Microb Ecol (2013) 65:541–554
DOI 10.1007/s00248-012-0108-9

MICROBIOLOGY OF AQUATIC SYSTEMS

Arcellacea (Testate Lobose Amoebae) as pH Indicators in a Pyrite Mine-Acidified Lake, Northeastern Ontario, Canada

R. Timothy Patterson · Edouard D. R. Lamoureux · Lisa A. Neville · Andrew L. Macumber

Received: 3 April 2012 / Accepted: 3 August 2012 /

Abstract Arcellacea (testate lobose amoebae) were examined in 24 sediment–water interface samples collected over two late August field seasons in 2010 and 2011, from James and Granite lakes, Temagami Region, Northeastern Ontario. The work was carried out to quantitatively test species–environment relationships in a lake system known to be characterized by a significant pH gradient, partially the result of contamination from the early twentieth century Northland Pyrite Mine Co., located on the shoreline in the southern basin of James Lake. Redundancy analysis confirmed that arcellacean assemblage structure was most strongly controlled by pH, explaining 14.06 % ($p<0.002$) of the total variance. Q- and R-mode cluster analysis supported by detrended correspondence analysis yielded two major faunal assemblages. The Oligotrophic Assemblage (1) had a Shannon Diversity Index (SDI) ranging up to 2.45, typical of healthy boreal lakes. This assemblage characterized samples collected from higher pH stations within James and Granite lakes away from the immediate area of the mine site, while the Low pH Assemblage 2010 (2a) and Low pH Assemblage 2011 (2b) groupings were from the very low pH environments of James Lake adjacent to the former mine site. Both low diversity assemblages (SDI ranging from 0.62 to 1.22) were characterized by *Arcella vulgaris*, a species known to thrive in hostile lacustrine environments. Differing depositional conditions during August 2010, a probable result of different prevailing wind patterns that summer, led to allochthonous specimens of the seasonally planktic *Cucurbitella tricuspis* dominating the Low pH Assemblage 2010 (2a) fauna.

Introduction

Arcellacea, also informally known as thecamoebians [1] or testate lobose amoebae [2], are a group of unicellular amoeboid testate rhizopods found preserved in lacustrine sediments throughout the world [3]. Found primarily in organic-rich lake sediment, they form small tests (5–500 μm) generally by agglutination of xenogenous mineral grains and other material (e.g., diatom frustules) within an autogenous cement. Some taxa are also known to secrete autogenous platelets [3]. Their rapid reproduction rate of days to weeks makes them particularly useful for monitoring lakes vulnerable to contaminant loading. For example, broad population changes have been detected between years at the same location in controlled experiments designed to test the effectiveness of remediation efforts in oil sands tailings ponds [4, 5]. As organic-rich surface sediments may contain 500–3,000 specimens per ml [7], little material is required for analysis. In addition, the samples are very easy to prepare for examination [1]. The development of sophisticated ordination methods in statistical paleoecology has allowed for a better understanding of species–environmental interactions [9]. Unfortunately only a handful of systematic studies have been carried out to date that test the response of arcellaceans to specific environmental controls and gradients in lacustrine environments. These include assessments of total phosphorus loading [10, 11], temporal variation in seasonality [12], salinity [13], effectiveness of oil sands tailings pond remediation efforts [5], metal contamination [14–17], pH [18], and climate [8].

Electronic supplementary material The online version of this article (doi:10.1007/s00248-012-0108-9) contains supplementary material, which is available to authorized users.

R. T. Patterson (✉) · E. D. R. Lamoureux · L. A. Neville · A. L. Macumber
Department of Earth Sciences and Ottawa-Carleton Geoscience Center, Carleton University,
1125 Colonel By Drive,
Ottawa, ON, Canada
e-mail: tim_patterson@carleton.ca

Previous research involving a qualitative assessment of arcellacean faunal response to various environmental variables has been carried out in Northeastern Ontario. The results have indicated that these organisms respond to both metal contamination [19, 20] and pH changes [21, 22] at both the species and assemblage level. James Lake, in the Temagami Region of Northeastern Ontario (Fig. 1), was found to be notable as there was a considerable north–south pH gradient observed that was hypothesized to be related to early twentieth century pyrite mining activity on the southern shore [21, 23]. The purpose of this research is to utilize this natural laboratory to quantitatively assess arcellacean species and assemblage response to pH in a contiguous environment where levels of other environmental variables are relatively consistent.

James and Granite Lake Physical Overview

Most lakes in the Temagami Region are categorized as oligotrophic to mesotrophic. James Lake is a mesotrophic lake, located ~21 km south of the town of Cobalt along Highway 11 [23]. The lake is elongated in a north–south direction with an area of 45.3 ha, and is divided into a large northern basin (80 % area) and a small southern basin (20 % area), separated by a narrows (Supplementary Fig. 1; [22]). In August 2010, surface water temperatures ranged from 20.3 to 23.2 °C in the southern basin, and from 19.9 to 20.2 °C in the northern basin. Temperature dropped slowly with depth to an 8-m thermocline, below which temperatures rapidly dropped to 8.0 °C at the bottom. The lake is fed by a small inflow stream at the north end and drains

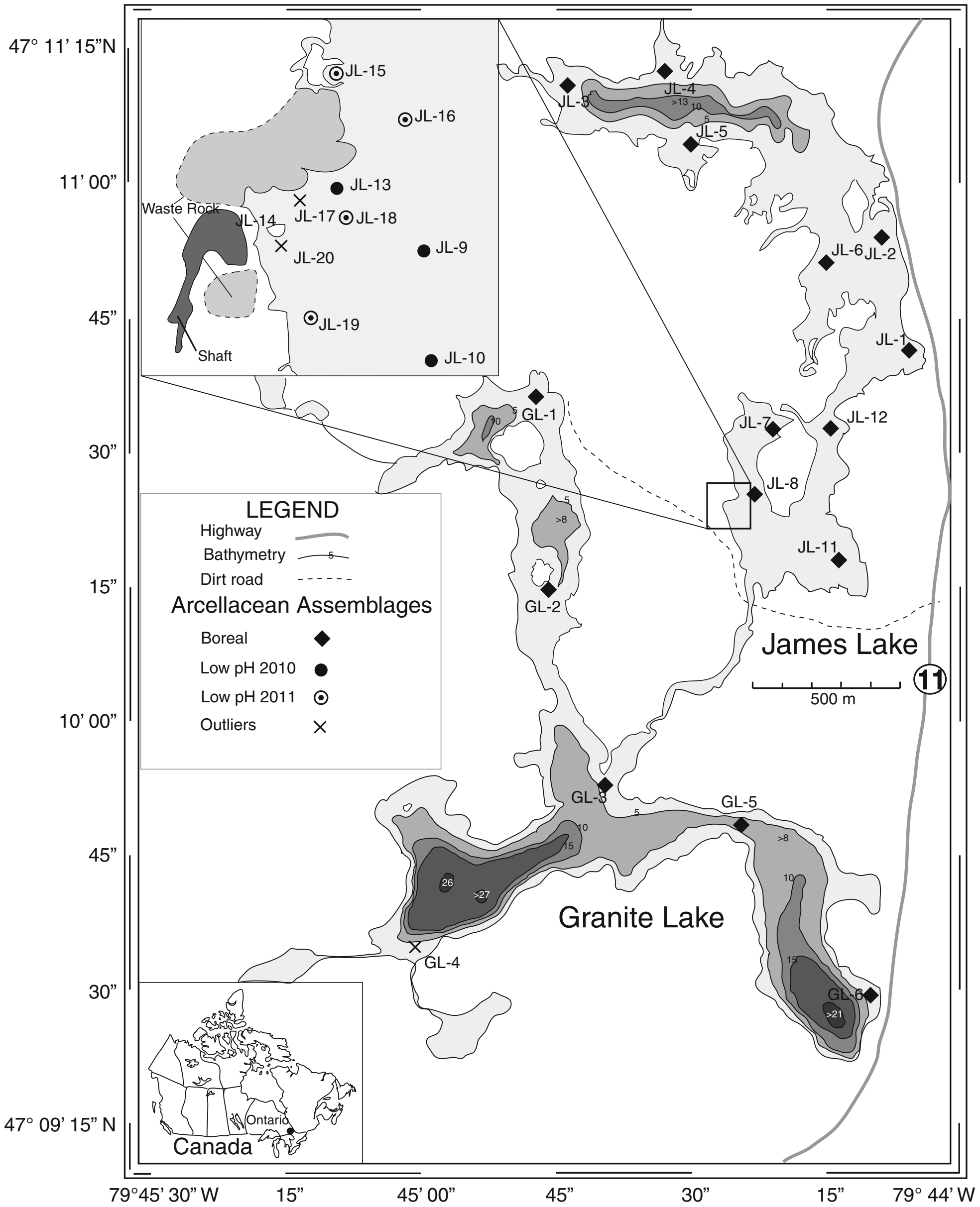


Fig. 1 Location of James and Granite lakes, including sites sampled with assemblage type. Contour interval is set at 5 m

through an equally small outflow stream at the south end, which flows into the adjacent oligotrophic Granite Lake [23]. The underlying geology of the area, primarily comprised Keewatin age volcanic rocks of felsic and mafic composition, and the surrounding vegetation, is made up of a mixed boreal forest of balsam fir (*Abies balsamea*), white birch (*Betula papyrifera*), white pine (*Pinus strobus*), and trembling aspen (*Populus tremaloides*).

