Palaeontologia Electronica
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SEASONAL ENVIRONMENTAL AND CHEMICAL IMPACT ON THECAMOEBIAN COMMUNITY COMPOSITION IN AN OIL SANDS RECLAMATION WETLAND IN NORTHERN ALBERTA

Lisa A. Neville, Francine M.G. McCarthy, and Michael D. MacKinnon

ABSTRACT

Thecamoebian (testate amoeba) communities appear to respond to a variety of chemical parameters in aquatic ecosystems impacted by oil sands operations. A seasonal study, conducted over four seasons from May 2008 to March 2009 (spring, summer, fall and winter) in a constructed aquatic environment at the Mildred Lake site of Syncrude Canada Ltd. in northeastern Alberta, identified species and strain-level variation among living (i.e., Rose Bengal-stained) thecamoebians. The changes in this epibenthic community appeared to reflect seasonal and micro-environmental changes, as little change in the porewater chemistry, composition of sediments or bottom waters was observed over the study interval. The total (living + dead) thecamoebian test assemblage remained relatively constant over the course of the study, suggesting that the fossil assemblage reflects time-averaged conditions. Some variability was, however, observed among the species composing the difflugiid population. In addition, the speed at which they respond to environmental changes emphasizes their potential usefulness as environmental indicators. This has important implications for the use of thecamoebians as paleoenvironmental indicators. The difference between living and total assemblages reflects taphonomic skewing presumably resulting from variations in preservation potential and/or selective predation of species and strains.

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Keywords: thecamoebians, oil sands, reclamation, testate amoebae, seasonality, living vs. total assemblages

INTRODUCTION

Thecamoebians (also called testate amoebae) are protists that form a diverse and important component of the microbial trophic level within the

PE Article Number: 13.2.13A
Copyright: Palaeontological Association July 2010
Submission: 18 January 2010. Acceptance: 19 May 2010

Neville, Lisa A., McCarthy, Francine M.G., and MacKinnon, Michael D., 2010. Seasonal Environmental and Chemical Impact on Thecamoebian Community Composition in an Oil Sands reclamation wetland in Northern Alberta. *Palaeontologia Electronica* Vol. 13, Issue 2; 13A:14p;
http://palaeo-electronica.org/2010_2/231/index.html

benthic community of lakes and wetlands, and play a critical role in food webs as the intermediate between bacterial and benthic invertebrate communities (Patterson and Kumar, 2000; Beyens and Meisterfeld, 2001). These epifaunal/shallow infaunal benthic protozoans, particularly those belonging to the Superfamily Arcellacea, produce a fossilizable test of pseudo-chitinous material that is variably agglutinated in different species (Medioli and Scott, 1983). Their fossilized tests are found in all freshwater aquatic and moist terrestrial sediments, although the preservation potential varies between species, with some rarely reported as fossils (e.g., *Difflugia amphora* Wallich, 1864), even though they may be common in community studies of surface sediments (Boudreau et al., 2005; Patterson and Kumar, 2002). Thecamoebians display a rapid generation time and a high degree of sensitivity to environmental conditions at the sediment-water interface and epibenthic zone, and their fossil remains preserve a record of their populations over time (Boudreau et al., 2005; Patterson et al., 2002; McCarthy et al., 1995). Unlike most microfossil groups, thecamoebians do not dissolve in low pH environments, and in comparison to other microfossil types with shells that preserve well (e.g., diatoms, spores and pollen) thecamoebians reflect depositional conditions at the sediment/water interface of lacustrine freshwater and peat environments (Patterson et al., 1985).

Thecamoebians have recently been used to investigate the impact of sulphide mining in acid-sensitive lakes in Ontario (Patterson et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000; Patterson and Kumar, 2002), the impact of road salt runoff (Roe et al., 2010) and reclamation options in the oil sands constructed wetlands in Northeastern Alberta (McCarthy et al. 2008; Neville, 2010). As part of the oil sands study, it was demonstrated that thecamoebians can be used as proxies to monitor varying degrees of impact of oil sands constituents (Neville, 2010). To better apply this tool, an understanding of the natural variability of the population was required. This included investigating thecamoebian assemblages in natural lakes in Northeastern Alberta, as well as identifying their population response characteristics in relation to seasonal environmental changes. The families Centropyxidae and Difflugiidae were found to exhibit different degrees of sensitivity to the major by-products of oil sands mining activity in the Suncor Wetlands, with most difflugiid taxa exhibiting high sensitivity and lower tolerance to oil sands constituents, whereas centropyxid taxa appeared to thrive in all but the most highly impacted sites. Major byproducts created during the extraction and processing of oil sands in Alberta include naphthenic acids and elevated levels of conductivity, both of which are leached from the oil sands during processing (Harris, 2007). Naphthenic acids (NAs), a family of low molecular weight, naturally occurring carboxylic acid surfactants are released from the bitumen into water under the elevated pH conditions used in the oil-sand extraction process. They are important because in process waters they have been shown to be responsible for most of the acute toxicity to aquatic organisms (MacKinnon and Boerger, 1986; Han et al., 2009). Oil sands process-affected water (OSPW) also contains elevated levels of ions relative to regional water bodies. Salt leaching from the oil sand during processing and addition of process chemicals adds to the ion load, so that conductivity in OSPW ranges from about 1000 to 5000 μS/cm, with the primary ions being Na, Cl, HCO_3 and SO_4 (FTFC, 1995).

