RELATIONSHIP OF HYPOLIMNETIC IRON, SEDIMENT BIOGEOCHEMISTRY, AND SULFATE IN THE PROMOTION OF CYANOBACTERIAL BLOOMS

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Abstract

Previous work suggests that a high rate of internal ferrous iron (Fe^{2+}) loading from anoxic sediments into overlying waters favours cyanobacteria dominance over eukaryotic algae. This conceptual model was assessed in four embayments along the Georgian Bay coastline. Cyanobacteria dominated all embayments in the relatively warmer summer of 2012 but not in the much cooler summer of 2014; internal Fe^{2+}loading was observed in both summers in all embayments. A large cyanobacteria bloom was observed only in warmer 2012 in the meso-eutrophic embayment. Results show that warm summer temperatures and internal Fe^{2+} loading are necessary preconditions for cyanobacteria dominance, while large blooms require high nutrient levels. Release of ferrous iron from sediments is restricted by sulfide production via the formation of insoluble ferrous sulfides, which reduces lake iron availability when they are buried in sediments. Increases in ferrous iron due to decreased sulfate levels may explain recent increases in bloom activity within Ontario and Quebec lakes. Sediment cores were collected from Lakes Erie and St. George and analyzed for historical trends in acid-volatile sulfides (AVS) and acid-extractable iron. Lake Erie seemed to closely follow the historical SO_2 emission trends of the region, except for an anomaly occurring in the mid 1970's. Neither of the two cores demonstrated a clear historical relationship between acid extractable iron and AVS, although the patterns in Erie are similar. Surficial sediments from Hamilton Harbour and Lake Erie were processed to form sediment incubation cores, which had additions of sulfate and/or organic carbon to determine their effects on the rate of internal loading and production of iron sulfides. The overall results indicate that heterotrophic bacterial metabolism of organic matter is the primary driver of internal loading and subsequent redox-associated events such as sulfide deposition. Soluble iron was not sequestered with even relatively high levels of sulfate until the latest stages anoxia, and it therefore seems unlikely that ferrous iron will be controlled by sulfide production in these natural systems during the course of one season, but long-term sequestration may be more likely after large blooms.

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Co-Authorship Statement

Chapter 2 was published in the Canadian Journal of Fisheries and Aquatic Sciences (reprint permission in Appendix A). Mark Jason Verschoor (MJV) was the first author and the co-authors were Christopher R. Powe (CRP), Eric McQuay (EM), Sherry L. Schiff (SLS), Jason J. Venkiteswaran (JJV), Jiahua Li (JL), and Lewis A. Molot (LAM).

MJV, CRP, EM, and JL conducted the fieldwork.

MJV, the University of Alberta Biogeochemical Analytical Service Laboratory, the Trent University laboratory in Dorset, the Water Quality Centre at Trent University, and the University of Waterloo conducted laboratory sample analyses.

JL conducted statistical analysis and contributed to the synthesis of ideas.

Each of the authors contributed to the study design, synthesis of ideas, and preparation of the final manuscript.

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AVS – acid-volatile sulfides

C – carbon

cHAB – harmful cyanobacteria bloom

DFe – dissolved iron

DIN – dissolved inorganic nitrogen

DO – dissolved oxygen

DOC – dissolved organic carbon

DP – dissolved phosphorus

DRFe – dissolved reactive iron

E_h – redox potential

Fe – iron

Fe^{2+} – ferrous iron

Fe^{3+} – ferric iron

GOE – great oxygenation event

H_2S – hydrogen sulfide

Mo – molybdenum

N – nitrogen

N_2 – nitrogen gas

NH_4 – ammonium

NO_3 – nitrate

O_2 – oxygen

OM – organic matter

ORP – oxidation-reduction potential

P – phosphorous

S – sulfur

SO_2 – sulfur dioxide

SO_4 – sulfate

SRP – soluble reactive phosphorous

SWI – sediment-water interface

TN – total nitrogen

TP – total phosphorous

WWTP – wastewater treatment plant

Chapter 1: Introduction and Literature Review

1.1 Relevance of Research

The cyanobacteria (commonly known as blue-green algae) are a morphologically and genetically diverse and successful group and comprise one of the largest sub-groups of Gram-negative prokaryotes (Rippka *et al.* 1979, Giovannoni *et al.* 1988). Cyanobacteria are considered the first major group of phototrophs to evolve oxygenic photosynthesis using water, and geochemical and fossil evidence indicates that during the Precambrian Era, this novel adaptation was responsible for the transition of the Earth's atmosphere from an originally anaerobic state to its current, aerobic condition, known as the "Great Oxygen Event" or GOE (Giovannoni *et al.* 1988). Molecular phylogenetic evidence also indicates that the chloroplasts of all green plants and algae were acquired as cyanobacterial endosymbionts of photosynthetic prokaryotes after the GOE (Ibid.). Cyanobacteria occupy a diverse array of habitats, including rock, soils, oceans, and fresh waters, as endolithic, epiphytic, or open water (pelagic) cells or colonies.

This last group garners the most public attention, because in eutrophic systems, potentially harmful cyanobacteria blooms (cHAB), dominated by large-bodied colonial and filamentous taxa tend to develop during the summer period, and can produce toxic or unpleasant taste and odour compounds, accumulation of surface scums, and anoxia (which leads to fish kills), all of which are expensive to mitigate (Wilhelm 1995, Havens 2008, Smith *et al.* 2008, Watson *et al.* 2008). The term 'bloom' is often used to refer to a visible surface accumulation of cyanobacteria; however, dominance can occur without visible surface accumulations. The word 'bloom' is defined here to mean dominance by cyanobacteria (> 50% of the phytoplankton biomass), regardless of whether surface accumulation occurs (Smayda 1997). Freshwater blooms of colonial cyanobacteria continue to be a significant global problem despite billions of dollars spent on nutrient abatement intended to reduce primary productivity (Steffensen 2008), and in some regions, the problem appears to be worsening and increasing in frequency (Codd *et al.* 2005, Carey *et al.* 2008, Winter *et al.* 2011, Michalak *et al.* 2013).

Although the risk is lower in lakes with total phosphorus (TP) concentrations below 20 μg L^{-1} (Downing *et al.* 2001), cHABs have recently been recorded in oligotrophic and mesotrophic lakes such as Lake Couchiching (Sherman 2005), mesotrophic parts of Lake of the Woods in northwestern Ontario (Chen *et al.* 2007, 2009), central Ontario (Verschoor *et al.* 2017), Norway (Halstvedt *et al.* 2008), Portugal (Galvão *et al.* 2008) and Greece (Vareli *et al.* 2009).

1.2 Ecology of Pelagic Cyanobacteria

Phytoplankton communities usually display significant seasonal trends, where relatively few taxa dominate for limited periods of time, followed by a succession of other taxa as the year progresses (Murrell and Lores 2004, Abrantes *et al.* 2006). For example, diatoms tend to dominate during the Spring and Fall when higher and more frequent wind velocities create well-mixed conditions. Diatoms require well mixed conditions to remain suspended in the water column due to their relatively heavy silica frustules (which in turn rely on solubilized silica made available through the same mixing process (Dokulil and Teubner 2000), yet are better adapted to fluctuating light regimes, because their cell division process is metabolically independent of photosynthesis (Cosper 1982, Reynolds 1994, Ibelings *et al.* 1994, Mitrovic *et al.* 2003).

The summer phytoplankton community in oligotrophic and mesotrophic lakes are typically dominated by eukaryotic algae including Chlorophyta (green algae) and Chrysophyta, which are subjected to heavy grazing by zooplankton communities, which transition from relatively large cladocera such as *Daphnia* to smaller cladocera (e.g. *Bosmina, Ceriodaphnia*), copepods, and rotifers, due to preferential fish predation. Typical successional cycles within the phytoplankton community can be disrupted by eutrophication and can lead to domination by cyanobacteria during warm periods (Dokulil and Teubner 2000), particularly in hard-water lakes (Reynolds and Walsby 1975). In eutrophic lakes, eukaryotic algae are replaced when water temperatures reach 20^{o}C by colonial and filamentous cyanobacteria, which are more difficult to graze due to their large size and have lower nutritional value (Alexander 2012). Additionally, cyanobacteria may tolerate lower light conditions better than other algal groups (Havens *et al.* 2003) and may even shade out competing groups with their biomass (Scheffer *et al.* 1997). Warmer temperatures tend to favour cyanobacteria growth (Joehnk *et al.* 2008, Kosten *et al.* 2012) along with stable, stratified water columns, and these factors will likely become more influential in the wake of climate change (O'Neil *et al.* 2012). Hence, in eutrophic temperate lakes, cyanobacteria generally appear as the dominant component of summer blooms. During the period of general autumn decline, there are frequent periods of rapid decrease in algal standing crop, often of the order of 50% over a period of 10 days to 2 weeks, likely due to increasingly adverse light or temperature conditions (Stauffer 1974). There is increased formation of akinetes - thick-walled dormant cells that function as survival structures for filamentous cyanobacteria (Wolk 1965, Rother and Fay 1977) – and dormant vegetative cells produced by colonial cyanobacteria such as *Microcystis* as evidenced by sedimentation trap experiments (Reynolds 1976, Fallon and Brock 1980).

1.3 Cyanobacteria Bloom interactions with Nutrients

Primary producers require a range of elements to grow, including macronutrients such as nitrogen (N), phosphorous (P), iron (Fe), and micronutrients such as cobalt, copper, manganese, magnesium, calcium and others. It is generally accepted among limnologists that P is the primary nutrient that determines the productivity and trophic status of most lakes, although some lakes are N-limited (Schindler 2012). The eutrophication of water bodies is a significant problem around agricultural and urban areas, but has been mitigated to various degrees since the early 1970s via reduction of P inputs by diversion of nutrient point sources from advanced wastewater treatment facilities (Dolan 1993, Rast and Thornton 1996, Jeppesen *et al.* 2005). In some lakes, these restorative measures have been very effective (Cooke *et al.* 1993), but in other lakes, the predicted improvement of water quality following restoration measures has either been delayed, or failed to occur (Welch *et al.* 1986, Cooke *et al.* 1993) because of release of legacy P from sediments during periods of anoxia (i.e., internal P loading) (Cooke *et al.* 1993, Jeppesen *et al.* 2007).

The risk of blooms is substantially high when open water total P (TP) concentrations exceed 35 μg L^{-1} (Downing *et al.* 2001). The current guideline for maximum acceptable TP concentration in Ontario is 20 μg L^{-1} (Ontario Ministry of Environment and Energy 1994), which is deemed preventative for algal blooms in Ontario, based on historical data. However, increasing incidences of blooms in oligotrophic and mesotrophic lakes in recent years (Winter *et al.* 2011) suggest that this target is no longer valid.

Another macronutrient which has been and continues to be implicated in algal blooms is N, available as nitrate, ammonium, and organic N (including urea), which decomposes to ammonium. When the ratio of N:P falls below 11:1, nitrogen-fixing cyanobacteria predominate (Hecky and Kilham 1988). Attempts to control algal blooms in freshwaters by reducing N inputs are generally unsuccessful if P inputs are not controlled first, due to cyanobacterial N fixation (Schindler *et al.* 2008). However, nitrate can play an important role if concentrations are high enough to delay the onset of internal P and ferrous Fe (Fe^{2+}) loading via raising the redox at the sediment/water boundary (Molot *et al.* 2014, Beutel et al. 2016, Verschoor *et al.* 2017), whereas ammonium can be released in significant quantities from sediments that have become anoxic (have low redox) (Whitmire and Hamilton 2005).

A nutrient of particular importance to formation of cyanobacteria blooms is Fe (Wilhelm 1995, Molot *et al.* 2010, 2014). Cyanobacteria have higher Fe requirements relative to eukaryotic photoautotrophs (especially N-fixers (Kustka 2003)) and can only transport the reduced form of iron, Fe^{2+} directly across their cytoplasmic membranes (Kranzler *et al.* 2013). In contrast,

eukaryotic algae have lower Fe requirements and can transport the more abundant oxidized ferric forms of iron Fe^{3+} (Morel *et al.* 2008, Shaked and Lis 2012). Fe^{3+} is sparingly soluble as the free ion in the presence of dissolved oxygen but chelation to dissolved organic carbon (DOC) can raise dissolved Fe concentrations (Stumm & Lee 1960), lowering the loss rate of Fe^{3+} to sediments (Molot and Dillon 2003).

In oxygenated waters (operationally defined here as >1 mg/L dissolved O_2), Fe^{2+} is rapidly oxidized to insoluble ferric hydroxides, to which phosphate adsorbs quite strongly (Stumm & Lee 1960). Consequently, Fe^{2+} is virtually absent from oxygenated waters. Under anoxic conditions, iron in ferric hydroxide-phosphate complexes and ferric phosphate minerals (e.g., strengite, $Fe(PO_4).2H_2O$) is microbially reduced, liberating highly soluble Fe^{2+} ions, which are readily available to most phytoplankton (Harvey 1937, Bostrom *et al.* 1988, Gachter and Muller 2003, Hopkinson and Morel 2009). Cyanobacteria blooms are preceded by development of sediment anoxia and release of Fe into overlying waters at depths that previous studies have shown are accessible to migrating cyanobacteria (Molot *et al.* 2014, Verschoor *et al.* 2017). Development of sediment anoxia is seen by these authors to be a key driver of bloom formation.

Cyanobacteria can acquire iron via ferric iron scavenging mechanisms such as siderophores and extracellular capsular polysaccharides (Wilhelm 1995; Rose *et al* 2005; Li 2016; Sorichetti *et al.* 2016), but they appear unable to transport Fe^{3+} across their cell membranes without first reducing it to Fe^{2+} via ferric reductases at the cell surface, which is inefficient due to possible reoxygenation at the cell membrane prior to transport, and energetically expensive (Lis *et al.* 2015). However, when the top layer of sediment becomes anoxic and Fe^{2+} diffuses upwards into overlying waters to depths accessible to migrating cyanobacteria, it appears that Fe limitation can be overcome, and a bloom may occur.

In shallow lakes and shoreline (littoral zone) areas where stratified or calm water exists, Fe^{2+} may be found immediately adjacent to populations of cyanobacteria in the euphotic zone. Cyanobacterial blooms have been observed in eutrophic polymictic systems which do not thermally stratify for any great duration, but do become episodically hypoxic (Gulati and van Donk 2002, Smith *et al.* 2011, Giles *et al.*, 2016, Jabbari *et al.*, 2019) and likely have boundary layers at the sediment-water interface (SWI) that contain accessible Fe^{2+}. Internal P loading occurs in polymictic systems (Jensen & Andersen 1992, Ramm & Scheps 1997) suggesting that the SWI is anoxic at least periodically when winds are calm and the boundary layer thickness increases, perhaps developing at night when photosynthetic production of dissolved oxygen stops, and thus may also contain Fe^{2+} (Loewen *et al.* 2007, Bryant *et al.* 2010).

Cyanobacteria blooms in western and central Lake Erie appear to originate inshore in areas that have polymictic behaviour (shallow and not stratified, e.g., Maumee Bay, Sandusky Bay) (Millie *et al.* 2009). In fact, the western basin does occasionally stratify for up to 5 days, and the bottom waters can even become anoxic (Bridgeman et al., 2006, Jabbari et al. 2019).

Many cyanobacteria can obtain N for protein synthesis and other uses via atmospheric nitrogen (N_2) fixation, but the nitrogen fixing enzyme, nitrogenase, requires two metal cofactors, Fe and molybdenum (Mo) (Dixon and Kahn, 2004), and limitations of either of these can therefore limit bloom formation (Downs *et al.*, 2008; Romero *et al.*, 2013). With the apparent cellular uptake threshold of Mo being approximately 1-5 nM (Glass *et al.*, 2012), and freshwater lake Mo concentrations ranging from 0.1 nM in the headwater lakes of Quebec to 890 nM in California lakes (Downs *et al.*, 2008, Smedley and Kinniburgh, 2017), Mo limitation of nitrogen-fixing blooms clearly varies geographically. Unsurprisingly, in urbanized and industrialized areas, Fe and Mo are highly elevated above naturally occurring levels (Neal and Robson, 2000; Chappaz *et al.*, 2008; Yuan, 2017). N_2 fixation is therefore unlikely to be limited in these watersheds, thus increasing the likelihood of filamentous cyanobacteria blooms where N/P ratios are low and P levels are high enough to generate sediment anoxia.

