Investigating the Potential of Thecamoebians (Testate Amoebae) as Bio-indicators of impact of Oil Sands Mining Operations on freshwater environments in Northeastern Alberta, Canada

by

Lisa Ann Neville, Hons. B.Sc.

Department of Earth Science

Submitted
in partial fulfillment of the requirements
for the degree

Master of Science

Faculty of Mathematics and Science, Brock University
St. Catharines, Ontario

Master of Science (2009)
(Earth Science)

Brock University
St. Catharines, ON, Canada

TITLE: Investigating the Potential of Thecamoebians (Testate Amoebae) as Bio-indicators of impact of Oil Sands Mining Operations on freshwater environments in Northeastern Alberta, Canada

AUTHOR: Lisa Ann Neville, Honours B.Sc.
(Brock University, St. Catharines, Ontario, Canada, 2007)

SUPERVISOR: Professor, Dr. Francine M.G. McCarthy

NUMBER OF PAGES: xi, 174 pp.

Abstract

Thecamoebian (testate amoeba) species diversity and assemblages in reclamation wetlands and lakes in northeastern Alberta respond to chemical and physical parameters associated with oil sands extraction. Ecosystems more impacted by OSPM (oil sands process-affected material) contain sparse, low-diversity populations dominated by centropyxid taxa and *Arcella vulgaris.* More abundant and diverse thecamoebian populations rich in difflugiid species characterize environments with lower OSPM concentrations. These shelled protists respond quickly to environmental change, allowing year-to-year variations in OSPM impact to be recorded. Their fossil record thus provides corporations with interests in the Athabasca Oil Sands with a potential means of measuring the progression of highly-impacted aquatic environments to more natural wetlands. Development of this metric required investigation of controls on their fossil assemblage (e.g. seasonal variability, fossilization potential) and their biogeographic distribution, not only in the constructed lakes and wetlands on the oil sands leases, but also in natural environments across Alberta.

Acknowledgments

I have many people to thank for their contribution to allowing me to finish both my undergraduate and graduate degrees.

A sincere thank you to Dr. Francine McCarthy, for creating a project with no microscope work then allowing me to move in a completely different direction. Francine your guidance, patience and encouragement made this project and my masters experience a success and a lot of fun.

I would like to thank Dr. Michael D. MacKinnon for his insight, teachings and support while allowing Dr. McCarthy and myself the opportunity to work with Syncrude and Suncor in the Oil Sands of Alberta. I could not have dreamed of a better project.

Extended thanks to all the faculty and staff in the Brock University Earth Science department, for all their assistance and support during this project. A special thank you to: Dr. Dan McCarthy and Dr. Keith Tinkler for being members of my committee and continually providing a different perspective, Mike Lozon the Department draftsman for his helpfulness and creativity in producing the diagrams in my thesis, Diane Gaboury for assisting me with all the little things.

Of course none of this would have been possible without the support of my family and friends. Words cannot describe how much my parents have sacrificed to allow me to attain this level of education. I love you and thank-you for everything you have done. Michelle, my little sister, your intelligence never ceases to amaze me, I believe you can do anything you dream of, so continue to dream big (at least one of us in this family should own a Porsche). I thank Curtis Webb for his continued optimism and support in everything I do. I would especially like to thank my Nanny in Nova Scotia for always being there when I need her. You are my favorite person to talk to, as well as very supportive in everything I decide to do.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Photo Plates

List of Appendices

List of Abbreviations

AENV	Alberta Environment
ASRD	Alberta Sustainable Resource Association
CCME	Canadian Council of the Ministers of the Environment
CEAA	*Canadian Environmental Assessment Act*
CEPA	*Canadian Environmental Protection Act*
C/N	Carbon/ Nitrogen
CT	Coarse Tailings
DFO	Department of Fisheries and Oceans
EPEA	Environmental Protection and Enhancement Act
EPL	End Pit Lake
EUB	Alberta Energy and Utilities Board
IP	In-Pit Tailings
MFT	Mature Fine Tailings
MLSB	Mildred Lake Settling Basin
NWPA	*Navigable Waters Protection Act*
OSPM	Oil Sands Process Effected Material
OSPW	Oil Sands Process Effected Water
RSDS	Regional Sustainable Development Strategy
SDI	Shannon Diversity Index
SWSS	South West Sand Storage
TFT	Thin Fine Tailings

Chapter 1

Introduction

1.1. The Oil

The bitumen in the Alberta Oil Sands is contained within the McMurray Formation (Figure 1.1) (Vigrass, 1968). The most widely accepted theory of the source of the oil is that during the building of the Rocky Mountains, organic-rich Paleozoic rocks were shifted down to depths where pressure and temperature conditions metamorphosed organic sediment to oil (Figures 1.1 & 1.2). The oil then migrated through the permeable strata to the McMurray Fm. where it degraded into heavy, viscous bitumen (Pelly, 2007).

The three major Oil Sands reserves in Alberta are the Peace River, Athabasca and Cold Lake, together comprising 77,699 km^2 (Syncrude Canada Ltd., 2003). The combined estimated reserve potential exceeds one trillion barrels of bitumen (Mossop, 1980). The Athabasca Formation is the largest of the three areas in both size and reserve potential (Syncrude Canada Ltd., 2003).

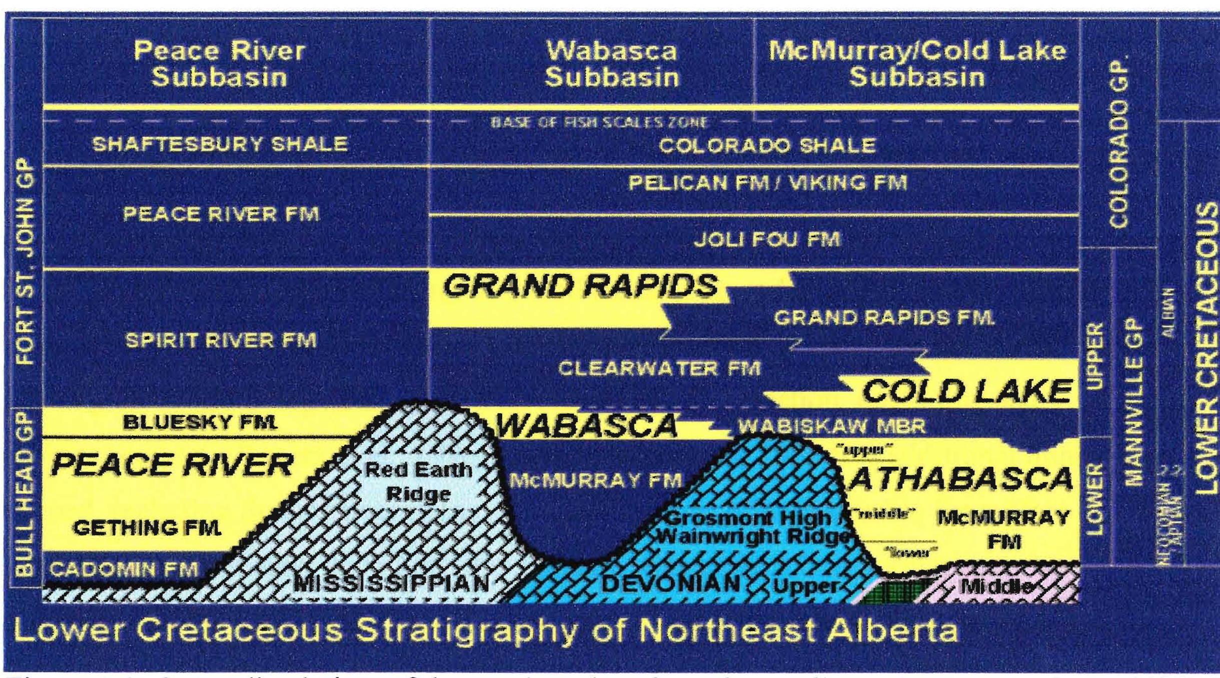


Figure 1.1: Generalized view of the stratigraphy of Northern Alberta (Proctor et al., 1983)

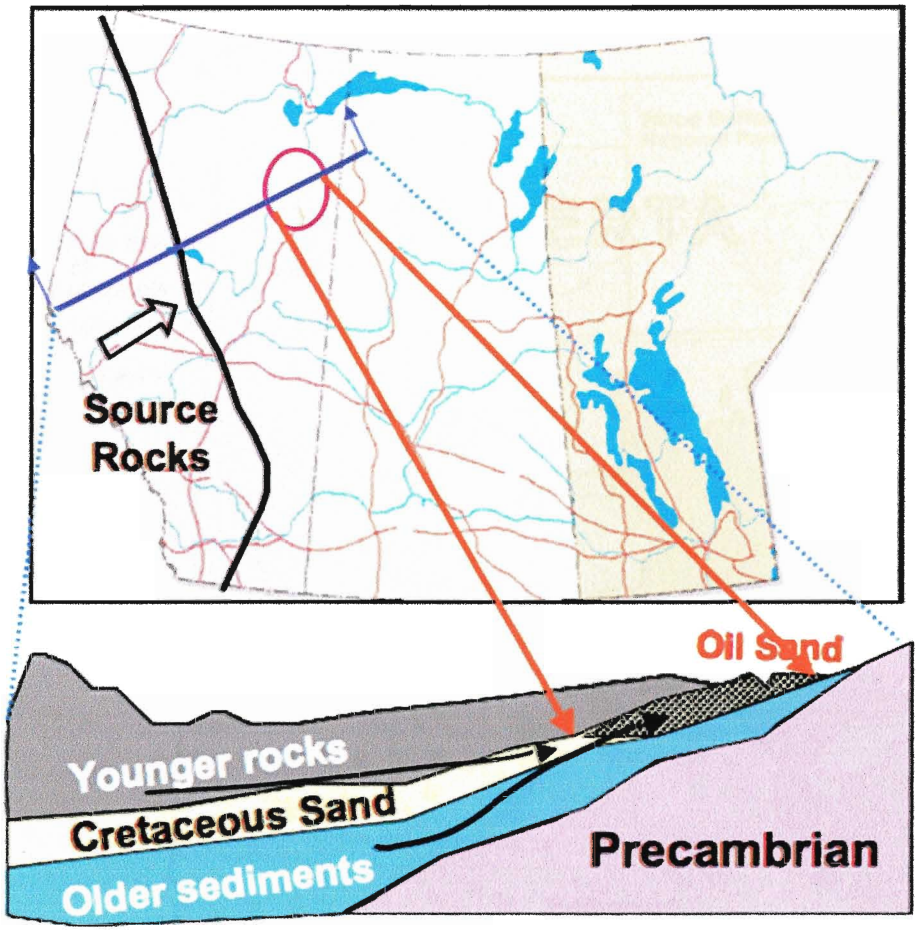


Figure 1.2: Migration path of the oil to the Oil Sands (Priece, 2004)

1.2. Oil Production

1.2.1. Oil Companies

Large –scale surface mining of the oil sands (Figure 1.3 & 1.4) began in 1978 about 20 km north of Fort McMurray (Harris, 2007) with Syncrude Canada Limited opening the world's largest integrated oil sand mining, extraction, upgrading and utilities complex (Rogers et al., 1996). Between 1978 and 2002 five additional mines opened, including Suncor Energy Inc. The total area in Alberta suitable for surface mining is approximately 2535 km^2 (253,500 ha). Currently active development is occurring on over 250 km^2 (25,000 ha) and production now exceeds over one million barrels of crude oil per day (Harris, 2007). When fully developed Syncrude alone will have over 250 km^2 of land requiring reclamation (Rogers et al., 1996).

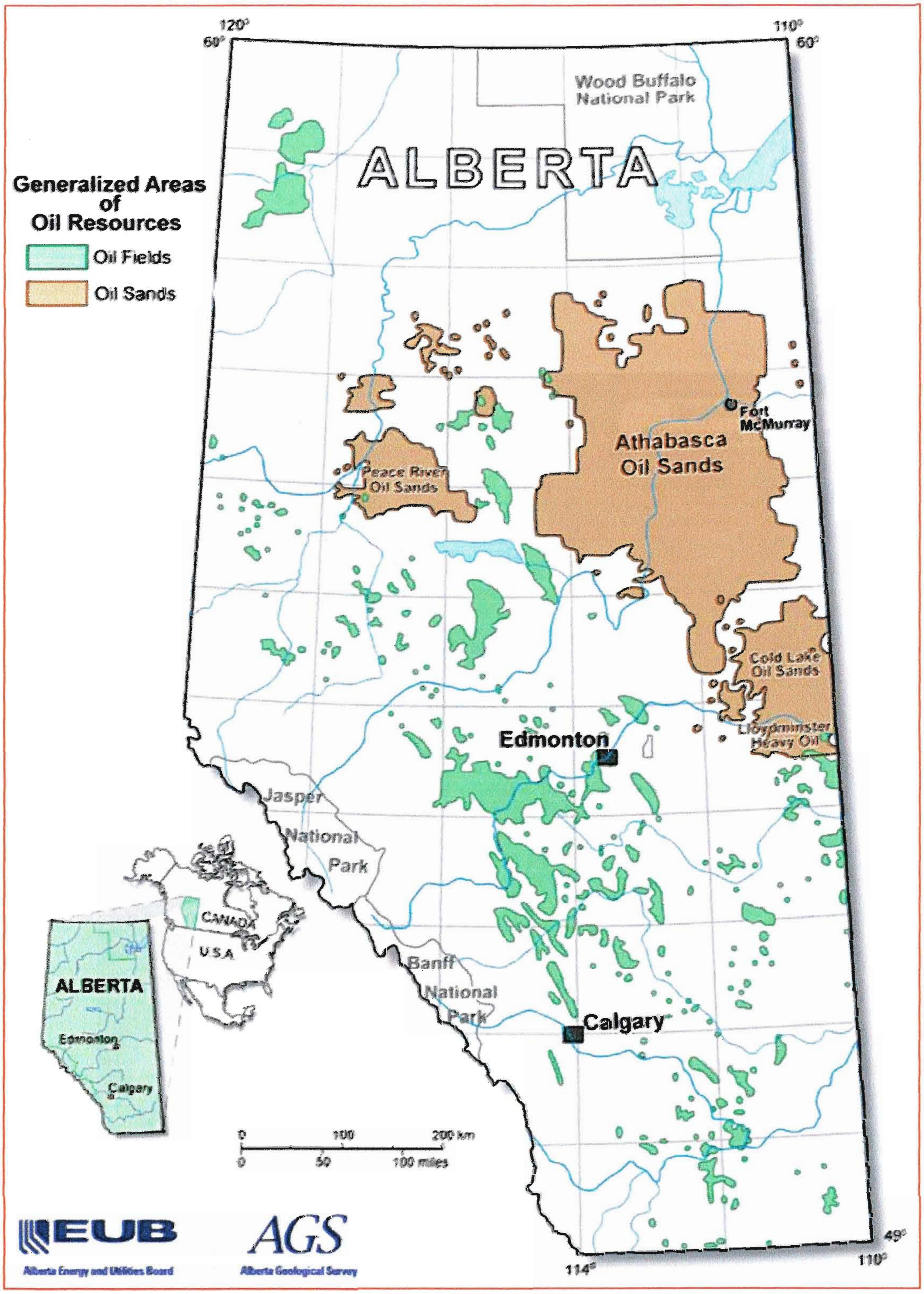


Figure 1.3: Map showing the location of Athabasca oil sands in Alberta Canada (Alberta Energy and Utilities Board et al., 2000).

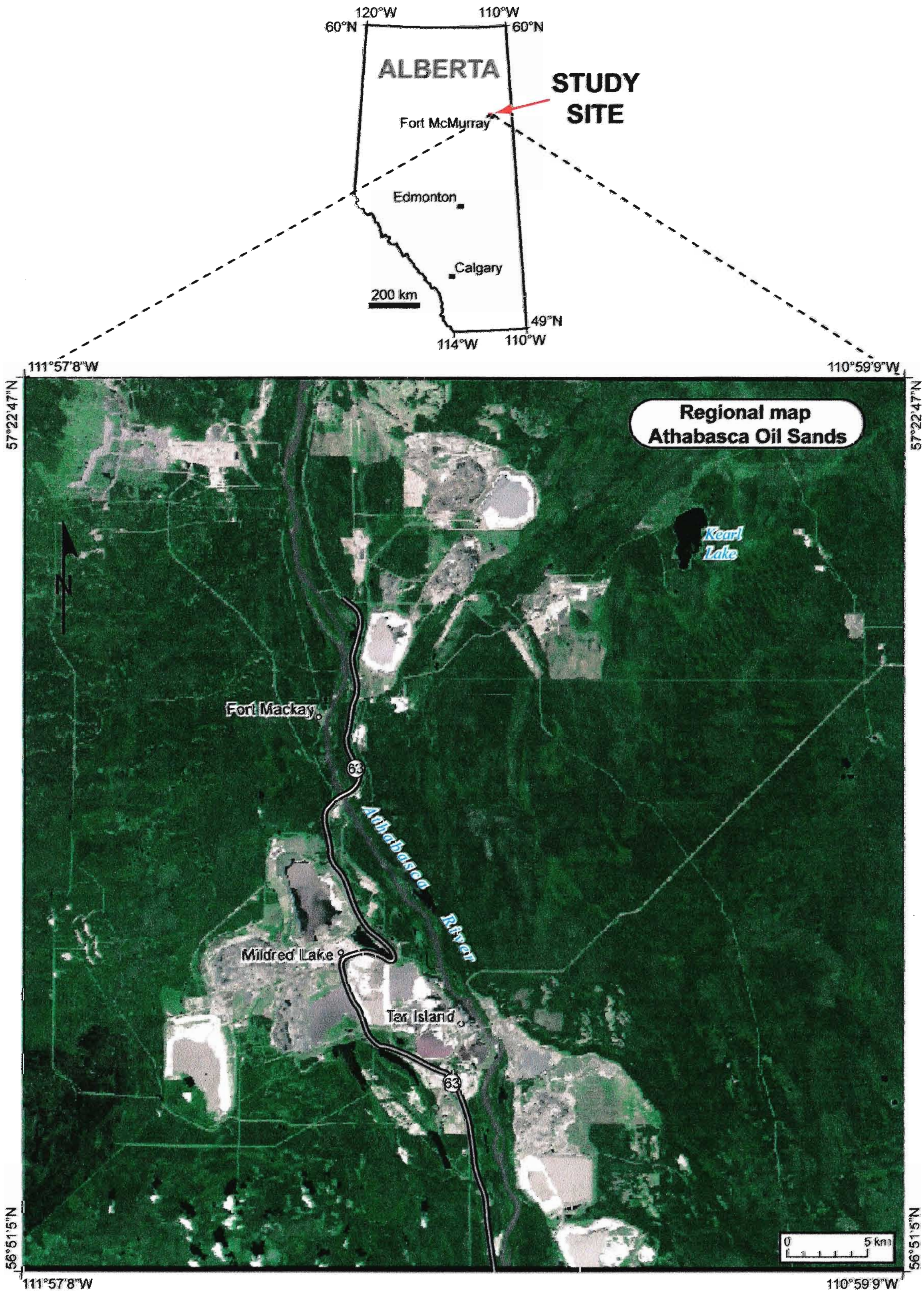


Figure 1.4: Satellite image of the mining operation in the Athabasca oil sands of Alberta Canada, North of Fort McMurray (Figure 1.3).

Oil sand (Figure 1.5) is composed of sand, bitumen, mineral rich clays and water. In its raw state bitumen is black and asphalt-like (Syncrude Canada Ltd., 2003). It requires upgrading to become transportable by pipeline and useable by conventional refineries. The upgraded bitumen product (synthetic crude) is a combination of naphtha, light and heavy gas oils (Masliyah, 2006).

Oil sands production takes place in a couple of ways. In-situ bitumen production uses steam-assisted gravity drainage (SAGD). This method of production injects steam into the ground, the steam rises and heats the bitumen, and the heated bitumen then flows into the well. The more common method of production used in the Athabasca Oil Sands is open pit mining, where the top layer of sediment (overburden) is removed and stockpiled allowing access to the underlying oil sand (Masliyah, 2006). Open pit mining is the preferred method because it allows for 90% recovery while *in-situ* mining only allows for 25-60% recovery (Hirsch, 2005). Open pit mining does, however, disturb a large area of land that must be reclaimed, and for that various reclamation techniques are being explored. The oil sands companies need a biomonitoring tool that allows them to monitor the progress of various reclamation options, and this study explores one group of protists (thecamoebians, also called testate amoebae) as a potential tool (see section 1.5).

During the processing of oil sand (Figure 1.6), the bitumen is extracted from the sand by digestion and flotation in a suspension of hot water aided by the addition of caustic NaOH (sodium hydroxide) (Harris, 2007). This liberation process uses large volumes of water (0.8 – 0.9 m^3 per tonne of oil sand). It takes about two tonnes of mined oil sand and 14-20 barrels (42 US gallons, 34.972 Imperial gallons, 158.987 L) of water to produce one barrel of synthetic crude oil (FTFC- Fine Tailings Fundamentals Consortium, 1995). About 80% of the required water is recycled (reused) and 20% is drawn from the Athabasca River (Masliyah, 2006). The extracted bitumen is upgraded into a sweet crude oil by fluid coking, hydroprocessing, hydrotreating and reblending (Harris, 2007).

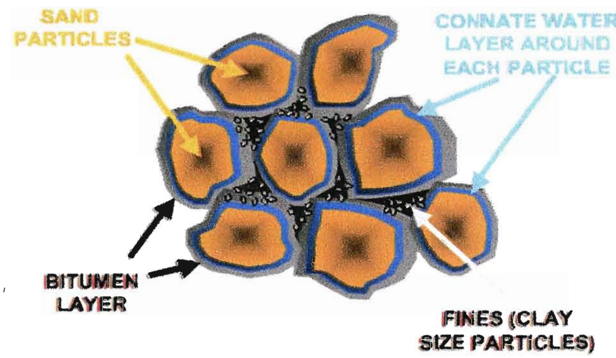


Figure 1.5: A diagram of oil sand ore (MacKinnon, 2007).

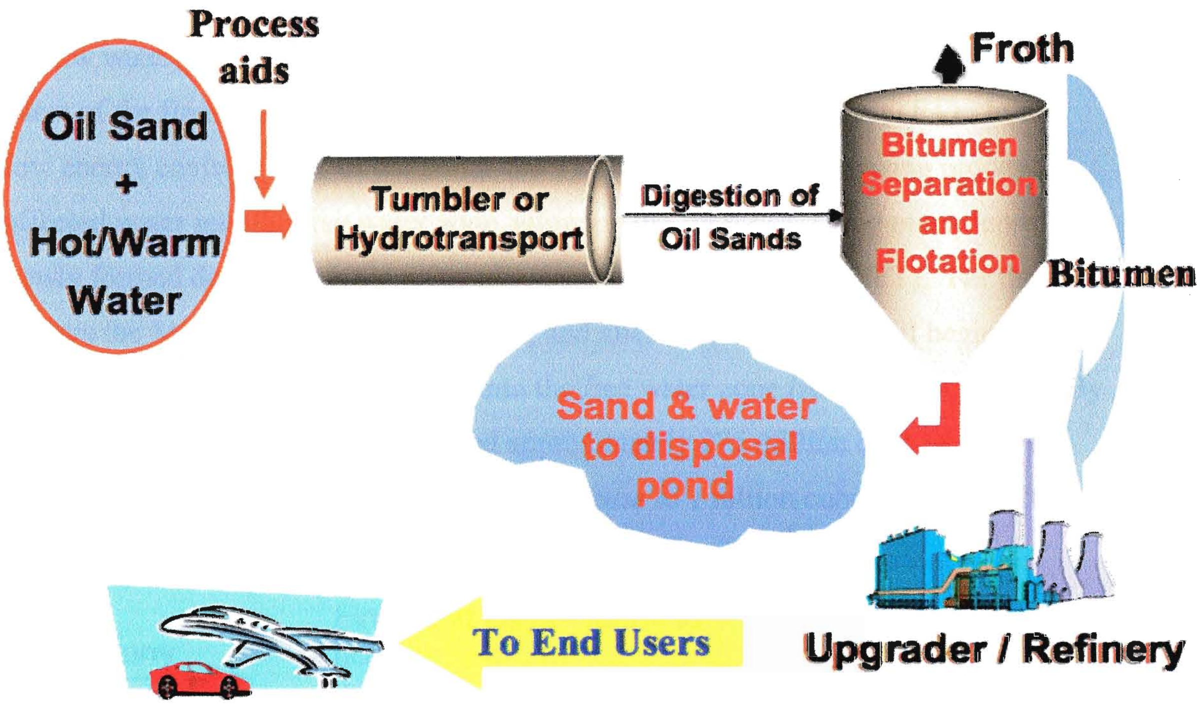


Figure 1.6: A diagram depicting the main processes that take place during oil sands refinement, before oil can reach end users. The sand and water disposal pond is the focus of the remediation efforts (MacKinnon, 2007).

1.2.2. Tailings and Tailings Management

By weight the oil sands are composed of 82-85% mineral solids, 6-16% bitumen, 2-5% connate water and associated salts, therefore creating large amounts of tailings (Masliyah, 2007). After the extraction process there are three types of remaining materials (coarse tailings- CT, flotation tails and Plant 6 tails), characterized based on the ratio of the main components remaining. Coarse tailings are the most significant in terms of volume; CT consist of water, coarse solids (sand and coarse to medium silt), fines (fine silt and clay) and un-recovered bitumen (Allen, 2008). Oil sands process affected material (OSPM) is an umbrella term used to characterize the remaining material. Figure 1.6 depicts the main components of the extraction process. Table 1.1 characterizes the materials involved during the various processing stages and how they influence the remaining water (Rogers et al., 1996).

During deposition (storage) the coarse and fine solids segregate, the coarse particles settle out and are deposited as either sub aerial (above water) or sub aqueous (below water) beach deposits around the tailings ponds (Figure 1.7) (Harris, 2007). Most of the fines continue into the tailings pond as thin fine tailings (TFT). In the low energy environments of the ponds the TFT begins to settle releasing process affected water into the free water zone, allowing the fines to become more dense. The newly formed denser fines are referred to as mature fine tailings (MFT). For the next 5 years the MFT will continue to densify, and after 5 years the rate will begin to decrease all the time releasing water into the free water zone (Rogers et al., 1996). By 1995 Syncrude alone had accumulated approximately 275 million cubic meters of MFT, it is predicted that this number will increase to 1 billion cubic meters by 2025 (Rogers et al., 1996).

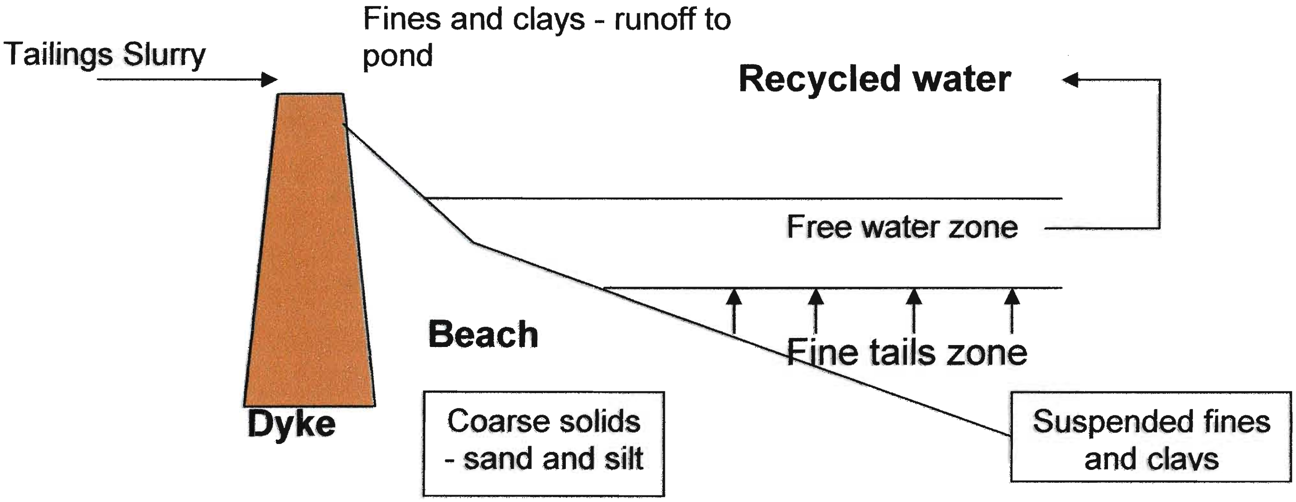


Figure 1.7: Cross section of oil sand tailings settling basin (modified from Fine Tailings Fundamental Consortium, 1995).

The bedrock geology of the oil sands affects water quality in the tailings and reclamation ponds and wetlands when the oil-containing material has been processed. Ions in the soil pore water will be released during aqueous digestion of extraction: main ions released will be Na, Cl, SO_4, Ca, Mg. The amount and rate depend on the geologic history. Cation exchange in clays from high sodium environments leads to Na exchange of Ca and Mg (Harris, 2007; Vitt et al., 1996):

$$2Na^{+} + Ca\text{-}Clay \leftrightarrows Na\text{-}Clay + Ca^{+2}$$

1.3. Alberta's Wetlands

Alberta contains approximately 114,000 km^2 of wetlands, representing 18% of the province's land base (Figure 1.8 and 1.9) (Vitt et al., 1996). Wetlands cover approximately half of the natural landscape in the oil sands region and are therefore a major component of the natural Boreal Forest ecosystem (Harris, 2007).

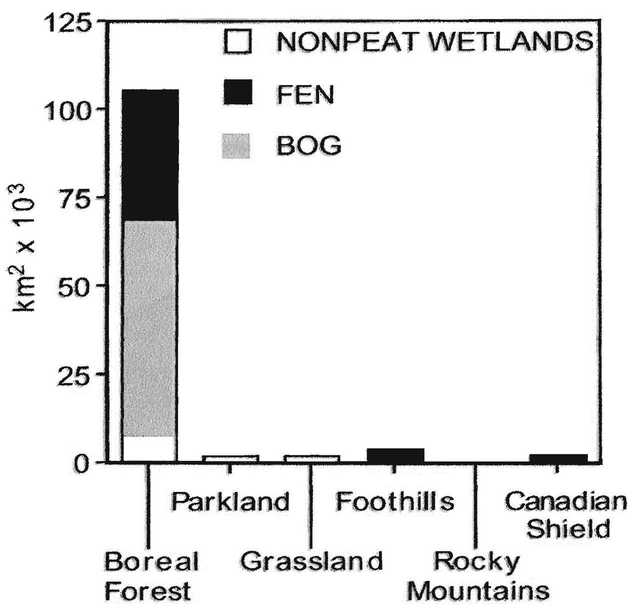


Figure 1.8: Land coverage of wetlands in Alberta by natural region (Vitt et al., 1996)

Natural wetlands are defined as areas where the land is saturated with water for long enough periods to support wet adapted processes including vegetation and aquatic invertebrates (Canadian Wildlife Services, 2009). They are shallow (<2 m) with stagnant or slowly moving water. Types of wetlands found in the Boreal Forest ecosystem of the oil sands region are bogs, fens, marshes, shallow open water wetlands and swamps (Figure 1.8) (Halsey, 2007). Natural Boreal wetlands are a critical habitat for many important wildlife species, and they are significant to the traditional way of life of the local Aboriginal people (Harris, 1996). Figures 1.9 and 1.10 show the range of types of wetlands occurring in Canada, specifically Alberta and the Fort McMurray area. Wetland vegetation type and potential succession of wetlands is controlled by climate, human activity, water source, rate of water flow and water table fluctuations which influence nutrient and alkalinity availability as well as substrate decomposition and accumulation (Halsey, 2007). Water and nutrient availability are further affected by the geologic setting which influences the wetland interaction with the surrounding environment (Devito and Mendoza, 2007). Furthermore, in Alberta in particular, it has been found that the presence or absence of natural salts associated with the bedrock geology is a significant variable explaining wetland variability (Halsey, 2007; Vitt et al., 1996).

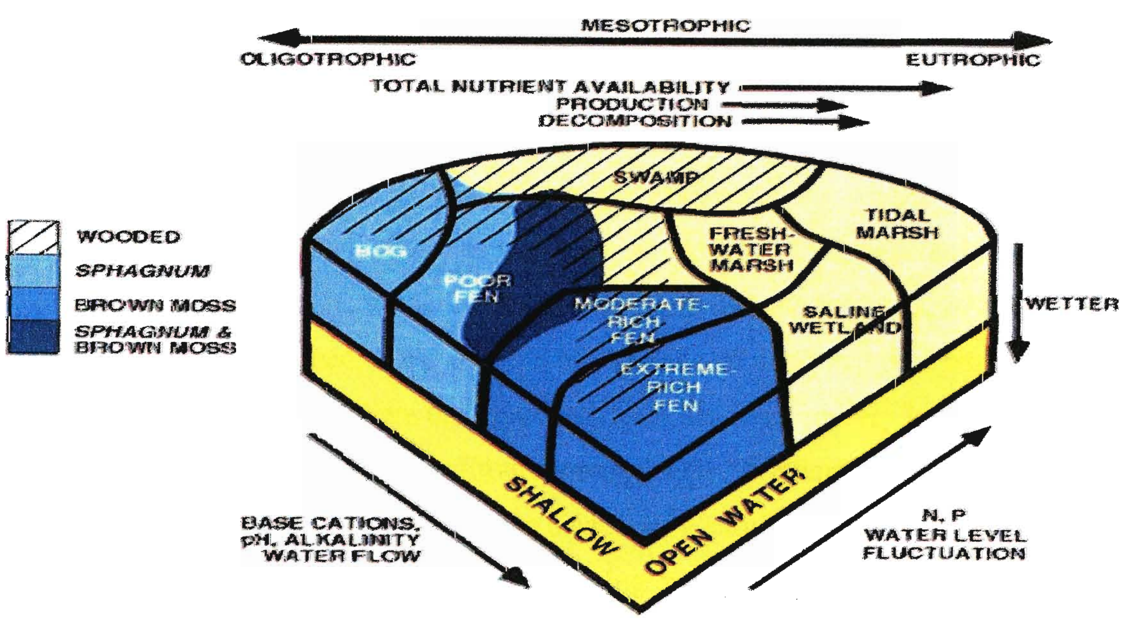


Figure 1.9: Types and characteristics of wetlands (Vitt et al., 1996).

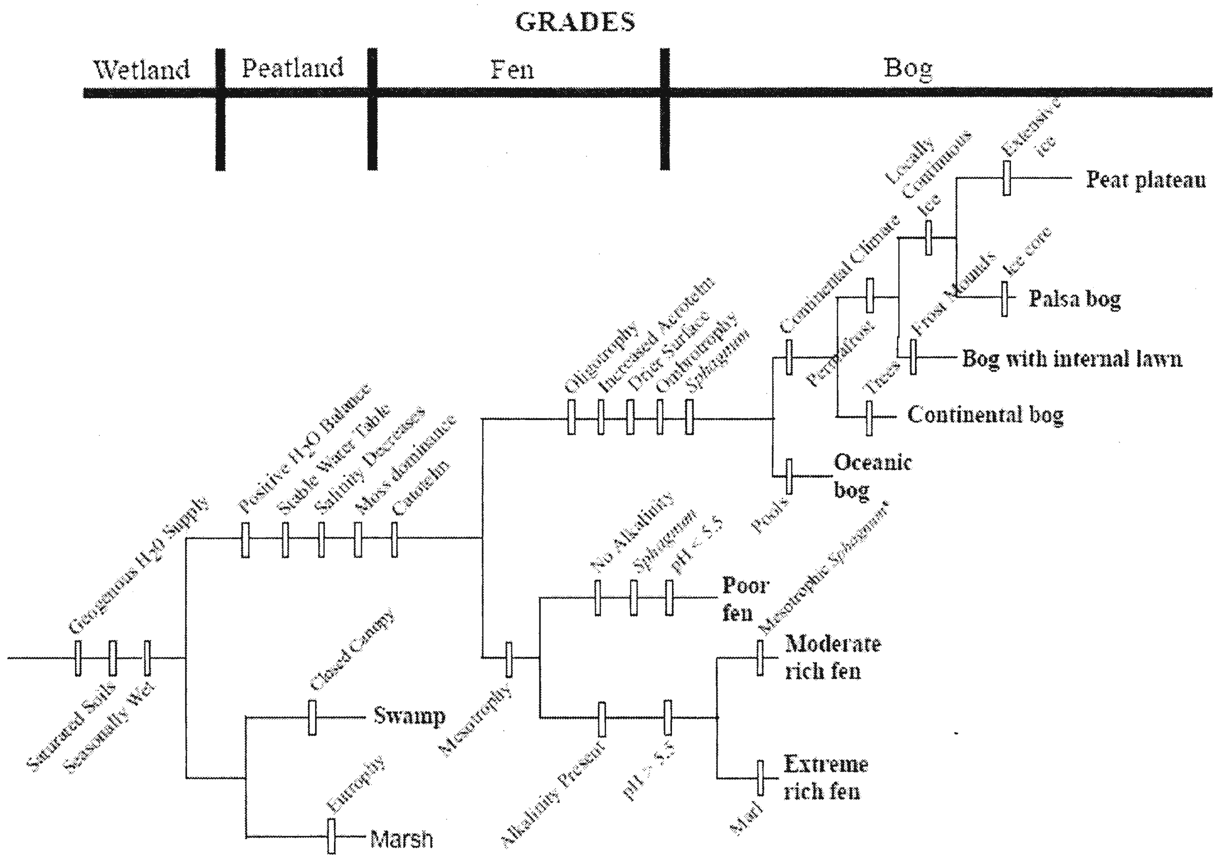


Figure 1.10: Characteristics composing different grades of wetlands in Alberta (Vitt et al., 1996).

1.4. Reclamation and Legislation

1.4.1. Reclamation

Reclamation efforts in the oil sands are large-scale, involving whole landscapes and watersheds, thus oil sands wetlands are fundamentally different from many of the wetland projects documented in the published literature (Harris, 2007). Wetland reclamation is defined as the creation of wetlands on disturbed land where they did not formerly exist or where their previous form has been entirely lost (Canadian Council of Ministries of the Environment, 1996). Wetland restoration is a process of returning a remnant wetland site to a capability similar to that before the disturbance (Leung and Smith, 1999). When working on a restoration project in the Oil Sands region the limiting factor of the reclamation process is the time-scale for natural succession, which tends to be in hundreds of years, however succession in constructed areas tends to happen in shorter periods because various elements such as phosphate and magnesium can be introduced, aiding in the reclamation process. Nutrients act like a catalyst by increasing productivity and resulting detrital deposition (Harris, 2007).

The procedures used for surface mining operations of the oil sands produces several byproducts that are relevant for wetland reclamation (list modified from Harris, 2007):

- mining excavation produces pits, and leaves behind overburden
- extraction of bitumen from oil sands using an aqueous process produces process-affected tailings containing sand, silt and clay in suspension, soluble organic chemicals such as naphthenic acids, hydrocarbons, ammonia, heavy metals and salts.
- Upgrading of bitumen produces by-products like sulphur and coke that are stockpiled for later retrieval. These piles may directly or indirectly affect wetlands chemically.

These changes alter the geochemistry, topography and hydrology of the land, causing reclamation efforts to incorporate and accommodate these post-mining changes.

One of the main difficulties with reconstruction of wetlands in the oil sands region of Alberta is due to the thick layered marine-derived (saline) soils, which complicates the hydrogeochemical design of wetlands by naturally increasing the conductivity (Vitt et al., 1996). The overburden may be of coarse-grained sand or shale, fine-grained silt or clay, non-saline, saline or sodic depending on whether it originated from soils derived from the Clearwater Formation or the Pleistocene layer (Figure 1.1) (Ciborowski amd Whelly, 1997). The challenge with using these soils (containing saline and sodic leachates) for reconstructed wetlands is that many species naturally found in Boreal wetlands are very sensitive to the elevated conductivity and sodium (Table 1.1) (Harris, 2007).

An additional difficulty faced during reclamation is elevated pH levels (Ciborowski and Whelly, 1997). The pH of water after oil sands extraction is typically between 8-9. To further compound the issue, bitumen contains surfactants (soap) made up of a carbon and hydrogen chain that terminates in an acidic group. When these surfactants are introduced to water they affect surface wettability, interfacial tension, and surface electric charge (Masliyah, 2006).

Table 1.1: Constituents affecting water chemistry in reclaimed wetlands in the oil sands region of Alberta (modified from Harris 2007).

Nutrients	• Nitrogen is a limiting nutrient in natural Boreal marshes and fens • Phosphorous may be the limiting nutrient in sub-saline reclaimed marshes • Phosphorus availability increases with catchment size • Adding phosphorus enhances initial water treatment rates, but may favor weedy vegetation
Naphthenic acids	• Saturated, polycyclic and acyclic carboxylic acids that naturally occur in petroleum deposits • Can be toxic to aquatic organisms
Salinity	• High in sodium and sulphate ions rather than calcium and bicarbonate ions • Limits organisms to those which can tolerate the wetland conductivity
Ammonia	• Inorganic form of nitrogen. • Concentrations may increase as dissolved oxygen decreases • Under specific conditions of temperature and pH, the un-ionized component of ammonia can be toxic to aquatic life. The un-ionized component of ammonia increases with pH and temperature. • Open water aeration and a healthy bacterial population promotes removal
Hydrocarbons	• Mostly substrate and sediment bound
Dissolved Oxygen	• Supersaturation or undersaturation can sometimes be harmful for organisms and cause sickness or death

The long-term reclamation plan outlined by oil companies includes constructed wetlands and End Pit Lakes (EPLs). The use of both natural and constructed wetlands as a means to treat contaminated water is emerging as an important technology. The technology had its origins in observations of improvements in the quality of waters flowing through natural wetlands (Fine Tailings Fundamentals Consortium, 1995).

EPLs will be established in the mined-out pit of an extraction area. Approximately 27 EPLs have been planned to date within the Athabasca oil sands area (Westcott and Watson, 2007). They will consist of bottom substrate capped with water, soft tailings or other process-related materials may be placed on top of the bottom substrate (Golder Associates Ltd. 2001). Modeling and relevant background studies have been the basis of research, but a fully realized EPL had not yet been constructed. Some potential issues with EPLs include impact to aquatic life, the bioaccumulation of compounds within food webs and the development and sustainability of the ecosystem (Golder Associates Ltd. 2002). The success of the EPLs is unknown so ongoing research such as this project is critical for developing the reclamation plan. These projects give sort-term indication of potential long-term results (Westcott and Watson, 2007).

1.4.2 Legislation

Federal agencies involved in the oil sands include Environment Canada and the Canadian Environmental Assessment Agency administer of the *Canadian Environmental Assessment Act* (CEAA) (Westcott and Watson, 2007). CEAA is a federally legislated environmental assessment process designed to integrate environmental considerations in project planning. It is responsible for the overall administration of the federal environmental assessment process. Oil sands projects are subject to environmental assessment under CEAA (Canadian Council for Ministries of the Environment, 1999).

The *Canadian Environmental Protection Act* (CEPA) is also administered by Environment Canada (in conjunction with Health Canada). CEPA protects the environment, including water quality, sediment quality, aquatic organisms and human health, from toxic substances or other pollutants (Environment Canada and Health Canada, 2001). The Canadian Environmental Quality Guidelines have set recommended levels for several substances within water, sediment and tissue (CCME 1999, updated 2006) (Alberta Environment, 1999; Canadian Council for Ministries of the Environment, 1999).

Environment Canada also administers Sections 36(3) of the *Fisheries Act*, which prohibits the discharge of deleterious substances into water frequented by fish unless otherwise authorized. While the department of Fisheries and Oceans Canada (DFO) manages the rest of the *Fisheries Act* (Fisheries and Oceans Canada, 1986).

Alberta Environment (AENV) manages Alberta's *Environmental Protection and Enhancement Act* (EPEA), which is intended to integrate the protection of air, water and land (Alberta Environmental Protection, 1993). Specifically Alberta's *Water Act* regulates activities affecting water bodies in Alberta. Alberta's Sustainable Resource Development (ASRD) ensures a balance between the economic, environmental and social values within Alberta (Alberta Environmental Protection, 1995).

Part of the License to Operate under the provincial *Clean Water Act*, issued by the Government of Alberta to oil companies operating in the oil sands, states that there will be no discharge of process affected waters or of surface runoff from the plant site and mining area to the surrounding watershed (Rogers et al., 1996). Additionally, a component of the federal *Environmental Protection and Enhancement Act* (EPEA) outlines the reclamation objective of returning disturbed landscapes to 'equivalent land capability', which is defined as the ability of the land to support various land uses after reclamation that are similar but not necessarily identical to those that existed before mining.

Several additional guidelines and planning initiatives, should be followed at both federal and provincial levels. Federal guidelines include the Canadian Environmental Quality Guidelines (CCME 1999, updated 2006) and the Habitat Conservation and Protection Guidelines (DFO 1998). Provincial regulations include the Surface Water Quality Guidelines (AENV 1999c) and the Fort McMurray-Athabasca Oil Sands Sub-regional Integrated Resource Plan (AEP 1996). These parameters have forced the oil companies to invest in research regarding reclamation. It is predicted that surface mining will be in operation until beyond 2050, making reclamation an ongoing project (Harris, 2007).

The need for a biomonitoring tool that is sensitive to, but can tolerate fairly high levels of oil sands process affected material (OSPM), led to the current study of thecamoebians (testate amoebae).

1.5. Thecamoebians

1.5.1. Biology and Taxonomic Classification

The term "thecamoebians" (shelled/ testate amoebae) is an informal one used to characterize a very diverse "group" of protists belonging to two different classes and several orders within the Phylum Sarcodaria, Subphylum Sarcodina, Class Rhizopoda (Table 1.2) (Scott et al., 2001). The systemic taxonomy of thecamoebian species identified in this study can be found in Table 1.3. This polyphyletic group of amoeboid protozoans is characterized by the presence of lobose pseudopods, an amoeboid sarcodine cell, and a very simple sac-like, decay-resistant outer shell (Kumar and Dalby, 1998; Charman, 2001). The tests are morphologically distinct, typically allowing species-level identification (Booth and Zygmunt, 2005).

Thecamoebians, with some exceptions, reproduce once every two to eleven days (Ogden and Hedley, 1980) predominantly by simple asexual fission of the parent cell, although some are known to possess different forms of sexual reproduction (Schonborn, 1996). Sexuality appears to be rare (Valkanov, 1962).

These organisms occur abundantly in Quaternary to Recent lacustrine sediments (Kumar and Patterson, 2000) although they range back to the Carboniferous (Thibaudeau et al., 1986) or possibly even Cambrian (Scott et al., 2001). Average abundance estimates are greater in peat, reaching 16,000,000 per m^2 compared to oligotrophic lakes, which reach 226,000 per m^2 (Heal, 1962). Although thecamoebians inhabit a wide variety of environments (Figure 1.11), fossilization occurs almost exclusively in species found in late Quaternary/Holocene lakes, peatlands and rivers (Medioli and Scott, 1988). Only a small fraction of one order, the Order Arcellinida appears to commonly fossilize in lacustrine sediments (Medioli and Scott, 1983; Van Hengstum et al., 2008). Medioli et al. (1990) suggest that only ~20-25 species have been reported as fossils in lacustrine sediments, but this statement is subjective, as different workers employ very different taxonomic schemes. Medioli and coworkers are typically "lumpers", while other workers, such as Ogden and Hedley (1980), Charman (2001), and Mitchell et al., (2007) are "splitters". In addition to this fundamental difference in philosophy, there are two very different protocols for processing and analyzing samples: the protocol employed in this thesis, primarily employed by micropaleontologists working on lacustrine sediments (the "splitters") (Collins et al., 1990; Kumar and Dalby, 1998; Patterson and Kumar, 2000; Scott and Medioli, 1983) and another methodology, primarily employed by biologists working on peatlands (Bobrov and Mazei, 2004; Booth and Zygmunt, 2005; Hendon and Charman, 1997; Warner et al., 1990). A comparison study of the two methods has not yet been completed; differing methods are employed by the groups researchers because they study different types of substrate.

Table 1.2: Taxonomic position of thecamoebians (Medioli and Scott, 1983).

Phylum Sarcodaria

Superclass Rhizopoda

Class Lobosa	Class Filosa
Order Thecolobosa	Order Testacealobosa
(= Arcellinida)	(= Gromida)

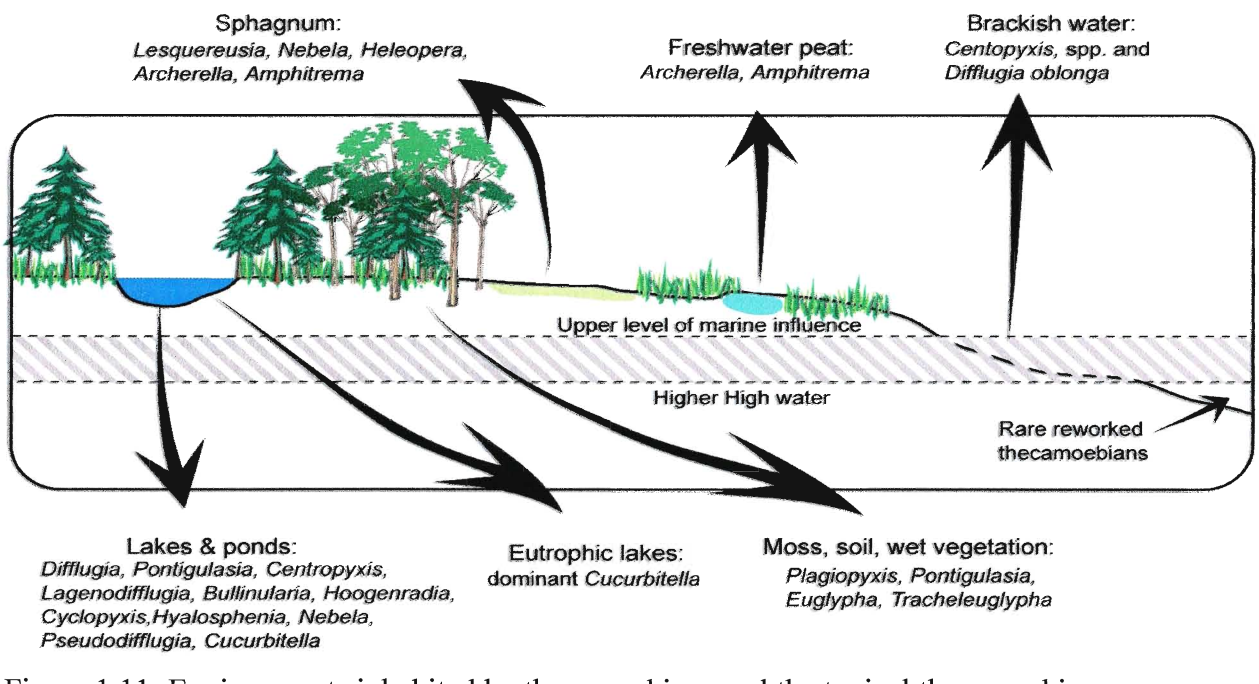


Figure 1.11: Environments inhabited by thecamoebians and the typical thecamoebian found in each environment (modified from Scott et al., 2001).

