# TESTATE AMOEBAE (THECAMOEBIAN) BASED RECONSTRUCTION OF LAKE SIMCOE FRINGE WETLAND PALEOENVIRONMENTS

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#### Abstract

Lake Simcoe, Ontario, Canada, a large southern Ontario glacial lake, has been affected by multiple environmental stressors throughout its history. This study uses testate amoebae paleodistribution to reconstruct the environmental conditions of Lake Simcoe's fringe wetlands before and during the Anthropocene.

Testate amoebae are unicellular shelled protists that inhabit almost every environment in the world from sandy marine beaches to all freshwater and terrestrial habitats.

Their rapid asexual reproduction makes testate amoebae sensitive indicators of short-lived environmental change.

Paleolimnological and paleoecological investigations using testate amoebae have been used to describe hydrology, pH, trophic status, land use, climate change, forest fires, oxygen concentrations, metal contamination and other variables.

Using compound microscopy techniques, testate amoebae assemblages were identified and tabulated from processed sedimentary cores extracted from fringe wetlands in Lake Simcoe and Tub Lake, with the latter lake used as a reference to assess anthropogenic impact during the Anthropocene. One core, taken from the outlet area of Lake Simcoe, was used to reconstruct the past variability of the paleoenvironment while

all four cores were used to reconstruct Anthropocene paleoenvironments.

Prior to European settlement three distinct testate amoebae communities inhabited the Victoria Point wetland area based on hydrologic variability: an arctic-like, oligotrophic testate amoebae community dominated by alkaline, lacustrine species; a CaCo<sub>3</sub>-based community dominated by mesotrophic calcipiles; and an "early fringe wetland community" dominated by aquatic and soil-based species.

The anthropogenic activities of the last ~200 years lead to a rapid paludification of the shoreline in all Lake Simcoe wetland locations. Anthropogenic activities have also produced a constant state of disturbance along the shoreline where the mean rate of change in the testate amoebae community since ~AD 1300 (0.46) is greater than the rate of change in the preceding record (0.40). Some of this change is also attributable to recent climate change particularly in the winter and spring, and possibly to the introduction of the Zebra mussel in the mid 1990s.

While similarities in the response of testate amoebae assemblages across wetlands do exist, this study has also found that local activities have affected testate amoebae assemblage paleodistributions, species richness and diversity.

# Lay Summary

Testate amoebae were used as a proxy to reconstruct Lake Simcoe fringe wetland paleoenvironments with the objectives of elucidating any possible changes to Lake Simcoe fringe wetland biological communities over time and to provide an assessment of any long-term changes if found. This research contributed to greater knowledge of the use of testate amoebae as a proxy in fringe wetlands, the diversity of testate amoebae, and the distribution and abundance of testate amoebae across time and space.

Testate amoebae were found to be a good proxy for fringe wetland environmental conditions, indicating both lacustrine and peat-based environments as well as changes in hydrology, pH, and temperature. The diversity and abundance of testate amoebae paleodistribution was found to increase through time at all locations commensurate with paludification and nutrient enrichment of the sampling sites. Testate amoebae community distribution was also found to be sensitive to various and unique anthropogenic and natural perturbations including land clearance, water level stabilization, rapid increases in precipitation, nutrient enrichment, and climate change.

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# Testate Amoebae species abbreviations

Abbr Species	Abbr Species
AA - Arcella arenaria	DPU - Difflugia pulex
AC - Arcella catinus	DR - Difflugia rubescens
ACR - Arcella crenulata	DS - Difflugia styla
AD - Arcella dentata	DY - Difflugia yorkui
ADI - Arcella discoides	DV - Difflugia viscidula
AE - Arcella excavata	EC - Eugylpha compressa
AG - Arcella gibbosa	EF - Euglypha filifera
AH - Arcella hemispherica	EL - Euglypha laevis
AI - Arcella intermedia	ER - Eugylpha rotunda
AME - Arcella megastoma	ES - Euglypha stringosa
AR - Arcella robusta	ET - Eugylpha tuberculata
AV - Arcella vulgaris	HE - Hyalosphenia elegans
AS - Assulina seminulum	HP - Hyalosphenia papilio
CE - Cathrulina elegans	HPU - Hyalosphenia punctata
CL - Cyphoderia laevis	HPE - Hyalosphenia petricola
CM - Clypeolina marginata	HPA - Hyalosphenia petricola var. amethystea
CAC - Centropyxis aculeata	HS - Hyalosphenia subflava
CAE - Centropyxis aerophila	HSP - Heleopera sphangi
CCO - Centropyxis constricta	LV - Lagendifflugia vas
CD - Centropyxis delicatula	LE - Lesquereusia epistenium
CDI - <i>Centropyxis discoides</i> (aculeata strain)	LM - Lesquereusia modesta
CEC - Centropyxis ecornis	LS - Lesquereusia spiralis
CO - Centropyxis orbicularis	NB - Nebela barbata
CP - Centropyxis platystoma	NC - Nebela collaris

CS - Centropyxis spinosa (aculeata strain)	NG - Nebela galeata
CEU- Cyclopyxis eurystoma	NGR - Nebela gracilis
CK - Cyclopyxis kahli	NO - Netzelia oviformis
CI - Cyclopyxis intermedia	PSpp - Penardiophrys spp.
COV - Cryptodifflugia oviformis	PF - Pseudodifflugia fulva
CSSp - Cryptodifflugia spp.	PC - Pyxidicula cymbalum
CSA - Cryptodifflugia sacculus	PO - Pyxidicula operculata
CCR - Cryptodifflugia crenulata	PP - Pyxidicula patens
DSpp - Difflugia spp.	PA - Phryganella acropodia
DA - Difflugia acuminata	PI - Paraquadrula irregularis
DB - Difflugia biddens	PG - Physochila griseola
DBR - Difflugia bryophila	PASPP - Paraeuglypha spp.
DG - Difflugia glans	QS - Quadrella symmetrica
DGL - Difflugia globulosa	SL - Sphenoderia lenta
DL - Difflugia lancelota	TE - Trinema enchelys
DLI - Difflugia <i>lithophilia</i>	TL - Trinema lineare
DLU - Difflugia lucida	TA - Trigonopyxis arcula
DLIT - Difflugia lithoplates	TD - Tracheuglypha dentata
DO - Difflugia oblonga	

### Chapter 1. Introduction and Literature Review

## 1.1 Aquatic ecosystems and multiple stressors

#### 1.1.1 <u>Freshwater Lakes</u>

Multiple stressors on freshwater ecosystems are having deleterious effects on global water availability and quality through changes in climate, land use, hydrology, nutrient cycling, pH, and biota (Heathwaite 2010; Davis et al. 2010; Kolar and Lodge 2000). By far, the accelerated nutrient enrichment of freshwater bodies through anthropogenic activities, termed cultural eutrophication, is the most ubiquitous effect with nearly 50% of the world's freshwater being found in a eutrophic state (Ansari et al. 2011). In addition to the major influence of changing nutrient cycles on the health of freshwater bodies, cumulative impacts of multiple stressors are complex and unpredictable posing continuous challenges to research and management of these systems (Strayer 2010; Christensen et al. 2006; Schindler 2001).

Canadian lakes, mainly in the highly populated 300km wide band that borders the United States, have been subjected to multiple anthropogenic stressors over the last ~200 years also known as the "Anthropocene" (Crutzen 2002). They have been dammed, impounded, and diverted. Their biota and that of their

watersheds have been exploited and they have been polluted by airborne contaminants, livestock, industrial and human waste, and agricultural fertilizers (Schindler 2001). With the addition of climate change, Canadian lakes are undergoing degradation "on a scale that was not comprehensible to the average Canadian at the end of the twentieth century" (Schindler 2001).

Much of the freshwater ecosystem degradation is a result of climate altered hydrology including increased water temperature and changing patterns of stratification, precipitation, evaporation, flow, snow-pack, ice-melt, and storm events (Schindler 2001; Yan et al. 1996). A decline in water flow, coupled with increased residence time, evaporation, diffuse nutrient loading, and thermal stratification is resulting in a rise in nutrient concentrations, exacerbating eutrophication and altering the frequency and magnitude of cyanobacterial blooms (Moss et al. 2011; O'Neil et al. 2011; Schindler 2001).

Climate change is also intensifying the effects of nonnative species on aquatic ecosystems. 75% of non-native species in the Great Lakes are of Ponto-Caspian origin giving them a distinct competitive advantage over native species as temperatures increase, and nutrient and precipitation cycles are altered (EPA 2008; Rahel and Olden 2008; Schindler 2001). Climate change is also enabling rapid range expansion and large increases in the biomass of non-native species (EPA 2008).

#### 1.1.2 Freshwater wetlands and shallow lakes

Somewhat unique effects of multiple stressors are also being noted in wetlands and shallow lakes worldwide. The greatest concern for the health of these systems under climate change scenarios are shifts in hydrological regimes leading to altered geochemistry and ecological functioning (Hobbs et al. 2012; Mortsch 1998; Cox and Campbell 1997; Mortsch and Quinn 1996; Schindler et al. 1996).

Under such stress, shallow lakes and wetlands can exhibit "catastrophic shifts" described by the alternative stable state theory (Bayley and Prather 2003; Gundersen 2000; May 1977; Holling 1973). For shallow lakes, two alternative stable states are understood to exist - one dominated by clear water and rooted macrophytes, and the other by turbid water and planktonic algae (Gundersen 2000). Transitions between the states are "thought to center around the interaction between submerged vegetation and turbidity" which is ultimately controlled by nutrient availability (Scheffer et. al 1993). Freshwater wetland stable states are also controlled by

nutrient availability where an increase in soil nutrient content is characterized by a shift in dominant emergent plant species, usually following a disturbance (Gundersen 2000).

Bayley and Prather (2003) noted that the stable state of shallow wetland lakes undisturbed by anthropogenic activities was controlled primarily by nutrient status but depth, surface area and nitrogen concentrations may also play a role.

#### 1.1.3 Multiple stressors in Ontario, Canada

Recently, Hadley et al. (2012) sampled 53 lakes with variable anthropogenic disturbance, previously studied by Hall and Smol (1996), to assess changes in 14 water quality variables between 1990-1992 and 2007-2008. The authors found that despite impacts of previous and ongoing anthropogenic stress to the south-central Ontario lakes (acidification and nutrient enrichment from industry and development), the overarching driver of diatom community change was climate-related (longer ice-free season, longer growing season, and enhanced lake stratification).

Ontario has a history of numerous aquatic invasions of non-native species, mainly in the Great Lakes Basin. As of 2009, 186 non-native species were present in the basin (OMNR 2013), a situation referred to as a "fish zoo" by Schindler (2001). The introduction or expansion of many species, such as the smallmouth bass (*Micropterus dolomieu*), have had negative

impacts on native aquatic communities (Vander Zanden and Olden 2008; OMNR 2012). A recent invader, the Zebra Mussel (Dreissena polymorpha), was first discovered in Lake St. Clair (42.445755, -82.654481) in 1988 and is thought to have originated from the ballast water of an ocean going ship (OMNR 2013). Since 1988, they have spread throughout the Great Lakes, the Trent Severn waterway, the Rideau Canal, and many other inland Ontario lakes and rivers (OMNR 2013). D. polymorpha is considered the most aggressive freshwater invader worldwide, rapidly colonizing and populating waters with biomass exceeding 10 times that of native invertebrates (GISD 2013). The impacts of D. polymorpha are extensive: it is an "ecosystem engineer" that alters phytoplankton communities, benthic communities, habitats, and invertebrate competition (GISD 2013). They are also responsible for threats to endangered species and significant economic costs are associated with their control (GISD 2013; OMNR 2013).

#### 1.2 Study system: Lake Simcoe, Ontario

#### 1.2.1 Lake and Watershed Properties

Lake Simcoe (44.440153,-79.358422) (Figure 1) is the sixth largest inland lake in Ontario with a total watershed area of  $3634 \, \mathrm{km^2}$  (2912km² terrestrial and  $722 \, \mathrm{km^2}$  lake surface), a perimeter of 303km, and an approximate volume of 11 x 109

m³(Palmer et al. 2011; Stainsby et al. 2011; Eimers et al. 2005). Part of the Trent-Severn waterway, Lake Simcoe is a hard-water, dimictic, and relatively shallow lake (mean depth 16m) (Figure 2), with the nearshore zone (0-20m) covering 67% of the lake area (Ginn 2011). Rocky substrates predominate in shallow areas of the nearshore zone (<8-9m) but sandy areas and finer sediments occur as well (Ozersky et al. 2011). Soft sediments are found at littoral depths greater than 8-9m (Ozersky et al. 2011), as well as in the deeper zones of the lake (Todd et al. 2007).

Overall, the lake is divided into three basins: the oligomesotrophic main basin in the northeast (area 643km², mean depth 14m, maximum depth 33m), mesotrophic Cook's Bay in the south (area 44km², mean depth 13m, maximum depth 15m), and oligo-mesotrophic Kempenfelt Bay in the west (area 34km², mean depth 20m, maximum depth 42m) (Palmer et al. 2011; Ozersky et al. 2011). 35 tributaries drain the terrestrial watershed, with five (Beaver, Black, Talbot, and Holland rivers and Pefferlaw Brook) contributing 60% of the total drainage area (Eimers et. al 2011).

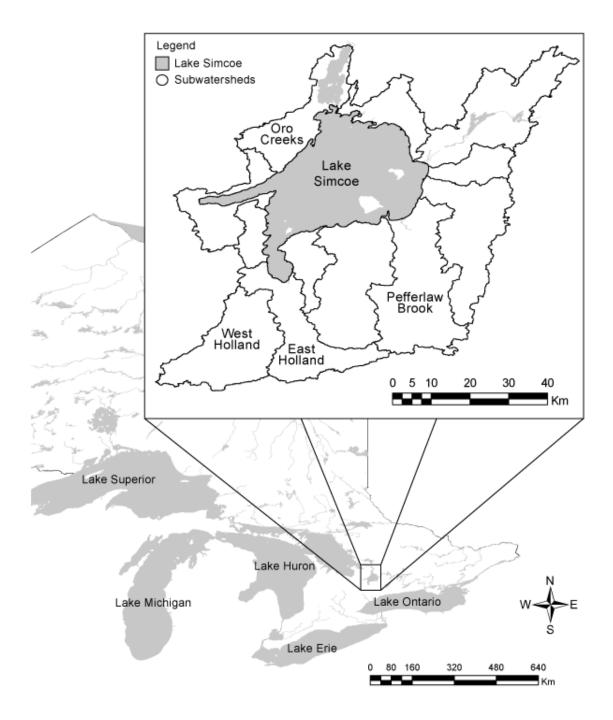


Figure 1: Lake Simcoe watershed. (OMNR 2010a, 2012b, 2008, 2006 and DMTI 2012; ESRI 2013; Adobe 2013)

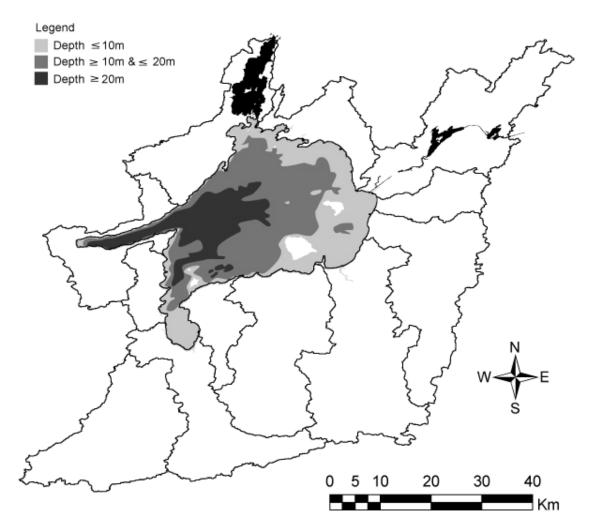


Figure 2. Simplified Lake Simcoe bathymetry. (Adapted from Baird & Associates 2006; Adobe 2013)

## 1.2.2 <u>Lake and watershed geology</u>

Lying within the western portion of the St. Lawrence lowlands, Lake Simcoe is surrounded by clay and sand plains deposited from proglacial lakes and ponds that formed about 11,200 BP by the retreat of the Laurentide ice sheet (Todd et al. 2004; Jackson et al. 2000; Johnson 1997). Wisconsinan

deposits of undifferentiated till, glaciofluvial outwash, and glaciolacustrine sediments form a thin layer over the eastern and northern portions of the watershed and cover the bedrock in the western and southern portions (Todd et al. 2004; Johnson 1997).

Beneath the majority of glacial deposits lies Middle
Ordovician limestone with a small northern portion of the
watershed overlying Precambrian metasedimentary and gneissic
rock characteristic of Canadian Shield geology directly to the
north of the watershed (Todd et al. 2004; Johnson 1997). The
southern portion of the lake is bounded by the Oak-Ridges
Moraine formed of glaciofluvial and glaciolacustrine deposits
overlying drumlins created from Newmarket till deposited by
the Ontario lobe of the Laurentian ice sheet (Barnett et al.
1998; Gwynn and DiLabio 1973).

The in-lake sediments of Lake Simcoe are divided into four seismostratigraphic sequences: post-glacial, late-glacial, glaciofluvial, and Newmarket Till (Todd and Lewis 2007). The most recent post-glacial (Holocene) sediments are up to 8m thick in some areas and do not extend over the entire lake. Underlying post-glacial sediments, late-glacial sediments of up to 30m thick extend over the entire lake and were likely deposited during the occupation of the basin by Lake

Algonquin. Underlying late-glacial sediments are rapidly deposited glaciofluvial sediments. These sediments are widespread in the lake and are generally less than 10m thick. The final sequence, Newmarket Till, underlies the glaciofluvial sediments and is characterized by 10-30m high peaks which are also seen in drumlin fields mapped around Lake Simcoe. The Newmarket Till layer was recorded in all lake areas except Cook's Bay and Kempenfelt Bay but this is likely due to the thickness of overlying sediment (Todd and Lewis 2007).

#### 1.2.3 <u>Prehistoric use</u>

The use of the Lake Simcoe watershed began approximately 11,000 years ago in the Paleoindian time period (Table 1) by First Nation groups settling along tundra-like "glacial lake strand lines" after the formation of Lake Algonquin which encompassed the Lake Simcoe watershed from ~11,200 BP to ~10,400 BP (Jackson et al. 2000; Storck 1984). Early settlements were situated to the east and south of the current Lake Simcoe margins (Storck 1984). A large number of archaeological sites excavated in the eastern Simcoe Lowlands were found to be mostly small, seasonal settlements (Storck 1984). A number of other sites of similar and larger size were located in the vicinity of the Lower and Upper Holland River

(Stewart 2004).

As the Simcoe Lowlands drained due to reduced water levels in Lake Algonquin, likely caused by the isostatic rebound of the lake's outlets, a new time period known as the Archaic began (Table 1) (Digging Ontario n.d.). Warming temperatures of the Archaic period in southern Ontario brought deciduous forests and an increasingly diverse flora and fauna to the region (OAS n.d.). Early and Middle Archaic sites (Table 1) were located near the Lower Holland River and in the southwestern portion of the watershed. The settlements mark the start of a shift in settlement location over time from southerly to north-westerly direction. The shift is thought to be an artifact of changing shorelines as warming temperatures proceeded or a reduced reliance on wetland resources over time (Stewart 2004). It was during the Late Archaic (Table 1) that the fish weirs at the Atherley Narrows (Lake Simcoe outlet) were constructed (OAS n.d.; Swayze 1983).

The next time period, known as the Woodland, spanned from ~3000 BP to European contact (Table 1). Rough population estimates for the hunter-gatherers of the Early/Middle Woodland time period in southern Ontario are between 2,000 to 2,500 people (Warrick 1990). A greater number of archaeological sites dated (Table 1) to the Middle Woodland

period suggested that a population increase occurred from the Late Archaic to the Early and then Middle Woodland time periods. However, Warrick (1990) found no evidence of such a population increase. In the Simcoe area, just one band of approximately 450 people was clustered in the Eastern Lake Simcoe/Victoria County area.

It was in the later part the Woodland period that the Wendat (Huron) peoples living in horticultural settlements inhabited Lake Simcoe's watershed in the area known as Wendake ("the land surrounded by water") (Ramsden 1996; Innisfil Library n.d.). Wendake was bounded by Georgian Bay to the west, Lake Simcoe to the east, Precambrian shield to the north, and the Oak Ridges Moraine to the south. While the construction of the Atherley Narrows fish weirs are attributed to the Wendat peoples (Ontarioplaques.com n.d.), over a century of archaeological research has failed to uncover a Wendat settlement pre-dating ~ AD 1300 in Simcoe County (Warwick 2008, 1990). The only Wendat village of the Uren Phase (Table 1) of the Late Woodland period was located at the tip of Kempenfelt Bay. The village was 0.01km<sup>2</sup> and roughly supported a population of 400-500 people (Warrick 2007; Sioui 1999).

The Middleport Phase (Table 1) of the Late Woodland period

witnessed a Wendat population explosion from approximately 11,000 to 29,000 people (Warrick 1990). Most of the increased population is believed to have occurred in Simcoe County around the initial Uren settlement. The presence of twelve villages, including the Uren settlement, have been confirmed (Warrick 1990). Villages in the Middleport period doubled in size from the Uren, holding approximately 1000 people each (Warrick 1990).

By the Late Contact period (AD 1475), Wendat populations stabilized to about 30,000 people and larger settlements of 2,500 appeared (Warrick 1990). Settlements expanded throughout western and northern Simcoe County with some northern settlements clustered between Bass Lake (44.60318,-79.509113) and Lake Simcoe (Warrick 1990).

Between AD 1550 and 1625, Wendat populations remained stable at around 30,000 people. Unlike earlier times where individual populations were divided amongst various small and large settlements, Late Contact populations were aggregated into higher density regional settlements defended by natural and human-made barriers (Warrick 1990).

Between AD 1634 and 1647, Wendat populations dropped to 8,600 through the spread of European disease and possible settlement abandonment. In 1649, the Wendat burned and

abandoned their last 13 villages and the remaining population migrated to French held Christian Island. In 1650-51, after devastating starvation and disease on Christian Island, the surviving 600 Wendat migrated with the Jesuits to Quebec (Warrick 1990).

Table 1. Prehistoric time periods and land use/population.

The second of th						
Time Period	<sup>14</sup> C dates	Use of the watershed	Archaeological sites	Reference		
Paleoindian	12000-	concentrated, small,possib ly seasonal settlements in the southeastern area	Draper, Prideaux locality 14, Deavitt, Boyington, Cowieson, Cryderman, Perry, Sister, McMillan, Zander, Boag, Hunt, Udora, McDonald, Banting, Hussey, Fowler,	Anderson 2013;Carr 2012; Storck 1982,1984 ; Stewart 1984		
Early Archaic	10000-	small, possib ly seasonal settlements in the south and southwestern area	Creek, Muirhead, Goodring, Verkalik, Kilmorlie-	Anderson 2013; Storck 1984,1982 ,1978; Stewart 1984;		
Middle Archaic	8000- 5000	small, possib ly seasonal settlements in the south and southwestern area	Fraser, Gooding, Verkalik, Froud-			
Late Archaic	5000- 3000	small, possib ly seasonal settlements in the south and	Atherley Narrows fish weirs, Muirhead, Kilmorlie, McMillan,	Anderson 2013;OAS n.d.; Stewart 2004		

			southwestern area; meeting place	Aitchison, Boag	
Early W	oodland	3000- 1500	~450people	Eastern Lake Simcoe /Victoria County	Warrick 1990
Middle	Woodland	1500- 1000	~450people; meeting place	Eastern Lake Simcoe /Victoria County; Atherley Narrows	
Late Woodla nd	- 1	1000- 650	meeting place	Atherley Narrows	Warrick 2007; Wright 1972
	Uren	650-620	~450 people	Kempenfelt Bay	Warrick 2007,1990
	Middleport	620-530	~13000 people	Barrie area	Warrick 2007,1990
	Late contact	530-416	~22000 people	North & South Simcoe	Warrick 2007,1990

#### 1.2.4 <u>Historic use</u>

## 1.2.4.1 Initial European and Aboriginal settlement

Human use of the Simcoe area in the transition from prehistory to history after the eradication of the Wendat is not clear. There are reports that Algonkian speaking groups lived or may have lived in the Orillia area in the 17<sup>th</sup> century (Hunter 1909; Ramsden 1988). Hunter (1904) describes 22 occupation sites in Orillia of unknown, but supposed Algonkian (Ojibwe), origin. These sites were never dated. Most of the sites are above the Narrows in the Couchiching basin but a few are located in the Simcoe basin, namely at Shingle Bay, Smiths

Bay, and the west Narrows peninsula (Hunter 1903; Wright 1972). It is believed that the Ojibwe of Georgian Bay and farther north expanded into Southern Ontario in about AD 1701, but there is no specific mention of the Simcoe area (Schmalz 1991; Ontario Heritage Trust 2012). It is not until the naming of the lake (Lake Simcoe) in 1793 by Lieutenant Governor Simcoe as well as the extension of Yonge St. to Holland Landing (1796) that the area reappears in the record (Hunter 1909a).

In 1818, a final treaty for the surrender of lands (644,261 ha) in the Georgian Bay/Simcoe area is signed between several Ojibwe Chiefs and the Crown (Ontario Heritage Trust 2012; Hunter 1909a). It is noted that after the treaty, the Ojibwe under Chief Yellowhead (Musquakie) continue to use their hunting grounds in the Simcoe watershed (Ontario Heritage Trust 2012).

The first wave of European settlers began to arrive to the county in 1819 (Hunter 1909a) although there are indications of limited European occupation dating back to the original settler, a farmer named Smith, in 1794 (Wilson and Ryan 1988). Additional early settlers were Mr. St. George who operated a trading post at the Atherley Narrows from 1802 until 1820, and a "Soldier's Landing" in the east branch of the Holland River

during the war of 1812 (Hunter 1909a; Hunter 1909b). This
Landing was the site of trade between "Indians" and fur
traders and a small town arose from that in 1828 (Hunter
1909b). Holland Landing itself (44.098434,-79.485839) began
with a single residence/mill in 1821 and became a village in
1835 (Hunter 1909b). The main settlers in 1819 were
"fugitives" from Lord Selkirk's Red River Settlement in
Manitoba who began the first community (Scotch Settlement at
44.089743,-79.615529) in West Gwillimbury along the Holland
River (Hunter 1909b).

Settlement progressed quickly, expanding to New Tecumseth (44.152652,-79.858346), Adjala-Tosorontio (44.133928,-79.92907), Innisfil (44.303704,-79.599481), Essa (44.28257,-79.780018), and Penetanguishine (44.769771,-79.936033) in the 1820s. (Hunter 1909b). Population statistics for New Tecumseth indicate 546 people in 1829 (Hunter 1909b). Hunter (1909b) states that the northward spread of settlers was slow due to the "Big Swamp" that cut off communication and access to the south (Hunter 1909b).

In 1820, the township of Oro (44.475931,-79.618707) was surveyed but settlement did not progress until the 1830s.

Hunter (1909b) also mentions the intent of the Government in 1819 to establish a "negro slave settlement" in Oro. This

settlement was eventually created in 1830 on the second line (Wilberforce Street) in Oro. By 1836, the community grew to about 100 residents (York University 2013).

It was also in 1830 that the current Lieutenant Governor, Sir John Colborne, established the Coldwater-Narrows Reserve (10,000 acre narrow strip between Orillia and Matchedash Bay along the old trading route ridge line (now Hwy. 12)) as exclusive lands for three First Nations groups under the leadership of Chief's Yellowhead, Snake, and Aisance (AANDC 2010; Hunter 1909a). Between 1830 and 1832, the bands under Chief's Yellowhead and Snake were settled at the Atherley Narrows while the band under Chief Aisance settled at Coldwater (AANDC 2010). Settlement by both Chief Yellowhead's band and European settlers in the 1830s was mainly north of the ridge line (Hwy. 12) on the shores of Lake Couchiching. It is reported that a house for Chief Yellowhead was built in 1831 on the lot now occupied by the St. James Anglican rectory grounds on Front St. (St. James Anglican Church n.d.; Hunter 1909a). The first European settlers were concentrated in the West St. & Colborne St. area, on the outskirts of the Aboriginal settlement.

In 1834, a few European settlers attempted to establish a town (Innisfallen) at a landing place on Shingle Bay. A few

cabins were built on the shoreline but did not expand beyond that (Hunter 1909b).

In 1836, it is said that the three Chiefs surrendered the Coldwater-Narrows reserve (although this is disputed) and by 1838 had moved to new and old lands on Beausoliel and Snake Islands and at Rama (Hunter 1909a; Ontario Heritage Trust 2012; AADNC 2010).

The town of Orillia remained contained within the North Orillia area (above King Street) until at least 1867 when the town became incorporated (Fowlie 1867). In 1871, the Northern Railway into Orillia was completed following along the shoreline. By 1881, roads had been constructed below Kings Street throughout the South Orillia area (Macdonald 1881).

Settlement of the eastern side of the lake followed a similar trajectory with rare European occupation occurring prior to 1819 (Georgianmaps.ca n.d.), and initial settlement taking place between 1819 and the 1830s. Keswick, Sutton, Pefferlaw, Jackson's Point, Beaverton, and Brechin all experienced their first European settlers within this time period (Town of Georgina n.d.; BDCC 2010; Ramara Historical Society n.d.).

1.2.4.2 Agriculture, Livestock and Lumber

Agriculture was a major occupation of initial settlers in

all areas within Simcoe County from the very beginning (Hunter 1909b). Hunter (1909b) indicates that at least one farmer in the area, specifically West Gwillimbury, was progressive, utilizing newly patented mechanized equipment (McCormick Reaper) on his farm.

Wilson and Ryan (1988) describe three distinct clusters of agricultural land use in the basin: 1801 to 1891(A), 1891-1941(B), and 1941-1981(C). Total agricultural land in cluster A grew rapidly, so that by 1851 and 1881, the percentage of watershed transformed into farmland was 43 and 86% respectively. There was a slight decrease in unimproved farmland in 1891, dropping the total to just about 85%. Cluster B total agricultural land decreased slightly before climbing to it's maximum of 87.9% in 1941. There were decreases in improved farmland and cropland but increases in unimproved farmland during this time. During the cluster C stage, all types of farm land steadily decreased to a total of ~57% of the watershed in 1976. There was a slight increase (~59%) between 1976 and 1981 which is attributed to the switch to corn agriculture in 1961. Within these agricultural totals, specific subwatersheds account for much of the agricultural land use. Between 1891 and 1981, 55 to 70% of the Holland River area, and about 42 to 65% in the Black, Pefferlaw and Beaverton areas was utilized for agriculture (Wilson and Ryan

1988). The poor soils of Northern Lake Simcoe kept most agriculture within the southern area.

Throughout this same period (1801 to 1981), heads of livestock increased almost steadily to a maximum of ~82,000 in 1961 after which the only recorded decline to ~68,000 in 1981 takes place.

Alongside clearing activities for early agricultural use, the Simcoe basin was actively logged in the early years of settlement. Sawmills (Table 2) dotted the landscape beginning in  $\sim$  AD 1825. Between 1825 and  $\sim$ 1830, most sawmills were small, water-powered endeavours constructed in conjunction with grist mills (Hunter 1909b) suggesting local use by pioneer settlers and farmers. Hunter's (1909b) first specific mention of a commercial sawmill was one built by Mark Scanlon in 1832 on what is now known as Scanlon's Creek. Following his commercial success, other entrepreneurs constructed four more mills along the creek (Hunter 1909b). Beginning in the 1850s with the opening of the Northern Railway in 1853 (Hunter 1893; Cooper 2013), a 10 fold increase in logging by large commercial sawmills began (Hunter 1909a). Two of the largest sawmills in the southern Simcoe County were those located at Bradford and Bell Ewart. The mill at Bradford was capable of producing 150,000 board feet per day or 20 million per year

(Hunter 1893) and Bell Ewart 15 million per year (Hunter 1909a). Logging did not reach great importance in north Lake Simcoe and beyond until the waning of timber resources in the south in the 1860s (Hunter 1893) and the extension of the railway to Orillia in 1871. By 1893, most lumber activities had ceased in the watershed (Hunter 1893).

Table 2. Location and dates of Sawmills in Simcoe County.