In deeper lake areas, some species of cyanobacteria may be able to escape the summer periods of low nutrient levels in the epilimnion and access rich hypolimnetic sources of P, Fe^{2+}, ammonium, and other nutrients by controlling their buoyancy via gas vesicles (Ganf and Oliver 1982, Oliver 1994, Walsby 1994). Other studies have confirmed that certain species indeed vertically migrate below the thermocline (Reynolds 1976), and even into anoxic waters (Walsby *et al.* 1983, Camacho *et al.* 1996, Head *et al.* 1999). Furthermore, several species of cyanobacteria have been shown to translocate P from the sediment–water interface to the epilimnion, and can have a significant effect on the internal phosphorous loading of lakes, further prolonging cyanobacterial dominance (Osgood 1988, Barbiero and Welch 1992, Pettersson *et al.* 1993, Barbiero and Kann 1994, Head *et al.* 1999).

In eutrophic lakes, cyanobacteria are often the dominant primary producers (Fallon and Brock 1980), and can have a very significant effect on nutrient loading: in deep lakes, their biomass contributes to the sediments, and in shallower lakes, it is often recycled (Pennington 1974, Pennington *et al.* 1976). At the end of the growing season, significant amounts of decomposition occur at the sediment-water interface (Bortleson 1970). In one study of eutrophic Lake Mendota for example, methane evolution accounted for about 40-50% of the total organic material reaching the sediments, 60-70% of the organic matter produced by cyanobacteria was decomposed in the water column, while 30-40% of the organic material sedimented to the mud

surface where further decomposition took place to yield only about 11% permanently sequestered as lake sediments (Fallon and Brock 1980). Nutrients are cycled along with the organic carbon. This indicates that when anthropogenic P inputs are eliminated and organic matter production falls, sediment nutrients will gradually be released into overlying water and discharged downstream, the extent depending on the degree of anoxia. The extent of post-control anoxia explains the persistence and legacy of nutrient additions to lakes.

1.4 Redox Biogeochemistry of Lake Sediments

Lake productivity is closely linked to hypolimnetic dissolved oxygen (DO) concentrations in thermally stratified lakes: a productive lake creates greater quantities of organic matter (OM), which are deposited onto the sediments and deplete DO as they decompose. Maximum DO is about 8 mg L^{-1} in cold hypolimnetic water but high respiration rates will cause concentrations to decline. This can lead to hypoxic conditions (<4 mg L^{-1} oxygen) or anoxia in the hypolimnion, which often restricts the habitat of cold-water fishes (Gudimov *et al.* 2016).

Bottom waters may be permanently oxygenated or anaerobic, but usually oscillate seasonally between these extremes in most water bodies (Yakushev *et al.* 2016). Under anaerobic conditions, redox-driven reactions strongly influence mobilization of P (Mortimer, 1941, 1942) as well as the accumulation of metals and other toxic substances (e.g., methane, ammonium, and sulfide) near the lake bottom (Gudimov *et al.* 2016). Such internal loading occurs during seasonal stratification, as the hypolimnion becomes increasingly anoxic, and redox and microbial processes mediate the release of P adsorbed to Fe^{3+} complexes or salts (Mortimer 1941, 1942, Bostrom *et al.* 1988, Brunberg and Bostrom 1992). The increased availability of this mobilized P and other nutrients can increase the risk of harmful algal blooms (Doan *et al.* 2016). Consequently, there has been a great deal of interest in understanding these biogeochemical processes and attempting to control internal loading within lakes.

One suggested method of controlling internal loading is to maintain an oxidized sediment-water interface (SWI), based upon the "classical model" first proposed by Einsele (1936, 1938), later refined by Mortimer (1941, 1942), and briefly described above (Katsev *et al.* 2006, Hupfer and Lewandowski 2008). The goal is to maintain a target hypolimnetic DO level (e.g. 2-4 mg L^{-1} O_2), by reducing external nutrient loading, supplying deep water oxygenation, inducing artificial destratification, or removing sediments containing high amounts of organic matter (Hupfer and Lewandowski 2008, Gudimov *et al.* 2016).

The classical model was very successful in describing seasonal P release in a variety of environments (Katsev and Dittrich 2013), was instrumental in several modelling approaches (e.g. mass balance models using 'anoxic factors' to predict P accumulation), and for decades it

was widely accepted that aerobic conditions at the sediment-water interface prevented phosphorus release to the overlying water column (Hupfer and Lewandowski 2008). However, over time, numerous exceptions were reported, including controlled laboratory experiments, and despite some reports of success using hypolimnetic oxygenation in lakes, most management strategies utilizing the oxygenation paradigm failed to increase long-term P retention (Katsev *et al.* 2006, Hupfer and Lewandowski 2008, Katsev and Dittrich 2013).

It has been suggested that because the classical model was appealing in its simplicity and was supported by evidence from aquatic systems suffering from extensive eutrophication at the time, that earlier studies which suggested more complicated interactions were virtually ignored (Hupfer and Lewandowski 2008). More recent studies show that the mechanisms of P release from sediments are indeed much more complicated, and involve many other species and processes, such as manganese (Mn), nitrates (NOx), sulfate (SO_4), sulfides, carbonates, organic matter, apatite precipitation, pH, microbial zones, bioturbation, rooted plant activity, frequency and duration of mixing events, precipitation, water column stability, heterotrophic respiration, and photosynthetic oxygen production (Katsev *et al.* 2006, Melton and Stief *et al.* 2014, Giles *et al.* 2015, Han *et al.* 2015). Consequently, it has recently been argued that the relative importance of P mobilization mechanisms varies with the time scale of interest (Katsev and Dittrich 2013, Giles *et al.* 2015), and thus new models are being developed that account for the combined influence of multiple microbial communities and redox reactions. A discussion of these factors follows.

1.5 Sediment Redox Chemistry and Microbial Zones

1.5.1 Surficial Organic Matter

In eutrophic systems, high lake productivity can lead to the deposition of large amounts of organic matter (OM) in the form of fresh algal detritus to the lake bed. Algae can be a major nutrient reservoir during its decomposition, directly releasing significant quantities of P, N and S into the water column and creating an anaerobic environment during the mineralization process, which facilitates the reduction and dissolution of Fe^{3+} oxyhydroxides, and increases the release of Fe^{2+} into the water column (Han *et al.* 2015). Under these strongly reducing conditions, the redox interface can enter the water column itself, accelerating P release and even creating a “black bloom” or black water event of iron sulfide precipitates on the sediment surface (Han *et al.* 2015). High levels of OM significantly slow down the oxidative cycling of nutrients, and its own remineralization (Yakushev *et al.* 2016), and is a more important driver of sediment P flux than hypolimnetic O_2 concentrations (Katsev and Dittrich 2013).

1.5.2 The Sediment-Water Interface

Sediments can be conceptually partitioned into distinct redox zones, where increasingly reducing environments occur with depth. The first zone occurs at the SWI within the first few millimeters and is defined by oxidative processes that are chiefly aerobic but can vary seasonally. Below the aerobic SWI, denitrification takes place (~6-12mm), followed by manganese (IV) reduction, then ferric iron reduction (~12-23mm), then sulfate reduction, and finally carbon dioxide reduction (methanogenesis) (Melton and Stief *et al.* 2014). Fe and N are of major importance in profundal lake sediments in terms of nutrient cycling, and are biologically connected through nitrate-reducing Fe^{2+} oxidizers and chemically by NOx species (see Table 1.1) (Melton and Stief *et al.* 2014).

The oxygen penetration depth below the SWI is determined chiefly by sediment porosity and overlying oxygen concentration, and determines the overall profile (Gudimov *et al.* 2016). Sediments physically limit diffusion of DO from oxygenated overlying water, creating a diffusion gradient with a sharp redox gradient just millimeters deep (Frindte *et al.* 2015, Yakushev *et al.* 2016).

1.5.3 Nitrates and Ammonium

Within a few millimeters below the SWI in unproductive systems, nitrate becomes the dominant oxidizer in place of oxygen (Dittrich *et al.* 2009, Yakushev *et al.* 2016). High nitrate levels maintain a relatively high redox level, playing a critical role in suppressing Fe^{2+} and sulfide release into the water column and controlling methane release rates (Frindte *et al.* 2015). In the absence of oxygen, nitrate also becomes the primary electron acceptor for many species of microbes in denitrification (with a characteristic redox or oxidation reduction potential (ORP) of -40 mV (Frindte *et al.* 2015)), Fe^{2+} oxidation, anaerobic ammonium oxidation (anammox), and organic matter oxidation (Melton and Stief *et al.* 2014). Conversely, inorganic nitrogen species produced during denitrification can be organically fixed by microbial processes into ammonium, which can also be produced through dissimilatory nitrate reduction to ammonium (DNRA) in addition to organic matter degradation (Melton and Stief *et al.* 2014).

Table 1.1 Microbially and chemically mediated reactions that form the biogeochemical Fe cycle, listed in decreasing redox potential. Reactive oxygen species (ROS) include superoxide ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$). Organic ligands (L) bind Fe (III) and photoreduce it to Fe (II). Nitrate (NO_3^-)-dependent Fe (II) oxidation is restricted to anoxic zone, below the surface-water interface (SWI, bold line). The different gradients of O2, light, NO_3^-, Fe^{2+}, and Fe^{3+} in a redox-stratified environmental system are shown, as well as where the different biotic and abiotic Fe redox transformations are expected to take place. Adapted from Melton and Swanner *et al.* (2014) with supporting data from Melton and Stief *et al.* (2014), Jobin and Namour (2016), and Rincón-Tomás *et al.* (2016).

Microbially mediated reactions	**Redox Zone & Eh**	**Chemically mediated reactions**
Microaerophiles $4Fe^{2+} + 10H_2O + O_2 \rightarrow 4Fe(OH)_3 + 8H^+$ *Gallionella* spp., *Leptothrix* spp., *Mariprofundus* spp., *Sideroxydans* spp.	Aerobic > +500 mV	Reactive Oxygen Species Oxidation $Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$ $Fe^{2+} + O_2^{\bullet -} + 2H^+ \rightarrow Fe^{3+} + H_2O_2$ $Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + OH^{\bullet} + H_2O$ $Fe^{2+} + OH^{\bullet} + H^+ \rightarrow Fe^{3+} + H_2O$ Reduction: $O_2^{\bullet -} + Fe(III) \rightarrow O_2 + Fe^{2+}$
Photoferrotrophs $HCO3^- + Fe^{2+} + 10H_2O \xrightarrow{hv} (CH_2O) + 4Fe(OH)_3 + 7H^+$ *Rhodopseudomonas palustris* TIE-1 *Rhodobacter* sp. SW2 *Chlorobium ferrooxidans* (KoFox) *Thiodictyon* sp. F4	Photic > +100 mV SWI 0-8mm	Light reactions Photoreduction $Fe^{3+}–L \xrightarrow{h\upsilon} Fe^{2+}–L$
NO_3^--reducing Fe(II)-oxidizers $10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+$ *Acidovorax* spp., KS, 2002 *Thiobacillus denitrificans*	Nitrate 6-12mm +400 to +250 mV	Nitrogen species Oxidation of Fe $4Fe^{2+} + 2NO_2^- + 5H_2O \rightarrow 4FeOOH + N_2O + 6H^+$ $Fe^{2+} + NO_2^- + 2H^+ \rightarrow FeOOH + NO + H_2O$ $Fe^{2+} + NO + H^+ \rightarrow FeOOH + ½N_2O + ½H_2O$
Fe-ammox $NH_4^+ + 6FeOOH + 10H^+ \rightarrow NO_2^- + 6Fe^{2+} + 10H_2O$ Unknown	Manganese ~12.5mm +250 to 0 mV	Manganese Oxidation of Fe $2Fe^{2+} + MnO_2 + 2H_2O \rightarrow Mn^{2+} + 2FeOOH + 2H^+$
Fe(III)-reducing organic C and/or H_2-oxidizers $4FeOOH + CH_3CHOHCOO^- + 7H^+ \rightarrow 4Fe^{2+} + CH_3COO^- + HCO_3^- + 6H_2O$ $2Fe(OH) + H_2 \rightarrow 2Fe^{2+} + 2H_2O$ *Geobacter* spp., *Shewanella* spp, *Albidoferax ferrireducens*, *Geothrix* spp.	Iron ~22.5mm 0 to -100 mV	Humic Substance Reduction of Fe $Fe^{3+} + HumS^- \rightarrow HumS + Fe^{2+}$ Phosphorus Retention $3Fe^{2+} + 2PO_4^{3-} \rightarrow Fe_3(PO_4)_2$ (vivianite)
Dissimilatory sulfate reduction $SO_4^{2-} + ATP \rightarrow APS \rightarrow SO_3^{2-} \rightarrow HS^{2-}$ *Desulfovibrio vulgaris, Desulfotomaculum* spp.*, Desulfosporomusa* spp.*, Desulfosporosinus* spp., Thermodesulfovibrio spp., *Archaeoglobus, Thermocladium, Caldivirga*	Sulfur -100 to -300 mV	Sulfur species Reduction of Fe $2FeOOH + 3H_2S \rightarrow 2FeS + S^0 + 4H_2O$
Methanogenesis $CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$ $CH_3COOH \rightarrow CH_4 + CO_2$ *Methanoarchaeota*	Methane < -300 mV	

1.5.4 Iron

Because of its ability to form complexes with carbon (C), oxygen, N and sulfur (S) species, and its redox sensitivity, iron plays a fundamental role in the metabolism of nearly all living organisms (Melton and Swanner *et al.* 2014, Han *et al.* 2015).

The two main redox states of Fe have different solubilities: oxidized Fe^{3+} is poorly soluble at circumneutral pH in oxygenated waters, while reduced Fe^{2+} is highly soluble and therefore more bioavailable in anoxic waters (Melton and Swanner *et al.* 2014). Because the redox potential of the Fe^{3+}– Fe^{2+} couple lies between the redox potentials of the major C, N, O or S species redox couples, Fe drives global biogeochemical cycles to a significant degree (Weber *et al.* 2006, Melton and Swanner *et al.* 2014). For example, P lability in sediments is primarily controlled by Fe redox cycling, owing to the dissolution of Fe^{3+} oxyhydroxides under reducing conditions (Han *et al.* 2015). Because Fe^{2+} is more mobile than the oxidized form, it diffuses upwards through the sedimentary porewater and can act as a reductant in many microbial and chemical processes in each layer (Melton and Stief *et al.* 2014).

1.5.5 Fe-NOx Interactions

Fe can be microbially oxidized and reduced through processes coupled to N species in the denitrification zone, when Fe^{2+}, nitrate, and an organic co-substrate are available (Melton and Stief *et al.* 2014). *Thiobacillus denitrificans* is best known for this ability, but other groups such as *Geobacter,* anammox bacteria, and some species within the genera *Dechloromonas* and *Paracoccus* are also known (Melton and Stief *et al.* 2014). Although the reactions and biogeochemical cycles linking Fe and N are not well studied (Melton and Stief *et al.* 2014), the widespread occurrence of biological nitrate-dependent Fe^{2+} oxidation in natural systems suggests that this reaction may play a significant role in the coupling of Fe and N redox cycles in sedimentary environments. (Weber *et al.* 2006).

1.5.6 Fe-Ammox

Ammonium can be oxidized to dinitrogen gas (N_2) in anoxic sediments through the microbial process of anaerobic ammonium oxidation (ammox) followed by denitrification. Microbial Fe-ammox reduces Fe^{3+} hydroxides coupled to ammonium oxidation. This metabolism produces nitrate, nitrite, or N_2, depending on the ambient pH (Melton and Stief *et al.* 2014). Additionally, ammonium produced during N_2 fixation or dissimilatory nitrate reduction to ammonium (DNRA) can act as an electron donor to microbial Fe-ammox in the iron cycle as well as nitrification reactions in the nitrogen cycle (Melton and Stief *et al.* 2014).