Biogeographical and ecological research on thecamoebians has been limited by the different taxonomic approaches of various research groups. To overcome these barriers comparisons between regions and environments can be made if taxonomic lumping of similar taxa is applied (Booth and Zygmunt, 2005). The morphotypic names used in this thesis (Table 1.3) are based on those established and illustrated by Medioli and Scott (1983) and more recently by Kumar and Dalby (1998) who illustrate ecophenotypic strains.

Table 1.3: Systematic taxononomy of thecamoebians, outlining the species observed in this study.

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)

1.5.2. Test Composition & Morphology

Thecamoebian tests are either flattened or rounded with an aperture located on or near the tapered end, or a donut-shaped test with an invaginated aperture on the ventral side, which is more or less flattened (Medioli & Scott, 1983), although substantial morphological variability has been observed between these two broad groups (Kumar and Dalby, 1998).

Most thecamoebians build xenogenous tests by agglutinating foreign particles (xenosomes) in an autogenous/organic cement (Boudreau et al., 2005; Medioli and Scott, 1988), usually mucopolysaccharide. The nature of the xenosomes is entirely controlled by the composition of the substrate, and may consist of sand/mineral grains (Patterson and Kumar, 2000; Scott et al., 2001) and/or diatom frustules (Kumar and Patterson, 2000). In most cases, the nature of the xenosomes depends on the availability of inorganic particles and not on genome-based selectivity. McCarthy (1984) demonstrated that clones of *Centropyxis aculeata* incorporated grains of carborundum into their tests in the laboratory and Patterson et al. (1996) found that various species incorporated shiny metallic particles from contaminated lakes in Northern Ontario (Patterson et al., 1996). Collins et al. (1990) found that thecamoebian tests in the Arctic tended to be very coarsely agglutinated while specimens in Florida tended to incorporate higher numbers of diatom frustules. Tests can also be entirely secreted by the organism, using idiosomes (particles secreted by the organism) (Medioli and Scott, 1983). Autogenous tests are usually smooth, proteinaceous, sometimes made of siliceous (or rarely calcareous) platelets (idiosomes) (Kumar and Dalby, 1998).

The morphological diversity of the tests reveals important taxonomic characteristics such as presence or absence of spines, nature and shape of xenosomes and idiosomes, shape and composition of tests and morphological features associated with the apertures such as diaphragms, collars, lobes and teeth (Kumar and Dalby, 1998). Variability of natural thecamoebian populations is expressed either in the varying shell sizes (correlated), or in the varying sizes of separate parameters (non-correlated) (Bobrov et al., 2004). Some researchers feel that size is of no taxonomic importance because it may be determined at the time of fission by the volume of cytoplasm available in the parent test (Medioli and Scott, 1983). It is thought that cytoplasmic volume may be controlled by the availability of food in the period prior to reproduction (Kumar and Dalby, 1998). The size and shape of a test aperture demonstrates the greatest variability (Patterson and Kumar, 2000). The character of variability (its amplitude and correlativity) differs not only on different species, but also in different population of the same species (Bobrov et al., 2004). Some research has suggested that within a species "morphing" of the asexually reproducing organisms can take place in response to environmental stresses (conditions outside the preferred tolerances of the species) (Reinhardt et al., 1998). These infrasubspecific variants are called "strains" (Kumar & Dalby, 1998).

Thecamoebian tests are resistant to dissolution, giving them a higher preservation potential than most organisms, and their rapid generation time allows them to respond to environmental changes faster than surrounding vegetation (Patterson and Kumar, 2002; Jauhaininen, 2002). This makes thecamoebians excellent indicators of both short and long-term environmental trends (outlined in sections 1.5.3 & 1.5.4) (Kumar and Dalby, 2000).

1.5.3. Ecology and Potential as Paleoenvironmental Indicators

Thecamoebians can be found in a wide range of geographic settings, ranging from tropical to arctic latitudes (Collins et al., 1990; Asioli et al., 1996). These protists depend on water to live because they posses an unprotected cell membrane for feeding (Warner, 1990), but they are found in most areas where there is sufficient moisture, for example soils, mires, peat bogs, freshwater to brackish ponds and lakes, forest floor litter, damp soil, and occasionally mossy habitats (Heal, 1962; Medioli et al., 1990). Most of the lacustrine taxa prefer oligotrophic lakes with mildly acidic water and are found in reduced numbers in eutrophic lakes (Medioli et al., 1990). They play an important role in the food web of lacustrine and wetland ecosystems (Figure 1.12). Although their biology and trophy remain poorly understood, it is recognized that some taxa, e.g. *Centropyxis aculeata*, are bacteriophage (McCarthy, 1984) and others, such as *Cucurbitella tricuspis*, graze on algae (Patterson and Kumar, 2000; Warner et al., 1990). They are in turn preyed upon by higher organisms in the food chain (Patterson and Kumar, 2000).

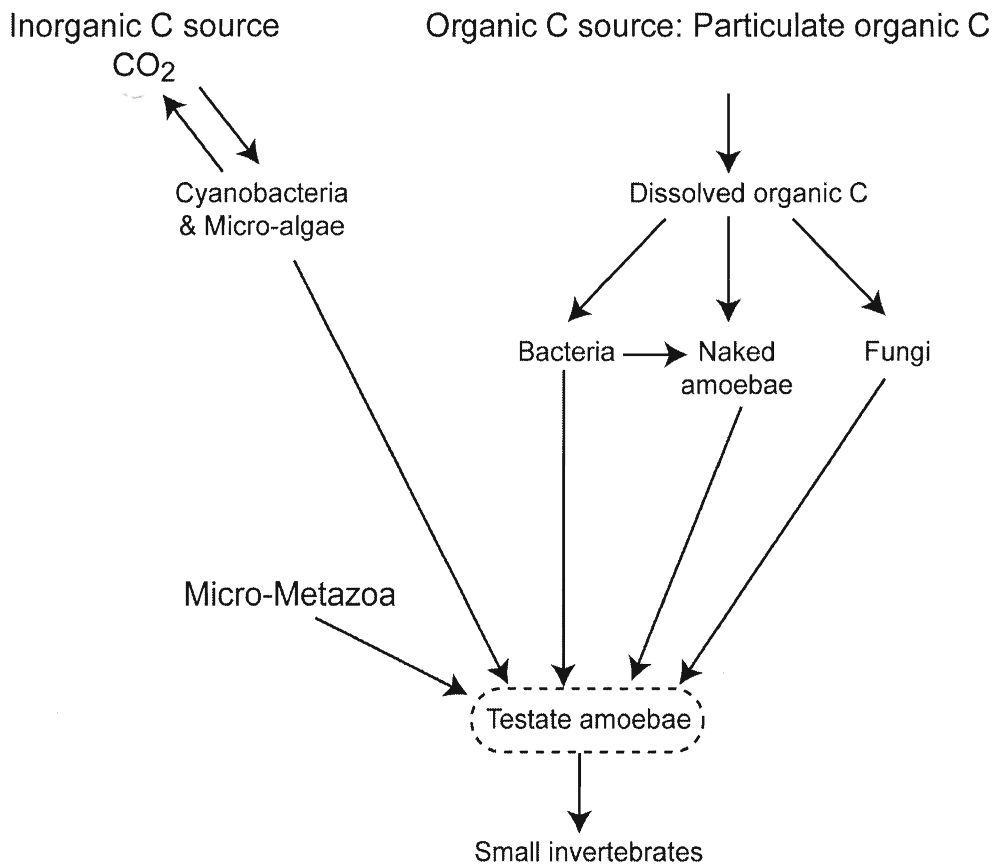


Figure 1.12: The role of thecamoebians (testate amoebae) in the aquatic food web (modified from, B. Warner personal communication).

It is accepted that various thecamoebian species preferentially inhabit specific ecological niches (Medioli and Scott, 1983). More recent studies have shown that infrasubspecific variants (strains) can be particularly sensitive to differing environmental variations (see Chapter 2) (Asioli et al., 1996; Reinhardt et al, 1998; Kumar and Dalby, 1998), potentially making them good environmental indicators, specifically with a high degree of sensitivity to conductivity and naphthenic acids.

Relatively little work has been done to understand the ecology of these protists that play a significant role in freshwater ecosystems. Ecological factors thought to be important in controlling the distribution pattern of thecamoebians include: dissolved oxygen content, dystrophy-grade of the lake (C/N ratio in sediment which depends upon the existence of humus compounds), pH, water temperature, salinity, the grain size of sediment, and the existence of *Sphagnum* carpet around the lake, although as yet there is no predictable response trend (Patterson and Kumar, 2002).

Thecamoebians react quickly to environmental change due to their rapid generation time and sensitivity to various environmental parameters. Because they have a high preservation potential, are resistant to low pH conditions, are small (<300 μm), and are present in significant numbers in small paleontological samples, they are important tools in environmental monitoring and paleoenvironmental reconstruction (McCarthy et al., 1995). Other commonly used benthic bioindicators, such as ostrocods and mollusks, tend to dissolve in lower pH environments typical of freshwater deposits. Thecamoebians are autochthonous, preserved *in situ*, thus avoiding some of the problems associated with the interpretation of pollen, which are largely allochthonous (Jauhaininen, 2002). Since they live at the sediment-water interface, only a very thin sample of the topmost sediment is needed, allowing high-resolution studies.

The importance of understanding continental and sub-continental patterns of thecamoebian distribution has been noted, in order to allow accurate environmental interpretation of changes in modern and fossil assemblages (Booth and Zygmunt, 2005; Collins et al., 1990). Nonetheless, only a few studies have examined the biogeographic distribution of thecamoebians in North American lakes (Scott and Medioli, 1983; Patterson et al., 1985; Honig and Scott, 1987; Collins et al., 1990) and wetlands (Charman 2001; Booth and Zygmunt, 2005). Most lacustrine studies have found climate to be a dominant control on species distribution (e.g. McCarthy et al., 1995), while in oligotrophic peatlands the distribution of taxa is thought to be primarily controlled by substrate moisture (Charman, 2001).

1.5.4. Previous Studies using Thecamoebians as Proxies of Ecosystem Health

Thecamoebians have proven to be valuable paleoenvironmental tools in many applications, such as ecology and paleoecology of peatlands, paleoclimatic reconstruction in lacustrine settings (Boudreau et al., 2005; McCarthy et al., 1995), Holocene climate changes (Patterson and Kumar, 2000), settlement history and land use changes (Reinhardt et al., 2005), distinguishing natural versus anthropogenic acidification/eutrophication (Patterson and Kumar, 2000; Patterson and Kumar, 2002; Reinhardt et al., 2005), paleo-sea level reconstructions (Scott et al., 2001), paleohydrological changes in lakes (Patterson and Kumar, 2000; Patterson and Kumar, 2002; McCarthy et al., 2007). Booth (2008) used thecamoebians to infer water-table depth as well as develop transfer functions which will be used for understanding temporal relationships between vegetation and hydrology within raised bogs, delimiting special and temporal patterns of past centennial and sub-centennial scale drought events, monitoring and informing bog restoration efforts and assessing the response of terrestrial vegetation to past climate variability and change.

More recently, research has concentrated on their potential as indicators in environmental, remediation and anthropogenic impact studies (Patterson et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000; Patterson and Kumar, 2002; Kauppila et al., 2006). Specific examples of such studies include thecamoebians indicating changes in lake bottom acidity (Kumar and Patterson, 2000), mine tailings in near neutral pH lakes and contaminated low pH lakes (Reinhardt et al., 1998; Patterson and Kumar, 2002). Because they are lower on the food chain, they have been found to accumulate arsenic, making them excellent indicators of arsenic contamination (Patterson and Kumar, 2002).

Their ability to reproduce rapidly (generation times of only a few days- Ogden and Hedley, 1980) makes them excellent ongoing indicators of an ecosystem's health. In most polluted environments researchers have found a dramatic reduction in diversity of thecamoebian species or "strains", and typically one or two species or strains will dominate the population (Reinhardt et al., 1998). Patterson and Kumar (2000) has found that infrasubspecific strains sometimes discriminate among environments better than species units and recommends their use when studying lake microenvironments, pollutants, and rates of lake remediation. Their potential for high-resolution sampling makes it possible to not only analyze the pre-impact species compositions in restoration applications, but also to follow recovery using sediment samples (Kauppila et al., 2006).

Very little work has been done using thecamoebians to monitor remediation, but because they have shown the ability to respond to differing levels of pollution in an environment, the potential exists (Patterson and Kumar, 2002) and in some cases they have provided data on rates of remediation by providing fossil record evidence of environmental condition changes (Patterson et al., 1996; Reinhardt et al., 1998). Patterson and Kumar (2000) found that in most the aquatic environments it was possible to observe natural remediation taking place as evidence by the return of vegetation and "normal" arcellacean faunas in parts of the environments. The return of vegetation accelerates the rate of natural remediation by stabilizing cover material that in effect "caps" the tailings. Patterson and Kumar (2002) suggest that lake remediation in polluted lakes is best achieved by leaving the tailings undisturbed to be buried naturally or by speeding the process by addition of an allochthonous sediment cap. Reinhardt et al. (1998) observed that a thecamoebian fauna from a trench had become very stressed due to dredging as a previous remediation attempt. It appeared that degrading the tailings only served to nullify any natural remedial effects that have already occurred. In addition, when tailings are removed a new location must be found to store them (Patterson and Kumar, 2002; Reinhardt et al., 1998).

1.6. This Study and Methodology

This study investigates the potential of thecamoebians as bioindicators of aquatic environmental health in lakes and wetlands constructed by companies operating in the Oil Sands of Alberta (see Section 1.4). Oil companies are obligated to reclaim all land disturbed by mining activity, and as part of the reclamation plan, they require a means of monitoring aquatic environmental health to assess the progression to more natural environments from those highly impacted by OSPM, and thus profoundly disturbed. As summarized above in Section 1.5, thecamoebians are ideal environmental proxies, and they have been used in several similar studies of anthopogenic impact (Kauppila et al., 2006; Patterson and Kumar, 2000; Reinhardt et al., 1998). These protists respond quickly to environmental change and their remains fossilize readily. Because thecamoebians play an intermediate role in the food chain, eating bacteria and fungi and being consumed by larger micro-metazoa (Figure 1.12), thecamoebian populations can be used to infer the health of the directly related biotic populations.

The first step in this study was to determine whether a relationship exists between thecamoebian assemblages and the byproducts of oil sands extraction and processing (OSPM/OSPW). Twenty two samples collected from the sediment-water interface in the oil sands Lease 86 constructed wetland test facility by employees of Suncor Energy Inc. in August 2007 were analyzed for thecamoebians. The samples were processed for thecamoebian analysis at Brock University by sieving, retaining the >63 and 45-63 um size fractions separately. Samples were counted wet (in water) in a gridded Petri dish at 50X magnification using a Leica MZ 12.5 microscope. Species were identified to strain, using the key of Kumar and Dalby (1998) and tabulated. Plots were then generated, generally lumping strains together to simplify the figures. The data were then compared with analysis (performed by staff at the Edmonton Research Station of Syncrude Canada Ltd.) of a large suite of chemical and physical parameters in the water column immediately above the sediment-water interface.

Major ionic and trace metal contents were determined at the Syncrude's Edmonton Research facility using Syncrude Canada's standard analytical methods (Syncrude, 1995). The analytical methods used included:

- pH: measured using pH meter on whole samples after calibration of meter with buffers at pH= 4, 7 and 10;
- conductivity: determined on whole samples using a YSI conductivity meter after calibration conductivity standard- 1000uS/cm of KCl.

Prior submission for general water analyses, samples were filtered using 0.45µm Millex® disposable filters. Analyses reported include:

- Cations and minor elements: determined by ICP-OES (Inductively coupled plasma optical emission spectrometry using a Varian Vista-PRO RL ICP-OES).
- Ammonium (NH_4^+) concentrations: cation analysis by ion chromatography (Dionex Corporation, Sunnyvale, CA, USA Model DI-300 IC) using Dionex-DX 300 Series Chromatographic System, with Ion-Pac CS16 4mm Guard column (P/N: 057574) with Ion-Pac CS16 4mm Analytical Column (P/N: 057573) and a cation Self-Regenerating Suppressor (SRS) (P/N: 061563). Gradient program with Methanesulfonic Acid (MSA) was used.
- Anions (Cl^- and $SO_4^=$): determined by ion chromatography (Dionex Corporation, Sunnyvale, CA, USA Model DI-300 IC) using Dionex-DX 600 Series Chromatographic System. A Ion-Pac AG4A-SC Guard Column (P/N 043175) and Ion-Pac AS4A-SC Analytical Column (P/N 043174) were used with a gradient program with 3 mM sodium bicarbonate/2.4 mM sodium carbonate eluents .
- Alkalinity (HCO_3^- and $CO_3^=$): measured by auto-titration using a Metrohm Titriono Model 751 titrator.
- Total Naphthenic Acid (NAs): concentrations were obtained using the FTIR method, described by Jivraj *et al.*, 1996, in which the carboxylic acids were extracted from H_2SO_4 acidified (pH 2-2.5) water samples with methylene chloride, and absorption at wave numbers 1706 and 1745cm^{-1} were measured with a Thermo Instruments (Canada) Inc. Nicolet Model 8700 FT-IR spectrometer.

Chapter 2 discusses the strong inverse relationship between the more sensitive difflugiid thecamoebians and the major constituents in OSPM that impact aquatic ecosystem health: naphthenic acids and sodium chloride. Conversely, aquatic environments with high concentrations of these constituents at the time of collection were characterized by low-diversity assemblages, and were strongly dominated by centropyxid taxa (*Centropyxis aculeata* and *Centropyxis constricta*) together with *Arcella vulgaris*. The thecamoebian taxa in the constructed wetland test facility were also common in natural lakes in the Boreal Forest region of Alberta (Appendix 2).

The second step was to determine whether the correlations between thecamoebian assemblages and the major byproducts of oil sands extraction found during the first year of study were reproducible, and whether thecamoebian populations responded sufficiently quickly to be used in assessing reclamation management options. Chapter 3 discusses the comparison between eight sites in the Suncor constructed wetlands test facility re-sampled in June 2008 with the data from August 2007. The June 2008 samples processed in the same manner as the August 2007 dataset, but prior to counting, samples were stained with Rose Bengal after being fixed with formaldehyde, following the methodology of Scott and Medioli (1980). Assemblages of thecamoebian tests at sites with no change to reclamation strategy (and therefore little change in conditions, except for those relating to climate) were almost identical to those analyzed the previous year. At sites where the input of OSPW was reduced, the relative abundance of difflugiid taxa (and therefore the species diversity, measured using the Shannon Diversity Index, SDI) increased markedly year-to-year. Species diversity at most sites in the constructed wetland test facility exceeded that found in the twelve natural lakes analyzed from a transect from near Canmore in SW Alberta to Fort McMurray in NE Alberta (Appendix 3, Table 3.8, 3.9, 3.14).

The relative abundance of stained tests, interpreted as containing cytoplasm at the time of collection (i.e. "living"), differed substantially from species to species in the June 2008 dataset (Chapter 3) as well as in the natural lakes sampled in July 2008 (Appendix 4). This was tentatively interpreted to mean that some taxa have a lower fossilization potential. If so, they would be under-represented in the fossil assemblage (thanatoceonosis). It was also possible that different species bloom during different months. Samples collected from the Syncrude Demo Pond called Big Pit (a mock End Pit Lake constructed by Syncrude Canada Ltd., potentially to be used for long term storage) over several months were stained and analyzed in order to assess variations in the biocoenosis (living assemblage) through the growing season. These data are in Appendix 2 & 3 and referred to briefly in the General Discussion and Conclusions (Chapter 4).

Observations of thecamoebians in all samples except for those with the highest concentrations of oil sands process affected material, and of specimens with bitumen incorporated into their tests (Plate 4.1), suggest that thecamoebians seem to tolerate moderate levels of OSPM/OSPW. It is possible that they use low concentrations of these by-products of oil sands extraction and processing as building materials, and perhaps even as a source of nutrients. The general discussion in Chapter 4 examines the contributions of this study to develop a better understanding of thecamoebian biology, ecology and biogeography.

1.6.1. Study Sites

The samples investigated for this project were collected from the Suncor Constructed Wetlands (Figure 1.13 & 1.14) (CT demo site, Sustainable Lake North, Sustainable Lake South, High Sulphate Wetlands, Crane Lake) (Chapter 2 & 3), the Syncrude Demo Pond (Figure 1.14 & 1.15) (Chapter 4), and fifteen natural lakes in a NE-SW transect across Alberta from Gregoire Lake near Fort McMurray to the Spray Lakes Reservoir near Canmore (Chapter 5). Samples from the Suncor constructed wetland and Syncrude Big Pit sites were collected by employees of Syncrude and Golder Associates Inc. at the sediment water interface (approximately the top centimeter of sediment) by core or Ekman grab sampler. No preference was given to the sampling tool; the tool was selected based on the requirements of each environment and the availability of a floating dock at Big Pit allowing for the use of a corer. Samples from outside the oil sands were collected by myself, Dr. Francine McCarthy, and Dave Christie MSc. candidate, using an Ekman grab sampler to collect sediment from the sediment water interface (Chapters 2,3,4,5).

Set One (Table 1.5) from the Suncor wetlands was collected by employees of Golder Associates on August 29th, 2007. Set Two (Table 1.5) from the Suncor wetlands was collected by employees of Golder Associates on June 13th, 2008, from eight of the same sites, at the same location sampled the previous year. Approximately 50 g of sediment was collected from the sediment water interface, 50 ml of water was collected from above each sample and pH, dissolved oxygen and temperature were recorded at time of collection. Water samples were transported to Syncrude Canada Ltd. Edmonton Research facility for water analysis of conductivity, major ions, trace metals and napthenic acids using their standard protocols (Syncrude, 1995). Sediment samples were transported to Brock University in glass gars and stored at $4^{O}C$ until processed for thecamoebian analysis (chapter 2 & 3). Set Two was collected with the intention of assessing reproducibility of the biomonitoring technique, In addition, since the flux of OSPW was reduced to certain parts of the Suncor Constructed Wetlands, it allowed us to investigate the reponse of the thecamoebian fauna to environmental changes after approximately one year. See appendix 3 for additional site information.

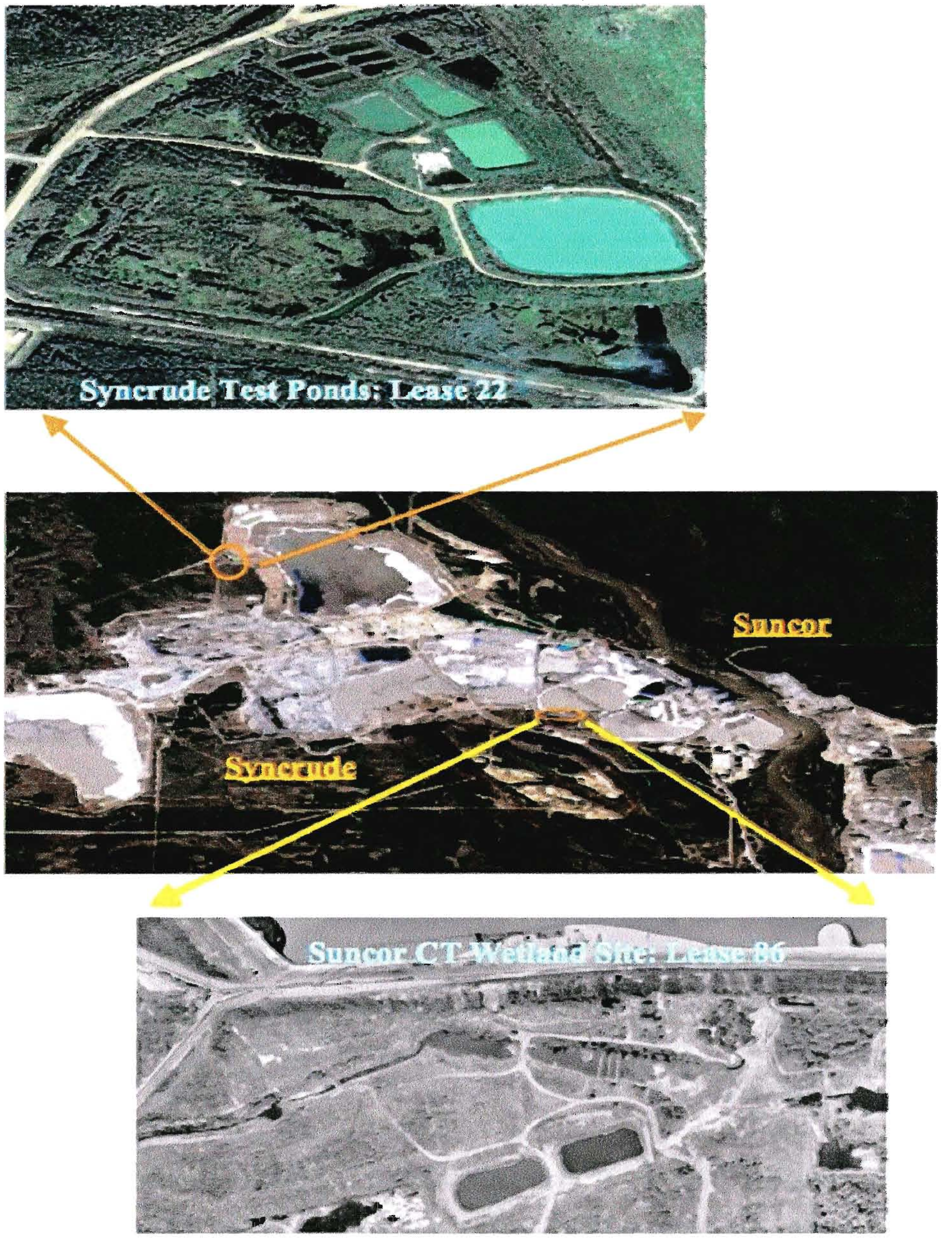


Figure 1.13: Satellite photo of the Athabasca Oil Sands mining operation, with excerpts of the Syncrude and Suncor test ponds (M. MacKinnon, personal communication). For exact coordinates of sites please see Figures 1.14 & 1.16.

1.6.2. Suncor Constructed Wetlands

Suncor Energy Inc. (Oil Sands) has implemented the use of Consolidated Tailings (CT) as the basis of tailings management. Current and future mine pits will be backfilled with CT material and reclaimed as terrestrial or wetland landscapes (Golder Associates Ltd., 2006). Figure 1.14 shows the Suncor constructed wetlands and the location of all wetlands observed on the Suncor property. The Suncor

Constructed Wetlands were developed as a means of testing and monitoring reclamation practices that are used for the remediation of current and future mine pits. The construction and implementation of each wetland composing the Suncor Constructed Wetlands varies and is summarized below (Golder Associates Ltd., 2006).

Between the completion of the Set One (2007) and Set Two (2008) study, modifications were made to the various Suncor Constructed Wetlands causing increased or decreased inflow of OSPW to various wetlands (C. Daly of Suncor Energy Limited, personal communication). Modifications to wetlands investigated as part of this study are outlined in Chapter 3. The individual sample sites Suncor Constructed Wetlands were numbered when they were constructed, and they are constantly monitored. The sample numbers assigned refer to these sample sites, and will be used throughout the thesis. The sample numbers for the second year of study (Set Two) have been assigned a (-2) behind the original sample number. The sites from the Suncor Constructed Wetlands have been characterized and continuously monitored by Golder Associates (Golder Associated Ltd., 2006). Examples of site description are included below.

1.6.2.1. Suncor CT Demo Site (Sites 2,,3,4,5,8,9,10,11,12,13,16,17,18,19,20,23)

UTM 465700E, 6315841N

CT Demonstration Study Site (CT Demo site) at Reclamation Area 11 is a 34 ha series of wetlands. This study site was created in 1999/2000 to assess various approaches for reclaiming a CT landscape (Figure 1.14). The study site was created by flooding an area with 1m of CT, while a water-filled trench was filled with 4m of CT. Some of the areas received a muskeg cap (Golder Associated Ltd., 2006).

The amount of hydrocarbon (oil) in the CT release water is dependent on the efficiency of Suncor's extraction plant. The CT release water occasionally contains visible amounts of oil (Leung et al., 1999).

1.6.2.2. Sustainable Lake North Test Pond (Site 14)

UTM 467500E, 6316502N

One of two sustainable lake test ponds established on Waste Area 14 in 1992 as a field-scale water capped fine tails pond. This pond is 123 x 54 m with a depth of 8m (Figure 1.14). The pond was filled with approximately 14,700m^3 of fine tails, and 24,000m^3 of tailings recycle water. Total volume 39,000m^3 (Golder Associates Ltd., 2006).

1.6.2.3. Sustainable Lake South Test Pond (Site 15)

UTM 467500N, 6316502N

One of two sustainable lake test ponds established on Waste Area 14 in 1992 as a field-scale water-capped fine tails ponds (Golder Associates Ltd., 2006). This pond is 111 x 49 m with a depth of 8 m (Figure 1.14). The 29,000 m^3 volume was filled with about 14,700m^3 of fine tails and 14,000 m^3 of tailings recycle water. A yearly addition of phosphate was added to this pond during the spring until 1996 (Golder Associates Ltd., 2006).

1.6.2.4. High Sulphate Wetlands (Site 22)

UTM 466387E, 6317227N

A small wetland located on the north side of Crane Lake (Figure 1.14). It had surface water sulphate levels of 1,130 mg/L in 1995 and sediment sulphate values of 13,100 mg/L. As a consequence of this naturally high level of sulphate, these wetlands have now been described as High Sulphate Wetlands. It is approximately 0.17 ha of wetlands that consists of an open water area that reaches a maximum depth of 0.95 cm. Three main habitat types were found along a moisture gradient in the wetlands: floating, aquatic plants, found in open water area; emergent plants (shore marsh), along the perimeter; and shrub-grasses found in the drier areas (Golder Associates Ltd., 2006).

1.6.2.5. Crane Lake (Site 21)

UTM 466403E, 6316924N

This site consists of lake and wetland environments, which lies just outside the active Suncor mining area (Figure 1.14). Originally this was a small wetland area that was ringed by overburden and stockpiled muskeg. The result was the restriction of drainage; hence water levels within the wetlands increased resulting in what is now Crane Lake. The lake is approximately 24.3 ha and is surrounded by grass, shrubs and emergent macrophytes. It supports a large bird population, high concentrations of phytoplankton, zooplankton and benthic invertebrates. This site can be considered slightly disturbed from runoff from the surrounding land that has been reclaimed from previous mining activities. Classified as eutrophic based on phytoplankton species composition. The berms around this lake were reclaimed between 1983 and 1990 (Golder Associates Ltd., 2006).

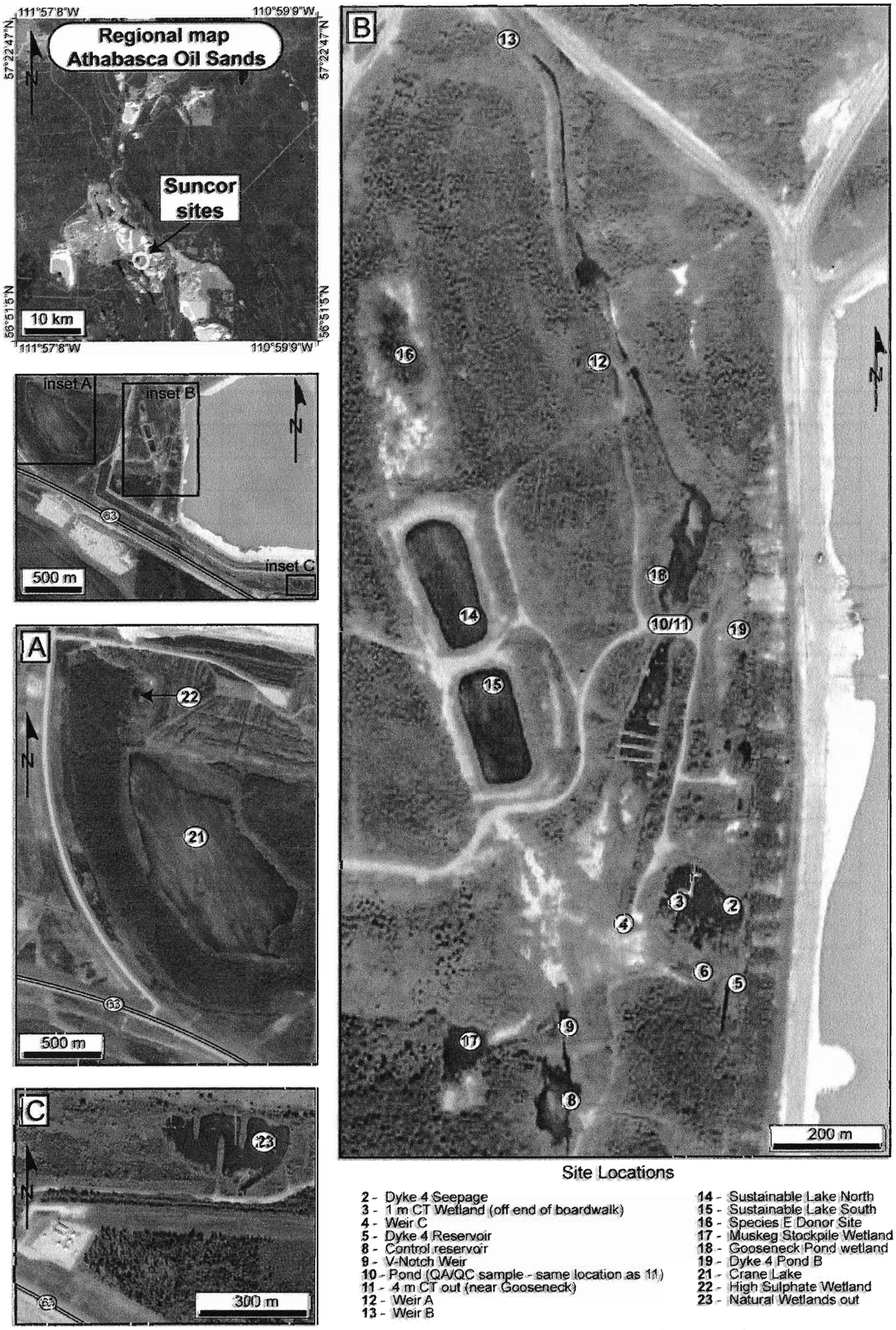


Figure 1.14: Satellite image depicting the location of the Set One and Two sites on the Suncor property in the Athabasca oil sands (Google Earth, 2009). Coordinates can be found around map in top left corner.

Table 1.4: Sample numbers and locations of the sites collected from the Suncor wetlands. Sample numbers for the second year of study (Set Two) have been assigned a (-2) behind the original sample number (Figure 1.15).

Set One (sampled in 2007)	Set Two (sampled 2008)	
Sample Number		Sample Location
2	2-2	Dyke 4 seepage
3	-	1m CT
4	-	Weir C
5	5-2	Dyke 4 Reservoir
8	8-2	Control Reservoir
9	9-2	V-notch Weir
10	-	Pond A
11	11-2	4m CT out (Gooseneck)
12	-	Weir A
13	-	Weir B
14	14-2	Sustainable Lake North
15	15-2	Sustainable Lake South
16	-	Sodic Wetland
17	-	Muskeg stockpile wetland
18	18-2	Gooseneck Wetland (Jan's Pond)
19	-	Dyke 4 Pond B
20	-	WA 14, Pond A
21	21-2	Crane Lake
22	22-2	High Sulphate Wetlands (HSW)
23	-	Natural wetland out (NWL out)

1.6.3. Syncrude Demo Ponds

The Syncrude Demo Ponds were developed as a means of testing and monitoring reclamation practices that are used for the remediation of current and future mine pits. The construction and implementation of each wetland composing the Suncor Demo Ponds varies (Golder Associates Ltd., 2006). Information regarding the Demo Pond investigated as part of this study is outlined below.

Samples were collected from Demo Pond (Figure 1.15) by Syncrude employees on May 21, July 22, August 19 and September 30 2008 (Table 1.5). Samples were collected from the same locations during different months with the intention of investigating thecamoebian populations during different seasons. See appendix 3 for additional site information.

1.6.3.1. Demo Pond (Big Pit/ BPIT)

UTM 458352E, 6326665N

Demo Pond is a large-scale test pond which was constructed in 1993 (Figure 1.15). It was constructed in saline overburden and contains 9m of fine tailings overlain by 2.5m of diverted local surface stream flow. Demo Pond is approximately 4 to 5 acres in size and a depth of 2.9m, it contains 70,000m^3 of MFT with 70,000m^3 of surface run-off water. Demo Pond is an artificial pond with parallel shores and no shoreline aquatic vegetation. The bottom consists of fine tailings from the CT used to fill the pond initially (Golder Associates Ltd., 2006).

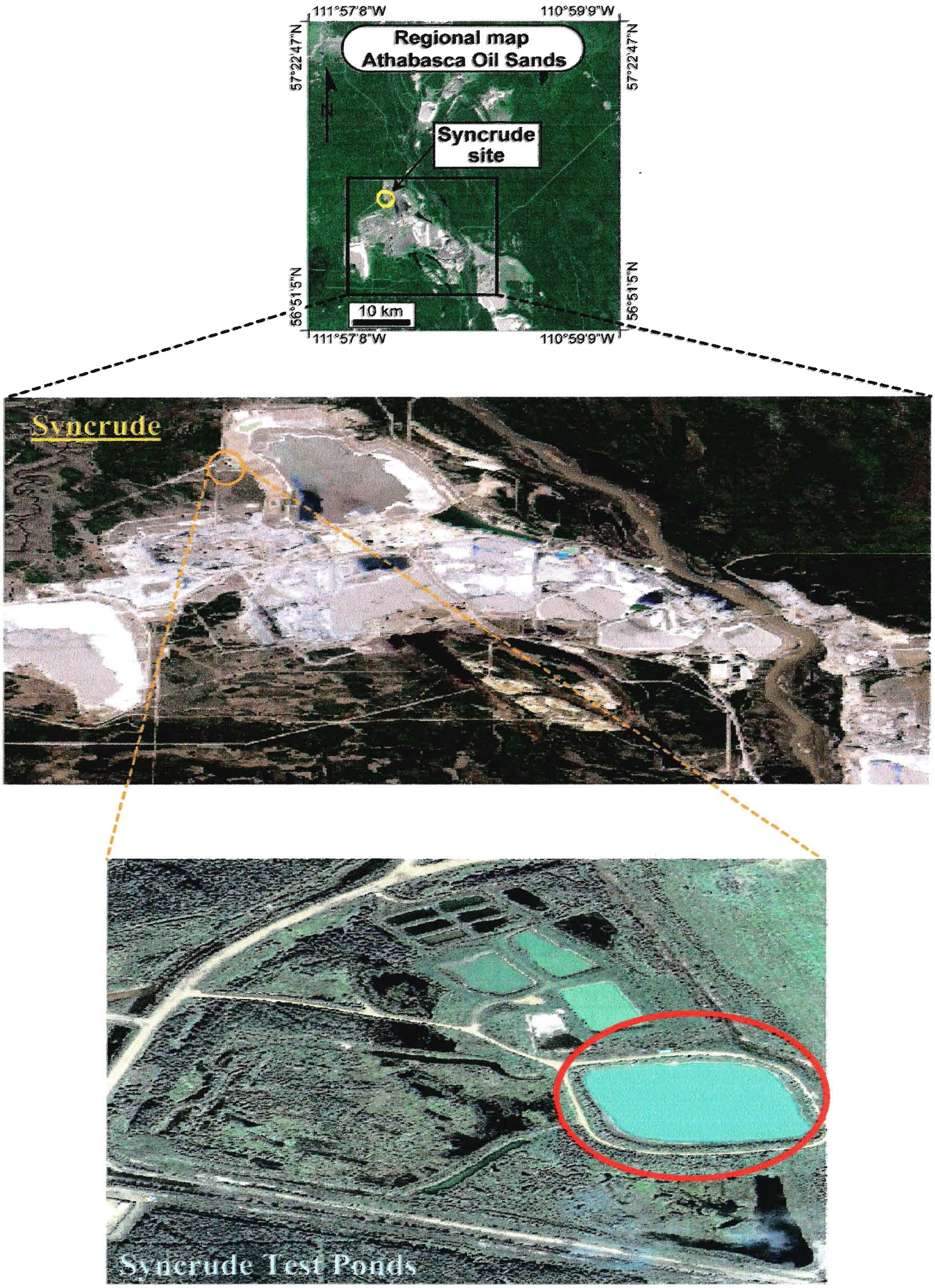


Figure 1.15: Satellite image indicating the location of the Demo pond (red circle) study sites on Syncrude property in the Athabasca oil sands (Google Earth, 2009). Coordinates can be found around map in top left corner.

Table 1.5: Samples collected from the Syncrude Demo Pond, between three or four replicates were collected from the same location in each month.

May	July	August	September
Sample Number			
BPIT1-M	BPIT1-J	BPIT1-A	BPIT1-S
BPIT2-M	BPIT2-J	BPIT2-A	BPIT2-S
BPIT3-M	BPIT3-J	BPIT3-A	BPIT3-S
PBIT4-M	PBIT4-J		

1.6.4. Alberta Lakes

Lakes sampled along the transect across Alberta can be found in Table 1.6. Most of the lakes (except for Jasper (ponded glacial melt) and Islet Lake) were selected using the *Atlas of Alberta Lakes* because it contained detailed information on each lake (Crosby et al., 1990). Only samples where a minimum count of 100 was achieved are included in this table.

Table 1.6: Site location and information for the Alberta Lakes investigated.

	Date Sampled	**Northing**	**Easting**	**Vegetation Zone**	**Drainage Basin**
Spray Lakes Reservoir	25/07/08	5101249	11523914	Rocky Mountain	Bow River
Jasper (ponded glacial melt)	24/07/08	5310426	11758320	Rocky Mountain	Bow River
Baptiste Lake	19/07/08	5443613	11334129	Boreal Forest	Athabasca River
Island Lake	21/07/08	5451399	11333301	Boreal Forest	Athabasca River
Gregoire Lake	21/07/08	5628787	11111264	Boreal Forest	Athabasca River
Lac St. Anne	18/07/08	5340593	11421516	Boreal Forest	North Saskatchewan R.
Wabamun Lake	18/07/08	5333552	11426508	Boreal Forest	North Saskatchewan R.
Islet Lake	19/07/08	5327421	11249369	Boreal Parkland	North Saskatchewan R.
Buffalo Lake	27/07/08	5262438	11256558	Boreal Parkland	Red Deer River
Miquelon Lake	27/07/08	5315609	11252204	Boreal Parkland	Battle River
Chestermere L.	27/07/08	5163155	11349359	Grassland	Bow River Basin
Eagle Lake	27/07/08	5059211	11317506	Grassland	Bow River Basin

1.7. References

Alberta Energy and Utilities Board (EUB), Alberta Geological Survey (AGS). 2000. http://www.ercb.ca/portal/server.pt?

Alberta Environment (AENV). 1999. Regional Sustainable Development Strategy for the Athabasca Oil Sands Area. Pub. No. 1/754. ISBM: 0-7885-0680-0.

Alberta Environmental Protection (AEP). 1993. A Decision Making Process for the Evaluation of Fish Introductions in Alberta.

Alberta Environmental Protection (AEP). 1995. Water Quality Based Effluent Limits Procedures Manual. Environmental Protection. Edmonton, Alberta.

Allen, E. W. 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. Journal of Environmental Engineering Science, 7: 123-138.

Asioli, A., Medioli, F.S., Patterson, R.T. 1996. Thecamoebians as a tool for reconstruction on paleoenvironments in some Italian lakes in the foothills of the southern Alps (Orta Varese and Candia). Journal of Foraminiferal Research, 26: 248-263.

Bobrov, A., Mazei, Y. 2004. Morphological Variability of Testate Amoebae (Rhizopoda: Testacealobosea: Testaceafilosea) in Natural Populations. Acta Protozoologica, 43: 133 – 146.

Booth, R.K. 2008. Testate amoebae as proxies for mean annual water- table depth in Sphagnum- dominated peatlands of North America. Journal of Quaternary Science, 23: 43- 57.

Booth, R. K., Zygmunt, J.R. 2005. Biogeography and comparative ecology of testate amoebae inhabiting Sphagnum- dominated peatlands in the Great Lakes and Rocky Mountain regions of North America. Diversity and Distributions, 11: 577- 590.

Boudreau, E.A., Galloway, J.M., Patterson, R.T., Kumar, A., Michel, F.A. 2005. A paleolimnological record of Holocene climate and environmental change in theTemagami region, northeastern Ontario. Journal of Paleolimnology, 33: 445- 461.

Canadian Council of Ministries of the Environment (CCME). 1996. Guidance manual for developing site-specific soil quality remediation objectives for contaminated sites in Canada. National Contaminated Sites Remediation Program, Canadian Council of Ministers of the Environment, Winnipeg.

Canadian Council of Ministries of the Environment (CCME). 1999. Updated 2006. Summary of Existing Canadian Environmental Quality Guidelines.

Canadian Wildlife Services (CWS). 2009. Environment Canada. Quebec Region. last updated, 2006-07-06. http://www.qc.ec.gc.ca/faune/AtlasTerresHum.htm

Carter, H.J. 1856. Notes on the freshwater Infusoria of the island of Bombay. No. 1. Organization. Annals and Magazine of Natural History, series. 2, 18(105):221-249.

Carter, H.J. 1864. On freshwater Rhizopoda of England and India. Annals and Magazine of Natural History, series 3, 13:18-39.

Charman, D.J. 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. Quaternary Science Reviews, 20: 1753-1764.

Ciborowski, J., Whelly, M. 1997. Chironomidae as Indicators of Wetland Viability: Report on Field Work in Wetlands of the Fort McMurray Alberta Area. Technical Report No. 4. University of Windsor. Windsor, ON.

Collins, E.S., McCarthy, F.M.G., Medioli, F.S., Scott, D.B., Honig, C.A. 1990. Biogeographic distribution of modern thecamoebians in a transect along the Eastern North American coast. p. 783 – 792. In C. Hemleben and others (Eds). Paleoecology, Biostratigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera. Kluwer Academic Publishers. Amsterdam.

Crosby, J.M., Bradford, M.E., Mitchell, P.A., Prepas, E.E., McIntyre, L.G., Hart Buckland-Nicks, L., Hanson, J.M. 1990. Atlas of Albert Lakes. Mitchell, P., Prepas, E. (Eds). The University of Alberta Press. Edmonton, Alberta.

Deflander, G. 1953. Ordres des Testaceolobosa (De Saedeleer, 1934), Testaceofilosa (De Saedeleer, 1934), Thalamia (Haeckel, 1862) ou Thecamoabians (Auct.) (Rhizopoda Testacea), in Grassa, P., ed., Traite de Zoologie, Tome I, fasc.2, p. 97-148, pl 1; Masson Paris.

Devito, K., Mendoza, C. 2007. Maintenance and Dynamics of Natural Wetlands in Western Boreal Forest: Synthesis of Current Understanding from the Utikuma Research Study Area. Appendix C. In Harris, M.L. Guideline for wetland establishment on reclaimed oil sands leases (revised second edition). Prepared by Lorax Environmental for CEMA Wetlands and Aquatics Subgroup of the Reclamation Working Group, Fort McMurray, AB. Feb/07.

Dujardin, F. 1835. Observations nouvelles sur les pr6tendus Cephalopodes microscopiques: Ann. Sci. Nat., ser. 2, 3: 312-314.

Ehrenberg, C.G. 1830. Organisation, systematik und geographisches Verhältnis der Infusionsthierchen. Druckerei der Königliche Akademie der Wissenschaften, Berlin.

Ehrenberg, C.G. 1832. Über die Entwicklung und Lebensdauer der Infusionsthiere, nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen, 1831, 1-154.

Ehrenberg, C.G. 1840. Das grössere Infusorienwerke. Königliche Preussischen Akademie der Wissenschaften zu Berlin Bericht, 198-219.

Ehrenberg, C.G. 1843. Verbreitung und Einfluss des mikroskopischen Lebens in Südund Nord Amerika. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen, 1841, 291-446.

Environment Canada and Health Canada. 2001. Priority Substances List Assesment Report. Ammonia in the Aquaic Environment. Canadian Environmental Protection Act, 1999. ISBN 0-666-29192-1.