Area	Location	Date	Quantity	Reference
Midhurst	"Oliver's Mills"	1825	1	Hunter 1909b
Coldwater	?	~1825	1	Hunter 1909b
Tollendal	?	~1829	1	Hunter 1909b
Big Bay Point	?	1830	1	Hunter 1909b
New Tecumseth	a creek	~1832	1	Hunter 1909b
Lefroy	?	~1832	1	Hunter 1909b
Bradford	Scanlon Creek	1832	6	Hunter 1909b
Vespra	Wicken's Creek	1847	1	Hunter 1909b
Bradford	Bradford bridge	1848	1	Hunter 1909b
Bell Ewart	?	1852	1	Hunter 1893, 1909b
Lakeland	?	?	1	Hunter 1909b
Big Bay Point	Hewson's creek	1852	1	Hunter 1909b
Marchmont	?	>1852	1	Hunter 1909b
Washago	Lake Couch.	1852	1	Hunter 1909b
Orillia	?	>1852	2	Hunter 1909b
Alliston	?	1853	1	Hunter 1909b

Angus	?	1854	1	Hunter 1909b
Bradford	?	1858	1	Hunter 1893
Ramara	Atherley	1859	1	MacDonald 1909
Bradford	Holland River	1869	1	Hunter 1909b
Ramara	Longford	1869	1	Hale 1929
Morrison	Severn	>1870	?	Hunter 1909b
North Adjala	?	1878	1	Hunter 1909b

#### 1.2.4.3 Population

Statistics Canada census population data for Simcoe County (Table 3) show an initial population in 1824 of 868 people. By 1830, the population had grown 174% to 2,301, and between 1830-40, 402% to 11,572 people. Just before the opening of the Northern Railway in 1853, the population of Simcoe County had swelled to 27,165 people.

Population statistics compiled by Wilson and Ryan (1988) from Census Canada data indicate that by 1881 there were approximately 60,000 people in Simcoe County, a population that persisted until 1941. After 1941, the population rapidly expanded to just under 110,000 in 1961 and by 1981 was over 200,000. This period of rapid growth is attributed to an influx into the urban centres of Aurora, Barrie, Newmarket, and Orillia (Wilson and Ryan 1988).

Table 3. Population of Simcoe County 1824-1861 (Queens U. 2013; Wilson and Ryan 1988)

Population	Date	Growth(%) from previous
838	1824	0
926	1825	10.5
1117	1826	20.63
1378	1827	23.37
1716	1828	24.53
1790	1829	4.31
2301	1830	28.55
3451	1831	49.98
3429	1832	-0.64
5883	1833	71.57
7737	1834	31.51
7829	1835	1.19
10115	1836	29.2
10825	1837	7.02
9829	1838	-9.2
11246	1839	14.42
11572	1840	2.9
12778	1841	10.42
23050	1848	80.39
27165	1851-1852	17.85
44720	1860-1861	64.62

# 1.2.5 <u>Current anthropogenic use</u>

Current land use in the watershed (Figure 3 & Table 4) is predominantly agriculture and related activities ( $\sim 65.1\%$ ). A

further ~25.11% of the landscape is a combination of wetlands(0.04%), woodlands and reforested areas (25%), and significant water courses (1%). The remaining watershed area is given over to urban development (6%), and the aggregate industry, aboriginal reserves, and unknown use (~1.3%). Within the developed areas of the watershed exists a road network of 5193.47km in length (all values calculated from OMNR 2010b). Included in the urban land use (population of 350,000 in 2008), is the recreational use of the watershed by approximately 50,000 cottagers (LSRCA 2008). Lake Simcoe also has a substantial tourism industry centred around camping and fishing (Wilson 2008). Visitation to the lakes three provincial parks adds over 300,000 people to the watershed each year while visitation by anglers adds 144,000 people (Wilson 2008).

Table 4. Land use in the Lake Simcoe watershed.

Land Use	Area (km²)	Percentage of	
		Total (2823.29km <sup>2</sup> )	
Wetland	14.02	0.04	
Woodland &	730.05	25	
Reforested			
Water	20.46	0.07	
Urban	171.89	6	
Agriculture	1832.95	65.1	
Aggregate & other	37.39	1.3	

 $0.0085 \, \text{km}$ )

Lake Simcoe is a part of the 386km long Trent-Severn waterway that connects Lake Ontario to Lake Huron via Georgian Bay. The human-made waterway tracks an ancient transportation and migration route in use since ~11,0000BP, connecting natural waters by a series of locks and canals (Parks Canada 2009). When finally completed in 1920, commercial vessels were larger than the locks and canals could accommodate and most of the goods traffic to Southern Ontario had shifted to road and rail (PFTSW, 2007). Accordingly, use of the waterway shifted to recreational purposes with an average of ~151,000 boats per year (1994-2007) traversing the waterway (TrentSevern.com n.d). The lock at Gamebridge (44.486762,-79.149232) connecting Balsam Lake to Lake Simcoe was completed in 1904 while the connection between Lake Simcoe's receiving waters, Lake Couchiching (44.661327, -79.379017), and Georgian Bay (44.808635,-79.826367) was completed in 1920. When the waterway was opened in 1920, water levels in Lake Simcoe rose 3m, flooding the eastern portion of the lake along with the wild cranberry and wild rice fields vital to the Chippewa of Georgina Island (ABG 2007). The residents of Georgina Island report that prior to the opening of the waterway, the trek

from the island to the main land could be made on foot due to ankle-deep water (ABG 2007). Water levels in Lake Simcoe (mean 10.173m, range 10.06-10.25m: 1960-1995) are regulated by Environment Canada, the governing body of the Trent-Severn system (Environment Canada 2010; Parks Canada 2009).

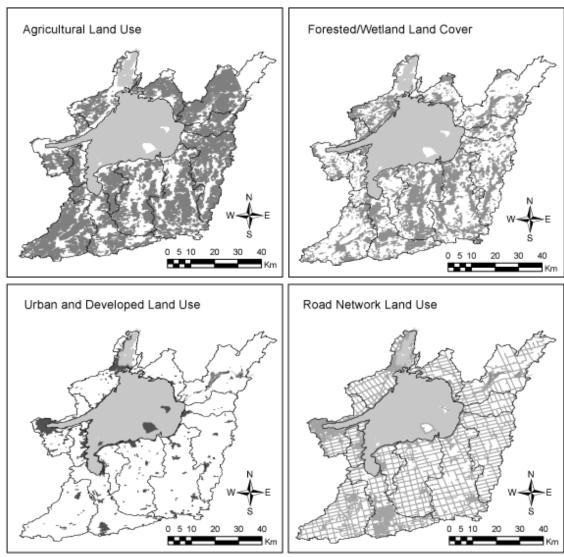


Figure 3.Lake Simcoe watershed land use. (OMNR 2010b; ESRI 2013; Adobe 2013)

# 1.3 Multiple stressors on Lake Simcoe

#### 1.3.1 <u>Glacial and post-glacial influences</u>

As a glacial lake, Lake Simcoe has been influenced by many climatic stressors since its formation by the oscillatory retreat of the Laurentide ice sheet. The margins of last glacial maximum (21,000-18,000 BP) stretched as far south as Chicago, Illinois (Lewis et al. 1994). Deglaciation between 21,000 and 7,500 BP produced large proglacial lake impoundments from meltwater along the southern margins of the sheet.

Initial exposure of the Lake Simcoe area occurred at 13,200 BP during the Mackinaw interstade period (Lewis et al. 1994). Ice-marginal meltwater flowed over south-central Ontario draining into Lake Iroquois in the area of Lake Ontario. Re-advance of the ice sheet covered Simcoe from 13,000 to 12,000 BP. The return of glacial retreat around 12,000 BP exposed the southern portion of the Simcoe area to the meltwaters of glacial Lake Schomberg along the margin of the retreating ice sheet. From ~11,900-11,200 BP (early Kirkfield Phase of Lake Algonquin), the entire Simcoe area was once again exposed and flooded by marginal meltwaters that drained through Fenelon Falls and into Lake Iroquois. The transition into the main stage of Lake Algonquin (11,200-

10,400 BP) is characterized by isostatic uplift of the Fenelon Falls outlet, shifting drainage away from the Simcoe area. With the rapid lowering of Lake Algonquin leading to the formation of Lakes Stanley and Hough in about 10100BP, the entire Lake Simcoe terrestrial watershed was fully exposed (Lewis et al. 1994). The final collapse of the Laurentide Ice Sheet in ~8,000 BP removed any possibility of meltwater supply to the Simcoe watershed, marking the transition to local water sources.

From ~7,900 to 7,500 BP, Lake Simcoe may have been a closed system with water levels much lower than today's depths as evidenced by the hydrologic state of the Great Lakes at this time (Lewis et. al 2008; Edwards et. al 1996). Increasing temperature and precipitation from ~7,500 to ~4,000 BP may have been the cause of modern lake levels but a direct link cannot be reliably inferred (Edwards et. al 1996).

Precipitation and temperature declined after 4,000 BP, reaching present levels at ~1,500 BP, establishing the temperate climate of today (Edwards et. al 1996).

While no Lake Simcoe-specific research exists for its formative ecology (apart from terrestrial pollen reconstructions), recent investigations into limestone dominated Glacier Bay National Park and Reserve (Milner et. al

2007) in southeastern Alaska provides an idea of the combined lake and landscape evolution following deglaciation. Although the description of formation and succession following deglaciation is specific to southeastern Alaska during the late Holocene, the general terrestrial species succession is mirrored well in both a core taken from Lake Simcoe (Todd and Lewis 2007), and a core from Graham Lake in southeastern Ontario (Fuller 1997). This suggests that a similar process of lake succession may have occurred in the Lake Simcoe basin following the retreat of the Laurentide ice sheet although the timing of succession may be very different as a result of climate during the Alaskan (1800s to present) and Laurentide retreats.

The first 5 years of lake and watershed formation in the National Park was dominated by physical processes - water accumulation and sedimentation. Within 5 to 15 years after deglaciation, terrestrial biological process took over as fine-grained carbonate-rich but nitrogen (N) limited, high pH terrestrial sediments were colonized by cyanobacteria, bryophytes, horsetail (Equisetum spp.), and lichens, forming the first soils. As the glacier continued its retreat, species living along the previous glacial margins such as dwarf shrubs (Dryas spp.), willows (Salix spp.), alders (Alnus spp.), and spruce (Picea spp.) began to colonize the new territory

(Milner et. al 2007; Fuller 1997).

From 20 to 50 years after exposure, early communities began to influence subsequent colonization through facilitative and competitive interaction; for instance, dwarf shrub abundances reduced alder germination and early spruce seedling growth while enabling older spruce seedling growth. In this way, early non-vascular and vascular colonizers shaped subsequent terrestrial successions (Milner et. al 2007).

Early lake ecology followed much the same path. Glacial lake sediments were high in pH (7.9 - 8.3) and low in N and dissolved organic carbon (DOC), limiting initial productivity to a few species of attached algae (Gyrosigma spp., Amphora spp., and Achnanthes spp.) (Milner et. al 2007). As lake water turbidity from glacial silts reduced and UV penetration increased, a few highly tolerant zooplankton (e.g. Daphnia spp., Cyclops spp.) colonized the lake. Many lakes remained fishless due to migration barriers or hydrologic isolation. As the first 50 years after deglaciation progressed, linkages between terrestrial and aquatic ecosystems began. Increases in terrestrial soil stabilization and productivity added to the organic matter of the lakes, providing the potential for enhanced aquatic food chains (Milner et. al 2007).

There was increasing biotic control over terrestrial and

aquatic ecosystems between 50 and 150 years after deglaciation (Milner et. al 2007). On the terrestrial side,  $N_2$ -fixation by alder lead to N accumulation in sediments which differentially inhibited spruce growth and lead to a spruce forest of differing age, density and size. On the aquatic side, steady increases in lake water DOC and N were correlated to organic matter from the terrestrial environment, especially where early the terrestrial environment was dominated by alder. In high N lakes, benthic diatoms (Fragilaria spp.) increased in abundance. pH remained high, facilitating the colonization by alkaliphilous macrophytes (Potamogeton spp. and Chara spp.) which provided increased habitat for attached algae, cladocerans and other invertebrates (Milner et. al 2007).

Terrestrially, if N pools were sufficiently created by early alder colonization then spruce forests continued to dominate the terrestrial environment for a time (Milner et. al 2007). On the flip side, limited N pools left spruce susceptible to disease and opened the landscape to recolonization by other species (e.g. Pinus spp.) (Milner et. al 2007; Fuller 1997). In the aquatic environment, pH began to decline as DOC increased about 200 years after deglaciation. This was due to reduced ground water inputs that hardened sediments, limiting penetration of precipitation, and

increasing surface run-off. This chemical change altered the biologic communities such that acid tolerant diatom species (e.g. Cymbella spp., Aulacoseira spp.), and macrophytes (e.g. Nuphar spp., Menyanthes spp., Hippuris spp.) replaced the alkaliphilous species. Additionally, diversity of zooplankton increased during this time. Another significant change was the reduction of benthic diatom species and the increase in planktonic species as N concentrations become stable (Milner et. al 2007).

## 1.3.2 <u>Prehistoric anthropogenic influences</u>

For much of the history of the Lake Simcoe watershed, anthropogenic activities were related to hunting and gathering. The Holland Marsh area was the site of multiple occupations through the early Paleoindian to Woodland period (~12,000 to 416 BP) (Anderson 2013; Stewart 2004). This long occupation history is credited to the stable supply of resources offered by the marsh to early peoples as changing climactic conditions may have influenced flora, fauna and water resources elsewhere.

Literature on the environmental impacts from North

American hunter-gatherer land use is sparse. More attention is
paid to the reverse causal relationship in which the
environment impacts hunter-gatherer social complexity

(Sassaman 2004). However, growing interest in the former relationship is producing evidence that suggests huntergatherer activity is not as environmentally benign as once thought and may have had considerable impact through resource exploitation (Rick and Erlandson 2009).

Specific research on the environmental impacts of huntergatherers in the Holland Marsh area has yet to be conducted but Nicholas (2006a, 2006b, 1998a, 1998b) indicates that prehistoric land use impacts in wetlands around the world may be determined by sedentary-like behaviour and socio-political organization. There are indications that hunter-gatherer mobility decreases as wetland productivity increases (Nicholas 2006b) though this is both spatially and temporally specific and may not be applicable to all wetland land use. Indicators of long-term occupation through socio-political organization include population size (numerous substantial structures, site size), territoriality (weapons, barriers, or cemeteries), public works (mounds, canals and other earthworks), and social differentiation (grave goods) (Nicholas 2006b). Longer huntergatherer occupations are more likely to impact the surrounding environment.

Unfortunately, no references to any material culture (apart from lithics) or site components are provided in the

Holland Marsh site reports summarized by Stewart (2004) nor in the report on the Zander site which is the largest excavated site (Stewart 1984). Stewart (2004) does suggest via lithic frequencies that site sizes increased in the Middle Archaic period and that the marsh may have played a role in territoriality as a natural defence barrier. However, such limited evidence cannot be used to suggest resource exploitation or land use alterations by Holland Marsh huntergatherers.

A little more certainty around the land use effects of later horticultural populations exists. Early maize horticultural practices (slash-and-burn) are thought to have had an impact on forest succession and lake health in southern Ontario although this has been debated (Munoz and Gajewski 2010; Clark and Royall 1995; Burden et al. 1986). Munoz and Gajewski (2010) looked at the pollen and charcoal histories of 20 sites in southern Ontario to assess the source of changes in forest abundances: prehistoric horticultural impacts or climate-related. Five sites were found to have statistically significant (p < 0.10) impacts from horticultural land use between AD 1000 and 1600 showing a reduction in the abundances of Acer spp. and Fagus spp. and an increase in Quercus spp. and Pinus spp. None of these significant sites were in the Simcoe watershed. Additionally, Lake Simcoe pollen diagrams

from Todd et al. (2004) do not display this genus shift during prehistoric horticultural times.

A diatom response to prehistoric horticultural land clearance activities was noted in cores retrieved from Second Lake in Awenda Provincial Park on the shores of Georgian Bay, (Burden et al. 1986). Abundances of Peridinium wisconsinense and Pediastrum increased from greater in nutrient input from erosion. Cores from recent paleolimnolgical studies of Lake Simcoe (Hawryshyn 2010; Rode 2009) did not reach depths sufficient to evaluate prehistoric impacts on the lake.

The prehistoric anthropogenic influences on the Lake Simcoe watershed, if any exist, have yet to be elucidated.

## 1.3.3 <u>Historic and current anthropogenic influences</u>

Anthropogenic influences since European colonization have received far greater attention than influences from previous periods. This may partially be a result of the climate change discourse begun in the 1970s that increasingly focused the attention of the scientific, governmental, media, and general public communities on the influences of industrialization on the biosphere (Risbey 2008; Keller 2012).

The following sections outline research conducted thus far on nutrient enrichment, non-native species introductions, and climate warming affecting Lake Simcoe.

#### 1.3.3.1 Nutrient enrichment

As previously described (section 1.3.1), under nonanthropogenic influence, lakes receive their nutrients
primarily from overland drainage. Additional nutrients are
also introduced via groundwater or atmospheric deposition.
Under the influence of anthropogenic watershed use, culturally derived nutrient inputs are also loaded into lakes.

Research by scientists involving whole-lake experiments in the Experimental Lakes Area (ELA) of northwestern Ontario during the mid-1960s to early 1970s established the connection between phosphorus (P) and increased algal growth, and a low N:P ratio and increased dominance of algal communities by cyanobacteria (Schindler 1974 and 1977). The most influential conclusion from his 1974 phosphorus paper was that P abatement leading to a proportional abatement in phytoplankton blooms might "simply and swiftly" (p.898) solve symptoms of eutrophication. Schindler recommended that the first step in P abatement would be to ban or greatly reduce phosphates in detergents. In 1970, the Canada Water Act called for the immediate reduction of phosphorus in detergents to 8.7% and to 2.2% by 1971 (Knud-Hansen 1994). Despite these significant measures, scientists soon discovered that there was far more involved in eutrophication than a single point-source of excess nutrients (Fee 1979) and that anthropogenic sources of

nutrients are many (Lee et al. 1978). The term "cultural eutrophication" has been used to describe these anthropogenic excess nutrient inputs from point and non-point sources such as untreated sewage and other effluents, urban and agricultural run-off and the atmosphere (Smol 2002).

Two studies (Johnson and Nicholls 1989; Wilson and Ryan 1988) investigated sedimentation in the Simcoe basin from ~ AD 1800 to the early 1980s, elucidating the period prior to active lake monitoring programs. Wilson and Ryan (1988) collected available Canadian census data to evaluate agricultural land use changes and the potentially associated erosion using Trimble's erosive land use index (ELU) (Trimble 1974). The authors found two general trends in erosive land use: a general erosive increase since 1880 and three distinct periods of change showing increases prior to 1901, decreases from 1911 to 1961, and further increases after 1961. Increases prior to 1901 are attributed to increasing land use for agriculture and agricultural intensification. Decreases in the ELU index from 1911-1961 are attributed to an increase in agricultural yields, a reduction in cropland, and an increase in pasture. After 1961, the ELU index value climbed to its maximum value as a result of cropland increases and the expansion of corn and soybean cultivation. While the authors made no causal link between erosive land use and nutrient

enrichment, erosion from agricultural lands has shown greater nutrient concentrations in eroded sediment than in remaining terrestrial soil stocks (Majaliwa et al. 2012) so there is a strong possibility that erosion from Simcoe agricultural lands did impact nutrient availability in Lake Simcoe from 1800 to 1981.

The study by Johnson and Nicholls (1989) took sedimentation from accelerated erosion one step further by linking it to phosphorus loading through time. The authors estimated pre-settlement and present day sedimentation and P loads for four sub-basins and the total basin (Table 5).

Additionally, peak sedimentation rates were determined for each sub-basin (Main = 1890-1901 AD; Cook's Bay & Outlet = 1936; Kempenfelt = 1921), showing "delayed" sedimentation in

Table 5. Sedimentation and P loading from Johnson & Nicholls (1989)

Basins	Pre- 1800's P (t y <sup>-1</sup> )	1986 P $(t y^{-1})$	Pre-1800's Sedimentation (t y <sup>-1</sup> )	1986 Sedimentation (t y <sup>-1</sup> )
Total	28.23 (100%)	74.6 (100%)	27669 (100%)	63896 (100%)
Outlet	1.47 (5%)	12.2 (16.2%)	2102 (7.7%)	9669 (15.1%)
Kempenfelt	2.04 (7%)	3.0 (4.1%)	1837 (6.7%)	2241 (3.5%)
Cook's Bay	1.5 (5%)	19.0 (26.1%)	1680 (6.1%)	16834 (26.3%)

Main	23.22	40.4	21699 (79.5%) 35152 (55.5%	)
	(83%)	(53.6%)		

all but the Main sub-basin which is likely linked to the draining of the Holland Marsh in 1930 and increased urbanization. The results suggest that anthropogenic land use has increased sedimentation loads 130% and P loads 164% from pre-settlement to the mid-1980s. There has also been a shift in the sub-basin sources for these changes - sedimentation and P in Cook's Bay and the lake Outlet have both increased substantially with a commensurate decrease in the Main and Kempenfelt sub-basins.

The 1980s was the beginning of active monitoring and research of Lake Simcoe to determine the sources of excess phosphorus for abatement purposes related to cold-water fishery loss (Welch and Perkins 1979). The Lake Simcoe Environmental Management Strategy (LSMES) steering committee final report in 1985, outlined the current state of the basin and made recommendations about implementation of P abatement initiatives.

Estimates of P loading in 1982, 1983, and 1984 were 102.94, 67.54, and 81.56 t  $y^{-1}$  respectively (LSMES 1985). Percentage of total average 1982-1984 loadings of from tributary, septic, sewage effluent, stormwater runoff, and

atmospheric deposition were 64.1%, 3.7%, 13.8%, 2.6%, and 15.7% respectively. It was thus determined that a target of 75 t y<sup>-1</sup> of total phosphorus loading from all anthropogenic sources would achieve a 5mg L<sup>-1</sup> end-of-summer hypolimnetic dissolved oxygen (DO) concentration that, while not ideal for cold-water fish, was a substantial improvement over previous DO concentrations that hovered between 0 and 1 mg L<sup>-1</sup> in 1985 (Ramkellawan et al. 2009; Snodgrass and Holubeshen 1993).

Mean annual total phosphorus (TP) loadings from the late 1980s to 2003 fluctuated between 51.74 and 113.06 t  $y^{-1}$  (Winter et al. 2007b; Scott et al. 2001; Snodgrass and Holubeshen 1993) with the 1998-2003 mean value at 67 t  $y^{-1}$  being mostly attributed to tributary reductions from LSEMS initiatives (Winter et al. 2007b). While 67 t  $y^{-1}$  of total phosphorus is well below the initial target of 75 t  $y^{-1}$ , Winter et al. (2007) note that DO concentrations (mean 4.3 mg  $L^{-1}$ ) still remain substantially below the 7 mg  $L^{-1}$  required for lake trout protection. Between 2004 and 2009, the mean annual TP loadings rose to 84.6 t  $y^{-1}$  (O'Connor et al. 2013; O'Connor et al. 2012).

In 2009, the Lake Simcoe Protection Plan created by the Government of Ontario threw its support behind the primary goal of P abatement for Lake Simcoe by ushering in a new total

phosphorus target of 44 t y<sup>-1</sup> to achieve hypolimnetic DO concentrations of 7 mg L<sup>-1</sup> to restore the cold-water fishery in Lake Simcoe (North et al. 2013). But as Marsden (1989) and others (e.g. Hupfer and Lewandowski 2008; Scheffer and van Nes 2007; Sondergaard et al. 2001; Schindler 2001; Jeppesen et al. 1997; Fee 1979) have concluded, lake restoration is complex and reduction of external phosphorus loads is often not the only action needed.

## 1.3.3.2 Non-native species

Movement of species into new areas is a part of the dynamic nature of ecology (Milner et. al 2007). However, the modern era has experienced species movements as a direct result of globalization, climate change, and other anthropogenic influences (EPA 2008). Non-native (alien, exotic or non-indigenous) species have the potential to become invasive (i.e. "likely to cause economic or environmental harm or harm to human, animal, or plant health" (ISAC 2006)) when ecosystem conditions are optimal for their reproductive success.

Multiple non-native species have been introduced into Lake Simcoe over the last century including fish (common carp, rainbow smelt, black crappie, bluegill and round goby), invertebrates (zebra mussel, spiny water flea, quagga mussel, rusty crayfish and Eurasian amphipod), and aquatic plants

(Eurasian watermilfoil and curly-leaf pondweed) (Province of Ontario 2009; Ozerky et al. 2011). More than half of these introductions have occurred in the last two decades (Province of Ontario 2009).

A recent and very aggressive invader into Lake Simcoe, the Zebra Mussel (*Dreissena polymorpha*), was likely introduced in 1991 and was well established by 1996 (GISD 2013; Ozersky et al. 2011). Since then, *D. polymorpha* are suspected of decreasing algal biovolume, increasing transparency, increasing macrophyte biomass and production, and changing nutrient dynamics and benthic communities (Ozerky et al. 2011).

In 2006 to 2008, Ozersky et al. (2011) conducted a videobased survey of the Main basin and Kempenfelt Bay, and a grabmethod survey of Cook's Bay to estimate *D. polymorpha* biomass and distribution in the lake. Lake wide biomass was estimated at 11,879 tonnes with Cook's Bay accounting for 3.5%. In Kempenfelt Bay and the Main basin, biomass was distributed along a depth gradient with 25.6% in 0-3.5m, 32.1% in 3.5-8m, and 0.1% at depths greater than 20m. Overall, *D. polymorpha mean* biomass was greatest at depths of ~5m in all surveyed areas. The authors attribute this distribution to disturbance by water movement in the very shallow areas and incompatible

substrates at greater depths.

Ozersky (2010) also found through a before/after (1993/2008) study of littoral benthos in Lake Simcoe, that considerable change in the abundance and community composition had occurred. The mean density in 1993 was 367.9 individuals/m³ compared to the 2008 density of 22,192.4 individuals/m³. A total of 60 and 85 taxa were identified in 1993 and 2008 respectively. Ozersky found that the greatest change was an increase in the relative abundance of detritivores and omnivores and a decrease in native filter feeders.

Other changes in Lake Simcoe since the invasion of D.

polymorpha have been noted: the abundance of profundal benthos has decreased (Jimenez et al. 2011), as well as phytoplankton volume (Eimers et al. 2005), and macrophyte biomass and areal coverage has increased in Cook's Bay (Dewpew et al. 2011; Ginn 2011). From a theoretical perspective, these changes are indicative of a what Hecky et al. (2004) call the "nearshore phosphorus shunt." The "nearshore phosphorus shunt." The "nearshore phosphorus shunt" hypothesizes that Dreissenid mussels engineer their environment by increasing the retention and recycling of nutrients in the nearshore, causing eutrophication of the zone while simultaneously depriving the pelagic zone of nutrients. This can be seen even when external phosphorus loads are

stable (reduced Lake Simcoe P loads in the 1990s may qualify here).

# 1.3.3.3 Climate change

Climate change in the Great Lakes region is expected to be considerable over the next century (Hayhoe 2010). Temperatures are expected to rise 1.4°C by 2039, 2.0 to 3.0°C by 2069, and 3.0 to 5.0°C by 2099 relative to 1961-1990 temperatures. These increases in temperature may be more pronounced in the winter months until 2039, shifting to the summer months after 2039. Precipitation is expected to increase between 20 and 30% during the spring and winter seasons. Climate-related changes to the Great Lakes water levels, temperature and ice-cover are also anticipated. Modelling of Lake Erie ice cover suggests that ice-free winters could be experienced by 2020. The effects of temperature and precipitation are likely to balance each other out until the end of the century such that lake levels are not projected to drop significantly until then (Hayhoe 2010). Summer surface water temperatures in Lake Superior are expected to rise as much as 6.0°C by the end of the century and maximum summer stratification by as much as 90 days (Trumpickas et al. 2009).

A multi-impact study assessing end-of-century climate change and land use (Barlage et al. 2002) in a Great Lakes watershed has determined that the percentage of precipitation

that results in surface runoff will increase 4.3% based reference conditions (1994-2003), with 1.6% attributed to land use change and 2.5% to climate change.

Climate change projections have also been conducted on a Lake Simcoe watershed (Black River) to assess the overall change to hydrology and water quality (Crossman et al. 2013). By the end of the century, flows in the river are expected to increase in response to increases in winter temperature and precipitation which would be falling as rain rather than snow. These precipitation increases (72 - 101% in January to March) are expected to have large corresponding increases in TP between 51 and 81%. Alternatively, the incidence of drought in the summer months is expected to rise yet still produce an increase of 25% in TP concentrations. Overall, TP loads from the Black River are expected to increase between 14 and 32% by the end of the century (Crossman et al. 2013).

The implications of all these changes on Lake Simcoe are great. Warmer air and water temperatures, greater precipitation, shifts in the timing of these climate changes, longer growing seasons and stratification, and continued land use alterations could drastically exacerbate the already strained ecology and water quality of Lake Simcoe.

Increased temperatures during the winter and spring months

have been shown to have a substantial effect on spring phytoplankton blooms and zooplankton communities (Sommer and Lewandowaska 2011; Sommer and Lengfellner 2008). Warming conditions accelerate spring phytoplankton bloom peak by 1 day °C<sup>-1</sup>, and decrease phytoplankton biomass and cell size. Sommer's recent study (2011) finds that these changes in phytoplankton under warming conditions are linked to the overwintering of zooplankton predators, mainly copepods, that at higher densities due to warm winters, are placing increased grazing pressures on the phytoplankton communities. Such changes in primary productivity and herbivory could result in changes at higher trophic levels as well.

Of specific concern to Lake Simcoe is that of possible climate change effects on the already stressed cold-water fish habitat. Fang et al. (2004) investigated the effects of climate change on cold-water fish in 209 shallow ( $Z_{max}4m$ ), medium ( $Z_{max}13m$ ), and deep ( $Z_{max}24m$ ) lakes of differing lake status (eutrophic, mesotrophic, and oligotrophic) in the US. Results for deep mesotrophic lakes show reductions in the mean number of days providing a) good growth conditions (-16 days), b)good-growth habitat (-2 days), and c) good-growth habitat lake water volume (-5 days) from reference conditions (1962-1979). The number of deep mesotrophic lakes with the ability

to support cold-water fish populations is expected to drop by 50% from the reference total of 148 under climate change scenarios. For Lake Simcoe, whose coldwater fishery has already collapsed, such predictions do not bode well for habitat recovery.

# 1.4 Paleolimnology

One of the most common challenges faced by aquatic scientists is the lack of background and natural variability data on ecosystems of interest (Smol 2002). More often than not, active monitoring of an ecosystem does not begin until systems are already impaired. Lack of background data severely limits the ability to determine the severity of degradation or recovery, set realistic mitigation goals, or gauge the timing of impairment (Smol 2002). The use of paleolimnolgical techniques can provide the needed data on pre-disturbance conditions to ecosystem managers.

Paleolimnology is a multidisciplinary science using lake sediment archives of past biological, chemical and geological events and processes to reconstruct environmental conditions (Cohen 2003; Smol 2002). The ideal location in an aquatic environment to conduct paleolimnological studies is in the "accumulation zone" of a lake (Smol 2002). This zone, normally found in the deepest parts of a lake basin, is

characterized by little wind-driven turbulence and other factors that may hinder sedimentation (Smol 2002).

Accumulation zones often provide the most complete, continuous and reliable record of past environmental change. The fundamental attribute of accumulated sediments that make them ideal for environmental reconstruction is the geologic Law of Superposition which states that "for any undisturbed sedimentary sequence, the deepest deposits are the oldest, since these are progressively overlain by younger material" (Smol 2002).

Wetlands are also considered "accumulation zones" where "sediment accumulation exceeds erosion, thereby providing the potential for the accumulation of long and nearly continuous depositional records" (Williams 2011). Not only do wetlands have long depositional records, but they also hold a wealth of bioindicators and geochemical indicators due to their productivity. Macro remains, such as seeds, found in sediment cores remain identifiable in deep deposits due to a wetland's low-oxygen environment that preserves organics. Preserved micro remains, usually made of siliceous material, are also abundant in wetlands provided the salinity concentration remains below 20g L<sup>-1</sup> and carbonate concentration below 250 mg L<sup>-1</sup> (Ryves et al. 2006). Other factors affecting the

preservation of siliceous microfossils are bioturbation and other physical mixing within the soil; physical mixing of the sediment surface can result in the fragmentation of fossils. Despite the stated limitations of microfossil preservation, they still provide some of the "most detailed sources of information for environmental reconstruction available to the paleolimnologist" (Cohen 2003). A fairly new microfossil proxy in paleolimnology is the testate amoebae.

## 1.4.1 <u>Testate amoebae in paleolimnology</u>

Testate amoebae (also referred to as Thecamoebians,
Arcellaceans, and Rhizopods) are a group of single celled
organisms including lobose and filose amoebae (Beyens and
Meisterfeld 2001; Charman 2001). About 1900 species and
subspecies have been described thus far. The lobose order
Arcellinida is the largest group and contains about threequarters of all known species of testate amoebae. These
amoebae are commonly referred to as "testate" because they
form an exterior, decay resistant test made from metabolized
silica, protein, calcium or an agglutinated matrix of
siliceous material such as sand grains and diatom frustules.
It is this test that is preserved in the sedimentary record
and used to identify past specimens, in most cases to the
species level. Taxonomic identification is based on aperture

details and morphological characteristics of the chamber (Beyens and Meisterfeld 2001).

