Use of Arcellinida (testate lobose amoebae) Arsenic tolerance limits as novel tool for biomonitoring Arsenic contamination in lakes

Use of Arcellinida (testate lobose amoebae) Arsenic tolerance limits as a novel tool for biomonitoring Arsenic contamination in lakes

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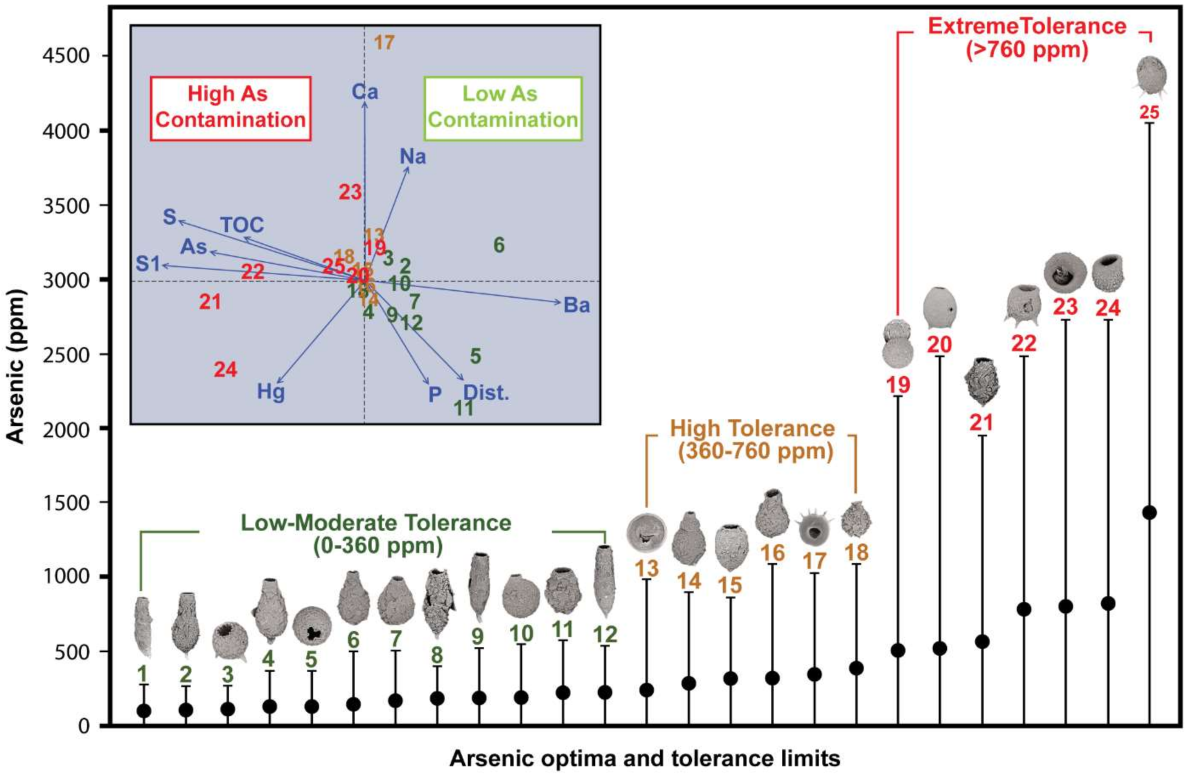
ABSTRACT GRAPHIC



ABSTRACT

Arsenic (As) contamination from legacy gold mining in subarctic Canada poses an ongoing threat to lake biota. With climatic warming expected to increase As bioavailability in lake waters, developing tools for monitoring As variability becomes essential. Arcellinida (testate lobose amoebae) is an established group of lacustrine bioindicators that are sensitive to changes in environmental conditions and lacustrine ecological health. In this study, As-tolerance of Arcellinida (testate lobose amoebae) in lake sediments (n = 93) in subarctic Northwest Territories, Canada was investigated. Arcellinida assemblage dynamics were compared with the intra-lake As distribution to delineate the geospatial extent of legacy As contamination related to the former Giant Mine (Yellowknife). Cluster analysis revealed five Arcellinida assemblages that correlate strongly with ten variables (variance explained = 40.4%), with As (9.4%) and S1-carbon (labile organic matter; 8.9%) being the most important (p-value = 0.001, n = 84). Stressed assemblages characterized proximal lakes <10 km from the former mine site, consistent with a recently identified, geochemically-based zone of high As impact. The weighted average tolerance and optima (WATO) analysis led to identification of three arcellinidan groups based on the As-sensitivity: Low-Moderate Tolerance Group (As=0–350 ppm); High Tolerance Group (As=350–760 ppm); and, Extreme Tolerance Group (As>750 ppm). The predictive capability of the Low-Moderate and Extreme tolerance groups is particularly strong, correlating with As concentrations in 66.6% (n = 20/30) of a test dataset. We propose that As influences the spatial distribution of the more nutrient-sensitive Arcellinida taxa (e.g., *Cucurbitella tricuspis* and *Difflugia oblonga* strain "oblonga") through suppression of preferred microbial food sources. These findings, which indicate that there is a variable species-level arcellinidan response to As contamination, showcases the potential of using the group as a reliable tool for inferring

historical variability in As concentrations in impacted lakes, not possible using As itself due to the redox driven sensitivity of the metalloid to post-depositional remobilization. Arcellinida can also provide insight into the overall impact of As contamination on the ecological health of lakes, a metric not readily captured using instrumental analyses. Lakes with As-stressed arcellinidan faunas and high As concentrations may then be targeted for further As speciation analysis to provide additional information for risk assessment.

Key words: Arsenic contamination, subarctic, gold mining, lake sediments, Arcellinida, As tolerance limits

1. INTRODUCTION

Arsenic (As) is a ubiquitous metal(loid), averaging 5 mg/kg^{-1} in the Earth's crust (USDHHS, 2007). While As has various industrial applications (Wang and Mulligan, 2006), it is also globally recognized as an element of environmental concern and is often linked to several ecological and human health hazards (Caussy and Priest, 2008). Due to the mineralization of gold with As-rich sulfides, gold mining mineral processing activities are a primary anthropogenic source of As in lake sediments and waters in mining districts worldwide (e.g., Borba et al., 2003; Oyarzun et al., 2004; Palmer et al., 2015; Galloway et al., 2017). Contamination of lake sediments and waters by As is of particular concern due to the substantial recreational, ecological, and traditional values of lakes. Lake sediments can serve as a repository for As that can be liberated to the overlying water column under certain environmental conditions (e.g. seasonably variable redox conditions; Martin and Pedersen, 2002; Palmer et al., 2019).

The latent risk of gold mining-associated As contamination in lakes has been the impetus behind numerous studies focused on characterizing As in lake sediments and waters (Azcue et al., 1994; Bright et al., 1996; Andrade et al., 2010; Palmer et al., 2015; Galloway et al., 2015, 2017). Several instrumental techniques, like Instrumental Neutron Activation Analysis (e.g. Salzsauler et al., 2005), Inductively Coupled Plasma (ICP) - Atomic Emission Spectrometry (e.g., Ryu et al., 2002), and ICP - Mass Spectrometry (e.g., Galloway et al., 2017), are routinely used as means to quantify the spatio-temporal variability of total As concentrations in sediments of impacted lakes. However, results generated by such methods do not provide information on the ecological response of lacustrine ecosystem to As contamination. Additionally, several studies have highlighted limitations associated with utilizing elemental concentration profiles of redox-sensitive elements such as As, due to the potential of post-depositional remobilization

(Couture et al., 2008; Andrade et al., 2010; Schu et al., 2017). The long-term stability of As in lake systems is dependent on its interaction with Fe-, Mn-, and Al-(oxy)hydroxides, organic matter (OM), and sulfides, which in turn are mediated by factors like seasonal ice cover, pH, redox condition, and biotic functions (e.g. microbial activity; Toevs et al., 2006; Du Ling et al., 2009). Limnological drivers can remobilize sedimentary As, and due to associated seasonal changes in redox conditions, can result in cycling between the highly toxic inorganic species (e.g. As^{+3} and As^{+5}) and compounds of As (e.g. arsenic trioxide (As_2O_3), arsenite (AsO_3^{3-}), and arsenate (AsO_4^{3-}); Martin and Pedersen, 2002; Palmer et al., 2019). Climate variability has also been shown to have a profound impact on the stability of physical, chemical and biological properties of lake systems (Rosenzweig et al., 2007). Computer model-predicted climate warming and associated changes in redox conditions may lead to the release of As from sediments into overlying surface waters in impacted lakes. There is thus a need to better understand the long-term spatio-temporal variability of As in lake ecosystems to better predict future geochemical trajectories and the potential impact on biota.

Benthic meiofaunal communities are sensitive to environmental change in lakes. The subfossil remains of such communities can be used to document the impact of contamination on lacustrine ecosystems through time (e.g., Dixit et al., 1989; Cattaneo et al., 2004). Arcellinida (i.e. testate lobose amoebae) are well established freshwater benthic bioindicators and are routinely used to provide insights into both sediment character and the general ecological health of lakes (Patterson and Kumar, 2002). This cosmopolitan group of shelled protists is found across a wide geographical range that extends from the tropics to the poles (Beyens and Chardz, 1995; Dalby et al., 2000) living in fresh and brackish aquatic systems (Charman, 2001; Patterson and Kumar, 2002; van Hengstum et al., 2008). The importance of Arcellinida as bioindicators is mainly attributed to their: 1) reproduction (1–11 days) that enables rapid community response to ecological change (Medioli and Scott, 1983); 2) high preservation potential owing to their decay-resistant tests (shells); and, 3) sensitivity to a wide range of environmental parameters (e.g., Kumar and Patterson, 2000; Neville et al., 2011; Patterson et al., 2013; Roe et al., 2010; Prentice et al., 2017). Such attributes offer a high degree of resolution of environmental interpretation, which is imperative for monitoring the ecological health of lakes impacted by anthropogenic contamination (Neville et al., 2011; Patterson et al., 2013). Several recent studies have applied statistical techniques to evaluate the response of Arcellinida to mine-induced contamination (e.g., Kumar and Patterson, 2000; Kihlman and Kauppila, 2012), but only a few have considered the impact of As contamination on assemblage composition (Patterson et al., Reinhardt et al., 1998; Nasser et al., 2016; Patterson et al. 2019). In 2016, As was identified as a significant control on arcellinidan distribution in lakes differentially impacted by As contamination associated with legacy gold mining operations and mineral processing at the Giant Mine site (1948-1999) in the Yellowknife area, Northwest Territories, Canada (Nasser et al., 2016; **Figure 1**). The findings of this proof-of-concept study provided new insight into the sensitivity of Arcellinida to As contamination and identified the potential of using the group as a tool for monitoring changes in As concentrations and ecological health in impacted lakes.