1.5.7 Fe-OC

Deep within the iron reduction zone (down to about 15 cm (Dittrich *et al.* 2009)), Fe^{3+} oxyhydroxide reduction is coupled to organic carbon and hydrogen oxidation via dissimilatory iron reducing bacteria (DIRB). The two main genera of bacteria responsible are *Shewanella* and *Geobacter*, which can also utilize humic substances, microbially produced flavins, or biological nanowires to shuttle electrons to ferric compounds (Lovley *et al.*1996, Melton and Stief *et al.* 2014). This process operates on both organic and inorganic compounds and affects the distribution of natural and contaminant metals, hydrocarbons, and radionuclides (Weber *et al.* 2006).

1.5.8 SO_4 and H_2S

Still further down into the sediment, strictly anaerobic microorganisms exist which can utilize more recalcitrant electron acceptors such as sulfate and carbon dioxide (Frindte *et al.* 2015). Sulfur diagenesis is apparently neglected in freshwater environments because of the low sulfate concentrations in freshwaters (29 mM marine vs. 25-300 μM freshwater), but sulfate reduction rates in eutrophic lakes can approach those in brackish environments (Katsev *et al.* 2006, Melton and Stief *et al.* 2014).

Dissolved sulfate can cause P mobilization from sediments when sulfur diagenesis creates hydrogen sulfide (H_2S) as a byproduct. H_2S reductively dissolves ferric oxyhydroxides and thereby releases iron-bound phosphate, and prevents P from immobilizing into ferrous minerals, such as vivianite ($Fe_3(PO_4)_2$), by depleting porewater iron via iron monosulfide (FeS) precipitation (Katsev and Tsandev *et al.* 2006, Katsev and Dittrich 2013, Han *et al.* 2015). Additionally, H_2S can directly dissolve vivianite, thereby mobilizing even more phosphorus (Katsev and Tsandev *et al.* 2006).

Manganese oxides in sediments can act as a redox buffer and delay the upward movement of sulfide toward the SWI (Frindte *et al.* 2015). Both Mn and Fe oxides react with H_2S to produce insoluble metal sulfides, thiosulfate ($S_2O_3^{2-}$), and elemental sulfur (S^0). Thus, all sulfur species diffuse out of the sediments in a specific order: thiosulfate first, then S^0, and then H_2S. (Yakushev *et al.* 2016).

After oxygen levels are depleted at the sediment-water interface to 5 μM (usually by late summer), thiosulfate and S^0 begin to be released into the overlying water column (Yakushev *et al.* 2016). Several days later, H_2S emerges after all Fe and Mn oxides have been dissolved (Ibid.). During this time, H_2S, Mn(II), PO_4, Fe(II), $S_2O_3^{2-}$, and NH_4 all coexist in the water-sediment column and can cause the precipitation of metal sulfides on the sediment surface (i.e. a “black bloom”) (Han *et al.* 2015, Yakushev *et al.* 2016).

1.5.9 Methane

At the deepest layers of sediment, beyond N and Fe cycling and where sulfate has been depleted, methanogenesis begins ((Dittrich *et al.* 2009, Melton and Stief *et al.* 2014, Frindte *et al.* 2015). Although there is a concentration gradient of methane extending all the way to the sediment-water interface, anaerobic oxidation of methane (AOM) using nitrate as the electron acceptor, followed by oxidation by aerobic methanotrophic bacteria effectively prevents methane enrichment of overlying waters until long after DO depletion of the hypolimnion, when methane shows a non-uniform accumulation in overlying waters (Frindte *et al.* 2015). Because these deep sediments are not under the influence of light or mechanical mixing (unlike littoral sediments), their redox stratification is very stable (Melton and Stief *et al.* 2014).

1.5.10 Microbial Zones

Early investigators assumed that Fe cycling was chiefly an abiotic process involving oxygen, nitrites, manganese, sulfur species, and organic carbon. We now know that most redox processes are microbially mediated (Melton and Swanner *et al.* 2014, Yakushev *et al.* 2016). The formation of redox zones in sediments follows a distinct succession of genera and species, uniquely adapted to process the changing chemical profiles encountered with increasing sediment depth. Below the hypoxic and anoxic conditions of the SWI, aerobic heterotrophic bacteria disappear (or become inactive) and aerobic autotrophic and anaerobic heterotrophic bacteria become more abundant and may form bacterial mats (Yakushev *et al.* 2016).

At least three distinct microbial zones were noted in one study (Wurzbacher *et al.* 2016):

1) A redox-stratified zone at 0-5 cm deep, where oxygen down to sulfate act as electron acceptors, and ammonia and soluble reactive phosphorous (SRP) increase. Bacteria dominate and there is high microbial activity, cell numbers, taxa turnover, and spatial variability.

2) A transition zone at 5-14 cm deep, where methane concentrations rise, and CO_2 has a local minimum. Eukaryotic abundance approaches zero, microbial cell numbers drop off and activity decreases, diversity decreases, taxa turnover stays high and is maximized, and bacteria decline, being rapidly replaced by archaea.

3) A “depauperate horizon” at 14 cm and below, where methane and CO_2 concentrations reach saturation, and most chemical parameters remain constant. Microbial cell numbers and activity are low, as are spatial variability, evenness, and diversity; *Archaea* are dominant.

1.6 Thesis questions, hypotheses and objectives

The following research questions are addressed in this thesis:

1) Can cyanobacteria blooms be predicted in freshwater lakes based on seasonal fluctuations of hypolimnetic phosphorus and ferrous iron levels released from sediments?

2) Does the generation of insoluble iron sulfides sequester biogeochemically significant levels of iron, which may limit cyanobacterial growth? Is there historical evidence within the sediments that suggests such a relationship?

3) What levels of sulfate are adequate to suppress ferrous iron levels, and how important is organic carbon (such as that following a bloom) for promoting the anoxic conditions necessary for release of ferrous iron and/or production of sulfides?

The working hypotheses proposed to answer the above questions were therefore:

1) Internal Fe^{2+} loading from anoxic sediments is a necessary pre-condition for cyanobacterial dominance: if cyanobacterial dominance occurs in the absence of internal Fe^{2+} loading, the ferrous iron→ bloom model must be modified or rejected.

2) Because sulfur emissions peaked in the 1970s then declined dramatically with growing environmental awareness and legislation in developed countries (Garmo *et al.* 2014), ferrous iron levels in lakes may have been increasing due to reduced iron sulfide sequestration, thus leading to increased incidences of cyanobacterial blooms. If iron sequestration has changed over the decades, then the concentration of iron sulfides buried in sediments should coincide with known historical changes in sulfur dioxide emissions.

3) Addition of sulfate to the water overlying incubating sediments should increase the production of iron sulfides and thereby decrease ferrous iron levels under anoxic conditions. Addition of organic carbon to sediments should intensify the aforementioned biogeochemical reactions in a manner similar to that seen in eutrophic and hypereutrophic systems.

To test the above hypotheses, the following investigations were carried out:

1) The relative abundance of large filamentous and colonial cyanobacteria in phytoplankton communities in several embayments along Georgian Bay were measured and compared with hypolimnetic phosphorus and soluble iron profiles collected over several summers (as proxies for internal loading) and sediment chemistry fractions.

2) Sediment cores from lakes within industrialized areas which received high levels of acid precipitation for several decades in the mid-20th century (Lake Erie and Lake St. George) were collected, sectioned, and the relative changes in concentration of iron sulfides and acid extractable iron measured downcore, and compared to historical sulfur dioxide emission data.

3) Surficial sediments from Hamilton Harbour and Lake Erie were incubated with sulfate and organic carbon additions, and changes in redox, conductivity, and soluble sulfate, phosphorus and iron (products of internal loading) followed.

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Chapter 2: Internal iron loading and warm temperatures are preconditions for cyanobacterial dominance in embayments along Georgian Bay, Great Lakes

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2.1 Abstract

Previous work suggests that a high rate of internal ferrous iron (Fe^{2+}) loading from anoxic sediments into overlying waters favours cyanobacteria dominance (>50% of the phytoplankton biomass) over eukaryotic algae. This Cyanobacteria–Ferrous conceptual model was assessed along the Georgian Bay coastline of Lake Huron, Ontario, in one meso-eutrophic and three oligotrophic embayments that experience natural hypolimnetic anoxia. Cyanobacteria dominated all embayments in the relatively warmer summer of 2012 but not in the much cooler summer of 2014, although hypolimnetic anoxia and internal Fe^{2+} loading were observed in both summers in all embayments. A cyanobacteria bloom large enough to turn the lake visibly green was observed only in warmer 2012 in the meso-eutrophic embayment. Results show that warm summer temperatures and internal Fe^{2+} loading are necessary preconditions for cyanobacteria dominance, while high nutrient levels are needed to form large blooms. There were no consistent patterns between dominance and total and dissolved phosphorus (P), total nitrogen, ammonium, and nitrate. Internal P loading was not a necessary precondition for dominance. While P removal programs will decrease phytoplankton biomass in eutrophic waters, oxidized surficial sediments must be maintained throughout an aquatic system to prevent cyanobacteria dominance.

2.2 Introduction

Some of the primary goals of eutrophication management include lowering the risk of algal and cyanobacteria bloom formation and maintaining deepwater oxygen levels above critical levels for fish communities. These goals are typically accomplished at the watershed scale by lowering productivity through nutrient loading controls. The link between excessive phosphorus (P) loading and higher productivity has been known since the 1960s (Sakamoto 1966) with conclusive experimental confirmation published over 40 years ago (Schindler and Fee 1974; Schindler *et al.* 2008), providing the basis for widespread and often successful management actions to lower productivity by decreasing total P (TP) loading (Dolan 1993; Schindler *et al.* 2016). Regardless of whether eutrophic systems are nitrogen (N), P, or light-limited, point-source P controls are viewed as one of the primary means to induce nutrient limitation, thereby lowering productivity and, hence, phytoplankton biomass. New nutrient reduction targets for Lake Erie are focused on P loading only (International Joint Commission 2013). Some researchers advocate removing both N and P as a means of mitigating freshwater cyanobacteria blooms (Paerl *et al.* 2011; Gobler *et al.* 2016), an idea that has been vigorously disputed (Schindler 2012; Schindler *et al.* 2008, 2016). In spite of these criticisms, support exists for dual nutrient controls (e.g., US EPA 2015).

One of the hallmark characteristics of eutrophic systems is the high risk of developing potentially harmful cyanobacteria blooms. Here, we use the term "dominant" to describe cyanobacteria populations comprising >50% of th e total phytoplankton biomass. Further, the phrase "cyanobacteria bloom" is used in this paper to describe cyanobacteria populations with visible surface accumulations or in which the surface waters are clearly green regardless of whether cyanobacteria are dominant. Empirical among-lakes data show that the risk of cyanobacteria dominance increases with increasing average summer epilimnetic TP and total N (TN) concentrations (i.e., with increasing productivity) and decreasing TN/TP ratios; nevertheless, dominance can occur at most TP concentrations, TN concentrations, and TN/TP ratios (Downing *et al.* 2001), including systems that are not eutrophic (Chen *et al.* 2007, 2009; Carey *et al.* 2008; Vareli *et al.* 2009). Clearly, TN and TP are important risk factors for cyanobacteria dominance, but other factors must be investigated to consistently explain cyanobacteria dominance over their eukaryotic algal competitors along a trophic gradient.

Molot *et al.* (2014) hypothesized that much of the unexplained variance and the increased risk of cyanobacteria dominance with nutrient concentrations in Downing *et al.*'s (2001) meta-analysis might be accounted for by variation in internal ferrous iron (Fe^{2+}) loading, a process dependent on development of sediment anoxia. Anoxia is generally correlated with productivity

(i.e., with high nutrient levels as demonstrated by the relationship between TP and anoxic factor; Nürnberg 1995). Molot *et al.* (2014) presented a conceptual model (referred to here as the Cyanobacteria–Ferrous or Cyano-Fe model) describing cyanobacteria bloom formation as a function of sediment anoxia, P, N, Fe^{2+}, and sulfate (SO_4^{2-}). The central thesis of this conceptual model is that the magnitude of summer phytoplankton biomass is typically controlled by macronutrient concentrations (usually P or N but sometimes light in hypereutrophic systems), but dominance by eukaryotic algae or cyanobacteria is influenced by the availability of Fe^{2+}, regardless of what limits total phytoplankton biomass.

The unknown link between TP and TN and risk of cyanobacteria dominance across a macronutrient concentration gradient (Downing *et al.* 2001) may be explained to some extent by an increasing risk of surface sediment anoxia and internal Fe^{2+} loading with nutrient enrichment (Molot *et al.* 2014). Cyanobacteria have high Fe requirements relative to eukaryotic photoautotrophs and can only transport Fe^{2+} directly across the inner membrane (e.g., Kranzler *et al.* 2013; see review in Molot *et al.* 2014; C. Powe and L. Molot, unpublished data). In contrast, eukaryotic algae have lower Fe requirements and can transport Fe^{3+} (ferric Fe) directly across their inner membranes (C. Powe and L. Molot, unpublished data). Cyanobacteria demand for Fe is to some extent mitigated by Fe^{3+} scavenging mechanisms such as siderophores and extracellular capsular polysaccharides (Wilhelm 1995; Rose *et al.* 2005; Li *et al.* 2016; Sorichetti *et al.* 2016), but they are unable to transport Fe^{3+} across their cell membranes without prior reduction. While Fe^{2+} can be generated by biological and photoreduction of Fe^{3+}, Molot *et al.* (2014) hypothesized that rapid reoxidation to Fe^{3+} before transport across the inner membrane lowers cell supply rates.

Thus, Molot *et al.* (2014) hypothesized that large cyanobacteria populations that turn surface waters green (i.e., large blooms) must be supported by high external supply rates of Fe^{2+}, and the most likely source of this would be internal Fe^{2+} loading from anoxic sediments. Buoyancy-controlling cyanobacteria can migrate to at least 10–12 m below the surface to acquire nutrients (Camacho *et al.* 1996, 2000; Head *et al.* 1999; Gervais *et al.* 2003), suggesting that in eutrophic systems they may migrate into or adjacent to anoxic bottom waters to acquire Fe^{2+}. Thus, diffusion of Fe^{2+} from anoxic sediments into overlying waters may play a role in the success of these taxa. If, on the other hand, the external supply rate is low, then the Cyano-Fe model predicts that a system with a low supply rate of Fe^{2+} will be dominated by eukaryotic species.

The Cyano-Fe model suggests that the timing of a cyanobacteria bloom may in part be related to the seasonal onset of internal Fe^{2+} loading, which in turn is controlled by anoxia, reducible Fe content of surface sediments, and SO_4^{2-} reduction rate (Molot *et al.* 2014) in waters with

suitable temperatures and light. Evidence to date from eutrophic systems supports the Cyano-Fe model in thermally stratified lakes (Molot *et al.* 2010, 2014), with more limited evidence from polymictic systems (Smith *et al.* 2011). What constitutes a suitable temperature for cyanobacterial bloom formation during the summer is not clear. Cyanobacteria exceeded 20% of the phytoplankton biovolume in eutrophic Lake St. George, Ontario, only when the temperature at 1 m exceeded 21 °C (McQueen and Lean 1987). Several studies have examined the effects of temperature on photosynthesis and growth rate in laboratory cultures of cyanobacteria. The results are difficult to compare because of different growing conditions (e.g., culture media, irradiance, photoperiods, mixing conditions), but in general they describe maximum growth rates between 20 and 30 °C with much lower growth rates below 15 °C (Konopka and Brock 1978; Robarts and Zohary 1987; Butterwick *et al.* 2005). Paerl and Huisman (2009) suggested that maximum growth rates for eukaryotic algae are lower than those for cyanobacteria in warm waters and that cyanobacteria dominance is favoured in eutrophic waters above 25 °C. However, others have reported that some, but not all, eukaryotic species grow as rapidly as cyanobacteria at 25 to 30 °C in culture (Cole and Christian 2000; Butterwick *et al.* 2005). Tropical eukaryotic species are inherently adapted to high water temperatures, and transplanted species may not survive cold winters. Confounding our understanding of the impact of temperature on cyanobacterial bloom formation is the impact of climate warming on sediment anoxia via higher respiration rates and longer icefree seasons (Stainsby *et al.* 2011; North *et al.* 2014), which promote internal Fe^{2+} loading, thereby favouring cyanobacteria (Molot *et al.* 2014). Therefore, the temperature-mediated effects on cyanobacterial growth must be separated from those on sediment anoxia and internal Fe^{2+} loading.