The study of thecamoebian response in the Suncor Wetlands did not isolate environmental parameters and only focused on water chemistry of the test systems (Neville, 2010). Recognizing that these protists respond to physical as well as chemical aspects of their environment, a study to assess the response of thecamoebians to seasonal environmental variations (temperature, dissolved oxygen (DO), nutrients) under consistent chemical conditions (salinity, NAs) was undertaken. The majority of seasonality studies have been conducted using foraminiferal populations (Murray, 1973; Boltovskoy and Wright, 1976). The first investigation of seasonality using living vs. total populations was conducted by Scott and Medioli (1980). Much less research, however, has been conducted using thecamoebians, the only studies of seasonality to date were conducted on peatlands by Heal (1964) and Warner et al. (2007).

METHODS AND MATERIALS

A study of thecamoebian assemblages was conducted on samples collected during the spring, summer, fall and winter from the Syncrude Demo Pond (Figure 1), a large-scale test pond on the Mildred Lake site (458352E, 6326665N) in northeastern Alberta. Demo Pond was chosen for this study because there has been relatively little change in its chemical constituents since its construction. It was constructed in 1993 in an excavation within the local clay overburden materials, with no recharge or discharge of either surface or ground

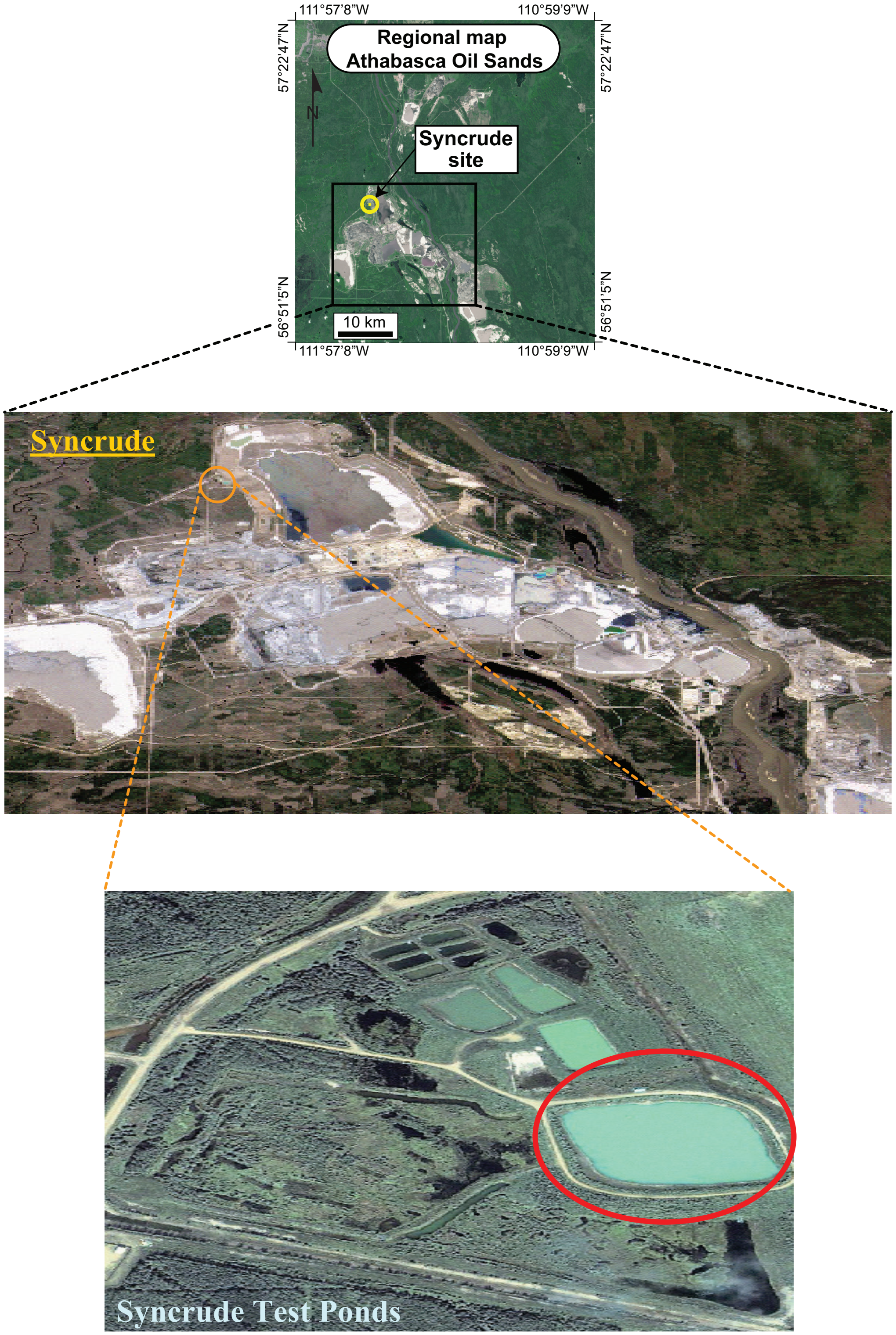

FIGURE 1. Satellite photos showing the location of the Demo Pond on Syncrude property in the Athabasca Oil Sands (Google Earth, 2009). The yellow dot in Demo Pond indicates the sampling location (floating dock).

waters. The small 4-Ha pond was originally filled with a soft tailings slurry, known as mature fine tails (MFT consisting of about 30 wt% solids and 1.5 wt% hydrocarbon), to a depth of up to 12 m, and then capped with about 3 m of non-process runoff water from the surrounding muskeg. In this typically steady state system impacts from NAs, salinity and oxygen demand maintain the benthic community under stress, but below the threshold for acute toxicity. The seasonal effects on this water body follow the seasonal cycle of a northern environment. The ice-cover period extends from November to April (ice thickness of 80-110 cm). Other than at the surface DO levels are low enough for the system to be anoxic, and DO levels within 5-10 cm of water are classified as anaerobic. This system provided an opportunity to test thecamoebian community response in a slightly stressed habitat to a range of seasonal environmental changes from climate factors, while maintaining a stable chemical environment. It has been argued that if seasonal changes are not significant in smaller aquatic environments that are subject to greater climate extremes, then they are not likely to be significant under more stable lacustrine environments (*c.f.,* Scott and Medioli, 1980).