Fine Tailings Fundamentals Consortium (FTFC). 1995. "Vol., Title," In: Advances in Oil Sands Tailings Research, Alberta Department of Energy, Oil Sands and Research Division, Publisher.

Fisheries and Oceans Canada (DFO). 1986. Policy for the Management of Fish Habitat. Fish Habitat Management Branch.

Golder Associates Ltd. 2001. Consolidation Tailings (CT) Integrated Reclamation Landscape Demonstration Project: Technical Report #1, Year 2000: Summary Report. Prepared for Suncor Energy Inc. 20 p.

Golder Associates Ltd. 2002. Wetlands Study Sites Associated with Oil Sands Research and Monitoring. Prepared for, CONRAD Environmental Research Group.

Golder Associates Ltd. 2006. Consolidated Tailings (CT) Integrated reclamation landscape project technical report #7. Prepared for, Suncor Energy Inc., Oil Sands.

Google Earth. 2009. Athabasca Oil Sands, Suncor and Syncrude Sites. Neville. Google Earth, http://earth.google.com/ (generated March 2009)

Haeckel, E. H. 1862. Die Radiolarien (Rhizopoda Radiaria), Pt. 1; Berlin, 1-572.

Halsey, L. 2007. Natural Wetlands in the Oil Sands Region. Appendix B. In Harris, M.L. Guideline for wetland establishment on reclaimed oil sands leases (revised second edition). Prepared by Lorax Environmental for CEMA Wetlands and Aquatics Subgroup of the Reclamation Working Group, Fort McMurray, AB. Feb/07.

Harris, M.L. 2007. Guideline for wetland establishment on reclaimed oil sands leases (revised second edition). Prepared by Lorax Environmental for CEMA Wetlands and Aquatics Subgroup of the Reclamation Working Group, Fort McMurray, AB. Feb/07.

Heal, O.W. 1962. The abundance of microdistribution of testate amoebae (Rhizopoda:Testacea) in *Sphagnum*. Oikos, 13: 35-47.

Hempel, A. 1898. A list of the Protozoa and Rotifera found in the Illinois River and adjacent lakes at Havana, Ill. Illinois State Laboratory of Natural History Bulletin, 5:301-388.

Hendon, D., Charman, D.J. 1997. The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. The Holocene, 7: 199- 205.

Hirsch, T. 2005. An Overview of Alberta's Oil Sands Resources. University of Alberta. Edmonton, Alberta.

Honig, C.A., Scott, D.B. 1987. Postglacial stratigraphy and sea-level change in southern New Brunswick. Canadian Journal of Earth Science, 24: 354- 364.

Jauhaininen, S. 2002. Testacean amoebae in different types of mire following drainage and subsequent restoration. European Journal of Protistology, 38: 59- 72.

Kauppila, T., Kihlman, S., Makinen, J. 2006. Distribution of Arcellaceans (tesate amoebae) in the sediments of a mine water impacted bay of lake Retunen, Finland. Water, Air, and Soil Pollution, 172: 337- 358.

Kumar, A., Dalby, A.P. 1998. Identification Key for Holocene Lacustrine Arcellacean (Thecamoebian) Taxa. Paleontological Society.

Kumar, A., Patterson, R.T. 2000. Arcellaceans (thecamoebians): new tools for monitoring long- and short-term changes in lake bottom acidity. Environmental Geology, 39: 689- 697.

Lamarck, J.B. 1816. Histoire naturelle des animaux sans vertebres. Verdiere, Paris, 2:1-568.

Leidy, J. 1874. Notice of some new fresh-water rhizopods. Proceedings of the Academy of Natural Sciences of Philadelphia, series 3:77-79.

Leidy, J. 1879. Fresh water rhizopods of North America. United States Geological Survey of the Territories Report, 12:1-324.

Leung, S., Smith, R. 1999. Impacts of Process Affected Waters on Phytoplankton Ecology. Prepared for Syncrude Canada Ltd.

Masliyah, J. 2006. Bitumen Extraction from oil sands: Fundamentals ad Processing. Special presentation to Syncrude operators. Department of Chemical and Materials Engineering. University of Alberta. Edmonton, Alberta.

MacKinnon, M. 2007. Development of Alberta's Athabasca Oil Sands and some of the Reclamation Challenges. Syncrude Research Department, Edmonton. Presentation to Brock University.

McCarthy, F.M.G., McAndrews, J.H., Blasco, S.M., Tiffin, S.H. 2007. Spatially discontinuous modern sedimentation in Georgian Bay, Huron Basin, Great Lakes. Journal of Paleolimnology, 37: 53-70.

McCarthy, F.M.G., Collins, E.S., McAndrews, J.H., Kerr, H.A., Scott, D.B., Medioli, F.S. 1995. A Comparison of Postglacial Arcellacean ("Thecamoebian") and Pollen Succession in Atlantic Canada, Illustrating the Potential of Arcellaceans for Paleoclimatic Reconstruction. 1995. Journal of Paleontology, 69: 980-993.

McCarthy, F.M.G. 1984. Infraspecific variation in Arcelacea (thecamoebians) from Eastern Canada and a selected biostratigraphic study. Unpublished honours thesis, Dalhousie University, Halifax, Nova Scotia, 41 p.

Medioli, F.S., Scott, D.B., and Abbott, B.H. 1987. A case study of protozoan interclonal variability: taxonomic implications. Journal of Foraminiferal Research, 17:28-47.

Medioli, F.S., Scott, D.B. 1988. Lacustrine thecamoebians (mainly Arcellaceans) as potential tools for paleolimnological interpretations. Paloeogeography Paloeoclimatol Paloeoecol, 62: 361- 386.

Medioli, F.S., Scott, D.B. 1983. Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Special Publication No. 21.

Medioli, F.S., Scott, D.B., Collins, E.S., McCarthy, F.M. 1990. Fossil thecamoebians: Present status and prospects for the future, in: Paleoecology, Biostrstigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera (Hemleben, G., Kaminski, M.A., Kuhnt, W., Scott, D.B., eds.), NATO Advaced Study Institute Series C. Mathematical and Physical Science, 327: 813-840.

Milne-Edwards, H., Haime, J. 1850. A monograph of the British fossil corals, Pt. I. - Palaeontograph. Soc. London, I-XXVfl-71.

Mitchell, E.A.D., Charman, D.J., Warner, B.G. 2007. Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodivers Conserv, 10: 1-22.

Mignot, J.P., Raikov, I.B. 1992. Evidence for meiosis in the testate amoeba *Arcella*. Journal Protozool., 39: 287-289.

Mossop, G.D. 1980. Geology of the Athabasca Oil Sands. Science, 207: 145-149.

Ogden, C.G. 1980. Notes on some Diffugiidae from Norfolk (Rhizopodea, Protozoa). British Museum of Natural History Bulletin, (Zoology), 39: 125-138.

Ogden, C.G., Hedley, R.H. 1980. An atlas of freshwater Testate Amoebae. British Museum (Natural History). Oxford University Press, pp. 1-222.

Patterson, R.T., MacKinnon, K.D., Scott, D.B., and Medioli, F.S. 1985. Arcellaceans (Thecamoebians) in small lakes of New Brunswick and Nova Scotia: modern distribution and Holocene stratigraphic changes. Journal of Foraminiferal Research, 15:114-137.

Patterson, R.T., Fishbein, E. 1989. Re-Examination of the statistical methods used to determine the number of point counts needed for micropaleontological quantitative research. Journal of Paleontology, 63: 245- 248.

Patterson, R.T., Kumar, A. 2000. Use of Arcellacea (Thecamoebians) to gauge levels of contamination and remediation in Industrially Polluted Lakes. p. 257- 278. In Martin, R.E. (Eds). Environmental Micropaleontology, The Application of Microfossils to Environmental Geology, Kluwer Academic/ Plenum Publishers, New York, Boston, Dordrecht, London, Moscow.

Patterson, R.T., Kumar, A. 2002. A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeograppy, Palaeoclimatology, Palaeoecology, 180: 225- 251.

Patterson, R.T., Baker, T., Burbidge, S.M. 1996. Arcellaceans (thecamoebians) as proxies of arsenic and mercury contamination in Northeastern Ontario Lakes. Journal of Foraminiferal Research, 26: 172-183.

Patterson, R.T., MacKinnon, K.D., Scott, D.B., Medioli, F.S. 1985. Arcellaceans ("Thecamoebians") in small lakes of New Brunswick and Nova Scotia:

modern distribution and Holocene stratigraphic changes. Journal of Foraminiferal Research, 15: 114- 137.

Pelley, J. 2007. Alberta's Oil Sands. Environmental Science and Technology, 41: 4838-4839.

Penard, E. 1902. Faune Rhizopodique du Bassin du Lèman. Henry Kundig, Geneve.

Perty, M. 1849. Über vertikale Verbreitung mikroskopischer Lebensformen. Naturforschende Gesellschaft in Bern Mittheilungen, 153-176.

Priece, J. 2004. Overview of Oil Sands Geology. Presentation to Canadian Institute of Mining (CIM), Montreal, Québec.

Proctor, R.M., Taylor, G.C., Wade, J.A. 1983. Oil and Natural Resources of Canada: GSC Paper 83-31, 59p.

Reinhardt, E.G., Dalby, P.A., Kumar, A., Patterson, T. 1998. Arcellaceans as Pollution Indicators in Mine Tailing Contaminated Lakes near Cobalt, Ontario, Canada. Micropaleontology, 44: 131-148.

Reinhardt, E.G., Little, M., Donato, S., Findlay, D., Krueger, A., Clark, C., Boyce, J. 2005. Arcellacean (thecamoebian) evidence of land-use change and eutrophication in Franchman's Bay, Pickering, Ontario. Environmental Geology, 47: 729-739.

Rhumbler, L. 1895. Beitrage zur Kenntins der Rhizopoden. (Beitrag III, IV und V): Zeitschrift fur wissenschaftliche Zoologie, 61: 38 -110, pls. 4,5.

Rogers, M.E., Ferguson, D., MacKinnon, M. 1996. Water management challenges at the worlds largest integrated oil sands mining and refining complex. Corrosion 96, The NACE International Annual Conference and Exposition, No.568.

de Saedeleer, H. 1934. Beitrag zur Kenntnisder Rhizopoden: morphologische und systematische Untersuchungen und ein Klassifikationsversuch: M6m. Museum Roy. d'Hist. Nat. Belgique, No. 60, p. 1-112, pls. 1-8.

Sarvis, A., P. 2000. Postglacial water levels in the Great Lakes region in relation to Holocene climate change: thecamoebian and palynological evidence. MSc thesis. Brock University. Ontario.

Schlumberger, P., 1845. Observations sur quelques nouvelles especes d'Infusoires de la famille des Rhizopodes: Annales des Sciences Naturelles, Zoologie, 3:254-256.

Schonborn, W. (1996). Beschalte Amnben (Testaceae). Wittenberg, Lutherstadt.

Schulze, F.E. 1877. Rhizopodenstudien VI: Archiv fur Mikroskopische Anatomie, 13: 9-30, pls. 2, 3.

Scott, D.B., Medioli, F.S. 1980. Living vs. Total Foraminiferal Populations: Their Relative Usefulness in Paleoecology. Journal of Paleontology, 4: 814-831.

Scott, D.B., Medioli, F.S. 1983. Agglutinated Rhizopods in Lake Erie: Modern Distribution and Stratigraphic Implications. Journal of Paleontology, 4: 809- 820.

Scott, B.D., Medioli, F.S., Schafer, C.T. 2001. Monitoring in coastal environments using foraminifera and thecamoebian indicators. Cambridge University Press. Cambridge.

Smol, J.P. 1992. Paleolimnology: an important tool for effective ecosystem management. Journal of Aquatic Ecosystem Health, 1: 49-58.

Stein, S.F.N.von. 1859. Uber die ihm aus eigener Untersuchung bekannt gewordenen Suswasser-Rhizopoden: Konigliche Bohmishce Gesellschaft der Wissenschaften Abhandlungen, ser. 5, v.10, Berichte der Sectionen, p. 41-43.

Steinecke, F. (1927). Leiformen und Leitfossilen des Zehlaubruches: Die Bedeutung der fossilen Mikroorganismen fur die Erkenntnis der Nekrozonosen eines Moores. Bot. Arch., 19: 327-344.

Syncrude Canada Ltd. 1995. Syncrude Analytical Methods: Updated 2006. Syncrude Edmonton Centre.

Syncrude Canada Ltd. 2003. Syncrude Fact Book. Agecoutay, L. (ed.). Published by Syncrude Canada.

Thibaudeau, S.A., Medioli, F.S. 1986. Carboniferous thecamoebians and marsh foraminifera: new stratigraphic tools for ancient paralic deposits. Abstract to the Geological Society of America, Annual Meeting, San Antonio, pp. 771.

Valkanov, A. 1962. Uber die Kopulation der Testaceen (Rhizoposa-Testacea). Reports of the Bulgarian Academy of Science, 15: 305-308.

Van Hengstum, P.J., Reinhardt, E.G., Beddows, P.A., Huang, R.J., Gabriel, J.J. 2008. Thecamoebians (testate amoebae) and foraminifera from three anchialine cenotes in Mexico: low salinity (1.5 – 4.5 psu) faunal transitions. School of Geography and Earth Sciences, McMaster University, 1280 Main Street West, Hamilton, ON, Canada.

Vigrass, L.W. 1968. Geology of Canadian Heavy Oil Sands. AAPG Bulletin, 52.

Vitt, D.H., Halsey, L.A., Thormann, M., Martin, T. 1996. Peatland Inventory of Alberta. Prepared for the Alberta Peat Task Force, National Center of Excellence in Sustainable Forest Management, University of Alberta, Edmonton, 117pp.

Vitt, D.H., Halsey, L.A., Thormann, M., Martin, T. 1996. Peatland Inventory of Alberta Phase 1: Overview of Peatland resources of the natural regions and subregions of the province. National Centers of Excellence in Sustainable Forest Management, University of Alberta, Edmonton, Alberta.

Wallich, G.C. 1864. On the extent, and some of the principal causes, of structural variation among the difflugian rhizopods: Annals and Magazine of Natural History, series 3, 13:215-245.

Warner, B. G. 1990. Testate Amoebae. In: Warner, B.G., (ed.). Methods in Quaternary Ecology. Pg 65 – 74.

Warner, B.G., Asada, T., Quinn, N.P. 2007. Seasonal Influences on the Ecology of Testate Amoebae (Protozoa) in a Small Sphagnum peatland in Southern Ontario, Canada. Micobial Ecology, 54: 91- 100.

Westcott, F., Watson, L. 2007. End Pit Lakes, Technical Guidance Document. Report prepared for The Cumulative Environmental Management Association End Pit Lakes Subgroup, 50pp.

Yudina, T.A., Sukhanova, K.M. 2000. Cell biology and life cycle of the testate amoeba *Corythion delamarei*. Cytology, 43: 613-623.

Chapter 2

Using thecamoebians (testate amoebae) as proxies of ecosystem health in constructed wetlands in the Oil Sands of Alberta, Canada

Lisa A. Neville[1], Francine M.G. McCarthy[1], Michael D. MacKinnon[2], Patricia Marlowe[3]

[1] Dept. of Earth Studies, Brock University, 500 Glenridge Ave, St. Catharines, ON L2S 3A1
[2] Syncrude Canada Limited, Research Dept., 9421-17 Ave, Edmonton, AB T6N 1H4
[3] Golder Associates Ltd., 340 MacLennan Cst. Fort McMurray, AB T9H 5C8

This manuscript has been submitted to the Journal of Environmental Earth Science and generally follows the style required by the publisher of this journal.

2.1. Introduction

Large –scale surface mining operations within the Athabasca Oil Sands deposit in northeastern Alberta (Fig. 1) began in the mid 1960's north of Fort McMurray (Alberta Energy 2008). Currently three commercial operations are in production. Associated surface mining and tailings management has led to the disturbance of large areas of Boreal Forest (Fig. 1). Current mine development has disturbed over 500 km^2, as oil production now exceeds over 600,000 barrels of crude oil per day. If development meets expectation, production will approach 2Mbbls/day and the reclamation challenge will include both terrestrial and aquatic (lake and wetland) habitats. The oil sands industry is committed to meeting this challenge but will need metrics to document that reclaimed areas are progressing towards natural ecosystems in the region.

In oil sands processing, a hot-water caustic digestion method is used to separate the bitumen from the oil sands. During the processing and subsequent recycling of the water on the operational sites, ionic and dissolved organic concentrations are elevated in both the surface and substrate pore waters relative to natural non-OSPM (oil sands process-affected material) impacted waters. The general salinity of the OSPW (oil sands process-affected water) will be elevated through the addition of Na^+, Cl^- and SO_4^{-2} ions, while dissolved organic acids (naphthenic acids) and ammonia (NH_4^+) are added to the waters during processing, the former through release from the bitumen and the latter as a by-product of upgrading. The OSPW character differs with the major by-products of oil sands extraction and therefore a range of properties can be expected in reclamation options.

As part of the license to operate, oil companies cannot release process-affected water into the natural ecosystem (Masliyah 2006). This means that the water is stored on site, recycled, and will affect reclamation strategies. During the extraction process, fluid tailings consisting of water, fines particles (<44 μm), unrecovered bitumen, salts and naphthenic acids are also produced. OSPW and the tailings materials require reclamation, and part of the strategy will include constructed wetlands and lakes. Both the water and the substrates will be affected by OSPW.

Oil sands operators have committed to reclaiming the disturbed areas to a level of capability equivalent to that of pre-development. These reclaimed landscapes, comprising of upland vegetation, wetlands, and lakes will contain varying types of OSPM and will require time to mitigate the effects associated with such materials. Placement of OSPW over soft tails (clay and silt), such as the fine tails associated the oil sands extraction tailings, will produce wetland and lake environments, where benthic biota will colonize. The benthic community will range from bacteria to benthic invertebrate animals, and their development will likely be influenced by the properties of the OSPM, as well as the composition of overlying water. Water capping of soft tailings was first conceptualized as a reclamation strategy to deal with the huge volumes of extraction bi-products (Allen 2008).

Wetland habitats, both constructed and opportunistic, will be important components in the lease-closure of the oil sands, and the rate of progression from OSPM-stressed systems to more natural functions will be an important factor for gauging reclamation success. Simple and effective tools to monitor the early stages of remediation of these wetlands need to be developed to demonstrate a trajectory towards natural processes. The role of the benthic community in ascertaining this will be critical, and this study investigates the use of microfossils to assess the rate and effectiveness of the development of natural wetland ecosystems.

Thecamoebians (also called testate amoebae) are single-celled organisms (protists), found in moist soils, wetlands, and lakes. They are a diverse and important component of the microbial trophic level within the 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, 2002; Beyens and Meisterfeld, 2003). 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 by different species (Medioli et al., 1983). Their fossilized tests are found in all aquatic and moist terrestrial sediments although the preservation potential varies between species. The fossil remains of thecamoebians preserve a record of their populations over time (with length of time averaged inversely proportional to the rate of sedimentation).

Thecamoebians display a rapid generation time and a high degree of sensitivity to environmental conditions at the sediment-water interface and epibenthic zone. As a result, they have proven to be useful paleoecological indicators in studies of paleoclimate (Boudreau et al., 2005; McCarthy et al., 1995), sea level change (Scott and Medioli, 2001), and anthropogenic impact, including the impact of sulphide mining in acid-sensitive lakes in Ontario (Patterson et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000; Patterson et al., 2002) and in Finland (Kauppila et al., 2006). These latter studies led us to investigate their sensitivity to the byproducts of oil sands production, particularly because the analytical methods are relatively simple and inexpensive, and these microfossils are one of the most sensitive indicators of short-term ecosystem change in aquatic ecosystems.

Our observations suggest that thecamoebians provide a valuable proxy of wetland and end pit lake ecosystem health (Neville et al., 2008, 2009). This paper shows that they possess the ability to indicate ecosystem health by rapidly responding to chemical concentrations in the benthic zone of wetlands.

2.2. Methods

Sediment samples were collected from constructed wetlands located on the Suncor Energy Inc. oil sands operation (Lease 86) in August 2007 by Golder Associates. The sites were chosen to reflect varying degrees of OSPM influence. The samples were transferred to glass jars, and were stored at 4°C prior to shipping to Brock University. At the same time, water samples were collected and transported to Syncrude Canada Ltd. Edmonton Research facility for water analysis (conductivity, major ions, trace metals and naphthenic acids) using their standard protocols (Syncrude, 1995).

Samples were prepared for thecamoebian analysis following the standard micropaleontological methods described in Scott et al. (2001). Processing was completed by wet sieving subsamples of 5cc through 63 and 45μm sieves, taking care not to mechanically break the tests. A 500μm sieve was used to remove coarse detritus from some samples, and dish detergent was used to disagreggate clays in stiff, muddy samples. Thecamoebians smaller than 45μm are lost using this protocol (Beyens and Meisterfeld, 2001), but this is offset by the advantages of very rapid and inexpensive processing, and the requirement of only 50X magnification to identify the specimens.

For quantitative analysis the samples were placed in a gridded Petri dish and wet counted at 50X magnification. The 45-63μm and > 63μm size fractions were examined separately to decrease the likelihood of missing specimens obscured by large particles. Thecamoebians were primarily identified using the key of Kumar and Dalby (1998), although reference was also made to photoplates and descriptions in various publications, notably Medioli and Scott (1983). Specimens were identified to strain level because strains have been found to convey useful information on aquatic subenvironments (Kumar and Patterson, 2000; Kauppila et al. 2006), and as they did not appear to be significant in the Suncor wetlands, the data is reported in this paper as species without subdivision to strain/ subspecies/morphotype level. Species diversity was calculated using strains, which provide useful information whether they are true species or simply ecophenotypes, an ongoing debate among researchers (e.g. Medioli and Scott, 1983). When the number of thecamoebians counted in the 5cc subsample did not reach 100, an additional 5cc subsample was processed and counted, since, for statistical analysis, the absolute number of specimens generally examined varies between 100 and 1,000 per sample (Patterson et al., 1989). Minimum sample size used in this study was 101.

Species diversity was calculated using the Shannon-Weaver Diversity index (SDI) (Shannon and Weaver, 1949). This equation is used to calculate the environmental stability for each site. 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 et al., 2002). The SDI is calculated using the following formula, where S is the species richness for each sample:

$$SDI = -\sum_{I}^{S}\left(\frac{Fi}{Ni}\right) * \ln\left(\frac{Fi}{Ni}\right)$$

2.3. Results

Twelve of the 20 sites examined from the first set of samples (Set One, collected in August of 2007) contained sufficient numbers of tests to allow meaningful comparisons of assemblages (McCarthy et al., 2008). Water samples from these 12 Suncor wetland sites showed a range in conductivities from 611 to 2680 μS/cm (Table 2.1), with most of the variability changes being seen in Na^{+}, Cl^{-} and SO_4^{-2} concentrations (Figures 2.2 and 2.3). Naphthenic acids are considered a strong indicator of OSPW/ OSPM character and ranged from 3.6 to 55.4 mg/L.

The 6 samples from wetlands with relatively low OSPM impact (Sites 8, 9, 16, 17, 20 and 21- average naphthenic acid concentration 7.95 mg/L, conductivity 1398.5μS/cm) contained a relatively abundant (N= 137/5cc) and diverse fauna (av. SDI= 2.00). Difflugiid species dominated the assemblages, primarily *Difflugia oblonga* and *Cucurbitella tricuspis* (Figure 2.2). Other difflugiid taxa include *Difflugia urceolata, Difflugia corona, Difflugia protaeiformis, Difflugia globulus, Difflugia urens, Difflugia bacilliarum, Difflugia bidens, Pontigulasia compressa, Lagenodifflugia vas,* and *Lesquereusia spiralis.* Centropyxid taxa are relatively less abundant at these sites, and *Centropyxis constricta* is usually the most common centropyxid thecamoebian. *Arcella vulgaris* is vertually absent from these sites.

The 6 samples from the high OSPW character wetlands (Sites 2, 5, 11, 13, 14 and 18- average naphthenic acid concentration 39.0 mg/L, conductivity 1946.67μS/cm) contain a generally less diverse (av. SDI= 1.24), and typically less abundant fauna (N= 111/5cc) dominated by the genera *Arcella* and *Centropyxis* (Figure 2.3). Difflugiid taxa are rare in these samples, and generally consist only of *Difflugia oblonga* or *Cucurbitella tricuspis*. Sites that display stronger OSPW character than those reported in this paper are essentially barren of thecamoebian tests (McCarthy et al., 2008).

Regression and correlation analysis performed on the data (Figure 2.4) showed a strong relationship (r^2=0.772) between naphthenic acid concentrations and the relative abundance of centropyxid thecamoebians (*Centropyxis aculeata* + *Centropyxis constricta*) and a strong inverse relationship (r^2=0.7171) with the relative abundance of difflugiid thecamoebians. Conductivity also shows a relationship (r^2=0.6878) with the relative abundance of centropyxid thecamoebians and an inverse relationship (r^2=0.5774) with the relative abundance of difflugiid thecamoebians (Figure 2.5).

2.4. Discussion

Diverse difflugiid- dominated thecamoebian assemblages in samples from the constructed wetland sites 8, 9,16, 17, 20 and 21 have low OSPW character (naphthenic acid concentrations <11mg/L and conductivity values <1300μS/cm, except for an outlier, Site 9- V-notch weir). These sites can be considered healthier, more natural wetlands, although it should be noted that most of these sites displayed elevated salinity (conductivities >1000 μS/cm) relative to the surrounding natural lakes and wetlands (Neville et al., 2008). Abundant and diverse difflugiid-rich thecamoebian assemblages have been shown to typically characterize unstressed freshwater environments in North America (Collins et al., 1990).

Sites where recharge of OSPW occurs on a regular basis (2, 5, 11, 13, 14 and 18), through seepage from sand dykes or through addition of fresh OSPW to the wetland complex as part of a larger study being conducted by Suncor Energy Inc., have elevated naphthenic acid concentrations >36mg/L and conductivity values >1860μS/cm. These are characterized by a low diversity and typically less abundant fauna dominated by the genus *Centropyxis.* High relative abundances of centropyxid taxa at sites with high conductivity water are consistent with the dominance of these taxa in marginal marine environments, such as salt marshes and estuaries (Scott et al., 2001). *Centropyxis aculeata*, an opportunistic taxon, has been found able to tolerate hostile conditions (including industrial impact) better than most thecamoebian taxa (Patterson and Kumar, 2002).

Naphthenic acid concentrations >40mg/L together with conductivity values >1900 μS/cm appear required to allow the colonization of the hardiest species, *Arcella vulgaris*. The stressed nature of the *Arcella vulgaris*- bearing samples is evident in the very low species diversity associated with these samples (Average SDI ~1.28). When *Arcella vulgaris* is present in a sample, typically more than 50% of the remaining sample is composed of centropyxids. Kumar and Patterson (2000) showed that *Arcella vulgaris* was able to thrive even in the most hostile parts of James Lake, which was impacted by the dumping of waste rock from a pyrite mine in northeastern Ontario.

The ratio of difflugiid vs. centropyxid taxa was found to be a useful and robust metric of ecosystem health in this study- it merely requires genus-level identification, and thus does not require taxonomic expertise. However, some difflugiid taxa are found in most samples, even those relatively highly impacted by OSPM. The presence of *Difflugia oblonga* is not unexpected, since it is the most ubiquitous difflugiid thecamoebian (Collins et al., 1990). *Cucurbitella tricuspis* is also a common taxon in most freshwater environments, and its presence in samples highly impacted by OSPM might be due in part to the unusual ecology of this species. It is possible that hostile bottom water conditions might have less impact on this taxon, which has a planktonic phase in its life cycle (Schonborn 1984; Medioli et al., 1987), as the rainwater may dilute the concentration of chemicals at the surface.

In contrast, some difflugiid taxa, such as *Difflugia urceolata* and *Difflugia corona*, appear to be especially sensitive to OSPM impact, and are rare when naphthenic acid concentrations exceed 36 mg/L. These are considered equivalent to canaries in the coalmine. The presence of these sensitive taxa is reflected by higher species diversity, illustrated by the Shannon-Wiener diversity index.

2.5. Conclusions

Thecamoebians (testate amoeba) assemblages are sensitive proxies of environmental quality in the constructed wetlands test facility, and their chemical sensitivity is sufficient to respond to changes in ecosystem health. *Arcella vulgaris* and centropyxid taxa are able to tolerate the high conductivity and high concentrations of naphthenic acids associated with oil sands extraction. Difflugiid thecamoebian taxa are generally more sensitive to oil sands process-affected materials, and are used as "canaries in the coalmine" to indicate the threshold at which ecosystem health is improving. Difflugiid-dominated assemblages are generally restricted to samples with < 11mg/L naphthenic acids and conductivity values <1500 μS/cm. The use of thecamoebians as an inexpensive and effective analytical technique for monitoring the progression of remediation may be possible. The preliminary success of this microfossil proxy within OSPM wetlands warrants further investigation to determine if the thecamoebian community is indeed responding to changes in chemical concentrations at contaminated sites as well as to verify how quickly contaminated wetlands can progress to natural ecosystems.

2.6. Acknowledgments

We thank Christine Daly from Suncor Energy Limited for allowing us access to the Suncor wetlands and resources, Mike Lozon for his assistance with drafting, and Syncrude Canada Limited for financial support of this study.

2.7. List of Table and Figures

2.8. Tables

Table 2.1.

Sample	Site Location	Naphthenic Acids (mg/L)	Conductivity (μS/cm)	Thecamoebian Species Diversity (SDI)	% *Arcella vulgaris*	% centropyxid	% difflugiid
2	Dyke 4 seepage	55.4	2570	1.37	1.9	78.2	19.8
5	Dyke 4 Reservoir	36.1	1860	2.12	0.63	61.0	38.9
8	Control Reservoir	10.9	1070	2.13	0	16.9	81.8
9	V-notch Weir	10.7	2450	2.16	0	42.8	55.9
11	4m CT out (Gooseneck)	47.9	2530	1.19	9.5	78.4	11.4
13	Weir B	50.6	2680	1.68	1.0	61.1	37.8
14	Sustainable Lake North	44.2	2040	1.1	24.7	72.1	3.1
16	Sodic Wetland	5.2	1350	2.07	4.9	52.2	42.9
17	Muskeg stockpile wetland	3.6	611	2.44	1.7	20.7	77.5
18	Gooseneck Wetland (Jan's Pond)	51.2	2650	1.08	8.4	82.3	9.2
20	WA 14, Pond A	8.9	1610	1.36	0.3	40.0	59.7
21	Crane Lake	8.4	1300	1.85	0	36.3	63.7
Average High OSPW		**39.0**	**1946.67**	**1.24**	**6.29**	**58.47**	**18.5**
Average Low OSPW		**7.95**	**1398.5**	**2.00**	**1.15**	**34.82**	**63.58**

2.9. Figures

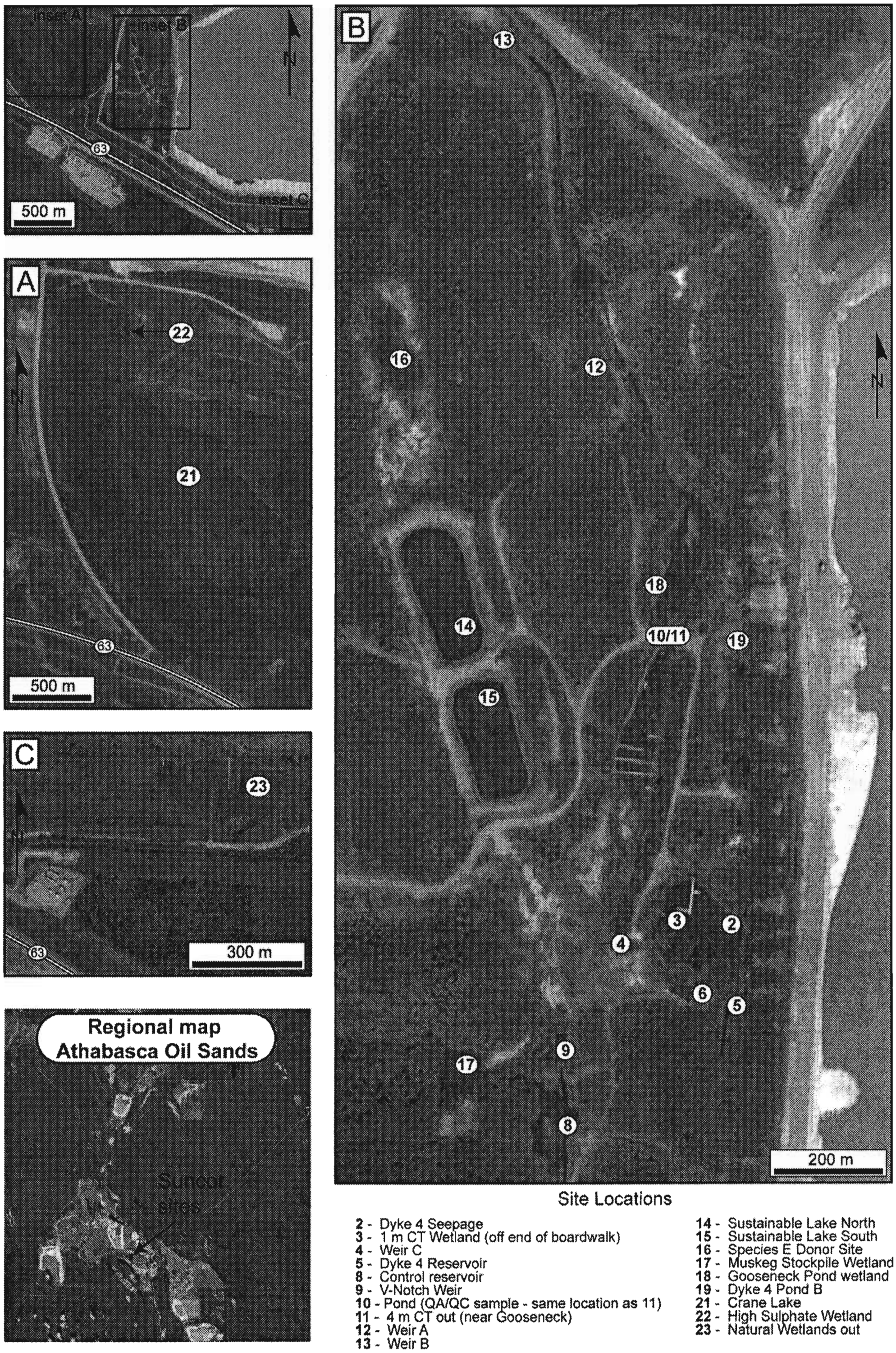


Figure 2.1.

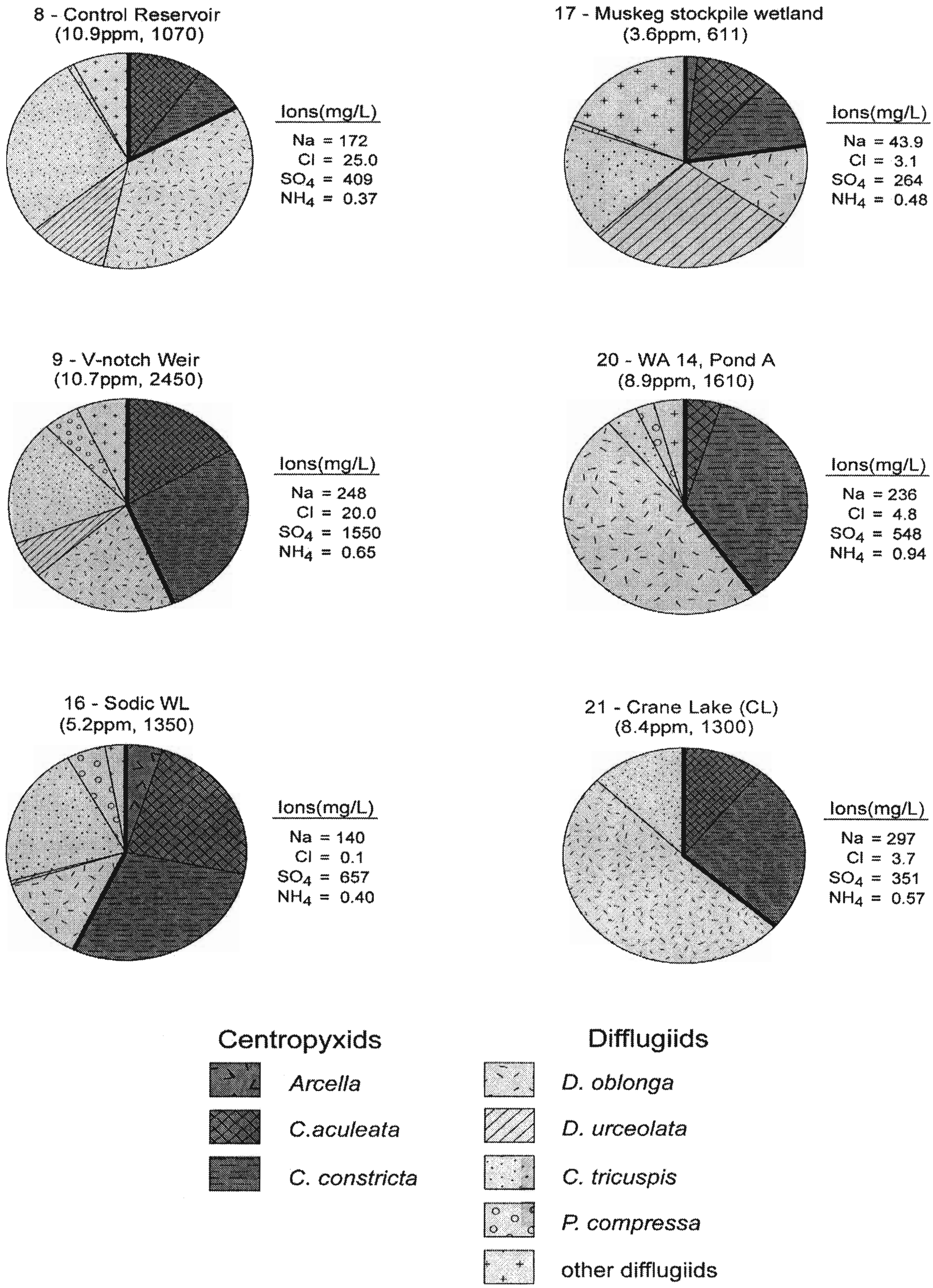
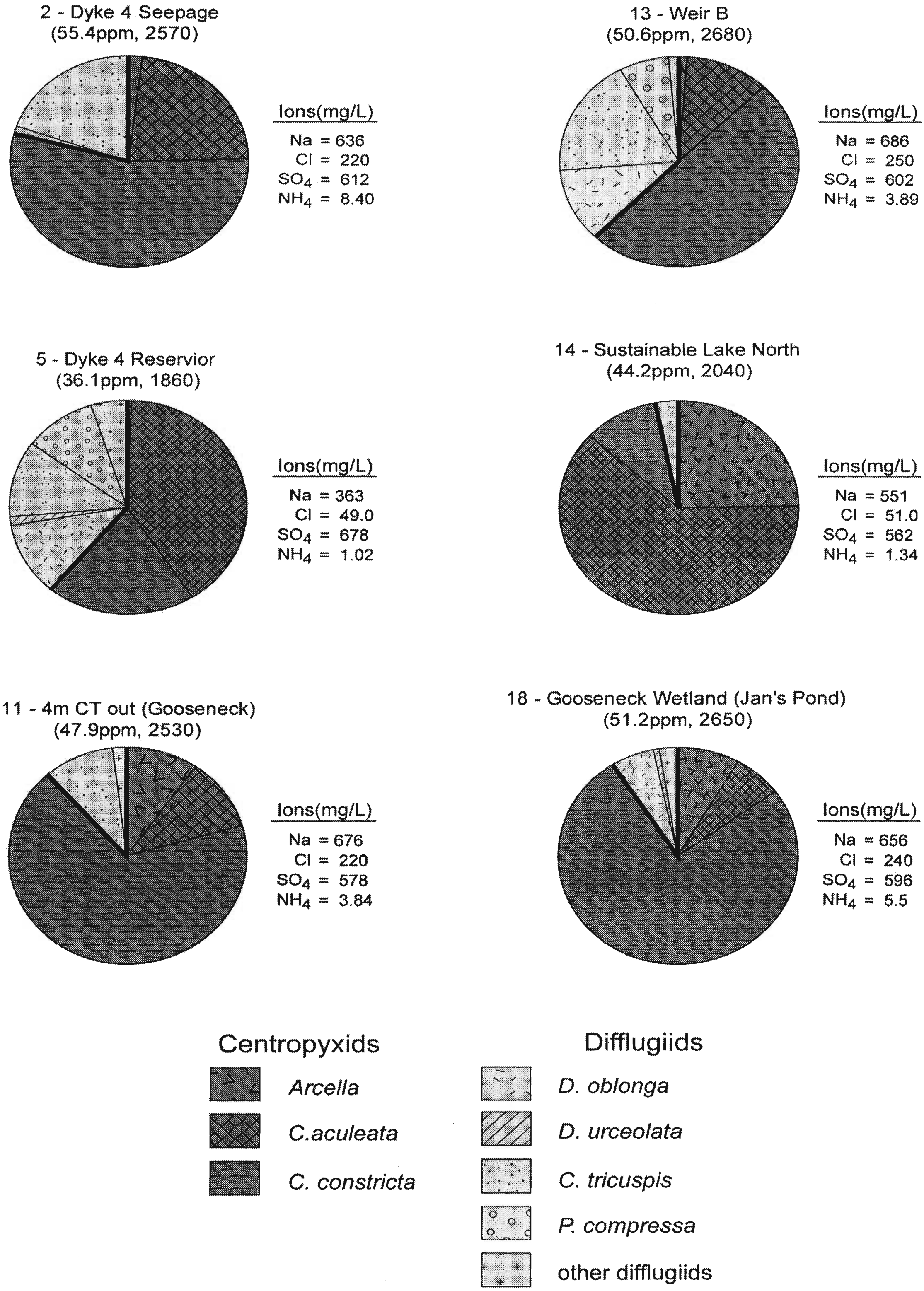
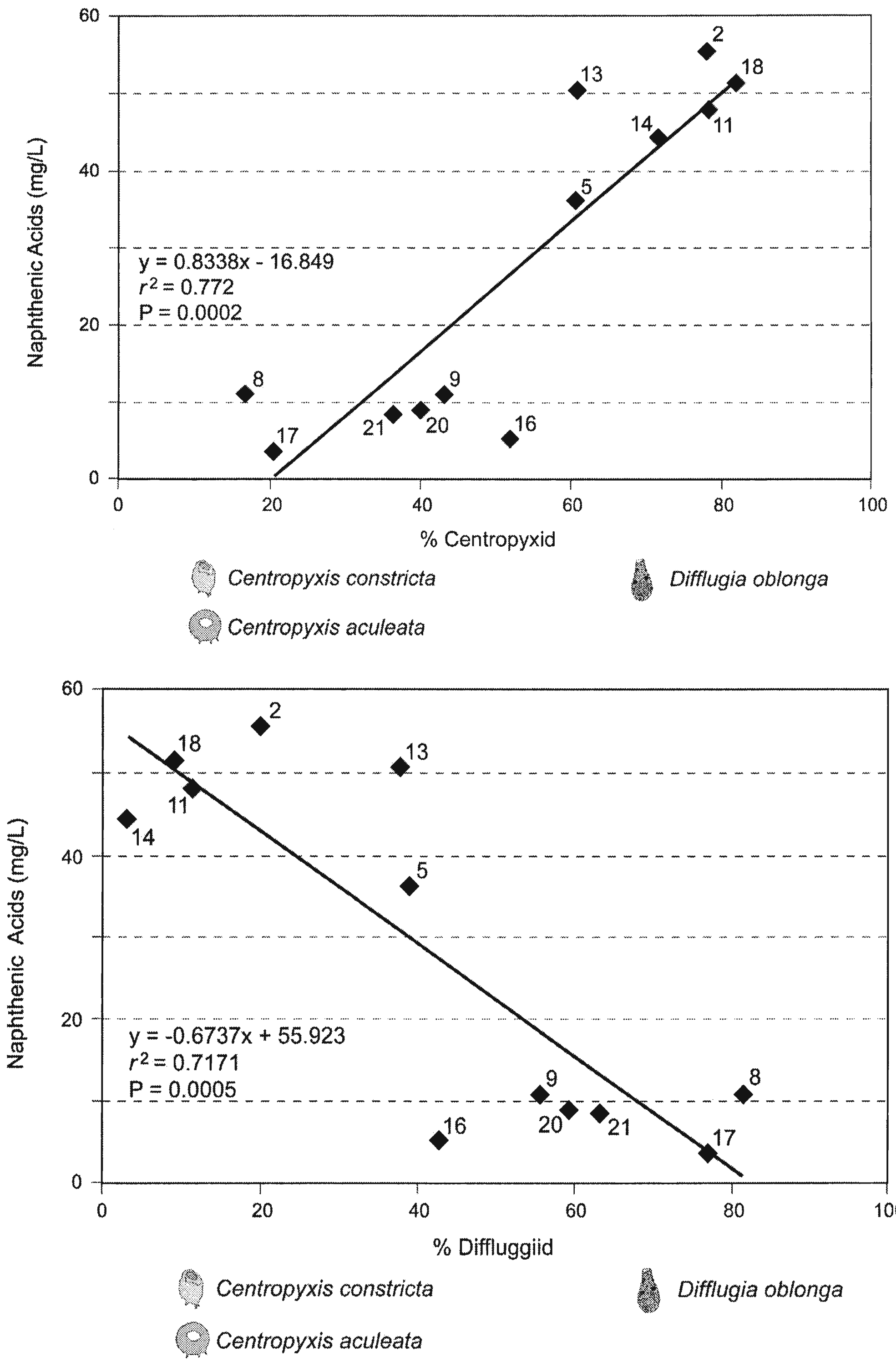

8 - Control Reservoir
(10.9ppm, 1070)
Ions(mg/L)
Na = 172
Cl = 25.0
SO4 = 409
NH4 = 0.37
17 - Muskeg stockpile wetland
(3.6ppm, 611)
Ions(mg/L)
Na = 43.9
Cl = 3.1
SO4 = 264
NH4 = 0.48
9 - V-notch Weir
(10.7ppm, 2450)
Ions(mg/L)
Na = 248
Cl = 20.0
SO4 = 1550
NH4 = 0.65
20 - WA 14, Pond A
(8.9ppm, 1610)
Ions(mg/L)
Na = 236
Cl = 4.8
SO4 = 548
NH4 = 0.94
16 - Sodic WL
(5.2ppm, 1350)
Ions(mg/L)
Na = 140
Cl = 0.1
SO4 = 657
NH4 = 0.40
21 - Crane Lake (CL)
(8.4ppm, 1300)
Ions(mg/L)
Na = 297
Cl = 3.7
SO4 = 351
NH4 = 0.57
Centropyxids
Arcella
C.aculeata
C. constricta
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
P. compressa
other difflugiids

Figure 2.2.



2 - Dyke 4 Seepage
(55.4ppm, 2570)
Ions(mg/L)
Na = 636
Cl = 220
SO_4 = 612
NH_4 = 8.40
13 - Weir B
(50.6ppm, 2680)
Ions(mg/L)
Na = 686
Cl = 250
SO_4 = 602
NH_4 = 3.89
5 - Dyke 4 Reservior
(36.1ppm, 1860)
Ions(mg/L)
Na = 363
Cl = 49.0
SO_4 = 678
NH_4 = 1.02
14 - Sustainable Lake North
(44.2ppm, 2040)
Ions(mg/L)
Na = 551
Cl = 51.0
SO_4 = 562
NH_4 = 1.34
11 - 4m CT out (Gooseneck)
(47.9ppm, 2530)
Ions(mg/L)
Na = 676
Cl = 220
SO_4 = 578
NH_4 = 3.84
18 - Gooseneck Wetland (Jan's Pond)
(51.2ppm, 2650)
Ions(mg/L)
Na = 656
Cl = 240
SO_4 = 596
NH_4 = 5.5
Centropyxids
Arcella
C.aculeata
C. constricta
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
P. compressa
other difflugiids

Figure 2.3.



Naphthenic Acids (mg/L)
% Centropyxid
y = 0.8338x - 16.849
r2 = 0.772
P = 0.0002
0
20
40
60
80
100
2
13
18
14
11
5
8
17
21
9
20
16
Centropyxis constricta
Centropyxis aculeata
Difflugia oblonga
Naphthenic Acids (mg/L)
% Diffluggiid
y = -0.6737x + 55.923
r2 = 0.7171
P = 0.0005
Centropyxis constricta
Centropyxis aculeata
Difflugia oblonga

Figure 2.4.

