SEASONAL AND LONG-TERM TRENDS IN THE DIATOM AND PHYTOPLANKTON COMMUNITY COMPOSITION OF LAKE SIMCOE, ONTARIO

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Abstract

Lake Simcoe and the Laurentian Great Lakes are among the freshwaters of the world that have collectively experienced accelerated rates of qualitative and quantitative degradation from multiple anthropogenic influences. Recent stressors include the introduction of invasive species, eutrophication and climate change. This study is two-fold: 1) a short-term study (2009-2011) monitoring the diatom relative abundance during the ice-free and ice-covered periods using sediment traps and pelagic sampling methods and 2) a long-term study (1980-2011) monitoring trends in the total phytoplankton and diatom spring blooms. Seasonal phytoplankton relative abundances were analyzed for changes in dominant species and correlated to environmental variables.

In the short term study diatom community structures of the ice-free and ice-covered sediment traps were statistically different ($p = 0.0003$) as were the ice-free pelagic and adjacent sediment traps ($p = 0.0001$): the diatom assemblages of the four sites, however, were not statistically different. Overall, the pelagic samples were dominated by *Stephanodiscus minutulus/parvus* and *Fragilaria crotonensis*. The ice-free sediment traps were dominated by *Stephanodiscus binderanus* and *Fragilaria crotonensis* and the ice-covered sediment traps were dominated by *Stephanodiscus minutulus/parvus*. Although total phosphorus (TP) was significantly related to the ice-free pelagic diatom community, this was not the case in the sediment traps. Silica (Si) concentrations were significantly 'drivers' in all sampling methodologies and chloride concentrations were also important in shaping the ice-free pelagic and ice-covered sediment communities.

In the long-term study, there were no significant changes in the total phytoplankton or diatom biovolumes in the spring between the 1980s and the 2000s. There was however, evidence of a shift from larger *Stephanodiscus* species to *Cyclotella*, and smaller *Stephanodiscus* species. There is also evidence that the spring bloom may begin under the ice. A shorter ice-covered period lengthens the ice-free season which could cause earlier spring blooms.

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List of Abbreviations

ANOSIM	Analysis of Similarities
CA	Correspondence Analysis
CCA	Canonical Correspondence Analysis
DCA	Detrended Correspondence Analysis
LSEMS	Lake Simcoe Environmental Management Strategy
LSPP	Lake Simcoe Protection Plan
LSRCA	Lake Simcoe Region Conservation Authority
OMOE	Ontario Ministry of the Environment
PCA	Principal Components Analysis
RDA	Redundancy Analysis
SIMPER	Simplicity Percentage
ALKTI	Alkalinity
Cl^-	Chloride
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
$NH_3 + NH_4^+$	Ammonia and ammonium
$NO_3^- + NO_2^-$	Nitrate and nitrite
TKN	Total Kjeldahl nitrogen
Si	Reactive silicate
TP	Total phosphorus
VWHDO	volume-weighted hypolimnetic dissolved oxygen

Chapter 1

Introduction and Literature Review

The Current State of Freshwater Ecosystems

The surface freshwaters of the world (rivers, reservoirs and lakes) have collectively experienced accelerated rates of qualitative and quantitative degradation from anthropogenic influences (Wetzel, 1992a). Humans depend on this resource for drinking, sanitation, irrigation, industry, transportation, recreation and fisheries (Carpenter et al., 2006). These precious resources are now among the most intensively altered ecosystems on Earth (Carpenter et al., 2011). Despite the necessity of water to all life, it has not been sufficiently protected as a valuable resource. Canada contains approximately 7% of the globe's renewable freshwater (Environment Canada) and perhaps such abundance has caused Canadians to disregard our degrading water resources. Canadian freshwaters have experienced many stresses including: changes in physical morphology, loss of native species, acidification, bioaccumulation of pesticides, pathogens and eutrophication (Schindler, 2001). Drivers influencing these changes are: climate change, UV radiation, land-use changes, altered hydrologic flows and water withdrawals, aquatic invasive species, chemical and nutrient inputs and over harvesting (Carpenter et al., 2011). The complex interactions of variables make predicting future changes and managing freshwater ecosystems a challenge. It has been predicted that the expanding human population and future changes in global climate will further stress our valuable lake ecosystems (Carpenter et al., 2006; Hawryshyn, 2010). Lake Simcoe is an example of an economically important lake due to its size and location, which has undergone major land use changes pressuring the lake and its watershed (Young et al., 2010).

Description of Study Area: Lake Simcoe

Lake Simcoe characteristics

Lake Simcoe is the largest lake in south central Ontario next to the Great Lakes with a surface area of 722 km^2 and a terrestrial watershed area of 2,899 km^2 (Fig. 1.2; LSRCA and MOE, 2009). It is located north of Lake Ontario and east of Georgian Bay of Lake Huron (latitude 44°25′N and longitude 79°20′W) (Evans et al., 1996; Eimers et al., 2005). It is also an important link in the Trent-Severn Waterway, which extends from Lake Ontario to Georgian Bay (Fig. 1.1).

Lake Simcoe is a shallow, dimictic, hard-water lake (Winter et al., 2007) (recent ice-free season alkalinity from 2009-2011 ranged from 113-116 mg/L $CaCO_3$). The lake is currently classified as mesotrophic with total phosphorus (TP) concentrations ranging from 11.2 to 17.5 μg/L from 2009 – 2011. The lake is composed of the main basin (mean depth of 14 m, maximum depth of 33 m), Cook's Bay (mean depth 13 m, maximum depth of 15 m) and Kempenfelt Bay (mean depth 20 m, maximum depth of 42 m) (Fig. 1.3; Young et al., 2010). It has a flushing rate of approximately 11 years and drains north through a single outflow at the Atherley Narrows into Lake Couchiching (Young et al., 2010).

Lake Simcoe as an important economic resource

Each year the lake generates approximately $200 million in revenues from its fishing, recreation and tourism industry indicating that Lake Simcoe is an economically

important natural resource (Young et al., 2010). Its watershed encompasses twenty-three municipalities and provides drinking water to eight of them. It also assimilates wastewater from fourteen water pollution control plants (LSRCA and MOE, 2009). Its location is within commuting distance of the Greater Toronto Area, which is the fifth largest metropolitan area in North America. Rapid urbanization has resulted in land use changes and a more than doubling of the population in the Lake Simcoe watershed within the last two decades making it increasingly vulnerable to anthropogenic influences (Eimers et al., 2005).

Fig. 1.1 Map of Lake Simcoe relative to Ontario and the Laurentian Great Lakes (source: Eavan O'Connor, Lake Simcoe Region Conservation Authority).

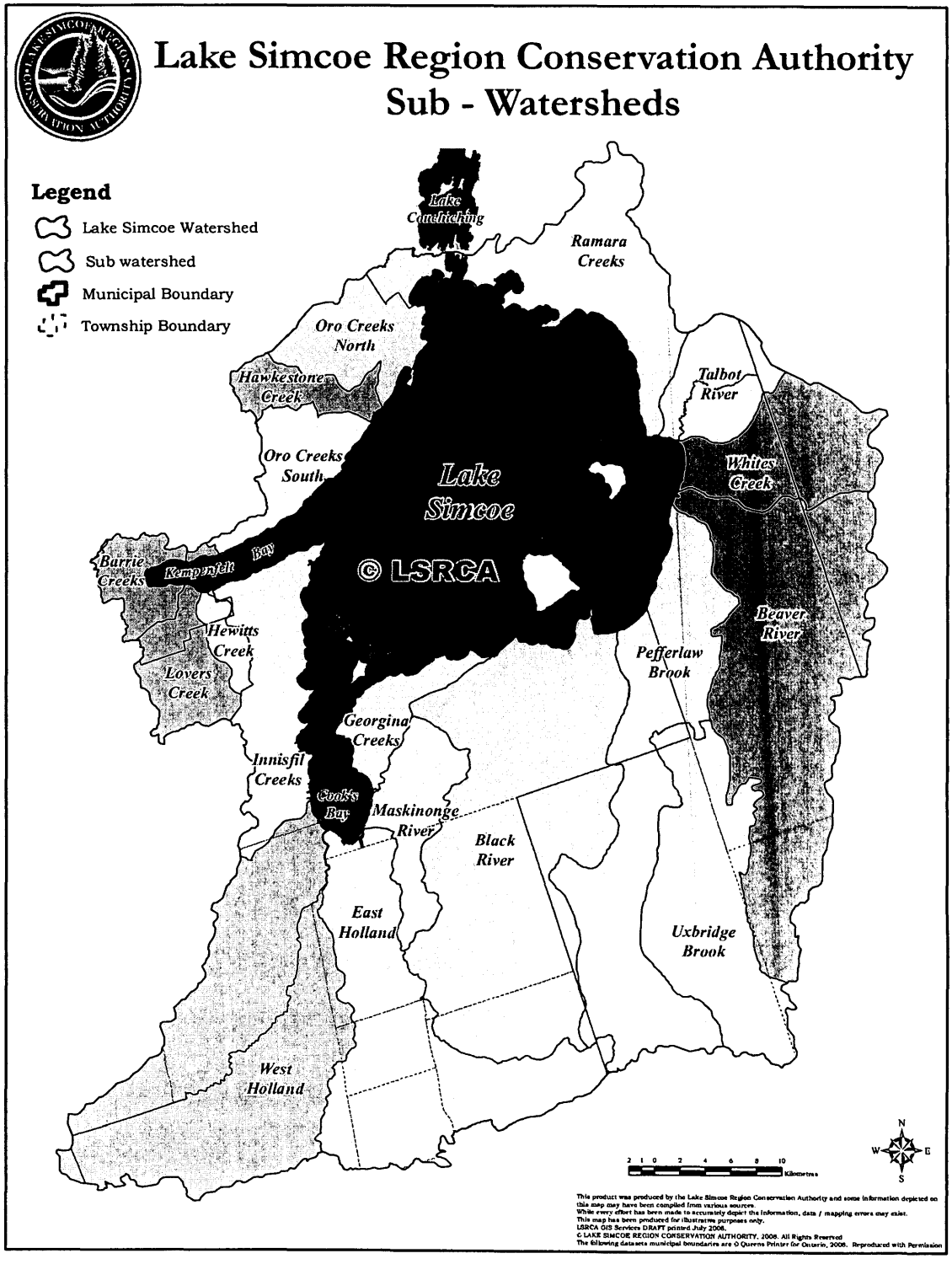

Fig. 1.2 Watershed map of Lake Simcoe including its sub watersheds (source: www.lsrca.on.ca).

Historical Review of Lake Simcoe Research

Lake Simcoe and its watershed began changing in the 1800s due to human induced land use changes such as increased agriculture and urban development (Evans et al., 1996; Eimers et al., 2005; Lake Simcoe Science Advisory Committee, 2008). Donald Rawson did the first published study of Lake Simcoe in 1926 and reported that the lake was cool, clear and alkaline with plenty of oxygen at the bottom (Rawson, 1928). However, it was mentioned in an annual report of the Ontario Department of Game and Fisheries (1907-1910) that the lake was already yielding a very small harvest of commercial fish and that game fishing was exceptionally poor. As a result of increased anthropogenic influences, water quality issues became a major concern in the 1970s (Winter et al., 2002; Young et al., 2010).

In the 1970s, information on phytoplankton of Lake Simcoe was limited. The first set of water quality data was collected during 1971-1974 (Ralston et al., 1975). Nicholls and MacCrimmon (1974) also responded with several studies and showed evidence of high nutrient loading in the lower Holland River (Holland Marsh) and Cooks Bay. The mean concentration of inorganic nitrogen in the subsurface water under the cultivated land was about 10 times higher than under uncultivated marsh during the growing season. In addition, the combined effects of fertilization and drainage, which create oxidizing and nitrifying conditions, produced run off from a cultivated plot that yielded 4 to 5 times more TP (1.56 kg P/ha) and 40 to 50 times more nitrate (4.1 kg N/ha) compared to the runoff water from an uncultivated plot (Nicholls and MaCrimmon, 1974). Also, more than 90% of the TP in runoff to Cook's Bay was in the soluble reactive form (as opposed

to only 45% from the uncultivated marsh) and was, therefore, readily available for aquatic macrophyte and phytoplankton growth (Nicholls and MacCrimmon, 1974). With such high nutrient loading in the Holland Marsh, chlorophyll *a* concentrations in Cook's Bay were as high as 358 μg/L, highly indicative of eutrophic conditions (Nicholls, 1976).

In the early 1980s, The Lake Simcoe Environmental Management Strategy was implemented to address concerns involved in the loss of the coldwater fishery (LSEMS, 1985), improve water quality, reduce phosphorus loads and protect natural heritage features and functions (LSEMS, 1992; Nicholls, 2001). Studies beginning in 1981 were initiated to measure sources of phosphorus in the watershed, identify potential point sources and recommend remedial procedures to reduce phosphorus inputs (Winter et al., 2007). For example, Nicholls and his coauthors (1985) reported that the phytoplankton community composition and biomass during 1980-1982 showed a clear gradient in trophic status among the nine sampling sites across the lake. The highest average biomass was almost 1.4 mm^3/L at site C1 near the mouth of the Holland River in Cook's Bay. Evidence of point source nutrient inputs were found at the inner Kempenfelt Bay and Cook's Bay with the largest nutrient loading occurring in the spring. There was a strong correlation ($r = 0.76$) between TP and total algal biomass in Lake Simcoe (Nicholls et al., 1985). In late 1984, two major sewage treatment sources (Aurora and Newmarket) were diverted out of the watershed in order to reduce nutrient loading (Nicholls et al., 1985).

By the 1990s, attention was focused on the declining water quality and the near extirpation of lake whitefish and lake trout (Johnson and Nicholls, 1989). Remedial strategies included point source nutrient load reductions and improved agricultural practices (Winter et al., 2007). At the same time, urban development within the watershed increased such that it was one of the fastest growing urban areas in Canada. For example, the City of Barrie's population grew by approximately 30% from 1991-2001 consequently increasing urban land cover by 200% (LSEMS, 2003). The population in the entire watershed is expected to further increase from 400,000 (2009) to 600,000 in 2031 (LSRCA, 2009). Further lake assessments increased in complexity due to impact of other stressors (Winter et al., 2011). The invasion of the zebra mussel (*Dreissena polymorpha*) in the early 1990s became widespread throughout the lake by 1995 and likely reduced phytoplankton biomass and changed the community composition (Winter et al., 2011). Since these reductions in nutrient inputs, the average TP levels were lower between 2000 and 2003 as compared to 1980-1983 (Eimers et al., 2005). TP concentrations as well as phytoplankton biovolumes varied two-fold across the lake with the highest concentrations maintained in Cook's Bay (Eimers et al., 2005). Following these reductions, excessive plant growth continued and average volume-weighted hypolimnetic dissolved oxygen (VWHDO) increased from 3.6 in 1996 to 5.6 mg/L in 2008. However, VWHDO still remains below the acceptable level of 7 mg/L which therefore continues to limit the recruitment of cold-water fish species (Young et al., 2010).

It was for these reasons that the government of Ontario developed the Lake Simcoe Protection Act and the Lake Simcoe Protection Plan (LSPP, 2008). The Lake Simcoe Protection Act received Royal Assent in December 2008, and the Lake Simcoe Protection Plan was approved in June 2009. The Lake Simcoe Protection Plan's objectives are to protect, improve and restore the elements that contribute to the ecological health of the Lake Simcoe watershed, including water quality, hydrology, key natural heritage features and their functions. More specifically, the objectives are to restore a self-sustaining cold water fish community, to reduce inputs of TP and other nutrients of concern and pollutants; to respond to adverse effects related to invasive species and to prevent invasive species from entering the Lake Simcoe watershed. The plan also states important future goals are to promote environmental sustainability for land and water uses, to improve the watershed's capacity to adapt to climate change; and to provide for ongoing scientific research and monitoring related to the ecological health of the Lake Simcoe watershed. The federal government also recently invested $30 million in a five year Lake Simcoe Clean-Up Fund (2007-2011). The purpose of this initiative was to implement high impact projects such as rehabilitation of habitats to reduce TP inputs, restore the cold-water fishery and also to enhance research and monitoring essential for the restoration of the lake and its watershed.

Current Stressors of Lake Simcoe

Lake Simcoe has been identified as a multiple stressor system (Hawryshyn et al., 2012). A report by the Lake Simcoe Science Advisory Committee described eight lake stressors affecting the ecological health of the lake and its watershed including pollutants

such as pharmaceuticals and other organics; pesticides and metals; pathogens; excessive water withdrawal; land use changes; nutrient enrichment; climate change and invasive species (Lake Simcoe Science Advisory Committee, 2008). Although some of the contaminants come from atmospheric deposition, the terrestrial watershed contains important point and non-point sources. These stressors do not necessarily act independently and can interact with each other, and can affect the lake and its watershed in unexpected ways (Hawryshyn, 2010).

Nutrients

Phosphorus is the most common limiting nutrient for biotic production in freshwater lakes including Lake Simcoe (Eimers et al., 2005; Young et al., 2010). Loading of P promoted the growth of aquatic macrophytes and phytoplankton and their decomposition has led to reduced concentrations of deep-water dissolved oxygen. This oxygen deficit is a contributing factor to the decline of deep-water fish populations such as lake trout and lake white fish (Eimers et al., 2005). Urban, rural and agricultural uses of P provided the majority of TP loading to the lake (Lake Simcoe Science Advisory Committee, 2008). Across the lake, concentrations of TP have significantly decreased, occurring mainly in the 1990s. Prior to human settlement, P loads were estimated at 32 tonnes/year (Johnson and Nicholls, 1989; Nicholls, 1997). The estimated mean load from 2002-2007 was 72 tonnes/year as compared to more than 100 tonnes/year in the 1980s and early 1990s (LSRCA and MOE, 2009; Winter et al., 2010). Recent concentrations have remained fairly constant or have slightly increased at some stations (Young et al.,

2010). Spring TP concentrations have also decreased over the past 30 years, however, slight increases have occurred recently (Young et al., 2010).

Phosphate, total ammonia, nitrate and nitrite are soluble and more biologically available forms of nutrients. Phosphate has significantly decreased over the past 40 years due to restrictions imposed on phosphates in detergents, the diversion of sewage out of the watershed and improvements to waste water treatment plants. Total ammonia also decreased and is currently lower than in 1980 although occasionally concentrations rise above Provincial Water Quality Objectives (PWQO). Nitrate and nitrite recently have slightly increased, however, large interannual variation existed (Young et al., 2010).

In addition to nutrient loading, increases in conductivity, sodium and chloride concentrations have occurred in the last 30 years. Chloride concentrations are also increasing at a faster rate than in the past. This is evidence of the cumulative effects of road salt application and increased impervious surfaces within the watershed on water column chemistry (Young et al., 2010).

Invasive species

Lake Simcoe has experienced many introductions of non-indigenous species. The impacts of these species are often unpredictable and can be devastating involving the loss of native species and changes in nutrient and energy cycles (Lake Simcoe Science Advisory Committee, 2008). Examples of invasive species include fish species (common carp, rainbow smelt, bluegill, black crappie, round goby), zooplankton (spiny waterflea), crayfish (rusty crayfish) and aquatic macrophytes (curly pond weed and Eurasian

watermilfoil). One of the most recent invasive species is the zebra mussel (*Dreissena polymorpha*), which had become established in Lake Simcoe by 1994 (Evans et al., 2001). Because of their widespread establishment throughout the lake, zebra mussels may have contributed to increased water clarity, reduced algal biovolumes and decreased alkalinity and calcium concentrations (Young et al., 2010).

Diatoms: Bio Indicators of Water Quality

Phytoplankton form the base of the food chain and play an essential ecological role in energy transfer to higher trophic levels. They are short lived (i.e. short generation time) and thus can respond rapidly to changing environmental conditions (Kilham et al., 1996). Because of this, analyses of their community composition over time provide information on the crucial link between physical, chemical and climatic variables and their biological and ecological impacts. They are indicators of the ecological health of the lake (Kirilova et al., 2008) and as early responders to change, they are useful in predicting future implications to subsequent trophic levels such as zooplankton and fish (Kerfoot et al., 2010). This is why phytoplankton monitoring is a vital component of environmental assessment (Dillon and Rigler, 1974; Nicholls et al., 1985; Woodbridge and Roberts, 2010).

Diatoms are a group of aquatic, photosynthetic, unicellular algae of the division Bacillariophyceae (Wehr and Sheath, 2003). They are abundant in most aquatic habitats (marine and freshwater) throughout the world and are significant contributors to carbon fixation (Round et al., 2007; Smetacek, 1999; Reynolds, 2006). Diatoms are preferred

high quality food for primary consumers and are a biological mechanism for carbon export from the surface to deep waters thereby playing a crucial role in nutrient cycling (Brett and Muller-Navarra, 1997; Treguer, 2002).

A diatom is enclosed in a unique cell wall called a frustule composed mainly of silica (Round et al., 2007). Consequently, the cell wall is rigid, preserves in sediments and experiences a unique form of reproduction, which involves successive reduction in the mean size of the daughter cells (Battarbee et al., 2001). The cell wall consists of two valves; the epitheca, the newer and larger valve, and the hypotheca, which is overlapped by the epitheca. These two thecae are linked together by girdle bands and a thick layer of organic material, which coats the entire structure. The frustule can have one of two forms, which correspond to two evolutionary lineages given by the orders; centrales (radially symmetrical) or pennales (bilaterally symmetrical) (Wehr and Sheath, 2003; Round et al., 2007). Exchange of materials between the cell and its aqueous environment occurs through pores and slits in the frustule. The morphology (i.e. size, shape, organization of puncta/areolae, and presence or absence of raphes or spines) of the frustule is useful in microscopic taxonomic identification (Round et al., 2007).

Diatom Seasonality

The water column is a dynamic system and consequently, phytoplankton species composition is variable (Harris, 1983). This means that depending on the combined physical, chemical and biological conditions of the aqueous environment, the relative abundance (cell numbers) or biovolume of each algal species is affected and, hence,

community composition varies with time (Lund, 1965). Factors such as light, air and water temperature, nutrients (e.g., P, nitrogen, silica), pH, alkalinity, predation (Lund, 1965), water column stability, stratification (Winter et al., 2011), maximum water depth, precipitation and oxygen saturation (Kirilova et al., 2008) influence the dominance and absence of algal species (Tilman, 1976). The direct and indirect effects of changes in these environmental and climatic variables make for complex interactions between variables and algal species such as diatoms. For example, a study done by Tilman (1976) showed that coexistence of two freshwater diatom species (*Asterionella formosa* and *Cyclotella meneghiniana*) was possible when the growth rate of each species was limited by a different nutrient (SiO_2 and PO_4 respectively). Reynolds (1980) found that phytoplankton groups exhibit patterns of seasonal succession that are both general and predictable.

Diatoms generally dominate the phytoplankton in the spring (often referred to as a spring bloom, even in unproductive lakes) and again in the fall when the water column is mixing and when nutrient availability is high (Reynolds, 1980; Nicholls, 1976; Blomqvist et al., 1994). Since diatoms possess heavy, silica frustules and are not able to swim through the water column, they depend on the mixing of the water column to maintain their position in the photic zone (Richardson et al., 1983). Consequently, when the water column becomes stable or stratifies (as in the summer or winter), dinoflagellates, which are motile, and cyanobacteria which regulate their buoyancy with gas-vacuoles tend to outcompete the diatoms, as they are able to vertically migrate within the water column to optimize their use of light and nutrients (Richardson et al., 1983). Margalef (1978)

determined preferred degrees of turbulence that were quite different at 2-100 cm^2s^{-1} for diatoms and 0.02-1 cm^2s^{-1} for dinoflagellates. As a result of their inability to travel independently throughout the water column, diatoms are able to tolerate a wider range of light conditions than many other algae because diatoms are continually transported through a range of light conditions (Richardson et al., 1983). Diatoms also have high silica requirements due to the composition of their frustules (Wetzel, 2001). Consequently, their growth can be limited during periods of stratification as in the summer and winter seasons when the water column has a low dissolved silica (SiO_2) concentration (Reynolds, 1980). Silica concentrations are usually higher in the spring and/or fall when a mixing water column brings up available silica from the lake bottom. Turbulent conditions combined with high silica concentrations help explain the seasonality of diatoms. Such generalizations are not always accurate, as smaller diatoms inhabit stratified lakes as well as turbulent lakes, presumably because smaller diatoms are more easily suspended than larger diatoms. However, the generalizations of preferred habitat are widely accepted (Fogg, 1991).