In this study, the objective was to examine relationships among water level, temperature, sediment anoxia, nutrient levels, and internal Fe^{2+} and P loading on cyanobacteria dominance and bloom formation in one meso-eutrophic and three oligotrophic embayments along Georgian Bay (northeast Lake Huron in Ontario; Fig. 2.1). These embayments offer an excellent opportunity to test the validity of the Cyano-Fe model, since they span a range of nutrient regimes and some experience natural hypolimnetic anoxia during the ice-free season that is not linked with anthropogenic nutrient loading (TP concentrations are very low, <10 $\mu g \cdot L^{-1}$). Incomplete mixing during early spring means that deep waters begin the stratification season with a dissolved oxygen (DO) deficit, which can lead to anoxia even in oligotrophic systems (Molot *et al.* 1992). Naturally occurring anoxia is not uncommon in small, oligotrophic forested lakes with a maximum fetch <1.4 km in central Ontario (Molot *et al.* 1992).

In addition, one meso-eutrophic embayment in particular (Sturgeon Bay) has experienced frequent blooms of *Anabaena* since 1990 (Schiefer 2003). Water level was included in this study because there is a public perception that declining water levels in the Great Lakes in recent years (Georgian Bay Association 2016) has somehow been responsible for the increasing incidence of cyanobacteria blooms.

We hypothesized that warm temperatures, high nutrient levels, and internal Fe^{2+} loading are necessary preconditions for a bloom to form, while warm temperatures and internal Fe^{2+} loading are required for dominance across a trophic range. The study was conducted in a relatively warm (2012) and a much cooler (2014) summer, allowing us to distinguish between the potential impact of temperature and Fe^{2+} availability on cyanobacteria dominance. Seasonal time series data were collected to assess whether anoxia and internal Fe^{2+} loading preceded cyanobacterial dominance, as required by the Cyano-Fe model. If dominance occurs in the absence of either anoxia or internal Fe^{2+} loading, then the Cyano-Fe model must be either modified or rejected.

2.3 Materials and Methods

2.3.1 Study sites

Four embayments along the Georgian Bay coast (Lake Huron, Ontario) were the focus of this study. Three of these included the north basin of Sturgeon Bay (45°36'51.1236"N, 80°26'1.7052"W), Deep Bay (45°23'41.3982"N, 80°13'25.7916"W), and Twelve Mile Bay (45°5'0.927"N, 79°56'44.088"W) (Fig. 2.1). In 2014, North Bay (the northern bay of Honey Harbour; 44°53'7.1"N, 79°48'39.8"W) was sampled instead of Twelve Mile Bay. These embayments are located between Honey Harbour in the south and Sturgeon Bay Provincial Park near Pointe au Baril in the north, a distance of about 100 km.

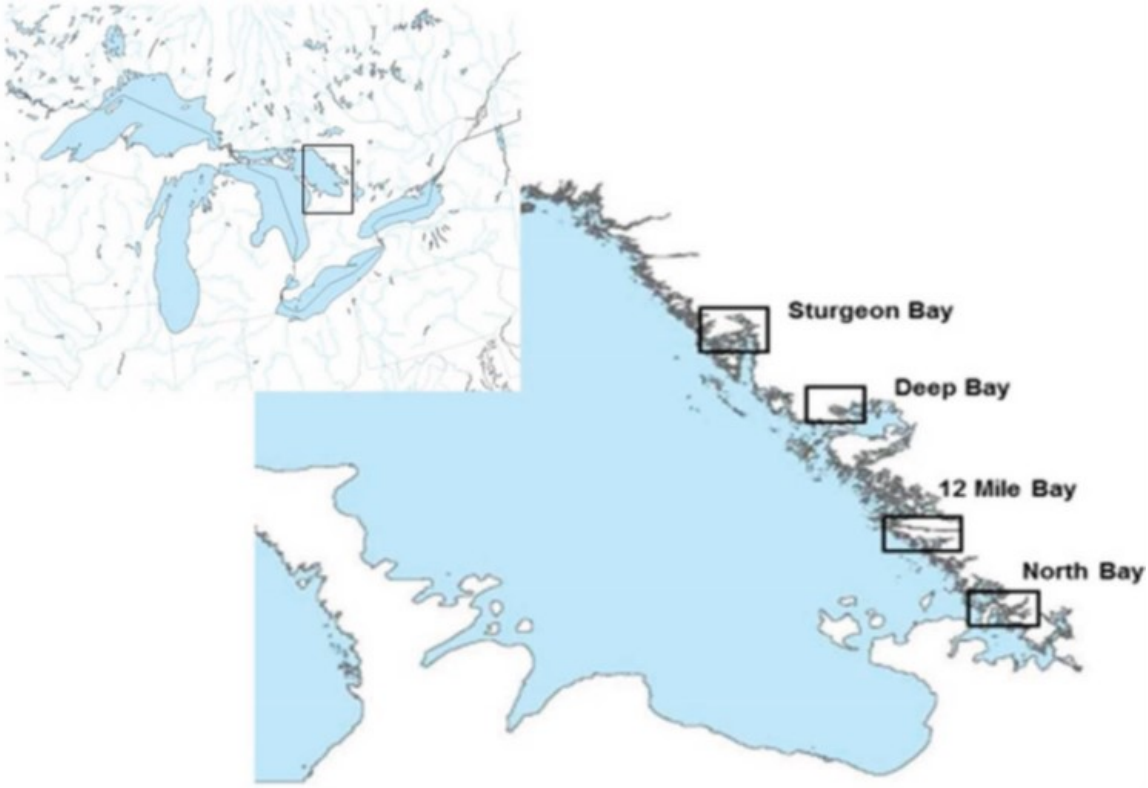

Figure 2.1 Location of study sites along Georgian Bay, Lake Huron. Inset shows Great Lakes region. Sturgeon Bay is meso-eutrophic, while the other embayments are oligotrophic. (Figure made from Natural Earth: http://www.naturalearthdata.com/.)

Physical characteristics of the study sites are listed in Table 2.1. The embayments are described in more detail in Schiefer (2003; Sturgeon Bay), Schiefer and Schiefer (2010; Twelve Mile Bay and North Bay), and Schiefer (2005; Deep Bay). The study area is located on the Precambrian Shield, with largely undeveloped forest catchments and wetlands. Many of the embayments along the Georgian Bay coast, including those studied here, have cottage development with numbers, size, and usage increasing over the past few decades from small summer occupancy units to multi-use, year-round establishments (Schiefer *et al* 2007; Schiefer and Schiefer 2010).

Table 2.1 Physical characteristics of the study embayments along the Georgian Bay, Lake Huron coast.

	Mean depth (m)	Max. Depth (m)	Surface area (km^2)	Watershed area (km^2)
Sturgeon Bay (N basin)	6	14	2.95	21.9
Deep Bay	10	21	2.85	26.8
Twelve Mile Bay	12	24	2.98	27.2
North Bay	–	22	2.20	11.2

Deep Bay, Twelve Mile Bay, and North Bay are oligotrophic, while the north basin of Sturgeon Bay is meso-eutrophic. Late summer blooms of filamentous cyanobacteria have been reported intermittently for many years in the north basin of Sturgeon Bay (Schiefer 2003). Reports of Sturgeon Bay blooms have increased from relatively infrequent occurrences prior to 1990 to annual events over the past few years. While Sturgeon Bay's relatively high TP concentration and blooms are unusual for the region where most embayments are oligotrophic, there is concern among community members that blooms may spread to other embayments currently unaffected.

2.3.2 Water sample collection

Sampling was biweekly. Sturgeon Bay and Deep Bay were sampled between mid-June and early September in 2012 and between mid-June and early October in 2014. Twelve Mile Bay was sampled between mid-June and early September in 2012, and North Bay was sampled between mid-June and early October in 2014. Embayments were sampled at their deepest points (Table 2.1). Profiles of conductivity, temperature, and DO were taken using a YSI 6600v2 Sonde connected to a YSI 650 display and data logging system (YSI Inc., Ohio). Prior to recording these measurements, the sonde was allowed time to stabilize between each 0.5 m vertical sampling interval.

Water samples were collected using a horizontal sampler without metal parts at 2 m intervals to a depth of 0.5 m above the sediment. Equal sample volumes from discrete depths were pooled to create integrated epilimnetic and metalimnetic samples, with the depths of the layer boundaries identified each time from temperature profiles; boundaries between the epilimnion, metalimnion, and hypolimnion were defined by the intersection of straight lines drawn through the temperature–depth profile of adjacent layers. To minimize the risk of contamination from previously collected samples, samples were collected from top to bottom of the water column, since surface samples generally have lower nutrient concentrations than deeper waters. Epilimnetic and metalimnetic samples were filtered through 400 μm acid-washed mesh to remove large zooplankters and debris and stored in 4 L acid-washed carboys before pooling. To minimize oxygen contamination after collection, hypolimnetic samples were transferred directly to an acid-washed syringe from the horizontal sampler through a customized T-fitting, which allowed samples to be carefully drawn into and flushed out of the syringe repeatedly until air bubbles were no longer visible in the apparatus. Five millilitres of sample was then used to flush the filter of possible contaminants. The syringe was then filled and filtered into acid-washed 50 mL snap cap vials through 0.2 μm syringe-tip filters (Sartorius cellulose acetate with glass fibre prefilter). Integrated epilimnetic, metalimnetic, and discrete hypolimnetic samples were

subsampled for ammonium (NH_4^+ measured as $NH_3^+ + NH_4^+$), nitrate (NO_3^- measured as $NO_2^- + NO_3^-$), SO_4^{2-}, total dissolved Fe, and total dissolved P (DP) and filtered through 0.2 µm syringe-tip filters into acid-washed vials. Dissolved inorganic N (DIN) is defined here as $NO_3^- + NH_4^+$. Subsamples were also collected for TN and TP and stored unfiltered in acid-washed vials.

Fe^{2+} was not measured directly because of the difficulty of quantifying this form accurately in remote locations and the likelihood of autoreduction of Fe^{3+} to Fe^{2+} by reagents in the presence of dissolved humic matter (Verschoor and Molot 2013). Instead, we assumed that where water column profiles showed large, increasing concentrations of dissolved Fe with increasing depth in anoxic waters relative to metalimnetic and epilimnetic concentrations, such large increases indicated release of Fe^{2+} from anoxic sediments similar to internal loading of P from anoxic sediments (Nürnberg and Dillon 1993; Molot and Dillon 2003). While some dissolved Fe may include other forms such as Fe sulfide, settling ferric hydroxides, and organic Fe compounds (e.g., Cook 1984; Rickard and Morse 2005), for the purposes of this study it was not necessary to quantify Fe^{2+} accurately, only to confirm that an internal loading profile in anoxic waters was present. We also assumed that increasing DP profiles with depth in anoxic waters was generated by internal P loading (Nürnberg 1984; Nürnberg *et al.* 1986). Internal P and Fe loading was assumed present in anoxic water if concentrations at the top of the anoxic profile were at least 50% higher than the highest of the metalimnetic or epilimnetic value. Groundwater influxes to these Precambrian Shield systems were assumed confined to the nearshore epilimnetic sediments and thus negligible contributions to the hypolimnetic zone. Subsamples for phytoplankton identification and enumeration were collected from the integrated epilimnetic and metalimnetic samples and preserved with Lugols iodine (4 mL per 96 mL of sample) in screw-cap centrifuge tubes (Jasprica 2002). Samples were gently mixed and stored in the dark until analysis.

2.3.3 Water chemistry methods

In 2012, TN, NO_3^-, NH_4^+, and SO_4^{2-} were analyzed at the University of Alberta Biogeochemical Analytical Service Laboratory in Edmonton. NH_4^+ (in 2012 and 2104) was analyzed using the Berthelot reaction method (Method 4500-NH4 F; American Water Works Association 1999) with a detection limit of 2 $\mu g \cdot L^{-1}$. NO_3^- was measured colourimetrically after reduction to nitrite (Method 4500-NO3 I; American Water Works Association 1999) with a detection limit of 1 $\mu g \cdot L^{-1}$. TN was determined by combustion–oxidation to nitrite with chemiluminescence detection (conformed to method D5176; American Society for Testing Materials 2008) with a detection limit of 10 $\mu g \cdot L^{-1}$. SO_4^{2-} was measured using ion chromatography with a detection limit of 0.04 $mg \cdot L^{-1}$.

In 2012, total and DP were analyzed at the Trent University laboratory in Dorset, Ontario, using ascorbic acid–molybdate colourimetry after autoclave digestion in sulfuric acid (Method 4500-P F; American Water Works Association 1999) with a detection limit of 1 $\mu g \cdot L^{-1}$. Total dissolved Fe was measured at the Water Quality Centre at Trent University in Peterborough, Ontario, with inductively coupled plasma – mass spectrometry (ICP-MS; Thermo XSeries-2) with a detection limit of 1 $\mu g \cdot L^{-1}$.

In 2014, NO_3^- and SO_4^{2-} were analyzed at the University of Waterloo with ion chromatography on a Dionex ICS-2100, total DP and total dissolved Fe were measured at the Trent University Water Quality Centre with ICP-MS (Agilent 8800 Triple Quadrupole), and TN and NH_4^+ were analyzed at the University of Alberta Biogeochemical Analytical Service Laboratory.

2.3.4 Phytoplankton analyses

Phytoplankton samples were identified at the genus level where possible and counted using the Utermöhl method (Lund *et al.* 1958) and an inverted microscope (Nikon DIAPHOT-TMD, Mississauga, Ontario) with phase-contrast optics. One millilitre of a well-mixed sample was pipetted into a 10 mm diameter, 1 cm deep Utermöhl slide and settled for a minimum of 8 h. Large individual and colonial eukaryotic phytoplankton and colonies and filaments of cyanobacteria were enumerated by counting the contents of the entire chamber at 100× magnification. Very small (~10 µm greatest axial linear dimension) individual eukaryotes and cyanobacteria were enumerated in 20–50 uniformly distributed fields with a minimum of 200 total individuals counted. All phytoplankton were classified by shape and size (Prescott 1970; Wehr and Sheath 2003). Measurements from 10–20 individual cells or colonies for each phytoplankton size–shape classification were taken at the highest practical magnification (up to 1600×) and averaged. Data were organized into as many as 50 distinct size–shape classifications, and the cyanobacterial and eukaryotic phytoplankton biovolumes were calculated by multiplying cell count values by their respective geometric cell volumes (Hillebrand *et al.* 1999; Olenina *et al.* 2006; Vadrucci *et al.* 2007). Biovolume was converted to biomass by assuming an approximate cell density of 1 $g \cdot mL^{-1}$. Population growth rate was calculated as the slope of ln(biomass) versus time.

2.3.5 Water level and meteorological data

Monthly water level data for Lake Michigan – Lake Huron were available online from Fisheries and Oceans Canada at http://www.tides.gc.ca/C&A/network_means-eng.html. Mean monthly water levels were derived from observations at six monitoring stations: Thessalon and Tobermory in Ontario; and Milwaukee (Wisconsin), Ludington, Mackinaw City, and Harbor Beach (Michigan) in the US. Levels are referenced to the International Great Lakes Datum 1985. Mean daily air temperatures at Muskoka A station (44°58'00.000"N, 79°18'00.000"W) between 1939 and 2004 were available online at http://climate.weather.gc.ca/historical_data/search_historic_data_e.html.

2.3.6 Statistical analyses

Redundancy analysis (RDA) was used to determine statistically significant relationships between phytoplankton community composition and environmental factors (epilimnetic and metalimnetic temperature, dissolved Fe at 10 m, redox potential at 10 m, DP at 10 m, epilimnetic TP, epilimnetic NH_4^+, and epilimnetic NO_3^-). Transformations were applied to environmental variables (z score standardization) and phytoplankton composition data (Hellinger transformation). Phytoplankton were excluded when their abundance was less than 10% of the total biomass to avoid a cluttered and unreadable diagram and because the focus of the study was on taxa that were dominant. Only data from Sturgeon in 2012 were used because this was the only lake–year to experience a bloom and cyanobacteria dominance. RDA modeling based on forward selection was performed in R using "rda" in the "vegan" package and "forward.sel" functions in the "packfor" package (R Core Team 2015; http://www.R-project.org). Pearson correlation coefficients were calculated using Microsoft Excel.