In 1903, sulfide-rich deposits were discovered in lenses of greenschist on the southwest shore of James Lake. The Northland Pyrite Mine Co. (NPMC) operated a mine there from 1906 to 1911, excavating a deep trench and producing 38,000 tonnes of pyrite, which were sent to cobalt for production of sulfuric acid used in the extraction of silver from mine operations there [22]. Spoil piles of low-grade ore containing pyrite, pyrrhotite, and chalcopyrite were disposed of along the shore of the lake (Supplementary Fig. 1). The disposal of sulfide-rich rock resulted in leaching and subsequent metal-rich acid mine drainage (AMD) into the lake, which is a common and widespread problem in similar settings elsewhere (e.g., [15–17]). At the NPMC site, AMD was subsequently blamed for acidification of the lake substrate and the blanketing of the adjacent lake bottom with clay- and sand-sized particles of iron oxides, which both consumed the available oxygen and smothered benthic organisms [21]. As a result of the AMD, metal concentrations in the sediments adjacent to the spoil piles also became very high, further impacting benthic organisms. Although mining activity impacted lake health in the area adjacent to the mine site, the presence of low diversity, stressed arcellacean assemblages in a core collected in the lake adjacent to the mine tailing piles indicates that a low-pH environment was in place in the southern basin of James Lake by at least 1,300 years before present [21]. This was not an unusual finding as background metal concentrations can often be high in lake sediments near mining operations ([17]). The results of Kumar and Patterson [21] thus suggested that natural exposure of the sulfide-rich ore body to lake water at the shoreline was already impacting the lake long before mining activity commenced.

An unpublished Ontario Ministry of the Environment (OMOE) study carried out in 1990 noted low pH values in James Lake near the mine site and significantly also found concentrations of Fe, Al, and SO_4 in lake water from the southern basin of the lake to be above acceptable drinking water limits as determined by the Ontario Water Resources Act and its replacement, the Ontario Safe Drinking Water Act of 2002 [24]. As a remediation measure, a limestone causeway was built to separate the highly contaminated and water-filled mine trench and an adjacent artificial tailings pond from the lake. The presumed reason for using crushed limestone to build the causeway was that it would act to buffer the AMD low-pH water that was fed by ground water flow, continually emanated from the trench. At present, AMD water from the trench freely flows through the porous causeway and into the lake. Although the highest levels of contaminants are now restricted to the pond and trench, they are still well above OMOE guideline levels in the area of James Lake immediately adjacent to the mine spoil piles [24]. The pH is still very low in the lake waters adjacent to the mine site (pH=2.0–3.9; Table 1) as well, at least partially related to the underlying geology of the site. “Green holes”, lake bottom depressions approximately 0.5 m across and characterized by a distinctive green and blue-green algae flora, are found scattered across the lake bottom adjacent to the mine tailing piles [22]. The green holes are interconnected by a series of linear green features, several centimeters across, which are the lake bottom expression of faults in the underlying bedrock. The faults and green holes are additional conduits for the discharge of low pH AMD hydrogen sulfide-rich groundwater into the lake, which creates localized low-pH reduced environment ideal for algal blooms. As natural massive sulfide deposits are still exposed to the lake, Kumar and Patterson [21] questioned the design of the causeway as a remediation strategy, particularly as trench water still freely flows into the lake and pH in the lake near the old mine site is naturally very low due to groundwater flow.

Materials and Methods

Field and Laboratory Methods

Six stations from the James Lake northern basin, 14 from James Lake southern basin, and 6 downstream stations in Granite Lake, were sampled over two seasons in August 2010 and August 2011. Stations were broadly distributed, although there was a higher concentration of stations sited near the Northland Pyrite mine site, including four from green holes. A Garmin 76CSx GPS unit was used to record sampling coordinates. A YSI Professional Plus handheld multi-parameter instrument equipped with quatro cables was used to record pH, temperature (in degree Celsius), conductivity (in microsiemens), and dissolved oxygen (in milligrams per liter) at 0.5 m depth intervals through the water column and at the sediment–water interface.

In the laboratory, 5 cc subsamples of sediment for micropaleontological analysis were wet sieved through a 37-μm mesh to remove clay and fine silt and subsequently separated into aliquots for counting using a wet splitter [25]. Arcellaceans were quantified using an Olympus SZH10 dissecting light binocular microscope (typically at ×40–80 magnification). Loss on ignition was carried out to measure total organic carbon and carbonate in the sediment (after

Table 1 Water properties (pH, depth (in meters)); nutrient concentration (in parts per million) of NH3, TKN, and OP; geochemistry (in parts per million) for metals; loss on ignition (in percent) for water, organics, and carbonate; grain size data (in percent) for sand, silt, clay, and magnetic susceptibility (low frequency; mag) results

	Depth (m)	pH	NH3	TKN	P	Al	Ba	Ca	Cu	Fe	Pb	Mg	K	Si	Na	Ti	Zn	Sand (%)	Silt (%)	Clay (%)	Water (%)	Organics (%)	Carbonates (%)	mag
JL-1	2	6.33	401	18,500	726	8,860	229	10,600	109	31,900	43	2,400	540	345	1930	121	317	56.5	35.2	8.4	91.4	3.5	0.4	5.5
JL-2	2	6.55	471	18,800	762	10,100	214	11,800	91	34,300	35	2,010	610	302	1280	100	231	18.0	68.3	13.7	94.4	1.7	0.1	1.5
JL-3	4.25	6.73	8.93	5,520	459	12,700	54	5,090	28	26,100	11	6,680	180	351	130	474	112	76.1	21.0	2.9	81.5	5.6	0.2	1.8
JL-4	2.5	6.51	64.9	16,100	534	7,680	72	7,970	68	12,800	21	1,140	250	268	450	<100	124	41.7	50.7	7.7	94.3	2.0	0.3	1.2
JL-5	4.25	6.51	11.2	12,600	524	11,000	60	7,810	84	19,900	50	1,300	230	328	370	104	166	67.3	28.4	4.2	94.0	2.1	0.4	1.8
JL-6	2.5	6.55	76.1	11,000	332	8,390	72	6,820	76	16,200	26	1,020	190	304	390	103	150	65.5	29.5	5.0	95.3	1.7	0.3	0.8
JL-7	1.9	6.9	76.7	14,800	714	18,900	37	3,360	579	275,000	63	<1,000	210	408	280	<100	475	9.1	79.9	11.0	92.0	2.9	0.3	10.6
JL-8	2.5	6.7	136	8,200	741	18,000	52	2,150	458	381,000	43	<1,000	170	2,200	790	<100	273	29.5	62.5	8.0	95.1	1.3	0.4	2.7
JL-9	3	3.9	171	4,460	916	19,200	5	1,020	812	555,000	48	<1,000	30	2,980	110	600	534	85.8	12.8	1.4	92.3	1.7	0.5	3.2
JL-10	4	3.9	144	5,820	908	18,100	5	<1,000	499	504,000	52	<1,000	40	2,890	150	579	357	74.0	23.9	1.8	93.6	1.3	0.4	2.5
JL-11	2	6.7	34.8	11,500	502	23,000	40	4,160	575	317,000	68	<1,000	200	509	250	<100	678	65.4	29.1	5.5	92.9	2.0	0.5	7.7
JL-12	2	6.31	112	19,400	812	11,000	68	6,800	280	96,800	53	1,210	300	345	340	111	477	9.5	72.3	18.2	94.6	2.4	0.3	2.8
JL-13	1.25	2.12	225	17,800	950	15,500	35	4,620	996	60,400	28	1,800	70	394	190	1,750	121	60.9	35.8	3.3	94.6	3.6	0.2	6.3
JL-14	0.75	2.07	8.2	1,370	383	2,050	30	<1,000	167	347,000	36	<1,000	80	286	20	1,250	<100	34-8	48.0	17.2	62.1	6.6	1.4	14.8
JL-15	1	2.69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
JL-16	1	2.49	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
JL-17	1	2.12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
JL-18	2	3.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
JL-19	2.5	2.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
JL-20	1.75	2.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GL-1	2.25	6.5	28.1	10,800	465	8,190	106	15,900	142	28,000	49	2,130	310	384	530	157	231	71.6	24.1	4.4	91.2	5.3	0.2	4.5
GL-2	2.9	6.5	2.07	1,180	353	7,660	17	2,400	20	19,100	10	4,230	180	420	110	393	<100	73.3	22.5	4.2	53.8	1.6	0.3	32
GL-3	4.95	6.5	2.38	1,460	372	6,290	23	1,980	26	23,000	11	3,490	190	435	90	326	113	79.8	14.7	5.5	46.9	1.9	0.3	27.5
GL-4	1.2	6.5	4.34	327	364	12,000	35	1,850	8	27,700	<5	6660	140	530	60	585	<100	97.5	1.9	0.5	29.2	1.1	0.5	18
GL-5	7	6.5	4.65	1,700	680	9,770	42	10,300	58	27,200	13	10,500	470	612	180	566	<100	12.1	77.6	10.4	52.6	1.8	1.1	79
GL-6	4.75	6.5	64.7	2,900	479	10,400	73	3,090	73	32,300	49	5,760	450	444	250	566	192	73.4	22.1	4.6	63.2	2.3	0.3	60.6