This study aims to further develop Arcellinida as a tool for biomonitoring variability in As concentrations and lacustrine ecological health by determining the tolerance limits of various taxa to varying As concentrations to identify specific As-indicator taxa or assemblages. The study represents the largest inter-lake assessment of the influence of mine-induced As contamination on Arcellinida assemblage dynamics in lake systems under take to date, as well as the first attempt to quantify the species-level response of Arcellinida to As concentration variability. A secondary objective of this study is to assess whether As-controlled arcellinidan assemblage dynamics vary as a function of distance from Giant Mine, likely due to downwind roaster stack-derived As aerial fallout. To achieve these objectives, the spatial distribution of Arcellinida in 93

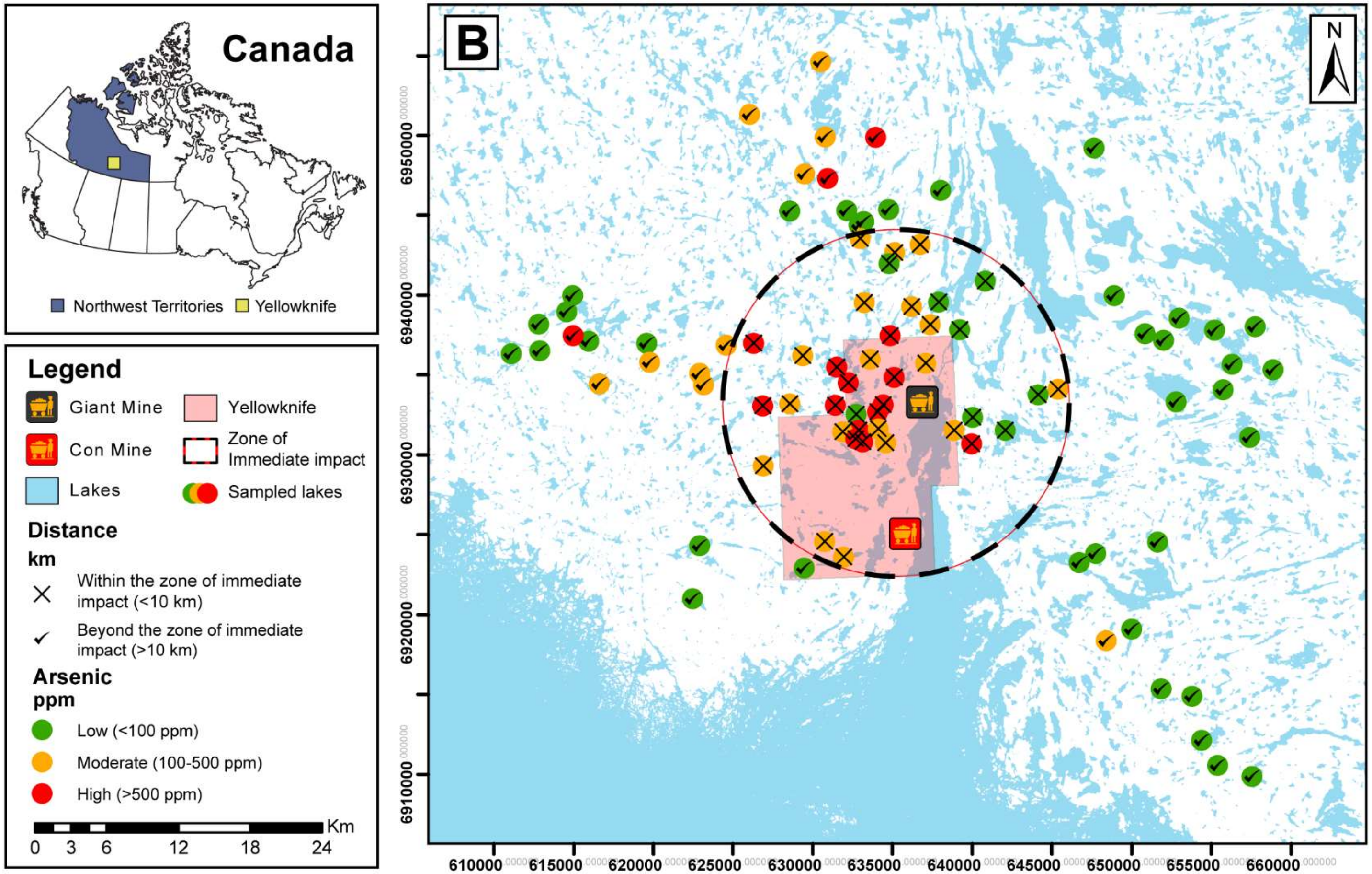


Figure 1. Map of sampling sites showing the locations of the 93 near-surface sediment samples examined in the study (colored circles). The color-coding of the circles reflects the spatial distribution of As in the Yellowknife area, which is divided into three categories: high (red circles), moderate (yellow circles), and low (green circles). The dashed circle represents the outer limits of the Giant Mine zone of immediate airborne As contamination impact.

sediment-water interface samples from 90 lakes within a radius of ~30 km around the Giant Mine site was examined. The publication of several recent studies on the regional distribution of As in lake waters (Palmer et al., 2015) and sediments (Galloway et al., 2012, 2015) in the Yellowknife area has provided valuable information about the geospatial extent of the As contamination zone of impact. Palmer et al. (2015) delineated the limit of the zone of significant aerial As fallout to be ~17 km away from the historic roaster stack at Giant Mine, based on an assessment of the As concentration in 98 lake surface water samples. In an investigation of As levels in near-surface sediment samples, Galloway et al. (2017) identified a similar zone of influence as well as a zone of immediate influence within a radius of 11 km around Giant Mine. The dataset (n=93) used in this study is a subset of the data used by Galloway et al. (2017; n = 105) and is thus directly comparable to that research.

2. Study Area

The study was carried out in lakes within a radius of ~30 km around the Giant Mine, a former gold mine located ~5 km northeast of the city of Yellowknife **(Figure 1)**. Detailed information pertaining to the history of gold mining in the Yellowknife area is provided in **Supplementary Document 1**.The study area is characterized by a gradual change in elevation, from 157 m above mean sea level (MASL) close to Great Slave Lake to 350-400 m above MSAL to the north of Thistlethwaite Lake (Kerr and Wilson, 2000). The primary drainage in the river catchment is via the Yellowknife River, which flows southward into Yellowknife Bay, Great Slave Lake. Yellowknife has a subarctic, continental climate characterized by short, dry, cool summers with a mean annual temperature of −4.3° C and a mean annual precipitation of 170.7 mm

(Environmental Canada, 2019). The wind direction is variable throughout the year but blows primarily from the east and south (Pinard et al., 2007).

Lakes investigated in this study are underlain by rocks assigned to the Yellowknife Supergroup of the southern Slave structural province of the Canadian Shield. These include Archean metavolcanic and metasedimentary rocks intruded by younger granitoids and diabase dykes (Yamashita et al., 1999; Cousens, 2000). The most prevalent surficial sediments in the study region are fine clastic lacustrine sediments from Glacial Lake McConnell and glacigenic sediments that form a thick (<2 m) discontinuous veneer (Kerr and Wilson, 2000). Accumulations of Holocene-aged peat also occur in the study region and can be greater than 1 m thick in bogs and wetlands (Kerr and Wilson, 2000).

3. MATERIALS AND METHODS

3.1 Field methods

A total of 93 surface sediment samples (upper ~ 1 cm) were collected from 90 lakes around the sites of Giant and Con Mines in 2012 (sample ID: B12; *n* = 61) and 2014 (sample ID: Y14; *n* = 32**; Figure 1)**. Lakes located within a radius of 30 km from the mines were targeted to ensure coverage of areas beyond the Airborne As fallout zones of impact (~17km) and immediate impact (~11 km) proposed by Palmer et al. (2015) and Galloway et al. (2017). Lakes were accessed via a pontoon-equipped Bell Long Ranger helicopter. Surface sediment samples were collected by Ekman Grab approximately 1 cm of sediment from the top of each grab, where Arcellinida populations are often abundant, was retained using an inert plastic laboratory spoon for arcellinidan, sedimentological and geochemical analyses. The location of each sampling station was recorded by Global Positioning System (GPS; accuracy ± 3 m) (Galloway et al., 2015, 2017). The water sampling depth at each station was determined using a HONDEX Honda portable handheld depth sounder (model: PS-7; optimal depth range: 0.6–80 m; beam angle: 24°; Galloway et al., 2015, 2017). Where possible, muddy substrates from the middle of each lake were selected for sampling because arcellinidan populations are typically reduced on nutrient-poor silt to sand substrates (Patterson and Kumar, 2002). Water property data (pH, water temperature, dissolved oxygen and conductivity) was collected from each sample site using a YSI Professional Plus handheld multi-parameter unit with quatro-cable (Galloway et al., 2015, 2017).