2.4 Results

Runoff to the embayments is soft water (generally with conductivity <0.08 $mS \cdot cm^{-1}$), but there are occasional intrusions of harder water through narrow channels connecting the embayments to the open waters of Georgian Bay where the conductivity is typically >0.18 $mS \cdot cm^{-1}$ (Schiefer *et al.* 2007; Schiefer and Schiefer 2010). During the summer of 2012, surface conductivity was 0.14–0.18 $mS \cdot cm^{-1}$ in Twelve Mile Bay, 0.09–0.11 $mS \cdot cm^{-1}$ in Deep Bay, and 0.08–0.10 $mS \cdot cm^{-1}$ in the north basin of Sturgeon Bay, suggesting varying mixtures of Georgian Bay water and Precambrian Shield runoff. Surface conductivity in 2014 ranged from 0.12 to 0.13 $mS \cdot cm^{-1}$ in North Bay. These measurements indicate that Sturgeon Bay and Deep Bay are less susceptible to water exchange with Georgian Bay than Twelve Mile Bay and North Bay. SO_4^{2-} concentrations in the embayments reflect a mixture of soft water Precambrian Shield inputs and Georgian Bay water. Concentrations in nearby Lake Huron are 15.9 $mg \cdot L^{-1}$ (Chapra *et al.* 2012), while in 2012 epilimnetic SO_4^{2-} ranged from 6.8 to 8.6 $mg \cdot L^{-1}$ in Sturgeon Bay, 7.5 to 8.9 $mg \cdot L^{-1}$ in Deep Bay, and 10.9 to 12.9 $mg \cdot L^{-1}$ in Twelve Mile. The higher SO_4^{2-} concentrations in Twelve Mile correspond to higher conductivity in this embayment than in the others, reflecting a greater water exchange rate with Georgian Bay. In June 2012, hypolimnetic SO_4^{2-} concentrations near the sediments were similar to epilimnetic concentrations in all embayments, but hypolimnetic concentrations began to decline in July probably because of SO_4^{2-} reduction, with differences of more than 1.5 $mg \cdot L^{-1}$ from top to bottom in mid-July in Sturgeon Bay and Twelve Mile and in late July in Deep Bay. SO_4^{2-} reduction rates were not high enough to prevent internal Fe^{2+} loading (latter is discussed below).

Mean summer (June–August) water levels in Lake Michigan – Lake Huron between 1918 and 2014 ranged from a low of 175.76 m in June 1964 to a high of 177.39 m in July and August 1986, a difference of 1.63 m (Fig. 2.2). The mean summer water level for the 25 year periods between 1964 and 1989 and 1990–2014 were 176.8 and 176.4 m, respectively, a difference of only 0.4 m. Therefore, lake levels in the period after 1990, which were associated anecdotally with a high incidence of blooms, were on average only slightly lower than the preceding period. However, water levels after 1999 were consistently low, averaging 176.2 m.

Mean summer air temperatures between 1939 and 2004 at Muskoka A station near Bracebridge, Ontario (the nearest weather station with a continuous record but ending in 2004) ranged from 15.1 °C in 1992 (about 1 year after Mount Pinatubo erupted in the Philippines) to 19.5 °C in 1955, with a mean of 17.4 °C. The anecdotal period of blooms (1990–2004) was slightly warmer than the 15-year prebloom period preceding it (1975–1989); means were 17.6 and 17.3 °C and medians were 17.8 and 17.0 °C, respectively. An alternative way of looking at

temperatures is to consider the frequency of a warm years; 41% of the summers were warmer than 17.5 °C during 1939–1989 compared with 67% during 1990–2004 (Fig. 2.2). The mean summer air temperature was 1.7 °C cooler in 2014 compared with 2012 at nearby Parry Sound Canadian Coast Guard meteorological station (the Parry Sound station lacks a long-term record). The temporal sequences of chemical and biological changes are an important aspect of the Cyano-Fe model and are examined next.

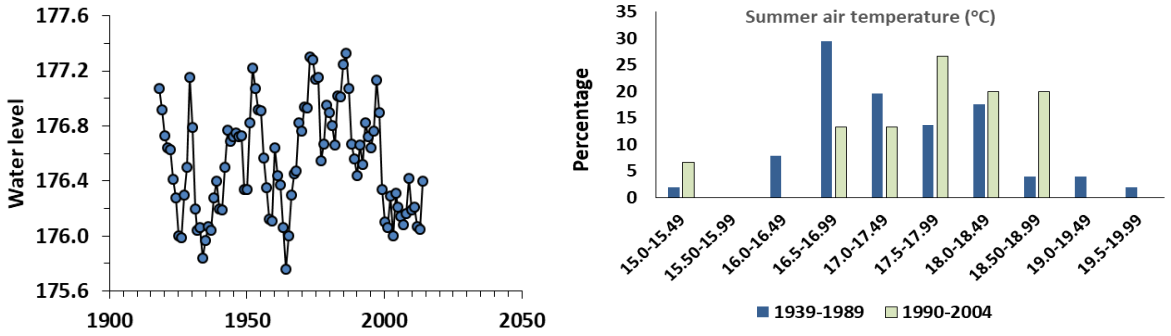

Figure 2.2 (A) Mean June–August water level (m) in Lake Michigan – Lake Huron between 1918 and 2014 and (B) frequency distribution of mean summer air temperatures for 1939–1989 and 1990–2014 at Muskoka A weather station.

The difference in summer air temperatures in 2012 and 2014 had a major effect on surface water temperatures in Sturgeon Bay. The number of days when the top 3 m of the water column were above 20 °C was twice as high in the latter half of June and three times higher in July in 2012 compared with 2014 (Figs. 2.3A and 2.3B). In both years, the north basin was thermally stratified when sampling began in mid-June and remained stratified until sampling ended in early September, although the hypolimnion disappeared by mid-July in 2012 and mid-September in 2014, having been entrained into the metalimnion. Although anoxia is in principle defined to be 0 mg·L^{-1} DO, internal loading of Fe^{2+} and P was observed above 0 mg·L^{-1}. Indeed, the lowest value recorded in Sturgeon Bay with the YSI was 0.045 mg·L^{-1}, perhaps because of YSI calibration issues. Since the redox potential declined sharply below 0.5 mg·L^{-1} (Fig. 2.3C), we used the redox–DO relationship to operationally define the top boundary of the anoxic zone as 0.5 mg DO·L^{-1}. The top of the anoxic zone moved upwards from the bottom (10–12 m depending on where the anchor was dropped) to a depth of 7 m towards the end of July in both years (Fig. 2.3D).

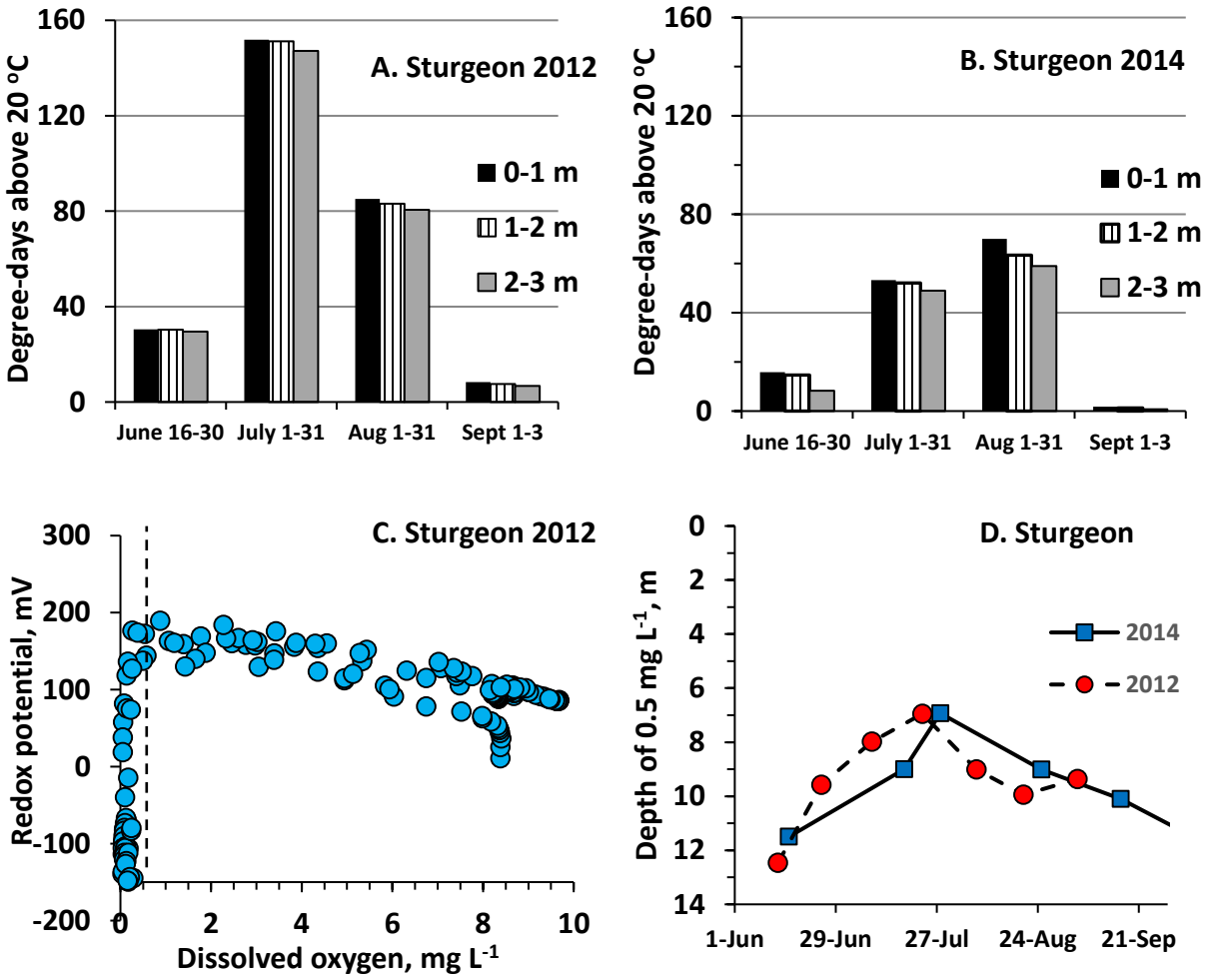

Figure 2.3 Degree-days above 20 °C in the top 3 m in (A) 2012 and (B) 2014, (C) dissolved oxygen versus redox (dashed line is 0.5 mg·L–1) in 2012, and (D) the depth at which 0.5 mg·L–1 dissolved oxygen occurred in Sturgeon Bay in 2012 (solid line, squares) and 2014 (dashed line, circles).

Dissolved Fe concentrations at 10 m (2 m above the bottom) ranged more than 20-fold from 45 to 2290 µg·L^{-1} in 2012 and 147 to 3053 µg·L^{-1} in 2014 (Table 2.2). Dissolved Fe concentrations increased between 9 and 12 m after these strata became anoxic. Concentrations reached 1000 µg·L^{-1} at 10 m by the end of July in both years compared with epilimnetic concentrations <60 µg·L^{-1} in 2012 and <150 µg·L^{-1} in 2014 (Figs. 2.4A and 2.4B). When the 9 m stratum became oxic in August, dissolved Fe concentrations declined from 535 µg·L^{-1} to much lower levels similar to those found in the epilimnion due to dilution with low Fe water and oxidation of dissolved Fe^{2+} to Fe^{3+} and precipitation. DP also increased in the hypolimnion by late July in both years when anoxia developed, but the increases were not as large as the

increases in dissolved Fe (Table 2.2; Figs. 2.4C and 2.4D). Epilimnetic DP levels ranged from 6 to 10 µg·L^{-1} in 2012 and 6 to 12 µg·L^{-1} in 2014. Hypolimnetic DP reached 55 µg·L^{-1} at 10 m in 2012 and 44 µg·L^{-1} in 2014, exceeding 180 µg·L^{-1} at 12 m near the sediments in both years.

Table 2.2 Mean nutrient concentrations ± 1 standard deviation in the epilimnion (total phosphorus (TP), NH_4^+, and NO_3^-) and 2 m off the bottom (dissolved P (DP), dissolved iron (DFe)) in the embayments June–September.

	Sturgeon		Deep		Twelve Mile	North
	2012	2014	2012	2014	2012	2014
TP	13.1±2.9 (9.2-16.9)	12.8±3.0 (9.6-17.1)	7.5±2.0 (3.5-9.6)	15.4±4.5 (10.9-20.3)	9.0±2.4 (5.2-11.2)	10.9±3.3 (6.2-14.8)
NH_4^+	18±18 (1-49)	19±12 (1-31)	33±68 (1-186)	29±12 (13-41)	20±26 (1-70)	21±28 (1-63)
NO_3^-	4±5 (0.5-15)	87±71 (0.5-197)	7±5 (0.5-15)	92±45 (30-145)	10±12 (1-35)	132±50 (7-202)
DP	18.7±17.6 (6.5-54.7)	19.4±16.6 (6.7-43.8)	5.2±0.6 (4.5-6.0)	12.9±7.3 (5.0-23.8)	4.2±0.6 (3.6-5.2)	6.8±2.4 (4.2-9.7)
DFe	70±838 (45-2290)	1249±1321 (147-3053)	1065±1736 (88-4803)	792±237 (162-669)	37±8 (27-52)	163±209 (49-537)

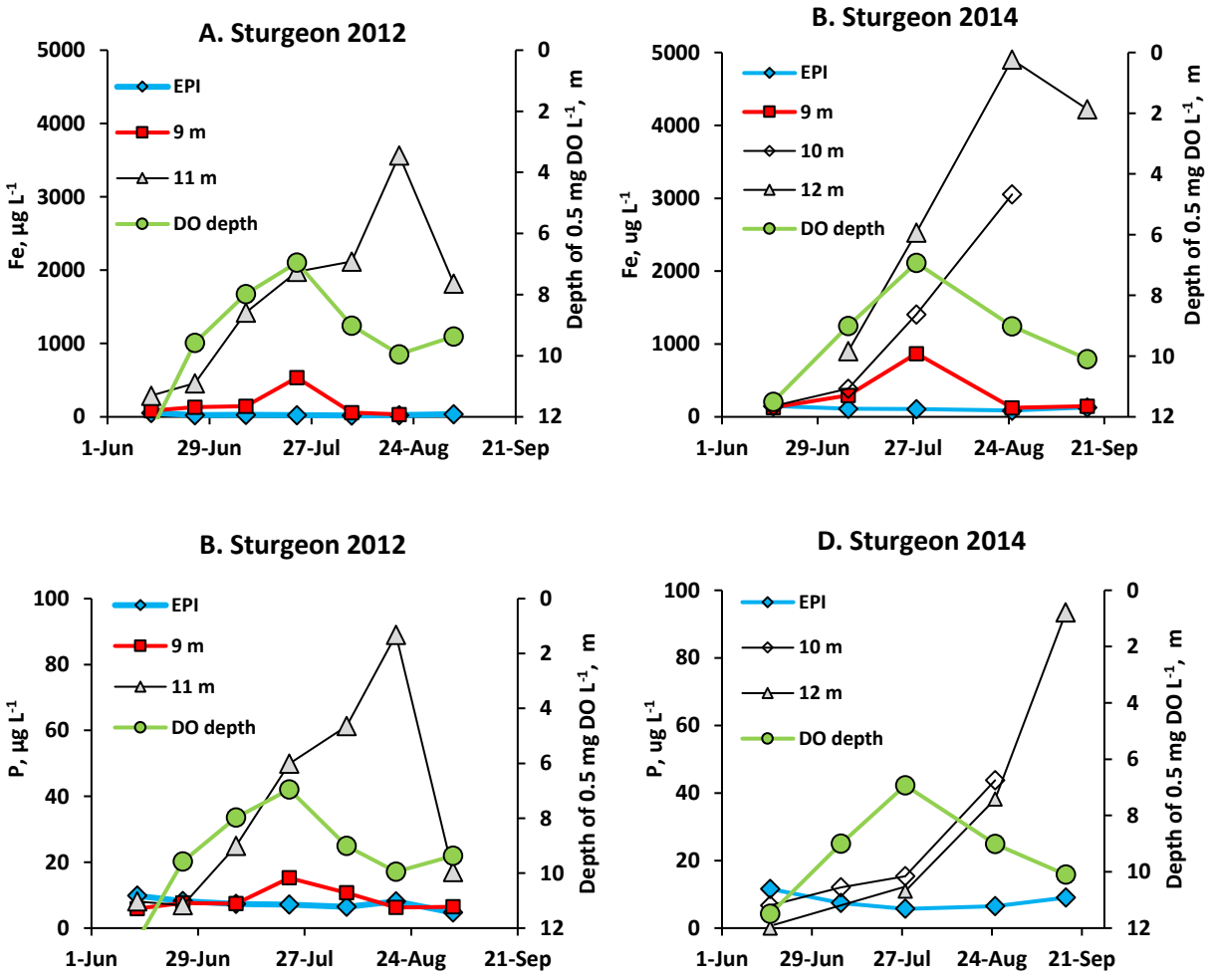

Figure 2.4 Dissolved Fe (panels A and B) and dissolved P (panels C and D) in Sturgeon Bay epilimnion and several hypolimnetic depths and the depth at which 0.5 mg·L^{-1} dissolved oxygen occurred in 2012 and 2014.