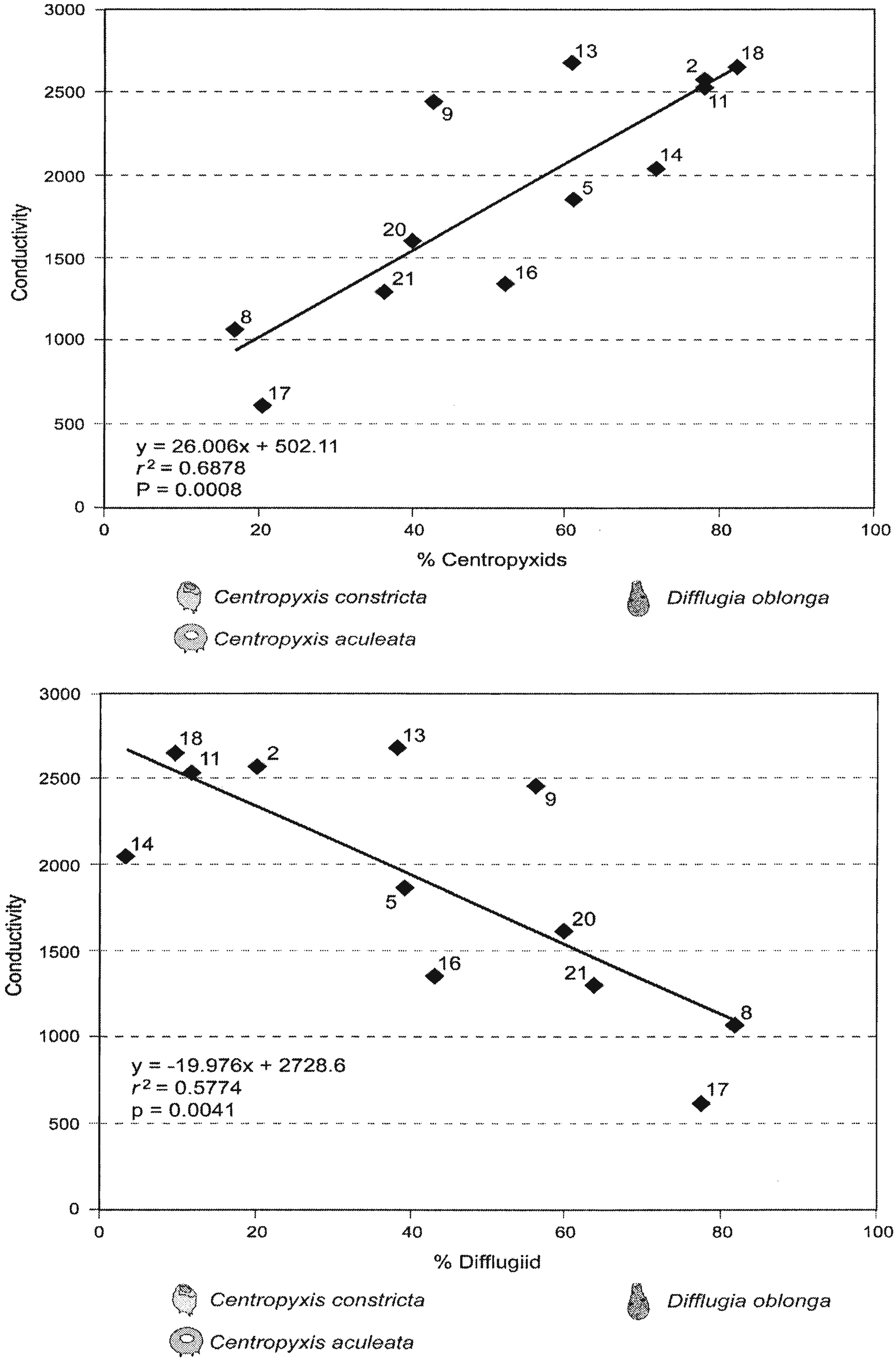

Conductivity
% Centropyxids
y = 26.006x + 502.11
r^2 = 0.6878
P = 0.0008
Centropyxis constricta
Centropyxis aculeata
Difflugia oblonga
Conductivity
% Difflugiid
y = -19.976x + 2728.6
r^2 = 0.5774
p = 0.0041
Centropyxis constricta
Centropyxis aculeata
Difflugia oblonga

Figure 2.5.

2.10. References

Alberta Energy (2008) Alberta Energy Homepage, www.energy.gov.ab.ca. Last reviewed April 15, 2008.

Allen EW (2008) Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. J. Environ. Engineering Sci. 7:123-138.

Beyens L, Meisterfeld R (2001) 'Protozoa: Testate amoebae'. p. 121-153. In Smol JP, Birks HJB, Last, W.M (Eds). Tracking Environmental Change Using Lake Sediments. Volume 3: Terrestrial, Algal, and Siliceous Indicators. Kluwer Academic Publishers. The Netherlands.

Boudreau EA, 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.

Collins ES, McCarthy FMG, Medioli FS, Scott DB, Honig CA (1990) Biogeographic distribution of modern thecamoebians in a transect along the Eastern North American coast. p. 783-792. In C. Hemleben and others (Eds). Paleoecology, Biostratigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera Kluwer Academic Publishers. Amsterdam.

Google Earth. 2009. Athabasca Oil Sands, Suncor Sites. Neville L A. Google Earth, http://earth.google.com/ (generated March 20).

Kauppila T, Kihlman S, Makinen J (2006) Distribution of Arcellaceans (testate amoebae) in the sediments of a mine water impacted bay of Lake Retunen, Finland. Water, Air, and Soil Pollut. 172: 337- 358.

Kumar A, Dalby AP (1998) Identification Key for Holocene Lacustrine Arcellacean(Thecamoebian) Taxa. Paleontological Society.

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.

Masliyah J (2006) Bitumen Extraction from oil sands: Fundamentals ad Processing. Special presentation to Syncrude operators. Department of Chemical and Materials Engineering. University of Alberta. Edmonton, Alberta.

McCarthy FMG, Collins E.S, McAndrews JH, Kerr HA, Scott DB, Medioli FS (1995). A comparison of postglacial Arcellacean ("Thecamoebian") and pollen succession in Atlantic Canada, illustrating the potential of arcellaceans for paleoclimatic reconstruction. J. Paleontol 69: 980-993.

McCarthy FMG, Neville LA, MacKinnon MD (2008) Annual variations in the Suncor Experimental Wetlands and seasonal variations in thecamoebian assemblages in Syncrude's BPIT test site: implications for biomonitoring. Report prepared for Syncrude,Canada Ltd.

Medioli FS, Scott DB (1983). Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Special Publication No. 21.

Medioli FS, Scott DB, Abbott BH (1987) A case study of protozoan interclonal variability: taxonomic implications: J. Foraminiferal Res. 17: 28– 47.

Neville LA, McCarthy FMG, MacKinnon MD, Marlowe P, Penner T (2009) Using thecamoebians (testate amoebae) as proxies of ecosystem health in Oil Sands impacted regions of NE Alberta: *Difflugia* spp. as canaries in the coalmine. *NAMS Microfossils II, Houston TX, March 16-18, 2009.*

Neville LA, McCarthy FMG, MacKinnon MD, Marlowe P (2008) Thecamoebians (testate amoebae) as proxies of ecosystem health in the Oil Sands wetlands: reclamation options. Oral presentation, Annual Meeting of the Geological Society of America, Houston, TX.

Patterson RT, Barker T, Burbidge SM (1996) Arcellaceans (Thecamoebians) as Proxies of Arsenic and Mercury Contamination in Northeastern Ontario Lakes. J. Foraminiferal Res. 26: 172-183.

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. Paleontol. 63: 245- 248.

Patterson RT, Kumar A (2002) A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeog, Palaeecolimatol., Palaeoecol. 180: 225- 251.

Reinhardt EG, Dalby PA, Kumar A, Patterson T (1998) Arcellaceans as Pollution Indicators in Mine Tailing Contaminated Lakes near Cobalt, Ontario, Canada. Micropaleontology 44: 131-148.

Schonborn WS (1984) Studies on remains of Testacea in cores of the Great Woryty Lake (NE-Poland): Limnologica (Berlin) 16:185–190.

Scott BD, Medioli FS, Schafer CT (2001) Monitoring in Coastal Environments Using Foraminifera and Thecamoebian Indicators. Cambridge University Press. Cambridge, p. 177.

Shannon CE, Weaver W (1949) The Mathematical Theory of Communication: Universityof Illinois Press, Urbana, 55 p.

Syncrude Canada Ltd. 1995. Syncrude Analytical Methods: Updated 2006. Syncrude Edmonton Centre.

Chapter 3

INVESTIGATING THECAMOEBIANS (TESTATE AMOEBAE) AS BIO-INDICATORS OF RECLAMATION SUCCESS IN THE OIL SANDS OF ALBERTA

LISA A. NEVILLE[1], FRANCINE M.G. MCCARTHY[1], MICHAEL D. MACKINNON[2], DAVE G.J. CHRISTIE[1]

[1] Dept. of Earth Studies, Brock University, St. Catharines, ON L2S 3A1

[2] Syncrude Canada Limited, Edmonton, AB, T6N 1H4

This manuscript has been submitted to the Journal of Paleontology and generally follows the style required by the publisher of this journal.

INTRODUCTION

THECAMOEBIANS (TESTATE amoebae) are a diverse and important component of benthic community of lakes and wetlands (Patterson and Kumar, 2002; Beyens and Meisterfeld, 2003), and they play a critical role in food webs as the intermediate between bacteria and benthic invertebrates. This polyphyletic group of protists, characterized by the presence of lobose pseudopods, an amoeboid sarcodine cell, and a simple sac-like decay-resistant organic test, includes species belonging to two different classes and several orders within the Phylum Sarcodaria, Subphylum Sarcodina, Class Rhizopoda

(Kumar and Patterson, 2000; Charman, 2001; Scott et al., 2001) (See Abbreviated Systematic Taxonmomy).

Their tests, which are variably agglutinated in different species, are morphologically distinct, allowing species and subspecies/ strain-level identification (Reinhardt et al., 1998; Kumar and Dalby, 1998; Booth and Zygmunt, 2005). They are found preserved in all fresh to slightly brackish aquatic and moist terrestrial sediments. Thecamoebians display a high degree of sensitivity to environmental conditions at the sediment-water interface and epibenthic zone, leading to their use as paleoenvironmental indicators (McCarthy et al., 1995; Patterson et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000; Scott et al., 2001; Patterson and Kumar, 2002; Boudreau et al., 2005; Kauppila et al., 2006).

Neville et al. (submitted) demonstrated the potential of thecamoebians as bio-indicators of impact by the by-products of oil sands mining and extraction on aquatic ecosystems near Fort McMurray, Alberta, comparing assemblages of these protists with levels of naphthenic acids (NA) and conductivity in the bottom waters. In oil sands operations, water is used in the extraction process, where the bitumen is separated from the oil sand ore using a hot-water process. As a result, fluid tailings consisting of water, sand, fines, and unrecovered bitumen are produced, and will be referred to as oil sands process-affected material (OSPM). The tailings are transported to retention ponds where they settle and densify, releasing process-affected water (OSPW). Released water is recycled back into the extraction (Mikula et al., 1996). OSPW contains elevated ionic and dissolved organic acid concentrations relative to natural water. The salinity of the OSPW is elevated through the addition of Na^+, Cl^- and SO_4^{-2} ions from the ore and processing chemicals, while dissolved organic acids (naphthenic acids - NAs) result from their dissolution from bitumen during extraction. NAs account for most of the dissolved organic carbon in OSPW (Schramm et al, 2000). In addition, OSPW may also contain elevated ammonia (as NH_4^+) as a by-product of the upgrading processes. The OSPW character will vary with differing ore sources and extraction conditions used, but in general it will stress biota through elevated ionic content and presence of low molecular weight organic acid constituents (Harris, 2007).

Neville et al. (submitted) examined thecamoebians in samples collected in August, 2007 from the sediment-water interface at an oil sands constructed wetland test facility (Suncor Energy Inc. Lease 86), which is used by the oil companies to research reclamation options (Fig. 3.1). Sites with relatively low OSPW impact (NA < 11 mg/L) contained a relatively abundant (N= 137/5 ml) and diverse thecamoebian fauna (av. SDI= 1.97) dominated by the Family Difflugidae ("difflugiids"). Samples from high OSPW character wetland sites (NA >35 mg/L) generally contained a less diverse (av. SDI= 1.42), and typically less abundant fauna (N= 111/5 ml) dominated by the genera *Centropyxis* Stein, 1859 (Family Centropyxidae; "centropyxids") and *Arcella* Ehrenberg, 1830 (Family Arcellidae; "arcellids"). A strong correlation (r^2=0.688) between conductivity and the relative abundance of centropyxid thecamoebians also illustrated a response to OSPM/OSPW.

Since commercial mining operations of the Athabasca Oil Sands in northeastern Alberta began in the mid 1960's, surface mine developments and their resulting tailings retention have led to the profound disturbance of large areas (currently >500 km^2) of northern Boreal Forest (Alberta Energy, 2008). As part of the License to Operate issued by the Government of Alberta to companies operating in the oil sands under the provincial *Clean Water Act*, process-affected water (OSPW) is not released into the natural ecosystem (Masliyah, 2006). Currently, OSPW is retained on site within secure retention ponds, from which it is recycled for operational needs. OSPW and associated tailings materials both require reclamation, and options including constructed wetlands and lakes where exposed to OSPM will occur (Holroyd et al., 2009). As these aquatic reclamation systems develop over time, it is expected that they will progress toward a more natural state, as a detrital layer builds at the sediment interface, providing habitat for increased development of biota (Harris, 2007).

Successful reclamation will include the development of a viable benthic community, ranging from bacteria to benthic invertebrates in the wetland and lake environments containing OSPW over soft tails associated with the oil sands extraction (OSPM) (FFTC, 1995). The development of the benthic community will be influenced by the properties of the OSPM, as well as the composition of overlying water- OSPW (Allen, 2008). Wetland and lake habitats, both constructed and opportunistic, are important components in the lease-closure of the oil sands, and the rate of progression from OSPW-stressed systems to more natural functions is an important factor for gauging success (Harris, 2007; SCL, 2003). To meet these challenges, the oil sands operators are looking for sensitive metrics to assist in assessing the rate and degree to which these reclaimed areas are progressing towards natural ecosystems. This will allow them to meet their goal of returning the disturbed areas to pre-disturbance levels of productive capability.

The rapid generation time of thecamoebians (typically reproducing once every two to eleven days according to Ogden and Hedley, 1980) should allow communities to respond quickly to environmental change, hopefully at rates sufficient to provide input for selecting remediation strategies. This study assesses the sensitivity of thecamoebians to common by-products of oil sands mining and extraction (namely salts and organic acids), and investigates how quickly populations of these benthic protists respond to changes in OSPW content. By utilizing the Constructed Wetland Test Facility (CWTF) located at Suncor Energy Inc. Lease 86, thecamoebian assemblages were compared over a two year period, as the level of recharge of OSPW decreased and its relative influence on the wetland water quality was reduced (Figure 3.1). This study thus adds a temporal and operational aspect to the initial surveys that were described in Neville et al. (submitted), together with increased statistical rigor.

METHODS

Eight of the 22 sites collected in August 2007 from constructed wetlands located on the oil sands operation (Lease 86) were re-sampled in June 2008. The location of these sites with reference to the point sources of water into the wetlands is shown in Fig. 3.1. In addition to atmospheric input, the Suncor wetland sites received OSPW from adjacent settling basins, episodically by pumping and continuously by seepage from the sand dykes (Sites 2 and 5, Fig. 3.1). In addition, natural runoff enters the wetland near Site 8, and flows through the constructed wetland toward the northwest (site 18). Sites 8 and 9 (Fig. 3.1) are upstream from the inflow of OSPW (Sites 2 and 5). Sites such as Crane Lake (Site 21), located over one km from the OSPW input point, appear to be recharged by waters in contact with non-disturbed materials, and only minor OSPW character (low NA content and major ion distribution) is noted; these sites do display elevated salinity (conductivities >1000 μS/cm) relative to the surrounding natural lakes and wetlands, likely natural salt leaching from clays of the area. Sites 14 and 15 (Sustainable Lake North and Sustainable Lake South) contain aged/ mature OSPW, and do not have water recharge or discharge, other than from hydrology within their limited watersheds, that is sufficient to maintain the water in these isolated ponds.

Grab samples of 50-200 ml from the sediment-water interface at Sites 2, 5, 8, 9, 14, 15, 18 and 21 (Table 3.1) were placed in glass jars and stored at 4°C prior to shipping to Brock University. At the same time, water samples from above the sediment at each site were collected and transported to Syncrude Canada Ltd. Edmonton Research facility for water analysis (conductivity, pH, major ions, trace metals and naphthenic acids) using their standard protocols (Syncrude, 1995; Jivraj et al., 1996).

Samples from both the August 2007 and June 2008 datasets ("Sets One and Two", with samples from Set Two identified with –2 next to the site number in Table 3.1) were prepared for thecamoebian analysis following the standard micropaleontological methods described in Scott et al. (2001): wet sieving subsamples of 5 ml volume through 500, 63 and 45 μm sieves, using dish detergent to disaggregate clays in muddy samples. For quantitative analyses, the samples were placed in a gridded Petri dish and wet counted at 50x magnification using a Leica MZ12.5 microscope. The 45-63 μm and >63 μm size fractions were examined separately to decrease the likelihood of missing specimens obscured by large particles. When the number of thecamoebians counted in the 5ml subsample did not reach the minimum of 100, an additional 5ml subsample was processed and counted, in order to allow statistical analysis of the data (Patterson and Fishbein, 1989). The thecamoebian communities observed in subsamples were virtually identical to each other, suggesting that counts of ~100 individuals are sufficient to characterize these wetlands. The standard error of estimate for each sample is shown in Appendix 4.

Thecamoebians were identified using the key of Kumar and Dalby (1998) as a primary reference, although reference was also made to photoplates and descriptions in various publications, notably Medioli and Scott (1983). Specimens were identified to strain level because these intraspecific variants/ morphotypes have been found to convey useful information on aquatic sub-environments (Appendix 4) (Asioli et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000; Kauppila et al. 2006). While the data are generally reported in this paper as species without subdivision to strain/subspecies/morphotype level to simplify the discussion, strain-level determinations were used in calculating species diversity. The Shannon-Weaver Diversity index (SDI) (Shannon and Weaver, 1949) was chosen for this study because it is commonly used in micropaleontological studies (Patterson and Kumar 2000; 2002; Reinhardt, et al., 2005). The SDI is calculated using the following formula, where S is the species richness for each sample:

$$SDI = -\sum_{I}^{S}\left(\frac{Fi}{Ni}\right) * \ln\left(\frac{Fi}{Ni}\right)$$

RESULTS AND OBSERVATIONS

Selected water chemistry data for the eight sites sampled in both September 2007 ("Set One") and June 2008 ("Set Two") are shown in Table 3.1 (for a full report of water chemistry analysis, see Appendix 4). Average conductivity and naphthenic acid (NA) concentrations were 1656 μS/cm and 23.8 mg/L in June 2008, while values of 1970 μS/cm and 33 mg/L, respectively, were reported in the samples collected in August 2007. The greatest year-to-year change in these indicators of OSPW influence was noted at Site 2 (Dyke 4 Seepage), which saw NA concentrations fall from 55.4 to 28.9 mg/L and conductivities from 2570 to 1620 μS/cm. This site is closest to the input of the episodic recharge of OSPW to the CWTF (Fig. 3.1). The direct addition of OSPW water from the adjacent settling basin was markedly reduced between August 2007 and June 2008. Meanwhile, the continuous influx of seepage waters (OSPW character) via Site 5 and surface runoff waters (low OSPW character) from Site 9 were not altered, so that the overall impact on the CWTF from OSPW would be reduced by the lower import through Site 2. As a result, Site 18 (Gooseneck Wetland/ Jan's Pond), which is downstream of the OSPW outfall, would effectively receive a more diluted OSPW. This is what was observed, with a reduction in NA concentrations from 51.2 to 33.2 mg/L, and conductivity from 2650 to 2130 μS/cm. Very little change in water quality was measured at Site 21 (Crane Lake), where NA concentrations were measured at 8.4 mg/L in both years, while conductivities increased slightly from 1300 to 1440 μS/cm between August 2007 and June 2008. This site is not part of the CWTF watershed, but reflects the runoff from non-OSPM materials. Another site also isolated from the CWTF hydrology, but with strong OSPW character was Site 15 (Sustainable Lake South). It also experienced only a slight decrease in NA concentrations from 41.5 to 36.2 mg/L and no change in conductivity, which remained at 1820 µS/cm (Table 3.1). Neither Site 21 or 15 would have been directly affected by the change in water quality in the CWTF resulting from the decrease in OSPW flux near Site 2 (Dyke 4 Seepage).

Thecamoebian assemblages in Set Two (June, 2008) were nearly identical to those analyzed in Set One (Sept, 2007) at Sites 8, 9 and 21 (Fig. 3.2; Table 2S). The assemblages at these sites, which had low OSPW impact and little year-to-year variation, were dominated by difflugiid taxa, primarily *Difflugia oblonga* Ehrenberg 1832 and *Cucurbitella tricuspis* (Carter 1856), with *Centropyxis constricta* Ehrenberg 1843 as the most common centropyxid taxon. Full species assemblage data for both the August 2007 and June 2008 sample sets can be found in Appendix 4. The majority of tests in the samples collected in June 2008 were stained by Rose Bengal, suggesting that a large portion of the sample was living (or recently alive, i.e. tests contained cytoplasm) at the time of collection (Scott and Medioli, 1980). The determination of living vs. dead protists using Rose Bengal is not universally accepted (Bernard et al., 2006), but since the wetlands were only constructed in 1999-2000, the potential problem of staining very old tests is less of a concern.

A marked change in the thecamoebian assemblage was noted in Set 2 relative to Set 1 at Sites 2, 5 and 18, where the relative abundance of difflugiid thecamoebians and species diversity increased (Fig. 3.3; Table 3.1). At Site 18 (Gooseneck Wetland/ Jan's Pond), for instance, a 35% decrease in NA concentration and 20% decrease in conductivity is associated with an increase in species diversity (SDI= 1.53 from 1.08) and an increase in the relative abundance of difflugiid species from 9.2 to 45.0% of the total assemblage (Table 3.1). In addition, no specimens of *Arcella vulgaris* Ehrenberg 1830 were found in the June 2008 sample from Site 18, whereas 10 specimens (7.2% of the assemblage) were found in the August 2007 sample. At Site 2, a 37% decrease in NA concentration and 48% decrease in conductivity are associated with an increase in species diversity (SDI= 1.45 from 1.37) and an increase in the relative abundance of difflugiid species from 19.8 to 41.9% of the total assemblage. The relative abundance of *Arcella vulgaris* also decreased (Table 3.1). A chi-square test performed on counts from samples 2-1 and 2-2 revealed a statistically significant difference between the August 2007 and June 2008 samples, with a p-value = 0.002.

A more subtle and mixed change in water quality at Site 5 (a 12% decrease in the NA concentration and a 6% increase in conductivity) is associated with an increase in the relative abundance of difflugiid taxa from 38.9 to 47.2% of the assemblage, but with a slight decrease in diversity (SDI= 2.01 from 2.12) (Fig. 3.3; Appendix 4). Unlike Sites 2 and 18, Site 5 is not downstream from the OSPW input point of the imported OSPW, and would not have been directly impacted by the reduced volume of recharge between August 2007 and June 2008, but would still receive OSPW from the seepage waters being continuously added to Site 5 along the base of sand containment dykes of the settling basin.

Thecamoebian assemblages from the two of the larger water bodies at the CWTF, Sustainable Lake North (Site 14) and Sustainable Lake South (Site 15), differed substantially from each other, even though the chemical characteristics of the overlying water differed only slightly. These sites do not receive OSPW recharge or discharge, so any change in water quality would result from climatic parameters and time, as the constituents in the water mature (Fig. 3.4; Table 3.1). High concentrations of NA and high conductivity values at both sites remained fairly constant between August 2007 and June 2008, but a more diverse and difflugiid-rich thecamoebian assemblage characterized Site 15 in both sample years, and a much greater change in thecamoebian assemblage characterized Site 14, with an increase in the relative abundance of difflugiid taxa from 3.1 to 23.5% if the total assemblage, and a change in SDI from 1.1 to 1.76 (Table 3.1).

DISCUSSION

Samples from the constructed wetland facility (CWTF) at Suncor Energy Inc. (including eight sites that were resampled ten months after the initial dataset) and analyzed for thecamoebians and chemistry of the overlying water column appear to demonstrate the sensitivity of these protists to the major by-products of oil sands mining and processing. Samples with low OSPW character, i.e. low concentrations of salt (conductivity <1300μS/cm) and dissolved organics (mainly naphthenic acids- NA <11 mg/L) contain diverse difflugiid-dominated assemblages that are similar to those found in five natural lakes in the Boreal Forest region of Alberta. (Appendix 4 & Figure 3.5). The relatively diverse (SDI 1.61-2.07, av. 1.73) thecamoebian assemblages in these natural lakes were dominated by *Difflugia oblonga* (37.9%), *Centropyxis constricta* (24.2%), *Cucurbitella tricuspis* (15.6%), and *Centropyxis aculeata* (14.6%), and contained <1% *Arcella vulgaris.* By comparison, the thecamoebian assemblage in Crane Lake (Site 21) was dominated by *Difflugia oblonga* (51.5%), *Centropyxis constricta* (25.3%), *Cucurbitella tricuspis* (13.1%), and *Centropyxis aculeata* (10.1%), and that at the v-notch weir (Site 9) dominated by *Difflugia oblonga* (19.62%), *Centropyxis constricta* (27.22%), *Cucurbitella* *tricuspis* (18.99%), and *Centropyxis aculeata* (16.45%). The species diversity at the low OSPW sites in the Suncor wetland was slightly higher than in the 5 natural lakes analyzed (av. SDI= 2.06 compared with av. SDI= 1.73 in Boreal Forest Lakes) (Appendix 4), although both means fall within the range of SDI (1.5 - 2.5) for thecamoebian populations normally characterizing intermediate conditions according to Patterson and Kumar (2002). The relatively high species diversity in constructed wetlands on the oil sands properties, together with the observation that thecamoebians often appear to selectively incorporate bitumen into their tests (Chapter 4), suggest that at low concentrations, the by-products of oil sands extraction may be a resource for thecamoebians.

Sites adjacent to, and downstream from, the settling pond containing fresh OSPW, contain high concentrations of salts and organic acids. A deliberate decrease in OSPW at the outfall near Site 2 was part of a larger study being conducted by Suncor Energy Inc. NA levels at Site 2 fell from 55.4 to 28.9 mg/L and conductivity decreased from 2570 to 1620 μS/cm between August 2007 and June 2008 (Table 3.1). The reduction in OSPW character in bottom waters at Site 2, and at sites downstream from this outfall (e.g. Site 18), reflects the dilution over the intervening 10-month period, presumably by atmospheric water during the wetter, less evaporative part of the year.

During the first year of study (Set One, 2007) Sites 2, 5, & 18 were considered highly impacted by OSPW (average NA levels 47.5 mg/L and conductivity 2360 μS/cm) (Fig. 3.3). Samples from these sites were characterized by a low diversity and typically less abundant thecamoebian fauna dominated by the genus *Centropyxis.* High relative abundances of centropyxid taxa at sites with high conductivity water are consistent with the dominance of these taxa in marginal marine environments, such as salt marshes and estuaries (Scott et al., 2001). Most lakes in North America, in contrast, are dominated by difflugiid species (Patterson et al., 1985; Collins et al., 1990; McCarthy et al., 1995). (For a more thorough explanation of this work and the significance of species presence, see Chapter 4). The abundance of *Arcella vulgaris* at some of these sites with high OSPW impact is consistent with the presence of this taxon in highly stressed lakes in northeastern Ontario impacted by sulphide mining (Patterson et al., 1996; Reinhardt et al., 1998; Kumar and Patterson, 2000).

In response to the deliberate decrease in OSPW to these sites a decrease in NA and conductivity is observed during the second year of study (Set Two) (Fig. 3.3). With this change in water chemistry we observe a shift in the thecamoebian fauna from a low diversity (average SDI 1.52) centropyxid dominated assemblage to a more diverse (average SDI 1.66) community composed of both centropyxid and difflugiid thecamoebians. This apparent rapid response of the thecamoebian community to the decrease in OSPW is consistent with the rapid generation time reported in the literature (c.f. Ogden and Hedley, 1980).

Sites 8, 9 and 21 that were characterized by relatively low OSPW impact during the first year of study (average NA levels 10 mg/L and conductivity 1606 μS/cm) experienced little to no change in water chemistry in June 2008 (average NA levels 6.9 mg/L and conductivity 1240 μS/cm), being far from the OSPW outfall (Fig. 3.2, Table 3.1). No significant change occurred in the thecamoebian high diversity (average SDI 2.06) difflugiid dominated thecamoebian faunas year to year. The small changes in water chemistry at sites 8, 9 and 21 may reflect seasonal differences to some degree, with higher runoff from spring freshette occurring in the June 2008 samples. Small changes in the thecamoebian assemblages from month to month were observed in the Syncrude Demo Pond (Big Pit), over a four-month period in 2008, from May to September (Table 3.2, Chapter 4, Appendix 4). Big Pit is a pond located on Syncrude property that does not receive OSPW, so the water chemistry remains relatively constant (other than seasonal climatically-induced variations) (Table 3.2). While the proportion of difflugiid vs. centropyxid thecamoebians stayed relatively consistent in Big Pit, the species composition of the assemblage varied throughout the study, with particularly large variation in the difflugiid species (Fig. 3.6)(Neville et al., in prep).

The relative abundance of centropyxid thecamoebians shows a strong correlation with NA concentrations (r^2= 0.743) and conductivity (r^2= 0.707) in the combined August 2007 and June 2008 sample sets, and an inverse correlation exists with difflugiid thecamoebians (Figs. 3.7, 3.8). Re-sampling eight sites further confirmed the sensitivity of thecamoebians to by-products of oil sands mining and extraction (Neville et al., 2008), with the additional data slightly improving the r^2 values for three of the four graphs plotted based on the 2007 dataset alone. The only exception is a slightly lower r^2 value for the relationship between the concentration of NA and the relative abundance of centropyxid thecamoebians (Neville et al., submitted). The high degree of similarity between the two sample sets at sites 8, 9 and 21, where no marked change in water quality was measured between August 2007 and June 2008, suggests that this metric is reproducible (Figs. 3.3, 3.7 & 3.8).

The line of best fit created by plotting the relative abundance of difflugiid or centropyxid thecamoebians against the concentration of NA or conductivity (Figs. 3.7 &

3.8) may be used to extrapolate how quickly the health of an aquatic ecosystem undergoing remediation is improving (future work will establish baselines to estimate endpoints). A decrease in OSPW impact is reflected by a shift along the line of best fit in a favorable direction (increasing relative abundance of difflugiids/ decreasing relative abundance of centropyxids in the thecamoebian community). The nature of this relationship merits further examination.

The comparison between the two largest water bodies in the constructed wetland facility, Sustainable Lake North (Site 14) and South (Site 15), illustrates the potential value of thecamoebian assemblages in biomonitoring to assess reclamation management options. These small lakes, constructed at the same time of approximately the same dimensions and immediately adjacent to one another, have similar concentrations of OSPW (Table 3.1) in both sample sets. The thecamoebian assemblage at Site 15 in both the August 2007 and 2008 sample sets was more diverse and difflugiid-rich than would be expected from the high conductivity and naphthenic acid concentration measured there, and is an outlier on the plots against OSPW constituents (compared with Site 14, which plots very close to the line of best fit in Figs. 3.7 and 3.8). These lakes have been managed differently since their construction in 1992, with intensive nutrient loading practised at Sustainable Lake South (Site 15) but not at Sustainable Lake North (Site 14). The properties of the sediment in each site also reflects this, with Site 15 showing higher organic content from detrital build-up on the underlying OSPM resulting from its higher productivity, while Site 14 shows a more OSPM-dominated character. Our analysis suggests that nutrient loading may have sped up the remediation process through higher productivity and resulting detrital deposition rates, even if the site remains highly impacted by OSPM constituents.

CONCLUSIONS

Thecamoebian (testate amoeba) assemblages have been shown to be sensitive proxies of environmental quality in the Suncor Energy Inc. Constructed Wetlands Test Facility near Fort McMurray, Alberta. This benthic protist fauna seems to potentially provide a relatively easy and inexpensive metric for assessing remediation practices and their effectiveness in oil sands aquatic reclamation systems. Thecamoebian community structure and composition responded to year-to-year changes in water chemistry, produced in large part by a deliberate reduction of OSPW flux to the eastern part of the wetland test facility. Difflugiid thecamoebians are particularly sensitive to the common by-products of oil sands mining and extraction (salts, NA), while *Arcella vulgaris* and centropyxid taxa are able to tolerate the higher conductivity and elevated concentrations of NA associated with oil sands process affected waters (OSPW). Difflugiid-dominated assemblages appear to be generally restricted to samples with <11 mg/L NA and conductivity <1300 μS/cm, although adding nutrients to the ecosystem appears to allow the more sensitive difflugiid species to colonize highly impacted sites, possibly accelerating the remediation process. Because the rapid generation time of these protists allows them to respond quickly to changes in water quality resulting from natural environmental changes or deliberate reclamation management decisions, they can be used to assess various reclamation strategies, in addition to monitoring the overall health wetland and lake habitats, which are important components in the lease-closure of the oil sands. The preliminary success of this microfossil proxy in the oil sands region warrants further investigation of how best to apply the thecamoebian community to evaluate aquatic reclamation in and outside the oil sands region.

ACKNOWLEDGMENTS

We thank Christine Daly from Suncor Energy Limited for allowing us access to the Suncor wetlands and resources, Mike Lozon for his assistance with drafting, Brian Calvert for his advice on statistics, and Syncrude Canada Limited for financial support of this study.

LIST OF TABLES AND FIGURES

Table 3.1: Summary of chemical and micropaleontological data for samples collected from the Suncor constructed wetland test facility (CWTF) in August 2007 ("Set 1": Sites x-1) and in June 2008 ("Set 2": Sites x-2). The change (percentage or absolute) from August 2007 to June 2008 for each site sampled during both sample years is shown in italics.

Table 3.2: Summary of chemical and micropaleontological data for samples collected from Syncrude Demo Pond (Big Pit) in May, July, August and September.

Figure 3.1: Satellite photo showing the location of the Suncor Constructed Wetlands Test Facility (CWTF) in the Athabasca Oil Sands (Google Earth, 2009). Fresh oil sands process-affected water (OSPW) held in the settling pond that forms the eastern boundary, enters the wetlands at Site 2 (Dyke 4 Seepage) and flows northwestward as shown by the arrows.

Figure 3.2: The similarity in thecamoebian assemblages in samples collected from the CWTF in August 2007 to those collected in June 2008 is evident in the pie diagrams from sites with low OSPW character. Samples with low values of naphthenic acids (5.7-10.9 mg/L) and conductivity (1060- 2450μS/cm) contain diverse thecamoebian assemblages (SDI 1.69-2.25) dominated by difflugiid taxa, particularly *Difflugia oblonga* and *Cucurbitella tricuspis*.

Figure 3.3: Thecamoebian assemblages in samples collected in August 2007 differ significantly from those collected from the CWTF in June 2008 at sites closer to the outfall of OSPW. Although high OSPW character is illustrated by the high values of naphthenic acids and conductivity in samples from both years, a marked reduction in OSPW impact occurred between August 2007 and June 2008. This is reflected in the thecamoebian record by an increase in diversity and an increase in the relative abundance of difflugiid taxa at the expense of *Arcella vulgaris*, *Centropyxis aculeata* and *Centropyxis constricta.*

Figure 3.4: Thecamoebian assemblages in samples collected from the two large water bodies at the experimental wetland facility differ substantially from one another in both sample years, despite the strong similarity in water chemistry at both sites, and in both sample years. The higher relative abundance of difflugiid taxa and greater species diversity in Sustainable Lake South (Site 15) probably reflects differences in reclamation strategy employed at the two sites, with large volumes of nutrients added to Sustainable Lake South but not to Sustainable Lake North. Fertilization appears to be a successful reclamation strategy.

Figure 3.5: Summary pie diagram showing the average thecamoebian species that comprise assemblages in natural lakes in the Boreal Forest region of Alberta. The assemblages are similar to those found in low OSPW-character samples from the CWTF (see Figure 3.2).

Figure 3.6: Substantial variation was noted in samples analyzed from the Syncrude Demo Pond (Big Pit) from sediment collected in May, July, August, and September of 2008, particularly in the difflugiid species present, although the relative abundance of difflugiids vs. centropyxids remained relatively constant.

Figure 3.7: Naphthenic acid concentration from year one (2007, solid diamonds) and year two (2008, hollow diamonds) show a strong correlation with the percentage of centropyxid thecamoebians, yielding an r^2 value of 0.743. The inverse correlation exists with difflugiid thecamoebians with an r^2 value of 0.727. The values from year two have shifted down the line of best fit compared to the values from year one.

Figure 3.8: Conductivity from year one (2007, solid diamonds) and year two (2008, hollow diamonds) show a strong correlation with the percentage of centropyxid thecamoebians, yielding an r^2 value of 0.707. The inverse correlation exists with difflugiid thecamoebians with an r^2 value of 0.602. The values from year two have shifted down the line of best fit compared to the values from year one.

ONLINE SUPPLEMENTARY MATERIAL

Table 3.1S: Percent abundance, standard error, depth, species diversity for each sample analyzed from Set One (August 2007) and Set Two June 2008).

Table 3.2S: Percent abundance, standard error, depth, species diversity for each sample analyzed from natural lakes from the Boreal Forest region of Alberta: Baptiste Lake, Island Lake, Gregoire Lake, Lac St. Anne, and Wabamun Lake.

TABLES

Table 3.1.

Site Number	Site Location	Naphthenic Acids (mg/L)	Conductivity (μS/cm)	Thecamoebian Species Diversity (SDI)	% centropyxid	% difflugiid	% *Arcella vulgaris*
2-1	Dyke 4 seepage	55.4	2570	1.37	78.2	19.8	2.0
2-2		28.9	1620	1.45	52.9	41.9	1.45
	Change: Aug. 07 to June 08	***-26.5***	***-950***	***+ 0.08***	***- 25.3***	***+ 22.1***	***- 0.55***
5-1	Dyke 4 Reservoir	36.1	1860	2.12	61.0	38.9	0.63
5-2		31.9	1975	2.01	52.7	47.2	0
	Change: Aug. 07 to June 08	***-4.2***	***+115***	***- 0.11***	***- 8.3***	***+ 8.3***	***-0.63***
8-1	Control Reservoir	10.9	1070	2.13	16.9	81.8	0
8-2		6.6	1060	2.25	18.9	81.1	0
	Change: Aug. 07 to June 08	***-4.3***	***-10***	***+ 0.12***	***+10.6***	***- 0.07***	***0***
9-1	V- Notch Weir	10.7	2450	2.16	42.8	55.9	0
9-2		5.7	1220	2.24	41.2	58.8	0
	Change: Aug. 07 to June 08	***-5***	***-1230***	***+ 0.08***	***-3.7***	***+4.9***	***0***
14-1	Sustainable Lake North	44.2	2040	1.1	72.1	3.1	24.7
14-2		40.1	1980	1.76	52.9	23.5	23.5
	Change: Aug. 07 to June 08	***-4.1***	***-60***	***+ 0.66***	***-26.6***	***+ 2.9***	***1.2***
15-1	Sustainable Lake South	41.5	1820	1.8	26.2	73.7	0
15-2		36.2	1820	2.15	30.5	69.5	0
	Change: Aug. 07 to June 08	***-5.3***	***0***	***+ 0.35***	***+14.1***	***-5.69***	***0***
18-1	Gooseneck Wetland	51.2	2650	1.08	82.3	9.2	8.4
18-2		33.2	2130	1.53	55.0	45.0	0
	Change: Aug. 07 to June 08	***-18***	***-520***	***+ 0.45***	***-33.2***	***+ 35.8***	***- 8.4***
21-1	Crane Lake	8.4	1300	1.85	36.3	63.7	0
21-2		8.4	1440	1.69	42.9	57.1	0
	Change: Aug. 07 to June 08	***0***	***+140***	***- 0.16***	***+15.4***	***- 6.6***	***0***

Table 3.2.

Date (2008)	**Naphthenic Acids (mg/L)**	**Conductivity (μS/cm)**	**pH**	**Average Monthly Temperature (C^0)**	**Total Monthly Precipitation (mm)**	**Thecamoebian Species Diversity (SDI)**	**% Difflugiid**
May	-	1825	7.8	10.1	7.8	2.22	80.6
July	37.5	2266	7.92	16.2	56.6	2.32	85.9
August	26	2140	8.25	15.9	137.6	2.03	74.6
September	34	1850	8.31	9.8	27.4	2.21	84.1
Average	*26*	*12*	*2*	*26*	*57.4*	*2.2*	*81.3*

111°57'8"W
110°59'9"W
57°22'47"N
56°51'5"N
Regional map
Athabasca Oil Sands
Suncor sites
10 km
N
inset A
inset B
inset C
Crane Lake
settling basin
63
500 m
A
22
21
500 m
C
23
63
300 m
B
13
16
12
18
14
10/11
19
15
3
2
OSPW
4
DSW
6
5
9
17
8
RO
200 m
settling basin
Site Locations
2 - Dyke 4 Seepage
3 - 1 m CT Wetland (off end of boardwalk)
4 - Weir C
5 - Dyke 4 Reservoir
8 - Control reservoir
9 - V-Notch Weir
10 - Pond (QA/QC sample - same location as 11)
11 - 4 m CT out (near Gooseneck)
12 - Weir A
13 - Weir B
14 - Sustainable Lake North
15 - Sustainable Lake South
16 - Species E Donor Site
17 - Muskeg Stockpile Wetland
18 - Gooseneck Pond wetland
19 - Dyke 4 Pond B
21 - Crane Lake
22 - High Sulphate Wetland
23 - Natural Wetlands out

Figure 3.1

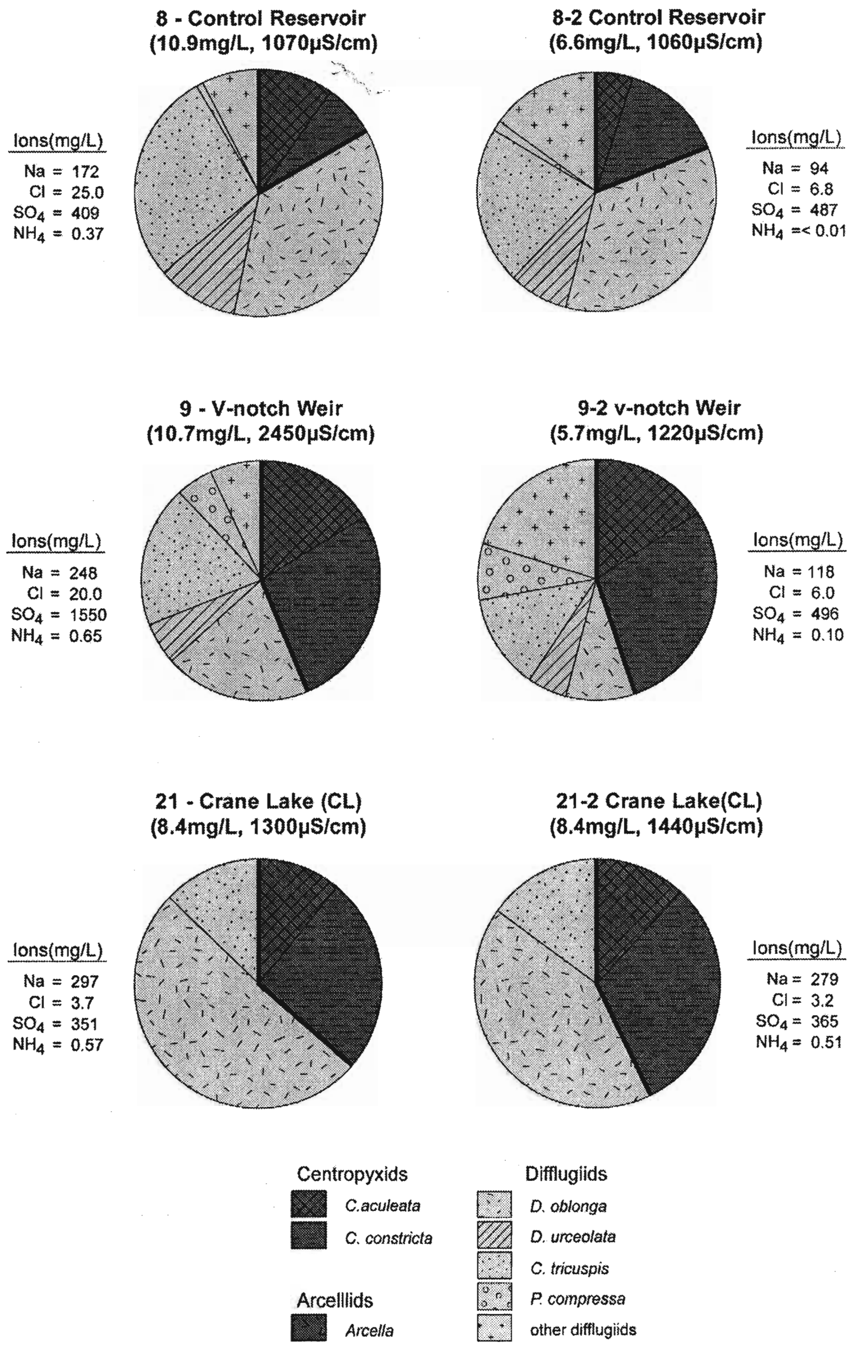

8 - Control Reservoir
(10.9mg/L, 1070μS/cm)
Ions(mg/L)
Na = 172
Cl = 25.0
SO4 = 409
NH4 = 0.37
8-2 Control Reservoir
(6.6mg/L, 1060μS/cm)
Ions(mg/L)
Na = 94
Cl = 6.8
SO4 = 487
NH4 =< 0.01
9 - V-notch Weir
(10.7mg/L, 2450μS/cm)
Ions(mg/L)
Na = 248
Cl = 20.0
SO4 = 1550
NH4 = 0.65
9-2 v-notch Weir
(5.7mg/L, 1220μS/cm)
Ions(mg/L)
Na = 118
Cl = 6.0
SO4 = 496
NH4 = 0.10
21 - Crane Lake (CL)
(8.4mg/L, 1300μS/cm)
Ions(mg/L)
Na = 297
Cl = 3.7
SO4 = 351
NH4 = 0.57
21-2 Crane Lake(CL)
(8.4mg/L, 1440μS/cm)
Ions(mg/L)
Na = 279
Cl = 3.2
SO4 = 365
NH4 = 0.51
Centropyxids
C.aculeata
C. constricta
Arcelllids
Arcella
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
P. compressa
other difflugiids

Figure 3.2.

