10 were considered highly colinear and removed from subsequent statistical analysis (clay, DO, Temperature, Co, and Mg). The remaining variables (n = 19), along with the arcellinidan taxa present in sufficient counts, were used in redundancy analysis (RDA) and were further reduced to arsenic (As), total organic content (TOC), iron (Fe), strontium (Sr), calcium (Ca), and water depth.

4.1.4.3. Cluster analysis and non-metric multidimensional scaling. Q-mode cluster analysis was used to group the arcellinidan species and strains results for each sample using Ward's Minimum variance method (Ward, 1963). Euclidean distance was employed in this method (after Fishbein and Patterson, 1993). R-mode cluster analysis was used to group species that were closely related to each other. Q-mode and

R-mode cluster analysis were performed on 25 arcellinidan species and strains preserved in the 30 sediment-water interface samples that were deemed to have statistically significant Arcellinida counts. Using the heatmap function of the Stats package in RStudio (R Team, 2020), the results of Q- and R-modes clustering are displayed as a two-way heat map. Non-metric multidimensional scaling (NMDS; Kruskal, 1964) was carried out to compare the similarity between assemblages in multiple dimensions.

4.1.4.4. Redundancy analysis. Redundancy analysis (RDA; Van Den Wollenberg, 1977) was used to quantify the relationship between the samples, the identified assemblages, and possible controls identified as Fe, Sr, Ca, As, TOC, and water depth. A Hellinger transformation was used to satisfy the assumption of linearity used in RDA (Rao, 1995). Variance partitioning was then performed on partial RDA results to determine the proportion of the variance explained by the identified controls over the distribution of Arcellinida. Controls with p-value <0.05 were deemed to be significant driver of the faunal distribution.

5. Results

5.1. Cluster analysis

Q-mode cluster analysis was carried out on 25 arcellinidan species and strains preserved in 30 sediment-water interface. Three unique Arcellinidan assemblages were identified with their Approximately Unbiased (AU) p-value : (1) "Elevated Arsenic Assemblage (EAA; AU p-value = 97 %; n = 9)" ; (2) "Centropyxid-Dominated Assemblage (CDA; AU p-value = 87 %; n = 9)"; and (3) "Difflugid-Dominated Assemblage (DDA; AU p-value = 95 %; n = 12)" (Fig. 2). Assemblages were named based on a distinct feature of their assemblage such as a dominant species or a controlling variable.

5.2. Non-metric multidimensional scaling

Results from the NMDS analysis corroborated the findings of the cluster analysis; sample assemblage groupings were similar in each approach (Fig. 3). The NMDS biplot shows that there is no overlap between the EAA assemblage and the other two assemblages, but a slight overlap between the CDA and DDA assemblages (Fig. 3). Sample L76C was an outlier in the NMDS analysis (Fig. 3).