Samples from the sediment to water interface in Syncrude's Demo Pond were collected on May 21st, July 22nd, August 19th, September 30th 2008 and again on March 12th 2009 by employees of Syncrude Canada Ltd. Four replicates were collected in May and July, and three replicates were collected in August, September and March. The samples were taken using an Ekman grab or a corer, from a floating dock located in an area of the pond that was underlain by the MFT zone (Figure 2), water depths averaged 2.65 m. During each month of sampling, 2 surface samples (0-2 cm) were collected and the remaining samples were collected from between 5-15 cm in the core. The sediment samples were transferred to glass jars and were stored at 4°C prior to shipping to Brock University. At the same time, water samples from above the sediment were collected and transported to Syncrude Canada Ltd. (SCL) Edmonton Research facility. Water analysis was performed using SCL standard protocols (Syncrude, 2005). In addition, substrate samples were analyzed for solids, bitumen and particle size distribution using SCL methods.

Samples were prepared for thecamoebian analysis following the standard micropaleontological methods described in Scott et al. (2001). Subsamples of 5cc were sieved through 500, 63 and 45μm mesh. Samples were stained with Rose Bengal to determine the presence of cytoplasm in tests (Scott and Medioli, 1980; Bernhard, 2000). The assumption that tests stained using this method were living at the time of collection has been called into question (Bernhard et al., 2006). Since the Demo Pond has only existed since 1993, the problem of spuriously old stained tests is minimal, so the new technique proposed by Bernhard was not employed. For quantitative analysis, the samples were placed in a gridded Petri dish and wet counted using a dissecting binocular microscope. Thecamoebians were identified primarily using the key by Kumar and Dalby (1998), although reference was also made to photoplates and descriptions in various publications, notably Medioli and Scott (1983). Specimens were identified and species diversity was calculated using strains, because strains have been found to convey useful information on aquatic subenvironments (Kumar and Patterson, 2000; Kauppila et al., 2006).

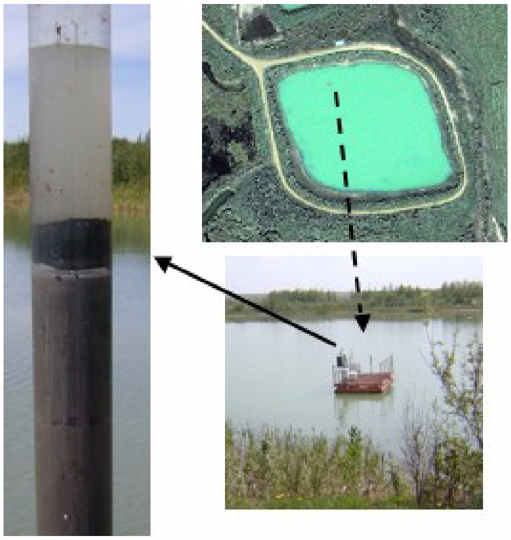

FIGURE 2. Exact sample location and example of core method of sampling. An abbreviated taxonomy of the species can be found in the Appendix.

Species diversity was calculated using the Shannon-Weaver Diversity index (SDI) (Shannon and Weaver, 1949). Harsh, unfavorable environmental conditions are normally characterized with an SDI between 0.5 - 1.5, intermediate conditions rage from 1.5 - 2.5 and favorable/stable conditions have an SDI >2.5 (Patterson and Kumar, 2002). The SDI was calculated using the following formula, where S is the species richness for each sample:

$$SDI \quad \overset{S}{\underset{I}{}} \; \frac{Fi}{Ni} * \ln \frac{}{}$$

The relative fractional abundance (F_i) was calculated for each taxonomic unit using:

$$Fi \quad \frac{Ci}{Ni}$$

where C_i is the species count, and N_i is the number of individuals (total population) in the sample (Patterson and Fishbein, 1989).

Data analysis was preformed using the computer program Minitab version 15 (Minitab Inc. USA). To examine the factors that influence thecamoebian taxon richness, linear regression analysis was preformed between the environmental variables and the independents (% difflugiid and % living) (Table 1). Lower P-values indicate a higher degree of influence by the given variable on the independent. Canonical Corrrespondence Analysis (CCA) was used to examine the population relationships between thecamoebian taxa and the measured environmental variables.

The coefficient of variance (CV) was used to calculate the variance among each parameter recorded for each month of study (Table 1), where s is the standard deviation and $\overline{X}$ is the mean. The coefficient of variance is a dimensionless measure of variability expressed as a fraction of the mean. When comparing between data sets with different units or widely different means the coefficient of variation generates comparable values unlike standard deviation. Similarity among a data set is expressed by lower covariant values (Davis, 2002).

$$Cv \quad \frac{s}{\overline{X}}$$

RESULTS

Parameters such as average sediment to water interface temperature, air temperature, total precipitation, and stained tests versus unstained tests (% living) varied (coefficient of variance values all <0.58) over the study period from May-September 2008 and March 2009 (Table 1). Parameters such as naphthenic acid concentration, conductivity, pH and dissolved oxygen content (DO), species diversity index (SDI) and the percent of difflugiid taxa composing the overall population remained relatively consistent, in contrast (coefficient of variance values all >0.17). Neither SDI or percent difflugiid changed by more than 10% over the course of the study period, while the percent of the sample living at the time of collection (represented by stained tests) varied substantially, with the greatest fraction of stained (living) tests during July and August, the wettest and warmest months (Table 1, Figure 3). The thecamoebian population remained relatively consistent between samples collected within the same month (P-values <0.05).