ICP-MS data were not available for samples JL-15, JL-16, JL-17, JL-18, JL-19, and JL-20

[26]). Grain size analysis (percent clay, silt, and sand) was carried out on each sample using a Beckman Coulter LS 13 320 Particle Size Analyzer. Environmentally available metals known to be important paleoenvironmental indicators were commercially analyzed from the sediments using inductively coupled plasma mass spectrometry (ICP-MS) following US EPA 6010-C methodology ([27]; Supplementary Table 1). Sedimentary phosphorus was measured to determine the lake trophic status, using the Olsen's phosphorus (sedimentary Olsen P (OP)) extraction method (after [28]). This approach provides a measure of bioavailable phosphorus and is a suitable extraction method for samples of neutral to alkaline pH but is less reliable in low pH regimes such as exists in James Lake near the NPMC site [29]. The phosphomolybdate colorimetric technique was used to measure phosphorus concentrations (after [30]).

Taxonomy

Thecamoebians, also known as testate amoebae, are an artificial polyphyletic grouping of unrelated testate amoeboid protozoans within the subphylum Sarcodina Schmarda, 1871 that includes the orders Arcellinida Kent, 1880, and Gromida Claparède and Lachmann, 1859, along with part of the suborder Allogromiina Loeblich and Tappan, 1961, of the order Foraminiferida Eichwald, 1830 [31]. Paleontologists and limnologists primarily use the term thecamoebian, while testate amoebae is used by soil scientists and peatlands researchers [1]. Although there is a long tradition of usage in the literature, neither the terms thecamoebian nor testate amoebae have validity under the provisions of the International Commission on Zoological Nomenclature [32]. The orders Arcellinida, Gromida, and Allogromiina are valid though [31]. At higher taxonomic levels, these protozoans are classified based on the nature of their pseudopodia [31]. The Superfamily Arcellacea Ehrenberg, 1832 within the Arcellinida (subclass Lobosia Carpenter, 1861; class Rhizopodea von Siebold, 1845) are characterized by lobose pseudopods. Genetic analysis recently confirmed the taxonomically distinct nature of Arcellacea [2]. As species found in wet-seived preparations are almost exclusively attributable to species of Arcellacea, this is the most appropriate term to apply in lacustrine studies.

Arcellacean species and strains were identified following Roe et al. [11] and Patterson et al. [10] with reference to standard limnological reference keys (e.g., [33, 34]). As lacustrine arcellacean species can display a significant amount of environmentally controlled morphological variability (e.g., [34, 35]), the accepted practice by researchers has been to designate informal infrasubspecific "strain" names for these ecophenotypes [1, 36]. Infrasubspecific level designations have no status under the International Zoological Code of Nomenclature [32]. However, they are useful for delineating environmentally significant populations within lacustrine environments [6, 15, 17, 18, 20, 22, 37]. Scanning electron micrograph images of common species and strains were obtained using a Tescan Vega-II XMU VP scanning electron microscope at the Carleton University SEM facility.

Statistical Methods

Thirty arcellacean species and strains were identified in the 26 sediment–water interface samples collected. The probable error (pe) was calculated for each sample using the formula:

$$\mathrm{pe} = 1.96\left(\frac{s}{\sqrt{X_i}}\right) \tag{1}$$

where s is the standard deviation of the population count and x_i is the fractional abundance [23]. A sample was deemed statistically insignificant if probable error exceeded the total count for a sample. Sample GL-4 was barren and sample JL-14 contained a statistically insignificant population. Twenty-four samples were thus utilized in the subsequent multivariate analysis. Standard error (S_{Xi}) was calculated for each species using the following formula:

$$S_{X_i} = 1.96\sqrt{\frac{F_i(1-F_i)}{N_i}} \tag{2}$$

A species was considered to be present in insignificant numbers if the standard error exceeded the total count for that species in all samples [38]. All species identified in the study were found to be significant and were thus included in the subsequent multivariate analysis.

Shannon diversity index (SDI) was calculated to assess the faunal diversity in each sample and provides an indication of relative lake ecosystem health [39]. SDI is defined as:

$$\mathrm{SI.} = -\sum_{i=1}^{S}\left(\frac{X_i}{N_i}\right) \times \ln\left(\frac{X_i}{N_i}\right) \tag{3}$$

where X_i is the abundance of each taxon in a sample, N_i is the total abundance of the sample, and S is equal to the species richness of the sample. SDI between 2.5 and 3.5 is considered stable, between 1.5 and 2.5 is in transition, and below 1.5 is considered stressed [6, 40].

R-mode cluster analysis was carried out using PC-Ord to find characteristic species associations. With the same software, Q-mode cluster analysis was carried out using Ward's minimum variance method and recorded as squared-Euclidean distances (after [41]). Q-mode and R-mode cluster analyses were carried out on the 30 arcellacean species and strains in the 22 statistically significant samples and organized into a hierarchical dendrogram. Detrended correspondence analysis (DCA) was used to plot assemblages in multidimensional space to compare similarity. A two-way Q-mode versus R-mode cluster analysis plot (Fig. 2) and DCA (Fig. 3) were used to assign assemblage groups to samples and view species composition.

The relationships between arcellacean assemblages and measured environmental variables were assessed by means of several statistical techniques using CANOCO version 4.5 and CANODRAW [42–44, 68]. Redundancy analysis (RDA) was carried on the 18 samples that underwent environmental analysis, to explain the observed clustering using measured variables. As ICP-MS analysis was not carried out on samples JL-15, JL-16, JL-17, JL-18, JL-19, and JL-20, they were excluded from the RDA. No data transformations were applied to the fractional abundance data since DCA revealed that the gradient length of the species data represented a linear response (<2.00). Gradient lengths greater than 2.00 represent a unimodal response, and since RDA is a linear-based analysis. If that had been the case, the species data would have been transformed (Hellinger transformation) from unimodal to linear [45, 46]. Pearson correlation analysis was used to determine the intercorrelations between environmental variables to determine the degree of redundancy in the data set [47]. Forward selection was used to test the percent variance explained by each environmental variable and at what level of significance. A variable was included if its *p* value was