In 2012, cyanobacteria dominated from first half of July through the summer, reaching over 80% of the phytoplankton biomass in the epilimnion by late July. In contrast they did not become dominant in 2014, although they had reached 33% of the biomass by mid-September (Figs. 2.5A and 2.5C). Growth of cyanobacteria began after sediment anoxia developed in early summer in both years, reaching "bloom" proportions in 2012 (i.e., the lake was visibly green) but not in 2014 (Figs. 2.5B and 2.5D). The epilimnetic biomass peaked at 9300 $\mu g \cdot L^{-1}$ in early September 2012 when it was predominantly *Dolichospermum*, a filamentous N_2 fixer. The most common cyanobacteria in 2014 were *Woronichinia* and *Dolichospermum*. The net summer growth rates for the epilimnetic populations were 0.054 day^{-1} in 2012 and 0.016 day^{-1} in 2014 when it was cooler (Fig. 2.5E).

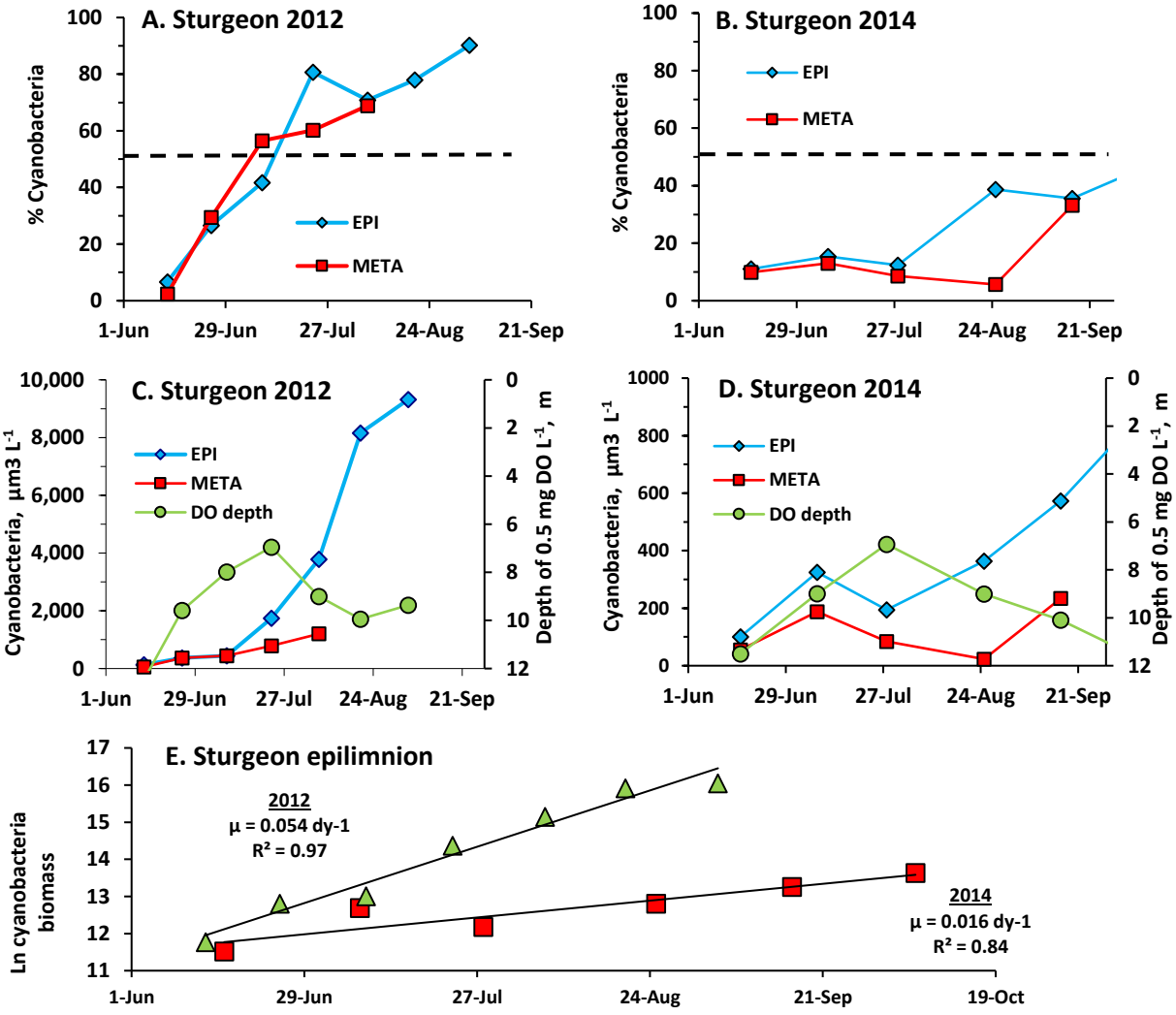

Figure 2.5 Cyanobacteria percent (panels A and B), biomass (panels C and D), and summer growth (panel E) in Sturgeon Bay in 2012 and 2014. The horizontal dashed line in panels A and B is the 50% criterion for defining cyanobacteria dominance. The net growth rates, µ, are shown in panel E with the regression R^2.

Mean epilimnetic and metalimnetic nutrient concentrations ranged from 9 to 17 µg·L^{-1} TP (Table 2.2) and 372 to 432 µg·L^{-1} TN. TN/TP ratios by mass in the metalimnion and epilimnion ranged from 18 to 73 in 2012 (means 37 and 38, respectively) and 20 to 56 in 2014 (means 29 and 36, respectively), which are generally indicative of P deficiency (Healey and Hendzel 1979). However, while TN/TP ratios were high, epilimnetic and metalimnetic NH_4^+ and NO_3^- concentrations were occasionally undetectable, especially immediately before cyanobacteria growth began, indicating that the bulk of the N was present in dissolved organic or particulate form, which may have favoured diazotrophs such as *Dolichospermum*. Twelve taxa exceeding 10% of the phytoplankton biomass on at least one occasion (excluding picophytoplankton) were used in the RDA of Sturgeon Bay data in 2012: *Dolichospermum, Coelomoron, Microcystis, Planktothrix, Woronichinia, Peridinium, Ceratium, Asterionella, Fragilaria*, unknown coccoids, unknown diatom "centrics", and unknown algal "medium rounds". *Dolichospermum*, the most abundant of the cyanobacteria, was closely associated with high dissolved Fe at 10 m and to a lesser degree high epilimnetic NH_4^+ during late summer as well as low redox at 10 m and low epilimnetic NO_3^- (Fig. 2.6). Variance inflation factors for epilimnetic TP, epilimnetic NO_3^-, and redox potential at 10 m depth were high (45.8–84.9), indicating a high degree of multicollinearity, while variance inflation factors for dissolved Fe and DP at 10 m and epilimnetic NH_4^+ were relatively low (2.0–7.2). The proportion of variance explained by RDA axis 1 was 49.3%, and the proportion explained by axis 2 was 27.5%. Epilimnetic NH_4^+ was removed without loss of adjusted R2, which was 70%. Global tests of the RDA were significant ($p < 0.001$), and the first two canonical axes were significant ($p < 0.01$) with and without epilimnetic NH_4^+ included.

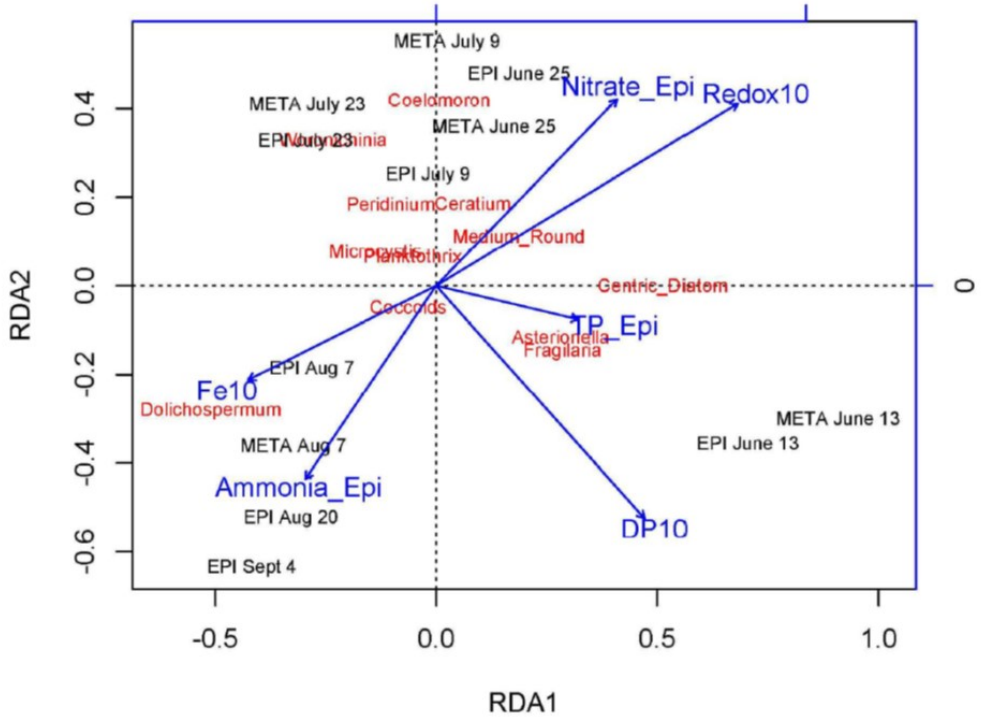

Figure 2.6 Redundancy analysis (RDA) triplot of Sturgeon Bay 2012 phytoplankton response variables, environmental predictor variables, and sampling dates (objects).

2.4.2 Deep Bay (2012 and 2014)

The anoxic zone in Deep Bay extended from the sediments upwards to a depth of 7.2 m in early August 2012, while in 2014 it had only extended upwards to 14.5 m by late August (Fig. 2.7). Dissolved Fe responded to development of anoxia in both years (Table 2.2) but increased over time at a slower rate than in Sturgeon Bay. In comparison, DP did not increase as bottom waters became anoxic in 2012 but increased by about 7 µg·L^{-1} in 2014 (Table 2.2; Fig. 2.7).

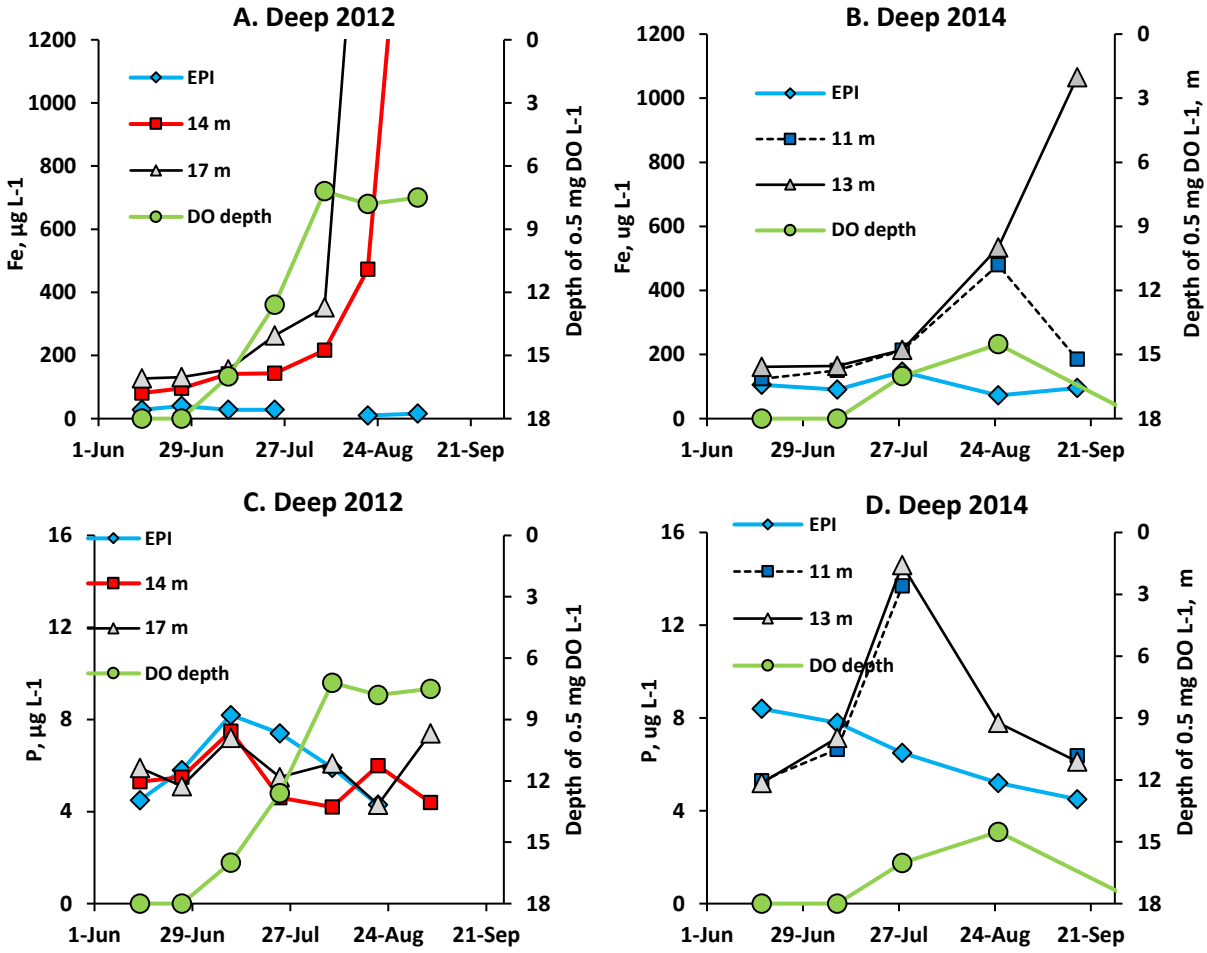

Figure 2.7 Dissolved Fe (panels A and B) and dissolved P (panels C and D) in the epilimnion and several hypolimnetic depths and the depth at which 0.5 mg·L^{-1} dissolved oxygen occurred in Deep Bay in 2012 and 2014.

Cyanobacteria populations were small in this oligotrophic embayment: <400 µg·L^{-1} in both years (Fig. 2.8). Cyanobacteria growth began after the appearance of anoxic sediments. Cyanobacteria dominated the epilimnion at the end of the summer in 2012 but did not dominate the metalimnion in 2012 or either layer in 2014 (Fig. 2.8). In 2012, the metalimnetic population consisted primarily of the non-N_2-fixing colonial cyanobacteria, Microcystis. The epilimnetic population consisted primarily of Dolichospermum, which also was the most important cyanobacterium in both layers in 2014. Mean epilimnetic and metalimnetic TP and TN concentrations ranged from 4 to 20 µg·L^{-1} TP (Table 2.2) and 270 to 480 µg·L^{-1} TN. TN/TP ratios by mass in the metalimnia and epilimnia ranged from 19 to 74 in 2012 (means 38 and 45, respectively) and 19 to 49 in 2014 (means 32 and 42, respectively).