Rationale for Study

A recent paper published on Lake Simcoe phytoplankton (Winter et al., 2011) assessed the long-term trends in average, annual ice-free phytoplankton biomass and community composition from 1980 to 2007. Since the 1980's, the total algal biovolume and diatom abundance have decreased significantly in Cook's Bay and the main basin. These changes corresponded to point source TP load reductions and the invasion of the zebra mussel (*Dreissena polymorpha*) in the mid-1990s. TP load reductions resulted

from improved sewage treatment, the diversion of sewage effluent out of the watershed from Newmarket and Aurora and various actions to reduce non-point source loads. Interestingly, the total algal biovolume increased after 2004 despite the fact that dreissenid mussels were still widespread in the lake (Ozersky et. al., 2010). This increase may have been related to a slight increase in phosphorus load (Winter et al., 2011). It has also been suggested that the abundance of dreissenid mussels in the lake has decreased from levels observed during the 1990s (David Barton, University of Western Ontario, personal communication), which may have reduced their effect on phytoplankton abundance. There was also a significant change in the community composition of pelagic diatoms from 1980 to 2008. There was a decrease in the abundance of *Stephanodiscus* spp. and an increase in *Fragilaria* spp. This sustained shift coincided with decreased nutrient loading, increased water clarity, increased silica concentration, increased water column stability and the invasion of the lake by zebra mussels (Winter et al., 2011).

There has been a considerable amount of work published on the seasonality of phytoplankton in many systems, however, to date there has been no work published on trends in the seasonality of diatoms of Lake Simcoe. A study of benthic diatoms in four Lake Erie estuaries using canonical correspondence analysis (CCA) suggested that seasonal changes in temperature and/or flow, nutrients, pH and alkalinity were the most influential factors in the variability of diatom species composition temporally and spatially (Sgro et al., 2006). It has been suggested by Winter et al. (2011) that a detailed, seasonal analysis of the diatom species community composition would help explain changes in annual diatom peaks through time.

Phytoplankton sampling for Lake Simcoe began in 1971, however, several different methods of sample collection were used. Lake water quality monitoring began in 1980, and since then, the same methods for sampling phytoplankton during the ice-free season have been used. Overtime the number of sampling sites/ stations has increased to include as many as twelve sites, however, only the pelagic phytoplankton during the ice-free seasons were sampled. Historically these samples have been pooled to form a composite "yearly" sample. This raises questions regarding the most suitable method for capturing a realistic view of the lake phytoplankton ecology during the whole year. Although the ice-covered winter season poses difficulties in sampling, sediment trap sampling could be a useful alternative providing insight to settling overwintering species and capturing a more holistic view of the lake primary productivity. Perhaps the presence of overwintering species caught in the sediment traps will help explain the reason for later summer hypolimnetic dissolved oxygen depletion.

The presence and relative size of phytoplankton blooms greatly impact the ecosystems water quality for the successive season. Since the 1970s, eutrophication causing excessive algal growth was of particular concern for Lake Simcoe and strict recommendations were made to reduce TP loading at point sources (Nicholls et al., 1985). Since such efforts to reduce TP concentrations and the invasion of the dreissenid mussels in the mid-1990s, algal biomass has been reported to have decreased, notably in Cook's Bay, site E51 and K42 (Eimers et al., 2005). A similar trend pre and post-*Dreissena* was observed in the Laurentian Great Lakes including Lake Erie (Nicholls and Hopkins, 1993). Species level shifts including a decrease in diatom production were reported for

Lake Erie (Barbiero et al., 2006). Recent literature was published on the disappearance of the winter bloom (Kerfoot et al., 2010) and the loss of the spring bloom in Lake Michigan (Fahnenstiel et al., 2010; Vanderploeg et al., 2010). Such questions concerning the relative changes in spring bloom size over time for Lake Simcoe have not been addressed.

Research Objectives and Questions

Chapter 2 presents spatial and seasonal analyses of diatom relative abundances in Lake Simcoe at four sites (K42, C9, S15 and N31/E51) from 2009 – 2011 (objectives 1-4). Chapter 3 presents a temporal analysis of the long-term spring changes in phytoplankton and diatom community composition from 1980 – 2011 at station K42 for Lake Simcoe (objectives 5-8).

Chapter 2

1. What were the short-term seasonal trends in diatom species composition between the ice-covered seasons and ice-free seasons at stations N31, K42, C9 and S15 from 2009 – 2011 as determined by diatoms collected in sediment traps? Were the diatom assemblages for the ice-covered seasons significantly different from the ice-free seasons during 2009 – 2011? If so, what species were responsible for these differences? Were the diatom assemblages for the ice-covered seasons significantly different between years 2009 – 2010? Were the diatom assemblages for the ice-free seasons significantly different between years 2010 – 2011? If so, what species were responsible for these differences?

2. Was Lake Simcoe phytoplankton composition spatially homogeneous at the four sampling sites (K42, C9, S15 and N31/E51) within a season during 2009 – 2011? Were there significant differences in diatom composition between sites during the ice-free and ice-covered seasons using diatoms collected in sediment traps? Were there significant differences in diatom composition between sites in the ice-free pooled pelagic samples? Were there significant differences between the physic-chemical variables at the four sample sites?

3. Were there differences between the ice-free diatom species composition of the pelagic zone (at sites K42, C9, S15 and E51) and their adjacent sediment traps (at sites K42, C9, S15 and N31) in 2010 and 2011? If so, what species were responsible for these differences?

4. Which chemical and physical variables were correlated with the taxa in each group of samples: 1) the ice-free pooled pelagic samples (at sites K42, C9, S15 and E51) and 2) the ice-free sediment trap samples (at sites K42, C9, S15 and N31) in 2010 and 2011?

Chapter 3

5. How did the total phytoplankton biovolumes compare to the diatom biovolumes over time (1980-2011)? Did the changes in biovolumes correlate to the physical and chemical variables of the water column?

6. How has the amount of phytoplankton (biovolume) changed at station K42 over time (1980-2011)?

7. Has there been a shift in the dominant taxa of phytoplankton (relative biovolume) at station K42 over time (1980-2011)?

8. Which physical and chemical variables correlated with spring phytoplankton and diatom community composition?

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Chapter 2

Comparison of Ice-free and Ice-covered Sediment Trap Diatom Communities at Four Stations in Lake Simcoe, Ontario 2009-2011

Abstract

Monitoring phytoplankton is critical to understanding and assessing changes in water quality of Lake Simcoe and is an integral part of the Ontario government's Lake Simcoe Protection Plan (2009). Sediment trap and pelagic sampling techniques were used to assess spatial and temporal trends in the diatom communities at four stations during the ice-covered and ice-free seasons (2009-2011). The relative species abundances of the ice-free and ice-covered sediment traps were statistically different. The diatom assemblages of the ice-free pelagic and adjacent sediment traps were also statistically different. However, the diatom assemblages of the four sites were not statistically different from each other irrespective of the sampling method. Overall, the pelagic samples were dominated by *Stephanodiscus minutulus/parvus* and *Fragilaria crotonensis*. The ice-free sediment traps were dominated by *Stephanodiscus binderanus* and *Fragilaria crotonensis* and the ice-covered sediment traps were dominated by *Stephanodiscus minutulus/parvus*. The community structures of each sampling method and season were then related to environmental variables. Although TP was a significant variable in the ice-free pelagic diatom community, it was not the case in the sediment traps. Si concentrations were drivers in all sampling methodologies and chloride concentrations were also important in shaping the ice-free pelagic and ice-covered sediment communities.

Introduction

Like so many freshwaters, Lake Simcoe has been subjected to anthropogenic pressures causing concerns over water quality and aquatic biota. Such pressures include excessive total phosphorus (TP) loading, introductions of non-native species and climate change (Mills et al., 2003; Lake Simcoe Science Advisory Committee, 2008). A three-fold increase in phosphorus loading to the lake (Evans et al., 1996) led to eutrophication marked by excessive growth of macrophytes and algae, decreased oxygen availability in the water column and recruitment failure of cold water fish (lake trout, lake whitefish and lake herring) (Winter et al., 2011).

In response, phytoplankton monitoring became important in tracking the response of the lake to decreased TP loading (Nichols et al., 1985; Winter et al., 2007). A recent study of Lake Simcoe focused on annual changes in the average, ice-free biovolume of the phytoplankton community from 1980-2007 in relation to TP reductions, nutrient levels, timing of the zebra mussel invasion and stability of the water column (Winter et al., 2011). Since the 1990s, the total algal biovolume and diatom abundance decreased significantly in Cook's Bay and the main basin. These changes corresponded to point source TP load reductions and the invasion of the zebra mussel (*Dreissena polymorpha*) in the mid-1990s which in turn corresponded to increased transparency of the water column, increased silica (Si) concentration and increased water column stability (Winter et al., 2011). TP load reductions resulted from improved sewage treatment, the diversion of sewage effluent out of the watershed from the towns of Newmarket and Aurora and various other actions to reduce non-point source loads. Interestingly, the total algal

biovolume increased again after 2004 although it has been suggested that the abundance of dreissenid mussels in recent years may have decreased from levels observed during the late 1990s (David Barton, University of Western Ontario, personal communication), which may have reduced their effect on phytoplankton abundance. There was also a significant change in the community composition of pelagic diatoms from 1980 to 2008 with a decrease in the abundance of *Stephanodiscus* spp. and an increase *Fragilaria* spp.

A paleolimnological analysis of sediment diatoms in Lake Simcoe found modest changes in the diatom community coinciding with clearing of the watershed for agriculture in the late 1800s and early 1900s and also found that community changes accelerated starting in the 1930s with increased eutrophication and later with climate change and invasion of zebra mussels (Hawryshyn et al., 2012). The authors argued that it will be challenging to assign ('disentangle') causality to community changes in a complex, multiple-stressor environment and that other approaches would be necessary. Hawryshyn et al. (2012) suggested that plankton studies using sediment traps combined with seasonal-resolution paleolimnological research could be useful to elucidate the seasonal dynamics of diatoms and other algae and disentangle causality. Sediment traps can provide autecological information integrated over short periods of time on diatom seasonality allowing for reliable interpretations of changes in these communities (Battarbee et al., 2005) especially when assemblages are identified to the species level.

This study examines short-term seasonal trends in diatom species composition in sediment traps deployed during ice-free and ice-covered periods as well as ice-free

pelagic samples from four lake stations. Interannual comparisons for each of the seasons were also examined. Specifically this study asked:

1) What were the short-term seasonal trends in diatom species composition between the ice-covered seasons and ice-free seasons from 2009 – 2011 as determined by diatoms collected in sediment traps? Were the diatom assemblages for the ice-covered seasons significantly different from the ice-free seasons (2009 – 2011)? If so, what species were responsible for these changes?

2) Was Lake Simcoe phytoplankton composition spatially homogeneous among the four sampling sites (K42, C9, S15 and N31/E51) within a season during 2009 – 2011? Were there significant differences in diatom composition between sites during the ice-free and ice-covered seasons using diatoms collected in sediment traps? Were there significant differences in diatom composition between sites in the ice-free pooled pelagic samples? Were there significant differences between the physico-chemical variables at the four sample sites?

3) Were there differences between the ice-free diatom species composition of the pelagic zone and their adjacent sediment traps in 2010 to 2011? If so, what species were most responsible for these changes?

4) Which physico-chemical variables were correlated with the taxa in each group of samples: 1) the ice-free pooled pelagic samples and 2) the ice-free sediment trap samples in 2010 and 2011?

Description of Study Area: Lake Simcoe

Lake Simcoe is the largest lake in south central Ontario next to the Great Lakes with a surface area of 722 km^2 and a terrestrial watershed area of 2,899 km^2 (LSRCA and MOE, 2009). It is located north of Lake Ontario and east of Georgian Bay of Lake Huron (latitude 44°25'N and longitude 79°20'W)(Evans et al., 1996; Eimers et al., 2005). It is also an important link in the Trent-Severn Waterway, which extends from Lake Ontario to Georgian Bay.

Lake Simcoe is a shallow, dimictic, hard water lake (Winter et al., 2007) (recent ice-free season alkalinity from 2009-2011 ranged from 113-116 mg/L $CaCO_3$). The lake is currently classified as mesotrophic with TP concentrations ranging from 11.18 to 17. 47 ug/L from 2009 – 2011. The lake is composed of the main basin (mean depth of 14 m, maximum depth of 33 m), Cook's Bay (mean depth 13 m, maximum depth of 15 m) and Kempenfelt Bay (mean depth 20 m, maximum depth of 42 m) (Young et al., 2010; Fig. 2.1). It has a flushing rate of approximately 11 years and drains north through a single outflow at the Atherley Narrows into Lake Couchiching (LSRCA and MOE, 2009).

Methods

The Ontario Ministry of the Environment began routinely sampling Lake Simcoe for water quality beginning in 1980 (Eimers et al., 2005). Four sites were chosen to represent the lake as a whole: K42, C9, S15 and N31/E51 (Fig. 2.1). Sediment traps were deployed at the north end of the lake around 1 km from site N31. Pelagic phytoplankton collected from E51 were compared with the sediment trap data in the analyses.

Sediment trap sampling periods and sampling sites

Duplicate sediment trap samples were taken from Lake Simcoe at four sites (K42, C9, S15 and N31) (Fig. 2.1). Traps were deployed during ice-covered and ice-free seasons during the following dates: December 9, 2009 – May 27, 2010 and November 24, 2010 – May 2, 2011 (ice-covered) and July 19/20, 2010 – November 23/24, 2010 and May 2, 2011 – November14/15, 2012 (ice-free) (Table 2.1). Site N31 was later added after the first set of sediment traps were deployed and therefore data from this site are absent from the ice-covered period of December 9, 2009 to May 27, 2010. Site N31 sediment traps were deployed about 1 km from station N31 to ensure that the traps were deployed in water greater than 15 m deep.

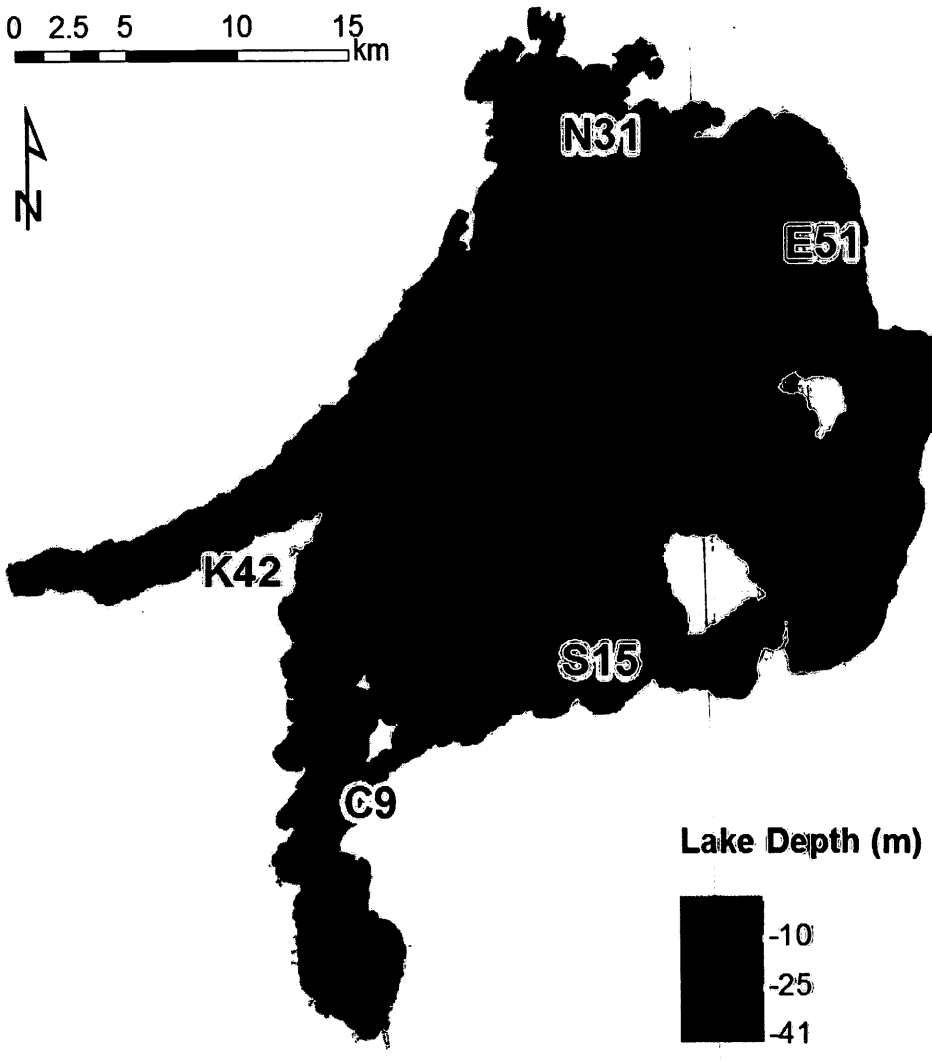

Fig. 2.1 Bathymetry map of Lake Simcoe showing the site locations of the sediment trap sample sites (K42, C9, S15 and N31) and the pelagic sample sites (K42, C9, S15 and E51) (Eavan O'Connor, Lake Simcoe Region Conservation Authority). The map data may have been compiled from various sources. While every effort has been made to accurately depict the information, data/mapping errors may exist. The map has been produced for illustrative purposes only.

Table 2.1 Summary of Lake Simcoe sediment trap deployments. Site N31 was later added with the second set of samples (July 19, 2010- November 23, 2010). Two replicates were taken at each site.

Date	Site	Site Depth (m)	Trap Depth (m)	Latitude (N)	Longitude (W)
09/12/2009-	S15	20.0	17.5	44° 21' 51.4"	79° 23' 18.9"
27/05/2010		19.7	17.2	44° 21' 50.1"	79° 23' 12.8"
	C9	18.8	16.3	44° 17' 34.6"	79° 30' 00.1"
		18.9	16.4	44° 17' 38.7"	79° 30' 02.1"
	K42	38.8	n/a	44° 23' 53.4"	79° 34' 16.4"
		38.8	n/a	44° 23' 56.6"	79° 34' 11.2"
19/07/2010-	N31	16.3	13.3	44° 32' 14.3"	79° 22' 46.4"
23/11/2010		16.4	13.4	44° 32' 10.5"	79° 22' 51.4"
	S15	18.3	15.3	44° 21' 35.8"	79° 22' 55.2"
		18.4	15.4	44° 21' 31.8"	79° 22' 49.9"
	C9	18.6	15.6	44° 17' 20.9"	79° 29' 59.2"
		18.6	15.6	44° 17' 18.7"	79° 29' 53.5"
	K42	40.1	37.1	44° 23' 52.1"	79° 34' 33.5"
		39.9	36.9	44° 23' 58.4"	79° 34' 32.7"
24/11/10-	N31	16.0	13.0	44° 32' 14.3"	79° 22' 47.0"
02/05/2011		16.1	13.1	44° 32' 10.8"	79° 22' 50.3"
	S15	18.7	15.7	44° 21' 33.8"	79° 22' 55.2"
		18.7	15.7	44° 21' 32.0"	79° 22' 49.0"
	C9	18.7	15.7	44° 17' 21.8"	79° 30' 00.5"
		18.8	15.8	44° 17' 21.4"	79° 29' 51.3"
	K42	39.5	36.5	44° 23' 58.7"	79° 34' 31.9"
		39.6	36.6	44° 23' 51.8"	79° 34' 33.1"
02/05/2011-	N31	16.6	13.6	44° 32' 10.6"	79° 23' 01.7"
14/11/2011		16.6	13.6	44° 32' 10.6"	79° 22' 51.0"
	S15	20.5	17.5	44° 21' 48.7"	79° 23' 18.4"
		20.5	17.5	44° 21' 50.9"	79° 23' 12.2"
	C9	19.4	16.4	44° 17' 33.9"	79° 30' 01.7"
		19.6	16.6	44° 17' 36.5"	79° 29' 55.8"
	K42	41.0	38.0	44° 23' 53.3"	79° 34' 17.4"
		40.9	37.9	44° 23' 55.6"	79° 34' 07.8"

Sediment traps

Each sediment trap consisted of four beakers (A-D) held together in a metal stand extending upward supporting 4 transparent, plastic cylinders (each about 1.5 m in length) (Fig. 2.2). A grate was placed over the top of the trap to prevent fish and other large organisms from entering the trap. The sediment trap was kept vertical using a submerged sediment trap float (50 lb. buoyancy) on a line attached to the trap. The trap was secured 1 m from to the lake bottom with 120 lb. steel weights. Attached to the weights was a 5/8" polypropylene "drag line" connected to 180 lb. steel weight. A surface buoy with a light and radar reflector attached to the 180 lb. weights was used to mark the sediment trap location and attached using 3/16" stainless steel aircraft cable (Fig. 2.3).

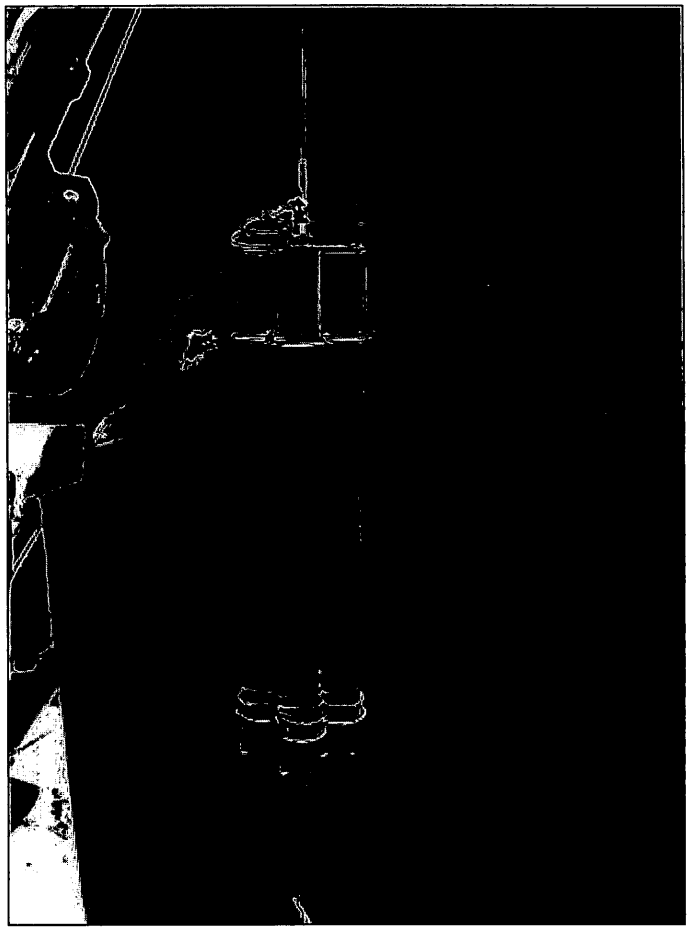

Fig. 2.2 Photo of sediment trap used for collection of seasonally sedimented diatoms. Courtesy of Mike Mueller (Ontario Ministry of Environment).

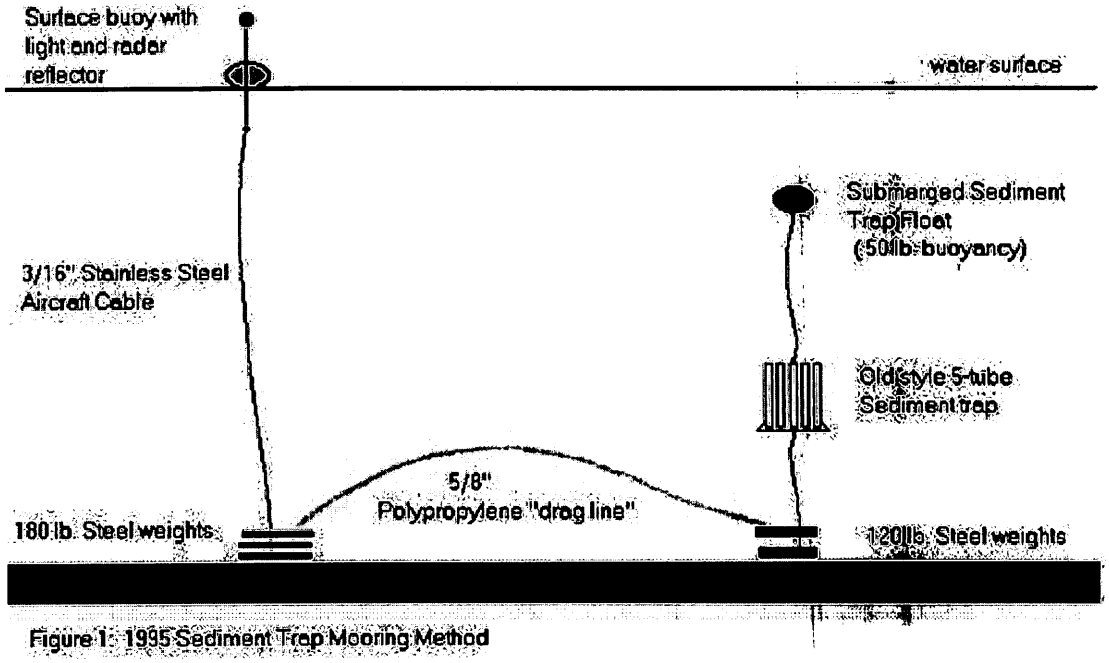

Fig. 2.3 Diagram of sediment trap set up for collection of seasonally sedimented diatoms. Courtesy of Wendy Page (Ontario Ministry of the Environment).

