Assessment of toxic cyanobacterial abundance at Hamilton Harbour from analysis of sediment and water

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The western embayment of Lake Ontario, Hamilton Harbour, is one of the most polluted sites in the Laurentian Great Lakes and in recent years has seen a reoccurrence of cyanobacterial blooms. This study uses a multidisciplinary approach to examine the presences of toxic Cyanobacteria in the harbour in order to gain insight into these recurrent blooms.

Microscopic analyses of phytoplankton samples collected during the 2009 summer-fall sampling season from two locations within the harbour showed the spatial and seasonal diversity of the contemporary cyanobacterial community. *Microcystis* colonies relative abundances in relation to total algal numbers were estimated. The lowest and highest relative abundances of *Microcystis* in the phytoplankton population were 0.6% and 9.7%, respectively, and showed seasonal variability between stations. Fourteen cyanobacterial genera comprising six families and three orders were identified and for which the most abundant filamentous genera during the summer-fall sampling season were *Planktothrix*, *Aphanizomenon* and *Limnothrix.* Potential microcystin producers *Microcystis, Planktothrix*, *Aphanizomenon* and *Dolichospermum* were also present and during the sampling period *Microcystis* was recorded at both stations on all dates, however, its relative abundance was below 10 % throughout the study period. The composition and abundance of filamentous cyanobacteria were observed to be positively statistically correlated to water quality environmental parameters dissolved nitrates (NO_3/NO_2), dissolved inorganic carbon (DIC), and conductivity. Redundancy analysis (RDA) found that 53.35% total variance of *Aphanizomenon* was correlated to low water column NO_3/NO_2 and conductivity, and higher

water column DIC. 58.13% of the relative abundance of *Planktothrix* was correlated to high concentrations of dissolved nitrates, while 51.69% of total variance of *Limnothrix* was correlated to higher DIC and lower water column dissolved nitrate concentrations.

Information about past cyanobacterial communities was obtained from the sediment core analysis, using paleolimnological and modern molecular methods. The age of the 100.5 cm long sediment core retrieved from the deepest part of Hamilton Harbour was established to be 140 years (1869-2009), using the Constant Rate of Supply (CRS) ^{210}Pb age model. This age was not sufficient to provide information of harbour's environmental conditions, presence of the blooms, and triggers for their occurrence before European settlement in the area. Results of the HPLC analysis of fossil pigments indicated that the dominant members of the algal community have not changed over the 140 years and that cyanobacteria were regular members of the phytoplankton community. The composition of the major chlorophyll pigments indicated high presence of Chlorophyta and Bacillariophyta in the harbour at all times. The main algal groups identified on the basis of marker pigments presence, besides the Chlorophyta and Bacillariophyta, were the Dinophyta and the Cryptophyta. The presence of a scytonemin derivative, compound B, indicated that cyanobacterial blooms were occurring in past, before the first officially recorded blooms in the 1960s. Cyanobacterial pigments presence indicated that Cyanobacteria have been a regular but not dominant feature of Hamilton Harbour phytoplankton in the past. To our knowledge, this study is the first one examining fossil pigments from Hamilton Harbour.

Results of the PCR-DGGE molecular analysis of 16S rRNA-V3 gene fragments from sedimentary DNA revealed the presence of thirteen cyanobacterial genotypes. The temporal change in the cyanobacterial community composition was indicated by the increasing number of species over time, from the oldest to the most recent sediment layers. The deepest sediment strata showed the lowest number (two bands) and intensity of bands. The most recent sediment layer had the greatest numbers (11) and intensity of bands. This increased diversity indicated changing environmental conditions in the harbour, primarily nutrient pollution and worsening water quality. Results of the PCR-DGGE molecular analysis of *mcy*E-AMT gene fragments showed that *Microcystis aeruginosa* and *Planktothrix rubescens* were two microcystin producers present in Hamilton Harbour over the last 80 years. The persistent presence and resilience of these two genera indicated a more serious and longer-term issue of toxic blooms than previously recognized.

Historical records show that noticeable anthropogenic impact on Lake Ontario environment has been measurable since the 1780s, the first dramatic impact on the Lake Ontario watershed was evident from the mid1880s, the earliest evidence of eutrophication in the lake occurred between 1820 and 1850, while human induced environmental changes in Hamilton Harbour date back *ca.* 350 years. In the 1960s, cyanobacterial blooms were first officially recognized in the harbour and the lower Great Lakes. The present research is the first report of the *mcyE* module and AMT domain of microcystin genes being amplified from sediment of North American lakes, and showed that toxic Cyanobacterial have been regular members of Hamilton Harbour phytoplankton community for almost a century.

This research considerably deepened the knowledge of the past toxic cyanobacterial blooms in Hamilton Harbour and their possible causes. It also showed that in the absence of historical records, both the PCR-DGGE method and the *mcy*E-AMT gene may be used for reconstruction of the past toxic blooms not only in the Laurentian Great Lakes, but also in other aquatic regions of the world impacted by toxic cyanobacterial blooms. Also, it demonstrated the utility of the combined molecular and paleolimnological analyses, which might become a useful tool in the determination of the bloom causes factors and in the mitigation of the future production of toxic blooms.

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Table of Contents

List of Figures

List of Tables

.

Chapter 1

General Introduction

1.1 Lake Ontario and anthropogenic impacts on the lake

1.1.1. Lake Ontario

The Laurentian Great Lakes comprise the world's largest freshwater ecosystem. It consists of five large lakes: Superior, Michigan, Huron, Erie and Ontario. Due to its geographical position, the first of the Great Lakes influenced by the activities of European settlers in North America was Lake Ontario (Fig. 1.1) (Stoermer *et al.*, 1985). According to Schelske (1991), paleolimnological records of nutrient loading, and biological, trophic and geochemical responses to increased nutrient loads, show four stages of nutrient enrichment. The first stage, the early 1800s, was before European settlement (pristine phosphorus loads). After settlement, *circa* 1850, the second period began, with moderate phosphorous increase; the third phase spanned thirty years (1940-1970) when an exponential increase from urban sources was recorded. Beginning in the mid-1970s the final phase was distinguished by decreased phosphorus loading, due to phosphorus reduction strategies. Furthermore, a paleolimnological analysis of the Bay of Quinte sediment core provided confirmation of nutrient enrichment prior to 1700, during the period of French settlement (1669-1784) (Schelske, 1991).

Figure 1.1. Map of Lake Ontario.

Currently, over 8 million people live around and within the watershed of Lake Ontario and depend on the lake for recreational activities and drinking water (Fuller *et al.*, 1995). The primary inlet to Lake Ontario is the Niagara River (Fig.1.1). This river is also the main outlet for Lake Erie and supplies approximately 80% of the water entering the lake. The major outflow (93%) for Lake Ontario is via the St. Lawrence River that leads to the Atlantic Ocean (Fuller *et al.*, 1995). Hence, Lake Ontario is impacted not only by the surrounding human activities, but also largely influenced by material loadings and human activities from the upper lakes including Lakes Superior, Michigan, Huron, and Erie (Stoermer *et al.*, 1985). Lake Ontario water levels are also greatly impacted by the inflow from Lake Erie, which is in turn mainly affected by the upper Great Lakes since within the Lake Erie basin precipitation gains are balanced by evaporation losses. Because Lake Ontario's surface area is only 7.8% of the Great Lakes total area, the effect of the upper Great Lakes loadings are even more pronounced (Sly, 1991).

1.1.2. History of anthropogenic impacts on Lake Ontario

Human activities and increased population growth around the Laurentian Great Lakes have greatly increased the environmental impacts on these basins. These influences have been especially harsh in Lake Ontario: beginning as early as 1780 it was the first lake reached by Europeans (Sly, 1991). The presence of the Europeans resulted in major water quality and biological community changes due to the cumulative effects of a number of stressors. These stressors were forest clearance (Christie, 1974), agricultural expansion (Yang *et al.*, 1993), intensive fishing (Christie, 1974; Fuller *et al.* 1995)), industrial/urban development (Rodgers, 1989; Fuller *et al.* 1995; Crossett *et al.* 2009), management of lake

level (Sly, 1991), introductions of nonnative species (Christie, 1974; Duggan *et al.*, 2003), contaminants inputs (nutrients and toxins), climatic variation and microclimate modifications and their interactions (Sly, 1991).

Before European arrival, numerous semi-permanent Aboriginal villages existed south of the lake and a small number existed along the north shore. Yet their presence had negligible impacts on the lake (Sly, 1991). However, beginning in the 1670s there was an increasing population of European explorers, traders and missionaries and the establishment of fortified posts. As a result, noticeable anthropogenic impacts on the lake's environment have been measurable since the 1780s (Sly, 1991). These early settlements were basically autonomous with agriculture and trading as the dominant activities. It was the lumber industry that caused the first significant settlement growth and the necessary shift from sustenance to commercial agriculture occurred in the 1820s (Sly, 1991). Further development such as canal construction, especially in New York State (mid-1820s) and the building of railroads (mid-1840s) directed population growth in important commercial and industrial areas.

1.1.2.1. Impact of deforestation

The first dramatic impact on the Lake Ontario watershed, evident from the mid 1880s, was from watershed deforestation. This deforestation and its subsequent soil erosion began with the earliest settlers and occurred for agricultural purposes (Schelske, 1991). The primary effects of this deforestation were faster snowpack melt in the spring, increased runoff levels, and more prominent and more extreme discharge of nutrients; the loss of forest canopy increased summer surface water temperatures and changed ratios of spring heating

and fall cooling. This, in turn, altered aquatic plant and related fish communities. These changes due to deforestation resulted in extremely high sediment accumulation rates (Sly, 1991). The increase in sediment rates and run-off from terrestrial sources directly impacted the total dissolved solids concentrations in such a manner, that Lake Ontario to date has the greatest concentrations of total dissolved solids of all the Great Lakes. Lake Ontario also has the highest concentrations of chlorine, sodium, potassium, and calcium (Sly, 1991), which have been rising since approximately 1910 (Beeton, 1965, 1969). Before the Great Lakes Water Quality Agreement in 1972, the general increase of ion concentrations was approximately 4% per decade (International Lake Ontario – St Lawrence River Water Pollution Board, 1969), but since this agreement was implemented a notable decrease in this rate has been observed (Sly, 1991). Currently, most trace elements (Pb, Cd, Ni, Zn, Cu, Cr, Fe, Mn) show extensive spatio-seasonal variations with values mainly highest at the lake's west end and lowest in the centre (Chau *et al.*, 1970).

1.1.2.2. Impact of nutrient loading and toxic contaminants

The earliest evidence for eutrophication in Lake Ontario occurred between 1820 and 1850 as a result of nutrient enrichment consequent to the agricultural expansion of early settlement and forest clearance (Schelske *et al.*, 1983). One of the most obvious effects of increased nutrient inputs was a change in productivity, *i.e.* increased phytoplankton biomass that may have caused algal blooms followed by increased sedimentation of organic matter (Schelske *et al.*, 1983). The increased productivity was accompanied by changes in species composition and diversity of the phytoplankton community (Skei *et al.*, 2000). Besides eutrophication, Lake Ontario's ecosystem was exposed to persistent pollutants like PCBs

(polychlorinated biphenyls), DDT (dichlorodiphenyltrichloroethane), PAHs (poly-aromatic hydrocarbons), dioxins, and toxic trace metals (Poulton, 1987; Murphy *et al.*, 1990). Despite concerted efforts to reduce primary inputs of these contaminants, secondary sources like contaminated sediments, dump sites, contaminated land and the environment modified by humans supplied the aquatic environment with contaminants. Additionally, volatile contaminants which can be atmospherically transported long distances from the sources may also be a source of contaminants to the aquatic environment (Skei *et al.*, 2000). The cultural eutrophication consequences for Lake Ontario were the total system biomass increase, changes in pelagic and benthic species, and the occurrence of more pollution-tolerant species (Allan and Zarull, 1995). The reduced total number of sensitive species resulted in a reduction of trophic levels and less complex food webs (Allan and Zarull, 1995).

1.1.2.3. Impact of introduced and invasive species

Exacerbating the impact of nutrient loading and toxic contaminant**s** is the more than 170 years of extensive introduction of non native species in the Great Lakes (Mills *et al.*, 1993; Ricciardi and Rasmussen, 1998; Ricciardi, 2001; Grigorovich *et al.*, 2003) . This 170 year period has resulted in the cumulative number of approximately 170 non-indigenous species throughout the Great lakes (Duggan *et al.*, 2003). During the 1870s the rate of invasion began to increase and the increase intensified in the 1950s after the St. Lawrence Seaway opening (Duggan *et al.*, 2003). At the present, approximately 60 non-indigenous species are established in Lake Ontario, including fish (15 species), invertebrates (19 species) and algae (14 species) (Duggan *et al.*, 2003). These species are endangering the lake's biological integrity (Sala *et al.*, 2000), as they may disturb the naturally established

ecosystem balance and interact with other ecosystem stressors (toxic contaminants, climate change), possibly increasing their negative effects (e.g. Mazak, *et al.* 1997; Stachowicz *et al.*, 2002).

1.2 Study Area: Hamilton Harbour

Hamilton Harbour (also called Burlington Bay) is a triangular-shaped bay located at the western end of Lake Ontario (43^{o} 17' 20" N - 79^{o} 50' 2" W) (Fig. 1.2). It has surface area of 21.5 km^{2}, is 8 km long (east-west) and 5 km wide (north-south). The maximum depth is about 23 meters in the centre of the bay with an additional deep area at the east end and a 10 m deep channel connects it to Lake Ontario.

Repeated glaciations formed the present shape of Lake Ontario, the last of which ended some 11000 years ago (Sly and Prior, 1984; Anderson and Lewis, 1985; Muller and Prest, 1985; Sly, 1986).

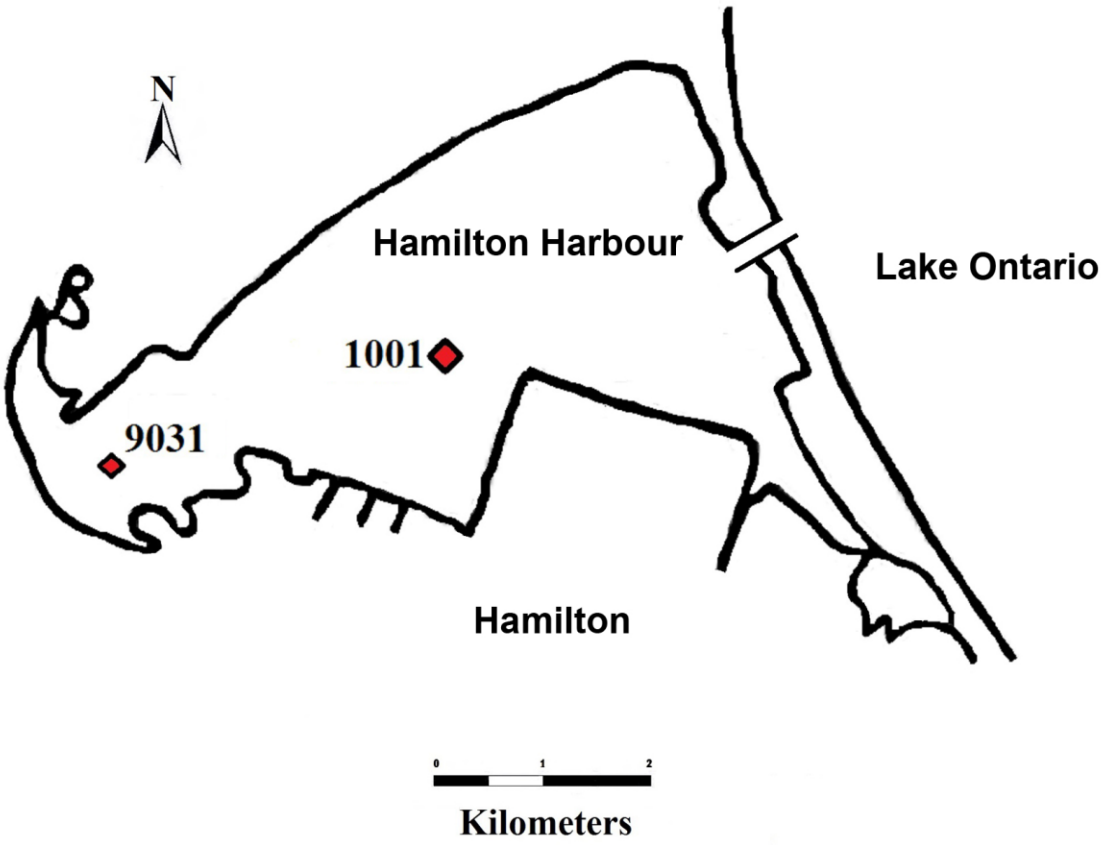

Figure 1.2. Map of Hamilton Harbour with sampling sites 1001 (Central) and 9031 (Western)**.**

According to Duthie *et al.* (1996), in the past 8300 years the Hamilton Harbour site has changed from a shallow water body separated from the lake, to a deep embayment of Lake Ontario. From 8300 BP-7000 BP, Hamilton Harbour was a moderately alkaline mesotrophic pond, warmer than today, and most likely with substantial marginal wetland (Duthie *et al.*, 1996). An initial transitory connection with Lake Ontario was probably present in *ca.* 7000 BP, while a permanent connection was formed *ca*. 6200 BP, causing a decline in water temperatures and inferred trophic levels. In the period between 3200 BP to 280 BP, Hamilton Harbour was a moderately alkaline embayment, oligotrophic to mesotrophic, and comparatively colder than present (Duthie *et al.*, 1996).

Present-day interaction between Hamilton Harbour and Lake Ontario is via a ship canal through the Burlington Bar (Wolfe *et al.*, 2000); the ship canal is 820 m long, 107 m wide and 9.5 m deep (Nriagu *et al.*, 1983). A consequence of the canal excavation is a 30-100% greater mixing level between lake and harbour waters compared to the natural hydrologic state (Wolfe *et al.*, 2000) and a lake inflow as big as 72–87% of the harbour's daily water budget (Versteeg *et al.*, 1995). Both of these physical processes have a major influence on the dilution and dispersion of contaminants within the Harbour.

The result of the past century of urbanization has been the direct discharge of municipal and industrial untreated sewage and industrial effluents from Hamilton and Burlington cities into the harbour (Nriagu *et al.*, 1983; Wolfe *et al.*, 2000). Also, heavy metals (Nriagu *et al.*, 1983; Poulton, 1987; Poulton *et al.*, 1988), nutrients (MOE, 1985) and organic and bacterial contaminants (IJC, 1985; Rodgers, 1989) have also been discharged. Because of this, Hamilton Harbour is one of the most polluted sites in the Laurentian Great

Lakes (Wolfe *et al.*, 2000) as was identified as such by the Water Quality Board of the International Joint Commission in 1985 (IJC, 1985). With more than a hundred years of industrial activities around the Hamilton Harbour and contaminated water and sediments, this area is identified as a Great Lakes Area of Concern (AOC) in need of cleanup and restoration (Taylor and Blokpoel, 1996).

Since Hamilton Harbour water quality greatly impacts the water quality of Lake Ontario at the western end of the basin, this area is also of particular concern (Duthie *et al.*, 1996). Before the construction of the Burlington Ship Canal, Hamilton Harbour was a natural embayment (called Makassa lake) with limited water exchange (Forde, 1979). The consequent interaction between waters of the harbour and the lake reduced the harbour's residence time by 60%, diluting concentrations of pollutants and oxygenating its hypolimnetic waters (Barica, 1989). A major change in the water budget of Hamilton Harbour was due to large amounts of oligotrophic Lake Ontario water moving into the harbour and mixing with its more stable and presumably mesotrophic water (Barica, 1989). This water exchange process became of crucial importance for improving water quality of the harbour; at the same time, the more polluted harbour water discharged to the lake adversely affects water quality of western Lake Ontario to about 5 km offshore (MOE, 1986).

As one of the 42 areas of concern identified by the International Joint Commission within the Great Lakes Basin (Halfon, 1996), Hamilton Harbour is the focus of a multidisciplinary environmental investigation and Remedial Action Plan (RAP) (Rogers, 1989). Due to past uncontrolled loadings of nutrients and toxic contaminants, beneficial uses of the harbour have been impaired despite reduction in pollutant loads (Mayer and Johnson,

1994). The sewage treatment plants of Hamilton and Burlington cities, as well as the steel industries located on the south shore are the main sources of nutrients and toxic contaminants (Mayer and Johnson, 1994). In 1989 the RAP for rehabilitation of the harbour was developed and implemented and it continues today. Nonetheless, in order for this plan to succeed Mayer and Johnson (1994) have stated that a better understanding of past pollution trends is necessary.

Information on the past activities in the harbour can be obtained from benthic sediments (Duthie *et al.*, 1996). In this situation paleoecological investigations are especially important since the long-term history can be obtained by determining predisturbance limnological conditions (Smol, 2008), as well as by defining long-term trends, and evaluating the significance of environmental stressors (Smol, 2008). Many lake management issues have been studied by the paleolimnological method. By using long-term biological, geological and chemical indicators accumulated in lake sediments, past environmental conditions can be inferred (Smol, 2008) and the state of the present ecosystem can be resolved (Duthie *et al.*, 1996).

1.3 Cores as chronological records of past and present communities

1.3.1. Lake Ontario sediments

Since lakes, climate and lake organisms are directly and indirectly connected, paleoclimate proxies from lake sedimentary records are an important tool in the investigation of past lake environments (Smol *et al.* 1991; Battarbee, 2000) as well as the history of the

airshed and watershed (Smol *et al.* 1991). This information can provide data on the long-term natural dynamics and the impacts of human activities on the lake (Itkonen *et al.*, 1999). Smaller basins tend to be more susceptible to local or short-term changes than larger ones (Smol *et al.*, 1991).

The lake surface water physical properties are directly altered by climate effects that are most likely as fast in large lakes as in small ones (Smol *et al.*, 1991). They correspond to the lake ice breakup timing (Weyhenmeyer, 2008) and can also involve alternations in biological variables, such as development of spring phytoplankton (Weyhenmeyer *et al.*, 1999; Gerten and Adrian, 2000; Weyhenmeyer, 2001). However, climate effects that alter physico-chemical conditions in the catchment considerably differ in large and small lakes (Weyhenmeyer, 2008). Due to a strong dilution effect, small changes in the catchments do not impact concentrations in lakes with large water volumes, while greater changes in the catchments have an effect on concentrations even in large lakes, but with a substantial time gap (Weyhenmeyer, 2008). These effects, and other influences over time, can be detected in water sediments.

Aquatic sediments are defined as deposits of solid inorganic and organic material that move under the influence of gravity through a water column (Wheeler Alm and Stahl, 2004). Sediment composition can be very complex and variable due to its inorganic and organic content, biomass, mineralogy, environment etc. Sediment mineralogy influences both the organic matter and the biomass of the sediment (Wheeler Alm and Stahl, 2004).

Mineralogically, most lake sediments are dominated by the non-clay silicates, quartz and feldspar; dominant carbonate minerals are calcite and dolomite, with the significant clay

minerals being steatite and illite. In the oxygenated environments of most lakes, oxide coatings on sediments are common (Jones and Bowser, 1978). The sediment inorganic content is important for DNA yield since nucleic acids can bind to sediment particles (especially clay minerals) quickly and in large amounts. Sediments with high silicate mineral content can absorb from 16 µg (Greaves and Wilson, 1969) to more than 200 µg of DNA per gram of sediment (Ogram *et al.*, 1988). DNA also binds to sand but in much lower quantity (1.8 µg /g) (Aardema *et al.*, 1983).

According to Thomas *et al.*, (1972) the three major sediment units in the surface sediments of Lake Ontario are (1) glacial till and bedrock; (2) glaciolacustrine clay; (3) postglacial mud, while sand and silt represent minor units in the Ontario surficial sediments. The surface sediments of Lake Ontario contain variable amounts of quartz, feldspar, clay minerals, organic carbon and calcite. Quartz and feldspar contents are greatest in the coarser inshore sediments while clay minerals and organic carbon are greatest in the finer offshore sediments. Carbonate is generally low throughout the lake and illite is the dominant clay mineral with lesser amounts of chlorite and kaolinite (Thomas *et al.*, 1972).

Organic matter content in sediments can range from less than 0.1% in oceanic red clays to almost 100% in certain humic and algal coals (Hue, 1988). This variability depends on many factors, both biotic and abiotic. Primarily, lakes will have rich organic sediments because of high levels of primary productivity and terrestrial inputs. Approximately 50-80% of the organic matter in sediments is contained in heterogeneous organic substances - sedimentary humic substances (Rashid, 1985), which are one of the major contaminants found in DNA extracts from aquatic sediments. Since humic substances often interfere with

enzymatic activities, they can inhibit restriction enzyme digestion and PCR of DNA extracted from sediments (Rashid, 1985; Stefan *et al.*, 1988; Rochelle *et al.*, 1992; Tsai and Olson, 1992) and are very difficult to remove from DNA extracts because they are ubiquitous and vary in molecular size (Wheeler Alm and Stahl, 2004). Humic substances and lipids are present in larger proportions than other organic matter components and increase with depth in sediments of Lakes Ontario, Erie, and Huron (Kemp and Johnston, 1979). Biomass present in the initial sediment varies with by location and with depth within the sediment which influences the yield of extracted DNA, and results in deeper sediments giving a lower DNA yield (Wheeler Alm and Stahl, 2004).

Assessments based on paleolimnology have been applied in many studies worldwide. Paleolimnological investigations have been used in eutrophication (Hall *et al.*, 1999; Finsinger *et al.*, 2006; Weckström, 2006), urban development (Meriläinen *et al.*, 2003), and land use (Augustinus *et al.*, 2006; Davis *et al.*, 2006). Acidification (Ginn *et al.*, 2007a, b), impacts of mining (Salonen *et al.*, 2006), transport of contaminants (Donahue *et al.*, 2006), and climate change (Laird *et al.*, 2003; Moos *et al.*, 2005; Harris *et al.*, 2006; Westover *et al.*, 2006) have also been investigated by this method.

The Great Lakes and Hamilton Harbour are subject to intensive paleolimnological research as well. For instance, proxy data from sediment cores and biogenic silica analysis i.e. diatom production peaks (Schleske *et al.*, 1983) have shown that an increase in nutrients related to early settlement and forest clearance caused early eutrophication in the lower Great Lakes. Duthie *et al.*, (1996) used proxy evidence from sedimentary diatoms, pollen, and oxygen and carbon isotope analyses in a radiometrically dated sediment core (HH26comp) to

demonstrate that Hamilton Harbour has changed from a shallow, unconnected water body, to a deep embayment of Lake Ontario over the past 8300 years. Yang *et al.* (1993) used short sediment core and siliceous microfossil analyses to demonstrate human induced environmental changes in Hamilton Harbour over the past 345 years. Sources of genotoxic contamination in Hamilton Harbour were detected from chemical and biological profiles of bottom and suspended sediment samples (Marvin *et al.*, 1999), while analysis of bottom sediments by Brassard *et al.* (1997) showed elevated metal and organic contaminants concentrations due to industrial and municipal discharge since 1920s.

All of the taxa used as ecological and paleolimnological indicators tend to have specific environmental optima and tolerances, and can therefore be used in paleoenvironmental reconstructions (Kling, 1998). The most common algal remains that can be recovered from sediments and used in the interpretation of environmental changes are siliceous microfossils (diatoms and chrysophyte cysts). Less frequently, akinetes of cyanobacterial algae *Aphanizomenon* and *Anabaena/Dolichospermum* and remains of green algae can be observed. Analysis of diatoms (silicate algae, Phylum Bacillariophyta) is a principal tool in environmental reconstruction of the acidification of surface waters, eutrophication and climate change (Hall and Smol, 1995). Diatoms grow in almost all aquatic environments throughout the world and are often found in considerable abundance in the benthos and as plankton in the water column, forming the principal communities of the sediment record (Battarbee, 2002). Diatom ecology and distribution and their change in time and space are indicators of environmental change and are used to identify diatom-environment relationships in paleolimnology.

Useful indicators of paleolimnological environments are nonsiliceous algal remains, especially in the absence of diatom microfossils or under eutrophic conditions (Wolfe *et al.*, 1994). Since vegetative cells of cyanobacteria preserve poorly in lake sediments, they have not been extensively studied (Räsänen *et al.*, 2006). Certain cyanobacterial species produce akinetes, which are resting spores, filled with storage products, and as such are significant for cyanobacterial long-term survival. Stored starch and lipid droplets are used as an energy source while cyanophycin granules are used as a source of nitrogen (Castenholz and Waterbury, 1989). Compared to vegetative cells, akinetes are larger with thicker cell walls, which make them resistant to decomposition. The density of akinetes is higher than that of water and they tend to separate from trichomes and deposit in the sediments (Hori *et al.*, 2003) and highly organic sediments often contain remains of cyanobacteria (Korde, 1996). Since deeper sediments are unaffected by wave action, akinetes that have settled there are protected from resuspension (Livingstone and Reynolds, 1981), and can consequently be used as a paleolimnological proxy (Cronberg, 1986; van Geel *et al.*, 1994; Kling, 1998; van Geel, 2001; Eilers *et al.*, 2004; Bradbury *et al.*, 2004, Räsänen *et al.*, 2006).

The presence of akinete-forming cyanobacteria in a lake is reliably recorded by the appearance of akinetes in the sediments (Wolfe *et al.*, 1994). *Anabaena/Dolichospermum* and *Aphanizomenon* are the main nitrogen-fixing akinete producers whose remnants can be identified from sediments. In the study of Lake Winnipeg's past and recent plankton using algal fossil remains (Kling, 1998) the progressive increase in abundance of planktonic cyanobacterial remnants in sediments was connected with increase in phosphorous levels, summer temperatures and summer nitrogen limitation in the lake. Increased occurrence of

nitrogen fixing species near the top of the core indicated increased anthropogenic eutrophication (Kling, 1998).

1.4 Toxic cyanobacterial blooms

1.4.1. Causes of blooms

Excessive anthropogenic inputs of nutrients (from discharge of municipal wastewater and agricultural land runoff) have many adverse effects on the biology, chemistry, and physical characteristics of lakes and reservoirs. Planktonic shifts towards dominance of the blue-green algae (Cyanobacteria) are common characteristics of eutrophic lakes. Some Cyanobacteria produce toxins for instance *Microcystis, Anabaena/Dolichospermum, Aphanizomenon, Gloeotrichia, Lyngbya, Oscillatoria* (Carmichael, 1991). Recently, the genus *Anabaena* has been taxonomically revised and separated into two genera: *Anabaena* (benthic-type cluster) and *Dolichospermum* Ralfs ex Bornet et Flahault (planktonic-type cluster) based on molecular phylogeny (*Wacklin et al.*, 2009). In this research, the generic name *Dolichospermum* will be used to refer to the organisms that we know are planktonic.

Although Cyanobacteria occur in lakes over the whole year, it is usually only in late summer and early autumn that they reach bloom proportions (Lee, 2008). One of the most significant events diminishing the usefulness of eutrophicated waters are cyanobacterial blooms (Räsänen *et al.*, 2006). Surface blooms, including toxic ones, are a natural phenomenon as they have been documented since ancient times (McGowan & Britton, 1999), yet Cyanobacteria typically dominate in nutrient-rich waters and anthropogenic

eutrophication is marked by recurrent blooms (Reynolds, 1984; Whitton, 1992). Other planktonic algae that can bloom in nutrient-enriched waters belong to other phyla including Chlorophyta, Euglenophyta, Bacillariophyta, Chrysophyta and Dinophyta. These algal blooms, along with population shifts and dominance, indicate deterioration in water quality.

Algal blooms in freshwater reservoirs, lakes, and streams are of great concern since their occurrence has ecological, aesthetic, and human health impacts. Cyanobacterial blooms occur under conditions that include high nutrient concentrations (usually phosphorus and nitrogen compounds), water temperature above 25°C, low light intensity in water, low N:P ratio, high pH, low CO_2 and low grazing rate by zooplankton (McGowan & Britton, 1999; Mankiewicz *et al.*, 2003; Zurawell *et al.*, 2005). According to Hallegraeff (1993) harmful algal blooms are increasing due to anthropogenic influences, while Sellner *et al.* (2003) point out that climate variability may be an equally critical factor that contributes to this problem.

Many of the cyanobacterial bloom-forming species have gas vesicles and/or specialized nitrogen-fixing heterocysts, which may be the reason for the higher competitiveness of Cyanobacteria compared to other bloom-forming species (Oliver and Ganf, 2000). Cells and colonies with gas vesicles are buoyant in the water column and can optimize their vertical position (Mankiewicz *et al.*, 2003). In some Cyanobacteria, buoyancy prevents sedimentation in water and enables subsequent formation of the surface scum (Mankiewicz *et al.*, 2003). Also, a competitive cyanobacterial advantage over eukaryotic microalgae results from higher phosphorus and nitrogen affinity, considerable capacity for phosphorus storage, and low losses to grazing by zooplankton resulting from the formation of large colonies (Reynolds, 1987).

Although algal blooms are often associated with eutrophication, not all of them result from anthropogenic influence on lakes. Some blooms may occur in pristine conditions, though this ecological fact has largely been ignored (McGowan and Britton, 1999). Evidence given by McGowan and Britton (1999) for Whitemere lake in the UK can be applied to similar postglacial kettle hole lakes in North America and Eurasia that are thermally stratified, with long retention times and which are groundwater fed. Analyses of fossil carotenoids from dated sediment cores have shown that cyanobacterial blooms may be a normal characteristic of these lakes (McGowan and Britton, 1999).

1.4.2. Importance of blooms

Blooms can cause drinking water taste and odor problems (Downing *et al.*, 2001), while toxic blooms can be serious threats to animals and humans. Under certain conditions, Cyanobacteria and other freshwater algae (diatoms, synurophytes, chlorophytes, euglenoids, and dinoflagellates) are able to cause various nuisance effects, such as excessive buildup of foams, scum, unpleasant smell and water discoloration. Thick scum formed on the water surface can accumulate downwind but can also be blown close to shore (Chorus and Bartram, 1999) and in this way humans and animals can come into contact with this material. Repeated or severe blooms can cause depletion of dissolved oxygen because of the decay of the dead algae (Chorus and Bartram, 1999). In highly eutrophic lakes, summer anoxia and fish kills are the consequences of this mass algal development (Zaccaroni and Scaravelli, 2008). Algal blooms also affect zooplankton through disruption in the trophic food chain caused by changes in planktonic populations due to accumulation of toxins (Zaccaroni and Scaravelli, 2008). Upon exposure to toxic Cyanobacteria or their toxins, zooplankton growth

has been directly inhibited (Zaccaroni and Scaravelli, 2008).

1.4.3. Cyanobacterial blooms and toxins

Certain species in harmful algal blooms can impact other organisms through synthesis of toxins (Sellner *et al.*, 2003). The most damaging effects of harmful algal blooms include fish, bird, and mammal mortalities, human health problems, and a loss of coastal resources. (Sellner *et al.*, 2003). The major cyanobacterial toxin producers include genera *Microcystis, Dolichospermum/Anabaena, Aphanizomenon, Gloeotrichia, Lyngbya, Cylindrospermopsis, Nodularia, Oscillatoria and Planktothrix*. No clear correlation exists between the composition and density of a specific bloom and the toxins concentration in the cyanobacterial cells or in the surrounding water, since not all species or strains are toxic (Elder *et al.*, 1993).

Cyanobacterial blooms can be unpleasant both visually and due to the release of volatile organic compounds. These secondary metabolites, such as malodorous metabolites geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and MIB (2-methylisoborneol) (Watson *et al.*, 2000), can cause unpleasant tastes. Also, some cyanobacterial species produce certain metabolites that create a more serious problem (Carmichael, 1994). Due to their harmful effects and toxicity on higher organisms, these molecules are referred to as cyanotoxins and include several classes of substances (Codd, 1995; Chorus and Bartram, 1999). According to their chemical structures cyanobacterial toxins can be classified as cyclic peptides (microcystins and nodularins), alkaloids (anatoxin, saxitoxin, cylindrospermopsin, aplysiatoxin, lyngbyatoxin) and lipopolysaccharides. These toxins are usually studied in relation to their toxicity to animals (Kaebernick and Neilan, 2001) and are referred to as

hepatotoxins (affect the liver), neurotoxins (affect the nervous system), cytotoxins (affect cellular structure), dermatotoxins (affect the skin) and irritant toxins (Fitzgerald, 2001; Mankiewicz *et al.*, 2003). Toxins form at all stages of organisms' growth and can be either membrane that are bound or exist freely inside the cells. Cyanotoxins are usually contained within the cyanobacterial cells, but substantial amounts are released through passive flow in the aquatic environment upon the cell death, during cell lysis (Sivonen and Jones, 1999).

One of the features of toxic Cyanobacteria is that they can produce different toxins within the same genus or the same toxins among different genera. Hence, the ability to produce toxins does not appear to be monophyletic when compared to various gene phylogenies. Another characteristic is the variability in the level of toxicity between different strains of the same species (Dow and Swoboda, 2000). Also, cyanotoxins may be injurious only at higher concentrations (Codd, 1995). The ability of planktonic genera to form scums and benthic genera to form mats can result in high local concentrations of cyanobacterial biomass of toxins per liter. If animals come into contact with this toxic material adverse and even fatal effects can occur (Codd *et al.*, 2005).

1.4.4. Cyanobacterial toxin microcystin (*myc*)

Hepatotoxic microcystins are of greatest concern since chronic low-level exposures to them (as low as 1 μg/l) can produce adverse effects on human health (Chorus and Bartram, 1999). These toxins are produced by strains of distantly related cyanobacterial genera including *Microcystis, Anabaena/Dolichospermum, Planktothrix, Oscillatoria,* and more rarely *Nostoc, Anabaenopsis,* and *Hapalosiphon* (Carmichael, 1992; Skulberg *et al.*, 1993; Sivonen and Jones, 1999). Microcystins accumulate in vertebrate hepatocytes due to active

transport by a highly expressed unspecific organic anion transporter (bile acid carrier transport system) and inhibit eukaryotic protein phosphatases type 1 and type 2A (PP-1 and PP2A) (Goldberg *et al.*, 1995; MacKintosh *et al.*, 1990). As a result, excessive phosphorylation of cytoskeletal elements occur (Carmichael, 1992). The primary reaction of hepatocytes to microcystin exposure starts with cytoskeleton function disruption, causing swelling of the plasma membrane, oxidative degradation of lipids, DNA damage, programmed and premature death of cells and tissues and ultimately death by extensive bleeding into the liver (Miura *et al.*, 1989; MacKintosh *et al.*, 1990; Hermansky and Stohs, 1991; Kondo *et al.*, 1992; Thompson and Pace, 1992; Wickstrom *et al.*, 1995; Rao and Bhattacharya, 1996; Fischer *et al.*, 2000). Vertebrates (including humans) die mostly because of consequences of severe liver damage.

Microcystins are cyclic heptapeptides (Fig. 1.3) consisting of seven amino acids and share the common cyclo(-Adda-D-Glu-Mdha-D-Ala-L-X-D-MeAsp-L-Z) structure, where X and Z represent variable L-amino acids, Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10 phenyl-4,6-decadienoic acid, D-MeAsp is 3-methylaspartic acid, and Mdha is *N* -methyldehydroalanine (Botes *et al.*, 1985). To date, more than 80 microcystin isoforms have been reported (Hotto *et al.*, 2007) primarily differing in L-amino acids together with alterations to side chains (Sivonen and Jones, 1999). Microcystin-LR (MC-LR) is the most frequently found and the most studied variant, with the variable amino acids leucine (L) and arginine (R). Other frequently found variants are MC-RR, MC-YR and MC-LA (de Figueiredo *et al.*, 2004). Each microcystin variant possesses different degrees of toxicity; toxic and nontoxic cyanobacterial populations can coexist in a single ecosystem,

indistinguishable by microscopy (Hotto *et al.*, 2007). That is why the toxicity or non toxicity of blooms in the same body of water can vary from year to year. Also, certain species will exhibit high or low toxicity under different laboratory conditions (such as temperature, light intensity, nutrients and trace metals) (Kaebernick and Neilan, 2001).

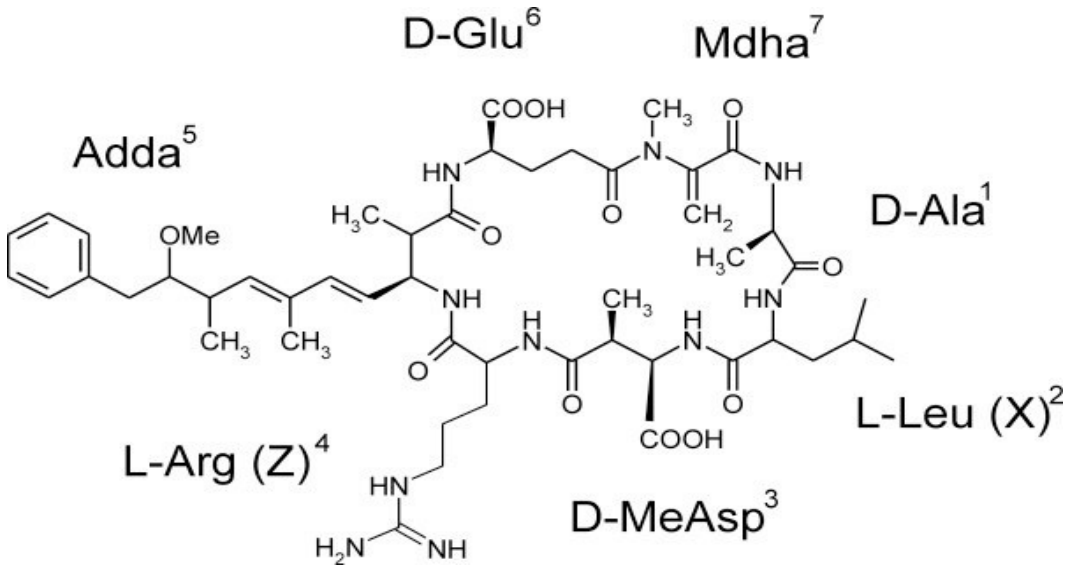

Figure 1.3. Molecular structure of microcystin-LR (after Fewer *et al.*, 2007).

1.4.5. Microcystin genes and biosynthesis

Microcystins are synthesized nonribosomally by a multifunctional enzyme complex and their synthesis is an energy (ATP)-dependent process (Bickel and Lyck, 2001). This enzymatic

complex consists of peptide synthetases, polyketide synthases (PKSs), and additional modifying enzymes. Both prokaryotes and eukaryotes have nonribosomal peptide synthetases (NRPSs), whose function is catalyzation of peptides formation by use of a thiotemplate mechanism (Nishizawa *et al.*, 1999; Nishizawa *et al.*, 2000; Tillett *et al.*, 2000).

The synthesis of the enzymatic complex is coded by the *mcy* genes cluster (Fig. 1-4) composed of two operons (*mcy* A–C and *mcy* D– J) (Kaebernick and Neilan, 2001). Although toxic and non-toxic microcystin-producing strains are morphologically indiscernible, they differ genetically. Since all toxic strains of the genera *Microcystis*, *Anabaena/Dolichospermum*, *Nostoc*, and *Planktothrix* carry microcystin synthetase (*mcy*) gene cluster (Neilan *et al.*, 1999) polymerase chain reaction (PCR) methods for its detection directly from environmental samples have been developed (Tillett *et al.*, 2001; Pan *et al.*, 2002).

The first fully sequenced complex metabolite gene cluster from *M. aeruginosa* PCC7806 (Fig. 1.4) was the microcystin biosynthesis gene cluster, *mcy*S (Tillett *et al.*, 2000). The *mcy*S genomic locus is 55 kb long and consists of 10 genes organized in two divergently transcribed operons (*mcy*A–C and *mcy*D–J), where each gene is coding for a specific enzyme involved in the synthesis, tailoring and transport of the toxin (Dittmann *et al.*, 1997; Tillett *et al.*, 2000). Also, the ORFs in the *mcy*S cluster are differently organized in *Microcystis*,

Anabaena and *Planktothrix* since in *Anabaena mcy*S cluster adheres to the NRPS pathways 'co–linearity' rule (Kleinkauf and von Dohren, 1996), while this rule does not apply to the *Microcystis* and *Plankothrix* clusters.

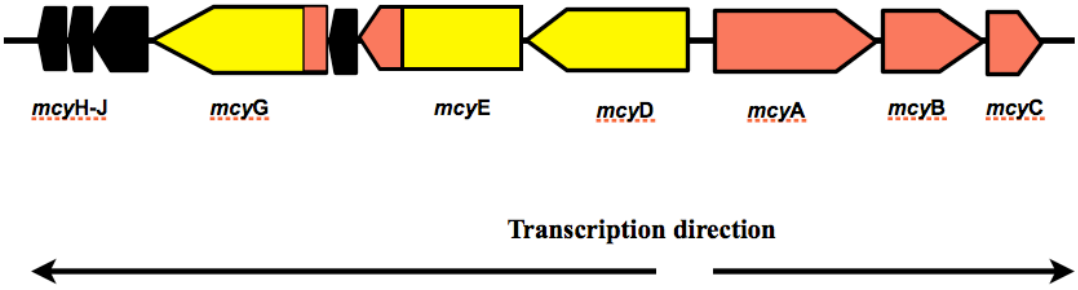

Figure 1.4. Organization of the microcystin (*mcy*) gene cluster from Microcystis aeruginosa PCC7806. Yellow: polyketide synthase encoding genes. Red: peptide synthetase encoding genes. Black: modifying enzymes encoding genes.

1.4.6. Factors that influence microcystin synthesis and ecological roles of microcystin

Laboratory studies on environmental parameters and toxin production have generally demonstrated that the highest toxin concentrations were observed under optimal cell growth conditions (Kaebernick and Neilan, 2001), possibly indicating a direct connection between microcystin production levels and cell division (Orr and Jones, 1998). Generally, lower toxin concentrations have been observed at low light intensities while increased cellular iron uptake has been observed at high light intensities; this may ultimately be responsible for higher toxin production (Utkilen and Gjolme, 1995). Low phosphorus concentrations decrease microcystin production in *Dolichospermum*/*Anabaena*, *Microcystis* and *Oscillatoria* (Sivonen and Jones, 1999). The effect of nitrogen on cyanotoxin synthesis in nitrogen-fixing and non-nitrogen-fixing strains is different: nitrogen-free medium strains that can fix nitrogen (*Dolichospermum*/*Anabaena*, *Cylindrospermopsis, Nodularia* and *Aphanizomenon*) show the highest levels of cyanotoxins (Rapala *et al.*, 1993; Rapala *et al.*, 1997; Lehtimäki *et al.*, 1997; Saker *et al.*, 1999), while at high levels of nitrogen, non-nitrogen fixing strains (*Oscillatoria* and *Microcystis*) show the highest levels of toxin (Sivonen and Jones, 1999).

Temperature affects toxin levels similarly in most cyanobacterial cultures; at temperatures between 18 °C and 25°C microcystin and nodularin concentrations were highest in *Microcystis, Dolichospermum*/*Anabaena* and *Nodularia*, while lower toxin concentrations were observed at higher or lower temperatures (Sivonen and Jones, 1999). Also, different chemical forms of produced toxin correspond to different temperatures (Rapala *et al.*, 1997) so that below 25°C, *Dolichospermum*/*Anabaena* spp. produce microcystin-LR instead of

microcystin-RR whose synthesis is favored at higher temperatures (Rapala *et al.*, 1997).

Responses of procaryotes and eucaryotes (phytoplankton and zooplankton) to cyanotoxins are various and may range from selective feeding and positive attraction to cyanobacterial cells to death by the toxins (Kaebernick and Neilan, 2001). Cyanobacteria and cyanotoxins affect population structure of zooplankton (cladocerans and rotifers), which can lead to cyanobacterial ecological success. As Cyanobacteria are not the usual source of food for zooplankton (DeMott, 1991), it feeds on phytoplankton in competition with cyanobacteria and generally selectively avoids Cyanobacteria (DeMott, 1991). The presence of contaminant heterotrophic bacteria positively affects growth of many bloom-forming Cyanobacteria. For example, a mutualistic relationship appears to exist between *Pseudomonas aeruginosa* and *Dolichospermum/ Anabaena* and they both share the fixed nitrogen (Paerl and Millie, 1996). *Microcystis aeruginosa* cells show increased cell-specific CO_2 fixation rates when associated with bacteria and protozoan grazers (Paerl and Millie, 1996). Therefore, cyanotoxins may function as beneficial hosts attractants and antagonistic microbes and grazers repellents (Paerl and Millie, 1996). An allelopathic function of microcystins has also been suggested as they inhibit growth of different algal species (Keating, 1978). Also, the possible microcystin function in inhibition of protein phosphatases against other cyanobacteria has been suggested (Shi *et al.*, 1999).

The ecological or cellular function of microcystin is still unclear. Being secondary metabolites, these peptides are not utilized in primary metabolism (Carmichael, 1992; Kurmayer and Christiansen, 2009), since microcystin-deficient strains and engineered mutants can survive without it. However, non-toxic forms can produce non-toxic substitutes

with the analogue function to toxic counterparts, directly connected to growth rates of non-toxic strains (Orr and Jones, 1998; Neilan *et al.*, 2013).