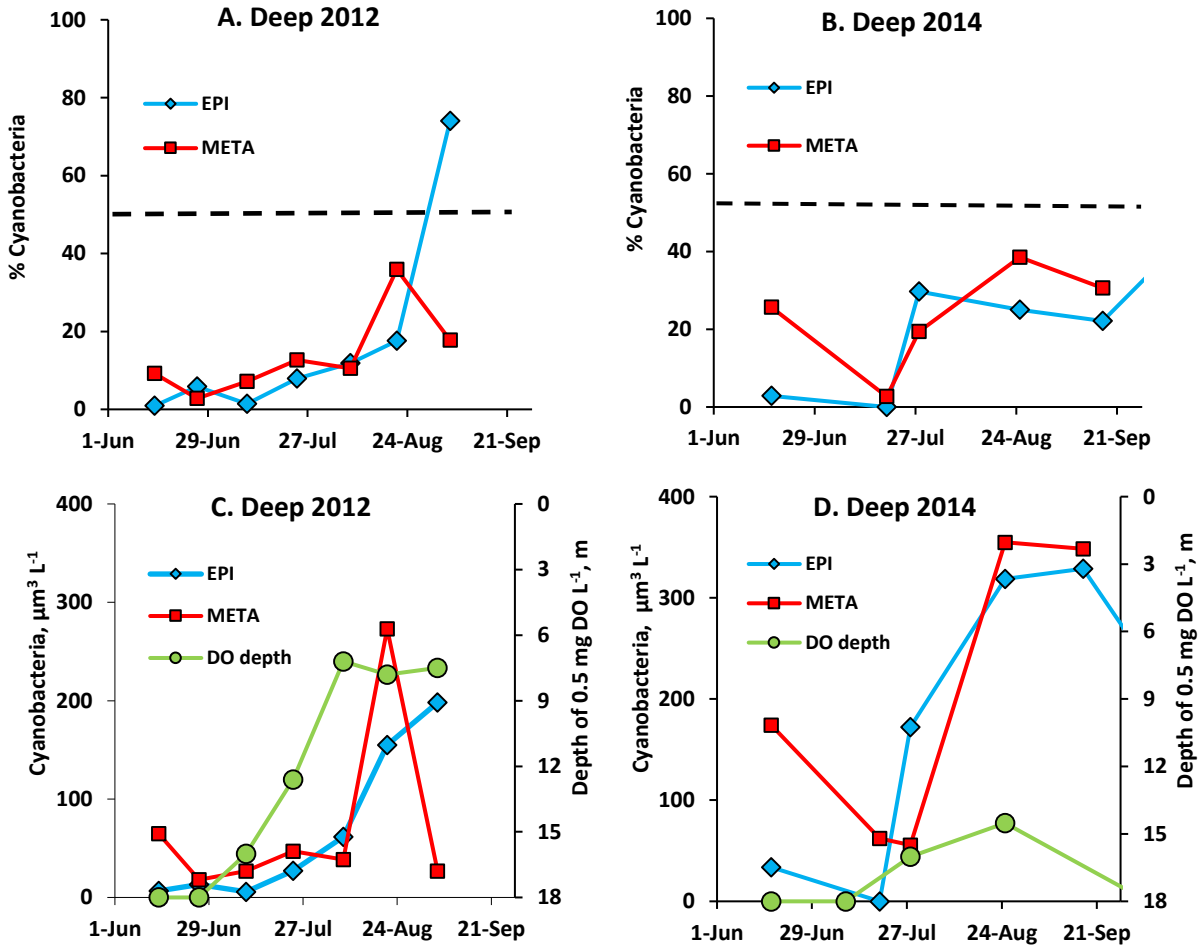

Figure 2.8 Cyanobacteria percent (panels A and B) and biomass (panels C and D) in Deep Bay in 2012 and 2014. The horizontal dashed line in panels A and B is the 50% criterion for defining cyanobacteria dominance.

2.4.3 Twelve Mile Bay (2012)

Anoxia was first detected in bottom waters (10.5 m) in late June 2012 in Twelve Mile Bay (Fig. 2.9A). As the summer progressed, the top of the anoxic zone gradually extended upwards to 9m by early August but was less than 2 m from the sediments, in contrast with the anoxic zones in Sturgeon Bay (Fig. 2.5) and Deep Bay (Fig. 2.7), which were thicker. Internal Fe^{2+} loading was clearly detectable at 10m in early August (Fig. 2.9A), having increased to 141 from 48 $\mu g \cdot L^{-1}$ 2 weeks earlier, but the increase at 9 m was much smaller (Table 2.2). The maximum difference in DP between the bottom and the top of the hypolimnion on any given day was quite small: <2.3 $\mu g \cdot L^{-1}$ (Table 2.2). This small concentration gradient indicates that internal P loading and contributions from mineralization of settling particulate P in the hypolimnion were minimal after anoxia developed. Epilimnetic TP ranged from 5 to 11 $\mu g \cdot L^{-1}$ (Table 2.2), epilimnetic TN ranged from 208 to 267 $\mu g \cdot L^{-1}$, and epilimnetic TN/TP ratios ranged from 19 to 44.

Clear differences in epi- and metalimnetic cyanobacteria biomass emerged by late August after anoxia and internal Fe loading developed (Fig. 2.9B). The non-N_2 fixer *Planktothrix* was the dominant taxon in metalimnetic samples collected in late August, reaching 1600 $\mu g \cdot L^{-1}$ in early September, comprising 89% of the phytoplankton biomass. In contrast, the cyanobacteria biomass was less than 60 $\mu g \cdot L^{-1}$ in the epilimnion during the summer, never exceeding 8% of phytoplankton biomass.

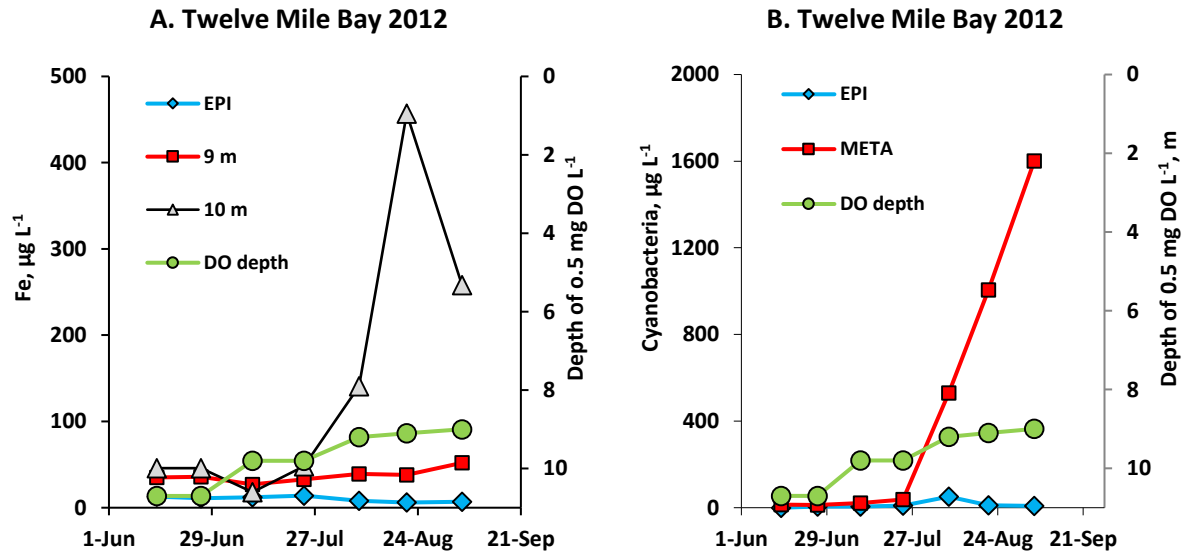

Figure 2.9 The depth at which 0.5 $mg \cdot L^{-1}$ dissolved oxygen occurred and dissolved Fe concentration (A) and cyanobacteria biomass (B) in Twelve Mile Bay in 2012.

2.4.4 North Bay (2014)

DO exceeded 0.5 mg·L^{-1} throughout the water column to the bottom at 16 m until the end of July (Fig. 2.10A). The top of the anoxic zone reached its shallowest depth of 8 m at the end of August and then began to move downwards. There was a small increase in dissolved Fe of 9 and 20 µg·L^{-1} at 14 and 15 m between late July and late August (Fig. 2.10A), then much larger increases occurred at these depths (463 and 739 µg·L^{-1}, respectively) by mid-September (Table 2.2). There was no evidence of internal P loading after anoxia was detected with hypolimnetic concentrations less than 10 µg P·L^{-1} throughout the sampling period except very close to the sediments late in the season in September and October (Table 2.2). Epilimnetic TP ranged from 6 to 15 µg·L^{-1} (Table 2.2), epilimnetic TN ranged from 316 to 450 µg·L^{-1}, and epilimnetic TN/TP ratios ranged from 29 to 73. Epilimnetic NH_4^+ was below the detection limit throughout the study period, while epilimnetic NO_3^- was low in mid-June (7 µg·L^{-1}), increasing to higher levels in July and August (184–202 µg·L^{-1}). Cyanobacteria growth began between late July and late August coincident with development of anoxia and small increases in internal Fe^{2+} loading (Fig. 2.10B). Cyanobacteria biomass was low and never dominated the phytoplankton community in the epilimnion and the metalimnion. Maximum biomass and proportions were reached in the epilimnion in mid-September (244 µg·L^{-1}, 23% of total phytoplankton biomass) and in the metalimnion in early October (309 µg·L^{-1}, 41% of total phytoplankton biomass). Cyanobacteria consisted primarily of *Dolichospermum* and *Aphanizomenon*.

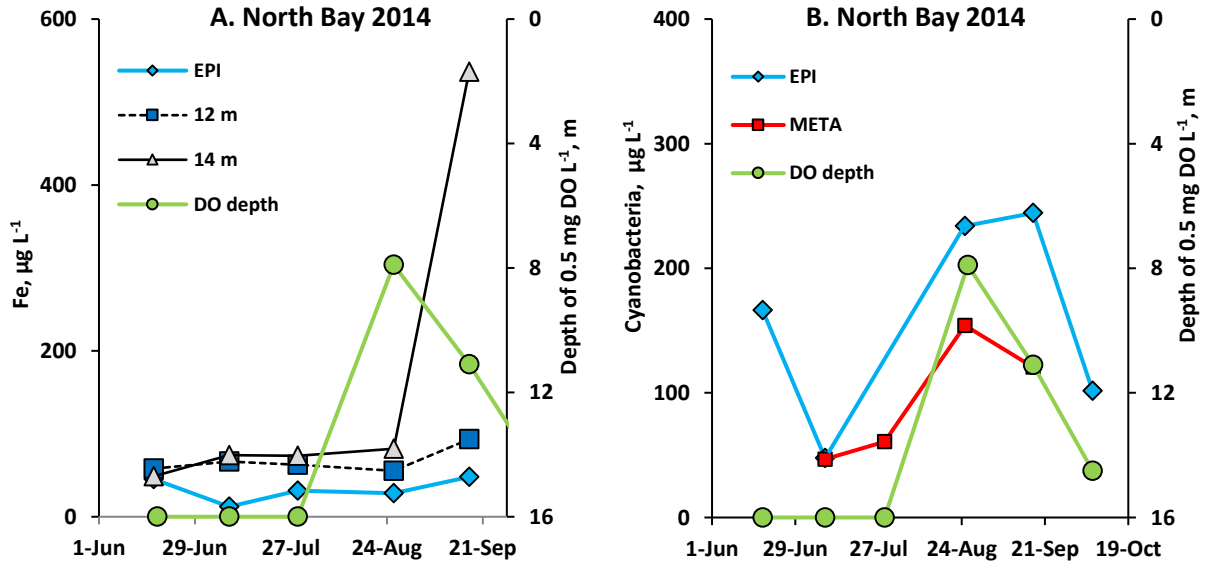

Figure 2.10 The depth at which 0.5 mg·L^{-1} dissolved oxygen occurred and dissolved Fe concentration (A) and cyanobacteria biomass (B) in North Bay in 2014.

2.4.5 TN and TP correlations with biomass

Mean epilimnetic and metalimnetic cyanobacteria biomass was highly correlated with mean epilimnetic and metalimnetic TN ($r = 0.91$) and mean TP ($r = 0.94$) ($n = 5$, (3 epilimnia and 2 metalimnia)) in 2012 but not in 2014 ($r = -0.02$ for TP and -0.26 for TN, $n = 6$, (3 epilimnia and 3 metalimnia)).

2.4.6 DIN

In 2012, epilimnetic NO_3^- and NH_4^+ concentrations were depleted (i.e., below the detection limit) by the end of June in Sturgeon Bay and Deep Bay (Fig. 2.11; Table 2.2), and subsequently, the dominant cyanobacteria in both lakes was N_2-fixing *Dolichospermum*. In Twelve Mile Bay, metalimnetic NH_4^+ was depleted by early July, while NO_3^- was available until the end of July. The metalimnion was dominated by non-N_2-fixing *Planktothrix*. NH_4^+ peaked briefly in Sturgeon Bay and Twelve Mile Bay as N_2-fixing cyanobacteria became dominant and peaked 1 month before dominance in Deep Bay. NO_3^- and NH_4^+ concentrations were higher in Sturgeon Bay and Deep Bay in 2014 than in 2012 (Fig. 2.11). The positive relationship between *Dolichospermum* and epilimnetic NH_4^+ illustrated in the RDA diagram (Fig. 2.6) was influenced in large part by the buildup in midseason of NH_4^+ from leakage or senescence associated with the large population of *Dolichospermum* (Fig. 2.11).

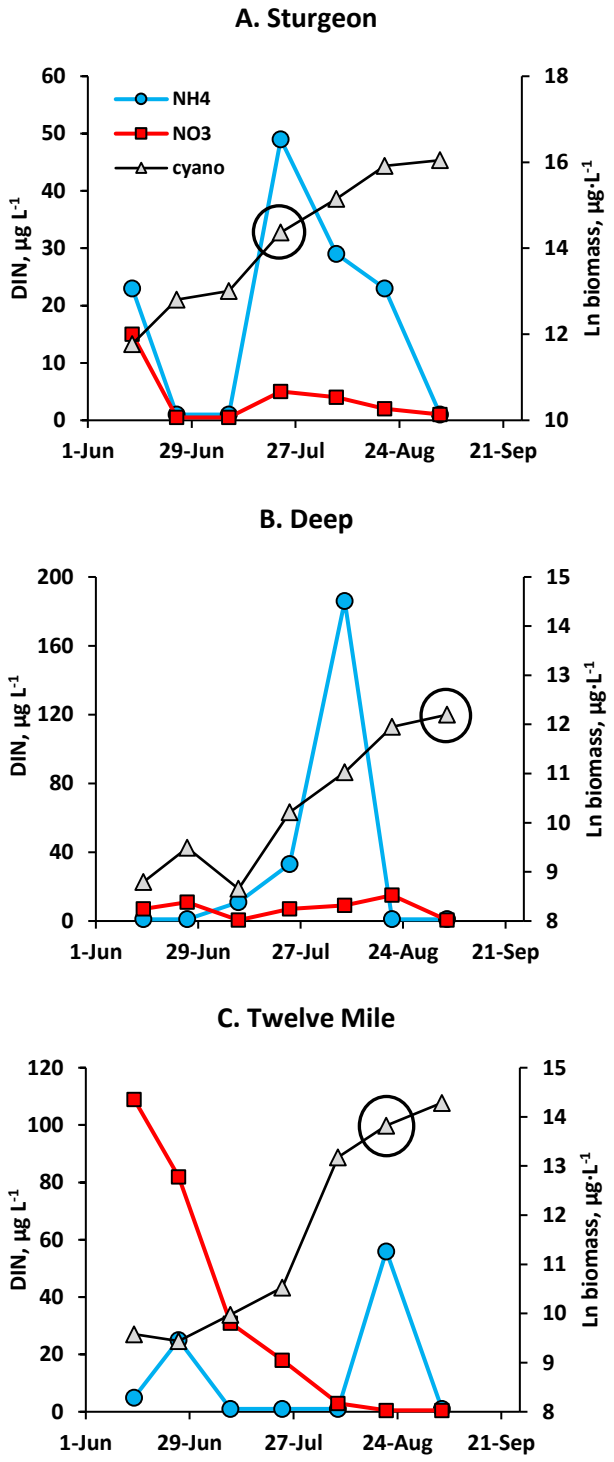

Figure 2.11 NH_4^+ and NO_3^- (i.e., dissolved inorganic nitrogen, DIN) and cyanobacteria biomass ($\mu g \cdot L^{-1}$) in 2012 in (A) Sturgeon Bay (epilimnion), (B) Deep Bay (epilimnion), and (C) Twelve Mile Bay (metalimnion). Circles denote when cyanobacteria biomass first exceeded 50% of total phytoplankton biomass.