Sediment trap sample digestion and slide preparation

Following collection, the samples were digested in order to remove the organic matter for optimum viewing of the diatom frustules. Approximately 0.5 g wet weight of sediment was measured into 20 mL disposable scintillation vials and 15 mL H_2O_2 (30%) was added to each vial. The vials were then placed in a hot water bath (54.4 – 65.6°C) and stirred until all organic matter had been mineralized, then left to settle out for 24 hours. The supernatant was then manually aspirated off and 15 mL of distilled water was added to each vial. The diatoms were further purified by removing the supernatant and refilling the vials with distilled water an additional five times. Each time, the samples were left for 24 hours to settle between each removal of supernatant (Battarbee et al., 2001).

Using a glass pipette, 1 mL of suspended diatoms was added to 9 mL of distilled water in a test tube. A series of serial dilutions was then carried out producing four different dilutions (A-D). The solutions were plated onto pre-cleaned glass coverslips (22x22 mm, #2). The coverslip samples were then placed in a fume hood at room temperature for 24 hours in order for the water to evaporate and the diatoms to settle onto the coverslips. The coverslips were mounted onto glass slides (length x width, 7.62 x 2.54 cm and thickness, 0.96 to 1.06 mm) by first placing the glass coverslips on a heated hot plate (approximately 170°C). One to two drops of prepared Naphrax© and toluene solution were added to each slide and the coverslips were inverted and placed on the slides. The Naphrax© solution is used due to its high refractive index of 1.73. In this case, two glass coverslips were mounted on each slide. The slides were then placed onto

the hot plate to boil off the toluene and remove the bubbles and then left to dry for at least 24 hours. The slides were checked to ensure that the concentration of diatoms for viewing was appropriate (three to four diatoms per field of view was ideal) (Battarbee et al. 2001).

Pooled pelagic diatom samples

All sites were sampled by the Ontario Ministry of the Environment (OMOE) every two weeks during the ice-free season (usually April through November) as follows: April 10, 2009 – November 4, 2009, April 20, 2010 – November 14, 2010 and April 27, 2011 – November 25/26 2011. Composite vertical samples were taken through the euphotic zone (defined as 2.5 times the Secchi disc depth) to a maximum depth of 15 m using a polyvinyl chloride (PVC) hose (Winter et al., 2011). Pelagic diatom samples were fixed and preserved by the OMOE using Lugol's iodine solution and two drops of 37% formalin after concentration to 25 mL. The samples from all of the ice-free sampling periods were then combined to create one ice-free pooled samples for each of the four sites (K42, C9, S15 and E51). There were some difficulties in identifying some of the archived diatoms because of degradation. Some of the features of the diatom frustules had been digested making identification to the species level impossible. Many of the species were identified as "centrics unknown". Archived samples were digested similarly to the sediment samples. Pelagic phytoplankton collected from E51 were compared with the sediment trap data collected at N31.

Sediment and pelagic diatom identification

The diatom slides were counted and identified to species (or the lowest taxonomic level possible) using a Nikon eclipse 80i inverted microscope at 1000x magnification under oil immersion (nD = 1.515 at 23°C). In order to ensure that a representative proportion of the coverslip was observed, diatom identification began along three or four continuous transects. A Nikon DS-Fi1 digital camera along with NIS-Elements D3.1 Imaging Software was used to photograph, and save diatom sample images. The images were used to ensure that the identification of diatom species remained accurate and consistent throughout the project.

Diatom frustules were counted if at least 50% of the frustule was present. Also, *Asterionella* was counted only if the larger end was present; only the middle section of *Tabellaria* was counted; and centric diatoms such as *Cymbella*, *Navicula*, *Amphora* and *Achnanthes* were counted only if the central nodule was present. The number of chrysophyte cysts per sample was also recorded. All samples were archived for future use.

For the sediment trap samples, up to 500 diatoms were counted in the first samples in order to determine the minimum number of diatom valves to count. It was determined that after 300 diatom valves were counted per sample, changes in relative abundances were small. The relative species abundance of major species continued to decrease slightly (2-3%) between 300 and 500 total diatoms counted, probably because more rare species were being counted as the total count grew. Based on a rarefaction curve, 300

cells were deemed appropriate, as the focus of the study was on general trends and not rare species. Duplicate samples were taken at each site and therefore at least 600 diatoms were counted per site per season (ice-free, ice-covered seasons).

For the pooled pelagic samples, approximately 300 individual diatom valves were also counted. These samples were compared to the sediment trap samples and hence the same number of frustules was counted; however, duplicate samples were not counted.

Water chemistry variables

Integrated water samples (2.5 x Secchi depth) were collected at the same time as the ice-free pelagic phytoplankton samples using methods outlined by Ingram and Young (2010). The samples were analyzed using standard Ontario Ministry of the Environment methods (Janhurst, 1995; 1996; 1998). The variables included: Gran alkalinity (ALKTI), chloride (Cl^-), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), ammonia and ammonium (NH_4), nitrate and nitrite (NO_3), total Kjeldahl nitrogen (TKN), pH, reactive silicate (Si) and total phosphorus (TP). Secchi disc depth, which was measured by lowering an 8-inch diameter disc with alternating black and white quadrats into the water until it is no longer visible, was also measured for each of the samples. TP measurements represented an average of two replicate samples.

Statistical analyses – diatom data

Ordinations were performed on the diatom species abundance data using CANOCO version 4.5 for Windows (terBraak and Šmilauer, 2002). A detrended correspondence

analysis (DCA) was performed in order to determine the gradient length (beta diversity). The beta diversity was relatively low (gradient lengths were approximately 2) indicating that the species had a linear distribution along gradients. Ordination techniques such as principal components analysis (PCA) perform best at low beta diversity. PCA was performed on the relative species abundances of sediment trap data and the pooled pelagic data to summarize the general trends of variation (Davis, 1986; Harper, 1999). Relative species abundances were calculated as a proportion of the total species abundance in order to directly compare between methodologies. Species abundances were divided by the standard deviation and the square root taken to remove large effects created by dominant species. Rare species (those with abundances of less than 5%) were downweighted because rare species have an inordinate influence on multivariate analyes (ter Braak and Šmilauer, 2002). For all of the ordinations, two axes accounted for the majority of the variation.

To test if various groups of diatom samples had statistically different species compositions, a one-way analysis of similarities (ANOSIM) (Clark, 1993) was performed on the relative abundances of the following groups: 1) ice-free versus ice-covered sediment trap assemblages, 2) ice-free pooled pelagic versus sediment trap assemblages, and 3) the four sites (K42, C9, S15 and N31/E51). To determine which species were primarily responsible for the differences seen between the groups using the ANOSIM test, a similarity percentage test (SIMPER) was performed (Clark, 1993). The SIMPER test identifies which species account for a large percentage of the differences seen

between groups. ANOSIM and SIMPER non-parametric tests were performed using PAST version 2.15 for Windows.

Statistical analyses – water chemistry

The environmental variables were tested for normality using the Shapiro-Wilk test. Variables that did not approximate the normal distribution ($p>0.05$) were transformed using $\log_{10}$, ln, square rooted, squared or inverted as appropriate. The correlation coefficients and variance inflation factors (VIF's) quantified collinearity among variables. All variables were then plotted on a PCA ordination diagram.

Combined diatom and water chemistry statistical analyses

The length of the gradients representing the diatom species abundances as determined by the detrended correspondence analyses (DCA) were relatively short. Consequently, redundancy analyses (RDA) were used to determine if the differences in species composition over time was related to changes in physico-chemical variables. Specifically, the physico-chemical variables were tested with three groups of samples 1) ice-covered sediment trap samples, 2) ice-free sediment trap samples and 3) ice-free pooled pelagic samples. Monte-Carlo permutation tests were conducted independently for each physico-chemical variable to determine its significance among the diatom species abundance data. A final RDA ordination diagram was produced using only the significant variables. Variables with high VIFs (variance inflation factors) were also eliminated as these show co-linearity between variables. Variables with VIFs higher than 5 were eliminated from the analyses. In cases where few variables were significant (≤ 2),

variables with higher VIFs (<13) were kept as explanatory variables and those with higher VIFs ($\geq$ 13) were eliminated from the analysis. The high VIF variables were added as supplementary or "passive" variables which allows them to be part of the ordination diagram and therefore their relationship to other variables is visible (terBraak and Šmilauer, 2002).

Results

Short-term (2009-2011) seasonal trends in ice-covered and ice-free sediment traps

The PCA ordination diagram summarizes the general trends in the variation of the ice-covered sediment trap diatom assemblages (2009-2010 and 2010-2011) and the ice-free diatom assemblages (2010 and 2011) (Fig. 2.4). An ANOSIM test confirmed that the ice-free assemblage was significantly different from the ice-covered assemblage ($p = 0.0003$). The total amount of variation in the diatom community composition explained by the first two axes in the ordination diagram was 75.8%.

The five species responsible for 72.3% of the differences between ice-covered and ice-free seasons were *Stephanodiscus minutulus/parvus* (32.33%), *Stephanodiscus binderanus* (19.65%), *Fragilaria crotonensis* (11.55%), *Tabellaria floccuolsa* (4.95%) and *Asterionella formosa* (3.78%).

The ice-covered period was dominated largely by *Stephanodiscus minutulus/parvus* (38.06%) with smaller amounts of *Tabellaria flocculosa* (4.95%), *Stephanodiscus binderanus* (2.93%), *Fragilaria crotonensis* (2.37%), *Fragilaria capucina* (2.21%) and *Asterionella formosa* (1.97%) (Fig. 2.5).

The ice-free period was composed primarily of *Stephanodiscus binderanus* (18.76%), *Fragilaria crotonensis* (12.76%) and *Tabellaria floccuolsa* (6.73%) and less so of *Stephanodiscus minutulus/parvus* (8.81%) and *Asterionella formosa* (4.14%) (Fig. 2.5).

The diatom assemblages for each ice-covered season (2009-2010 and 2010-2011) were also statistically different from each other ($p = 0.028$) as were the diatom assemblages for each ice-free season (2010 and 2011) ($p = 0.029$).

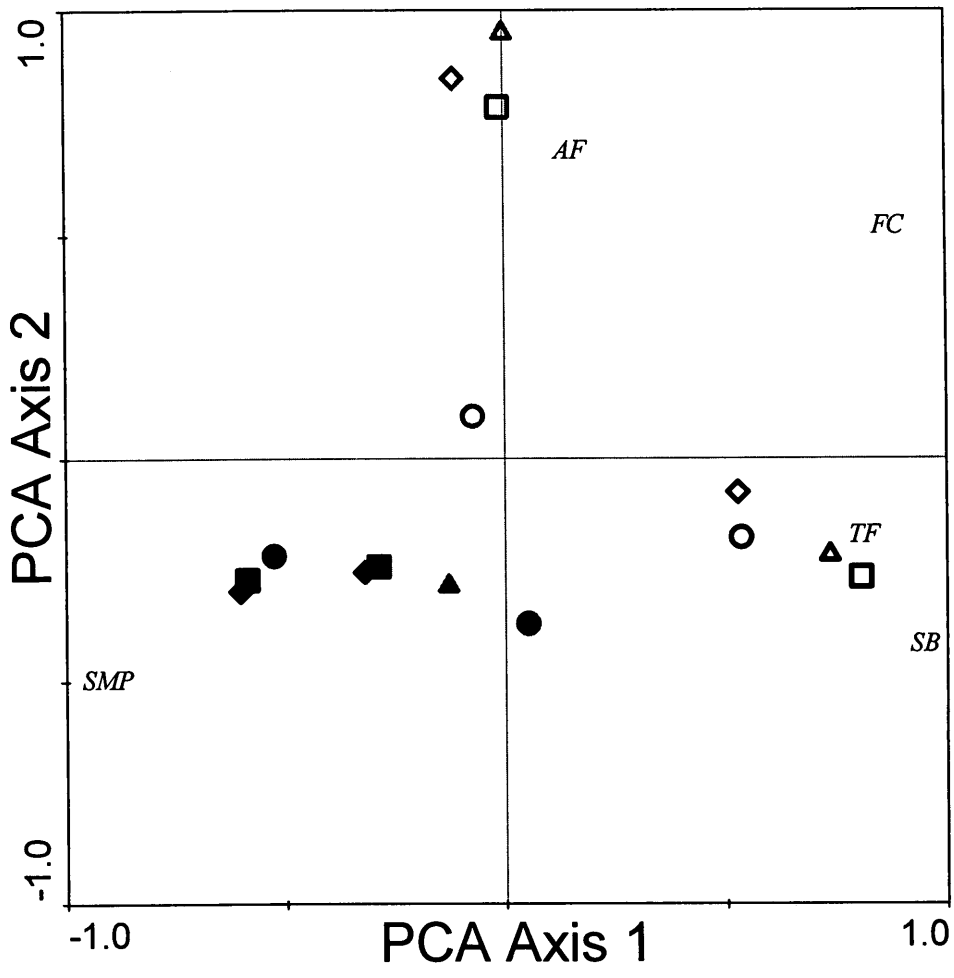

Fig. 2.4 PCA ordination biplot sample scores for ice-covered diatom samples from 2009 (solid black) and 2010 (solid gray); and ice-free samples from 2010 (open black) and 2011 (open grey) using the 15 sediment trap samples and all 108 species for sites K42 (circle), C9 (diamond), S15 (square) and N31 (triangle). The 5 species that contribute most to seasonal differences are shown. Legend: 2010 (black) and 2011 (grey). Species acronyms are listed in Appendix B. The percent variation explained by the first two axes was 75.8%.

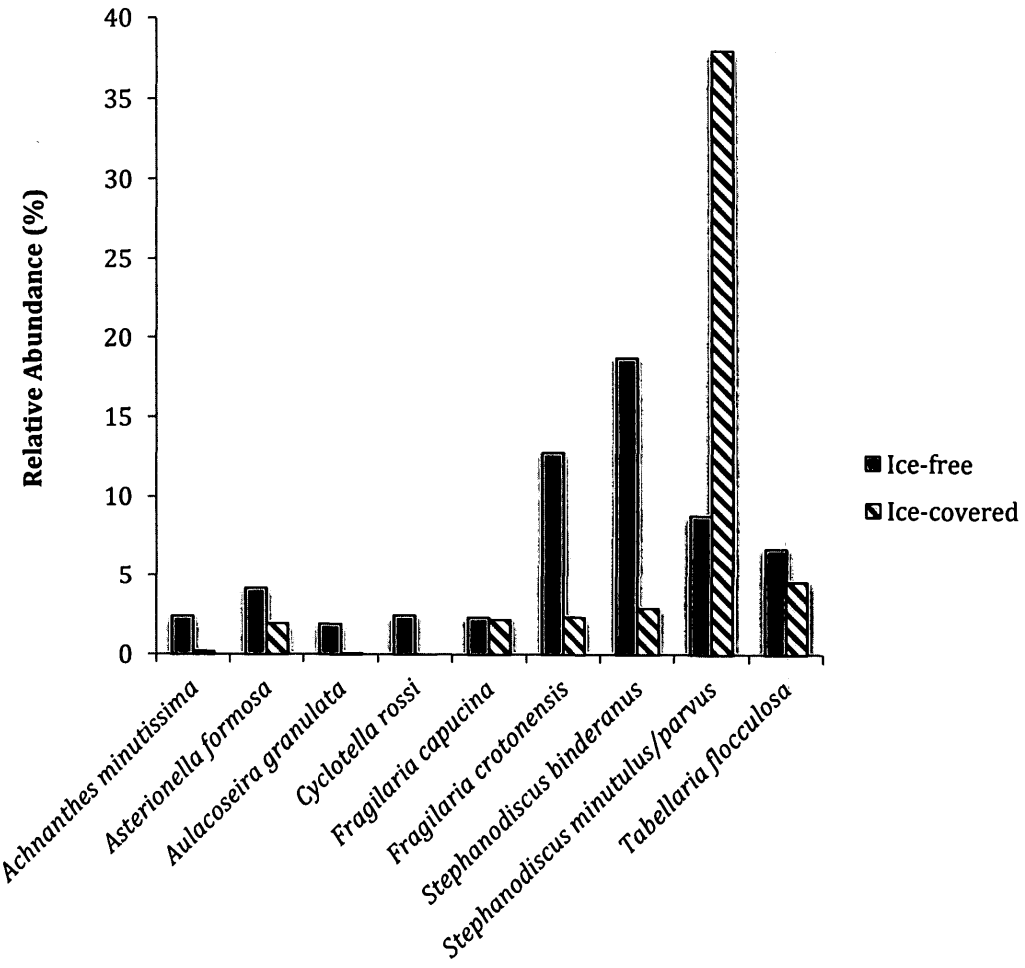

Fig. 2.5 Comparison of mean relative species abundance (%) for most common species sampled in the ice-free and ice-covered sediment trap samples (2009-2011).

Spatial comparison 2009-2011

The PCA ordination diagram summarized the general trends in spatial variation of the diatom assemblages of sites K42, C9, S15 and E51 using the ice-covered sediment trap samples (Fig. 2.4), the ice-free sediment trap samples (Fig. 2.4) and the ice-free pooled pelagic samples (Fig. 2.6). The ANOSIM test revealed that community composition among the four sites within a season was not statistically different based on the three sampling methods (Tables 2.2; 2.3; 2.4). The cumulative percent variance in the diatom communities explained by the first two axes of the sediment trap samples and the ice-free pooled pelagic samples were 75.8% and 48.9% respectively.

The physico-chemical variables for the ice-free season were also tested for similarity among sites (Fig. 2.7). The ANOSIM test confirmed that the sites were not statistically different within a season based on their physico-chemical variables for the years 2009-2011 (Table 2.5).

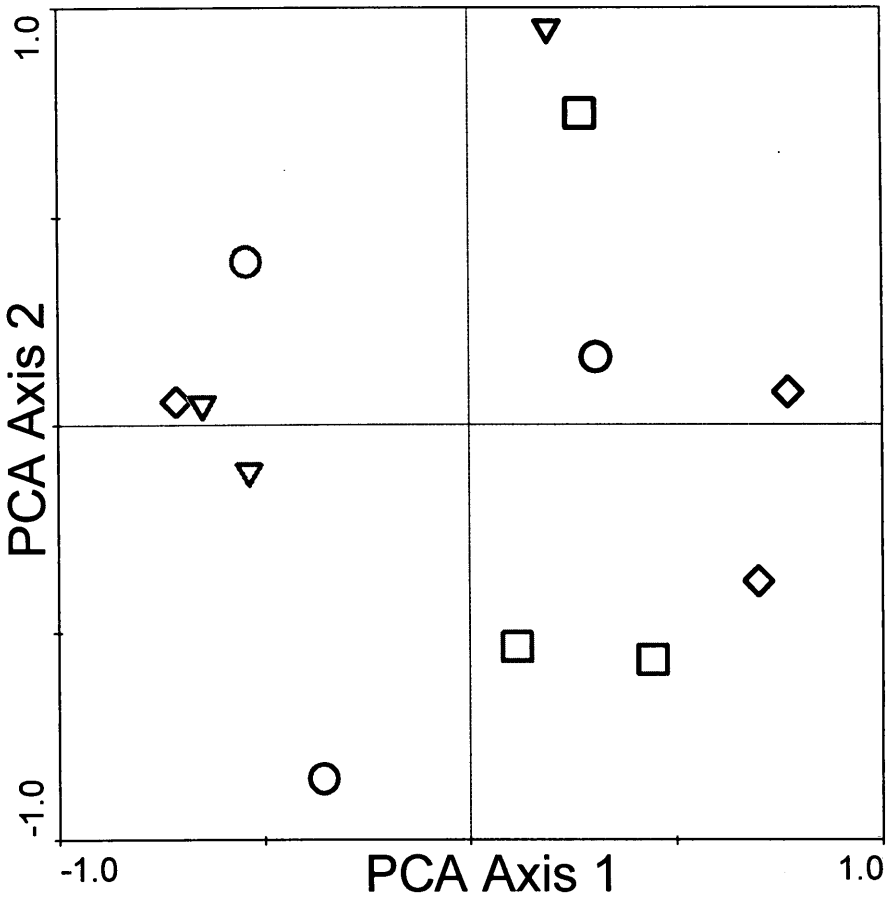

Fig. 2.6 PCA ordination sample scores for the site comparison from 2009-2011 using the ice-free pooled pelagic diatom samples. The sites were: K42 (circle), C9 (diamond), S15 (square) and E51 (down triangle) for pooled pelagic data. Legend: 2009 (light grey), 2010 (grey) and 2011 (black). The percent variation explained by the first two axes was 48.9%.

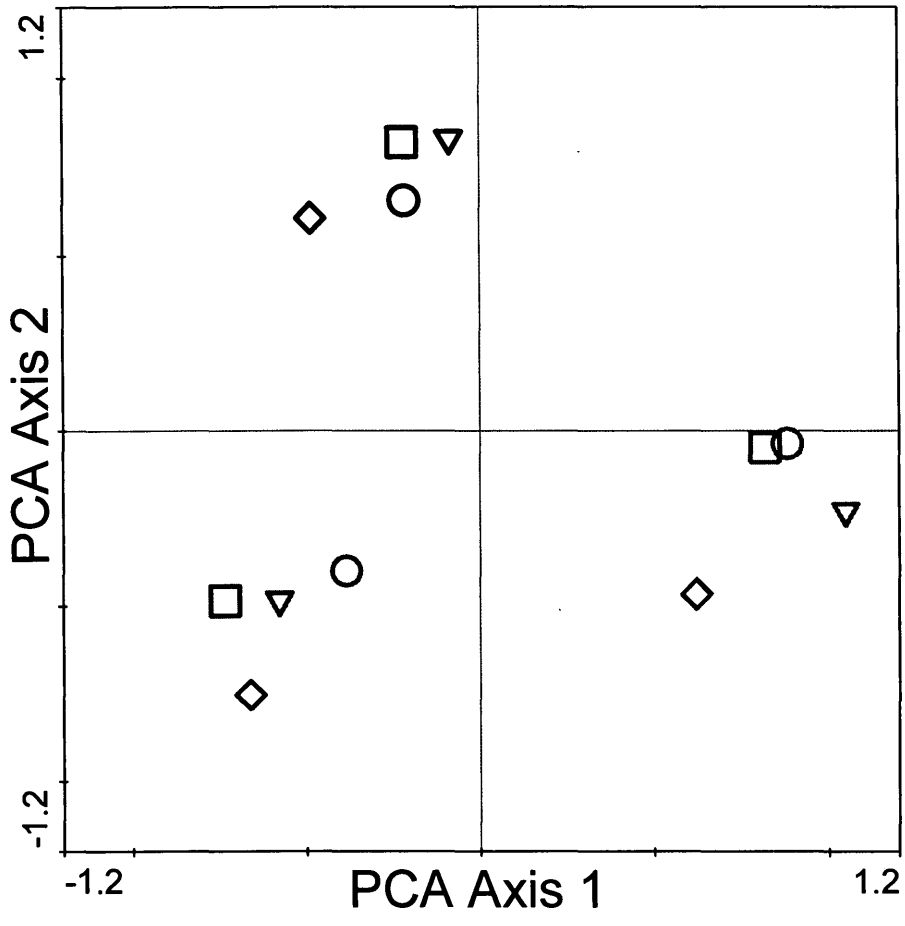

Fig. 2.7 PCA ordination sample scores for the site comparison from 2009-2011 for the pooled pelagic environmental variables. The sites were: K42 (circle), C9 (diamond), S15 (square) and E51 (down triangle) for pooled pelagic data. Legend: 2009 (light grey), 2010 (grey) and 2011 (black).

Table 2.2 P-values from ANOSIM test comparing the four sites K42, C9, S15 and N31 using the averaged ice-covered sediment trap relative diatom community abundances (2009-2010). None of the values were significant at the 0.05 level.

	K42	C9	S15	N31
K42		0.665	0.669	1
C9	0.665		1	0.666
S15	0.669	1		0.670
N31	1	0.666	0.670	

Table 2.3 P-values from ANOSIM test comparing the four sites K42, C9, S15 and N31 using the averaged ice-free sediment trap relative diatom community abundances (2010-2011). None of the values were significant at the 0.05 level.

	K42	C9	S15	N31
K42		1	1	0.665
C9	1		0.669	1
S15	1	0.669		1
N31	0.665	1	1	

Table 2.4 P-values from ANOSIM test comparing the four sites K42, C9, S15 and E51 using the ice-free pooled pelagic relative diatom community abundances (2009-2011). None of the values were significant at the 0.05 level.

	K42	C9	S15	E51
K42		0.595	0.491	1
C9	0.595		0.604	0.305
S15	0.491	0.604		0.203
E51	1	0.305	0.203	

Table 2.5 P-values from ANOSIM test comparing the four sites K42, C9, S15 and E51 using the physico-chemical data (2009-2011). None of the values were significant at the 0.05 level.