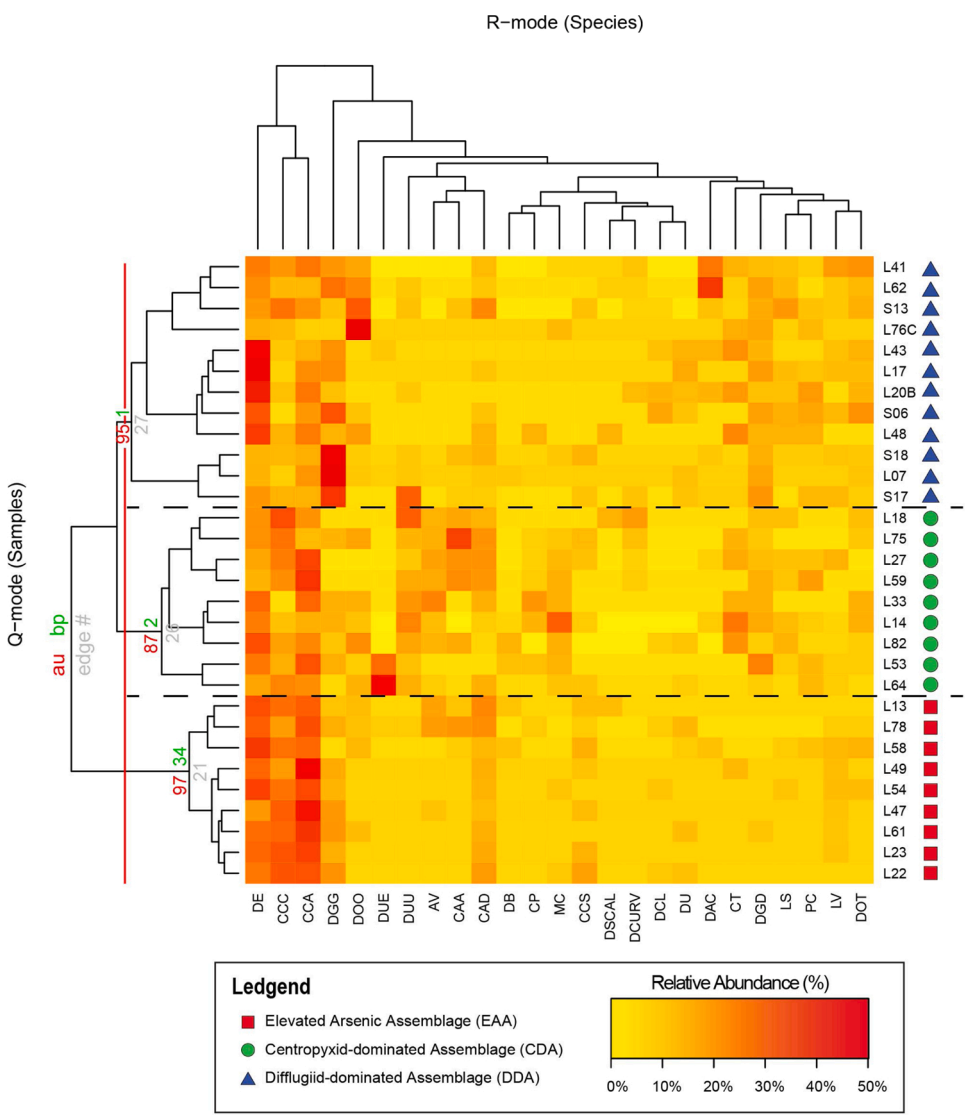


Fig. 2. Heat map of combined Q-mode and R-mode cluster dendrogram for 30 samples and 25 species and strains within three distinct faunal assemblages.

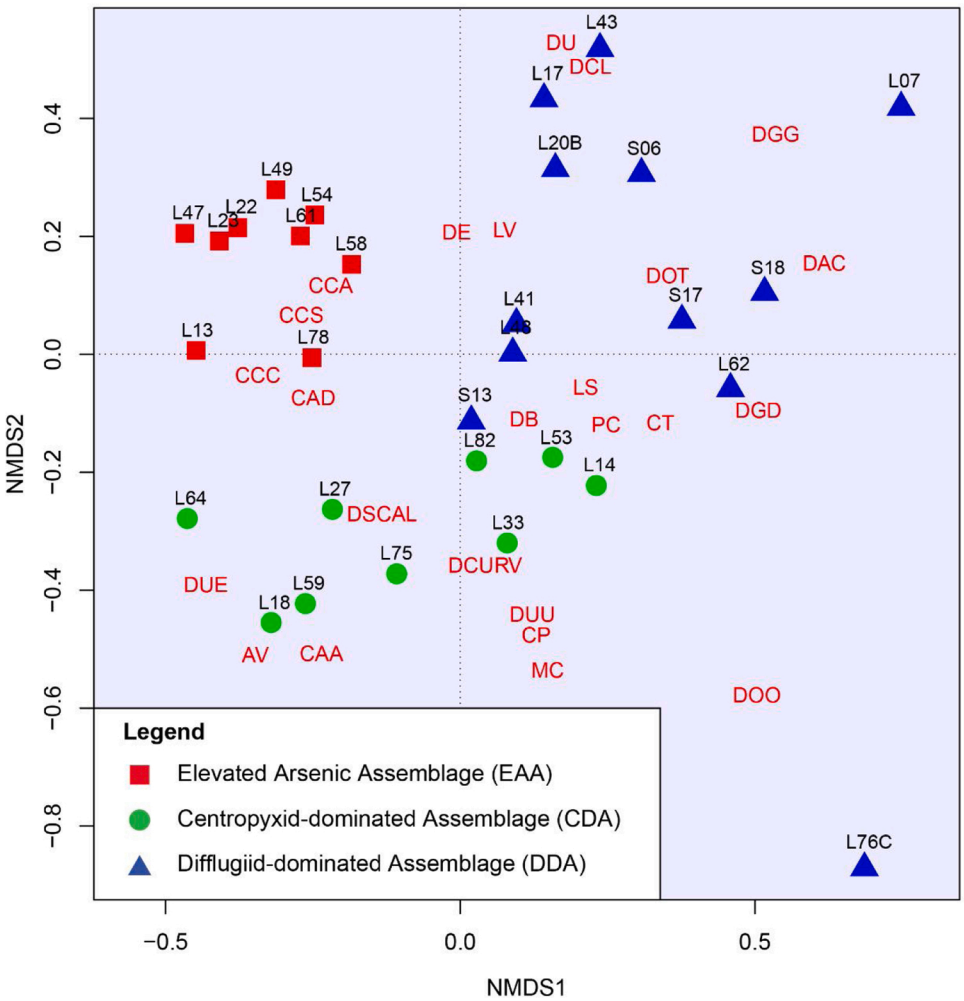
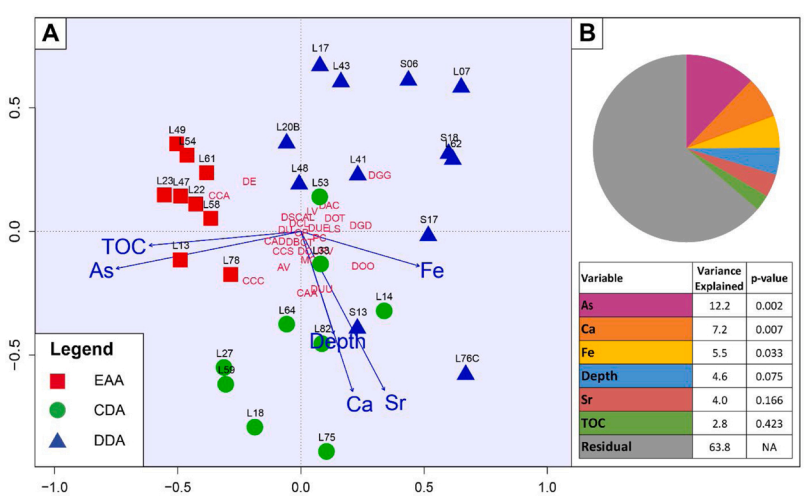