Testate amoebae are found in almost every environment in the world from sandy marine beaches to all freshwater and terrestrial habitats (Beyens and Meisterfeld 2001). Many species are ubiquitous while others are habitat-specific. The same can be said for distribution: many are cosmopolitan while others are geographically restricted. In terms of wetlands habitat, they are the most common protists with a biomass up to 1 g m<sup>-2</sup>. Testate amoebae with siliceous tests are most abundant in wetlands and shallow water bodies (Beyens and Meisterfeld 2001) but specimens of all test types can be found.

The biology and reproductive strategies of testate amoebae make them ideal for use in paleolimnolgical research (Charman 2001). Reproduction is mostly by asexual binary fission with sexual reproduction being rare. Rates of reproduction are rapid with field observations recording 10-27 generations per year and laboratory studies reporting population doubling in 2 to 3 days. Generation times are dependent on many factors such as temperature, nutrient availability, and population density (Beyens and Meisterfeld 2001). This rapid asexual reproduction makes testate amoebae sensitive indicators of short-lived

environmental change with the possibility of rapid morphological changes (Charman 2001; Beyens and Meisterfeld 2001).

Paleolimnological and paleoecological investigations using testate amoebae have been used to describe hydrology, pH, trophic status, land use, climate change, forest fires, oxygen, metal contamination and other variables (Patterson et al. 2012; Beyens and Meisterfeld 2001). The most common freshwater paleoecological investigation locations that use testate amoebae are peatlands and lacustrine environments.

In peatland environments, the major use of testate amoebae is for the inference of moisture conditions. Quantitative transfer functions have been developed that link water levels and testate amoebae assemblages. For example, in 2002, Booth conducted a study investigating moisture levels and testate amoebae assemblages in 139 microsites located in 11 Michigan peatlands. Booth found that "depth to water table can be reconstructed from fossil data with a mean error of ±7.5 cm, although predictive ability deteriorates in extremely dry environments (<0.30 cm water table depth)" (Booth 2002).

A relatively new quantitative reconstruction is lacustrine sedimentary phosphorus. Patterson et al. (2012) analyzed 73 sediment-water interface samples from 33 urban and rural lakes

in southern Ontario to assess the possibility of a link between water quality variables and testate amoebae assemblages. The authors found a strong correlation between testate amoebae and sedimentary phosphorus (Olsen P) and thus created a training set for the quantitative reconstruction of Olsen P in freshwater sediment fossil assemblages in southern Ontario. Comparison of the training set to a sediment core from Haynes Lake was able to link anthropogenic watershed use to sedimentary phosphorus concentrations throughout the last 200 years.

Far less often, testate amoebae are used in transition zones such as lacustrine fringe wetlands. In 2001, Booth investigated two Lake Superior coastal wetlands to assess their use as environmental and paleoenvironmental indicators and to determine if morphological variation was related to microenvironmental conditions. Like other wetland/peatland studies, Booth found that pH and substrate moisture were the main predictors of community assemblages. It is important to note that pH was the only chemical variable assessed in the study.

Previous studies reconstructing Holocene environments using testate amoebae have been carried out on lakes from northeastern Ontario (Boudreau et al. 2005), eastern Ontario (Elliott et al. 2012), northwestern Ontario (Warner and

Charman 1994), Newfoundland and Nova Scotia (McCarthy et al. 1995), and Arctic Siberia (Muller et al. 2009) with a focus on climate-related community changes. The multi-proxy study conducted by McCarthy et al. (1995) compared pollen succession for temperature and precipitation reconstructions to testate amoebae assemblages from deglaciation to the present (McCarthy et al. 1995). The authors found that arcellacean assemblages tracked well with pollen inferred climate changes throughout the last 13,000 BP and even responded to short-lived climactic changes that were not recorded by pollen assemblages.

Additional studies (e.g. Sonnenburg et al. 2013,2009; van der Linden et al. 2008; Patterson et al. 2002; Burbidge and Schroder-Adams 1998; Scott and Medioli 1983), at both the Holocene and Anthropocene timescales have focused on or included testate amoebae response to anthropogenic influences.

Finally, a recent study (Watchorn et al. 2013) focused on both climate-related and anthropogenic disturbances since deglaciation using testate amoebae assemblages to conclude that both climatic changes and European settlement activities impacted lake heath. In fact, European settlement activities had a greater impact on lake health (pH and trophy) than did the Hypsithermal Climatic Optimum.

## 1.4.2 <u>Elemental and isotopic analysis</u>

Carbon and nitrogen elemental and isotopic composition of

organic matter are useful proxies for reconstructing past environmental conditions, assessing climate change, and evaluating anthropogenic impacts on ecosystems by providing evidence of past productivity and organic matter origin (Koff 2012; Meyers 2003).

Plants are the dominant source of organic matter to lake or wetland sediments and can be divided into two geochemically significant groups based on their biochemical composition (Meyers 2003). Group one consists of nitrogen-rich non-vascular plants containing little to no cellulose or lignin such as phytoplankton (Meyers 2003). Group two are carbon-rich vascular plants containing large proportions of woody tissues such as grasses, shrubs, trees and emergent macrophytes (Meyers 2003). Both groups retain their biochemical composition after deposition enabling the distinction of organic matter sources to the sediment even after microbial degradation (Meyers 2003).

The percentage of organic carbon (%C) contained in sediments is directly proportional to the abundance of organic matter deposited (Koff 2012). Typical plant organic matter is composed of 40-50 %C (Koff 2012; Meyers 2003). An increase in %C can indicate increased organic matter input (autochthonous or allochthonous) while a decrease may indicate greater inputs of inorganic matter or increased microbial degradation (Koff

2012).

The molar carbon to nitrogen ratio (C:N) can be used to identify the proportions of algal and land plant matter composition of the sediment. Algae typically has a molar C:N value between 4 and 10 while land plants have ratios over 20. Intermediate levels of C:N values (~15) are typical of nearshore environments where a mix of terrestrial and aquatic derived organic matter is found (Koff 2012). An increase in sediment C:N ratios may be an indicator of increased land plant organic matter and land derived sediment from erosion after land clearing activity (Meyers 2003). Alternately, a drop in the C:N ratio may be an indicator of increased deposition from autochthonous algae and macrophyte growth.

Stable carbon isotopic composition ( $\delta^{13}C$  and  $\delta^{15}N$ ) within sediments is useful for further differentiating the type of organic matter deposited and productivity (Meyers 2003). The ratio of  $^{13}C$  to  $^{12}C$ , commonly reported as  $\delta^{13}C$ , is affected by the concentration of  $^{13}C$  in the environment and the photoshynthetic pathway ( $C_3$ ,  $C_4$ , CAM) of the plant (Koff 2012). Phytoplankton and  $C_3$  land plants (mostly shrubs, trees and emergent macrophytes), have similar  $\delta^{13}C$  values (-25 to -30%) wile  $C_4$  plants (mostly grasses in tropical and subtropical regions), have values up to -10% (Koff 2012). Taken alone, the

 $\delta^{13}$ C present in lacustrine or wetland environments in temperate regions does not provide evidence of the type of organic matter deposited. However,  $\delta^{13}$ C values considered in conjunction with C:N values and  $\delta^{15}$ N values can narrow down the likely origin of the organic matter (Figure 4).

Like  $\delta^{13}\text{C}$  ,  $\delta^{15}\text{N}$  values represent a ratio of two different isotopes,  $^{15}\text{N}$  and  $^{14}\text{N}$  in this case. The use of  $\delta^{15}\text{N}$  values alone to interpret either organic matter origin or productivity is not widely used due to the complexity of the nitrogen cycle (Meyers 2003). However, some differences in the ratio are detectable. The  $\delta^{15}N$  available to plants in aquatic and terrestrial environments differ, providing a means to differentiate organic matter sources. The nitrogen commonly used by algae, NO<sub>3</sub>, has a 7-10% greater  $\delta^{15}N$  value that the atmospherically derived  $N_2$  used by land plants. Even so, there are many biological processes within sediments and anthropogenic N inputs from agricultural and sewage sources that complicate the basic differences of aquatic and terrestrial  $\delta^{15}N$  values found in sediments. Increases in  $\delta^{15}N$ may indicate anthropogenic inputs or denitrification from anoxia. An abundance of cyanobacteria will reduce  $\delta^{15}N$  values (N-fixation). Combining  $\delta^{13}$ C and  $\delta^{15}$ N can assist with differentiation between organic matter sources (Figure 4).

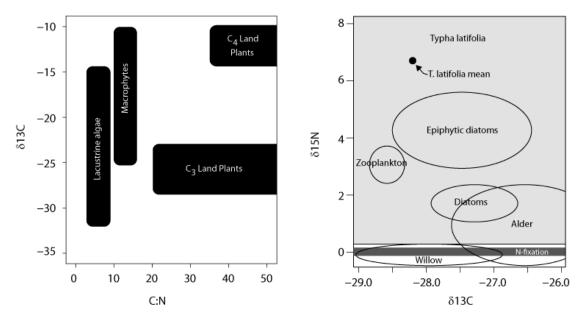


Figure 4. Generalized  $\delta^{13}C$  and C:N values (left plot) and  $\delta^{13}C$  and  $\delta^{15}N$  values (right plot) of major and example sources of plant organic matter (left plot). Left plot adapted from Meyers 2003 and Koff (2012). Right plot derived from Keough et al. (1996) data.

# Chapter 2. Rationale and Objectives

# 2.1 Rationale for the Study

The health of Lake Simcoe is a concern for numerous stakeholders including residents, industry, and governmental organizations.

The Lake Simcoe watershed is home to over 400,000 people living in both rural and urban settings (Province of Ontario 2010) with population 2031 projections at 667,100 residents (shs inc. 2007). Seven municipalities within the watershed draw their drinking water directly from the surface waters of

Lake Simcoe (Province of Ontario 2011).

Considered part of Southern Ontario's "recreational heartland," in 2008, 6.2 million people visited Simcoe county for tourism and recreation purposes, many of them participating in nature-based activities (OMNR 2012c). Total spending for recreational activities in 2008 was over \$34 million dollars with an economic impact of \$630 million and 8,000 jobs (OMNR 2012c). Scott et al. (2002) report that in 1999, tourism across the entire "recreational heartland" (Muskoka, Bruce, Grey, Simcoe and Haliburton counties) contributed \$814 million in economic impact and 26,000 full time jobs. Ontario residents seeking outdoor recreational experiences made up 93% of tourists (Scott et al. 2002).

In terms of natural resources, the watershed contains parts of the Oak Ridges Moraine and Greenbelt and is home to provincially significant wetlands, woodlands, prime agricultural areas, and aggregate resources (Province of Ontario 2009).

Over the last century, anthropogenic use of the watershed's natural resources has led to impairments to the lake's water quality and ecology. It is this impairment and the potential for increased impairment that threatens the continued enjoyment and economic benefits offered by the above

ecosystem services.

While an enormous amount of scientific research has been conducted on Lake Simcoe, there is a distinct gap in knowledge about the changes experienced in the littoral areas of the lake. A number of studies (e.g. Ginn 2011; Ozersky et al. 2011; Depew et al. 2011; Stantec 2007; Neil et al. 1985,1991; Millard and Veal 1971) have assessed changes in the nearshore environment within the active monitoring period (~1970 to present) but none of these assessed longer-term influences in this zone.

In their 2005 publication, Eimers et al. highlighted the importance of long-term Lake Simcoe data sets to understand the drivers of water-quality variation and any relationships with multiple influences that might exist. Three paleolimnological studies were conducted between 2009 and 2011 using sedimentary diatoms, chironomids, and P fractions to elucidate long-term trends in Lake Simcoe water quality (Hiriart-Baer et al. 2011; Hawryshyn 2010; Rode 2009), however none focused specifically on littoral areas. One very recent study (Danesh et al. 2013), assessed a number of proxies for a response to water quality changes from a lacustrine sediment core taken in Cook's Bay, Lake Simcoe. Danesh et al. (2013) found that three distinct transitions occurred in the time

period investigated: initial European colonization (mid 1800s), the start of intensive agriculture in the subwatershed (AD 1920-1930), and the urbanization/industrialization of the subwatershed following World War II (AD 1950). The changes in non-pollen palynomorph assemblages are attributed to increased nutrient loads to Lake Simcoe from anthropogenic activities (Danesh et al. 2013).

Considering the large proportion of the basin under 20m (67%) water depth (Ginn 2011), the recent invasion of the littoral zone by *D. polymorpha* (Evans et al. 2011), increased biomass in shallow Cook's Bay (Ginn 2011), and recent quantification of internal P load (Nurnberg et al. 2013), a long term data set elucidating littoral changes prior to monitoring is needed to inform any littoral management decisions.

# 2.2 Study Objectives

Testate amoebae were used as paleolimnological/paleoecological indicators to reconstruct ecological changes to lacustrine wetland habitats from climate, nutrient, and non-native species influences.

Sedimentary core analysis of fossil testate amoebae assemblage composition was carried out at high resolution for the historic period (> AD 1800) and low-resolution for the

prehistoric period (< AD 1800) on three cores from fringe wetland locations in the south, east, and north shores of Lake Simcoe. These locations were chosen based on historic and present subwatershed influences: the south core wetland is representative of areas of significant land use change and agricultural influence, the east core wetland is representative of provincially protected and minimal anthropogenic influence, and the north core wetland is representative of historic urban and whole-lake influence. A fourth core, taken from a private nature reserve just north of Lake Simcoe, was also analyzed for testate amoebae to provide a minimally anthropogenic influenced reference for comparison.

This study was conducted to elucidate any possible changes to Lake Simcoe fringe wetland biological communities over time and to provide an assessment of any long-term changes under the following lines of inquiry:

- 1) Have nearshore fringe wetland testate amoebae communities changed since European settlement?
- 2) If so, has there been a change in richness, a shift in species abundance or community composition?
- 3) Are any of these changes related to temporal trophic status, land use, climate, or non-native species introductions?

- 4) Are the changes unique to each wetland or are they lake-wide?
- 5) Can a comparison with a reference wetland pin-point the source of these changes as anthropogenic?
- 6) What is the past variability of the testate amoebae communities prior to European settlement?

Site locations, and materials and methods are presented in Chapter 3 and 4 respectively. The results of this study are presented in Chapter 5 and 6. Chapter 5 will address questions 1 through 5 using all four cores together with carbon and nitrogen elemental and isotopic analyses. Chapter 6 will address question 6 using the extended core extracted from the northern lake-outlet site. Chapter 7 will summarize the main findings of this thesis project. It is our hope that the results from this project shed some light on the ecological history of Lake Simcoe nearshore wetlands.

# Chapter 3. Site Locations

The coring locations (Figure 5) were chosen based on prehistoric, historic and present subwatershed influences including First Nations settlement, urban development, whole-lake nutrient impacts. Three cores from the fringe wetland locations in the south, east, and north shores of Lake Simcoe were extracted in the late autumn 2011, and in spring 2012. A fourth core was extracted from a private nature reserve just north of Lake Simcoe in late autumn 2011, to be used as reference wetland.

#### 3.1 Victoria Point

The Victoria Point (VP) sediment core was extracted from a fringe wetland located on the shoreline of Smith's Bay just south of the Atherley Narrows (44.593844,-79.386331) within the boundaries of urban Orillia. The core is representative of prehistoric and historic land use change and whole-lake influence (lake outlet). Much of urban Orillia is contained within the Oro Creeks North subwatershed which drains into Shingle Bay, south of the coring site. The coring site itself is located in a wetland receiving waters only from Lake Simcoe.

The prehistory of the area, presented in sections 1.2.3, 1.3.1, and 1.3.2, details a climatic history beginning in

11,900 BP and anthropogenic history beginning in 4,600 BP. In summary, the area was one of the last areas of the Simcoe watershed to be exposed following glacial retreat.

The main stage of Lake Algonquin formed glaciolacustrine deposits up to 2.5m thick around the outlet of Lake Simcoe. Archaeological surface surveys have noted a number of sites near the wetland, the closest just 500m north and the largest (~34 acres) just 4km northwest of the wetland. The Atherley Narrows Fish Weirs (1km northeast) have been dated to 4,600 BP. Archaeological finds of stone stone and metal axes near the wetland suggest a long use history of the area.

The history and present use of the area are presented in sections 1.2.4, 1.2.5, and 1.3.3. The early history of the Orillia area began in 1830 as First Nations and European settlers began inhabiting the shore of Lake Couchiching just above the Atherley Narrows. Settlement and land clearing progressed slowly, gaining momentum after the construction of the railway in 1871. By 1881, a road had been constructed along the coring site's peninsula (Macdonald 1881). Geologic surveys listed the coring site as a swamp of "mainly muck" (Deane 1950) and "muck and peat" Finamore and Bajc 1984).

Population growth and the urbanization of the Orillia area continued at a steady pace, growing to ~30,000 people by 2011.

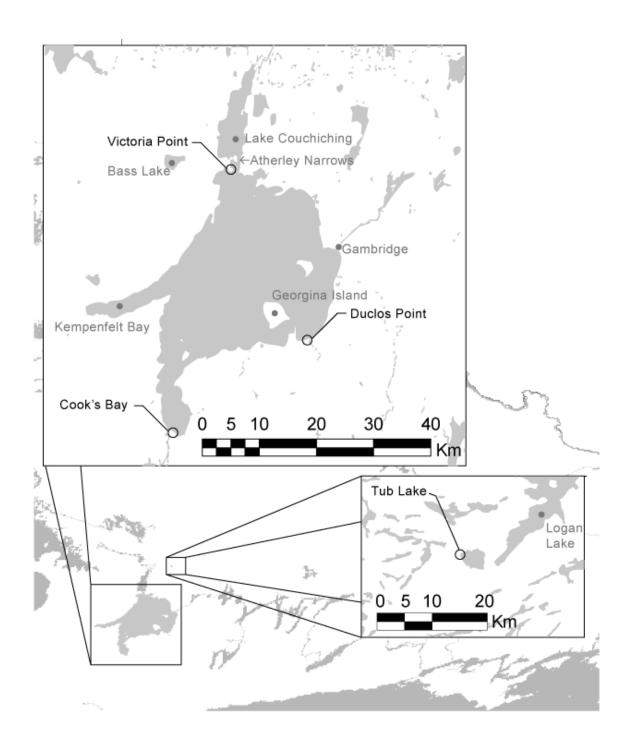


Figure 5. Site locations. Black text = coring sites & grey text = other locations mentioned in the thesis.

## 3.2 Cook's Bay

The Cook's Bay (CB) sediment core was extracted from a fringe wetland located on the shoreline of Cook's Bay just outside the mouth of the Holland River (44.203097,-79.511711) in an area known as the "Simcoe Lowlands." The core is representative of significant land use change, agricultural, and urban influences on the lake.

Two subwatersheds drain into Lake Simcoe via The Holland River: The East Holland (EHR) and West Holland River (WHR).

The EHR is described as one of the most populated subwatersheds in the Simcoe basin including the towns of Aurora, King, Newmarket, East Gwillimbury, Georgina,

Whitchurch-Stouffville, and Uxbridge which accounts for 17.3% of the subwatershed's land use. Agriculture accounts for an additional 30.7% of land use. The WHR is primarily an agricultural area with 57% attributed to that land use and only 3% to urban use. The agricultural area includes a portion of "The Holland Marsh," a large wetland area drained in 1920-1930.

The prehistory of the area, presented in sections 1.2.3, 1.3.1, and 1.3.2, details a climatic and anthropogenic history beginning in 13,200 BP. In summary, the area currently defined as the East and West Holland River subwatersheds was one of

the first areas of the Simcoe watershed to be exposed following glacial retreat. For thousands of years, the area was inundated with glacial meltwaters until post-glacial rebound altered the outlet location of Lake Algonquin, drawing water away from the area. The area remained "wet" throughout the next 10,000 years (Karrow et al. 1975), providing valuable resources for First Nations groups over the entire time period.

The history and present use of the area are presented in sections 1.2.4, 1.2.5, and 1.3.3. There are two interconnected events in the history of the area that best define the subwatersheds. The first is The Holland Marsh drainage project that created 7,000 acres of farmland from wetland space, drying the land through a series of man-made canals that also served to drain 64,300 acres of watershed area and maintain the Holland River at a constant water level to prevent floods. The second event was the rapid urbanization of the EHR subwatershed that brought sewage effluent into Cook's Bay through the Holland Marsh canal system.

#### 3.3 Duclos Point

The Duclos Point (DP) sediment core was extracted from a fringe wetland located on the shoreline of Duclos Point Nature Reserve (44.336833,-79.242289). The core is representative of

agricultural land use and, as a Provincially Significant wetland in a Provincial Park, of minimal recent anthropogenic impact.

The wetland is contained within one subwatershed: The Pefferlaw River (PR). The PR is considered to be a rural watershed with just 5.5% of land being used in an urban context. 48% of the subwatershed is classified as rural/agricultural. The wetland sampled within the nature reserve is considered to contain a variety of provincially significant wetland communities representing Ontario's ecological and species diversity. There are very few permissible uses of the park and its resources. The head waters flowing into the wetland begin a short distance from Lake Simcoe in an area of rural/agricultural land use.

The prehistory of the Simcoe area, presented in sections 1.2.3, 1.3.1, and 1.3.2, details a climatic history beginning sometime between 13,200 BP and 11,900 BP. Like Victoria Point, the terrestrial area of Duclos Point was flooded by the main stage of Lake Algonquin indicating a glacial history similar to Victoria Point. The Ontario Ministry of Natural Resources (2007) reports that artifacts and burial grounds have been discovered in the nearby area but not in the nature reserve itself. There has been no other indication of First Nations

land use prior to the historic period.

The history and present use of the Simcoe area are presented in sections 1.2.4, 1.2.5, and 1.3.3. There is very little detailed information regarding the early history of the area (Georgina, ON). What is known is that settlement followed a similar land clearing and agricultural trajectory to the Cook's Bay area from 1819 to the 1830s (Town of Georgina n.d.; BDCC 2010; Ramara Historical Society n.d.). The nature reserve was a significant First Nations site used as a camping and hunting ground for the Chippewas of Georgina Island First Nation (OMNR 2007).

## 3.4 Tub Lake

The Tub Lake (TL) sediment core was extracted from a fringe wetland located on the shoreline of Tub Lake in a privately owned nature reserve (44.8632,-79.008075), just north of the Queen Elizabeth II Wildlands Provincial Park. The core is representative of early land clearing activities followed by no anthropogenic use beyond minimal hunting and fishing activities.

The Longford Reserve is 45,000 acres in size and contains 22 lakes all draining into the Black River which merges with the Severn River, forming the Black-Severn watershed (Steve Clark, personal communication). The land was given as grant

to the British-Canadian Land and Emigration Company for timber harvesting in the 1860s and was actively logged until the 1890s (Hale 1929; Steve Clark, personal communication). The land was purchased in 1920 by the current owners. At the time of purchase, the area was barren of any vegetation following the earlier logging activity and two forest fires, the last one occurring in 1918 (Steve Clark, personal communication). Upon purchase, the new owners declared the area for recreation purposes only. The first recreational cabins were built in the north end of the reserve near the Town of Vankoughnet in the District of Muskoka. In the 1960s, a group from Toronto built 18 primitive cabins on Logan Lake near Tub Lake. These two "settlements" and the dirt roads that connect them to the surrounding lands are the extent of anthropogenic use of the area since land clearing in the late 1800s (Steve Clark, personal communication).

## Chapter 4. Materials and Methods

## 4.1 Field and laboratory methods

A 2.8m sediment core from a hummock/hollow interface zone (De Vleeschouwer et al. 2010) was obtained from Victoria Point (VP) wetland in November 2011. Three other cores no less than 1m were extracted from wetlands at Cook's Bay (CB), Duclos Point (DP), and Tub Lake (TL) between November 2011 and May

2012. The upper 30cm of each core from VP and DP were extracted as a 20x20x30cm monolith using a simple galvanized steel cutting box based on Cuttle and Malcolm (1979). The lower 1-2.5m of the cores were extracted using a 50x5cm "D" corer (Russian Peat corer/borer) with extension rods (EPA 1999; De Vleeschouwer et al. 2010). The sediment compaction at CB and TL prevented use of the "D" corer so the length of both cores were extracted as monoliths in 30cm increments.

The "D" sediment cores were covered in 6mm polyethylene plastic, placed in 50cm long, 5cm diameter ABS half-tube cradles and wrapped with plastic stretch film. The monoliths were gravity extruded from the steel box, covered with the same 6mm plastic and securely wrapped with stretch film (De Vleeschouwer et al. 2010). In the laboratory, the monoliths and cores were refrigerated at ~4°C until subsampled.

With regard to the Victoria Point core, high resolution photographs were taken of the core sections immediately after extraction to document oxidation sensitive colour and stratigraphy (De Vleeschouwer et al. 2010). Lithology via photography and subsampling was assessed using a digital version of the Munsell Book of Color (Irtel n.d.) with display colour set to North America General Purpose 2, and the LRC lacustrine sediment classification scheme (Schnurrenberger et

al. 2003). Sediments were also assessed for the presence of carbonates using the dilute hydrochloric acid field method (USDA n.d.) and classified based on the FAO classification scheme (2006) reproduced in Table 6. Due to the qualitative nature of the classification scheme, the scheme was supplemented with a video-captured "extreme" reaction used as a reference point (Harter 2011).

Two hundred and ten sediment samples were subsampled at 1cm intervals in the top 30cm and at 4cm intervals in the bottom of the cores. The subsamples were 1-2cc in volume and were stored in small plastic self-locking bags and refrigerated at  $\sim$ 4°C until processed.

Table 6. FAO (2006) classification scheme for soil carbonate concentrations.

90	Category	Category Name	Description
0	N	Non-calcareous	No detectable visible or audible effervescence
0 to 2	SL	Slightly calcareous	Audible effervescence but not visible
2 to 10	MO	Moderately calcareous	Visible effervescence
10 to 25	ST	Strongly calcareous	Strong visible effervescence. Bubbles form a low foam.
>25	EX	Extremely calcareous	Extremely strong reaction. Thick foam forms quickly.

Subsamples for micropaleontological analysis of testate amoebae were first spiked with one Lycopodium clavatum tablet (Lund 2011) to calculate absolute concentrations (Stockmarr 1971). Subsamples were then treated following preparation A (Hendon and Charman 1997) in which the spiked subsamples, contained in 15ml centrifuge tubes and submerged in deionized water, were placed in a boiling water bath for 10 minutes, followed by 300µm and 15µm nylon mesh sieving to remove coarse and fine fractions respectively. Residues retained by the 15µm mesh were washed into 15ml centrifuge tubes and spun at 2,500rpm for 5 minutes to remove excess water. Course fractions were washed into 4 dram vials for analysis of macro remains where refinement of core events was necessary.

Extracts of the subsamples were mounted on glass slides in a glycerin medium for identification under a generic trinocular biological microscope with infinity plan achromatic objectives at x200 and x400 magnification. A minimum of 150 tests were counted where possible (Payne and Mitchell 2009). Micrographs (Appendix A) were obtained using a 3 megapixel CMOS trinocular digital camera with ToupView® software.

Testate amoebae identification to species level followed keys, descriptions, illustrations, and micrographs found in Siemensma (2013), Booth and Sullivan (2007), McAndrews et al.

(2005), Charman et. al (2000), Kumar and Dalby (1998), Ogden and Headley (1980), and Corbet (1973). Multiple keys were used due to the presence of both lacustrine and peat-based testate amoebae in the cores. Species abbreviations are provided beginning on page xviii of this thesis.

A total of seventeen samples from the VP core were shipped to MyCore Scientific Ltd., Deep River, Ontario, for age determination using alpha spectrometry to detect polonium-210 (210Po) concentrations as a proxy for lead-210 (210Pb) concentrations under the assumption of isotope secular equilibrium (Edgington and Robbins 1975). Prior to shipment, samples were dried at ~60°C to a constant weight, ground with a mortar and pestle to a fine powder, and stored in glass vials.

Two separate shipments were sent to MyCore Scientific Ltd. Samples for the first shipment, selected for greatest resolution of initial European settlement and recent events, were every centimetre from 0-4cm and from 27-29cm, and every second centimetre in between. Samples for the second shipment were selected from lower sediments to refine background <sup>210</sup>Pb measurements.

The Constant Rate of Supply Model (CRS) was used to date the  $^{210}{
m Pb}$  sediment measurements. The CRS assumes 1) a constant

rate of unsupported <sup>210</sup>Pb (atmospherically derived) is supplied to sediments through time, 2) the initial <sup>210</sup>Pb concentration is variable, and 3) the influx rate of sediment is variable (Noller 2000; Appleby and Oldfield 1983, 1978). The CRS model provides a 95% confidence interval of 1-20 years proportional to the inferred age (Binford 1990).

Ambrosia pollen abundances, commonly and reliably used as an anthropogenic marker to support <sup>210</sup>Pb dates (Blais et al. 1995), were identified in the VP core using testate amoebae extracts to assess the most likely depth related to European land clearing. Ambrosia pollen abundances were also identified for the CB, DP, and TL cores to enable coarse comparison of all cores. Extrapolation of <sup>210</sup>Pb dates down-core for VP was not possible due to a lack of dates near background (~150 years before sediment extraction) and a highly variable sedimentation rate (Noller 2000).

#### 4.2 Statistical methods

Quantitative microfossil analysis was conducted on sixtythree samples (every 2cm) from the top 30cm of each core
(monoliths) and on forty samples beneath 30cm at intervals of
lithologic interest prior to European settlement (VP) and at
progressively lower resolutions down each core (CB,DP and TL)
for the Anthropocene timescale. Specific sample depths for

each core are recorded in the Appendix B.

To obtain taxa abundances for comparison across samples, absolute abundance values of testate amoebae counts (  $AA_{\it TA}$  ) as tests/cm³ were calculated using

$$AA_{TA} = \left(\frac{L_t * \sum TA}{\sum L_s}\right) / V \tag{1}$$

where  $L_t$  is equal to the number of Lycopodium spores added to each sample (i.e. 20848  $\pm$  1546),  $T\!A$  is the total number of tests counted on each slide,  $L_s$  is the total number of Lycopodium spores counted on each slide, and V is the volume in cm<sup>3</sup> of the sample processed for testate amoebae analysis (Taylor et al. 2012).

To assure the statistical reliability of quantitative assessment (Payne and Mitchell 2009; Patterson and Fishbein 1989), samples and the taxa within each sample were analyzed to assess the statistical significance of the population counts obtained during microscopic analysis.

Statistical significance of each sample (SSP) was judged to be true if the total count for each taxon was greater than the probable error (pe), calculated by

$$pe = 1.96 \left( \frac{s}{\sqrt{X_i}} \right) \tag{2}$$

where s is the standard deviation of the all taxa counts in the sample, and  $X_i$  is the total sample count (Boudreau et al. 2005). Where pe was greater than the total count for each taxon, the sample was not included in subsequent analysis.

Statistical significance of each species within each sample (SSPS) was assessed by first calculating the relative fractional abundance  $\left(F_i\right)$  of each taxon in each sample using

$$F_i = \frac{C_i}{N_i} \tag{3}$$

where  $C_i$  is the taxon count and  $N_i$  is the sum of all taxa counts in the sample. The fractional abundance was then used to calculate the standard error  $\left(S_{F_i}\right)$  of each taxon using

$$S_{F_i} = 1.96 \sqrt{\frac{F_i(1 - F_i)}{N_i}}$$
 (4)

where  $\,F_{i}\,$  is the fractional abundance of the taxon, and  $\,N_{i}\,$  is the sum of all taxa counts.

Taxa were deemed to be significant if the fractional abundance was greater than the standard error (Boudreau et al.

2005) and included in subsequent analysis. All calculations were conducted using R open source software (R Core Team 2013).

Five samples, VP230, VP242, TL54, TL62, and TL90 were void of testate amoebae and were removed from further analysis. SSP analysis indicated that sample TL4 was not significant.

Examination revealed a large dominance by one taxon (P. acropodia at 84.86%) with other taxa representing ≤5% each.

Additional counts of testate amoebae in TL4 (increasing the total count from 162 to 284) did not change fractional abundances or species richness. Despite the non-significance determined by SSP analysis, the sample was included in further analysis as single species dominance appears to be a characteristic of the sample rather than a sampling error.

Analysis of all taxa in each sample yielded statistically significant populations of testate amoebae in more than one sample so no taxa were excluded from subsequent multivariate analysis (Patterson et al. 2012).

# 4.2.1 <u>Bicluster analysis</u>

Bicluster (aka cocluster or two-way cluster) analysis, used to define any biofacies present in the cores and to compare assemblages and distribution across cores, was conducted on relative frequencies of  $AA_{TA}$  counts.

Biclustering is a two-dimensional grouping of matrix interactions of similar activity under similar conditions commonly used in gene expression research (Madeira and Oliveira 2004) and successfully used in paleolimnological research to elucidate similar species assemblages in core samples regardless of stratigraphic position (Patterson et al. 2013; Boudreau 2005).

R-mode (for taxa grouping) and Q-mode (for sample grouping) hierarchical cluster analyses via Ward's Minimum Variance method using euclidean distance were combined to form an ordered matrices of fractional species abundances in R (R Core Team 2013; Boudreau 2005).