	K42	C9	S15	E51
K42		0.806	0.708	0.495
C9	0.806		1	0.692
S15	0.708	1		0.699
E51	0.495	0.693	0.699	

Ice-free pelagic and adjacent sediment trap sample comparison 2010-2011

A PCA ordination diagram showed the ice-free diatom assemblages of the pelagic zone and the sediment traps for the years 2009-2011 (Fig. 2.8). The first two axes of the PCA explained 52.5% of the total variation among communities captured in the pelagic and sediment trap samples. This figure shows that the type of method used (pelagic compared to sediment trap) is more important than interannual variation. The methodology separates on axis 1. An ANOSIM test was used to confirm that statistical differences exist overall (all years combined) between the diatom assemblages of the pelagic zone and the adjacent sediment traps ($p = 0.0001$) (Table 2.6). The SIMPER analysis was used to determine that nine of 124 taxa contributed 75.67% of the variance between the two groups: *Stephanodiscus minutulus/parvus* (24.71%), *Stephanodiscus binderanus* (16.02%), *Fragilaria crotonensis* (14.65%), unknown centrics (4.77%), *Asterionella formosa* (4.18%), *Tabellaria floculosa* (3.74%), *Fragilaria spp.* (2.97%), *Cyclotella rossi* (2.54%) and *Achnanthes minutissima* (2.11%) (Fig. 2.8 B).

Comparing within years, the relative diatom species abundances for the 2011 ice-free sediment trap samples were not statistically different from the 2011 ice-free pelagic samples ($p = 0.059$) (Table 2.6). However, the 2010 ice-free sediment trap samples were statistically different from the 2010 ice-free pelagic samples ($p = 0.029$) (Table 2.6). The SYMPER analysis determined that eight species out of 124 were responsible for the variance between the 2010 adjacent samples; *Stephanodiscus binderanus* (28.96%), *Stephanodiscus minutulus/parvus* (19.23%), *Fragilaria crotonensis* (17.68%), *Fragilaria*

spp. (4.97%), *Asterionella formosa* (4.68%), *Tabellaria floculosa* (4.12%), *Aulcoseira spp.* (4.12%) and *Asterionella / Tabellaria* (2.95%) (Fig.2.8; Table 2.6).

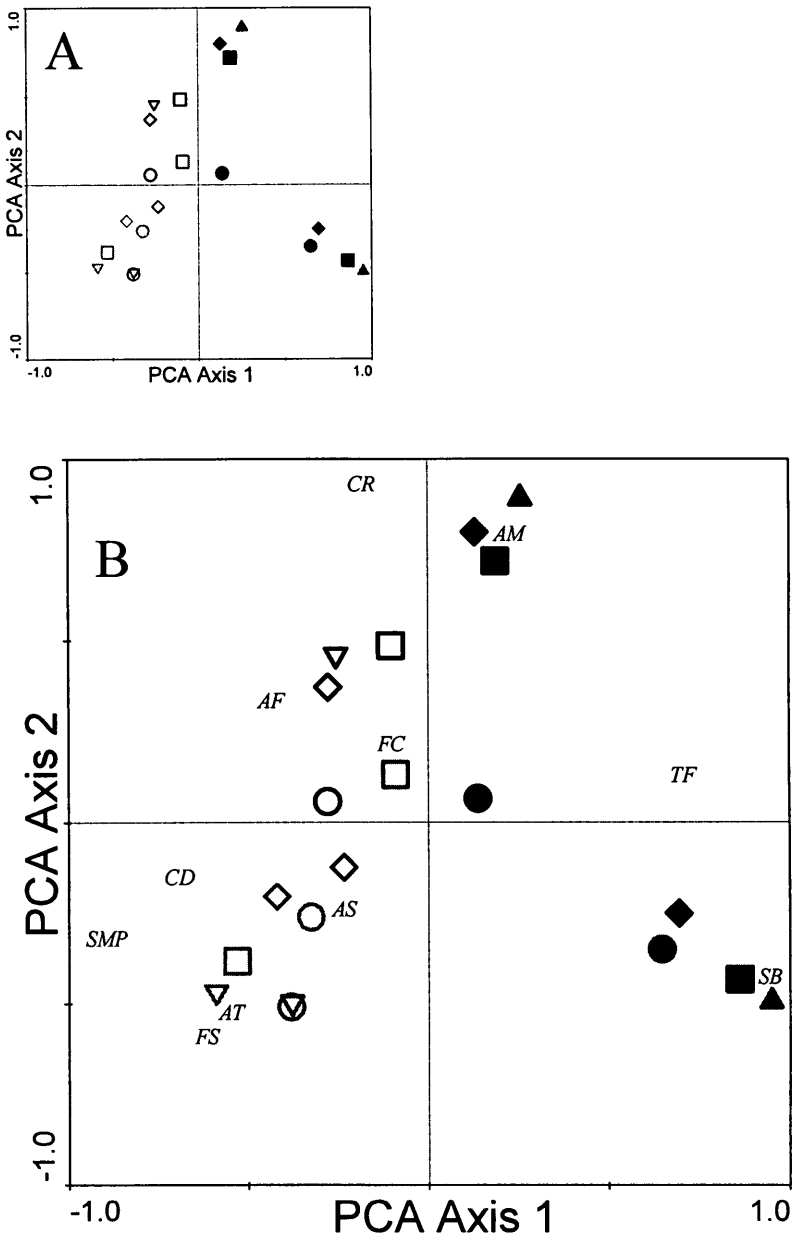

Fig. 2.8 PCA ordination of ice-free sediment (closed) and ice-free pooled pelagic diatom sample scores (open) for sites K42 (circle), C9 (diamond), S15 (square), N31 (triangle) and E51 (down triangle). (A) sites only and (B) adds major species. Legend: 2009 (light grey), 2010 (grey) and 2011 (black). Species acronyms are listed in Appendix B. The percent variation explained by the first two axes was 52.5%.

Table 2.6 P-values from SIMPER analysis comparing ice-free 2010 and 2011 sediment trap data to ice-free 2009, 2010 and 2011 pooled pelagic data. Significant values are shown in bold face.

	Sediment 2010	Sediment 2011	Pelagic 2009	Pelagic 2010	Pelagic 2011
Sediment 2010		**0.031**	**0.027**	**0.029**	**0.028**
Sediment 2011	**0.031**		**0.029**	**0.027**	0.059
Pelagic 2009	**0.027**	**0.029**		0.293	0.082
Pelagic 2010	**0.029**	**0.027**	0.293		0.224
Pelagic 2011	**0.028**	0.059	0.082	0.224	

Relative abundance comparison of the three sample types

The six dominant diatom taxa among the ice-covered sediment trap, ice-free sediment trap and ice-free pelagic samples were *Asterionella formosa,* unknown centrics, *Fragilaria crotonensis, Stephanodiscus binderanus*, *Stephanodiscus minutulus/parvus* and *Tabellaria floculosa* (Fig. 2.9). Overall, *Stephanodiscus minutulus/parvus* was the dominant taxa especially in the pelagic ice-free samples and the ice-covered sediment traps. *Fragilaria crotonensis* also dominated the ice-free pelagic samples. The ice-free sediment trap samples were primarily dominated by *Stephanodiscus binderanus* and *Fragilaria crotonensis*.

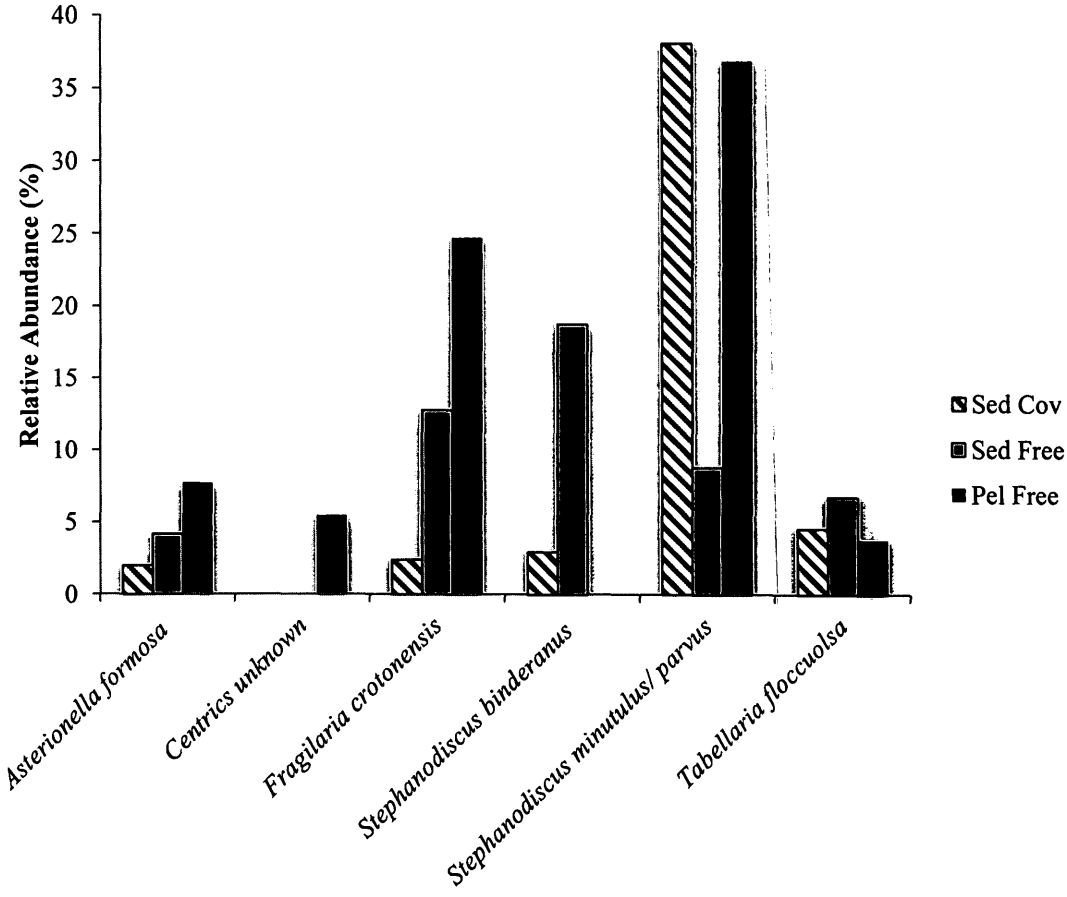

Fig. 2.9 Comparison of yearly averages (2009-2011) for all sites (K42, C9, S15 and N31/E51) for relative diatoms species abundances (%) for the three types of samples: sediment trap ice-covered (Sed Cov), sediment trap ice-free (Sed Free) and ice-free pooled pelagic samples (Pel Free).

Environmental drivers of relative diatom species abundance using ice-free pooled pelagic data

Previous analyses showed that there were differences in diatom abundances ($p < 0.05$) between samples (i.e., between ice-free and ice-covered sediment trap samples and between ice-free sediment trap samples and ice-free pelagic samples). In this section, we look at relationships between physico-chemical variables and relative species abundance over time within each of these samples: 1) pooled pelagic samples (2009-2011), 2) ice-free sediment trap samples (2010-2011) and 3) ice-covered sediment trap samples (2009-2010).

All 11 of the physico-chemical variables were tested for normality before conducting the multivariate analysis (Table 2.7). Five of the variables (ALKTI, Cl, TKN, Si and TP) were significant in the RDA analysis of the pelagic relative species abundances collected in the ice-free seasons of 2009-2011 (Table 2.8). However, Si had a high VIF value and was removed from the analysis but was retained as a passive variable (Table 2.10). The ordination diagram using environmental variables ALKTI, Cl, TKN and TP explained 34.0% of the variation in the pelagic diatom species data (Fig. 2.10).

Tabellaria flocculosa and *Fragilaria crotonensis* tended to be found in sites with high concentrations of Si and low TKN (Fig. 2.10). Comparatively, *Cyclotella comensis* and *Stephanodiscus minutulus/parvus* were found in high abundances in sites with high

TKN and TP and low Si. *Cyclotella cyclopuncta* and *Cyclotella rossi* tended to be found in sites with high alkalinity and chloride concentrations.

Table 2.7 Results for Shapiro-Wilk normality tests for four sites (K42, C9, S15 and E51) from 2009-2011 (N = 12). Significant values are shown in bold face.

Variable	Transformation	W-value	P-value
ALKTI	Log_{10}	0.650	**0.0003**
Cl	Log_{10}	0.829	**0.020**
DIC	None	0.836	**0.025**
DOC	Log_{10}	0.900	0.160
NNH	Inverse	0.608	**0.0001**
NNO	Inverse	0.751	**0.003**
TKN	None	0.826	**0.019**
pH	None	0.911	0.217
Si	None	0.802	**0.010**
TP	Squared	0.861	**0.051**
Secchi	Inverse	0.966	0.861

Table 2.8 Monte-Carlo permutation tests for significance (999 permutations) for RDA using the ice-free pooled pelagic relative abundance species data with 11 transformed environmental variables. Significant values are shown in bold face. Variance inflation factors (VIF) are expressed for significant variables that were use in the subsequent RDA.

Variable	P-value	VIF	VIF
ALKTI	**0.052**	**1.7318**	**1.566**
Cl	**0.014**	**1.9473**	**1.818**
DIC	0.173		
DOC	0.609		
NNH	0.499		
NNO	0.650		
TKN	**0.044**	**19.611**	**1.709**
pH	0.275		
Si	**0.035**	**23.494**	**Removed**
TP	**0.039**	**2.826**	**2.337**
Secchi	0.444		

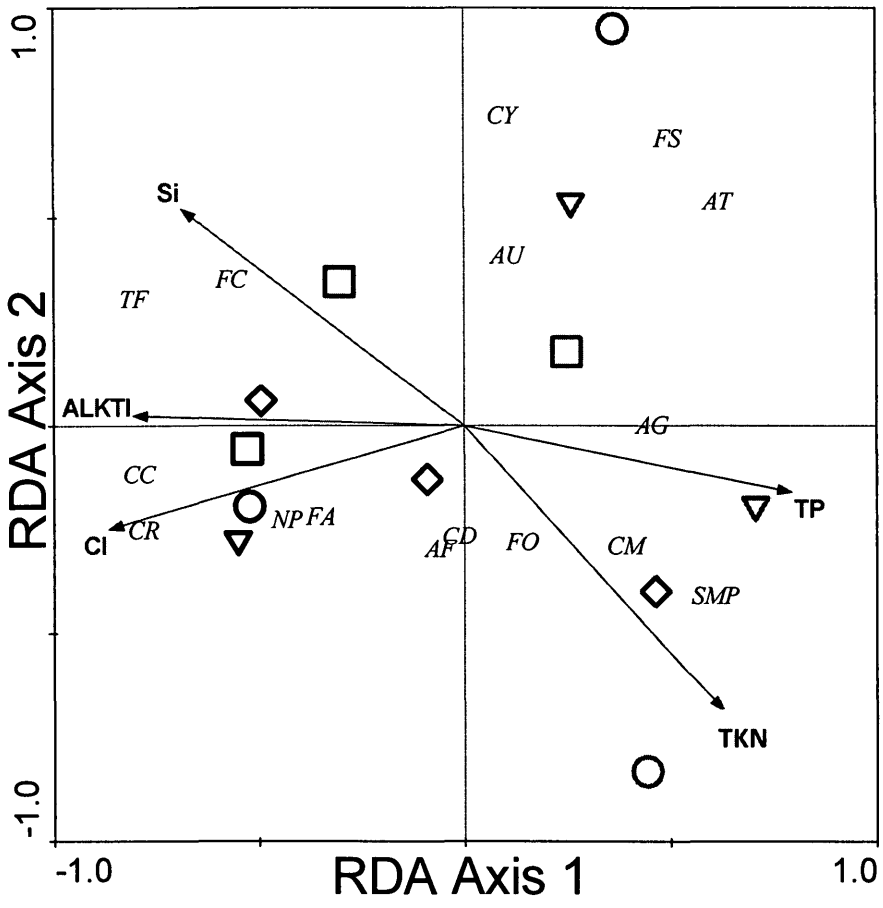

Fig. 2.10 RDA ordination diagram showing ice-free pelagic diatom species scores with the 5 significant variables (alkalinity, Cl, TKN, TP and Si) with relative diatom species abundances (N = 63) for sites K42 (circle), C9 (diamond), S15 (square) and E51 (down triangle). The species gradient length was 1.541. The significance of all canonical axes in the ordination using all 5 significant variables was p = 0.037. The significance of all canonical axes in the ordination using 4 significant variables was p = 0.049 with Si added as a supplementary variable. Only dominant species are shown (>2% of the relative abundance of each sample). Legend: 2009 (light grey), 2010 (grey) and 2011 (black). Species acronyms are listed in Appendix B. The percent variation explained by the first two axes was 34.0%.

Environmental drivers of relative diatom species abundance using ice-free sediment trap data

All 11 of the physico-chemical variables were tested for normality before conducting the multivariate analysis (Table 2.9). Four of the variables (Cl, DIC, pH and Si) were significant to the RDA analysis of the relative species abundances collected in the sediment traps during the ice-free seasons of 2010-2011 (Table 2.10). The significance of all canonical axes (using the four variables) was $p = 0.06$. Variables with VIF's higher than 5 were removed. pH and dissolved inorganic carbon were added as a passive variables due to their high VIF values. The ordination diagram using environmental variables Cl and Si explain 42.9% of the variation in the ice-free diatom species data (Fig. 2.11).

Stephanodiscus binderanus was highly correlated with sites with high concentrations of DIC, pH and Si (Fig. 2.11). *Fragilaria spp., Achnanthes minutissima, Amphora spp., Fragilaria tenera* tended to be negatively correlated with DIC, pH and Si. *Cyclotella rossi* and *Fragilaria nanana* were highly correlated with sites having high concentrations of Cl (mainly in 2011).

Table 2.9 Results for Shapiro-Wilk normality tests for four sites (K42, C9, S15 and N31) from 2010-2011 (N=8). Significant values are shown in bold face.

Variable	Transformation	W-value	P-value
ALKTI	Log_{10}	0.641	**0.0005**
Cl	Log_{10}	0.815	**0.041**
DIC	None	0.742	**0.007**
DOC	Log_{10}	0.931	0.522
NNH	Inverse	0.641	**0.0005**
NNO	Inverse	0.806	**0.033**
TKN	Log_{10}	0.804	**0.032**
pH	None	0.909	0.346
Si	ln	0.863	0.130
TP	Squared	0.707	**0.003**
Secchi	Inverse	0.982	0.975

Table 2.10 Monte-Carlo permutation tests for significance (999 permutations) for RDA using the ice-free sediment trap relative abundance species data with 11 transformed environmental variables singly constrained. Significant values are shown in bold face. Variance inflation factors (VIF) are expressed for significant variables that were used in the subsequent RDA using all significant variables.

Variable	P-value	VIF	VIF
ALKTI	0.075		
Cl	**0.004**	**6.700**	**2.031**
DIC	**0.002**	**13.034**	**Removed**
DOC	0.147		
NNH	0.075		
NNO	0.133		
TKN	0.131		
pH	**0.012**	**16.756**	**Removed**
Si	**0.012**	**8.596**	**2.031**
TP	0.226		
Secchi	0.293		

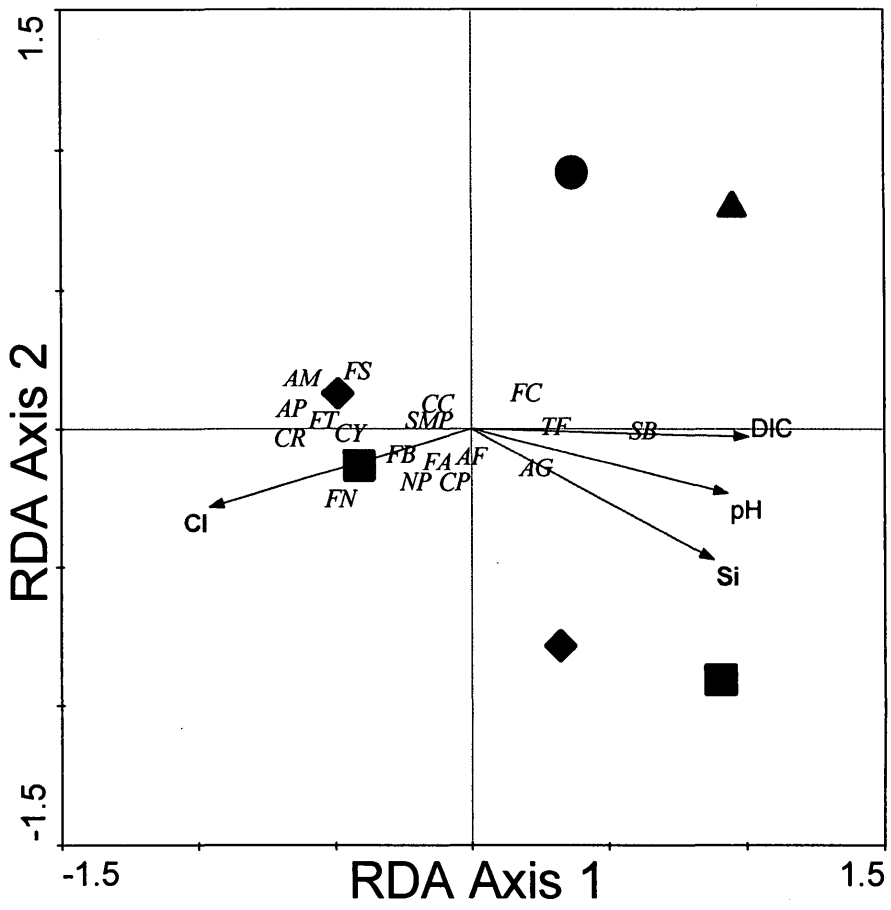

Fig. 2.11 RDA ordination triplot of sediment trap ice-free diatom species scores (N=8) with the 4 significant environmental variables with VIFs lower than 5 (chloride and silicate) and with the collinear variables added as passive variables (pH and dissolved inorganic carbon). Sites are as follows: K42 (circle), C9 (diamond), S15 (square) and N31 (triangle). The species gradient length was 3.086. The overall significance of all canonical axes in the ordination is p = 0.0600. Only dominant species are shown (>2% of the relative abundance of each sample). Legend: 2010 (black) and 2011 (grey). Species acronyms are listed in Appendix B. The percent variation explained by the first two axes was 42.9%.

Environmental drivers of relative diatom species abundance using ice-covered sediment trap data

All 11 of the physico-chemical variables were tested for normality before conducting the multivariate analysis, and transformed to normality if necessary (Table 2.11). Three of the 11 environmental variables (DIC, TKN and Si) were significant to the RDA analysis of the relative species abundances collected in the sediment traps during the ice-covered seasons of 2009-2010 (Table 2.12). The significance of all canonical axes (using the three variables) was $p = 0.007$. Variables with VIF's higher than 13 were removed. Silica was added as passive variable due to the high VIF value. The ordination diagram using environmental variables DIC and TKN explain 65.1% of the variation in the ice-covered diatom species data (Fig 2.12).

The 2010 sites and species (*Asterionella formosa* and *Stephanodiscus minutulus/parvus*) were highly correlated with TKN and low DIC and Si. Comparatively, the 2011 sites were highly correlated with DIC and Si and low TKN. *Tabellaria flocculosa, Stephanodiscus binderanus, Frangilaria capucina and Fragilaria crotonensis* were correlated with DIC and Si.

Table 2.11 P-values for Shapiro-Wilk normality tests for four sites (K42, C9, S15 and E51) from 2009-2010 (N = 7). Significant values are shown in bold face.

Variable	Transformation	W-value	P-value
ALKTI	Log_{10}	0.600	**0.0003**
Cl	Log_{10}	0.840	0.010
DIC	None	0.739	**0.010**
DOC	Inverse	0.664	**0.002**
NNH	Inverse	0.664	**0.002**
NNO	Inverse	0.805	**0.046**
TKN	Squared	0.823	0.069
pH	None	0.958	0.804
Si	ln	0.790	**0.033**
TP	Squared	0.859	0.147
Secchi	Inverse	0.866	0.172

Table 2.12 Monte-Carlo permutation tests for significance (999 permutations) for RDA using the ice-free sediment trap relative abundance species data with 11 transformed environmental variables singly constrained. Significant values are shown in bold face. Variance inflation factors (VIF) are expressed for significant variables that were use in the subsequent RDA using all significant variables.