1.4.7. Microcystin producers of Hamilton Harbour

For decades, cyanobacterial harmful algal blooms (HABs) have been observed in the Great Lakes. Massive blooms of different cyanobacteria (*Dolichospermum*/*Anabaena*, *Aphanizomenon* and *Microcystis*) occurred during summer water column thermal stratification, in the 1960s and 1970s (Bierman *et al.*, 1984; Brittain *et al.*, 2000), but have declined since then due to strategies of phosphorus reduction. A reappearance of cyanobacterial harmful algal blooms has been observed in the Great Lakes since the mid-1990s, accounting for 50% or more of all blooms in the peak years in Central regions of Ontario (Ontario Ministry of Environment, 2013). In 2001 Hamilton Harbour developed a prominent cyanobacterial bloom in late August and September composed of a number of blue-green taxa dominated by several species of *Microcystis* [*M. viridis* (A.Braun) Lemmermann, *M. botrys* Teiling, and *M. wesenbergii* (Komárek) Komárek] and *Aphanizomenon* [*Aphanizomenon flos-aquae* (Linneaus) Ralfs ex Bornet et Flahault]. The bloom was toxic and microcystin concentration as high as 400 μg/l was reported in surface scums (Murphy *et al.*, 2003). In the research conducted by Allender and colleagues (Allender *et al.*, 2009) water samples collected from Hamilton Harbour in August of 2002 and 2006 were tested for presence of *mcy* genes. This research confirmed previous findings of Murphy *et al.* (2003) that *Microcystis was* the harbour's microcystin producer.

1.5 Microcystin fate in water and sediments

There are several mechanisms that determine the environmental fate of microcystins once they are released from cyanobacterial cells. As any biological molecule, microcystin is susceptible to degradation. Since microcystins are compounds stable over a range of pH and temperatures, chemical or thermal degradation is considered to be only a minor process influencing microcystin fate in aquatic ecosystems (Welker *et al.* 2001). Because the absorbance spectra of microcystins does not overlap with solar spectrum (Welker *et al.* 2001), direct photodegradation of microcystins by sunlight is insignificant (Tsuji *et al.,* 1994), but indirect microcystin photooxidation can take place via sunlight absorbing substances occurring in natural waters that act as photosensitisers, such as various pigments (Tsuji *et al.,* 1994) or humic substances (Welker & Steinberg 1999, 2000). The key process responsible for microcystin breakdown in the environment is most likely degradation by indigenous microorganisms (Jones *et al.,* 1994; Jones & Orr 1994; Rapala *et al.,* 1994). Biodegradation of microcystins (or nodularins) by heterotrophic bacteria in various media has been investigated as well, and microcystin-degrading bacteria have been isolated from natural waters or sediments (Babica, 2006).

In 1994, Rapala *et al.* demonstrated that when lysed toxic cyanobacterial laboratory strains raw extracts were exposed to natural populations of microorganisms from lakes, cyanobacterial hepatotoxins were degraded and that the toxins loss was partially due to adsorption by lake sediments (Rapala *et al.*, 1994). However, there are important variations in the degradation rate and the half-life (D1/2) of microcystin from water bodies with and without previous bloom history. In water bodies with no prior microcystin contamination, a

lag period before the start of degradation was observed (Edwards *et al.*, 2008), due to period of adaptation (Jones and Orr 1994; Christoffersen *et al.*, 2002), while in water bodies exposed to microcystins in the past, significantly faster degradation occurred (Jones *et al.*, 1994; Cousins *et al.*, 1996; Christoffersen *et al.*, 2002) with much lower half-lives of microcystin degradation than in previously unexposed water bodies (Rapala *et al.*, 1994; Lahti *et al.*, 1997; Christoffersen *et al.*, 2002).

Physico-chemical interactions between sediments and toxin molecules also affect microcystins environmental fate, while only minor adsorption of microcystins onto suspended particulate matter and sand sediments occurs (Rivasseau *et al.,* 1998; Hyenstrand *et al.,* 2003), natural clay particles, which are most likely the active binding components in soils and sediments, adsorb microcystins from aqueous phase (Morris *et al.,* 2000; Miller *et al.,* 2001; Chen *et al.*, 2006b) with efficiency of about 80% (Morris *et al.,* 2000). The adsorption mechanism is most likely hydrophilic interactions between ionizable groups (carboxylic- or amino-) of microcystins and clay particles (Tsuji *et al.,* 2001) or even microcystin chelatation with the metal ions in clays (Chen *et al.*, 2006b).

Cyanobacterial toxins (either from benthic cyanobacterial mats or from settling biomass of planktonic cyanobacteria) can be accumulated and biodegraded in sediments (Rapala *et al.,* 1994; Cousins *et al.,* 1996; Holst *et al.,* 2003). Several studies have investigated the adsorption of microcystins on natural sediments (Tsuji *et al.,* 2001; Ihle *et al.,* 2005; Babica *et al.,* 2006; Chen *et al.,* 2006a, 2006b), but the knowledge of microcystin sedimentary concentrations and their spatial or temporal variability is limited.

1.6 16S rRNA gene and identification of Cyanobacteria

Due to overlapping characteristics and phenotypic plasticity of microorganisms, traditional classification based on morphological, developmental, and nutritional similarities does not necessarily correlate well with their natural and evolutionary relationships, as has been shown by sequence comparisons (Lane *et al.*, 1985). This makes microscopic identification of Cyanobacteria to the species level very time consuming and laborious. Comparison of gene sequence data has proved to be much faster and a more precise tool for identification of taxa, particularly within the Cyanobacteria (Lyra *et al.*, 2001). Since divergence of the primary lineages of bacteria occurred early in biotic history, highly conserved genes, such as SSU rRNAs, are frequently used for reconstruction of bacterial phylogenies (Woese, 1987). 16S rRNA genes (SSU rRNA gene) analyses have clearly demonstrated that a phylogenetically reliable taxonomy does not always reliably correlate to morphological characters analysis (Giovannoni *et al.*, 1988; Wilmotte, 1994).

The 16S rRNA has been shown to be a suitable gene to study the evolution and phylogeny of Cyanobacteria in marine and freshwater environments since a broad range of relationships, from phylum to species, can be determined from its sequence analysis (Urbach *et al.*, 1992; Neilan *et al.*, 1997). By targeting cyanobacterial-specific 16S ribosomal RNA gene sequence, cyanobacterial community composition and distribution can be studied directly without the need for cultivation; the use of PCR assay detection of *Microcystis*-specific 16S rRNA gene sequence allows the study of occurrence of toxigenic *Microcystis* spp. (Ouellette and Wilhelm, 2003). In this way, an analysis of the distribution of genotypes based on the presence or absence of target genes in the samples was enabled (Ouellette and

Wilhelm, 2003).

Ribosomal RNAs and the genes that encode them are among the most highly conserved cellular molecules due to being integral elements of the ribosomal protein synthesizing apparatus. Hence rRNAs are the basic components present in all domains of life, yet contain sufficient sequence variability to determine relationships between closely related groups (Devereux and Wilkinson, 2004). Small ribosomal subunit 16S rRNA gene analysis has been used as one of the molecular tools for detection of microcystin producing cyanobacteria since the 16S rRNA gene can distinguish not only broad taxonomic groups but also strains within a species. (Neilan *et al.*, 1997; Otsuka *et al.*, 1998, 1999; Baker *et al.*, 2001; Tillet *et al.*, 2001).

1.7 *mcy* E gene and identification of Cyanobacteria

The molecular characterization of toxic and nontoxic cyanobacteria is based on the presence or absence of various gene loci that have a potential to produce toxin. Microcystin synthetases encoding genes are always present in microcystin-producing Cyanobacteria such as *Microcystis*, *Dolichospermum*/*Anabaena* and *Planktothrix,* and are constitutively expressed (Dittmann *et al.,* 1997; Tillet *et al.,* 2000; Christiansen *et al.,* 2003; Hisbergues *et al.,* 2003; Rouhiainen *et al.,* 2004; Börner and Dittmann, 2005). A genetic marker of toxigenic producers is the *mcy*E gene region (Rantala *et al.*, 2008) used for detection of potential microcystin-producers in different environmental samples (Vaitomaa *et al.,* 2003; Mbedi *et al.,* 2005; Jungblut and Neilan, 2006; Rantala *et al.,* 2006). To date, one study has used *mcy*E for molecular identification of toxic cyanobacteria from lake sediment samples

(Dadheech *et al.,* 2009).

1.8 Cyanobacterial sedimentary pigments

Pigments are organic compounds that give a characteristic color to plant tissues and are involved in the photosynthetic processes by capturing of light energy during the light-dependent reactions of photosynthesis. The primary pigment in plants, algae, and Cyanobacteria is chlorophyll *a*, which absorbs blue (maximal absorption at 665 nm) and red light (465 nm). Other light wavelengths are absorbed by carotenoids and other accessory pigments, which absorb light energy and pass it on to the chlorophyll *a* molecules.

Extensive array of anthropogenic influences on water ecosystems, such as acidification, eutrophication, fisheries and land use management and practices and climate change, are indicated by fossil pigments (Leavitt and Hodgson, 2001). The majority of lake sedimentary pigments originate from algal communities (planktonic and benthic), phototrophic bacteria and aquatic plants with a small input of undegraded terrestrial plants pigments (Leavitt, 1993). Pigment degradation occurs in the water column and in the sediments following their deposition and is often less rapid, especially under conditions of sediment anoxia (Patoine and Leavitt, 2006)

To reconstruct aquatic communities and processes from lake sediments, isolation, identification and quantification of fossil pigments and their derivatives (isomers, allomers, epimers, etc.) as well as photoprotectant compounds and other lipid-soluble pigments are used (Leavitt and Hodgson, 2001). Important indicators of total algal abundance are widely present pigments such as β-carotene, Chl *a*, pheophytin *a*. More useful in investigation of

historical changes in algal taxa (classes and divisions) or functional groups (flagellates, colonial, N_2-fixing, etc.) are unchanged carotenoids, such as alloxanthin, lutein, echinenone, fucoxanthin, and peridinin (Leavitt and Hodgson, 2001). Measuring the concentrations of the main cyanobacterial pigments, such as echinenone, zeaxanthin, and myxoxanthophyll, in sediment samples (Leavitt and Hodgson, 2001), is a useful tool for estimating the occurrence and intensity of cyanobacterial blooms (Poutanen and Nikkilä, 2001). Echinenone of zeaxanthin are also indicators of total cyanobacterial biomass (Patoine and Leavitt, 2006).

Oscillaxanthin and myxoxanthophyll are two main cyanobacterial carotenoids that increase in lake sediments during eutrophication. Family Oscillatoriaceae is usually the first to dominate when eutrophic conditions develop; presence of oscillaxanthin, observed only in this group, can indicate the onset of trophic shifts (Swain, 1985; Feuillade *et al.*, 1995). A general indicator of past existence of cyanobacterial population is sedimentary myxoxanthophyl since it is present in the majority of the species (Swain, 1985), but it is also less stable than other cyanobacterial sedimentary pigments (Leavitt, 1988). Pigment markers of colonial Cyanobacteria are canthaxanthin, myxoxanthophyl and scytonemin, while aphanizophyll is characteristic for N_2-fixing forms (Nostocales) (Leavitt and Hodgson, 2001).

1.9 Aims of the study

The aims of this study are to determine the paleolimnological record of temporal diversity and presence of toxic genera in cyanobacterial assemblages of Hamilton Harbour sediment, and to gain a better understanding of seasonal distribution of toxigenic

Cyanobacteria in relationship to the environmental factors of the Harbour.

1.10 Thesis Objectives and Hypotheses

Objectives important to resolve hypotheses questions focus on the following:

Objective 1. Identification of potentially toxic Cyanobacteria and their seasonal dynamics in Hamilton Harbour in 2009.

To identify potential microcystin producers of Hamilton Harbour, morphological identification of planktonic cyanobacteria and a count of filamentous forms and *Microcystis* colonies from environmental samples will be performed using inverted microscopy. Measurements of the main physico-chemical parameters (water temperature, conductivity, pH, dissolved oxygen, inorganic carbon, chlorophyl *a*, N and P dissolved and total forms, etc.) and their seasonal vertical profiles will be included in this study. The statistical analysis of environmental variables and their relationship to diversity and abundances of filamentous cyanobacterial assemblages will be evaluated. *Microcystis* percentage in relation to total algal numbers will be evaluated to test the following hypothesis:

Hypothesis 1. Environmental factors influence the abundance of cyanobacterial assemblages.

Objective 2. Isolation and identification of sedimentary pigments from Hamilton Harbour using reverse phase-HPLC.

To infer past cyanobacterial blooms, stratigraphic distribution and concentration of fossil cyanobacterial pigments will be used, while distribution and concentration of other sedimentary pigments will be used to infer past composition of photosynthetic communities in the Harbour, and to test the following hypothesis:

Hypothesis 2: Cyanobacterial blooms happened historically in Hamilton Harbour.

Objective 3. Analysis of cyanobacterial assemblages in Hamilton Harbour sediment using 16S-rRNA gene fragments.

To assess genotypic diversity/similarity and shifts in cyanobacterial communities over time, results of PCR-DDGE amplification of V3 region of the 16S rRNA will be used. Stratigraphic profiles of cyanobacterial assemblages will be inferred from Hamilton Harbour sediment DNA extracts to test the following hypothesis:

Hypothesis 3: Genetic fingerprints of cyanobacterial assemblages from different stratigraphic intervals are different at different time periods, especially between the oldest and the most recent parts of the sediment.

Objective 4. Investigation of the presence of microcystin producers in Hamilton Harbour sediments using mcyE gene fragments.

To determine occurrence and diversity of toxic cyanobacterial genera in the past, PCR amplification of *mcy*E gene and PCR-DDGE analysis of its AMT region will be employed. Stratigraphic profiles of toxic cyanobacterial assemblages will be inferred from Hamilton Harbour sediment DNA extracts to test the following hypothesis:

Hypothesis 4: Microcystin-producing cyanobacterial genotypes were present in the past before documented cyanobacterial blooms.

Chapter 2

Microscopic identification and seasonal distribution of planktonic Cyanobacteria in Hamilton Harbour during 2009 summer-fall season

2.1. Overview

Phytoplankton are an important component of any aquatic ecosystem and a base for its food web (Lampert and Sommer, 2007). The species composition, diversity, biomass and seasonal succession of phytoplankton in freshwater ecosystems depends on abiotic (chemical, physical) and biotic factors (Reynolds, 1986). The relationships among these parameters and phytoplankton communities is complex; the combined role of environmental variables such as nutrients, light intensity, competition, and zooplankton density have a major influence on the phytoplankton's community ecology (Reynolds, 1986). Understanding the factors and correlations that determine diversity, abundance, and biomass of phytoplankton is of considerable importance for lake management decisions. The same environmental variables that influence phytoplankton communities also influence water quality and nuisance algae development (Schindler, 1977). Remediation of water quality and algal blooms problems requires understanding of mechanisms that favour cyanobacterial development and in some cases, dominance in lakes (Downing *et al.*, 2001). The anthropogenic addition of nutrients to water bodies have caused the enrichment of freshwater environments leading to cultural eutrophication. Some of the consequences of eutrophication

are deterioration of water quality and decreased uses of water bodies for domestic, industrial and recreational purposes (Paerl and Huisman, 2009). Biological consequences of eutrophication are changes in plankton populations including decreased species diversity and changes in dominant biota with significant shifts towards Cyanobacteria (Paerl and Huisman, 2009).

Cyanobacterial blooms are promoted by additions of phosphorus (Fogg, 1969; Smith, 1986; Downing *et al.*, 2001). Phosphorus is the primary limiting nutrient in freshwaters determining the abundance of phytoplankton (Schindler, 1977). Even minute increases in phosphorus concentrations may lead to substantial increases in the production of Cyanobacteria (Sivonen, 1990; Larsson *et al.*, 2001). However, the importance of nitrogen in the production of blooms comprised of non-nitrogen fixing cyanobacteria has also been suggested (O'Neil, 2012). High concentrations of total phosphorus and nitrogen have been correlated with the occurrence of the microcystin producing genera *Dolichospermum, Microcystis* and *Planktothrix* in Canadian lakes, implying that nutrient rich waters offered ideal conditions for the existence of toxin producing Cyanobacteria (Kotak *et al.*, 1996; Giani *et al.*, 2005). Inshore areas of the Great Lakes have experienced outbreaks of toxin-producing cyanobacteria since 1990s (Watson *et al.*, 2008). Blooms have been reported from inshore areas such as Saginaw Bay (Lake Huron), Maumee River and Sandusky Bay (eastern Lake Erie), and western and southern Lake Ontario embayments (*e.g.*, Brittain *et al.*, 2000; Ouellette *et al.*, 2006; Hotto *et al.*, 2007).

Cyanobacterial growth has also been linked to iron availability (Twiss *et al.*, 2000; Hyenstrand *et al.*, 2001). Cyanobacteria appear to require more cellular iron than eukaryotic

algae (Fay and van Baalen, 1987), hence increases in iron have stimulated cyanobacterial growth over that of green algae in batch cultures (Morton and Lee, 1974). In addition, several authors have shown a correlation between changes in toxicity at different iron concentrations (Utkilen and Gjølme,1995; Imai *et al.*, 1999; Martin-Luna *et al.*,2006b; Nagai *et al.*, 2007; Sevilla *et al.*, 2008). In addition, differences in the growth of toxic and nontoxic strains of *M. aeruginosa* have been observed regarding iron availability, with microcystin-producers remaining viable for longer periods under conditions of iron stress (Utkilen and Gjolme, 1995; Imai *et al.*, 1999; Martin-Luna *et al.*, 2006b; Sevilla *et al.*, 2008). An iron-binding protein, the ferric uptake ABC-transporter (Fut), which facilitates iron transport in Cyanobacteria, has been identified (Katoh *et al.*, 2001). Also, a ferrous iron transporter (FeoB) that has an accessory iron uptake function is only expressed under conditions of severe iron stress (Katoh *et al.*, 2001). Therefore, the hypothesis that microcystin may allow toxic cells to take up or store iron more efficiently may be supported (Utkilen and Gjolme, 1995; Sevilla *et al.*, 2008).

Microcystin synthesis may also be regulated by iron availability (Drechsel and Jung, 1998, Martin-Luna *et al.*, 2006a), based on a fact that the binding of the ferric uptake regulator FurA to the *mcyS* gene cluster provides transcriptional control over toxin synthesis (Martin-Luna *et al.*, 2006a, 2006b). In an iron-replete environment FurA acts as a transcriptional repressor and its control on the toxin promoter would lead to increased microcystin production in iron-deficient cells, consistent with findings of Sevilla *et al.* (2008). One of the triggers for the microcystin synthesis could be iron deficiency, since iron starvation caused an increas e of *mcyD* transcription, correlative to the increase of

microcystin-LR levels in *Microcystis aeruginosa* PCC7806 strain grown in iron-replete and iron-deplete media (Sevilla *et al.*, 2008). The cyanobacterial genom encodes for three Fur homologues (A, B and C) (Kaneko *et al.*, 2007; Frangeul *et al.*, 2008). In *Anabaena* sp. PCC 7120, FurB was shown to have a DNA protective function (Hernandez *et al.*, 2004, Lopez-Gomollon *et al.*, 2009), while the function and regulatory targets in cyanobacteria remain unclear for FurC protein (Hernandez *et al.*, 2004).

Cyanobacterial growth has also been linked to nitrogen availability, which affects not only the growth but also the synthesis of microcystin in toxic Cyanobacteria (Downing *et al.*, 2005, Van de Waal *et al.*, 2009). Assimilation and control of nitrogen are subject to fine control in Cyanobacteria. NtcA is a global nitrogen regulator found in prokaryotes (including Cyanobacteria) that helps regulation of nitrogen-responsive genes (Ginn and Neilan, 2010). NtcA also has a role of a transcriptional activator of toxin genes in *M. aeruginosa* PCC 7806, since it binds to the *mcyA*/*D* promoter (Ginn and Neilan, 2010). Nitrogen levels affect microcystin production rates by contoling the transcription levels of ntcA (Ginn and Neilan, 2010). It has been shown that under nitrogen-limited and nitrogen-starved conditions *ntcA* transcription increased 4.07-fold and 2.36-fold, respectively, compared to the control (nitrogen-excess) (Ginn and Neilan, 2010). Under the nitrogen-limited condition the transcription of the toxic *mcyB gene* increased 14.09 fold compared to nitrogen-sufficient conditions (Ginn and Neilan, 2010).

Water quality issues of Hamilton Harbour

Hamilton Harbour's water quality issues began in the late 1700s when European settlers arrived to the region (Campbell, 1966). Since then, dramatic anthropogenic impacts

have been observed on the harbour ecosystem (Versteeg *et al.*, 1995). In the 1800s, with the increase of the population and economy of the region, the harbour's shorelines became sites of industrial and commercial development (Hall, 2006). With the construction of the Burlington Ship Canal in 1823 and the raw sewage discharge directly into the harbour until 1964 (RAP, 1992) bacterial contamination, excessive nutrient and ammonia loadings have been important water quality issues in the harbour. At the beginning of the 20th century steel production in the area began with discharge of untreated effluents containing several contaminants including metals, aromatic hydrocarbons, and cyanide into Hamilton Harbour (Versteeg *et al.*, 1995). The consequences of these inputs were the closures of the Harbour beaches to recreational purposes by the 1940s and by the 1960s, severe water quality problems further limited public access to the shorelines (Hall *et al.*, 2006). Hamilton Harbour was identified as an area of concern by the Water Quality Board of the International Joint Commission in 1985 (IJC, 1985) and a Remedial Action Plan (RAP) for the Hamilton Harbour region was implemented in 1987 (Hall *et al.*, 2006). Over the years of remediation efforts, nutrient and contaminant loadings have been markedly reduced (Hamilton Harbour RAP, 1992). However, eutrophication continues to be a concern, mostly due to the phosphorus, ammonia and suspended particles loadings that make their way into the harbour from the point as well as non point sources (Hiriart-Baer *et al.*, 2009).

One of the consequences of harbour's eutrophication are re-ocurring cyanobacterial blooms and potential microcystin production. Since 1990s, more frequent and severe outbreaks of noxious and toxic Cyanobacteria have been recorded (Watson *et al.*, 2008). However, there is an information gap regarding long-term data of cyanobacterial blooms and

toxins in the harbour. Long term research interest on harmful cyanobacterial blooms both in USA and Canada has been historically focused on marine environments (Boyer, 2006). In 1993, American national research agendas, such as Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) and Monitoring and Event Response for Harmful Algal Blooms (MERHAB), recognized the importance of inland harmful cyanobacterial blooms and included the Laurentian Great Lakes in their research and monitoring plans (Boyer, 2006).

As one of the ubiquitous cyanobacterial genera, the genus *Microcystis* (Chroococcales) is found in freshwater as well as in brackish environments (Codd, 2000). Because of its common occurrence in water bodies and its ability to form toxic blooms, *Microcystis* is one of the most studied freshwater Cyanobacteria in the world (Carmichael, 1996). *Microcystis* cells are characterized by spherical cells (1-6 μm) that form colonies which can produce massive accumulations in surface blooms (Komarek, 2003). Gas vesicles (intracellular inclusions present in many planktonic species) enable *Microcystis* cells to float on the water surface and form distinctive surface scums (Komarek, 2003). The *Microcystis* life cycle includes both pelagic and benthic stages and there are no morphological differences between them (Brunberg and Blomqvist, 2002). However, the benthic stage is mainly considered a physiological resting stage due to poor light conditions that restrict photosynthetic activity (Brunberg and Blomqvist, 2002). During unfavorable growth conditions, benthic (resting) stages of the *Microcystis* colonies can accumulate at the bottom of the waterbody and survive for longer periods *i.e*. overwinter in sediments (Brunberg and Blomqvist, 2002). These benthic colonies can migrate back to the water column

and serve as an inoculum for the planktonic population that develops during summer (Reynolds *et al.*, 1981; Trimbee and Harris, 1984; Head *et al.*, 1999; Ihle *et al.*, 2005). In Hamilton Harbour, *Microcystis* has been identified as the main microcystin producer by Murphy *et al. (*2003) and Allender *et al.* (2009). Although the role of *Microcystis* in harbour toxin production is well established, it is not clear if it dominates the algal assemblages as well.

The objectives of this study were:

a) microscopic identification of potential microcystin-producing filamentous Cyanobacteria and their seasonal dynamics in Hamilton Harbour in 2009;

b) microscopic identification of *Microcystis* colonies and estimation of its relative abundance in relation to total algal numbers.

c) statistical analysis of the relationship between environmental variables (the main water physico-chemical parameters) and abundances of filamentous cyanobacterial assemblages.

2.2. Materials and Methods

2.2.1. Sampling sites, phytoplankton sampling and analysis

Samples were collected by the National Water Research Institute, Environment Canada, using a Van Dorn bottle in the period June-November 2009. To assess the horizontal and vertical variability in water quality conditions in Hamilton Harbour two stations were sampled: 1001 (Central: 43.28750 N, 79.83833 W) and 9031 (Western: 43.27722 N,

79.87833 W) (Fig 1.2). Even though absolute water quality conditions differ spatially, temporal changes measured at the center station reflect changes across the harbour (Hiriart-Baer *et al.*, 2009). Also, to capture average conditions within the epilimnion of the harbour and to relate them to external nutrient inputs, an integrated water sample was used (Hamilton Harbour Remedial Action Plan, 2010b). Western station, as one of the corner stations systematically sampled and close to Grindstone creek and Cootes Paradise, was sampled to capture seasonal variability in harbour water quality conditions. At stations 1001 and 9031 samples were collected from 1 m depth and an additional integrated sample (top of the thermocline to the surface) was collected from station 1001 during the stratified months on following dates: June 30, July 7, July 20, August 6, August 31, September 16, October 8, November 3, and one sample was collected between August 17 and 24. Phytoplankton samples were immediately preserved in Lugol's iodine solution and stored at room temperature for future analyses.

Phytoplankton identification was conducted on a 25 ml aliquot of a well mixed sample. The aliquot was settled in a graduated glass cylinder over a 48 hrs period and a 2.5 ml concentrated sub-sample was transferred into an Utermöhl chamber. Both coccoid and filamentous cyanobacterial taxa were identified to the genus level (Table 2.1) using an inverted microscope (Zeiss Axio-vert 35). The taxonomic key employed in the identification included freshwater algal identification key (Wehr and Sheath, 2003). The morphological identification of Cyanobacteria was performed at 400x magnification. Cyanobacterial filaments were counted at 100 x magnification, over the entire chamber bottom to determine the relative abundance of filamentous forms. Cyanobacterial community richness during the

season was estimated for the harbour. Species richness, together with evenness, is one of the two basic components of species diversity. A number of cyanobacterial genera at two sampling stations during the season (richness) was estimated based on the number of genera present in phytoplankton samples (Fromin *et al.*, 2002).

Microcystis colonies and total algal units in samples were counted at 100 x magnification, using a random field counting method to determine the relative abundance of *Microcystis* colonies during the sampling period. The number of the algal units and *Microcystis* colonies was estimated according to the formula:

$C=N \cdot A/n \cdot v \cdot a$

where: C =number of algal units /l; N = total number of units counted in all fields; A = surface area of the bottom of the sedimentation chamber (in mm^2); n = number of fields used for cell counts; v = volume (in ml) of the sample; a = area of the visual field (in mm^2).

2.2.2. Physico-chemical parameters

Physical and chemical parameters as well as phytoplankton composition samples were determined on water samples collected at 1m depth from two sites in Hamilton Harbour, the central (CS) and western (WS) stations (1001 and 9031, respectively) (Fig 1.2). Using a YSI 6600 (Hoskin Scientific, Burlington, ON) dissolved oxygen, pH, specific conductivity and water temperature were measured, and Secchi disc depth was determined. Concentrations of nutrients including total phosphorus (TP), total dissolved phosphorus (TDP*),* soluble reactive phosphorus (SRP), ammonia (NH_3-N-F), nitrate/nitrite ($NO_3/_2$) and chlorophyll (chl) *a* were determined by the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario. Ammonia was measured by colorimetry (indophenol blue)

on filtered water samples and $NO_3/_2$ was determined by colorimetry (azo dye) following a copper–cadmium column reduction on filtered water samples (American Public Health Association, 2005). Concentrations of phosphorus were determined by colorimetry (chloride–molybdate complex) on unfiltered (TP) and filtered (TDP, SRP) water samples following acidic persulfate digestion (TP and TDP, only). Spectrophotometry following an acetone extraction (Unesco, 1969) was used for the determination of chlorophyll (chl) *a* concentration. Concentrations of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined by NLET, following NLET methodology. Concentrations of DOM, reported as C content (DOC), were determined using a UV-persulfate TOC analyzer (Pheoniz 8000TM). Briefly, filtered (0.45 μm) water samples were acidified with phosphoric acid to pH of 3 or lower, mixed with sodium persulphate and phosphoric acid solutions in a UV reactor chamber, and the CO_2 produced was sent to a nondispersive infrared (NDIR) detector to calculate DOC concentrations in mg/l. Concentrations of DIC were determined using a UV-persulfate TOC analyzer (Pheoniz 8000TM). In short, filtered (0.45 μm) water samples were acidified with phosphoric acid to pH of 3 or lower to convert organic carbon to carbon dioxide, sparged with a stream of inert nitrogen gas in the IC sparger, and then oxidized in a series of steps in which moisture and chlorine were removed. Finally, the CO_2 concentration was measured by a non-dispersive infrared (NDIR) detector. Concentrations of particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined from filtered suspended solids, which were acidified, dried, and analyzed by high-temperature combustion (1000°C) using a PE 2400 CHN analyzer.

2.2.3. Statistical analyses

Physico-chemical data sets were statistically analyzed using Principal Components Analysis (PCA) technique. PCA is one of the most commonly used multivariate statistical techniques which deals with analysis of multiple variables. It reduces big data sets with many variables to smaller data sets with fewer, newly derived variables, while simultaneously summarizing the original information so it can be used for further analysis, *i.e.* variable reduction (Quinn and Keough, 2002). It also identifies patterns in data, and reveals similarities and differences in the data that could not be found by analyzing each variable separately (Quinn and Keough, 2002). Here, principal components analysis (PCA) was used to explore major differences in the water quality conditions among Hamilton Harbour water samples collected from the central (CS) and western (WS) stations during 2009 (Figure 1.2). All samples were collected by National Water Research Institute, Environment Canada, Burlington, Ontario. Some sample collections had missing data: SRP, TPF and TPUF from the central and western stations on Aug 6 and TPF from the western station on Sept 16. These missing values were substituted with average values from the two closest sampling periods. NH_3-N-F was below detection on July 7 and July 20 for the western station, and the detection limit value (0.005 mg/l) was used as a substitute for these two dates. Lastly, due to several missing data values, the June 30 and Nov 3 central and western station samples were omitted from analysis. This left a total of 14 samples for PCA which was performed using CANOCO software version 4.5 (ter Braak and Šmilauer, 2002). Scaling focussed on inter-variables correlations and variables were divided by their standard deviation, centered and standardized prior to ordination.

Statistical technique used to explore relations between the relative abundance (%) of six genera of filamentous Cyanobacteria (*Planktotrix, Aphanizomenon, Limnotrix, Pseudoanabaena, Dolichospermum,* and *Lyngbya*) and Hamilton Harbour water quality data collected in 2009 was Redundancy Analysis (RDA). This technique constrains the scaling of objects and variables to a linear combination of covariates in a way that differences in a variance for each variable contribute to the analysis (Quinn and Keough, 2002). RDA is useful for ecological data, since it directly incorporates environmental variables in the scaling/ordination of species abundance data (Quinn and Keough, 2002). Relative abundance of filamentous cyanobacteria was square root transformed prior to multivariate analysis. RDA is a form of direct gradient analysis constrained by the PCA ordination of environmental variables and is suited to the analysis of short species gradients (≤2 SD). Detrended Correspondence Analysis (DCA, performed using CANOCO version 4.5) of the filamentous cyanobacteria abundance data revealed that gradient lengths of species composition along both DCA axis 1 and 2 were < 1 SD, indicating RDA was the preferred method of direct species-environment ordination. Such short species gradient lengths also point to little species turnover between samples, which is not unexpected given that they are from repeated sampling of the same water body during one season. RDA was performed using CANOCO software version 4.5 (ter Braak and Šmilauer, 2002). Scaling focused on inter-species correlations, and species variables were divided by their standard deviation prior to ordination and centered by species. Manual selection of significant ($p \leq 0.10$) environmental variables explaining variation in species data was performed using 999

unrestricted Monte Carlo permutations (reduced model). The minimum environmental variables significance of $p \leq 0.10$ was selected due to the small sample size in this research.

2.3. Results

2.3.1. Identification of Cyanobacteria from Hamilton Harbour using inverted microscopy

A total of 14 cyanobacterial genera comprising six families and three orders were identified in Hamilton Harbour phytoplankton samples (Table 2.1.). Both coccoid/colonial (Fig. 2.1 and 2.2) and filamentous forms (Fig. 2.3) were observed. Presence or absence of certain genera in both vertical and horizontal profiles of cyanobacteria communities accounted for differences in spatio-temporal diversity during the sampling season. *Microcystis* (Fig. 2.2 b-e) was the only genus present during the entire sampling season, occasionally with *Rhabdogloea* colonies endogloeically present in its mucilage (Fig. 2.2 e). Genera *Aphanizomenon* (Figure 2.3 e), *Pseudoanabaena* (Figure 2.3 c) and *Merismopedia* (Fig. 2.1 e-f) were not observed at sampling station 1001 at 1 m depth (Table 2.3). At sampling station 1001 in the depth integrated samples *Pseudoanabaena* (Fig. 2.3 c) and *Merismopedia* were not present (Table 2.4). *Gloeocapsa* (Fig. 2.3 a) and *Gomphosphaeria* (Fig. 2.2 d) were absent from station 9031 at 1 m depth while the presence of *Merismopedia* was recorded in one sample (Table 2.5). At sampling station 1001 at depth 10-0 m, presence of heterocysts and akinetes was observed in *Dolichospermum* on one date during the sampling season: on August 31, one akinete and one heterocyst per one filament was recorded. At sampling station 9031 at 1 m depth, presence of heterocysts and akinetes was

observed in *Dolichospermum* on three dates during the sampling season: on July 20, two akinetes per filament; on August 6, two heterocysts per filament; and on September 16, two heterocysts and one akinete per 13 filaments. The genus *Aphanizomenon* was also producing akinetes and heterocyst, and their presence was generally increasing toward late summer and fall.

Based on the number of filamentous and colonial/coccoid genera present in Hamilton Harbour phytoplankton samples, cyanobacterial genus richness was estimated. The number of genera during the season ranged from 1 to 9. At western station, the highest number of cyanobacterial genera (9) was on August 17-24. At central station, integred samples showed a maximum number of genera (8) on two dates in late summer (August 31 and September 16) and while the 1m depth samples showed the highest number of cyanobacterial genera (6) on three dates during the season (July 7, August 17-24, and September 16).

Table 2.1. Cyanobacteria identified in phytoplankton samples from CS and WS sampling stations from Hamilton Harbour during 2009 summer-fall period.

Order	Family	Genus	Morphology
Chroococcales	Synechococcaceae	***Aphanothece***	Coccoid/colonial
		Rhabdogoea	
	Merismopediaceae	***Aphanocapsa***	
		Coelosphaerium	
		Gomphosphaeria	
		Merismopedia	
	Microcystaceae	***Gloeocapsa***	
		Microcystis	
Nostocales	Nostocaceae	***Aphanizomenon***	Filamentous
		Dolichospermum	
Oscillatoriales	Pseudoanabaenaceae	***Limnothrix***	
		Pseudoanabaena	
	Phormidiaceae	***Planktothrix***	
		Lyngbya	

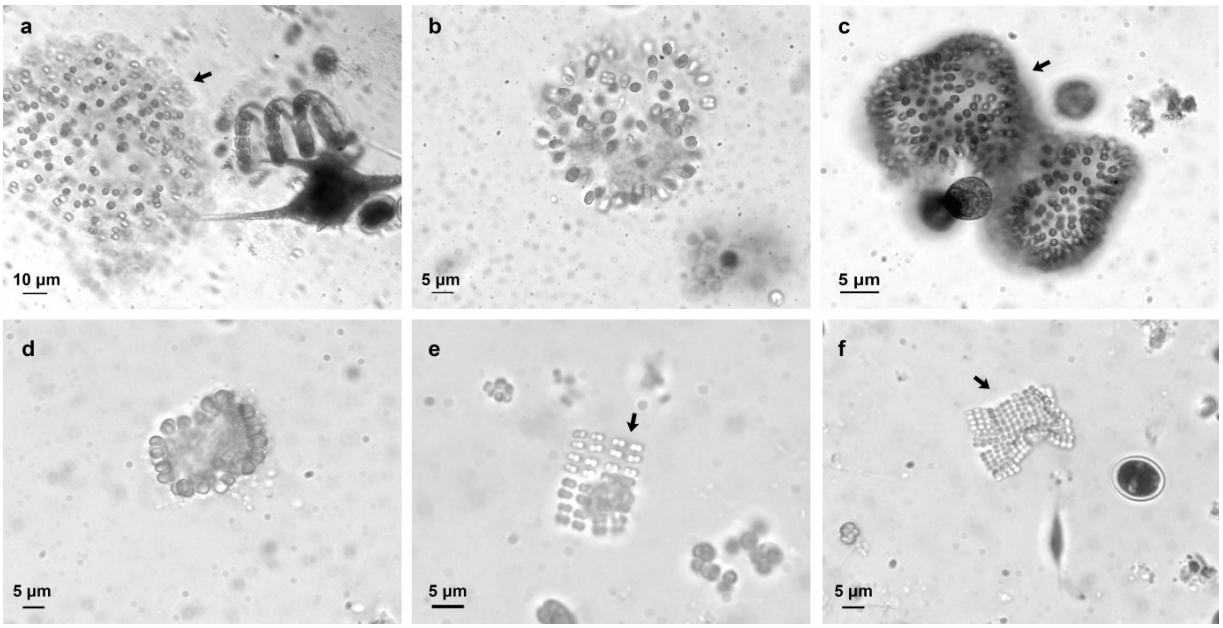

Figure 2.1. Inverted microscope images of coccoid/colonial Cyanobacteria identified from Hamilton Harbour water samples. *Aphanocapsa* sp. (arrow) **(**a); *Aphanothece* sp. (b); *Coelosphaerium* sp. (arrow) (c); *Gomphosphaeria* sp. (d); *Merismopedia* sp. (arrow) (e-f).

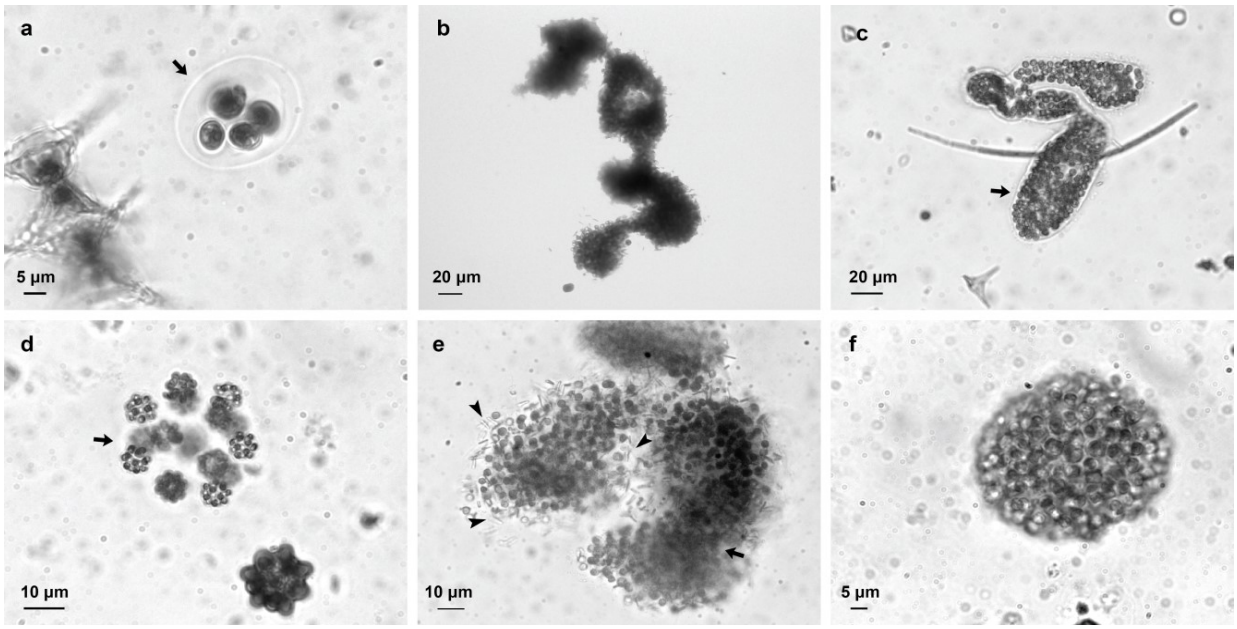

Figure 2.2. Inverted microscope images of coccoid/colonial Cyanobacteria identified from Hamilton Harbour water samples. *Gloeocapsa* sp. (arrow) **(**a); *Microcystis* sp. (arrow) (b-f); *Rhabdogloea* sp. (arrowheads) (e) present endogloeically in *Microcystis* sp. colony.

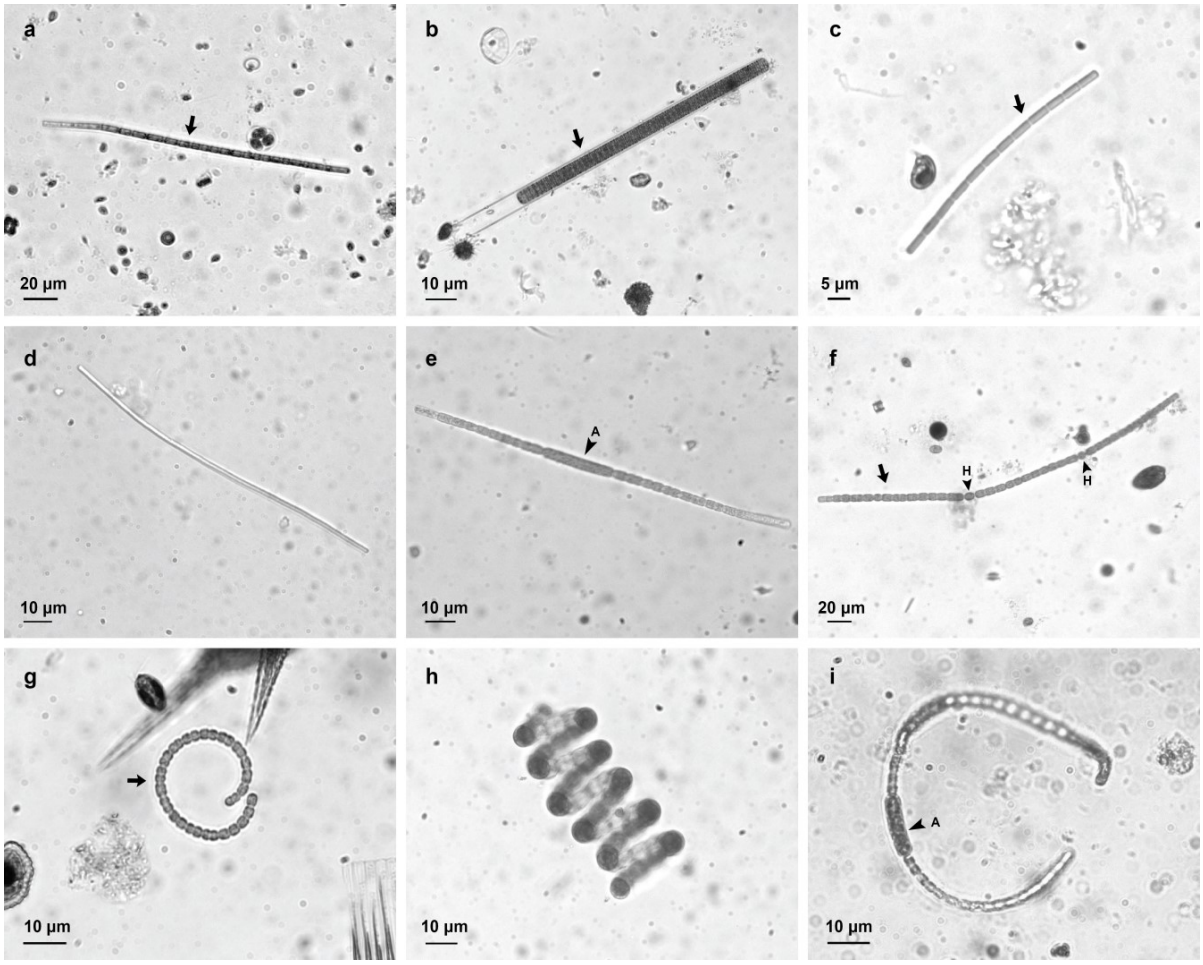

Figure 2.3. Inverted microscope images of filamentous Cyanobacteria identified from Hamilton Harbour water samples. Planktothrix sp. (arrow) (a); Lyngbya sp. (arrow) (b); Pseudoanabaena sp. (arrow) (c); Limnothrix sp. (arrow) (d); Aphanizomenon sp. (e); Dolichospermum sp. (arrow) (f-i). Heterocysts are labeled as "H" and akinetes as "A".

Table 2.2. Presence of cyanobacterial genera identified during 2009 at different dates (June-November) at CS at 1m depth

		Genus												
Station/ Depth (m)	**Sampling date**	**APZ**	**DL**	**LM**	**LB**	**PL**	**PAN**	**APC**	**APT**	**CS**	**GC**	**GS**	**MP**	**MC**
		Filamentous						**Coccoid/colonial**						
1001 1 m	**Jun 30**													+
	Jul 07					+		+		+	+	+		+
	Jul 20			+	+	+								+
	Aug 06					+								+
	Aug 17-24		+			+		+	+	+				+
	Aug 31			+	+	+		+						+
	Sep 16		+		+	+		+		+				+
	Oct 08		+			+								+
	Nov 03					+								+

Legend for cyanobacterial genera abbreviations: *Aphanizomenon*-APZ; *Dolichospermum*-DL; *Limnothrix*-LM; *Lyngbya*-LB; *Planktothrix*-PL; *Pseudoanabaena*-PAN; *Aphanocapsa*-APC; *Aphanothece*-APT; *Microcystis*-MC; *Coelosphaerium*-CS; *Gomphosphaeria*-GS; *Gloeocapsa*-GC; *Merismopedia*-MP.

Table 2.3. Presence of cyanobacterial genera identified during 2009 at different dates (June-November) at CS from integrated water sample (0-10 m depth).

Station/ Depth (m)	Sampling date	Genus												
		APZ	DL	LM	LB	PL	PAN	APC	APT	CS	GC	GS	MP	MC
		Filamentous						Coccoid/colonial						
1001 10-0 m	Jun 30													+
	Jul 07													+
	Jul 20													+
	Aug 06				+	+		+			+			+
	Aug 17-24		+	+		+				+				+
	Aug 31	+	+			+		+	+	+		+		+
	Sep 16		+	+	+	+		+	+	+				+
	Oct 08	+				+			+					+
	Nov 03	+				+			+					+

Legend for cyanobacterial genera abbreviations: *Aphanizomenon*-APZ; *Dolichospermum*-DL; *Limnothrix*-LM; *Lyngbya*-LB; *Planktothrix*-PL; *Pseudoanabaena*-PAN; *Aphanocapsa*-APC; *Aphanothece*-APT; *Microcystis*-MC; *Coelosphaerium*-CS; *Gomphosphaeria*-GS; *Gloeocapsa*-GC; *Merismopedia*-MP.

Table 2.4. Presence of cyanobacterial genera identified during 2009 at different dates (June-November) at WS at 1m depth

Station/ Depth (m)	Sampling date	Genus												
		APZ	DL	LM	LB	PL	PAN	APC	APT	CS	GC	GS	MP	MC
		Filamentous						Coccoid/colonial						
9031 1 m	Jun 30													+
	Jul 07													+
	Jul 20		+	+		+								+
	Aug 06		+	+		+	+	+	+				+	+
	Aug 17-24	+		+	+	+		+	+	+			+	+
	Aug 31			+	+	+	+	+	+	+				+
	Sep 16	+	+		+	+				+				+
	Oct 08	+	+											+
	Nov 03	+				+								+

Legend for cyanobacterial genera abbreviations: *Aphanizomenon*-APZ; *Dolichospermum*-DL; *Limnothrix*-LM; *Lyngbya*-LB; *Planktothrix*-PL; *Pseudoanabaena*-PAN; *Aphanocapsa*-APC; *Aphanothece*-APT; *Microcystis*-MC; *Coelosphaerium*-CS; *Gomphosphaeria*-GS; *Gloeocapsa*-GC; *Merismopedia*-MP.

2.3.2. Filamentous Cyanobacteria relative abundances and diversity

During the sampling period from June to November six filamentous genera of Cyanobacteria were identified in Hamilton Harbour water samples. Filamentous community composition for each sampling station and sampling depth is expressed as percent abundance (Figures 2.4, 2.5, and 2.6).