The resulting data sets are displayed as heatmaps using the R-packages *lattice* (Sarkar 2008) and *latticeExtra* (Sarkar and Andrews 2013) and Adobe® Illustrator® CS, version 5.5, for placement of cluster division lines based on euclidean distance.

With regard to the Anthropocene timescale, initial attempts at biclustering analysis using data from all cores simultaneously, constructed six biofacies with one group distinct from all others. The distinct group consisted of VP samples (214 to 90cm) and a TL sample (78cm). The VP samples are characterized by *Charophyte* encrustations and high

abundances of *C. aerophila*. Similar macro and micro fossils were also observed in the TL78 sample (and in no other TL sample) suggesting a pre-1800 origin for the Tub Lake sediment. Thus, the VP samples from 274 to 90cm and the TL sample at 78cm were removed from further Anthropocene timescale analysis to enable comparison of only recent deposits.

# 4.2.2 <u>Species Richness and Diversity</u>

Diversity was calculated using  $AA_{\it TA}$  counts and Hill's family of diversity numbers for effective number of species (Hill 1973)

$$N0=S$$
 (5)

where S is the total number of species,

$$NI = e^{H'}$$
 (6)

where H' is Shannon's Diversity index (Shannon 1948), and

$$N2 = 1/\lambda$$
 (7)

where  $\lambda$  is Simpson's Diversity index (Simpson 1949). The  $\bar{N}l:N0$  ratio was also calculated to assess species rarity related to ecosystem stress (Odum 1985). Calculations for Nl and N2 were completed using functions from the vegan and rioja R packages respectively (Oksanan et. al 2013;

Juggins 2012).

## 4.2.3 <u>Stratigraphic displays</u>

Testate amoebae fractional abundances based on bicluster analysis, Hill's diversity, sample absolute abundances, bicluster divisions, and age-depth data are plotted stratigraphically for each core using the *rioja* R package (Juggins 2012).

# 4.3 Statistical methods specific to Anthropocene analysis

## 4.3.1 Ordination-based rate of change

Detrended Correspondence analysis (DCA) with downweighting of rare species was conducted on untransformed testate amoebae data (  $AA_{TA}$  ) from all four cores to summarize major patterns of assemblage variation (Birks 2010) using the decorana() function from the vegan R package (Oksanen et al. 2013).

Ordination-based rate of change using the scores from axis one, two, and three of the DCA analysis was conducted on all cores by first interpolating species data along the length of each core to every 0.5cm using the <code>interp.dataset()</code> function (spline method) in the <code>rioja</code> R package, and extracting the off-diagonal values from the distance matrix of the DCA scores (Simpson 2013; Birks 2012). Rate of change was plotted against depth for all cores. Summary statistics for rate of change

after European settlement depths were also calculated for each core to assess the stability of testate amoebae communities since initial land clearing.

## 4.3.2 <u>Environmental records</u>

Long term climate records were available for the region from Environment Canada (2013). Records of air temperature and precipitation were collected from climate stations near to Lake Simcoe from 1866 to 2011 (Table 7). As a continuous record of air temperature and precipitation was not available from a single climate station, climate data was retrieved from multiple stations no further than 50km from the lake centre (44.4367, -79.3392) to construct a nearly continuous record.

Table 7. Environment Canada climate station locations and dates used.

Climate Station	Years	Coordinates
Barrie	1866-1873; 1877-1887; 1893-1900; 1909- 1917;1919-1921; 1931- 1935;1954-1957	44.4, -79.683333
Sutton West	1874; 1905	44.316667, -79.3
Orillia	1875; 1901-1902; 1904; 1906-1907;1918; 1926- 1927;1929-1930;1936-1946; 1949-1952	44.616667, -79.4
Newmarket	1876	44.066667, -79.433333
Coldwater	1888-1892;1903; 1908	44.033333, -79.666667

Beeton	1922-1925;1928;1947	44.1, -79.783333
Midhurst	1948;1953;1958-1962;1964- 1972	- 44.45 <b>,</b> -79.766667
EssaOntHydro	1963	44.35, -79.816667
Shanty Bay	1973-2011	44.399444, -79.632778

Non-continuous lake-ice records were available from various sources (IceWatch Canada 2013; Environment Canada and Lake Simcoe Fisheries Assessment Unit data as cited in Stainsby and MacRitchie 2011; Youtube videos) for the years 1853 to 2011. Data obtained from Stainsby (2011) from 1970 to 2008 was supplemented with IceWatch data recorded at Kempenfelt Bay (1853-1969) by volunteers, and Youtube videos documenting ice freeze and thaw in southern Lake Simcoe (2009-2011).

Trend analyses on climate and lake-ice data were completed using linear regression models: ordinary least squares, generalized least squares, and generalized linear model, depending on the parametric assumptions violated by the data being analyzed. Annual and seasonal means for maximum, minimum, and mean air temperatures as well as total rainfall, snowfall, and precipitation were assessed for trends.

Total phosphorus load data (kg yr<sup>-</sup>1) from ~ AD 1850 was also retrieved from multiple sources (O'Connor et al.

2013,2012; Peat and Waiters 1994; Scott and Winter 2006; Snodgrass and Holubeshen 1992; Scott et al. 2001; Wilson 1986) for use with Victoria Point testate amoebae assemblage variation. The data arises as a result of both statistical estimation and direct measurement. Total phosphorus load for the entire lake was chosen based on the available data and the location of Victoria Point at the outlet of the lake. Phosphorus measurements specific to the Orillia area did not begin until the late 1990s while total lake phosphorus has been estimated for as far back as 1782.

## 4.3.3 <u>Elemental and Isotopic analysis</u>

Dried and ground samples of every other cm (0 to 28cm) from Victoria Point, Cook's Bay, and Tub Lake were sent to MBL Stable Isotope Laboratory in Wood's Hole, MA for analysis of the percentage of carbon (%C) and Nitrogen (%N), and  $\delta^{13}$ C and  $\delta^{15}$ N isotopes for the determination of changes to the abundance and origin of deposited organic matter.

## 4.3.4 Climatic and nutrient analysis

As  $^{210}\text{Pb}$  dating was conducted on sediments only from the Victoria Point core, additional statistical analyses were conducted on the core to assess the influence of measured and estimated environmental variables on testate amoebae distribution since  $\sim$  AD 1850.

Principle component analysis (PCA) using testate amoebae assemblage, air temperature, total rainfall, snowfall, precipitation, and phosphorus load data was used to assess correlations between environmental variables and testate amoebae assemblages using the prcomp() function with the correlation matrix option in R (R Core Team 2013). PCA scores from axes one to three were plotted in an interactive three dimensional biplot using the rgl:3D visualization device system (Adler et al. 2013) graphics package in R. Screenshots of various angles of the plot are presented.

PCA was also conducted on only testate amoebae assemblages for comparison of PCA assemblage scores from axis one, two and three to environmental variables using Pearson correlation analysis. Correlations were also run on the fractional abundances of dominant taxa identified through bicluster analysis.

# Chapter 5. Lake Simcoe's shoreline during the Anthropocene

#### 5.1 Results

## 5.1.1 Age-Depth modelling

Results from <sup>210</sup>Pb analysis (Table 8) of the Victoria Point core indicate supported <sup>210</sup>Pb background lies within the 36 - 44cm depth profile range. *Ambrosia* pollen abundances (Figure

19) within the span provide a depth of 42cm as a likely candidate for the year 1871 when railway construction was completed in the vicinity of the sampling site (Hunter 1893).

Table 8. 210 Pb analysis results using the CRS model.

Table 8. 210 Pb analysi	is results using the	e CRS model.
Depth (cm)	Age (AD)	Sediment Accumulation Rate (cm y <sup>-1</sup> )
0	2011.8 ± 0	1.1
1	2010.9 ± 0.1	1.3
2	2010.1 ± 0.1	0.94
3	2009.1 ± 0.1	0.65
4	2007.5 ± 0.2	0.83
5	2006.3 ± 0.1	0.54
6	2004.4 ± 0.4	0.5
7	2002.5 ± 0.2	0.51
9	1999 ± 0.7	0.4
10	1996 ± 0.3	0.51
12	1992 ± 1.0	0.76
13	1991 ± 0.4	0.37
15	1985 ± 1.4	0.3
16	1982 ± 0.6	0.03
18	1976 ± 2.3	0.39
19	1973 ± 0.9	0.44
21	1969 ± 2.7	0.53
22	1967 ± 1.2	0.57
24	1963 ± 3.8	0.43
25	1961 ± 1.6	0.45

27	$1957 \pm 4.8$	0.59
28	1955 ± 2.1	0.57
29	1953 ± 5.4	1.02
30	1952 ± 2.7	0.23
35	1931 ± 9.8	0.11
36	1922 ± 10	0.12
44	<1861	

## 5.1.2 <u>Environmental records</u>

Trend analysis of annual climate variables shows a rise in mean, maximum, and minimum annual air temperatures from 1866 to 2011 with the greatest increase in minimum temperatures (2°C,  $R^2$ =0.23,  $p < 1 \times 10^{-8}$ ) (Figure 6). Total annual precipitation shows a positive trend ( $R^2$ =0.25 and 0.21) resulting in a 248.6mm increase since 1866 with most of the contribution coming in the form of rain (200.1mm). Annual total snowfall also increased though not as conclusively (44.4mm,  $R^2$ =0.03) (Figure 7).

Air temperature trends show increases in both mean and minimum values across all seasons (Figures 8,10,12,14). Mean temperatures rose significantly in the winter and spring by  $1.3^{\circ}\text{C}$  (p=0.0123) and  $2.5^{\circ}\text{C}$  ( $p<1x10^{-5}$ ) respectively. The change in minimum temperatures for winter ( $2.6^{\circ}\text{C}$ ) ( $p<1x10^{-4}$ ) and spring ( $2.8^{\circ}\text{C}$ ) ( $p<1x10^{-7}$ ) were also highly significant.

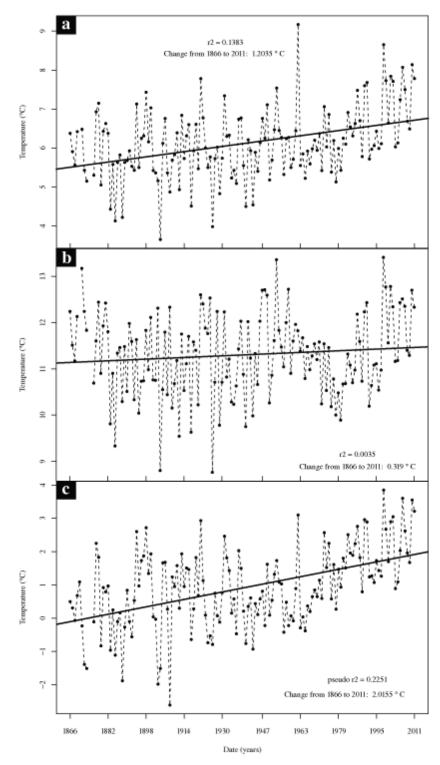


Figure 6.Trend analysis plots of a) mean annual air temperatures, b) maximum annual air temperatures, c) minimum annual air temperatures

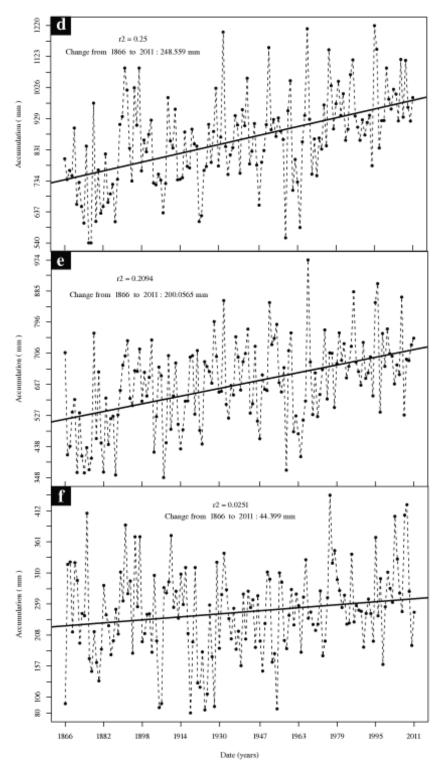


Figure 7.Trend analysis plots of d)annual total precipitation, e)annual total rain, f)annual total snow

Maximum temperatures have both slightly decreased (winter and summer) and increased (spring and autumn) with spring showing the largest increase at  $1.7\,^{\circ}\text{C}$ .

Total precipitation (Figures 9,11,13,15) has increased in all seasons. More specifically, rainfall and snowfall have increased throughout the seasons with the exception of spring snowfall, which has decreased slightly by 6.88mm and summer snowfall which remains unchanged (no snow). The greatest seasonal rainfall increase are the spring rains, increasing by 64.59mm since 1866. Winter snowfall showed the largest increase of 50.13mm.

Lake-ice trends (lake water freeze and thaw dates) indicate an increase of 22.88 days to the ice-free season (Figure 16).

### 5.1.3 Lake Simcoe-wide testate amoebae assemblages

Preservation of testate amoebae tests was very good throughout the cores with very few broken tests and representatives present from each test type: proteinaceous (e.g. Hyalospheniidae, Arcellidae), agglutinated (e.g. Centropyxidae, Difflugiidae), siliceous (e.g. Euglyphidae, Trinematiidae), and calcareous (e.g. Paraquadrulidae). Mean test counts in the top 30cm were 208 per sample and 71 per downcore sample due to decreased test density.

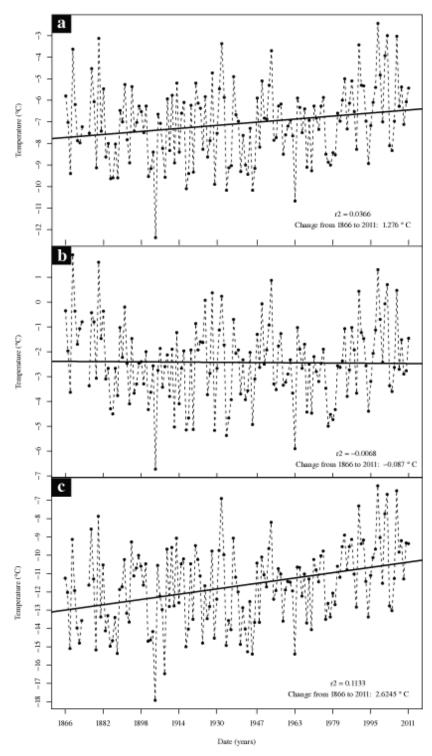


Figure 8.Trend analysis plots of a) mean winter air temperatures, b) maximum winter air temperatures

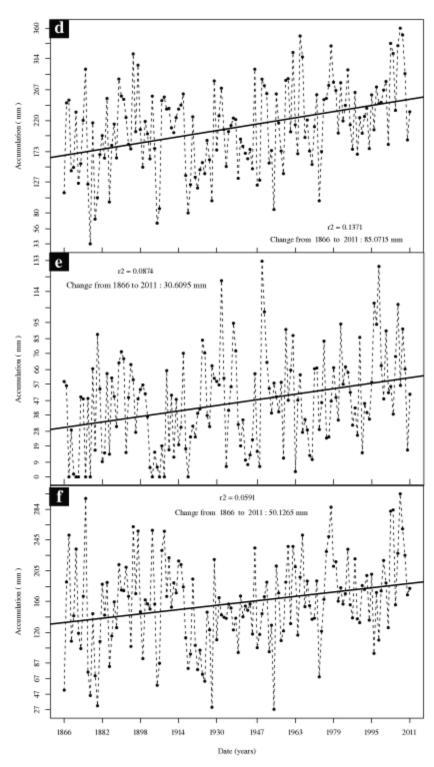


Figure 9.Trend analysis plots of d)winter total precipitation, e)winter total rain, f)winter total snow

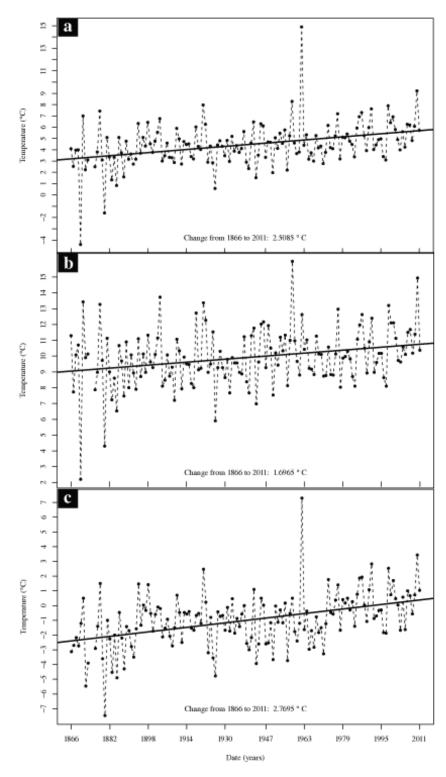


Figure 10.Trend analysis plots of a) mean spring air temperatures, b) maximum spring air temperatures, c) minimum spring air temperatures

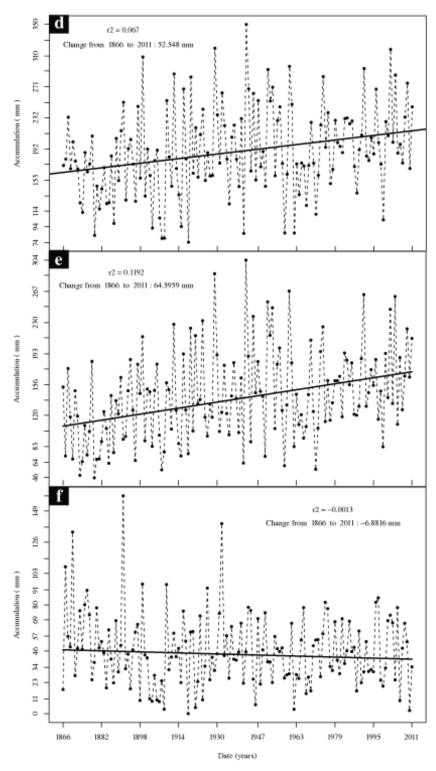


Figure 11. Trend analysis plots of d) spring total precipitation, e) spring total rain, f) spring total snow

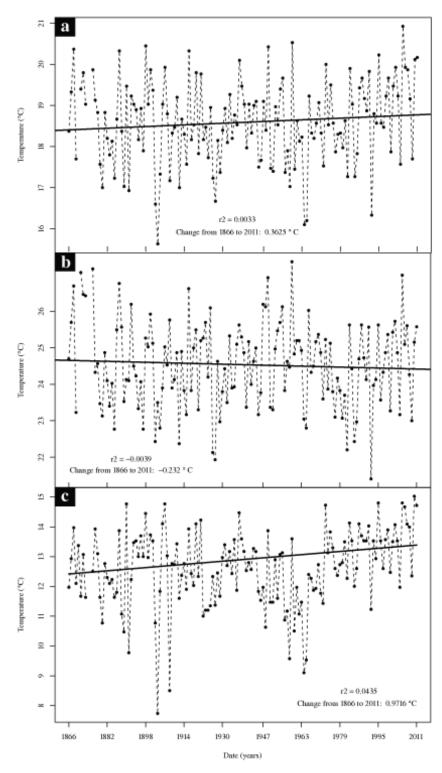


Figure 12. Trend analysis plots of a) mean summer air temperatures, b) maximum summer air temperatures, c) minimum summer air temperatures

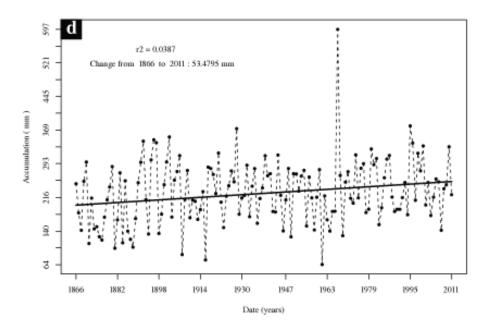


Figure 13. Trend analysis plot of d) summer total precipitation/rain (there was no snowfall throughout the record)

Simple species richness ( N0 ) ranged from 1 to 26 with a mean of 9.95 and maximum richness in the Victoria Point core. Species diversity, in terms of Hill's NI (abundant effective species), increased up the cores with the exception of Tub Lake. All three cores from Lake Simcoe had the greatest values of NI in the top 8cm. Six NI species were recorded at 18cm in the Tub Lake core before falling to 3 at 0cm. N2 diversities (very abundant effective species) also increased up-core but were more subtle than NI, ranging from 1.0 in TL to 9.8 in CB. Summary values of each Hill number from combined core results are displayed in boxplot format (Figure

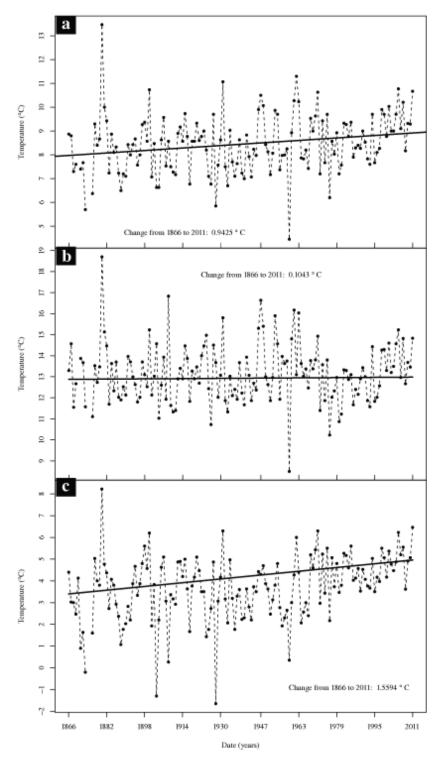


Figure 14. Trend analysis plots of a) mean autumn air temperatures, b) maximum autumn air temperatures

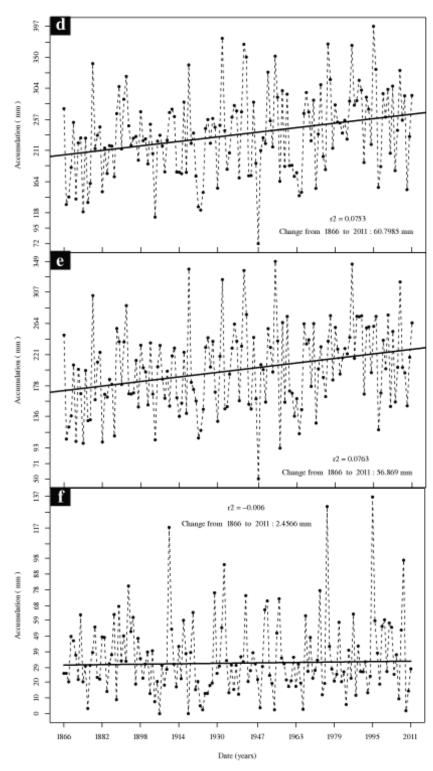


Figure 15. Trend analysis plots of d) autumn total precipitation, e) autumn total rain, f) autumn total snow

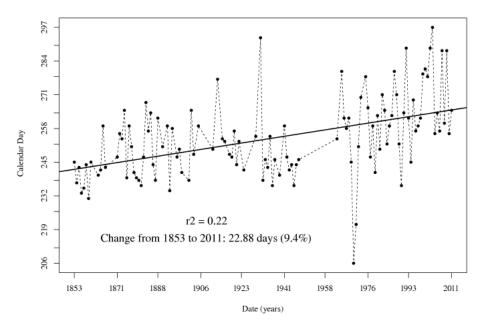


Figure 16. Trend analysis plot of ice-free days.

17) and detailed richness and diversity results are displayed stratigraphically (Figures 19-22) and described (see 5.1.5).

Although eighty-five taxa of testate amoebae across all cores were included, bicluster analysis indicated that only fifteen taxa in twoseparate assemblages in four, mostly distinct, biofacies described the samples based on Euclidean distance (Figure 18). Assemblage I consists of P. acropodia, C. delicatula, C. platystoma, C. spinosa(aculeata strain), and P. cymbalum, all highly dominant taxa. Assemblage II consists of less dominant taxa: C. aerophila(soil type), C. aculeata, P. fulva, D. globulosa, C. kahli, H. elegans, C. orbicularis, A. discoides, A. arenaria, and C. discoides. A third assemblage, Assemblage III, was identified to be composed of

the remaining rare taxa and will not be referenced in further analysis.

Assemblage I: Biofacies I and II have only subtle changes based on dominance fluctuations of highly abundant taxa throughout all Lake Simcoe cores. Biofacies III and IV show distinctions between themselves and from Biofacies I and II. Biofacies III, containing only DP and VP samples, is uniquely

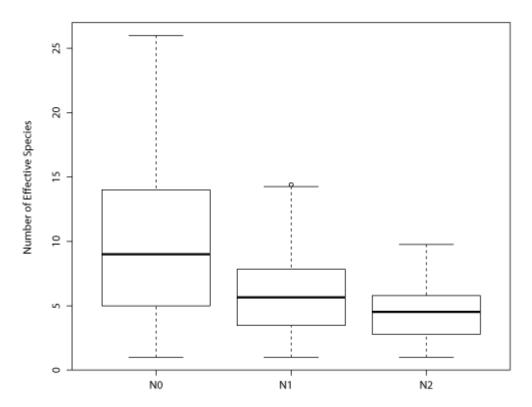


Figure 17.Boxplot of Hill's Family of Diversity numbers for all cores combined.

dominated by C. delicatula to the near exclusion of the taxa dominant in the other Biofacies. Biofacies IV containing all TL samples and one CB sample is highly dominated by P.

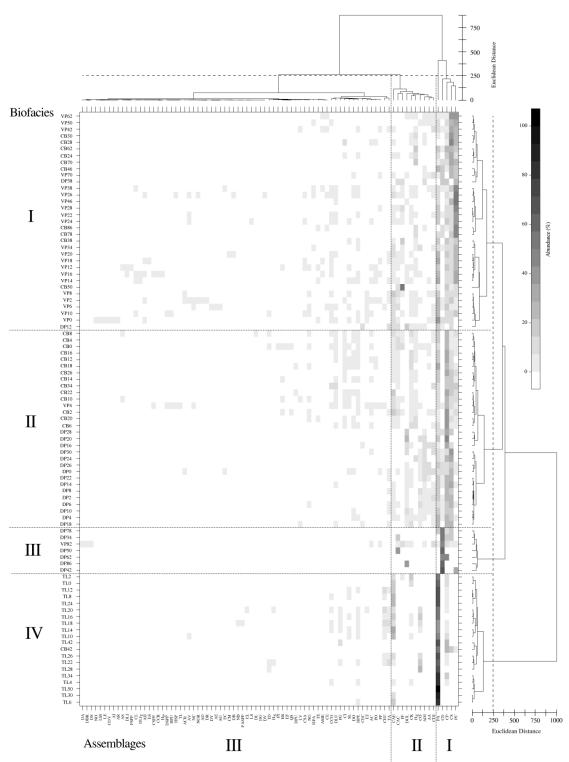


Figure 18.Bicluster heatmap of biofacies and associated assemblages for all cores combined. See page xviii for taxa abbreviations.

acropodia, reaching 100% abundance in one sample. Fractional abundance ranges of the taxa characterizing each Biofacies from Assemblage I are shown in Table 9.

Assemblage II: This assemblage of less abundant taxa reveals a similar pattern to the distribution of Assemblage I: subtle changes are evident between Biofacies I and II while greater distinction exists in Biofacies III and IV. The sum of fractional abundances of the taxa found in each Biofacies from Assemblage II are shown in Table 10.

Table 9. Fractional abundances of taxa characteristic of biofacies in Assemblage I. See page xviii for taxa abbreviations.

Biofacies	Taxa	Minimum Abundance (%)	Maximum Abundance (%)			
I	P. cymbalum	0	55.57			
	C. spinosa	0	42.95			
	P. acropodia	0	36.63			
	C. platystoma	0	21.86			
II	C. platystoma	8.33	43.9			
	C. spinosa	0	40			
	P. acropodia	4.54	23.98			
III	C. delicatula	43.16	71.26			
	C. platystoma	0	50			
IV	P. acropodia	41.61	100			

Table 10. Sum of fractional abundances of taxa characteristic of biofacies in Assemblage II. See page xviii for taxa abbreviations.

	Assemblage II Taxa										
Biofacies	CAE	CAC	PF	DGL	CK	HE	CO	ADI	AA	CDI	
I	157.4	68.8	128.1	28.4	41	108.4	16.2	50.42	10.85	39.3	
II	112.1	69.4	22.7	107.8	93.4	59.8	73.4	101.1	54.6	89.1	
III	0	60.2	9.6	42.06	1.37	0	0	1.37	0	8.23	
IV	245.1	0	5.9	0	34.5	0	93.8	0	0	0	

## 5.1.4 <u>Testate amoebae assemblages by core</u>

#### 5.1.4.1 Victoria Point

Bicluster analysis of the Victoria Point profile identified three Q-mode clusters (Figure 19) of species included in both assemblages identified in the main bicluster analysis (see 5.1.3). The three clusters are separated into six zones showing assemblage change at 70cm, 46cm, 42cm (AD 1871), and 10cm (AD 1996). Biofacies I is characterized primarily by the dominance of P. cymbalum (36.04  $\geq F_i \leq$  55.57) followed by P. acropodia (9.06  $\geq F_i \leq$  19.76). In Biofacies II, primary dominance shifts from P. cymbalum (20.00  $\geq F_i \leq$  37.41) to P. spinosa(aculeata strain) (24.29  $\geq F_i \leq$  35.78) although dominance is near equal. Biofacies III located at the top and bottom of the profile, is dominated by P. acropodia (1.37  $\geq F_i \leq$  36.63), followed by P. platystoma

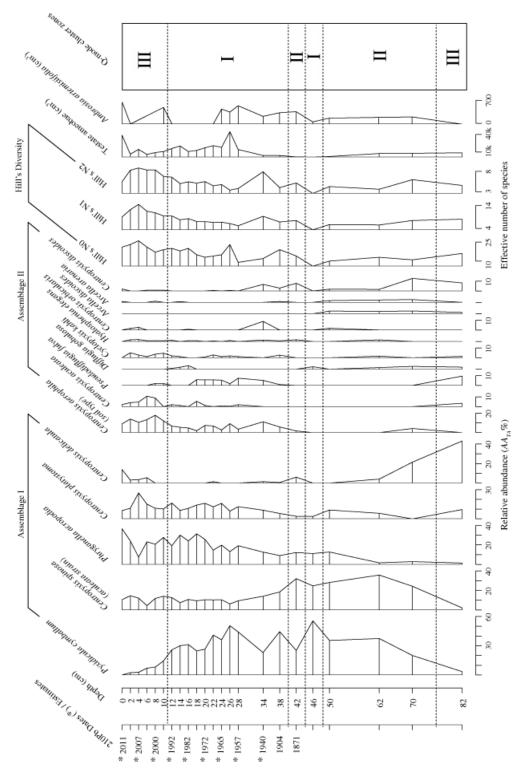


Figure 19.VP stratigraphic plot of Biofacies assemblages relative frequencies, Q-mode cluster zones,  $^{210}{\rm Pb}$  & Ambrosia abundances, and richness & diversity.

(8.43  $\geq$   $F_i$   $\leq$  26.66), and C. aerophila(soil type) (0  $\geq$   $F_i$   $\leq$  17.82).

### 5.1.4.2 Cook's Bay

Three Q-mode clusters (Figure 20) of species included in both assemblages identified in the main bicluster analysis were also identified in the Cook's Bay core profile. The three clusters are separated into eight zones showing assemblage change at 50cm (~ AD 1830), 46cm, 42cm, 30cm, 26cm, and 24cm.

Biofacies I is defined at three separate core locations characterized primarily by the dominance of C. platystoma  $(6.25 \geq F_i \leq 42.86)$  followed by P. acropodia  $(4.54 \geq F_i \leq 51.38)$ . Biofacies II is dominated by a single species, P. fulva with a fractional abundance of 52.65. Like Biofacies I, Biofacies III is interspersed throughout the core (86-62cm, 46cm, 30-28cm, and 24cm). P. cymbalum  $(13.99 \geq F_i \leq 45.86)$  is closely followed by C. spinosa(aculeata strain)  $(11.11 \geq F_i \leq 42.95)$  in dominance of this zone.