The dominant centropyxid taxon in May and July was *Centropyxis constricta* (Ehrenberg, 1843), whereas *Centropyxis aculeata* (Ehrenberg, 1832) increased in abundance during August and September (Figure 3). *Arcella vulgaris* Ehrenberg, 1830 was present in low numbers in May and increased in numbers in September. *Cucurbitella tricuspis* (Carter) Medioli et al., 1987 remained an important component of the thecamoebian population from May to August. In August the highest proportion of the *C. tricuspis* population was alive, while in September its numbers decreased significantly.

The relative abundance of difflugiid (vs. centropyxid) thecamoebians comprising the monthly populations remained relatively consistent over the course of the study (ranging from 74.6 to 87.8%) (Table 1 and Figure 3). However, the thecamoebian taxa composing the difflugiid population varied greatly, with P-values > 0.05.

Difflugia oblonga Ehrenberg, 1832 remained relatively ubiquitous throughout the study (Fig 3), its living population peaked during the warmest months of July and August, then its total numbers decreased significantly in September. *Difflugia urceolata* (Carter, 1864) was present (stained and empty tests) during both May and September but was virtually absent in July and August. Substantial increases of both *D. urceolata* and *Pontigulasia compressa* (Carter, 1864) occur in the month of September (Figure 3).

The thecamoebians composing the group "Others" in Figure 3 are *Lagenodifflugia vas* (Leidy, 1874)*, Difflugia protaeifomis* Lamarck, 1816*, Difflugia bidens* Penard, 1902*, Diffligia corona* Wallich, 1864*, Difflugia bacillaliarum* Perty, 1849 and *Difflugia globula* (Ehrenberg, 1848). The proportion of the species grouped as "Others" remains relatively consistent throughout the study, except for the month of July. An increase in the number of "Others" in July is due to an increase in the number of living *D.globula*. The dominant thecamoebian in July was *D. amphora.*

The thecamoebian taxa observed in the month of March are common species observed in

TABLE 1. Average naphthenic acid, conductivity, pH, dissolved oxygen (DO) and temperature at the sediment water interface (Temp 1), air temperature (Temp 2) and precipitation for each month. As well as thecamoebian population specific information, such as average species diversity (SDI), percent of the population composed of difflugiids and percent of the population composed of living (stained by Rose Bengal at time of collection) tests. The coefficient of variation indicates similarity among each parameter, and P-values indicate the degree of influence each parameter (variable) had on the independents.

Date	Naphthenic Acids (mg/L)	Conductivity (μS/cm)	pH	DO	Temp 1 (C°)	Temp 2 (C°)	Precipitation (mm)	Thecamoebian Species Diversity (SDI)	% Difflugiid	% Living
May, 08	38.4	1825	7.8	<0.6	12.5	10.1	7.8	2.22	80.6	43.3
July, 08	37.5	2266	7.92	<0.5	17.9	16.2	56.6	2.32	85.9	76.0
August, 08	26	2060	8.25	<0.4	17.8	15.9	137.6	2.03	74.6	71.0
September, 08	34	1810	8.31	<0.4	9.5	9.8	27.4	2.21	84.1	32.8
March, 09	20	1680	8.45	<0.5	1.3	-0.9	19.8	2.35	87.8	7.63
Coefficient of variance	0.206	0.101	0.034	0.169	0.583	0.587	1.048	0.056	0.063	0.611
	P-values comparing parameters to % difflugiid									
	0.210	0.745	0.791	0.655	0.674	-	0.568			
	P-values comparing parameters to % living									
	0.702	0.068	0.335	0.783	0.002	-	0.014			

the fossil record with very few stained tests (Boudreau et al., 2005; McCarthy et al., 1995) (Figure 3). The majority of the population observed in March was composed of encysted thecamoebians

DISCUSSION

Seasonal variations of the living (stained) thecamoebian populations and variations among the species composing the difflugiid population can be related to environmental changes (temperature and precipitation) (Table 1 and 2, Figure 3). The monthly consistency of the total assemblage composition in terms of difflugiids to centropyxids can be attributed to water chemistry, as the proportion of difflugiids (vs. centropyxids) did not change with changing environmental parameters (Table 1, Figure 3). Scott and Medioli (1980) observed similar results while conducting a seasonality study using *foraminiferal* populations. Their results indicated a highly variable living foraminiferal population with insignificant changes to the total assemblage (Scott and Medioli, 1980).

The warmer spring and summer months of May to August harbored high numbers of *C. tricuspis, D. oblong, D. amphora* and *D. globula* (Figure 3). The substantial increase in *D.globula* in July appears to be an anomaly because *D.globula* is typically considered an indicator of cool to cold climates (Collins et al., 1990). *C. tricuspis* is a common taxon recorded in most freshwater environments, due in part to the unusual ecology of this species, which has a planktonic phase in its life cycle (Schonborn, 1984; Medioli et al., 1987). It is possible that September does not harbor a sufficient amount of algal food source to support its planktonic phase. The high presence of *D. oblonga* in the warmer summer months suggests that it can somewhat tolerate climate extremes (Collins et al., 1990; McCarthy et al., 1995), but it lives in higher numbers in temperatures above 10ºC (Table 1).