At sampling station 1001 at 1 m depth four filamentous genera were recorded beginning in July 7 (Fig. 2.4). The genus *Planktothrix* dominated at the 1001-1m sampling station during the whole sampling period and was the only genus present on July 7, August 6, and November 3. The second most abundant genus was *Limnothrix,* which was present on only two dates (July 20 and August 31) with 22.2% and 11.1% abundance, respectively. Genera *Dolichospermum* and *Lyngbya* were present sporadically in samples, with under 5% abundance, except on July 20 when *Lyngbya* had 11% abundance. The genera *Aphanizomenon* and *Pseudoanabaena* were absent from all samples.

The integrated water sample taken from 0-10 m depth at sampling location 1001 (Fig. 2.5) showed greater diversity of filamentous Cyanobacteria than the sample from 1 m . The presence of filamentous Cyanobacteria was detected on August 6 in which the genus *Planktothrix* (60%) was dominant followed by the genus *Limnothrix* (30%). Maximum relative abundance of *Planktothrix* was on October 8 (93%). The genus *Aphanizomenon* was recorded on three sampling dates with highest abundance (44%) on November 3. On August 17-24, *Dolichospermum* constituted 31% of the filamentous community. Lastly, the presence of *Lyngbya* was recorded on two dates (beginning of August and September) and *Pseudoanabaena* was absent from all samples.

The greatest diversity of filamentous genera was recorded at sampling station 9031 at 1 m depth (Fig. 2.6). Three genera were present from July 20. *Planktothrix*, although present throughout the season was less relatively less abundant then at station 1001 and absent from the October 8 sample. Its lowest relative abundance was on September 16 (less than 5%), while it dominated the community on July 20 and August 31, with 95.1% and 83.6%, respectively. *Pseudoanabaena* was present on two occasions, on August 6 and 31, with relatively low abundances (approximately 6% and 10 %, respectively). *Aphanizomenon* was present toward the end of the season, being dominant on September 16 (90.1%) and October 8 (99.7%). *Lyngbya* and *Dolichospermum* were present on 3 and 4 dates, respectively, but in low relative abundances ranging from 3.37% to 0.04%. The western station experienced a severe *Limnothrix* bloom between August 17-24, when the number of filaments exceeded $87x10^3$/l, although this was not apparent from its relative abundance of 92.7%.

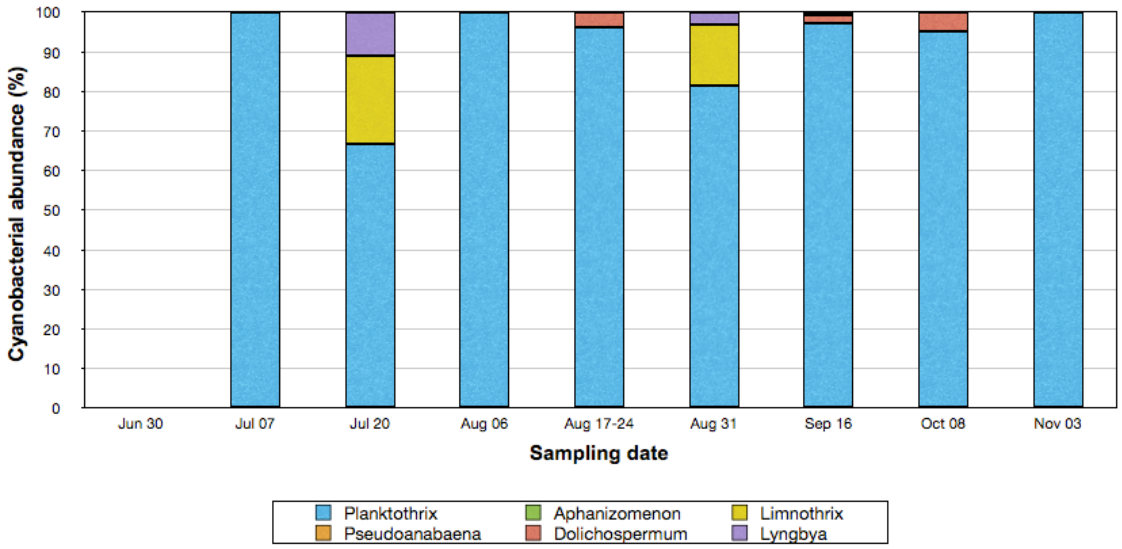

Figure 2.4. Percent abundance of filamentous cyanobacterial genera identified from Hamilton Harbour water samples during 2009 at different dates (June-November) CS at 1m depth.

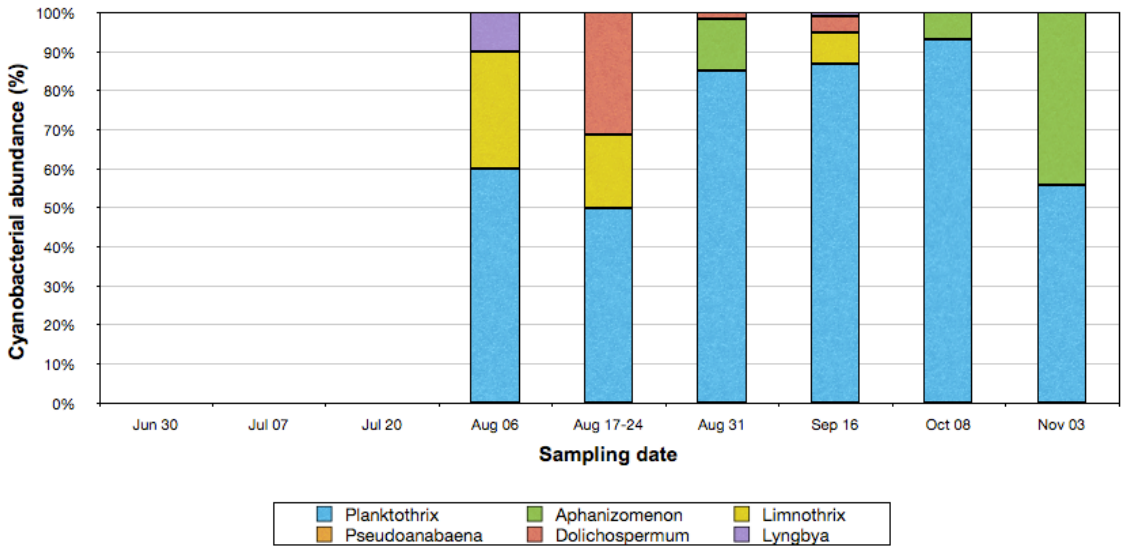

Figure 2.5. Percent abundance of cyanobacterial filamentous genera identified from Hamilton Harbour water samples during 2009 at different dates (June-November) at CS from integrated water sample (0-10 m depth).

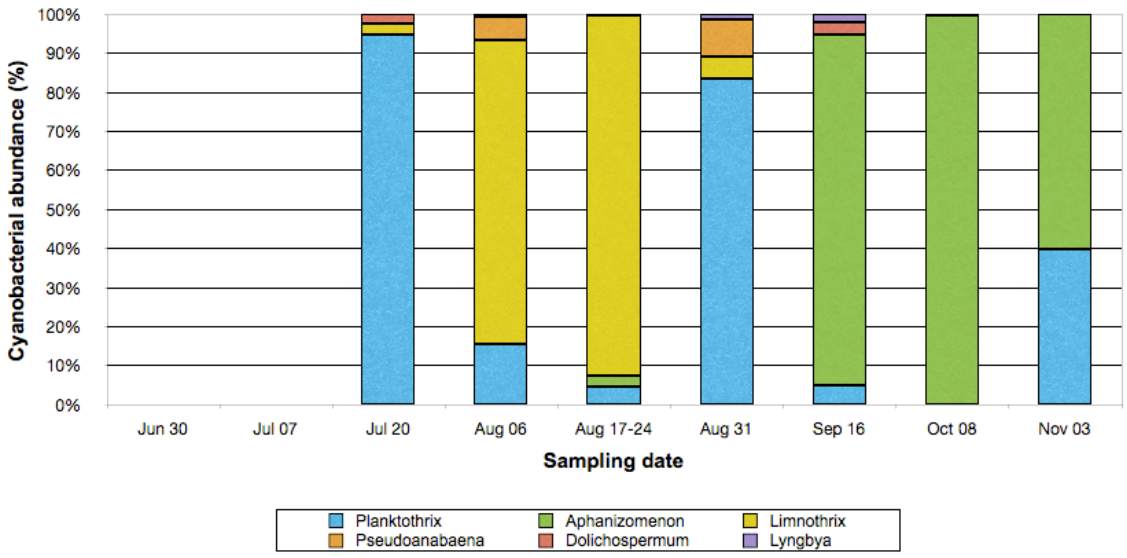

Figure 2.6. Percent abundance of cyanobacterial filamentous genera identified from Hamilton Harbour water samples during 2009 at different dates (June-November) at WS at 1 m depth.

2.3.3. *Microcystis* relative abundance

During the sampling period from June 30 to November 3, presence of *Microcystis* was recorded at all stations/depths and at all dates. The algal unit numbers and *Microcystis* colonies numbers data were used to determine the relative abundances of *Microcystis* colonies in phytoplankton population of Hamilton Harbour (Figures 2.7-2.9).

At CS at 1 m depth an average number of algal units was 10102 x 10^3/l (ranging from 4825 x 10^3/l to 16389 x 10^3/l) while an average number of *Microcystis* colonies of 445 x 10^3/l (ranging from 54 x 10^3/l to 837 x 10^3/l) accounted for its average 4.3 % relative abundance (0.6-6.2 %).

The integrated water sample taken from CS had an average of 10613 x10^3/l of algal units (ranging from 5069 x 10^3/l to 18598 x 10^3/l). An average number of *Microcystis* colonies of 578 x 10^3/l (ranging from 65 x 10^3/l to 1386 x 10^3/l) accounted for its average 5.5 % relative abundance (1-7.9 %).

Finally, WS at 1 m depth an average of 23148 x10^3/l algal units (ranging from 5787 x 10^3/l to 45819 x 10^3/l) was recorded. An average number of *Microcystis* colonies was 463 x 10^3/l (ranging from 245 x 10^3/l to 1304 x 10^3/l) and accounted for average relative abundance of 2.6 % (0.9-6.8 %).

In the present study, the relative abundance of *Microcystis* stayed below 10 % throughout the study period. The lowest relative abundance of *Microcystis* in the phytoplankton population was on June 30 CS at 1 m depth, and highest on August 17-24 at the same station in the integrated water sample. The relative abundance of *Microcystis* in the

phytoplankton population showed seasonal variability between stations.

A paired-samples t-test was conducted to compare numbers of algal units and *Microcystis* colonies collected from central and western stations. A significant difference in the number of algal units/l was found at 1 m depth between CS (10102±5612 x 10^3/l) and WS (23148±13038 x 10^3/l) stations; t (8) = -2.73, p =0.025

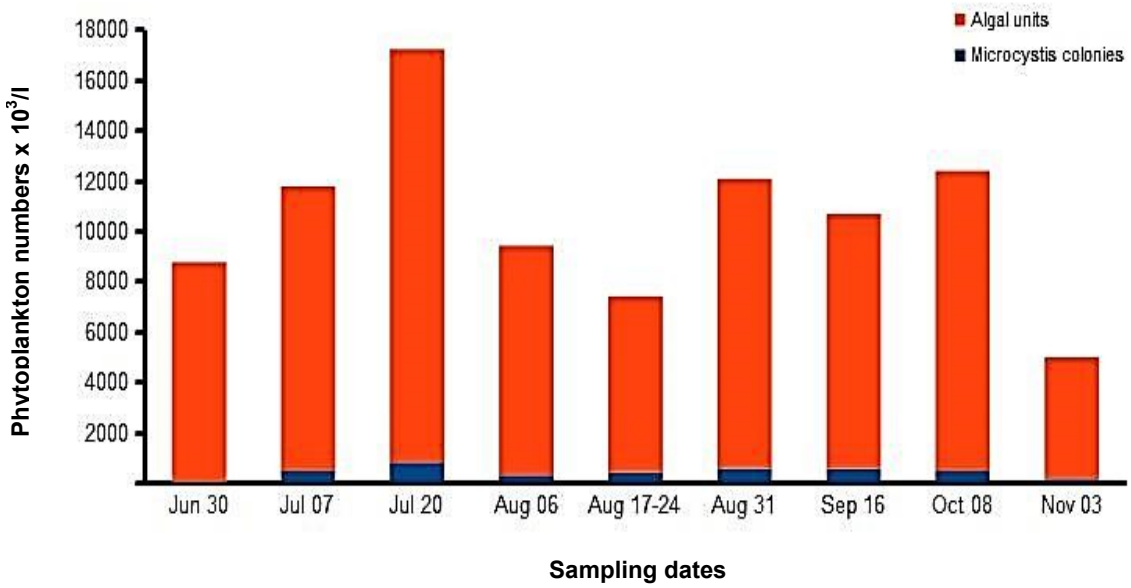

Figure 2.7. The algal unit numbers and *Microcystis* colonies numbers identified from Hamilton Harbour water samples during 2009 at different dates (June-November) at CS at 1m depth.

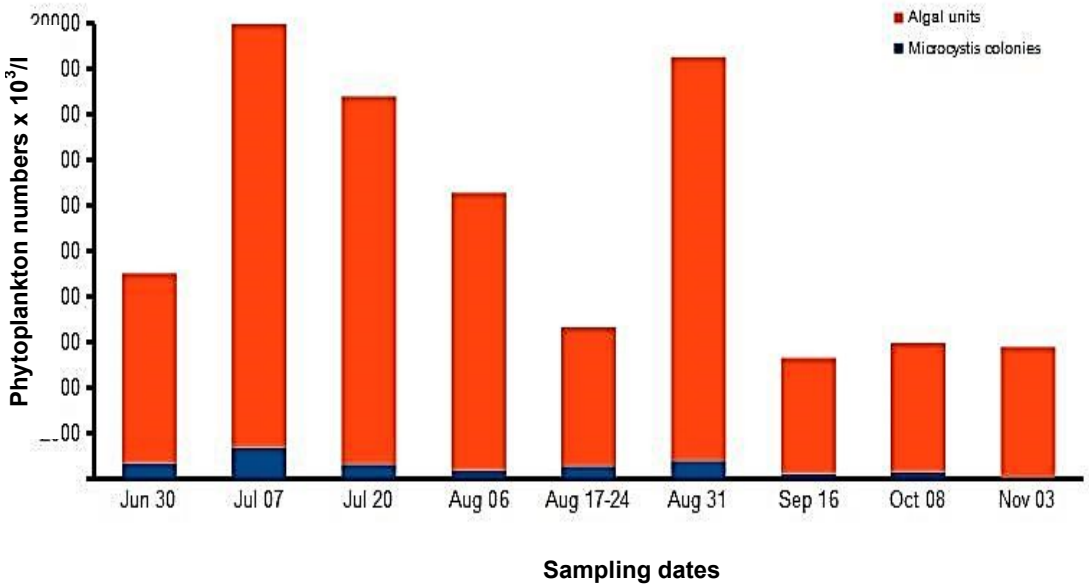

Figure 2.8. The algal unit numbers and *Microcystis* colonies numbers identified from Hamilton Harbour water samples during 2009 at different dates (June-November) at CS from integrated water sample (0-10 m depth).

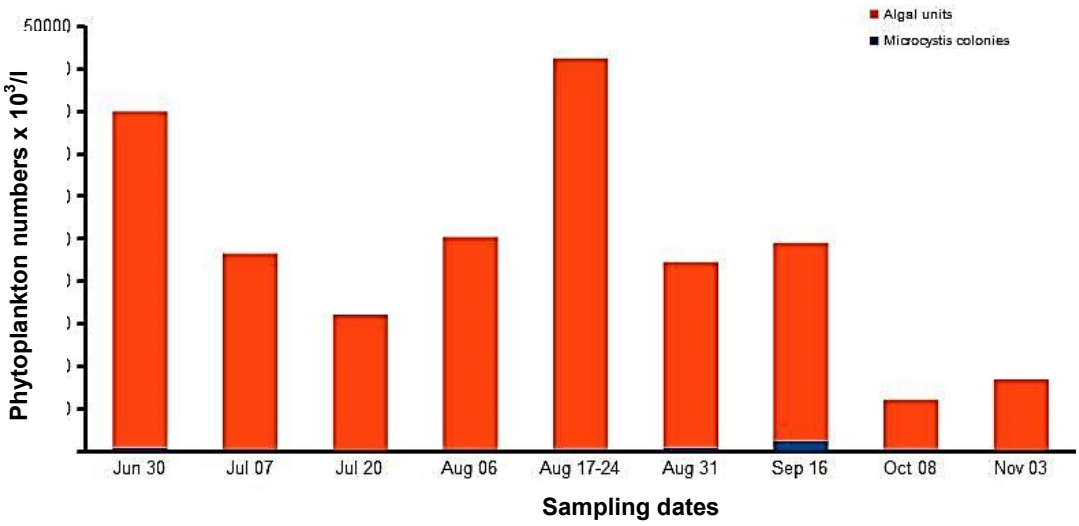

Figure 2.9. The algal unit numbers and *Microcystis* colonies numbers identified from Hamilton Harbour water samples during 2009 at different dates (June-November) at WS at 1 m depth.

2.3.4. Physico-chemical parameters

Physico-chemical parameters of the Hamilton Harbour water samples collected from June to November 2009 from 1 m depth are shown in Tables 2.5-2.7. During the sampling period the difference between lowest and highest water temperature in Hamilton Harbour was approximately 10 ^{o}C for both stations. The water was alkaline (pH > 8 for both stations), with conductivity decreasing during the sampling period (Table 2.5). Nitrate/nitrite (NO3/NO2) concentrations were higher at the beginning of th e sampling period for both stations and decreased towards late summer and fall, with slightly higher mean values for the CS (Tables 2.6-2.7) compared to WS. An ammonia content (NH_3-N-F) increase was recorded with the season's progress (0.016 mg/l on average for both stations), with the exclusion of the June 30 values, which were 5-6 times higher than for the rest of the season. As for the total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) concentrations, there was generally low variation during the season, with decreasing trend toward the end of the sampling period (Tables 2.6-2.7). Chlorophyll (chl) *a* concentrations ranged from 6.6 µg/l to 16.6 µg/l for CS and 10.3 µg/l to 19.9 µg/l for WS. The mean concentration was higher for WS compared to CS (Tables 2.6-2.7). The mean dissolved organic carbon (DOC) concentrations did not differ much for CS and WS (Tables 2.6-2.7). Dissolved inorganic carbon (DIC) concentrations ranged between 25.4-30.6 mg/l for CS and 13.7-33.6 mg/l for WS. Particulate organic carbon (POC) concentrations for CS and WS did not vary much throughout the sampling period (Tables 2.6-2.7). The mean concentrations of particulate organic nitrogen (PON) were very similar for both stations (approximately 0.2 mg/l) (Tables 2.6-2.7).

A paired-samples t-test was conducted to compare concentrations of environmental parameters between the two sampling stations. Statistically significant differences were found for the NO_3-NO_2 -F, DOC, and SRP-P-F scores. For NO_3-NO_2 -F, a significant difference in the concentration scores was found between CS (2.07±0.36 mg/l) and WS (1.86±0.38 mg/l) stations; $t(7)=2.71$, $p = 0.005$. For DOC, a significant difference in the concentration scores was found between CS (3.66±0.30 mg/l) and WS (3.90±0.35 mg/l) stations; $t(7)=3.99$, $p = 0.005$. Also, there was a significant difference for SRP-P-F at CS (0.003±0.002 mg/l) and WS (0.002±0.001 mg/l) stations; $t(7)=3.22$, $p = 0.015$. Other environmental parameters did not significantly differ ($p > 0.05$).

Table 2.5. Hamilton Harbour water quality physical parameters from from June-November 2009 at 1m depth for CS and WS sampling stations.

Station/Depth (m)	Parameter	Max	Min	Mean	SD
CS 1m	Secchi depth (m)	4.0	1.0	2.5	0.9
	Water Temperature (^{o}C)	22.2	11.8	18.6	3.3
	pH	9.05	8.26	8.70	0.26
	Conductivity (µs/cm)	719	538	609	71
	Dissolved Oxygen (mg/l)	10.3	6.6	8.7	1.2
WS 1m	Secchi depth (m)	3.5	1.8	2.4	0.7
	Water Temperature (^{o}C)	21.5	11.1	18.5	3.5
	pH	8.97	8.34	8.74	0.19
	Conductivity (µs/cm)	722	539	618	74
	Dissolved Oxygen (mg/l)	9.8	6.4	8.4	1.0

Table 2.6. Hamilton Harbour water quality chemical parameters from June-October 2009 for CS at 1 m depth.

Station/Depth	Parameter	Max	Min	Mean	SD
CS 1m	NO_3-NO_2-F (mg/l)	2.63	1.69	2.07	0.357
	NH_3-N-F (mg/l)	0.108	0.010	0.028	0.033
	Chl *a* (µg/l)	16.6	6.6	11.1	4.4
	DOC (mg/l)	4.1	3.3	3.7	0.3
	DIC (mg/l)	30.6	25.4	27.6	1.8
	POC (mg/l)	2.05	1.03	1.50	0.40
	PON (mg/l)	0.275	0.139	0.209	0.060
	SRP-P-F (mg L^{-1})	0.0071	0.0020	0.0034	0.0018
	TP-P-F (mg L^{-1})	0.026	0.013	0.017	0.005
	TP-P-UF (mg L^{-1})	0.0597	0.0357	0.0422	0.0082

Table 2.7. Hamilton Harbour water quality chemical parameters from from June-October 2009 for WS at 1 m depth.

Station/Depth	Parameter	Max	Min	Mean	SD
WS 1m	NO_3-NO_2-F (mg/l)	2.50	1.43	1.86	0.39
	NH_3-N-F (mg/l)	0.135	0.006	0.039	0.049
	Chl *a* (µg/l)	19.9	10.3	15.4	3.6
	DOC (mg/l)	4.5	3.4	3.9	0.3
	DIC (mg/l)	33.6	13.7	27.4	6.2
	POC (mg/l)	2.47	1.23	1.65	0.44
	PON (mg/l)	0.400	0.205	0.267	0.069
	SRP-P-F (mg L^{-1})	0.0053	0.0016	0.0024	0.0013
	TP-P-F (mg L^{-1})	0.023	0.013	0.016	0.004
	TP-P-UF (mg L^{-1})	0.0688	0.0326	0.0440	0.0127

2.3.5. Statistical analyses of correlation between filamentous Cyanobacteria relative abundances and environmental parameters

A PCA of 2009 water quality samples from western and central stations of Hamilton Harbour are shown in Figure 2.10. The 1st and 2nd PCA axes captured 35.7% and 21.0% of the data variation, respectively. Early summer samples (July 7 and 20) from both the CS and WS plot strongly to the upper left quadrant in PCA space , which is indicative of water conditions being higher in conductivity, nitrates, dissolved oxygen, DOC, TP-F, and chl *a* (included passively).As the season progresses in to late summer (August), water samples from both the central (1001) and western (9031) station plot increasingly to the right on PCA axis 1 and negatively on PCA axis 2, which is indicative of a decrease in the water quality parameters listed above and an increase in Secchi depth, ammonia content (NH_3-N), soluble reactive phosphorous (SRP-P-F), and declining DIC and TP in both central and western stations. The very last sampling period (October 7) sees a shift towards more positive PCA axis 1 and 2 scores. This sampling date is similar to that of late summer but with a further increase in both water clarity (Secchi depth) and ammonia concentrations.

For the RDA, a manual selection of environmental variables under Monte Carlo permutations identified three significant ($p \leq 0.10$) environmental variables (DIC, Conductivity, and NO_3/NO_2-) which explained the variance in species data and these were retained for ordination (Figure 2.11). The variance inflation factor was <7 for all variables indicating no excessive co-linearity between retained variables. The genus-environment correlation between genus axis 1 and canonical axis 1, and genus axis 2 and canonical axis 2 was 0.850 and 0.604, respectively. The cumulative percentage variance of genus data and that

of the genus-environment relations was 72.5% and 95.3% for the first two canonical axes and did not appreciably improve with additional higher order axis (Table 2.8). Tables 2.9 and 2.10 contain further characteristics of the fitting of the environmental and genus variables respectively to RDA canonical axis.

The relative abundance of *Planktothrix* was correlated with high water column dissolved nitrates (NO_3/NO_2-F). *Aphanizomenon* was relatively more abundant during times of low water column dissolved nitrates and conductivity and higher water column DIC content. Similarly, *Pseudoanabaena* and to a lesser extent *Limnothrix* were more abundant during times of higher water column DIC and lower water column dissolved nitrates. The variance in *Lyngbya* and *Dolichospermum* abundance was not well correlated to the environmental data set (Table 2.10, Figure 2.11).

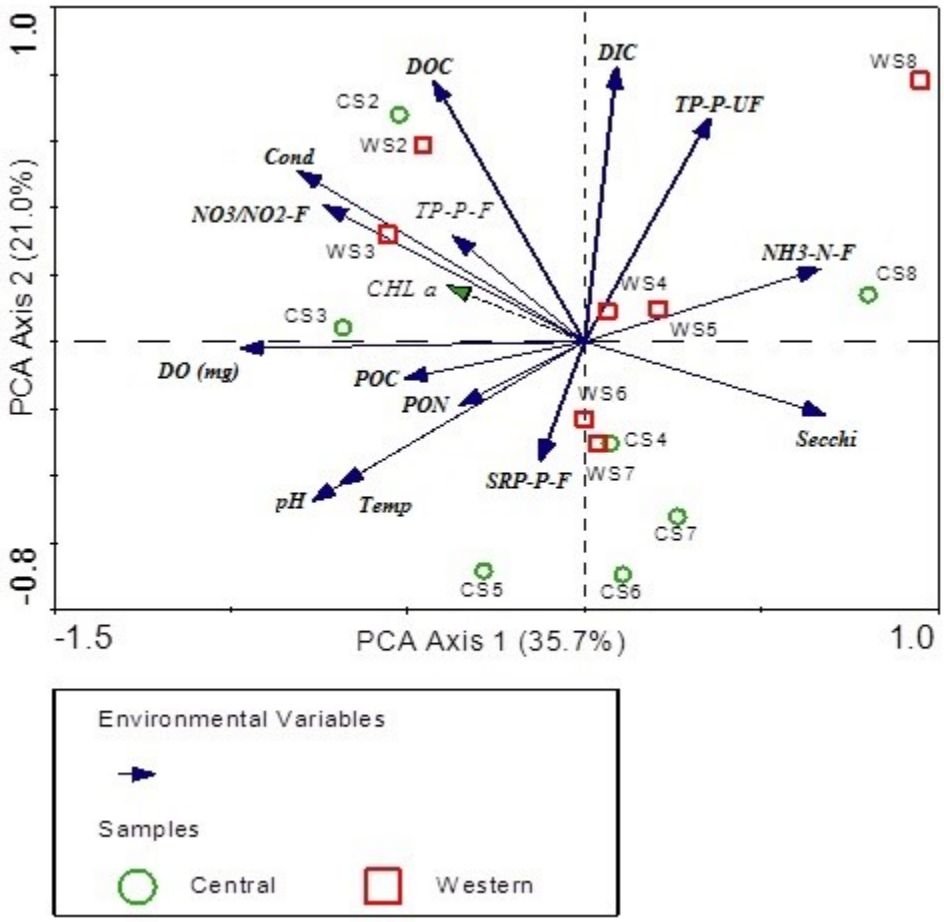

Figure 2.10. Principal components analysis (PCA) of water quality data collected in 2009 from Hamilton Harbour central (1001; circles) and western (9031; squares) stations. The symbol (i.e. circle) followed by a letter-number combination (i.e. CS2) denotes the sampling date; 2=July 7, 3=July 20, 4=Aug 6, 5=Aug 17-24, 6=Aug 31, 7=Sept 16, 8=Oct 8. Environmental variables codes: CHL *a*-Chlorophyll *a* (added passively to the analysis); Cond-Conductivity; DIC-Dissolved inorganic carbon; DO-Dissolved oxygen; DOC-Dissolved organic carbon; NH_3-N-F-Ammonia nitrogen filtered sample; $NO_3/_2$-F-Nitrate/nitrite nitrogen filtered sample; POC- Particulate organic phosphorus; PON-Particulate organic nitrogen; Secchi-Secchi depth; SRP-P-F-Soluble reactive phosphorus-phosphorus-filtered; Temp-Temperature; TP-P-F-total phosphorus phosphorus filtered sample; TP-P-UF-total phosphorus phosphorus unfiltered sample.

Table 2.8. Redundancy Analysis (RDA) summary results showing eigenvalues, genus-environment correlation for each canonical axis and the cumulative percentage variance of genus data and genus-environment relation of first four RDA axes

	Axis 1	Axis 2	Axis 3	Axis 4
Eigen values	0.379	0.120	0.024	0.261
Genus-environment correlations	0.850	0.604	0.548	0.000
Cumulative percentage variance of genus data	37.9	49.9	52.3	78.5
Cumulative percentage variance of genus-environment relation	72.5	95.3	100.0	0.0

Table 2.9. RDA regression coefficients, interest correlations and associated t-values for retained environmental variables for RDA Axis 1 and 2

	Regression coefficients		Interset correlations		*t* values	
Environmental variables	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Cond	0.786	-2.444	-0.146	-0.236	0.305	-0.95
NO_3/NO_2-F	-1.243	2.066	-0.429	-0.056	-0.498	0.8268
DIC	0.649	-0.454	0.664	0.179	0.575	0.705

Table 2.10. Cumulative fit per genus as fraction of variance of genera explained from RDA canonical axis.

Fraction fitted	Axis 1	Axis 2	Axis 3	Axis 4	% total variance explained
PL	0.557	0.558	0.581	0.778	58.13
APZ	0.395	0.501	0.534	0.918	53.35
LM	0.1784	0.505	0.517	0.759	51.69
PA	0.172	0.1739	0.187	0.589	18.71
DL	0.041	0.104	0.211	0.265	21.08
LB	0.046	0.08	0.106	0.107	10.55

Legend for cyanobacterial genera abbreviations: APZ = *Aphanizomenon*
DL = *Dolichospermum*; LB = *Lyngbya*; LM = *Limnothrix*; PAN = *Pseudoanabaena*.

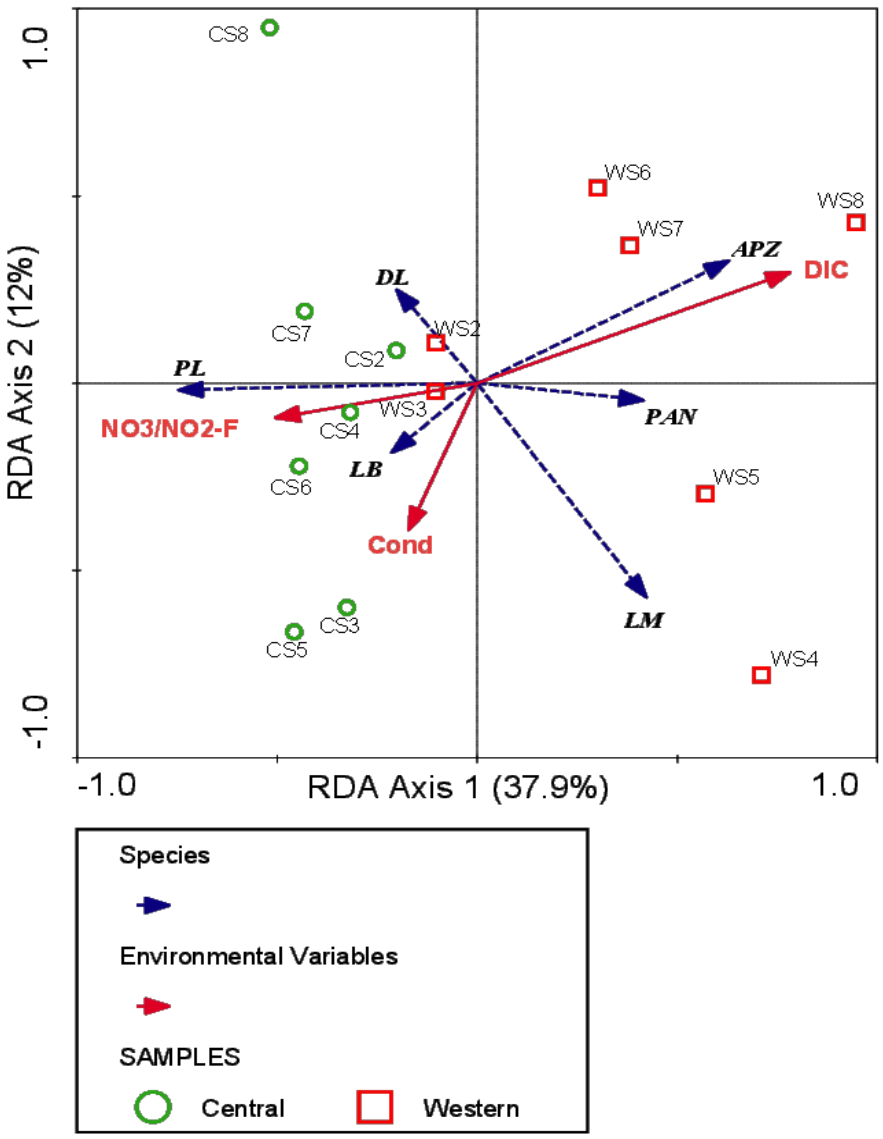

Figure 2.11. Redundancy analysis (RDA) of selected environmental variables (solid arrows) and abundance of filamentous cyanobacterial genera (dashed arrows) from water sampled in 2009 from Hamilton Harbour central (1001; circles) and western (9031; squares) stations. The symbol (i.e. circle) followed by a letter-number combination (i.e. CS2) denotes the sampling date; 2=July 7, 3=July 20, 4=Aug 6, 5=Aug 17-24, 6=Aug 31, 7=Sept 16, 8=Oct 8. The cyanobacterial genera are as follows: APZ = *Aphanizomenon;* DL = *Dolichospermum;* LB = *Lyngbya;* LM = *Limnothrix;* PAN = *Pseudoanabaena;* PL = *Planktothrix.*

2.4. Discussion

The phytoplankton assemblages both in marine and freshwaters are diverse, and are an ecological response to naturally dynamic environments (Reynolds, 2012). Species composition of phytoplankton assemblages are shaped predominantly by local environmental conditions (Beisner *et al.,* 2006). As local environment influences nutrient/food uptake, competition, and risk of predation, the environment also indirectly controls growth, reproduction, movement, and survival of the individual organisms so that all ecological influences are spatial in their nature (Franklin and Mills, 2007).

In studies of freshwater phytoplankton, special attention has been directed toward large colony-forming Cyanobacteria because they commonly dominate the phytoplankton in eutrophic lakes (e.g. *Anabaena/Dolichospermum, Aphanizomenon, Oscillatoria and Microcystis)* (Reynolds, 1984; Smith, 1986). Certain cyanobacterial species are typically found together in the epilimnion due to local conditions such as water turbulence and oxygen conditions (Dokulil and Teubner, 2000). The epilimnetic group consists of turbulent species (*Limnothrix redekei*, *Planktothrix agardhii*) and species that prefer high water column stability (*Microcystis* spp.) (Reynolds, 2006). Genera such as *Aphanizmenon* and *Microcystis* are usually more frequent in warm and stable water column (Dokulil and Teubner, 2000), and can also migrate vertically in search for the best environmental conditions, mostly light and nutrients (Reynolds, 1984). However, the cyanobacterial tolerance for site conditions is not species–specific, since the species like *Microcystis viridis*, *Planktothrix agardhii*, *Limnothrix planctonica*, and *L. redekei*, can both occur in the epilimnion, in turbulent and aerobic conditions, and in the metalimnion, in more stable, anaerobic conditions (Wojciechowska *et*

al., 2004).

Environmental factors that also affect phytoplankton community production and biomass are nutrients and their availability (Jackson and Hecky, 1980; Carpenter *et al.*, 1998). Different concentrations of nutrients differently affect phytoplankton. Most long term research of nutrients effect on cyanobacterial blooms has been done on limiting nutrients, nitrogen (N) and phosphorus (P), and their ratio in water. Phosphorus often controls the proliferation of freshwater cyanobacterial blooms because many cyanobacteria have the ability to fix nitrogen (Paerl, 1988; Paerl *et al.*, 2001). However, many bloom forming cyanobacterial genera that can not fix nitrogen may be nitrogen limited (Davis *et al.*, 2010), and it has been hypothesized that both N and P control harmful cyanobacterial blooms (Paerl *et al.*, 2008; Paerl and Huisman, 2009). Both of these elements have inorganic and organic pools in water. Inorganic nitrogen is present in the forms of nitrate (NO_3), nitrite (NO_2), ammonia (NH^{4+}), or gaseous atmospheric di-nitrogen (N_2) (Kalff, 2002). Phosphorus (P) can be found as inorganic dissolved phosphorus (PO^{4-}) or bound in either organic (*i.e.* phospholipids) or inorganic (*i.e.* insoluble phosphate-iron flocs) compounds (Kalff, 2002). Much of the soluble N and P pools consist of various organic dissolved forms (Kolowith *et al.*, 2001) which many cyanobacteria can use as important nutrient sources (Glibert and O'Neil, 1999; Davis *et al.*, 2010). Inorganic carbon is essential to normal photosynthetic production, and its supply is one of the several critical factors in development of algal blooms in response to lake enrichment (Kuentzel, 1969). Inorganic carbon in the aquatic environments is plentiful and renewable and is not a limiting nutrient (Reynolds, 1986). Dissolved inorganic carbon (DIC) occurs in water as carbon dioxide, bicarbonate, and

carbonate. However, dissolved carbon dioxide concentrations are highly variable and can be separated from the pH-dependent bicarbonate system (Reynolds, 1986). Typical eutrophic phytoplankton species, including many Cyanobacteria, can maintain growth at relatively high pH and low concentrations of free carbon dioxide in water (Reynolds, 1986). Cyanobacteria are able to use both free CO_2 and bicarbonate ions as inorganic carbon sources in photosynthesis, and this ability gives them a competitive advantage over other phytoplankton (Pick and Lean, 1987). That is why carbon may influence the relative proportion of cyanobacteria (Shapiro, 1973), migration behaviour (Klemer *et al.*, 1982) and scum formation (Paerl and Ustach, 1982).

Many culturally eutrophic embayments of the Laurentian Great Lakes are prone to summer algal and cyanobacterial blooms. Lake Erie experienced toxic cyanobacterial blooms since 1990s (Brittain *et al.*, 2000), and Bay of Quinte (Lake Ontario) also has a long history of eutrophication (Nicholls, 1999). A study of cyanobacterial community composition monitored during the summer months in 2006 (Chunn, 2007), confirmed that Bay of Quinte (Lake Ontario) and Maumee Bay (Lake Erie) had potentialy hepatotoxic Cyanobacteria present. At both sites increases in cyanobacterial cell concentration were observed from early to late summer, with Bay of Quinte containing higher concentration of cyanobacterial cells than Maumee Bay (Chunn, 2007). Also, toxin microcystin was detected and its concentrations corresponded to the increase in hepatotoxic cell concentrations. However, the research did not take into consideration environmental factors that could have affected seasonal changes in the community structure, the occurrence of blooms and microcystin production.

2.4.1. Seasonal distribution of planktonic cyanobacterial assemblages and filamentous cyanobacterial abundance in Hamilton Harbour

Hamilton Harbour is a highly stressed system, subject to strong physical disturbances (Munawar and Fitzpatrick, 2007). In this study, rapidly changing cyanobacterial assemblages emphasized the seasonal differences in composition of the communities and abundance of filamentous Cyanobacteria. Based on the presence of different genera over the sampling period, the identified dominant filamentous cyanobacterial genera in Hamilton Harbour were *Planktothrix*, *Aphanizomenon* and *Limnothrix*. The potentially toxic *Planktothrix* is not widespread in Lake Ontario according to Hotto *et al.* (2007), while *Aphanizomenon* has been identified as the bloom-forming genus and one of dominant microcystin producers in Hamilton Harbour in 2001 (Murphy *et al.*, 2003). To date, *Limnothrix* has not previously been identified as a bloom-forming genus in Hamilton Harbour. The dominant coccoid colonial cyanobacterial genus was *Microcystis*, which is in agreement with previous findings for Hamilton Harbour (Murphy *et al.*, 2003; Allender *et al.*, 2009) and Lake Ontario (Hotto *et al.*, 2007). Of the four dominant genera, three were potential microcystin producers (*Microcystis, Planktothrix* and *Aphanizomenon*).

The results of the analysis of the planktonic cyanobacterial assemblages at central station (1001) at 1m depth indicated that *Microcystis* and *Planktothrix* were dominant plankton cyanobacterial genera, with co-dominance with *Limnothrix* (Table 2.2, Figure 2.4). Integrated over the epilimnion, this station showed greater diversity of filamentous genera, as well as the presence of akinetes and heterocysts in *Dolichospermum* on one date (August 31).

The results of the western station cyanobacterial analysis at 1 m depth (Table 2.4,

Figure 2.6) showed the greatest richness in filamentous cyanobacterial genera. The bloom of the genus *Limnothrix* coincided with the highest water temperature during the sampling period (21.4 ^{o}C), concentrations of total phosphorus, particulate organic nitrogen, dissolved inorganic carbon, and ammonia very close to the mean values for the sampling period, and concentrations of chlorophyll *a*, dissolved organic carbon, nitrate/nitrite, dissolved oxygen, and water transparency slightly below mean values. Under conditions of generally high nutrient supply, it would have been possible for other cyanobacterial genera to bloom as well. Therefore, the main chemical factors that may have promoted a bloom of *Limnothrix*, could not be determined. However, *Limnothrix* dominance at that time could have been caused by the influence of wind-induced increased turbulence and high irradiance levels as well as the selective grazing of larger zooplankton. The occurrence of akinetes in *Dolichospermum* was observed on July 20, heterocysts on August 6, and on September 16 both akinetes and heterocysts were observed in the filaments. Irregular occurrences of cyanobacterial heterocysts could be reactions to ammonia concentrations on previous dates. The concentrations of ammonia (NH_3) were indeed very low (<0.005 mg/l) on July 7 and July 20, and that could have been a trigger for heterocyst differentiation; the same could be true for September 16, since the concentration of ammonia on a previous sampling date was 0.006 mg/l (Table 2.8). This finding is consistent with literature data that heterocyst formation in Cyanobacteria is stimulated in conditions of combined nitrogen deprivation (Wolk, 1996; Adams, 2000).

A decrease in NH_3 concentration in the water column before the observed heterocysts presence (August 31) may also have been the cause for their differentiation observed in the

integrated samples at CS. Plankton respond to temperature and nutrient changes in a particular system over a 3–7 days period (Lampert and Sommer, 2007) and an increase in the number of heterocysts can be a natural response for organisms sensitive to NH_3 concentrations in the water column.

The presence of akinetes of planktonic *Dolichospermum* at the western station was recorded on two occasions: in the early summer (July 20) and in the late summer (September 16). The abundance of akinetes in filaments was higher in July than in September indicating more intensive akinete production at the beginning of the season. However, the relative abundance of filaments in the water column was higher in late summer (3.4%), compared to abundance in July (2.4%). Environmental factors which are known to initiate akinete formation are a decrease in light intensity and water temperature, nutrient depletion, the breakdown of thermal stratification, grazing pressure and/or microbial attack (Paerl, 1988). Other triggers include high light intensity and super-saturated oxygen concentrations (Baker, 1999). An increase in dissolved oxygen concentrations during the 20 day period (June 30-July 20) could have triggered akinete production in *Dolichospermum*, and the continual decrease in the same environmental variable could have depressed production of akinetes on the later date. This conclusion is in accordance with findings of Kravchuk *et al.* (2006), who observed a one-week lag response of *Dolichospermum* cells to the environmental factors, such as water temperature, dissolved oxygen content, inorganic phosphorus concentration and pH.

The observed discontinued occurrence of the genus *Aphanizomenon* at western station (1 m depth), when it was absent on August 31 (Figure 2.6) but present on the presiding and

following sampling dates, could be a consequence of physical disturbance (wind, cargo ships) and/or micro patchiness of available nutrients. Also, since the sampling is only a snapshot of the community in time, absence/presence of some genera could be an indication of physical disturbance of the system at the time of sampling.

2.4.2. Seasonal abundance of *Microcystis* in Hamilton Harbour

In the present study, the relative abundance of *Microcystis* stayed below 10 % throughout the study period. The lowest relative abundance of *Microcystis* in the phytoplankton population was lowest on June 30 at CS at 1 m depth (Figure 2.7), even though the concentrations of nutrients such as NO_3/NO_2, NH_3-N-F, DOC, DIC, SRP-P-F, TP-P-F and TP-F-UF were the highest recorded in the season, and the water temperature was relatively high. The reason for low abundance could have been due to the fact that part of *Microcystis* population overwinters vegetatively in the sediment and has to be repopulated to the water column before establishment of the summer bloom (Verspagen *et al.*, 2004). A study on the Lake Volkerak (Netherlands) recruitment of benthic *Microcystis* to the water column showed that in deeper parts of the lake colonies on the sediment surface (beyond 5m depth) were almost in the dark and in anoxic conditions (Verspagen *et al.*, 2004) and that extended periods of darkness caused degradation of light-harvesting complexes and photosynthetic reaction centers in phytoplankton (including Cyanobacteria) and macroalgae (Murphy and Cowles, 1997; Lüder *et al.*, 2002). The consequence of this decreased photochemical vitality of *Microcystis* colonies may have been the reduced *Microcystis* ability to repopulate the water column (Verspagen *et al.*, 2004). On the other hand, physiologically

less damaged colonies from shallower parts of the lake were better adapted to inoculate the water column than colonies from deeper sites (Verspagen *et al.*, 2004). In the case of Hamilton Harbour, it is probable that newly recruited benthic *Microcystis* colonies had to be transported to the central part of the harbour from shallower locations and that with the progression of the season, transported and benthic colonies from the central station both acted as an inoculum for the epilimnetic *Microcystis* population. Also, the lowest transparency of the season (value of Secchi depth was 1 m) on the June 30 might have been a contributing factor to the lowest relative abundance of the *Microcystis*. Finally, the influence of physical disturbance of the location at the time of sampling should be taken into consideration when discussing lowest relative abundance of the season. The integrated sample from the same date had much higher relative abundance of the *Microcystis* (8.6 %) (Figure 2.8). Higher relative abundance could have been caused by less turbulent conditions in deeper parts of the water column, which are beneficial for development of *Microcystis* colonies.

The highest relative abundance of *Microcystis* was recorded on August 17-24 at the CS in integrated water sample, when it reached almost 10% of the total phytoplankton population (Figure 2.8). This abundance coincided with the warmest water temperature recorded during the season (22.2 ^{o}C) and nutrient concentrations close to or below average values for the season. A well established epilimnetic *Microcystis* population and approximately 40% lower phytoplankton population numbers (compared to the season average) most probably contributed to the highest relative abundance of *Microcystis*.

The phytoplankton community of the harbour was highly abundant throughout the season, which was expected for a eutrophic ecosystem such is Hamilton Harbour. Throughout the study period, the dominant algal groups were Chlorophyta and Bacillariophyta, followed by Dinophyta and Cryptophyta but were not identified to lower taxonomic levels nor enumerated as separate groups. A seasonal peak in the abundance of total algae of 45819 x 10^3/l algal units was recorded on August 17-24 at western station at 1 m depth, when *Limnothrix* bloom was also observed. Statistically significant differences in algal unit abundances found between central and western stations indicate that local seasonal differences in environmental conditions could have influenced algal densities at two locations in the harbour.

Even though pico cyanoplankton have not been identified in the present study, it is worth noting that Chroococcoid Cyanobacteria (pico and nano) have an important role in primary production in Lake Ontario (Caron *et al*, 1985), and are responsible for almost 38% of primary production of the lake (Caron *et al*, 1985). Carrick and Schleske (1997) showed that picoplankton production constituted on average 30% of total phytoplankton production in Lake Apopka (Florida), and that the most abundant taxon was *Synechococcus* sp.

2.4.3. Physico-chemical parameters in Hamilton Harbour during 2009 summer-fall season

Hamilton Harbour is an extremely degraded waterbody with contaminated sediments and high nutrient levels. As a major industrial port with a long history of pollution from industrial and municipal waste (Dermott, 2007), Hamilton Harbour was identified as an Area of Concern (IJC, 1988) and Remedial Action Plan targets for water clarity, phosphorus,

ammonia, and chlorophyll concentrations were set (RAP, 1992). Results of the water quality analysis are shown in Tables 2.5-2.7.

Despite an initial decline of the surface TP concentrations in the period between 1987 and 1997 that followed the remediation process of Hamilton Harbour, TP concentrations have not improved much since then. An average TP concentration of 39.7±3.4 μg $l^{-}1$ has been recorded between 1997 and 2012 (Hiriart-Baer *et al.*, unpublished data). In the 2009 summer-fall season, Hamilton Harbour was eutrophic, as mean total phosphorus (TP-UF) values at both stations were approximately 43.0 μg l^{-1} (42.2±8.2 μg l^{-1} for CS and 44.0±12.7 μg l^{-1} for WS) (Tables 2.6 and 2.7). During the sampling season the TP minimal concentration of 32 μg l^{-1} was well above 10-15 μg l^{-1} that is considered a threshold concentration after which the cyanobacterial development can be explained by physical factors (water column stability, for instance) (Steinberg and Hartmann, 1988). In this study, phosphorus was not likely the limiting factor of cyanobacterial growth.