Variable	P-value	VIF	VIF
ALKTI	0.656		
Cl	0.867		
DIC	**0.009**	**28.378**	**12.593**
DOC	0.216		
NNH	0.058		
NNO	0.096		
TKN	**0.027**	**16.063**	**12.593**
pH	0.618		
Si	**0.034**	**34.458**	**Removed**
TP	0.165		
Secchi	0.709		

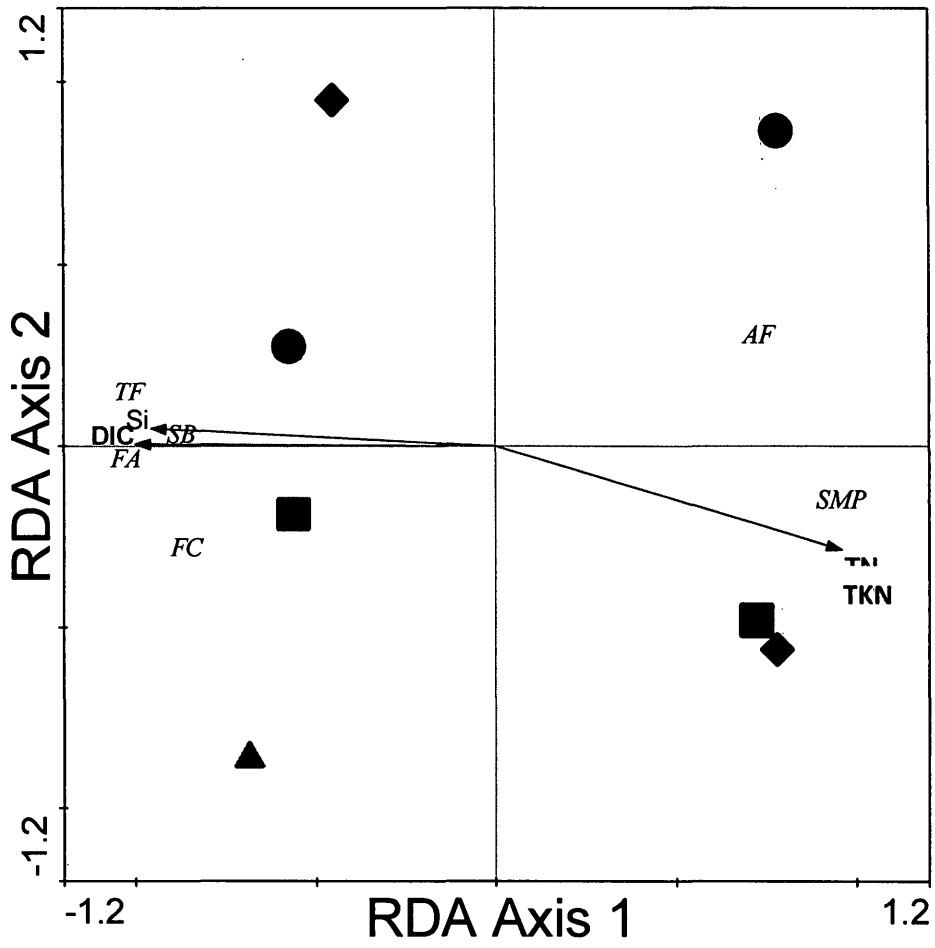

Fig. 2.12 RDA ordination triplot of sediment trap ice-covered diatom species scores (N = 7) for 2009 and 2010. The 3 significant environmental variables with VIFs lower than 5 (TKN and DIC) are shown with the collinear variables added as passive variables (Si). Sites are as follows: K42 (circle), C9 (diamond), S15 (square) and N31(triangle). The species gradient length was 1.261. The overall significance of all canonical axes in the ordination is p = 0.007. Si was added as a passive variable. Only dominant species are shown (>2% of the relative abundance of each sample). Legend: 2010 (Black) and 2011 (Grey). Species acronyms are listed in Appendix B. The percent variation explained by the first two axes was 61.5%.

Discussion

Overall, the most abundant diatom taxa (2009-2011) for all three sample methods, ice-covered sediment trap, ice-free sediment trap and ice-free pelagic, were *Stephanodiscus minutulus/parvus*, *Stephanodiscus binderanus*, *Fragilaria crotonensis*, *Tabellaria flocclolsa*, *Asterionella formosa* and unknown centrics. A recent paleolimnological study also found similar diatom taxa present in the Lake Simcoe sediments post-1990 including *Stephanodiscus minutulus* (dominated the sediments post-1900) and a mixture of centrics including *Stephanodiscus spp.* and *Cyclotella comensis/gordonensis* and pennates including *Tabellaria flocculosa, Asterionella formosa and Fragilaria crotonensis* (dominated the sediments post-1970) (Hawryshyn et al., 2012). These findings suggest that the species were correctly identified and their presence remained consistent across sampling and laboratory methodologies. This also supports the notion that biological indicators such as diatoms can be used to infer past ecological conditions and historical biological assemblages (Smol, 2008). However, there were significant differences among sampling methods, sampling years and seasons.

Statistically significant seasonal differences were present among diatom assemblages in sediment traps from the ice-covered (2009 and 2010) and ice-free (2010 and 2011) seasons. Although seasonal sediment trap work had not been previously explored for Lake Simcoe, diatom seasonality has been well documented (Lund, 1965; Reynolds, 1973; Harris, 1983). Low water column stability as well as high mixing conditions favours establishment of diatoms making them the dominant group in the

spring and the fall seasons (Reynolds, 1980). This study found that the ice-covered diatom assemblages were significantly different from the ice-free assemblages. Differences in light (Foy et al., 1976), temperature (Hammer, 1964) and mixing energy (sinking rate) (Lund et al., 1965) between the two seasons probably influenced the diatom communities. The ice-free season tends to have brighter light intensity (due to the lack of snow and ice on the surface), intense mixing of the water column and higher temperatures in comparison to the ice-covered period. Five species were responsible for almost 73% of the variation were *Stephanodiscus minutulus/parvus*, *Stephanodiscus binderanus*, *Fragilaria crotonensis*, *Tabellaria flocculosa* and *Asterionella formosa*. *Stephanodiscus minutulus/parvus* dominated the ice-covered samples especially in 2009-2010. This taxon was also highly abundant in the ice-free pelagic samples, possibly due to its small size which allows for a slower sinking rate, efficient uptake of nutrients and more rapid dividing times compared to larger diatom cells (Litchman et al., 2006). The sediment traps of the ice-free season were dominated by *Stephanodiscus binderanus*, *Fragilaria crotonensis*, *Tabellaria flocculosa* and smaller abundances of *Stephanodiscus minutulus/parvus*. *Stephanodiscus binderanus* is a small, centric, heavily silicified species, which forms long chains. As a result it settles out of the water column quickly explaining why it was found in the ice-free sediment trap samples with relatively high abundance. *S. minutulus/parvus* (small in size) dominated over-winter sediment traps and Hawryshyn et al. (2012) described a shift from *Fragilaria spp.* to *S.minutulus* since the mid-1990s. These findings disagree with a study by Winter et al. (2011) who described a declining trend in *Stephanodiscus spp.* and an increase in *Fragilaria spp.*

Paleolimnological sediment cores provide an integrated sample of winter and summer diatoms from the pelagic water column and littoral zone, hence, the cores may have captured *S.minutulus* from the ice-covered seasons which would explain a higher overall abundance of *S.minutulus*. The ice-free pelagic sampling done by Winter et al. (2011), would not have captured the high abundance of *S.minutulus* from the ice-covered period possibly explaining their overall decrease in *Stephanodiscus spp.*. Perhaps the decline in *Stephanodiscus* in pelagic samples involves larger species such as *S.niagaria* and *S.alpinus* which were present in higher abundances prior to 1996 coinciding with the invasion of the zebra mussels (Winter et al., 2011).

There were also differences between years for the ice-covered and ice-free seasons. The ice-free period for 2010 was characterized by relatively higher abundances of *Tabellaria flocculosa* and *Stephanodiscus binderanus* and 2011 by *Asterionella formosa* and *Fragilaria crotonensis*. A study of Elk Lake by Bradbury et al. (2002) found that in some years the short-term variability of individual diatom species was greater than long-term patterns of distribution. Such differences may be attributed to annual changes in the physical, chemical and climate variables (Lund, 1965; Paterson et al, 2008). Differences may also be a result of the difference in dates of deployment for the ice-free samples, which was July 19 in 2010 and May 5 in 2011. Some of the spring species may not have been collected in the 2010 ice-free sediment traps as they were deployed relatively late and diatoms tend to peak in early spring.

The diatom community assemblages did not significantly differ among the four sites (K42, C9, S15 and N31/E51). No significant differences were found between the

sites in the ice-free sediment trap samples, the ice-covered sediment trap samples, the ice-free pelagic samples and the physico-chemical samples. There appears to be more variation among years than among sites within a season and among years as depicted in the ordinations than spatial variation. Diatoms exhibit rapid assemblage shifts in accordance with environmental changes (Smol, 2008). The similarity of physico-chemical variables among sites for each ice-free season mirrored diatom assemblage similarity among sites. Such similarity in physico-chemical variables may be a result of intense horizontal mixing in the upper waters during the ice-free season. Since the implementation of the LSPP, the number of monitoring stations has increased in an attempt to better understand how differences in point source loadings of P affect P concentrations spatially within in the lake. Despite the lack of significant differences in the diatom assemblages and physico-chemical variables found among sites, the number of sites monitored should be maintained because they improve reliability and create a more accurate, holistic understanding of the Lake Simcoe ecosystem.

There were significant differences between the diatom assemblages of the pelagic zone and their adjacent sediment traps during the ice-free seasons in 2010 and and 2011. Four taxa contributed over 60% of the variance: *Stephanodiscus minutulus/parvus* (24.71%), *Stephanodiscus binderanus* (16.02%), and *Fragilaria crotonensis* (14.65%) and unknown centrics (4.761%). Overall, the pelagic samples were dominated by *Stephanodiscus minutulus/parvus, Fragilaria crotonensis* and unknown centrics. The ice-free sediment traps were dominated by *Fragilaria crotonensis* and *Stephanodiscus binderanus*. *Fragilaria crotonensis* is a benthic species with an optimal TP range of 12-

16 µg /L which suggests that the lake is well mixed, mesotrophic and has a considerable littoral area (Paterson et al., 2007). *Fragilaria crotonensis* is also a euplanktonic species and forms long chains (observed in the samples), which reduces its sinking rate (Padisák et al., 2003; Wehr and Sheath, 2003), explaining its presence in the ice-free sediment traps. Small centrics including *Stephanodiscus minutulus/parvus* are expected in the pelagic zone of the water column in eutrophic waters due to their nutrient requirements and their size and morphology. While dominant in the pelagic ice-free samples, it is possible that they settled out of the pelagic zone from the preceding ice-free period and were later captured in the ice-covered sediment traps, which explains their high abundances in both samples. The differences between the sediment traps and the pelagic samples may be exaggerated because some diatoms in the pelagic samples were only identified as unknown centrics whereas they were identified in the sediment trap samples.

RDA analyses compared the physico-chemical variables of the water column to the diatom taxa assemblages collected by each of the methods for each season to determine possible drivers for relative abundance (although correlation is not causation). The RDA ordination of the ice-covered sediment trap samples revealed that the diatom species composition was significantly related to Si and TKN; composition in the ice-free sediment trap samples was related to Cl, DIC, pH and Si and the ice-free pelagic community samples were related to alkalinity, Cl, TKN, Si and TP.

Si was the only variable that was significantly related to community composition in all three sampling methods. This supports earlier studies of diatoms suggesting that they require high concentrations of silica to construct their frustules (Tilman, 1977).

Fragilaria was common in all methods of sampling and is associated with higher Si:P ratios (Kilham et al.,1986). TKN does not appear to add much in terms of ecological understanding to the presence of each diatom taxa. Changes in TKN are probably representative of changes in organic nitrogen (because ammonium concentrations are much lower than organic N) which in turn may represent changes in cell biomass. TP was found to be a main driver of the pelagic ice-free communities, which is to be expected as phosphorus is one of the main nutrients limiting phytoplankton growth (Lund, 1965; Reynolds, 1980; Young et al., 2010). Interestingly, *S.minutulus/parvus* which is eutrophic was associated with higher TP concentrations. An interesting study by Bradbury et al., (2002) found *Stephanodiscus minutulus*, *Fragilaria crotonensis*, and *Asterionella formosa* to be the dominant and most persistent planktonic diatoms in Elk Lake for the past 1500 years and suggested that rapid alternations between *Fragilaria crotonensis* and *Stephanodiscus minutulus* track climate changes related to the timing of ice out and the strength of spring circulation (Bradbury, 1988). Bradbury and his colleagues found that a rapid transition to summer, cold temperatures, a late ice-out date and weak spring circulation promotes the dominance of *Fragilaria crotonensis* whereas a slow transition to summer; a longer period of circulation which increases nutrient availability and low light levels favour blooms of *Stephanodiscus minutulus*. (Bradbury, 1988).

Cl was identified in the RDA as significant in influencing the spring diatom communities of both the ice-free periods as sampled by the sediment traps and the pelagic samples. Increased urbanization and road salt application were likely responsible for the

increased Cl concentration (Chapra et al., 2009). This has been a recent concern and these results suggest that diatom species composition may be responding to this change in major ion chemistry. A long-term study examining chloride concentrations in Lake Simcoe tributaries found significantly increased Cl concentrations (1993-2007) (Winter et al., 2011). Concentrations from tributaries draining subwatersheds with the highest percentage of urban areas exceeded the water quality guideline to protect aquatic life for British Columbia, however, concentrations have not yet been exceeded Ontario guidelines (Winter et al., 2011). In another study, Winter reported that chloride concentrations were nearly significantly correlated with total snow depth and total precipitation (Winter et al., 2011). Winter et al., 2011 also reported that chloride concentrations in Lake Simcoe ranged from 36-40 mg/L (2007) and this study found concentrations 4 years later (2011) were about 13-23% higher, ranging from 44.4-45.1 mg/L. Lake Ontario and other lakes and reservoirs in the northeastern United States also found marked increases in chloride levels (Winter et al., 2011; Dixit et al., 1999). Dixit's study found that diatom species were closely related to pH, TP and Cl for July and August (Dixit et al., 1999). Evidence shows that Cl inputs must be reduced to avoid shifts in the diatom community structure in the future excluding chloride sensitive species.

Overall, the purpose of this study was to compare diatom assemblages collected during the ice-free and ice-covered seasons as well as compare the ice-free pelagic assemblages to the ice-free sediment trap assemblages at the four sampling sites. There were significant differences between the ice-covered and ice-free sediment trap assemblages as well as significant differences between the pelagic and adjacent sediment

trap samples. The pelagic samples although only preserved for at most a year in Lugol's iodine, were identified largely to genus and/or other general categories such as "unknown centric" due to degradation of the frustules. This raises a question about the phytoplankton preservation method for Lake Simcoe. Other questions: are we grasping the full picture of Lake Simcoe's primary production if we sample only part of the lake for part of the year? If the sites are relatively similar in diatom abundance, why are at least 8 pelagic sample sites enumerated separately? Since ice-free sediment traps capture late winter species (depending on deployment date) and benthic species, would sediment traps provide a better comparison to recent sediments in cores? It is arguable that using multiple sampling methods can provide a more comprehensive view of lake ecology as along as we are aware of what causes potential differences in the data.

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Chapter 3

Long-term Trends in Spring Diatom and Phytoplankton Biovolume and Community Composition for Lake Simcoe, Ontario 1980-2011

Abstract

The changes in magnitude and species composition of the phytoplankton and diatom spring blooms were analyzed for Lake Simcoe (1980-2011). There were no significant changes in the total phytoplankton or diatom biovolumes in the spring between the 1980s and the 2000s. Late sampling dates post-ice-out show evidence that the bloom may begin under the ice. There was also evidence of a shift from larger *Stephanodiscus* species to other genera such as *Cyclotella*, unknown centrics and smaller *Stephanodiscus* species. Average spring air temperatures in the two-week period following ice-out increased 2.4°C from 1994–2012 but it is unclear if warming temperatures have been detrimental to diatom growth. Spring diatom and total phytoplankton biovolumes were significantly correlated to total phosphorus (TP) and total Kjeldahl nitrogen (TKN) but not reactive silica (Si). However, Si concentrations were much higher in late winter when diatoms were not sampled. Si concentrations gradually declined until early summer when drawdown ended abruptly at temperatures between 15 and 20°C suggesting an abrupt termination of diatom growth. Abrupt termination may be caused by thermal stratification

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Introduction

Lake Simcoe has experienced multiple, interrelated stressors since the 1980s including nutrient enrichment, climate change and invasive species which have altered the phytoplankton biovolume and community composition (Young et al., 2010; Barbiero et al., 2006). This can have important consequences, as diatoms are an essential component of nutrient cycling supplying nutrients to the upper trophic levels and deeper waters and sequestering carbon from the atmosphere (Smetacek, 1999; Brett and Muller-Navarra, 1997). Diatoms dominate the phytoplankton biovolume in Lake Simcoe especially in the spring and the fall due to high silica concentrations and their morphological dependence on turbulent conditions to remain in the euphotic zone of the water column (Reynolds, 1980; Nicholls, 1976; Richardson et al., 1983).

Lake Simcoe is located geographically close to both the Laurentian Great Lakes (about 40 km from Georgian Bay) and Precambrian Shield lakes and is experiencing a number of similar stresses including climate change, excessive nutrient loading and invasive species (Evans et al., 1997; Lake Simcoe Science Advisory Committee, 2008; Fahnenstiel et al., 2010; Winter et al., 2011). Due to their relatively large size, proximity to each other and connectivity via the Trent-Severn waterway, the impacts of these stresses on Lake Simcoe may be similar to neighboring Great Lakes (Paterson et al., 2008). The surface-mixed layer of chlorophyll *a*, phytoplankton biomass and primary productivity in the water column decreased by 66%, 87% and 70% respectively in 2007-2008 compared to 1983-1987 in Lake Michigan (Fahnenstiel et al., 2010). Further,

diatoms in the deep chlorophyll layer decreased from >50% in 1983-1987 to < 5% in 2007-2008 (Fahnenstiel et al., 2010). The decline in the size of the spring diatom bloom may have been due to high rates of consumption by invasive mussels (*Dreissena bugensis* and *D. polymorpha)* (Fahnenstiel et al., 2010). The filtering rates of *Dreissena bugensis* were found to be 5 times the phytoplankton growth rates (Vanderploeg et al., 2001). Water chemistry changes such as increases in silica (57%) and nitrate (42%) concentrations are consistent with a reduction in utilization by phytoplankton (Fahnenstiel et al., 2010). Similarly, the phytoplankton biovolume of Lake Erie has also decreased by 80% since 1996 (Barbiero et al., 2006). Since the introduction of the *dreissenids,* April silica concentrations in the water column have doubled since 1960-1980 causing a decrease in diatom species richness and shifting the community composition from pennate and large centric diatoms to three dominant species with high silica requirements: *Aulacoseira islandica, Stephanodiscus hantschii and Stephanodiscus parvus* (Barbiero et al., 2006). The small softwater Precambrian Shield lakes (Blue Chalk, Chub, Crosson, Dickie, Plastic, and Red Chalk Main) have also experienced relative increases in chrysophyte algae in the lake driven by significant declines in diatom biovolumes from 1981-2003 (Paterson et al., 2008). However, these diatom declines were not caused by *dreissenids* whose expansion northward is limited by low calcium levels (Neary and Leach 1991; Ramcharan 1997). These recent changes in phytoplankton may have dramatic implications for higher trophic levels involving zooplankton and fish communities and may slow energy transfer to higher trophic levels thereby reducing system resilience to stressors (Conroy and Culver, 2004).

The main objective of this study was to analyze long-term trends in spring phytoplankton and diatom community composition from 1980 – 2011 at station K42 in Lake Simcoe. Specific questions include:

1) Did the total phytoplankton biovolume change significantly over time?

2) Did diatom biovolume change significantly over time? Did the relative abundance of diatoms change significantly? Did biovolumes correlate with physical and chemical variables?

3) Has there been a shift in the dominant taxa of phytoplankton at station K42 over time (1980-2011)?

4) How did the physical and chemical variables of the water column correlate with spring phytoplankton and diatom community composition?

Description of study area: Lake Simcoe

Lake Simcoe characteristics

Lake Simcoe is the largest lake in south central Ontario next to the Great Lakes with a surface area of 722 km^2 and a terrestrial watershed area of 2,899 km^2 (LSRCA and MOE, 2009). It is located north of Lake Ontario and east of Georgian Bay of Lake Huron (latitude 44°25'N and longitude 79°20'W) (Evans et al., 1996; Eimers et al., 2005). It is also an important link in the Trent-Severn Waterway, which extends from Lake Ontario to Georgian Bay.

Lake Simcoe is a, dimictic, hard water lake (Winter et al., 2007) (recent ice-free season alkalinity from 2009-2011 ranged from 113-116 mg/L $CaCO_3$). The lake is currently classified as mesotrophic with TP concentrations ranging from 11.2 to 17. 5 ug/L from 2009 – 2011. The lake is composed of the main basin (mean depth of 14 m, maximum depth of 33 m), Cook's Bay (mean depth 13 m, maximum depth of 15 m) and Kempenfelt Bay (mean depth 20 m, maximum depth of 42 m) (Fig. 3.1; Young et al., 2010). It has a flushing rate of approximately 11 years and drains north through a single outflow at the Atherley Narrows into Lake Couchiching (LSRCA and MOE, 2009).

Lake Simcoe as an important economic resource

Each year the lake generates approximately $200 million in revenues from its fishing, recreation and tourism industry indicating that Lake Simcoe is an economically important natural resource (Young et al., 2010). Its watershed encompasses twenty-three municipalities and provides drinking water to eight of them. It also assimilates wastewater from fourteen water pollution control plants (LSRCA and MOE, 2009). Its location is within commuting distance of the Greater Toronto Area, which is the fifth largest metropolitan area in North America. Rapid urbanization has resulted in land use changes and a more than doubling of the population in the Lake Simcoe watershed within the last two decades making it increasingly vulnerable to anthropogenic influences (Eimers et al., 2005).

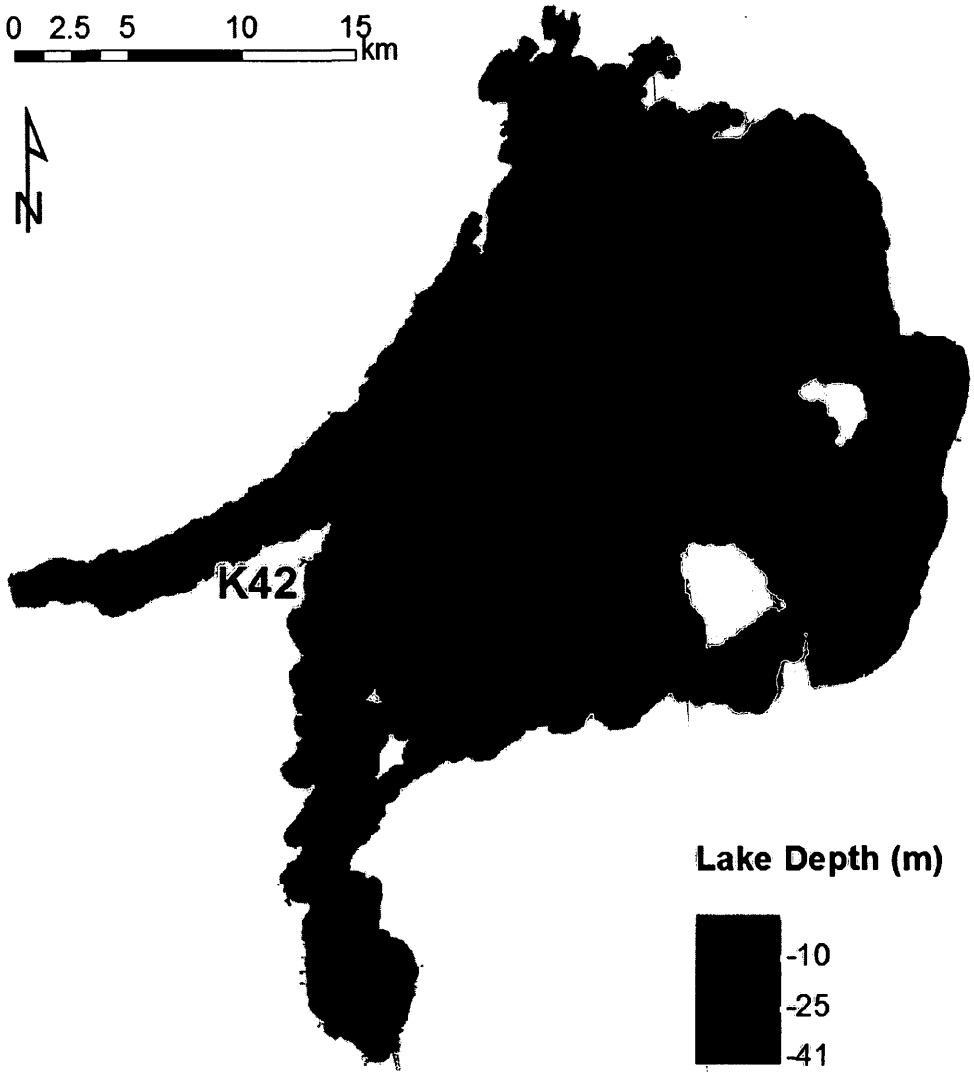

Fig. 3.1 Bathymetry map of Lake Simcoe showing the sub watersheds and site K42 sampling location (Eavan O'Connor, Lake Simcoe Region Conservation Authority). The map data may have been compiled from various sources. While every effort has been made to accurately depict the information, data/mapping errors may exist. The map has been produced for illustrative purposes only.

Methods

Phytoplankton sampling for Lake Simcoe began in 1971; however, samples were not consistently collected every year. Many of the samples were amalgamated into an annual ice-free composite sample and hence data for the first sample collected after ice-off are unavailable. Phytoplankton samples from the 1970s do not have the corresponding water chemistry data available and several different methods of sample collection were used. Prior to 1972, phytoplankton samples were analyzed by the municipal government and concentrated by sand filtration. Phytoplankton samples taken after 1972 were analyzed by the provincial government (the Ontario Ministry of the Environment) and concentrated by sedimentation, fixed with Lugol's iodine and preserved with formalin. Both methods of sampling involved phytoplankton taxa enumeration using the Segwick-Rafter cell method and compound microscopes at 200x magnification (Nicholls and Hopkins, 1993). For these reasons, data from the 1970s were eliminated from the trend analyses.