Fig. 3. Non-metric Multidimensional Scaling (NMDS) bi-plot. AV – *Arcella vulgaris,*", CAA – *Centropyxis aculeata* "aculeata", CAD – *Centropyxis aculeata* "discoides", CCA – *Centropyxis constricta* "aerophila", CCC – *Centropyxis constricta* "constricta", CCS – *Centropyxis constricta* "spinosa", CP – *Conicocassis pontigulasiformis*, CT – *Cucurbitella tricuspis*, MC – *Mediolus corona*, DB – *Difflugia bidens*, DGG – *Difflugia glans* "glans", DGD – *Difflugia glans* "distenda", DOO – *Difflugia oblonga* "oblonga", DOT – *Difflugia oblonga* "tenuis", DE – *Difflugia elegans*, DAC – *Difflugia protaeiformis* "acuminata", DCL – *Difflugia protaeiformis* "claviformis", DCURV – *Difflugia "curvicaulis*", DSCAL – *Difflugia protaeiformis* "scalpellum, DU – *Difflugia urens*, DUU – *Difflugia urceolata* "urceolata", DUE - *Difflugia urceolata* "elongata", PC – *Pontigulasia compressa*, LS – *Lesquereusia spiralis*, LV – *Lagenodifflugia vas*.

5.3. Redundancy analysis and partial redundancy analysis

Redundancy analysis (Fig. 4) grouped samples into three distinct assemblages that are similar to the groups observed with the NMDS and cluster analyses. Variance partitioning confirmed that there were five key controls over the distribution of Arcellinida that explained 36.2 % of the total variance; As (12.2 %; p-value = 0.002), Ca (7.2 %; p-value = 0.007), Fe (5.5 %; p-value = 0.033), Sr (4%; p-value = 0.166), water depth (4.6 %; p-value = 0.075), and TOC (2.8 %; p-value = 0.423; Fig. 4B). Variance partitioning also revealed that three out of the six variables analyzed (As, Ca, and Fe) were statistically significant ($p < 0.05$). In contrast, the statistical significance of Depth (p-value = 0.07), Sr (p-value =0.16), and TOC is low (p-value = 0.42). Of the variables analyzed, As (12.1 %) had the highest proportion of variance explained and TOC (2.8 %) the least (Fig. 4B).