Soluble reactive phosphorus (SRP) as the inorganic form orthophosphate is the measure of biologically available phosphorus (used by phytoplankton and bacteria during photosynthesis). Its two concentration peaks (7.01 μg l^{-1} and 4.2 μg l^{-1}) were recorded at the beginning and the end of the sampling period (early summer and fall) for the central station, most probably linked to spring/fall overturn periods. For the western station, however, only one peak (5.3 μg l^{-1}) was recorded at the beginning of the sampling season. The average chlorophyll *a* concentrations at both stations were higher than the water quality target of 10 μg l^{-1} indicating high total algal biomass and phytoplankton density during the season. Although no significant change has been observed for surface water chl *a* concentrations

since 1987 (Hiriart-Baer *et al.*, 2009) a recent study has suggested that light limitation, rather than supply of nutrients (especially phosphorus), may be of greater importance for chl *a* production in Hamilton Harbour (Pemberton *et al.,* 2007). However, taking into consideration that algal growth depends on the interplay of many environmental factors, and that a positive long term correlation between decrease in chl *a* and decrease in TP was observed (Hiriart-Baer *et al.*, 2009), it is certain that nutrient load reductions (phosphorus in particular) would be an effective strategy for algal growth limitation (including Cyanobacteria) and improvement of water quality conditions in the harbour.

Currently, there are no water quality targets set for nitrate or nitrite concentrations. Nitrate/nitrite (NO_3/NO_2) concentrations in Hamilton Harbour have been increasing since 1987 (Barica, 1990), and at an exponential rate since 1998 (Hiriart-Baer *et al.*, 2009). The average filtered NO_3/NO_2 concentrations of 2.06 mg/l (central station) and 1.85 mg/l (WS) mg/l were much below the limit of 13 mg/l set by the Canadian Water Quality Guideline, but were approximately four fold higher than mean concentrations of the Lake Ontario surface water (0.5 mg/l) in 2007 (Dove, 2008). Compared to the long term results of NO_3/NO_2 concentrations in surface waters of the harbour for the period 1987-2007 (Hiriart-Baer *et al.*, 2009), average concentrations for the 2009 season were very similar to those observed for long term seasonal averages.

The average NH_3-N concentrations in 2009 were 0.027 mg/l and 0.030 mg/l, for the central and western stations, respectively. The long-term trend suggests that total NH3 values in surface waters of Hamilton Harbour are on the decline since 2000 (Hiriart-Baer *et al.*, 2009).

The mean seasonal Secchi disc depths (water clarity) in the harbour have generally improved since 1987, with an increasing trend in water clarity until 1997 (Hiriart-Baer *et al.*, unpublished data). Since then, no improvement in water clarity has been observed and Secchi disc depths remain below the water quality target of 2.5 m. However, during 2009 season, the mean Secchi depth for central station was 2.5 m, meeting the water quality target.

Conductivity for both stations decreased as the season progressed. During the winter Hamilton Harbour receives a high road salt loading from Redhill Creek that greatly elevates conductivity values in surface waters (Hamblin and He, 2003). Higher readings at the beginning of the season were probably due to the flushing of the remaining road salt used over the winter. On the other hand, lower values observed later in the season were likely in part a result of the diluting effect of Lake Ontario water that enters the harbour through ship channel, as well as from inputs from creek and wastewater treatment plants. According to long term monitoring data, an increase in surface conductivity has been observed in the harbour since 2003 (Hiriart-Baer *et al.*, unpublished data).

2.4.4. Statistical correlations between filamentous cyanobacterial abundance and environmental factors

Principle components analysis (PCA) revealed that approximately 67% of the variation within the 2009 water quality data was due to seasonal trends in water quality which were represented by PCA Axis 1 and 2 (Figure 2.10). The early summer water samples were characterized by higher specific conductivity, nitrates, dissolved oxygen, DIC, DOC, TP-F and TP-P-UF and in general, as the season progressed, there was a relative decline in these parameters while there was a relative increase in Secchi depth, ammonia (NH3-N) and

soluble reactive phosphorus (SRP-P-F).

Redundancy analysis (RDA) found a good correlation between environmental gradients (water quality) and the relative abundance of *Planktothrix, Aphanizomenon* and *Limnothrix*; water quality parameters explained a significant portion of the observed variability in these genera. On the other hand, water quality gradients were only moderately successful in accounting for the variability observed in the abundance of the filamentous cyanobacteria *Pseudoanabaena, Lyngbya* and *Dolichospermum* (Table 2.10). 53.35% total variance of *Aphanizomenon* was correlated to low water column dissolved nitrates (NO_3/NO_2) and conductivity, and higher water column DIC (Figure 2.11, Table 2.10). The co-occurrence of higher relative abundances of *Aphanizomenon* and low dissolved nitrate concentrations toward the end of the season can be related to the ability of *Aphanizomenon* to fix nitrogen during N limited periods (Smith, 1983, 1990; Schindler *et al.*, 2008). Contrastingly, the relative abundance of *Planktothrix* was correlated with high concentrations of dissolved nitrates (58.13%) in the water column. As a genus incapable of N_2 fixation, *Planktothrix* would benefit from higher water dissolved nitrate concentrations. Seasonal trends in lower conductivity toward the end of the season can be associated with decrease in nutrients during the stratified period. Lower primary production can lead to an accumulation of DIC concentrations as consumption is reduced, which is generally beneficial for the growth of phytoplankton. Other non-N_2 fixing genera, *Pseudoanabaena* and *Limnothrix*, were more abundant during times of higher DIC and lower water column dissolved nitrate concentrations. The possible explanation for the high relative abundance of these two genera during the low nitrate concentration period is “luxury consumption of nutrients”. This

phenomenon refers to the absorption and storage of non-limiting nutrients within plant cells, when nutrients are freely available (Reynolds and Walsby, 1975; Oliver *et al.*, 2012). The internally stored nutrients can sustain algal, including Cyanobacteria, growth over two or three doublings despite the apparent exhaustion of nutrients in the water column (Reynolds and Walsby, 1975). The correlation between environmental variables and species abundance was 2.7 times higher for *Limnothrix* (51.69 %) compared to *Pseudoanabaena* (18.71 %). The lack of correlation between the abundance of *Dolichospermum* and any of the three analyzed environmental parameters indicates that other environmental factors were also affecting its growth As a N_2-fixer it should be tolerant of low nitrogen conditions and grow better than non-fixers, which was not the case. However, it does show this species has a tolerance to low DIC and is more abundant towards the end of the season.

2.5. Conclusions

Seasonal variability in distribution of the cyanobacterial genera and abundance of colonial *Microcystis* and filamentous Cyanobacteria from phytoplankton samples was observed. During the 2009 sampling season fourteen cyanobacterial genera belonging to six families and three orders were identified. The dominant genera in Hamilton Harbour were *Microcystis*, *Planktothrix*, *Aphanizomenon* and *Limnothrix.* Genus *Microcystis* was the only genus present on both stations throughout 2009 sampling season. Its abundance varied from less than1% to almost 10%. Results of the statistical analyzes showed that the abundance of filamentous genera was primarily correlated with water quality parameters NO_3/NO_2 and DIC. Positive correlations were found between high concentrations of dissolved nitrates

NO_3/NO_2 and genera *Planktothrix and Dolichospermum.* High concentrations of DIC correlated positively with abundances of *Aphanizomenon* and *Limnothrix.* Water quality parameters for the summer-fall season showed that Hamilton Harbour was a eutrophic ecosystem during that time based on high concentrations of TP. Concentrations of other water quality parameters generally followed the pattern of a long term trend for Hamilton Harbour.

Chapter 3

140 years of Hamilton Harbour phytoplankton history inferred from analysis of sedimentary pigments

3.1. Overview

3.1.1 Sediments of Hamilton Harbour

Sediments are depositories of biological and physical detritus as well as sinks for different chemicals (Hoagland *et al.*, 1996). The settling of different sized particles out of the upper water column to the bottom water layers is referred to as primary flux (Bloesch, 1994), while the redistribution of previously settled sediment particles back into the water column is referred to as the secondary flux (Bloesch, 1994). Sediment resuspension may take place after initial deposition due to strong winds, dredging operations (Bloesch, 1994), sediment focusing (lateral sediment transportation of fine material from shallower waters into the profundal zone) (Davis *et al.,* 1984) and bioturbation (mixing of sediments due to activity of organisms, by burrowing and/ or ingestion) (Bloesch, 1994). As an integral component of the aquatic ecosystem, sediments provide many aquatic organisms with habitat including areas to feed, spawn and rear young. However, sediments are also a repository for contaminants that originate from a number of sources (e.g. atmospheric deposition, urban/agricultural runoff, municipal/industrial discharges, port operations) and a potential source of pollutants to aquatic organisms and ultimately human consumers through food or water consumption

(Catallo *et al.*, 1995).

Hamilton Harbour sediments properties

Hamilton Harbour is one of the most severely degraded areas in the Laurentian Great Lakes (Wolfe *et al.*, 2000). Elevated level of metals and organic compounds in the harbour's bottom sediments was one of the reasons for the Water Quality Board of the International Joint Commission to identify Hamilton Harbour as a Great Lakes Area of Concern (AOC) (IJC, 1985).

Hamilton Harbour bottom sediments have been excessively disturbed both by anthropogenic processes (dredging, dumping and shipping) (Holmes, 1986; Rukavina *et al.*, 1994) as well as nutrient and chemical pollution (sewage and industrial discharges) (Mayer and Johnson, 1994). At the present, sediments are highly contaminated as the result of both past and present day contaminant loadings of heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls (Dermott and Bonnell, 2010). Physically, sediments of Hamilton Harbour show differences depending on location (Rukavina and Versteeg, 1996). In depths of less than 8 m, nearshore sediments mostly consist of sand (70%) and silt and clay in equal proportions (30%); the central basin deep-water sediments are predominantly muddy, predominantly made of silt and clay (Rukavina and Versteeg, 1996). Results of the mapping of Hamilton Harbour sediments suggest that the entire deep-water (8 - 25 m depth) area of the harbour is covered with black fine-grained sediments (Poulton, 1987; Rodgers *et al.*, 1992; Rukavina and Versteeg, 1996).

Extracts of Hamilton Harbour sediments are a complex mixture of organic compounds, many of which are pollutants (Balch *et al., 1*995) that consistently exceed

regulatory guidelines (Harlow and Hodson, 1988). The pollution history of the harbour sediments have been well-researched and high levels of chemical contaminants such as heavy metals (Nriagu *et al.,* 1983; Mayer and Johnson, 1994), polychlorinated biphenyls (PCBs) (Metcalfe *et al.,* 1990*;* Marvin *et al.,* 2000), organochlorine compounds (Poulton, 1987), polycyclic aromatic hydrocarbons (PAHs) (Mayer and Nagy, 1992; Murphy *et al.*, 1990) and coal tar (Murphy *et al.*, 1990) have been extensively documented. All of these pollutants are potential genotoxins that can induce mutations and cancer through chemical interaction with cellular DNA (Maurici *et al.*, 2005). Genotoxic compounds associated with sediments can have adverse effects on aquatic biota even at levels that are non-acute (Kosmehl *et al.*, 2004) and have been investigated by several research groups. For instance, Metcalfe *et al.* (1988) used contaminated sediment from the harbour to experimentally induce liver tumors in rainbow trout (*Salmo gairdneri*). Metcalfe *et al.*, 1990 also confirmed carcinogenic and genotoxic effects of Hamilton Harbour sediment extracts by inducing malignant hepatic tumors in rainbow trout (*Oncorhynchus mykiss*). Balch *et al,* (1995) demonstrated that organic extracts of Hamilton Harbour sediments might be responsible for the high prevalence of fish tumors in the harbour as demonstrated by an *in vivo* carcinogenicity assay with rainbow trout (*Oncorhynchus mykiss*). However, other organic compounds beside PAHs (such as nitrogen-containing aromatic compounds) may contribute to sediment carcinogenicity (Balch *et al.,* 1995), bioaccumulate in benthic and pelagic organisms (Morrison *et al.*, 1997) and extend this contamination to mammals and birds that feed on water-column biota (Mackay and Toose, 2004).

Siliceous fossils (diatoms and chrysophytes) of Hamilton Harbour sediments were investigated by Yang *et al.* (1993) and Duthie *et al.* (1996). Yang *et al.* (1993) conducted a study on the sedimentary siliceous fossils and showed that both the eutrophication and contamination of Hamilton Harbour were the outcomes of anthropogenic activities during the past 330 years, and that the harbour's historical environmental changes closely matched the environmental changes observed in Lake Ontario. The results of the study suggested that in the period between 1660-1770, Hamilton Harbour was a pristine oligotrophic environment. This conclusion was supported by findings of several paleolimnological studies of Lake Ontario offshore regions. In these studies nutrient enrichment (Schelske, 1991), effects of land use and cultural development (Sly, 1991) as well as ecological changes (Stoermer *et al.,* 1985) were investigated. Between 1770 and 1820, a large-scale settlement of the harbour's watershed caused the beginning of harbour environmental disturbance, and in this period, eutrophication coincided with early European settlers activities (deforestation, farming, etc.) (Yang *et al.*, 1993). The eutrophication trend was also recorded in Lake Ontario sediments shortly after 1770, to a lesser extent (Stoermer *et al.,* l985). In the period between 1820-1926 urban and industrial development continued, as was evident from increasing nutrient load and water contamination. The pollution of the harbour was highest in the early 1900s, based on trophic status inferred from diatoms and the abundance of polysaprobic taxa. The period between 1926-1961 was characterized by an abrupt increase in eutrophic taxa indicating that both eutrophication and water contamination continued to accelerate due to continual increases in industrial effluent and urban sewage. In the period between 1961-1970, Hamilton Harbour continued to be eutrophic. The sediment diatom record showed that the

1970-1986 period was characterized by the worst water pollution in history; a finding corroborated by published reports of greatly increased contaminant loads (IJC, 1972; IJC, 1985).

Limnological changes in Hamilton Harbour over the Holocene were investigated by Duthie *et al.* (1996) by analysis of parameters such as stratigraphic changes in lake pH, water level changes, changes in the trophic status, paleotemperature and other paleoclimatic factors. This study confirmed a marked anthropogenic influence on eutrophication trend in the last 280 years of the sedimentary record.

The benthic macroinvertebrate communities abundance and distribution in Hamilton Harbour were researched by Johnson and Matheson (1968). They noted that the fauna of the harbour was less diverse compared to that of Lake Ontario sediments (thirteen and thirty species, respectively). Out of thirteen species observed in the harbour nine were oligochaetes and four were chironomid larvae. The latter group was present mostly along the northern shore and occurred in very small numbers in shallower sediments far from the southern industrial shore. Two species of oligochaetes were abundant in the profundal sediments (*Limnodrilus hoffmeisteri* and *Tubifex tubifex*), while five *Limnodrilus* species occupied organically poorer sublittoral sediments, albeit in much lesser numbers. The presence of oligochaetes suggests that bioturbation is the dominant mixing process in the harbour surficial sediments (Mayer and Johnson, 1994) aiding to the aeration, oxygenation and homogenization of the sediments. In this way loosened sediment are easily transported to other parts of the harbour.

3.1.2. Sedimentary pigments

Pigments are chemical compounds present in all photosynthetic organisms that function as light harvesting agents for photosynthesis and photoprotection (Porra *et al.*, 1997). There are three principal classes of pigments: greenish chlorophylls; red, orange, or yellow carotenoids; and red (phycoerythrins) or blue (phycocyanins) phycobilins. Chlorophyll *a*, which absorbs blue (maximal absorption at 665 nm) and red light (465 nm) is the primary pigment in plants, algae, and Cyanobacteria. The light available to phytoplankton in the water column fluctuates during the day due to changes in solar radiation, cloud cover and surface reflectance (Reynolds, 2006). Hence, the presence of different accessory pigments in photoautotrophs allows light absorption at different wavelengths which in turn optimizes their carbon fixation at sub-saturating light intensities (Reynolds, 2006). For instance, accessory xanthophylls (present in Bacillariophyta, Chrysophyta, and Haptophyta) and phycobilins (present in Cyanobacteria, Rhodophyta, Glaucophyta and Cryptophyta), increase the harvesting of light in the middle parts of the visible spectrum (400 nm-700 nm) (Reynolds, 2006). By increasing the complement of accessory photosynthetic pigments, phytoplankton are able to widen the range in the absorbance spectrum so that they can effectively capture photons and transfer them to chlorophyll *a* (Reynolds, 2006). At the same time, the result of a widened spectral absorption is a shift in color known as 'chromatic adaptation' and the green color of chlorophyll is masked with blue, brown or purple (Tandeau de Marsac, 1977). The importance of this trait is reflected in the fact that due to changes in the proportions of different photosynthetic pigments, optimum absorption of the available wavelengths of light is enabled.

Since 1944, when fossil pigments were first isolated and identified from aquatic sediments (Fox, 1944; Fox *et al.*, 1944), fossil pigments were used as paleoenvironmental biomarkers due to their taxonomic specificity (Jeffrey *et al.*, 1997; Leavitt and Hodgson, 2001). Fossil pigments analyses provide a comprehensive indication of the past aquatic conditions, such as primary production and phototrophic community responses and status (Leavitt and Hodgson, 2001), and can be used to infer the course of long-term changes in algal and bacterial populations (Sanger, 1988; Leavitt and Hodgson, 2001) and past cyanobacterial blooms (Griffiths and Edmondson, 1975; Water, 1978; Zullig, 1981; Engstrom *et al.*, 1985; Poutanen and Nikkilä, 2001). Paleoenvironmental studies regarding the production (Vallentyne, 1957; Belcher and Fogg, 1964; Harris *et al.*, 1995) and composition of phototrophic communities in water environments (Züllig, 1981; Yacobi *et al.*, 1990; Schubert *et al.*, 1998), as well as past changes in the physical environment of lakes (Villaneuva *et al.*, 1993; Koopmans *et al.*, 1996; Hodgson *et al.*, 1997, 1998) used fossil pigments analyses.

Leavitt (1993) noted that more than 90% of pigments are degraded to colourless compounds before permanent sediment deposition. Hence, a small part of the water column phototrophic production actually ends up at the bottom of the water body and gets buried as the fossil record. However, preserved pigments and their derivatives are identifiable from sediments and sedimentary pigment concentration is directly proportional to abundance of algae in the water column (Guilizzoni *et al.,* 1983). Therefore, sedimentary pigments, as biological indicators contained within the fossil record, can be used as paleolimnological proxies. There are three identified primary phases of algal pigment degradation (Leavitt,

1993). The first is selective pigment loss in the water column and is the most rapid; the second is deposition of materials at the sediment-water interface shortly after sedimentation. The third phase of pigments degradation takes place in sediments and is much slower than the previous two phases. The first two processes have the greatest influence on recent sediments pigment concentrations. Generally, the loss of pigments in the sinking detrital material is affected by photooxidation, grazing by herbivores, cell senescence (Leavitt, 1993) and by bacterial, viral, and autolytic cell lysis (Bianchi and Findlay, 1991). Material deposited at the sediment-water interface also degrades but at a reduced rates, depending on the amount of pigment that was lost during the passage through the water column, chemical conditions of both the sediments and the water, and faunal composition of the interface (Hurley and Armstrong, 1991).

The primary factors that enhance pigment preservation in sediments are low temperature, light and oxygen concentration, low sediment resuspension, high sedimentation rates, absence of benthic organisms and increased eutrophy of the system (Sanger, 1988). Besides being markers of different algal, bacterial and plant taxa, pigments have unique degradation and transformation products such as isomers, allomers and the fragmented pieces of the original molecules that are useful indicators of past paleoecological conditions (Leavitt and Hodgson, 2001). Due to the ubiquitous presence of chlorophyll in photosynthetic organisms, chlorophylls and their derivatives commonly occur in the greatest abundance in freshwater and marine sediments (Sanger, 1988).

All groups of photosynthetic organisms (except for some bacteria) contain chlorophyll *a,* together with its derivatives pheophytin *a*, pheophorbide *a*, and chlorophyllide

a, is the most abundant pigment in sediments (Sanger, 1988). Other chlorophylls similarly occur in one derivative form. Carotenoids are also universally present in lake sediments and all photosynthetic organisms (Sanger, 1988). Some of the most common pigments that occur in the water column and sediments of the freshwater environments were reviewed by Leavitt and Hodgson (2001). According to these authors, β-carotene is the marker of plants, algae and some phototrophic bacteria (including Cyanobacteria), α-carotene is an indicator of some members of the Chlorophyta, Cryptophyta, Dinophyta and Chrysophyta; alloxantin of Cryptophyta; fucoxanthin of Bacillariophyta, Prymnesiophyta, Chrysophyta, and several Dinophyta; peridinin of Dinophyta; lutein of Chlorophyta, Euglenophyta and plants; diadinoxanthin of Bacillariophyta, Dinophyta, Prymnesiophyta, Chrysophyta, Raphidophyceae, Euglenophyta, and Cryptophyta; violaxanthin of Chlorophyta, Euglenophyta; diatoxanthin of Bacillariophyta, Dinophyta, Chrysophyta, while marker pigments of Cyanobacteria are canthaxanthin, zeaxanthin, echinenone, oscillaxanthin, aphanizophyll, and myxoxanthophyll (Leavitt and Hodgson, 2001). Chlorophylls and carotenoids are water-insoluble and membrane bound while anthocyanins and phycobilins, as water-soluble, are destroyed before deposition in sediments (Sanger, 1988).

Recently, fossil pigment analyses have been used for the assessment of anthropogenic effects such as urbanization and agriculture (Hall *et al.*, 1999); effects of climate on the water quality of the Northern Great Plains (Hall *et al.*, 1999) and on subarctic lakes in northern Sweden (Reuss *et al.*, 2010); cultural eutrophication of the Upper Klamath Lake (Oregon, USA) (Eilers *et al.*, 2004), Lake Okeechobee (Florida, USA) (Engstrom *et al.*, 2006), and Lake Peipsi (Estonia/Russia) (Leeben *et al.*, 2008). Sedimentary pigments were

also used in the reconstruction of past phosphorus concentrations (Guilizzoni *et al.*, 2011), primary production (Romero-Viana *et al.*, 2010), and main algal taxa (Borghini *et al.*, 2011) in lakes. Concentrations of the Cyanobacteria-specific pigments were used to estimate past occurrences and intensities of cyanobacterial blooms in the Baltic Sea (Poutanen and Nikkilä, 2001), Holocene cyanobacterial community structure (Fernandez-Carazo *et al.*, 2013), and for the estimation of total algal biomass changes and the proportion of Cyanobacteria before and after modern agriculture of the Sand Hills Lakes (Nebraska, USA) (Efting *et al.*, 2011). To our knowledge no research on sedimentary photosynthetic and/or cyanobacterial pigments has been attempted for Hamilton Harbour, and this study is the first one of this kind for that area.

The objectives of this study were:

a) sediment chronology establishment

b) inferring of stratigraphic distribution of past cyanobacterial blooms in Hamilton Harbour using cyanobacterial pigments presence and concentrations

c) inferring of past composition of photosynthetic communities in the Hamilton Harbour using distribution and concentration of other sedimentary pigments.

3.2. Materials and Methods

3.2.1. Sediment sampling and processing

A 100.5 cm long sediment core was retrieved from Hamilton Harbour on October 2009, from the deepest part of the harbour (Central station: N43.28750 -W79.83833) (Figure 1.2) using a gravity corer (diameter of a Plexiglas tube was 6.7 cm) at 23 m water depth. The

gravity core was sectioned into 0.5-cm stratigraphic intervals using a close-sectioning upright extruder. Core sections were stored in Whirlpak® bags and kept at -20 °C until further analyses. The sediment was analyzed for: LOI (loss on ignition), ^{210}Pb and other radioisotope activity, and presence of photosynthetic and cyanobacterial pigments.

3.2.2. LOI procedure

Sequential loss-on-ignition (LOI) analysis (Dean, 1974; Heiri *et al.,* 2001) was performed on subsamples (0.5 g wet sediment) of each sediment interval. Well-mixed, wet sediment subsamples were added to pre-weighed crucibles, dried at 90°C in an oven overnight (Fischer Isotemp® Oven Model 637). Samples were removed from the drying oven and allowed to equilibrate in a desiccator for ≥2 hours prior to weighing on mass balance (±0.0001g). After this, samples were combusted in a muffle furnace (Fischer Isotemp® Programmable Muffle Furnace) at 550°C for 3 hours, after which they were equilibrated in a dessicator prior to weighing. Similarly, the samples were then heated at 1000°C for 2 hours and weighed after a period of ≥2 hours in the dessicator. The loss in sediment mass after each heating/combustion step allowed for the determination of water content, weight percent of organic matter and carbonate content.

3.2.3. ^{210}Pb-based sediment core dating

The activities of ^{210}Pb, ^{226}Ra (via daughter products ^{214}Bi, ^{214}Pb), ^{137}Cs, ^{40}K, ^{238}U (via ^{234}Th) and ^{232}Th (via ^{228}Ac, ^{212}Pb) were measured on 63 sediment intervals (0.5 cm thick) spanning the 100.5 cm long sediment core using gamma-ray spectrometry. Approximately 2.0-5.0 g of freeze-dried sediment was weighed and packed into pre-weighed tubes

(SARSTEDT product No. 55.524) to a height of 35 mm. A TFA Silicone Septa (Supelco™) was placed overtop the sediment which was then sealed with 1 cm^3 of epoxy resin (Devcon® product No. 14310). Sealed samples were not analyzed until after 3 weeks had passed in order to establish equilibrium between ^{226}Ra and ^{222}Rn, which yields the daughter isotopes ^{214}Pb and ^{214}Bi. Samples were analyzed at the University of Waterloo gamma-ray spectrometry facility using an Ortec co-axial HPGe Digital Gamma Ray Spectrometer (Ortec GWL-120-15) and Maestro 32 software (version 5.32). Sample count time varied between 1-4 days depending upon sample activity and sample mass so that net ^{210}Pb gamma rays detected was ≈ 1000 or more events. See Hall *et al.* (2012) for further methodological details.

The Constant Rate of Supply (CRS) ^{210}Pb age model (Appleby, 2001; Sanchez-Cabeza and Fernández, 2012) was applied to the Hamilton Harbour sediment core. The CRS model is generally accepted as the most robust of the commonly used ^{210}Pb age models, and is especially suited for sites of variable sedimentation rates (Appleby, 2001; Sanchez-Cabeza and Fernández, 2012). The repeated dumping of large volumes of dredging spoils within the deep-waters of Hamilton Harbour complicates the creation of an accurate sediment age model as well as the general interpretation of the sediment record. The dredging spoils are effectively foreign sediment units inserted within the local sediment sequence accrued within the deep-waters of Hamilton Harbour. As they are also relatively young sediment likely to carry some unsupported ^{210}Pb activity, they serve to violate the assumption of constant unsupported ^{210}Pb flux to the coring location and will therefore lead to erroneous age model results. To circumvent this complication, CRS age model calculations were performed after identifying and removing portions of the sediment sequence judging to be dredging spoils

(see Wiklund *et al.*, in prep.). The anthropogenic fallout radionuclide ^{137}Cs activity with sediment depth was used as a stratigraphic age-maker to help assess the veracity of the ^{210}Pb-based age model.

3.2.4. Pigment analysis

Thirteen Hamilton Harbour sediment sub-samples from various depth intervals (Table 3.2.1) were analyzed for pigment concentrations using the reverse phase high performance liquid chromatography (HPLC) method described by Mantoura and Llewellyn (1983) and modified by Leavitt *et al.* (1989). 0.3-0.5 g sub-samples of the Hamilton Harbour core were freeze-dried prior to pigment extraction in 5 ml of extraction solvent mixture of acetone:methanol:water (80:15:5, by volume) (Leavitt *et al.*, 1989). After 24 hours each sample was filtered through a 0.22 µm Whatman PVDE filter and dried under a nitrogen stream. The dried pigments were recovered in 2 ml of calibration solution (acetone: IPR:ethanol, 70:25:5), containing 3.2 mg/l of Sudan II, as an internal standard. Pigment analysis was performed using WATERS 2695 HPLC system, at the University of Waterloo WATER Lab. Pigments were detected at 435 nm and 665 nm with spectra from 190–700 nm being collected continuously (0.5 sec intervals). Chromatograms were integrated using Empower™ 2 Chromatography Data Software (Waters Corporation, USA). Identification of individual pigments was based on a combination of retention time and absorbance spectra compared with authentic pigment standards obtained from DHI Water & Environment, Denmark or literature data (Wright *et al.*, 1991, Jeffrey *et al.*, 1997). Quantification of the pigments was done in a custom-made macro program including the Sudan II slope for corrections. Concentrations of pigments were expressed as mMol of pigment per gram of

organic matter (mMol/g OM). Organic matter content was determined as loss-on-ignition (LOI) after 3 h at 550°C for sediment samples. Identified pigments included pigments from algae (chlorophylls and their derivates and carotenoids) and Cyanobacteria, and pigment concentrations were graphed against sediment age using software C2 (Version 1.5., Newcastle University, Newcastle upon Tyne, UK).

Table 3.1. The age and depth of the sediment sub-samples taken for pigment analysis.

Pigment sample number	Sediment depth (cm)	Sediment age (year)
1	**5.25**	**2003**
2	**7.75**	**2000**
3	**10.25**	**1998**
4	**15.25**	**1992**
5	**20.25**	**1978**
6	**25.25**	**1968**
7	**30.25**	**1964**
8	**35.25**	**1957**
9	**40.25**	**1951**
10	**45.25**	**1940**
11	**70.25**	**1919**
12	**90.25**	**1904**
13	**100.25**	**1872**

3.3. Results

3.3.1. LOI results

Stratigraphic profiles of sediment LOI results and radioisotope activities are presented in Figure 3.1.1. Highly variable water content was observed down-the core showing strong covariations with other sediment bulk properties and naturally occurring radioisotopes. Changes in sediment properties shown in the sediment profile were large and repetitive, expected to be due to past sediment dredging events within Hamilton Harbour (Holmes, 1986). The practice of dumping dredging spoils from shallow, nearshore sediments in deep water would have the biggest influence on sediment properties (Holmes, 1986). Many coincident shifts between sediment water, organic and carbonate content are observable from the LOI stratigraphic profile (Figure 3.1.1). These shifts are consistent with the deposition of coarser-grained dredging spoils. Since the LOI profile was done on every sediment interval from the core, it was used for identifying occurrence of dredging spoils. Sediment intervals that showed ≥ 10% decrease in water content compared to the overlying interval presumably indicated the start of a dredging-spoil deposit. Likewise, sediment intervals that showed ≥ 10% increase in water content compared to the overlying sediment interval, presumably indicated the end of the dredging spoil deposit. If these observations were corroborated by a pronounced decline and then rise in unsupported ^{210}Pb activity, the sediment interval was considered as a confirmed dredging spoil. In the core, ten sub-sections were identified as dredging spoils. The thickness of the spoils ranged from as little as 0.5 cm to as much as 16 cm. The horizontal grey sections in Figure 3.1. A-C, denote sediment intervals identified as dredging spoils using the above approach. Eleventh suspected dredged spoil deposit found

between 98-99.5cm in the core was below the depth where it could be corroborated by unsupported ^{210}Pb activity.

3.3.2. ^{210}Pb chronology and sediment regime of Hamilton Harbour

The ^{210}Pb and ^{226}Ra stratigraphic profiles reconstructed from sediment sequences considered to be locally produced sediment are shown in Figure 3.2. The resulting total unsupported ^{210}Pb inventory in reconstructed stratigraphic profile was 87.55 dpm cm^2 ± 2.01(1 SD), which was 72% higher than total unsupported ^{210}Pb inventory expected from the relationship observed between direct annual atmospheric ^{210}Pb fallout and latitude in Canada (Muir *et al.,* 2009). Based on this result, an estimated sediment focusing factor value (ff) for the centre station coring site (in absence of dredging effects) was 1.72 ₤. This estimate provided some assurance that we have not been overzealous in identifying and removing dredging spoil sequences in the sediment record.

CRS-age model (Appleby, 2001; Sanchez-Cabeza and Fernández, 2012) results modelled from the reconstructed sediment record are shown in Figure 3.2. In CRS age model the dredged spoils intervals are treated as near instantaneous fluxes of sediments. The depth where supported ^{210}Pb (^{226}Ra) equals total ^{210}Pb activity is called background depth. The background depth for the core was found to be at 96 cm, and the time when sediment interval was deposited was calculated to be 1890.2 ± 9.3 years (2 SD). However, the age model error estimation (±2 SD) shown in Figure 3.1.2 is likely an underestimate of the true age uncertainty due to the need to identify and remove the effects of dredged spoils in the sediment record. The basal date of the core at 100.5 cm was inferred to be ~1869 assuming the sedimentation mass accumulation rate below 96 cm is equivalent to that observed in the

lower ^{210}Pb datable portion of the sediment core.

The ^{137}Cs activity profile from the Hamilton Harbour sediment core and the inferred dry mass sedimentation rate were compared to the recorded dredging history of Hamilton Harbour (Figure 3.3). The long-term average sedimentation rate for the center of the harbour was found to be 0.19 g m^{-2} $year^{-1}$ ± 0.06 (1 SD), with the exclusion of dredged spoils. Cesium 137 is a nuclear fission product first released into the environment in sizable quantities with the testing of the first thermonuclear weapon (November of 1952 by the USA). Following a large series of atmospheric thermonuclear weapon tests conducted by the Soviet Union in the early 1960s, ^{137}Cs peak fallout in North America occurred in ~1963 (Crusis and Anderson, 1995; Rhodes, 1995; Robins *et al.*, 2000; Appleby, 2001). The ^{137}Cs profile in Figure 3.1. appears to fit well with the expected timeline of ^{137}Cs fallout when allowances are given for the variable dilution of atmospheric fallout imparted upon the coring location by episodic dredged spoils disposal.

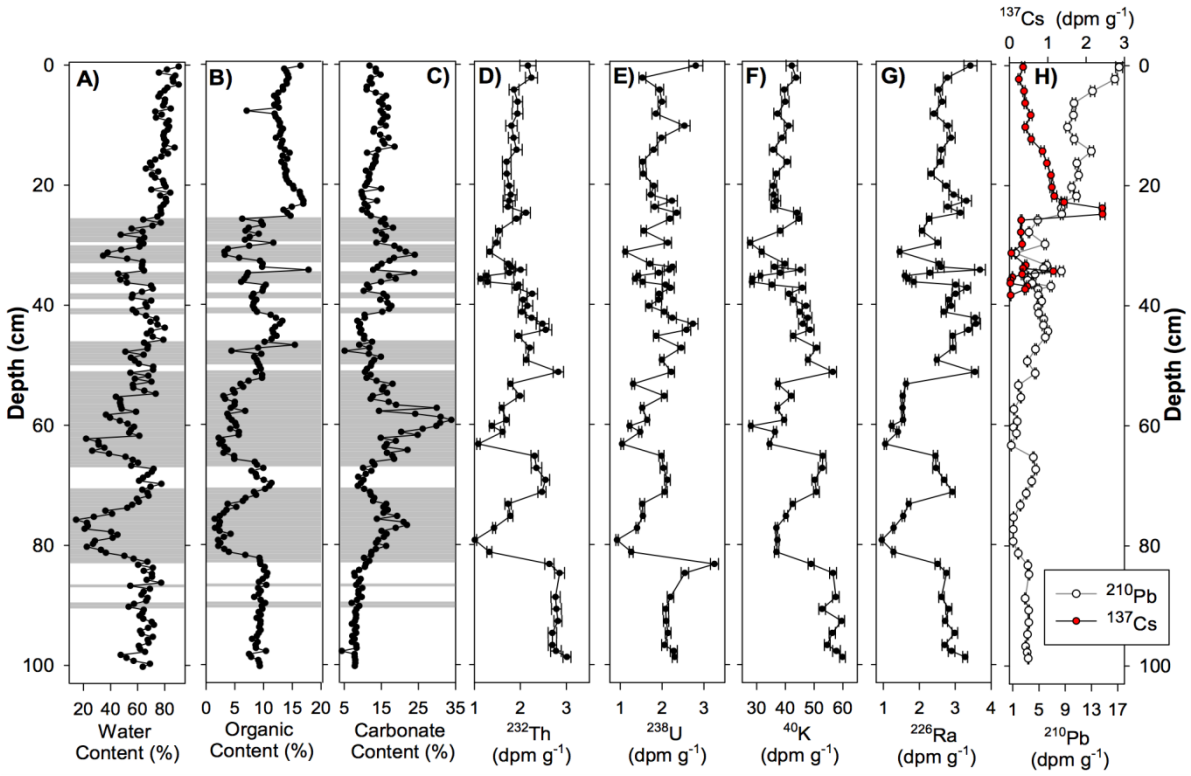

Figure 3.1. Stratigraphic profiles of **A)** % water content, **B)** % organic matter, **C)** % carbonate, and radioisotope activities of **D)** ^{232}Th, **E)** ^{238}U, **F)** ^{40}K, **G)** ^{226}Ra (=supported ^{210}Pb = mean of ^{214}Bi and ^{214}Pb) and **H)** total ^{210}Pb and ^{137}Cs for the center station core in Hamilton Harbour. The horizontal grey zones in **A)-C)** denote sections of the core that were dominated by dredging spoils.

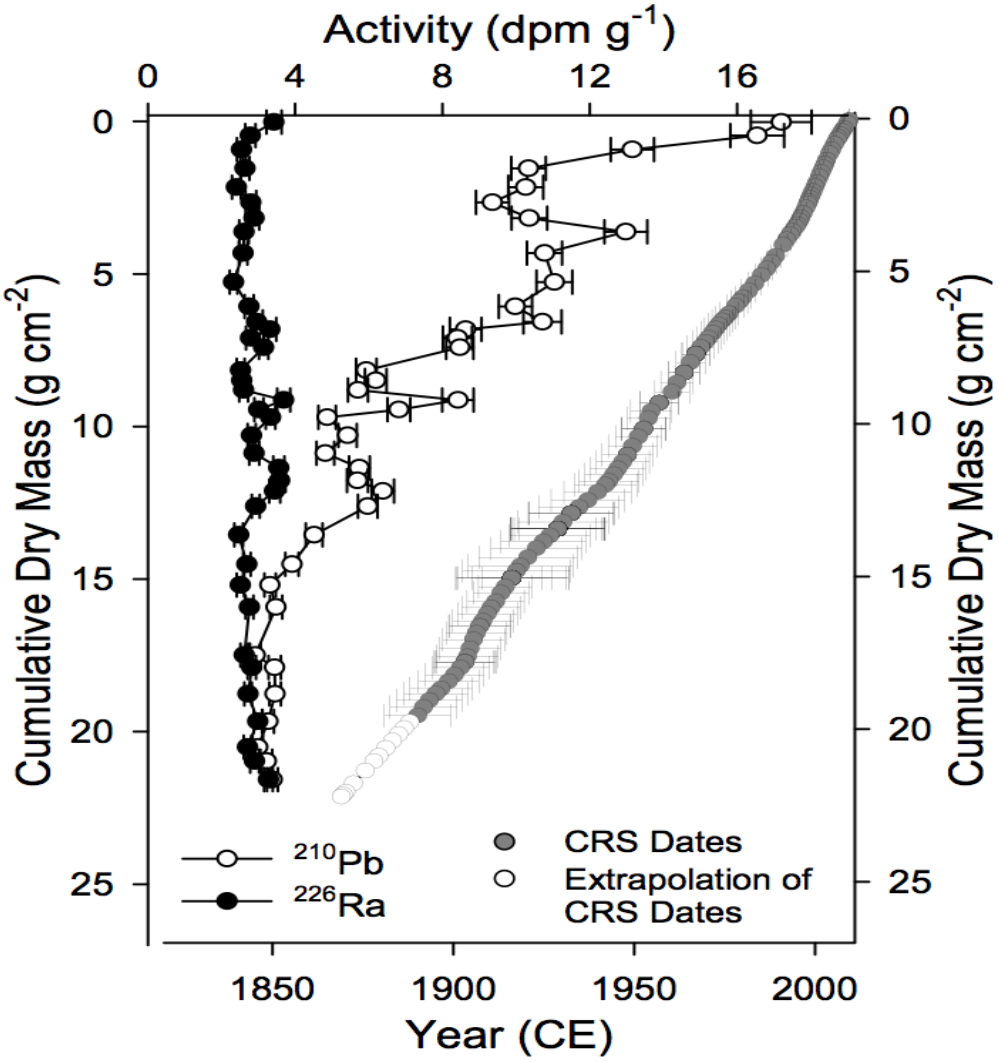

Figure 3.2. Total ^{210}Pb and ^{226}Ra activities (±1 SD) and the depth-age profile for the modified (dredging spoil intervals removed) sediment record taken from Hamilton Harbour (age error bars ±2 SD).

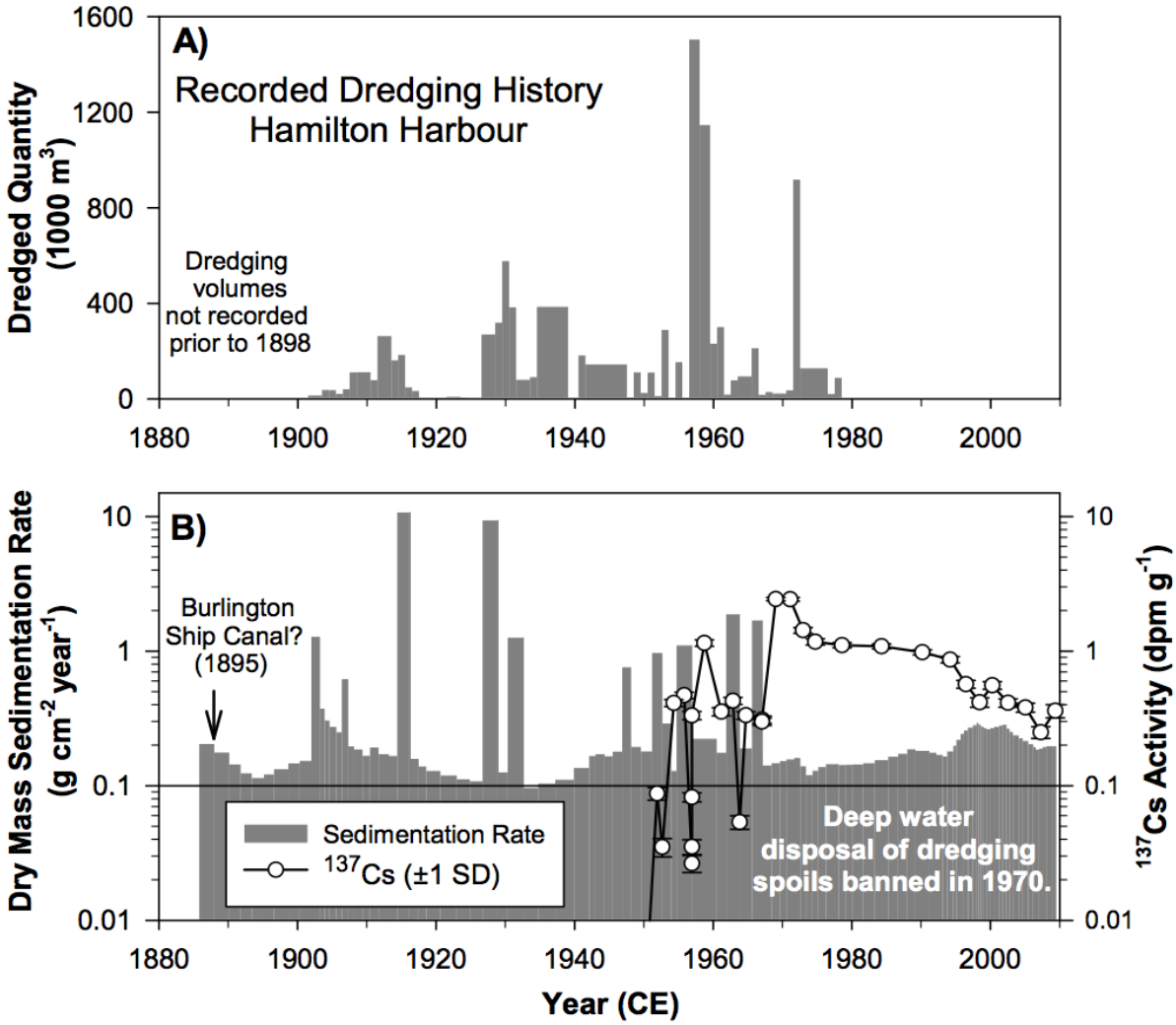

Figure 3.3. The sediment history of Hamilton Harbour. **A)** Recorded dredging volumes supported by Public Works in Hamilton Harbour (from Holmes, 1986). While deep water disposal was common prior to 1970, dredging spoils were also frequently disposed of in near shore areas adjacent to dredging sites. **B)** The inferred sedimentation rate history and ^{137}Cs activity (±1 SD) profile from the centre station Hamilton Harbour sediment core. ^{137}Cs activities < 0.1 dpm g^{-1} are below our detection limit ($L_{D;}$ α, β = 0.05; Currie, 1968) however, these shown here illustrate the apparent trends in ^{137}Cs activity during the critical 1950-60s period. The ten sediment intervals identified as dredging spoils in Figure A correspond to the ten events of massive sedimentation in figure B (vertical bars).

3.3.3. Pigment analysis

HPLC analysis of Hamilton Harbour sedimentary pigments provided information on past changes in abundance and composition of the algal community during the past ~140 years. Profiles of the main chlorophyll and carotenoid pigments showed variable stratigraphic patterns (Figures 3.4-3.5).

Analysis of fossil pigments suggests that the gross algal community composition has been highly variable in the past, with dominance of different algal groups at different times. Mean concentration of chlorophyll *a* (17 mMol g^{-1} OM) indicated high productivity typical of eutrophic lake (Carlson and Simpson, 1996) (Figure 3.4). Pigments of green alge, Chl *b* and lutein were present in majority of the samples (85% and 92%, respectively). Analysis of fossil carotenoids suggested that Chlorophyta, Bacillariophyta, Dinophyta, and Cryptophyta were present in the past, but their abundances showed little trend with depth. Relatively constant presence of diatoxanthin and alloxanthin indicated that diatoms and cryptophytes were abundant components of algal assemblages at all times. Several pigments showed increased concentrations in periods from 1918-1963 and again from 1998-2003.

Analysis of Cyanobacteria-specific carotenoids (Figure 3.5) showed that Cyanobacteria were common members of phytoplankton community of Hamilton Harbour. The pigment scytonemin was not recorded in the Hamilton Harbour sediment but its derivative, compound B (Figure 3.5), is related to presence of cyanobacteria exposed to high UV radiation was (Leavitt *et al.*, 1997). The presence of canthaxanthin indicated occurrence of colonial Cyanobacteria in higher abundances in the past than in the more recent sediments.

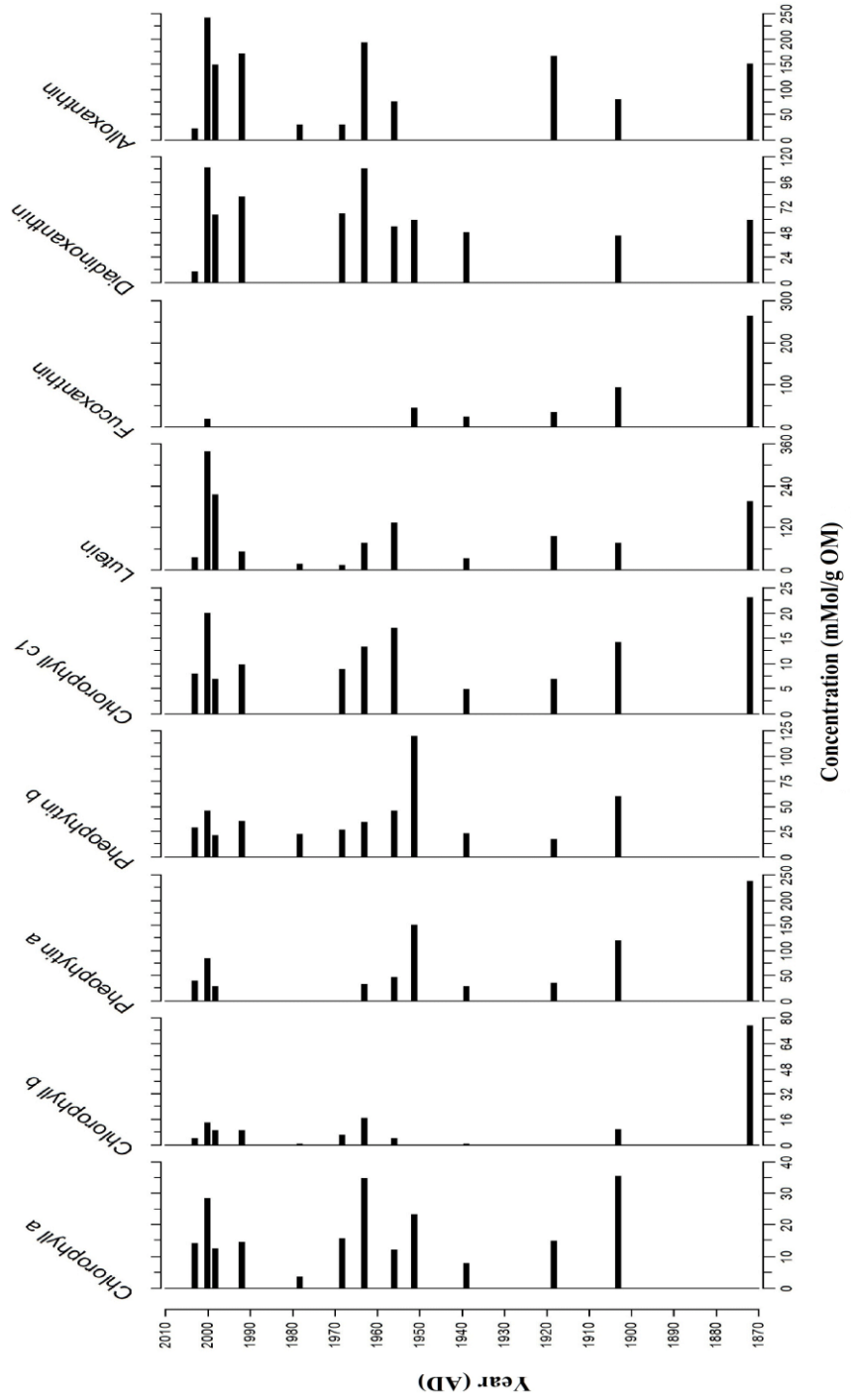

Figure 3.4. Stratigraphic profiles of the chlorophyll and carotenoid fossil pigments from the Hamilton Harbour core. Concentrations of pigments are expressed as mMol g-1 OM.