Spring phytoplankton and water chemistry were monitored in Lake Simcoe at site K42 for the years 1980, 1981, 1986, 1997, 2006, 2007, 2008, 2009, 2010 and 2011 by the Ontario Ministry of the Environment and the Lake Simcoe Region Conservation Authority (Fig. 3.1). The term "spring" was defined as the first sample taken after ice-off with the collection occurring in either April or May. The samples were taken approximately two to three weeks after ice-off; however, the date of collection varied and sampling times ranged from six to 30 days after ice-out (Table 3.1). Water samples from 1980-2011 were collected using a PVC hose through the euphotic zone with the

maximum collection depth determined as 2.5 times the Secchi disc depth (Winter et al., 2010).

Phytoplankton

Phytoplankton were fixed in the field using Lugol's iodine solution, concentrated to 25 ml via sedimentation and preserved using two drops of 37% formalin. They were counted using Utermöhl-like counting chambers and their biomass was expressed as a volume (mm^3/m^3) (Nicholls and Carney, 1979). Phytoplankton identification was primarily to the genus level, however, some were easily identified to the species level (Winter et al., 2010). Two measurements of phytoplankton community abundance were used in this study 1) actual biovolume (mm^3/m^3) and 2) relative biovolume (%).

Water chemistry variables

Integrated water samples (2.5 x Secchi depth) were collected at the same time as the ice-free pelagic phytoplankton samples using methods outlined for sampling Lake Simcoe by Ingram and Young (2010). The samples were analyzed using standard Ontario Ministry of the Environment methods (Janhurst, 1998; 1995; 1996; 2009 Performance Report: General Chemistry and Microbiology Section, published in 2010). The variables included nitrate and nitrite ($NO_3^- + NO_2^-$, abbreviated as NO), total Kjeldahl nitrogen (the sum of total organic nitrogen, ammonia and ammonium; (TKN)) (mg/L), silica (Si), total phosphorus (TP), conductivity and Secchi depth.

Climate variables

Kempenfelt Bay ice-out dates (1980-2011) were consistently made by Alex Mills (unpublished data) who made his observations in Barrie. The thaw date (i.e., ice-out) is the date in which there is no fixed ice within the end of the bay bounded by the city (there may be some floating sheets which are short-lived due to wind action). The mid-basin ice-free portion of the western bay associated with the (warmer) sewage treatment outflow was ignored for ice-out date observations.

Tributary flow data (total discharge m^3/s) was measured at the Beaver River station near Clarksburg Ontario (44° 31' 11" N, 80° 28' 4" W) 6 weeks prior to the ice-out date (http://www.ec.gc.ca/rhc-wsc/).

The average air temperature for the two-week period following ice-out from 1994-2011 was measured at the Lagoon City station (44° 32' 50" N, 79° 13' 0" W) (www.climate.weatheroffice.gc.ca).

Table 3.1 Sample dates for ice-off, pelagic phytoplankton and water chemistry samples taken at site K42 from 1980-2011. The number of days between the ice-off date and sampling date are shown.

Year	Ice-off	Phytoplankton	Physico-chemical	Difference (# days)
1980	April 12	April 30	May 20	18/38
1981	April 1	April 22	April 22	21
1986	April 6	May 5	May 22	29/46
1997	April 30	May 7	May 7	7
2006	April 13	May 8	May 6	25/23
2007	April 20	May 14	May 14	24
2008	April 20	May 20	May 6	30/16
2009	April 4	April 10	April 10	6
2010	April 4	April 20	April 20	16
2011	April 11	April 27	April 27	16

Pearson correlation analyses were conducted using Sigma Stat (version 3.1) statistical software to determine which of the six water chemistry variables were significantly related to the diatom and total phytoplankton biovolumes. Separate analyses were run with the diatom biovolumes including the data from 2009 (which was unusually high), excluding the data from 2009 and with the total algal biovolumes (diatom biovolume added to the other phytoplankton biovolumes).

Ordinations were performed on the first spring phytoplankton samples for each year using CANOCO version 4.5 for Windows (terBraak and Šmilauer, 2002). A detrended correspondence analysis (DCA) was performed in order to determine the gradient length (or beta diversity). The beta diversity was relatively low; gradient lengths were approximately 2 indicating that the species had a linear distribution along gradients. Ordination techniques such as PCA perform best at low beta diversity, hence, principal components analysis (PCA) was performed on the phytoplankton community

composition using 1) biovolumes for each taxa and 2) the relative abundances for each taxa to summarize the general trends of variation (Davis, 1986; Harper, 1999). The species scores were divided by the standard deviation and the square root taken to remove large effects created by dominant species. Rare species (those with abundances of less than 5% were downweighted because rare species have an inordinate influence on multivariate analyses (ter Braak and Šmilauer, 2002). For all of the ordinations, two axes accounted for the majority of the variation.

Physical and chemical variables were tested with the phytoplankton community biovolumes and relative abundances to determine if the species drift over time in the dominant species was related to changes in variables. Due to the short gradient lengths of the species data as determined by DCA, an RDA was chosen as the appropriate test for the linear constrained data sets. An RDA was performed singly constrained by each physico-chemical variable, which determines the significance of each variable to the species data set. Running an RDA using only the significant variables determines the significance of the overall ordination. Each analysis was also checked for variables with high VIFs (variance inflation factors) as these show co-linearity between variables. Variables with high VIFs were eliminated from the analysis so as to not overestimate the amount of variance explained.

Results

Changes in spring phytoplankton abundance (1980-2011)

Diatoms dominated the spring phytoplankton community samples in 1980, 1981, 2006, 2008, 2009, 2010 and 2011 (Fig 3.2; 3.3). In the spring of 2009, total phytoplankton and diatom biovolumes (mm^3/m^3) were unusually high; hence, this sample was treated as an outlier and was removed from several subsequent analyses. However, diatom relative abundance, while high, was not anomalously so (Fig. 3.3.) Overall from 1980-2011, the total phytoplankton biovolume increased very slightly ($r = 0.103$ including 2009 and 0.078 excluding 2009) (Table 3.2). The diatom biovolumes also increased slightly over time ($r = 0.097$ including 2009 and $r = 0.030$ excluding 2009), as well as diatom relative abundance ($r = 0.044$ including 2009 and $r = 0.0007$ excluding 2009) (Table 3.2).

Table 3.2 Pearson linear correlation coefficients (r) for spring diatom, other phytoplankton and total phytoplankton abundance for 1980-2011 at site K42.

	Including 2009	Excluding 2009
Diatom biovolume (mm^3/m^3)	0.097	0.030
Diatom relative abundance (%)	0.044	0.001
Total phytoplankton biovolume (mm^3/m^3)	0.103	0.078

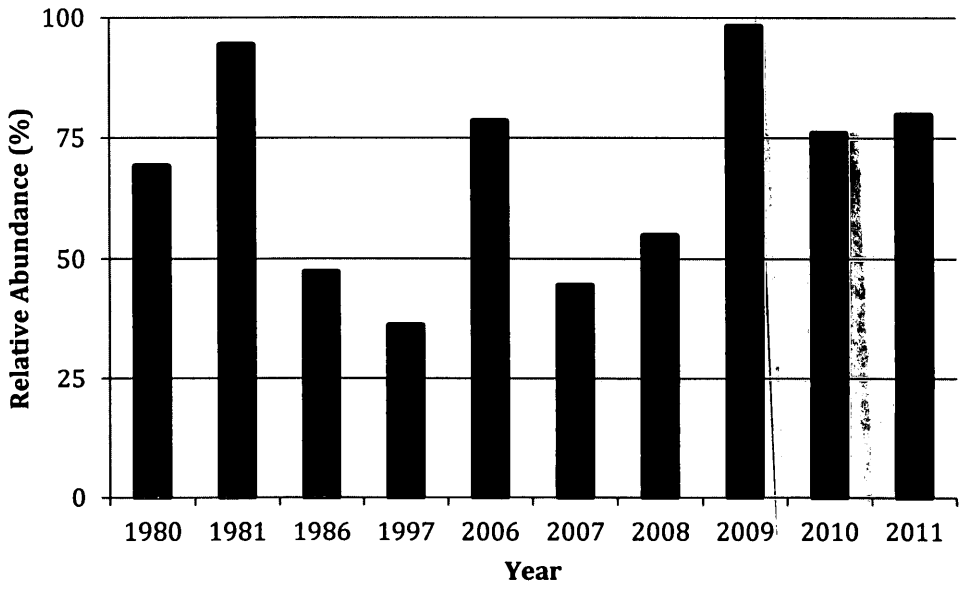

Fig. 3.2 Relative biovolume abundance (%) of diatoms for site K42 in the spring using the first sample collected after ice-off (typically in April or May for each of the sample years) from 1980-2011.

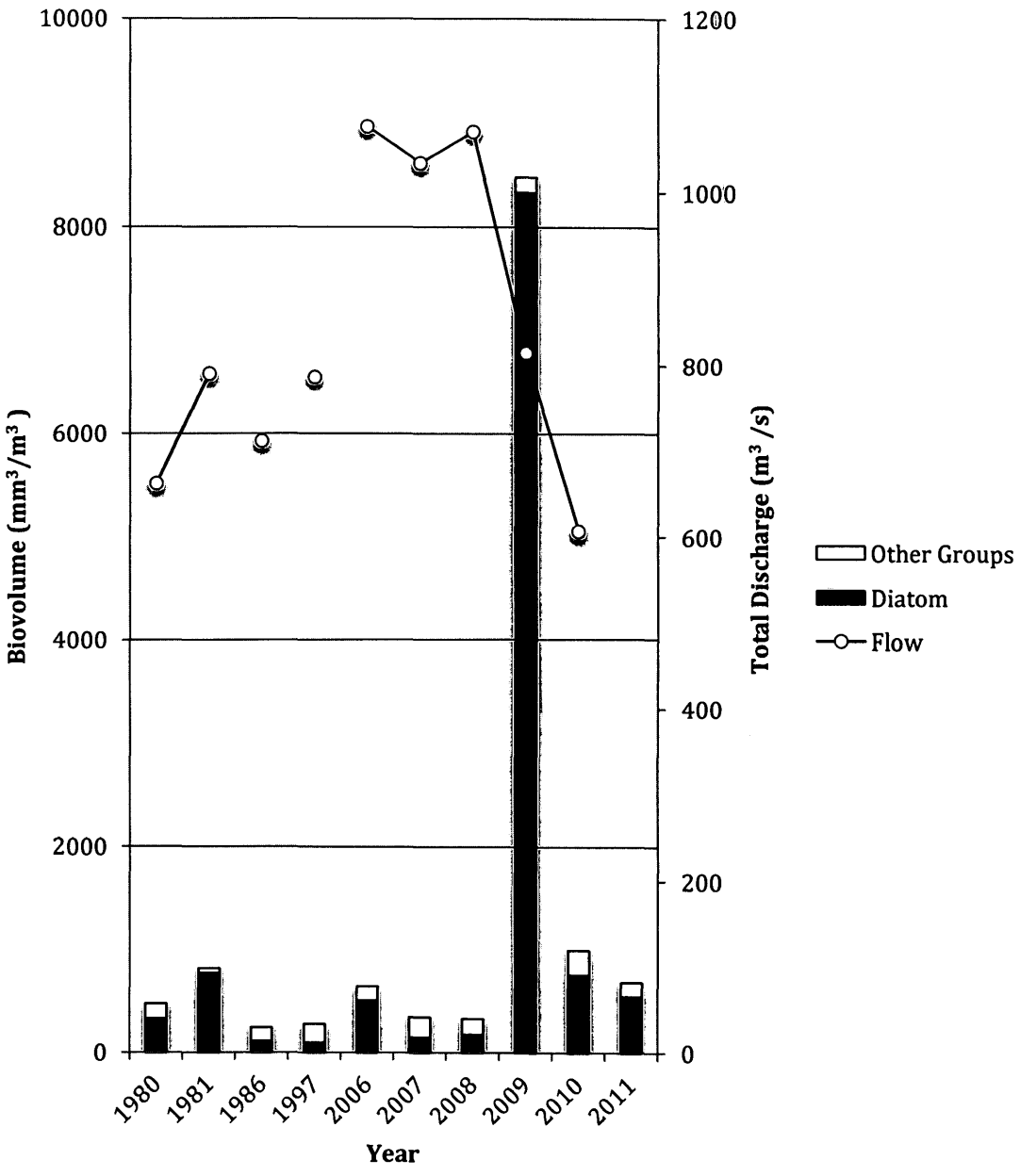

Fig. 3.3 Total phytoplankton biovolume (mm^3/m^3) and diatom biovolume (mm^3/m^3) for site K42 in the spring using the first sample after ice-off (typically in April or May) from 1980-2011. Total water discharge (m^3/s) for 6 weeks before ice-out at the Beaver River station was shown as a line.

Principal components analysis (PCA) revealed that the phytoplankton biovolumes of each taxa for 2009 were different from the other years, however, the SIMPER analysis revealed that the 2009 assemblage composition was not statistically significant from the other years ($p = 0.099$) (Fig. 3.4 A and B). The 2009 sample was removed solely for the purpose of viewing the other clustered years (Fig 3.4 C). When grouped, 1980s compared to 2000s, phytoplankton taxa biovolumes were not statistically different from each other ($p = 0.583$ including 2009; $p = 0.326$ excluding 2009) (Fig. 3.4 C and D).

The same analyses (PCA and SIMPER) were carried out using the relative abundance data and similar results were found. When compared independently, the relative phytoplankton taxa biovolumes of each year (1981-2011) were not statistically different from the other years ($p = 1$ for all years) (Fig. 3.5 A). A total of 76 taxa were identified in the 10 samples (1980-2011). Species representing < 10% of the relative abundance for each sample were eliminated from the figures for clarity resulting in 14 taxa remaining (Fig 3.4 B and D; Fig 3.5 B). Of the 14, 10 were diatoms (*Asterionella* (AS), *Cyclotella* (CY), unknown centric diatom (CD), *Fragilaria* (FR), *Stephanodiscus* spp (ST), *Stephanodiscus binderanus* (SB), *Stephanodiscus* large (SL), *Stephanodiscus* small (SS), *Synedra* (SY) and *Tabellaria* (TA)) and the other four were cryptomonads (*Cryptomonas* (CR) and *Rhodomonas* (RH)) and dinoflagellates (Dinophyceae (DI) and *Gymnodinium* (GY)).

When grouped, 1980s compared to 2000s, the phytoplankton relative abundances were not statistically different ($p = 0.753$ including 2009; $p = 0.264$ excluding 2009) (Fig. 3.5 A). The diatom genus, *Stephanodiscus* (large species), was dominant throughout the

1980s constituting up to 86% of the spring sample. However, this taxon was present as a dominant species only once in 2006-2011. From 2006 to 2011 the dominant species were as follows: 2006 (38.2% *Synedra*), 2007 (23.1% *Fragilaria* and 12.4% *Tabellaria*), 2008 (21.9% *Asterionella* and 15.7% *Stephanodiscus* (small)), 2009 (78.1% *Stephanodiscus* (large) and 19.9% *Cyclotella*), 2010 (64.1% *Stephanodiscus* (small)) and 2011 (42.5% *Stephanodiscus* (small) and 22.3% unknown centrics). Overall, there was a shift from larger *Stephanodiscus* species to other genera such as *Cyclotella*, unknown centrics and smaller *Stephanodiscus* species over time.

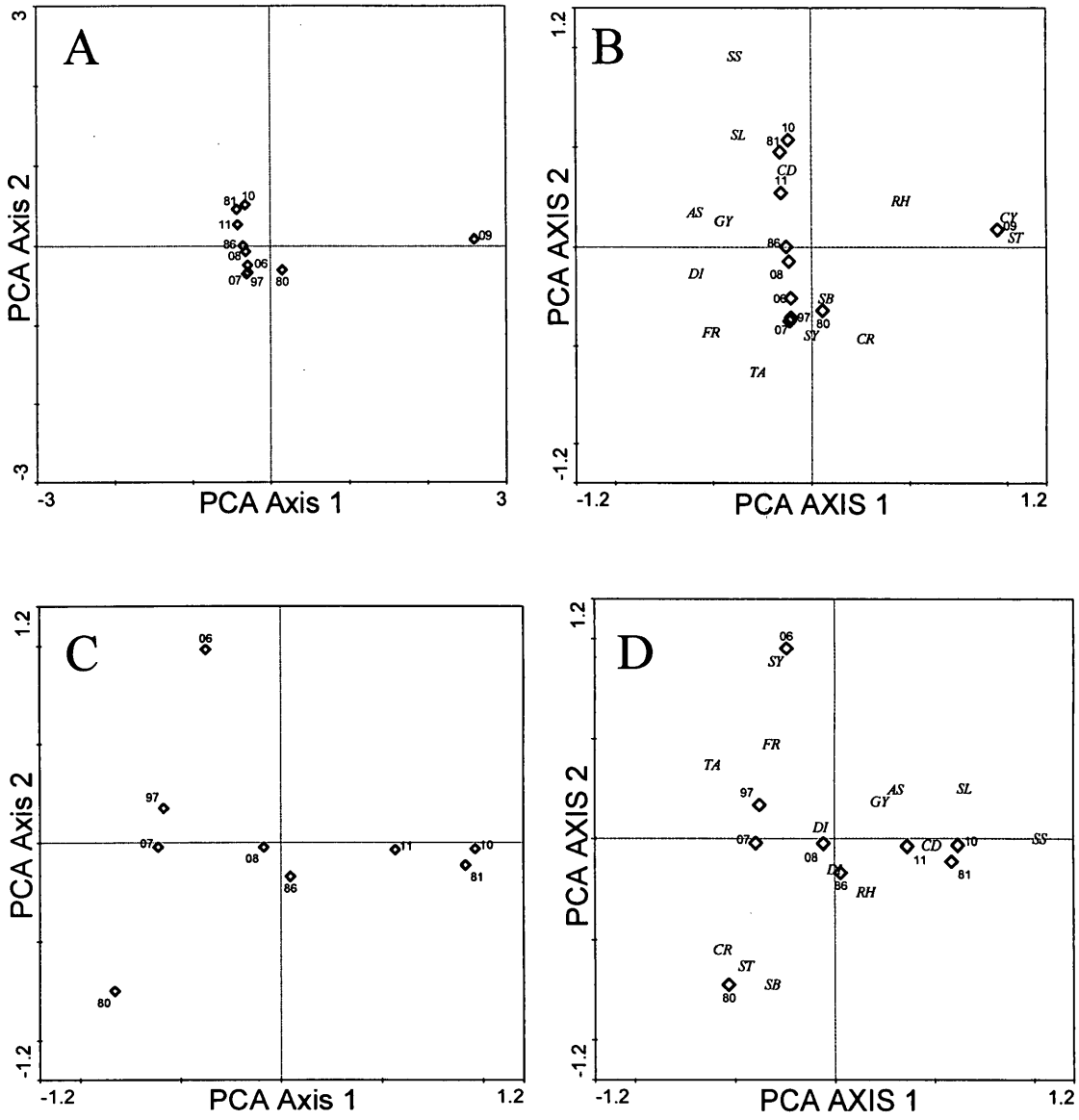

Fig. 3.4 PCA comparing biovolumes (mm^3/m^3) for all spring phytoplankton taxa of station K42 A) for all years including 2009, B) for all years including 2009 with the phytoplankton taxa shown, C) excluding 2009 and D) excluding 2009 with the phytoplankton taxa shown. Species representing greater than 10% of a sample are included. Species acronyms are listed in Appendix B. The percent variation explained by the first two axes for all years including 2009 and excluding 2009 was 86.7% and 58.2% respectively.

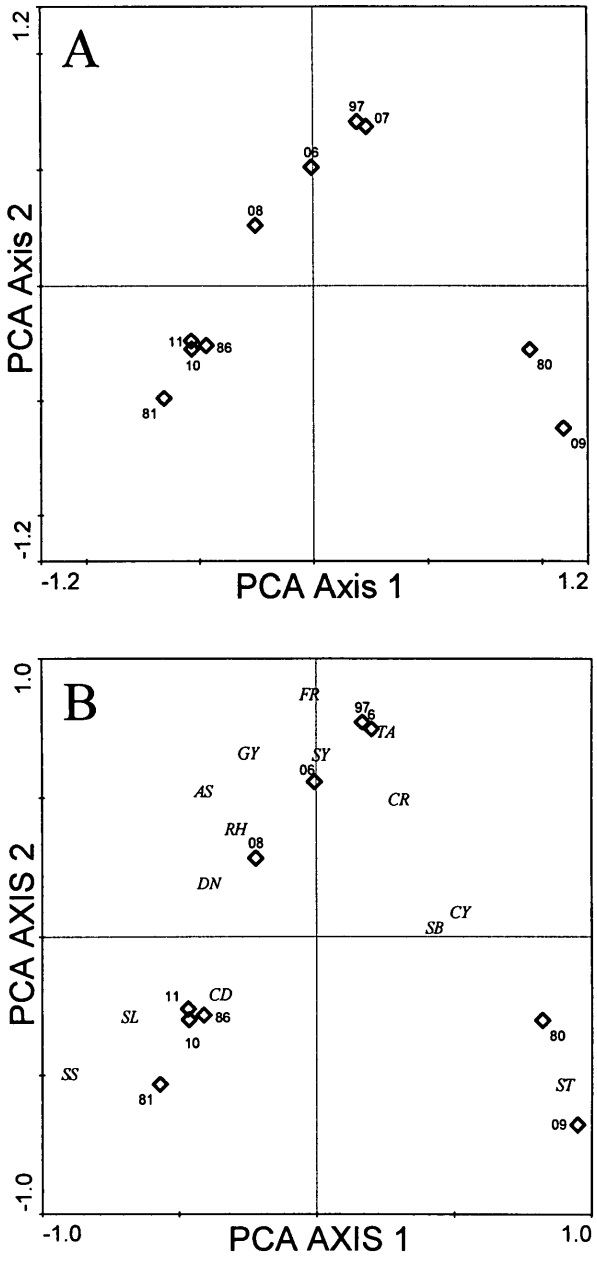

Fig. 3.5 PCA comparing relative abundances (%) for all spring phytoplankton taxa of station K42 for all available years (1980-2011), A) sample years shown and B) biplot showing sample years and phytoplankton taxa shown. Species representing greater than 10% of a sample are shown. Species acronyms are listed in Appendix B.

Spring phytoplankton abundance in response to physico-chemical variables

Six physico-chemical variables were included in the analyses to attempt to explain the variation in the phytoplankton taxa abundances over time (Table 3.3). Of the six variables, TKN was significantly correlated with total phytoplankton biovolume ($r = 0.708$ and 0.668, respectively, when the 2009 data were included) (Table 3.4). TKN was significantly correlated with diatom biovolume ($r = 0.035$ and 0.033, respectively, when the 2009 data were excluded) (Table 3.5). TKN is primarily organic N (Table 3.3) and the high correlation may be an indicator of high planktonic biomass in clear waters. TP was significantly correlated with both total phytoplankton biovolume and diatom biovolume ($r = 0.728$ and 0.735, respectively, with the 2009 data removed, (Table 3.4; Table 3.5).

Table 3.3 Summary table of the 6 measured physico-chemical variables for the first spring sample (taken approximately 2 weeks after ice-off) at site K42 for years 1980-2011.

Year	$NO_2 + NO_3$ (µg/L)	TKN (µg/L)	Si (mg/L)	TP (µg/L)	Secchi (m)	Conductivity (uS/cm)
1980	0.03	0.40	0.65	20.0	5.5	335
1981	0.05	0.35	1.00	28.0	3.3	350
1986	0.04	0.40	0.44	13.0	7.3	336
1997	0.15	0.46	1.80	16.0	6.0	
2006				12.4	8.0	
2007	0.07	0.42	0.92	12.3	8.0	378
2008	0.14	0.40	1.58	16.6	6.0	407
2009	0.17	0.51	1.32	18.0	3.0	403
2010	0.07	0.37	1.96	28.0	5.5	403
2011	0.07	0.41	1.90	15.0	6.0	405

Table 3.4 Summary table of Pearson's linear correlation analysis (r) of total phytoplankton biovolumes and physic-chemical variables including (top row) and excluding (bottom row) the 2009 data. For pairs with P values greater than 0.050, there is no significant relationship between the two variables. Significant values are shown in boldface.

	$NO_2 + NO_3$	TKN	Si	TP	Secchi	Conductivity
Correlation Coefficient	0.540 -0.305	**0.708** -0.679	0.062 0.413	0.075 **0.728**	-0.622 -0.485	0.350 0.311
P Value	0.133 0.463	**0.033** 0.064	0.876 0.309	0.838 **0.027**	0.055 0.185	0.395 0.497
Number of Samples	9 8	9 8	9 8	10 9	10 9	8 7

Table 3.5 Summary table of Pearson's linear correlation analysis (r) of diatom biovolume and physic-chemical variables including (top row) and excluding (bottom row) the 2009 data. For pairs with P values greater than 0.050, there is no significant relationship between the two variables. Significant values are shown in boldface.