Variable	Variance Explained	p-value
As	12.2	0.002
Ca	7.2	0.007
Fe	5.5	0.033
Depth	4.6	0.075
Sr	4.0	0.166
TOC	2.8	0.423
Residual	63.8	NA

Fig. 4. A. Redundancy analysis (RDA) tri-plot of 30 sediment-water interface samples, 25 Arcellinidan species and strains, and six environment variables. As – arsenic, Ca – calcium, Fe – iron, depth – water depth, Sr – strontium, and TOC – total organic carbon. AV – *Arcella vulgaris,*", CAA – *Centropyxis aculeata* "aculeata", CAD – *Centropyxis aculeata* "discoides", CCA – *Centropyxis constricta* "aerophila", CCC – *Centropyxis constricta* "constricta", CCS – *Centropyxis pontigulasiformis*, CT – *Cucurbitella tricuspis*, MC – *Mediolus corona*, DB – *Difflugia bidens*, DGG – *Difflugia glans* "glans", DGD – *Difflugia glans* "distenda", DOO – *Difflugia oblonga* "oblonga", DOT – *Difflugia oblonga* "tenuis", DE – *Difflugia elegans*, DAC – *Difflugia protaeiformis* "acuminata", DCL – *Difflugia protaeiformis* "claviformis", DCURV – *Difflugia "curvicaulis*", DSCAL – *Difflugia protaeiformis* "scalpellum, DU – *Difflugia urens*, DUU – *Difflugia urceolata* "urceolata", DUE - *Difflugia urceolata* "elongata", PC – *Pontigulasia compressa*, LS – *Lesquereusia spiralis*, LV – *Lagenodifflugia vas*. B. Variance partitioning test results including percentage variance in the arcellinidan data explained by environment variables and the *p*-values.

5.4. Arcellinida assemblages

5.4.1. Assemblage 1 – elevated arsenic assemblage (EAA; n = 9)

The faunal composition of the Elevated Arsenic Assemblage (EAA) was dominated by *Centropyxis constricta* (Ehrenberg, 1843) strain "aerophila" (median relative abundance = 31.8 %; range = 20.9–60.6 %), *Difflugia elegans* Penard, 1890 (median relative abundance = 27.4 %; range = 7.4–39.9 %) and *Centropyxis constricta* strain (Ehrenberg, 1843) "constricta" (median relative abundance = 17.8 %; range = 6.7–29.2 %). *Centropyxis aculeata* Ehrenberg, 1832 strain "discoides" (median relative abundance = 2.8 %; range = 1.3–11.2 %), *Difflugia glans* strain Penard, 1902 "glans" (median relative abundance = 3.1 %; range = 0–8.9 %) and *Centropyxis constricta* strain (Ehrenberg, 1843) "spinosa" (median relative abundance = 1.1 %; range = 0–3.3 %) were also common in this assemblage. The calculated SDI for the assemblage (median SDI = 1.6; range = 1.2–2.1) is reflective of stressed to transitional environmental conditions (Patterson and Kumar, 2002).

The EAA was found in shallow lakes with a median water depth of 1 m in organic-rich substrates (median TOC = 76 %; range = 37–85 %). The ICP-MS results revealed that all samples analyzed exceeded the interim sediment quality guidelines for As (ISQG =5.9 mg/kg), while 96 % of the samples had As concentrations beyond the probable effect levels guideline (PEL =17 mg/kg; Canadian Council of Ministers of the Environment (CCME, 2002). Samples hosting the EAA assemblage are characterized by the highest As concentrations (median As =272.4 mg/kg; range = 142.1–1353 mg/kg).

The NMDS results revealed that samples hosting the EAA closely grouped together and close to stress-indicating Arcellinida taxa (e.g., *C. constricta* strain "aerophila"; C. *constricta* strain "spinosa"; *C. constricta* strain "constricta"; *C. aculeata* strain "discoides" Fig. 3). The results of the RDA analysis revealed that EAA is positively associated with As and organic content, and negatively with Fe along the first RDA axis (Fig. 4A).