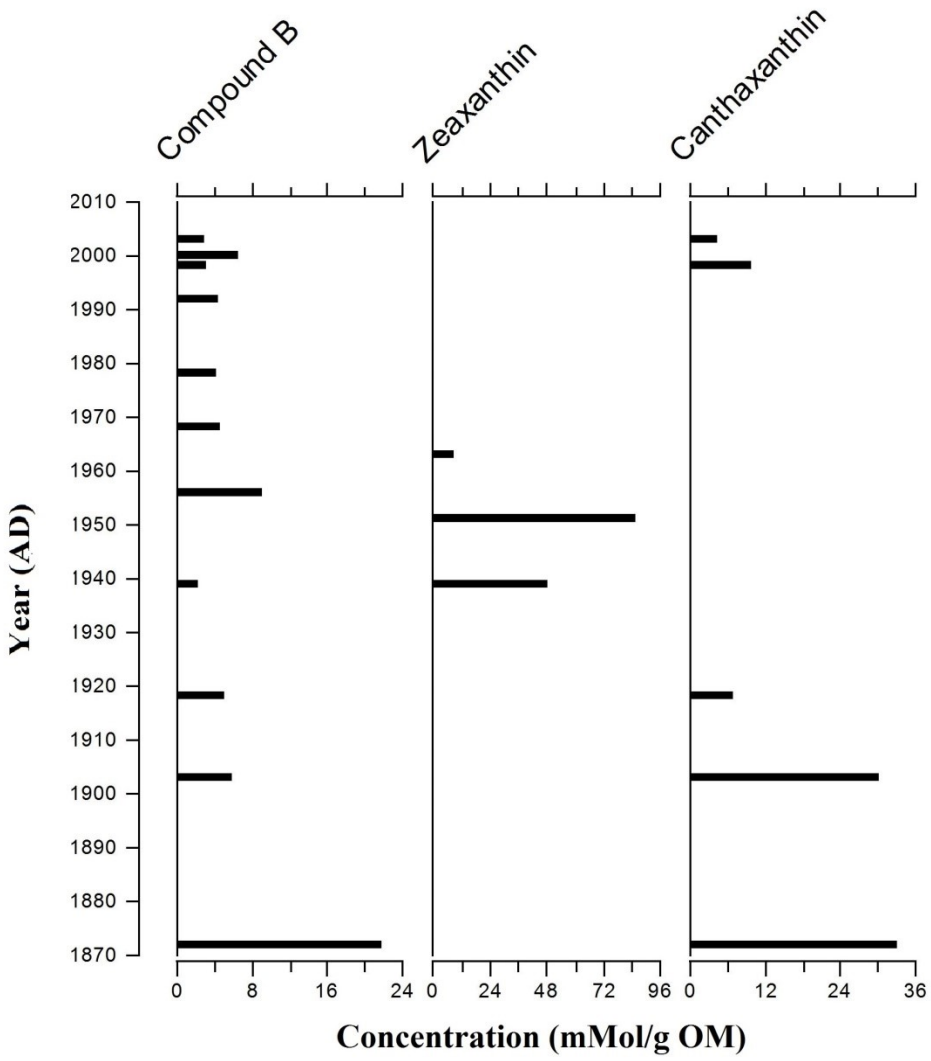

Figure 3.5. Stratigraphic profiles of the cyanobacterial fossil pigments from the Hamilton Harbour core. Concentrations of pigments are expressed as mMol g-1 OM

3.4. Discussion

3.4.1. Sediment chronology

A major confounding challenge for creating accurate sediment-age models for the sediment core from Hamilton Harbour was expected to be past dumping of dredging spoils in deep harbour waters (Holmes, 1986) i.e. near the coring location (Mayer and Johnson, 1994). The result of dredging spoils dumping is that older sediment lie atop younger sediment in the sediment core sequence, thereby violating the assumption of superposition. Radioisotope activities would be expected to have depressed unsupported ^{210}Pb activities in the older sediment spoils compared to the sediment deposited both above and below the dredging spoils layer. The degree of the ^{210}Pb activity depression observed in a sediment interval will be dependent on what proportion of the sediment in the interval is indeed dredging spoils (versus normal) and the relative difference in age between the flanking "normal sediment" and that of the dredged sediment spoils (how much if any residual unsupported ^{210}Pb activity is present in the spoils?). Transient increases in the rate of supply of the "contemporary" sediment will also lead to a depression of the unsupported ^{210}Pb activity via dilution of the atmospheric sourced ^{210}Pb fallout (Appleby, 2001). As such, a depression in ^{210}Pb activity below that of the expected monotonically declining trend cannot alone be used as evidence of dredging spoils.

^{210}Pb dated sediment records from Hamilton Harbour have been published previously by Nriagu *et al.* (1983) and Mayer and Johnson (1994). While difficulties of performing ^{210}Pb dating on a sediment sequence containing dredging spoils were acknowledged in both publications, only Mayer and Johnson (1994) identified and removed dredging spoil-dominated portions of the sediment sequences from the age model calculations. Removal of

sediment subsections derived from dredging spoils should render the remaining sediment sequence as one for which the assumption of superposition is valid, and for which a constant supply of unsupported ^{210}Pb is maintained in keeping with the assumption of the CRS model (Appleby, 2001; Sanchez-Cabeza and Fernández, 2012). The most significant problem with dredging spoils within the sediment sequence with respect to constructing an accurate Constant Rate of Supply (CRS) ^{210}Pb chronology is that if the dredging spoil materials have some residual unsupported ^{210}Pb activity, then its presence within the sediment sequence will violate the assumption of constant supply of unsupported ^{210}Pb (Appleby, 2001; Sanchez-Cabeza and Fernández, 2012). This will lead to erroneous age model results unless steps are taken to assess and account for dredging spoils within the sedimentary sequence.

The effect of dredging spoils on ^{137}Cs activities profiles can potentially be more complex and is dependent on the effective average age of the dredging spoils as well as what point in time they were dumped in deep waters. For example, dredging spoils which were originally deposited in the 1930s, then dredged and dumped in deeper waters in ~1960 would greatly depress ^{137}Cs activities in the sediment sequence of their new location. In contrast, dredging spoils that were originally deposited in the early 1960s and then dredged and dumped in deeper waters in later years would elevate ^{137}Cs activities in the sediment sequence of their new location.

Sediment intervals identified as dredging spoils had depressed water content (Figure 3.1 A) and also tend to show synchronous reductions in % organic matter content and naturally occurring radioisotopes (including ^{210}Pb). These features are in agreement with the expected properties of coarser grained and denser near shore sediments (Rukavina and

Versteeg, 1996) that were episodically dumped on-masse in the deep-water basin. Coarser grain size fractions generally have lower quantities of trace metals (Loring, 1991; Kersten and Smedes, 2002); also, dredging spoils contain primarily older sediments with lower ^{210}Pb activity, than those typically deposited in deep water before or after the disposals event.

Records of dredging volumes in Hamilton Harbour began in 1898 CE until deep-water disposal of dredging spoils was banned in 1970 CE. During that time period approximately 8 million m^3 of sediments were dredged from Hamilton Harbour and were discarded, either in other locations within the harbour, or onto adjacent wetlands (Holmes, 1986). These dredging records are only for those done by publicly funded operations and do not include private-sector operations. However, the dredging volumes of the latter are projected to have been small compared to that done by public works (Holmes, 1986).

Within the original 100.5 cm long core (62.42 g dry wt cm^{-2}) dredging spoils appear to account for 45 cm and 40.20 g dry wt cm^{-2} of the original sediment record. If the coring location is broadly reflective of the deep-water portion of Hamilton Harbour (~half of the total 22 km^2 harbour area), then as a rough estimate (0.45 m X $11x10^6$ m^2), approximately 5 million m^3 of dredging spoils have been deposited in the deep-water area of Hamilton Harbour between 1898 and 1970. This estimate represents ~60% of the total dredging volume (~8 million m^3) recorded by public works for this time period, for which a large, but unknown volume, was not disposed of in the deep center basin of Hamilton Harbour (Nriagu *et al.*, 1983; Holmes, 1986). Given the corroboration of this study's approximated dredged spoils volume with the recorded dredging history of Hamilton Harbour, both our identification of dredging spoil intervals and our resulting ^{210}Pb chronology appear to be a

sound interpretation of the sediment record of Hamilton Harbour over the last 100+ years.

3.4.2. Sedimentary pigments

Anthropogenic eutrophication is a major environmental concern in aquatic environments because nutrient additions dramatically change lake conditions (Conley *et al.*, 2009). There are many negative consequences of lake eutrophication, such as an increase in primary productivity that leads to an increase in organic matter, depletion of hypolimnetic dissolved oxygen (DO), anoxia, death of benthic fish and invertebrates, release of reduced substances from the sediments (including potentially toxic substances and biologically available phosphorus) (Nürnberg, 1995). Additional negative outcomes linked with eutrophication include decreased water usability, taste and odour problems, decreased recreational and aesthetic values of the waterbodies, and decreased biological diversity (Carpenter *et al.,* 1998).

Water quality problems linked to eutrophication are a long time issue of Hamilton Harbour. The history of the harbour's eutrophication began with the arrival of European settlers in 1786 (Campbell, 1966). The shift of the harbour ecosystem towards eutrophy started in 1860s, due to land use alternation and cultural development (Yang *et al.*, 1993). Pollution of the harbour accelerated in the 1930s and reached its peak in the second half of the 20th century (1970-1986) (Yang *et al.*, 1993). However, due to remediation efforts to control input of nutrients, water quality trends showed improvements since the end of 1980s (Hiriart-Baer *et al.*, 2009). Today, Hamilton Harbour remains a polluted, eutrophic body of water subject to strong physical disturbances, with a highly variable and rapidly changing phytoplankton community (Munawar and Fitzpatrick, 2007).

In the present study, the temporal changes in composition and abundances of the major algal groups were analyzed from sedimentary pigments in a sediment core from Hamilton Harbour. The extreme variability observed in the phytoplankton community composition of Hamilton Harbour indicated a highly disturbed system. These disturbances occurred due to human land-use activities which affected the water quality, but also due to dredging events that were happening in the past.

Sediment intervals identified as dredging spoils had depressed water and % organic content and contained primarily older sediments. Dilution of organic material due to presence of dredging spoils in the core could be the reason for decreased concentrations and absence of some pigments from samples. Although pigment concentrations were highly variable throughout the core, concurrent increase of some pigments was noted in periods 1919-1964 and 1998-2003. The increases from 1919-1964 were probably due to accelerated nutrient pollution. The 1998-2003 increases could be the consequence of increased water clarity due to invasion of dreissenid mussels (zebra mussel, *Dreissena polymorpha* and quagga mussel, *Dreissena bugensis*), which have colonized the docks and breakwalls in the harbour since 1990 (Dermott and Bonnell, 2007). Concentrations of chl *a* (although highly variable) were indicative of eutrophic conditions and variable primary production. Although chlorophyll *a* is not class-specific and is usually used as a general indicator of phytoplankton biomass, the transformation products formed during its decay can be used as indicators of heterotrophic processes (Bianchi *et al.*, 2002). Phaeopigments, as derivatives of chloropigments, are a result of bacterial/autolytic cell lysis, and of grazing activities (Sanger and Gorham, 1970; Welschmeyer and Lorenzen, 1985; Bianchi *et al.*, 1991). Pheophytine *a* and pheophytine *b*

sedimentary concentrations were on average 3.7 and 3.2 times higher than chl *a* and chl *b* concentrations, respectively, indicating algal grazing by zooplankton (Carpenter *et al.*, 1986; Leavitt *et al.* 1994). Different concentrations of pheophytine pigments could be attributed to differences in zooplankton grazing activity over time.

The composition of the major chlorophyll pigments indicated high presence of Chlorophyta and Bacillariophyta in the harbour at all times (Figure 3.4). The main algal groups identified on the basis of marker pigments presence were Chlorophyta, Bacillariophyta, Dinophyta, and Chrysophyta. These findings are consistent with phytoplankton observations for Hamilton Harbour, where these four algal groups were identified as dominant in 2002 and 2004 (Munawar and Fitzpatrick, 2007), and also in 2009 (present research, Chapter 2).

Cyanobacterial pigments were also detected in the Hamilton Harbour core (Figure 3.5). In almost all samples, the scytonemin derivative, compound B, was recorded. Scytonemin is a unique cyanobacterial extracellular sheath pigment whose function is protection against ultraviolet (UV) radiation (Leavitt *et al.*, 1997; Dillon and Castenholz, 1999). Upper layers of surface cyanobacterial blooms are exposed to high solar irradiance and high concentrations of scytonemin are observed. Hence, the presence of pigment scytonemin derivative compound B suggests that bloom-forming Cyanobacteria were members of the harbour algal communities over the past 140 years. Presence of colonial Cyanobacteria is indicated by sporadic occurrences of canthaxanthin and zeaxanthin. The absence of pigment aphanizophyll, suggests that presence of N2-fixing Cyanobacteria was not common in the harbour. Cyanobacterial pigments presence indicated that Cyanobacteria

have been a regular but not dominant feature of Hamilton Harbour phytoplankton in the past.

Very low concentrations and absence of some pigments from some of the samples were observed. Taking into consideration that all analyzed samples were from the time period when the harbour was already recognized as eutrophic (Yang *et al.*, 1993; Duthie *et al.*, 1996; Wolfe *et al.*, 2000, Munawar and Fitzpatrick, 2007; Hiriart-Baer *et al.*, 2009), the shifts in pigments concentrations may have been caused by environmental factors other than increase of eutrophication of the ecosystem. One of the reasons for the dilution of pigment concentration could be the interaction with Lake Ontario aided by an artificially deepened ship canal through the Burlington Bar (Wolfe *et al.*, 2000). Construction of the Burlington ship canal during 1823-1827 resulted in a major change in the water budget of Hamilton Harbour and was a major physical disturbance to the system (Barica, 1989). The consequence of enhanced water exchange was a decrease in hydraulic retention time to less than 40%, a displacement of harbour water by Lake Ontario water more than three times a year (Barica, 1989) and mixing levels 30–100% greater than the natural hydrologic state (Wolfe *et al.*, 2000). In such conditions epilimnetic exchange was more significant than hypolimnetic one (Klapwijk and Snodgrass, 1985) and it can be assumed that great proportion of the plankton was displaced together with water (Wolfe *et al.*, 2000) through the canal into the Lake Ontario. The water exchange events diluted the amount of photosynthetic pigments in the water column, and consequently, in sediments. Possible reason for variable pigments concentrations could have been the mixing of the surface sediments by high density of the oligochaetes and other macro invertebrates (boturbation), who completely homogenized the upper layers of benthic sediments in the depositional basin of the harbour (Mayer and

Johnson, 1994; Dermott and Bonnell, 2007). The passage of organic matter through digestive tract of detritus feeding oligochaetes and larval chironomids living in the benthic sediments resulted in organic molecules alteration (Brinkhurst and Jamieson, 1971). Also, herbivore grazing severely degrades both chlorophylls and carotenoids (Leavitt, 1993). Herbivore grazing pressure was confirmed from presence of the grazing indicator phaeophorbide *a* (Leavitt and Hodgson, 2001), which was present throughout the core but was not quantified. Finally, low temporal resolution i.e. small number of sediment samples taken for analysis could be the main reason for inconsistencies in pigments concentrations. Long temporal gaps between the samples clearly did not capture all fine changes in planktonic algal communities over time.

3.5. Conclusions

Changes in phytoplankton community composition in Hamilton Harbour during the past 140 years have been inferred from the stratigraphic analysis of sedimentary pigments. The vertical profile of fossil pigments showed high temporal variability. Two periods of elevated pigments concentrations coincided with historical acceleration of eutrophication and establishment of invasive dreissenid mussels in the harbour. Chlorophyta, Bacillariophyta, Cryptophyta and Dinophyta were identified as the most abundant algal groups over time. The temporal response of the phototrophic paleo communities could not be identified, since physical and chemical variability of the environment differently affected phytoplankton community composition. The presence of cyanobacterial pigment scytonemin derivative (compound B) and its stratigraphic distribution and concentrations, indicated that cyanobacterial blooms were the regular feature of Hamilton Harbour in the past 140 years. Different algal groups of Hamilton Harbour responded to environmental changes with different

temporal lags, generally resulting in variable phytoplankton crop levels and unpredictable shifts in composition. In this research sedimentary pigments of Hamilton Harbour have provided useful qualitative estimates of dominating phytoplankton communities over time.

Chapter 4

Assessment of the cyanobacterial communities and characterization of toxic Cyanobacteria from Hamilton Harbour sediment using PCR-DGGE

4.1. Overview

The phylum Cyanobacteria comprises a highly diverse bacterial group in relation to form, function, and habitat (Neilan *et al.*, 1995). Hence, an understanding of the current cyanobacterial systematics is necessary for the accurate identification of species. In the early 19th century, the Cyanobacteria were recognized and treated as members of the plant world (Castenholz and Waterbury, 1989) and its taxonomy has been based on morphology under the International Code of Botanical Nomenclature (ICBN) (Oren, 2004). Plasticity of morphological characters used to distinguish cyanobacterial species and loss of original morphological features under culture conditions (Laamanen *et al.*, 2001) has led to difficulties in species delineation (Palinska *et al.*, 1996; Komárek and Komarkova, 2004). That is why the taxonomic usefulness of morphological traits alone had to be re-evaluated. In the last four decades the criteria for ICBN classification of Cyanobacteria have been considerably revised (Komárek, 2012), following the application of molecular methods. Almost all recent nomenclature revisions (in the past 10 years) have integrated molecular data from molecular phylogenetic analyses based on the rRNA genes and other relevant genetic markers (Whitton and Potts, 2010). Since Cyanobacteria are a group of

photosynthetic gram-negative prokaryotes, bacteriologists use physiological and biochemical characteristics of cultured strains for taxonomic identification (Castenholz and Waterbury, 1989; Castenholz, 2001). The identification of cyanobacterial taxa using the International Code for Nomenclature of Bacteria (the Bacteriological Code or ICNB) was initially proposed by Stanier *et al.* (1978) and Rippka *et al.* (1979). However, under current classification cyanobacterial relationships have not been completely resolved and different approaches are being used for taxonomic determination.

Phylogenetic classification is a system that identifies species in relation to their evolutionary history. In this approach species are identified using monophyly of taxons. Many phylogenetic studies are using nucleic acid sequencing for construction of phylogenetic trees. However, nucleic acids are prone to lateral (horizontal) gene transfer, which is assumed to be an important factor in shaping prokaryotic genomes (Philippe and Douady, 2003). The evolution of cyanobacteria is based on horizontal transfer of DNA between cyanobacterial strains (Rudi *et al.,* 1998, 2000; Barker *et al.,* 1999) and within populations (Hayes *et al.,* 2006). A result of lateral exchanges is the difficulty of obtaining congruent gene phylogenies (Daubin *et al.*, 2002).

Molecular biological techniques have become an important tool in inferring phylogenetic relationships of cyanobacteria (Wilmotte, 2004) and identification of community species composition and dynamics (Ouellette and Wilhelm, 2003). From isolated community DNA, certain genes (also known as genetic or molecular markers) can be detected (Alberts *et al.*, 2002) and amplified using PCR. These PCR amplicons can then be used in monitoring of the microbial diversity and the successive changes in community

structure (Muyzer *et al.*, 1993). Genetic markers can also be used for characterization of microorganisms, including Cyanobacteria, both from cultures and environmental samples (Schäfer and Muyzer, 2001).

One of the most useful phylogenetic markers used in environmental prokaryotic diversity studies is the universally present small ribosomal subunit rRNA or 16S rRNA gene (Kirk *et al.*, 2004). The 16S rRNA gene is widely used for several reasons: it is present in all prokaryotic organisms; it has well defined regions for taxonomic classification; these regions are not prone to horizontal gene transfer; and it has sequence databases available to researchers (García-Martínez *et al.*, 1999). As a structural part of the 30S ribosomal small subunit (SSU), the 16S rRNA is approximately 1550 base pairs long and consists of eight highly conserved regions (U1-U8) and nine variable regions (V1-V9) (Jonasson *et al.*, 2002). The 16S rRNA gene generally has sufficient heterogeneity between species (or strains) to provide an accurate determination due to its hypervariable unconserved regions (Case *et al.*, 2007), that can be used to identify species (Van de Peer *et al.*, 1996).

In 1993, Muyzer *et al.* used PCR-DGGE of ribosomal DNA as a microbial ecology tool. Since then, PCR-DGGE molecular fingerprinting, based on electrophoretic separation of PCR amplicons that have the same size but different sequences, has become a widely used technique that provides microbial community genetic profiles from different environments. DGGE 16S rRNA gene analysis has been successfully used in studies of cyanobacterial communities in both freshwater and marine sediments. In the most recent studies, different cyanobacterial phylotypes were detected from sediments of northern Chiles's high-altitude saline wetland (Dorador *et al.*, 2008), Hong Kong's Victoria Harbor polluted sediments

(Zhang *et al.*, 2008), Kenyan Rift Valley lakes (Dadheech *et al.,* 2009), Nankai Trough sediment (Mills *et al.*, 2012), and late Holocene sediments of maritime Antarctic lakes (Fernandez-Carazo *et al.*, 2013). In the present study, PCR-DGGE analysis of 16S rRNA V3 hypervariable region was used to detect Cyanobacteria from mixed-community sediment samples of Hamilton Harbour.

Detection and characterization of toxin producing cyanobacteria is not possible using microscopy-based techniques, since toxic and nontoxic strains are morphologically identical and their populations can coexist in the same ecosystem (Hotto *et al.*, 2007) and even in the same bloom (Vezie *et al.*, 1997, 1998; Kurmayer *et al.*, 2004). Cyanobacteria that are genetically able to produce toxins but only under certain conditions (toxigenic) (Ouellette and Wilhelm, 2003) can also be components of the bloom. Also, different cyanobacterial genera are capable of producing the same toxin, and different toxins can be produced within the same genus, and the level of toxicity may be influenced by environmental factors, such as water temperature, light intensity, pH, nutrients and trace metals (Kaebernick and Neilan, 2001).

The study of microcystin-producing genera using molecular tools has been enabled by the detection and characterization of the microcystin synthetase gene cluster (*mcy*), which is present in all major microcystin producers *Microcystis*, *Anabaena*/*Dolichospermum* and *Planktothrix* (Tillet *et al.*, 2000; Christiansen *et al.*, 2003; Rouhiainen *et al.*, 2004). A molecular marker used for the detection and identification of toxic cyanobacteria, the *mcy*E gene region (Rantala *et al.*, 2008) is more conserved than the other *mcy* regions and therefore more reliable for the detection of the microcystin producers (Rantala *et al.* 2006). This region

is also necessary for microcystin production (Dittmann *et al.*, 1997) since it is involved in the synthesis of the structural amino acid Adda (Chapter 1, Figure 1.3) and its integration in the microcystin molecule. The Adda moiety is associated with the binding of the toxin to the hepatic protein phosphatase (Goldberg *et al.*, 1995) and as such is essential for the toxicity of the microcystin molecules (Jaiswal *et al.*, 2008). The transfer of an amino group (NH_2) on the Adda moiety is enabled by the aminotransferase (AMT) domain, which has a crucial role in the biosynthesis of all known microcystin congeners (Tillett *et al.*, 2000). Its location in the *mcy*E gene (Jungblut and Neilan, 2006) indicates that the AMT domain will only be present in toxic strains (Sivonen and Jones, 1999).

PCR detection of the species-specific *mcy* gene cluster has been used for the identification of the microcystin ecotypes in toxic cyanobacteria of the genus *Planktothrix* (Kurmayer *et al.*, 2005; Mbedi *et al.*, 2005), *Microcystis* and *Anabaena/Dolichospermum* (Vaitomaa *et al.*, 2003), *Microcystis* (Via-Ordorika *et al.*, 2004; Ouellette *et al.*, 2006). Potential microcystin-producing cyanobacteria in Lake Ontario were characterized using the genes *mcy*A, -B, -D and -E (Hotto *et al.*, 2005). Genus-specific microcystin synthetase gene E (*mcy*E) was used in detection of the microcystin-producing cyanobacteria in lakes in Finland (Vaitomaa *et al.*, 2003; Rantala *et al.*, 2006), Kenya (Dadheech *et al.,* 2009), Greece (Vareli *et al.,* 2009), Russia (Belykh *et al.,* 2011) and the Mediterranean Sea (Vareli *et al.*, 2012), as well as in the Laurentian Great Lakes (Ontario and Erie) (Chunn, 2007).

In 2006, the Bay of Quinte (Lake Ontario) and Maumee Bay (Lake Erie), two embayments of the Great Lakes prone to toxic cyanobacterial blooms, were investigated for the presence of microcystin and nodularin producing cyanobacterial genotypes (Chunn,

2007). The Cyanobacteria genotypic community was assessed using DNA-based analyses of the 16S rRNA V3 hypervariable region and the aminotransferase (AMT) region of the microcystin (*mcyE*) and nodularin (*nda*) genes. Results of DGGE analysis showed that microcystin producing species, most likely from the genus *Microcystis,* were occurring at all study sites at some point during the sampling season (Chunn, 2007).

To date, PCR-DGGE of the *mcy*E region has been used for the identification of toxic cyanobacteria from lake sediment samples of different Kenyan lakes (Rift Valley) (Dadheech *et al.,* 2009). In the present study, PCR-DGGE analysis of AMT domain of the *mcy*E gene is used for the identification of microcystin producers from Hamilton Harbour sediment samples.

The objectives of this study were:

a) to assess the genotypic diversity/similarity and shifts in cyanobacterial communities over time using 16S rRNA and its V3 region from sedimentary DNA;
b) to determine the occurrence and diversity of toxic cyanobacterial genera in the past using *mcy*E gene and its AMT region from sedimentary DNA.

4.2. Materials and Methods

4.2.1. Sampling

The sediment core used for molecular analysis was taken from the central part of Hamilton Harbour at the central monitoring station 1001 (Fig. 1.2) and sectioned into 0.5 cm stratigraphic intervals (Table 4.1). Before molecular analysis, steps regarding prevention of

samples contamination were taken. To prevent contamination during coring, the coring equipment was cleaned using 10% bleach solution. The acrylic tube was submerged in the bleach solution and soaked for ~30 min, after which tube was rinsed with distilled water. To prevent contamination of molecular samples during DNA isolation, molecular samples were obtained from the center of each sediment sub sample. 38 eight Hamilton Harbour sediment core sub-samples from various depth intervals were used for extraction of sedimentary DNA and subsequent molecular analysis.

Table 4.1. The depth and age of the sediment sub-samples taken for molecular analysis

Molecular sample number	Sediment depth (cm)	Sediment age (year)
1	0.25	2009
2	0.75	2009
3	2.25	2007
4	4.25	2004
5	5.25	2003
6	6.25	2002
7	7.75	2000
8	8.25	2000
9	10.25	1998
10	12.25	1996
11	14.25	1994
12	15.25	1992
13	15.75	1991
14	16.25	1989
15	18.25	1983
16	20.25	1978
17	22.25	1974
18	24.25	1971
19	25.25	1968
20	26.25	1967
21	28.25	1967
22	30.25	1964
23	34.25	1962
24	35.25	1957
25	38.25	1954
26	40.25	1951
27	42.25	1948
28	45.25	1940
29	46.25	1935
30	50.25	1933
31	50.75	1933
32	54.25	1929
33	58.25	1929
34	60.25	1929
35	62.25	1929
36	66.25	1929
37	70.25	1919
38	75.25	1916
39	78.25	1916
40	80.25	1916
41	80.75	1916
42	85.75	1912
43	90.25	1904
44	95.25	1892
45	100.25	1872
46	101.75	1869

4.2.2. DNA extraction from sediment sub-samples

DNA was isolated from sediment sub-samples using the FastDNA® SPIN Kit for Soil and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA) according to the manufacturer's protocol. For each extraction, 0.5 g (wet weight) of sediment was placed into 2.0 ml tube containing lysing matrix E, a mixture of ceramic and silica particles; 122 µl of MT buffer and 978 µl of sodium phosphate buffer were added and samples were homogenized for 40 seconds in the FastPrep® Instrument at a speed setting of 6.0. After the lysis, samples were centrifuged at 14,000 x g for 5-10 minutes to pellet the cell debris, sediment and lysing matrix. The supernatant was transferred to a clean 2.0 ml microcentrifuge tube, 250 µl PPS (protein precipitation solution) was added and mixed by shaking the tubes by hand 10 times. To pellet the precipitate, tubes were centrifuged at 14,000 g for 5 minutes and the supernatant was transferred to clean 15 ml tubes, in which 1 ml of binding matrix suspension was added. To allow the binding of DNA, tubes were vortexed for 2 minutes and then placed in a rack for 3 minutes to allow settling of silica matrix. After this, 500 µl of the supernatant was removed and the binding matrix was resuspended in the remaining amount of supernatant. Approximately 600 µl of the mixture was transferred to a SPIN™ Filter and centrifuged at 14,000 x g for 1 minute. The catch tubes were emptied and the remaining mixture was added to the SPIN™ Filter and centrifuged as before. The pellet was resuspended in 500 µl of SEWS-M wash solution (Tris-HCl and 100% ethanol), and the tubes were centrifuged twice-at 14,000 g for 1 minute and at 14,000 x g for 2 minutes. The tubes were replaced with clean catch tubes, the SPIN™ Filters were air dried for 5 minutes at room temperature, the binding matrix pellet was resuspended

in 50-100 µl of DES (DNase/Pyrogen-Free Water) and centrifuged at 14,000 g for 1 minute to bring eluted DNA into the clean catch tube. Isolated DNA was stored at -20°C. Concentration of each sample was checked using NanoDrop ND-1000 spectrophotometer (Thermo Fisher, MA). After testing different dilutions for the presence/absence of 16S rRNA and *mcy*E genes, samples were 10x diluted for PCR-DGGE amplifications.

4.2.3. PCR Amplification of Cyanobacteria-specific gene fragments

4.2.3.1. PCR Amplification of 16S rRNA gene-V3 region

Thirty-eight DNA extracts from different sediment depths were used to check for the presence of cyanobacteria-specific V3 region of 16S rRNA gene. To amplify the 161 bp fragment and at the same time to reduce the amplification of non-cyanobacterial and to obtain as much as possible pure cyanobacterial 16S rRNA genes, nested PCR was used. Both PCR reactions were performed in a 25 µL volume, with 2.5 µl 10x Taq polymerase buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0 (at 25°C), 1.0% Triton® X-100) (Promega; W.I., U.S.A.), 2 µl (25mM) MgCl2, 0.5 µl (10mg/ml) of bovine serum albumin (BSA), 0.625 U Taq DNA polymerase (Promega; W.I., U.S.A.), 0.5 µl (10 µM) dNTPs (Promega, Madison, W.I., U.S.A.), 2 µl DNA extract and 0.5 µl (10 µM) of each cyanobacterial specific primer (first amplification) and general bacterial primer (second amplification) (Table 4.2). PCR amplifications were done using the Eppendorf Mastercycler® Gradient 5331 (Eppendorf, C.A., U.S.A.).

The first cyanobacteria-selective round of amplification was performed with the 20 cycle touchdown procedure as follows: initial denaturation step at 95°C for 1 min 30 s was followed by 20 cycles at 94°C for 1 min, 65°C for 1min, and 72°C for 1 min. In each cycle the annealing temperature decreased by 0.5°C to end at 55°C. The final extension was at 72°C for 10 min. According to Muyzer *et al.* (1993), this touchdown procedure ca be used to reduce the formation of non-target DNA fragments during amplification.

The second round of amplification was the same touchdown 20 cycle PCR amplification procedure as in the first round, with five additional cycles performed subsequently at 94°C for 1 min, 55°C for 1min, and 72°C for 1 min. A final extension step at 72°C for 10 min similarly concluded the nested round of the procedure. Amplified products were electrophoresed through EtBr stained 0.7 % agarose gel in 1x TBE buffer for 45 min at 100 V along with a DNA ladder (Hae III-ΦX174 DNA) and visualized using the Syngene Bioimaging System (Synoptics Ltd., United Kingdom).

Table 4.2. Primers used to amplify 16S rRNA gene - V3 region of cyanobacteria

Primer name	Primer sequence	Amplification round	Reference
Cya-b-F371	5'-CCTACGGGAGGCAGCAGTGGGGAATTTTCCG-3'	1	Zwart *et al.*, 2005
Cya-R783	5'-GACTACWGGGGTATCTAATCCW-3'		Zwart *et al.*, 2005
(GC)-F357*	5'-CCTACGGGAGGCAGCAG-3'	2	Zwart *et al.*, 2005
R518	5'-CCAGCAGCCGCGGTAAT-3'		Zwart *et al.*, 2005

* 40 bp GC clamp (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC-3') added to the 5' end of primer F357GC to increase the separation of DNA bands in DGGE gel (Muyzer *et al.*, 1993)

4.2.3.2. Amplification of *mcy*E aminotransferase (AMT) region

The 32 sediment DNA extracts used in the 16S rRNA-V3 amplification were also used to check for presence of a 472 bp fragment of the AMT domain of the PKS in the *mcy*E gene. The amplification reagents and their concentrations were the same as above except that different primers were used, (GC)-HEPF (TTTGGGGTTAACTTTTTTGGGCATAGTC) and HEPR (AATTCTTGAGGCTGTAAATCGGGTTT) (Jungblut and Neilan, 2006). To increase the separation of DNA bands in the DGGE gel (Muyzer *et al.*, 1993), a 40 bp GC-clamp was also added to the 5' end of the forward primer (GC)-HEPF. The thermal cycle program was as follows: initial denaturation step at 92°C for 2 min, 35 cycles of 92°C for 20 s, 52°C for 30 s, and 72°C 1 min, with a final extension step at 72°C for 5 min. After amplification PCR products were visualized as above.

4.2.4. DGGE Profiling of 16S rRNA gene-V3 and mcyE-AMT amplicons

The PCR products were analyzed by denaturing gradient gel electrophoresis (DGGE) using the D-Code universal mutation detection system following the manufacturer's instructions (Bio-Rad, USA). Twenty-five µl of each PCR product was loaded on a parallel DGGE gel. The products were resolved on 8% (w/v) polyacrylamide gels (acrylamide/bisacrilamide 37.5:1;1.0 mm, 16 x16 cm) containing a 35-55% denaturing gradient of urea and formamide for V3 and 40-55% denaturing gradient for AMT amplicons. Electrophoresis was performed for 16 hours in 1x TAE buffer (40 mM tris–HCl, 20 mM acetic acid, 1 mM EDTA) of pH 7.6 at constant voltage (60 V) and temperature (60 °C). The gels were stained for 60 min in 1x TAE buffer containing ethidium bromide (25 µL of 1

μg/mL), destained in 1 x TAE buffer for 10 min and visualized using the Syngene Bioimaging System (Synoptics Ltd.; Cambridge, United Kingdom).

Determination of toxic cyanobacteria genotypes from sediment samples was achieved through comparison with a standard marker developed by Chhun (2007). The standard consisted of amplified *mcy*E AMT fragments from three known microcystin-producing species: NIVA CYA-83/1 (*Dolichospermum lemmermannii* var. *minor*), UTCC 299 (*Microcystis aeruginosa*) and UTCC 507 (*Planktothrix rubescens*).

4.2.5. Estimation of cyanobacterial community richness using DGGE bands profiles

Species richness, together with evenness, is one of the two basic components of species diversity. A number of cyanobacterial species in the respective sediment layer (richness) was estimated based on the number of bands present within the DGGE profiles (Fromin *et al.*, 2002). Since the total number of bands ('band richness') in each sample is related to the number of dominant phylotypes, it was used for the comparison of samples (Müller *et al.*, 2001; van der Gucht *et al.*, 2001).

4.3. Results

4.3.1. DGGE 16S rRNA gene-V3 genetic profiles of sedimentary Cyanobacteria

The DGGE profiles of the 16S rRNA gene fragments amplified from sediment sub-samples are shown in Figures 4.1-4.3. V3 domains of the 16S rRNA gene revealed the presence of thirteen cyanobacterial genotypes (since each band was assumed to be an individual genotype) and their temporal dynamics (Fig. 4.1). Cyanobacterial genotypes were detected based on the position of their 16S rRNA gene -V3 amplicons on the DGGE gels.

The banding pattern showed that the number of bands decreased with depth in the sediment core. The highest number of bands (11) was recorded in the most recent sediment layer (2009); one band was detected in samples from 1992, 1983, 1935, and 1929 D. Two bands could be detected in majority of samples and they were most prominent from 2009 to 1948. Their presence was also detected in older sediment layers, but the intensity of the bands was greatly reduced. The intensity of bands was highest in the most recent sediment samples, coinciding with higher DNA concentrations. Sub-samples from the same year but from different sediment sources (autochthonous sediments and dredged spoils) showed variable number of bands. Such cases were particularly obvious in sub-samples from 1931 and 1929. In three out of four subsamples from the dredged material from 1929, no bands were detected in one sample while two bands were present in three samples (Fig. 4.2). In non-dredged sediment from the same year, one band was observed. In four sub-samples from dredged material from 1916 (Fig. 4.3) the number of bands varied from 1-3 while one sub-sample had no bands. A similar situation was observed in the 1932 layer where either no bands or two were present in dredged and non-dredged material (Fig. 4.2 and 4.3). The deepest sediment strata showed the lowest number (two bands) and intensity of bands. Nevertheless, the amplification of cyanobacterial genes was successful, despite very low DNA concentrations from the oldest sediment parts.

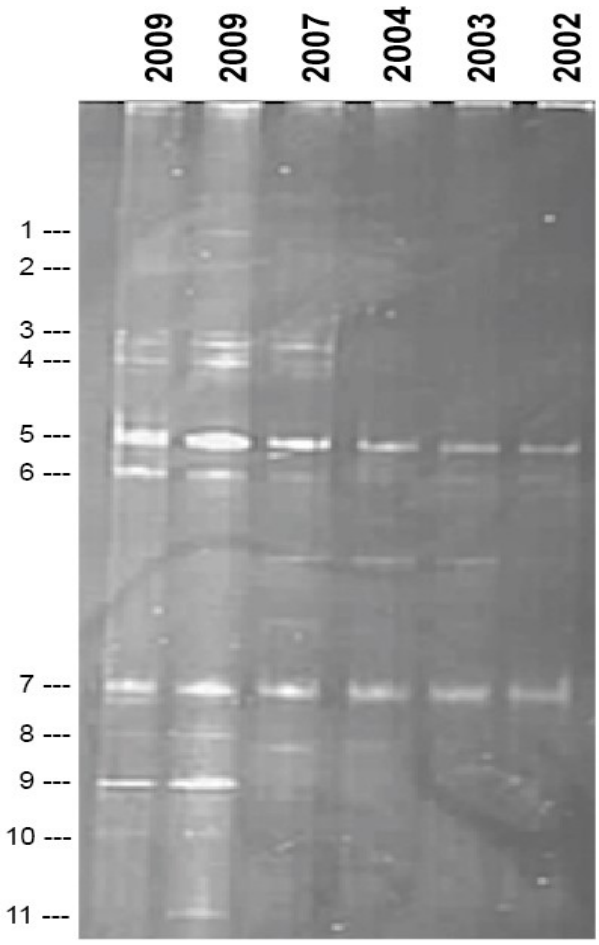

Figure 4.1. DGGE profile of 16S rRNA V3 domain amplicons obtained from Hamilton Harbour sediment sub-samples 1-5 (2009-2002). 1-11: DGGE bands in the most recent sample from 2009

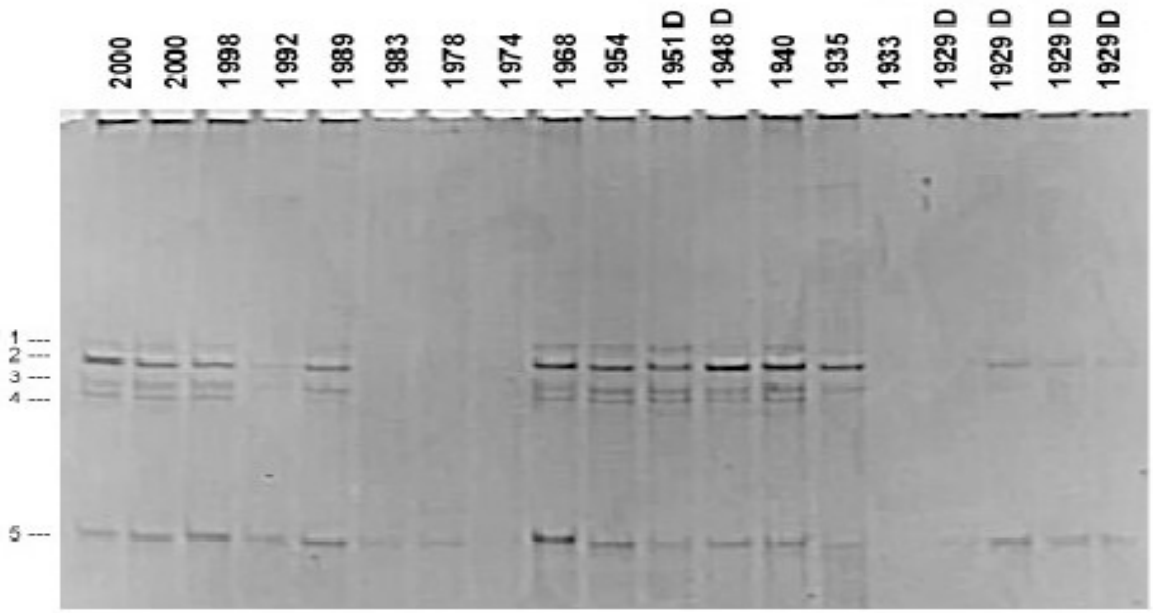

Figure 4.2. DGGE profile of 16S rRNA V3 domain amplicons obtained from Hamilton Harbour sediment sub-samples 7-35 (2000-1929). Letter D denotes samples from the dredged material. 1-5: DGGE bands in samples. Negative image of ethidium bromide stained gel.

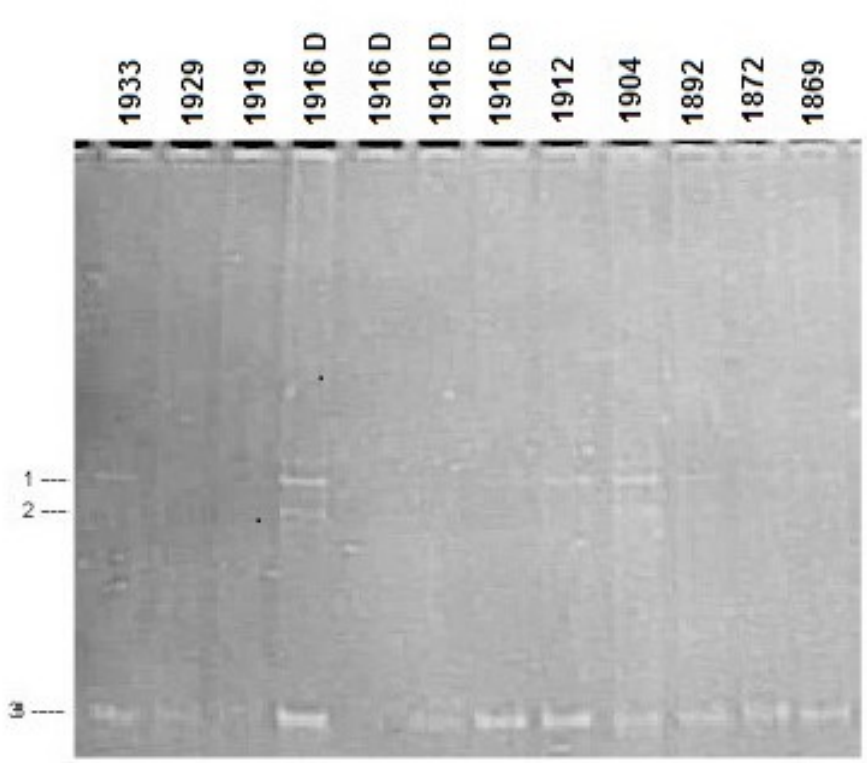

Figure 4.3. DGGE profile of 16S rRNA V3 domain amplicons obtained from Hamilton Harbour sediment sub-samples 36-46 (1929-1869). Letter D denotes samples from the dredged material. 1-3: DGGE bands in samples.

4.3.2. DGGE *mcy*E-AMT genetic profiles of sedimentary Cyanobacteria

PCR-amplification of the aminotransferase domain from bulk genomic DNA using HEP primers was successful from all analyzed sediment sub-samples (Figures 4.4 and 4.5). In order to avoid inconsistencies, all DNA samples were PCR amplified and run on the DGGE system at the same time. The identification of each cyanobacterial species was achieved by comparing band position on the gel to the position of bands in the standard marker. DGGE fingerprints of PCR-amplified *mcy*E genes of cyanobacterial communities from sediments showed 2 bands, indicating the constant presence of two microcystin producing Cyanobacteria (*Microcystis aeruginosa* and *Planktothrix rubescens*) over the 80 years period (1929-2009). *Planktothrix rubescens* provided clear bands in all samples while *Microcystis aeruginosa* provided strong signal only in the most recent sediment (sample 1 corresponding to year 2009) and fainter bands in subsequent sub-samples. The third microcystin producing organism, *Dolichospermum lemmermannii*, was not detected in any of the samples.

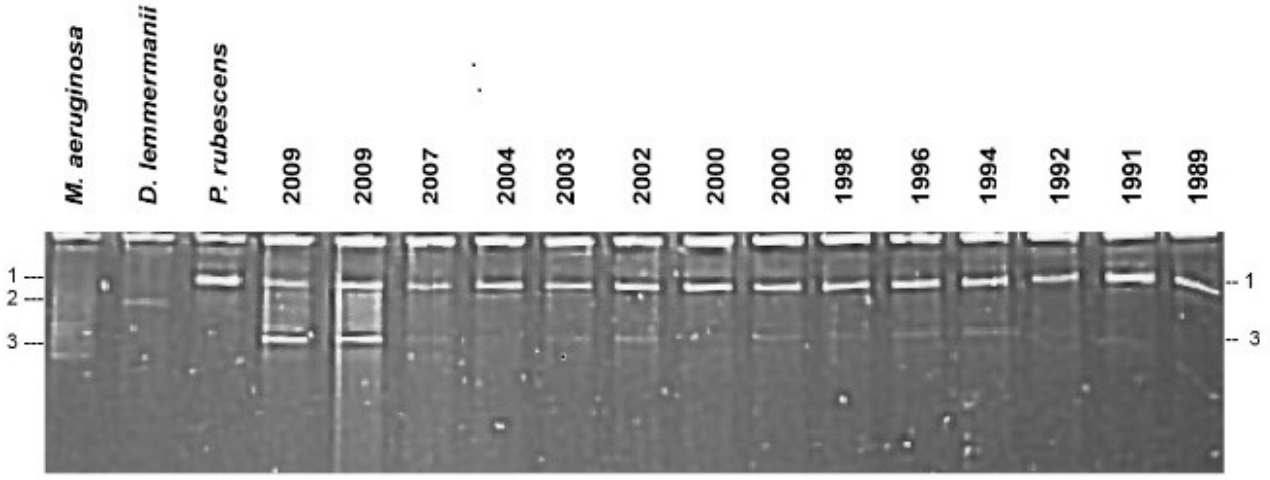

Figure 4.4. DGGE profile of *mcy*E-AMT domain amplicons obtained from Hamilton Harbour sediment sub-samples 1-14 (2009-1989). The two bands in samples correspond to bands from *Planktothrix rubescens* (first) and *Microcystis aeruginosa* (third) in the standard marker. Standards used (left to right) were : 1. *Planktothrix rubescens* UTCC 507, 2. *Dolichospermum lemmermannii* NIVA-CYA 83/1, and 3. *Microcystis aeruginosa* UTCC 299.