3.2 Laboratory methods

Samples used in this study were subsampled and analyzed for element and organic geochemical, sedimentological, and micropaleontological analysis. Elemental concentrations of the sediment subsamples were analyzed using ICP-MS following *aqua regia* digestion (ICP-MS 1F/AQ250 package) at Bureau Veritas, Vancouver **(Supplementary Table 1)**. *Aqua regia* digestion was used instead of complete digestion as the former provides the total concentration of metal(loids) that could potentially become bioavailable, while the latter can volatilize As (Parsons et al., 2012). Analytical precision was assessed using three Pulp duplicates. Calculated Relative Percent Difference (RPD) was less than 5% for As (RPD range = 1.47%-4.31%). Analytical accuracy was assessed using three standard reference materials: 1) STD DS9 (n = 9); 2) STD D10 (n = 2); and, 3) STD OREAS45EA (n = 11). Mean As concentration measured in STD DS9 was 27.4 ppm ± 1.42 (n = 9) compared to an expected *aqua regia* concentration of 25.5 ppm (mean RPD = 7.806% ± 3.95). Mean As concentration measured in STD DS10 was 45.6 ppm ± 0.1 (n = 2) compared to an expected *aqua regia* concentration of 46.2 ppm (mean

RPD = 1.307% ± 0.3101). Mean measured As concentration for STD OREAS45EA was 9.7 ppm ± 1.16 (n = 11) compared to an expected *aqua regia* As concentration of 10.3 ppm (mean RPD = 11.1% ± .7.27). Analyzing eleven laboratory methods blanks resulted in detecting As in only two blanks (detected As concentrations = 0.2 ppm and 0.1 ppm).

Particle size analysis (PSA) was performed on the sediment subsamples to recognize sedimentological patterns across the study area that may influence the distribution of Arcellinida and element concentrations. Subsamples were prepared for PSA by digesting subsamples in a heated bath (70 C°) with 10% HCl and 30% H_2O_2 to remove carbonate and organic content, respectively (Murray 2002; van Hengstum et al., 2007). Following digestion, sedimentary grain size in each subsample was analyzed using a Beckman Coulter LS13 320 laser diffraction analyzer fitted with a universal liquid medium (ULM) sample chamber over a measurement range between 0.4 and 2000lm. Samples were loaded into the instrument until an obscuration level of 10±3% was attained. GRADISTAT (Version 8; Blott and Pye, 2001) was used to compile the results **(Supplementary Table 1)**. Garnet15 (mean diameter 15 µm: ± 2 µm), an accuracy standard supplied by Beckman Coulter, was run once per month. An in-house mud sample (Cushendun Mud; mean diameter = 20.5 µm: ± 0.76 µm) was run at the start of every session as a precision control.

Sediment subsamples were also analyzed for organic matter content using the Rock-Eval® 6 instrument at the Geological Survey of Canada, Calgary. Rock-Eval® 6 Analysis uses heat to break down large organic matter molecules to smaller and chemically more identifiable molecules (Lafargue et al., 1998). Quantitative measurements of total organic carbon (TOC) and other organic geochemical variables, including S1 carbon, S2 carbon, and S3 carbon were produced **(Supplementary Table 1).** S1-carbon represents the quantity of free hydrocarbon in sediments (mg hydrocarbons/g) that is devolatilized during pyrolysis at 300 ˚C. In sediment-water interface sample, S1 mainly consists of readily degradable geolipids and pigments predominantly derived from autochthonous organic matter such as algal-derived lipids (Carrie et al., 2012). S2-carbon represents the quantity of large molecules, kerogen-derived hydrocarbons released through thermal cracking of the organic matter, in sediment samples (mg hydrocarbons/g) near 650 ˚C. The S2 compounds in sediment generally correspond to highly aliphatic biomacromolecule structures of algal cell walls (Meyers and Teranes, 2001. S3 represents the amount of carbon dioxide released during pyrolysis of kerogen, while in sediment samples it represents lignins, terrigenous plant materials, humic and fulvic acids (Carrie et al., 2012). The quantity of all organic matter released during pyrolysis and oxidation heating accounts for TOC (wt.%) in sediment samples. Analyses of standard reference material (IFP 160000, Institut Français du Pétrole and internal 9107 shale standard, Geological Survey of Canada, Calgary; Ardakani et al., 2016) show accuracy and precision to be greater than 5% relative standard deviation.

Sediment subsamples (3 cm^3) were used for micropaleontological analysis. Subsamples were first wet sieved through a coarse (297 µm) and fine (37 µm) sieves to remove any coarse debris (e.g. grass and sticks) and retain Arcellinida tests, respectively. A wet splitter (Scott and Hermelin, 1993) was used to subdivide each subsample into six aliquots for quantitative analysis. Aliquots were identified and enumerated wet for total Arcellinida tests (live plus dead) on a gridded petri dish using an Olympus SZH dissecting binocular microscope (7.5-64X magnification) until, whenever possible, a statistically significant number of specimens were quantified (**Supplementary Table 1**; Patterson and Fishbein, 1989). Although living Arcellinida specimens may have been present at the time of sampling the samples were not stained so the enumerated Arcellinida analysis was carried out on live plus dead Arcellinida specimens (i.e., arcellinidan tests). Identification of Arcellinida primarily followed the illustrations and descriptions found in various key papers where specimens are well illustrated (e.g. Reinhardt et al., 1998; Roe et al., 2010; Patterson et al., 2013). Arcellinidan species can display considerable environmentally controlled infraspecific morphological variability (e.g., Medioli and Scott, 1983). To deal with this phenotypic plasticity, the accepted practice has been to designate informal infrasubspecific "strain" names for these ecophenotypes (Asioli et al., 1996; Reinhardt et al., 1998; Patterson and Kumar, 2002). While infrasubspecific level designations have no status under the International Zoological Code of Nomenclature (art. 45.5; 4th edition, 1999; ICZN, 1999), they have been extensively used in the literature for defining environmentally significant populations within lacustrine environments (e.g. Reinhardt et al. 1998; Kumar and Patterson, 2000; Patterson and Kumar, 2002; Roe et al., 2010; Steele et al., 2018). Scanning electron microscope images of common species and strains were obtained using a Tescan Vega-II XMU VP scanning electron microscope (SEM) in the Carleton University Nano Imaging Facility. All SEM plates were digitally produced using Adobe Photoshop™ CC 2018 **(Figure 2; Figure 3)**.

3.3 Data screening, variables reduction

The data were screened to remove samples or variables characterized by >25% missing values and values below the lower method detection limit (MDL; e.g., As lower MDL = 0.1 ppm) or above the upper MDL (e.g., As upper MDL = 10000 ppm; Reimann et al., 2008). Samples with geochemical results below the lower MDL were converted to ½ lower MDL (e.g., 0.05 ppm for As). Values that exceeds the upper MDL are changed to upper MDL (e.g., 10000 ppm for As;

applicable only to sample BC19; Reimann et al., 2008). These criteria resulted in the removal of five samples from the analyses (B44, B56, B59, Y56, Y59).

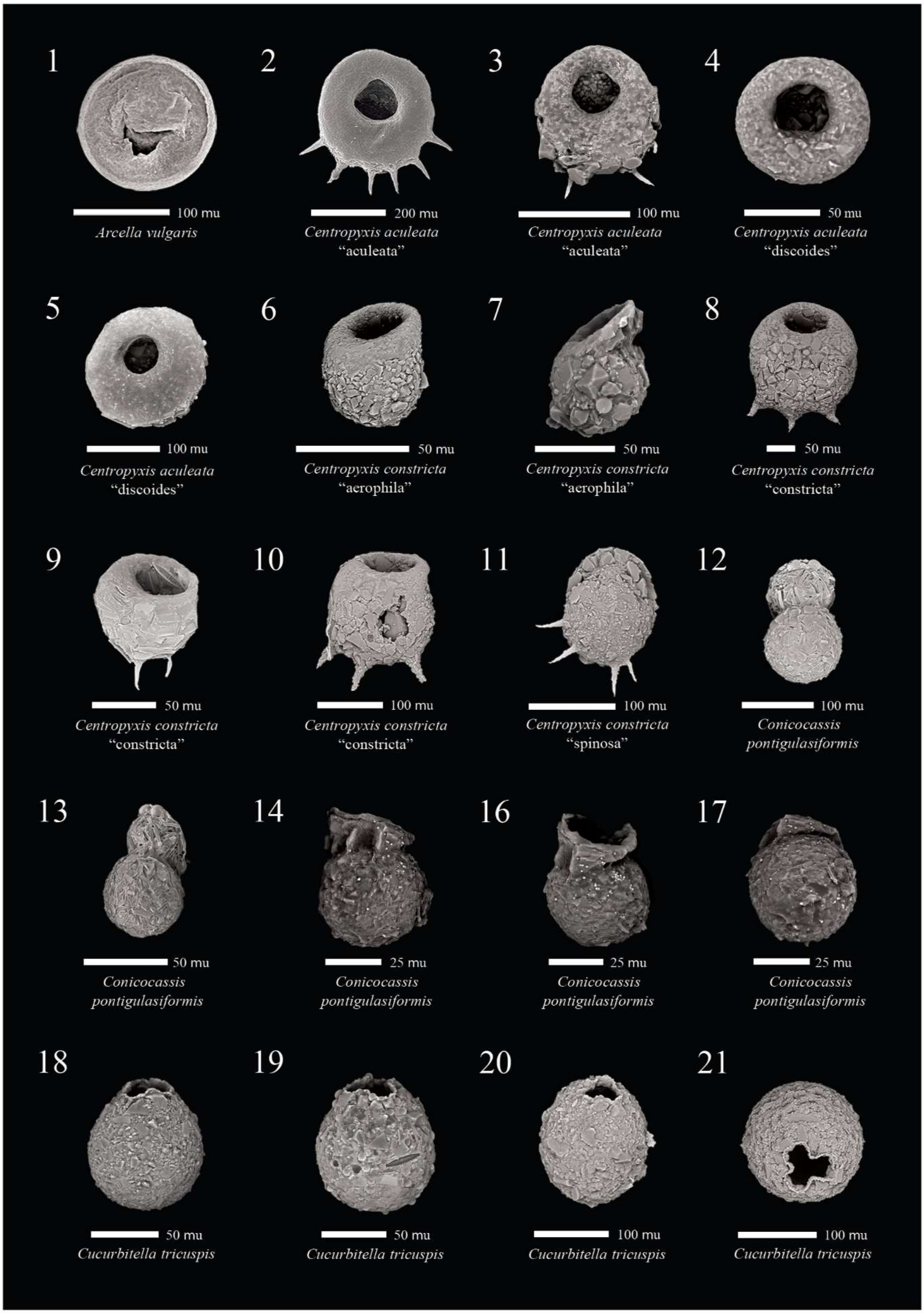
Because the inclusion of all measured variables in ordination analyses (e.g. redundancy analysis) creates clutter that can mask meaningful patterns generated by these methods, we used the Spearman's Rank correlation and Variance Inflation Factor (VIF) to reduce the number of variables used in the analyses. Spearman's Rank correlation served to remove highly correlated variables ($r_s > 0.7$), while VIF was employed to ensure the removal of highly collinear variables (VIF >10) **(Supplementary Table 2)**. Although TOC had collinear features with a number of variables (e.g. As and S1-carbon) it was retained for statistical analyses as this variable is known to influence the distribution of several key arcellinidan taxa as well as sediment chemistry (Patterson and Kumar, 2002).