#### 5.1.4.3 Duclos Point

The Duclos Point core was also composed of three separate Q-mode clusters (Figure 21) of species included in both assemblages identified in the main bicluster analysis. The three clusters are separated into twelve zones showing assemblage change at 62cm, 38cm (~ AD 1830), 34cm, 30cm, 28cm,

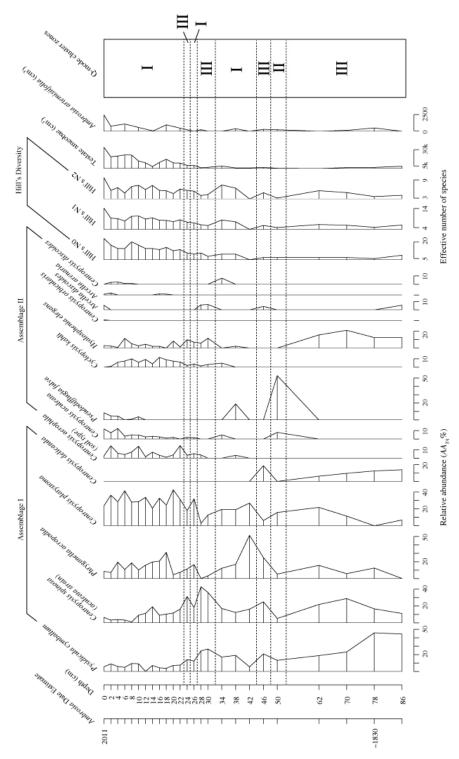


Figure 20.CB stratigraphic plot of Biofacies assemblages relative frequencies, Q-mode cluster zones, Ambrosia estimates & abundances, & richness & diversity.

26cm, 20cm, 18cm, 16cm, and 14cm.

Biofacies I, defined at five separate core locations, is characterized primarily by the dominance of C. platystoma  $(18.98 \ge F_i \le 50.00)$  followed by C. delicatula  $(0 \ge F_i \le 50.00)$ . Biofacies II (four separate zones) is dominated by C. spinosa(aculeata strain)  $(12.12 \ge F_i \le 40.00)$ , C. platystoma  $(0 \ge F_i \le 27.96)$ , and P. acropodia  $(8.33 \ge F_i \le 23.98)$ . Biofacies III (three separate zones) is dominated most strongly by C. delicatula  $(18.98 \ge F_i \le 60.00)$ .

#### 5.1.4.4 Tub Lake

Bicluster analysis defined two Q-mode clusters in the Tub Lake core (Figure 22) of species included in both assemblages identified in the main bicluster analysis. The two clusters are separated into six zones showing assemblage change at 42cm, 34cm, 28cm, 6cm, and 2cm. Biofacies I and II are defined at three separate core locations each (I: 42cm,28-8cm, and 2-0cm; II:50cm, 34-30cm, and 6-4cm) (Figure 22). Additionally, both Biofacies are characterized primarily by the dominance of P. acropodia which is more abundant in Biofacies I (81.36  $\geq$   $F_i$   $\leq$  100.00) than Biofacies II (41.61  $\geq$   $F_i$   $\leq$  71.27). The second most dominant species in both biofacies is C. aerophila(soil type) with abundances of  $0 \geq$ 

 $F_i \leq$  17.37 and 0  $\geq$   $F_i \leq$  31.79 for Biofacies I and II respectively.

## 5.1.5 <u>Species richness and diversity</u>

The following descriptions of Hill's family of diversity results focus on results from one sample prior to *Ambrosia*-based background in each core. Complete results are displayed graphically in the stratigraphic plot for each core (Figures 19-22).

#### 5.1.5.1 Victoria Point

Simple species richness ( N0 ) ranged from 6 to 26 with the maximum richness at 4cm, and minimum richness at 46cm. Species richness increased to  $\geq$  20 at 26,16,12,6,4,2, and 0cm. Species diversity, in terms of Hill's NI (abundant effective species), increased up the core, with greatest diversity between 8 and 2cm deep. N2 diversity (very abundant effective species) also increased up-core but was more subtle than NI, ranging from 2.6 (46cm) to 8.377 (4cm). The lowest N1:N0 ratio was recorded at 26cm deep (0.26) while the highest was at 34cm (0.77). The mean N1:N0 ratio was 0.49.

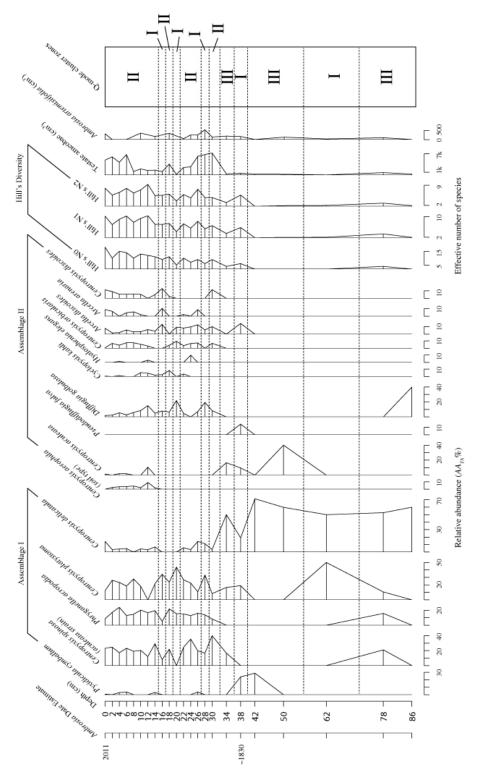


Figure 21.DP stratigraphic plot of Biofacies assemblages relative frequencies, Q-mode cluster zones, Ambrosia estimates & abundances, and richness & diversity.

# 5.1.5.2 Cook's Bay

Simple species richness ( N0 ) ranged from 4 to 23 with the maximum richness at 1cm, and minimum richness at 42cm. Species richness increased to  $\geq$  20 at 8, and 0cm. Species diversity, in terms of Hill's NI (abundant effective species), increased up the core, with maximum values at 10cm (10.1) and 0cm (14.2) deep. N2 diversity (very abundant effective species) also increased up-core but was more subtle than NI, ranging from 2.7 (42cm) to 9.76 (0cm). The lowest N1:N0 ratio was recorded at 18cm deep (0.52) while the highest was at 62cm (0.94). The mean N1:N0 ratio was 0.66.

#### 5.1.5.3 Duclos Point

Simple species richness ( N0 ) ranged from 4 to 18 with the maximum richness at 1cm, and minimum richness at 34cm. Species richness consistently increased to  $\geq$  10 between 14 and 0cm. Hill's NI (abundant effective species) diversity increased up the core, with maximum values at 12, 6, and 0cm (10.2-10.3) deep. N2 diversity (very abundant effective species) also increased up-core but was more subtle than NI, ranging from 3.0 (34cm) to 9.3 (12cm). The lowest N1:N0 ratio was recorded at 0cm deep (0.57) while the highest was at 38cm (0.96). The mean N1:N0 ratio was 0.76.

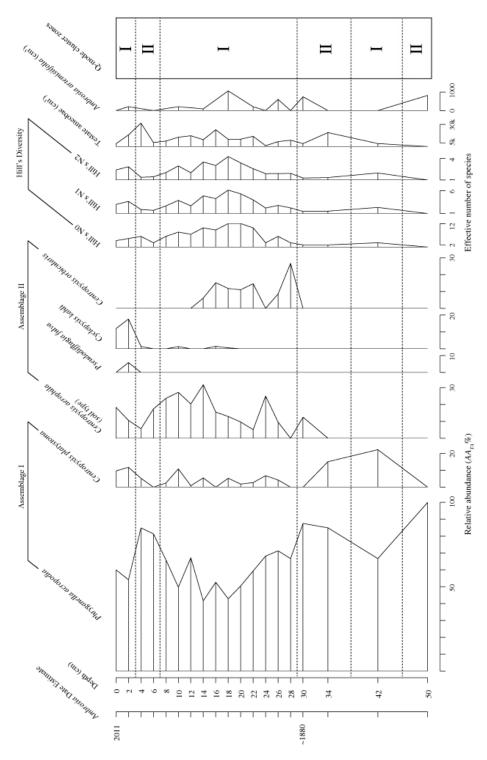


Figure 22.TL stratigraphic plot of Biofacies assemblages relative frequencies, Q-mode cluster zones, Ambrosia estimates & abundances, and richness & diversity.

#### 5.1.5.4 Tub Lake

Simple species richness ( N0 ) ranged from 2 to 12 with maximum richness at 18 and 20cm and minimum richness at 30 and 34cm. Species richness increased up the core, reaching  $\geq$  10 from 22 to 18, and against 14cm deep before declining to 4 by 0cm. Hill's NI (abundant effective species) diversity fluctuated between 1.45 (30cm) and 6.12 (18cm), averaging 3.18 for the core. N2 diversity (very abundant effective species) was similar to NI but with a mean of 2.35 with the greatest reductions at 18 and 20cm. The lowest N1:N0 ratio was recorded at 4cm deep (0.31) while the highest was at 50cm (1.0) with a mean of 0.60.

# 5.1.6 Ordination-based rate of change

Rate of change analysis shows increases of change near Ambrosia-based background date estimates (Figure 23,24). Cook's Bay has a substantial delayed rate of change following initial Ambrosia abundances from settlement.

The greatest rate of change associated with European settlement in any core, 0.88, is found in the Duclos Point core at 34.5cm. The mean rate of change following settlement range from 0.069 to 0.086 (VP and DP). All three Simcoe cores show increased rates of change within the upper 6cm; the rate of change peak in Victoria Point beginning in ~AD 2000 (8cm)

is the greatest change since the late Holocene began at ~82cm. Change in Tub Lake was fairly stable between land clearing and ~8cm where change peaks to 0cm come close to the levels of change associated with land clearance.

Rate of change summary statistics (Figure 25) of postsettlement samples confirm that Duclos Point has experienced the greatest rates of change in all aspects (minimum, maximum, median, mean and the quartiles).

## 5.1.7 <u>Elemental and Isotopic analysis</u>

The %C measured by MBL Stable Isotope Laboratory differed between the Tub Lake sample and the Lake Simcoe samples (Figure 26). The TL %C measurements exhibited an general decline from the highest value (45%) at 26cm to the lowest values of 7.4 and 8.1% at 18 and 0cm respectively. A peak of 31.7% at 8cm was also measured. In contrast, %C measurements in VP and CB remained fairly stable with a range of measurements from 37.8 to 48.5. Stability of %C increased in the CB core from 14 to 0cm and in the VP core from 8 to 0cm. The TL %N measurements followed a similar trajectory to %C, falling from 2.31% at 26cm to a low of 0.48% at 18cm and 0cm, with an additional peak at 8cm of 2.09% (Figure 30). The %N measurements at CB also followed %C fluctuations until 18cm at which point %N became stable until 8cm. %N then increased

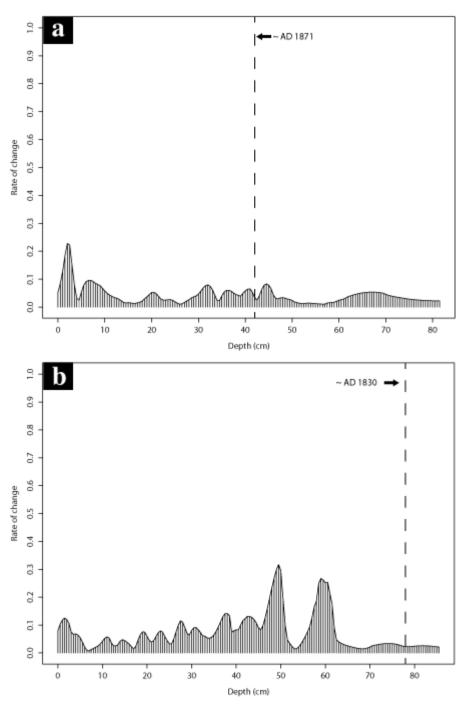


Figure 23.Ordination based rate of change plots for a) Victoria Point and b) Cook's Bay

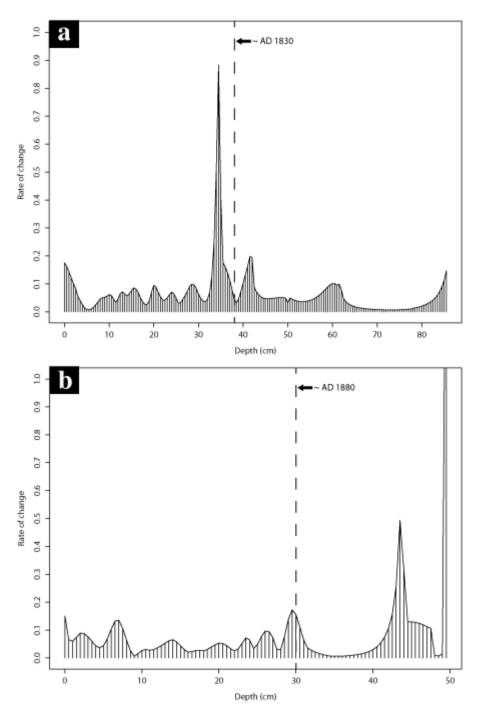


Figure 24.Ordination based rate of change plots for a)Duclos Point and b)Tub Lake

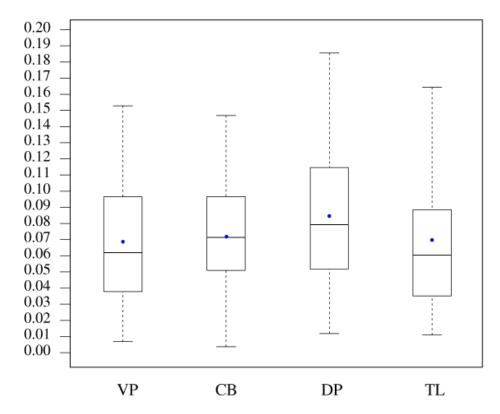


Figure 25.DCA-based rate of change summary statistics of post-Ambrosia spike samples in each core.

from 2.33% to 3.07% after which it remained between 3.0 and 3.39%. The %N in the VP core fluctuated between 2.01 and 2.90% with a small peak at 12cm and a larger decline at 2cm.

The C:N ratio of Tub Lake begins in the low 20's at 28 and 26cm (23.54 and 22.43) before declining sharply to 18.62 at 24cm (Figure 26). From 24 to 2cm, the C:N ranged between 17.35 and 18.62 with a mean of 17.98. Finally, a slight increase to 19.73 at 0cm in the TL core is evident. The Cook's Bay core shows initial C:N ratios of 18.2 and 18.5 at 28 and 26cm

followed by an increase to 21.77 at 24cm. From 24 to10cm, the CB C:N ratio had a mean of 21.24 and a range of 19.63 to 22.98. At 8cm there is a large decline from 22.06 (10cm) to 16.8. Between 8 and 0cm, there is a steady decrease in the ratio, ending at 14.78. The C:N ratio of Victoria Point is fairly stable from 28cm to 10cm with a mean of 18.27. From 8 to 0cm is again stable at a mean of 19.99 with the exception of a large peak at 2cm of 27.15.

The  $\delta^{13}C$  and  $\delta^{15}N$  values in the TL core are fairly stable with a general decline towards 0cm (Figure 26). Values range between -26.1 and -27.5 ( $\delta^{13}C$ ) and 1.2 and 3.4 ( $\delta^{15}N$ ). There is a peak of  $\delta^{13}C$  from 24 to 22cm and a  $\delta^{15}N$  decline at 2cm. The  $\delta^{13}C$  and  $\delta^{15}N$  values of VP also remain fairly stable until 8cm where there is a decline in both isotopes ( $\delta^{13}C = -27.8$  to -28.9,  $\delta^{15}N = 1.2$  to -0.1), increasing again towards 2cm. CB shows a unique isotope signature with a general increase in  $\delta^{15}N$  beginning at 18cm followed by a sharp increase at 10cm ending in a measurement of 7.9 at 0cm. For  $\delta^{13}C$  there is a near opposing profile as  $\delta^{13}C$  declines throughout the core beginning it's sharp decrease at 10cm followed by a slight recovery from 4 to 0cm.

### 5.1.8 <u>Climatic and nutrient analysis</u>

Exploratory Pearson correlation analysis of numerous

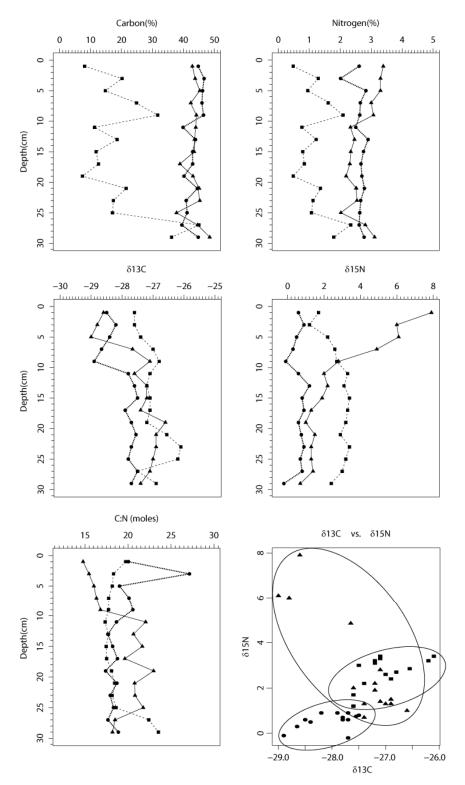


Figure 26.Elemental and isotopic measurements of VP (circle), CB (triangle), and TL (square) cores.

environmental variables - annual and seasonal minimum, maximum, and mean air temperatures, annual and seasonal total rain, snow, and precipitation, the number of ice-free days, C:N ratios, and phosphorus loads - indicated strong correlations (e.g. r=0.88 at p<0.000001) between a number of similar variables (e.g. minimum and maximum temperatures to mean temperatures). Therefore, seasonal maximum and minimum temperatures, and total precipitation were removed from further analysis. Additionally, C:N ratio and ice-free days data were incomplete for the time period (AD 1871-2011) and were removed from principle component analysis involving both assemblage and environmental data.

While annual variables did not consistently show significant correlations to seasonal variables, studies suggest a strong seasonal growth pattern for testate amoebae (Davidova and Vailev 2013; Warner et al. 2007; Gilbert et al. 2003; Velho et al. 1999), thus, all annual variables (maximum, minimum, and mean temperatures, and total rain, snow, and precipitation) were removed from principle component analysis and Pearson correlation analyses.

Principle component analysis of Victoria Point testate amoebae assemblages and the remaining environmental variables returned a 0.47 cumulative proportion of variance for the

first three components (Figures 27-30 showing various angles of the PCA results). Additionally, all three components accounted for >0.1 of the proportion of variance (PC1=0.21, PC2=0.14, PC3=0.13). Application of Horn's Parallel Analysis (Horn 1965) using the paran() function in the paran R package (Dinno 2012) retained the first three components as relevant.

Visualization of PCA sample variation in three dimensions showed most variation is associated with quadrants four and three (Figure 31). Three samples with increased variation appear at the extremes of quadrant eight (0cm depth), quadrant five (26cm depth), and quadrant six(4cm depth). Five of twelve environmental variables plotted with directionality show direct positive and negative associations with a number of samples (Table 11).

Pairwise Pearson correlations of PCA components one, two, three, with fourteen environmental variables including C:N ratios and ice-free days (Table 12) show strong significant correlations with variables from all seasons. Component one is negatively correlated to mean temperatures during the spring, summer, and autumn seasons, and positively correlated to spring snowfall. Component two is negatively correlated to lake-wide phosphorus loads and component three is positively

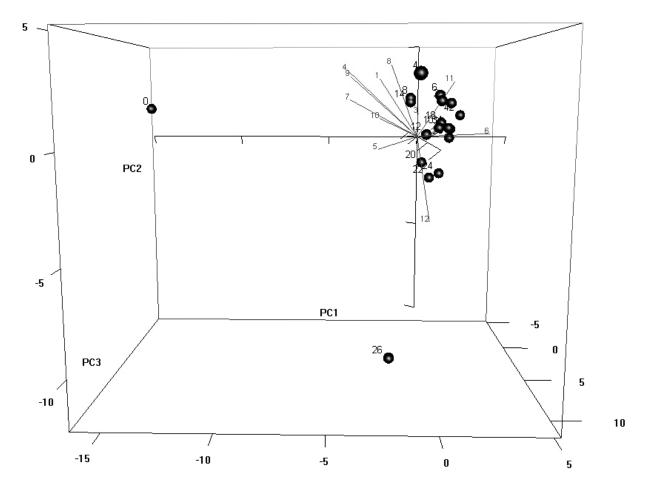


Figure 27. Three dimensional plot of Principal Component Analysis. 3D points are VP samples - numbers correspond with the top depth (cm) of each sample, i.e. 26 = 26cm deep. Lines emanating from the axes center correspond to environmental variables where 1=winter mean temp., 2=winter rain, 3=winter snow, 4=spring mean temp., 5=spring rain, 6=spring snow, 7=summer mean temp, 8=summer precipitation/rain, 9=autumn mean temp., 10=autumn rain, 11=autumn snow, 12=phosphorus.

correlated to winter mean temperature and rainfall and negatively correlated to winter snowfall. Weaker positive associations also exist for spring mean temperatures and summer rainfall. Pairwise Pearson correlations of Assemblage I

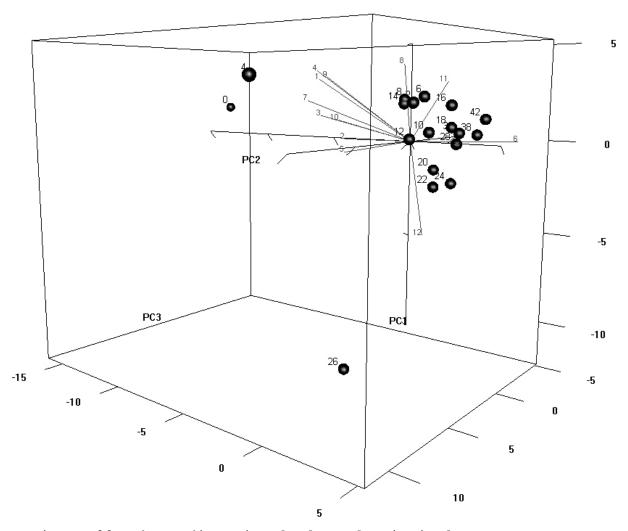


Figure 28. Three dimensional plot of Principal Component Analysis. Point and line information is presented with Figure 27.

taxa identified in bicluster analysis and environmental variables (Table 13) shows a strong significant, consistent, negative

correlation with spring snowfall in four of five highly abundant species. Positive correlations at p < 0.05 exist for

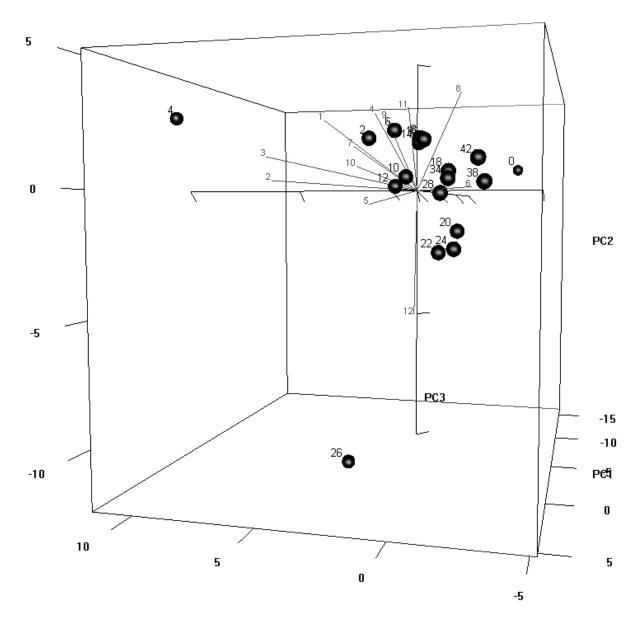


Figure 29. Three dimensional plot of Principal Component Analysis. Point and line information is presented with Figure 27.

spring (*P. acropodia*), summer, and autumn (*C. delicatula*) mean temperatures, and phosphorus loading (*P. cymbalum* and *C. platystoma*). Other less significant correlations indicate a

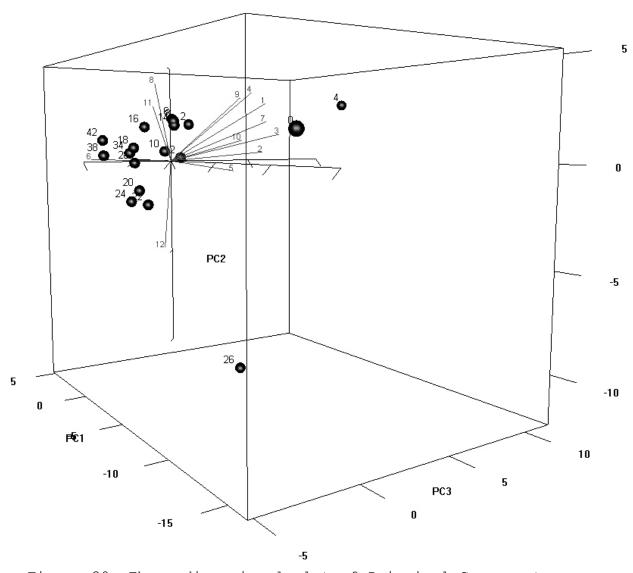


Figure 30. Three dimensional plot of Principal Component Analysis. Point and line information is presented with Figure 27.

relationship mainly with seasonal precipitation. Pairwise

Pearson correlations of Assemblage II taxa with environmental variables (Table 14) resulted in variable correlations by species. C. aculeata, and P. fulva are the most highly correlated species. P.fulva is positively correlated to

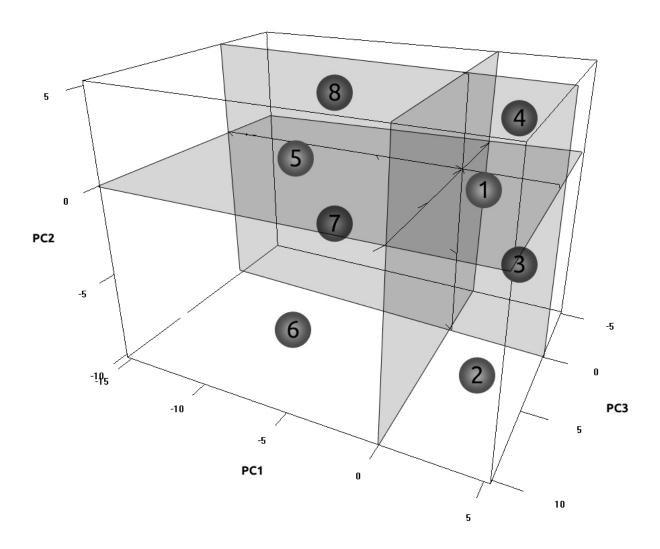


Figure 31. Three dimensional PCA quadrants and associated components/axes (PC1 = principal component 1; PC2 = principal component 2; PC3 = principal component 3).

phosphorus loading and negatively correlated to winter and summer mean temperatures, summer rainfall, and C:N ratios. *C. aculeata is* positively correlated to spring, summer, autumn, and winter mean temperatures, and ice-free days and negatively

Table 11. Direct correlations of PCA analysis of combined testate amoebae assemblage and environmental variables

Plot number	3D plot quadrant	Environmental variable	Samples: positive associations (cm)	Samples: negative associations (cm)
1	5	Winter mean temp.		
2	1	Winter total rain		
3	5	Winter total snow		4
4	5	Spring mean temp.		24
5	6	Spring total rain		
6	3   4	Spring total snow	18,28,34,38, 42	
7	5	Summer mean temp.		
8	8	Summer total rain		
9	5	Autumn mean temp.		
10	5	Autumn total rain		
11	1	Autumn total snow	10	
12	3	Phosphorus loads	~20,22,24	8,14

correlated to spring snowfall. Two other species also show significant correlations: *H. elegans* is positively correlated with winter rainfall while *A. arenaria* is negatively

Table 12. Pairwise Pearson correlations of PCA scores with environmental variables. r coefficients denoted with \* indicate p<0.1, \*\* p<0.05, \*\*\* p<0.005, \*\*\*\* p<0.005. See page xviii for taxa abbreviations.

	PCA comp. 1	PCA comp. 2	PCA comp. 3
Winter mean temp.	-0.34	-0.34	0.55**

Winter total rain	0.03	0.1	0.69***
Winter total snow	-0.14	-0.07	-0.68***
Spring mean temp.	-0.57**	0.40*	0.34
Spring total rain	-0.34	-0.08	0.29
Spring total snow	0.66**	-0.02	-0.32
Summer mean temp.	-0.54**	0.22	0.43
Summer total rain	-0.2	0.48*	-0.14
Autumn mean temp.	-0.54**	0.37	0.26
Autumn total rain	-0.33	0.11	0.37
Autumn total snow	0.29	0.36	0.01
Phosphorus loads	0.11	-0.57**	-0.04

correlated with spring snowfall and positively with autumn mean temperatures. As with Assemblage I species, less significant correlations indicate relationships mainly with seasonal precipitation although five out of the seven species in Assemblage II show correlations with mean summer temperatures as well.

Table 13. Pairwise Pearson correlations of fractional abundances with environmental variables in Assemblage I. r coefficients denoted with \* indicate p < 0.1, \*\* p < 0.05, \*\*\* p < 0.005. See page xviii for taxa abbreviations.

	Assemblage I taxa					
	PC	CS	PA	СР	CD	
Winter mean temp.	-0.25	0.19	0.1	0.24	0.26	
Winter total rain	0.06	-0.06	-0.17	0.11	-0.17	
Winter total snow	-0.02	-0.04	-0.06	-0.39	-0.06	
Spring mean temp.	-0.3	0.34	0.48**	0.29	0.46*	
Spring total rain	0.07	0.2	0.31	0.21	0.26	
Spring total snow	-0.02	-0.7***	-0.57**	-0.67***	-0.51**	
Summer mean temp.	-0.26	0.24	0.33	0.15	0.51**	
Summer total rain	-0.41*	-0.09	0.19	-0.4	0.19	
Autumn mean temp.	-0.44*	0.27	0.32	0.26	0.51**	
Autumn total rain	0.03	0.4*	0.35	0.41*	0.22	
Autumn total snow	-0.33	-0.41*	-0.22	-0.37	-0.26	
Ice free days	-0.05	0.02	0.07	0.24	0.04	
C:N ratio	-0.38	-0.21	-0.12	-0.38	0.13	
Phosphorus loads	0.55**	0.37	0.11	0.52**	-0.2	

Table 14. Pairwise Pearson correlations of fractional abundances with environmental variables in Assemblage II. r coefficients denoted with \* indicate p<0.1, \*\* p<0.05, \*\*\* p<0.005, \*\*\*\* p<0.0005. See page xviii for taxa abbreviations.

	Assemblage II taxa							
	CAE	CAC	PF	CK	HE	CO	AA	CDI
Winter mean temp.	0.25	0.62**	-0.46*	0.15	0.28	0.23	0.33	0.09
Winter total rain	0.28	0.32	-0.29	0.44*	0.55**	0.45*	-0.09	-0.15
Winter total snow	-0.22	-0.41*	0.11	-0.25	-0.37	-0.22	0.01	0.17

Spring mean temp.	0.32	0.74**	-0.39	-0.06	0.01	0.09	0.6	0.17
Spring total rain	0.41*	0.46*	-0.26	0.09	0.3	-0.07	0.12	0.15
Spring total snow	-0.6	-	-0.01	-0.24	-0.14	-0.01	-	-0.29
cocar bhow		0.52**					0.53**	
Summer mean temp.	0.46*	0.57**	-0.45*	0.002	0.26	0.33	0.42*	0.46*
Summer total rain	0.01	0.19	-0.45*	-0.22	-0.27	-0.04	0.41*	0.14
Autumn mean temp.	0.28	0.65**	-0.34	-0.12	-0.13	0.01	0.52**	0.24
cemp.		*						
Autumn total rain	0.34	0.17	-0.06	0.28	0.21	0.22	0.31	0.12
Autumn total snow	-0.32	0.15	-0.06	-0.12	-0.2	-0.06	-0.22	-0.32
Ice free days	0.21	0.62**	-0.21	0.29	0.34	-0.03	0.14	-0.21
C:N ratio	-0.001	0.15	-0.41*	-0.03	-0.07	-0.14	0.09	0.03
Phosphorus loads	0.17	-0.25	0.75**	0.39	0.24	-0.14	-0.36	-0.19
3 3 3 5			**					

## 5.2 Discussion

## 5.2.1 <u>Lake Simcoe-wide testate amoebae trends</u>

Results from bicluster analysis show some consistency in lake-wide testate amoebae assemblages. All three cores from Lake Simcoe show strong abundances of *C. spinosa*(aculeata strain) and *C. aerophila*(soil type) after initial logging activity in the early 1800s.

In general, centropyxids are found in wet, well developed

soils and mosses, commonly *Sphagnum* (Flagstad 2007). They are also known to be opportunistic and highly tolerant (Holcova 2008; Scott et. al 2004; Patterson et al. 2002; Kihlman and Kauuppila 2010), allowing them to colonize and thrive in unstable environments. The presence of *C. spinosa* (aculeata strain) and *C. aerophila*(soil type) in all cores confirms that all three locations have been fringe wetlands since the early 1800s.

In particular, *C. spinosa*(aculeata strain), a strain of *C. aculeata*, is commonly considered an opportunistic (Burbidge and Schroder-Adams 1998) and highly tolerant taxa of elevated levels of conductivity (Dallimore et al. 2000, Patterson et al. 1985), low temperatures (Boudreau et al. 2005; Dallimore et al. 2000), and low trophy (Burbidge and Schroder-Adams 1998). *C. spinosa*(aculeata strain) has been reported to inhabit submerged plants during the dry season of The Pantanal wetland in South America (Heckman 1998). Such a habitat preference may explain the difference seen between test morphology of *C. aculeata* (xenogenous) and *C. spinosa*(aculeata strain) (autogenous) through access, or lack thereof, to the raw materials needed for agglutination.