During the cooler month of September a change in the dominant species composing the thecamoebian population is observed in relation to species tolerances. In response to a change in environmental parameters an increase in *A. vulgaris, D. urceolata* and *P. compressa* is observed. *D. urceolata* was also present during May, its absence in July and August, may be due to predation or because it prefers to live in lower temperatures (Collins et al., 1990; McCarthy et al., 1995) than those averaging 16ºC as were observed in the mid summer months. *A. vulgaris* is typically considered an indicator of a drop in water body pH (Patterson and Kumar, 2000) or consistently low pH conditions (Boudreau et al., 2005; Kumar and Patterson, 2000). In this study, however, the increase of *A. vulgaris* in September coincides with the highest pH values recorded (pH=8.31), which is unusual as Patterson and Kumar (2000) and Bou-

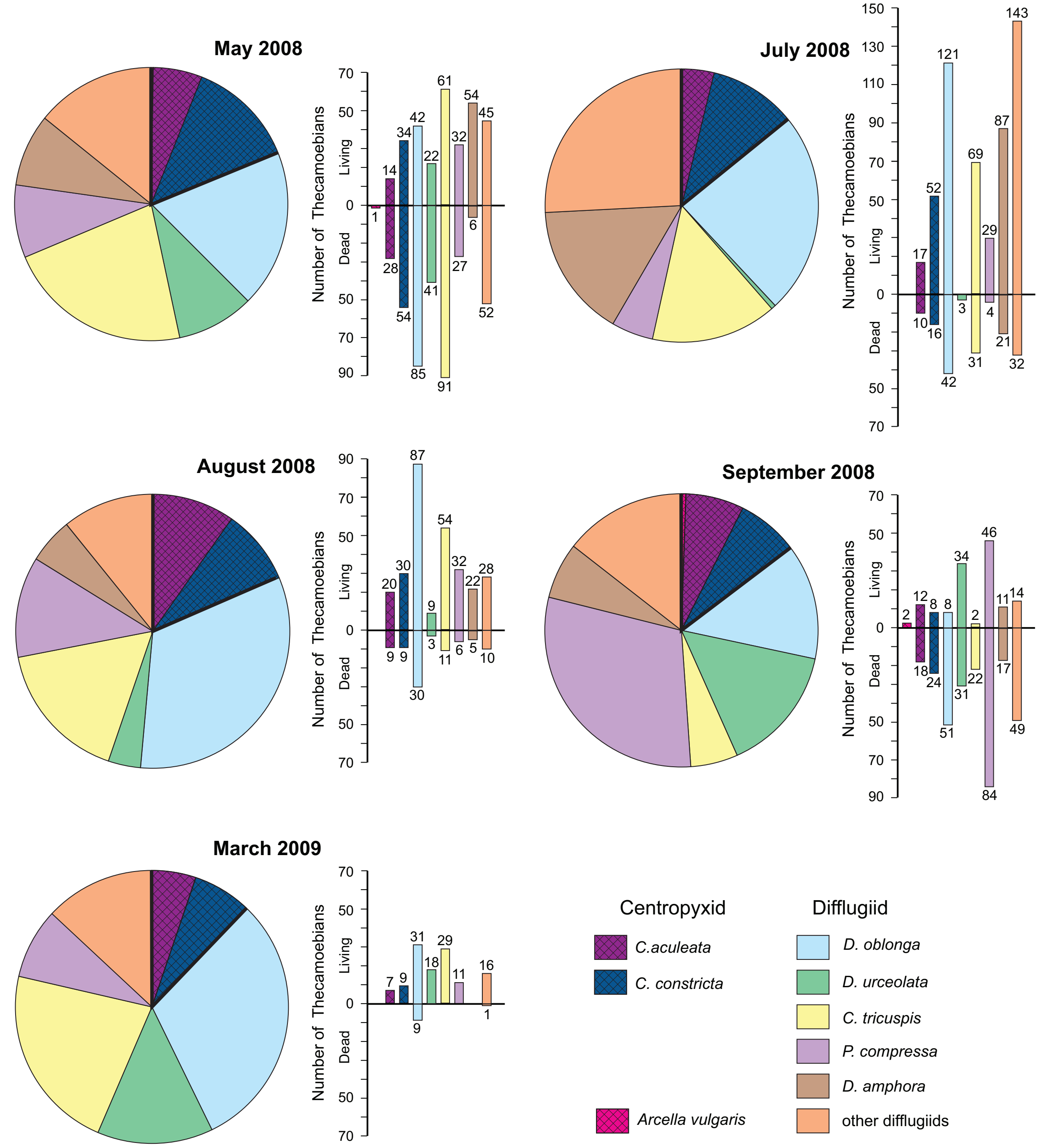

FIGURE 3. Thecamoebian population composition for May, July, August, September 2008 and March 2009, with associated bar graphs representing proportions of stained versus unstained test.

dreau et al., (2005) suggest *A. vulgaris* is an indicator of a low pH environment. Typically *P.compressa* appears to tolerate climate extremes (McCarthy et al., 1995), and is often found in high numbers where other species, more sensitive to climate conditions, can't thrive. It is common in all ponds except those undergoing eutrophication (Collins et al., 1990). This may explain its significant increase in total and living numbers during September, when we observe the lowest proportion of *C. tricuspis*.

TABLE 2. Percent abundance (% A), standard error (Error), tests per cc and sum of thecamoebians observed for each month of study.