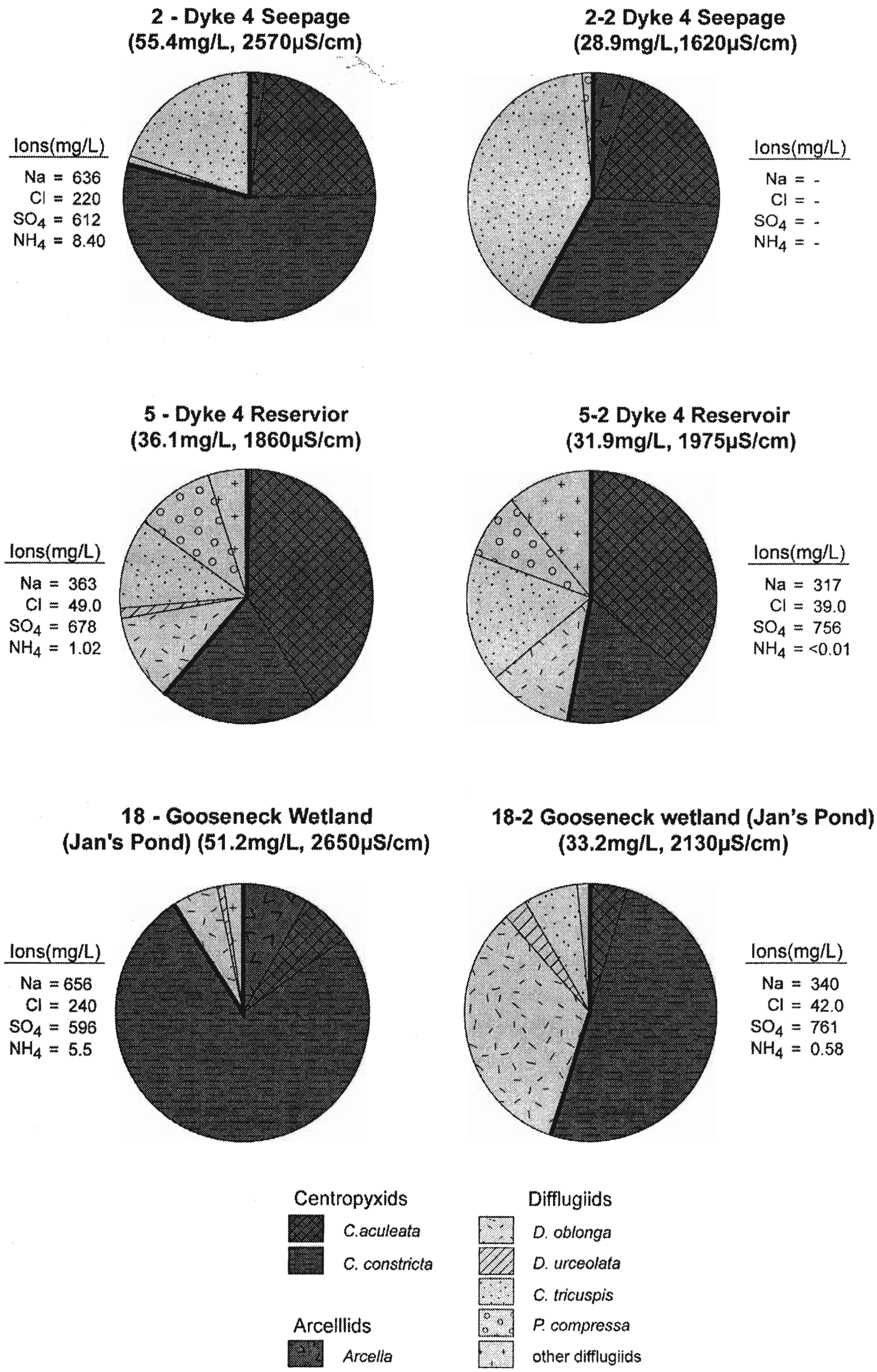
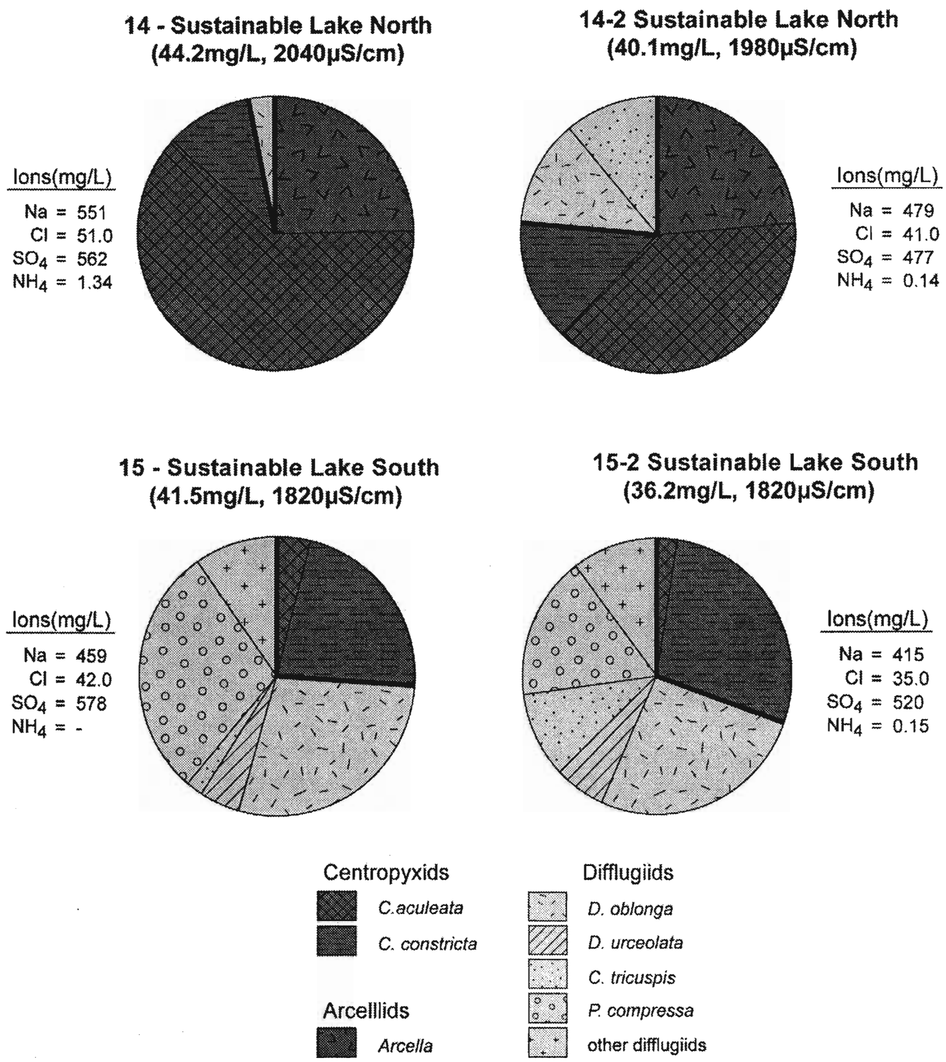

2 - Dyke 4 Seepage
(55.4mg/L, 2570µS/cm)
Ions(mg/L)
Na = 636
Cl = 220
SO4 = 612
NH4 = 8.40
2-2 Dyke 4 Seepage
(28.9mg/L,1620µS/cm)
Ions(mg/L)
Na = -
Cl = -
SO4 = -
NH4 = -
5 - Dyke 4 Reservior
(36.1mg/L, 1860µS/cm)
Ions(mg/L)
Na = 363
Cl = 49.0
SO4 = 678
NH4 = 1.02
5-2 Dyke 4 Reservoir
(31.9mg/L, 1975µS/cm)
Ions(mg/L)
Na = 317
Cl = 39.0
SO4 = 756
NH4 = <0.01
18 - Gooseneck Wetland
(Jan's Pond) (51.2mg/L, 2650µS/cm)
Ions(mg/L)
Na = 656
Cl = 240
SO4 = 596
NH4 = 5.5
18-2 Gooseneck wetland (Jan's Pond)
(33.2mg/L, 2130µS/cm)
Ions(mg/L)
Na = 340
Cl = 42.0
SO4 = 761
NH4 = 0.58
Centropyxids
C.aculeata
C. constricta
Arcelllids
Arcella
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
P. compressa
other difflugiids

Figure 3.3.



14 - Sustainable Lake North
(44.2mg/L, 2040µS/cm)
Ions(mg/L)
Na = 551
Cl = 51.0
SO4 = 562
NH4 = 1.34
14-2 Sustainable Lake North
(40.1mg/L, 1980µS/cm)
Ions(mg/L)
Na = 479
Cl = 41.0
SO4 = 477
NH4 = 0.14
15 - Sustainable Lake South
(41.5mg/L, 1820µS/cm)
Ions(mg/L)
Na = 459
Cl = 42.0
SO4 = 578
NH4 = -
15-2 Sustainable Lake South
(36.2mg/L, 1820µS/cm)
Ions(mg/L)
Na = 415
Cl = 35.0
SO4 = 520
NH4 = 0.15
Centropyxids
C.aculeata
C. constricta
Arcelllids
Arcella
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
P. compressa
other difflugiids

Figure 3.4.

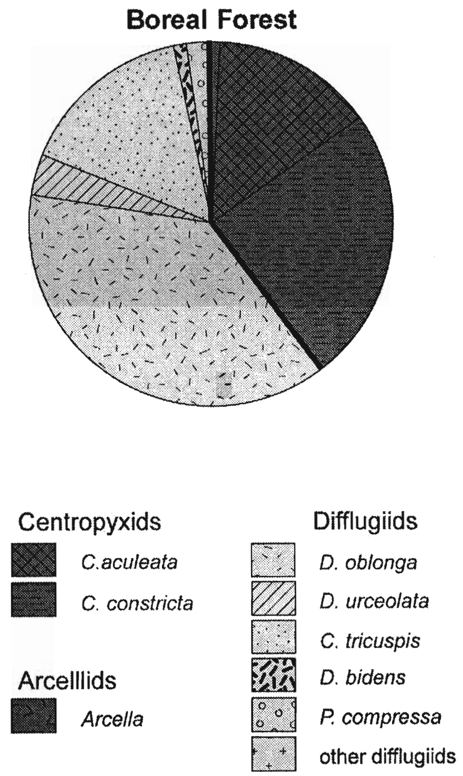

Boreal Forest
Centropyxids
C.aculeata
C. constricta
Arcelllids
Arcella
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
D. bidens
P. compressa
other difflugiids

Figure 3.5.

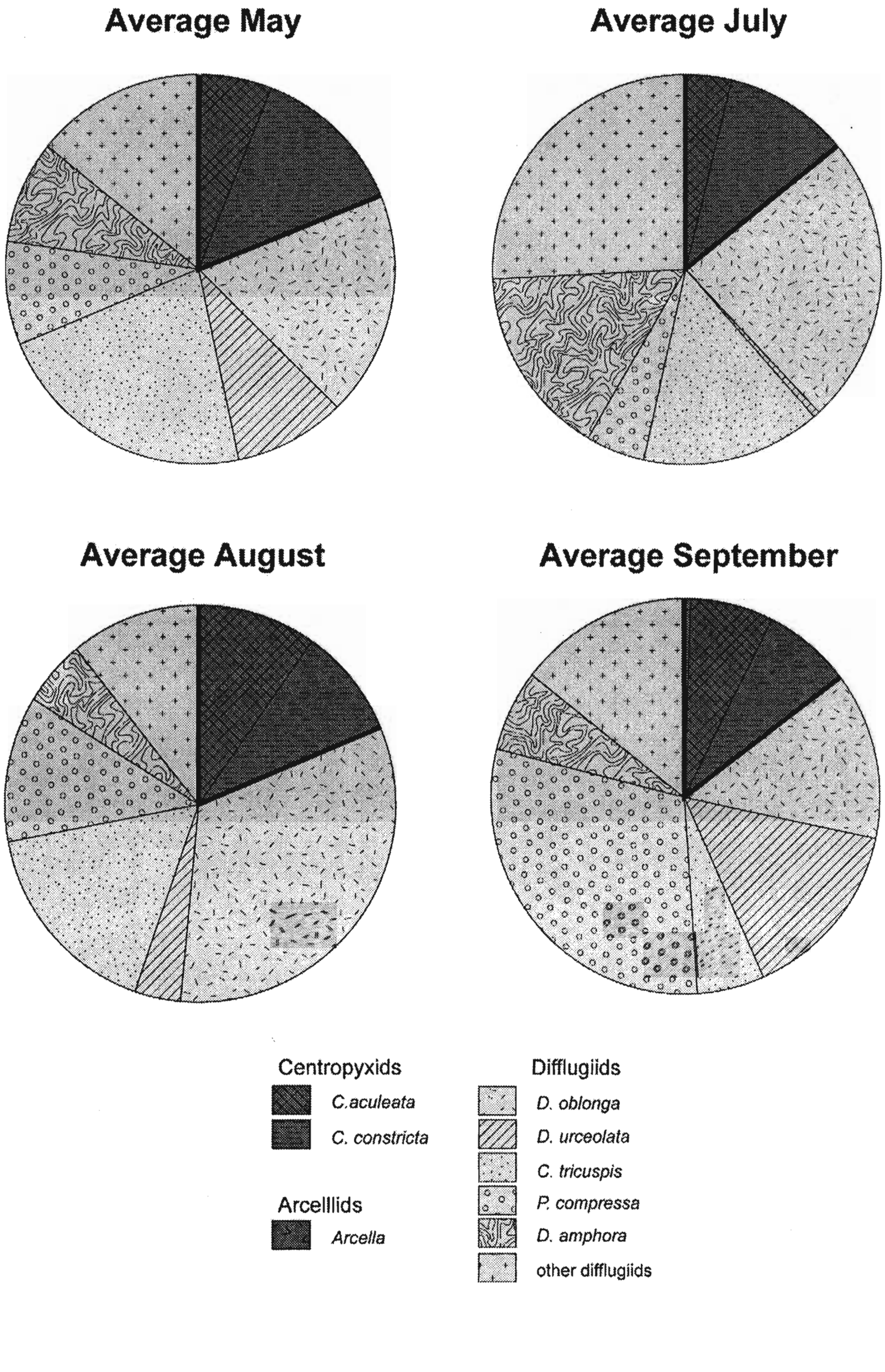
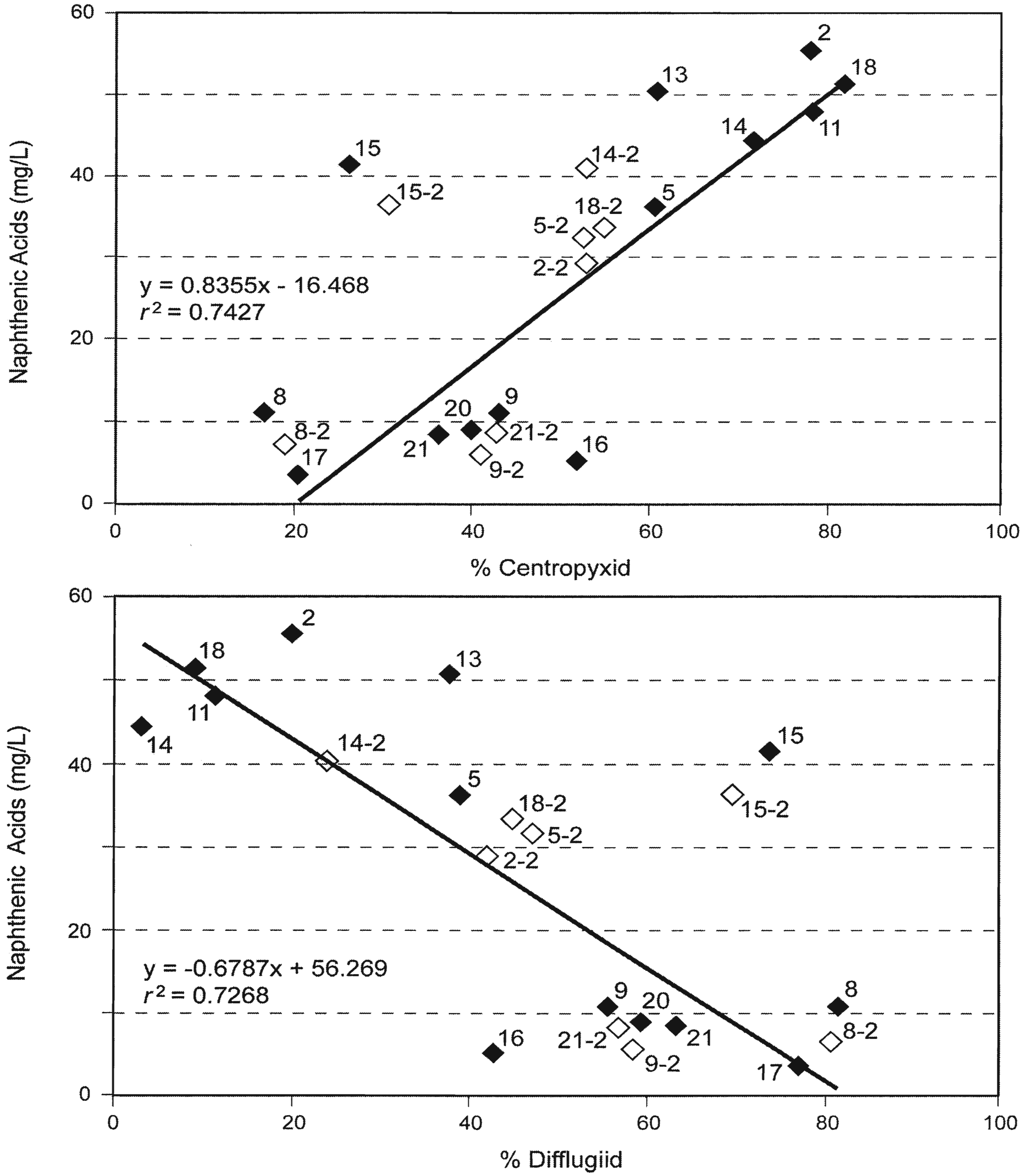
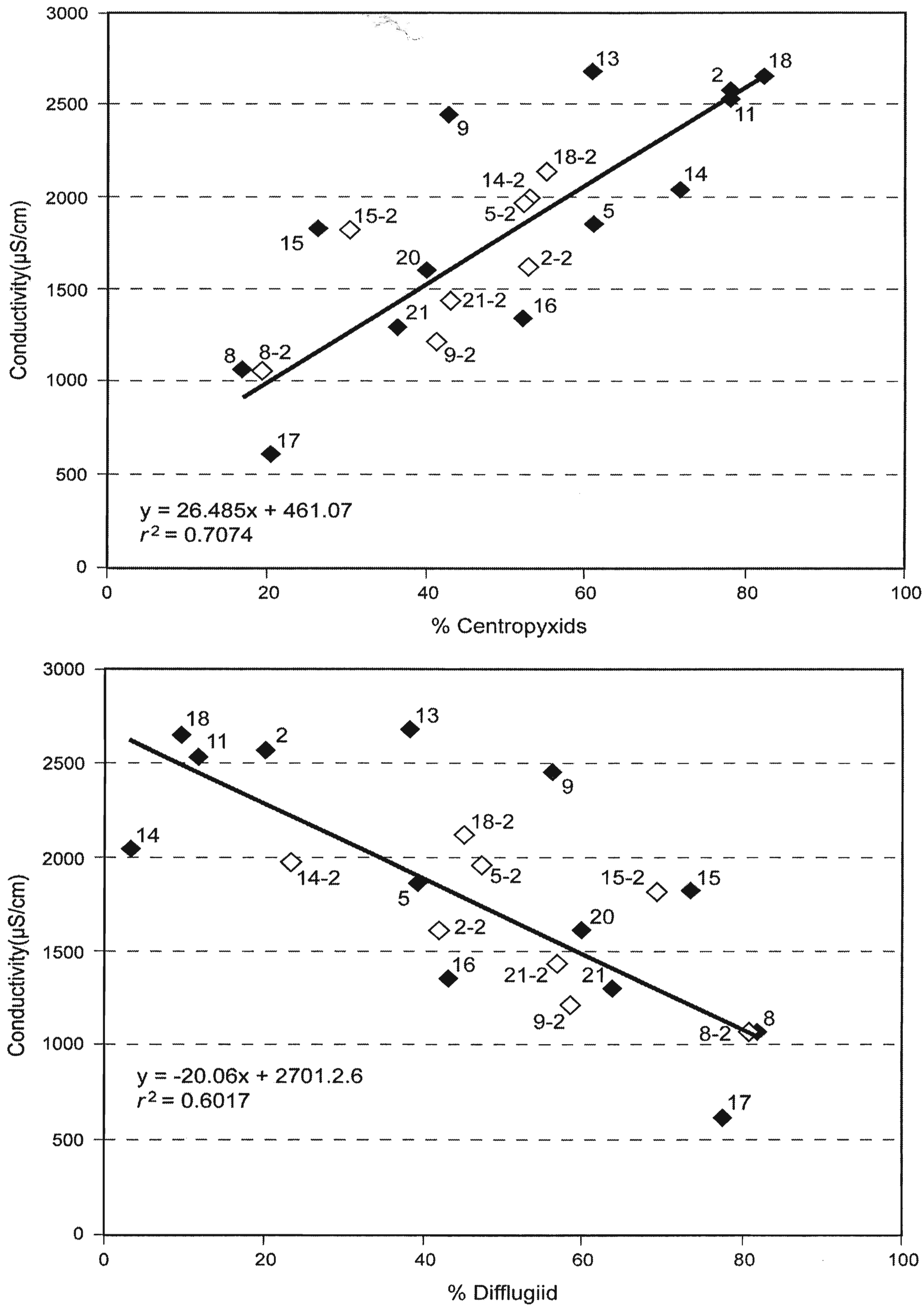

Average May
Average July
Average August
Average September
Centropyxids
C.aculeata
C. constricta
Arcelllids
Arcella
Difflugiids
D. oblonga
D. urceolata
C. tricuspis
P. compressa
D. amphora
other difflugiids

Figure 3.6.



Naphthenic Acids (mg/L)
% Centropyxid
y = 0.8355x - 16.468
r² = 0.7427
2
18
13
11
14
14-2
15
15-2
5
18-2
5-2
2-2
8
8-2
17
20
9
21
21-2
9-2
16
% Difflugiid
y = -0.6787x + 56.269
r² = 0.7268

Figure 3.7.



y = 26.485x + 461.07
r² = 0.7074
Conductivity(µS/cm)
% Centropyxids
y = -20.06x + 2701.2.6
r² = 0.6017
Conductivity(µS/cm)
% Difflugiid

Figure 3.8.

REFERENCES

ALBERTA ENERGY. 2008. Alberta Energy Homepage, www.energy.gov.ab.ca. Last reviewed April 15, 2008.

ALLEN, E. W. 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. Journal of Environmental Engineering Science, 7:123-138.

BERNHARD, J. M. 2000. Distinguishing live from dead foraminifera: methods review and proper applications: Micropaleontology 46: Supplement 1, 38-46.

BERNHARD, J.M., OSTERMANN, D.R., WILLIAMS, D.S., AND J.K. BLANKS. 2006. Comparison of two methods to identify live benthic foraminifera: A test between Rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions. Paleoceanography, 21: 1-8.

BEYENS, L. AND R. MEISTERFELD. 2001. 'Protozoa: Testate amoebae'. p. 121-153. In J.P Smol, H.J.B. Birks, W.M. Last (eds). Tracking Environmental Change Using Lake Sediments. Volume 3: Terrestrial, Algal, and Siliceous Indicators. Kluwer Academic Publishers. The Netherlands.

BOUDREAU, E.A., J.M. GALLOWAY, R.T. PATTERSON, A. KUMAR, F. AND A. MICHEL. 2005. A paleolimnological record of Holocene climate and environmental change in the Temagami region, northeastern Ontario. Journal of Paleolimnology, 33:445- 461.

CARTER, H.J. 1856. Notes on the freshwater Infusoria of the island of Bombay. No. 1.Organization. Annals and Magazine of Natural History, series. 2, 18(105):221-249.

CARTER, H.J. 1864. On freshwater Rhizopoda of England and India. Annals and Magazine of Natural History, series 3, 13:18-39.

COLLINS, E.S., F.M.G. MCCARTHY, F.S. MEDIOLI, D.B. SCOTT, AND C.A. HONIG. 1990. Biogeographic distribution of modern thecamoebians in a transect along the Eastern North American coast. p. 783 – 792. In C. Hemleben and others (Eds). Paleoecology, Biostratigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera Kluwer Academic Publishers. Amsterdam.

DEFLANDRE, G. 1953. Ordres des Testaceolobosa (De Saedeleer, 1934), Testaceofilosa (De Saedeleer, 1934), Thalamia (Haeckel, 1862) ou Thecamoabians (Auct.) (Rhizopoda Testacea), in Grassa, P., ed., Traite de Zoologie, Tome I, fasc.2, p. 97-148, pl 1; Masson Paris.

DUJARDIN, F. 1835. Observations nouvelles sur les pretendus Cephalopodes microscopiques: Annals de Sciences Naturelles, serie 2, 3:312-314.

EHRENBERG, C.G. 1830. Organisation, systematik und geographisches Verhältnis der Infusionsthierchen. Druckerei der Königliche Akademie der Wissenschaften, Berlin.

EHRENBERG, C.G. 1832. Über die Entwicklung und Lebensdauer der Infusionsthiere, nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen, 1831, 1-154.

EHRENBERG, C.G. 1840. Das grössere Infusorienwerke. Königliche Preussischen Akademie der Wissenschaften zu Berlin Bericht, 198-219.

EHRENBERG, C.G. 1843. Verbreitung und Einfluss des mikroskopischen Lebens in Süd-und Nord Amerika. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen, 1841, 291-446.

FTFC (Fine Tailings Fundamentals Consortium). 1995. Advances in Oil Sands Tailings Research, Vol 1-4. Alberta Department of Energy, Oil Sands and Research Division, Edmonton, Alberta.

GOOGLE EARTH. 2009. Athabasca Oil Sands, Suncor Sites. Neville L.A. Google Earth, http://earth.google.com/ (generated March 2009).

HARRIS, M.L. 2007. Guideline for wetland establishment on reclaimed oil sands leases (revised second edition). Prepared by Lorax Environmental for CEMA Wetlands and Aquatics Subgroup of the Reclamation Working Group, Fort McMurray, AB. Feb/07.

HAECKEL, E. H. 1862. Die Radiolarien (Rhizopoda Radiaria), Pt. 1; Berlin, 1-572.

HEMPEL, A. 1898. A list of the Protozoa and Rotifera found in the Illinois River and adjacent lakes at Havana, Ill. Illinois State Laboratory of Natural History Bulletin, 5:301-388.

HOLROYD, P. AND T. SIMIERITSCH. 2009. The Waters That Bind Us: Transboundary Implications of Oil Sands Development. (Ed. L. Fauth, Publ. The Pembina Institute, Drayton Valley, AB). Report. February, 2009. 49pp.

JIVRAJ, M.N., M. MACKINNON, AND B. FUNG, B. 1996. Naphthenic acid extraction and quantitative analysis with FT-IR Spectroscopy. Research Report, Syncrude Canada Ltd. 12pp.

KAUPPILA, T., S. KIHLMAN, AND J. MAKINEN. 2006. Distribution of Arcellaceans (testate amoebae) in the sediments of a mine water impacted bay of Lake Retunen, Finland. Water, Air, and Soil Pollution, 172:337- 358.

KUMAR, A. AND A.P. DALBY. 1998. Identification Key for Holocene Lacustrine Arcellacean (Thecamoebian) Taxa. Paleontological Society.

KUMAR, A. AND R.T. PATTERSON. 2000. Arcellaceans (thecamoebians): new tools for monitoring long- and short-term changes in lake bottom acidity. Environmental Geology, 39:689- 697.

LAMARCK, J.B. 1816. Histoire naturelle des animaux sans vertebres. Verdiere, Paris, 2:1-568.

LEIDY, J. 1874. Notice of some new fresh-water rhizopods. Proceedings of the Academy of Natural Sciences of Philadelphia, series 3:77-79.

LEIDY, J. 1879. Fresh water rhizopods of North America. United States Geological Survey of the Territories Report, 12:1-324.

MASLIYAH, J. 2006. Bitumen Extraction from oil sands: Fundamentals and Processing. Special presentation to Syncrude operators. Department of Chemical and Materials Engineering. University of Alberta. Edmonton, Alberta.

MCCARTHY, F.M.G., E.S. COLLINS, J.H. MCANDREWS, H.A. KERR, D.B. SCOTT, D.B., AND F.S. MEDIOLI. 1995. A comparison of postglacial Arcellacean ("Thecamoebian") and pollen succession in Atlantic Canada, illustrating the potential of arcellaceans for paleoclimatic reconstruction. Journal of Paleontology, 69: 980-993.

MCCARTHY, F.M.G., L.A. NEVILLE, AND M.D. MACKINNON. 2008. Annual variations in the Suncor Experimental Wetlands and seasonal variations in thecamoebian assemblages in Syncrude's BPIT test site: implications for biomonitoring. Report prepared for Syncrude,Canada Ltd., 35 p.

MEDIOLI, F.S. AND D.B. SCOTT. 1983. Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Special Publication No. 21.

MEDIOLI, F.S., D.B. SCOTT, D.B., AND B.H. ABBOTT. 1987. A case study of protozoan interclonal variability: taxonomic implications. Journal of Foraminiferal Research, 17:28-47.

MILNE-EDWARDS, H., J. HAIME. 1850. A monograph of the British fossil corals, Pt. I. - Palaeontograph. Soc. London, I-XXVfl-71.

MIKULA, R. J., K.L. KASPERSKI, R. BURNS, M.D. MACKINNON. 1996. The natural and fate of oil sands fine tailings. Suspensions: Fundamentals and Applications in the Petroleum Industry. L. L. Schramm. Washington, D.C., ACS. 251:677-723.

NEVILLE, L.A., submitted. Investigating the Potential of Thecamoebians (Testate Amoeba) as Bio-indicators of Impact of Oil Sands Mining Operations in Northeastern Alberta, Canada on freshwater environments. MSc thesis, Brock University, St.

Catharines, ON.

NEVILLE, L.A., F.M.G. MCCARTHY, M.D. MACKINNON, AND P. MARLOWE submitted. Using thecamoebians (testate amoebae) as proxies of ecosystem health in constructed wetlands in the oil sands of Alberta, Canada. Manuscript submitted to Environmental Earth Science, April 2009.

NEVILLE, L.A., F.M.G. MCCARTHY, M.D. MACKINNON, P. MARLOWE, AND T. PENNER. 2009. Using thecamoebians (testate amoebae) as proxies of ecosystem health in Oil Sands impacted regions of NE Alberta: *Difflugia* spp. as canaries in the coalmine. *NAMS Microfossils II, Houston TX, March 16-18, 2009.*

NEVILLE, L.A., F.M.G. MCCARTHY, M.D. MACKINNON, AND P. MARLOWE. 2008. Thecamoebians (testate amoebae) as proxies of ecosystem health in the Oil Sands wetlands: reclamation options. Oral presentation, Annual Meeting of the Geological Society of America, Houston, TX.

PATTERSON, R.T., AND E. FISHBEIN. 1989. Re-Examination of the statistical methods used to determine the number of point counts needed for micropaleontological quantitative research. Journal of Paleontology, 63:245- 248.

PATTERSON, R. T., AND A. KUMAR. 2000. Assessment of Arcellacean (Thecamoebian) assemblages, species, and strains as contaminant indicators in James Lake, Northeastern Ontario, Canada. Journal of Foraminiferal Research, 30: 310- 320.

PATTERSON, R. T., AND A. KUMAR. 2002. A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeography, Palaeoclimatology, Palaeoecology, 180:225- 251.

PATTERSON, R.T., K.D. MCKINNON, D.B. SCOTT, AND F.S. MEDIOLI. 1985. Arcellaceans (Thecamoebians) in small lakes of New Brunswick and Nova Scotia: modern distribution and Holocene stratigraphic changes. Journal of Foraminiferal Research, 15:114-137.

PATTERSON, R.T., T. BARKER, AND S.M. BURBIDGE. 1996. Arcellaceans (Thecamoebians) as Proxies of Arsenic and Mercury Contamination in Northeastern Ontario Lakes. Journal of Foraminiferal Research, 26:172-183.

PENARD, E. 1902. Faune Rhizopodique du Bassin du Lèman. Henry Kundig, Geneve.

PERTY, M. 1849. Über vertikale Verbreitung mikroskopischer Lebensformen. Naturforschende Gesellschaft in Bern Mittheilungen, 153-176.

REINHARDT, E.G., LITTLE, M., DONATO, S., FINDLAY, D., KRUEGER, A., CLARK, C. and J. BOYCE. Arcellacean (thecamoebian) evidence of land-use change and eutrophication in Frenchman's Bay, Pickering, Ontario. Environmental Geology, 47: 729 -739.

REINHARDT, E.G., A.P. DALBY, A. KUMAR, AND R.T. PATTERSON. 1998. Arcellaceans as Pollution Indicators in Mine Tailing Contaminated Lakes near Cobalt, Ontario, Canada. Micropaleontology, 44:131-148.

RHUMBLER, L. 1895. Beitrage zur Kenntins der Rhizopoden. (Beitrag III, IV und V): Zeitschrift fur wissenschaftliche Zoologie, 61:38 -110, pls. 4,5.

SAEDELEER, H. DE. 1934. Beitrag zur Kenntnisder Rhizopoden: morphologische und systematische Untersuchungen und ein Klassifikationsversuch: Museum Royal d'Histoire Naturelle Belgique, No. 60, p. 1-112, pls. 1-8.

SCHLUMBERGER, P. 1845. Observations sur quelques nouvelles especes d'Infusoires de la famille des Rhizopodes: Annales des Sciences Naturelles, Zoologie, 3:254-256.

Schramm, L. L., E.N. Stasiuk, M. MacKinnon. 2000. Surfactants in Athabasca Oil Sands Slurry Conditioning, Flotation Recovery, and Tailings Processes. In Surfactants, Fundamentals, and Applications in the Petroleum Industry. (ed. L. L. Schramm). Cambridge, U.K., Cambridge University Press: 365-430.

SCHULZE, F.E. 1877. Rhizopodenstudien VI: Archiv fur Mikroskopische Anatomie, 13: 9-30, pls. 2, 3.

SCOTT, D.B., AND F.S. MEDIOLI. 1980. Living vs. total foraminiferal populations: their relative usefulness in paleoecology. Journal of Paleontology, 4:814-831.

SCOTT, B.D., F.S. MEDIOLI, AND C.T. SCHAFER. 2001. Monitoring in Coastal Environments Using Foraminifera and Thecamoebian Indicators. Cambridge University Press. Cambridge, p. 177.

SHANNON, C.E., AND W. WEAVER. 1949. The Mathematical Theory of Communication: University of Illinois Press, Urbana, 55 p.

STEIN, S.F.N. VON. 1859. Uber die ihm aus eigener Untersuchung bekannt gewordenen Suswasser-Rhizopoden: Konigliche Bohmishce Gesellschaft der Wissenschaften Abhandlungen, ser. 5, v.10, Berichte der Sectionen, p. 41-43.

SYNCRUDE CANADA LTD. 1995. Syncrude Analytical Methods: Updated 2006. Syncrude Edmonton Centre.

SYNCRUDE CANADA LTD. 2003. Syncrude Fact Book. Agecoutay, L. (ed.). Prepared by Dept of Government and Public Affairs, published by Syncrude Canada Ltd., Fort McMurray, AB, 63 p.

WALLICH, G.C. 1864. On the extent, and some of the principal causes, of structural variation among the difflugian rhizopods: Annals and Magazine of Natural History, series 3, 13:215-245.

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 Centropyxidae 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)

Chapter 4

General Discussion and Conclusions

The Oil Sands of Alberta is a classic example of human impact and development. The need to establish a quantifiable mechanism capable of monitoring the progression of aquatic environments undergoing remediation is more important than ever, and would potentially benefit reclamation programs worldwide. Generally information included in a reclamation strategy includes knowledge of baseline conditions and natural variability, identification of the time when conditions in the lake first began to change, and a determination of possible outcomes of remediation (Ford, 1988). The temporal component requires long-term data so that realistic targets for remediation efforts can be set, anthropogenic activity can be discerned and measured, and future scenarios inferred. A core sample study would provide much of this information (Smol, 1992; Patterson and Kumar, 2000).

Companies operating in the Oil Sands require a means of monitoring the progression of an aquatic ecosystem highly impacted by OSPM (oil sands process-affected material) to less impacted, more natural aquatic systems, as part of their legal obligations to the Province of Alberta (Chapter 1, section 1.4). The studies of the impact of eutrophication and sulfide mining on thecamoebian assemblages (Reinhardt et al., 1998; Kumar and Patterson, 2000; Patterson and Kumar, 2000; Kaupilla et al., 2006) led to the proposal to investigate their use as biomonitors, even though no work had previously been published on their sensitivity to the by-products of oil sands extraction. Although relatively little is yet known about their ecology and biogeography (Collins et al., 1990; McCarthy et al., 1995; Charman, 2001; Booth and Zygmunt, 2005), their position in the food web, their rapid generation time, and their high fossilization potential, made thecamoebians optimal candidates as biomonitors.

A preliminary investigation of the use of thecamoebians as proxies of oil sands impact began in oil sands Lease 86 constructed wetland test facility (Figure 1.14), Fort McMurray Alberta, where optimum re-habilitation practices for the creation of ecologically viable sustainable wetland environments are sought by

Suncor Energy Inc. Information available from this facility allows researchers to address the need for further studies required to demonstrate successful reclamation practices (Golder Associates Ltd., 2006). This thesis by no means attempted to fully characterize the ecology of the constructed wetlands, nor did it exhaustively examine the ecology of thecamoebians. Its aim was to determine whether thecamoebian assemblages reflected variations in water quality in different parts of the constructed wetland, both closer to and farther from point sources of oil sands process-affected water.

In August 2007, ~50 ml of sediment from the sediment-water interface and ~50 ml of water were sampled by Suncor employees from the Suncor constructed wetlands. The sediment samples were shipped to Brock and prepared for thecamoebian analysis. At the beginning of the study, in September 2007, a processing and analysis protocol was developed. Processing of sediments for thecamoebian analysis typically follows one of two protocols depending on the type of research. One group of researchers follows the "lacustrine"/ "micropaleontological" method (Scott et al., 2001), while the other group follows the "wetland"/ "biological" method of processing (Hendon and Charman, 1997; Tolonen, 1966 & 1986; Warner, 1990). Both methods produce viable numbers of thecamoebians but unfortunately they do not observe the same species so researchers cannot compare results

Samples of 5-10 cm^3 are wet sieved using the lacustrine method and the >63, 45-63, and 38-45μm size fractions are counted wet at 50X magnification in a gridded Petri dish to ensure sampling without replacement (i.e. no double-counting of specimens). In the wetland method, samples are prepared using 1 cm^3 of peat and one *Lycopodium* tablet (Stockmarr, 1971), mounted on glycerol and counted at x400 magnification using the wetland method and focus is on the size fraction between 15μm and 300μm. Although the facilities exist in the Palynology Laboratory at Brock University to process the samples using the wetland method, the lacustrine method was employed as it appears to be less destructive, and the sulfide mining impact studies (Patterson and Kumar, 2000; Reinhardt et al., 1998) had used this protocol in Canada, allowing us to compare our results with theirs. A student at the

University of Waterloo, Alison Legg used the wetland protocol in a similar parallel study in the Oil Sands region (B. Warner, U. Waterloo, pers. comm.). A comparison study identifying the similarities and differences between the species observed using the different protocols, suggesting ways to compare the data sets. Future research is needed to address the differences that differing protocols and the use of different taxonomies would have on the results of thecamoebian/ testate amoeba studies, but this is beyond the scope of the current thesis.

The lacustrine/ micropaleontological protocol summarized in Scott et al. (2001) was modified slightly during the analysis of samples collected for the "Set One" (August 2007 Suncor dataset) investigation, when it was observed that the proportion of thecamoebians found in the 38 - 45 μm size fraction was insignificant (usually <10%) in comparison to numbers observed in the larger size fractions (Figure 6.1). The 38 - 45μm size fraction was not included in subsequent investigations. The loss of these thecamoebians was offset by the advantages of very rapid processing and the requirement of only 50x magnification to identify the specimens.

Thecamoebian analysis of this dataset, called "Set One" (Chapter 2), suggested a relationship between thecamoebian assemblages and the chemical composition of water in the wetlands (Figure 2.2 & 2.3), which was analyzed by Syncrude Canada Ltd., following their standard protocols (Syncrude, 1995). Some of the original wetlands sampled for the Set One data series (Sites 3, 4, 10, 12, 19, 22, 23) were not included in the evaluation of the data in Chapter 2 because they did not contain sufficient numbers of tests for meaningful comparison (i.e. < 100 tests).

A strong positive correlation was found between the relative abundance of centropyxid thecamoebians (*Centropyxis aculeata* and *Centropyxis constricta*) and concentrations of the common constituents of OSPM, such as napthenic acid concentrations (r^2= 0.772) and conductivity (r^2= 0.6878) (Figure 2.4 & 2.5). These taxa have previously been reported as common in stressed environments (Asioli et al. 1996; Boudreau et al., 2005; Collins et al., 1990; Decloitre, 1956; McCarthy et al., 1995; Patterson and Kumar, 2002; Scott et al., 2001). Conversely, a strong negative correlation exists between oil sands constituents and the relative abundance of difflugiid thecamoebians mainly, *Difflugia oblonga, Difflugia urceolata, Cucurbitella tricuspus and Pontigulasia compressa*. Difflugiid-dominated assemblages were restricted to samples with < 11mg/L naphthenic acids and conductivity values <1500 μS/cm (Figure 2.2). Samples from wetlands with relatively low OSPM character (average naphthenic acid concentration 7.95 mg/L, conductivity 1398.5μS/cm) contained a relatively abundant (N= 137/5cc) and diverse thecamoebian fauna (av. Shannon Diversity Index= 2.00). In thecamoebian studies, 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).

Some thecamoebians (both stained and empty tests) found not only in Set One, but in all samples from the oil sands constructed wetlands and lakes, had incorporated black grains of bitumen into their tests. It was thought that in most cases, the nature of the xenosomes depends on the availability of inorganic particles and not on genome-based selectivity (Medioli and Scott, 1983; Patterson and Kumar, 2002). It is possible that the black particles incorporated in the test of *Cucurbitella tricuspis* may have been incorporated during its planktonic stage (Schonborn, 1984; Medioli et al., 1987), based strictly on the high availability of floating bitumen. However, this does not explain the incorporation of bitumen in the tests of other species. Bitumen grains were mainly observed on the tests of centropyxid species (Plate 4.1), *Difflugia oblonga* and *Pontigulasia compressa.* In most cases it appeared that the grains found on *P. compressa* were selectively placed around the collar of the thecamoebian (Plate 4.1).

Approximately one year later, in June 2008, several of these sites were re-sampled and called "Set Two". The reinvestigation confirmed that the relationship between thecamoebian assemblages and the degree of OSPM impact were robust and reproducible. Although sampled at slightly different locations, and earlier in the summer season, thecamoebian assemblages differed only slightly at sites with little year-to-year change in the concentrations of naphthenic acids and conductivity (Sites 8-2, 9-2, 15-2, 21-2 that receive natural runoff but lie outside the drainage of OSPW) (Figure 3.2). Those sites with more marked differences in thecamoebian assemblage

(Sites 2-2, 5-2, 18-2) were found to have received less OSPW (oil sands process-affected water) due to a deliberate decrease in outflow to the southeastern part of the constructed wetland (OSPW/CTRW in Figure 2.1 (Figure 3.3). This showed that thecamoebians respond quickly (i.e. within 10 months) to variations in OSPW, making them excellent potential biomonitors to assess various reclamation options.

Their potential to gauge the success of reclamation options is most evident in a comparison of the two largest water bodies in the Suncor constructed wetland facility, Sites 14 and 15. At site 15, (Sustainable Lake South), the thecamoebian assemblage in both the August 2007 and 2008 sample sets was more diverse and difflugiid-rich than would be expected from the high conductivity (1820μS/cm) and naphthenic acid (10.7ppm) concentration measured (Figure 3.2). Sustainable Lake South was virtually identical to the adjacent sustainable Lake North (Site 14) (Figure 1.15), and measurements of naphthenic acid concentration and conductivity were very similar (Table 3.1, Figures 3.2 & 3.3). Although these lakes were created at the same time, they have been managed differently, with nutrient loading practiced at Sustainable Lake South but not at Sustainable Lake North. The more diverse, difflugiid-dominated thecamoebian fauna found in both the August 2007 and June 2008 samples from Site 15 suggests that nutrient loading may have sped up the remediation process through higher productivity and resulting detrital deposition rates, even if the site still appears to be highly impacted by OSPM constituents. Future work should examine the potential of nutrient loading in these systems.

A slight modification to the protocol was made when the June 2008 samples ("Set Two") were processed. The samples were stained using Rose Bengal, a common technique in fossil protist research (Scott and Medioli, 1980). Tests stained using this method are generally reported to have been living at the time of collection, but Bernhard (2000) has called this conclusion into question, citing staining of cysts in older sediments. The study proposed a new technique to better distinguish cytoplasm, but it has yet to become standard (Bernhard et al., 2006). The relative abundance of stained specimens vs. empty tests was recorded during analysis of Set Two, bearing in mind the controversy surrounding the interpretation of the data, but since the wetlands were constructed within the last few decades, the assumption of living vs. dead was thought to be valid.

It was noted that the living assemblage (biocoenose) in Set Two differed somewhat from the fossil assemblage (thanatoceonose), although the total assemblage ("living + dead") compared very closely with the 2007 data set ("Set One"). The observed differences between the living and total assemblage may reflect a combination of temporal/ seasonal differences and differential preservation potential. A small separate study was conducted to see how reproducible the fossil thecamoebian assemblage data from various sites within a pond is from month to month. Variations in the living fraction of the assemblage could also provide insights into thecamobian ecology, by aiding our understanding of tolorences.

Sediment samples collected in May through September 2008 by Syncrude employees from the sediment-water interface at several locations within Demo Pond (Figure 1.16) were examined. This large-scale test pond, approximately 4 to 5 acres in size and 2.9 m deep, was constructed in 1993 on the Syncrude Canada Ltd. research study site (UTM 458352E, 6326665N) in northeastern Alberta. Over the five-month period, parameters such as naphthenic acid concentration, conductivity, average temperature, total precipitation and living (stained) fraction of sample, pH, species diversity index (SDI) and percent difflugiid taxa varied slightly (Table 4.1). The slight variability of naphthenic acids concentrations, conductivity and pH can be attributed to natural environmental variability from month to month. Neither SDI nor the relative abundance of difflugiid or centropyxid taxa changed by more than 10% over the course of the study period. The fraction of the sample living (i.e. stained) at the time of collection varied by 35%, with the greatest fraction living during the month of July and August (Table 4.1). As temperature and the amount of precipitation increased from May to August the percentage of the thecamoebian population living also increased (Table 4.1). Additional parameters were investigated but no correlation was apparent (Appendix 3, Section 3.3). The future use of various multivariate techniques to assess the data is recommended.

The dominant centropyxid taxon in May and July was *Centropyxis constricta*, whereas *Centropyxis aculeata* increased in abundance during August and September

(Figure 4.3). *Arcella vulgaris* was present in low numbers in May and increased in numbers in September (Figure 4.3). *Arcella 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). Interestingly, in this study, the increase of *Arcella vulgaris* in September coincides with the highest pH values recorded (8.31) (Table 4.1).

The species comprising the difflugiid population varied slightly throughout the five-month study. It is possible that predation is in part responsible for the variations in the dominant difflugiid species during this study. Certain thecamoebians may be more susceptible to predation due to their test composition (Kumar and Dalby, 1998; Medioli and Scott, 1983). *Difflugia oblonga* remained relatively ubiquitous throughout the study, its living population peaking during the summer months of July and August, then decreasing significantly in September. This suggests that *D. oblonga* can tolerate climate extremes (Collins et al., 1990; McCarthy et al., 1995), but it that it thrives in higher numbers in temperatures above 10^0C. *Difflugia urceolata* was present (stained and empty tests) during both May and September but was virtually absent in July and August. This absence 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 16^0C as were observed in the mid summer months. It also probably has a lower preservation potential, being relatively large, coarsely agglutinated and thin walled. Substantial increases in both *Difflugia urceolata* and *Pontigulasia compressa* occurred in September. Typically *P.compressa* is common in all ponds except those undergoing eutrophication (Collins et al., 1990). This explains its significant increase in total and living numbers during September. *Cucurbitella tricuspis* 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. *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 in September the amount of sunlight was not sufficient to support its planktonic phase.

The thecamoebians grouped together as "other difflugiids" in figure 4.3 are *Lagenodifflugia vas, Difflugia protaeifomis, Difflugia bidens, Diffligia corona, Difflugia bacillaliarum, Difflugia globulus*. The proportion of the species grouped as "others" remains relatively consistent throughout the five-month study period, except for the month of July. An increase in the numbers of others in July is due to an increase in the number of living *D.globulus*. The increase in *D.globulus* appears to be an anomaly because it is typically considered an indicator of cool to cold climates (Collins et al., 1990). In July the dominant thecamoebian was *Difflugia amphora. D. amphora* is not typically found in the fossil records, and the fact that they are generally more abundant as stained than empty tests suggests that they have a reltively low fossilization potential (Figure 4.3). The literature on *D. amphora* suggests that it is typically found in eutrophic environments (Ellison, 1995), consistent with increased presence during summer months.

The variations within the living population as well as the total species variation observed over the summer months suggests that thecamoebians respond rapidly to shifts in environmental parameters. Studies of seasonality using foraminiferal populations (Murray, 1973; Boltovskoy et al., 1976), including the first investigation using living vs. total populations Scott and Medioli, (1980) also indicated a highly variable living foraminiferal population with insignificant changes to the total assemblage. Paleoecology studies using thecamoebians should focus on the species with high preservability, however finding a thecamoebian with low preservation potential in the fossil record would be an indicator of a very specific environment.

A common question when the preliminary results of thecamoebian analysis of oil sands constructed wetlands were presented was "How do the thecamoebian assemblages in the oil sands compare with natural populations in Alberta?" Few thecamoebian studies have been done in Alberta to date (Booth and Zygmunt, 2005), and none using the micropaleontological protocol.Field work was thus conducted in July 2008 to collect samples from natural sites, on the assumption that a better understanding of their geographic distribution and of the environmental parameters influencing these common protists would provide a natural baseline to compare the data from the Suncor wetlands. The *Atlas of Alberta Lakes* (Crosby et al., 1990) was useful in choosing 15 sites from a variety of geographical settings representing a range of environmental parameters, including precipitation, temperature, evaporation, water budget, elevation, bedrock geology, surficial sediment and trophic status (Figure 4.4, Table 1.6). The distribution of the lakes studied ranged northeast-southwest from Gregoire Lake (56° 28.787 N, 111° 11.264W) to the Spray Lakes Reservoir (51° 01.249N, 115°23.9414W).Thecamoebian data were compared with limnological and chemical data to investigate the parameters that control thecamoebian distribution in natural aquatic systems in the province of Alberta. Thecamoebian assemblages in these natural lakes not impacted by oil sands mining and processing are compared with assemblages found in constructed lakes and wetlands on oil sands leases near Fort McMurray (Chapter 2, 3).

Sediment samples were collected from fifteen locations across the province of Alberta, from four different vegetation zones (Rocky Mountain, Boreal Forest, Boreal Parkland, Grassland; Dyke et al. 2004) and five different drainage basins (Athabasca River, North Saskatchewan River, Battle River, Red Deer River, and Bow River basins) (Table 1.6). Approximately 100 mL of sediment and water were collected over a nine-day period in July 2008, using an Ekman grab sampler. Temperature, total dissolved solids (TDS) and dissolved oxygen (DO) were recorded at the time of sampling using a Hydro Lab (Tables 4.2 & 4.3). To avoid complications introduced by lake stratification (Cole, 1979), all lakes were sampled within the first two meters of the shoreline. The sediment samples were analyzed for thecamoebians at Brock University using standard protocols reported in Chapter 3, while water samples were transported to Syncrude Canada Ltd. Edmonton Research facility for water analysis (conductivity, major ions, trace metals and naphthenic acids) using their standard protocols (Syncrude, 1995).