C. aerophila(soil type) is commonly found in well aerated
wetland soils and has been found in both alkaline and acidic

conditions (Couteaux 1969; Smith and Headland 1983). The species is highly variable in both geography and ecology (Chardez 1979).

The distinction between Biofacies I and II is established by the abundances of *P. cymbalum* to Biofacies I and *C. platystoma* in Biofacies II. Biofacies I represents all but two VP samples (82 and 4cm), and early CB samples. Biofacies II contains the remainder of the CB samples (except 42cm) and the mid to upper DP samples. Together, the Biofacies represent all but one post-land clearing sample.

Although the literature on *P. cymbalum* is nearly non-existent (one study records that *P. cymbalum* is exclusively associated with aquatic macrophyte habitat (Lansac-Toha et al. 2000)), ecological investigations on another member of the genus, *P. operculata*, offer insight into the nature of the genus. An ecological investigation by Jax (1985) suggests that like *C. aculeata*, *P. operculata* is an early colonizer (R-selected species), living most successfully on submerged aquatic vegetation and consuming diatoms, green algae, bacteria, and detritus. Jax (1985) also found that abundances of *P. operculata* plummeted in mature systems as a result of competition. Abundances of *P. cymbalum* seem to follow this general successional trajectory in both VP and CB cores,

populations reducing through time. However, the finding that *P. operculata* is an early colonizer does not translate to the histories of *P. cymbalum* in Lake Simcoe where the species has been highly abundant for hundreds of years. An alternate explanation for the decline in *P. cymbalum* in both locations from about 26cm is a reduction in habitat in the coring locations as a function of increasing peat depth and vegetation change.

As with all centropyxids, *C. platystoma* is a cosmopolitan species. There is not much known of the ecological preferences of this species but like *C. spinosa*(aculeata strain), it was found inhabiting submerged wetland plants of The Pantanal wetland in South America (Heckman 1998), submerged plants in glacial South American lakes (Grabandt n.d.), marl lakes in Ontario and also minerotrophic waters (Legg 2009).

In contrast to the Lake Simcoe Biofacies of post-land clearing, Biofacies III, containing all pre-land clearing DP samples and the VP sample at 82cm, is dominated mainly by *C. delicatula*. Only three studies of modern identification exist: Andrews (2012) and Davidova (2012,2013). Andrews (2013) did little else than identify and describe the species. Davidova (2011,2012) identified just two individuals in newly built (1996) mesotrophic Ovcharitsa reservoir in southeastern

Bulgaria from shoreline vegetation (<1m) and the sediment-water interface between 5 and 10m depths. In contrast, *C. delicatula* was found to be a dominant species in littoral (0.5-1m water depth) samples from Durankulak Lake, northeastern Bulgaria (Davidova 2013) with a late summer seasonal relative abundance of 77%. Durankulak Lake is described as a shallow, natural reservoir with eutrophic to hypereutrophic nutrient status and a strongly seasonal hydrologic regime (high-water in Spring, low in Summer/Autumn).

Despite the differences between abundant species from VP, CB, and DP, the general trend of testate amoebae communities across the lake since land clearing indicates a dominance by species preferring submerged vegetation habitat, well-developed wetland soils, unstable ecological conditions, and possibly oligotrophic to mesotrophic nutrient concentrations.

In CB and VP, these conditions were present in varying degrees prior to European settlement, while in DP, a complete ecosystem shift occurred after land clearing. This extreme shift is evident through the DCA based rate of change analysis showing a large peak of change associated with land clearing (Figure 23,24) and a change in dominance from C. delicatula to C. platystoma.

### 5.2.2 Victoria Point

Overall, Victoria Point wetland exhibits a shift from a shallow, possibly seasonally flooded shoreline with neutral to slightly acidic pH, and mesotrophic to eutrophic nutrient concentrations (82 to 62cm) to a deeper shoreline inhabited by submerged aquatic vegetation from 62 to 42cm. Following that, an environment with increasing peat depths, and reduced open water in the coring area pervades. These transitions are highlighted by Q-mode divisions of testate amoebae assemblages with shifting abundances of dominant species.

#### 5.2.2.1 Shallow shoreline - 82 to 62cm

At depths between 82 and 62cm (Zones III & II) a seasonally flooded wetland transitions into a deeper shoreline with reduced hydrologic fluctuations.

The sample at 82cm deep is dominated by *C. delicatula*, a species with a strong late summer growth pattern. It is indicative of shallow (0.5-1m), mesotrophic to eutrophic littoral zones (Davidova 2013). The less abundant *C. discoides* (aculeata strain) (~10%) is associated with high moisture content (95% commonly), stressed environments, pH of ~4.5-6.5 (Charman et al. 2007; Parent et al. 2009; Booth 2001) and seasonal high water periods (Heckman 1998).

Also present, though far less abundant ( $\sim 10\%$  each), are C.

platystoma, and P. fulva. As stated earlier, C. platystoma is a known inhabitant of submerged vegetation and its abundance at less than 10% suggests submerged vegetation was limited at the time. P. fulva is known to be both an aquatic and soil species, preferentially inhabiting soil depths between 3 and 10cm (Vincke et al. 2006). They are also known to inhabit nutrient rich, pH neutral environments with high moisture content (87% commonly) (Charman et al. 2007; Booth & Zygmunt 2005).

### 5.2.2.2 Deeper water shoreline - 62 to 42cm

From 62 to 42cm, encompassing Zones I and II (Figure 19), the abundances of *P. cymbalum*, *C. spinosa* (aculeata strain) and *P. acropodia* define the time period ending with the clearing of land by European settlers. Based on the abundances and habitat preferences of *P. cymbalum* and *C. spinosa* (aculeata strain), submerged aquatic vegetation likely increased throughout the zones. The increase in dominance of *P. acropodia* beginning at 62cm, may be a indication of increasing paludification (peat formation), and nutrient enrichment along the shoreline. Ecologically, *P. acropodia* dwells most successfully at the soil-litter interface, feeding on fungi spores and bacteria (Ogden and Pitta 1990). The species is quantitatively described as preferring a moist habitat with a near neutral pH range (Booth 2008; Payne et al. 2006), and has

been shown to thrive in high nutrient environments (Mitchell 2004).

A rapid increase in *P. cymbalum* at 46cm, dated just prior to European settlement, along with a slight decrease in *P. acropodia*, and small showing of *D. globulosa*, may reflect a brief episode of high nutrient concentrations, possibly from an erosional episode. As stated previously, *P. operculata* is believed to be an opportunistic species of disturbed habitats – a characteristic that may also define *P. cymbalum*, which shows a brief increase in abundance. This characteristic combined with *P. acropodia's* preference for a leaf-litter, soil based habitat, and *D. globulosa* preference for an alkaline (pH 8.0 to 8.1), lacustrine habitat (Qin et al. 2013), suggests that erosion of the shoreline wetland and inputs from calcareous sediments likely occurred at this time.

There is evidence of Ambrosia pollen (though in limited abundance) at this time as well. Species richness dropped to its lowest value (6) in any sample during the late Holocene, which may be a result of increased water input into the wetland (Tsyganov et al. 2013).

Whether the erosion is from a brief climatic event or from the occupation of "Mount Slaven," an Algonkian and possibly Wendat settlement of  $\sim 34$  acres situated approximately  $4\,\mathrm{km}$ 

Northwest from the coring site (Hunter 1904), cannot be determined as no  $^{14}\mathrm{C}$  date exists for the archaeological site.

The sample at 42cm is marked by changes in testate amoebae assemblage and an increase in Ambrosia abundance. Assemblages indicate a reduction in the submerged vegetation preferred by P. cymbalum, an increase in ecosystem instability (C. spinosa(aculeata strain)), a change in hydrologic regime (C. delicatula and C. discoides), and a decrease in nutrient concentrations (C. spinosa(aculeata strain)). These results are consistent with a disturbed habitat likely caused by European settlement of the area.

#### 5.2.2.3 Peat dominated shoreline - 38 to 0cm

Following the disturbances at 46 and 42cm, Zone I persists until ~10cm in depth, with increasing absolute abundances, species richness and diversity. Declines in *P. cymbalum* and *C. spinosa*(aculeata strain) (aquatic habitat), along with increases of *P. acropodia*, *C. aerophila*, and *P. fulva* (soil habitat) suggest the recommencement of paludification, and increased stability.

Following the decrease of *C. spinosa* (aculeata strain), the species abundance quickly stabilized for the remainder of the core (~ AD 1904-2011). This stabilization appears to be a strong indicator of anthropogenic influence on the lake

through reduced water level fluctuations.

As previously stated, *C. spinosa*(aculeata strain) is an early colonizer of disturbed environments, and preferentially inhabits low trophy environments. The opening of the Trent-Severn waterway in AD 1920 and the subsequent regulation of water levels has removed hydrologic fluctuation from the lake. Testate amoebae analysis of an artificially inundated kettle hole mire shows increases in *C. aculeata* abundances following two inundation events (Lamentowicz and Obremska 2010), suggesting water level fluctuations are needed by the species to thrive.

The disturbances at 46 and 42cm may have increased erosion and nutrient input to the coring site location, kick-starting paludification and contributing to the decline of C. spinosa (aculeata strain).

The final Zone (III) from 10cm to 0cm sees the complete disappearance of *P. cymbalum*, an increase in *C. aerophila* and the reintroduction of *C. delicatula*. Anecdotal evidence from observations during 2009, 2010, and 2011 suggests that seasonal fluctuations in water levels along the shoreline (~1.5m early spring depth to ~15cm late fall depth) in conjunction with peat depth are providing the necessary environment for *C. delicatula* growth.

#### 5.2.2.4 Elemental and isotopic analysis

Overall, elemental and isotopic values from the top 28cm of the VP core indicate that organic matter is derived from both autochthonous and allochthonous sources which is consistent with fringe wetland locations (Koff 2012).

Minor fluctuations of organic and inorganic inputs into the shoreline are evident until 10cm. C:N values, hovering between 17.5 and 18.5, together with  $\delta^{13}$ C values, about -28, indicate a mix of autochthonous and allochthonous organic matter (OM) inputs (Koff 2012).  $\delta^{13}$ C and  $\delta^{15}$ N values begin a decline at 14cm as C:N increases >20, indicating a possible increase in terrestrial derived OM (Torres et al. 2012). From 8 to 6cm  $\delta^{13}$ C and  $\delta^{15}$ N increase slightly while C:N decreases - a sign of decreased allochthonous OM. At 4cm a spike in C:N (19.02 to 27.15) suggests a large influx of terrestrial derived OM.

#### 5.2.3 Cook's Bay

Overall, the testate amoebae communities of Cook's Bay wetland suggest an unstable environment throughout most of the core. Species rise and fall quickly, responding to hydrologic and nutrient disturbances. An environment with relative stability and reduced disturbance is prevalent beginning 22-18cm.

### 5.2.3.1 Unstable environment - 86 to 24cm

The rise and fall of *C. spinosa* (aculeata strain) on multiple occasions from 86 to 24cm indicates that an unstable hydrologic regime and nutrient concentration existed throughout the time period (Lamentowicz and Obremska 2010). This is supported by abundances of the late summer species *C. delicatula*, the wet indicator, *Hyalosphenia elegans* (Lamentowicz et al. 2006), and by *C. platystoma*, associated with environmental conditions similar to *C. spinosa* (aculeata strain) (Heckman 1998).

Q-mode cluster analysis highlighted the sample at 50cm deep as the only representative of Biofacies II in this core. The sample is dominated by *P. fulva* at 52.63%, an aquatic and soil species, preferentially inhabiting soil depths between 3 and 10cm (Vincke et al. 2006) in nutrient rich, pH neutral, high moisture content environments (Charman et al. 2007; Booth & Zygmunt 2005). Its presence at over 50% abundance suggests an influx of sediment and nutrients. A situation that repeated at 38cm though to a lesser extent.

The variability and general decline of the *P. cymbalum* population suggests a reduction in its submerged vegetation habitat. Since *P. acropodia* is most successful in high nutrient environments at the litter-soil interface (Mitchell 2004; Ogden and Pitta 1990), the large spike in *P. acropodia* 

abundance beginning at 46cm indicates a continuation of high nutrient loading. The N1:N0 ratio of 0.86 indicating limited species richness and high dominance, supports an inference of a stressed ecosystem (Odum 1985).

# 5.2.3.2 Relatively stable environment - 22 to 0cm

Following the instability between 82 to 24cm, Zone I stability from 22 to 0cm reflects a reduction in stress and hydrologic change, and an increase in nutrient concentrations (C. spinosa(aculeata strain)) with moderate water table depths (H. elegans). A strong submerged aquatic component (C. platystoma) rose replacing the community once dominated by P. cymbalum. A well-developed, nutrient rich, well-drained soil environment developed (P. acropodia, C. aerophila).

Beginning at 34cm, a new species, *Cyclopyxis kahli*, forms part of the Assemblage II structure. *C. kahli* is reportedly a terrestrial species commonly found in soils and forest litter that can be transported into water bodies through erosion (Roe and Patterson 2006). The presence of *C. kahli* indicates that sediments and nutrients continue to enter the system even during times of greater stability.

#### 5.2.3.3 Anthropogenic impacts

While no geochronological analysis was conducted on the core, a similar study (Danesh et al. 2013) was conducted on a core from inner Cook's Bay in 2013, provides a sediment

chronology very similar to the Ambrosia estimate determined here (Figure 20), with mid-1800s land clearing at ~75cm.

Danesh et al. (2013) place the Holland Marsh canal construction between ~55 and 42cm, and the 1950s urbanization boom at ~28cm. These chronological depths are consistent with the changes to testate amoebae communities, Q-mode zonation, and increases in Ambrosia pollen in the Cook's Bay core.

Therefore, the instability from 50 to 24cm and increased stabilization beginning at 22cm, may be a result of numerous dated factors including the Holland Marsh drainage and canal construction from 1924 to 1930, increased urbanization (starting in the 1950s), the flood event of Hurricane Hazel (1954), the dramatic rise of corn as a crop (~1960), the diversion of sewage effluent in the 1980s, and the introduction of the Zebra mussel in the mid-1990s.

The Holland Marsh project (~50cm sample) created 7,000 acres of farmland from wetland space, draining the land through a series of human-made canals that also served to redirect the Schomberg Branch of the Holland River to flow around the farmland. The canals were also designed to drain 64,300 acres of watershed area, which now includes the urban areas of several townships. A pump system was installed to maintain the river at 2.5m below the level of Lake Simcoe (OMAFRA 2010).

After 1941, the population of Simcoe County rapidly expanded to just under 110,000 in 1961 and by 1981 was over 200,000 (~34 to 20cm samples). This period of rapid growth is attributed to an influx into the urban centres of Aurora, Barrie, Newmarket, and Orillia (Wilson and Ryan 1988). Both Newmarket and Aurora discharged their sewage effluent into the East Branch of the Holland River until 1984 (LSEMS 1985).

Hurricane Hazel swept through the Holland Marsh area in October of 1954 (~38cm sample), flooding drainage canals and agricultural land (OMAFRA 2010). An increase in Ambrosia pollen and P. fulva abundance, and a substantial decrease in P. acropodia may indicate erosion at the core location.

Beginning in about 1961, Wilson and Ryan (1988) report a large increase in corn as a crop in the watershed. In 1961, corn occupied ~2% of all cropland but by 1981 it accounted for 35%. Corn crops (row crop) leave the ground between the rows bare and unprotected from rain and snowmelt enabling the erosion of soil. The rise in corn crops almost doubled the erosive land use index from 1961 to 1981 (Wilson and Ryan 1988). The shift to Zone III and subsequent reappearance of *P. acropodia* around this time may indicate increased nutrient inputs as a result.

The Zebra Mussel (Dreissena polymorpha), likely introduced

in 1991 and well established by 1996 (GISD 2013; Ozersky et al. 2011), are suspected of decreasing algal biovolume, increasing transparency, increasing macrophyte biomass and production, and changing nutrient dynamics and benthic communities (Ozerky et al. 2011). A before/after study (Ozersky 2010) of littoral benthos in Lake Simcoe found that D. polymorpha mean density increased from 367.9 individuals/m² in 1993 to 22,192.4 individuals/m² in 2008. The reappearance of P. fulva at 10cm may indicate nutrient enrichment of the shoreline by D. polymorpha in a process known as the "nearshore phosphorus shunt" (Hecky et al. 2004).

An increase in nutrient input from the above sources and the anthropogenic stabilization of water levels in the Holland River may be responsible for the instability of the testate amoebae community from 50 to 24cm and the enriched stability above 24cm in depth.

#### 5.2.3.4 Elemental and isotopic analysis

Elemental and isotopic values from the top 28cm of the CB core indicate that organic matter was derived from a mix of autochthonous and allochthonous sources for most of the time period with C:N ratios ranging between 14.78 and 22.98.  $\delta^{13}$ C and  $\delta^{15}$ N values support a phytoplankton origin for the autochthonous OM from 28 to 12cm (Keough et al. 1996). From 10

to 0cm, a large increase in  $\delta^{15}N$  combined with a small decrease in  $\delta^{13}C$  points towards a progressive change from phytoplankton OM to emergent vascular OM possibly from a progressive increase in Typha spp. (Cloern et al. 2002).

The increase in Typha spp. may signal a shift to an alternative stable state, i.e. the ecosystem resilience threshold was surpassed, in the wetland (Gunderson 2000). Alternative stable states for freshwater wetlands are controlled by nutrient availability such that an increase in soil nutrient content is characterized by a shift in dominant emergent plant species (Gundersen 2000). Koster et al. (2005) report similar trends in  $\delta^{13}$ C and  $\delta^{15}$ N (decline vs. increase) lake sediment measurements, attributing the shift to greater nutrient supply from land clearing. The possible increase in Typha spp. abundances since 1930 agrees with an assessment of increased nutrient supply leading to the  $\delta^{13}C$  and  $\delta^{15}N$  shift for this core. Both T. latifolia and T. angustifolia are known to prefer disturbed environments with greater nutrient availability (Thiebaut 2008) and stable hydrologic regimes (Vaccaro 2005).

## 5.2.4 <u>Duclos Point</u>

Similar to Cook's Bay, Q-mode divisions in Duclos Point highlight rapid shifts in testate amoebae communities over

time resulting in great instability. The change to Duclos point wetland testate amoebae communities induced by land clearing activities resulted in an ecosystem shift from a seasonal hydrologically variable environment to an unstable deep water, alkaline, environment with both submerged aquatic and emergent wetland communities.

### 5.2.4.1 Seasonal wetland - 86 to 42cm

Beginning at 86cm, *C. delicatula* is the dominant species representing between 50 and 71.43% of Zone III and I assemblages. As in both VP and CB, *C. delicatula* is indicative of seasonal hydrologic regimes, reaching maximum abundances in late summer (Davidova 2013) when nutrient concentrations increase due to decreasing water levels. Seasonal periods of higher water levels are likely responsible for the variable increases in the hygrophilic species, *D. globulosa*, *C. platystoma*, *C. spinosa*(aculeata strain), *C. aculeata*, and *P. cymbalum* until 42cm.

### 5.2.4.2 Deeper water wetland - 42 to 0cm

From 42 to 16cm, testate amoebae communities fluctuate rapidly between Biofacies I, II, and III dominated by C. platystoma, C. spinosa(aculeata strain) and C. delicatula respectively, resulting in a consistently stressed environment. Following land clearance at ~38cm, P. acropodia, D. globulosa, and Arcella discoides are established with

moderate abundance indicating consistently high nutrient status and water levels (Charman et al. 2007). Absolute abundance calculations pinpoint this period as highly suitable for rapid population growth. However, high population counts decline after 26cm, not reaching similar abundances until 6cm in depth.

Some consistency in testate amoebae assemblage is evident in the final Zone (II) from 14 to 0cm. Species richness is consistently above 10 species with decreasing dominance (0.57  $\geq$  H1:H0  $\leq$  0.86). Limited seasonal fluctuations returned (C. delicatula) and C. spinosa(aculeata strain) and C. platystoma have mostly stable populations showing a decrease in ecosystem stress.

## 5.2.5 <u>Tub Lake</u>

Overall, Tub Lake wetland shows very little change in testate amoebae assemblage. Land clearing activities introduced additional species to the community, both short and long-term, but did not have a long term effect on the primary species in the soil community.

## 5.2.5.1 Pre-land clearing - 50 - 30cm

P. acropodia is most successful testate amoebae throughout the core, but during pre-disturbance times, the species reached a maximum of 100% abundance. It's great abundance

indicates high litter deposition and soil nutrient concentrations (Mitchell 2004; Ogden and Pitta 1990). Variable declines in *P. acropodia* are mirrored by increased abundances in *C. platystoma* suggesting brief periods of higher water levels with increased submerged aquatic vegetation.

## 5.2.5.2 Post-land clearing 30 - 0cm

Ambrosia abundances pinpoint 30cm deep as the most likely depth of land clearing from logging activities ( $\sim$  AD 1880). There is a general reduction in C. platystoma and associated habitat, along with an introduction of C. aerophila and C. orbicularis in the post-distrubance record.

C. orbicularis has been reported from small stream environments with positive correlations to fine organic debris in low flow water columns (Holcova 2008). C. orbicularis is also known to be a grassland species inhabiting water-filled crevice microenvironments and increasing in abundance with greater water availability (Esteban et al. 2006; Finlay et al. 2000). The opposing abundance of this species to C. aerophila(soil type), common to well aerated soils (Couteaux 1969; Smith and Headland 1983), suggests that the area was subject to wet and dry episodic events with possible erosion acting as a transport mechanism of these non-aquatic species (Roe and Patterson 2006).

As area vegetation patterns matured and erosional episodes decreased, *C. orbicularis* disappeared from the record and *P. acropodia* reestablished its dominance (>80%) by 4cm. The persistence of *C. aerophila* might be attributed to the persistence of dry grassland conditions still in evidence today around the wetland. The appearance of *P. fulva* and *C. kahli* in the upper 2cm of the core indicate a recent environment of nutrient rich, moist, deep soils with increasing encroachment by terrestrial plant cover or erosional episodes (Charman et al. 2007; Vincke et al. 2006; Roe and Patterson 2006; Booth & Zygmunt 2005).

### 5.2.5.3 Elemental and isotopic analysis

C:N values support an influx of terrestrial based OM from 28 to 26cm (*C. orbicularis* peak) followed by a mixed aquatic and terrestrial derived OM from 24 to 6cm. The top three samples show an increase in terrestrial OM.

 $\delta^{13}$ C and  $\delta^{15}$ N values are very consistent throughout the core, indicating both phytoplankton and terrestrial derived OM. There is a brief increase in  $\delta^{13}$ C values following land clearing that might be attributable to an increase in terrestrial based OM during early succession (Wang and Woller 2006).

# 5.2.6 <u>Climatic and nutrient analysis</u>

The testate amoebae communities of Victoria Point have suggested that following land clearance and water level stabilization activities, increasing peat depths and reduced open water were quickly established. This paludification resulted in the loss or reduction of species reliant on submerged aquatic vegetation and hydrologic fluctuations for success.

Principle component analysis of variability in both assemblage and environmental measurements through time (PCAone) indicates that increases in spring snowfall and phosphorus loading measurements have the largest direct effect on assemblage variation.

Compression of only assemblage variation through PCA (PCAtwo) and subsequent association through Pearson correlation analyses reveals that 20% of variation is positively correlated to spring snowfall and negatively correlated to spring, summer, and autumn mean temperatures. 14% of PCA variation is negatively correlated to lake-wide phosphorus loading and an additional 13% is negatively correlated to winter snow and positively to winter rain and mean temperatures.

#### 5.2.6.1 Phosphorus loading

Samples from 20,22, and 24cm (P<sub>samples</sub>) are most highly correlated to changes in phosphorus loads to the lake, with the 22cm sample at the closest distance (PCAone). These samples are dated from 1965 to 1970 and show a reduction in species richness from 26 at 26cm to 13-15 between 20 and 24cm. The N1:N0 ratio also increases for these samples from 0.27 at 26cm to 0.45-0.54. Phosphorus loads at this time were estimated and measured at 132,500 and 144,100 kg yr<sup>-1</sup> for 1960 and 1972AD. These values represented the largest loads to the lake since AD 1800. The other minor influence on these samples was a moderate winter snowfall (182.3mm), more typical of the Lake Simcoe area prior to European settlement than snowfall from 2000 to 2011 (213mm).

The relative abundances of three species were strongly and positively correlated to increasing phosphorus load values to the lake:  $P.\ cymbalum\ (P_{samples}\ range=26.46-40.97\%,\ overall$  mean=24.29%),  $C.\ platystoma\ (P_{samples}\ range=12.32-15.95\%,\ overall$  mean=11.86%), and  $P.\ fulva\ (P_{samples}\ range=5.43-5.84\%,\ overall$  mean=2.33%).

All three are known inhabitants of lacustrine environments with *P. cymbalum* and *C. platystoma* associated with submerged vegetation. Submerged vegetation monitored in Cook's Bay has

shown a substantial increase since 1984 (1.2 to 3.1 kg m<sup>-2</sup>), attributed to increased phosphorus concentrations and water clarity (LSRCA 2011). Of particular interest is steady increase in the eutrophic indicator (Nichols and Shaw 1986) Myriophylum spicatum representing 11.9 to 60.7% of the community between 1984 and 2008 respectively. The increase in submerged vegetation biomass in conjunction with the increase in eutrophic M. spicatum supports the assessment that positive correlations between P. cymbalum and C. platystoma, associated with submerged vegetation habitat, are indirect indicators of increased phosphorus concentrations in the lake.

As  $P.\ fulva$  is known to inhabit both aquatic and soil environments, its strong correlation (r=0.75 at p<0.0005) may be linked to increases in either soil or water based phosphorus concentrations. The presence and distribution of the species in the core, directly after land clearing, and during high phosphorus loading time of the 1960s-1970 and mid to late 1990s suggests that nutrient loading to the lake water itself rather than a build-up of phosphorus in peat is the likely factor affecting species distribution.

The samples at 8cm (AD 2000) and 14cm (AD 1988) were exclusively negatively correlated to lake-wide phosphorus loads. Mean phosphorus loads for time periods were the lowest

since European settlement in the mid 1800s (67,375 and 70,613  $$\rm kg\ yr^{-1})$  .

#### 5.2.6.2 Climatic influences

From PCA component one (PCAtwo), the most strongly correlated variable is total spring snowfall (r=0.66 at p=0.002). Samples associated with this variable include 18,28,34,38,42cm with 28cm (AD 1957) resulting in the strongest correlation (PCAone). These samples are dated from 1871 to 1957 and 1977. Species richness for these samples range from 9 to 19 with a mean of 13.8. N1:N0 ratio is between 0.36 and 0.77 with a mean of 0.53. The mean snowfall for correlated samples is 55.9mm and 39.9mm for uncorrelated samples. While no significant correlation between spring snowfall and the number of ice-free days exists for all samples, the mean number of ice-free days for the correlated samples is 250 and 264 for uncorrelated samples.

Interestingly, there exists no positive correlation between spring snowfall and any of the abundant species from Assemblage I or II, defined as representative of the samples through bicluster analysis. There are, however, six species with negative correlations >0.51: C. spinosa(aculeata strain), C. platystoma, C. delicatula, C. aerophila, C. aculeata, P. acropodia, and A. arenaria. The largest negative correlation is among the centropyxids. No literature exists that directly

discusses snow cover and testate amoebae abundance or ecology.

However, with general knowledge of centropyxid and snow cover ecology, inferences can be made regarding the correlation.

Centropyxids are highly tolerant R-strategists that thrive on disturbance, particularly hydrologic disturbance, and low nutrient concentrations. Snow is a thermal insulator that maintains moderate soil temperatures (0°C at 50cm snow depth) preventing deep freezing of the soil and reducing disturbance at the soil-snow interface (Callaghan et al. 2011). Snow cover also allows microbial processes to continue over the cold season, increasing nutrient availability. Increased soil temperatures provide greater opportunities for infiltration of water and nutrients during the spring thaw (Jones and Pomeroy n.d.). Therefore, greater snow cover depth fosters a rich, stable the soil environment which may rob centropyxids of the necessary microenvironment for population maintenance. Soil stability may also enhance competitor success, which could account for the overall positive correlation.

Correlations with PCA axis three (PCAtwo) contains two strong correlations - a negative correlation with winter snow fall, and a positive correlation with winter rainfall. No specific samples were definitively associated with these variables in PCA analysis (PCAone). A plot of PCAtwo results

(not shown) indicates that samples 2,4, and 6 all fall within the axis space of the negative winter snow correlation with the remainder of samples in the axis space of the positive winter rain and mean temperature correlations. The mean winter snow in negatively correlated samples is 52mm greater than in non-correlated samples.

Ordination-based rate of change analysis indicate large magnitudes of change within the upper 10cm of all cores.

Victoria Point change is larger than that recorded during land clearing activities. These changes were only visible with the addition of the third axis of DCA, included based on Horn's Parallel analysis results. The relationship of the third axis to winter climate variables suggests that climate change, particularly winter climate change, is the main driver behind the recent change in all four wetlands.

The recent diatom-based paleolimnological study by
Hawryshyn et al. (2012) found that increased air temperatures
and reduced ice cover from the 1950s to the 1970s produced a
lake-wide algal community shift. This change in community is
not recorded in the testate amoebae record likely as a result
of substrate moisture and water chemistry being the dominant
controls on species distribution and abundance (Booth 2002).

5.2.6.3 Summary of nutrient and climate impacts

Changes in spring climate measurements since the mid 1800s

had the greatest impact on testate amoebae assemblage variation from 1871 to 1957 and again in 1977. Greater levels of spring snowfall created a stable environment that reduced disturbance and the abundance of centropyxid community members and may increased favourable conditions for other species.

From 1965 to 1970, increased in phosphorus loading to the lake negatively impacted the community, decreasing species richness and increasing dominance. Those testate amoebae that inhabit submerged aquatic vegetation increased in abundance likely as an indirect result of increased habitat availability. The reappearance of *P. fulva* in the mid to late 1990s may be a response to increases in lake-wide phosphorus loading or an increase in phosphorus concentrations as a result of the "nearshore phosphorus shunt" following the establishment of the Zebra mussel.

Variation in the remainder of the samples is not strongly linked to specific environmental variables or phosphorus loading to the lake. There is an indication that samples from 2004 to 2011 are linked to an increase in winter snowfall.

### 5.2.7 <u>Lake Simcoe and Tub Lake comparison</u>

Testate amoebae species composition of the reference wetland included many of the same cosmopolitan species identified in Lake Simcoe wetlands (*P. acropodia, C.* 

platystoma, C. aerophila, P. fulva, C. kahli, and C. orbicularis). Land clearing activities in Lake Simcoe and the reference wetland introduced C. aerophila and C. orbicularis into each community. C. orbicularis is a grassland species likely transported to the lacustrine systems through erosive rains (Holcova 2008; Esteban et al. 2006; Finlay et al. 2000) while C. aerophila(soil type) is common to well aerated soils (Couteaux 1969; Smith and Headland 1983).

The presence of *C. orbicularis* in all wetlands, though in differing abundances and paleodistributions, after land clearing activities suggests its use as an indicator of community succession following landscape disturbance. In Tub Lake, this species is present in decreasing abundances from 28 to 14cm (~30cm = land clearing) suggesting that Tub Lake has recovered from the disturbance, progressing past the successional community. This is not the case in Lake Simcoe.

Victoria Point records the species 8cm after land clearing (~70 years) and again in the most recent sediments. Duclos Point also records the species 8cm after land clearing but abundances continue to the present with one small disappearance during a disturbance event. Finally, Cook's Bay fails to record the species until the top sample. The continued presence of the species, or initial appearance,

indicates that the ecosystems surrounding Lake Simcoe wetlands have not fully recovered from land clearing and have been in a continued state of disturbance for the last ~200 years.

The testate amoebae community of Tub Lake also shows a strong primary species continuity along the length of the core - a feature not seen in any of the Lake Simcoe cores. Just prior to disturbance, the relative abundance of *P. acropodia* was ~88%, dropping to ~41% during the post-land clearing recovery period. By 4cm in depth, *P. acropodia* had recovered to ~84%.

The primary species in Victoria Point and Cook's Bay wetland (*P. cymbalum*) prior to land clearing held abundances of ~38% and ~47% respectively. Its abundance has diminished to ~6% in Cook's Bay and has disappeared completely from Victoria Point wetland. The same holds true for Duclos Point - *C. delicatula* diminished from ~71% to ~4%, recovering slightly in the top sediment.