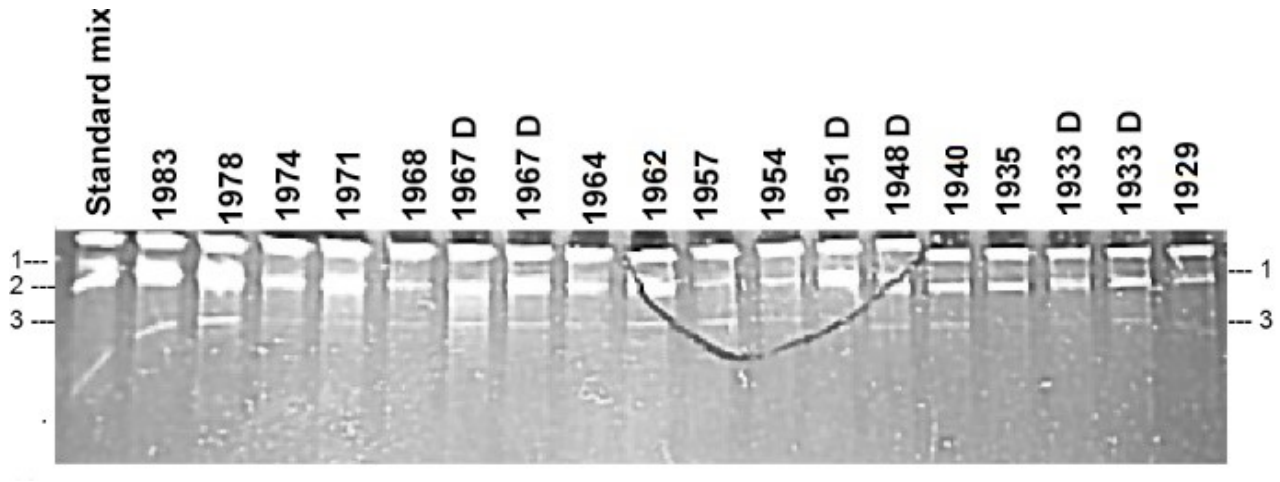

Figure 4.5. DGGE profile of *mcy*E-AMT domain amplicons obtained from Hamilton Harbour sediment sub-samples 15-33 (1983-1929). The two bands in samples correspond to bands from *Planktothrix rubescens* (first) and *Microcystis aeruginosa* (third) in the standard marker. Standard mix (top to bottom) consisted of: 1. *Planktothrix rubescens* UTCC 507, 2. *Dolichospermum lemmermannii* NIVA-CYA 83/1, and 3. *Microcystis aeruginosa* UTCC 299.

4.3.3 Estimation of cyanobacterial community richness using DGGE bands profiles

Based on the number of bands present in the 16S rRNA-V3 DGGE profiles, cyanobacterial species richness was estimated. The number of DGGE bands ranged from 2 to 11. Phylotype (band) richness of the cyanobacterial communities was highest in the samples from the top of the sediment core (11) and lowest in the deepest sediment strata where 2 bands were observed. The total number of the DGGE bands in the sediment core was 13.

4.4. Discussion

Harmful algal blooms (HABs) are rapidly increasing throughout the world with growing incidence since the 1960's (Carmichael, 2008). Increases in algal and cyanobacterial blooms on the global scale have mostly been ascribed to nutrient enrichment (Heisler *et al.,* 2008) and climate change (Paerl and Huisman, 2008; Paerl *et al.*, 2011). Due to global warming, an increase in surface water temperatures could play a role in the proliferation of cyanobacterial blooms (Paerl and Huisman, 2008).

The increase of algal bloom reports in Ontario from 1994 to 2009 was investigated by Winter *et al.* (2011), who noted a statistically significant increase in the number of blooms dominated by chlorophytes and Cyanobacteria, and the greatest increase was in cyanobacterial blooms (>50% of the total on average) (Winter *et al.*, 2011). The expansion of human activities on and around the lakes as well as the region's warming over the last several decades has likely played a major part in the rise of cyanobacterial blooms in Ontario's lakes (Winter *et al.*, 2011). The same authors reported that in 2009, 66% of

cyanobacterial blooms were toxic, with presence of microcystin confirmed by ELISA testing (Winter *et al.*, 2011).

The occurrence of toxic cyanobacterial blooms in the Laurentian Great Lakes have become more frequent since mid-1990s (Watson *et al.*, 2008) and in the last decade, these blooms, comprised primarily of *Microcystis* spp., have become annual events (Brittain *et al.*, 2000; Hotto *et al.*, 2005; Rinta-Kanto *et al.*, 2005; Ouellette *et al.*, 2006; Conroy *et al.*, 2007). However, reports of toxic cyanobacterial blooms in the Great Lakes are more prevalent in the lower, more populated Great Lakes (Watson *et al.*, 2008). Additionally, sporadic high toxin levels have been reported in inshore areas of Lakes Michigan, Huron, Erie, and Ontario (Brittain *et al.,* 2001; Vanderploeg *et al.,* 2001; Hotto *et al.*, 2005; Rinta-Kanto *et al.*, 2005). A rise in toxic cyanobacterial blooms has been observed in Lake Erie, which in 2011 experienced the largest harmful algal bloom in its recorded history (Michalak *et al.*, 2013). The bloom began in the western basin in mid-July, spread into the central basin, extended eastward and persisted until mid-October; it was estimated that the bloom covered more than 5,000 km^2 of water surface (Michalak *et al.*, 2013). Phytoplankton analysis confirmed that bloom was almost entirely composed of *Microcystis* until late August, after which it was replaced by nitrogen-fixing *Anabaena* (secondary bloom) (Michalak *et al.*, 2013). *Microcystis* DNA analysis from various sampling locations showed that similar strains occurred in the western and central basins; microcystin concentrations ranged from 0.1 μg/L to 8.7 μg/L and were detected at the most western basin sampling sites (Michalak *et al.*, 2013). Authors hypothesized that the most probable reason for such a large bloom was a combination of land use, agricultural practices, and meteorological conditions (Michalak *et*

al., 2013). Lake Erie and Lake Ontario blooms were investigated using DNA analyses on several occasions. For instance, Rinta-Kanto *et al.* (2009a) analysed dynamics of *Microcystis* genotypes in Lake Erie, using *Microcystis*-specific 16S rDNA copies and *mcy*D gene copies and correlated the abundance of the genes with relative abundance of toxic *Microcystis* cells and microcystin concentrations. The results of the study showed that microcystin-producing genotypes coexisted with non-toxic genotypes and that the the proportion of microcystin-producing *Microcystis* cells accounted for no more than 8% of the total cyanobacterial abundance. Also, *Microcystis* was identified as the most important contributor to the microcystin concentrations but other potential microcystin producing genera were present as well (*Planktothrix*, *Aphanizomenon*, *Anabaena/Dolichospermum*) (Rinta-Kanto *et al.*, 2009a). The analysis of Lake Erie sediments showed the presence of viable toxic Microcystis species, genetically similar to bloom forming populations (Rinta-Kanto *et al.*, 2009b). Analysis from sedimentary *mcy*A fragments showed that microcystin-producing cyanobacteria, beside *Microcystis*, were *Nostoc*, *Anabaena/Dolichospermum* and *Planktothrix* (Rinta-Kanto *et al.*, 2009b). Waters along the southern and eastern shores of the Lake Ontario were analyzed for the presence of potential microcystin-producing Cyanobacteria (Hotto *et al.*, 2007). Molecular analysis has shown that the genetic potential for toxin production was widespread, while the actual levels of toxin microcystin were generally low (Hotto *et al.*, 2007). Based on *mcy*A and *mcy*E primer sets *Microcystis* was identified as the major microcystin producer in Lake Ontario, while potentially toxic *Planktothrix* and *Anabaena/Dolichospermum* species were not widespread (Hotto *et al.*, 2007). Also, molecular analysis revealed that the *mcy* genes were sporadically distributed

along the shoreline indicating that separate genotype and phenotype microcystin-producing populations were present in different embayments of the lake (Hotto *et al.*, 2007). Comparative analysis of Bay of Quinte (Lake Ontario) and Maumee Bay (Lake Erie) cyanobacterial genotypic community was performed in 2005 and 2006 on water samples by Chunn (2007). 16S rRNA-V3 hypervariable region and aminotransferase (AMT) region of the microcystin (*mcyE*) and nodularin (*nda*) gene were analysed using denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR (qPCR). Results of the analysis showed that in both bays cyanobacterial genetic populations differed between early and late summer, based on profiles of the 16S rRNA-V3 amplicons (Chunn, 2007). Bay of Quinte DGGE analysis of AMT amplicons showed that one or two toxic genetic populations occurred in early and late summer. In Maumee Bay no toxic genes were detected in early summer, but by late summer, multiple AMT genotypes were detected (>5). Positions of bands on the gel, compared to DGGE standard, revealed that *M. aeruginosa and A. lemmermannii* were present in all samples, but at different times during the summer season (Chunn, 2007).

Hamilton Harbour, as one of the Lake Ontario embayments, is susceptible to cyanobacterial blooms because of the long history of eutrophication caused by anthropogenic nutrient pollution. Bloom formations have been observed since the mid-1990s with the harbour phytoplankton community being dominated by species of *Microcystis* (Erdner *et al.*, 2008). In 2001 (late August-September), microcystin was reported in Hamilton Harbour in concentrations as high as 400 µg/l in some wind concentrated scums (Murphy *et al.*, 2003).

Several species of *Microcystis* dominated the toxic bloom together with *Aphanizomenon flos-aquae* towards the end of the summer (Murphy *et al.*, 2003). In the study of Allender *et al.* (2009) water samples collected from Hamilton Harbour in August of 2002 and 2006 were tested for presence of *mcy* genes. This research confirmed previous findings of Murphy *et al.* (2003) that *Microcystis was* the harbour's microcystin producer.

In the present research, temporal response of the cyanobacterial community of Hamilton Harbour, Lake Ontario, was analyzed from sedimentary DNA, using PCR-DGGE of *mcy*E-AMT gene.

4.4.1. DGGE Analysis

4.4.1.1. DGGE 16S rRNA-V3 genetic analysis of sedimentary Cyanobacteria

Results of DGGE analysis of PCR amplified 16S rRNA V3 amplicons showed historical changes of the cyanobacterial composition in Hamilton Harbour over 140 years. The separation patterns of DGGE bands indicated distinctive cyanobacterial populations from different sediment layers. The highest diversity occurred in 2009 surface layers (0-1 cm) where 11 DGGE bands were detected. The diversity generally increased from the oldest to the most recent sediment layers, indicating changing environmental conditions in the harbour. Based on the number of DGGE bands in sediment samples, three general time periods can be inferred: period I (1869-1931; 100.5-45.5 cm) with presence of mostly two bands (Figure 4.3), period II (1939-2000) with presence of 5 bands in majority of samples (Figure 4.2) and period III (2002-2009) with presence of 3-11 bands (Figure 4.1). The

absence of bands in some of the dredged sediment layers could be due to dumping of allochtonous material (i.e. sediment from other parts of the harbour) and dilution of the DNA. Also, organic molecules such as humic acids and pollutants could have had a negative influence on the amplification of V3 regions both in dredged and non-dredged samples. Finally, low abundance of total DNA (<1%) could have been a contributing factor for bands' absence from those samples.

The constant number of bands in samples from 1869-1933 time range indicates a relatively constant environment in the harbour. Based on historic records inferred from diatoms (Yang *et al.*, 1993), excessive nutrient loadings (due to agricultural practices and industrialization) have caused eutrophication of the harbour since 1860s, with eutrophication at its peak in the early 1900s. Those conditions were favourable and relatively stable for the cyanobacterial development. Three bands in the 1916 sample are an indication of different conditions in different parts of the harbour at different times, since all samples from 1916 were from the dredged material.

Increased cyanobacterial diversity in sediment samples from the 1935-2000 period (Figure 4.2) is evident from the presence of five bands. The observed increase may have been due to increase in nutrients and increase of temperature over time. According to the siliceous fossil record, 1926-1961 period was characterized by accelerated eutrophication and water contamination and by an abrupt increase of eutrophic diatom taxa (Yang *et al.*, 1993). Starting in the early1930s an increase in industrial effluents and municipal wastes, together with substantial dredging, accelerated the nutrient pollution, which culminated in the worst water pollution in history of the Harbour (1970-1986) (Yang *et al.*, 1993). The sedimentary

history determined from the core, is supported by Hamilton Harbour water quality historical records. For instance, total nitrogen levels increased from 1-2 mg/ l in 1949 to up to 3 mg/l in 1976 (Matheson, 1958; Piccinin, 1977); total phosphate was up to 0.08 mg/l in 1950 (Matheson, 1958). Globally rising temperatures resulted in surface water warming and prolonged duration of stratification, which favour cyanobacterial growth and reproduction, and occurrence of cyanobacterial blooms (Paerl and Huisman, 2008; Paerl *et al.*, 2011). Synergistic effects of nutrient and temperature increases may have promoted cyanobacterial diversity during this period.

In the third period (2002-2009) the greatest variation in number of bands (3-11) was observed (Figure 4.1). There are several possible causes for the observed change in the cyanobacterial community composition toward increased number of species. One of them is the change in water quality. The water quality of Hamilton Harbour has been improving since 1987 (Hiriart-Baer *et al.*, 2009), since phosphorous abatement was introduced in order to control eutrophication and eventually improve the ecosystem's health. Further reduction of phosphorus inputs was implemented again in the 1990s (Gerlofsma *et al.*, 2007). However, the recent establishment of invasive dreissenid mussels (zebra mussel, *Dreissena polymorpha* and quagga mussel, *Dreissena bugensis*) caused additional water quality issues. Dreissenid mussels are filter feeders and therefore remove suspended particles, increasing light penetration and water clarity of the harbour (Horgan and Mills, 1997). Increased water clarity changes the UV light penetration, which is known to stimulate the growth of toxic bloomers and microcystin production (Wulff, 2001). In such environment, microcystin producing species can resist grazing by herbivores (Paerl, 1988) and can minimize the adverse effects of

UV irradiation by forming surface scums. Finally, elevated iron concentrations due to use of enhanced ferric iron chloride in wastewater treatment process (Mederios and Molot, 2006) may be another cause for the increase in toxic cyanobacterial blooms in Hamilton Harbour. Cyanobacteria require more cellular iron than eukaryotic algae (Fay and van Baalen, 1987) and cyanobacterial growth has also been linked to iron availability (Twiss *et al.*, 2000; Hyenstrand *et al.*, 2001).

4.4.1.2. DGGE mcyE-AMT genetic analysis of sedimentary Cyanobacteria

The results of the DGGE analysis of PCR amplified AMT amplicons indicated the presence of two hepatotoxic genotypes throughout the most of the core, over a much longer time period than previously thought (Figures 4.4 and 4.5). The detected toxic genotypes were consistent with species of the genera *Microcystis* and *Planktothrix*. The occurrence of *Microcystis* as one of the main hepatotoxin producers of Hamilton Harbour was expected and was in agreement with previous findings of Murphy *et al.* (2003) for Hamilton Harbour, Hotto *et al.* (2007) for Lake Ontario and Chunn (2007) for Bay of Quinte (Lake Ontario) and Maumee Bay (Lake Erie). The second potentially toxic genus, *Planktothrix*, has previously been confirmed as microcystin producer from phytoplankton samples of Lake Ontario (Hotto *et al.*, 2007) but not from Hamilton Harbour. The third potential microcystin producer, *Dolichospermum*, was not detected in any of the tested samples. The research conducted on phytoplankton samples from the southern and eastern shores of Lake Ontario in 2001 and 2003 (Hotto *et al.*, 2007) showed that potentially toxic *Planktothrix* and *Anabaena/Dolichospermum* species were not widespread in lake Ontario. However, both

Anabaena/Dolichospermum and *Planktothrix* were detected from Lake Erie sediment samples (Rinta-Kanto *et al.*, 2009b)

The results of the DGGE analysis using *mcy*E species-specific primers clearly showed that potentially toxic and/or toxic cyanobacteria have been a component of the phytoplankton community of Hamilton Harbour for at least 80 years (since 1929), and that the toxic population has shown no change in species composition over this time period. Regardless of the variable, generally decreasing DNA concentrations in sediment from top to bottom, a notably weeker signal was observed for *Microcystis aeruginosa* genotype (band 3 on DGGE gels, Figures 4.4 and 4.5) compared to *Planktothrix rubescens* (band 1 on DGGE gels). Also, samples from 2004 and 1935 showed no presence of the *Microcystis* genotype. The lack of detectable *Dolichospermum* genes in any of the samples indicates that *Dolichospermum* had: too low abundance for amplification, low DNA preservation, or that there was no toxigenic *D. lemmermannii* present in the past. Since two out of three toxic genes were detected, DNA seems to have been preserved well enough for the detection of the third gene. Low *Dolichospermum* abundance (<1% of total DNA) could have been a contributing factor for its non-detection, rather than its absence in the past. Presumed low abundance is in accord with findings of Hotto *et al.* (2007) for Lake Ontario's low abundance of *Dolichospermum*. It is also in agreement with results of this study (Chapter 2) of Hamilton Harbour phytoplankton samples for the period June-November 2009 (Figures 2.4-2.6). The lack of *Microcystis* genes presence in two samples may be the result of the genes' low concentrations or non reproducible amplification which is not uncommon for the toxic genes from environmental samples, rather than its absence in those years (Ouellette *et al.*, 2006).

The presence of cyanobacterial pigment scytonemin derivative, compound B (Figure 3.5), related to presence of cyanobacteria exposed to high UV radiation (Leavitt *et al.*, 1997), and indicative of cyanobacterial blooms, was detected in 11 sediment samples taken for pigments analysis (Table 3.1). Toxic *mcy*E genes were present in all sediment strata corresponding to years of inferred cyanobacterial blooms, indicating their potential toxicity.

4.4.2. Estimation of cyanobacterial richness based on DGGE profiles

As a component of biodiversity, species richness (number of present species) is often combined with molecular profiling methods in order to compare microbial communities. The estimation of cyanobacterial richness from different sediment sub-samples was based on 16S rRNA-V3 profiles *i.e*. number of bands on DGGE gels (Figures 4.1-4.3). It was observed that over time (1869-2009) the number of DGGE bands increased, as did a relatively stable number of bands during three time intervals identified from the core. However, richness analysis based on DGGE bands positions can underestimate the true community richness. Bands that migrate to the same position on the gel do not always originate from the same organism or have the same sequence (Muyzer *et al.*, 1993; Jackson *et al.*, 2000). It is almost certain that in this study richness underestimation happened due to low abundance of target DNA (<1%) in the total sedimentary genomic DNA mixture (Muyzer *et al.*, 1993), at least in some of the samples. In such cases, no DNA amplification was possible by PCR-DGGE method, and consequently no bands were detected from samples.

4.5. Conclusions

The vertical profile of cyanobacterial assemblages detected from Hamilton Harbour sediment core using 16S-V3 region showed high temporal variability. Sedimentary stratigraphy showed a generally increasing trend of cyanobacterial species diversity from oldest to most recent sediment layers, as identified from the number of DGGE bands. Cyanobacteria of Hamilton Harbour responded to environmental changes with shifts in community structure, manifested as an increase in number of DGGE bands, but with notable time lags. The vertical profile of sedimentary toxic cyanobacterial assemblages showed no change over time. Two toxic genotypes, *Planktothrix rubescens* and *Microcystis aeruginosa*, were consistently detected from 1929-2009. Toxic genes were present in all sediment strata corresponding to years of pigments-inferred cyanobacterial blooms. Based on historical evidence of eutrophication and evidence of toxic genes present in the core, as well as current eutrophication and bloom formation data, it can be assumed that such blooms did occur in the past, at least for the time period covered by this analysis. All the sediment slices included in this analysis were from the time period already impacted by human activities. A more detailed reconstruction of the past would require a longer sediment core that goes to pre-settlement time, before the human influence on the harbour.

Chapter 5

General conclusions

The worldwide phenomenon of harmful algal blooms (HABs) is of increasing public concern in the Laurentian Great Lakes. Hepatotoxic microcystins from cyanobacteria are of considerable concern for human health as they can produce adverse effects at low threshold levels. The Laurentian Great Lakes, as the world's largest freshwater ecosystem, are greatly influenced by anthropogenic actions that contribute to such blooms. Furthermore, mechanisms that regulate toxin production in bloom-forming cyanobacteria are not well understood. As one of the most polluted sites in the Great Lakes ecosystem, Hamilton Harbour has been designated as an area in need of particular attention and concern due to recurring toxic cyanobacterial blooms and consequent high microcystin levels. Today, Hamilton Harbour is moderately eutrophic and together with the increased transparency, nutrient levels are still sufficient to stimulate development of severe late summer blooms of cyanobacteria, some of which are toxic.

This research sought to gain a better understanding of the Hamilton Harbour cyanobacterial community composition along temporal scale, with special emphasis on toxic/toxigenic genera, using a multidisciplinary approach. Information of contemporary cyanobacterial communities was obtained using environmental water samples. Information of historical changes in cyanobacterial communities was obtained from the sediment core

collected from the central part of the harbour, after sedimentary chronology was established using the Constant Rate of Supply (CRS) ^{210}Pb age model.

The aims of the first part of the study (Chapter 2) were to gain a better understanding of the seasonal distribution of contemporary toxigenic Cyanobacteria in relation to the environmental factors of the harbour and to estimate *Microcystis* relative abundance in relation to total algal numbers. To elucidate the relationships between environmental characteristics and biological communities in Hamilton Harbour, environmental (physico-chemical) and biological (phytoplankton) data were collected in the summer-fall season of 2009 from two sampling stations. Using microscopy, seasonal diversity of contemporary cyanobacterial communities was analyzed. Of the 14 genera identified from phytoplankton samples, the four dominant genera were *Microcystis*, *Planktothrix*, *Aphanizomenon* and *Limnothrix.* During the sampling period from June 30 to November 3, *Microcystis* presence was recorded at both stations and at all dates, while it's relative abundance remained below 10 % throughout the study period and showed seasonal variability between stations. Water quality analysis results indicated that though most of the water quality parameters generally followed the pattern of a long term trend for Hamilton Harbour, loads of some of the nutrients were still high and might have been factors in cyanobacterial blooms. The statistical analysis of environmental variables and their relationship to diversity and abundances of filamentous cyanobacterial assemblages were evaluated at the relevant seasonal scale. Results of the statistical RDA analysis correlated environmental variables NO_3/NO_2 and DIC and conductivity to seasonal changes in filamentous cyanobacterial community structure and abundance. Positive correlations were found between high concentrations of dissolved

nitrates NO_3/NO_2 and genera *Planktothrix* and *Dolichospermum*. High concentrations of DIC correlated positively with abundances of *Aphanizomenon* and *Limnothrix*. The results of this study supported the initial hypothesis that environmental factors influence the abundance of cyanobacterial assemblages in the harbour. Rapid temporal and spatial changes in cyanobacterial community in 2009 season confirmed previous findings (Munawar and Fitzpatrick, 2007) that Hamilton Harbour is a highly stressed ecosystem and that further efforts in the reduction of nutrient loads are necessary in remediation efforts.

The aim of the second part of the study (Chapters 3 and 4) was to determine historical changes in cyanobacterial communities. Determination of natural variability of past algal and cyanobacterial communities was established using a paleolimnological approach (Chapter 3). HPLC method was used to analyze fossil pigments concentrations and their stratigraphic distribution. Sedimentary pigments analyses revealed that algal and cyanobacterial communities were highly variable over time. The dominant and most abundant algal groups over the past 140 years were the Chlorophyta, Bacillariophyta, Dinophyta and Cryptophyta. Cyanobacteria were regular but not dominant members of the phytoplankton community. Stratigraphic distribution and concentrations of cyanobacterial pigments revealed the presence of bloom forming cyanobacteria. Bloom formation was indicated by presence of compound B, a derivative of the pigment scytonemin which is commonly observed in surface cyanobacterial blooms. The results of the study supported the hypothesis that cyanobacterial blooms occurred in the past, and it was shown that their frequency was at least once in every decade. However, a limited number of sedimentary samples used in the fossil pigments analysis indicated only a general pattern of change of planktonic algal communities over

time. To gain better understanding of phytoplankton/cyanobacterial dynamics over time, a finer temporal resolution, *i.e*. additional number of samples is necessary, preferably from a longer sediment core that goes farther back in time, before the human impact on the harbour.

An insight into the past genotypic composition and diversity of the cyanobacterial community was enabled using modern molecular tools such as polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) (Chapter 4). Sedimentary genomic DNA was examined for presence of two Cyanobacteria-specific gene fragments: V3 region of the 16S rRNA and AMT region of *mcy*E gene. Based on the number of DGGE bands of 16S rRNA-V3 region, it was shown that the cyanobacterial community was changing and that the number of species increased from 2 to 11 during 140 years, which confirmed hypothesis that the genetic fingerprints of cyanobacterial assemblages from different stratigraphic intervals differed at different time periods. Using *mcy*E-AMT gene fragment it was shown that toxic cyanobacterial genera were present in the harbor over the past 80 years and that their diversity did not change. Prior to this study, Murphy *et al.* (2003) and Allender *et al.* (2009) noted that *Microcystis aeruginosa* was the primary microcystin producer in Hamilton Harbour. In the present study it was shown that in addition to *Microcystis, Planktothrix rubescens* was also contributing to microcystin production in the harbour. Though the presence of *mcy*E genes does not necessarily indicate the toxic nature of present/past blooms, recent toxic bloom occurrences under similar (if not more moderate) levels of eutrophication indicate that toxic cyanobacterial blooms likely did occur in the past. However, the level of their toxicity is unknown. Results of this study confirmed initial hypothesis that microcystin-producing cyanobacterial genotypes were present in the past

before documented cyanobacterial blooms. The *mcy*E gene and aminotransferase domain proved successful in the identification of toxin producing cyanobacterial genera from sediment samples. In the absence of historical records, both the PCR-DGGE method and the *mcy*E-AMT gene employed in this study may be used for the reconstruction of past toxic blooms not only in the Laurentian Great Lakes, but also in other aquatic regions of the world impacted by cyanobacterial toxic blooms.

The present research is the first report on fossil pigments from Hamilton Harbour and the first on the *mcy*E module of microcystin genes being amplified from sediment of North American lakes. It has extended the knowledge of toxic cyanobacterial blooms history by showing that Hamilton Harbour has experienced potentially toxic cyanobacterial blooms since 19th century, long before the first official records in the 1960s, and that the blooms occurred parallel with the growing anthropogenic impacts, primarily nutrient and contaminant pollution of the harbor. Since sedimentary microcystin presence and concentrations were not tested, direct measurements of toxin should be undertaken parallel to molecular analyzes of *mcy* genes. The outcomes of such approach would provide an inference of past changes in microcystin production and its correspondence to the presence of toxic cyanobacterial species as well as a link to environmental conditions that most probably caused toxin production. The current understanding on cyanobacterial blooms in the harbour is limited by the age of the sediment core. The sedimentary age of 140 years leaves a gap in knowledge of the environmental conditions in the harbour before European settlers' impact. A longer core, which delves deeper into the past, would provide a better insight into the ecological conditions of the harbour before human influence, and would help to define the

time period of the beginning of the blooms and triggers for their occurrence.

The study approach of the present research, that combined contemporary analysis of phytoplankton and water chemistry with analysis of sedimentary pigments and DNA records, most certainly would prove a useful tool in determination of the bloom causes factors, mitigation of the future production of blooms, and remediation efforts not only in already impacted aquatic environments, but also in blooms prevention in water bodies that have not yet experienced toxic cyanobacterial blooms.

Appendix

Table 1. Number of algal units, *Microcystis* colonies and *Microcystis* percent abundance in phytoplankton samples from CS at 1 m depth.

Station/ Depth (m)	Sampling date	Algal units number (x10^3/l)	*Microcystis* colonies *number* (x10^3/l)	*Microcystis* colonies abundance (%)
1001 1 m	Jun 30	8737	54	0.6
	Jul 07	11290	500	4.4
	Jul 20	16398	837	5.1
	Aug 06	9117	326	3.6
	Aug 17-24	7009	435	6.2
	Aug 31	11508	598	5.2
	Sep 16	10117	587	5.8
	Oct 08	11921	489	4.1
	Nov 03	4825	185	3.8

Table 2. Number of algal units, *Microcystis* colonies and *Microcystis* percent abundance in phytoplankton samples from CS at 10-0 m depth.

Station/ Depth (m)	Sampling date	Algal units number (x10^3/l)	*Microcystis* colonies *number* (x10^3/l)	*Microcystis* colonies abundance (%)
1001 10-0 m	Jun 30	8313	717	8.6
	Jul 07	18598	1386	7.4
	Jul 20	16137	668	4.1
	Aug 06	12192	391	3.2
	Aug 17-24	6080	587	9.7
	Aug 31	17718	815	4.6
	Sep 16	5069	261	5.1
	Oct 08	5672	310	5.5
	Nov 03	5738	65	1.1

Table 3. Number of algal units, *Microcystis* colonies and *Microcystis* percent abundance in phytoplankton samples from WS at 1 m depth.

Station/ Depth (m)	Sampling date	Algal units number ($x10^3$/l)	*Microcystis* colonies *number* ($x10^3$/l)	*Microcystis* colonies abundance (%)
9031 1 m	Jun 30	39479	505	1.3
	Jul 07	22999	310	1.3
	Jul 20	15893	228	1.4
	Aug 06	24972	293	1.2
	Aug 17-24	45819	391	0.9
	Aug 31	21809	505	2.3
	Sep 16	23260	1304	5.6
	Oct 08	5787	391	6.8
	Nov 03	8313	245	2.9

Table 4. Hamilton Harbour raw water quality data for physical parameters from June-November 2009 at 1 m depth for Central (1001) and Western (9031) sampling stations

Station/Depth (m)	Sampling Date	Secchi Depth (m)	Water Temperature (^{0}C)	pH	Conductivity (µs/cm)	Dissolved Oxygen (mg/l)
CS 1 m	Jun 30	1.00	20.8	8.57	719	8.0
	Jul 07	1.50	20.2	8.77	702	9.6
	Jul 20	2.00	19.6	9.05	677	10.3
	Aug 06	3.00	19.9	8.83	605	9.1
	Aug 17-24	2.20	22.2	8.97	583	9.1
	Aug 31	2.50	19.5	8.71	548	8.2
	Sep 16	3.50	19.6	8.77	550	7.6
	Oct 08	3.00	14.5	8.37	538	6.6
	Nov 03	4.00	11.8	8.26	564	10.0
WS 1 m	Jun 30	n/a	21.5	8.76	722	8.6
	Jul 07	2.00	20.1	8.67	704	9.4
	Jul 20	2.25	19.0	8.88	673	9.8
	Aug 06	2.00	19.5	8.64	617	8.2
	Aug 17-24	1.90	21.4	8.73	589	7.9
	Aug 31	1.75	18.9	8.89	547	8.4
	Sep 16	2.50	20.3	8.97	549	8.6
	Oct 08	3.25	14.5	8.34	539	6.4
	Nov 03	3.50	11.1	n/a	n/a	n/a

Table 5. Hamilton Harbour raw water quality data for chemical parameters from June-October 2009 for Central Station (1001) at 1 m depth

Station/ Depth (m)	Sampling date	NO_3/NO_2 -F (mg/l)	NH_3-N-F (mg/l)	Chl *a* (µg/l)	DOC (mg/l)	DIC (mg/l)	POC (mg/l)	PON (mg/l)	SRP-P-F (mg/l)	TP-P-F (mg/l)	TP-P-UF (mg/l)
	Jun 30	2.63	0.108	6.6	4.1	30.6	n/a	n/a	0.0071	0.026	0.0597
	Jul 07	2.51	0.017	12.1	4.0	29.8	1.27	0.178	0.0030	0.019	0.0426
	Jul 20	2.27	0.012	15.8	3.9	27.6	2.05	0.275	0.0029	0.015	0.0426
WS	Aug 06	1.99	0.010	6.8	3.4	27.4	1.03	0.140	0.0027 *	0.014 *	0.0392*
1m	Aug 17-24	1.79	0.019	15.3	3.6	25.4	1.88	0.267	0.0024	0.013	0.0357
	Aug 31	1.69	0.014	16.6	3.3	25.7	1.70	0.265	0.0023	0.014	0.0357
	Sep 16	1.78	0.015	7.1	3.4	26.6	1.22	0.196	0.0042	0.018	0.0397
	Oct 08	1.89	0.026	8.2	3.6	27.6	1.16	0.139	0.0020	0.013	0.0392

* denotes missing values that were substituted with average values from the two closest sampling periods

Table 6. Hamilton Harbour raw water quality data for chemical parameters from June-October 2009 for Western Station (9031) at 1 m depth

Station/ Depth (m)	Sampling date	NO_3/NO_2-F (mg/l)	NH_3-N-F (mg/l)	Chl *a* (µg/l)	DOC (mg/l)	DIC (mg/l)	POC (mg/l)	PON (mg/l)	SRP-P-F (mg/l)	TP-P-F (mg/l)	TP-P-UF (mg/l)
	Jun 30	2.07	0.135	16.9	4.5	33.6	n/a	n/a	0.0053	0.023	0.0688
	Jul 07	2.50	< 0.005	18.0	3.9	30.2	1.42	0.205	0.0016	0.017	0.0399
	Jul 20	2.31	< 0.005	18.6	4.2	29.4	1.77	0.251	0.0019	0.014	0.0367
WS	Aug 06	1.66	0.010	10.3	3.7	29.6	1.23	0.220	0.0020 *	0.013 *	0.0396 *
1m	Aug 17-24	1.64	0.035	12.7	4.0	13.7	1.92	0.319	0.0021	0.013	0.0424
	Aug 31	1.65	0.006	11.3	3.4	25.7	1.30	0.234	0.0023	0.013	0.0348
	Sep 16	1.61	0.015	19.9	3.6	25.6	2.47	0.400	0.0017	0.013 *	0.0326
	Oct 08	1.43	0.037	15.6	3.9	31.4	1.43	0.238	0.0018	0.014	0.0525

* denotes missing values that were substituted with average values from the two closest sampling periods

Table 7. Hamilton Harbour LOI and ^{210}Pb chronology data

Depth interval (cm)		Mid point depth	Moisture Content	Organic matter	Mineral matter	CRS dates	Dry mass sedimentation	210Pb	214Bi	137Cs
top	bottom	(cm)	(%)	(%)	(%)		(g/cm2 yr)	(dpm/g)	(dpm/g)	(dpm/g)
0	0.5	0.25	90.41	16.46	11.84	2009	0.2	17.2	3.9881	0.3597
0.5	1	0.75	81.75	13.61	13.49	2009	0.2	17.03		
1	1.5	1.25	75.8	13.99	13.58	2008	0.19	16.87		
1.5	2	1.75	87.86	14.23	14.78	2008	0.19	16.71		
2	2.5	2.25	85.95	14.35	12.17	2007	0.19	16.55	2.7583	0.2495
2.5	3	2.75	86.29	14.01	11.9	2007	0.19	15.65		
3	3.5	3.25	90.37	14.06	12.43	2006	0.2	14.79		
3.5	4	3.75	81.98	13.37	11.07	2005	0.21	13.96		
4	4.5	4.25	79.9	13.4	11.04	2005	0.23	13.16	2.4717	0.3812
4.5	5	4.75	76.79	12.56	13.5	2004	0.24	12.41		
5	5.5	5.25	75.43	11.86	16.31	2003	0.25	11.69		
5.5	6	5.75	80.24	12.35	15.37	2003	0.27	11		
6	6.5	6.25	79.95	12.51	14.41	2002	0.28	10.34	2.5821	0.4121
6.5	7	6.75	78.87	11.88	15.37	2002	0.28	10.32		
7	7.5	7.25	84.5	12.68	16.86	2001	0.27	10.3		
7.5	8	7.75	73.1	7.09	14.86	2001	0.27	10.28		
8	8.5	8.25	77.83	11.95	15.49	2000	0.26	10.26	2.4879	0.5568
8.5	9	8.75	73.64	12.13	16.63	1999	0.27	10.03		
9	9.5	9.25	83	12.57	15.4	1999	0.27	9.8		
9.5	10	9.75	81.22	12.87	14.84	1999	0.28	9.58		
10	10.5	10.25	83.3	12.83	16.2	1998	0.29	9.36	2.7486	0.4171
10.5	11	10.75	82.26	13.38	13.11	1998	0.28	9.6		
11	11.5	11.25	79.65	13.04	12.91	1997	0.27	9.85		
11.5	12	11.75	79.04	12.85	14.71	1997	0.26	10.1		
12	12.5	12.25	79.57	12.17	16.92	1996	0.24	10.36	2.9869	0.5678
12.5	13	12.75	81.22	13.3	15.32	1996	0.22	10.98		
13	13.5	13.25	80.01	13.09	15.83	1995	0.2	11.62		
13.5	14	13.75	87.34	12.96	18.57	1995	0.18	12.29		
14	14.5	14.25	79.05	13.72	14.15	1994	0.16	12.98	2.5469	0.8636
14.5	15	14.75	82.43	14.55	11.18	1993	0.17	12.4		
15	15.5	15.25	77.59	13.35	13.61	1992	0.18	11.84		
15.5	16	15.75	73.04	14.14	13.35	1991	0.18	11.3		
16	16.5	16.25	69.24	13.12	13.45	1989	0.19	10.77	2.6907	0.9792
16.5	17	16.75	70.29	13.65	12.82	1988	0.17	10.84		
17	17.5	17.25	66.06	13.68	12.48	1987	0.16	10.9		
17.5	18	17.75	75.28	14.01	10.86	1985	0.15	10.97		

Depth interval (cm)		Mid point depth (cm)	Moisture Content (%)	Organic matter (%)	Mineral matter (%)	CRS dates	Dry mass sedimentation (g/cm2 yr)	^{210}Pb (dpm/g)	^{214}Bi (dpm/g)	^{137}Cs (dpm/g)
top	bottom									
18	18.5	18.25	70.61	13.81	11.83	1983	0.15	11.04	2.2698	1.0845
18.5	19	18.75	72.94	13.87	11.57	1982	0.14	10.77		
19	19.5	19.25	78.79	14.08	11.69	1980	0.14	10.5		
19.5	20	19.75	79.46	14.45	11.17	1979	0.14	10.24		
20	20.5	20.25	80.67	15.12	10.66	1978	0.14	9.98	2.7752	1.1068
20.5	21	20.75	70.15	15.27	14.97	1976	0.14	10.16		
21	21.5	21.75	84.14	16.3	9.68	1975	0.13	10.34		
21.5	22	22.25	76.69	16.38	9.8	1974	0.12	10.72	3.1322	1.1725
22	22.5	22.75	80.9	16.65	10.16	1973	0.14	9.64		
22.5	23	23.25	81.14	16.94	13.83	1973	0.16	8.63	3.2668	1.4276
23	23.5	23.75	79.28	16.91	10.5	1972	0.16	8.52		
23.5	24	24.25	76.99	14.94	11.32	1971	0.15	8.41	2.7129	2.4244
24	24.5	24.75	77.05	13.49	9.86	1970	0.15	8.44		
24.5	25	25.25	77.4	14.13	11.21	1968	0.14	8.47	3.0482	2.4309
25	25.5	25.75	74.69	14.71	12.17	1967	0.17	7.13		
25.5	26	26.25	64.17	6.32	15.82	1967	721.46	4.81	2.348	0.3099
26	26.5	26.75	77.18	9.6	14.92	1967	721.45	4.45		
26.5	27	27.25	71.05	9.86	16.14	1967	721.44	4.11		
27	27.5	27.75	55.6	7.43	18.16	1967	721.44	3.79		
27.5	28	28.25	63.73	6.87	13.52	1967	721.43	3.49	2.1741	0.2996
28	28.5	28.75	47.49	9.18	15.17	1967	721.42	4.84		
28.5	29	29.25	64.93	7.61	16.28	1967	721.4	6.51		
29	29.5	29.75	61.24	6.78	15.84	1966	0.19	6.22		
29.5	30	30.25	64.31	11.72	13.7	1964	0.19	5.93	2.6979	0.3335
30	30.5	30.75	61.42	7.52	18.53	1964	652.48	5.22		
30.5	31	31.75	47.79	3.81	19.93	1964	652.47	3.18		
31	31.5	32.25	37.86	3.23	21.58	1964	652.46	1.45	1.4606	0.0536
31.5	32	32.75	34.6	3.28	24.02	1964	652.43	2.23		
32	32.5	33.25	52.27	5.75	18.41	1964	652.41	3.26		
32.5	33	33.75	63.75	9.4	17.22	1964	652.4	4.57		
33	33.5	34.25	63.31	9.85	15.08	1962	0.18	6.19	2.6415	0.4273
33.5	34	34.75	63.18	9.78	14.02	1961	0.22	5.7	2.3451	0.355
34	34.5	35.25	64.72	17.83	12.8	1957	0.11	8.42	3.6984	1.1484
34.5	35	35.75	45.59	7.28	23.86	1957	526.52	4.41	2.2122	0.3328
35	35.5	36.25	51.51	7.06	17.09	1957	526.5	3.35	1.4958	0.0821
35.5	36	36.75	47.25	6.68	18.89	1957	526.49	3.28	1.6628	0.0352
36	36.5	37.25	52.07	6.12	14.92	1957	526.47	3.97	1.8322	0.0264
36.5	37	37.75	69.95	10.39	11.15	1955	0.13	6.82	3.0329	0.4691

Depth interval (cm) top	bottom	Mid point depth (cm)	Moisture Content (%)	Organic matter (%)	Mineral matter (%)	CRS dates	Dry mass sedimentation (g/cm2 yr)	^{210}Pb (dpm/g)	^{214}Bi (dpm/g)	^{137}Cs (dpm/g)
37	37.5	38.25	71.16	9.98	11.74	1954	0.29	4.87	3.2924	0.4111
37.5	38	38.75	63.29	9.84	10.25	1953	0.28	4.89		
38	38.5	39.25	56.28	8.26	15.57	1953	460.83	4.92	2.9634	0.0351
38.5	39	39.75	55.74	7.98	16.5	1953	460.81	5.17		
39	39.5	40.25	70.08	8.75	14.74	1951	0.18	5.42	2.8026	0.0874
39.5	40	40.75	65.86	8.31	16.68	1950	0.19	5.12		
40	40.5	41.25	66.85	8.15	17.73	1948	0.21	4.82	2.8007	0.0002
40.5	41	41.75	56.39	8.43	17.13	1948	400.32	4.9		
41	41.5	42.25	58.92	8.87	15.27	1948	400.31	4.98	2.7361	0.027
41.5	42	42.75	66.23	11.24	10.52	1947	0.18	5.35		
42	42.5	43.25	73.9	12.16	10.57	1945	0.17	5.74	3.3862	0.0073
42.5	43	43.75	69.51	13.28	8.56	1944	0.17	5.72		
43	43.5	44.25	74.78	12.85	9.29	1943	0.17	5.69	3.3335	-0.0561
43.5	44	44.75	80.12	12.03	9.04	1942	0.14	6.03		
44	44.5	45.25	69.87	11.56	9.61	1940	0.11	6.38	3.3734	0.0295
44.5	45	45.75	66.92	11.69	9.35	1937	0.1	6.17		
45	45.5	46.25	71.18	12.27	10.38	1935	0.1	5.97	3.1659	0.0138
45.5	46	46.75	79.04	11.41	10.34	1933	0.1	5.57		
46	46.5	47.25	64.83	10.19	12.53	1933	247.03	5.19		
46.5	47	47.75	67.7	15.46	9.04	1933	247.02	4.82		
47	47.5	48.25	67.36	9.06	11.8	1933	247.01	4.48	2.7958	-0.0179
47.5	48	48.75	50.99	4.45	5.11	1933	247	4.14		
48	48.5	49.25	64.43	9.63	11.63	1933	246.99	3.82		
48.5	49	49.75	55.05	8.38	14.88	1933	246.98	3.52		
49	49.5	50.25	57.96	8.8	13.05	1933	246.98	3.23	2.5164	-0.0495
49.5	50	50.75	60.96	9.01	12.39	1933	246.96	4.04		
50	50.5	51.25	71.53	9.3	12.18	1930	0.13	4.98		
50.5	51	51.75	71.42	9.53	11.14	1929	0.16	4.71		
51	51.5	52.25	54.71	8.67	10.6	1929	219.85	4.44	3.5611	0.0289
51.5	52	52.75	67.89	9.76	12.12	1929	219.84	3.66		
52	52.5	53.25	57.95	9.74	11.03	1929	219.83	2.97		
52.5	53	53.75	70.25	7.4	13.7	1929	219.82	2.38		
53	53.5	54.25	56.53	6	18.02	1929	219.81	1.87	1.6439	-0.0384
53.5	54	54.75	56.85	6.48	15.63	1929	219.8	1.96		
54	54.5	55.25	64.96	4.68	15.23	1929	219.79	2.05		
54.5	55	55.75	73.38	5	16.54	1929	219.78	2.15		
55	55.5	56.25	44.01	3.05	12.72	1929	219.77	2.25	1.6784	-0.0528
55.5	56	56.75	47.22	3.31	12.16	1929	219.76	1.94		

Depth interval (cm) top	bottom	Mid point depth (cm)	Moisture Content (%)	Organic matter (%)	Mineral matter (%)	CRS dates	Dry mass sedimentation (g/cm2 yr)	^{210}Pb (dpm/g)	^{214}Bi (dpm/g)	^{137}Cs (dpm/g)
56	56.5	57.25	46.98	5.02	17	1929	219.74	1.66		
56.5	57	57.75	47.55	4.96	18.97	1929	219.72	1.41		
57	57.5	58.25	48.22	4.32	29.94	1929	219.71	1.18	1.5808	-0.0315
57.5	58	58.75	58.83	6.82	14.37	1929	219.7	1.3		
58	58.5	59.25	36.63	3.73	24.14	1929	219.68	1.42		
58.5	59	59.75	39.71	3.95	30.99	1929	219.66	1.56		
59	59.5	60.25	46.94	4.48	33.88	1929	219.64	1.7	1.561	-0.0671
59.5	60	60.75	52.6	5.07	30.87	1929	219.63	1.4		
60	60.5	61.25	57.36	5.27	29.84	1929	219.62	1.14	1.2158	-0.0259
60.5	61	61.75	55.12	4.21	26.18	1929	219.61	1.35		
61	61.5	62.25	53.82	5.72	20.36	1929	219.59	1.58	1.4158	-0.0243
61.5	62	62.75	61.26	5.69	24.82	1929	219.58	1.35		
62	62.5	63.25	21.94	2.2	14.85	1929	219.57	1.14		
62.5	63	63.75	31.08	2.87	18.91	1929	219.54	0.95		
63	63.5	64.25	31.3	2.34	16.44	1929	219.52	0.79	1.0844	-0.0316
63.5	64	64.75	35.4	3.2	15.89	1929	219.51	1.31		
64	64.5	65.25	26.44	3.76	22.07	1929	219.49	2.02		
64.5	65	65.75	38.88	3.06	16.54	1929	219.46	2.96		
65	65.5	66.25	51.03	4.89	18.04	1929	219.45	4.16	2.4908	-0.0617
65.5	66	66.75	56.46	4.94	18.43	1929	219.44	4.25		
66	66.5	67.25	60.33	8.48	12.58	1929	219.43	4.34		
66.5	67	67.75	55.28	8.88	13.15	1929	219.42	4.43		
67	67.5	68.25	71.83	9.98	10.11	1927	0.11	4.52	2.4638	-0.0535
67.5	68	68.75	70.54	7.92	12.32	1925	0.11	4.36		
68	68.5	69.25	67.5	8.65	10.83	1923	0.12	4.21		
68.5	69	69.75	64	8.82	8.77	1921	0.13	4.06		
69	69.5	70.25	61.14	10.06	10.19	1919	0.14	3.91	2.7612	-0.0474
69.5	70	70.75	77.56	11.38	9.49	1917	0.16	3.76		
70	70.5	71.25	69.54	11.02	8.69	1916	0.19	3.61		
70.5	71	71.75	63.53	10.27	10.39	1916	148.29	3.34		
71	71.5	72.25	67.28	8.34	11.95	1916	148.29	3.08	3.0655	-0.0725
71.5	72	72.75	68.07	8.7	12.17	1916	148.28	2.83		
72	72.5	73.25	59.52	6.88	13.07	1916	148.27	2.6		
72.5	73	73.75	60.93	6.41	12.81	1916	148.26	2.38		
73	73.5	74.25	55.98	3.77	16.33	1916	148.25	2.17	1.7103	-0.055
73.5	74	74.75	52.35	5.28	15.57	1916	148.24	1.87		
74	74.5	75.25	36.26	3.57	16.71	1916	148.23	1.59		
74.5	75	75.75	41.18	3.08	15.62	1916	148.21	1.35		

Depth interval (cm)		Mid point depth (cm)	Moisture Content (%)	Organic matter (%)	Mineral matter (%)	CRS dates	Dry mass sedimentation (g/cm2 yr)	^{210}Pb (dpm/g)	^{214}Bi (dpm/g)	^{137}Cs (dpm/g)
top	bottom									
75	75.5	76.25	27.7	2.39	19.26	1916	148.2	1.13	1.5456	-0.0188
75.5	76	76.75	14.54	1.52	13.83	1916	148.17	1.12		
76	76.5	77.25	22.55	2.4	21.01	1916	148.14	1.11		
76.5	77	77.75	23.15	2.28	21.91	1916	148.11	1.1		
77	77.5	78.25	20.8	1.58	18.9	1916	148.08	1.09	1.325	-0.0164
77.5	78	78.75	40.43	2.38	15.06	1916	148.05	1.09		
78	78.5	79.25	45.08	4.28	16.41	1916	148.03	1.09		
78.5	79	79.75	41.55	3.11	15.71	1916	148.02	1.09		
79	79.5	80.25	28.47	2.11	14.17	1916	148	1.08	0.963	0.0014
79.5	80	80.75	27.04	2.48	13.6	1916	147.98	1.25		
80	80.5	81.25	22.5	2.14	16.29	1916	147.95	1.44		
80.5	81	81.75	32.97	3.18	13.54	1916	147.93	1.64		
81	81.5	82.25	36.57	3.98	12.35	1916	147.91	1.86	1.3768	-0.0032
81.5	82	82.75	50.2	6.85	12.25	1916	147.89	2.17		
82	82.5	83.25	57.01	9.3	10.34	1916	147.88	2.52		
82.5	83	83.75	67.25	9.4	11.78	1916	147.87	2.9		
83	83.5	84.25	60.43	9.47	10.82	1914	0.17	3.32	2.458	-0.0067
83.5	84	84.75	71.26	10.21	10.57	1913	0.17	3.36		
84	84.5	85.75	64.59	10.07	9.04	1912	0.19	3.4		
84.5	85	86.25	70.62	10.67	7.73	1910	0.17	3.48	2.8816	-0.0032
85	85.5	86.75	70.69	10.51	7.87	1909	0.19	3.41		
85.5	86	87.25	66.71	9.87	9.45	1908	0.2	3.33		
86	86.5	87.75	77.44	9.22	8.92	1907	0.21	3.26		
86.5	87	88.25	54.63	10.49	8.25	1907	112.33	3.19		
87	87.5	88.75	69.12	9.08	9.88	1906	0.25	3.12		
87.5	88	89.25	64.49	9.53	8.88	1906	0.27	3.05		
88	88.5	89.75	62.91	9.18	8.83	1905	0.31	2.98		
88.5	89	90.25	67.38	8.38	9.72	1904	0.37	2.92	2.6393	-0.0134
89	89.5	90.75	66.37	9.51	8.45	1903	0.27	3.04		
89.5	90	91.25	57.4	10.3	7.01	1903	98.89	3.17		
90	90.5	91.75	53.37	9.7	9.06	1903	98.88	3.31		
90.5	91	92.25	64.54	9.8	8.33	1902	0.15	3.44	2.832	-0.0598
91	91.5	92.75	63.59	9.18	7.96	1900	0.15	3.45		
91.5	92	93.25	61.47	9.51	8.2	1899	0.13	3.45		
92	92.5	93.75	64.16	8.81	8.17	1897	0.12	3.45		
92.5	93	94.25	70.43	9.48	7.9	1895	0.11	3.46	2.6535	-0.0312
93	93.5	94.75	72.1	9.46	7.06	1893	0.12	3.41		
93.5	94	95.25	67.76	9.26	8.29	1892	0.14	3.36		

Depth interval (cm)		Mid point depth (cm)	Moisture Content (%)	Organic matter (%)	Mineral matter (%)	CRS dates	Dry mass sedimentation (g/cm2 yr)	^{210}Pb (dpm/g)	^{214}Bi (dpm/g)	^{137}Cs (dpm/g)
top	bottom									
94	94.5	95.75	61.86	9.49	7.89	1890	0.18	3.31		
94.5	95	96.25	63.05	9.17	8.21	1888		3.27	3.1594	-0.0947
95	95.5	96.75	71.32	8.86	7.19	1886		3.19		
95.5	96	97.25	67.46	8.02	7.81	1885		3.12		
96	96.5	97.75	68.06	8.96	7.09	1883		3.05		
96.5	97	98.25	60.9	8.63	8.24	1881		2.98	2.8528	-0.0873
97	97.5	98.75	61.81	8.69	8.33	1879		3.1		
97.5	98	99.25	65.47	10.43	4.37	1878		3.22	2.6957	
98	98.5	99.75	47.57	7.49	7.81	1876		3.3		
98.5	99	100.25	51.73	7.85	7.83	1872		3.38	3.3019	-0.1363
99	99.5	100.75	57.05	9.1	7.99	1870				
99.5	100	101.25	69.11	9.31	7.99	1870				
100	100.5	101.75	63.88	9.37	7.87	1869				

Bibliography

Aardema, B.W,. Lorenz, M.G., Krumbein, W.E. (1983). Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. *Applied and environmental microbiology*. **46:** 417–420.