5.4.2. Assemblage 2 – centropyxid-dominated assemblage (CDA; n = 9)

The Centropyxid-Dominate Assemblage (CDA) was dominated by *C. constricta* strain "aerophila" (median relative abundance = 11 %; range = 2.8–40.9 %), *C. constricta* strain "constricta" (median relative abundance = 10.1 %; range = 6.7–60.6 %), and *D. elegans* (median relative abundance = 10 %; range = 3.1–24 %). Other common species include *Centropyxis aculeata* strain "aculeata" (median relative abundance = 5.4 %; range = 0–31.5 %), *Difflugia urceolata* (Carter, 1856) strain "urceolata" (median relative abundance = 5%; range = 1.3–23.2 %), *Mediolus corona* (Wallich, 1864) (median relative abundance = 3.6 %; range = 0–19.5 %), *C. aculeata* strain "discoides" (median relative abundance = 3.6 %; range = 0–9.2 %) and *Arcella vulgaris* Ehrenberg, 1830 (median relative abundance = 4.1 %; range = 0–12.5 %). The calculated SDI (median SDI = 2.2; range = 1.6–2.5) is reflective of environments transitioning towards less stressed conditions (Patterson and Kumar, 2002).

Compared to the other assemblages, samples of the CDA were collected from slightly deeper sample sites (median water depth =1.9 m) that were characterized by high organic content (median TOC = 65 %; range = 38–82 %) and relatively high proportions of minerogenic material (median minerogenic material = 32 %; range = 2–59 %) compared to EAA (median minerogenic material = 22 %; range = 10–34 %). The geochemical results revealed that the CDA had the second highest As concentrations (median As =169.7 mg/kg; range = 60.4–233.6 %). The results also show that the CDA samples have the highest median Sr concentrations of all samples (46.9 mg/kg; range = 33.2–150.4 mg/kg).

The results of NMDS analysis showed CDA samples clustering closely together, with some overlap with the Difflugid-Dominate Assemblage (Fig. 3). The NMDS bi-plot also shows a close association between samples of CDA and *D. urceolata* strain "elongata", *Arcella vulgaris*, *C. aculeata* strain "aculeata", *Difflugia protaeiformis* Lamarck, 1816 strain "scalpellum", *Difflugia curvicaulis* Penard, 1899, and to some extent *D. urceolata* strain "urceolata", *Conicocassis pontigulasiformis* (Beyens et al., 1986), and *Difflugia bidens* Penard, 1902. The RDA results show a positive association between CDA samples, Ca, Sr, and water depth along the second RDA axis (Fig. 4A).

5.4.3. Assemblage 3 – difflugid-dominated assemblage (DDA; n = 12)

Species in the Difflugid Dominated Assemblage (DDA) were primarily characterized by *D. elegans* (median relative abundance = 11.8 %; range = 1.8–63.5 %) and *D. glans* strain "glans" (median relative abundance = 10 %; range = 0–84.7 %). Other common species include *C. constricta* strain "aerophila" (median relative abundance = 7.7 %; range = 0–16 %), *Difflugia glans* Penard, 1902 strain "distenda" (median relative abundance = 4.4 %; range = 1.5–8.2 %). The calculated SDI (median SDI = 1.8; range = 0.6–2.5) is indicative of stressed systems that are transitioning towards healthier environmental conditions (Patterson and Kumar, 2002).

Samples of DDA were collected from shallow lakes with a median water depth of 1.5 m. The organic content of the substrates hosting DDA was still elevated (median TOC = 53 %; range = 10–74 %) but was lower than the proportions seen in the samples of EAA and CDA. In contrast, the minerogenic content for the DDA increased compared to the other assemblages (median minerogenic material = 43 %; range = 0–88 %). Of the three assemblages, DDA was found to have the lowest As concentrations (median As =61.3 mg/kg; range = 16.1–316.6 %). However, the relatively low concentrations of the DDA were still well above the acceptable guidelines (CMME, 2002). The geochemical results also revealed that the DDA samples have the highest concentration of Ca with a median of 12,850 mg/kg.

The NMDS results revealed that samples of the DDA grouped together but were more spread out compared to the samples of the EAA and CDA (Fig. 3). Samples of the DDA grouped with species *D. urens, D. claviformis, D. glans* strain "glans", *D. acuminata, D. oblonga* strain "tenuis", *Lagenodifflugia vas* (Leidy, 1874), and *D. elegans.* The RDA results showed an association between DDA and Fe and a negative association with As and organic content along the first RDA axis (Fig. 4A).