	$NO_2 + NO_3$	TKN	Si	TP	Secchi	Conductivity
Correlation Coefficient	0.536 -0.353	**0.703** **-0.749**	0.053 0.321	0.077 **0.735**	-0.629 -0.562	0.342 0.206
P Value	0.137 0.391	**0.035** **0.033**	0.892 0.438	0.833 **0.024**	0.052 0.116	0.407 0.657
Number of Samples	9 8	9 8	9 8	10 9	10 9	8 7

Data were transformed and tested for normality using the Shapiro-Wilk normality test before using RDA (Table 3.6). RDA revealed that out of the seven physico-chemical variables, TKN was the only variable significant to the analysis when tested against the actual phytoplankton biovolumes (mm^3/m^3) and the relative phytoplankton abundances (%) (Fig.3.6). Monte-Carlo permutation tests for significance (999 permutations) confirmed this ($p = 0.046$ and 0.05 respectively) (Table 3.7). Together axis 1 and 2 explain 86.2% of the variation in the phytoplankton biovolumes and 50.4% of the variation in the phytoplankton relative abundance (%) data respectively. Removing the 2009-phytoplankton abundance data resulted in no significant physic-chemical variables to explain the variation (Table 3.7).

Table 3.6 Results for Shapiro-Wilk normality tests for physic-chemical data for station K42 from 1980-2011including and excluding 2009. Variables with a normal distribution ($P < 0.05$) were shown in bold face.

Variable	Including 2009	Excluding 2009
Conductivity	**0.031**	0.082
NO_2+NO_3	**0.007**	**0.003**
Si	0.106	0.164
TKN	**0.044**	0.147
TP	**0.045**	0.058
Secchi	**0.034**	**0.031**

Table 3.7 P-values of Monte-Carlo permutation tests for significance (999 permutations) for RDA using the actual phytoplankton taxa biovolumes (mm^3/m^3) and relative abundance (%) for station K42 from 1980-2011 including 2009 and excluding 2009 data. Significant values were shown in bold face.

	Including 2009		Excluding 2009	
Variable	Actual Values	Relative Values	Actual Values	Relative Values
Conductivity	0.758	0.758	0.256	0.348
$NO_2 + NO_3$	0.107	0.107	0.735	0.391
Si	0.752	0.752	0.391	0.498
TKN	**0.046**	**0.046**	0.119	0.253
TP	0.670	0.493	0.141	0.573
Secchi	0.079	0.399	0.232	0.424

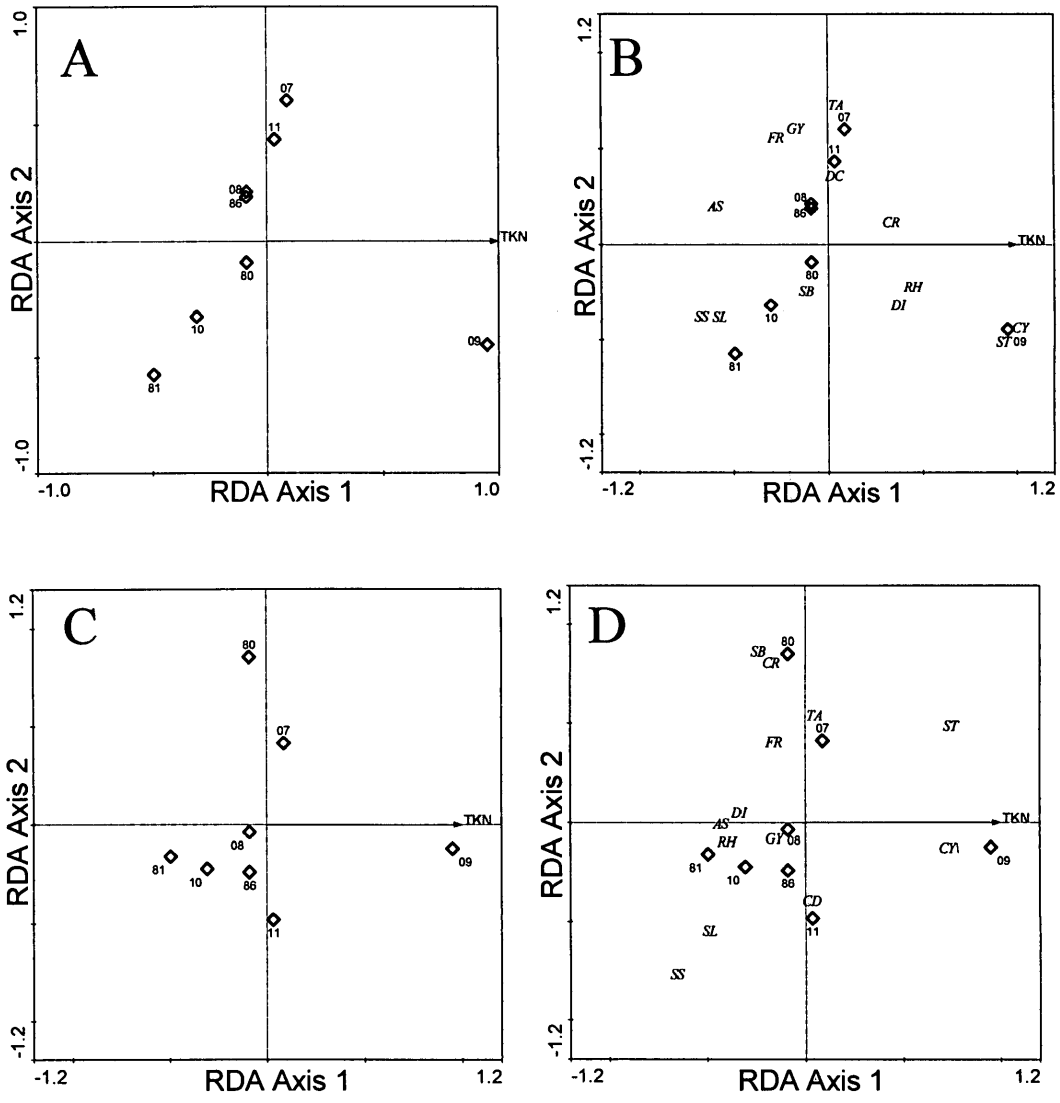

Fig. 3.6 RDA ordinations comparing spring physic-chemical variables to all phytoplankton data (1980-2011) for site K42 using A) taxa biovolume data (mm^3/m^3), B) taxa biovolume data (mm^3/m^3) and phytoplankton species shown, C) relative abundance data (%) and D) relative abundance data and phytoplankton species shown. The arrows indicate physic-chemical gradients that correlate with the distribution of phytoplankton data. Species representing >10% of the overall abundance are shown. Species acronyms are listed in Appendix B. The percent variation explained by the first two axes for the phytoplankton biovolumes and phytoplankton relative abundance was 82.6% and 50.4% respectively.

Spring phytoplankton biovolumes in response to sampling days after the ice-off dates and climate variables

In addition to the physicochemical variables, timing of the sampling after the ice-off date and climate variables such as changes in the dates of the ice-off period, flow rates, average water temperature (^{o}C) and silica concentrations under the ice were examined in order to further explore the unexplained variation in the spring phytoplankton.

Pre-ice out flow rates of Beaver River, a major tributary to Lake Simcoe, were not significantly correlated with phytoplankton or diatom biomass ($r = - 0.081$ and $r = - 0.079$, respectively, including the 2009 data, and $r = - 0.320$ and $r = - 0.292$, respectively, excluding the 2009 data). This indicates that large fluxes of material typically associated with high runoff during the spring did not influence the size of the bloom post-melt.

There was a negative relationship between total diatom biovolume and the number of sampling days between ice-out date and sampling date ($r^2 = 0.317$) (Fig. 3.7) although the negative slope was probably driven by one point. Similarly, there was a negative relationship between Si concentration and the number of sampling days between ice-out date and sampling date ($r^2 = 0.24$) (Fig. 3.8). Together, these suggest that diatom growth continues post-melt, which continues to deplete Si from the water column but the population size declines because settling and grazing exceed growth. In fact, many of the spring samples may have been collected too late to capture peak diatom biovolumes. The hypothesis that post-melt Si concentrations had already been reduced by late winter

diatom blooms is supported by high Si concentrations measured 1 m under the ice at 10 inshore stations during 2010/11 (Fig. 3.9). Concentrations reached 2.5 to 4 mg/L in January and February, declining to < 2 mg/L by late April. These high concentrations may have been caused by high concentrations in tributary inflows or exclusion from ice as it formed. While it is unclear whether high Si concentrations occurred deeper than 1 m, the euphotic zone is shallow under the ice and would have encompassed mostly Si-rich waters.

In 2007 and 2008 Si concentrations gradually declined after ice out, stopping in late June when the water temperature at 5 m reached approximately 15 to 20°C (Fig. 3.10). Since the Si analysis captures only extracellular Si, these results suggest that removal of Si by diatoms in Lake Simcoe is continuous during the spring, and is terminated during early summer.

Ice-out occurred earlier in 2006-2011 compared to 1980-1986 (Table 3.1). The average air temperature calculated for the two weeks after ice-out increased 2.4°C from 1994-2012 ($r^2 = 0.020$) (Fig 3.11) indicating that ice-out is occurring earlier and warming in the immediate post ice-off period has become more rapid.

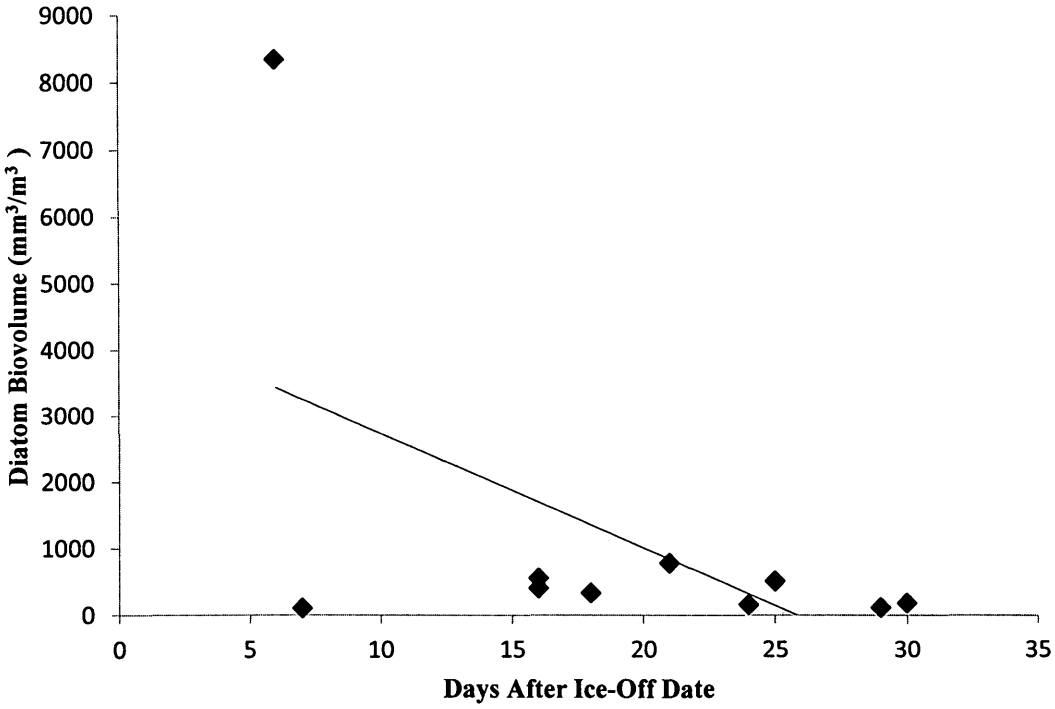

Fig 3.7 Diatom biovolume vs number of days between ice-off and the first sampling date ($r^2 = 0.317$).

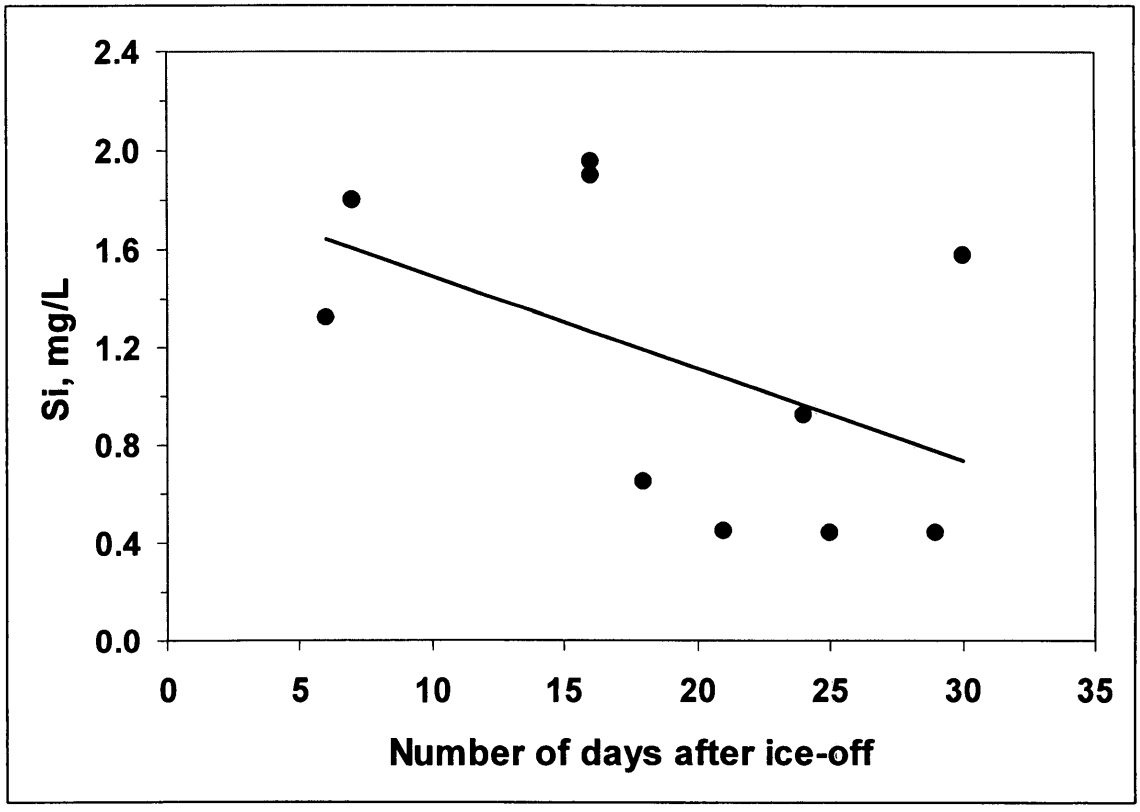

Fig 3.8 Silica concentration vs number of days between ice-out and number of sampling days between ice-out and the first sampling date for study years between 1980-2011. The Pearson correlation r = - 0.49.

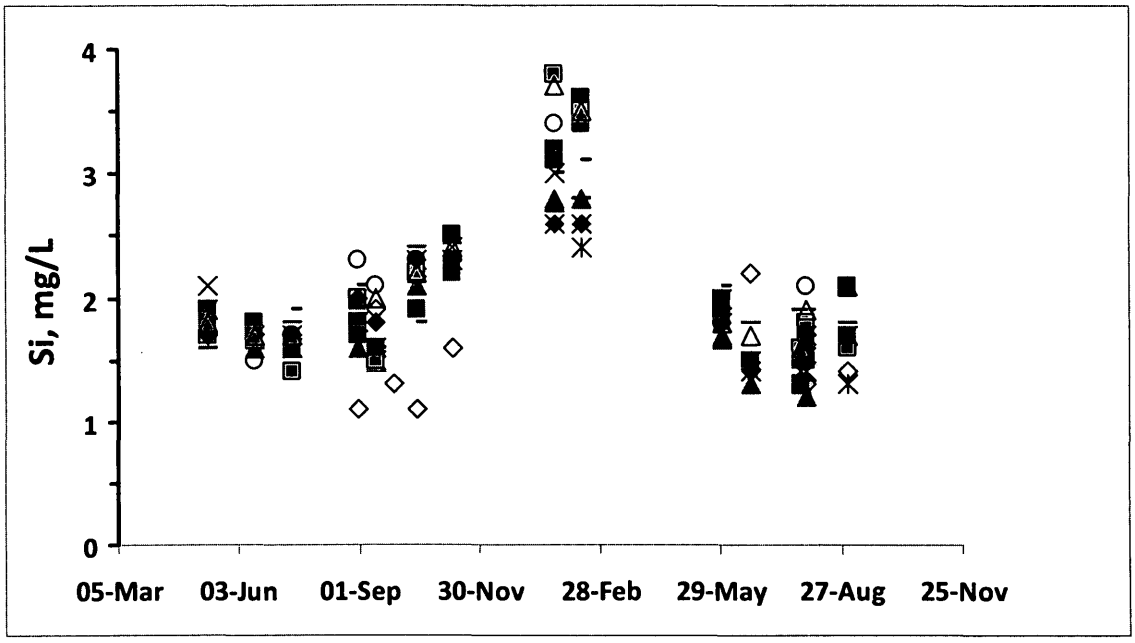

Fig. 3.9 Si concentration at 10 inshore stations in 2010/11.

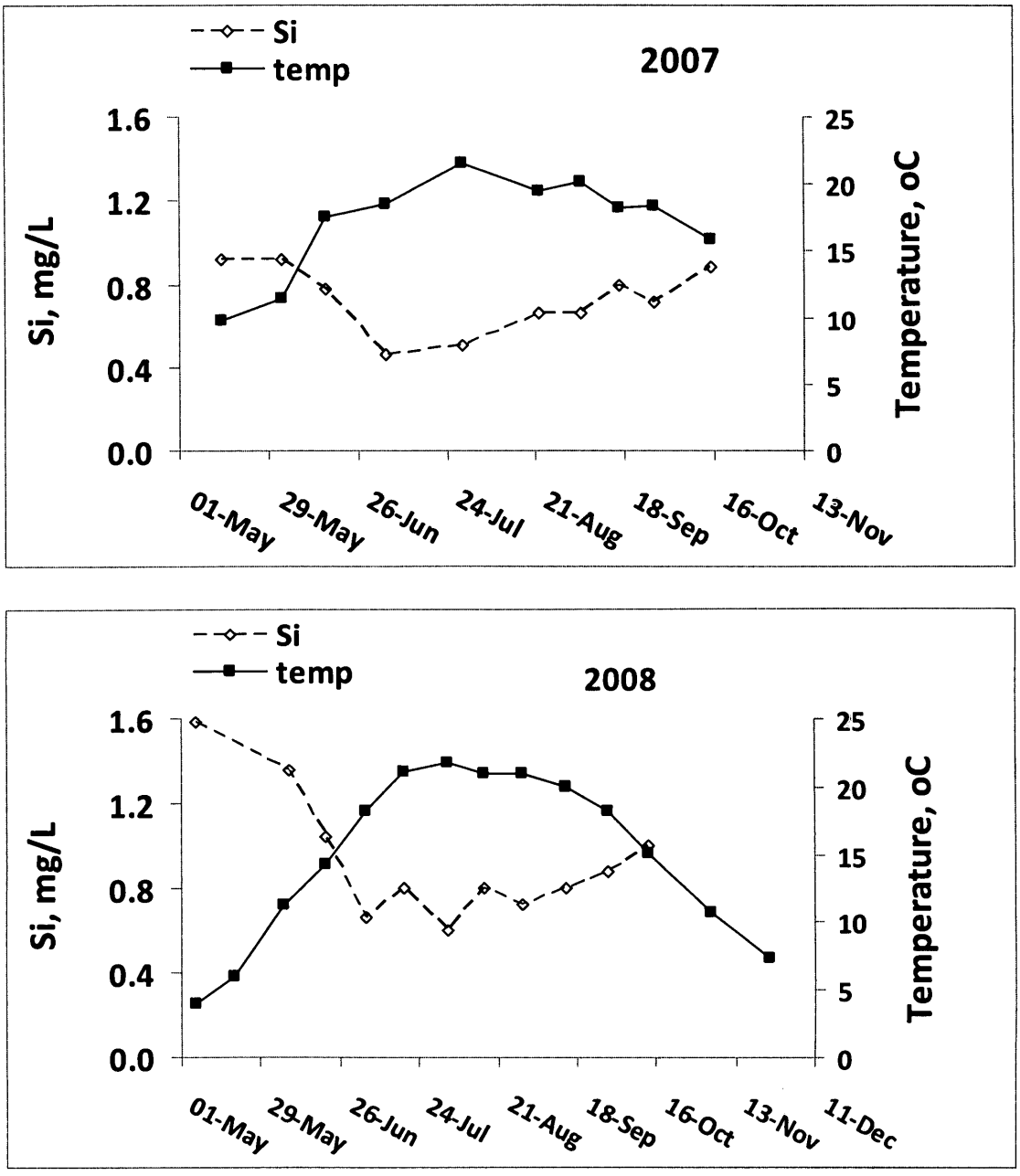

Fig 3.10 Si concentration during the ice-free period and water temperature at 5 m (°C) in 2007 and 2008 at station K42.

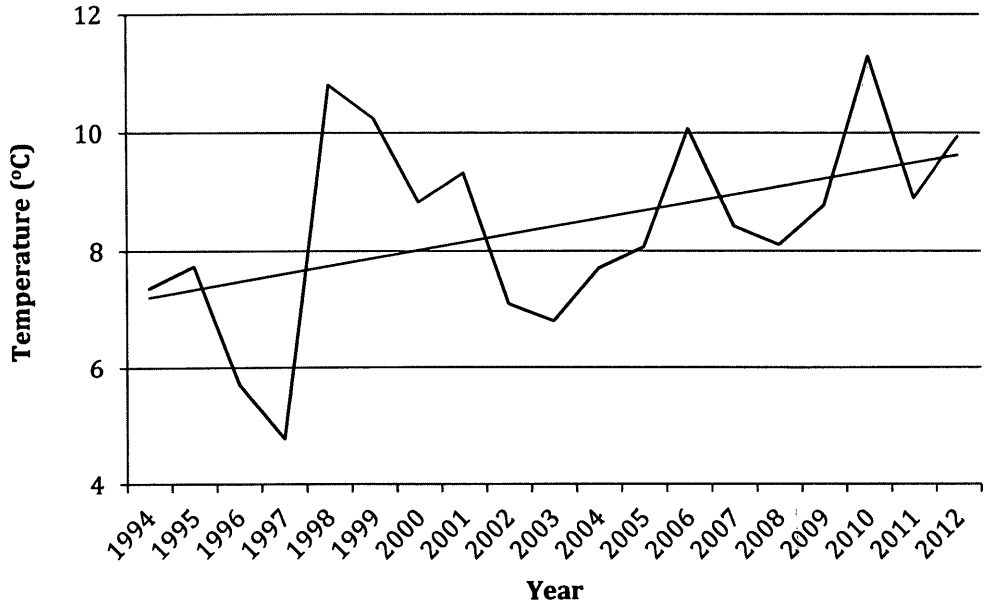

Fig 3.11 Average air temperature (°C) calculated for the two weeks after ice-out (1994-2012). A line of best fit is shown.

Discussion

While spring diatom blooms occurred annually (dominant in all years except 1986, 1997 and 2007) in Lake Simcoe, spring diatom blooms were reported absent from Lake Michigan in recent years (Fahnenstiel et al., 2010; Mida et al., 2010; Vanderploeg et al., 2010). This study found no significant trends over time in the spring total phytoplankton diatom abundance or diatom relative abundance in Lake Simcoe, which contrasts to the Great Lakes. Declines in phytoplankton densities have been reported in the western basin of Lake Erie (Nicholls and Hopkins, 1993), decreased total phytoplankton biovolumes (approximately 20%) in the eastern basin since 1996 (Barbiero et al., 2006) and significant decreases in bacilliariophyta and total phytoplankton biomass in the western, central and eastern basins of Lake Erie in the spring and summer since 1983 (Makarewicz et al., 1999). Fahnenstiel et al. (2010) and Mida et al. (2010) similarly reported large declines in spring phytoplankton abundance in southern Lake Michigan such that the offshore pelagic zone (historically mesotrophic) is now comparable to oligotrophic Lake Superior. Diatoms as well as other groups experienced dramatic reductions in 2007-2008 (Fahnenstiel et al., 2010).

Also, there also no significant changes in the dominant taxa (defined as >10% of the biomass) in Lake Simcoe from the 1980s to the 2000s. There was however, evidence of a shift from larger *Stephanodiscus* species to other genera such as *Cyclotella*, unknown centrics and smaller *Stephanodiscus* species. This is consistent with other studies claiming that lake warming will shift spring diatom dominant species from pennant and large centric diatoms to small centric forms such as small *Aulacoseira islandica*,

Stephanodiscus hantzschii, and *Stephanodiscus parvus* (Barbiero et al., 2006; Winder et al., 2009). These findings further support the notion that warmer temperatures favour small-sized diatoms (Winder et al., 2009) indicating that climate change may be influencing the spring phytoplankton assemblages.