Adams, D.G. (2000). Heterocyst formation in cyanobacteria. *Current opinion in microbiology*. **3:** 618-624.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. (2002). Molecular biology of the cell 4th edition. National Center for Biotechnology Information Bookshelf.

Allan, R.J. and Zarull, M.A. (1995). Impacts on biodiversity and species changes in the lower Great Lakes. Lakes and Reservoirs: *Research and Management*. **1:** 157-162.

Allender, C.J., LeCleir, G.R., Rinta-Kanto, J.M., Small, R.L., Satchwell, M.F., Boyer, G.L., Wilhelm, S.W. (2009). Identifying the source of unknown microcystin genes and predicting microcystin variants by comparing genes within uncultured cyanobacterial cells. *Applied and environmental microbiology*. **75:** 3598-3604.

American Public Health Association (2005). Standard methods for the examination of water and wastewater. American Public Health Association, Washington.

Anderson, T.H. and Lewis, C.F.M. (1985). Postglacial water level history of the Lake Ontario basin. In: Karrow, P.F. and Calkin, P. E. (eds), Quaternary evolution of the Great Lakes, pp. 231-253. Geol. Assoc. Can., Spec. Paper 30, St John's, Newfoundland.

Appleby, P.G. (2001). Chronostratigraphic techniques in recent sediments. In: Last WM, Smol JP, editors. Tracking environmental change using lake sediments: Basin analysis, coring, and chronological techniques. Dordrecht: Kluwer Academic Publishers. 171–203.

Augustinus, P., Reid, M., Andersson, S., Deng , Y., Horrocks, M. (2006). Biological and geochemical record of anthropogenic impacts in recent sediments from Lake Pupuke, Auckland City, New Zealand. *Journal of Paleolimnology*. **35:** 789–805.

Babica, P. (2006). Environmental and ecotoxicological aspects of cyanobacterial toxins – microcystins. PhD Thesis. Masaryk University, Faculty of Science, Brno, Czech Republic.

Babica, P., Kohoutek, J., Bláha, L., Adamovsky, O., Marsalek, B. (2006). Evaluation of extraction approaches linked to ELISA and HPLC for analyses of microcystin-LR, -RR and -YR in freshwater sediments with different organic material contents. *Analytical and bioanalytical chemistry*. **385:** 1545–1551.

Baker, J.A., Neilan, B.A., Entsch, B., McKay, D.B. (2001). Identification of cyanobacteria and their toxigenicity in environmental samples by rapid molecular analysis. *Environmental Toxicology*. **16:** 472–482.

Baker, P.D. (1999). Role of akinetes in the development of cyanobacterial populations in the lower Murray River, Australia. *Marine and FreshWater research*. **50:** 265-279.

Balch, G.C., Metcalfe, C.D., Huestis, S.Y. (1995). Identification of potential fish carcinogens in sediment from Hamilton Harbour, Ontario, Canada. *Environmental Toxicology and chemistry*. **14:** 79-91.

Barica, J. (1989). Unique limnological phenomena affecting water quality of Hamilton Harbour, Lake Ontario. *Journal of Great Lakes Research*. **15**: 519–530.

Barica, J. (1990). Ammonia and nitrite contamination of Hamilton Harbour, Lake Ontario. *Water Quality Research Journal of Canada*. **25**: 359-386.

Beeton, A.M. (1965). Eutrophication of the St. Lawrence Great Lakes. *Limnology and Oceanography*. **10:** 240-54.

Beeton, A.M. (1969). Changes in the environment and biota of the Great Lakes. In: Eutrophication: Causes, Consequences, Correctives. Proc. Nat. Acad. Sci., U.S.A., pp. 150-87.

Beisner, B.E., Peres-Neto, P.R., Lindström, E.S., Barnett, A., Longhi, M.L. (2006). The role of environmental and spatial processes in structuring lake communities from bacteria to fish. *Ecology*. **87:** 2985-2991.

Belcher, J.H., and Fogg, G.E. (1964.) Chlorophyll derivatives in the sediments of two English lakes. In: Miyaka, Y. and T. Koyama (eds.) Recent Researches in the Field of Hydrosphere, Atmosphere and Nuclear Geochemistry. Maruzen co. Tokyo: 39–48.

Belykh, O., Sorokovikova, E., Fedorova, G., Kaluzhnaya, O., Korneva, E., Sakirko, M., Sherbakova, T. (2011). Presence and genetic diversity of microcystin-producing cyanobacteria (*Anabaena* and *Microcystis*) in lake Kotokel (Russia, lake Baikal region). *Hydrobiologia*. **671:** 241-252.

Bianchi, T.S. and Findlay, S. (1991). Decomposition of Hudson estuary macrophytes: Photosynthetic pigment transformations and decay constants. *Estuaries*. **14:** 65-73.

Bianchi, T.S., Findlay, S. Fontvieille, D. (1991). Experimental degradation of plant materials in Hudson River sediments. *Biogeochemistry*. **12:** 171–187.

Bickel, H. and Lyck, S. (2001). Importance of energy charge for microcystin production. In: Chorus, I. (Ed.), Cyanotoxins—Occurrence, Causes, Consequences. Springer, Berlin, pp. 133–141.

Bierman, V.J., Dolan, D.M., Kasprzyk, R., Clark, F.L. (1984). Retrospective analysis of the response of Saginaw Bay, Lake Huron, to reductions in phosphorus loadings. *Environmental Science and Technology*. **18:** 23-31.

Biodiversity Science Assessment Team (BSAT) (1994). Biodiversity in Canada: A Science Assessment for Environment Canada. Environment Canada, Ottawa, 245 pp.

Bloesch, J. (1994). A review of methods used to measure sediment resuspension. *Hydrobiologia*. **284:** 13-18.

Borghini, F., Colacevich, A., Caruso, T., Bargagli, R. (2011). An Update on Sedimentary Pigments in Victoria Land Lakes (East Antarctica). *Arctic, Antarctic, and Alpine Research*. **43:** 22-34.

Botes, D.P., Tuiman, A.A., Wessels, P.L., Viljoen, C.C., Kruger, H., Williams, D.H., Santikarn, S., Smith, R.J., Hammond, S.J. (1984). The structure of cyanoginosin-LA, a cyclic peptide from the cyanobacterium *Microcystis aeruginosa. J. Chem. Soc., Perkin Trans*. **1:** 2311– 2318.

Boyer, G.L. (2008). Cyanobacterial toxins in New York and the lower Great Lakes ecosystems. In Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs (pp. 153-165). Springer New York.

Bradbury, J.P., Colman, S.M., Reynolds, R.L. (2004). The history of recent limnological changes and human impact on Upper Klamath Lake, Oregon. *Journal of Paleolimnology*. **31:** 151–165.

Brassard, P., Kramer, J.R., Collins, P.V. (1997). Dissolved metal concentrations and suspended sediment in Hamilton Harbour. *Journal of Great Lakes Research*. **23:** 86-96.

Brinkhurst, R.O. and Jamieson, G.G.M. (1971). Aquatic Oligochaeta of the World. University of Toronto Press, Toronto, 860 pp.

Brittain, S.M., Wang, J., Babcock-Jackson, L., Carmichael, W.W., Rinehart, K.L., Culver, D. A. (2000). Isolation and characterization of microcystins, cyclic heptapeptides hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. *Journal of Great Lakes Research*. **26:** 241–249.

Brunberg, A.K. and Blomqvist, P. (2002). Benthic overwintering of *Microcystis* colonies under different environmental conditions. *Journal of Plankton Research.* **24:** 1247-1252.

Burley, M. (2007). Water quality and phytoplankton photosynthesis. *Canadian Technical Report of Fisheries and Aquatic Sciences.* **2729:** 9-42.

Campbell, M.F. (1966). A mountain and a city: the story of Hamilton. McClelland and Stewart: Toronto, ON. p. 351.

Carlson, R.E. and Simpson, J.(1996). Trophic state. In: A Coordinator's Guide to Volunteer Lake Monitoring Methods. North American Lake Management Society. 96 pp.

Carmichael, W.W. (1991). Toxic freshwater blue-green algae (cyanobacteria): an overlooked health threat. *Health Environ. Digest*. **5:** 1-4.

Carmichael, W.W. (1992). Cyanobacteria secondary metabolites—The cyanotoxins. *Journal of Applied Microbiology*. **72:** 445– 459.

Carmichael, W.W. (1994). The toxins of cyanobacteria. *Scientific American*. **270:** 78-86.

Carmichael, W.W. (2008). A world overview—One-hundred-twenty-seven years of research on toxic cyanobacteria—Where do we go from here?. In Cyanobacterial harmful algal blooms: State of the science and research needs (pp. 105-125). Springer New York.

Caron, D.A., Pick, F.R., Lean, D.R. (1985). Chroococcoid cyanobacteria in Lake Ontario - vertical and seasonal distribution during 1982. *Journal of Phycology*. **21:** 171-175.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological applications*. **8:** 559-568.

Carpenter, S.R., Elser, M.M., Elser, J.J. (1986). Chlorophyll production, degradation, and sedimentation: Implications for paleolimnology. *Limnology and Oceanography*. **31:** 112-124.

Carrick, H.J. and Schelske, C.L. (1997). Have we overlooked the importance of small phytoplankton in productive waters?. *Limnology and Oceanography*. **42:** 1613-1621.

Case, R. J., Boucher, Y., Dahllöf, I., Holmström, C., Doolittle, W.F., Kjelleberg, S. (2007). Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. *Applied and environmental microbiology*. **73:** 278-288.

Castenholz, R. W. (2001). General characteristics of the cyanobacteria. *Bergey's manual of systematic bacteriology*. **1:** 474-487.

Castenholz, R.W. and Waterbury, J.B. (1989). Group I. Cyanobacteria. In Bergey's manual of systematic bacteriology, vol. 3. J.T. Staley, M.P. Bryant, N. Pfenning, and J.G. Holt (eds). Baltimore, MD, USA, Williams and Wilkins, pp 1710–1727.

Catallo, W.J., Schlenker, M., Gambrell, R.P., Shane, B.S. (1995). Toxic chemicals and trace metals from urban and rural Louisiana lakes: recent historical profiles and toxicological significance. *Environmental science and technology*. **29:** 1436-1445.

Charlton, M.N. and Le Sage, R. (1996). Water quality trends in Hamilton Harbour: 1987 to 1995. *Water Quality Research Journal of Canada*. **31:** 473-484.

Chau, Y.K., Chawla, V.K., Nicholson, H.F., Vollenwelder, R.A. (1970). Distribution of trace elements and chlorophyl a in Lake Ontario. Proc. 13th Conf. Great Lakes Res. 659-772.

Chen, W., Li, L., Gan, N., Song, L. (2006a). Optimization of an effective extraction procedure for the analysis of microcystins in soils and lake sediments. *Environmental Pollution*. **143**: 241-246.

Chen, W., Song, L., Gan, N., Li, L. (2006b). Sorption, degradation and mobility of microcystins in Chinese agriculture soils: risk assessment for groundwater protection. *Environmental Pollution*. **144:** 752–758.

Chorus, I. and Bartram, J. (1999). Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E and FN Spon, London, U.K. 416 pp.

Christiansen, G., Fastner, J., Erhard, M., Börner, T., Dittmann, E. (2003). Microcystin biosynthesis in Planktothrix: Genes, evolution, and manipulation. *Journal of bacteriology*. **185:** 564-572.

Christoffersen, K., Lyck, S., Winding, A. (2002). Microbial activity and bacterial community structure during degradation of microcystins. *Aquatic Microbial Ecology*. **27:** 125–136.

Chunn, A. (2007). Molecular characterization of toxic Cyanobacteria in North American and East African Lakes. MSc Thesis. Waterloo University, Ontario, Canada.

Codd, G.A. (1995). Cyanobacterial toxins: occurrence, properties and biological significance. *Water Science and Technology*. **32:**149-156.

Codd, G.A., Lindsay, J., Young, F.M., Morrison, L.F., Metcalf, J.S. (2005a): Cyanobacterial toxins. In: Harmful Cyanobacteria (eds. Huisman J., Matthijs H.C.P. and Visser P.M.), pp. 1-23. Springer-Verlag.

Codd, G.A., Morrison, L.F. Metcalf, J.S. (2005b): Cyanobacterial toxins: risk management for health protection. *Toxicology and Applied Pharmacology*. **203:** 264-272.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E. (2009). Controlling eutrophication: nitrogen and phosphorus. *Science.* **323:**1014-1015.

Conroy, J.D., Quinlan, E.L., Kane, D.D., Culver, D.A. (2007). *Cylindrospermopsis* in Lake Erie: testing its association with other cyanobacterial genera and major limnological parameters*. Journal of Great Lakes Research*. **33:** 519–535

Cousins, I.T., Bealing, D.J., James, H.A., Sutton A. (1996). Degradation of microcystins by indigenous mixed bacterial populations. *Water research*. **30:** 481-485.

Cronberg, G. (1986). Bluegreen algae, green algae and Chrysophyceae in sediments. In Berglund, B. E., John Wiley and Sons LTD (ed.), Handbook of Holocene Paleoecology. Chichester: 507–526.

Crossett, K.M., Culliton, T.J., Wiley, P.C., Goodspeed, T.R. (2004). Population trends along the coastal United States: 1980–2008. Coastal Trends Report Series. Washington, DC: National Oceanic and Atmospheric Administration, National Ocean Service Management and Budget Office.

Crusius, J. and Anderson, R.E. (1995). Evaluating the mobility of ^{137}Cs, $^{239+240Pu}$ and ^{210}Pb from their distributions in laminated lake sediments. *Journal of Paleolimnology*. **13:** 119-141.

Currie, L.A. (1968). Limits for qualitative detection and quantitative determination: application to radiochemistry. *Analytical Chemistry*. **40:** 586–593.

Dadheech, P.K., Krienitz, L., Kotut, K., Ballot, A., Casper, P. (2009). Molecular detection of uncultured cyanobacteria and aminotransferase domains for cyanotoxin production in sediments of different Kenyan lakes. *FEMS microbiology ecology*. **68:** 340–350.

Daubin, V., Gouy, M., Perriere, G. (2002). A phylogenomic approach to bacterial phylogeny: evidence of a core of genes sharing a common history. *Genome Research*. **12:** 1080-1090.

Davis, M.B., Moeller, R.E. Ford, J. (1984) Sediment focusing and pollen influx In: Haworth, E.Y. and Lund, J.W.G. (eds), Lake Sediments and Environmental History. Leicester University Press, Leicester, 261–93.

Davis, R.B., Anderson, D.S., Dixit, S.S., Appleby, P.G., Schauffer, M. (2006). Responses of two New Hampshire (USA) lakes to human impacts in recent centuries. *Journal of Paleolimnology*. **35:** 669–697.

Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful algae*. **8:** 715-725.

Davis, T.W., Harke, M.J., Marcoval, M.A., Goleski, J., Orano-Dawson, C., Berry, D.L., Gobler, C.J. (2010). Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during bloom events. *Aquatic Microbial Ecology*. **61:** 149–162.

de Figueiredo, D.R., Azeiteiro, U.M., Esteves, S.M., Fernando J.M., Gonçalves, F.J.M. Pereiraa, M.J. (2004). Microcystin-producing blooms—a serious global public health issues. *Ecotoxicology and Environmental Safety*. **59:** 151–163.

Dean, W.E. (1974). Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. *Journal of Sedimentary Research*. **44:** 242–248.

DeMott, W.R. (1991). Efects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of Daphnia. *Limnology and Oceanography*. **36:** 1346-1357.

Dermott, R. (2007). Assessment of Hamilton Harbour; Background. *Canadian Technical Report of Fisheries and Aquatic Sciences*. **2729:** 1-8.

Dermott, R. and Bonnell, R. (2007). Benthic fauna in Hamilton Harbour: 2002-2003. *Canadian Technical Report of Fisheries and Aquatic Sciences*. **2729:** 91-120.

Dermott, R., and Bonnell, R. (2010). Benthic fauna in Hamilton Harbour and adjacent Lake Ontario 2002–2005 in comparison to 1964. *Aquatic Ecosystem Health and Management*. **13:** 413-428.

Devereux, R. and Wilkinson, S.S. (2004). Amplification of ribosomal RNA sequences. Molecular Microbial Ecology Manual. 3.01: 509–522.

Dillon, J.G. and Castenholz, R.W. (1999). Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. *Journal of Phycology*. **35:** 673-681.

Dittmann, E., Neilan, B.A., Erhard, M., von Döhren, H., Börner, T. (1997). Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. *Molecular microbiology*. **26:** 779–787.

Dixit, S.S., Dixit, A.S., Smol, J.P. (1991). Multi-variable environmental inferences based on diatom assemblages from Sudbury (Canada) lakes. *Freshwater Biology*. **26:** 251-265.

Dobson, A.P., Rodriguez, J.P., Roberts, W.M., Wilcove, D.S. (1997). Geographic distribution of endangered species in the United States. *Science.* **275:** 550–553.

Dokulil, M. T. and Teubner, K. (2000). Cyanobacterial dominance in lakes. *Hydrobiologia*. **438:** 1-12.

Dokulil, M.T. and Padisak, J. (1994). Long-term compositional response of phytoplankton in a shallow, turbid environment, Neusiedlersee (Austria/Hungary). *Hydrobiology*. **275:** 125–137.

Donahue, W., Allen, E., Schindler, D. (2006). Impacts of coalfired power plants on trace metals and polycyclic aromatic hydrocarbons (PAHs) in lake sediments in central Alberta, Canada. *Journal of Paleolimnology*. **35**: 111–128.

Dorador, C., Vila, I., Witzel, K.P., Imhoff, J.F. (2008). Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion?. *FEMS microbiology ecology*. **64**: 419-432.

Dorador, C., Vila, I., Witzel, K.P., Imhoff, J.F. (2013). Bacterial and archaeal diversity in high altitude wetlands of the Chilean Altiplano. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*. **182:** 135-159.

Dove, A. (2009). Long-term trends in major ions and nutrients in Lake Ontario. *Aquatic Ecosystem Health and Management*. **12:** 281-295.

Dow, C.S. and Swoboda, U.K. (2000). Cyanotoxins. In: Whitton, B.A., Potts, M. (Eds.), The Ecology of Cyanobacteria. Kluwer Academic Publishers, The Netherlands, pp. 613–632.

Downing, J.A., Watson, S.B., McCauley, E. (2001). Predicting cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. **58:** 1905-1908.

Downing, T.G., Meyer, C., Gehringer, M.M., Van de Venter, M. (2005). Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. *Environmental Toxicology*. **20:** 257–262.

Drechsel, H. and Jung, G. (1998) Peptide siderophores. *Journal of Peptide Science.* **4:** 147–181.

Duggan, I.C., Bailey, S.A., Colautti, R.I., Gray, D.K., Makarewic, J.C., MacIsaac,, H.J. (2003). In: Munawar, M. (ed): Biological invasions in Lake Ontario: past, present and future. State of Lake Ontario (SOLO) – Past, Present and Future. Ecovision World Monograph Series.

Duthie, H.C., Yang, J.R., Edwards, T.W.D., Wolfe, B.B., Warner B.G. (1996). Hamilton Harbour, Ontario: 8300 years of limnological and environmental change inferred from microfossil and isotopic analyses. *Journal of Paleolimnology*. **15:** 79–97.

Edwards, C., Graham, D., Fowler, N., Lawton, L. A. (2008). Biodegradation of microcystins and nodularin in freshwaters. *Chemosphere*. **73:** 1315–1321.

Efting, A.A., Snow, D.D., Fritz, S.C. (2011). Cyanobacteria and microcystin in the Nebraska (USA) Sand Hills Lakes before and after modern agriculture. *Journal of Paleolimnology*. **46:** 17-27.

Eilers, J.M., Kann, J., Cornett, J., Moser, K., Amand, A.S. (2004). Paleolimnological evidence of change in a shallow, hypereutrophic lake: Upper Klamath Lake, Oregon, USA. *Hydrobiologia*. **520:** 7-18.

Elder, G.H., Hunter, P.R., Codd, G.A. (1993). Hazardous freshwater cyanobacteria (blue-green algae). *The Lancet*. **341:** 1519-1520.

Engstrom, D.R., Schottler, S.P., Leavitt, P.R., Havens, K.E. (2006). A reevaluation of the cultural eutrophication of Lake Okeechobee using multiproxy sediment records. *Ecological Applications*. **16:** 1194-1206.

Engstrom, D.R., Swain, E.B., Kingston, J.C. (1985). A palaeolimnological record of human disturbance from Harvey's Lake, Vermont: geochemistry, pigments and diatoms. *Freshwater Biology*. **15:** 261-288.

Erdner, D.L., Dyble, J., Parsons, M.L., Stevens, R.C., Hubbard, K.A., Wrabel, M.L., Moore, S.K., Lefebvre, K.A., Anderson, D.M., Bienfang, P., Bidigare, R.R., Parker, M.S., Moelle, P., Brand, L.E., Trainer, V.L. (2008). Centers for Oceans and Human Health: a unified approach to the challenge of harmful algal blooms. *Environmental Health*. **7**: S2.

Fay, P. and Van Baalen, C. (1987). The Cyanobacteria. Amsterdam: Elsevier Science Publishers B.V.

Fernandez-Carazo, R., Verleyen, E., Hodgson, D.A., Roberts, S.J., Waleron, K., Vyverman, W., Wilmotte, A. (2013). Late Holocene changes in cyanobacterial community structure in maritime Antarctic lakes. *Journal of Paleolimnology*. **50:** 15–31.

Fewer, D.P., Rouhiainen, L., Jokela, J., Wahlsten, M., Laakso, K., Wang, H., Sivonen, K. (2007). Recurrent adenylation domain replacement in the microcystin synthetase gene cluster. *BMC Evolutionary Biology*. **7:** 183.

Finsinger, W., Bigler, C., Krähenbühl, U., Lotter, A.F., Ammann, B. (2006). Human impacts and eutrophication patterns during the past ~ 200 years at Lago Grande di Avigliana (N. Italy). *Journal of Paleolimnology*. **36:** 55–67.

Fischer, W.J., Hitzfeld, B.C., Tencalla, F., Eriksson, J.E., Mikhailov, A., Dietrich, D.R. (2000). Microcystin-LR toxicodynamics, induced pathology, and immunohistochemical localization in livers of blue-green algae exposed rainbow trout (*Oncorhynchus mykiss*). *Toxicological Sciences*. **54:** 365– 373

Fogg, G.E. (1969). The physiology of an algal nuisance. *Proceedings of the Royal Society of London. Series B. Biological Sciences.* **173:** 175-189.

Forde, A. V. (1979). A historical study of pollution of Burlington Bay. Reg. Mun. Hamilton-Wentworth, Ham. Munic. Lab. Report, Hamilton.

Fox, D. L. (1944). Biochemical fossils. *Science.* **100:** 111–113.

Fox, D. L., Updegraff, D.M., Novelli, G.D. (1944). Carotenoid pigments in the ocean floor. *Arch. Biochem*. **5:** 1–23.

Frangeul, L., Quillardet, P., Castets, A.-M., Humbert, J.-F.,Matthijs, H.C., Cortez, D., Tolonen, A., Zhang, C-C. Gribaldo , S., Kehr, J., Zilliges, Y., Ziemert, N., Becker, S., Talla, E., Latifi, A., Billault, A., Lepelletier, A., Dittmann, E., Bouchier, C., Tandeau de Marsac, N. (2008). Highly plastic genome of *Microcystis aeruginosa* PCC 7806, a ubiquitous toxic freshwater cyanobacterium. *BMC Genomics*. **9:** 274–295.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., .Aragno, M., Rossi, P. (2002). Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environmental Microbiology*. **4:** 634-643.

Fuller, K., Shear, H., Wittig, J. (1995). The Great Lakes: an environmental atlas and resource book. Environment Canada, Ontario Region, Downsview, Ontario, and Environmental Protection Agency, Great Lakes National Program Office, Chicago.

García-Martínez, J., Acinas, S.G., Anton, A.I.,Rodríguez-Valera, F. (1999). Use of the 16S–23S ribosomal genes spacer region in studies of prokaryotic diversity. *Journal of Microbiological Methods*. **36:** 55-64.

Gerlofsma, J., Bowen, K., Johannsson, O. (2007). Zooplankton in Hamilton Harbour 2002–2004*. Canadian Technical Report of Fisheries and Aquatic Sciences*. **2729:** 65-90.

Gerten, D. and Adrian, R. (2000). Climate-driven changes in spring plankton dynamics and the sensitivity of shallow polymictic lakes to the North Atlantic Oscillation. *Limnology and Oceanography*. **45:** 1058–1066.

Giani, A., Bird, D.F., Prairie, Y.T., Lawrence, J.F. (2005). Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. **62:** 2100-2109.

Ginn, B.K., Cumming, B.F., Smol, J.P. (2007a). Assessing pH changes since pre-industrial times in 51 low-alkalinity lakes in Nova Scotia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*. **64:** 1043–1054.

Ginn, B.K., Cumming, B.F., Smol, J.P. (2007b). Long term acidification trends in high- and low-sulphate deposition regions in Nova Scotia, Canada. *Hydrobiologia*. **586:** 261–275.

Ginn, H.P. and Neilan, B.A. (2010) NtcA from *Microcystis aeruginosa* PCC 7806 is autoregulatory and binds to the microcystin promoter. *Applied and environmental microbiology*. **76:** 4362-4368.

Giovannoni, S.J., Britschgi, T.B., Moyer, C.L., Field, K.G. (1990). Genetic diversity in Sargasso Sea bacterioplankton. *Nature*. **345:** 60–63.

Giovannoni, S.J., Turner, S., Olsen, G.J., Barns, S., Lane, D.J., Pace, N.R. (1988). Evolutionary relationships among cyanobacteria and green chloroplasts. *Journal of bacteriology*. **170:** 3584-3592.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S. (2003.) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Applied and Environmental Microbiology.* **69:** 1800-1809.

Glibert, P.M., and O'Neil, J.M. (1999). Dissolved organic nitrogen release and amino acid oxidase activity by *Trichodesmium* spp. *Bull. de l'Oceanogr. Monaco*. **19:** 265–271 (special issue).

Goldberg, J., Huang, H.B., Kwon, Y.G., Greengard, P., Nairn, A.C., Kuriyan, J. (1995). Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. *Nature*. **376:** 745–753.

Greaves, M.P. and Wilson, M.J. (1969). The adsorption of nucleic acids by montmorillonite. *Soil Biology and Biochemistry*. **1:** 317–323.

Griffiths, M. and Edmondson, W. T. (1975). Burial of oscillaxanthin in the sediment of Lake Washington. *Limnology and Oceanography*. **20:** 945-952.

Grigorovich, I.A., Colautti, R.I., Mills, E.L., Holeck, K.H., MacIsaac, H.J. (2003). Ballast mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences*. **6:** 740–756.

Guilizzoni, P., Bonomi, G., Galanti, G., Ruggiu, D. (1983). Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. In Paleolimnology. pp. 103-106. Springer Netherlands.

Guilizzoni, P., Marchetto, A., Lami, A., Gerli, S., Musazzi, S. (2011). Use of sedimentary pigments to infer past phosphorus concentration in lakes. *Journal of Paleolimnology*. **45:** 433-445.

Haffner, G. D., Griffith, M., Hebert, P. D. N. (1984). Phytoplankton community structure and distribution in the nearshore zone of Lake Ontario. *Hydrobiologia*. **114:** 51-66.

Halfon, E. (1996). Volume visualization of temperature in Hamilton Harbour, Lake Ontario. *Journal of Great Lakes Research*. **22:** 15-25.

Hall, J.D., O'Connor, K., Ranieri, J. (2006). Progress toward delisting a great lakes area of concern: the role of integrated research and monitoring in the Hamilton Harbour remedial action plan. *Environmental monitoring and assessment*. **113:** 227–243.

Hall, R., Laevitt, P., Smol, J., Zirnhelt, N. (1997). Comparison of diatoms, fossil pigments and historical records as measures of lake eutrophication. *Freshwater Biology***. 38:** 401-417.

Hall, R.I., Leavitt, P.R., Quinlan, R., Dixit, A., Smol, J.P. (1999). Effects of agriculture, urbanization and climate on water quality in the Northern Great Plains. *Limnology and Oceanography*. **44:** 739–756.

Hall, R.I., Wolfe, B.B., Wiklund, J.A., Edwards, T.W.D., Farwell, A.J., Dixon, D.G. (2012). Has Alberta oil sands development altered delivery of polycyclic aromatic compounds to the Peace-Athabasca Delta? *PloS One* **7**: e46089.

Hallegraeff, G.M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia*. **32:**79–99.

Hamblin, P.F. and He, C. (2003). Numerical models of the exchange flows between Hamilton Harbour and Lake Ontario. *Canadian Journal of Civil Engineering*. **30:** 168-180.

Hamilton Harbour Remedial Action Plan (RAP). 1992. Remedial Action Plan for Hamilton Harbour, Goals, Options and Recommendations. RAP Stage 2 Report, ISBN 0-7778-0533-2, Canada Ontario Agreement. Nov. 1992. 327 pp.

Hamilton Harbour Remedial Action Plan T.T. (2010b). Hamilton Harbour RAP water quality goals and targets interim, Part 2 review, Burlington, ON, p. 156.

Harada, K.-I., Kondo, F., Lawton, L. (1999). Laboratory analysis of cyanotoxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water. E and F.N. Spon, London, pp. 369–405.

Harlow, H.E. and Hodson, P.V. (1988). Chemical contamination of Hamilton Harbour: A review. *Canadian Technical Report of Fisheries and Aquatic Sciences*. 1603.

Harris, M.A., Cumming, B.F., Smol, J.P. (2006). Assessment of recent environmental changes in New Brunswick (Canada) lakes based on paleolimnological shifts in diatom species assemblages. *Canadian Journal of Botany*. **84:** 151–163.

Harris, P.G., Carter, J.F., Head, M., Harris, R.P., Eglinton, G., Maxwell, J.R. (1995). Identification of chlorophyll transformation products in zooplankton fecal pellets and marine

sediment extracts by liquid-chromatography mass-spectrometry atmospheric-pressure chemical-ionization. *Rapid Communications in Mass Spectrometry*. **9:** 1177–1183.

Hayes, P.K., Batley, J., Jenkins, C. (2006). Gene exchange within populations of *Nodularia spumigena* in the Baltic Sea. In: Program and Abstract, 12th International Symposium of Phototrophic Prokaryotes, Pau: p. 87 (Abstract).

Head, R.M., Jones, R.I., Bailey-Watts, A.E. (1999). An assessment of the influence of recruitment from the sediment on the development of planktonic populations of cyanobacteria in a temperate mesotrophic lake. *Freshwater Biology*. **41:** 759-769.

Heiri, O., ,Lotter, A.F., Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology*. **25:** 101–110.

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful algae*. **8:** 3-13.

Hermansky, S.J. and Stohs, S.J. (1991). Effects of microcystin-LR (MCLR) on hepatic microsomal membrane fluidity. *Research communications in chemical pathology and pharmacology*. **72:** 213–222.

Hernandez, J.A., Lopez-Gomollon, S., Bes, M.T., Fillat, M.F., Peleato, M.L. (2004) Three fur homologues from *Anabaena* sp. PCC7120: exploring reciprocal protein–promoter recognition. *FEMS Microbiology Letters.* **236:** 275–282.

Hiriart-Baer, V.P., Milne, J., Charlton, M.N., (2009). Water quality trends in Hamilton Harbour: two decades of change in nutrients and chlorophyll *a*. *Journal of Great Lakes Research*. **35:** 293–301.

Hiriart-Baer, V.P., Boyd, D., Long, T., Charlton, M.N., Milne, J.E. (2014). Hamilton Harbour over the last 25 years: insights from a long-term comprehensive water quality monitoring program. *Unpublished manuscript*.

Hoagland, K.D., Carder, J.P, Spawn, R.L. (1996). Effects of organic toxic substances. In: Stevenson, R.J., Bothwell, M., Lowe, R.L. (Eds), Algal Ecology: Freshwater Benthic Ecosystems. pp. 469–97. San Diego, CA: Academic Press.

Hodgson, D.A., Wright, S.W., Tyler, P.A., Davies, N. (1998). Analysis of fossil pigments from algae and bacteria in meromictic Lake Fidler, Tasmania, and its application to lake management. *Journal of Paleolimnology*. **19:** 1-22.

Hodgson, D.A., Wright, S.W., Davies, N. (1997). Mass spectrometry and reverse phase HPLC techniques for the identification of degraded fossil pigments in lake sediments and their application in palaeolimnology. *Journal of Paleolimnology*. **18:** 335-350.

Holmes, J.A. (1986). The Impact of Dredging and Spoils Disposal on Hamilton Harbour Fisheries: Implications for Rehabilitation. Canada Dept. of Fisheries and Oceans. Canadian Technical Report of Fisheries and Aquatic Sciences. 1498. 146pp.

Holst, T., Jorgensen, N.O., Jorgensen, C., Johansen, A. (2003). Degradation of microcystin in sediments at oxic and anoxic, denitrifying conditions. *Water research*. **37:** 4748-4760.

Horgan, M.J. and Mills, E.L. (1997). Clearance rates and filtering activity of zebra mussel (Dreissena polymorpha): implications for freshwater lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. **54:** 249–255.

Hori, K., Okamoto, J., Tanji Y., Unno, H. (2003). Formation, sedimentation and germination properties of *Anabaena* akinetes. *Biochemical Engineering Journal*. **14:** 67–73.

Hotto, A., Satchwell, M., Boyer, G. (2005). Seasonal production and molecular characterization of microcystins in Oneida Lake, New York, USA. *Environmental Toxicology*. **20:** 243–248.

Hotto, A., Satchwell, M., Boyer, G. (2007). Molecular Characterization of Potential Microcystin-Producing Cyanobacteria in Lake Ontario Embayments and Nearshore Waters. *Applied and environmental microbiology*. **73:** 4570–4578.

Hue, A.Y. (1988). Sedimentology of organic matter. In: Frimmel, F.H. and Christman, R.F. (eds.). Humic Substances And Their Role in the Environment. New York: John Wiley, pp. 215–244.

Hurley, J.P. and Armstrong, D.E. (1991). Pigment preservation in lake sediments: a comparison of sedimentary environments in Trout Lake, Wisconsin. *Canadian Journal of Fisheries and Aquatic Sciences*. **48:** 472-486.

Hyenstrand, P., Blomqvist, P., Pettersson, A. (1998). Factors determining cyanobacterial success in aquatic systems: a literature review. *Archiv fur Hydrobiologie Spec. Issues: Advances in*,. **51:** 41-62.

Hyenstrand, P., Rohrlack, T., Beattie, K.A., Metcalf, J.S., Codd, G.A., Christoffersen, K. (2003). Laboratory studies of dissolved radiolabelled microcystin-LR in lake water. *Water research*. **37:** 3299-3306.

Hyenstrand, P., Rydin, E., Gunnerhed, M., Linder, J., Blomqvist, P. (2001). Response of the cyanobacterium *Gloeotrichia echinulata* to iron and boron additions— an experiment from Lake Erken. *Freshwater Biology*. **46:** 735–741.

Ihle, T., Jähnichen, S., Benndorf, J. (2005). Wax and wane of *Microcystis* (Cyanophyceae) and microcystins in lake sediments: A case study in Quitzdorf Reservoir (Germany). *Journal of Phycology*. **41:** 479-488.

IJC (International Joint Commission) (1972). Annual report of the water quality board to the International Joint Commission. International Joint Commission, Great Lake Regional Office, Windsor, Ontario.

IJC (International Joint Commission) (1985). Report of the water quality board to the International Joint Commission. IJC, Great Lakes Regional Office, Windsor.

IJC (International Joint Commission) (1988). Revised Great Lakes Water Quality Agreement of 1978, as amended by Protocol signed Nov. 18, 1987. Consolidated by the International Joint Commission of United States and Canada. 130 pp.

Imai, A., Fukushima, T., Matsushige, K. (1999). Effects of iron limitation and aquatic humic substances on the growth of *Microcystis aeruginosa*. *Canadian Journal of Fisheries and Aquatic Sciences*. **56:** 1929–1937.

Itkonen, A., Marttila, V., Jarmo J. Meriläinen J. J., Salonen, V-P. (1999). 8000-year history of palaeo productivity in a large boreal lake. *Journal of Paleolimnology*. **21**: 271–294.

Jackson, C.R., Roden, E.E., Churchill, P.F. (2000). Denaturing gradient gel electrophoresis can fail to separate 16S rDNA fragments with multiple base differences. *Molecular Biology Today*. **1:** 49-51.

Jackson, T.A. and Hecky, R.E. (1980). Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone. *Canadian Journal of Fisheries and Aquatic Sciences*. **37:** 2300-2317.

Jaiswal, P., Prasanna, R., Singh, P.K. (2008). Cyanobacterial bioactive molecules-an overview of their toxic properties. *Canadian Journal of Microbiology*. **54:** 701-717.

Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (1997). Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO, Paris.

Johnson, M.G. and Matheson, D.H. (1968). Macroinvertebrate communities of the sediments of Hamilton Bay and adjacent Lake Ontario. *Limnology and Oceanography*. **13:** 99–111.

Jonasson, J., Olofsson, M., Monstein, H. J. (2002). Classification, identification and subtyping of bacteria based on pyrosequencing and signature matching of 16S rDNA fragments. *Apmis*. **110:** 263-272.

Jones B.F. and Bowser, C.J. (1978). The mineralogy and related chemistry of lake sediments. In: Lerman, A. (ed.) Lakes Chemistry, Geology, Physics. New York: Springer-Verlag, pp. 179-227.

Jones, G.J., and Orr, P.T. (1994). Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as determined by HPLC and protein phosphatases inhibition assay. *Water research*. **28:** 871-876.

Jones, G.J., Bourne, D.G., Blakeley, R.L., Doelle, H. (1994). Degradation of the cyanobacterial hepatotoxin microcystin by aquatic bacteria. *Natural Toxins*. **2:** 228-235.

Jungblut, A.D. and Neilan, B.A. (2006). Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. *Archives of microbiology*. **185:** 107-114.

Jüttner, F., Leonhardt, J. Moehren, S. (1983). Environmental factors affecting the formation of mesityl oxide di methyl allylic alcohol and other volatile compounds excreted by *Anabaena cylindrica*. *Journal of General Microbiology*. **129:** 407-412.

Kaebernick, M., and Neilan, B.A. (2001). Ecological and molecular investigations of cyanotoxin production. *FEMS microbiology ecology*. 35: 1-9.

Kalff, J. 2002. Limnology: Inland Water Ecosystems. New Jersey, USA, Prentice-Hall, Inc.

Kaneko, T., Nakajima, N., Okamoto, S., Suzuki, I., Tanabe, Y., Tamaoki, M., Nakamura, Y., Kasai, F., Watanabe, A., Kawashima, K., Kishida, Y., Ono, A., Shimizu, Y., Takahashi, C., Minami, C., Fujishiro, T., Kohara, M., Katoh, M., Nakazaki, N., Nakayama, S., Yamada, M., Tabata, S., Watanabe, M.M. (2007). Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. *DNA research*. **14:** 247–256.

Katoh, H., Hagino, N., Grossman, A.R., Ogawa, T. (2001). Genes essential to iron transport in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Journal of bacteriology* **183:** 2779–2784.

Keating, K.I. (1978). Blue-green algal inhibition of diatom growth: Transition from mesotrophic to eutrophic community structure. *Science.* **199:** 971-973.

Kemp A.L.W. and Johnston, L.M. (1979). Diagenesis of organic matter in the sediments of Lakes Ontario, Erie, and Huron. *Journal of Great Lakes Research*. **5:** 1-10.

Kerr, J.T. and Cihlar, J. (2004). Patterns and causes of species endangerment in Canada. *Ecological Applications*. **14:** 743–753.

Kerr, J.T. and Deguise, I. (2004). Habitat loss and the limits to endangered species recovery. *Ecology Letters*. **7:** 1163–1169.

Kersten, M. and Smedes, F. (2002). Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. *Journal of Environmental Monitoring*. **4:** 109–115.

Kirk, J.L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomos, J. N., Lee, H., Trevors, J. T. (2004). Methods of studying soil microbial diversity. *Journal of microbiological methods*. **58:**169-188.

Klapwijk, A. and Snodgrass, W.J. (1985). Model for lake-bay exchange. *Journal of Great Lakes Research*. **11:** 43-52.

Kleinkauf, H. and Von Dohren, H. (1996). A nonribosomal system of peptide biosynthesis. *European Journal of Biochemistry*. **236:** 335–51.

Klemer, A. R., Feuillade, J., Feuillade, M. (1982). Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of *Oscillatoria. Science.* **215:** 1629-1631.

Kling, H.J. (1998). A summary of past and recent plankton of Lake Winnipeg, Canada using algal fossil remains. *Journal of Paleolimnology*. **19:** 297–307.

Kolowith, L.C., Ingall, E.D., Benner, R. (2001). Composition and cycling of marine organic phosphorus. *Limnology and Oceanography*. **46:** 309-320.

Komarek, J. (2003) Coccoid and colonial cyanobacteria. In: Freshwater algae of North America - Ecology and classification. Wehr, J.D. and Sheath, R.G. (eds): Academic Press, pp. 59-116.

Komárek, J. (2012). Nomenclatural changes in heterocytous Cyanoprokaryotes (Cyanobacteria, Cyanophytes). *Fottea*. **12**: 141-148.

Komárek, J. and Komárková, J. (2004). Taxonomic review of the cyanoprokaryotic genera *Planktothrix* and *Planktothricoides*. *Czech Phycology*. **4:** 1-18.

Kondo, F., Ikai, Y., Oka, H., Okumura, M., Ishikawa, N., Harada, K.I., Matsuura, K., Murata, H., Suzuki, M. (1992). Formation, characterization and toxicity of the glutathione and cysteine conjugates of toxic heptapeptide microcystin. *Chemical research in toxicology*. **5:** 591– 596.

Koopmans, M.P., Köster, J., Van Kaam-Peters, H.M., Kenig, F., Schouten, S., Hartgers, W.A., deLeeuw, J.W., Sinninghe Damsté, J.S. (1996). Diagenetic and catagenetic products of isorenieratene: Molecular indicators for photic zone anoxia. *Geochimica et Cosmochimica Acta*. **60:** 4467-4496.

Korde, N.V. (1966). Algal remains in lake sediments – a contribution to development history of lakes and the surrounding regions. *Ergebnisse der Limnologie*. **3:** 1–38. (English translation by Fish. Res. Bd Can. 1371).

Kosmehl, T., Krebs, F., Manz, W., Erdinger, L., Braunbeck, T., Hollert, H. (2004). Comparative genotoxicity testing of rhine river sediment extracts using the comet assay with permanent fish cell lines (rtg-2 and rtl-w1) and the Ames test. *Journal of Soils and Sediments*. **4:** 84-94.

Kotak, B.G., Zurawell, R.W., Prepas, E.E., Holmes, C.F. (1996). Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status. *Canadian Journal of Fisheries and Aquatic Sciences*. **53:** 1974-1985.

Kowalchuka, G.A., Hola, W.H.G., Van Veen, J.A. (2006). Rhizosphere fungal communities are influenced by *Senecio jacobaea* pyrrolizidine alkaloid content and composition. *Soil Biology and Biochemistry*. **38:** 2852-2859.

Kravchuk, E.S., Ivanova, E.A., Gladyshev, M.I. (2006). Seasonal dynamics of akinetes of *Anabaena flos-aquae* in bottom sediments and water column of small Siberian reservoir. *Aquatic Ecology*. **40:** 325-336.

Kuentzel, L.E. (1969). Bacteria, carbon dioxide, and algal blooms. *Journal* (*Water Pollution Control Federation*). 1737-1747.

Kurmayer, R., Christiansen, G., Fastner, J., Börner, T. (2004). Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium *Planktothrix* spp. *Environmental Microbiology*. **6:** 831-841.

Kurmayer, R., Christiansen, G., Gumpenberger, M., Fastner, J. (2005). Genetic identification of microcystin ecotypes in toxic cyanobacteria of the genus *Planktothrix*. *Microbiology*. **151:** 1525-1533.

Kurmayer, R. and Christiansen, G. (2009). The genetic basis of toxin production in cyanobacteria. *Freshwater reviews*. **2:** 31–50.

Laamanen, M.J., Gugger, M.F., Lehtimäki, J.M., Haukka, K., Sivonen, K. (2001). Diversity of toxic and nontoxic *Nodularia* isolates (cyanobacteria) and filaments from the Baltic Sea. *Applied and environmental microbiology*. **67:** 4638-4647.

Lahti, K., Rapala, J., Fardig, M., Niemela, M. Sivonen, K. (1997). Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. *Water research*. **31:** 1005-1012.

Laird, K.R., Cumming, B.F., Wunsam, S., Rusak, J., Oglesby, R.J., Fritz, S.C., Leavitt, P.R. (2003). Lake sediments record large-scale shifts in moisture regimes across the northern

prairies of North America during the past two millennia. *Proceedings of the National Academy of Sciences*. **100:** 2483–2488.