6. Discussion

6.1. Inter-lake As distribution

Arsenic concentrations in the near-surface lake sediments ranged from 16.1 to 1353 mg/kg (n = 30). Except for two samples (S06 and S18), these concentrations are above the lacustrine sedimentary background level for As for the Yellowknife area (20–30 mg/kg; Galloway et al., 2018). Such elevated As concentrations are expected given the downwind position of the GTRC property from the Giant Mine site within a zone of immediate influence 10–17 km from the historic roaster at the former mine (Galloway et al., 2018; Nasser et al., 2020b; Palmer et al., 2015). Weathering of As from bedrock and derived surficial materials on the GTRC property may have also contributed As to lakes within the study area. However, a detailed study of the primary and secondary As mineral hosts, as well as the prevalent As species in the sediments of the sampled lakes, will be a necessary next step to confirm the relative contributions of geogenic and anthropogenic As in these lakes.

The spatial distribution of As in the lakes analyzed for this study was characterized by a subtle, yet notable, spatial pattern that cannot be fully attributed to the atmospheric deposition of As from the Giant Mine roaster stack. The highest As concentrations (up to 1353 mg/kg) are documented in the near-surface sediments of lakes located close to the centre of the study area and declined in lakes that are located further away from the centre (Fig. 1A). The limited spatial coverage due to the relatively low number of samples (n = 30) used in this study may have contributed to the subtlety of the spatial pattern. However, the Arcellinida assemblages show a clear response to this As spatial pattern in the form of a biotic transition from stressed to healthier assemblages away

from the centre of the study area (Fig. 1B). This is an important finding as it demonstrates the sensitivity of Arcellinida to the inter-lake spatial variability in As concentrations. Interestingly, the Spearman Rank Correlation results show a strong positive association between TOC and As (R_s = 0.6; *p*-value = 0.0004), as well as TOC and stress-indicating Arcellinida taxa like *C. constricta* strains "aerophila" (R_s = 0.4; *p*-value = 0.02) and "constricta" (R_s = 0.43; *p*-value = 0.01; Supplementary Fig. 2). The inter-relationship between As and TOC in lake sediments has previously been reported in several studies (Galloway et al., 2018; Gough et al., 2008; Kelly and Tate, 1998; Nasser et al., 2020b; Valsecchi et al., 1995). The organic content of sediments, particularly labile organic matter, can provide a substrate suitable for bacterial-mediated incorporation of As in sulfide minerals (Galloway et al., 2018). Recently, Arcellinida communities have also been shown to respond to changes in both As and labile organic matter fraction, represented by the Rock Evaluation Pyrolysis S1-carbon, in lake sediments in the Yellowknife area (Nasser et al., 2020b). While the different types of organic matter were not investigated in this study, it is possible that the distribution of the labile fraction of organic carbon is partly responsible for the spatial pattern of As and Arcellinida observed in the study area.

6.2. Arcellinida distribution controls

6.2.1. Arsenic and TOC

The results of the RDA and variance partitioning analysis carried out here isolated six key drivers that influenced the faunal structure of the Arcellinida assemblages. Arsenic is the most significant control, explaining 12.2 % (*p*-value = 0.002) of the observed variance. A portion of the explained As variance (5.4 %) is shared with the organic content, which has the smallest proportion of variance of the variables studied (variance explained = 2.8 %; *p*-value = 0.423; Fig. 4B). The species comprising the EAA were dominated by members of the genus *Centropyxis* (e.g., *C. constricta* "aerophila", *C. constricta* "constricta"), as well as *D. elegans*. Centropyxids are opportunistic and have a high tolerance for environmental stressors (Kumar and Patterson, 2000; Patterson et al., 1996), including As contamination (Gavel et al., 2018; Nasser et al., 2016, 2020a, 2020b; Patterson et al., 1996; Reinhardt et al., 1998). *Difflugia elegans* has been reported to thrive in substrates impacted by high As concentrations (Nasser et al., 2016, 2020b; Reinhardt et al., 1998). Nasser et al. (2020b) quantified the As stress tolerance of various arcellinidan taxa and determined that of 25 species and strains evaluated, *C. constricta* "constricta" and *D. elegans* had very high tolerances to high As concentration (As tolerance > 760 mg/kg). The distribution of arcellinidan taxa in the EAA assemblage corroborate these previous findings and show that individual taxa may be used to assess elevated As concentrations in lake sediments. Additionally, the decline in the numbers of centropyxid species and strains and concurrent increase in the abundance of more stress-sensitive taxa in the CDA and DDA demonstrates the sensitivity of the group to changes in As concentrations in lake sediments.