Despite Tub Lake's recovery from initial anthropogenic disturbance, a new stressor may be impacting the ecosystem, as seen in the change marked by Q-mode cluster Zone I of the top 2cm and presence of *P. fulva*, not seen in the core previously. The change is mirrored in the Lake Simcoe cores but is more subtle than other disturbances and therefore not marked by

cluster analysis. PCA analysis (PCAone) of the Victoria Point core isolated the 0 and 4cm samples as being completely unique and without precedence in the core. PCAtwo analysis suggests that these two extremes are in some way related to climate - 4cm to increased winter snowfall, and 0cm increased spring, summer, and fall temperatures. Thus, climate change may be responsible for the recent changes recorded in all wetlands studied regardless of previous disturbance.

#### 5.3 Conclusion

The paleoenvironmental record of four wetlands in Central Ontario were reconstructed using testate amoebae and organic geochemistry as environmental proxies. To refine dating estimates and constrain analysis to the time period of interest, Ambrosia pollen was conducted on the core. The paleodistribution of testate amoebae were influenced by hydrology, sediment composition, climate, and anthropogenic activities.

All three Lake Simcoe wetlands show some similarity in post-settlement assemblage composition. Cook's Bay and Duclos Point show similarity in the rates of assemblage change following land clearing activities. Despite these similarities, each wetland started as a unique ecosystem and responded to unique stressors based on the anthropogenic

influences in their catchments.

The control wetland, Tub Lake, records a similar response to initial anthropogenic disturbance but differs in its lack of continued disturbance indicators which may be a result of the cessation of anthropogenic activity in its catchment.

All four wetlands responded to land clearing activities through a diminishment in primary species abundance, and the introduction or abundance increase of disturbance indicating species. The hydrologic variability and influx of sediment and nutrients through erosion changed Lake Simcoe wetlands from shallow aquatic, seasonally enriched environments dominated by submerged aquatic vegetation to environments defined by paludification and enriched sediments. Land clearing disturbance altered Tub Lake wetland through an influx of sediment and nutrients through erosion but did not have a long-term effect on primary species abundance.

Prior to European settlement and land clearing activities,

Victoria Point was in the early stages of fringe wetland

development, shifting from a shallow water, seasonally

nutrient rich shoreline to one of greater water depth,

decreasing trophy, and increasing submerged vegetation. After

European settlement, the wetland shifted to a peat-dominated

system with progressive declines in submerged vegetation and

increased nutrient concentrations. The opening of the Trent-Severn waterway in 1920 and subsequent lake water level regulation may be partly responsible for the stability recorded in recent sediments and for the disappearance of *P. cymbalum*, the primary species prior to European settlement. In 2007, the greatest rate of change linked to an increase in winter snowfall occurred in the wetland.

The testate amoebae record of Cook's Bay is a record of continual disturbance. The great depth of initial disturbance (~78cm) provides very little background testate amoebae data with which to assess the extent of wetland disturbance relative to itself. In the single pre-disturbance sample, Cook's Bay wetland reflected much the same environment as that found in Victoria point - an early stage, shallow water, seasonally nutrient rich fringe wetland. Post land clearing events unique to the catchment kept the wetland in a continual state of disturbance; the drainage of the Holland Marsh and subsequent land use change to commercial farming, canal construction that altered hydrology and became a conduit for urban sewage effluent, the flooding of the Marsh by Hurricane Hazel, the eventual diversion of the same urban effluent, and the introduction of the Zebra mussel all caused great instability in the wetland. In the last 30 years, a modicum of

stability has returned to the wetland though there is very little similarity to the wetland that existed prior to European settlement.

Land clearing in the area of Duclos Point wetland produced the largest rate of change of any wetland in this study. Prior to land clearing, Duclos point was a strongly seasonal wetland with periods of high and low water levels, resulting in low and high levels of nutrient concentrations respectively. After land clearing, the wetland was an unstable deep water, alkaline, environment with both submerged aquatic and peatbased wetland testate amoebae communities. As with both other Lake Simcoe wetlands, some stability returned to the wetland in recent times but did not return to pre-disturbance conditions.

Signs of a new stressor impacting all four ecosystems are recorded in the upper ~10cm of each core. Despite the lack of anthropogenic influence on Tub Lake, Q-mode cluster analysis and ordination-based rate of change analysis, indicate a magnitude of wetland change nearly on par with the land clearing activities of the late 1800s. A similar change spike is also recorded in the upper 10cm of all Lake Simcoe wetland cores. These changes were only visible with the addition of the third axis of DCA. The PCA derived relationship of the

third axis to winter climate variables suggests that climate change, particularly winter climate change, is the main driver behind the recent instability in all four wetlands.

## Chapter 6. Past variability of Lake Simcoe's shoreline

#### 6.1 Results

#### 6.1.1 <u>Lithology</u>

Sedimentary units are shown in detail in Figure 32. There are three main units consisting, generally, of peat (0-85cm), carbonate mud (85-220cm), and sapropel ("gyttja") (220-280cm).

Carbonate tests (Table 15) based on the FAO classification scheme (2006) (Table 6) shows an increase in carbonate concentrations up core within the carbonate mud unit (85-220cm) and no carbonate content within the sapropel or peat units aside from moderate concentrations at 242cm deep.

The lower-most thick-bedded, biogenic section is composed almost entirely of brown sapropel with a single woody fragment at 257cm. Microscopic analysis of the carbonate mud facies between 202-90cm indicate the presence of *Charophyte* encrustations, therefore encrustations have been indicated throughout the main carbonate unit. Within the carbonate unit, diffuse to sharp transitions occur frequently via changes to

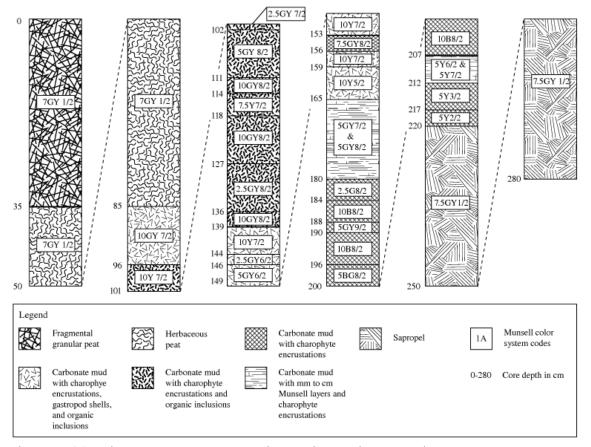


Figure 32.Lithology of the Victoria Point sediment core. (Created using Adobe 2013.)

colour or inclusions. Very thin laminated carbonate sediments defined by differing Munsell colour values occur in two different sections: 212-207cm and 180-165cm. There are also three distinct sections (165-156, 153-139, and 96-85cm) composed of carbonate mud, encrustations, gastropod shells, and fragmented organics. From 139-96cm smooth carbonate is randomly interspersed with very small fragmented organics while similar carbonate deposits at 207-180cm are void of organics.

The upper-most biogenic section is indistinctly separated between Typha dominated, brown fragmental granular peat and herbaceous peat at  $\sim 35\,\mathrm{cm}$ .

Table 15. Carbonate classification of the Victoria Point sediment core.

Sample number		Concentration (%)	Sample number	_	Concentration (%)
0	N	0	50	N	0
2	N	0	62	N	0
4	N	0	70	N	0
6	N	0	82	N	0
8	N	0	90	ST	10 to 25 (~10)
10	N	0	106	EX	>25
12	N	0	122	EX	>25
14	N	0	142	EX	>25
16	N	0	146	ST-EX	10 to 25 (~25)
18	N	0	170	ST-EX	10 to 25 (~25)
20	N	0	190	ST-EX	10 to 25 (~25)
22	N	0	202	ST	10 to 25 (~15)
24	N	0	214	ST	10 to 25 (~10)
26	N	0	222	ST	10 to 25 (~10)
28	N	0	226	N	0
30	N	0	230	N	0
34	N	0	242	MO	2 to 10 (~2)
38	N	0	258	N	0
42	N	0	274	N	0
46	N	0			

#### 6.1.2 <u>Testate amoebae assemblages</u>

As with the upper portions of the VP core, preservation of the tests was very good (very few broken tests and a variety of test types). Mean test counts were 65 per downcore sample (34 to 274cm). Only ten tests were recorded at 190 and 258cm depths. Despite such low numbers, SSP analysis of both samples assigned statistical significance to the samples due to low diversity.

Simple species richness (NO) ranged from 3 to 19 with minimum counts at 202 and 190cm, and the maximum count at 38cm. Generally, species counts remained <10 at  $\leq$  90cm and  $\geq$ 10 at  $\geq$ 80cm (the carbonate/peat transition). Species diversity, in terms of Hill's NI (abundant effective species), increased up the core, with greatest diversity at 34cm deep. N2 diversity (very abundant effective species) also increased up-core but was more subtle than NI, ranging from 2.04 (274cm) to 7.87 (34cm). Detailed richness and diversity results are displayed stratigraphically (Figure 34).

Although seventy-five taxa of testate amoebae were included, bicluster analysis indicated that only ten taxa significantly influence the assemblage composition in four, mostly distinct, Biofacies (Figure 8): Centropyxis platystoma, Centropyxis constricta, Centropyxis aerophila(lacustrine

type), Centropyxis spinosa(aculeata strain), Centropyxis discoides(aculeata strain), Centropyxis delicatula, Phryganella acropodia, Pyxidicula cymbalum, Difflugia globulosa, and Paraquadrulla irregularis.

Biofacies I, seen mostly in the upper 38cm of the core, has a  $\bar{N}I:N0$  ratio of 0.54. It is characterized by C. platystoma (0.0  $\geq$   $F_i$   $\leq$  27.25), P. acropodia (1.19  $\geq$   $F_i$   $\leq$  36.81), C. spinosa(aculeata strain) (0.0  $\geq$   $F_i$   $\leq$  24.39), and P. cymbalum (0.0  $\geq$   $F_i$   $\leq$  37.83), with P. acropodia and P. cymbalum dominating.

Biofacies II, located mostly between 42 and 62cm deep, is characterized by the same species found in I but with a change in dominance to  $C.\ spinosa$  (aculeata strain) and  $P.\ cymbalum$ . The NI:NO ratio is 0.57 for this group.

While Biofacies I and II are composed of similar species with differing abundances, Biofacies III and IV, differ distinctly in the presence/absence of calcareous *P. irregularis*. However, connections do exist through *C. aerophila*(lacustrine type) which abundantly spans both facies.

The species present in Biofacies III, found at various locations  $\geq$  90cm, contains *C. constricta* (0.0  $\geq$   $F_i$   $\leq$  26.67), *D. globulosa* (14.29  $\geq$   $F_i$   $\leq$  50.00), *C. delicatula* (0  $\geq$   $F_i$   $\leq$ 

12.50), C. discoides (aculeata strain)  $(0.0 \ge F_i \le 42.86)$ , and C. aerophila(lacustrine type)  $(33.34 \ge F_i \le 42.86)$  taxa with C. aerophila(lacustrine type) , D. globulosa and C. discoides (aculeata strain) as the dominant taxa. The  $\bar{N}1:N0$  ratio is 0.86.

Finally, Biofacies IV, between 106 and 170cm deep, is characterized by C. aerophila(lacustrine type) (30.44  $\geq$   $F_i$   $\leq$  42.86) and P. irregularis (28.69  $\geq$   $F_i$   $\leq$  55.07) with a  $\bar{N}I:N0$  ratio of 0.62.

#### 6.2 Discussion

The earliest phase of the shoreline environment (274 to ~220cm), characterized lithologically by a diffuse transition from sapropel to carbonate sediments, shows a strong initial dominance by *C. spinosa*(aculeata strain). At 258cm depth, *C. spinosa*(aculeata strain) was replaced by *P. cymballum*, *C. delicatula*, and *C. discoides*. This species change suggests that a hydrologic transition was underway at the coring location at that time.

As a hydrologic disturbance-based species with high tolerances for elevated levels of conductivity (Dallimore et al. 2000, Patterson et al. 1985), low temperatures (Boudreau et al. 2005; Dallimore et al. 2000), and low trophy (Burbidge

and Schroder-Adams 1998), C. spinosa(aculeata strain)

dominance and disappearance suggests that the wetland may have

been undergoing a transition from hydrologic inundations and

nutrient-poor waters to stabilized hydrology and increased

nutrient concentrations. This is supported by the appearance

of seasonal low-water indicator C. delicatula (Davidova 2013),

and seasonal high-water indicator C. discoides (Heckman 1998)

as well as an increase in P. cymballum a high-nutrient,

submerged vegetation indicator (see Chapter 5). There is also

an increase in high-nutrient, soil-dwelling (Mitchell 2004;

Ogden and Pitta 1990) P. acropodia abundance at 258cm.

The samples examined at 242 and 230cm were devoid of testate amoebae, a possible result of extended unfavourable environmental conditions although no acnanthamoebae cysts (resistant to temperature and pH fluctuations) were found as was the case in Elliott et al. (2011). Analysis of the pollen grain morphology within the samples found most grains to be either crumpled and broken. These preservation characteristics are the result of dry sediments, sediment compaction, and/or pollen abrasion during transport (Davis 2012). Therefore, the lack of tests within the samples may be a result of unfavourable dry conditions in the area rather than temperature or pH changes.

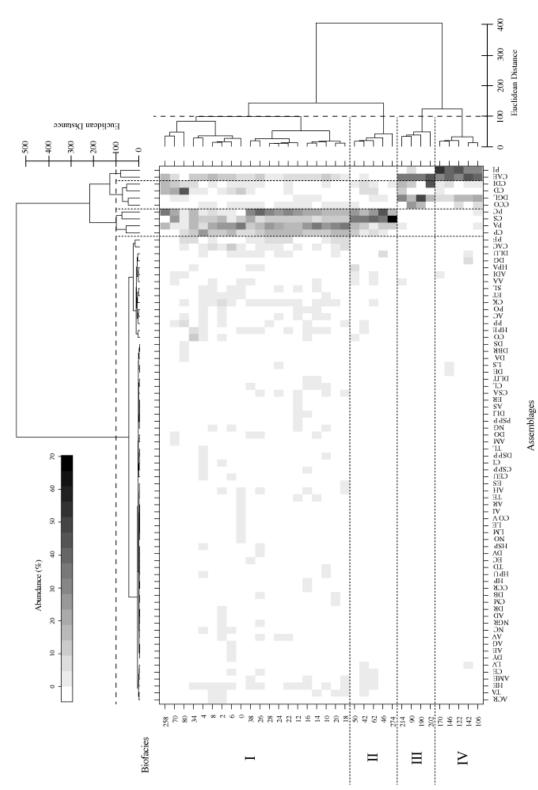


Figure 33.Bicluster heatmap of biofacies and associated assemblages. See page xiv and xv for taxa abbreviations.

Although testate amoebae did not return to the record until 214cm, the lithological shift at ~220cm to a strong carbonate concentration within the sediment (Table 15) indicates a return of wet conditions to the area (Dean and Fouch 1983). Carbonate sediments in a nearshore environment are typically deposited via diurnal and seasonal induced inorganic precipitation and bio-induced precipitation by algae and other aquatic vegetation (Dean and Fouch 1983). Dean and Fouch (1983) indicate that CaCO, precipitation is greater in a lake's littoral zone due to increased water temperatures and the greater presence of precipitating vegetation. The first observations of Charophyte encrustations at 202cm as well as a strong concentration of CaCO<sub>3</sub> (Table 15) at that depth suggests that CaCO, deposits were initially driven by inorganic precipitation followed by bio-induced precipitation as aquatic vegetation slowly became established following the dry period.

Additionally, samples from 212 to 207cm reveal laminations characteristic of annual or seasonal deposits suggesting reduced hydrologic perturbations and a supersaturation of carbonates in the water column (Anderson and Dean 1988).

The reemergence of testate amoebae along the shoreline at 214cm is observed in an assemblage dominated by agglutinated centropyxids and difflugids (Figure 32) namely, C.

aerophila(lacustrine type) , D. globulosa and C. discoides (aculeata strain) (Biofacies III). C. discoides (aculeata strain) and D. globulosa are often found to be the dominant species in many modern Arctic lakes (Collins et al. 1990; Bobrov et al. 2003) while C. aerophila(lacustrine type), with a preference for dry, low trophy conditions, has been identified as a dominant species at both the Pleistocene and Holocene timescales in a permafrost environment (Bobrov et al. 2003; Beyens and Chardez 1987; Beyens et al. 1986).

Additionally, centropyxids and difflugids are known to be opportunistic, highly tolerant genus' (Kihlman and Kauuppila 2010; Holcova 2008; Scott et. al 2004; Patterson et al. 2002), reinforcing the inference of a newly formed aquatic environment.

Although Charophyte encrustations were not observed until 202cm, a band of indistinct blue-green coloured laminated deposit is found from 200-196cm. This colouring was lost shortly after core extraction suggesting increased primary production through the presence of chlorophyll or derivatives (Kowalewska and Szymczak 2001; Laevastu 1958) at this time.

By 190cm, observed increases in Charophyte abundances coincide in an increase in sediment  $CaCO_3$  concentrations, an abrupt decline in  $C.\ discoides$  (aculeata strain), a moderate

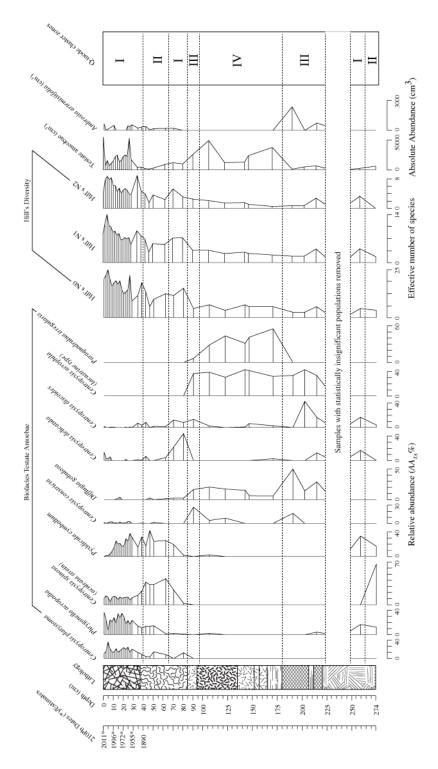


Figure 34.Stratigraphic plot of Biofacies assemblage relative frequencies & zones, <sup>210</sup>Pb & *Ambrosia* dates, richness & diversity, lithology, & *Ambrosia* abundances.

decline in C. aerophila (lacustrine type), and a substantial increase in D. globulosa. A sharp increase in Ambrosia pollen abundance is also found at this depth indicating terrestrial change was also underway.

The testate amoebae assemblage change may be related to lowering lake levels and further carbonate saturation of the water column. Qin et. al (2013) noted that D. globulosa was most abundant in alkaline lakes ranging from pH 8.0 to 8.1 while certain species of Charophytes (e.g. Chara contraria, C. vulgaris, C. globularis, C. halina) are well documented in low trophy, calcium rich waters (Pelechaty et al 2013; Apolinarska et al. 2011; Garcia 1994). Morphological analysis of a non-calcified Charophyte oospore at 106cm, suggests that C. globularis (Garcia 2013) may have been the species inhabiting Lake Simcoe acting to precipitate calcium through photosynthesis. The dramatic decrease in C. discoides (aculeata strain) may be attributable to a pH optima of ~4.5-6.5 for this species type (Parent et al. 2009; Booth 2001).

Following the community instability and limited biodiversity (  $\bar{N}I$  = = 3.63,  $\bar{N}I$ :N0 = 0.86) characteristic of stressed environments (Patterson et. al 2002; Magurran 1988) from 220-190cm, a complete shift in composition and dominance is seen as the assemblage of Biofacies IV (C.

aerophila(lacustrine type) and P. irregularis) enters the record at 170cm, and previously dominant species (C. discoides(aculeata strain) and D. globulosa) no longer define the community.

The assemblage of Biofacies IV is characteristic of alkaline, mesotrophic waterbodies (Beyens et. al 1991,1986). P. irregularis abundances rose dramatically from 0 to 55.07% of the assemblage between 190cm and 170cm depth. P. irregularis is a calciphile that forms an idiosomic calcareous test of rectangular calcite plates. Reports of the ecology of P. irregularis place this species in both lacustrine (Beyens et al. 1986) and peatland deposits (Opravilova and Hajek 2006; Muller et al. 2009), and all authors agree on its rarity in both live and fossil samples. In lacustrine habitats the species is highly indicative of mesotrophic, alkaline waters with high conductivity (Beyens et al. 1986) and in peatlands, it was found to be an indicator of calcareous fens, exclusively inhabiting bryophyte tufts (Opravilova and Hajek 2006), although it has been shown to also inhabit mosses (Nguyen-Viet et al. 2004). A study of calcium-rich Lake Lautrey in eastern France (Wall et al. 2010) noted a strong dominance by P. irregularis during warm climatic phases.

Commensurate with the peak in P. irregularis is an

increase in the agglomeration of sediments suggesting an increase in *Charophyte* carbonate precipitation activity.

Lithologically, a greenish-yellow laminated unit with mm sized vascular organics records increased lacustrine primary productivity and shoreline terrestrial productivity from 180-165cm, encompassing the *P. irregularis* peak.

From 165-139cm, a lithologic shift to large organic inclusions and small gastropod shells surrounded by reddishgrey to dark-grey carbonate mud deposits occurs. A brief survey of the course fractions from 154cm, show numerous mollusc species including Pisidium spp. (Pea clams), Amnicola limosa (Ordinary Spire snails), Gyraulus circumstriatus (Flatly Coiled Gyraulus), Gyraulus deflectus (Irregular Gyraulus), and Bakerilymnaea dalli (Small Pond snail) (identifications via Clarke 1981). Benthic analysis of the organically rich sediment-water interface of Victoria Point wetland from 2009-2010 (Kanavillil and O'Connor, unpublished) recorded all of these species in the mollusc communities during those years. The presence of G. deflectus (Clarke 1981) and the mollusc community itself may indicate a shift to a higher trophic status along the shoreline (Dillon Jr. 2004).

Anecdotal assessment of Pisidium dominance in this sample suggests that waters were calm, shallow (0.25-0.36m) and warm

(13.5-19.7°C) with carbonate concentrations ranging from 55-89 mg  $CaCO_3$  l<sup>-1</sup> based on *Pisidium* abundances >100 and >300 m<sup>-2</sup> (Dillon Jr. 2004; Kilgour and Mackie 1991).

Declines in *P. irregularis* from 122cm to 90cm revealed a drop in its relative abundance from 43.48 to 6.67% respectively, disappearing entirely by 82cm. The similar but more abrupt disappearance *C. aerophila*(lacustrine type) between 90 and 82cm (36.67% to 0%) coincides with the disappearance of *Charophyte* encrustations by 82cm. At the same time, diatoms become visible in the testate amoebae slides and the coarse macro fractions show a greatly reduced mollusc community.

Changes to water chemistry from precipitation induced rising lake water levels may have produced the biotic changes seen in the 90/82cm carbonate/peat transition. *P. irregularis*, *Pisidium*, and *Charophytes* are all calciphiles (calcicoles) suggesting a reduction in alkalinity in the water column.

Investigations into P. irregularis at "city sites" by Nguyen-Viet et al. (2004) proposed a link between the  $Ca^{2+}$  leaching effects of  $NO_2$  concentrations on the test and the absence of the species from atmospherically  $NO_2$  polluted sites. Lambert and Davy (2011) found that the Charophyte, C. globularis (identified in level 106cm), was extremely

sensitive to increases in Nitrate-N in both field and laboratory experiments, with concentrations of  $\geq 2 \text{mg } 1^{-1}$  negatively affecting growth rates. And finally, Hornbach and Cox (1987) as cited in Dillon Jr. (2004) found that populations of *Pisidium* exhibited optimal growth in naturally hard waters with laboratory experiments describing an optimal CaCO<sub>3</sub> concentration of 160mg  $1^{-1}$ .

Whole-lake experiments by Schindler et al. (1985) from 1971 to 1976 to test the effects of ammonium ( $\mathrm{NH_4}^+$ ) inputs on lake water chemistry offer significant findings related to the disappearance and reductions of calciphiles from the nearshore of Lake Simcoe. The authors found that the addition of ammonium + phosphorus rapidly acidifies lake waters through biological uptake (likely by phytoplankton), reducing pH values as low as 4.75 from a background of 6.95. Marked decreases in alkalinity were also observed .

The natural introduction of inorganic nitrogen into lakes takes place through nitrogen fixation, precipitation, groundwater flow, and surface run-off (Lambert and Sommer 2010). Certain microorganisms have the ability to transform organic atmospheric nitrogen to bioavailable inorganic forms  $(NO_3^-, NH_3^-, NH_4^+)$  (Lampert and Sommer 2010). In precipitation, ammonium is the second most important cation constituting 12-

25% of all protolytic cations (Schindler et al. 1985). Studies of streams and watersheds have found a strong retention of inorganic nitrogen by forests which is released upon decomposition and made available for transport to lakes via run-off and groundwater flow (Lambert and Sommer 2010; Gundersen and Bashkin 1994; Schindler et al. 1985).

Therefore, experimental evidence from various sources supports the hypothesis that increased precipitation likely played a major role in the acidification of Lake Simcoe by increasing inorganic N inputs which lead to the disappearance or reduction of calciphile species whose growth rates and surviorships are negatively correlated to increases in nitrogen.

The disappearance of *C. aerophila*(lacustrine type), a dominant species throughout the carbonate facies, may also be linked to acidification through a possible symbiotic relationship with *Charophytes* that may supply the raw materials for its agglomerated test construction, as well as an ecological preference for alkaline waters. Both the appearance and disappearance of *C. aerophila*(lacustrine type) are timed precisely with *Charophyte* growth. RDA analysis of environmental variables and modern testate amoebae assemblages recorded a positive correlation between pH (positively

correlated to alkalinity (Schindler et al. 1985)) and *C.*aerophila(lacustrine type) (Patterson et al. 2013).

Additionally, a reconstruction of lakes in the Temagami region of northeastern Ontario, found *C. aerophila*(lacustrine type) in association with *Charophytes* (Boudreau et al. 2005).

Within this same time frame of lake water alkalinity reduction, water level increases, and higher trophy lies the third largest testate amoebae abundance (48939 cm<sup>-3</sup>), surpassed only by recent climate correlated abundances in AD 1961 and 2011 (see Chapter 5).

Following the loss of calciphiles, lithology and testate amoebae assemblages are characterized by an increasingly terrestrial environment along the nearshore in the form of a fringe fen. From 85 to 40cm, there is an alternation from Biofacies I to Biofacies II assemblages. Connected to fen formation is a dramatic increase in rare species as shown by the N1:N0 ratios of 0.544 (Biofacies I) and 0.579 (Biofacies II) as opposed to 0.86 for the preceding Biofacies (III). Mean N1 values also record increases in biodiversity - 8.68, 5.18, 3.63 for Biofacies I, II, and III respectively.

While not included in the Biofacies I assemblage by bicluster analysis, C. delicatula is the most dominant species (44.19% at 80cm) in the early formation of the fen. As

described in section 5.2.1, *C. delicatula* has a late August distribution in shallow (0.5-1m), nutrient enriched (mesotrophic to hypereutrophic) waters. Less abundant species found in this sample include *C. platystoma*, *P. fulva*, and *C. discoides* (aculeata strain) (9.55%, 9.55%, 8.36% respectively). Optima, tolerances and habitat for all three taxa are provided in Table 16.

Table 16. Optima, tolerances and habitat for *C. platystoma*, *P. fulva*, and *C. discoides*(aculeata strain).

Taxa	Water table depth (cm) (low-common-high)	Moisture (%)	Habitat	Reference
C. platystoma	2.5-5-12.5	87.5- <i>92</i> -95	mosses, mineral rich, alkaline	Charman et al. 2007; Lamentowicz 2010
P. fulva	0-8-16	81-87-94	mosses, nutrient rich, neutral	Charman et al. 2007; Booth & Zygmunt 2005
C. discoides (aculeata strain)	-2-4-12	93- <i>95</i> -97	mosses, stressed environmen ts, pH: ~4.5-6.5	Charman et al. 2007; Parent et al. 2009; Booth 2001

Based on the ecological preferences of *C. delicatula* and the less abundant taxa, early fen formation is characterized by an unstable environment with hydrologic fluctuations,

increased organic content, and high productivity.

The final assemblage prior to European settlement,

Biofacies II from 65 to ~40cm (~ AD 1890), is dominated first

by *C. spinosa*(aculeata strain) followed by *P. cymbalum*.

Together, these species indicate an initially stressed,

oligotrophic environment with increasing sumberged aquatic

vegetation and nutrient concentrations (Farooqui et al. 2012;

Patterson and Kumar 2002; Dallimore et al. 2000).

#### 6.3 Conclusion

The paleoenvironmental record of Victoria Point wetland prior to European settlement was reconstructed using testate amoebae as an environmental proxy. To enrich interpretations and refine dating estimates, lithology and select pollen and macro analyses were conducted on the core. The paleodistribution of testate amoebae were influenced water quality (pH, trophic status, temperature), water quantity (inundation, drought and precipitation events), and sediment composition (carbonates, organics).

The earliest phase of Victoria Point wetland was characterized by hydrologic fluctuations and reductions which eventually lead to the disappearance of all testate amoebae at the location.

The return of wet conditions brought with it testate amoebae assemblages common in Arctic, oligotrophic, alkaline lakes and increased dissolved CaCO3 within the water column.

Decreasing water depths and further carbonate saturation of the lake waters increased alkalinity and nutrient concentrations leading to testate amoebae assemblages dominated by alkaline *D. globulosa* and the colonization of the area by *Charophytes*. A change in the terrestrial ecosystem of the area was also underway, increasing disturbance related *Ambrosia* pollen abundances.

As carbonate concentrations in the sediment reached greater than 25%, the appearance of *P. irregularis*, a mesotrophic calciphile, marked a new phase in the history of the location that lasted until the commencement of fringe wetland formation began at ~82cm.

The decline of the calciphiles (*P. irregularis*, *D. globulosa*, *C. aerophilia*, and *Charophytes*) was brought on by increasing water levels and lake acidification through an influx of nitrogen and phosphorus from precipitation, surface run-off, and possibly biotic sources.

Fringe wetland fen formation began under continual hydrologic disturbance leading to the gradual increase in submerged, and emergent aquatic vegetation along the

shoreline.

#### Chapter 7. Synthesis and Summary

#### 7.1 Synthesis

The health of Lake Simcoe has been extensively studied since excessive aquatic growth was first reported in the 1970's by shoreline residents. Most of this research has focused on elucidating, monitoring and controlling nutrient sources to reduce phosphorus loads to the limnetic zone of the lake to counteract the detrimental affects to the cold-water fishery. Some research has been conducted on the littoral zone of the lake though none, until very recently (Danesh et al. 2013), have attempted to understand the historic effects of stressors on littoral biological communities. The history of the Lake Simcoe fringe wetland communities have not been the focus of any study prior to the one presented here. My testate amoebae based paleolimnolgical/paleoecological study addresses the gap in knowledge surrounding the effects of multiple stressors on Lake Simcoe's fringe wetland communities at Anthropocene time scale with additional data from past variability.

Prior to the Anthropocene, Lake Simcoe's north shoreline
(Victoria Point) exhibited five distinct shifts in the

paleoenvironment evidenced by a testate amoebae response to changes in water quality (pH, trophic status, temperature), water quantity (inundation, drought, precipitation events), and sediment composition (carbonates, organics).

Proxy data indicate that Lake Simcoe's early shoreline lacustrine environment was similar to the environment found just prior to the start of the Anthropocene. Hydrologic perturbations encouraged the dominance of *C. spinosa* (aculeata strain) while soil and aquatic communities maintained a presence in the area.

A period of low water input to the area desiccated pollen grains and proved too unfavourable for continued testate amoebae growth.

The return of wet conditions brought with it a testate amoebae community similar to that found in arctic oligotrophic lakes where *C. discoides*(aculeata strain), *D. globulosa* and *C. aerophila*(lacustrine type) dominate.

The reduction of hydrologic disturbance and increased alkalinity as indicated by an increase in  $D.\ globulosa$ , and the introduction of Chara spp. (alkalinity &  $CaCo_3$  indicators).

A unique shift in Lake Simcoe's paleoenvironment is marked by further increases to  $CaCo_{\mbox{\tiny 3}}$  concentrations as well as

terrestrial and aquatic productivity. The shift is evident in the dramatic appearance and dominance of the calciphile *P*.

irregularis, a pronounced increase in *Charophyte*encrustations, and the appearance/preservation of vascular terrestrial detritus.

Increasing water levels from precipitation reduced CaCo<sub>3</sub> concentrations and acidified and enriched the lake waters (Lambert and Sommer 2010; Schindler et al. 1985) leading to the disappearance of the previously dominant calciphiles and the recommencement of a fringe wetland environment with increased abundances of submerged aquatic vegetation and organic matter.

Paludification of the shoreline is indicated by decreasing abundances of testate amoebae associated with submerged aquatic vegetation (*C. spinosa*(acueleata strain)) and increasing abundances of species that dwell at the soillitter interface (*P. acropodia*).