For statistical analysis, the absolute number of specimens generally examined varies between 100 and 1,000 per sample (Patterson and Fishbein, 1989). When the number of thecamoebians counted in the 5cc subsample did not reach 100, an additional 5cc subsample was processed and counted. Twelve of the lake samples (Table 1.6) contained sufficient numbers of thecamoebian tests to allow for meaningful comparisons of assemblages. Species diversity was calculated on the data at the strain level of identification using the Shannon-Weaver Diversity index (SDI) (Table 4.2)(Shannon and Weaver, 1949). No correlation appeared to exist among the stained and unstained tests within vegetation zones (Table 4.3)

Various strains of *Difflugia oblonga*, *Centropyxis constricta* and *Centropyxis aculeatea, Cucurbitella tricuspis* dominated the fauna in all of the natural lakes studied (Figure 4.5). *D. oblonga* can thrive in almost any climate and can tolerate climate extremes including extreme cold as long as the sediment is sufficiently organic (Collins et al., 1990; McCarthy et al., 1995). *Centropyxis constricta* and *Centropyxis aculeata* are the dominant species in many modern Artic lakes (Collins et al., 1990); they are considered to be opportunistic (Boudreau et al., 2005), tolerant of harsh environmental conditions including cold temperatures, marginally brackish waters and low nutrient availability (Collins et al., 1990; McCarthy et al., 1995). As an interesting aside the Athabasca Glacier melt water was also sampled. The melt water sample contained far too few thecamoebian specimens to be included in this study, but some thecamoebian species were observed: *Difflugia oblonga*, *Difflugia urceolata, Centropyxis aculeata, Centropyxis constricta, Difflugia bacillaliarum* and *Difflugia amphora.*

Species diversity was low throughout the transect, with SDI ranging from 1.25 to 2.07. Climate, as reflected in the vegetation zones, appears to exert the greatest control on species and strain distributions, although multiple sites sampled within one lake (Miquelon Lake) at different times yielded substantial differences. Low diversity assemblages strongly dominated by *Centropyxis aculeata* and *Centropyxis constricta* characterize both lakes in the Rocky Mountain region. Booth and Zygmunt (2005) also reported low thecamoebian species diversity from the Rocky Mountain region, but due to different sample protocol we cannot make a direct species comparison. Slightly more diverse assemblages dominated by *Difflugia oblonga* and *Cucurbitella tricuspis* characterize both lakes in the Grassland region. The Grassland contained the lowest proportion of centropyxids and the highest proportions of *C. tricuspis, D. urceolata*, and *D. bidens. D. urceolata* and *D. bidens* occur in environments with increased sediment input and sediment with high percentages of organic matter

(Patterson et al., 1996) and *C. tricuspis* is an indicator of eutrophication (Boudreau et al., 2005). The highest thecamoebian diversity was found in the Boreal Forest and Boreal Parkland zones. The Boreal Forest is dominated by *Difflugia oblonga* together with *Centropyxis constricta*, *Cucurbitella tricuspis*, and *Centropyxis aculeata*, while the Boreal Parkland is dominated by *Difflugia oblonga* together with *Centropyxis constricta* and *Centropyxis aculeata*. The Boreal Parkland contained the highest proportion of *Arcella vulgaris* possibly related to the high conductivity lakes found in this region. *Arcella vulgaris* is typically considered an indicator of extremely unfavorable environmental conditions. It has been found in low pH, high chemical content environments (Boudreau et al., 2005; Patterson and Kumar, 2000), as well as industrially impacted environments contaminated with Ag, Hg (Patterson et al., 1996) and other oil sands process affected material (Chapter 2, 3). Chemical analysis of water samples was performed by staff at Syncrude Canada Ltd. following their standard protocol (Syncrude, 1995). Lake chemistry (e.g. hardness and concentrations of Na^+, K^+) appears to exert an important secondary distribution controlling factor (Table 4.4). *Centropyxis constricta* and *Centropyxis aculeata* co-dominate oligotrophic hard water lakes while *Cucurbitella tricuspis* is abundant only in eutrophic lakes. Mesotrophic soft water lakes have the most diverse assemblages. Conductivity, DO at the sediment/water interface, water temperature, and pH had surprisingly little influence on the composition and diversity of thecamoebian assemblages. This quick study demonstrated that the assemblages in the Suncor constructed wetlands are similar to those found in Alberta, particularly in the Boreal Forest region (Figures 2.2, 2.3 & 4.5), and that diversity in the constructed wetlands generally exceeds that in the natural lakes. The ecological factors that were predicted to be important controls on thecamoebian biogeography typically appeared to exert little control on distributions in the natural lakes sampled in Alberta. Further research is required in order to better understand thecamoebian biogeography and ecology.

The studies conducted indicated that thecamoebians are highly sensitive to by-products of the oil sands mining operation. Grouping the thecamoebians as difflugiids or centropyxids is a quick, simple, and inexpensive means of obtaining a general understanding of the health of a given ecosystem at the time of sampling. Although the potential of thecamoebians as environmental indicators has been demonstrated, further work should increase the applicability of thecamoebians in addressing this and a wider range of environmental issues. Additional future research should include describing the value of incorporating bitumen in the tests of thecamoebians, and the investigation of cores from wetlands influenced by Oil Sands activity undergoing reclamation. To increase the usefulness of thecamoebians as bioindicators of ecosystem health, the annual study/investigation conducted as part of this project will have to be maintained.

4.1. Tables

Table 4.1: Average of data collected in May, July, August and September from the Syncrude Big Pit.

Date (2008)	Naphthenic Acids (mg/L)	Conductivity (µS/cm)	pH	Average Monthly Temperature (C^0)	Total Monthly Precipitation (mm)	Thecamoebian Species Diversity (SDI)	% Difflugiid	% living
May	-	1825	7.8	10.1	7.8	2.22	80.6	43.3
July	37.5	2266	7.92	16.2	56.6	2.32	85.9	76.0
August	26	2140	8.25	15.9	137.6	2.03	74.6	71.0
September	34	1850	8.31	9.8	27.4	2.21	84.1	32.8
Average	*26*	*12*	*2*	*26*	*75*	*8*	*9*	*35*

Table 4.2: Summary of limnological and micropaleontological data from the study sites in Alberta investigated for the thecamoebian biogeograhpic distribution study, grouped into vegetation zones.

	pH	Lake Temp (C^0)	Cond. (µS/cm)	DO sed/ water interface (mg C/L)	Mean Annual Precip. (mm)	Mean Annual Evap. (mm)	Mean Temp (C^0)	Total # tests (N)	# of Species (S)	SDI
Rocky Mtn:.										
Spray Lakes Reservoir	7.69	18.6	285	7.6	622	621	7.5	106	5	1.25
Jasper	7.59	18.4	420	6.6	620	620	8	103	4	1.26
Boreal Forest:										
Baptiste L.	8.06	22.1	340	12.7	493	638	12	130	7	1.61
Island L.	7.32	24.0	459	3.9	539	638	12	178	8	1.68
Gregoire L.	7.23	24.1	147	7.2	504	580	12	109	7	1.70
Lac St. Anne	7.81	16.6	343	8.5	549	642	12	183	7	1.61
Wabamun L.	8.06	23.2	570	9.8	534	642	12	454	11	2.07
B. Parkland:										
Islet L.	7.50	21.5	318	2.8	423	660	14	139	6	1.62
Buffalo L.	8.75	24.2	2350	10	413	665	13	124	7	1.80
Miquelon L.	9.32	24.2	2690	5.7	466	664	13	314	8	1.57
Grassland:										
Chestermere L.	8.23	20.6	400	3.2	416	712	12.5	117	6	1.46
Eagle L.	9.09	21.8	1548	8	376	712	12.5	125	9	1.66

Table 4.3: The total number of tests (and number of stained tests, interpreted as indicating the presence of cytoplasm in the test at the time of collection) and relative abundance of common thecamoebian species for each vegetation zone.

	Rocky Mountain		**Boreal Forest**		**Boreal Parkland**		**Grassland**	
	Total # of tests (# stained)	% of fauna stained	Total # of tests (# stained)	% of fauna stained	Total # of tests (# stained)	% of fauna stained	Total # of tests (# stained)	% of fauna stained
A. vulgaris	0 (0)	0	11 (0)	0	46 (3)	7	0 (0)	0
C. aculeata	79 (17)	22	195 (36)	16	227 (24)	11	2 (0)	0
C. constricta	76 (15)	20	324 (71)	20	242 (43)	18	6 (0)	0
D. oblonga	41 (4)	10	507 (86)	12	551 (105)	19	109 (15)	14
D. urceolata	0 (0)	0	49 (5)	9	3 (0)	0	13 (0)	0
C. tricuspis	4 (0)	0	209 (50)	24	49 (33)	67	89 (40)	45
D. bidens	0 (0)	0	12 (0)	0	8 (0)	0	6 (3)	50
P. compressa	0 (0)	0	26 (0)	0	0 (0)	0	10 (0)	0
Other difflugiids	0 (0)	0	5 (0)	0	12 (2)	0	7 (0)	0

Table 4.4: The results of chemical analysis of water at each site from Alberta included in the biogeographic distribution study of thecamoebians, ranked in descending order of diversity (SDI).

	$(Ca+Mg)/(CO_3+HCO_3)$	$Na/(Ca+Mg)$	Na	K	Mg	Ca	Cl	SO_4	CO_3	HCO_3	Ratio Cat:An	NH_4 ppm	S
Wabamun Lake	0.66	1.25	78.3	10.5	20.5	20.2	13	85.4	0	251	1.02	<0.01	29.0
Buffalo Lake	0.32	3.48	486	38.5	66	11.4	20	402	117	915	1.01	0.39	132
Gregoire Lake	1.06	0.13	3.9	1.0	5.0	17.9	2.1	7.2	0	75.6	1.04	<0.01	2.6
Island Lake	0.62	0.61	43.6	10.2	21.8	25.8	8.9	5.9	0	304	0.89	3.23	2.7
Eagle Lake	0.65	2.09	269	16.2	55.9	18.6	52	348	83	355	1.02	<0.01	115
Islet Lake	0.91	0.18	11.6	12.7	19.7	22.6	6.0	8.7	0	186	1.06	0.10	2.9
Baptiste Lake	0.73	0.60	32.1	5.1	13.1	25.0	4.0	15.2	0	196	1.06	0.17	5.5
Lac St. Anne	0.76	0.54	30.3	11.1	12.5	27.9	7.7	15.1	0	196	1.08	-	5.2
Miquelon Lake	3.62	1.07	356	62.8	145	48.7	31	1240	68	106	1.03	0.19	1700
Chestermere L.	1.34	0.33	24.8	1.0	17.4	36.5	9.7	70.1	0	149	1.05	<0.01	24.8
Jasper	1.48	0.05	4.6	0.5	18.8	53.5	6.7	69.8	0	175	0.99	<0.01	23.4
Spray Lakes Reservoir	1.23	0.03	2.0	1.0	10.5	41.2	0.9	37.8	0	145	0.96	0.11	12.6

4.2. Figures

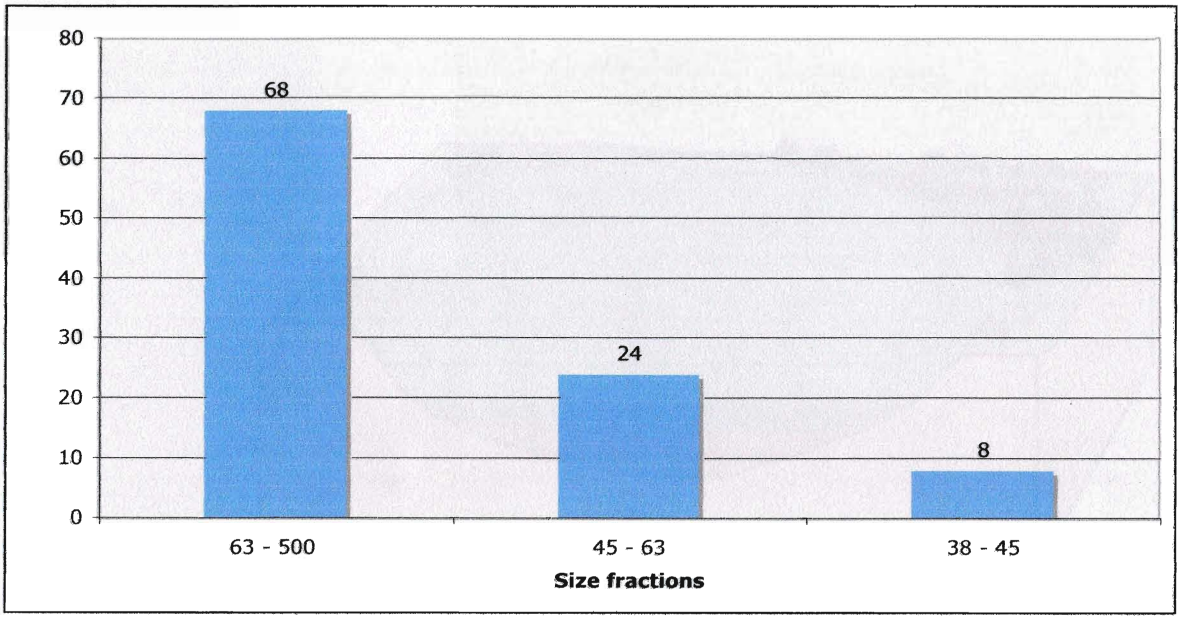


Figure 4.1: Percentage of tests observed in each size fraction averaged for all the sites collected as part of Set One (August 2007, Suncor constructed wetlands).

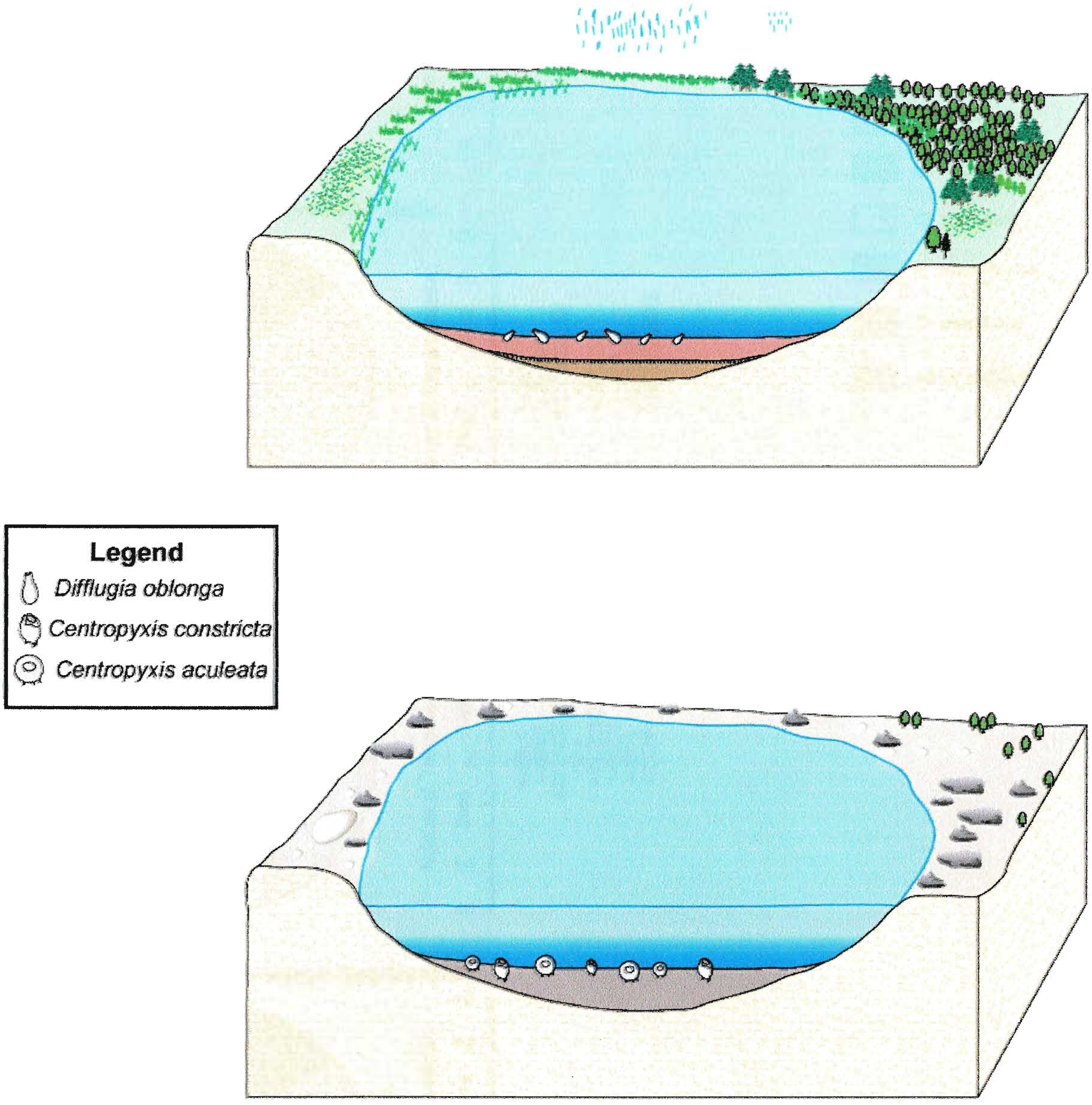


Figure 4.2: Diagram showing the thecamoebian populations found in varying types of Oil Sands impacted environments. The healthier environment (top) less impacted by OSPM is composed of mainly difflugids while the more highly impacted environment (bottom) contains mainly centropyxids.

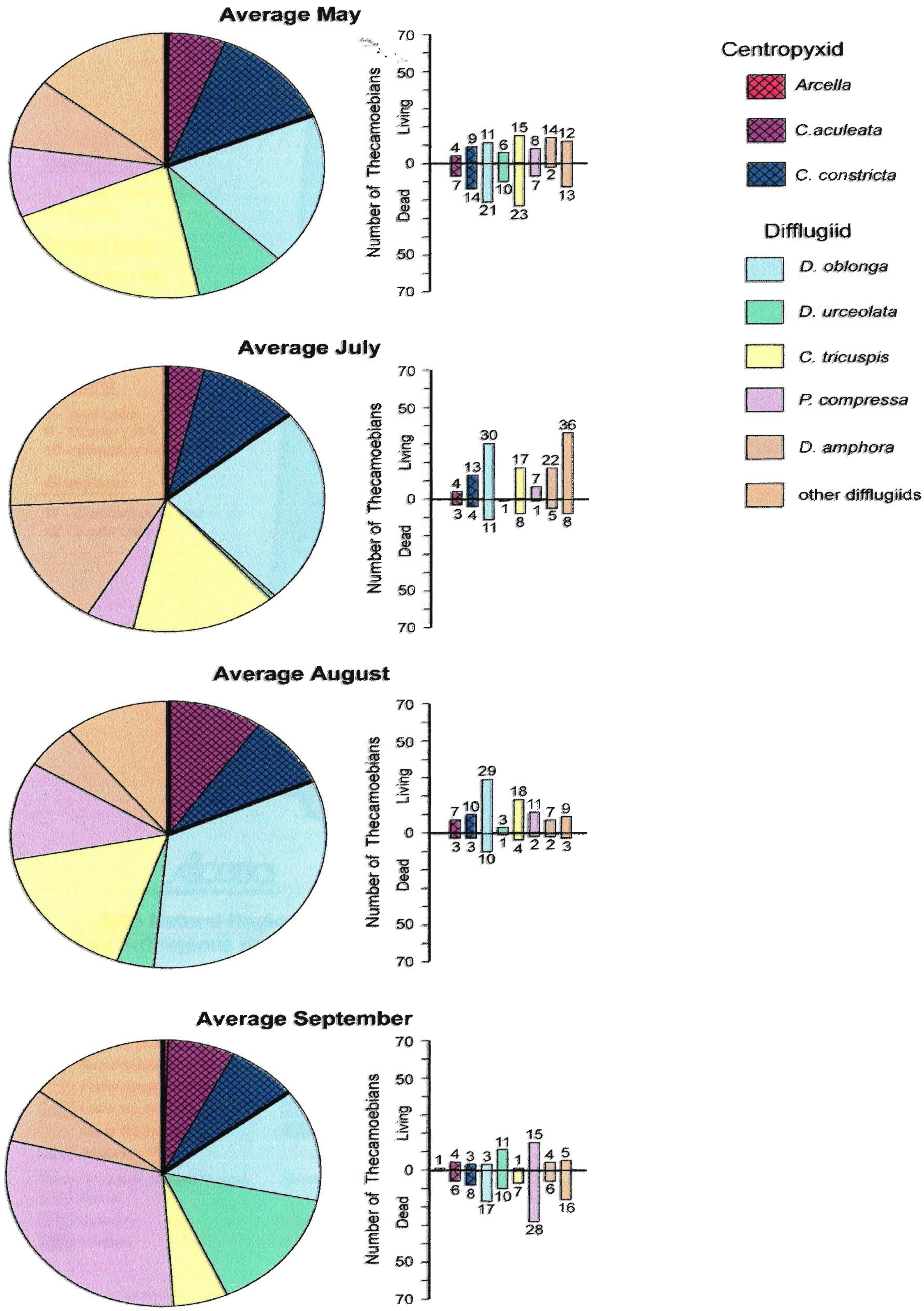


Figure 4.3: Thecamoebian population composition for May, July, August and September 2008, from the Syncrude Big Pit. Associated bar graphs represent stained versus unstained tests for each month.

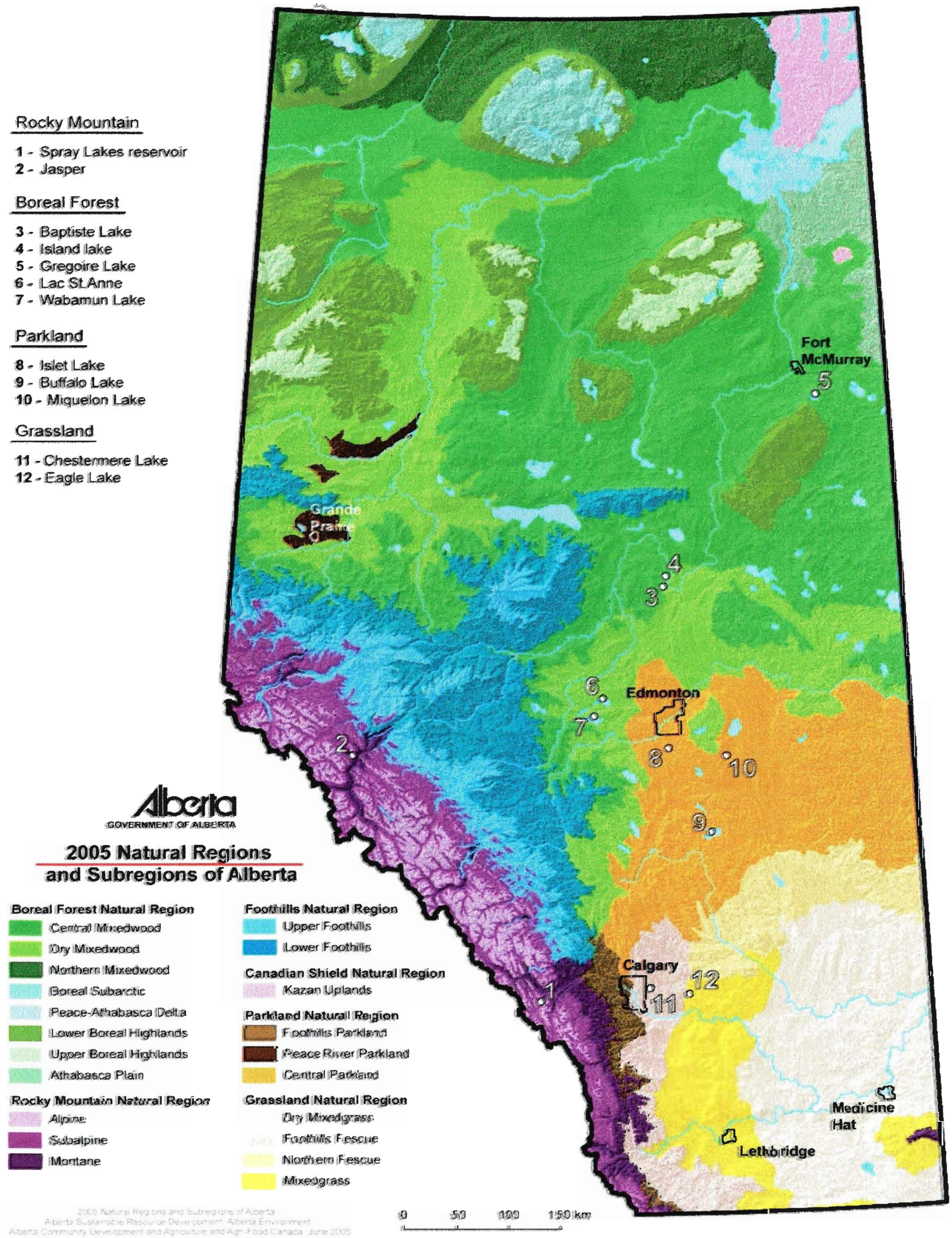


Figure 4.4: Vegetation map of Alberta indicating the location of the lakes studied in the biogeographic distribution of thecamoebian study (Government of Alberta, 2005)

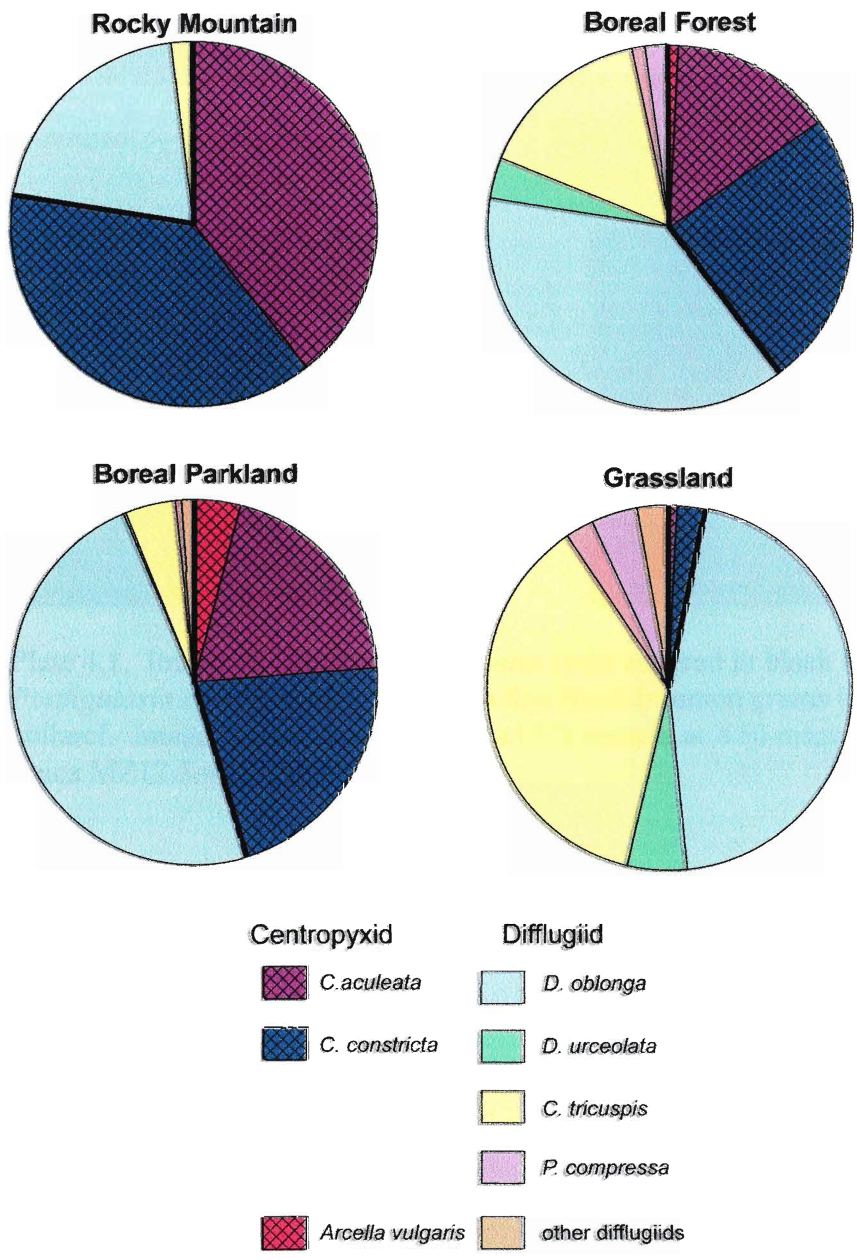


Fig. 4.5: Summary of the thecamoebian population found in each vegetation zone in Alberta, based on samples collected in July 2008 from the natural lakes.

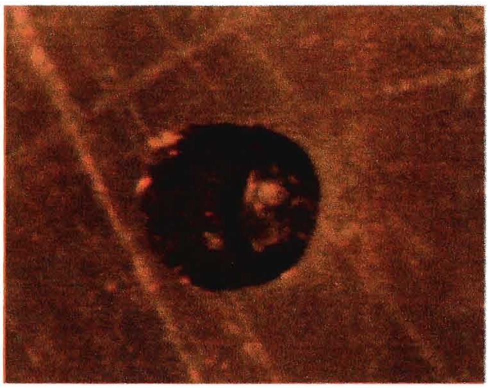
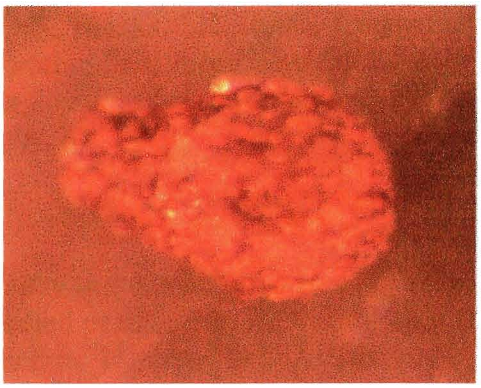
 

Plate 4.1. Image of *Centropyxis aculeata* (left) covered in black bitumen grains and *Pontigulasia compressa* (right) with a few black bitumen grains located around the collarcf. Image captured using a Leica EC3 camera at X50 magnification using a Leica MZ12.5 microscope.

4.6. References

Asioli, A., Medioli, F.S., Patterson, R.T. 1996. Thecamoebians as a tool for reconstruction on paleoenvironments in some Italian lakes in the foothills of the southern Alps (Orta Varese and Candia). Journal of Foraminiferal Research, 26: 248-263.

Bernhard, J. M. 2000. Distinguishing live from dead foraminifera: methods review and proper applications: Micropaleontology 46: Supplement 1, 38-46.

Bernhard, J.M., Ostermann, D.R., Williams, D.S., Blanks, J.K. 2006. Comparison of two methods to identify live benthic foraminifera: A test between Rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions. Paleoceanography, 21: 1-8.

Boltovskoy, E., Wright, R. 1976. Recent Foraminifera. The Hague, p. 515.

Booth, R. K., Zygmunt, J.R. 2005. Biogeography and comparative ecology of testate amoebae inhabiting Sphagnum- dominated peatlands in the Great Lakes and Rocky Mountain regions of North America. Diversity and Distributions, 11: 577- 590.

Boudreau, E.A., Galloway, J.M., Patterson, R.T., Kumar, A., Michel, F.A. 2005. A paleolimnological record of Holocene climate and environmental change in the Temagami region, northeastern Ontario. Journal of Paleolimnology, 33: 445- 461.

Charman, D.J. 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. Quaternary Science Reviews, 20: 1753-1764.

Cole, G.A. 1979. Texbook of Limnology. The C.V. Mosbe Company. St. Louis, p. 426.

Collins, E.S., McCarthy, F.M.G., Medioli, F.S., Scott, D.B., Honig, C.A. 1990. Biogeographic distribution of modern thecamoebians in a transect along the Eastern North American coast. p. 783 – 792. In C. Hemleben and others (Eds). Paleoecology, Biostratigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera Kluwer Academic Publishers. Amsterdam.

Crosby, J.M., Bradford, M.E., Mitchell, P.A., Prepas, E.E., McIntyre, L.G., Hart Buckland-Nicks, L., Hanson, J.M. 1990. Atlas of Albert Lakes. Mitchell, P., Prepas, E. (Eds). The University of Alberta Press. Edmonton, Alberta.

Decloltre, L. 1953. Les thecamoebians de l'Eqe (Groenland). Expeditions Polaires Francaises Missions Paul-Emile Victor VIII. Actualites Scientifiques et Industielles 1242, 100.

Dyke, A.S., Giroux, D., Robertson, L. 2004. Vegetation History, Glaciated North America. Geological Survey of Canada, Open File.

Ellison, R.L. 1995. Paleolimnological analysis of Ullswater using testate amoebae. Journal of Paleolimnology, 13: 51-63.

Ford, J. 1988. The effects of chemical stress on aquatic species composition and community structure, in: Ecotoxicology: Problems and Approaches (Lewin, S.A., Harwell, M.A., Kelly, J.R., Kimball, K.D. eds.), Springer-Verlag, New York, pp. 99-144.

Golder Associates Ltd. 2006. Consolidated Tailings (CT) Integrated reclamation landscape project technical report #7. Prepared for, Suncor Energy Inc., Oil Sands.

Hendon, D., Charman, D.J. 1997. The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. The Holocene, 7: 199- 205.

Kauppila, T., Kihlman, S., Makinen, J. 2006. Distribution of Arcellaceans (tesate amoebae) in the sediments of a mine water impacted bay of lake Retunen, Finland. Water, Air, and Soil Pollution, 172: 337- 358.

Kumar, A., Dalby, A.P. 1998. Identification Key for Holocene Lacustrine Arcellacean (Thecamoebian) Taxa. Paleontological Society.

Kumar, A., Patterson, R.T. 2000. Arcellaceans (thecamoebians): new tools for monitoring long- and short-term changes in lake bottom acidity. Environmental Geology, 39: 689- 697.

McCarthy, F.M.G., Collins, E.S., McAndrews, J.H., Kerr, H.A., Scott, D.B., Medioli, F.S. 1995. A Comparison of Postglacial Arcellacean ("Thecamoebian") and Pollen Succession in Atlantic Canada, Illustrating the Potential of Arcellaceans for Paleoclimatic Reconstruction. 1995. Journal of Paleontology, 69: 980-993.

Medioli, F.S., Scott, D.B. 1983. Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Special Publication No. 21.

Medioli, F. S., Scott, D. B., Abbott, B. H., 1987, A case study of protozoan interclonal variability: taxonomic implications: Journal of Foraminiferal Research, v. 17, p. 28– 47.

Murray, J.W. 1973. Distribution and Ecology of Living Benthic Foraminiferids. Crane, Russak, New York, p. 274.

Patterson, R.T., Fishbein, E. 1989. Re-Examination of the statistical methods used to determine the number of point counts needed for micropaleontological quantitative research. Journal of Paleontology, 63: 245- 248.

Patterson, R.T., Barker, T., Burbidge, S.M. 1996. Arcellaceans (Thecamoebians) as Proxies of Arsenic and Mercury Contamination in Northeastern Ontario Lakes. Journal of Foraminiferal Research, 26: 172-183.

Patterson, R.T., Kumar, A. 2000. Arcellaceans (thecamoebians): new tools for monitoring long- and short-term changes in lake bottom acidity. Environmental Geology, 39: 689- 697.

Patterson, R.T., Kumar, A. 2000. Use of Arcellacea (Thecamoebians) to Gauge Levels of Contamination and Remediation in Industrially Pollutes Lakes. p. 257- 278. In Martin, R.E. (Eds). Environmental Micropaleontology, The Application of Microfossils to Environmental Geology, Kluwer Academic/ Plenum Publishers, New York, Boston, Dordrecht, London, Moscow.

Patterson, R. T., Kumar, A. 2002. A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeograhpy, Palaeecolimatology, Palaeoecology, 180: 225- 251.

Reinhardt, E.G., Dalby, P.A., Kumar, A., Patterson, T. 1998. Arcellaceans as Pollution Indicators in Mine Tailing Contaminated Lakes near Cabalt, Ontario, Canada. Micropaleontology, 44: 131-148.

Schonborn, W. 1996. Beschalte Amnben (Testaceae). Wittenberg, Lutherstadt.

Schonborn, W. S., 1984, Studies on remains of Testacea in cores of the Great Woryty Lake (NE-Poland): Limnologica (Berlin), 16:185–190.

Scott, D.B., Medioli, F.S. 1980. Living vs. Total Foraminiferal Populations: Their Relative Usefulness in Paleoecology. Journal of Paleontology, 4: 814-831.

Scott, B.D., Medioli, F.S., Schafer, C.T. 2001. Monitoring in coastal environments using foraminifera and thecamoebian indicators. Cambridge University Press. Cambridge.

Shannon, C.E., Weaver, W. 1949. The Mathematical Theory of Communication: University of Illinois Press, Urbana, 55 p.

Smol, J.P. 1992. Paleolimnology: an important tool for effective ecosystem management. Journal of Aquatic Ecosystem Health, 1: 49-58.

Stockmarr, J. 1971: Tablets with spores used in absolute pollen analysis. Pollen et

Spores, 13: 615-21.

Syncrude Canada Ltd. 1995. Syncrude Analytical Methods: Updated 2006. Syncrude Edmonton Centre.

Tolonen, K. 1966: Stratigraphic and rhizopod analyses on an old raised bog, Varrussuo, in Hollola, South Finland. Annales Botanica Fennici, 3: 147-66.

Tolonen, K.1986: Rhizopod analysis. In Berglund, B.E., editor, Handbook of Holocene palaeoecology and palaeohydrology. Chichester: John Wiley, 12: 645-66.

Warner, B.G. 1990. Testate Amoebae. In: Warner, B.G (ed.). Methods in Quaternary Ecology. Pg 65 – 74.

Appendix 1

1.1. Ecological preferences and tolerances of main thecamoebian species, as reported in the literature.

Arcella vulgaris

- first appearance in a system indicates a possible drop in pH of the lake waters (Patterson and Kumar, 2000)
- low pH conditions with high nutrient (chemical) content (Boudreau et al., 2005)
- elevated metal concentrations and low pH (2- 5.5) and absent in pH of 6.5 – 7.5, pH was found to be more of a controlling factor then Fe and Al (Kumar and Patterson, 2000)
- found in sediment moderately to heavily contaminiated by Ag and Hg (Patterson et al. 1996).
- Can thrive in brackish, shallow pools (Loeblich and Tappan, 1953)

Centropyxis sp.

- appear to be opportunists tolerant of harsh environmental conditions including cold temperature, marginally brackish water, and low nutrient availability (Collins et al., 1990)
- found in oligotrophic perglacial lakes, immediately after deglaciation (Scott et al., 2001)
- are also adapted to eutrophic conditions (Scott et al., 2001)
- are usually dominate in coastal lakes occasionally affected by salt spray (Scott et al., 2001)
- can be found in pH as low as 5.5 (Patterson and Kumar, 2002)

Centropyxis aculeata

- are not restricted to but are the dominant species in many modern Arctic lakes (Collins et al. 1990)
- opportunistic (Boudreau et al., 2005)
- indicates a stressed environment (Asioli et al. 1996)
- ubiquitous (Collins et al., 1990)
- tolerate oligotrophic conditions and appear to tolerate climate extremes, and are often present in high numbers where other species more sensitive to climate conditions can't thrive (Collins et al., 1990)
- found this species relatively easy to culture in bacteria-rich pond water devoid of algae, suggesting that these organisms are bacteriophages – sediment used was low in organic matter (McCarthy et al., 1995)
- often dominate heavy metal contaminated sites (Reinhardt et al., 1998)
- can survive in sites heavily contaminated by mercury and arsenic (Patterson and Kumar, 2000)

Centropyxis aerophila
- an oligotrophic indicator in late glacial sediments (McCarthy, 1984)

Centropyxis constricta
- ubiquitous (Collins et al., 1990)
- tolerate oligotrophic conditions and appear to tolerate climate extremes, and are often present in high numbers where other species more sensitive to climate conditions can't thrive (Collins et al., 1990)
- common in polar regions, therefore tolerate low temperatures (Collins et al., 1990; McCarthy, 1984)

Cucurbitella tricuspis
- found in low pH conditions with high nutrient (chemical) content, can be an indicator of eutrophication (found in high levels of alga pediastrum (Boudreau et al., 2005)
- has a parasitic relationship with Spirogyra which was its food source and suggested that the same relationship may exist with other aquatic plants (Medioli and Scott,1987; Collins et al. 1990)
- has also been correlated to high concentrations of dissolved oxygen, nutrients and phytoplankton (Scott and Medioli 1983)
- has a plankonic stage in its life cycle, hence requires food in the water column as well as on the lake bottom (Patterson et al., 1985).

Difflugia amphora
- found in eutrophic waters (Ellison, 1995)

Difflugia bacillifera
- requires warm water and organic conditions to thrive (thermophilous) (Collins et al., 1990; McCarthy et al., 1995)
- The frequent incorporation of diatom frustules into the tests may indicate that this species selectively agglutinates diatom frustules if available (McCarthy, 1984)

Difflugia bidens
- its presence is related to increased sediment input, requires little vegetation and higher clastic input (Patterson et al., 1985). This species tends to agglutinate small xenosomes, so the presence of fine grains material is most likely a controlling factor (Scott et al., 2001).

Difflugia corona
- tolerate low temperatures (Collins et al., 1990; McCarthy et al., 1995)
- occur significantly in pH 6.5-7.5 (Patterson and Kumar, 2000)

Difflugia globulus
- large (>70mm in diameter), spheroidal or sub-spheroidal tests with aperture diameter usually greater than 0.75 of the test. The rim of the aperture is

usually armored with quartz grains, and large quartz grains are often abundant on the surface of the test. Aperture rim sometimes slightly raised above the surface of the test (Booth, 2008).
- is a good cool to cold climatic indicator (Collins et al., 1990)
- feeds on green and yellow-green algae (Schroder et al., 1897)

Difflugia fragosa
- distribution is controlled by warm temp rather than eutrophic conditions (Collins et al., 1990)

Difflugia oblonga
- ubiquitous, appears to tolerate climate extremes, and are often present in high numbers where other species more sensitive to climate conditions can't thrive (Collins et al., 1990)
- not sensitive to cold but will thrive in almost any climate as long as sediments are sufficiently organic (Collins et al., 1990; McCarthy et al., 1995)
- does not appear to do well in oligotrophic conditions (McCarthy, 1984)
- tolerates sandy environments (McCarthy et al., 1995)
- found mainly in envoronements with pH <6.2 (Ellison, 1995)

Difflugia protaeiformis
- requires warm water and organic conditions to thrive (Collins et al., 1990)
- found in pH of 3.9-7.5 and in industrial polluted environments (Asioli et al., 1996; Kauppila et al., 2006; Kumar and Patterson, 2000; Patterson and Kumar, 2000)
- Thermophilous (McCarthy et al., 1995)
- opportunistic and able to thrive in areas with high levels of pollutants (e.g. Hg, As, Cd, Cr, Cu, Pb) (Patterson and Kumar, 2000)
- is adapted to environments rich in organic matter, sulfides, sulfites, ammonia, nitrogen, nitric nitrongen and low oxygen (Asioli et al., 1996)

Difflugia urceolata
- tolerate oligotrophic conditions, and low temperatures (Collins et al., 1990; McCarthy et al., 1995)
- occur significantly in pH between 6.5-7.5 (Patterson and Kumar, 2000)
- occurs in sediment with high percentages of organic matter (Patterson et al., 1996)

Difflugia urens
- tolerate oligotrophic conditions (Collins et al., 1990)

Heleopera sphagni
- thrive in polar conditions (Collins et al., 1990)
- presence suggests a wetland community (McCarthy et al., 1995)

Lagenodifflugia vas

- indicates a stressed environment (Asioli et al. 1996)
- requires warm water and organic conditions to thrive (Collins et al., 1990)
- pH >6.2 (Ellison, 1995)

Lesquereusia spiralis

- Thermophilous (Collins et al., 1990) (McCarthy et al., 1995)
- occur significantly in pH 6.5-7.5 (Patterson and Kumar, 2000)

Nebella collaris

- presence suggests establishment of wetlands (McCarthy et al., 1995)

Pontigulasia compressa

- ubiquitous, appear to tolerate climate extremes, and are often present in high numbers where other species more sensitive to climate conditions can't thrive (Collins et al., 1990)
- common in all ponds except eutrophic (Collins et al., 1990)
- Thermophilous but also found in low percentages, coarsely aggluntinated in the arctic (McCarthy et al., 1995)

1.2. References

Asioli, A., Medioli, F.S., Patterson, R.T. 1996. Thecamoebians as a tool for reconstruction on paleoenvironments in some Italian lakes in the foothills of the southern Alps (Orta Varese and Candia). Journal of Foraminiferal Research, 26: 248-263.

Booth, R.K. 2008. Testate amoebae as proxies for mean annual water- table depth in Sphagnum- dominated peatlands of North America. Journal of Quaternary Science, 23: 43- 57.

Boudreau, E.A., Galloway, J.M., Patterson, R.T., Kumar, A., Michel, F.A. 2005. A paleolimnological record of Holocene climate and environmental change in the Temagami region, northeastern Ontario. Journal of Paleolimnology, 33: 445- 461.

Collins, E.S., McCarthy, F.M.G., Medioli, F.S., Scott, D.B., Honig, C.A. 1990. Biogeographic distribution of modern thecamoebians in a transect along the Eastern North American coast. p. 783 – 792. In C. Hemleben and others (Eds). Paleoecology, Biostratigraphy, Paleoceanography and Taxonomy of Agglutinated Foraminifera Kluwer Academic Publishers. Amsterdam.

Ellison, R.L. 1995. Paleolimnological analysis of Ullswater using testate amoebae. Journal of Paleolimnology, 13: 51-63.

Kumar, A., Patterson, R.T. 2000. Arcellaceans (thecamoebians): new tools for monitoring long- and short-term changes in lake bottom acidity. Environmental Geology, 39: 689- 697.

Kauppila, T., Kihlman, S., Makinen, J. 2006. Distribution of Arcellaceans (tesate amoebae) in the sediments of a mine water impacted bay of lake Retunen, Finland. Water, Air, and Soil Pollution, 172: 337- 358.

Loeblich, A.R.Jr., Tappan, H. 1953. Sarcodina, chiefly "thecamoebians" and foraminiferida. In Moore, R.C. (ed.), Treatise on Invertebrate Paleontology. Part C, Protista 2. Geological Society of America and University ok Kansas Press, v. 1, pp. i-xiii+cl, c510a.

McCarthy, F.M.G. 1984. Infraspecific variation in Arcellacea (Thecamoebians) from Eastern Canada and a selected biostratigraphic study. Unpublished honours thesis, Dalhousie University, Halifax, Nova Scotia, 41 p.

McCarthy, F.M.G., Kerr, H.A., Collins, E.S., McAndrews, J.H., Scott, D.B. 1995. Late glacial/postglacial thecamoebians and palynological succession: potential for climate change reconstruction.

Medioli, F.S., Scott, D.B. 1987. A case study of Protozoan intracolonial variability: taxonomic implications. Journal of Foraminiferal Research, 17: 28-47.

Patterson, R. T., Kumar, A. 2002. A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeograhpy, Palaeecolimatology, Palaeoecology, 180: 225- 251.

Patterson, T.R., Kumar, A. 2000. Use of Arcellacea (Thecamoebians) to Gauge Levels of Contamination and Remediation in Industrially Pollutes Lakes. p. 257- 278. In Martin, R.E. (Eds). Environmental Micropaleontology, The Application of Microfossils to Environmental Geology, Kluwer Academic/ Plenum Publishers, New York, Boston, Dordrecht, London, Moscow.

Patterson, R.T., MacKinnon, K.D., Scott, D.B., Medioli, F.S. 1985. Arcellaceans ("Thecamoebians") in Small Lakes of New Brunswick and Nova Scotia: Modern Distribution and Holocene Stratigraphic Changes. Journal of Foraminiferal Research, 15: 114- 137.

Patterson, R.T., Barker, T., Burbridge, S.M. 1996. Arcellaceans (thecamoebians) as proxies of arsenic and mercury contamination in Northeastern Ontario lakes. Journal of Foraminiferal Research, 26: 172-183.

Reinhardt, E.G., Dalby, P.A., Kumar, A., Patterson, T. 1998. Arcellaceans as Pollution Indicators in Mine Tailing Contaminated Lakes near Cabalt, Ontario, Canada. Micropaleontology, 44: 131-148.

Schroder, C.J., Scott, D.B., Medioli, F.S. 1987. Can smaller benthic foraminifera be ignored in paleoenvironmental analysis? Journal of Foraminiferal Research, 17: 101-105.