The RDA tri-plot shows As and TOC along RDA axis 1 closely associated with the EAA samples; this assemblage thus characterizes lakes where As concentrations and TOC were the highest (median As =272.4 mg/kg; median TOC = 76 %; *n* = 9; Fig. 4A). The close association between As and TOC in the tri-plot is also reflected by the strong positive correlation between these variables (R_s = 0.6; *p*-value = 0.0004; Supplementary Table 2). Arcellinidan communities are dependent on the abundance of their food sources; bacteria, algae and fungi (Nikolaev et al., 2005). The labile fraction of organic matter can serve as a habitat that can sustain healthy microbial communities (Sanei et al., 2005). Such habitat, however, can also mediate the authigenic precipitation of As, High As concentrations in sediments, pore waters, and surface waters may also inhibit basic cellular functions related to energy metabolism, enzyme activities and basal respiration in most bacteria (Baath, 1989; Walker et al., 2000). Thus, elevated As can cause a general reduction in microbial biomass in lacustrine environments (Gough and Stahl, 2011). A reduction in food sources could directly impact arcellinidan communities, particularly the relative abundance of nutrient-sensitive taxa such as *C. tricuspis* and Difflugiids (Nasser et al., 2020b).

6.2.2. Iron

Iron explained 5.5 % (*p*-value = 0.033) of the observed variance in the RDA analysis (Fig. 4B), plotted positively along Axis 1 on the RDA plot, and was mostly associated with the samples of CDA and DDA assemblage samples (Fig. 4A). This is not a surprising result as the median Fe concentrations for CDA (median Fe =11,100 mg/kg; *n* = 9) and DDA (median Fe =10,200 mg/kg; *n* = 12) are relatively similar and higher than that of the EAA (median Fe =5400 mg/kg; *n* = 9). While commonly associated with changes in redox conditions in lakes, Fe is a lithogenic element that has been linked to sediment influx into lakes via surface runoff (Kylander et al., 2011; Martín-Puertas et al., 2011; Evans et al., 2019). The spatial distribution of arcellinidan communities are sensitive to changes in the sedimentary composition of lake substrates, with healthy and diverse assemblages thriving in more nutrient-rich, silt- to mud-dominated substrates, and stressed-assemblages inhabiting nutrient-poor sandy substrates (Dalby et al., 2000; Roe and Patterson, 2006; Steele et al., 2018). The relatively low to moderate diversity of the DDA (mean SDI = 1.6; SDI range = 0.6–2.5) reflects stressed to transitional environmental conditions that may be attributed to the abundance of sand-sized sediments dominating the substrates hosting the assemblage. However the relatively high abundance of stress-indicating Arcellinida species [e.g., *D. elegans* (median relative abundance = 11.8; *n* = 12), *C. constricta* "aerophila" (median relative abundance = 7.7 %; *n* = 12)] may reflect an additional As concentration influence on the DDA bearing samples.

6.2.3. Calcium, water depth, and strontium

Other important controls on the distribution of Arcellinida in these samples include Ca (variance explained = 7.2 %; *p*-value = 0.007) and water depth (variance explained = 4.6 %; *p*-value = 0.075; Fig. 4B). The RDA tri-plot shows both variables negatively co-varying close to Axis 2, and in an association with CDA, and various arcellinidan species and strains (e.g., *D. urceolata* strain "urceolata", *C. aculeata* strain "aculeata", and to a lesser extent *D. oblonga* "oblonga"; Fig. 4A). Several studies have demonstrated the sensitivity of Arcellinida to changes in lacustrine water depth (Patterson et al., 1996; Reinhardt et al., 1998; Davidova and Boycheva, 2015; Nasser et al., 2016; Tsyganov et al., 2019). Arcellinida assemblages have previously been demonstrated to be indirectly sensitive to water depth in some lake systems where thermoclines isolate deeper water areas, resulting in seasonally dysoxic and anoxic conditions (e.g., Patterson et al., 1985, 1996). However, the presence of thermoclines was not a factor in the lakes under examination here due to the relatively shallow water depths (water depth range = 0.5–4.8 m; *n* = 30) and well oxygenated conditions observed (Dissolved oxygen (%) range = 60.4–120.7 %; *n* = 30). However, in many lakes water depth has a significant impact on several environmental variables and therefore may exert an indirect control on Arcellinida taxa, particularly in shallow water lakes where catchment hydrology has a predominant influence. Changes in Ca concentrations in lake sediments have previously been linked to changes in lake productivity related to the activity of photosynthesizing phytoplankton, microbial decomposition of organic matter, and physical mixing of lake deposits, with elevated Ca concentrations indicative of enhanced productivity (Behbehani et al., 1987; Kelts and Hsü, 1978; Otsuki and Wetzel, 1974; Woszczyk, 2016). Interestingly, the CDA is also associated with *Cucurbitella tricuspis* on the RDA tri-plot (Fig. 4A). This is expected because the assemblage has the highest median proportion of *C. tricuspis* (median relative abundance = 3.5 %; *n* = 9) compared to the EAA (median relative abundance = 0 %; *n* = 9) and DDA assemblages (median relative abundance = 0.7 %; *n* = 12; Supplementary Table 1). Several studies have linked the abundance of *C. tricuspis* to organic rich substrates and eutrophic conditions (Collins et al., 1990; Patterson et al., 2013). The relationship between the CDA