3.4 Statistical analyses

Thirty arcellinidan species and strains were identified in this study. Statistical analysis carried out on the Arcellinida dataset is described in Nasser et al (2016). Based on calculated Probable Error (pe) and Standard Error (Sxi), six samples (B10, B20, B48, B55, B59, and Y69) containing statistically insignificant populations and five statistically insignificant species were excluded from subsequent multivariate data analyses **(Supplementary Table 1)**.

RStudio statistical software (version 0.98.1028; R Core Team, 2014) was used to carry out several statistical and multivariate analyses on measured parameters and species data. As recommended by by Fishbein and Patterson (1993) Q-and R- mode cluster analysis, using Ward's Minimum variance method and Euclidean distance (Ward, 1963), was used to group samples containing similar Arcellinida assemblages and to determine which species were most closely associated (R packages: stats, cluster, and gplots). Non-metric multidimensional scaling

(NMDS; Kruskal, 1964) was used to further investigate the results of cluster analysis by assessing the similarity between identified assemblages in multidimensional space (R package: vegan). Redundancy analysis (RDA; van den Wollenberg, 1977) of the post-screening data sets (84 samples and 25 species and strains) was used to evaluate the relationship between arcellinidan assemblages and measured environmental variables (R package: stats). A series of partial RDAs (pRDA), coupled with variance partitioning tests, were carried out to identify the significance of the RDA axes and measured variables (R package: stats). Variables with a $p < 0.05$ were considered to be significant contributors to variance in the arcellinidan assemblage. Analysis of Arcellinida tolerance and optima to As spatial variability was carried out using Weighted Average Tolerance and Optima (WATO; Ter Braak and Barendregt, 1986) methods performed through the package 'analogue' in RStudio. The method produced ecological optima values and tolerance limits (upper and lower limits) for each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Cucurbitella tricuspis
20
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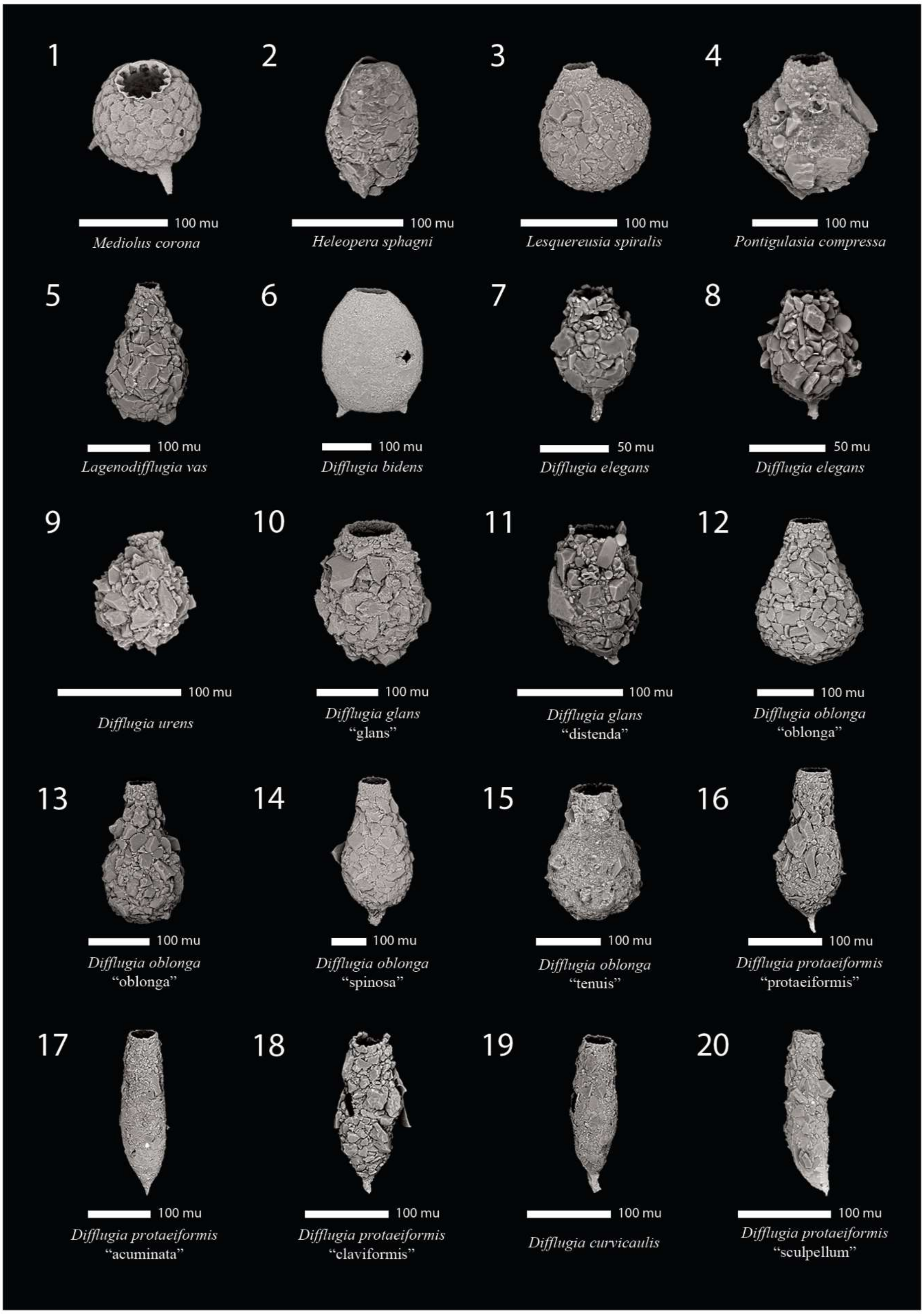
Cucurbitella tricuspis

Figure 2. Scanning electron microscope of selected arcellinidan tests from the study lakes. For more taxonomic information see Supplementary Materials Section 3.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2
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Heleopera sphagni
3
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Lesquereusia spiralis
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Pontigulasia compressa
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100 mu
Lagenodifflugia vas
6
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Difflugia bidens
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Difflugia elegans
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Difflugia elegans
9
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Difflugia urens
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Difflugia glans
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Difflugia glans
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12
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Difflugia oblonga
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Difflugia oblonga
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14
100 mu
Difflugia oblonga
"spinosa"
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100 mu
Difflugia oblonga
"tenuis"
16
100 mu
Difflugia protaeiformis
"protaeiformis"
17
100 mu
Difflugia protaeiformis
"acuminata"
18
100 mu
Difflugia protaeiformis
"claviformis"
19
100 mu
Difflugia curvicaulis
20
100 mu
Difflugia protaeiformis
"sculpellum"

344

Figure 3. Scanning electron microscope of selected Arcellinidan tests from the study lakes. For more taxonomic information see Supplementary Materials Section 3.

identified taxa, which is necessary for the identification of indicator-species and/or assemblages **(Supplementary Table 3)**.

4. RESULTS AND DISCUSSION

4.1 Spatial Distribution of As

Measured sedimentary As concentrations were significantly higher than the levels proposed by the interim sediment quality guidelines (ISQG; 5.9 ppm; CCME, 2002) and probable effect level guidelines (PEL; 17 ppm; CCME, 2002) in 94% (*n*= 84) of the samples (*n*=89**; Supplementary Table 1)**. This is particularly evident in lakes to the west (median As = 290.4 ppm; range = 30.2-4778.2 ppm *n*=27) and north (median As = 147 ppm; range = 16.1-10000 ppm *n*=24) of the Giant Mine. Median As levels in lakes to the east (As = 36.3 ppm; range = 9.7-553.9 ppm *n*=19) and south (median As = 31.3 ppm; range = 6.3-317.8 ppm *n*= 13) of the mine were comparatively lower, yet remain above the ISQG and PEL guidelines.

A negative Spearman's Rank coefficient between sedimentary As concentration and the distance from the roaster site (r_s = -0.5) indicates decreasing As concentrations in distal lakes **(Supplementary Table 3)**. This spatial pattern reflects the influence of the prevailing southeasterly winds (Pinard et al., 2007), which transported As-bearing stack emissions from the Giant and Con mines toward the northwest (Palmer et al., 2015; Galloway et al., 2017). The influence of prevailing wind direction may also explain the persistence of elevated levels of As in distal lakes to the north (B2; distance = 17 km, As=905.2 ppm) and west of the historic mining operations (Y15; distance = 21.5 km; As = 689.9). Recent studies confirm the persistence of As_2O_3 in lake sediments downwind of Giant Mine (Galloway et al., 2017; Schuh et al., 2017; Van den Berghe et al., 2017). Geogenic As is elevated in the Yellowknife area (background concentration = 150 ppm; Risklogic, 2002), yet its contribution of As to these lakes is minor.