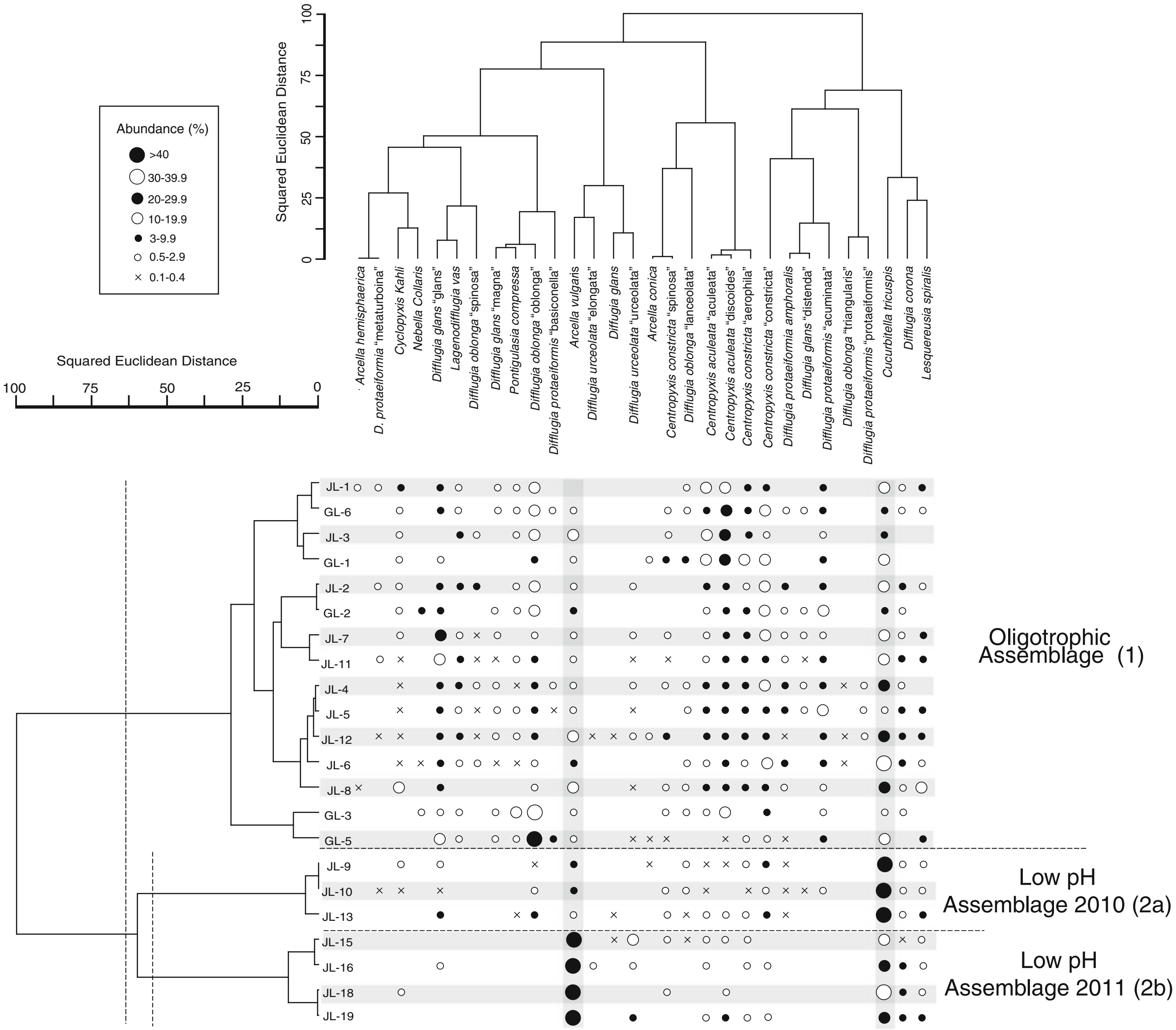


Fig. 2 R-mode vs. Q-mode cluster diagram for the 22 samples with statistically significant arcellacean counts. Two faunal assemblages are indicated, including one sub-subassemblage (a, b) for assemblage 2. The *dashed lines* discriminates clusters of samples with correlation coefficients greater than the selected level of significance. *JL* = James Lake; *GL* = Granite Lake

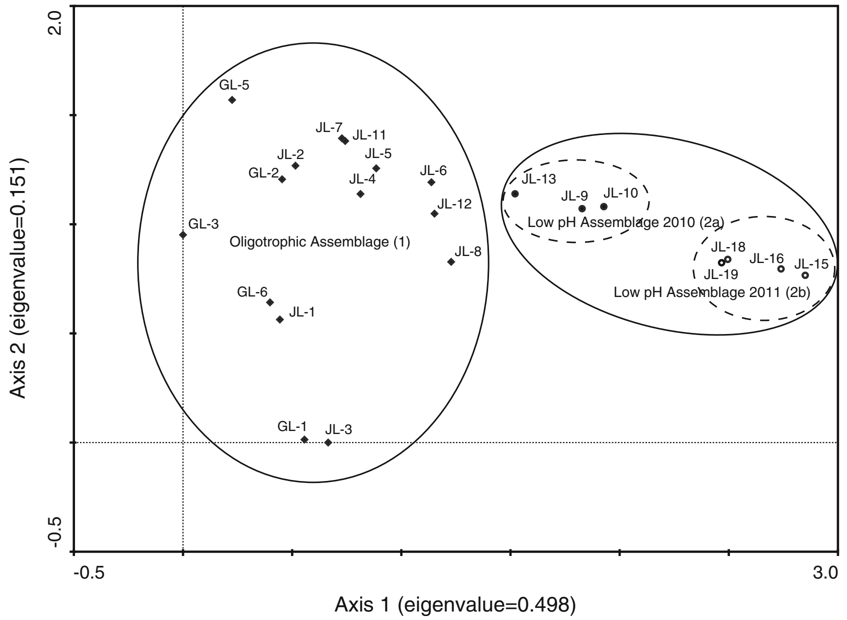


Fig. 3 Detrended correspondence analysis (DCA) results showing principal patterns of variation in the arcellacean populations for the analyzed samples. Clusters are *circled* with sub-clusters in *dashed circles*. *JL* = James Lake; *GL* = Granite Lake

less than 0.08 or if it has been shown to be influential in previous studies. The results of the RDA give the total variance explained by each variable, indicating which variables are strong drivers. SDI was plotted as a passive variable on the diagram and therefore did not impact the analysis (Figs. 4 and 5).

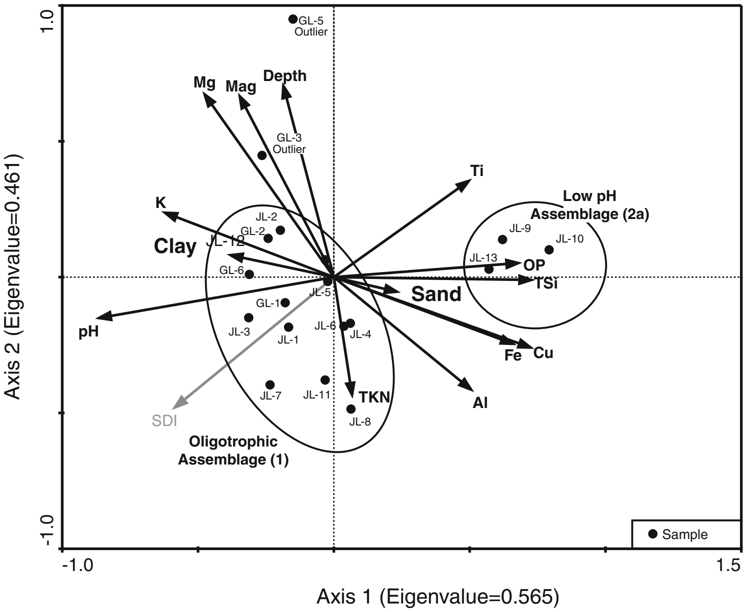
Results and Discussion

Arcellacean Zonation

The geography of James Lake plays an important role in the distribution of arcellacean assemblages. The north–south flow regime, coupled with the narrow bottleneck separating the north and south basins, results in any limnological influence from the vicinity of the Northland Mine site being restricted to the southern basin [21]. Interpretation of the Q- and R-mode cluster analysis resulted in recognition of two distinct arcellacean assemblages, one of which was subdivided: (1) "Oligotrophic Assemblage", (2a) "Low pH Assemblage 2010", and (2b) "Low pH Assemblage-2011" (Fig. 2). The DCA analysis produced identical results to that obtained by cluster analysis (Fig. 3). The RDA provides an additional quantification of the proportion of the variance in the arcellacean data set that can be attributed to measured environmental variables and provided confirmation that several factors influenced the faunal distribution (Figs. 4 and 5). RDA axes one (eigenvalue=0.565) and two (eigenvalue=0.461) explain 64.4 % of the total variance in the species data and 69.0 % of the species environmental relationship, while the measured environmental variables explain 69.4 % of the variation in the arcellacean data (Fig. 4). The Monte Carlo permutation tests show that the first and all canonical axes are significant at $p< 0.0002$. Based on the results of previous studies carried out in James Lake, it is not surprising that the most significant control on arcellacean distribution is pH, which explains a very high 14.1 % ($p<0.002$) of the total variance. Quantification of the sensitivity of arcellaceans to this important limnological variable, from highest values in the northern basin to very low values in the vicinity of the Northland Pyrite Mine site, is a noteworthy result. Other apparent controls include total silica (TSi at 7.9 %; $p<0.002$), OP (at 3.4 %; $p<0.002$), copper (Cu at 3.4 %; $p<0.002$), and iron (Fe at 1.9 %; $p<0.002$). The highest levels for these variables are similarly restricted to the southern basin (Table 1). The specific significance of these variables are discussed below in the context of the faunal assemblages discriminated by cluster and DCA analyses.