Sample	May		July		Aug		Sept		March	
Sum	**692**		**677**		**365**		**440**		**468**	
Individuals/cc	**35**		**34**		**24**		**29**		**23**	
	% A	Error	% A	Error	% A	Error	% A	Error	% A	Error
Arcella vulgaris (Av)	0.14	0.28	-	-	-	-	0.45	0.63	-	-
Centropyxis aculeate (Ca)	0.43	0.49	0.74	0.64	0.55	0.76	0.68	0.77	-	-
Centorpyxis aculeata "discoides" (Cad)	5.64	1.72	3.25	1.34	7.40	2.69	6.14	2.24	1.50	1.10
Centropyxis constricta "aerophila"(Caa)	11.85	2.41	7.98	2.04	9.86	3.06	7.27	2.43	1.92	1.24
Centropyxis constricta "constricta" (Ccc)	0.29	0.40	1.92	1.03	0.82	0.93	-	-	-	-
Centropyxis constricta "spinosa" (Ccs)	0.58	0.56	0.15	0.29	-	-	-	-	-	-
Lesquereusia spiralis (Ls)	0.43	0.49	-	-	-	-	1.59	1.17	0.43	0.59
Pontigulasia compressa (Pc)	8.53	2.08	4.87	1.62	17.81	3.92	29.55	4.26	2.35	1.37
Cucurbitella tricuspis (Ct)	21.97	3.08	14.77	2.67	10.41	3.13	5.45	2.12	6.20	2.18
Lagenodifflugia vas (Lv)	0.43	0.49	-	-	-	-	-	-	-	-
Difflugia protaeiformis "claviformis"(Dpc)	4.91	1.61	7.53	1.99	2.19	1.50	5.68	2.16	0.64	0.72
Difflugia protaeiformis "acuminata" (Dpa)	6.65	1.86	12.56	2.50	-	-	2.05	1.32	0.21	0.42
Difflugia bidens (Db)	1.01	0.75	1.18	0.81	0.82	0.93	2.27	1.39	-	-
Difflugia corona (Dc)	0.43	0.49	1.18	0.81	6.58	2.54	3.64	1.75	0.43	0.59
Difflugia bacillaliarum (Db2)	0.58	0.56	0.30	0.41	-	-	-	-	1.07	0.93
Difflugia urens (Du)	-	-	-	-	-	-	0.23	0.44	0.43	0.59
Difflugia urceolata "urceolata" (Duu)	-	-	0.15	0.29	1.37	1.19	-	-	0.21	0.42
Difflugia urceloata "elongata" (Due)	9.10	2.14	0.30	0.41	1.92	1.41	14.77	3.32	3.63	1.70
Difflugia globula (Dg)	-	-	3.10	1.31	0.82	0.93	0.45	0.63	0.85	0.83
Difflugia oblonga "glans" (Dog)	8.67	2.10	18.76	2.94	23.84	4.37	9.77	2.77	5.13	2.00
Difflugia oblonga "lanceolata" (Dol)	3.18	1.31	0.74	0.64	6.03	2.44	0.45	0.63	2.78	1.49
Difflugia oblonga "bryophila" (Dob)	1.01	0.75	0.15	0.29	2.19	1.50	-	-	0.21	0.42
Difflugia oblonga "tenuis" (Dot)	5.49	1.70	4.43	1.55	-	-	3.18	1.64	0.43	0.59
Difflugia amphora (Da)	8.67	2.10	15.95	2.76	7.40	2.69%	6.36	2.28	-	-
Thecamoebian cysts	-	-	-	-	-	-	-	-	71.58	4.09

Similar to the findings of Heal (1964), we observed maximum numbers of both the total (living + dead) and the living (stained) thecamoebians from spring to fall, after which time total numbers decreased as the organisms encysted in preparation for winter (Heal, 1964).

The species variation observed within the difflugiid population reflects seasonal changes in environmental parameters, as there was insignificant (P-values <0.05) fluctuation in chemical parameters during the study period (Table 1, Figure 3). Predation may be in part responsible for the monthly variations of the dominant difflugiid species, as certain thecamoebians may be more susceptible to predation due to their test composition (Kumar and Dalby, 1998; Medioli and Scott, 1983). It is also possible that chemical composition of the water body imposes one level of constraint (one controlling factor) on the thecamoebian population. Water chemistry influences the overall proportions of the thecamoebian populations in terms of proportions of centropyxids to difflugiids (Figure 4). Naphthenic acids have the greatest influence on population distribution, with the lowest P-value in comparison to the other variables (Table 1 and Figure 4). Temperature, precipitation and interesting