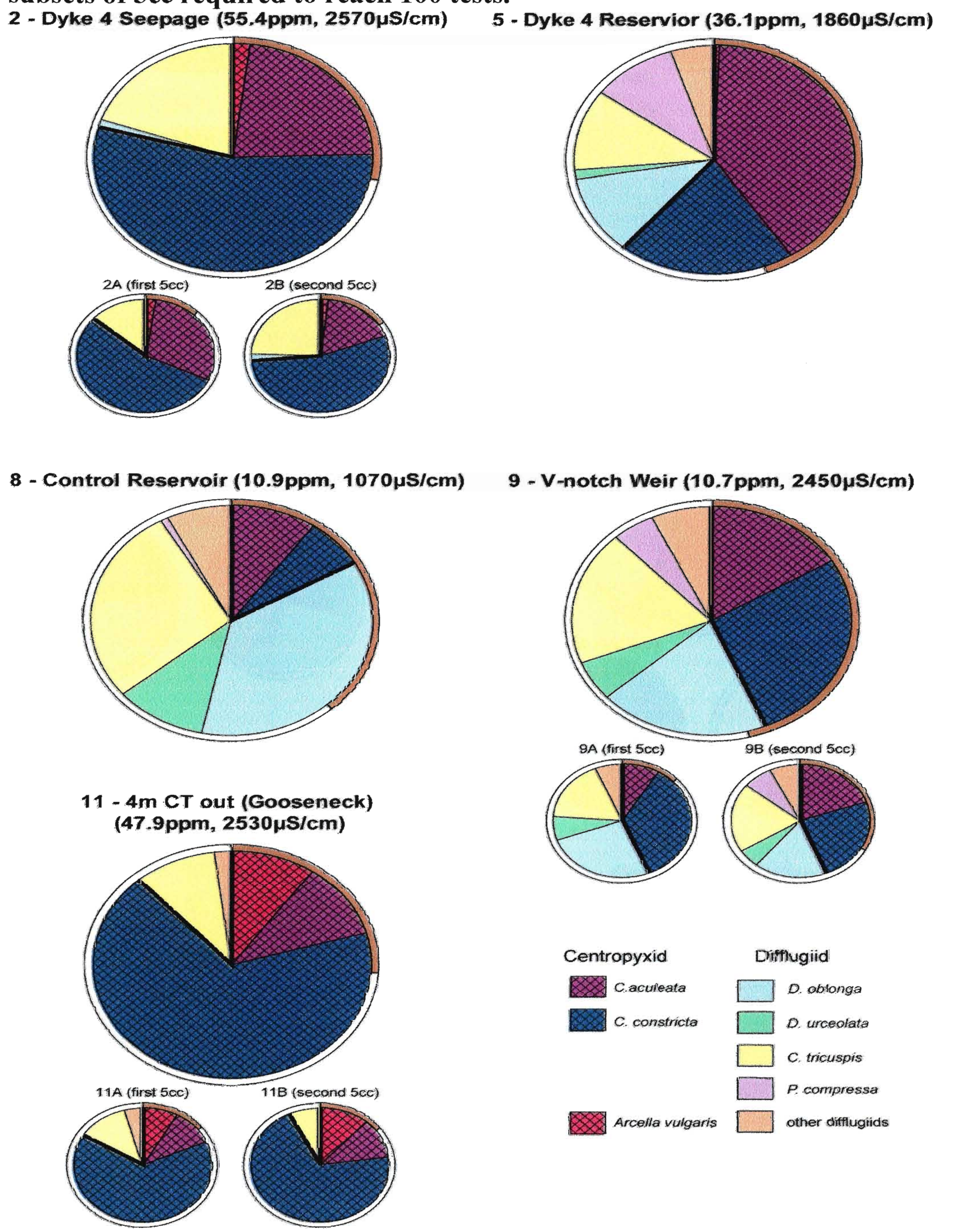
Scott, D.B., Medioli, F.S. 1983. Agglutinated Rhizopods in Lake Erie: Modern Distribution and Stratigraphic Implications. Journal of Paleontology, 4: 809- 820.

Scott, B.D., Medioli, F.S., Schafer, C.T. 2001. Monitoring in coastal environments using foraminifera and thecamoebian indicators. Cambridge University Press. Cambridge, 177 p.

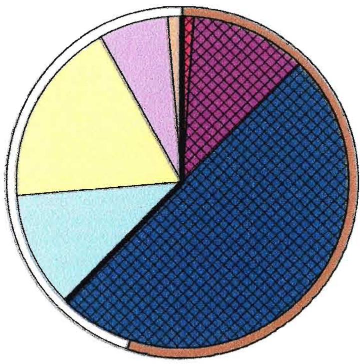
Appendix 2

For site pie diagrams the larger pie represents the entire sample while the smaller pies represent sub samples of 5cc's required to achieve 100 specimens for that site.

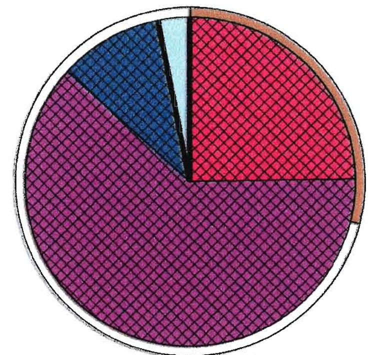
2.1. Pie Diagrams: Suncor Energy Inc. constructed wetlands, Set One (Aug, 2007). Large pie graphs represent entire sample and small pie graphs represent subsets of 5cc required to reach 100 tests.



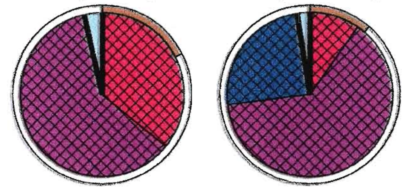
13 - Weir B (50.6ppm, 2680µS/cm)



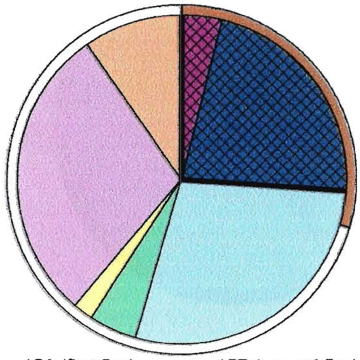
14 - Sustainable Lake North (44.2ppm, 2040µS/cm)



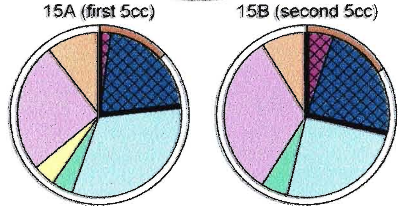
14A (first 5cc) 14B (second 5cc)



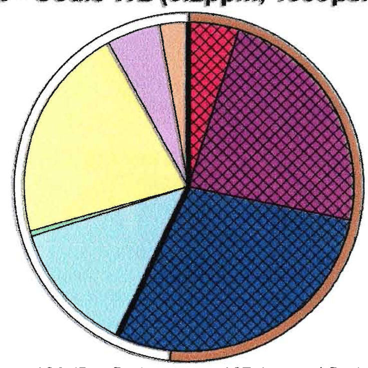
15 - Sustainable Lake South (41.5ppm, 1820µS/cm)



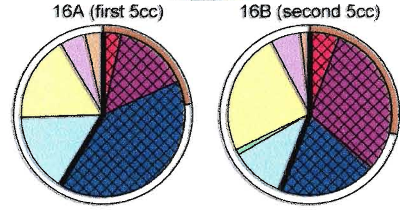
15A (first 5cc) 15B (second 5cc)



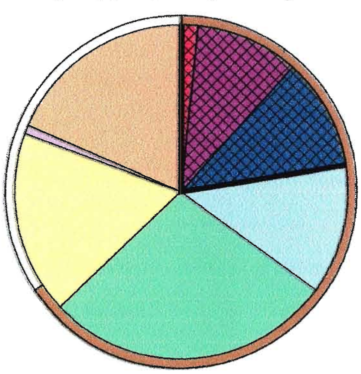
16 - Sodic WL (5.2ppm, 1350µS/cm)



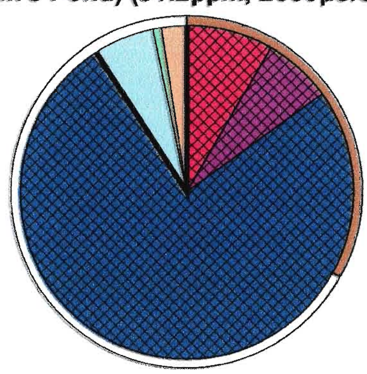
16A (first 5cc) 16B (second 5cc)



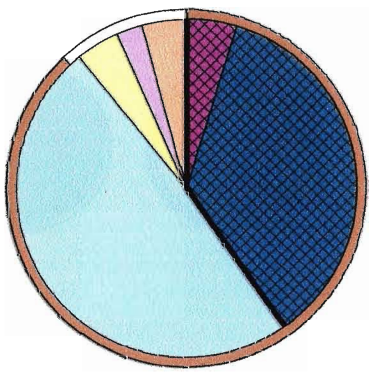
17 - Muskeg stockpile wetland (3.6ppm, 611µS/cm)



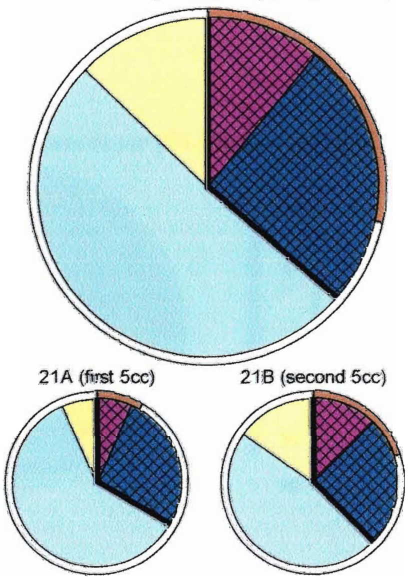
18 - Gooseneck Wetland (Jan's Pond) (51.2ppm, 2650µS/cm)



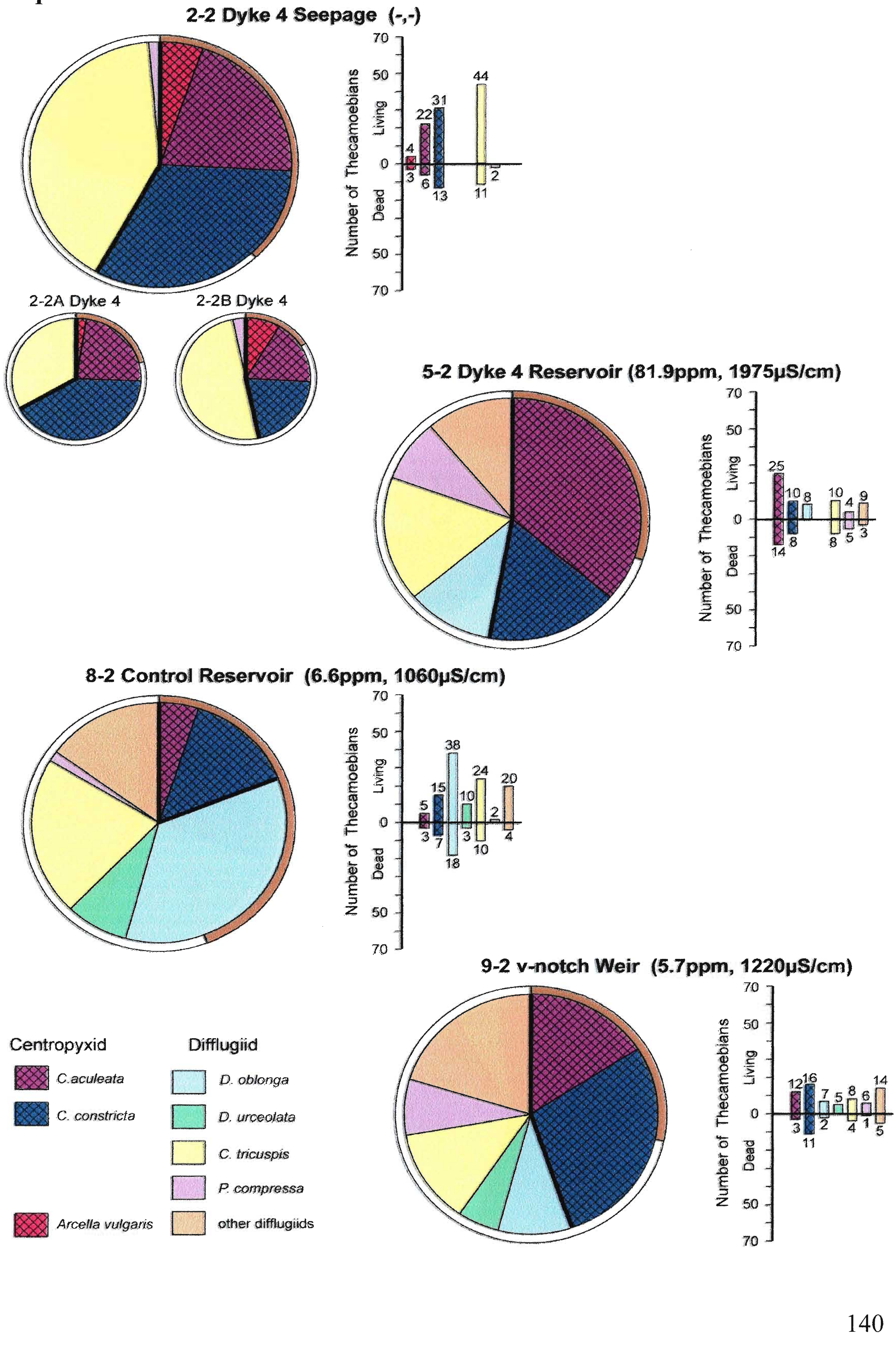
20 - WA 14, Pond A (8.9ppm, 1610µS/cm)



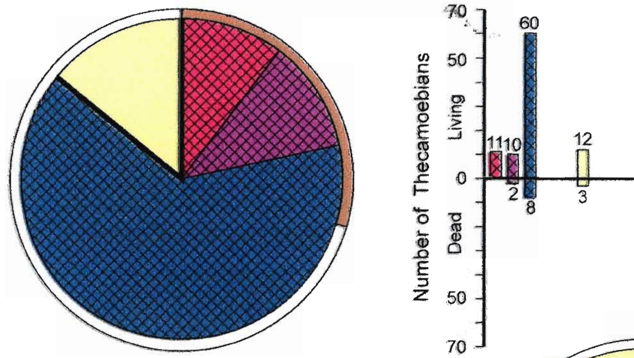
21 - Crane Lake (CL) (8.4ppm, 1300µS/cm)



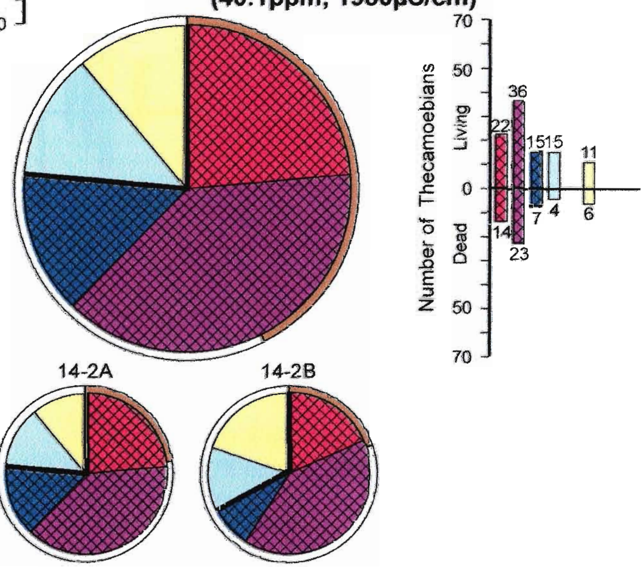
2.2. Pie Diagrams: Suncor Energy Inc. constructed wetlands, Set Two (June, 2008), including bar graphs of living (stained) and dead (empty) tests. Large pie graphs represent entire sample and small pie graphs represent subsets of 5cc required to reach 100 tests.



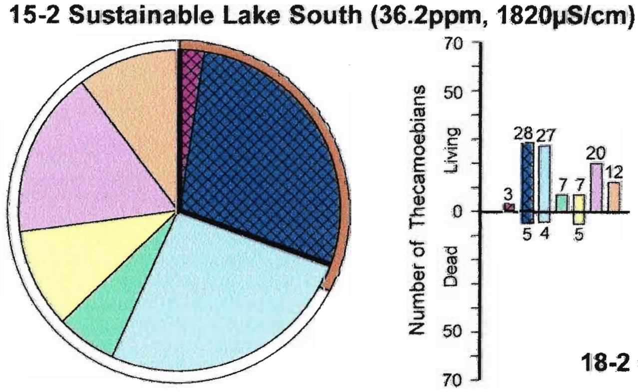
11-2 4m CT out (Gooseneck) (-, -)



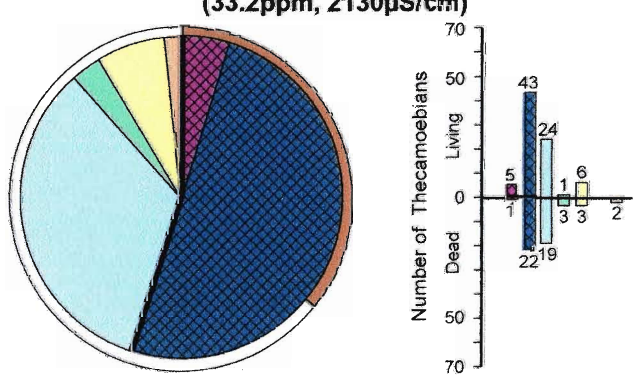
14-2 Sustainable Lake North (40.1ppm, 1980µS/cm)



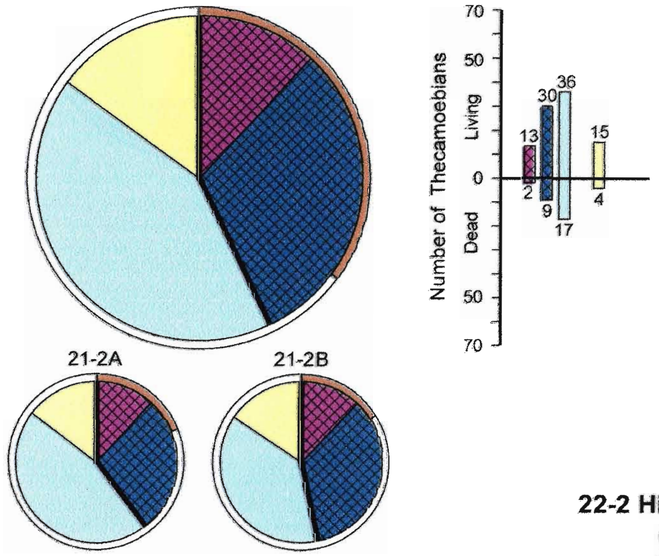
15-2 Sustainable Lake South (36.2ppm, 1820µS/cm)



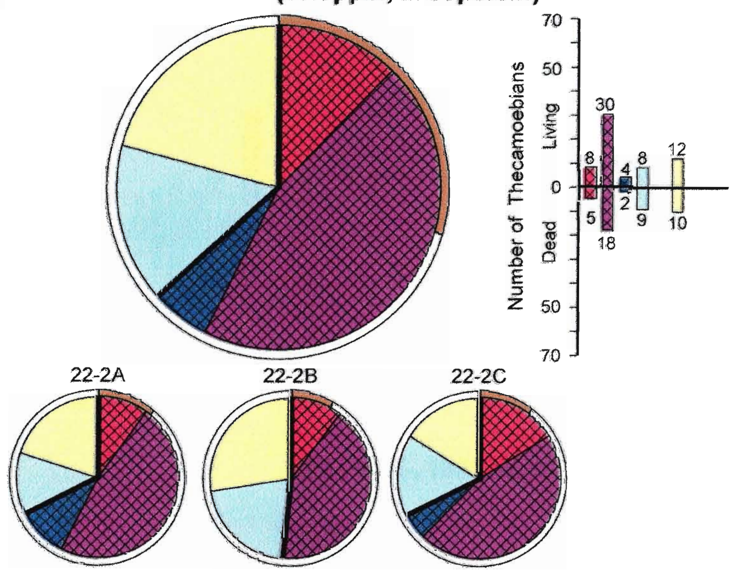
18-2 Gooseneck wetland (Jan's Pond) (33.2ppm, 2130µS/cm)



21-2 Crane Lake(CL) (8.4ppm, 1440μS/cm)

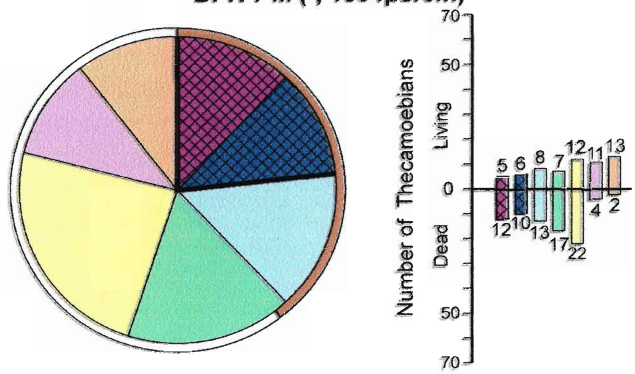


22-2 High Sulphate Wetlands (HSW)
(18.0ppm, 2780μS/cm)



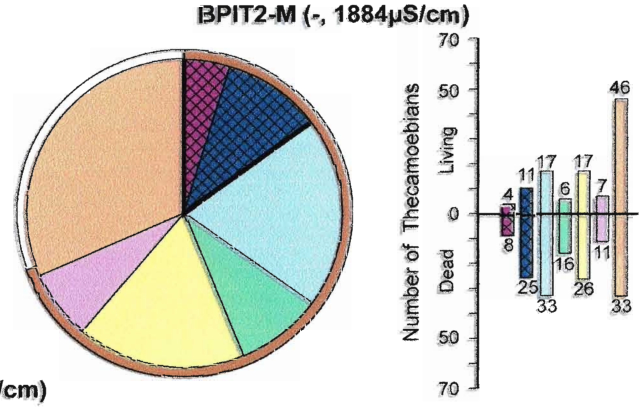
2.3. Pie Diagrams: Syncrude Canada Ltd. Big Pit samples, May, including bar graphs of living (stained) and dead (empty) tests.

BPIT1-M (-, 1884µS/cm)

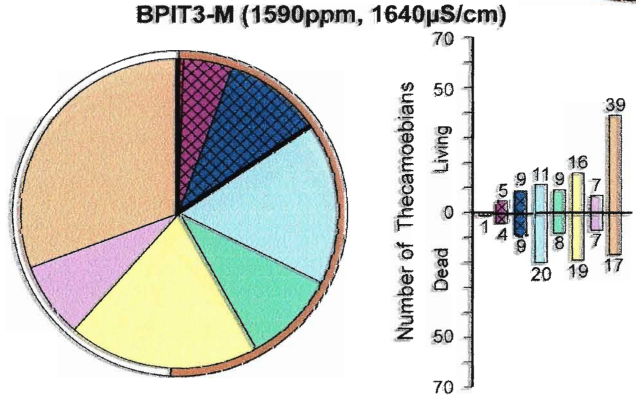


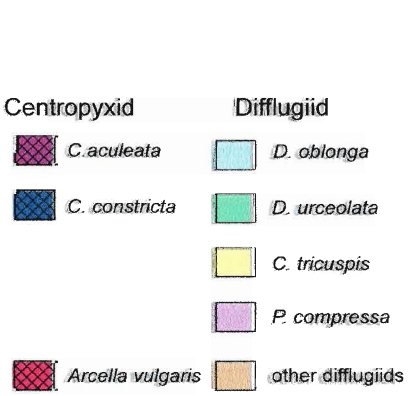
May 2008

BPIT2-M (-, 1884µS/cm)

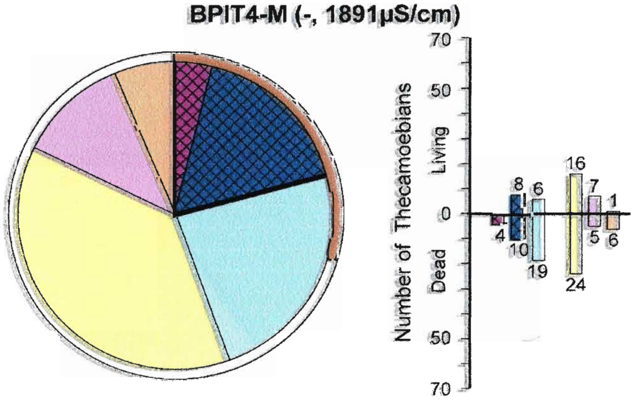


BPIT3-M (1590ppm, 1640µS/cm)





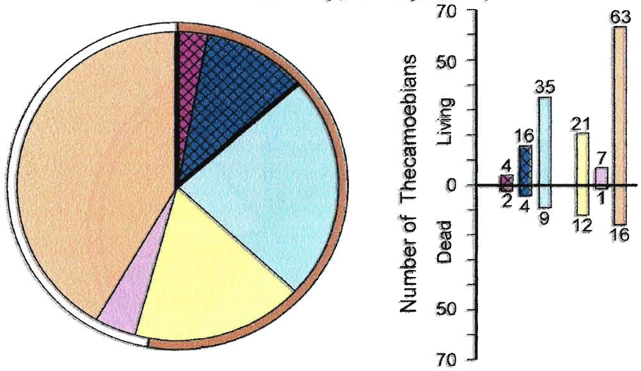
BPIT4-M (-, 1891µS/cm)



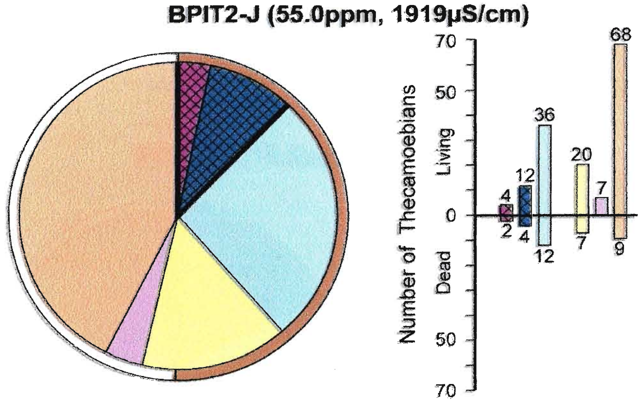
2.4. Pie Diagrams: Syncrude Canada Ltd. Big Pit samples, July, including bar graphs of living (stained) and dead (empty) tests.

BPIT1-J (-, 1919µS/cm)

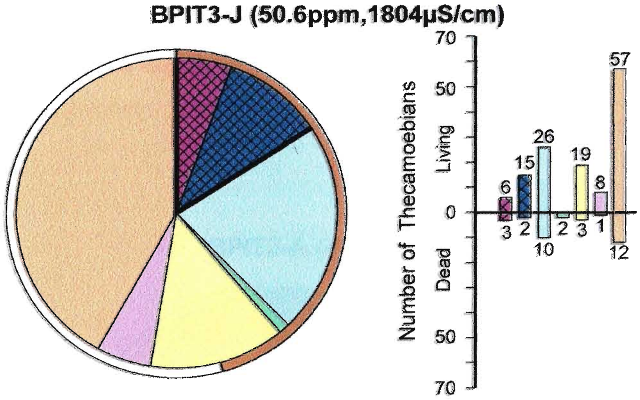
July 2008

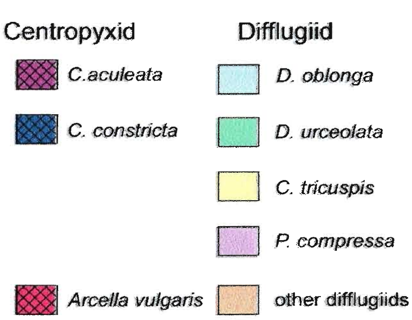


BPIT2-J (55.0ppm, 1919µS/cm)

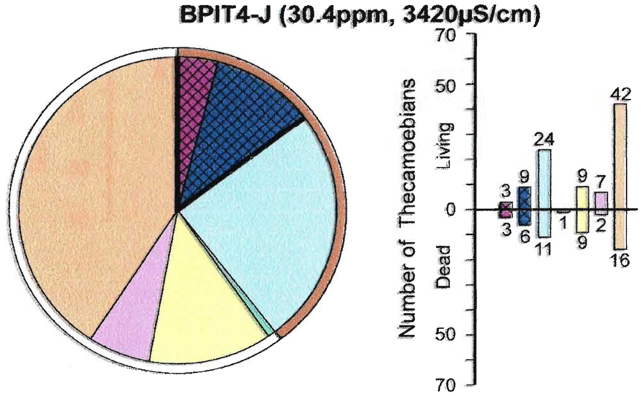


BPIT3-J (50.6ppm,1804µS/cm)





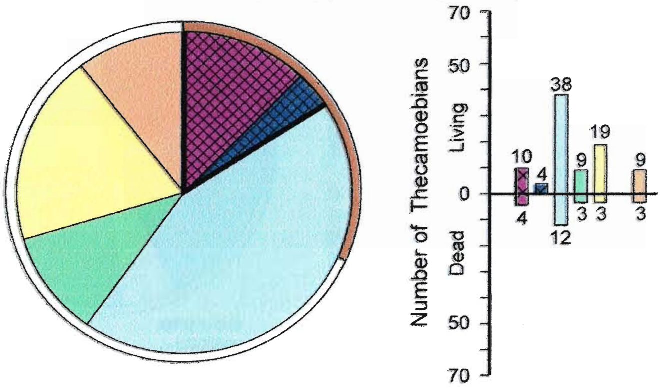
BPIT4-J (30.4ppm, 3420µS/cm)

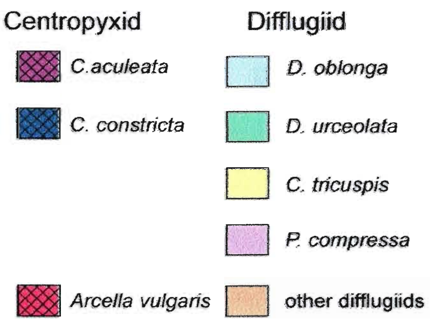


<u>2.5. Pie Diagrams:</u> Syncrude Canada Ltd. Big Pit samples, August, including bar graphs of living (stained) and dead (empty) tests.

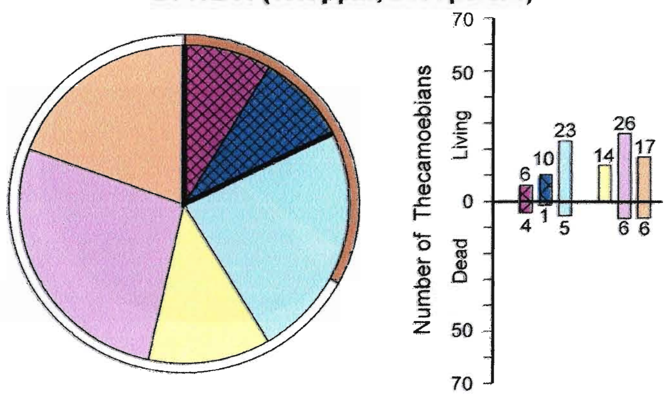
August 2008

BPIT1-A (14.5ppm, 2060µS/cm)

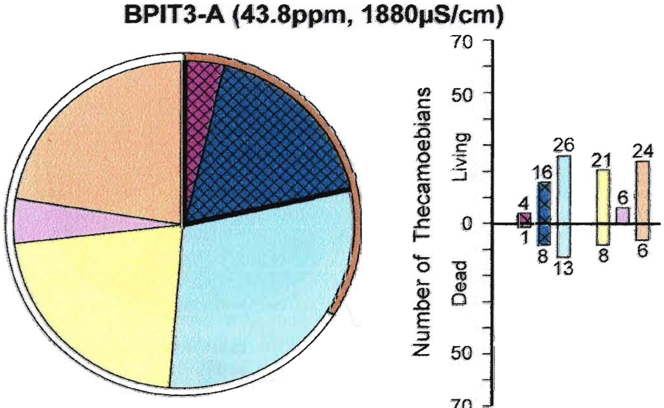


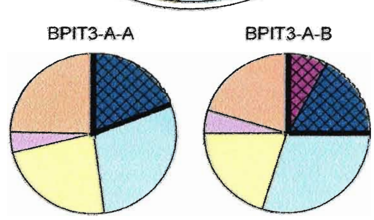


BPIT2-A (19.5ppm, 2480µS/cm)

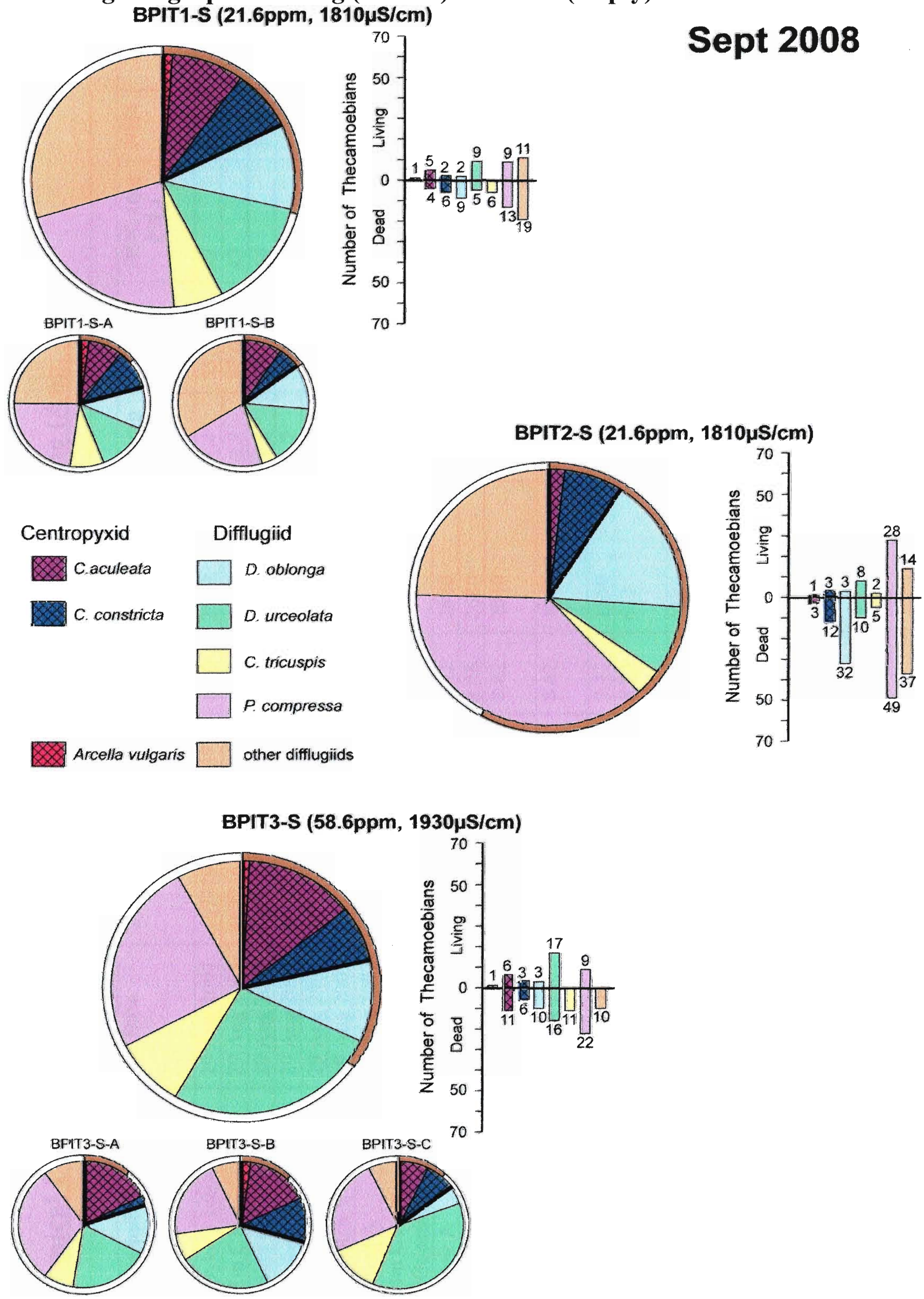


BPIT3-A (43.8ppm, 1880µS/cm)





2.6. Pie Diagrams: Syncrude Canada Ltd. Big Pit samples, September, including bar graphs of living (stained) and dead (empty) tests.



Appendix 3

3.1. Chemical Data: Suncor Energy Inc. constructed wetlands, Set One (Aug, 2007).

Set 1											
Sample #	Sample Location	Date	Northing	Easting	pH	Conductivity (µS/cm)	Temp (C^0)	Nap Acids (ppm)	DOC (mg C/L)	NH4	Na
2	Dyke 4 seepage	29 Aug 2007	467789	6316371	8.15	2570	11.4	55.4	42	8.40	636
3	1m CT	29 Aug 2007	467736	6316379	8.28	2620	12.5	57.0	43	-	670
4	Weir C	29 Aug 2007	467698	6316352	8.33	2590	11.8	55.1	44	5.94	670
5	Dyke 4 Reservoir	29 Aug 2007	467776	6316305	8.03	1860	11.7	36.1	47	-	363
8	Control Reservoir	29 Aug 2007	467741	6316208	8.23	1070	13.8	10.9	38	0.37	172
9	V-notch Weir	29 Aug 2007	67634	6316254	7.79	2450	11.5	10.7	31	-	248
10	Pond A	29 Aug 2007	467699	6316609	8.38	2530	13.3	46.2	43	3.32	672
11	4m CT out (Gooseneck)	29 Aug 2007	467699	6316609	8.39	2530	13.3	47.9	39	-	676
12	Weir A	29 Aug 2007	467657	6316854	8.32	2680	13.0	50.2	41	-	662
13	Weir B	29 Aug 2007	467513	6317175	8.38	2680	12.9	50.6	46	3.89	686
14	Sustainable Lake North	29 Aug 2007	467539	6316618	8.82	2040	15.3	44.2	71	-	551
15	Sustainable Lake South	29 Aug 2007	467536	6316575	8.62	1820	15.0	41.5	64	-	459
16	Sodic Wetland	29 Aug 2007	467444	6316831	7.72	1350	12.5	5.2	24	0.40	140
17	Muskeg stockpile wetland	29 Aug 2007	467560	6316240	8.23	611	13.4	3.6	20	-	44
18	Gooseneck Wetland (Jan's Pond)	29 Aug 2007	467689	6316652	8.35	2650	13.7	51.2	40	-	656
19	Dyke 4 Pond B	29 Aug 2007	467766	6316614	8.03	2180	11.5	56.2	51	1.65	417
20	WA 14, Pond A	29 Aug 2007	467094	6316476	8.03	1610	16.6	8.9	44	-	236
21	Crane Lake	29 Aug 2007	466418	6317035	8.93	1300	18.6	8.4	52	0.57	297
23	Natural wetland out (NWL out)	29 Aug 2007	469046	6315329	8.40	1590	21.5	68.9	68	8.46	386

Set 1 continued															
Sample #	K	Mg	Ca	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as HCO_3	Tot Cat/ Tot An	Na/Cl	(Ca+Mg)/ (CO_3+HCO_3)	B	S	Si	Sr
2	17.4	44.6	53.7	220	612	13.5	772	799	1.08	4.5	0.49	3.6	201	3.5	1.01
3	17.7	38.9	44.8	240	585	0.0	823	823	1.08	4.3	0.41	3.7	193	3.9	0.95
4	17.7	38.6	43.5	240	590	21.9	770	815	1.08	4.3	0.40	3.6	192	3.8	0.94
5	8.3	47.4	111	49	678	12.6	513	539	1.05	11.4	1.08	1.6	227	1.2	0.69
8	15.2	38.3	47.6	25	409	0.0	219	219	1.05	10.6	1.55	0.8	139	0.1	0.40
9	24.2	117.0	297	20	1550	0.0	193	193	1.00	19.1	7.78	1.3	500	-	1.88
10	18.1	40.1	41.2	230	585	31.5	768	832	1.09	4.5	0.40	3.5	186	3.1	0.87
11	18.3	40.3	41.0	220	578	48.0	785	883	1.08	4.7	0.37	3.5	187	3.1	0.87
12	18.1	41.7	46.9	240	597	17.7	810	846	1.06	4.3	0.42	3.6	200	3.7	0.92
13	18.3	40.6	37.3	250	602	33.9	774	843	1.06	4.2	0.38	3.7	202	2.8	0.84
14	13.2	21.7	16.5	51	562	60.0	628	750	1.06	16.7	0.21	3.3	184	0.6	0.27
15	13.5	24.2	21.7	42	578	23.7	509	557	1.05	16.9	0.34	2.6	189	0.2	0.42
16	9.8	47.2	150	0.1	657	0.0	242	242	1.01	2161	2.88	0.5	220		1.02
17	12.2	29.5	52.3	3.1	264	0.0	105	105	1.00	21.9	2.95	0.2	87	-	0.40
18	18.0	41.6	47.5	240	596	15.0	797	828	1.06	4.2	0.43	3.6	198	4.2	0.92
19	17.9	81.9	94	79	617	0.0	770	770	1.09	8.1	0.91	2.9	206	2.0	1.49
20	20.4	64.0	135	4.8	548	0.0	680	680	1.01	75.9	1.08	0.5	179	2.4	0.96
21	9.7	45.1	14.5	3.7	351	38.4	480	558	1.07	124	0.49	0.3	117	1.4	0.06
23	18.9	22.5	39.6	28	346	25.2	688	739	1.05	21.3	0.32	2.8	115	7.0	0.79

3.2. Chemical Data: Suncor Energy Inc. constructed wetlands, Set Two (June, 2008).

Set 2											
Sample Number	Sample Location	Date Sampled	Northing	Easting	pH	Conductivity (µS/cm)	Temp (C^0)	Nap Acids (ppm)	DOC (mg C/L)	NH4	Na
2-2	Dyke 4 seepage	27 Jun 2008	-	-	-	-	-	-	-	-	-
5-2	Dyke 4 Reservoir	27 Jun 2008	467776	6316305	7.79	1975	-	31.9		<0.01	317
8-2	Control Reservoir	27 Jun 2008	467741	6316208	8.27	1060	-	6.6		<0.01	94
9-2	V-notch Weir	27 Jun 2008	467634	6316254	7.47	1220	-	5.7		0.10	118
11-2	4m CT out (Gooseneck)	27 Jun 2008	-	-	-	-	-	-	-	-	-
14-2	Sustainable Lake North	27 Jun 2008	467539	6316618	8.62	1980	-	40.1		0.14	479
15-2	Sustainable Lake South	27 Jun 2008	467536	6316575	8.40	1820	-	36.2		0.15	415
18-2	Gooseneck Wetland (Jan's Pond)	27 Jun 2008	467689	6316652	7.90	2130	-	33.2		0.58	340
21-2	Crane Lake (CL)	27 Jun 2008	466413	6317033	7.98	1440	-	8.4		0.51	279
22-2	High Sulphate Wetlands (HSW)	27 Jun 2008	466390	6317220	7.93	2780	-	18.0		<0.01	396

Set 2 continued															
Sample #	K	Mg	Ca	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as HCO_3	Tot Cat/ Tot An	Na/Cl	(Ca+Mg)/ (CO_3+HCO_3)	B	S	Si	Sr
2-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-2	8.0	56.4	121	39.0	756	0	435	-	1.03	12.5	1.51	1.56	261	1.0	0.8
8-2	7.4	40.6	110	6.8	487	0	139	-	1.04	21.3	3.90	0.46	169	0.6	0.6
9-2	6.3	39.3	142	6.0	496	0	356	-	1.07	30.4	2.47	0.55	176	1.0	0.7
11-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14-2	11.9	19.9	18.7	41.0	477	33	599	-	1.08	18.0	0.24	2.91	171	0.3	0.3
15-2	12.8	22.4	23.5	35.0	520	25	466	-	1.06	18.3	0.36	2.44	181	0.2	0.4
18-2	13.2	80.9	102	42.0	761	0	545	-	1.04	12.5	1.33	1.71	267	5.2	1.3
21-2	8.3	49.1	30.8	3.2	365	0	571	-	1.05	134.6	0.60	0.30	126	1.1	0.2
22-2	14.2	116	190	0.5	1630	0	181	-	0.99	1222.4	6.46	0.79	513	0.3	2.0

3.3. Chemical Data: Syncrude Canada Ltd. Big Pit samples, May 2008.

May												
Sample Number	Date Sampled	Northing	Easting	Sample Depth (m)	Depth in substrate (cm)	Temp (C^0)	Conductivity (µS/cm)	pH	Nap Acids (ppm)	DO at sed/water interface (mg C/L)	NH_4	Na
BPIT1-M	21 May 2008	6326771	458197	2.6	0-2	-	1884	7.72	-	6	-	-
BPIT2-M	21 May 2008	6326771	458197	2.6	2-5	-	1884	7.72	-	6	-	-
BPIT3-M	21 May 2008	6326771	458197	2.6	0-5	13.2	1640	8.20	1590	6	-	-
BPIT4-M	21 May 2008	6326771	458197	2.6	5-15	-	1891	7.57	-	6	-	-

May continued																	
Sample #	K	Mg	Ca	F	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as HCO_3	Na/Cl	Na/(Ca+Mg)	Al	B	Fe	Mn	Si	Sr
BPIT1-M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BPIT2-M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BPIT3-M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BPIT4-M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

3.4. Chemical Data: Syncrude Canada Ltd. Big Pit samples, July 2008.

July												
Sample Number	Date Sampled	Northing	Easting	Sample Depth (m)	Depth in Substrate (cm)	Temp (C^0)	Conductivity (µS/cm)	pH	Nap Acids (ppm)	DO sed/water interface (mg C/L)	NH_4	Na
BPIT1-J	22 Jul 2008	6326775	458207	2.5	0-3	17.9	1919	8	-	0.6	-	-
BPIT2-J	22 Jul 2008	6326775	458207	2.5	5-10	17.9	1919	8	55.0	0.6	1.18	445
BPIT3-J	22 Jul 2008	6326775	458207	2.5	10-15	17.9	1804	8.05	50.6	0.6	1.29	442
BPIT4-J	22 Jul 2008	6326775	458207	2.5	5	17.9	3420	7.63	30.4	0.6	0.35	687

July continued																
Sample #	K	Mg	Ca	F	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as HCO_3	Na/Cl	Na/(Ca+ Mg)	Al	B	Fe	Mn	Si	Sr
BPIT1-J	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BPIT2-J	8.1	12.8	14.3	1.1	130	137	33	815	724	5.28	10.86	1.4	2.2	0.3	-	7.0	0.3
BPIT3-J	7.4	11.5	12.1	1.1	120	105	31	783	693	5.69	12.29	1.2	2.1	0.3	-	7.0	0.3
BPIT4-J	18.1	70.9	90.6	-	110	1440	19	516	454	9.64	2.86	2.1	2.3	0.5	-	8.2	0.3

3.5. Chemical Data: Syncrude Canada Ltd. Big Pit samples, August 2008.

August												
Sample Number	Date Sampled	Northing	Easting	Sample Depth (m)	Depth in Substrate (cm)	Temp (C^0)	Conductivity (µS/cm)	pH	Nap Acids (ppm)	DOC sed/water interface (mg C/L)	NH_4	Na
BIPT1-A	19 Aug 2008	6326776	458206	2.4	0-2	17.8	2060	8.20	14.5	0.35	-	485
BPIT2-A	19 Aug 2008	6326776	458206	2.4	2-5	17.8	2480	8.23	19.5	0.35	-	542
BPIT3-A	19 Aug 2008	6326776	458206	2.4	5-10	17.8	1880	8.33	43.8	0.35	-	443

August continued																
Sample #	K	Mg	Ca	F	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as HCO_3	Na/Cl	Na/(Ca+Mg)	Al	B	Fe	Mn	Si	Sr
BIPT1-A	9.7	32.5	24.8	0.3	110	107	28	1060	916	6.81	5.34	0.3	2.0	0.1	0.1	8.8	0.5
BPIT2-A	11.1	36.3	35.2	-	98	472	19	787	677	8.54	4.92	0.3	2.0	0.2	0.2	8.0	0.6
BPIT3-A	7.7	12.4	14.0	1.6	120	160	11	784	661	5.70	11.11	1.7	2.2	0.4	0.0	7.8	0.3

3.6. Chemical Data: Syncrude Canada Ltd. Big Pit samples, September 2008.

September												
Sample Number	Date Sampled	Northing	Easting	Sample Depth (m)	Depth in Substrate (cm)	Temp	Conductivity (μS/cm)	pH	Nap Acids (ppm)	DOC sed/water interface (mg C/L)	NH_4	Na
BIPT1-S	30 Sept 2008	6326776	458200	2.55	0-5	8.9	1810	8.32	21.6	0.41	-	400
BPIT2-S	30 Sept 2008	6326776	458200	2.55	5-10	8.9	1810	8.32	21.6	0.41	-	400
BPIT3-S	30 Sept 2008	6326776	458200	2.60	10-15	8.9	1930	8.31	58.6	0.41	-	453

September continued																
Sample #	K	Mg	Ca	F	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as HCO_3	Na/Cl	Na/(Ca+Mg)	Al	B	Fe	Mn	Si	Sr
BIPT1-S	8.4	20.8	21.1	0.5	110	113	0.0	865	709	5.61	6.24	1.2	1.7	0.5	0.1	9.7	0.4
BPIT2-S	8.4	20.8	21.1	0.5	110	113	0.0	865	709	5.61	6.24	1.2	1.7	0.5	0.1	9.7	0.4
BPIT3-S	7.7	7.7	11.3	1.4	130	70.7	11.4	923	776	5.38	16.28	0.9	2.3	0.2	-	6.0	0.3

3.7. Chemical Data: Natural Alberta Aquatic Environments, 2008.

Sample Name	Vegetation Zone	Date Sampled	Northing	Easting	pH	Temp (C^0)	Conductivity (µS/cm)	Nap Acids (ppm)	DO sed/water interface (mg C/L)	NH_4 (ppm)	Na/Cl
Spray Lakes Reservoir	Rocky Mountain	25 July 2008	5101249	11523914	7.69	18.6	285	0.18	7.6	0.11	3.65
Jasper	Rocky Mountain	24 July 2008	5310426	11758320	7.59	18.4	420	0.18	6.6	<0.01	1.06
Baptiste Lake	Boreal Forest	19 July 2008	5443613	11334129	8.06	22.1	340	2.4	12.7	0.17	12.39
Lac St. Anne	Boreal Forest	18 July 2008	5340593	11421516	7.81	16.6	343	0.30	8.5	-	6.07
Wabamun Lake	Boreal Forest	18 July 2008	5333552	11426508	8.06	23.2	570	0.25	9.8	<0.01	9.30
Island Lake	Boreal Forest	21 July 2008	5451399	11333301	7.32	24.0	459	1.1	3.9	3.23	7.56
Gregoire Lake	Boreal Forest	21 July 2008	5628787	11111264	7.23	24.1	147	0.2	7.2	<0.01	2.87
Buffalo Lake	Boreal Parkland	27 July 2008	5262438	11256558	8.75	24.2	2350	0.85	10	0.39	37.51
Islet Lake	Boreal Parkland	19 July 2008	5327421	11249369	7.50	21.5	318	0.95	2.8	0.10	2.98
Miquelon Lake	Boreal Parkland	27 July 2008	5315609	11252204	9.32	24.2	2690	1.09	5.7	0.19	17.73
Chestermere Lake	Grassland	27 July 2008	5163155	11349359	8.23	20.6	400	0.09	3.2	<0.01	3.95
Eagle Lake	Grassland	27 July 2008	5059211	11317506	9.09	21.8	1548	0.08	8	<0.01	7.98

Sample Name	(Ca+Mg)/ (CO_3+HCO_3)	Na/(Ca+Mg)	Na	K	Mg	Ca	Cl	SO_4	CO_3	HCO_3	Ratio Cat:An	B	S
Spray Lakes Reservoir	1.23	0.03	2.0	1.0	10.5	41.2	0.9	37.8	0	145	0.96	-	12.6
Jasper	1.48	0.05	4.6	0.5	18.8	53.5	6.7	69.8	0	175	0.99	-	23.4
Baptiste Lake	0.73	0.60	32.1	5.1	13.1	25.0	4.0	15.2	0	196	1.06	0.09	5.5
Lac St. Anne	0.76	0.54	30.3	11.1	12.5	27.9	7.7	15.1	0	196	1.08	0.06	5.2
Wabamun Lake	0.66	1.25	78.3	10.5	20.5	20.2	13	85.4	0	251	1.02	0.93	29.0
Island Lake	0.62	0.61	43.6	10.2	21.8	25.8	8.9	5.9	0	304	0.89	0.14	2.7
Gregoire Lake	1.06	0.13	3.9	1.0	5.0	17.9	2.1	7.2	0	75.6	1.04	-	2.6
Buffalo Lake	0.32	3.48	486	38.5	66	11.4	20	402	117	915	1.01	0.37	132
Islet Lake	0.91	0.18	11.6	12.7	19.7	22.6	6.0	8.7	0	186	1.06	0.05	2.9
Miquelon Lake	3.62	1.07	356	62.8	145	48.7	31	1240	68	106	1.03	0.10	1700
Chestermere Lake	1.34	0.33	24.8	1.0	17.4	36.5	9.7	70.1	0	149	1.05	-	24.8
Eagle Lake	0.65	2.09	269	16.2	55.9	16.6	52	348	83	355	1.02	0.18	115

3.8. Species Diversity: Suncor Energy Inc. constructed wetlands, Set One (Aug, 2007).

Set 1			
Sample	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
2	101	6	1.37
5	134	14	2.12
8	134	14	2.13
9	158	14	2.16
11	102	6	1.19
13	198	13	1.68
14	107	6	1.1
15	103	11	1.8
16	184	14	2.07
17	231	21	2.44
18	119	8	1.08
20	315	10	1.36
21	102	7	1.85

3.9. Species Diversity: Suncor Energy Inc. constructed wetlands, Set Two (Aug, 2007).

Set 2			
Sample	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
2-2	136	6	1.45
5-2	165	13	2.01
8-2	159	12	2.25
9-2	102	12	2.24
11-2	106	5	1.21
14-2	153	8	1.76
15-2	154	13	2.15
18-2	129	10	1.53
21-2	126	6	1.69
22-2	106	9	1.86

3.10. Species Diversity: Syncrude Canada Ltd. Big Pit samples, May, July, August and September 2008.

May			
Sample	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
BPIT1-M	106	9	1.85
BPIT2-M	183	19	2.48
BPIT3-M	142	10	2.1
BPIT4-M	261	16	2.45

July			
Sample	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
BPIT1-J	190	15	2.26
BPIT2-J	181	15	2.27
BPIT3-J	142	13	2.32
BPIT4-J	164	16	2.43

August			
Sample	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
BPIT1-A	114	11	2.01
BPIT2-A	118	10	2.04
BPIT3-A	133	10	2.05

September			
Sample	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
BPIT1-S	105	14	2.41
BPIT2-S	209	15	2.13
BPIT3-S	126	14	2.09

3.11. Species Diversity: Natural Alberta Aquatic Environments, 2008.

Natural Alberta Aquatic Environments				
Sample	**Vegetation Zone**	**Total Abundance**	**Species Richness**	**Shannon Diversity Index (SDI)**
Spray Lakes Reservoir	Rocky Mountain	106	5	1.25
Jasper	Rocky Mountain	103	4	1.26
Baptiste Lake	Boreal Forest	130	7	1.61
Lac St. Anne	Boreal Forest	183	7	1.61
Wabamun Lake	Boreal Forest	454	11	2.07
Island Lake	Boreal Forest	178	8	1.68
Gregoire Lake	Boreal Forest	109	7	1.70
Buffalo Lake	Boreal Parkland	124	7	1.80
Islet Lake	Boreal Parkland	139	6	1.62
Miquelon Lake	Boreal Parkland	314	8	1.57
Chestermere Lake	Grassland	117	6	1.46
Eagle Lake	Grassland	125	9	1.66

Appendix 4

Table 4.1: Set One raw data, with percent abundance and standard error, from the suncor wetlands, 2008.