Oligotrophic Assemblage (1)

This group consists of all populations found in the northern basin of James Lake, Granite Lake, and parts of the southern basin of James Lake further than 50 m from the pyrite mine site. Arcellacean species and strain makeup is variable but diverse. The calculated SDI ranges from 1.25 to 2.45, characteristic of a generally healthy boreal lake assemblage [6]. Samples within this assemblage are positively correlated with Mg, magnetic susceptibility, depth, K, clay, pH, TKN, and SDI (Fig. 4) and negatively correlated with Ti, sand, Olsen's P, TSi, Fe, Cu, and Al. All environmental variables are well fitted to the RDA, with both clay and sand having the weakest fit. Of all measured variables, pH is the most significant. The close correlation between pH and



Variable	%	P<
pH	14.06	0.002
Cu	3.422	0.002
TSi	7.984	0.002
OP	3.422	0.002
Fe	1.901	0.002
K	2.661	0.008
Mg	3.422	0.014
Al	2.281	0.008
Ti	5.323	0.086
mag	2.661	0.064
depth	3.422	0.118
clay	3.802	0.128
TKN	6.083	0.294
sand	4.942	0.43
Intercor.	69.40	n/a

Fig. 4 Redundancy analysis (RDA) sample–environment biplot for the 18 samples that yielded statistically significant arcellacean populations and were analyzed by ICP-MS. Assemblages identified by Q-mode cluster analysis and DCA are *circled*. Percentage variance explained by each environmental variable and its corresponding *p* value as calculated by variance partitioning (p=RDA) are shown in the table. Shannon Diversity Index (SDI) was plotted passively. *Mag* = low frequency magnetic susceptibility, *TKN* = total Kjeldahl nitrogen, *TSi* = total silica, *OP* = Olsen's phosphorus, *JL* = James Lake, and *GL* = Granite Lake

axis 1 indicates that it explains much of the observed species variation. The positive relationship with pH in these samples reflects their higher pH levels. The influence of pH on arcellacean assemblage structure will be discussed below in the context of the Low pH Assemblage (2a, 2b) (Fig. 4).

The oligotrophic assemblage generally comprised *Cucurbitella tricuspis* (Carter, 1856), *Difflugia oblonga* (Ehrenberg, 1832) strain "oblonga," *Difflugia glans* (Penard 1902) strain "glans," *Centropyxis aculeata* (Ehrenberg 1832) strain "discoides," *C. aculeata* (Ehrenberg 1832) strain "aculeata," *Centropyxis constricta* (Ehrenberg 1843) strain "aerophila," *C. constricta* (Ehrenberg 1843) strain "constricta," *Arcella vulgaris* (Ehrenberg 1830), and *Difflugia protaeiformis* (Ehrenberg 1830) strain "acuminata" as primary contributors in most samples (Fig. 2). Other species such as *Cyclopyxis kahli* (Deflandre, 1929), *Lagenodifflugia vas* (Leidy 1874), *Difflugia oblonga* (Ehrenberg 1832) strain "spinosa," *D. glans* strain "magna," *Pontigulasia compressa* (Carter 1864), *Difflugia urceolata* (Carter 1856) strain "urceolata," *C. constricta* (Ehrenberg 1843) strain "spinosa," *D. oblonga* strain "lanceolata," *Difflugia protaeiformia* (Ehrenberg 1830) strain "amphoralis," *Difflugia corona* (Wallich, 1864), and *Lesquereusia spiralis* (Ehrenberg 1840) occur commonly but not necessarily in all samples (Fig. 6).

Cucurbitella tricuspis is a dominant species in most James Lake stations, although significantly more so at sites from the southern basin. This species is most common in mesotrophic and eutrophic lakes and ponds where it has a symbiotic relationship with *Spirogyra* algae [34, 35, 48, 49]. A close correlation between the distribution of *C. tricuspis* and OP levels has been noted elsewhere ([10, 11, 68]). The correlation is further confirmed here based on the RDA analysis results where OP (Fig. 4) shows a strong positive correlation with *C. tricuspis* along axis 1 (Fig. 5). OP has a major impact on water quality as it acts as a limiting factor in controlling primary productivity in freshwater environments [50]. Algal blooms and eutrophication associated with excess OP deplete oxygen levels in aquatic environments, resulting in water quality degradation. In James Lake, *C. tricuspis* abundance is thus a proxy for algal productivity. The average OP concentration in the James Bay southern basin is 825 ppm, which increases to 879 ppm adjacent to the mine site. In contrast, OP concentrations are 557 ppm in the northern basin of James lake and 452 ppm in Granite Lake. It is therefore most likely that the source of OP is natural and related to groundwater input from either the mine site itself or the adjacent fault fractures on the lake bed. *C. tricuspis* is an excellent indicator species for characterizing the trophic state of lakes. The characterization of James Lake as a mesotrophic lake and Granite Lake as an oligotrophic lake could thus in large part be based on the relative abundance of *C. tricuspis,* without reliance on other analyses.

There is also a close correlation between OP and TSi in the RDA analysis along axis 1 (Fig. 4). An important component of TSi is amorphous biogenic silica (BSi), which is an important lacustrine nutrient [51]. Organisms such as diatoms extract dissolved BSi from the water column to build their frustules [52]. Diatoms are useful proxies for studies related to eutrophication and BSi has been shown to be a good proxy for diatom productivity [53]. The measured TSi values at stations on the faulted lake bottom adjacent to the mine site are very high, ranging up to 2,980 ppm. As discussed above, AMD seepage from the mine site and up through the fractures on the lake bottom

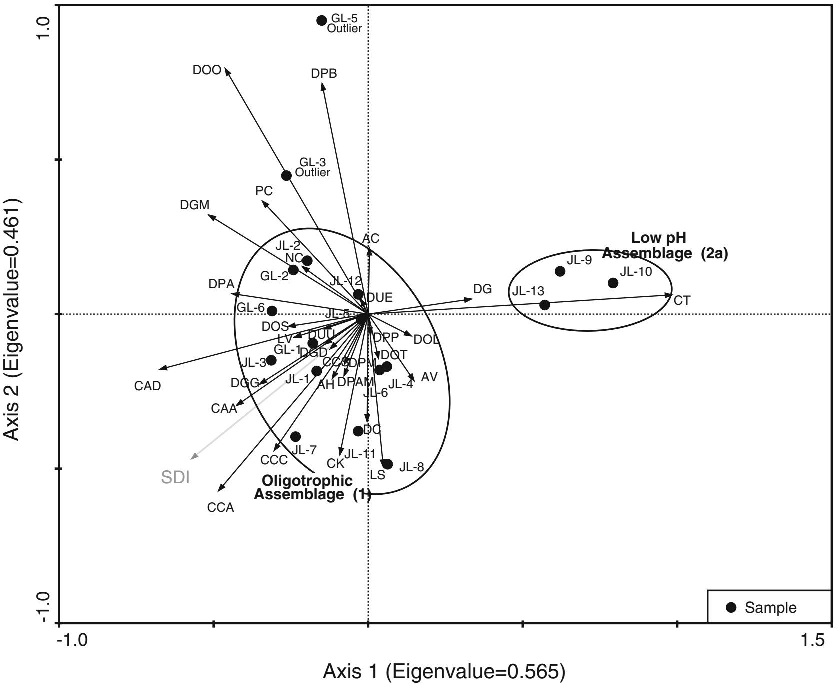


Fig. 5 RDA species–environment biplot. SDI (Shannon Diversity Index) is plotted passively. *AC* = *Arcella conica*, *AH* = *Arcella hemisphaerica*, *AV* = *A. vulgaris*, *CAA* = *C. aculeata* "aculeata," *CAD* = *C. aculeata* "discoides," *CAS* = *C. aculeata* "spinosa," *CCA* = *C. constricta* "aerophila," *CCC* = *C. constricta* "constricta," *CCS* = *C. constricta* "spinosa," *CK* = *C. kahli*, *CT* = *C. tricuspis*, *DPAM* = *D. protaeiformis* "amphoralis," *DC* = *D. corona*, *DGD* = *D. glans* "distenda," *DGG* = *D. glans* "glans," *DGM* = *D. glans* "magna," *DOL* = *D. oblonga* "*lanceolata*," *DOO* = *D. oblonga* "*oblonga*," *DOS* = *D. oblonga* "spinosa," *DOT* = *D. oblonga* "triangularis," *DPM* = *Difflugia metaturboina*, *DPA* = *D. protaeiformis* "acuminata," *DPB* = *D. protaeiformis "basiconella," DPP* = *D. protaeiformis "protaeiformis," DUE* = *D. urceolata "elongata," DUU* = *D. urceolata "urceolata," LV* = *L. vas, LS* = *L. spiralis, PC* = *P. compressa*

significantly influences the limnology of the lake. The elevated levels of TSi in this area are therefore probably derived from this groundwater flow. Similar groundwater influx into lakes has elsewhere been demonstrated to be a significant source of silica for diatom production ([54]). Although neither BSi or diatoms were specifically analyzed in this study, the close correlation of TSi with known eutrophication indicators OP and *C. tricuspis* is most likely related to diatom productivity. We interpret the apparent correlation between the arcellacea and TSi to be indirect, most likely representing a covariant response of both diatoms and arcellacea to OP fertilization.

Despite the outflow from James Lake to Granite Lake being very near the Northland Pyrite Mine site (Fig. 1), metal levels in Granite Lake were not elevated and the pH level (pH=6.5) was as expected for an oligotrophic lake in the area [23]. The arcellacean fauna was also very similar to that found in the northern basin of James Lake and distal areas of the southern basin away from the mine site. The protection of Granite Lake from low pH water and other contaminants originating from James Lake may be related to the nature of the outflow stream. The stream is only a few centimeters deep and, midway to Granite Lake, disappears temporarily in a heavily vegetated marshy area, which may to act as a pH buffer and metal sink. Unfortunately, no analyses were done on sediments from the marsh to test this hypothesis.