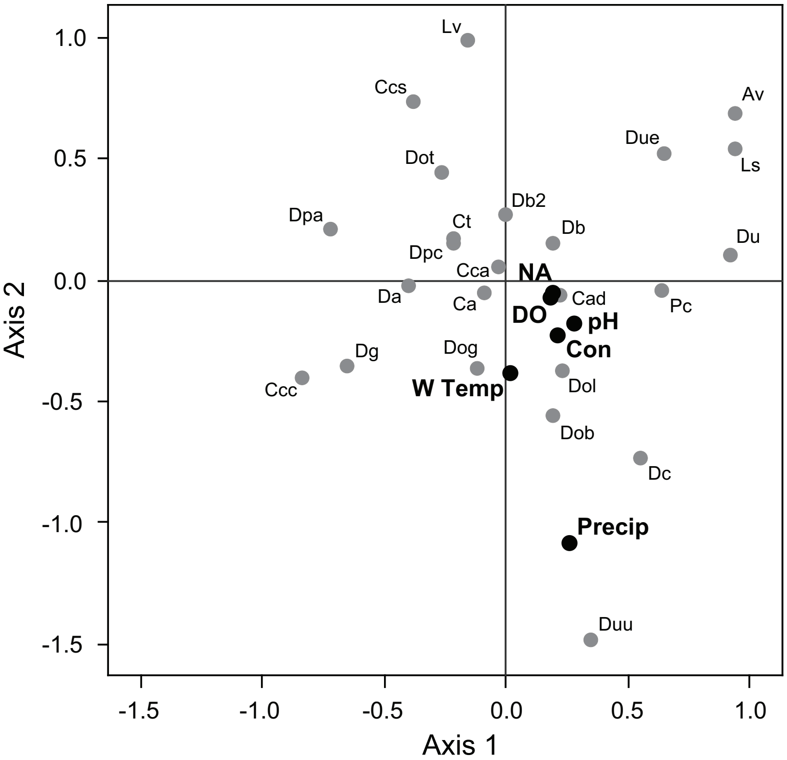

FIGURE 4. Canonical Correspondence Analysis (CCA) species-environment bi-plot. W Temp=water temperature, Precip=precipitation, DO=dissolved oxygen, Con=conductivity, NA=naphthenic acids. Species name abbreviations can be found in Table 2.

conductivity have the strongest influence on the living (stained) proportion of the thecamoebian population (Table 1 and Figure 5). P-values indicate with greater than 95% confidence that precipitation (P=0.014) and mainly water temperature (P=0.002) influence the living population, conductively is directly related to precipitation in a closed system such as Demo Pond.

Water chemistry influences the distribution of the total (living + dead) populations, in terms of the proportions of difflugiids to centropyxids while environmental factors such as temperature and precipitation influence the living population. The relative abundance of living (stained) thecamoebians is positively correlated with temperature and precipitation (Table 1), although the impact of these climatic parameters on benthic protists is probably indirect, possibly related to bio-productivity and the availability of food (Burbidge and Schroder-Adams, 1998). Environmental factors also influence the dominant species within the thecamoebian population and cause monthly taxon shifts within the difflugiid population (Figure 3) even though the proportion of the difflugiid population in relation to the centropyxid population remains relatively consistent. This is consistent with the observation that difflugiid taxa are very sensitive to environmental parameters (Collins et al., 1990). Neither Heal (1964) nor Warner et al., (2007) attributed seasonal changes they observed in testate amoebae communities directly to seasonal changes in environmental conditions. Heal (1964) considered changes in light conditions affecting symbiotic zoochlorellae in organisms to be one of the main driving factors in seasonal taxon shifts.

The literature suggests that *D. amphora* is typically found in eutrophic environments (Ellison, 1995), consistent with its increased presence during the summer months. *D. amphora* is not typically found in fossil records, probably because it has a low preservation potential. Typically when *D. amphora* is present it is observed as a stained (living) test. In May, July and August (the spring and summer months) we observe a higher frequency of stained in comparison to unstained tests, while in March *D. amphora* is completely absent both stained and unstained. This data strengthens the argument that *D. amphora* has a low preservation

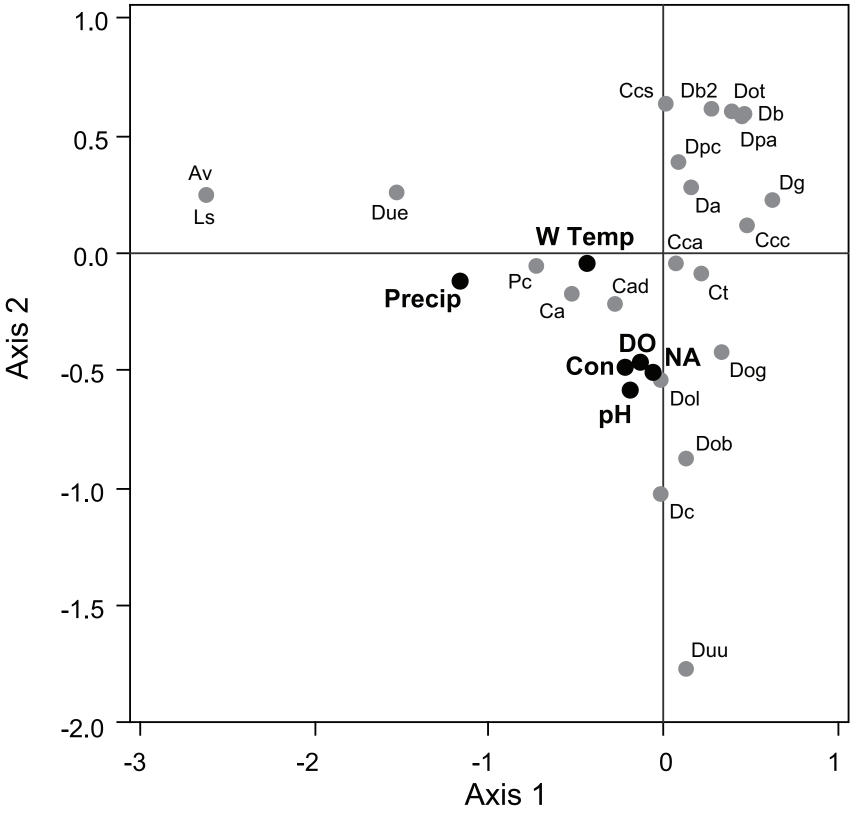

FIGURE 5. Canonical Correspondence Analysis (CCA) species-environment bi-plot, using the abundance of living species observed throughout the study. See Figure 4 caption to identify abbreviations.

potential, as it is mainly observed as a living (stained) tests and very rarely as an empty unstained test.