4.2 Arcellinida Assemblages

The results of Q-mode cluster analysis **(Figure 4),** and NMDS **(Figure 5)** revealed five distinct arcellinidan assemblages: 1) "High As Contamination Assemblage (HAC)"; 2) "As contamination Assemblage (AC)"; 3) "*Centropyxis aculeata* Assemblage (CA)"; 4) "Transitional Assemblage (T)"; and 5) "Healthy Assemblage (H)". Results of the R-mode cluster analysis suggests that only seven out of the 25 identified arcellinidan taxa (*Difflugia elegans* Penard, 1890, *Centropyxis constricta* (Ehrenberg, 1843) "constricta", *Centropyxis constricta* (Ehrenberg, 1843) "aerophila", *Centropyxis aculeata* (Ehrenberg, 1843) "aculeata", *Cucurbitella tricuspis* (Carter, 1856), *Difflugia oblonga* Ehrenberg, 1832 "oblonga" and *Difflugia glans* Penard, 1902 "glans") contributed significantly to defining the derived faunal assemblages (Figures 4, 5; **Supplementary Table 1**). The unique faunal structure of each assemblage reflects ecological conditions characteristic of stressed (e.g. HAC, AC, and CA; SDI values 1.3-2.2), transitional (e.g. T; SDI values 1.6-2.5) and relatively healthy lacustrine systems (e.g. H; SDI values 1.7-2.4). The variability reflected by the assemblages developed mostly in response to ten significant environmental parameters (As, S1 carbon, sulfur [S], sodium [Na], calcium [Ca], distance to Giant Mine, phosphorous [P], barium [Ba], mercury [Hg], and total organic content [TOC])identified by using partial RDA analysis that explain ~40% of the variance in the arcellinidan distribution **(Figure 6)**. Arsenic (9.4%) and S1-carbon (8.9%) exert the most influence over the composition of the identified assemblages and collectively explain 18.3% of the total variance. A detailed description of the location, taxonomic composition and primary environmental controls for each assemblage is provided in **Supplementary Material.**

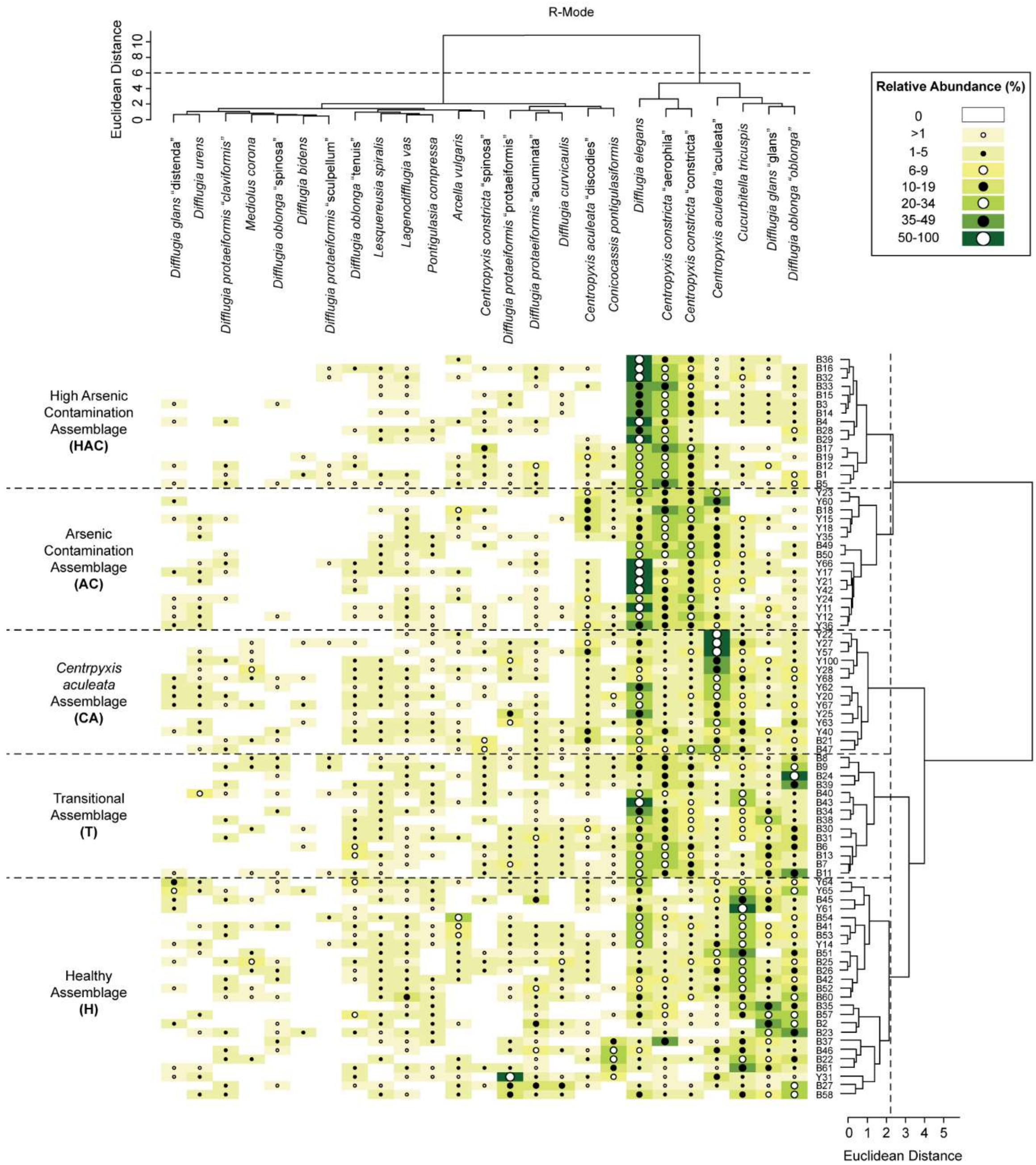


Figure 4. Combined Q-mode and R-mode cluster dendrogram for the 84 samples and 25 statistically significant species and strains. Five faunal assemblages are indicated.

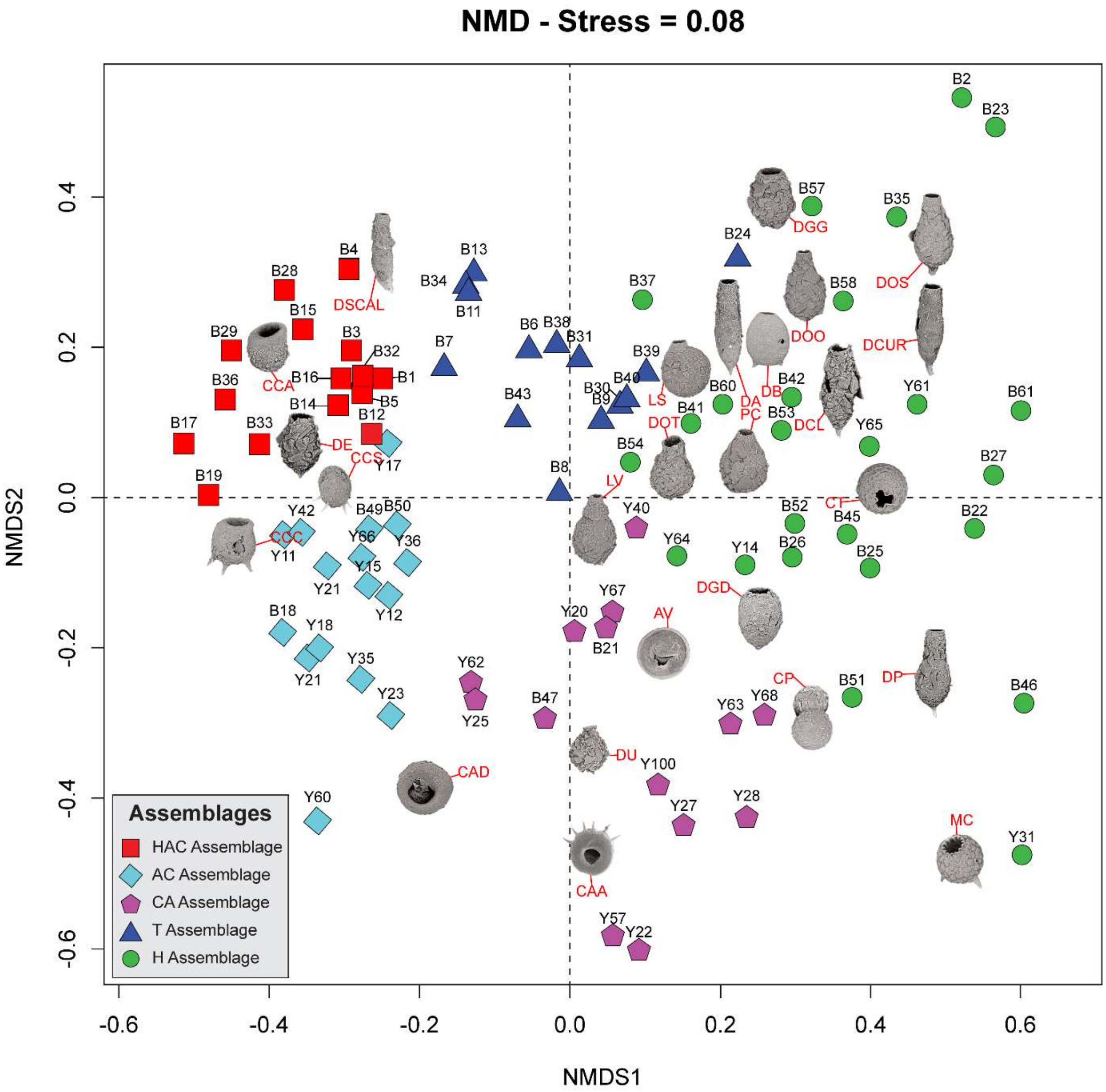
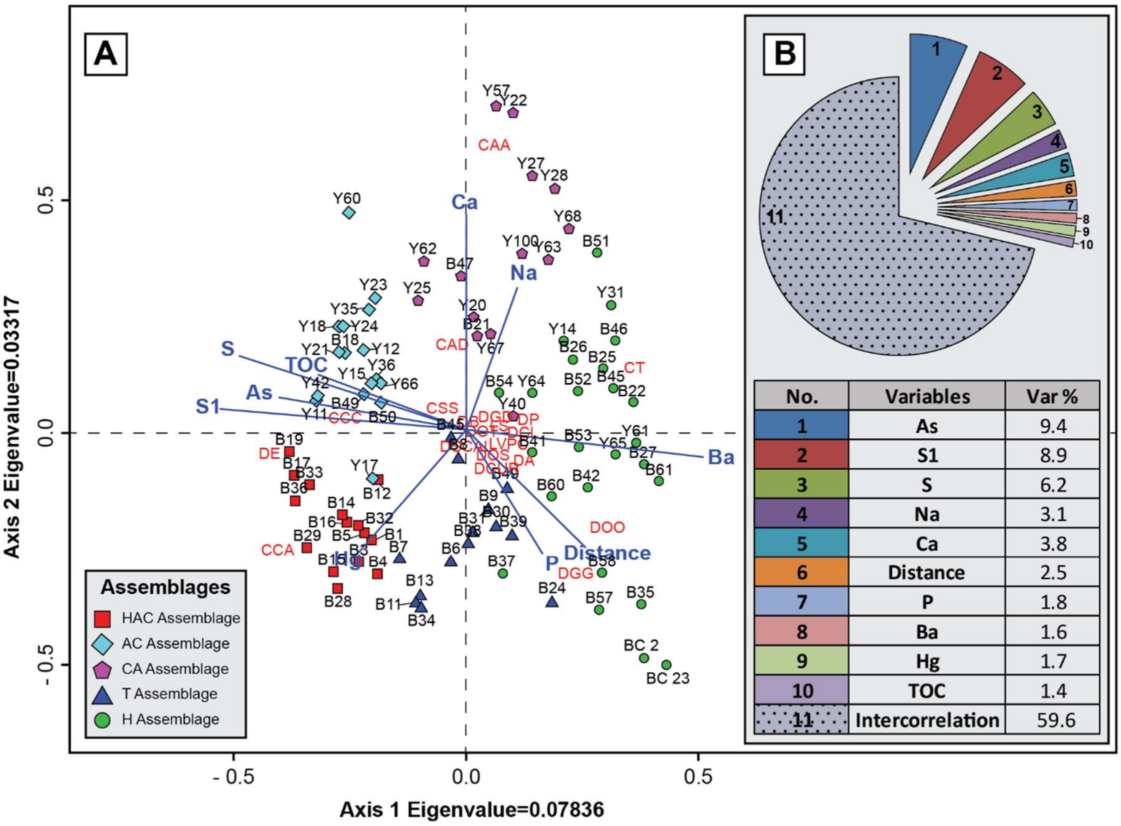