It is noted that samples in both the Oligotrophic Assemblage (e.g., JL-7, JL-8, and JL-11) and Low pH Assemblage (e.g., JL-9, JL-10) are characterized by relatively high Fe and Cu levels. Iron oxides in sediments are excellent scavengers for metals and are impacted by changes in sediment Eh and pH [55, 56]. Similarly, redox-sensitive trace metals such as Cu provide important information on redox conditions [57]. Although beyond the scope of this paper, the strong pH gradient within James Lake undoubtedly has a significant impact upon the speciation behavior of redox-sensitive elements such as Fe and Cu, which in turn has a potential impact upon organisms through alteration of redox-regulated biomolecules and enzymes involved in redox reactions. The impact of redox-sensitive elements on arcellacean faunas has been documented in several mine impacted lakes [14–17]. The competing influence on these samples may explain why Fe and Cu plot between the oligotrophic and low pH assemblages (Fig. 4). A larger data set encompassing several lakes is required to definitively determine the influence of Fe and Cu on arcellacean distribution in this region.

Two samples, GL-3 and particularly GL-5, both from Granite Lake, clustered anomalously from the other samples in the Oligotrophic Assemblage (Figs. 2, 4, and 5). The main difference in the faunal make up of these two samples was a very high proportion of *D. oblonga* “oblonga” that varied from 38 to 45 %. Such a high abundance is more typical of more nutrient-rich lakes (e.g., [10]). As the faunal makeup and relative abundance of the other species in GL-3 and GL-5, as well as the assemblages from the other samples from Granite Lake, are clearly typical of oligotrophic conditions, these samples are considered to be outliers.

Low pH Assemblage 2010 (2a)

All samples from the Low pH Assemblage 2010 (2a) subassemblage were from the area of the lake located immediately adjacent to, and just south of, the mine site. This subassemblage exclusively comprised samples collected during August 2010.

The low SDI (0.62 and 1.22) indicates that this fauna perseveres in very stressed environmental conditions, as might be expected under the very low pH conditions (2.2–3.9) that characterized these stations. The overwhelming dominance of *C. tricuspis* in these samples is most likely the result of currents transporting specimens of this seasonally planktic species from elsewhere in the lake to a hostile environment area normally characterized by an impoverished arcellacean fauna dominated by *A. vulgaris*. *A. vulgaris* is a generalist taxa capable of surviving in a variety of stressed environments, including low pH conditions, heavy metal contamination, and brackish conditions [3, 20]. Although the elevated OP levels in the area surrounding the waste rock piles might conceivably facilitate colonization of *C. tricuspis* there, the low pH conditions would preclude the presence of this species unless it were attached to floating algae on the surface. Kumar and Patterson [21] did not observe significant populations of *C. tricuspis* in a core spanning the last 1,300 years taken from the same area of the lake, suggesting that this species typically has not been a prominent feature of the arcellacean fauna in this part of the lake. In addition, samples collected from the same area during August 2011, as discussed below, did not have such high *C. tricuspis* populations. This suggests that localized lake current conditions prevailing during the summer of 2010 contributed to the high proportions of *C. tricuspis* found in the lake near the waste rock pile. Similar allochthonous occurrences of *C. tricuspis* environmenta have been reported from nearby Gillies Lake [19]. In Gillies Lake, high concentrations of *C. tricuspis* found in sediment–water interface samples from cold (6 °C) deep water, low oxygen (2 mg/L) environments below the thermocline were attributed to current derived transport of specimens from shallow vegetated areas of the lake. Of species actually inhabiting this hostile environment, *A. vulgaris*, *C. constricta* strain “constricta”, *D. corona*, and *L. spiralis* are all important, with both *A. vulgaris* and *C. constricta* strain “constricta” (Fig. 6) having been previously identified as indicative of low pH and metal contamination in the region [6, 22, 23]. Escobar et al. [18] also found that there was a tendency for *L. spiralis* to be more abundant in lower pH Florida lakes. Escobar et al. [18] questioned the utility of *A. vulgaris* as a pH indicator, reporting that the species never exceeded 8 % in a study on Florida lakes where the pH varied from 4.6 to 8.7. A taxonomic issue may explain the discrepancy as the species illustrated as *A. vulgaris* in Escobar et al. [18, p. 723] is actually a specimen of *Centropyxis aculeatea* strain “discoides”. Escobar et al. [18] may be in part correct though as there is a much closer correlation in the RDA of this study between *A. vulgaris* distribution (Fig. 4) and Fe, Cu, and Al (Fig. 5) than with pH, although it is still slightly negatively correlated with pH. In northern areas, the main characteristics of low pH lacustrine arcellacean faunas are very low SDIs coupled with *A. vulgaris* being an important component.

Low pH Assemblage 2011 (2b)

The Low pH Assemblage 2011 (2b) subassemblage was identified in samples collected near the waste rock pile during August 2011 and like the Low pH Assemblage 2010 (2a) subassemblage was characterized by a low SDI (0.83–0.96) and high abundances of *A. vulgaris* and *C. tricuspis* followed by *D. corona*, *D. urceolata*, and *L. spiralis* (Fig. 6). Although not included in the RDA due to a lack of sediment geochemistry for these samples, cluster analysis and DCA results indicate that this assemblage is directly related to subassemblage 2a and, as discussed above, is typical of the fauna expected in a low pH environment. The primary difference between the two is that the relative abundance of the two dominant taxa are reversed, with *A. vulgaris* being very abundant in these samples and *C. tricuspis* dominating in subassemblage 2a. As discussed above, seasonal variation in the transport of *C. tricuspis* to the area of the waste rock pile is most likely the cause for the year-to-year variation in assemblage composition. Sample JL-14 collected from a contaminated pond within the actual mine site (pH=2.6) would most likely have clustered with these samples but was excluded from ordination as it had a statistically insignificant population comprised only a few *A. vulgaris* specimens. Conditions within the pond are too stressed to support a significant population of even the most tolerant species.