Total phytoplankton and diatom biovolumes for 2009 were unusually large. The RDA and Pearson correlation analyses revealed that 2009 was defined by a significant nitrogen gradient (TKN). Most TKN is probably organic N and represented a high concentration of organic N in the bloom. TP concentrations were not exceptionally high in 2009 and neither were pre-ice-out flow rates in the Beaver River. This suggests that other variables not included in this study are responsible for the dramatic bloom in 2009.

It is interesting that the only significant relationships were correlations between TKN and total phytoplankton biovolumes (only if the 2009 data was included) and between TP and diatom biovolumes (only when the 2009 data was excluded). Phytoplankton biovolumes were not correlated with Si concentrations which isn't surprising since the type of analysis used does not measure cellular Si and the data suggest that much of the dissolved Si had already been removed by the diatom community before sampling began. Extracellular Si concentrations did increase since the introduction of the *Dreissena rostriformis bugensis* (zebra mussels) which has consistently recorded across the Lake Simcoe and Great Lakes literature (Winter et al., 2011; Barbiero et al., 2006; Mida et al., 2010). It has been well documented that nutrient concentrations (importantly Si and P) influence diatom assemblages (Tilman, 1977). Strong correlations have been found between pooled ice-free diatom assemblages and

variables including TP, TN and Si (1980-2007) (Winter et al., 2011). In contrast, the spring bloom (total phytoplankton and diatom biovolumes) at K42 was apparently not related to the measured physico-chemical variables and therefore did not explain the variance in spring diatom biovolumes over time.

There are several plausible explanations for the lack of correlations between physico-chemical and phytoplankton variables. First, the biovolume and chemistry data for each spring are derived from sampling on only one day at one station. Also, from 1980-2011, only 10 years of sampling data for both phytoplankton and chemistry were available. The data set was further constrained by missing data for the remaining years (i.e., conductivity data was missing for 1997 and all physico-chemical variables were missing for 2006 except TP and Secchi). Implicit in this design is the assumption that values collected six to thirty days after ice-out represent the peak or mean values for the bloom period, which may not be correct. Most samples were collected more than six days after ice-out. If spring blooms begin to diminish shortly after ice-out as the lake warms up, samples collected more than two weeks after ice-out may not be representative of either peak or mean spring biovolumes and water chemistry. Therefore, the large spring bloom of 2009 may not be an outlier but instead the most accurate representation of the magnitude of an average spring bloom because it occurred only six days after the ice-out date. Indeed, there is some evidence that the spring bloom in Lake Simcoe begins in late winter under ice (Rebecca North, pers. comm.). However, the phytoplankton biovolume was not unusually large in 1997 although sampling occurred within seven days of ice-out. Third, the average air temperature during the two weeks after ice off increased

2.4°C between 1994 and 2012. Hence, the duration of the spring bloom may have been shorter in recent years because of increasing water stability resulting from warmer air and water temperatures. Fourth, phytoplankton and physico-chemical sampling did not always occur on the same day. Physico-chemical samples were collected 20 days and 17 days after the phytoplankton sample in 1980 and 1986, respectively, and 14 days before in 2008. It appears that the sampling dates did not lend themselves well to the analysis of the spring phytoplankton or diatom blooms. This suggests that spring bloom sampling programs should include multiple sampling days, start in late winter while the ice is on if possible (logistically difficult and dangerous) and include the one-week period after ice-out.

The spring bloom probably contributes a large amount of organic matter to the sediment at the beginning of the ice-free season. Hence, factors that influence the size and duration of the spring bloom such as magnitude of spring runoff can potentially affect hypolimnetic oxygen concentrations later in the summer.

The abrupt end to the decline in Si concentration in early July in 2007 and 2008 (Fig 3.10) was likely due to a large and sudden drop in diatom growth rather than an increase in Si inputs to the lake counterbalancing loss to diatoms. The sudden halt to Si loss occurred when surface temperatures reached 15-20°C coinciding with onset of thermal stratification. Possible explanations for an abrupt end to spring diatom blooms include nutrient depletion, increased grazing pressure from zooplankton (Lund, 1965), enhanced aggregation during the late bloom phase (Engel, 2000), density and temperature

dependent parasite infections (Ibelings et al., 2011) and decreased mixing energy needed to keep diatoms suspended (Reynolds, 1973).

It was the intention of this project to analyze historical diatom samples to species in an attempt to explain more variation, however, they were identified only to genus because of sample degradation (Winter et al., 2011). The archived samples had been preserved in acidic Lugol's iodine and much of the diatom material was digested making the new counts less reliable than the historical counts.

To date, no work had been done on seasonality of Lake Simcoe's phytoplankton community. Despite the limitations described in this chapter, this study contributes to our understanding of Lake Simcoe. It also highlights the methodological limitations of the current sampling program and serves as a guide for improvements. Studies such as this emphasize the importance of long-term reliable data sets for scientific research purposes and ultimately future management decisions.

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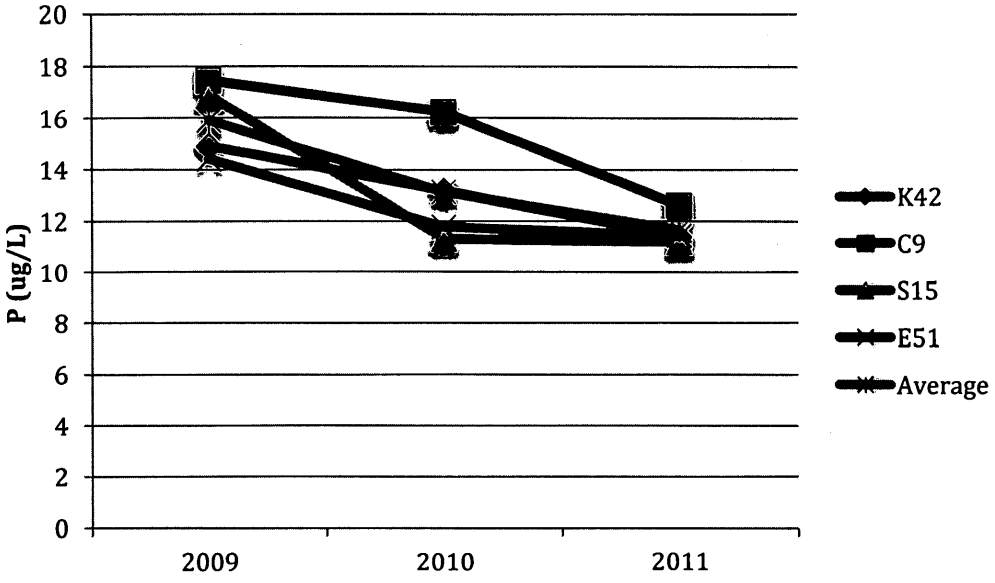
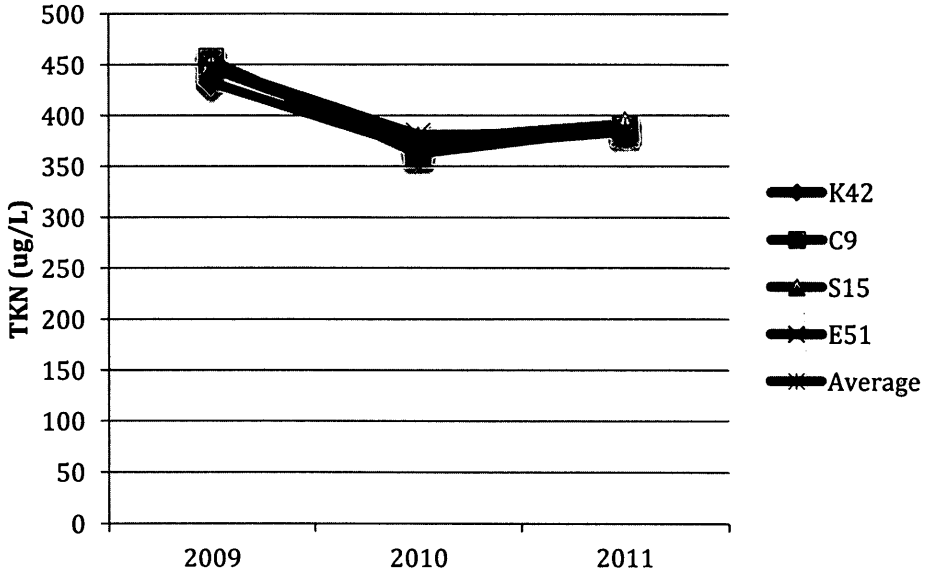
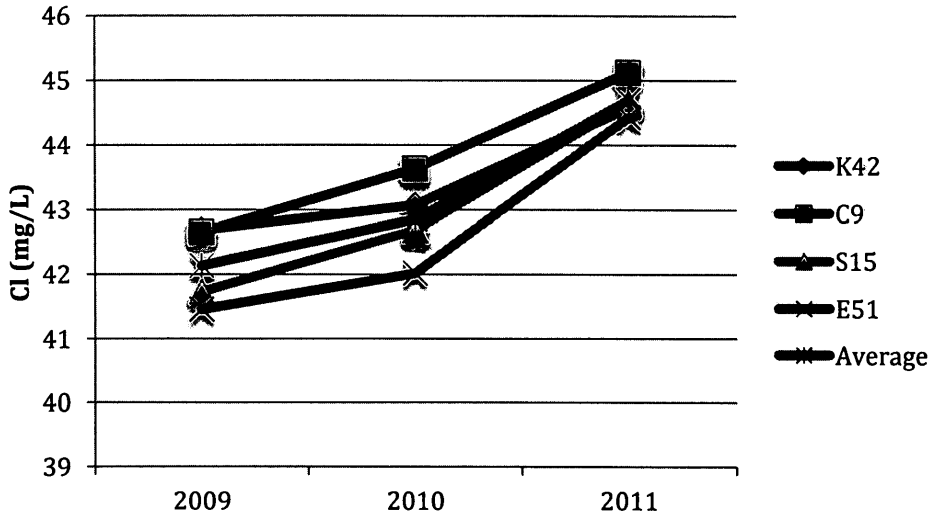
Appendix A. Plots of 11 physico-chemical variables for sites K42, C9, S15 and E51 from 2009-2011.



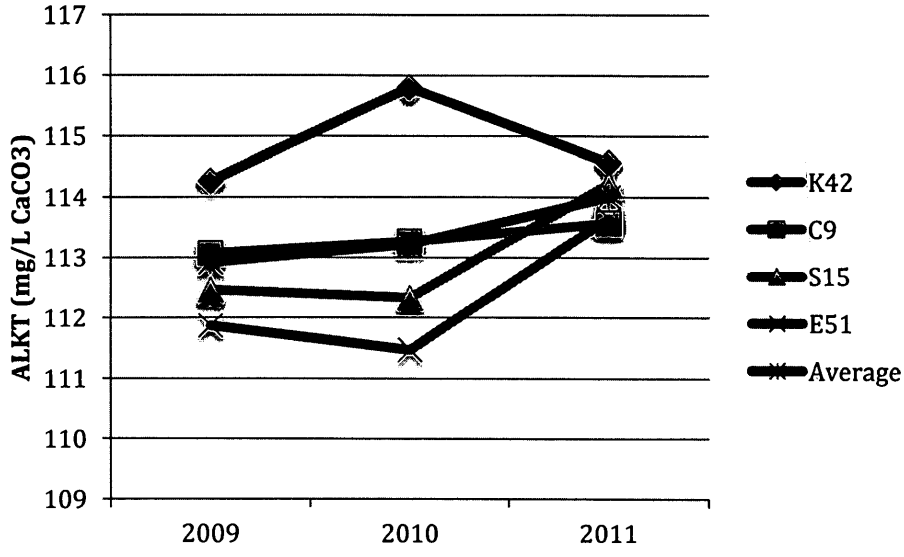
A. Phosphorus ug/L



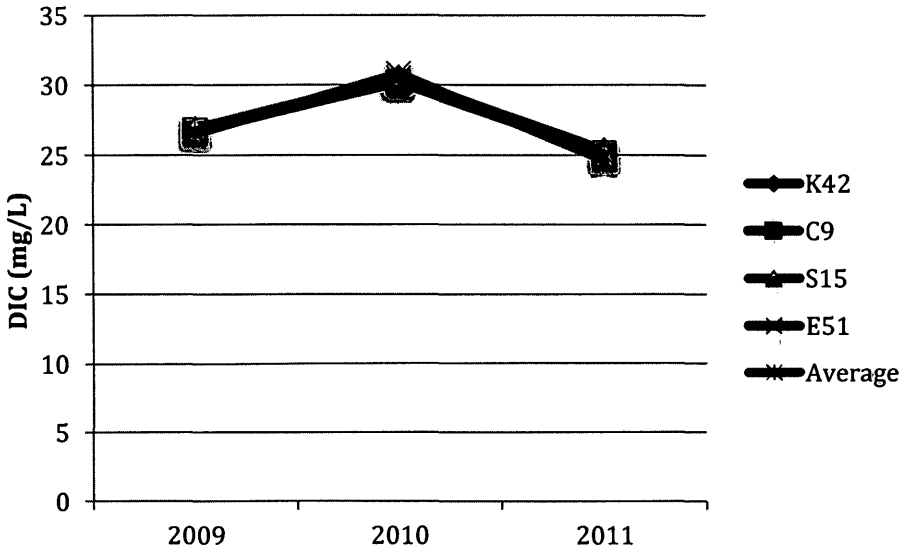
B. TKN ug/L



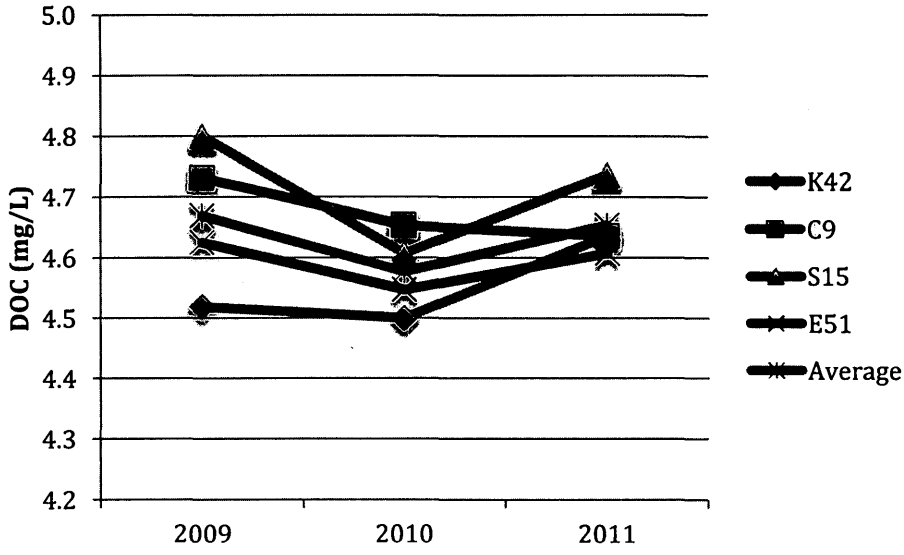
C. Chloride (mg/L)



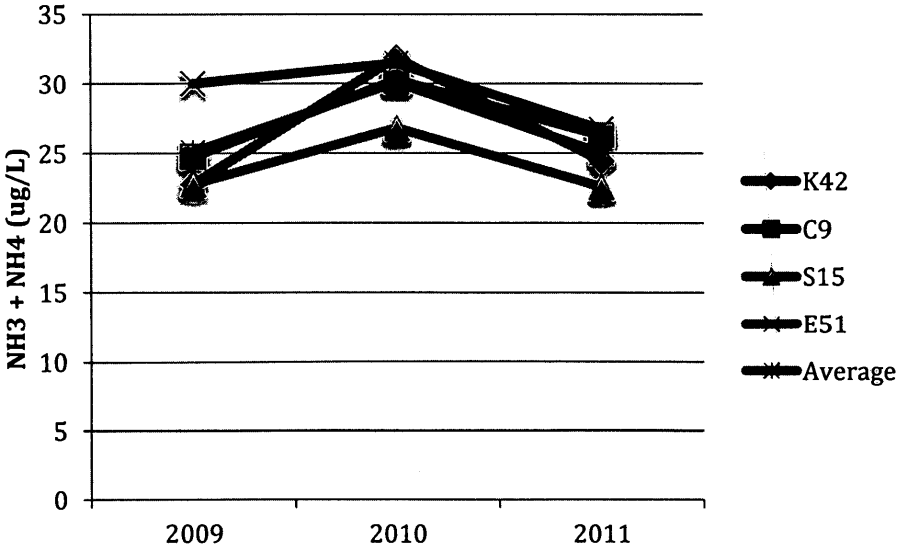
D. Alkalinity (mg/L CaCO3)



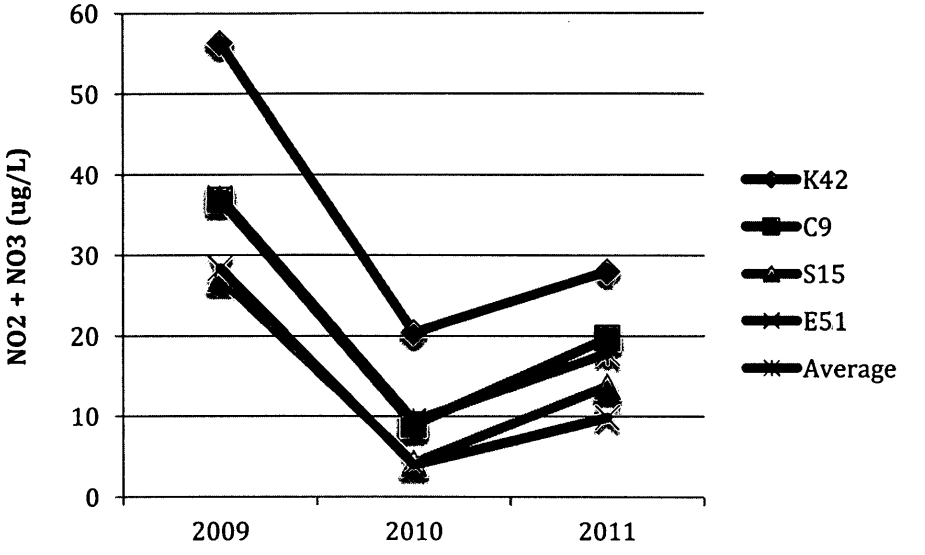
E. Dissolved inorganic carbon (mg/L)



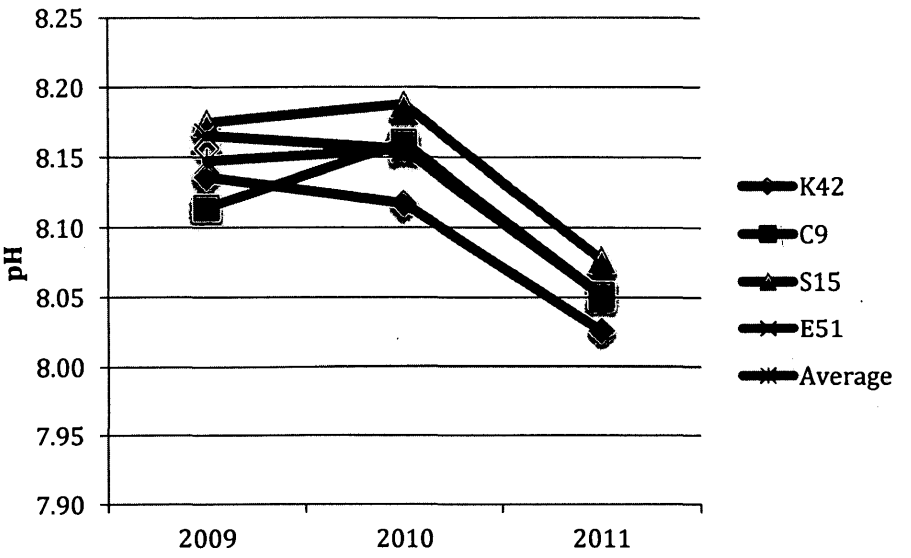
F. Dissolved organic carbon (mg/L)



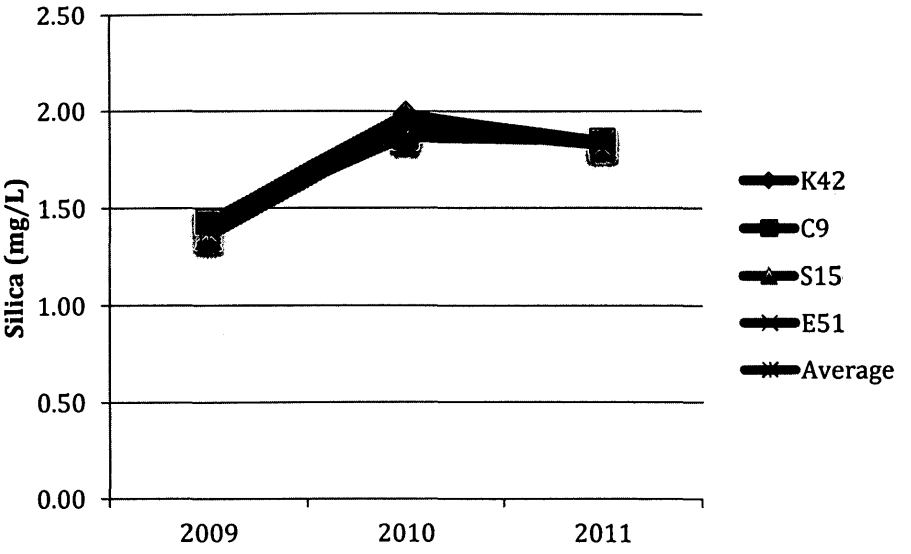
G. $NH_3 + NH_4$ (ug/L)



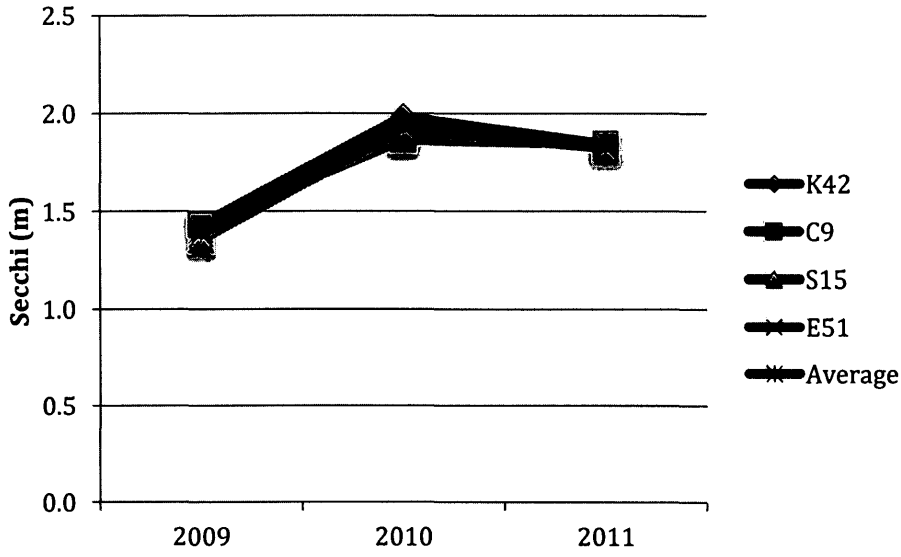
H. $NO_2 + NO_3$ (ug/L)


8.25
8.20
8.15
8.10
8.05
8.00
7.95
7.90
pH
2009
2010
2011
K42
C9
S15
E51
Average

I. pH


2.50
2.00
1.50
1.00
0.50
0.00
Silica (mg/L)
2009
2010
2011
K42
C9
S15
E51
Average

J. Si (mg/L)



K. Secchi (m)

Appendix B. Phytoplankton symbols and corresponding genera and species names.

Symbol	Genus and Species Name
AF	*Asterionella formosa*
AG	*Aulacosira gruanulata*
AP	*Amphora spp.*
AT	*Asterionella/Tabellaria*
AM	*Achnanthes minutissima*
AS	*Asterionella spp.*
AU	*Aulacoseira spp.*
CC	*Cyclotella cyclopuncta*
CD	unknown centric diatom
CM	*Cyclotella comensis*
CP	*Cryptomonas*
CP	*Cocconeis placentula*
CY	*Cyclotella spp.*
CR	*Cyclotella rossi*
DI	Dinophyceae
FA	*Fragilaria capucina*
FB	*Fragilaria brevistriata*
FC	*Fragilaria crotonensis*
FN	*Fragilaria nanana*
FO	*Fragilaria construens*
FS	*Fragilaria spp.*
FT	*Fragilara tenera*

GY	*Gymnodinium*
NP	*Nitzschia perminuta*
RH	*Rhodomonas*
SB	*Stephanodiscus binderanus*
SL	*Stephanodiscus* large
SMP	*Stephanodiscus minutulus/parvus*
SS	*Stephanodiscus* small
ST	*Stephanodiscus* spp
SY	*Synedra*
TA	*Tabellaria*
TF	*Tabellaria floculosa*