	2			3	5			8			9			10
	Tests counted	% Abundance	Standard Error	Tests counted	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted
Arcella vulgaris	2	1.98	1.39%	1	1	0.63	0.63%	0	0.00	0.00%	0	0.00	0.00%	1
Centropyxis aculeata	0	0.00	0.00%		49	30.82	3.66%	5	3.73	1.64%	1	0.63	0.63%	
Centorpyxis aculeata "discoides"	23	22.77	4.17%	1	15	9.43	2.32%	8	5.97	2.05%	25	15.82	2.90%	5
Centropyxis constricta "aerophila"	45	44.55	4.95%		24	15.09	2.84%	9	6.72	2.16%	41	25.95	3.49%	5
Centropyxis constricta "constricta"	10	9.90	2.97%		8	5.03	1.73%	0	0.00	0.00%	2	1.27	0.89%	
Centropyxis constricta "spinosa"	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Lesquereusia spiralis	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Heliopera sphagni	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Pontigulasia compressa	0	0.00	0.00%		16	10.06	2.39%	1	0.75	0.74%	8	5.06	1.74%	
Cucurbitella tricuspis	20	19.80	3.97%		18	11.32	2.51%	37	27.61	3.86%	30	18.99	3.12%	
Lagenodifflugia vas	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia protaeiformis "claviformis"	0	0.00	0.00%		4	2.52	1.24%	4	2.99	1.47%	3	1.90	1.09%	
Difflugia protaeiformis "acuminata"	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia bidens	0	0.00	0.00%		3	1.89	1.08%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia corona	0	0.00	0.00%		0	0.00	0.00%	6	4.48	1.79%	8	5.06	1.74%	
Diffugia fragosa	0	0.00	0.00%		0	0.00	0.00%	1	0.75	0.74%	0	0.00	0.00%	
Difflugia bacillaliarum	0	0.00	0.00%		1	0.63	0.63%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia urens	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia urceolata "urceolata"	0	0.00	0.00%		0	0.00	0.00%	1	0.75	0.74%	0	0.00	0.00%	
Difflugia urceloata "elongata"	0	0.00	0.00%		2	1.26	0.88%	13	9.70	2.56%	9	5.70	1.84%	
Difflugia globula	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia oblonga	1	0.99	0.99%		12	7.55	2.09%	22	16.42	3.20%	16	10.13	2.40%	
Difflugia oblonga "lanceolata"	0	0.00	0.00%		0	0.00	0.00%	2	1.49	1.05%	1	0.63	0.63%	
Difflugid oblonga "linearis"	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	
Difflugia oblonga "bryophila"	0	0.00	0.00%		1	0.63	0.63%	2	1.49	1.05%	4	2.53	1.25%	
Difflugia oblonga "oblonga"	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%	4	2.53	1.25%	
Difflugia oblonga "tenuis"	0	0.00	0.00%		5	3.14	1.38%	23	17.16	3.26%	6	3.80	1.52%	

Table 4.1 (con't)

	11			13			14			15			16	
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance
Arcella vulgaris	10	9.80	2.94%	2	1.01	0.71%	24	24.00	4.27%	0	0.00	0.00%	9	4.89
Centropyxis aculeata	0	0.00	0.00%	2	1.01	0.71%	1	1.00	0.99%	0	0.00	0.00%	24	13.04
Centorpyxis aculeata "discoides"	11	10.78	3.07%	21	10.61	2.19%	62	62.00	4.85%	4	3.88	1.90%	19	10.33
Centropyxis constricta "aerophila"	65	63.73	4.76%	91	45.96	3.54%	7	7.00	2.55%	23	22.33	4.10%	51	27.72
Centropyxis constricta "constricta"	4	3.92	1.92%	3	1.52	0.87%	3	3.00	1.71%	0	0.00	0.00%	2	1.09
Centropyxis constricta "spinosa"	0	0.00	0.00%	4	2.02	1.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Heliopera sphagni	0	0.00	0.00%	1	0.51	0.50%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Pontigulasia compressa	0	0.00	0.00%	13	6.57	1.76%	0	0.00	0.00%	30	29.13	4.48%	10	5.43
Cucurbitella tricuspis	10	9.80	2.94%	36	18.18	2.74%	0	0.00	0.00%	2	1.94	1.36%	39	21.20
Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia protaeiformis "claviformis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	1	0.97	0.97%	2	1.09
Difflugia protaeiformis "acuminata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia bidens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	8	7.77	2.64%	0	0.00
Difflugia corona	0	0.00	0.00%	2	1.01	0.71%	0	0.00	0.00%	1	0.97	0.97%	2	1.09
Diffugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	1	0.54
Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia urceloata "elongata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	5	4.85	2.12%	1	0.54
Difflugia globula	2	1.96	1.37%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia oblonga	0	0.00	0.00%	21	10.61	2.19%	0	0.00	0.00%	26	25.24	4.28%	16	8.70
Difflugia oblonga "lanceolata"	0	0.00	0.00%	1	0.51	0.50%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	1	0.97	0.97%	3	1.63
Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00
Difflugia oblonga "tenuis"	0	0.00	0.00%	1	0.51	0.50%	3	3.00	1.71%	2	1.94	1.36%	5	2.72

Table 4.1 (con't)

		17			18			19	20			21		
	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error
Arcella vulgaris	1.59%	4	1.73	0.86%	10	8.40	2.54%		1	0.32	0.32%	0	0.00	0.00%
Centropyxis aculeata	2.48%	4	1.73	0.86%	3	2.52	1.44%	1	0	0.00	0.00%	0	0.00	0.00%
Centorpyxis aculeata "discoides"	2.24%	19	8.23	1.81%	5	4.20	1.84%	2	14	4.44	1.16%	11	10.78	3.07%
Centropyxis constricta "aerophila"	3.30%	5	2.16	0.96%	86	72.27	4.10%	12	103	32.70	2.64%	26	25.49	4.32%
Centropyxis constricta "constricta"	0.76%	0	0.00	0.00%	4	3.36	1.65%		9	2.86	0.94%	0	0.00	0.00%
Centropyxis constricta "spinosa"	0.00%	20	8.66	1.85%	0	0.00	0.00%	3	0	0.00	0.00%	0	0.00	0.00%
Lesquereusia spiralis	0.00%	0	0.00	0.00%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Heliopera sphagni	0.00%	1	0.43	0.43%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Pontigulasia compressa	1.67%	2	0.87	0.61%	0	0.00	0.00%		8	2.54	0.89%	0	0.00	0.00%
Cucurbitella tricuspis	3.01%	41	17.75	2.51%	0	0.00	0.00%		13	4.13	1.12%	13	12.75	3.30%
Lagenodifflugia vas	0.00%	0	0.00	0.00%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia protaeiformis "claviformis"	0.76%	3	1.30	0.74%	0	0.00	0.00%		1	0.32	0.32%	0	0.00	0.00%
Difflugia protaeiformis "acuminata"	0.00%	0	0.00	0.00%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia bidens	0.00%	9	3.90	1.27%	0	0.00	0.00%	1	0	0.00	0.00%	0	0.00	0.00%
Difflugia corona	0.76%	15	6.49	1.62%	0	0.00	0.00%		10	3.17	0.99%	0	0.00	0.00%
Diffugia fragosa	0.00%	0	0.00	0.00%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia bacillaliarum	0.54%	1	0.43	0.43%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia urens	0.00%	1	0.43	0.43%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia urceolata "urceolata"	0.00%	11	4.76	1.40%	1	0.84	0.84%		0	0.00	0.00%	0	0.00	0.00%
Difflugia urceloata "elongata"	0.54%	53	22.94	2.77%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia globula	0.00%	13	5.63	1.52%	3	2.52	1.44%		2	0.63	0.45%	0	0.00	0.00%
Difflugia oblonga	2.08%	4	1.73	0.86%	7	5.88	2.16%		154	48.89	2.82%	14	13.73	3.41%
Difflugia oblonga "lanceolata"	0.00%	2	0.87	0.61%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugid oblonga "linearis"	0.00%	0	0.00	0.00%	0	0.00	0.00%		0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "bryophila"	0.93%	1	0.43	0.43%	0	0.00	0.00%		0	0.00	0.00%	7	6.86	2.50%
Difflugia oblonga "oblonga"	0.00%	1	0.43	0.43%	0	0.00	0.00%		0	0.00	0.00%	9	8.82	2.81%
Difflugia oblonga "tenuis"	1.20%	21	9.09	1.89%	0	0.00	0.00%		0	0.00	0.00%	22	21.57	4.07%

Table 4.1 (con't)

	22	23
	Tests counted	Tests counted
Arcella vulgaris		10
Centropyxis aculeata	4	
Centorpyxis aculeata "discoides"	1	7
Centropyxis constricta "aerophila"		
Centropyxis constricta "constricta"		
Centropyxis constricta "spinosa"		
Lesquereusia spiralis		
Heliopera sphagni		
Pontigulasia compressa		
Cucurbitella tricuspis		
Lagenodifflugia vas		
Difflugia protaeiformis "claviformis"		
Difflugia protaeiformis "acuminata"		
Difflugia bidens		
Difflugia corona		
Diffugia fragosa		
Difflugia bacillaliarum		
Difflugia urens		
Difflugia urceolata "urceolata"		
Difflugia urceloata "elongata"		
Difflugia globula		
Difflugia oblonga		2
Difflugia oblonga "lanceolata"		
Difflugid oblonga "linearis"	1	
Difflugia oblonga "bryophila"		
Difflugia oblonga "oblonga"		
Difflugia oblonga "tenuis"		

Table 4.2: Set Two raw data, with percent abundance and standard error, from the Suncor wetlands, 2008.

	2-2			5-2			8-2			9-2			11-2
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted
Arcella vulgaris	7	5.15	1.89%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	11
Centropyxis aculeata	0	0.00	0.00%	31	28.70	4.35%	1	0.63	0.63%	1	0.98	0.98%	0
Centorpyxis aculeata "discoides"	28	20.59	3.47%	8	7.41	2.52%	7	4.40	1.63%	14	13.73	3.41%	12
Centropyxis constricta "aerophila"	34	25.00	3.71%	18	16.67	3.59%	16	10.06	2.39%	25	24.51	4.26%	63
Centropyxis constricta "constricta"	10	7.35	2.24%	0	0.00	0.00%	0	0.00	0.00%	2	1.96	1.37%	5
Centropyxis constricta "spinosa"	0	0.00	0.00%	0	0.00	0.00%	6	3.77	1.51%	0	0.00	0.00%	0
Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Pontigulasia compressa	2	1.47	1.03%	9	8.33	2.66%	2	1.26	0.88%	7	6.86	2.50%	0
Cucurbitella tricuspis	55	40.44	4.21%	18	16.67	3.59%	34	21.38	3.25%	12	11.76	3.19%	15
Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	1	0.63	0.63%	0	0.00	0.00%	0
Difflugia protaeiformis "claviformis"	0	0.00	0.00%	6	5.56	2.20%	8	5.03	1.73%	3	2.94	1.67%	0
Difflugia protaeiformis "acuminata"	0	0.00	0.00%	2	1.85	1.30%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugia bidens	0	0.00	0.00%	0	0.00	0.00%	6	3.77	1.51%	7	6.86	2.50%	0
Difflugia corona	0	0.00	0.00%	1	0.93	0.92%	9	5.66	1.83%	0	0.00	0.00%	0
Difflugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	5	4.90	2.14%	0
Difflugia urceloata "elongata"	0	0.00	0.00%	0	0.00	0.00%	13	8.18	2.17%	0	0.00	0.00%	0
Difflugia globula	0	0.00	0.00%	3	2.78	1.58%	0	0.00	0.00%	9	8.82	2.81%	0
Difflugia oblonga "glans"	0	0.00	0.00%	6	5.56	2.20%	32	20.13	3.18%	9	8.82	2.81%	0
Difflugia oblonga " lanceolata"	0	0.00	0.00%	2	1.85	1.30%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	3	1.89	1.08%	0	0.00	0.00%	0
Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0
Difflugia oblonga "tenuis"	0	0.00	0.00%	4	3.70	1.82%	21	13.21	2.69%	8	7.84	2.66%	0

Table 4.2 (con't)

			14-2			15-2			18-2			21-2		
	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error
Arcella vulgaris	10.38	2.96%	36	23.53	3.43%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Centropyxis aculeata	0.00	0.00%	4	2.61	1.29%	0	0.00	0.00%	3	2.33	1.33%	0	0.00	0.00%
Centorpyxis aculeata "discoides"	11.32	3.08%	55	35.95	3.88%	3	2.54	1.45%	3	2.33	1.33%	15	11.90	2.89%
Centropyxis constricta "aerophila"	59.43	4.77%	15	9.80	2.40%	30	25.42	4.01%	60	46.51	4.39%	39	30.95	4.12%
Centropyxis constricta "constricta"	4.72	2.06%	7	4.58	1.69%	3	2.54	1.45%	5	3.88	1.70%	0	0.00	0.00%
Centropyxis constricta "spinosa"	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Lesquereusia spiralis	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Heliopera sphagni	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Pontigulasia compressa	0.00	0.00%	0	0.00	0.00%	20	16.95	3.45%	0	0.00	0.00%	0	0.00	0.00%
Cucurbitella tricuspis	14.15	3.39%	17	11.11	2.54%	12	10.17	2.78%	9	6.98	2.24%	19	15.08	3.19%
Lagenodifflugia vas	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia protaeiformis "claviformis"	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	2	1.55	1.09%	0	0.00	0.00%
Difflugia protaeiformis "acuminata"	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia bidens	0.00	0.00%	0	0.00	0.00%	10	8.47	2.56%	0	0.00	0.00%	0	0.00	0.00%
Difflugia corona	0.00	0.00%	0	0.00	0.00%	2	1.69	1.19%	0	0.00	0.00%	0	0.00	0.00%
Difflugia fragosa	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia bacillaliarum	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urens	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urceolata "urceolata"	0.00	0.00%	0	0.00	0.00%	4	3.39	1.67%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urceloata "elongata"	0.00	0.00%	0	0.00	0.00%	3	2.54	1.45%	4	3.10	1.53%	0	0.00	0.00%
Difflugia globula	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "glans"	0.00	0.00%	10	6.54	2.00%	23	19.49	3.65%	38	29.46	4.01%	20	15.87	3.26%
Difflugia oblonga " lanceolata"	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	3	2.33	1.33%	8	6.35	2.17%
Difflugid oblonga "linearis"	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	3	0.00	0.00%	25	19.84	3.55%
Difflugia oblonga "bryophila"	0.00	0.00%	0	0.00	0.00%	6	5.08	2.02%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "oblonga"	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	2	1.55	1.09%	0	0.00	0.00%
Difflugia oblonga "tenuis"	0.00	0.00%	9	5.88	1.90%	2	1.69	1.19%	0	0.00	0.00%	0	0.00	0.00%

Table 4.2 (con't)

	22-2		
	Tests counted	% Abundance	Standard Error
Arcella vulgaris	13	12.26	3.19%
Centropyxis aculeata	20	18.87	3.80%
Centorpyxis aculeata "discoides"	28	26.42	4.28%
Centropyxis constricta "aerophila"	5	4.72	2.06%
Centropyxis constricta "constricta"	1	0.94	0.94%
Centropyxis constricta "spinosa"	0	0.00	0.00%
Lesquereusia spiralis	0	0.00	0.00%
Heliopera sphagni	0	0.00	0.00%
Pontigulasia compressa	0	0.00	0.00%
Cucurbitella tricuspis	22	20.75	3.94%
Lagenodifflugia vas	0	0.00	0.00%
Difflugia protaeiformis "claviformis"	0	0.00	0.00%
Difflugia protaeiformis "acuminata"	0	0.00	0.00%
Difflugia bidens	0	0.00	0.00%
Difflugia corona	0	0.00	0.00%
Difflugia fragosa	0	0.00	0.00%
Difflugia bacillaliarum	0	0.00	0.00%
Difflugia urens	0	0.00	0.00%
Difflugia urceolata "urceolata"	0	0.00	0.00%
Difflugia urceloata "elongata"	0	0.00	0.00%
Difflugia globula	0	0.00	0.00%
Difflugia oblonga "glans"	12	11.32	3.08%
Difflugia oblonga " lanceolata"	2	1.89	1.32%
Difflugid oblonga "linearis"	3	2.83	1.61%
Difflugia oblonga "bryophila"	0	0.00	0.00%
Difflugia oblonga "oblonga"	0	0.00	0.00%
Difflugia oblonga "tenuis"	0	0.00	0.00%

Table 4.3: Seasonality raw data from Syncrude Demo Pond, May 2008.

	BPIT1-M			BPIT2-M			BPIT3-M			BPIT4-M		
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error
1) Arcella vulgaris	0	0.00	0.00%	1	0.55	0.54%	0	0.00	0.00%	0	0.00	0.00%
2) Centropyxis aculeata	0	0.00	0.00%	1	0.55	0.54%	2	1.41	0.99%	0	0.00	0.00%
3) Centorpyxis aculeata "discoides"	4	3.77	1.85%	8	4.37	1.51%	15	10.56	2.58%	12	4.60	1.30%
4) Centropyxis constricta "aerophila"	18	16.98	3.65%	15	8.20	2.03%	16	11.27	2.65%	33	12.64	2.06%
5) Centropyxis constricta "constricta"	0	0.00	0.00%	2	1.09	0.77%	0	0.00	0.00%	0	0.00	0.00%
6) Centropyxis constricta "spinosa"	0	0.00	0.00%	1	0.55	0.54%	0	0.00	0.00%	3	1.15	0.66%
7) Lesquereusia spiralis	0	0.00	0.00%	2	1.09	0.77%	0	0.00	0.00%	1	0.38	0.38%
8) Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
10) Pontigulasia compressa	12	11.32	3.08%	14	7.65	1.96%	15	10.56	2.58%	18	6.90	1.57%
11) Cucurbitella tricuspis	40	37.74	4.71%	35	19.13	2.91%	34	23.94	3.58%	43	16.48	2.30%
12) Lagenodifflugia vas	0	0.00	0.00%	1	0.55	0.54%	0	0.00	0.00%	2	0.77	0.54%
13) Difflugia protaeiformis "claviformis"	0	0.00	0.00%	7	3.83	1.42%	8	5.63	1.93%	19	7.28	1.61%
15) Difflugia protaeiformis "acuminata"	0	0.00	0.00%	18	9.84	2.20%	7	4.93	1.82%	21	8.05	1.68%
16) Difflugia bidens	3	2.83	1.61%	2	1.09	0.77%	0	0.00	0.00%	2	0.77	0.54%
17) Difflugia corona	0	0.00	0.00%	1	0.55	0.54%	0	0.00	0.00%	2	0.77	0.54%
18) Difflugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
19) Difflugia bacillaliarum	4	3.77	1.85%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
20) Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
21) Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
22) Difflugia urceloata "elongata"	0	0.00	0.00%	17	9.29	2.15%	24	16.90	3.14%	22	8.43	1.72%
23) Difflugia globula	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
24) Difflugia oblonga "glans"	12	11.32	3.08%	12	6.56	1.83%	16	11.27	2.65%	20	7.66	1.65%
25) Difflugia oblonga "lanceolata"	0	0.00	0.00%	7	3.83	1.42%	5	3.52	1.55%	10	3.83	1.19%
26) Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
28) Difflugia oblonga "bryophila"	7	6.60	2.41%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
29) Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
30) Difflugia oblonga "tenuis"	6	5.66	2.24%	12	6.56	1.83%	0	0.00	0.00%	20	7.66	1.65%
31) Difflugia amphora	0	0.00	0.00%	27	14.75	2.62%	0	0.00	0.00%	33	12.64	2.06%

Table 4.4: Seasonality raw data from Syncrude Demo Pond, July 2008.

	BPIT1-J			BPIT2-J			BPIT3-J			BPIT4-J		
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error
1) Arcella vulgaris	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
2) Centropyxis aculeata	3	1.58	0.90%	0	0.00	0.00%	0	0.00	0.00%	2	1.22	0.86%
3) Centorpyxis aculeata "discoides"	3	1.58	0.90%	6	3.31	1.33%	6	4.23	1.69%	7	4.27	1.58%
4) Centropyxis constricta "aerophila"	14	7.37	1.90%	14	7.73	1.99%	12	8.45	2.33%	14	8.54	2.18%
5) Centropyxis constricta "constricta"	5	2.63	1.16%	2	1.10	0.78%	3	2.11	1.21%	3	1.83	1.05%
6) Centropyxis constricta "spinosa"	1	0.53	0.52%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
7) Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
8) Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
10) Pontigulasia compressa	8	4.21	1.46%	7	3.87	1.43%	9	6.34	2.04%	9	5.49	1.78%
11) Cucurbitella tricuspis	33	17.37	2.75%	27	14.92	2.65%	18	12.68	2.79%	22	13.41	2.66%
12) Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
13) Difflugia protaeiformis	11	5.79	1.69%	15	8.29	2.05%	12	8.45	2.33%	13	7.93	2.11%
15) Difflugia protaeiformis "acuminata"	32	16.84	2.72%	23	12.71	2.48%	12	8.45	2.33%	18	10.98	2.44%
16) Difflugia bidens	4	2.11	1.04%	2	1.10	0.78%	0	0.00	0.00%	2	1.22	0.86%
17) Difflugia corona	2	1.05	0.74%	2	1.10	0.78%	0	0.00	0.00%	4	2.44	1.20%
18) Difflugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
19) Difflugia bacillaliarum	0	0.00	0.00%	2	1.10	0.78%	0	0.00	0.00%	0	0.00	0.00%
20) Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
21) Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%	1	0.70	0.70%	0	0.00	0.00%
22) Difflugia urceloata "elongata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	2	1.22	0.86%
23) Difflugia globula	4	2.11	1.04%	4	2.21	1.09%	9	6.34	2.04%	4	2.44	1.20%
24) Difflugia oblonga "glans"	38	20.00	2.90%	39	21.55	3.06%	23	16.20	3.09%	27	16.46	2.90%
25) Difflugia oblonga "lanceolata"	0	0.00	0.00%	2	1.10	0.78%	0	0.00	0.00%	3	1.83	1.05%
26) Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
28) Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	1	0.70	0.70%	0	0.00	0.00%
29) Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
30) Difflugia oblonga "tenuis"	6	3.16	1.27%	7	3.87	1.43%	11	7.75	2.24%	6	3.66	1.47%
31) Difflugia amphora	26	13.68	2.49%	29	16.02	2.73%	25	17.61	3.20%	28	17.07	2.94%

Table 4.5: Seasonality raw data from Syncrude Demo Pond, August 2008.

	BPIT1-A			BPIT2-A			BPIT3-A		
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Erro	Tests counted	% Abundance	Standard Erro
1) Arcella vulgaris	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
2) Centropyxis aculeata	0	0.00	0.00%	2	1.69	1.19%	0	0.00	0.00%
3) Centorpyxis aculeata "discoides"	14	12.28	3.07%	8	6.78	2.31%	5	3.76	1.65%
4) Centropyxis constricta "aerophila"	4	3.51	1.72%	11	9.32	2.68%	21	15.79	3.16%
5) Centropyxis constricta "constricta"	0	0.00	0.00%	0	0.00	0.00%	3	2.26	1.29%
6) Centropyxis constricta "spinosa"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
7) Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
8) Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
10) Pontigulasia compressa	0	0.00	0.00%	32	27.12	4.09%	6	4.51	1.80%
11) Cucurbitella tricuspis	22	19.30	3.70%	14	11.86	2.98%	29	21.80	3.58%
12) Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
13) Difflugia protaeiformis "claviformis"	0	0.00	0.00%	8	6.78	2.31%	0	0.00	0.00%
15) Difflugia protaeiformis "acuminata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
16) Difflugia bidens	3	2.63	1.50%	0	0.00	0.00%	0	0.00	0.00%
17) Difflugia corona	3	2.63	1.50%	10	8.47	2.56%	11	8.27	2.39%
18) Difflugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
19) Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
20) Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
21) Difflugia urceolata "urceolata"	5	4.39	1.92%	0	0.00	0.00%	0	0.00	0.00%
22) Difflugia urceloata "elongata"	7	6.14	2.25%	0	0.00	0.00%	0	0.00	0.00%
23) Difflugia globula	3	2.63	1.50%	0	0.00	0.00%	0	0.00	0.00%
24) Difflugia oblonga "glans"	36	31.58	4.35%	24	20.34	3.71%	27	20.30	3.49%
25) Difflugia oblonga "lanceolata"	14	12.28	3.07%	4	3.39	1.67%	4	3.01	1.48%
26) Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
28) Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	8	6.02	2.06%
29) Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
30) Difflugia oblonga "tenuis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
31) Difflugia amphora	3	2.63	1.50%	5	4.24	1.85%	19	14.29	3.03%

Table 4.6: Seasonality raw data from Syncrude Demo Pond, September 2008.

	BPIT1-S			BPIT2-S			BPIT3-S		
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error
1) Arcella vulgaris	1	0.95	0.95%	0	0.00	0.00%	1	0.79	0.79%
2) Centropyxis aculeata	2	1.90	1.33%	0	0.00	0.00%	1	0.79	0.79%
3) Centorpyxis aculeata "discoides"	7	6.67	2.43%	4	1.91	0.95%	16	12.70	2.97%
4) Centropyxis constricta "aerophila"	8	7.62	2.59%	15	7.18	1.79%	9	7.14	2.29%
5) Centropyxis constricta "constricta"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
6) Centropyxis constricta "spinosa"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
7) Lesquereusia spiralis	4	3.81	1.87%	2	0.96	0.67%	1	0.79	0.79%
8) Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
10) Pontigulasia compressa	22	20.95	3.97%	77	36.84	3.34%	31	24.60	3.84%
11) Cucurbitella tricuspis	6	5.71	2.27%	7	3.35	1.24%	11	8.73	2.51%
12) Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
13) Difflugia protaeiformis "claviformis"	7	6.67	2.43%	13	6.22	1.67%	5	3.97	1.74%
15) Difflugia protaeiformis "acuminata"	5	4.76	2.08%	2	0.96	0.67%	2	1.59	1.11%
16) Difflugia bidens	2	1.90	1.33%	5	2.39	1.06%	3	2.38	1.36%
17) Difflugia corona	6	5.71	2.27%	10	4.78	1.48%	0	0.00	0.00%
18) Difflugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
19) Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
20) Difflugia urens	0	0.00	0.00%	1	0.48	0.48%	0	0.00	0.00%
21) Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
22) Difflugia urceloata "elongata"	14	13.33	3.32%	18	8.61	1.94%	33	26.19	3.92%
23) Difflugia globula	0	0.00	0.00%	2	0.96	0.67%	0	0.00	0.00%
24) Difflugia oblonga "glans"	11	10.48	2.99%	25	11.96	2.24%	7	5.56	2.04%
25) Difflugia oblonga "lanceolata"	0	0.00	0.00%	0	0.00	0.00%	2	1.59	1.11%
26) Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
28) Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
29) Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
30) Difflugia oblonga "tenuis"	0	0.00	0.00%	10	4.78	1.48%	4	3.17	1.56%
31) Difflugia amphora	10	9.52	2.86%	18	8.61	1.94%	0	0.00	0.00%

Table 4.7: Natural Alberta aquatic environments, sampled in 2008.

	Baptiste Lake			Gregoire Lake			Island Lake			Lac St. Anne			Wabamun Lake		
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Erro
Arcella vulgaris	0	0.00	0.00%	7	6.42	2.35%	0	0.00	0.00%	4	1.16	0.57%	6	1.05	0.42%
Centropyxis aculeata	1	0.68	0.67%	10	9.17	2.76%	0	0.00	0.00%	6	1.73	0.70%	14	2.44	0.64%
Centorpyxis aculeata "discoides"	20	13.51	2.81%	0	0.00	0.00%	24	13.48	2.56%	51	14.74	1.91%	64	11.15	1.31%
Centropyxis constricta "aerophila"	50	33.78	3.89%	0	0.00	0.00%	39	21.91	3.10%	92	26.59	2.38%	22	3.83	0.80%
Centropyxis constricta "constricta"	11	7.43	2.16%	27	24.77	4.13%	0	0.00	0.00%	0	0.00	0.00%	56	9.76	1.24%
Centropyxis constricta "spinosa"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	3	0.52	0.30%
Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Pontigulasia compressa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	8	2.31	0.81%	12	2.09	0.60%
Cucurbitella tricuspis	25	16.89	3.08%	13	11.93	3.10%	17	9.55	2.20%	69	19.94	2.15%	38	6.62	1.04%
Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	4	0.70	0.35%
Difflugia protaeiformis "claviformis"	0	0.00	0.00%	0	0.00	0.00%	2	1.12	0.79%	0	0.00	0.00%	10	1.74	0.55%
Difflugia protaeiformis "acuminata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia bidens	3	2.03	1.16%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	6	1.05	0.42%
Difflugia corona	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	11	1.92	0.57%
Diffugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urceolata "urceolata"	0	0.00	0.00%	7	6.42	2.35%	0	0.00	0.00%	2	0.58	0.41%	0	0.00	0.00%
Difflugia urceloata "elongata"	7	4.73	1.74%	0	0.00	0.00%	5	2.81	1.24%	0	0.00	0.00%	4	0.70	0.35%
Difflugia globula	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga	11	7.43	2.16%	39	35.78	4.59%	18	10.11	2.26%	73	21.10	2.19%	184	32.06	1.95%
Difflugia oblonga "lanceolata"	20	13.51	2.81%	0	0.00	0.00%	68	38.20	3.64%	41	11.85	1.74%	105	18.29	1.61%
Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "oblonga"	0	0.00	0.00%	6	5.50	2.18%	0	0.00	0.00%	0	0.00	0.00%	17	2.96	0.71%
Difflugia oblonga "tenuis"	0	0.00	0.00%	0	0.00	0.00%	5	2.81	1.24%	0	0.00	0.00%	18	3.14	0.73%
Difflugia amphora	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%

Table 4.7 (con't)

	Buffalo Lake			Chestermere Lake			Eagle Lake			Spray Lakes Reservoir			Islet Lake		
	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error	Tests counted	% Abundance	Standard Error
Arcella vulgaris	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Centropyxis aculeata	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	11	7.91	2.29%
Centorpyxis aculeata "discoides"	22	17.74	3.43%	0	0.00	0.00%	2	1.60	1.12%	31	28.97	4.39%	31	22.30	3.53%
Centropyxis constricta "aerophila"	36	29.03	4.08%	0	0.00	0.00%	2	1.60	1.12%	45	42.06	4.77%	51	36.69	4.09%
Centropyxis constricta "constricta"	0	0.00	0.00%	0	0.00	0.00%	4	3.20	1.57%	0	0.00	0.00%	0	0.00	0.00%
Centropyxis constricta "spinosa"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Pontigulasia compressa	0	0.00	0.00%	7	5.98	2.19%	3	2.40	1.37%	0	0.00	0.00%	0	0.00	0.00%
Cucurbitella tricuspis	13	10.48	2.75%	53	45.30	4.60%	36	28.80	4.05%	4	3.74	1.83%	11	7.91	2.29%
Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia protaeiformis "claviformis"	0	0.00	0.00%	0	0.00	0.00%	7	5.60	2.06%	0	0.00	0.00%	0	0.00	0.00%
Difflugia protaeiformis "acuminata"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia bidens	8	6.45	2.21%	6	5.13	2.04%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia corona	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Diffugia fragosa	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urens	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%	7	5.60	2.06%	0	0.00	0.00%	0	0.00	0.00%
Difflugia urceloata "elongata"	0	0.00	0.00%	6	5.13	2.04%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia globula	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga	16	12.90	3.01%	20	17.09	3.48%	46	36.80	4.31%	10	9.35	2.81%	14	10.07	2.55%
Difflugia oblonga "lanceolata"	23	18.55	3.49%	25	21.37	3.79%	0	0.00	0.00%	17	15.89	3.53%	21	15.11	3.04%
Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "bryophila"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "tenuis"	6	4.84	1.93%	0	0.00	0.00%	18	14.40	3.14%	0	0.00	0.00%	0	0.00	0.00%
Difflugia amphora	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%	0	0.00	0.00%

Table 4.7 (con't)

	Jasper (ponded glacial melt)			Miquelon Lake		
	Tests counted	% Abundance	Standard Erro	Tests counted	% Abundance	Standard Erro
Arcella vulgaris	0	0.00	0.00%	0	0.00	0.00%
Centropyxis aculeata	21	20.79	4.04%	83	29.75	2.74%
Centorpyxis aculeata "discoides"	31	30.69	4.59%	0	0.00	0.00%
Centropyxis constricta "aerophila"	33	32.67	4.67%	33	11.83	1.93%
Centropyxis constricta "constricta"	0	0.00	0.00%	0	0.00	0.00%
Centropyxis constricta "spinosa"	0	0.00	0.00%	0	0.00	0.00%
Lesquereusia spiralis	0	0.00	0.00%	0	0.00	0.00%
Heliopera sphagni	0	0.00	0.00%	0	0.00	0.00%
Pontigulasia compressa	0	0.00	0.00%	0	0.00	0.00%
Cucurbitella tricuspis	0	0.00	0.00%	2	0.72	0.51%
Lagenodifflugia vas	0	0.00	0.00%	0	0.00	0.00%
Difflugia protaeiformis "claviformis"	0	0.00	0.00%	0	0.00	0.00%
Difflugia protaeiformis "acuminata"	0	0.00	0.00%	0	0.00	0.00%
Difflugia bidens	0	0.00	0.00%	0	0.00	0.00%
Difflugia corona	0	0.00	0.00%	0	0.00	0.00%
Diffugia fragosa	0	0.00	0.00%	0	0.00	0.00%
Difflugia bacillaliarum	0	0.00	0.00%	0	0.00	0.00%
Difflugia urens	0	0.00	0.00%	1	0.36	0.36%
Difflugia urceolata "urceolata"	0	0.00	0.00%	0	0.00	0.00%
Difflugia urceloata "elongata"	0	0.00	0.00%	0	0.00	0.00%
Difflugia globula	0	0.00	0.00%	13	4.66	1.26%
Difflugia oblonga	16	15.84	3.63%	124	44.44	2.97%
Difflugia oblonga "lanceolata"	0	0.00	0.00%	0	0.00	0.00%
Difflugid oblonga "linearis"	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "bryophila"	0	0.00	0.00%	23	8.24	1.65%
Difflugia oblonga "oblonga"	0	0.00	0.00%	0	0.00	0.00%
Difflugia oblonga "tenuis"	0	0.00	0.00%	0	0.00	0.00%
Difflugia amphora	0	0.00	0.00%	0	0.00	0.00%

Appendix 5

Chi-Square comparison of site 2 and site 2-2.

```
Skipping rows and/or columns filled with zeros.


Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

          C1      C2  Total
    1      2       7      9
        3.85    5.15
       0.886   0.661

    3     23      28     51
       21.79   29.21
       0.067   0.050

    4     45      34     79
       33.76   45.24
       3.742   2.792

    5     10      10     20
        8.55   11.45
       0.247   0.184

    9     20      55     75
       32.05   42.95
       4.531   3.382

Total    100     134    234

Chi-Sq = 16.542, DF = 4, P-Value = 0.002
1 cells with expected counts less than 5.
```

Appendix 6

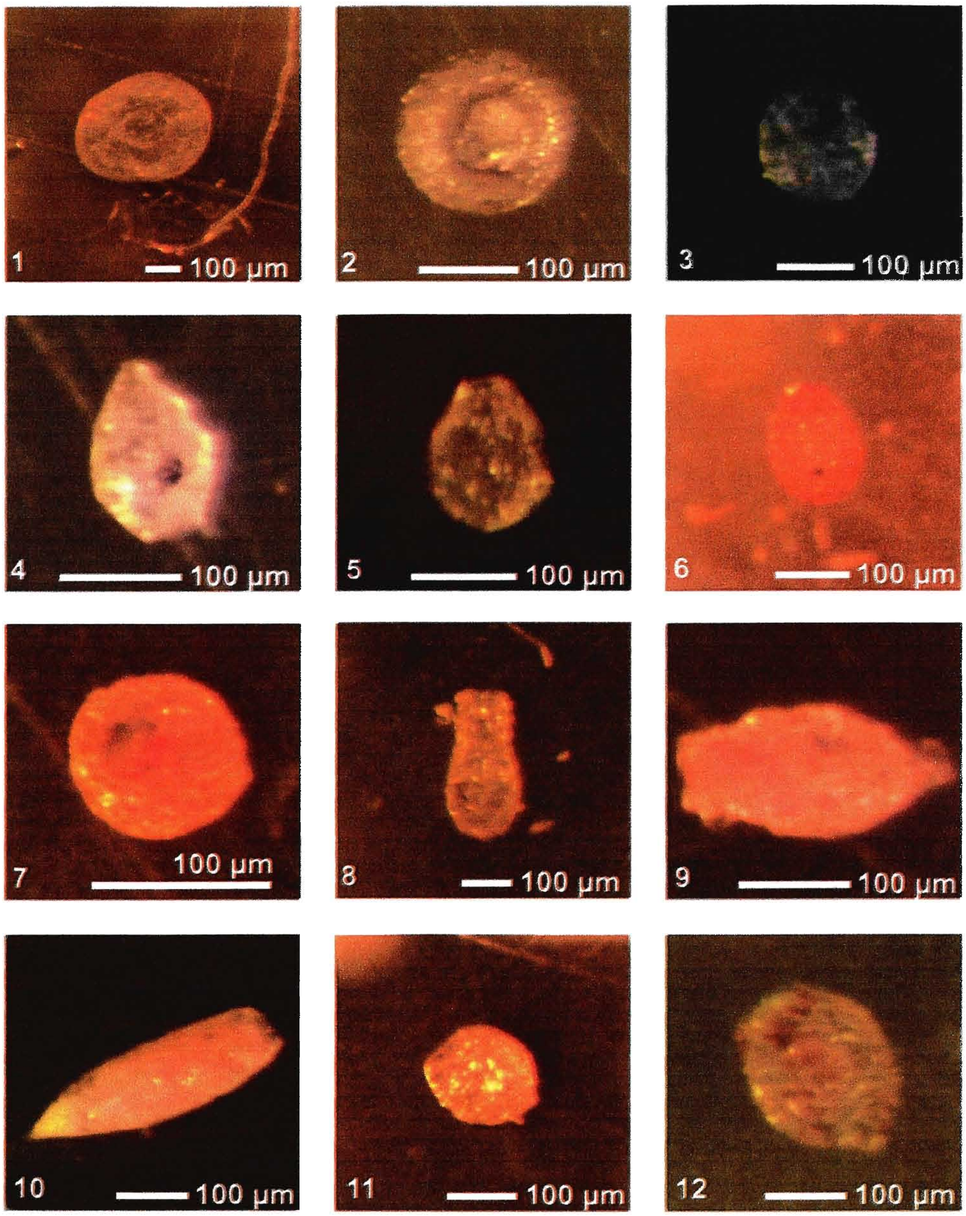


PLATE 1
Identification primarily using Kumar and Dalby (1998). **1** *Arcella vulgaris* Ehrenberg, 1930, 363 µm. **2** *centropyxis aculeate* "disoides" (Ehrenberg, 1832), 176µm. **3** *Centropyxis constricta* "aerphila" (Ehrenberg, 1843), 154 µm. **4** *Centropyxis constricta* "constricta" (Ehrenberg, 1843), 103 µm. **5** *Pontigulasia compressa* (Carter, 1864), 111 µm. **6** *Cucurbitella tricuspis* (Carter) Medioli et al., 1987, 120 µm. **7** *C. tricuspis*, apertural view, 97 µm. **8** *Lagenodifflugia vas* (Leidy, 1874), 105 µm. **9** *Difflugia protaeiformis* "amphoralis" Lamarck, 1816, 125 µm. **10** *Difflugia protaeiformis* "acuminata" Lamarck, 1816, 115 µm. **11** *Difflugia corona* Wallich, 1864, 153 µm. **12** *Difflugia bacillariarum* Perty, 1849, 173 µm.

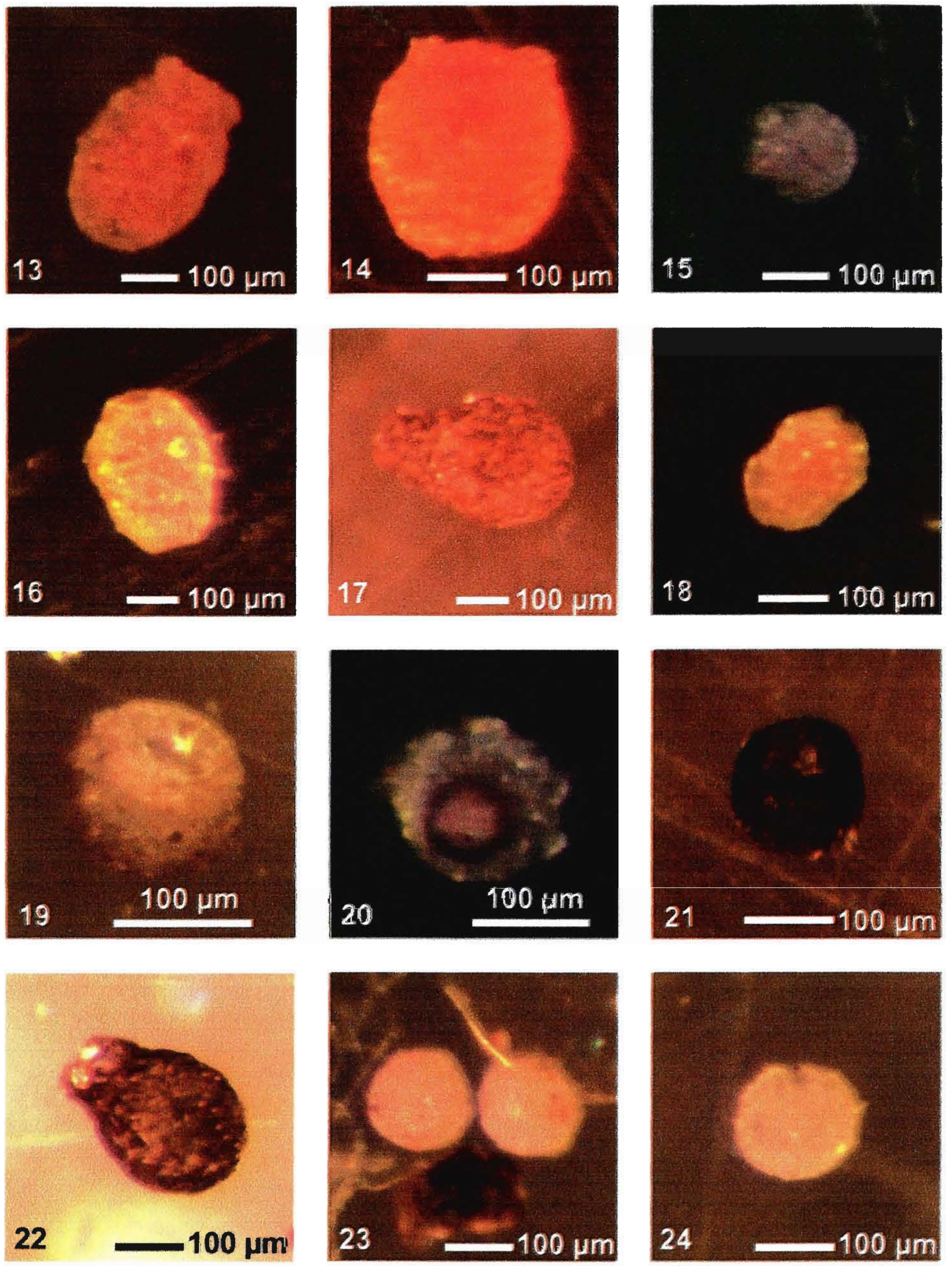


PLATE 2
Identification primarily using Kumar and Dalby (1998). **13** *Difflugia urceolata* "urceolata" Carter, 1864, 230 µm. **14** *Difflugia urceolata* "elongata" Carter, 1864, 246 µm. **15** *Difflugia globulus* (Ehrenberg, 1848), 166 µm. **16** *Difflugia oblonga* "glans" Ehrenberg, 1832, 225 µm. **17** *Difflugia oblonga* "tenuis" Ehrenberg, 1832, 238 µm. **18** unknown difflugiid species, 129µm. **19** *Centropyxis constricta* (Ehrenberg, 1843), side view, with black grains on the fundus, 140µm. **20** *Centropyxis constricta* (Ehrenberg, 1843), with black apertural grains, 118µm. **21** *Centropyxis constricta* (Ehrenberg, 1843), with black grains, 142µm. **22** *Difflugia oblonga* Ehrenberg, 1832, with black grains, 200 µm. **23** Cyst (x2), 148 µm. **24** Cyst (x2), 148 µm.