Figure 5. Non-Metric Multidimensional Scaling (NMDS) bi-plot. AV – *Arcella vulgaris*, CAA – *Centropyxis aculeata* “aculeata”, CAD – *Centropyxis aculeata* “discoides”, CCA – *Centropyxis constricta* “aerophila”, CCC – *Centropyxis constricta* “constricta”, CCS – *Centropyxis constricta* “spinosa”, CP – *Conicocassis pontigulasiformis*, CT – *Cucurbitella tricuspis*, MC – *Mediolus corona*, DB – Difflugia bidens, DOO – *Difflugia oblonga* “oblonga”, DOS – *Difflugia oblonga* “spinosa”, DOT – *Difflugia oblonga* “tenuis”, DGG – *Difflugia glans* “glans”, DGD – *Difflugia glans* “distenda”, DU – Difflugia urens, DE – *Difflugia elegans*, DPP – *Difflugia protaeiformis* “protaeiformis”, DPAC – *Difflugia protaeiformis* “acuminata”, DPCl – *Difflugia protaeiformis* “claviformis”, DCUR – *Difflugia protaeiformis* “curvicaulis”, DPSC – *Difflugia protaeiformis* “scalpellum”, LS – *Lesquereusia spiralis*, LV – *Lagenodifflugia vas*, PC – *Pontigulasia compressa*

4.3 Controls over the Distribution of Arcellinida

The RDA and pRDA results in **Figure 6** show that As is the dominant control on the distribution of Arcellinida. Stress-tolerant taxa such as *D. elegans,* and *C. constricta strains* ‘aerophila’ and ‘constricta’ dominate in HAC and AC assemblages (SDI range = 1.4-2.2). This is likely a response to the elevated sediment As levels (median As = 290.4 ppm; range = 21.1-10000 ppm; n = 31), which in turn is attributed to the downwind location (93.5% of lakes located to the west or north; n = 28) and close proximity to the historic Giant Mine roaster stack as a point source of As contamination (median distance to Giant Mine roaster stack = 9.85 km; n = 31). Levels of As were notably lower in samples associated with the CA assemblage (median As = 258.1 ppm; range = 33.4-921.1 ppm; n = 14), even though such lakes were relatively close to the mine (median distance to Giant Mine roaster stack = 6.6 km; n = 14). Most (57%; n=8) of the lakes hosting the CA assemblage were located upwind of the roaster, while the remaining lakes (43%; n=6) are situated downwind from the mine site **(Figure 1)**. The reduction in As levels is associated with a moderately diverse assemblage (SDI = 1.3-2.5; median SDI = 2) that is characterized by the emergence of *C. aculeata* “aculeata“ as a dominant member, a notably lower number of stress-indicating taxa, and a slight elevation in the number of healthy-lake species and strains (e.g. *C. tricuspis*, *D. oblonga* “oblonga” and *D. glans* “glans”). With greater distance from the Giant Mine site, the stress-indicating HAC and AC assemblages were less common and the lakes became dominated by a transitional assemblage (T; SDI = 1.6-2.5; median SDI = 2.1) that is comprised of lower proportions of stress-indicating species and higher



No.	Variables	Var %
1	As	9.4
2	S1	8.9
3	S	6.2
4	Na	3.1
5	Ca	3.8
6	Distance	2.5
7	P	1.8
8	Ba	1.6
9	Hg	1.7
10	TOC	1.4
11	Intercorrelation	59.6

Figure 6. Redundancy Analysis (RDA) species-environment-sample tri-plots for the 84 sediment-water-interface samples that yielded statistically significant arcellinidan populations and had no missing values. The five identified Arcellinida assemblages are: 1) the high As contamination assemblage (red square); 2) As contamination assemblage (light blue diamond); 3) *Centropyxis aculeata* assemblage (purple pentagon); 4) transitional assemblage (blue triangle); and 5) healthy assemblage (green circle).

numbers of healthy-lake indicating Arcellinida taxa. This result was expected since the transitional assemblage was observed most commonly in relatively distal lakes (median distance to Giant Mine roaster stack = 12.6 km; n = 14) and characterized by moderate to low As levels (median As = 76.5 ppm; range = 16.1-740.7 ppm; n = 14). The healthiest arcellinidan assemblage (H; SDI = 1.7-2.4; median SDI = 2.1) was found in lakes >10 km from the mine site (median distance = 19.6 km; n = 25) in lakes characterized by the lowest As concentrations (median As = 30.3 ppm; range = 6.3-905.2 ppm; n = 25). The observed faunal shift from stressed to healthy assemblages suggest well defined zones of impact (radius of ~ 20 km around the Giant Mine) and immediate impact (radius of ~ 10 km around the Giant Mine), which are consistent with the geospatial extent of the zones delineated by Palmer et al., (2015) and Galloway et al., (2017) (17 km and 11 km, respectively).

The RDA and pRDA results indicated that the labile fraction of total organic matter (S1-carbon) is also a significant control over the distribution of Arcellinida taxa in area lakes (**Figure 6**). The RDA tri-plot shows that S1-carbon and As are closely correlated, which is corroborated by a significant positive Spearman's Rank correlation between the two variables ($r_s = 0.5$; **Figure 6, Supplementary Table 2**). It is notable that the arcellinidan response to the spatial variability of S1-carbon was similar to that observed with the As concentrations. The highest average levels of S1-carbon in HAC (median S1-carbon = 50.4 HC/g rock; range = 33.9-66.5 HC/g rock; n = 15) and AC assemblages (median S1-carbon = 53.2 HC/g rock; range = 34.8-59.8 HC/g rock; n = 16) are associated with elevated levels of As and the dominance of stress-indicating taxa. The lowest median S1-carbon values are associated with low As levels and healthy lake taxa (HA; median S1-carbon = 18.9 HC/g rock; range = 0.4-36.4 HC/g rock; n = 25). Previous studies have similarly reported high organic matter content in metal-contaminated soils (Valsecchi et al., 1995; Kelly and Tate, 1998) and lakes (Gough et al., 2008; Gough et al., 2010). The relationship between organic matter and As may reflect: 1) organic matter mediatiation reduction of As^{5+} to As^{3+} and subsequent release of sediment-bound As into sediment pore water and the overlying water column (Van den berghe et al., 2017); 2) competition with As over for sorption sites (Redman et al., 2002); or 3) enhancement of As sequestration by providing an organic substrate with a large surface area for metal(loid)-organic matter complexation (Grafe et al., 2001). Galloway et al. (2017) reported a significant association between As, S1-carbon and S in Yellowknife area lakes. The relationship was interpreted to reflect S1-carbon as an organic substrate suitable for microbial growth, which in turn mediated the authigenic precipitation of As derived from roaster emission to As sulfides. The similar arcellinidan response to both variables in this study suggests that both influence faunal ecology.

While phosphorus (P) explains a small portion of the variance in Arcellinida distribution (1.8%), spatial variability of P in the study area seems to follow a relatively modest trend of increasing concentrations in distal lakes (median P = 1060 ppm; median distance = 16.7 km; n = 39) compared to lakes closer to the Giant Mine (median P = 880 ppm; median distance = 8.1 km; n = 45). As expected, the RDA tri-plot shows that P is positively associated with distance from the Giant Mine, and negatively with As and S1-carbon **(Figure 6)**. While organic matter is a limiting factor for bacterial growth, an increasing number of studies have documented the importance of several elements, particularly P, in controlling bacterial growth efficiency and attainable biomass within a wide range of aquatic systems (Toolan et al., 1991; Elser et al., 1995; Gurung and Urabe, 1999). In addition, the availability of P has been shown to influence As toxicity to primary producers in freshwater systems (Levy et al., 2005; Wang et al., 2013).