The few stained tests observed in March are the species *Difflugia oblonga* "glans". The staining of this test could possibly be attributed to the longevity error associated with Rose Bengal (Bernhard et al., 2006), or it could indicate that this strain of *D. oblonga* is particularly tolerant to cold conditions. Interestingly, in March we mainly find encysted thecamoebians (Table 2 and Figure 6). Thecamoebians contract their protoplasm and surround themselves within a spherical solid enclosure (cyst) as a means as a means of rest, dormancy or defense (Ogden and Hedley, 1980). Thecamoebian cysts are rarely reported in studies of fossil thecamoebians, possibly because this phase is relatively short-lived, and may result in encystment prior to fossilization. Their abundance (72% of the total population) in Demo Pond in March, but their absence from samples collected during the other months, suggests that conditions at the sediment-water interface were inhospitable, resulting in encystment. This may be a result of changes at the sediment water interface in response to surface icing. It does not appear to result from high impact of chemical constituents associated with OSPW, as no cysts were documented during the study of thecamoebian response to varying levels of oil sands by-products in a previous study where OPSW character of the exposure waters ranged from 0 to 100% (Neville, 2010).

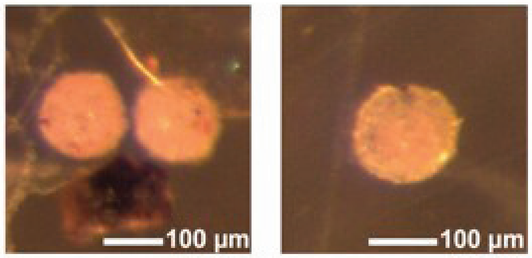

FIGURE 6. Thecamoebian cysts observed in March 2009. Images captured using a Leica MZ 12.5 image capture system.

In terms of strain level variability it does appear that some strains prefer certain conditions (Table 2). Among centropyxiids, it appears that *Centropyxis aculeata "discoides"* and *Centropyxis constricta "aerophila"* are the most opportunistic, more tolerant of climate extremes than other centropyxiids observed during the course of this study, as only the above mentioned were present in March. *Difflugia urceolata "urceolata"* appears to be more sensitive to cold in comparison to *Difflugia urceolata "elongata"* as *D. urceolata "urceolata"* was only present during the summer months when water temperatures were <17 C. *D. urceolata "urceolata"* was present in March but only as a member of the fossil record.

CONCLUSIONS

The variations observed over the study period (May, July, August, September and March) suggest that thecamoebians respond rapidly to seasonal variables such as temperature, light penetration, primary production and precipitation. Little variation in the relative abundance of total difflugiid vs. centropyxid thecamoebians was observed during the annual seasonal cycle, validating the use of this metric as an aquatic environment monitoring tool. However, the relative abundance of living thecamoebians (stained tests) and the species composition (particularly within the difflugiid taxa) varied substantially throughout the study. Depending on the time of year, some thecamoebian species appear to be more susceptible to predation or to have a low preservation potential as they were mainly observed as stained (live) tests. Paleoecological studies using thecamoebians should focus on the species with high preservation potential, however finding a thecamoebian with low preservation potential in the fossil record would be an indicator of a very specific environment.

Current environmental conditions should be gauged based only on the living population during time of collection. Looking at the total population is useful for paleoenvironmentalists who are typically interested in the overall marine lacustrine environment and not as interested in seasonal perturbations. The total population is a more accurate indicator of the general environmental conditions.

ACKNOWLEDGMENTS

We thank Syncrude Canada Limited for allowing us access to the wetlands and analytical resources, P. Marlow (Golder Associates) for providing information on the wetlands, M. Lozon (Brock University) for his graphic designs, B. Calvert for his statistical insight and D. Christie for collecting samples in March.

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ABBREVIATED SYSTEMATIC TAXONOMY

Phylum Sarcodaria Milne-Edwards,1850

Superclass Rhizopoda Dujardin, 1835

Class Lobosa Carpenter, 1861

Subclass Testacealobosa de Saedeleer, 1934

Order Thecolobosa Haeckel, 1878 (=Arcellinida *auctorum*)

Superfamily Arcellacea Ehrenberg, 1830

Family Difflugidae Stein, 1859

Genus *Cucurbitella* (Carter, 1856)

Cucurbitella tricuspis (Carter) Medioli et al., 1987

Genus *Difflugia* Leclerc *in* Lamarck, 1816

- *Difflugia amphora* Wallich, 1864
- *Difflugia bacillariarum* Perty, 1849
- *Difflugia bidens* Penard, 1902
- *Difflugia corona* Wallich, 1864
- *Difflugia fragosa* Hampel, 1898
- *Difflugia globulus* (Ehrenberg, 1848)
- *Difflugia oblonga* Ehrenberg, 1832

Difflugia protaeiformis Lamarck, 1816

- *Difflugia urceolata* Carter, 1864

Genus *Lagenodifflugia* (Leidy, 1874)

- *Lagenodifflugia vas* (Leidy, 1874)

Genus *Lesquereusia* (Schlumberger, 1845)

- *Lesquereusia spiralis* (Ehrenberg, 1840)

Genus *Pontigulasia* Rhumbler, 1895

- *Pontigulasia compressa* (Carter, 1864)

Family Centropyxididae Deflandre, 1953

Genus *Centropyxis* Stein, 1859

- *Centropyxis aculeata* (Ehrenberg, 1832)
- *Centropyxis constricta* (Ehrenberg, 1843)

Family Arcellidae Ehrenberg, 1830

Genus *Arcella* Ehrenberg, 1830

- *Arcella vulgaris* Ehrenberg, 1930

Family Hyalospheniidae Schulze, 1877

Genus *Heleopera* Leidy, 1879

- *Heleopera sphagni* (Leidy, 1874)