Lampert, W. and Sommer, U. (2007). Limnoecology: the ecology of lakes and streams. Oxford university press.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences*. **82:** 6955-6959.

Larsson, U., Hajdu, S., Walve, J., Elmgren, R. (2001). Baltic Sea nitrogen fixation estimated from the summer increase in upper mixed layer total nitrogen. *Limnology and Oceanography*. **46:** 811-820.

Leavitt, P.R. (1993). A review of factors that regulate carotenoid and chlorophyll deposition and fossil pigment abundance. *Journal of Paleolimnology*. **9:** 109–127.

Leavitt, P.R. and Carpenter, S.R. (1989). Effects of sediment mixing and benthic algal production on fossil pigment stratigraphies. *Journal of Paleolimnology*. **2:** 147–158.

Leavitt, P.R. and Hodgson, D.A. (2001). Sedimentary pigments. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal, and siliceous indicators. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 295-325.

Leavitt, P.R., Carpenter, S.R., Kitchell, J. (1989). Whole lake experiments: The annual record of fossil pigments and zooplankton. *Limnology and Oceanography*. **34:** 700–717.

Leavitt, P.R., Sanford, P.R., Carpenter, S.R., Kitchell, J.F. (1994). An annual fossil record of production, planktivory and piscivory during whole-lake experiments. *Journal of Paleolimnology*. **11:** 133–149.

Leavitt, P.R., Vinebrooke, R.D., Donald, D.B., Smol, J.P., Schindler, D.W. (1997). Past ultraviolet radiation environments in lakes derived from fossil pigments. *Nature*. **388:** 457-459.

Lee, R.E. (2008). Phycology. 4th Edition. Cambridge University Press.

Leeben, A., Tonno, I., Freiberg, R., Lepane, V., Bonningues, N., Makarõtševa, N., Heinsalu, A., Alliksaar, T. (2008). History of anthropogenically mediated eutrophication of Lake Peipsi as revealed by the stratigraphy of fossil pigments and molecular size fractions of pore-water dissolved organic matter. *Hydrobiologia*. **599:** 49-58.

Lehtimäki, J., Moisander, P., Sivonen, K., Kononen, K. (1997). Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. *Applied and environmental microbiology*. **63:** 1647-1656.

Livingstone, D. and Reynolds, C.S. (1981). Algal sedimentation in relation to phytoplankton periodicity in Rostherne Mere. *British Phycological Journal*. **16:** 195 – 206.

López-Gomollón, S., Sevilla, E., Bes, M.T., Peleato, M.L., Fillat, M.F. (2009). New insights into the role of Fur proteins: FurB (All2473) from *Anabaena* protects DNA and increases cell survival under oxidative stress. *Biochemical Journal*. **418:** 201–207.

Loring, D.H. (1991). Normalization of heavy-metal data from estuarine and coastal sediments. *ICES Journal of Marine Science.* **48:** 101-115.

Lüder, U.H., Wiencke, C., Knoetzel, J. (2002). Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta): a study to simulate Antarctic winter sea ice cover. *Journal of phycology*. **38:** 904–13.

Lynch, J.M., Benedetti, A., Insam, H., Nuti, M.P., Smalla, K., Torsvik, V., Nannipieri. P. (2004). Microbial diversity in soil: Ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. *Biology and Fertility of Soils*. **40:** 363-385.

Mackay, D. and Toose, L. (2004). Quantifying the fate of mercury in the Great Lakes Basin: toward an ecosystem approach. *Environmental research*. **95:** 298-304.

MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P., Codd, G.A., (1990). Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. *FEBS Letters*. **264:** 187–192.

Mankiewicz, J., Tarczynska, M., Walter, Z., Zalewski, M. (2003). Natural toxins from cyanobacteria. *Acta Biologica Cracoviensia Series Botanica*. **45**: 9–20.

Mantoura, R.F.C. and Llewellyn, C.A. (1983). The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Analytica Chimica Acta*. **151:** 297–314.

Martin-Luna, B., Hernandez, J.A., Bes, M.T., Fillat, M.F., Peleato, M.L. (2006b). Identification of a ferric uptake regulator from *Microcystis aeruginosa* PCC7806. *FEMS Microbiology Letters*. **254:** 63–70.

Martin-Luna, B., Sevilla, E., Hernandez, J.A., Bes, M.T., Fillat, M.F., Peleato, M.L. (2006a). Fur from *Microcystis aeruginosa* binds in vitro promoter regions of the microcystin biosynthesis gene cluster. *Phytochemistry*. **67:** 876–881.

Marvin, C.H., Allan, L., McCarry, B.E., Bryant, D.W. (1992). A comparison of ultrasonic extraction and Soxhlet extraction of polycyclic aromatic hydrocarbons from sediments and air particulate material. *International journal of environmental analytical chemistry*. **49:** 221-230.

Marvin, C.H., McCarry, B.E., Villella, J., Allan, L.M., Bryant, D.W. (2000). Chemical and biological profiles of sediments as indicators of sources of genotoxic contamination in Hamilton Harbour. Part I: Analysis of polycyclic aromatic hydrocarbons and thia-arene compounds. *Chemosphere*. **41:** 979-988.

Marvin, C.H., Smith, R.W., Bryant, D.W., McCarry, B.E. (1999). Analysis of high-molecular-mass polycyclic aromatic hydrocarbons in environmental samples using liquid chromatography- atmospheric pressure chemical ionization mass spectrometry. *Journal of chromatography A*. **863:** 13-24.

Matheson, D.H. (1958). A consolidated report on Burlington Bay. Dept. of Municipal Laboratories, City of Hamilton, Hamilton, Ontario.

Maurici, D., Aardema, M., Corvi, R., Kleber, M., Krul, C., Laurent, C., Loprieno, N., Pasanen, M., Pfuhler, S., Phillips, B., Sabbioni, E., Sanner, T., Vanparys, P. (2005). Genotoxicity and mutagenicity. *Alternatives to laboratory animals*. **33:** 117-130.

Mayer, T. and Johnson, M.G. (1994). History of anthropogenic activities in Hamilton Harbour as determined from the sedimentary record. *Environmental Pollution*. **86:** 341-347.

Mayer, T. and Nagy, E. (1992). Polycyclic aromatic hydrocarbons in suspended particulates from Hamilton Harbour. *Water Quality Research Journal of Canada*. **27:** 807-831.

Mazak, E.J., MacIsaac, H.J., Servos, M.R., Hesslein, R. (1997). Influence of feeding habits on organochlorine contaminant accumulation in Great Lakes waterfowl. *Ecological Applications*. **7:** 1133-1143.

Mbedi, S., Welker, M., Fastner, J., Wiedner, C. (2005). Variability of the microcystin synthetase gene cluster in the genus *Planktothrix* (Oscillatoriales, Cyanobacteria). *FEMS Microbiology Letters*. **245:** 299-306.

McElhiney, J. and Lawton, L.A. (2005). Detection of the cyanobacterial hepatotoxins microcystins. *Toxicology and Applied Pharmacology*. **203:** 219-230.

McGowan, S. and Britton, G. (1999). Ancient blue-green blooms. *Limnology and Oceanography*. **44:** 436–439.

Medeiros, A.S. and Molot, L.A. (2006). Trends in iron and phosphorus loading to Lake Ontario from waste water treatment plants in Hamilton and Toronto. *Journal of Great Lakes Research*. **32:** 788-797.

Meriläinen, J.J., Hynynen, J., Palomäki, A., Mäntykoski, K., Witick, A. (2003). Environmental history of an urban lake: a paleolimnological study of Lake Jyväsjärvi, Finland. *Journal of Paleolimnology*. **30:** 387–406.

Metcalfe, C.D., Balch, G.C., Cairns, V.W., Fitzsimons, J.D., Dunn, B.P. (1990). Carcinogenic and genotoxic activity of extracts from contaminated sediments in western Lake Ontario. *Science of the total environment*. **94:** 125-141.

Metcalfe, C.D., Cairns, V.W., Fitzsimons, J.D. (1988). Experimental induction of liver tumours in rainbow trout (Salmo gairdneri) by contaminated sediment from Hamilton Harbour, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*. **45:** 2161-2167.

Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., Chaffin, J.D., Cho,K., Confesor, R., Daloglu,I., DePinto, J.V., Evans, M.A., Fahnenstiel, G.L., He,L., Ho, J.C., Jenkinsg, L., Johengen, T.H., Kevin C. Kuo,K.C., LaPorte, E., Liu, X., McWilliams, M.R., Moore, M.R., Posselt, D.J., Richards, R.P., Scavia, D., Steiner, A.L., Verhamme, E., David M. Wright, D.M., Zagorski, M.A. (2013). Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proceedings of the National Academy of Sciences*. **110:** 6448-6452.

Miller, M.J., Critchley, M.M., Hutson, J., Fallowfield, H.J. (2001). The adsorption of cyanobacterial hepatotoxins from water onto soil during batch experiments. *Water research*. **35:** 1461-1468.

Mills, E.L., Leach, J.H., Carlton, J.T., Secor, C.L., (1993). Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research*.**19:** 1–54.

Mills, H.J., Reese, B.K., Shepard, A.K., Riedinger, N., Dowd, S.E., Morono, Y., Inagaki, F. (2012). Characterization of metabolically active bacterial populations in subseafloor Nankai Trough sediments above, within, and below the sulfate–methane transition zone. *Frontiers in microbiology*. **3:** 1-12

Miura, G.A., Robinson, N.A., Geisbert, T.W., Bostian, K.A., Whit, J.D., Pace, J.G. (1989). Comparison of in vivo and in vitro toxic effects of microcystin-LR in fasted rats. *Toxicon*. **27:** 1229–1236.

Moos, M.T., Laird, K.R., Cumming, B.F. (2005). Diatom assemblages and water depth in Lake 239 (Experimental Lakes Area, Ontario): implications for paleoclimatic studies. *Journal of Paleolimnology*. **34:** 217–227.

Morris, R.J., Williams, D.E., Luu, H.A., Holmes, C.F.B., Andersen, R.J., Calvert, S.E. (2000). The adsorption of microcystin-LR by natural clay particles. *Toxicon*. **38:** 303-308.

Morrison, H., Gobas, F.A.P.C., Lazar, R., Whittle, D.M., Haffner, G.D. (1997). Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in western Lake Erie. *Environmental science and technology* **31:** 3267–3273.

Morton, S. and Lee, T. (1974). Algal blooms—possible effects on iron. *Environmental science and technology* **8:** 673–674.

Muir, D.C.G., Wang, X., Yang, F., Nguyen, N., Jackson, T.A., Evans, M.S., Douglas, M., Köck, G., Lamoureux, S., Pienitz, R., Smol, J.P., Vincent, W.F., Dastoor, A. (2009). Spatial Trends and Historical Deposition of Mercury and Lead in Eastern and Northern Canada Inferred from Lake Sediment Cores. *Environmental Science and Technology*. **43:** 4802–4809.

Müller, A.K., Westergaard, K., Christensen, S., Sørensen, S.J. (2001). The effect of long-term mercury pollution on the soil microbial community. *FEMS microbiology ecology* **36:** 11–19.

Muller, E.H. and Prest, V. K. (1985). Glacial lakes in the Ontario basin. In: Karrow, P.F. and Calkin, P. E. (eds), Quaternary evolution of the Great Lakes, pp. 213-229. Geol. Assoc. Can., Special Paper 30, St John's, Newfoundland.

Munawar, M. and Fitzpatrick, M. (2007). An integrated assessment of the microbial and planktonic communities of Hamilton Harbour. *Canadian Technical Report of Fisheries and Aquatic Sciences*. **2729:** 43-63.

Murphy, A.M. and Cowles, T.J. (1997). Effects of darkness on multi excitation in vivo fluorescence and survival in a marine diatom. *Limnology and oceanography*. **42:**1444–53.

Murphy, T.P., Irvine, K., Guo, J., Davies, J., Murkin, H., Charlton, M., Watson, S.B. (2003). New microcystin concerns in the lower Great Lakes. *Water Quality Research Journal of Canada*. **38:** 127-140.

Murphy, T.P., Brouwer, H., Fox, M.E., Nagy, E., McArdle, L., Moller, A. (1990). Coal tar contamination near randle reef, Hamilton Harbour. NWRI contribution No. 90-17.

Muyzer, G., De Waal, E.C., Uitterlinden, G.A. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* **59**: 695-700.

Nagai, T., Imai, A., Matsushige, K., Fukushima, T. (2007). Growth characteristics and growth modeling of *Microcystis aeruginosa* and *Planktothrix agardhii* under iron limitation. *Limnology*. ***8*:** 261-270.

Neilan, B.A., Dittmann, E., Rouhiainen, L., Bass, R.A., Schaub, V., Sivonen, K.,and Börner, T. (1999). Nonribosomal peptide synthesis and toxigenicity of cyanobacteria. *Journal of bacteriology*. **181:** 4089-4097

Neilan, B.A., Jacobs, D., DelDot, T., Blackall, L.L., Hawkins, P.R., Cox, P.T., Goodman, A.E. (1997). rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic Bacteriology*. **47:** 693-697.

Neilan, B.A., Jacobs, D., Goodman, A.E. (1995). Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. *Applied and environmental microbiology*. **61:** 3875-3883.

Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E. (2013). Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environmental microbiology*. ***15*****:** 1239-1253.

Nicholls, K.H. (1999). Effects of temperature and other factors on summer phosphorus in the inner Bay of Quinte, Lake Ontario: Implications for climate warming. *Journal of Great Lakes Research*. **25:** 250-262.

Nishizawa, T., Asayama, M., Fujii, K., Harada, K. Shirai, M. (1999). Genetic analysis of the peptide synthetase genes for a cyclic heptapeptides microcystin in *Microcystis* spp. *Journal of Biochemistry*. **126:** 520–529.

Nishizawa, T., Ueda, A., Asayama, M., Fujii, K., Harada, K., Ochi, K., Shirai, M. (2000). Polyketide synthase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin. *Journal of Biochemistry*. **127:** 779–789.

Nriagu, J.O., Wong, H.K.T., Snodgrass, W.J. (1983). Historical records of metal pollution in sediments of Toronto and Hamilton Harbour. *Journal of Great Lakes Research*. **9:** 365–373.

Nürnberg, G.K. (1995). Quantifying anoxia in lakes. *Limnology and Oceanography*. **40:** 1100–1111.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful algae*. **14:** 313-334.

Ogram, A., Sayler, G.S., Gustin, D., Lewis, R.J. (1988). DNA adsorption to soils and sediments. *Environmental science and technology.* **22:** 982–984.

Oliver, R.L. and Ganf, G.G. (2000). Freshwater blooms. In The Ecology of Cyanobacteria: their Diversity in Time and Space, pp. 149–194. Edited by B. A. Whitton and M. Potts. Dordrecht: Kluwer Academic.

Oliver, R.L., Hamilton, D.P., Brookes, J.D., Ganf, G.G. (2012). Physiology, blooms and prediction of planktonic cyanobacteria. In Ecology of Cyanobacteria II (pp. 155-194). Springer Netherlands.

Ontario Ministry of the Environment (MOE) (1985). Hamilton Harbour technical summary and general management options. Water Resources Branch, Toronto.

Ontario Ministry of the Environment (MOE) (1986). Impact of Hamilton Harbour on Western Lake Ontario. Water Resources Branch, Ontario Ministry of the Environment.

Ontario Ministry of the Environment (MOE) (2013). Water Quality in Ontario 2012. Report. Environmental Monitoring and Reporting Branch, Ontario Ministry of the Environment.

Oren, A. (2004). A proposal for further integration of the cyanobacteria under the Bacteriological Code. International journal of systematic and evolutionary microbiology. 54: 1895-1902.

Orr, P.T. and Jones, G.J. (1998). Relationship between microcystin production and cell division rates in nitrogen limited *Microcystis aeruginosa* cultures. *Limnology and Oceanography*. **43:** 1604-1614.

Otsuka, S., Suda, S., Li, R., Watanabe, M., Oyaizu, H., Matsumoto, S., Watanabe, M.M. (1999). Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiology Letters*. **172:** 15-21.

Otsuka, S., Suda, S., Li, R.H., Watanabe, M., Oyaizu, H., Matsumoto, S., Watanabe, M.M. (1998). 16S rDNA sequences and phylogenetic analyses of *Microcystis* strains with and without phycoerythrin. *FEMS Microbiology Letters*. **164:** 119-124.

Ouellette, A.J.A. and Wilhelm, S.W. (2003). Toxic cyanobacteria: the evolving molecular toolbox. *Frontiers in Ecology and the Environment*. **1:** 359–366.

Ouellette, A.J.A., Handy, S.M., Wilhelm, S.W. (2006). Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. *Microbial Ecology*. **51:** 154–165.

Paerl, H.W. (1988). Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography*. **33:** 823-847.

Paerl, H.W. and Millie, D.F. (1996). Physiological ecology of toxic aquatic cyanobacteria. *Phycologia*. **35:** 160-167.

Paerl, H.W. and Huisman, J. (2008). Blooms like it hot. *SCIENCE-NEW YORK THEN WASHINGTON-*. 320(5872), 57.

Paerl, H.W. and Huisman, J. (2009). Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*. **1:** 27-37.

Paerl, H.W. and Ustach, J.F. (1982). Blue-green algal scums: an explanation for their occurrence during freshwater blooms. *Limnology and Oceanography*. **27:** 212-217.

Paerl, H.W., Hall, N.S., Calandrino, E.S. (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of the Total Environment*. **409:** 1739-1745.

Palinska, K.A., Liesack, W., Rhiel, E., Krumbein, W.E. (1996). Phenotype variability of identical genotypes: the need for a combined approach in cyanobacterial taxonomy demonstrated on *Merismopedia*-like isolates. *Archives of microbiology*. **166:** 224-233.

Pan, H., Song, L., Liu, Y., Borner, T. (2002). Detection of hepatotoxic *Microcystis* strains by PCR with intact cells from both culture and environmental samples. *Archives of microbiology*. **178:** 421–427.

Pemberton, K.L., Smith, R.E.H., Silsbe, G.M., Howell, E.T., Watson, S.B. (2007). Controls on phytoplankton physiology in Lake Ontario during the late summer: evidence from new fluorescence methods. *Canadian Journal of Fisheries and Aquatic Sciences*. **64:** 58-73.

Philippe, H. and Douady, C.J. (2003). Horizontal gene transfer and phylogenetics. *Current opinion in microbiology*. **6:** 498-505.

Piccinin, B.B. (1977). The biological survey of Hamilton Harbour 1976. Dept. of Biology, McMaster University. Tech. Report Series No. 2. 129 p.

Porra, R.J., Pfündel, E.E., Engel, N. (1997). Metabolism and function of photosynthetic pigments. In: Jeffrey SW, Mantoura RFC, Wright SW (eds) Phytoplankton pigments in oceanography UNESCO, Paris, pp 85-126

Poulton, D.J. (1987). Trace contaminant status of Hamilton Harbour. *Journal of Great Lakes Research*. **13:** 193–201.

Poulton, D.J., Simpson, K.J., Barton, D.R., Lum, K.R. (1988). Trace metal and benthic invertebrates in sediments of nearshore Lake Ontario at Hamilton Harbour. *Journal of Great Lakes Research*. **14:** 52–65.

Poutanen, E.L. and Nikkilä, K. (2001). Carotenoid pigments as tracers of cyanobacteria blooms in recent and post-glacial sediments of the Baltic Sea. *Ambio*. **30:** 179–183.

Quinn, G.P. and Keough, M.J. (2002). Experimental design and data analysis for biologists. Cambridge University Press. pp. 443-493.

Rantala, A., Rajaniemi-Wacklin, P., Lyra, C., Lepistö, L., Rintala, J., Mankiewicz-Boczek, J., Sivonen, K. (2006). Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (*mcy*E) PCR and associations with environmental factors. *Applied and environmental microbiology*. 72: 6101-6110.

Rantala, A., Rizzi, E., Castiglioni, B., De Bellis, G., Sivonen, K. (2008). Identification of hepatotoxin producing cyanobacteria by DNA-chip. *Environmental microbiology*. **10:** 653–664.

Rao, P.V.L. and Bhattacharya, R. (1996). The cyanobacterial toxin microcystin-LR induced DNA damage in mouse liver *in vivo*. *Toxicology*. **114:** 29–36.

Rapala, J., Lahti, K., Sivonen, K., Niemela, S.I. (1994). Biodegradability and adsorption on lake-sediments of cyanobacterial hepatotoxins and anatoxin-a. *Letters in Applied Microbiology*. **19:** 423-428.

Rapala, J., Sivonen, K., Luukkainen, R., Niemelae, S.I. (1993). Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena* strains: a laboratory study. *Journal of Applied Phycology.* **5:** 581-591.

Rapala, J., Sivonen, K., Lyra, C., Niemelae, S.I. (1997). Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. *Applied and Environmental Microbiology*. **63:** 2206-2212.

Rasanen, J., Kauppila, T., Vuorio, K. (2006). Sediment and phytoplankton records of the cyanobacterial genus *Anabaena* in boreal Lake Pyhajarvi. *Hydrobiologia*. **568:** 455–465.

Rashid, M.A. (1985). Geochemistry of Marine Humic Compounds. New York: Springer Verlag, 291 p.

Reuss, N., Leavitt, P.R., Hall, R.I., Bigler, C., Hammarlund, D. (2010). Development and application of sedimentary pigments for assessing effects of climatic and environmental changes on subarctic lakes in northern Sweden. *Journal of Paleolimnology*. **43:** 149-169.

Reynolds, C.S., Jaworsky, G.H.M., Cmiech, H.A. Leedale, G.F. (1981). On the annual cycle of the blue-green algae *Microcystis aeruginosa* Kütz. emend. Elenkin. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*. **293:** 419–477.

Reynolds, C.S. (1984). The Ecology of Freshwater Phytoplankton. Cambridge University Press, London.

Reynolds, C.S. (1987). Cyanobacterial water-blooms. In Callow, P. (ed.), Advances in Botanical Research 13. Academic Press, London: 67–143.

Reynolds, C.S. (2006). The ecology of phytoplankton. Cambridge University Press.

Reynolds, C.S. (2012). Environmental requirements and habitat preferences of phytoplankton: chance and certainty in species selection. *Botanica Marina*. **55:**1-17.

Reynolds, C.S. and Walsby, A.E. (1975). Water-blooms. *Biological reviews*. **50:** 437-481.

Rhodes, R. (1995). Dark Sun – The making of the hydrogen bomb. Simon and Schuster, Rockefeller Center, New York NY. 731pp.

Ricciardi, A. (2001). Facilitative interactions among aquatic invaders: evidence of 'invasional meltdown' in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. **58:** 2513-2525.

Ricciardi, A. and Rasmussen, J.B. (1998). Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Sciences*. **55:** 1759-1765.

Rinta-Kanto, J.M. and Wilhelm, S.W. (2006). Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. *Applied and environmental microbiology*. **72:** 5083–5085.

Rinta-Kanto, J.M., Konopko, E.A., DeBruyn, J.M., Bourbonniere, R.A., Boyer, G.L., Wilhelm, S.W. (2009a). Lake Erie *Microcystis*: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. *Harmful algae*. **8:** 665–673.

Rinta-Kanto, J.M., Ouellette, A.J.A., Boyer, G.L., Twiss, M.R., Bridgeman, T.B., Wilhelm, S.W. (2005). Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. *Environmental Science and Technology*. **39:** 4198–4205.

Rinta-Kanto, J.M., Saxton, M.A., DeBruyn, J.M., Smith, J.L., Marvin, C.H., Krieger, K.A., Sayler, G.S., Boyer, G.L., Wilhelm, S.W. (2009b). The diversity and distribution of toxigenic *Microcystis* spp. in present day and archived pelagic and sediment samples from Lake Erie. *Harmful algae*. **8:** 385–394.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*. **111:** 1-61.

Rivasseau, C., Martins, S., Hennion, M.C. (1998). Determination of some physicochemical parameters of microcystins (cyanobacterial toxins) and trace level analysis in environmental samples using liquid chromatography. *Journal of chromatography A.* **799:**155-169.

Robbins, J.A., Holmes. C., Halley, R., Bothner, M., Shinn, E., Graney, J., Keeler, G., tenBrink, M., Orlandini, K.A., Rudnick, D. (2000). Time-averaged fluxes of lead and fallout radionuclides to sediments in Florida Bay. *Journal of Geophysical Research: Oceans (1978–2012). 105*(C12): 28805-28821.

Rochelle, P.A., Fry, J.C., Parkes, R.J., Weightman, A.J. (1992). DNA extraction for 16S rRNA gene analysis to determine genetic diversity in deep sediment communities. *FEMS microbiology letters*. **100:** 59–66.

Rodgers, K. (Coordinator) (1989). Remedial Action Plan for the Hamilton Harbour, Stage 1 Report: Environmental conditions and problem definition. Ontario Ministry of the Environment, Toronto.

Romero-Viana, L., Keely, B.J., Camacho, A., Vicente, E., Miracle, M.R. (2010). Primary production in Lake La Cruz (Spain) over the last four centuries: reconstruction based on sedimentary signal of photosynthetic pigments. *Journal of Paleolimnology*. **43:** 771-786.

Rouhiainen, L., Vakkilainen, T., Siemer, B.L., Buikema, W., Haselkorn, R., Sivonen, K. (2004). Genes coding for hepatotoxic heptapeptides (microcystins) in the cyanobacterium *Anabaena* strain 90. *Applied and environmental microbiology*. **70:** 686-692.

Rudi, K., Skulberg, O.M., Jakobsen, K.S. (1998). Evolution of cyanobacteria by exchange of genetic material among phyletically related strains. *Journal of bacteriology*. **180:** 3453-3461.

Rudi, K., Skulberg, O.M., Skulberg, R., Jakobsen, K.S. (2000). Application of sequence-specific labeled 16S rRNA gene oligonucleotide probes for genetic profiling of cyanobacterial abundance and diversity by array hybridization. *Applied and environmental microbiology*. **66**: 4004-4011.

Rukavina, N.A. and Versteeg, J.K. 1996. Surficial sediments of Hamilton Harbour: Physical properties and basin morphology. *Water Quality Research Journal of Canada*. **31:** 529-551.

Rukavina, N.A., Keyes, D., Zeman, A.J. (1994). Side scan evidence of disturbed bottomsediments in Hamilton Harbour. Program with Abstract: Joint Meeting of the Geological Assoc. Canada and the Minaeralogical Assoc. Canada, May 1994.

Saker, M.L., Neilan, B.A. Griffths, D.J. (1999). Two morphological forms of Cylindrospermopsis raciborskii (cyanobacteria) isolated from Solomon dam, Palm Island, Queensland. *Journal of phycology*. **35:** 599-606.

Sala, O.E., Chapin III, F.S., Armesto, J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H. (2000). Global biodiversity scenarios for the year 2100. *Science.* **287:** 1770-1774.

Salonen, V., Tuovinen, N., Valpola, S. (2006). History of mine drainage impact on Lake Orijäryi algal communities, SW Finland. *Journal of Paleolimnology*. **35:** 289–303.

Sanchez-Cabeza, J.A. and Ruiz-Fernández, A.C. (2012). ^{210}Pb sediment radiochronology: An integrated formulation and classification of dating models. *Geochemica et Cosmochimica Acta*. **82:** 183-200.

Sanger, J.E. (1988) Fossil pigments in paleoecology and paleolimnology. *Palaeogeography, Palaeoclimatology, Palaeoecology.* **62:** 343-359

Sanger, J.E. and Gorham, E. (1970). The diversity of pigments in lake sediments and its ecological significance. *Limnology and Oceanography* **15:** 59–69.

Schäfer, H. and Muyzer, G. (2001). Denaturing gradient gel electrophoresis and marine microbial ecology. In: Paul J (ed.). Methods in Microbiology, vol 30. Academic Press, London, pp 425–468.

Schelske, C.L. (1991). Historical nutrient enrichment of Lake Ontario: paleolimnological evidence. *Canadian Journal of Fisheries and Aquatic Sciences*. **48:** 1529-1538.

Schelske, C.L., Stoermer, E.F., Conley, D.J., Robbins, J.A. (1983). Early Eutrophication in the Lower Great Lakes: New Evidence from Biogenic Silica in Sediments. *Science.* **222:** 320-322.

Schindler, D.W. (1977). Evolution of phosphorus limitation in lakes. *Science.* **195:** 260-262.

Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., Kasian, S.E.M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences*. **105:** 11254-11258.

Schubert, C.J., Villanueva, J., Calvert, S.E., Cowie, G.L., Von Rad, U., Schulz, H., Berner, U., Erlenkeuser, H. (1998). Stable phytoplankton community structure in the Arabian Sea over the past 200,000 years. *Nature*. **394:** 563-566.

Sellner, K.G., Doucette, G.J., Kirkpatrick, G.J. (2003). Harmful algal blooms: causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology*. **30:** 383–406.

Sevilla, E., Martin-Luna, B., Vela, L., Bes, M.T., Fillat, M.F., Peleato, M.L. (2008). Iron availability affects *mcy*D expression and microcystin-LR synthesis in *Microcystis aeruginosa* PCC7806. *Environmental microbiology*. **10:** 2476–2483.

Sheath R.G. and Wehr, J.D. (Eds.) (2003). Freshwater Algae of North America. Academic Press; New York, New York.

Shi, L., Carmichael, W.W., Kennelly, P.J. (1999). Cyanobacterial PPP family protein phosphatases possess multifunctional capabilities and are resistant to microcystin-LR. *Journal of Biological Chemistry*. **274:** 10039-10046.

Silliman, J.E., Meyers, P.A., Bourbonniere, R.A. (1996). Record of postglacial organic matter delivery and burial in sediments of Lake Ontario. *Organic Geochemistry*. **24:** 463-472.

Sivonen, K. (1990). Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Applied and environmental microbiology*. **56:** 2658-2666.

Sivonen, K. and Jones, G. (1999). Cyanobacterial toxins. In: Chorus, I. and Bartram, J. (Eds.) Toxic Cyanobacteria in Water. A Guide to their Public Health Consequences, Monitoring and Management. pp. 41-111. E and FN Spon, London.

Skei, J., Larsson, P., Rosenberg, R., Jonsson, P., Olsson, M., Broman, D. (2000). Eutrophication and contaminants in aquatic ecosystems. *Ambio*. **29:**184–194.

Skulberg, O.M., Carmichael, W.W., Codd, G.A., Skulberg, R. (1993). Taxonomy of toxic cyanophyceae (Cyanobacteria). In: Falconer, I.R. (Ed.), Algal Toxins in Seafood and Drinking Water. Academic Press, London, pp. 145– 164.

Sly, P.G. (1986). Review of postglacial environmental changes and cultural impacts in the Bay of Quinte. *Project Quinte: point source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario*, 7-26.

Sly, P.G. (1991). The effects of land use and cultural development on the Lake Ontario ecosystem since 1750. *Hydrobiologia*. **213**: 1-75.

Sly, P.G. and Prior, J.W. (1984). Late glacial and postglacial geology in the Lake Ontario basin. *Canadian Journal of Earth Sciences.* **21:** 802–821.

Smith, V.H. (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science.* **221:** 669–671.

Smith, V.H. (1986). Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. *Canadian Journal of Fisheries and Aquatic Sciences*. **43:** 148–153.

Smith, V.H. (1990). Nitrogen, phosphorus, and nitrogen fixation in lacustrine and estuarine ecosystems. *Limnology and Oceanography*. **35:** 1852-1859.

Smol, J.P. (1995). Paleolimnological approaches to the evaluation and monitoring of ecosystem health: Providing a history for environmental damage and recovery. In: Rapport, D.J., Gaudet, C.L., Calow, P. (Eds.). Evaluating and Monitoring the Health of Large-Scale Ecosystems, Springer, Berlin, pp. 301–318.

Smol, J.P. (2008). Pollution of Lakes and Rivers: A Paleoenvironmental Perspective 2nd Edition. Blackwell Publishing, Oxford. 383 pp.

Smol, J.P., Walker, I.R., Leavitt, P.R. (1991). Paleolimnology and hindcasting climatic trends. *Internationale Vereinigung fuer Theoretische und Angewandte Limnologie. Verhandlungen IVTLAP.* **24:** 1240-1246.

Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B., Osman, R.W. (2002). Linking climate change and biological invasion: ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences*. **99:** 15497-15550.

Stanier, R. Y., Sistrom, W.R., Hansen, T.A., Whitton, B.A., Castenholz, R.W., Pfennig, N., Gorlenko, V.N., Kondratieva, E.N., Eimhjellen, K.E., Whittenbury, R., Gherna, R.L., Trüper, H.G. (1978). Proposal to place the nomenclature of the cyanobacteria (blue-green algae) under the rules of the International Code of Nomenclature of Bacteria. *International Journal of Systematic Bacteriology*. **28:** 335-336.

Stefan, R.J., Goksøyr, J., Bej, A.K., Atlas, R.M. (1988). Recovery of DNA from soils and sediments. *Applied and environmental microbiology*. **54:** 2908–2915.

Steinberg, C. and Hartmann, H.M. (1988). Planktonic bloom-forming Cyanobacteria and the eutrophication of lakes and rivers. *Freshwater Biology*. **20:** 279-287.

Stoermer, E.F., Wolin, J.A., Schelske, C.L.; Conley, D.J. (1985). An assessment of ecological changes during the recent history of Lake Ontario based on siliceous algal microfossils preserved in the sediments. *Journal of Phycology*. **21:** 257-276.

Tandeau de Marsac, N. (1977). Occurrence and *Nature* of chromatic adaptation in Cyanobacteria. *Journal of bacteriology*. **130:** 82–91.

Taylor, M.E. and Blokpoel, H. (1996). Caspian Tern Nesting Raft in Hamilton Harbour, 1993-1995. Minister of Public Works and Government Services Canada. http:/sustainabilityfund.gc.ca/default.asp?lang=Enandn=7B1F70ED-1

ter Braak, C. and Šmilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5).

Thomas, R.L., Kemp, A.L.W., Lewis, C.F.M. (1972). Distribution, composition and characteristics of the surficial sediments of Lake Ontario. *Journal of Sedimentary Research*. **42:** 66-84.

Thompson, W.L. and Pace, J.G. (1992). Substances that protect cultured hepatocytes from the toxic effects of microcystin-LR. *Toxicology in vitro*. **6:** 568– 579.

Tillett, D., Dittmann, E., Erhard, M., von Döhren, H., Boerner, R., Neilan, B.A. (2000). Structural organisation of microcystin biosynthesis in *M. aeruginosa* PCC7806: An integrated peptide-polyketide synthetase system. *Chemistry & biology*. **7:** 753-764.

Tillett, D., Parker, D.L., Neilan, B.A. (2001). Detection of toxigenicity by a probe for the microcystin synthetase a gene (*mcy*A) of the cyanobacterial genus *Microcystis* : comparison of toxicities with 16S rRNA and phycocyanin operon (Phycocyanin Intergenic Spacer) phylogenies. *Applied and Environmental Microbiology* **67:** 2810–2818.

Trimbee, A.M. and Harris, G.P. (1984). Phytoplankton populations dynamics of a small reservoir; use of sedimentation traps to quantify the loss of diatoms and recruitment of summer bloom-forming blue green algae. *Journal of Plankton Research*. **6:** 897–918.

Tsai, Y-L. and Olson, B.H. (1992). Detection of low numbers of bacterial cells in soils and sediments by polymerase chain reaction. *Applied and environmental microbiology*. **58:** 754–757.

Tsuji, K., Masui, H., Uemura, H., Mori, Y., Harada, K.-I. (2001). Analysis of microcystins in sediments using MMPB method. *Toxicon*. **39:** 687–692.

Tsuji, K., Naito, S., Kondo, F., Watanabe, M.F., Suzuki, S., Nakazawa, H., Suzuki, M., Shimada, T., Harada, K.-I. (1994). A cleanup method for analysis of trace amounts of microcystins in lake water. *Toxicon*. **32:** 1251-1259

Tucker, A. (1948). The Phytoplankton of the Bay of Quinte. *Transactions of the American Microscopical Society*. **67:** 365-383.

Twiss, M.R., Auclair, J.C., Charlton, M.N. (2000). An investigation into iron stimulated phytoplankton productivity in the epipelagic Lake Erie during thermal stratification using trace metal techniques. *Canadian Journal of Fisheries and Aquatic Sciences*. **57:** 86–95.

UNESCO (1969). Determination of photosynthetic pigments in sea-water. Imprimerie Rolland-Paris, Paris, France.

Urbach, E., Robertson, D.L., Chisholm, S.W. (1992). Multiple Evolutionary Origins of Prochlorophytes within the Cyanobacterial Radiation. *Nature*. **355:** 267-270.

Utkilen, H. and Gjolme, N. (1995). Iron-stimulated toxin production in *Microcystis aeruginosa*. *Applied and Environmental Microbiology* **61:** 797-800.

Vaitomaa, J., Rantala, A., Halinen, K., Rouhiainen, L., Tallberg, P., Mokelke, L., Sivonen, K. (2003). Quantitative real-time PCR for determination of microcystin synthetase E copy numbers for *Microcystis* and *Anabaena* in lakes. *Applied and environmental microbiology*. **69:** 7289-7297.

Valdes-Weaver, L.M., Piehler, M.F., Pinckney, J.L., Howe, K.E., Rossignol, K., Paerl, H.W. (2006). Long-term temporal and spatial trends in phytoplankton biomass and class-level taxonomic composition in the hydrologically variable Neuse–Pamlico estuarine continuum, North Carolina, USA. *Limnology and Oceanography*. **51:** 1410–1420.

Vallentyne, J.R. (1957.). Carotenoids in 20,000-year old sediment from Searles Lake. California. *Archives of biochemistry and biophysics*. **70:** 29–34.

Van de Peer, Y., Chapelle, S., De Wachter, R. (1996). A quantitative map of nucleotide substitution rates in bacterial rRNA. *Nucleic acids research*. **24:** 3381-3391.

Van de Waal, D. B., Verspagen, J. M., Lürling, M., Van Donk, E., Visser, P. M., Huisman, J. (2009). The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. *Ecology letters.* **12:** 1326-1335.

van der Gucht, K., Sabbe, K., de Meester, L., Vloemens, N., Zwart, G., Gillis, M., Vyverman, W. (2001). Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. *Environmental microbiology* **3:** 80–690.

van Geel, B. (2001). Non-pollen palynomorphs. In: Smol J.P., Birks H.J.B. and Last W.M. (eds), Tracking Environmental Change Using Lake Sediments. Volume 3: Terrestrial, Algal, and Siliceous Indicators. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 99-119.

van Geel, B., Mur, L.R., Ralska-Jasiewiczowa, M. Goslar, T. (1994). Fossil akinetes of *Aphanizomenon* and *Anabaena* as indicators for medieval phosphate-eutrophication of Lake Gosciaz (Central Poland). *Review of palaeobotany and Palynology*. **83:** 97-105.

Vanderploeg, H.A., Liebig, J.R., Carmichael, W.W., Agy, M.A., Johengen, T.H., Fahnenstiel, G.L., Nalepa, T.F. (2001). Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences*. **58:** 1208-1221.

Vareli, K., Pilidis, G., Mavrogiorgou, M.C., Briasoulis, E., Sainis, I. (2009). Molecular characterization of cyanobacterial diversity and yearly fluctuations of microcystin loads in a suburban Mediterranean Lake (Lake Pamvotis, Greece). *Journal of Environmental Monitoring.* **11:** 1506-1512.

Vareli, K., Zarali, E., Zacharioudakis, G. S., Vagenas, G., Varelis, V., Pilidis, G., Briasoulis, E., Sainis, I. (2012). Microcystin producing cyanobacterial communities in Amvrakikos Gulf (Mediterranean Sea, NW Greece) and toxin accumulation in mussels (*Mytilus galloprovincialis*). *Harmful algae*. **15:** 109-118.

Verspagen, J.M., Snelder, E.O., Visser, P.M., Huisman, J., Mur, L.R., Ibelings, B.W. (2004). Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: internal buoyancy changes or resuspension? *Journal of Phycology*. **40:** 260–270.

Versteeg, J.K., Morris, W.A., Rukavina, N.A. (1995). Mapping contaminated sediment in Hamilton Harbour, Ontario. *Geoscience Canada*. **22:** 145–151.

Vezie, C., Brient, L., Sivonen, K., Bertru, G., Lefeuvre, J. C., Salkinoja-Salonen, M. (1997). Occurrence of microcystin-containing cyanobacterial blooms in freshwaters of Brittany (France). *Archiv für Hydrobiologie*. **139:** 401-413.

Vezie, C., Brient, L., Sivonen, K., Bertru, G., Lefeuvre, J. C., Salkinoja-Salonen, M. (1998). Variation of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). *Microbial Ecology*. **35:** 126-135.

Via-Ordorika, L., Fastner, J., Kurmayer, R., Hisbergues, M., Dittmann, E., Komarek, J., Erhard, M, Chorus, I. (2004). Distribution of microcystin-producing and non-microcystin-producing *Microcystis* sp. in European freshwater bodies: detection of microcystins and microcystin genes in individual colonies. *Systematic and applied microbiology*. **27:** 592-602.

Vincent, R.K., Qin, X.M., Mckay, R.M.L., Miner, J., Czajkowski, K., Savino, J., Bridgeman, T. (2004). Phycocyanin detection from LANDSAT TM data for mapping cyanobacterial blooms in Lake Erie. *Remote Sensing of Environment*. **89:** 381–392.

Water, E. (1978). Specific blue-green algal carotenoids in sediments of Esthwaite Water. *Limnology and Oceanography*. **23:** 777-784.

Watson, S.B., Brownlee, B., Satchwill, T., Hargesheimer, E.E. (2000). Quantitative analysis of trace levels of geosmin and MIB in source and drinking water using headspace SPME. *Water research*. **34:** 2818-2828.

Watson, S.B., Ridal, J., Boyer, G.L. (2008). Taste and odour and cyanobacterial toxins: impairment, prediction, and management in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. **65:** 1779-1796.

Weckström, K. (2006). Assessing recent eutrophication in coastal waters of the Gulf of Finland (Baltic Sea) using subfossil diatoms. *Journal of Paleolimnology*. **35:** 571–592.

Wehr, J.D. and Sheath, R.G. (2003). Freshwater algae of North America—Ecology and classification. Academic Press.

Welker, M. and Steinberg, C. (2000). Rates of humic substance photosensitized degradation of microcystin-LR in natural waters. *Environmental Science and Technology*. **34:** 3415-3419.

Welker, M. and Steinberg, C. (1999). Indirect photolysis of cyanotoxins: One possible mechanism for their low persistence. *Water research*. **33:** 1159-1164.

Welker, M., Steinberg, C., Jones, G. (2001). Release and persistence of microcystins in natural waters. In: Cyanotoxins - Occurrence, Causes, Consequences (ed. Chorus I.), pp. 83-101. Springer-Verlag, Berlin.

Welschmeyer, N.A. and Lorenzen, C.J. (1985). Chlorophyll budgets: zooplankton grazing phytoplankton growth in a temperate fjord and the Central Pacific gyres. *Limnology and Oceanography*. **30:** 1–21.

Weninger, J.M. and McAndrews, J.H. (1989). Late Holocene aggradation in the lower Humber River valley, Toronto, Ontario. *Canadian Journal of Earth Sciences*. **26:** 1842-1849.

Westover, K., Fritz, S., Blyakharchuk, T., Wright, H. (2006). Diatom paleolimnological record of Holocene climatic and environmental change in the Altai Mountains, Siberia. *Journal of Paleolimnology*. **35:** 571–592.

Weyhenmeyer, G.A. (2001). Warmer winters – are planktonic algal populations in Sweden's largest lakes affected? *Ambio*. **30:** 565–571.

Weyhenmeyer, G.A. (2008). Rates of change in physical and chemical lake variables – are they comparable between large and small lakes? *Hydrobiologia*. **599:** 105–110.

Weyhenmeyer, G.A., Blenckner, T., Pettersson, K. (1999). Changes of the plankton spring outburst related to the North Atlantic Oscillation. *Limnology and Oceanography*. **44:** 1788–1792.

Wheeler Alm, E. and Stahl, D.A. (2004). In: Kowalchuk, G.A.; de Bruijn, F.J.; Head, I.M.; Akkermans, A.D.; van Elsas, J.D. (Eds.). Molecular Microbial Ecology Manual. 2nd ed.1.06: 61-88. Kluwer Academic Publishers.

Wiklund, J.A., Jonlija, M., Hall, R.I., Müller, K.M., Hiriart-Baer, V. (2014). ^{210}Pb dating of a sediment core from a highly disturbed sedimentary environment: Lessons from Hamilton Harbour, Ontario, Canada. *For Submission to: Journal of Paleolimnology*

Whitton, B.A. (1992). Diversity, ecology, and taxonomy of the cyanobacteria. In: Mann, N. H. and Carr, N.G. (eds), Photosynthetic Prokaryotes. Plenum New York: 1–51.

Whitton, B.A. and Potts, M. (2000). Introduction to the cyanobacteria. In: Whitton, B.A., and Potts, M. (eds). Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 1–11, 689 pp.

Wickstrom, M.L., Khan, S.A., Haschek, W.M., Wyman, J.F., Eriksson, J.E., Saeffer, D.J., Beasley, V.R. (1995). Alterations in microtubules, intermediate filaments, and microfilaments induced by microcystin-LR in cultured cells. *Toxicologic pathology*. **23:** 326–337.

Wilmotte, A. (1994). Molecular evolution and taxonomy of the cyanobacteria. In The Molecular Biology of Cyanobacteria, pp 1-25. Edited by D. A. Bryant. Dordrecht: Kluwer.

Wilmotte, A. (2004). Molecular evolution and taxonomy of the cyanobacteria. In The molecular biology of cyanobacteria (pp. 1-25). Springer Netherlands.

Winter, J.G., DeSellas, A.M., Fletcher, R., Heintsch, L., Morley, A., Nakamoto, L., Utsumi, K. (2011). Algal blooms in Ontario, Canada: Increases in reports since 1994. *Lake and Reservoir Management*. **27:** 107-114.

Woese, C.R. (1987). Bacterial evolution. *Microbiological reviews*. **51:** 221–227.

Wojciechowska, W., Poniewozik, M., Pasztaleniec, A. (2004). Vertical distribution of dominant cyanobacteria species in three lakes – evidence of tolerance to different turbulence and oxygen conditions. *Polish Journal of Ecology*. **52:** 347–351.

Wolfe, B., Kling, H.J., Brunskill, G.J., Wilkinson, P. (1994). Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. *Canadian Journal of Fisheries and Aquatic Sciences*. **51:** 2274–2285.

Wolfe, B.B., Edwards, T.W.D., Duthie, H.C. (2000). A 6000-year record of interaction between Hamilton Harbour and Lake Ontario: quantitative assessment of recent hydrologic disturbance using ^{13}C in lake sediment cellulose. *Aquatic Ecosystem Health and Management*. **3:** 47–54.

Wolk, P.C. (1996). Heterocyst formation. *Annual review of genetics*. **30:** 59-78.

Wright ,S.W., Jeffrey, S.W., Mantoura, R.F.C., Llewellyn, C.A., Bjørnland, T., Repeta, D., Welschmeyer, N. (1991). Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Marine Ecology Progress Series*. **77:** 183–196.

Wulff, A. (2001). Is there a coupling between increased UVB radiation and toxic algal blooms. SIL 2001 XXVIII Congress, Melbourne, Australia, abstract.

Yacobi, Y.Z., Eckert, W., Trüper, H.G., Berman, T. (1990). High performance liquid chromatography detection of phototrophic bacterial pigments in aquatic environments. *Microbial Ecology*. **19:** 127-136.

Yang, J-R., Duthie H.C., Delorme, L.D. (1993). Reconstruction of the recent environmental history of Hamilton Harbour from quantitative analysis of siliceous microfossils. *Journal of Great Lakes Research*. **19:** 55-71.

Zaccaroni, A. and Scaravelli, D. (2008). Toxicity of Fresh Water Algal Toxins to Humans and Animals. In: Evangelista V., Barsanti, L., Gualtieri, P.,Frassanito, A.M., Passarelli, V. (eds). Algal Toxins: Nature, Occurrence, Effect and Detection, 45-91. NATO Science for Peace and Security Series. Springer Netherlands.

Zhang, W., Ki, J.S., Qian, P.Y. (2008). Microbial diversity in polluted harbour sediments I: bacterial community assessment based on four clone libraries of 16S rDNA. *Estuarine, Coastal and Shelf Science.* **76:** 668-681.

Zullig, H. (1981). On the use of carotenoid stratigraphy in lake sediments for detecting past developments of phytoplankton [Equipment]. *Limnology and Oceanography*. **26**.

Zurawell, R.W., Chen, H.R., Burke, J.M., Prepas, E.E. (2005). Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews*. **8:** 1-37.

Zwart, G., Hiorns, W.D., Methe, B.A., Van Agterveld, M.P., Huismans, R., Nold, S.C., Zehr, J.P., Laanbroek, H.J. (1998). Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. *Systematic and applied microbiology*. **21:** 546-556.

Zwart, G., Kamst-van A, M.P., van der Werff-Staverman, I., Hagen, F., Hoogveld, H.L., Gons, H.J. (2005). Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake. *Environmental Microbiology*. **7:** 365–377.