Arsenate (AsO_4^{3-}) and phosphate (PO_4^{3-}) are chemically analogous. This similarity allows arsenate to substitute for phosphate, when the availability of the latter is low, and pass into the cell via phosphate transporters and inhibit phosphorylation, which consequently impacts several protein functions and cellular growth (Meharg and Macnair, 1991). Therefore, the slightly reduced proportions of P in lakes closer to the Giant Mine may play a role in intensifying As toxicity to microbial and arcellinidan communities, influencing their distribution in the process.

4.4 Interaction between Arcellinida, As and S1

Healthy and active microbial communities thrive within organic substrates, especially the labile fraction (i.e. S1-carbon; Sanei et al., 2005). The development of such communities may provide an adequate source of nourishment for Arcellinida, which feed on bacteria, algae and fungi (Nikolaev et al., 2005). However, As is known to be toxic to most bacteria, except As-tolerant strains, as it can inhibit basic cellular functions linked to energy metabolism, basal respiration and enzyme activities (Baath, 1989; Walker et al., 2000). The effects of As toxicity have also been shown to be associated with a significant reduction in the microbial biomass in soil (Maliszewska et al., 1985; Hiroki, 1993; Simon, 2000), and, to a lesser extent, in lacustrine environments (Gough et al., 2008; Gough et al., 2010). Such a reduction in microbial biomass may induce sufficient environmental stress to impact nutrient-sensitive arcellinidan taxa (e.g. *C. tricuspis* and *D. oblonga* “oblonga”) as competition for dwindling food resources intensifies. An experimental study by Burnskill et al (1980) suggests that As may inhibit the activity of organic matter-reducing bacteria during the winter, while exerting no influence on bacterial productivity in the summer. A recent study by Palmer et al. (2019) in the Yellowknife area reported elevated levels of As^{5+} in well-mixed surface waters during the summer when oxic conditions dominate, and higher concentrations of As^{3+} during the winter when ice cover results in reducing conditions. Such seasonal inhibition may lead to a reduction in microbial biomass and a concurrent buildup of organic matter during the winter, which may decompose during the summer upon the recovery of microbial activities. A similar positive correlation between higher proportions of organic matter, represented by TOC, and elevated concentrations of heavy metal contamination have been reported by several studies (Kelly and Tate, 1998; Valsecchi et al., 1995; Gough et al., 2008). TOC is relatively high in our study (median = 25.6%), which may explain the association of stress-tolerant taxa with high levels of As, S1-carbon, and TOC, and less tolerant species with relatively lower As, S1-carbon, and TOC levels in our study. Based on our results we propose a mechanism whereby As has an indirect influence over the spatial distribution of Arcellinida in the study area through suppression of growth of their food resource (i.e. microbial communities).

The introduction of a contaminant into an aquatic system may directly or indirectly impact biota. Direct influences can increase mortality rates through toxicity and reduce populations, while indirect effects may either decrease (e.g., by limiting sources of nourishment) or increase (e.g., by lowering competition over food sources) the population. Unfortunately, studies designed specifically to assess the direct and indirect influence of As on Arcellinida are lacking. The results of this study provide information that explains indirect impacts of As on arcellinidan trophic function but it is important to note that the hypothesized relationship does not preclude any direct impact of As toxicity on arcellinidan species in the Yellowknife area lakes.

4.5 Tolerance of Arcellinida Taxa to As

In environmentally stressed lakes, the biotic components often display a range of tolerances in response to the introduction of contaminants into the system (Negro and de Hoyos, 2005). For instance, contamination is often associated with a massive reduction in the biomass of intolerant species, while resilient taxa may show little indication of ecological stress, and may in fact expand to fill the ecological void. Such variation in tolerance was captured by the WATO analysis performed on the 25 Arcellinida taxa, which exhibited a wide tolerance to changes in As concentration **(Figure 7, Supplementary Table 3)**. The optima values and upper tolerance limits exhibited a modest increasing trend accompanied by a taxonomic gradient from steno-metalloid (As) species (e.g. healthy lake taxa) to highly eury-metalloid (As) Arcellinida (e.g. stressed lake taxa). This association is in agreement with the findings of studies linking the abundance of healthy-lake and stressed-lake arcellinidan taxa to low or high levels of As, respectively (Patterson et al., 1996; Reinhardt et al., 1998; Nasser et al., 2016). However, the identification of robust As indicator species depends on quantitative characterization of species with both well-defined ecological optima and narrow tolerance ranges (Negro and de Hoyos, 2005). While the results of the analysis define the optima and upper tolerance As values for each taxon. The lower As tolerance limit for all the identified Arcellinida species was 0 ppm. This result is not surprising because the 25 arcellinidan taxa as would also be expected to be found in substrates where As is present in low concentrations, or is even absent.

While it was not possible to identify any individual high concentration steno-metalloid (As) indicator species in our study, the results of the WATO method reveal three groupings of taxa that reflect certain As concentration ranges: (1) “low-moderate tolerance group” (LMTG; As range = 0-350 ppm); (2) “high tolerance group” (HTG; As range = 360-760 ppm); and (3) "extreme tolerance group" (ETG; As range = >750 ppm**; Figure 7)**. The LMTG includes 12 arcellinidan species and strains with a relatively low to moderate As optima (99.3-225 ppm) and upper tolerance As range of 156.7-355.5 ppm. The species composition of the LMTG is primarily represented by *Difflugia* (*n*= 8) species and *C. tricuspis*, which are known to be abundant in relatively healthy lakes (Patterson et al., 1996; Neville et al., 2011). Therefore, members of this group are likely to be characteristic of assemblages from lakes were As levels do not exceed 360 ppm.

Species comprising the ETG are characterized by well-known stress-tolerant taxa (e.g. centropyxid species and stains and *D. elegans*), elevated optima range (507-1433.6 ppm) and upper tolerance range (1382.5-2613.5 ppm). Centropyxid species and strains are known for their opportunistic nature and ability to withstand a variety of severely stressed environmental conditions (Medioli and Scott, 1983; Kihlman and Kaupplia, 2012; Nasser et al., 2016; Gavel et al., 2018). In addition, *D. elegans* was previously reported (identified as *D. protaeiformis* "amphoralis") as being abundant in substrates with high As concentration (300-2100 ppm; Reinhardt et al., 1998). Therefore, arcellinidan assemblages dominated by these species are likely to be present under a wide range of As levels but expected to dominate when levels of As are extremely elevated (>750 ppm). The HTG (360-760 ppm), spanning As concentrations between the LMTG and ETG, is composed of six species (*A. vulgaris*, *L. vas* and *D. urens*) and strains (*D. glans* "distenda", *D. oblonga* "tenuis" and *C. aculeata* "aculeata"). These taxa include representatives of both the *Difflugia*-dominated LMTG and Centropyxid-dominated ETG. This explains why there is an overlap of As tolerance between the HTG [As optima (241-387.3 ppm] and upper tolerance limits [540.7-765.7 ppm]) and both the LMTG and ETG. Since the HTG is found both in lakes where As levels are below 360 ppm and those with

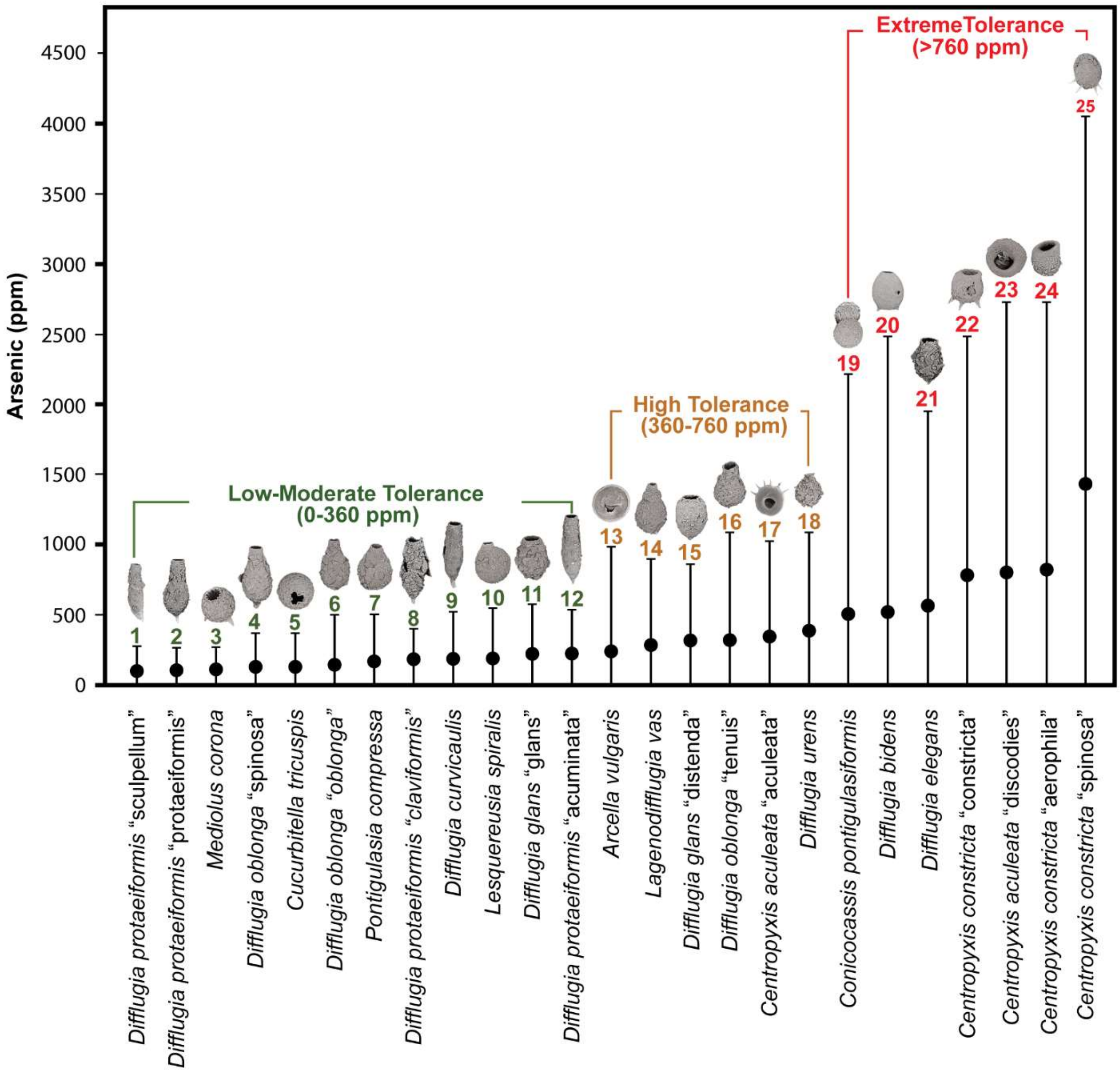


Figure 7. Results of the Weighted Average Tolerance and Optima Analysis (WATO) on the 25 statistically significant arcellinidan taxa.