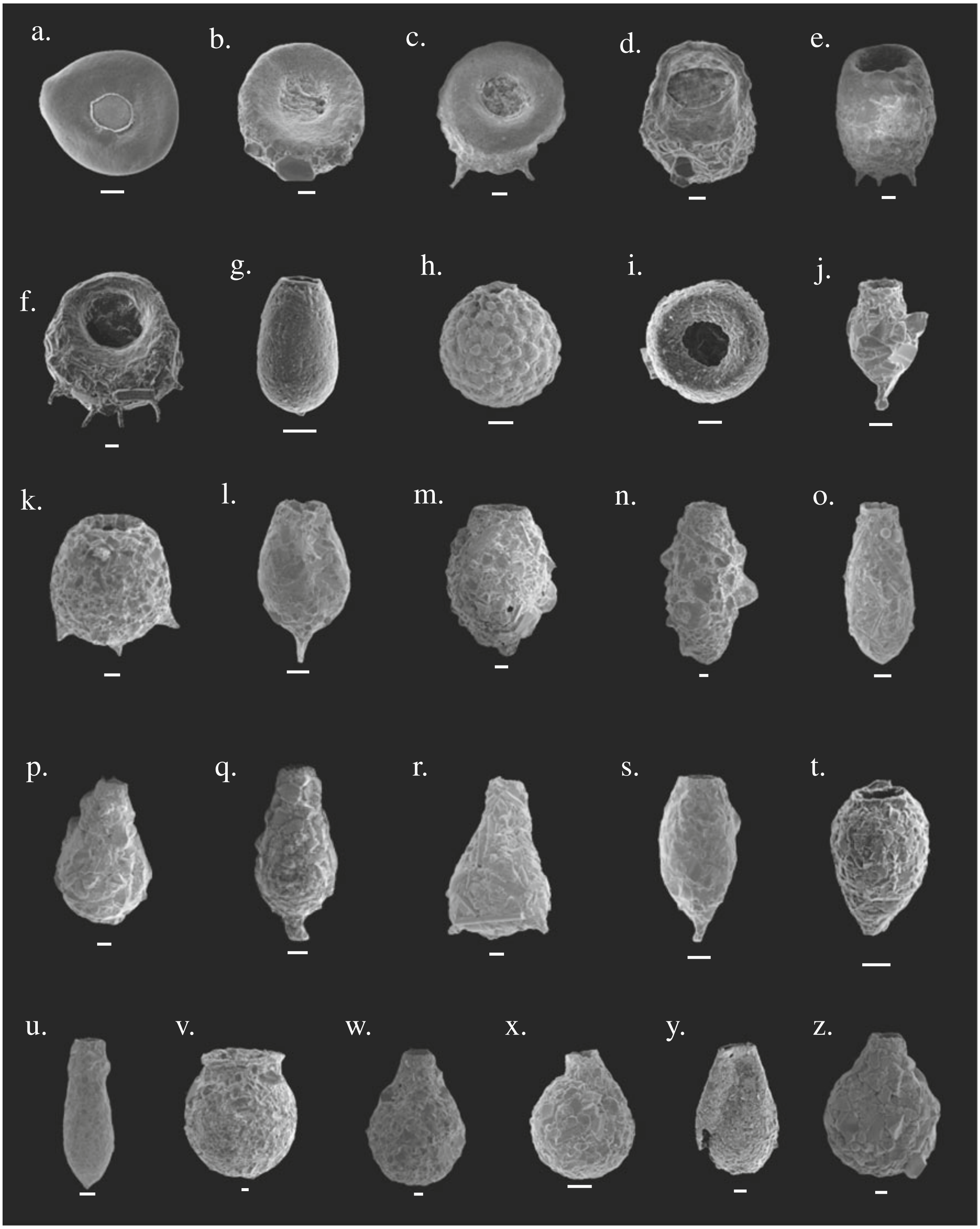


Fig. 6 **a** *A. vulgaris* (Ehrenberg 1830). **b** *C. aculeata* (Ehrenberg 1832) strain “discoides,” scale bar=20 μm. **c** *C. aculeata* (Ehrenberg 1832) strain “aculeata,” scale bar=20 μm. **d** *C. constricta* (Ehrenberg 1843) strain “aerophila,” scale bar=20 μm. **e** *C. constricta* (Ehrenberg 1843) strain “constricta,” scale bar=20 μm. **f** *C. constricta* (Ehrenberg 1843) “spinosa,” scale bar=10 μm. **g**, **h** *C. tricuspis* (Carter 1856), scale bars=20 μm. **i** *C. kahli* (Deflandre 1912). Scale bar=20 μm. **j** *D. protaeiformis* (Ehrenberg 1830) strain “amphoralis,” scale bar=20 μm. **k** *D. corona* (Wallich, 1864), scale bar=10 μm. **l** *D. glans* (Penard 1902) strain “distenda,” scale bar=10 μm. **m** *D. glans* (Penard 1902) strain “glans,” scale bar=10 μm. **n** *D. glans* (Penard 1902) strain “magna,” scale bar=10 μm. **o** *D. oblonga* (Ehrenberg, 1832) strain “lanceolata,” scale bar=20 μm. **p** *D. oblonga* (Ehrenberg, 1832) strain “oblonga,” scale bar=20 μm. **q** *D. oblonga* (Ehrenberg, 1832) strain “spinosa” (Ehrenberg, 1832), scale bar=20 μm. **r** *D. oblonga* (Ehrenberg, 1832) strain “triangularis,” scale bar=20 μm. **s** *D. protaeiformis* (Ehrenberg 1830) strain “acuminata,” scale bar=20 μm. **t** *D. protaeiformis* (Ehrenberg 1830) strain “basiconella,” scale bar=20 μm. **u** *D. protaeiformis* (Ehrenberg 1830) strain “protaeiformis,” scale bar=20 μm. **v** *D. urceolata* (Carter 1856) strain “urceolata,” scale bar=10 μm. **w** *L. vas* (Leidy 1874). Characteristic neck constriction not clear in SEM image. Scale bar=20 μm. **x** *L. spiralis* (Ehrenberg 1840), scale bar=20 μm. **y** *Nebela collaris* (Ehrenberg 1848), scale bar=10 μm. **z** *P. compressa* (Carter 1864), scale bar=10 μm

Conclusions

This research provides quantitative evidence that there is a strong link between arcellacean faunas and pH levels based on the analysis of 30 species and strains from 22 samples in James and Granite lakes. The stations were selected to span a significant pH gradient from higher pH environments in the northern basin of James and Granite lakes to very low pH environments adjacent to the Northland Pyrite Mine site in the southern basin of James Lake. Ordination (RDA) indicated that pH was the greatest influence on assemblage structure, explaining 14.1 % ($p<0.002$) of the total variance. Two primarily pH influenced arcellacean assemblages were also identified. The Oligotrophic Assemblage (1) typifies high pH environments characterized by diverse arcellacean faunas and high SDI values, while the depauperate Low pH Assemblage (2a, 2b) is characterized by very low SDI values.

Nutrient enrichment (TSi and P), originating from groundwater entering the lake from a trench at the old mine site and from faults visible on the adjacent lake bottom, influences productivity in the southern basin. As has been noted elsewhere [11], OP (here explaining 3.4 % of variance; $p<0002$) is an important control on assemblage structure and is closely linked with the relative abundance of the eutrophication indicator species *C. tricuspis*. The apparent close correlation between TSi (explaining 7.9 % of variance; $p<0002$) and *C. tricuspis* abundance is probably not directly linked to the arcellacean productivity though. It is more likely that TSi, as an indirect measure of BSi, is acting as a proxy for diatom productivity. As both the arcellaceans and diatoms are directly responding to OP enrichment, it is a covariant relationship that is observed. The strong pH gradient in the southern basin of James Lake has a strong influence on redox-sensitive elements such as Fe and Cu, which in turn potentially influences the distribution of benthic organisms in the basin. A much larger data set is required to quantify this relationship as the outcome of the RDA analysis where Fe and Cu plotted directly between the oligotrophic and low pH assemblages was indeterminate.

Acknowledgments This research was supported by a NSERC Discovery Grant to RTP. We thank the Carleton University Institute of Environmental Science for field support, Carley Crann for cartographic support, Graeme Swindles for assistance with the statistical analysis, Jianqun Wang for SEM photography and three anonymous reviewers for their constructive comments.

References

1. Patterson RT, Kumar A (2002) A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeogr Palaeocl Palaeoecol 180:225–251
2. Mitchell EAD, Charman DJ, Warner BG (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodivers Conserv 17:2115–2137
3. Medioli FS, Scott DB (1988) Lacustrine thecamoebians (mainly arcellaceans) as potential tools for palaeolimnological interpretations. Palaeogeogr Palaeocl Palaeoecol 62:361–386
4. Neville LA, McCarthy FMG, MacKinnon MD (2010) Seasonal environmental and chemical impact on thecamoebian community composition in an oil sands reclamation wetland in Northern Alberta. Palaeontol Electron 13.2.13A:14
5. Neville LA, McCarthy FMG, MacKinnon MD, Swindles GT, Marlowe P (2011) Thecamoebians (testate amoebae) as proxies of ecosystem health and reclamation success in constructed wetlands in the oil sands of Alberta, Canada. J Foramin Res 41:230–247
6. Patterson RT, Kumar A (2000) Assessment of arcellacean (thecamoebian) assemblages, species, and strains as contaminant indicators in James Lake, Northeastern Ontario, Canada. J Foramin Res 30:310–320
7. Wall A, Gilbert D, Magny M, Mitchell E (2010) Testate amoebae analysis of lake sediments: impact of filter size and total count on estimates of density, species richness and assemblage structure. J Paleolimnol 43:689–704
8. Wall AAJ, Magny M, Mitchell EAD, Vannière B, Gilbert D (2010) Response of testate amoeba assemblages to environmental and climatic changes during the Lateglacial–Holocene transition at Lake Lautrey (Jura Mountains, eastern France). J Quat Sci 25:945–956
9. Telford RJ, Birks HJB (2011) Is spacial correlation introducing biases in the apparent accuracy of paleoclimatic reconstructions? A rejoinder. Quaternary Sci Rev 30:3210–3213
10. Patterson RT, Roe HM, Swindles GT (2012) Development of an Arcellacea (testate lobose amoebae) based transfer function for sedimentary phosphorous in lakes. Palaeogeogr Palaeocl Palaeoecol. doi:10.1016/j.palaeo.2012.05.028
11. Roe HM, Patterson RT, Swindles GT (2010) Controls on the contemporary distribution of lake thecamoebians (testate amoebae) within the Greater Toronto area and their potential as water quality indicators. J Paleolimnol 43:955–975
12. Farooqui A, Kumar A, Swindles GT (2012) Thecamoebian communities as proxies of seasonality in Lake Sadatal in the Ganga-Yamuna Plains of North India. Palaeontol Electron 15.1.3A:19
13. Riveiros NAV, Babalola AO, Boudreau REA, Patterson RT, Roe HM, Doherty C (2007) Modern distribution of salt marsh foraminifera and thecamoebians in the Seymour-Belize Inlet Complex, British Columbia, Canada. Mar Geol 242:39–63
14. Kauppila T (2006) Sediment-based study of the effects of decreasing mine water pollution on a heavily modified, nutrient enriched lake. J Paleolimnol 35:25–38
15. Kihlman S, Kaupila T (2009) Mine water-induced gradients in sediment metals and arcellacean assemblages in a boreal freshwater bay (Petkellajti, Finland). J Paleolimnol 42:533–550
16. Kihlman S, Kaupila T (2010) Tracking the aquatic impacts of a historical metal mine using lacustrine protists and diatom algae. Mine Water Environ 29:116–134
17. Kihlman S, Kaupila T (2012) Effects of mining on testate amoebae in a Finnish lake. J Paleolimnol 47:1–15
18. Escobar J, Brenner M, Whitmore TJ, Kenney WF, Curtis JH (2008) Ecology of testate amoebae (thecamoebians) in subtropical Florida lakes. J Paleolimnol 40:715–731
19. Patterson RT, Barker T, Burbidge SM (1996) Arcellaceans (thecamoebians) as proxies of arsenic and mercury contamination in northeastern Ontario lakes. J Foramin Res 26:172–183