assemblage, Ca, and *C. tricuspis* therefore likely reflects an increase in lake productivity.

The RDA tri-plot shows Sr plotting closely to Ca along the second axis (Fig. 4A). This close association between the two elements is expected given their chemical similarities, both belonging to the Alkali-Earth metals group. Additionally, Sr is often associated with Ca-bearing minerals in lacustrine catchments like plagioclase and hornblende (Ekwere, 1985; Xu et al., 2013), with plagioclase found in volcanic, volcaniclastic, and granitoid rocks in the study area (Jenner et al., 1981). Both Sr and Ca have autochthonous (i.e., precipitation of $CaCO_3$ and $SrCO_3$) as well as erosional sources (i.e., terrigenous input from catchment; Kylander et al., 2011; Davies et al., 2015). While the source of both elements was not assessed in this study, a portion of the Sr and Ca flux to the sediments is attributed to terrigenous inputs via surface runoff. This assessment is supported by the findings of studies within the Canadian Shield that have confirmed the significant influence enhanced spring freshet events exert on seasonal and annual geochemical influx into lakes (Spence et al., 2011; Spence and Phillips, 2015). Knowledge about the nature of the relationship between Arcellinida species and strains and Sr is currently lacking. However, the low statistical significance of Sr (p-value $= 0.166$), coupled with its position along the second RDA axis, suggests a weak influence of Sr on the arcellinidan distribution in the investigated lakes (Fig. 4B).

7. Conclusions

Thirty sediment-water interface samples were analyzed from 30 lakes within the mineral claims of the GTRC adjacent to the former Giant Mine in Yellowknife, NT, to test the applicability of using Arcellinida as a cost-effective reconnaissance tool for assessing bioavailable As contamination. Cluster analysis and NMDS analysis were used to identify three arcellinidan assemblages: 1) Elevated Arsenic Assemblage (EAA); 2) Centropyxid-Dominated Assemblage (CDA); and 3) Difflugiid-Dominated Assemblage (DDA). The results of an RDA and partial RDA analysis revealed six key variables (As, Ca, Fe, Sr, Depth and Organic Content) that collectively explained 36.2 % of the total variance in the spatial distribution of Arcellinida in the study lakes. Of these variables, As was most statistically significant explaining 12.2 % of the total faunal variance (p-value <0.002). Although Ca, Fe, water depth and organic concentration were contributors to the arcellinidan assemblage structure it was the spatial variability of As concentration between lakes that was the most important factor in defining EAA, CDA and DDA, with EAA being prominent in lakes near the centre of the study area. The results of this study confirm the applicability of using the group as a tool for monitoring changes in sedimentary As concentrations and lacustrine ecological health. As arcellinidan taxa are immobile post-depositionally, future arcellinidan analysis can be used to provide a robust and tested analytical tool to determine past sedimentary As concentrations and the magnitude of any post-depositional re-mobilization of this metalloid.

CRediT authorship contribution statement

Louise Riou: Methodology, Software, Writing - original draft. **Nawaf A. Nasser:** Methodology, Software, Writing - review & editing, Supervision. **R. Timothy Patterson:** Conceptualization, Supervision, Writing - review & editing. **Braden R.B. Gregory:** Methodology, Writing - review & editing. **Jennifer M. Galloway:** Writing - review & editing. **Hendrik Falck:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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