As concentrations between 360-760 ppm, it is a generally less useful As indicator than the LMTG and ETG.

4.6 Paleoenvironmental Assessment Tool

The reliability of using the identified Arcellinida As tolerance groups to infer temporal changes in As levels in impacted lakes was assessed on a test dataset comprising arcellinidan assemblages and As concentrations (measured by ICP-MS) from 30 subsamples from a freeze core collected from Frame Lake, Northwest Territories, Canada (Gavel et al., 2018). We hypothesized that As ranges inferred by the relative abundance of taxa in the three tolerance groups derived from the inter-lake data set would represent measured total As concentrations from each core subsample independently measured using ICP-MS. Frame Lake sediments represented ideal test material as the well documented system was initially impacted by As contamination, then nutrient loading, and overprinted by redox-influenced remobilization of As upwards the stratigraphic column (Gavel et al., 2018).

Our results show that the measured As levels in 66.6% of the samples (n= 20) where remobilization of As fell within the As range suggested by the bioindicator groupings **(Figure 8; Supplementary Table 3)**. The identification of an LMTG assemblage (0-350 ppm As) in 16 samples was in line with measured As concentrations of 121.5-278 ppm. In addition, four samples characterized by very high levels of As (913.2-1473.5 ppm) were dominated by the ETG members. The two model mismatches (FL21, FL22) were dominated by high proportions of As-tolerant taxa from the HTG and ETG assemblages, even though the measured As concentrations of both samples is below 350 ppm. These results may be indicative of post-depositional remobilization of As out of these horizons **(Supplementary Table 3)**. Unidentified confounding environmental stressors

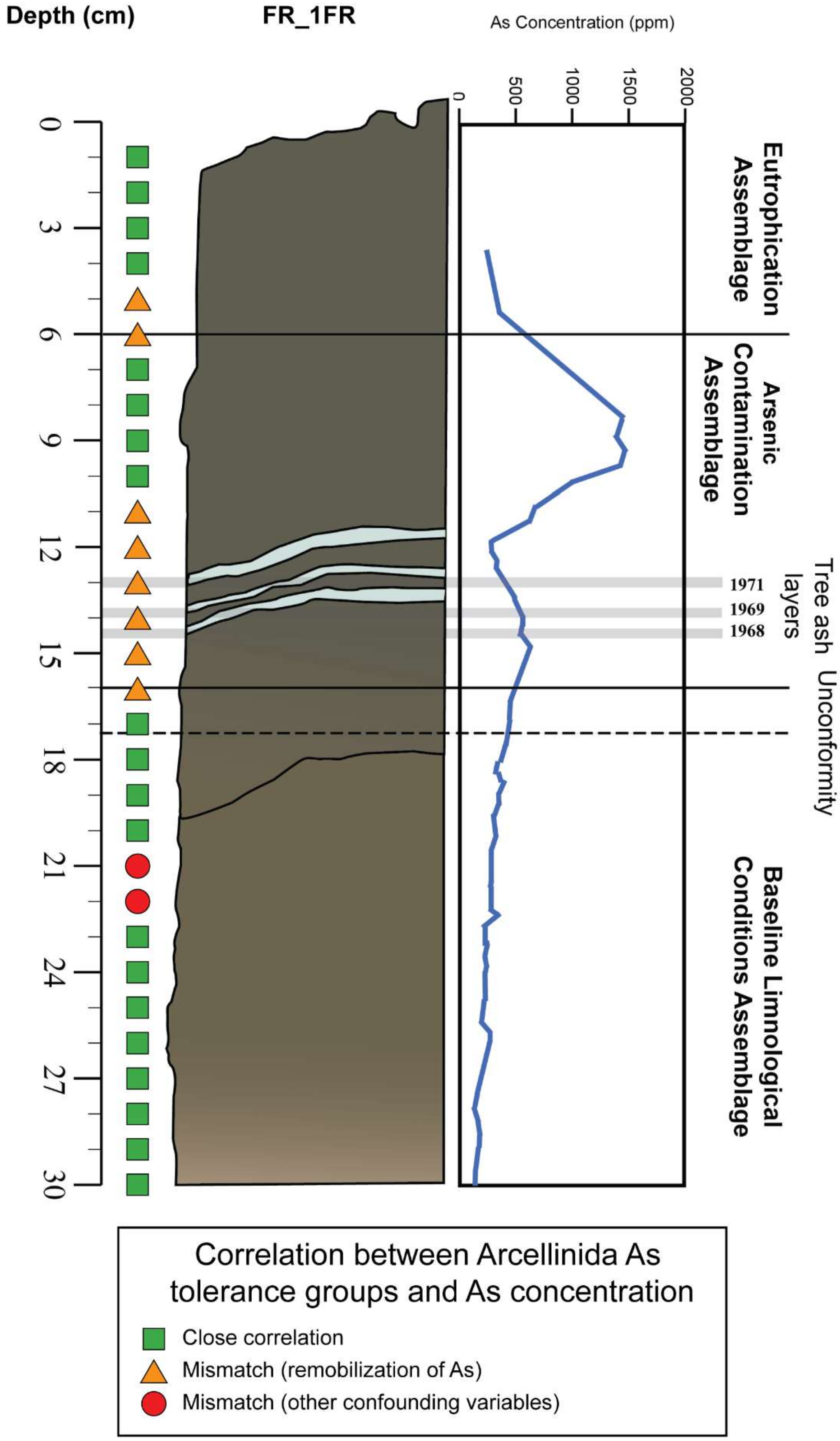

Depth (cm)
FR_1FR
As Concentration (ppm)
0
500
1000
1500
2000
0
3
6
9
12
15
18
21
24
27
30
Eutrophication Assemblage
Arsenic Contamination Assemblage
Tree ash layers
1971
1969
1968
Unconformity
Baseline Limnological Conditions Assemblage
Correlation between Arcellinida As tolerance groups and As concentration
Close correlation
Mismatch (remobilization of As)
Mismatch (other confounding variables)

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Figure 8. The correlation between Arcellinida As tolerance group and As concentrations of 30 freeze core samples from Frame Lake. The strength of the correlation is represented by three colored symbols, with the green square representing a strong correlation, the orange triangle representing a mismatch (weak correlation) attributed to the influence of As remobilization, and the red circle representing a mismatch attributed to the influence of other confounding variables (modified after Gavel et al., 2018).

may have also contributed to the observed fauna in these samples. The mismatches between the arcellinidan fauna and As concentrations in the other eight samples were likely attributable to variability in redox conditions, which resulted in As remobilization (Gavel et al., 2018). This result highlights the utility of these bioindicators as a tool to reconstruct As concentrations in sedimentary records, since the non-mobile arcellinidan assemblages provide a faithful paleoenvironmental record of As contamination at the time of deposition, regardless of any post-depositional remobilization of As.

5. Conclusions

Arcellinidan taxa (n = 25) in 84 sediment-water interface samples from the Yellowknife area responded to a decline in As concentrations further from the Giant Mine site by shifting from stressed assemblages near the mine to healthier assemblages in distant lakes (>10 km), thus corroborating the geographic extent of the airborne As contamination zone of immediate impact delineated by Palmer et al. (2015) and Galloway et al. (2017). The results also show that arcellinidan groups, based on As-tolerance limits, can be used to successfully infer As concentrations in 20 out of 30 freeze core samples impacted by mine-induced As regardless of As post-depositional remobilization. These results establish the utility of arcellinidan bioindicators as an independent proxy for monitoring changes in As concentrations and the ecological health in lakes impact by mine-induced As contamination. While the findings of this study confirm a strong relationship between Arcellinida and mine-derived As contamination in high latitude lakes (Yellowknife area), similar relationships are expected in As-contaminated lower latitude lakes. However, the impact of As contamination on the arcellinidan assemblage dynamics is likely to be more intense in low latitude lakes due to the warmer water conditions, which in turn increases the likelihood of As post-depositional mobility. More research assessing the relationship between Arcellinida and mine-induced As contamination in both higher and low latitude lakes is required to assess the consistency of the Arcellinidan response to As.

Faunal changes may provide insight into the nature of prevailing species of As, with highly stressed assemblages likely thriving when the more toxic tri-valent As^{3+} is dominant, while less-stressed assemblages likely associated with relatively less toxic penta-valent As^{5+} and more inert organic forms. Such insight is significant because accurate geochemical determination of concentrations of particular As species is a metric not captured during typical industry standard ICP-MS analysis. However, more research investigating direct and indirect effects of As contamination on Arcellinida and identify different As uptake mechanisms and pathways is required to validate the use of group as a tool for inferring the dominant As species in lakes sediments. Nevertheless, Arcellinida show great potential as a robust reconnaissance tool for identifying impacted lakes where As concentrations may be elevated prior to conducting As speciation geochemical analysis. The findings generated in this study have broad application to other As-impacted lacustrine systems and will provide valuable information to policy makers, environmental planners, mine developers, as well as potentially facilitating rehabilitation efforts in lakes impacted by As contamination.

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