20. Reinhardt EG, Dalby A, Kumar A, Patterson RT (1998) Utility of arcellacean morphotypic variants as pollution indicators in mine tailing contaminated lakes near Cobalt, Ontario, Canada. Micropaleontol 44:131–148
21. Kumar A, Patterson RT (2000) Arcellaceans (thecamoebians): new tools for monitoring long- and short-term changes in lake bottom acidity. Environ Geol 39:689–697
22. Patterson RT, Kumar A (2000) Use of arcellacea to gauge levels of pollution and remediation of industrially polluted lakes. In: Martin RE (ed) Environmental micropaleontology, vol 15, of topics in geobiology. Kluwer Academic/Plenum, New York, pp 257–278
23. Boudreau REA, Galloway JM, Patterson RT, Kumar A, Michel FA (2005) A paleolimnological record of Holocene climate and environmental change in the Temagami Region, northeastern Ontario. J Paleolimnol 33:445–461
24. Ontario Ministry of the Environment (2002) Safe Drinking Water Act. Available at http://www.ene.gov.on.ca/environment/en/legislation/safe_drinking_water_act/index.htm
25. Scott DB, Hermelin JOR (1993) A device for precision splitting of micropaleontological samples in liquid suspension. J Paleontol 67:151–154
26. Szava-Kovats R (2009) Re-analysis of the relationship between organic carbon and loss-on-ignition in soil. Comm Soil Sci Plant Anal 40:2712–2724
27. EPA (2011) SW-846 Online: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. US EPA. Available at http://www.epa.gov/osw/hazard/testmethods/sw846
28. Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorous in soils by extraction with sodium bicarbonate. US Dept Agric Circ 939, Washington
29. Zhou Q, Gibson CE, Zhu Y (2001) Evaluation of phosphorus bioavaiability in sediments of three contrasting lakes in China and the UK. Chemosphere 42:221–225
30. Watanabe FS, Olsen SR (1965) Test of an ascorbic acid method for determining phosphorous in water and $NaHCO_3$ extracts from soil. Soil Sci Soc Am Proc 29:677–678
31. Loeblich AR Jr, Tappan H (1964) Sarcodina, chiefly “Thecamoebians” and Foraminiferida. In: Moore RC (ed) Treatise on invertebrate paleontology, Part C, Protista 2. Geological Society of America and University of Kansas Press, Lawrence, pp 1–900
32. International Commission on Zoological Nomenclature (1999) International code of zoological nomenclature, 4th edn. The Natural History Museum, London, p 338
33. Kumar A, Dalby PB (1998) Identification key for Holocene lacustrine arcellacean (thecamoebian) taxa. Palaeontol Electron 1.1:34
34. Medioli FS, Scott DB (1983) Holocene arcellacea (thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Washington, p 63, Special publication 21
35. Medioli FS, Scott DB, Abbott BH (1987) A case study of protozoan intraclonal variability: taxonomic implications. J Foramin Res 1:28–47
36. Asioli A, Medioli FS, Patterson RT (1996) Thecamoebians as a tool for reconstruction of paleoenvironments in some Italian lakes in the foothills of the southern Alps (Orta, Varese and Candia). J Foramin Res 26:248–263
37. Roe HM, Patterson RT (2006) Distribution of thecamoebians (testate amoebae) in small lakes and ponds, Barbados, West Indies. J Foramin Res 36:116–134
38. Patterson RT, Fishbein E (1989) Re-examination of the statistical methods used to determine the number of point counts needed for micropaleontological quantitative research. J Paleo 63:245–248
39. Shannon CE (1948) A mathematical theory of communication. AT&T Tech J 27(379–423):623–656
40. Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton, p 185
41. Fishbein E, Patterson RT (1993) Error weighted maximum likelihood (EWML): a new statistically valid method to cluster quantitative micropaleontological data. J Paleo 67:475–486
42. Jongman RHG, ter Braak CJF, van Tongeren OFR (eds) (1987) Data analysis in community and landscape ecology. Pudoc, Wageningen, p 301
43. Šmilauer P (1992) CanoDraw 3.0 users guide, environmental change research centre. University College, London
44. ter Braak CJF (2002) CANOCO for Windows version 4.51. Biometris-Plant Research International, Wageningen
45. Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecol 129:271–280
46. Rao CR (1995) The use of Hellinger Distance in graphical displays of contingency table data. In: Tiit E-M, Kollo T, Niemi H (eds) New trends in probability and statistics, vol 3, Multivariate statistics and matrices in statistics. Brill, Leiden, pp 143–161
47. Birks HJB (1995) Quantitative palaenvironmental reconstruction. In: Maddy D, Brew JS (eds) Statistical modelling of quaternary science data. Quat Res Assoc, London, pp 161–251
48. Patterson RT, MacKinnon KD, Scott DB, Medioli FS (1985) Arcellaceans (thecamoebians) in small lakes of New Brunswick and Nova Scotia: modern distribution and Holocene stratigraphic changes. J Foramin Res 26:14–137
49. Schönborn W (1984) Studies on remains of Testacea in cores of the Great Woryty Lake (N.E. Poland). Limnologica 16:185–190
50. Correll DL (1999) Phosphorus: a rate limiting nutrient in surface waters. Poultry Sci 78:674–682
51. Conley DJ, Schelske CL (2001) Biogenic silica. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. v. 3: terrestrial, algal, and siliceous indicators. Kluwer Academic, Dordrecht, pp 281–293
52. Batterbee RW, Carvalho L, Juggins S (2001) Diatoms. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. v. 3: terrestrial, algal, and siliceous indicators. Kluwer Academic, Dordrecht, pp 155–202
53. Conley DJ (1988) Biogenic silica as an estimate of siliceous microfossil abundance in Great Lakes sediments. Biogeochemistry 6:161–179
54. Hurley JP, Armstrong DE, Kenoyer GJ, Bowser CJ (1985) Groundwater as a silica source for diatom production in a precipitation-dominated lake. Science 227:1576–1578
55. Levy DB, Barbarick KA, Siemer EG, Sommers LE (1992) Distribution and partitioning of trace metals in contaminated soils near Leadville, Colorado. J Environ Qual 21:185–195
56. Miao S, DeLaune RD, Jugsujinda A (2006) Influence of sediment redox conditions on release/solubility of metals and nutrients in a Louisiana Mississippi River deltaic plain freshwater lake. Sci Total Environ 371:334–343
57. Morford JL, Emerson S (1999) The geochemistry of redox sensitive trace metals in sediments. Geochim Cosmochim Acta 63:1735–1750
58. Ehrenberg CG (1832) Über die Entwicklung und Lebensdauer der Infusionstiere, nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. Abh Akad Wiss Berlin 1831:1–154
59. Carter HJ (1856) Notes on the freshwater Infusoria of the island of Bombay. Ann Mag Nat Hist 18(104):115–132
60. Penard E (1902) Faune Rhizopodique du bassin du lèman. Henry Kündig, Libraire de L’institut, Genève, p 712
61. Ehrenberg CG (1843) Verbreitung und Einfluss des mikroskopischen Lebens in Süd-und Nord Amerika. Königl Preufs Akad Wiss, Berlin, p 181
62. Ehrenberg CG (1830) Organisation, systematik und geographisches Verhältnis der Infusionstierchen. Königl Akad Wiss, Berlin

63. Deflandre G (1929) Le genre *Centropyxis* Stein. Arch Protistenkd 67:323–375
64. Leidy J (1874) Notice of some fresh-water and terrestrial rhizopods. P Acad Nat Sci Phila 26:86–88
65. Carter HJ (1864) On freshwater Rhizopoda of England and India. Ann Mag Nat Hist 13:18–39, ser 3
66. Wallich GC (1864) On the process of mineral deposit in the rhizopods and sponges, as affording a distinctive character. Ann Mag Nat Hist Ser 3(13):72–82
67. Ehrenberg CG (1840) Das grössere Infusorienwerke. Abh Akad Wiss, Berlin, pp 198–219
68. Patterson RT, Dalby A, Kumar A, Henderson LA, Boudreau EA (2002) Arcellaceans (thecamoebians) as indicators of land-use change: settlement history of the Swan Lake area, Ontario as a case study. J Paleolimnol 28:297–316
69. Smilauer P, ter Braak CJF (2002) CANOCO reference manual and canadraw for windows user`s guide: software for canonical community ordination (version 4.5). Micro-computer Power, Ithaca, p 500