A detailed investigation at the Anthropocene timescale of Victoria Point and two other Lake Simcoe wetlands (Cook's Bay and Duclos Point) shows a clear response to initial land clearing activities. All three wetlands exhibited a diminishment of primary species abundance, an introduction or abundance increase of disturbance indicating species, and an

increase in species richness and diversity.

Duclos Point showed the greatest rate of change associated with ~AD 1830 land clearing activities, changing from a stable, strongly seasonal shallow wetland indicated by C. delicatula, to an unstable, deeper, slightly alkaline wetland with submerged aquatic vegetation and enriched soil components.

Initial land clearing (~AD 1830) did not have the same pronounced affect on the environment of Cook's Bay wetland. It was not until the drainage of the Holland Marsh and canal construction (~AD 1920-1930) that a strong environmental response is recorded. Land use change to commercial farming along with water level regulation of the canal and its use as a receiving water for urban sewage effluent lead to a rapid increase in soil-based testate amoebae that thrive in high nutrient environments. The flood event of Hurricane Hazel (1954), the dramatic rise of corn as a crop (~1960) and the diversion of sewage effluent in the 1980's further changed the testate amoebae, shifting primary abundance to a submerged aquatic community (C. platystoma).

Just prior to European land clearing Victoria Point shows a clear change in proxy assemblage along with the presence of Ambrosia pollen. At 46cm, an increase in submerged aquatic

vegetation, alkalinity and soil enrichment records an erosional disturbance event that may be related to First Nations land clearing in the area or to a localized climatic event.

The land clearing activity of ~AD 1871, produced a marked but moderate change to the wetland, resulting in a gradual decrease of submerged aquatic vegetation and increase in soilbased testate amoebae that thrive in high nutrient environments. The opening of the Trent-Severn waterway in 1920 and subsequent lake water level regulation may be partly responsible for the stability recorded in recent sediments and for the disappearance of *P. cymbalum*, the primary species prior to European settlement.

An increase in phosphorus loads to the lake from 1965 to 1970 reduced species richness in the wetland while increasing or maintaining the dominance of species associated with submerged aquatic vegetation (*P. cymbalum*, *P. fulva*, and *C. platystoma*) positively correlated to phosphorus.

Changes in spring climate measurements since the mid 1800s had the greatest impact on testate amoebae assemblage variation from 1871 to 1957 and again in 1977. Greater levels of spring snowfall created a stable environment that reduced disturbance and the abundance of centropyxid community members

and may have increased favourable conditions for other species to thrive.

Land clearing disturbance altered the Tub Lake reference wetland through an influx of sediment and nutrients from erosion but did not have a long-term effect on primary species abundance.

A change in the testate amoebae communities from ~10 to Ocm is evident in all four wetlands despite a complete lack of anthropogenic influence on Tub Lake. Q-mode cluster analysis and ordination-based rate of change analysis, indicate a magnitude of wetland change nearly on par with the land clearing activities of the late 1800s in Tub Lake and a larger magnitude than land clearing in Victoria Point. A similar change spike is also recorded in the upper 10cm of Cook's Bay and Duclos Point wetland cores. These changes are linked to winter climate variables suggesting that climate change, particularly winter climate change, is the main driver behind the recent instability in all four wetlands. However, the addition of the Zebra mussel invasion of Lake Simcoe may also be influencing recent wetland change through an increase in nutrients via the "nearshore phosphorus shunt" hypothesis (Hecky et al. 2004).

#### 7.2 Summary

Testate amoebae were used as paleolimnological/paleoecological indicators to reconstruct paleoenvironmental changes to Lake Simcoe lacustrine fringe wetlands from climate, nutrient, and non-native species influences under the following lines of inquiry:

### 1) How have nearshore testate amoebae communities developed since deglaciation?

An arctic-like, oligotrophic, neutral pH testate amoebae community became established soon after deglaciation. The withdrawl of Lake Algonquin and the subsequent water level low-stand changed testate community dominance to mesotrophic calciphiles. The main Hypsithermal acidified and enriched lake waters enabling the establishment of slightly acidic to neutral testate amoebae typically found in wetland environments.

## 2) Have nearshore fringe wetland testate amoebae communities changed since European settlement?

Testate communities have shown a diminishment in the abundance of pre-settlement species and an introduction or abundance increase in disturbance indicating species.

3) If so, has there been a change in richness, a shift in species abundance or community composition? Species richness has increased in all three Lake Simcoe wetlands up-core from the point of European settlement. The largest change in species richness began in the mid-1990s, increasing the richness of all three wetlands to a mean of 17.3 species where the mean prior to European settlement was 6.8.

There has been a clear shift in both abundance and community composition since European settlement across all cores. There has been a 224% (DP), 262% (CB), and 281% (VP) mean increase in testate amoebae abundance since land clearing. All three communities have experienced a change in community composition such that pre-settlement species have been severely diminished or have disappeared entirely to be replaced by species either highly tolerant increased nutrient concentrations and disturbance or having a preferential affinity for such an environment.

# 4) Are any of these changes related to temporal trophic status, land use, climate, or non-native species introductions?

All three wetlands show a testate amoebae community change in relation to increased nutrient inputs from changes in land use around the watershed. The estimated

chronology of Cook's Bay land use change associated with the drainage of the Holland Marsh and construction of the canal system shows a strong qualitative relationship to testate amoebae community change. There is also a strong qualitative relationship between initial land clearing activities in Duclos Point and community change. The dated core extracted from Victoria Point provides evidence of community change associated with known dates of changes in land use, climate and non-native species. In Victoria Point, there are qualitative community changes associated with possible First Nations and European land clearing activities in ~AD 1306 and ~1871 respectively. Quantitatively, community change is correlated to increases in lake-wide phosphorus loading from 1965 to 1970, increases in spring snowfall between 1871 to 1957 and again in 1977, increases in winter snowfall between 2007 to 2010, and increases in winter rainfall for the remainder of the samples. Attributing community change to the introduction of Zebra mussels is difficult based on the co-occurrence of the introduction with climate change and lake-wide phosphorus increases. However, one species (P. fulva) has shown a consistent increase in abundance in relation to the influx of nutrients following land clearing activities and also

shows a very strong quantitative positive correlation to lake-wide phosphorus loading. With this in mind, its presence within the upper 10cm of both Cook's Bay and Victoria Point wetlands (~AD 1996 to 2011) may indicate an increase in nutrient concentrations to the wetlands as a result of the "nearshore phosphorus shunt" following the establishment of the Zebra mussel in Lake Simcoe in the mid-1990s and increased anthropogenic inputs.

## 5) Are the changes unique to each wetland or are they lake-wide?

The strongest lake-wide change is the paludification of the nearshore areas studied. Prior to land clearing activities, the areas were primarily aquatic environments with strong to moderate seasonally driven hydrologic regimes and fluctuating nutrient concentrations. After land clearing, the areas became increasingly dominated by the build up of organic matter and associated flora and fauna.

## 6) Can a comparison with a reference wetland pin-point the source of these changes as anthropogenic?

While the reference wetland lies within Canadian Shield geology and thus lacks species associated with the hard-water of Lake Simcoe, its history of community change can

still be compared to testate amoebae communities distributed over time in Lake Simcoe. The Tub Lake community shows great continuity across time with regard to its primary species which retained dominance throughout the entire core. After land clearing activity, the community succession indicator (C. orbicularis) reduced in abundance before finally disappearing suggesting that Tub Lake has recovered from the initial disturbance. This is not the case in Lake Simcoe where the primary species has changed a number of times throughout the length of each core and C. orbicularis continues its presence. The lack of anthropogenic influence on Tub Lake since ~AD 1890 and its community stability suggests that the factor affecting community instability within each of the Lake Simcoe wetlands is anthropogenic in origin.

7) What is the past variability of the testate amoebae communities prior to European settlement?

Prior to European settlement there were three distinct testate amoebae communities in Victoria Point wetland:

1) An "early fringe wetland community" dominated by aquatic and soil-based species; 2) An arctic-like, oligotrophic testate amoebae community dominated by

alkaline, lacustrine species; and 3)A CaCo<sub>3</sub>-based community dominated by mesotrophic calcipiles.

In closing, the results of my testate amoebae based paleolimnolgical/paleoecological study have provided insight into the paleoenvironments of Lake Simcoe's shoreline with regard to past variability and Anthropogenic influences. Lake Simcoe's shoreline has undergone changes in hydrology, trophic status, pH, and biological community composition mainly as a result of hydrologic changes. The acidification and nutrient enrichment of Lake Simcoe that instigated the paludification of the location was compounded by the anthropogenic activities of the last ~200 years quickly enriching the water and sediment of the shoreline. The mean rate of change in the testate amoebae community since ~AD 1300 (0.46) is greater than the rate of change in the preceding record (0.40).

It is my hope that the results of this study will provide Lake Simcoe stakeholders with the information necessary to make informed decisions regarding any future management or restoration efforts of the Lake Simcoe shoreline. I also hope that my use of testate amoebae supports the growing body of evidence showing its usefulness as a paleoenvironmental proxy for land use change, hydrology, climate, and nutrient

enrichment inferences.

Future research is required to fully understand the complexity of multiple stressors on testate amoebae communities as seen in the most recent samples of this study. Long-term seasonal monitoring of testate amoebae community composition with regard to physical and chemical environmental factors may provide the resolution needed to tease apart the effects of multiple stressors on the community. Additional research to evaluate the efficacy of using individual fringe wetland testate amoebae species as indicators of environmental change or disturbance may enable the creation of a protocol for rapid assessment of Lake Simcoe wetland health.

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## Appendix A - Testate amoebae & Chara micrographs



A. arenaria



C. aerophila (lacustrine
type)



C. aerophila (soil type)



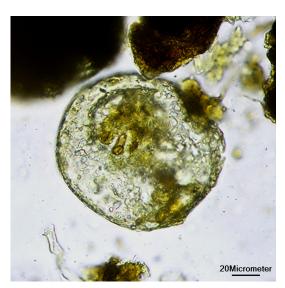
C. aculeata



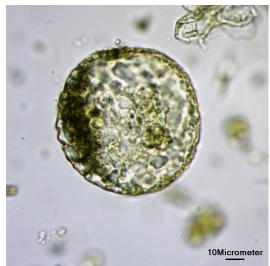


C. constricta

C. delicatula



C. discoides (aculeata C. orbicularis strain)





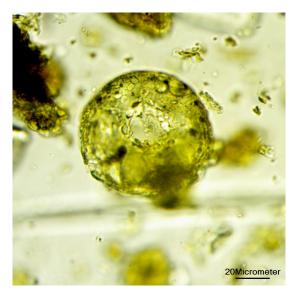
20<u>Micrometer</u>

C. platystoma

C. spinosa (aculeata
strain)



Chara globularis oogonia





C. kahli

D. globulosa



Chara spp. encrustation



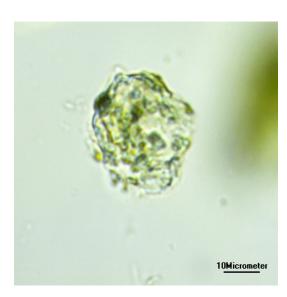
H. elegans





P. irregularis

P. acropodia





P. fulva

P. cymbalum

## Appendix B - Testate amoebae data

Site					Ambrosia		AA	AAT	AC	AC	R AD		ADI	ΑE	AG	Αŀ		
VP	C							3									3	2
VP	2					0				2	2	2					2	
VP	4									9					_			
VP	6			267		0					_				2	2		
VP	8							3			5							
VP	10					4				2								
VP	12						2			5								
VP	14					0		2		7								
VP	16																2	
VP	18					0											2	
VP	20																	
VP	22					0												
VP	24									3								
VP	26																2	
VP	28																	
VP	34			133														
VP	38			341				3										
VP	42																	
VP	46																	
VP	50							2						3				
VP	62							3						3				
VP	70							2						2				
VP	82					0				5				2				
VP	90					0												
VP	106					0												
VP	122					0												
VP	142			179		0												
VP	146					0												
VP	170					0								2				
VP	190																	
VP	202					0												
VP	214			41				2										
VP	230																	
VP	242																	
VP	258					0												
VP	274	273	175	41	(	0												

Site		Bott. A	ME AR		CE	CL	CN	1 (	CAC		ССО			CEC	СО	CP		S
VP	0	1		2					7	31	7	48	7				29	36
VP	2	3			2				12	41		9					32	43
VP	4	5							12	27	3	9			•		68	31
VP	6	7				2			29	36	7	15	2				41	12
VP	8	9							22	44	7		2				27	29
VP	10	11					2			31	2		2				26	36
VP	12	13							5	17	3						41	32
VP	14	15					2		3	14							22	19
VP	16	17	2				2			12	5				:		27	27
VP	18	19	2						14	5	2						36	24
VP	20	21						2	3	19	2						41	27
VP	22	23			2					24	3	5	2				43	36
VP	24	25			2				2	7	7						41	27
VP	26	27			3	2			2	48	12		3				41	32
VP	28	29							5	9							32	24
VP	34	35								15		2	9		1:		12	19
VP	38	39	2				3			20	2	2	10				19	63
VP	42	43	2			2				2		7	9				3	37
VP	46	47															2	20
VP	50	51									2		2		:	2	10	31
VP	62	63	3			2						5	2				7	44
VP	70	71								3		15	9					17
VP	82	83							5			63	12				14	3
VP	90	91								19	14		7					
VP	106	107								80	10		5					
VP	122	132								24	7							
VP	142	143								77			2					
VP	146	147								32			5					
VP	170	171								37		3	3					
VP	190	191								3	2							
VP	202	203								5			5					
VP	214	215								14		5	7					
VP	230	231																
VP	242	243																
VP	258	259								2		2	2					
VP	274	275											2					27

Site		ott. C	EU CK	CI	C	OV CS	SPFCS	SA C	CR D	SPFDA	, DB	DBI	R DG	DGL D	L DLI
VP	0	1				2									
VP	2	3		15	2										
VP	4	5	3	7	7		3			3					
VP	6	7	9	3											
VP	8	9		9											
VP	10	11		12				7 2		2					
VP	12	13		5				2						2	3
VP	14	15		3										5	
VP	16	17					3		2					9	2
VP	18	19						3							
VP	20	21		2								3			
VP	22	23		10											
VP	24	25		3				2							
VP	26	27		10				3				2			
VP	28	29		3											
VP	34	35													
VP	38	39		9											
VP	42	43													
VP	46	47												2	
VP	50	51													
VP	62	63		2										2	
VP	70	71												2	
VP	82	83		2							5		2	3	
VP	90	91												9	
VP	106	107												43	
VP	122	132												14	
VP	142	143											1	4 27	
VP	146	147												5	
VP	170	171												7	
VP	190	191												5	
VP	202	203												2	
VP	214	215												12	
VP	230	231													
VP	242	243													
VP	258	259													
VP	274	275													

```
Site
        Top Bott. DLU DLIT DO DPU DR DS DY DV EC EF EL ER ES ET HE
VΡ
                 1
VΡ
            2
                                            2
                                                                                            9
                                                                                                 5
                  3
VΡ
            4
                                                                                            5
                                                                                                 5
                  5
                       3
            6
                       2
                                                       2
                                                                                                 2
                 7
                                                                                           10
VΡ
\mathsf{VP}
           8
                                                                                           7
                                                                                                 2
                 9
                                                                                                 2
VΡ
           10
                                                                                            9
                 11
VΡ
                                                                                 3
                                                                                                 3
          12
                 13
\mathsf{VP}
          14
                            5
                 15
                                  2
                            2
VΡ
          16
                                                                                                 2
                 17
\mathsf{VP}
          18
                 19
                                                                                       3
                                                                                                 2
\mathsf{VP}
          20
                 21
                       7
\mathsf{VP}
          22
                 23
                       3
                                                                                                 2
VΡ
          24
                 25
                       2
          26
                       2
                                  2
                                                            2
                                                                                                 5
VΡ
                 27
          28
VP
                 29
VΡ
                                                                                                 2
          34
                 35
                                                                                                 2
          38
                                  5
                                                                 2
VΡ
                 39
                       2
                                                                                                 2
VΡ
          42
                 43
VΡ
          46
                 47
                       3
VΡ
          50
                 51
VΡ
                                                                                                 2
          62
                 63
                                  2
VΡ
          70
                 71
VΡ
          82
                 83
                                                 2
VΡ
          90
                 91
VP
         106
                107
\mathsf{VP}
         122
                132
VΡ
         142
                       2
                143
VP
         146
                147
\mathsf{VP}
         170
                171
\mathsf{VP}
         190
                191
VΡ
         202
                203
VΡ
         214
                215
VΡ
         230
                231
VΡ
         242
                243
VΡ
         258
                259
VΡ
         274
               275
```

Site VP		ott. HP	HS	HPU H		PA HS	SP LV	LE	LM 2		NB	NC	NG	NO	GR NO
VP VP	0 2	1			5				2	2			2		3
VP VP	4	3		2	2		2						3 2		2
VP VP	6	5 7		2	2		2							2	
VP VP	8	9										4		2 2	
VP VP	10	11		2	3									3	
VP VP	12	13		2	3									3	
VP	14	15			2									5	
VP	16	17	2		2										
VP	18	19	_		_										
VP	20	21													
VP	22	23			5										
VP	24	25									2				
VP	26	27			2	2	2								2
VP	28	29													
VP	34	35			7	2									
VP	38	39			10	2									
VP	42	43			2			2							
VP	46	47													
VP	50	51				5									
VP	62	63													
VP	70	71													
VP	82	83													
VP	90	91													
VP	106	107													
VP	122	132													
VP	142	143						2							
VP	146	147									2				
VP	170	171													
VP	190	191													
VP	202	203													
VP	214	215													
VP	230	231													
VP	242	243													
VP	258	259													
VP	274	275													

Site	Top E	Bott. P	SPFPF	PC PC	) PI	P F	PA P	l PG	PASFQS	SL	TE		TA	Т	)
VP	0	1					126				7	5			
VP	2	3		7	9	10	71							7	
VP	4	5		7	9	5	19				2		3		
VP	6	7		19			63				9				
VP	8	9	5	20			51				7			5	
VP	10	11	5	37			71								2
VP	12	13	5	65	3		48				3	2			
VP	14	15		77	7		77				5	2		2	
VP	16	17		78	7	2	60								
VP	18	19	15	63		2	80							2	
VP	20	21	15	68			66							2	
VP	22	23	20	143			51								
VP	24	25	14	93			51				2				
VP	26	27	9	262			68								
VP	28	29	19	113		2	49								
VP	34	35	7	31			17								
VP	38	39	3	152			31								
VP	42	43		29		2	14							2	
VP	46	47		45			9								
VP	50	51		39			14								
VP	62	63		46			2								
VP	70	71		14		2	2								
VP	82	83	14	5		7	2								
VP	90	91						3							
VP	106	107		5			5	60							
VP	122	132						34							
VP	142	143						56							
VP	146	147						32							
VP	170	171						65							
VP	190	191													
VP	202	203													
VP	214	215					2								
VP	230	231													
VP	242	243													
VP	258	259		3			2								
VP	274	275		7			5								

			-	ΓA Sum Am		S AA		AC A	CR AD	ADI AE	AG	АН	ΑI
CB	0	1	22	199	2482		2	5		11			
CB	2	3	54	335	772		8	7					
CB	4	5	39	256									
CB	6	7	50	329	955			16					
CB	8	9	52	424		3		5					
CB	10	11	57	297	539	2							
CB	12	13	73	195	298			3					
CB	14	15	149	183	0			4					
CB	16	17	59	232			3						
CB	18	19	31	192	931		2						
CB	20	21	34	154									
CB	22	23	43	147	428								
CB	24	25	86	150									
CB	26	27	61	187	0								
CB	28	29	163	135	212			3		8			
CB	30	31	127	94	0					6			
CB	34	33	156	41	0								
CB	38	39	702	89	338								
CB	42	43	261	37	0								
CB	46	47	323	48	235					2			
CB	50	51	406	38	174								
CB	62	53	514	32	0								
CB	70	71	382	52	138								
CB	78	79	149	24	619								
CB	86	87	264	72	0					4			
DP	0	1	124	281	267		25		2	22			
DP	2	3	104	122	0		8			2			
DP	4	5	125	146						3			
DP	6	7	108	108	0		5			6			
DP	8	9	213	68			4			2			
DP	10	11	174	94	336		2			3			
DP	12	13	128	66						4			
DP	14	15	188	102	148					4			
DP	16	17	111	38			4			5			
DP	18	19	129	123	2036								
DP	20	21	472	32						3			
DP	22	23	151	86	1209		2			7			
DP	24		1877	31	892					3			
DP	26		1499	56	1793		5			7			
DP	28	29	936	36	2935					2			
DP	30		1416	60	4731			2		6			
DP	34		546	12	178								
DP	38	37	716	21	152					3			
DP	42	41	518	7	0					-			
DP	50	49	329	5	120								
DP	62		1103	6	16								
DP	78	77	467	19	80								
DP	86	85	347	5	0								

Site	Ton Bo	ttom AME AR	ΑV	CE	CL	CM	CAC C	AF (	CO C	ח מ	בטו כ	CEC C	O (	CP C	cs
CB	0	1	, , ,	<u> </u>	0_	0	24	5	5	-	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	,	2	47	13
CB	2	3					25	49	·		8		_	122	11
СВ	4	5					32	15			7			73	10
СВ	6	7					11	14			3			137	11
СВ	8	9					21	31	21		4			119	5
CB	10	11					14	42			•			85	27
СВ	12	13					5	3						66	22
СВ	14	15					6	13						38	35
СВ	16	17					6	8						76	21
СВ	18	19					2	2						47	20
СВ	20	21					3	6						66	18
СВ	22	23						22						46	24
CB	24	25					3	5						27	47
CB	26	27					3	10						60	34
CB	28	29						5						4	58
CB	30	31												12	34
CB	34	33					2		3		3			8	7
CB	38	39						3						17	11
CB	42	43												10	6
CB	46	47								9				3	12
CB	50	51					3							6	2
CB	62	53								2				7	7
CB	70	71								5				6	15
CB	78	79								3					4
CB	86	87			2					10				5	8
DP	0	1					4			40	32		2	34	65
DP	2	3						3		4	11		8	32	30
DP	4	5					3	5		6	8		6	34	25
DP	6	7					2	4		5	6		8	20	25
DP	8	9						3			4		5	19	13
DP	10	11						2		4	5		5	17	19
DP	12	13					7	6		2		5	2		8
DP	14	15						2		7	5			22	30
DP	16	17									5	2	_	13	3
DP	18	19									3	7	5	29	27
DP	20	21								_			3	14	
DP	22	23								5			3	23	21
DP	24	25								1			2	7	11
DP	26	27								8			4	6	11
DP	28	29								4	-			12	6
DP	30	31					2			2	7		4	5	24
DP	34	33					2			6				2	2
DP	38	37					2			4				4	
DP	42 50	41					2			5 3					
DP	50	49 61					2							2	
DP DP	62 78	61 77								3 10				3 2	1
DP	78 86	85								3				2	4
חר	00	00								3					

Site		Bottom CEU	CK C	I COV	CSPP	CSA	CCR	DSPP	DA	DB	DBR DG	DGL DL	DLI
CB CB	0	1 3	3	2									
СВ	2 4	5 5	3 15	3									
CB	6	7	24	4									
CB	8	9	42	5									3
CB	10	11	17	6									0
CB	12	13	18	5									
СВ	14	15	7	8									
СВ	16	17	27	7									
CB	18	19	17	4									
CB	20	21	11	5									
CB	22	23	9	2									
CB	24	25	3	10									
CB	26	27	7	6									
CB	28	29	2	10									
CB	30	31	3										
CB	34	33	2										
CB	38	39											
CB	42	43											
CB	46	47											
CB	50	51											
CB	62	53											
CB	70 70	71 70											
CB CB	78 86	79 87											
DP	0	1	2									5	
DP	2	3										3	
DP	4	5	2									8	
DP	6	7	_									3	
DP	8	9										4	
DP	10	11	5									8	
DP	12	13	3									10	
DP	14	15	2									5	
DP	16	17	1									3	
DP	18	19	10									8	
DP	20	21										7	
DP	22	23	3									4	
DP	24	25											
DP	26	27										4	
DP	28	29										7	
DP	30	31										5	
DP	34	33											
DP	38	37											
DP	42 50	41											
DP DP	50 62	49 61											
DP	78	77											
DP	76 86	85										2	
DF	00	00										4	

		ttom HP	HS	HPU HPE HPA	HSP LV	LE	LM	LS	NB	NC	NG NGR NO
CB	0	1		2							2
CB	2	3		8							5
СВ	4	5		2							
CB	6	7		3							_
CB	8	9		5		_					5
CB	10	11		8		3					3
CB	12	13		9							
CB	14	15 17		2							
CB	16	17 10		11							2
CB CB	18 20	19		6							2 2
СВ	20 22	21 23		7							2
СВ	24	25 25		2							
СВ	26	25 27		2							
CB	28	29									
CB	30	31									
CB	34	33									
CB	38	39		2							
CB	42	43		_							
СВ	46	47									
СВ	50	51									
CB	62	53									
CB	70	71									
CB	78	79									
CB	86	87									
DP	0	1									
DP	2	3									
DP	4	5		3							
DP	6	7									2
DP	8	9									
DP	10	11									
DP	12	13									
DP	14	15									
DP	16	17				•					
DP	18	19				2					
DP	20	21									
DP	22	23									
DP DP	24 26	25 27									
DP	28	27 29									
DP	30	31									
DP	34	33									
DP	38	37									
DP	42	41									
DP	50	49									
DP	62	61									
DP	78	77									
DP	86	85									
٠.	50	-									

Site	•	ttom PSPP			PP	F	PA PI	PG	PASFQ			TL	TA	TD	
CB	0	1	17	11				9		2	2				3
CB	2	3	16	30			24			4				3	
CB	4	5	12	15			50								
CB	6	7		16			35								
CB	8	9	4	40			79								3
CB	10	11	11	27			30				2				
CB	12	13			9		31				8				
CB	14	15		13			36								
CB	16	17		10			48								
CB	18	19		7	2		60				9				
CB	20	21		11			7					5			
CB	22	23		11			11				2				
CB	24	25		21			17								
СВ	26	27		23	_		31								
CB	28	29		33	4		_								
CB	30	31		25			3								
CB	34	33		7			5				_				
CB	38	39	17	17			15				5				
CB	42	43		2			19								
CB	46	47		10			12								
CB	50	51	20	5			2								
CB	62	53		6			5								
CB	70	71		12			3								
CB	78	79		11			3					•			
CB	86	87		32		_	0.4					2		•	
DP	0	1		2		3	24				11			2	
DP	2	3		4			21								
DP	4	5		4			35								
DP	6	7		4			14							4	
DP DP	8	9					10							4	
DP	10	11 13					19							3 6	
DP	12 14	15 15		3			11							O	
DP	16	17		3			20 2								
DP	18	17					27							3	
DP	20	21					5							5	
DP	22	23					13							5	
DP	24	25 25					4							5	
DP	26	27		2			9								
DP	28	29		_			5								
DP	30	31					5								
DP	34	33					J								
DP	38	37	3	5											
DP	42	41	J	2											
DP	50	49		_											
DP	62	61													
DP	78	77					3								
DP	86	85													

	•		•		Ambrosia			СО	CP	CEU	(	CK	EL	Εī	ГН	HS
TL	0	1	325	300		5			29			36				
TL	2	3	40	170	203	1			20			30				
TL T	4	5	37	284		10		5	15			4				
TL	6	7	151	236		4			•		4.5					
TL	8	9	204	326		7		•	8		15	_				
TL	10	11	129	378		3 10			41		26	5				
TL TL	12	13 15	168 135	350		7 11		. 2	3 1 19		30 17					
TL	14 16	17	94	346 342		5		5 21 52			23	5				
TL	18	19	145	342	1042	4					28	2				
TL	20	21	62	218		2					27	_		2	2	11
TL	22	23	111	295	235	1		43			7			_	_	4
TL	24	25	290	44		1			3		,					7
TL	26	27	241	188		1		16			9					
TL	28	29	75	75	0			20			Ū					
TL	30	31	80	40			5	_`	•							
TL	34	35	120	265					40							
TL	42	41	105	45					10							
TL	50	49	255	5												
TL	54	53	0	0	0											
TL	62	63	0	0	0											
TL	78	79	22	5	0	;	5									
TL	90	91	0	0	0											
Sito	Ton Bo	ttom I	V/C	TA Sum	∧ mbrocio	ND	DE	DΛ	DC.	DV6E	ם מ	21	ТΛ	тг	`	
	-		-		Ambrosia	E NB	PF			PASF	P S	SL	TA	TE	)	
TL	0	1	325	300	0	NB		180	)	PASF	PP S	SL	TA	TE	)	
TL TL	0 2	1 3	325 40	300 170	0		PF 10	180 92	) 2	PASF	PP S	SL	TA	TC	)	
TL TL TL	0 2 4	1 3 5	325 40 37	300 170 284	0 203	5		180 92 241	) 2 1	PASF	P S	SL	TA	TC	)	
TL TL TL TL	0 2 4 6	1 3 5 7	325 40 37 151	300 170 284 236	0 203 0			180 92 241 192	) 2 1 2	PASF	PP S			Τ	)	
TL TL TL TL	0 2 4 6 8	1 3 5 7 9	325 40 37 151 204	300 170 284 236 326	0 203	5 3		180 92 241 192 215	) 2 1 2 5	PASF	PP S	8		ΤΣ	)	
TL TL TL TL TL	0 2 4 6 8 10	1 3 5 7 9 11	325 40 37 151 204 129	300 170 284 236 326 378	0 203 0	5 3 6		180 92 241 192 215 188	) 2 1 2 5 3	PASF	PP S	8			)	
TL TL TL TL TL TL	0 2 4 6 8 10 12	1 3 5 7 9 11	325 40 37 151 204 129 168	300 170 284 236 326 378 350	0 203 0 232	5 3 6 3		180 241 192 215 188 235	) 2 1 2 5 3	PASF	PP S	8		TE 5 14	)	
TL TL TL TL TL TL TL	0 2 4 6 8 10 12	1 3 5 7 9 11 13	325 40 37 151 204 129 168 135	300 170 284 236 326 378 350 346	0 203 0 232 117	5 3 6 3		180 92 241 192 215 188 235	) 2 1 2 5 3 5 4 10	PASF	PP S	8 6 3		5 14	)	
	0 2 4 6 8 10 12 14	1 3 5 7 9 11 13 15	325 40 37 151 204 129 168 135 94	300 170 284 236 326 378 350 346 342	0 203 0 232 117	5 3 6 3 3	10	180 92 241 192 215 188 235 144	) 2 1 2 5 3 3 5 4 10 3	PASF		8 6 3 3		5 14 19		
	0 2 4 6 8 10 12 14 16 18	1 3 5 7 9 11 13 15 17	325 40 37 151 204 129 168 135 94 145	300 170 284 236 326 378 350 346 342 341	0 203 0 232 117 1042	5 3 6 3 3 3		180 92 241 192 215 188 235 144 180	) 2 1 2 5 3 3 5 4 10 0 3 6 21	PASF	ЭΡ \$	8 6 3 3	3	5 14 19 31	)	
	0 2 4 6 8 10 12 14	1 3 5 7 9 11 13 15	325 40 37 151 204 129 168 135 94	300 170 284 236 326 378 350 346 342	0 203 0 232 117 1042	5 3 6 3 3	10	180 92 241 192 215 188 235 144	) 2 1 2 5 3 3 5 4 10 3 3 5 21 2	PASF		8 6 3 3		5 14 19	4	
	0 2 4 6 8 10 12 14 16 18 20	1 3 5 7 9 11 13 15 17 19 21	325 40 37 151 204 129 168 135 94 145 62	300 170 284 236 326 378 350 346 342 341 218	0 203 0 232 117 1042	5 3 6 3 3 3 2	10	180 92 241 192 215 188 235 144 180 146 110	2 1 2 5 3 5 4 10 0 3 6 21 2 2 5 3 3	PASF		8 6 3 3		5 14 19 31		
	0 2 4 6 8 10 12 14 16 18 20 22	1 3 5 7 9 11 13 15 17 19 21 23	325 40 37 151 204 129 168 135 94 145 62 111	300 170 284 236 326 378 350 346 342 341 218 295	0 203 0 232 117 1042 235	5 3 6 3 3 3 2	10	180 92 241 192 215 188 235 144 180 146 110	) 2 1 2 5 3 3 5 4 4 10 3 6 21 2 2 2 3 3 3 5 3 3 3 3 3 3 3 3 3 3 3 3 3	PASF		8 6 3 3		5 14 19 31		
	0 2 4 6 8 10 12 14 16 18 20 22 24	1 3 5 7 9 11 13 15 17 19 21 23 25	325 40 37 151 204 129 168 135 94 145 62 111 290	300 170 284 236 326 378 350 346 342 341 218 295	0 203 0 232 117 1042 235 0 648	5 3 6 3 3 3 2	10	180 92 241 192 215 188 235 144 180 146 110	) 2 1 2 5 3 3 5 4 10 3 6 21 2 5 3 3 6 21 2	PASF		8 6 3 3		5 14 19 31 10 33		
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