2.5 Discussion

2.5.1 Do the observations support the Cyano-Fe model?

An important feature of this study is that it interprets ecological and biogeochemical observations from several naturally anoxic, unproductive embayments as well as a meso-eutrophic embayment within a novel framework (the Cyano-Fe model) to explain cyanobacteria dominance and blooms across the productivity gradient. The framework tells us that the factors that control dominance operate in both oligotrophic and eutrophic lakes and that cyanobacteria dominance in oligotrophic lakes differs from blooms in more productive lakes only by the size of the populations. We cannot ignore N and P, but the framework forces us to look beyond them.

Two preconditions, warm temperatures and sediment anoxia with internal Fe^{2+} loading, were necessary for cyanobacteria dominance in all embayments regardless of TP, DIN, and TN concentrations. Cyanobacteria did not dominate in 2014 when temperatures were cooler although anoxia and internal Fe^{2+} loading occurred (but reached 30% of phytoplankton biomass in Sturgeon Bay). Three preconditions were needed to generate both cyanobacteria dominance and a large, visibly blooming population: high P levels, internal Fe^{2+} loading, and warm temperatures. These three preconditions were present only in Sturgeon Bay in 2012. These results support the Cyano-Fe conceptual model described by Molot *et al.* (2014) but modified to include the impact of water temperature on cyanobacteria growth rate.

Mean epilimnetic and metalimnetic cyanobacteria biomass was highly correlated with mean epilimnetic and metalimnetic TN and mean TP in 2012 but not in 2014. These correlations are consistent with the fact that higher nutrient levels support more biomass when temperatures are warm but cannot be used to infer that either N or P concentrations were responsible for cyanobacteria dominance in light of cyanobacteria dominance of the oligotrophic embayments in 2012.

There was no consistent pattern between cyanobacteria dominance and DIN in the embayments. Negative correlations between low NO_3^- and cyanobacteria biomass have been reported before (McQueen and Lean 1987; Nürnberg 2007) and have been interpreted by some as a causal relationship in which cyanobacteria purportedly have a competitive advantage at low NO_3^- and higher NH_4^+ (Hyenstrand *et al.* 1998). However, in 2012 both NH_4^+ and NO_3^- were depleted in the weeks preceding cyanobacteria population growth (conditions during this critical period determine the outcome of cyanobacteria versus eukaryotic algae completion) in Sturgeon Bay and Twelve Mile Bay, and in Deep Bay, NO_3^- was higher than NH_4^+ so it seems unlikely that a low NO_3^-/high NH_4^+ ratio regulated the outcome of competition between cyanobacteria and eukaryotic algae.

High TN/TP ratios suggest that the embayments were P-limited, but they could have been N-limited for short periods of time when DIN was undetectable in the euphotic zone, especially in Sturgeon Bay and Deep Bay, which were dominated by N_2-fixing *Dolichospermum*. DIN depletion may have contributed to growth of N_2 fixers in 2012. The NH_4^+ spike during the cyanobacteria growth periods may have been leakage from N_2 fixation or senescence. N_2 fixation is an Fe-rich process (see review of Fe requirements in Molot *et al.* 2014), so it is not surprising that growth of N_2 fixers occurred during periods of anoxia accompanied by internal Fe^{2+} loading.

Among the embayments and years, there were no consistent patterns in P concentration before initiation of cyanobacteria population growth and during the periods of cyanobacteria growth. For example, internal P loading occurred in Sturgeon Bay but not in Twelve Mile Bay or Deep Bay in 2012, although all three embayments were dominated by cyanobacteria and had anoxic hypolimnia. In Sturgeon Bay, cyanobacteria did not exceed 50% of the phytoplankton biomass in 2014 even in the presence of internal P loading.

The time series monitoring used in this study reinforces the conclusions of other studies (e.g., the experimental removal of Fe^{2+} in Lake 227 (Molot *et al.* 2010) and observations in Lake 227 and Hamilton Harbour (Molot *et al.* 2014)) that show an important role for internal Fe^{2+} loading in the competitive outcome between eukaryotic algae and cyanobacteria.

Episodic anoxia and internal Fe^{2+} loading may promote cyanobacteria bloom formation in shallow polymictic lakes. The sediment–water interface in eutrophic Missisquoi Bay, Lake Champlain, was hypoxic (~1 $mg \cdot L^{-1}$) at the beginning of a cyanobacteria bloom on 27 and 28 July 2008 (Smith *et al.* 2011). Fe^{2+} was detected 2–3 mm below the sediment surface, although the very high detection limit of the voltammetric method (~1100 $\mu g \cdot L^{-1}$ for Fe^{2+} compared with 1.0 $\mu g \cdot L^{-1}$ for total Fe using ICP-MS) does not exclude the possibility that biologically relevant concentrations occurred much closer to or even above the sediment surface. In late August, the full bloom in Missisquoi Bay was coincident with anoxic conditions and relatively high concentrations of Fe^{2+} (1100–2200 $\mu g \cdot L^{-1}$) at the sediment–water interface, suggesting that winds were light and water column mixing was not turbulent, which would allow an anoxic boundary layer to form at the sediment–water interface. In eutrophic, polymictic Clear Lake in California, where an Aphanizomenon bloom occurred each spring, the sediment surface was frequently anoxic but often of short duration (Wurtsbaugh and Horne 1983).

The Baltic Sea is a eutrophic, brackish water system with a long history of cyanobacteria blooms (Kahru *et al.* 2007; Funkey *et al.* 2014). In a paleolimnological study in the Baltic Sea, hypoxia and cyanobacteria abundance were correlated during an 8000-year period (Funkey *et al.* 2014). The increased abundance may have been caused by higher P concentrations from

internal P loading. Phosphate release is typically coupled to reduction of ferric hydroxides that release Fe^{2+} when sediments become anoxic (Jensen and Andersen 1992), so if internal P loading occurred during episodes of water column hypoxia, it is possible that internal Fe^{2+} loading also occurred.

Favourable conditions for cyanobacterial dominance may exist in other naturally anoxic oligotrophic embayments along the Georgian Bay coast, although TP concentrations in these embayments are not high enough to cause large surface blooms. Favourable growth conditions for cyanobacteria in these embayments include a combination of natural anoxia caused by incomplete spring turnover (Molot *et al.* 1992), an adequate source of easily reducible Fe in sediments, and SO_4^{2-} concentrations low enough not to completely inhibit internal Fe^{2+} loading via formation of Fe sulfides (Loh *et al.* 2013).

2.5.2 Water level, warmer waters, and incidence of cyanobacteria blooms

The publically perceived increase in cyanobacteria blooms in Sturgeon Bay after 1990 (Schiefer 2003; Georgian Bay Association 2016) may be related to a warming trend rather than to changes in land use or water level in Georgian Bay (Fig. 2.2). Water levels were consistently at the low end of the historical range between 1999 and 2014 but were at the higher end between 1990 and 1998 (Fig. 2.2) (Gronewold and Stow 2014); hence, it appears unlikely that changes in water levels alone explain all of the blooms reported after 1990. Diatom-inferred TP concentrations in Sturgeon Bay have been stable and relatively high (20–24 $\mu g \cdot L^{-1}$) since about 1800, suggesting that the TP-enriched state is natural (Cumming *et al.* 2006) and thus not a result of cottage development in recent decades.

Cumming *et al.* (2006) also reported increases in pigment concentrations of total diatoms and colonial cyanobacteria in Sturgeon Bay occurred after approximately 1950, which are consistent with public reports of increased incidence of cyanobacteria blooms. It was speculated that shifts in diatom species composition in recent decades to those that prefer more stable thermal stratification can be attributed to climate change and warmer surface waters. The Muskoka A weather records show a warming trend between 1950 and 2004, but the record does not go back far enough to compare periods before and after 1950. An analysis of Canadian weather records by Zhang *et al.* (2000) between 1900 and 1998 revealed a summer warming trend in the Georgian Bay area of about 1 °C per 99 years in daily maximum air temperature and over 2 °C per 99 years in daily minimum air temperature, most of which seem to have occurred after 1950. While the effect of warmer maximum daily air temperatures during the summer on lake warming is well understood, the increase in daily minimum air temperatures is no less important if it decreases overnight cooling (Lehman 2002).

While the extent of anoxia, nutrients, and implied availability of Fe^{2+} (from dissolved Fe profiles) were similar in 2012 and 2014 in Sturgeon Bay, temperatures were not, and we conclude that cyanobacteria growth was probably influenced by temperature. The net cyanobacteria growth rate in cooler 2014 was about 30% of the net growth rate in 2012. The warming regional climate is consistent with the public perception of increased incidence of cyanobacteria blooms in Sturgeon Bay and in Ontario in general (Winter *et al.* 2011).

While cyanobacteria may respond to warmer summers with higher growth rates, other climate-related mechanisms might also favour their dominance, such as longer ice-free seasons and thermal stratification periods (Stainsby *et al.* 2011). These longer periods will lengthen the extent of the hypolimnetic oxygen depletion season, which in turn could lead to an increase in the spatial and temporal extent of anoxia (Foley *et al.* 2012; North *et al.* 2014) and internal Fe^{2+} loading, especially in lakes that are susceptible to development of anoxia. Analysis of long-term oxygen and temperature records from Lake Simcoe in central Ontario shows that the lengthening of the ice-free season since 1980 by 30 days was accompanied by a lengthening of the oxygen depletion period in the hypolimnion (Li 2016).

2.5.3 Implications of the Cyano-Fe model for management

To prevent cyanobacteria blooms, the amount of substances contributing to exhaustion of dissolved oxygen must be lowered to levels that maintain surface sediments in an oxidized state. If a P removal program in a eutrophic system does little to improve sediment redox, then blooms will continue although productivity might be lower under the new loading regime.

The Cyano-Fe model can contribute to improved eutrophication management by leading us to design TP concentration and loading targets that prevent low redox levels in surficial sediments. For example, the current guideline for maximum acceptable TP concentration in Ontario is 20 $\mu g \cdot L^{-1}$ (Ontario Ministry of Environment and Energy 1994), which is assumed to prevent cyanobacteria blooms based on historical data. However, increasing incidences of blooms in Lake Erie (Conroy *et al.* 2005; Michalak *et al.* 2013) and in inland oligotrophic and mesotrophic lakes in recent years (Carey *et al.* 2008; Winter *et al.* 2011) suggest that this target is not universally applicable, and more stringent targets may now be required for some lakes. The relationship between sediment redox and bloom formation suggests that nutrients (and perhaps organic matter loading, especially to polymictic systems) should be managed with explicit objectives to decrease productivity and DO consumption to nutrient levels that will maintain DO concentrations above a critical level at the sediment–water interface.

If external TP loading criteria were based on a mechanistic understanding of the relationship among external P loading, anoxia, and internal Fe^{2+} loading for a given lake and climate regime,

lake-specific loading targets could be developed to decrease the spatial and temporal extent of anoxia. Similarly, in situ aeration might be more effective at preventing cyanobacteria blooms if systems were designed to deliver oxygen at a rate that leaves surficial sediments with a sufficiently high redox over a larger lake area, including shallow bays, than is currently considered necessary.

Schindler and his colleagues argued that N removal would have little impact on bloom formation in eutrophic lakes because N_2 fixation compensates for N losses sufficiently to allow cyanobacteria to dominate (Schindler 2012; Schindler *et al.* 2008, 2016). The amount of N_2 fixed will depend on the N debt, but we hypothesize that it is also dependent on the supply rate of Fe^{2+}. Schindler's argument is based in part on continued dominance by N_2-fixing cyanobacteria in Lake 227 for more than 20 years after experimental N additions ceased in 1990 while experimental P additions continued (Paterson *et al.* 2011; Schindler 2012; Schindler *et al.* 2008, 2016). The Lake 227 experiment clearly showed that N_2 fixation, even if operating only for a short but critical time, has been sufficient for cyanobacteria to continue dominating the lake with no significant decline in their biomass (Paterson *et al.* 2011). The Cyano-Fe model explains the post-1990 blooms in Lake 227 after N additions ceased and the lake became N-limited. Continued anoxia in the hypolimnion allowed high rates of internal Fe^{2+} loading (Molot *et al.* 2010) to support N_2 fixation, a process that has high Fe demands (see review in Molot *et al.* 2014) and is energetically costly (Stam *et al.* 1987). Scott and McCarthy (2010) argued that N_2 fixation rates are, in general, too low to alleviate N deficiency; however, N_2 fixation was clearly important in Clear Lake, California, providing over 40% of the annual N budget (Horne and Goldman 1972), even though N_2 fixation was at times Fe-limited (Wurtsbaugh and Horne 1983). The evidence to date and the Cyano-Fe model suggest that if managers want to shut off N_2 fixation, they will have to shut off internal Fe^{2+} loading.

Laboratory studies indicate that N removal will have a negligible impact on cyanobacteria growth in natural systems as long as sufficient micronutrients, including Fe^{2+}, are available. Growth rates and maximum biomass yields of heterocystous N_2 fixers in nutrient-replete, single-species laboratory cultures using only N_2 as a source of N were only slightly different (approximately 10%) than when grown with NH_4^+ and NO_3^- (Allen and Arnon 1955; Bagchi *et al.* 1985; Schlangstedt *et al.* 1987; Vargas *et al.* 1998). A lower growth rate caused by partial or whole dependence on N_2 implies that it will take longer for an N_2-fixing cyanobacteria population to reach its maximum yield. It is also possible that the lag phase of the growth curve might be longer in situ, which could affect the timing of bloom onset and maximum biomass.

Nevertheless, all of the NH_4^+ and NO_3^- had to be removed from these cultures to achieve a modest difference in maximum population size.

There is, however, one exception regarding the effectiveness of N removal. The Cyano-Fe model predicts that cyanobacteria dominance will cease with nutrient removal only if sediment redox is maintained at a level that suppresses reduction of Fe^{3+} to Fe^{2+}. In aquatic systems where P controls have not been able to sufficiently diminish internal Fe^{2+} loading rates (i.e., systems in which sediment redox remains low) and additional P controls are not feasible, nitrification of NH_4^+ without N removal in wastewater treatment plants (WWTP; i.e., no denitrification step) might be beneficial in cases where external N loading to watersheds is dominated by WWTP point sources because in situ nitrification by nitrifying bacteria can be a major oxygen sink in eutrophic lakes (Müller *et al.* 2012; Clevinger *et al.* 2014). WWTP nitrification will lower in situ oxygen demand and could raise the sediment redox level sufficiently in watersheds where point source N loads are relatively large. In addition, subsequent in situ NO_3^- -based respiration can raise sediment redox, thicken the oxidized layer in surficial sediments, and suppress internal P and Fe^{2+} loading (Jensen and Andersen 1992; Kleeberg and Kozerski 1997; Hemond and Lin 2010). Hence, WWTP nitrification before effluent discharge might be a beneficial course of action in some situations as long as aquatic health standards for NO_3^- are not exceeded. The emphasis here is on the impact that WWTP nitrification has on sediment redox, not on phytoplankton macronutrient supply, since the total external N supply will essentially be the same (although internal loading of N and P might be lowered by a higher sediment redox). Nutrient export from diffuse urban and agricultural sources is acknowledged as very important in many watersheds, but discussion of management of diffuse sources is beyond the scope of this paper.

If N removal fails to suppress a bloom because of a failure to improve sediment redox, it will shift cyanobacteria species composition to N_2 fixers if it shifts a lake from P to N deficiency. It may also affect cyanobacteria toxin production, although the impact on toxin production is unclear (Pick 2016). Low N appears to mitigate toxin production in non-N_2-fixing *Microcystis* (Harke *et al.* 2015; Gobler *et al.* 2016); however, N_2-fixing *Aphanizomenon* produces more toxin under moderate N stress (Gagnon and Pick 2012). These studies suggest that toxin production varies unevenly along an N gradient from strongly excessive N relative to P to severe N depletion.

A final management consideration is that WWTP N removal (both nitrification and denitrification) might be needed in some locations to help mitigate marine coastal eutrophication (i.e., reduce coastal productivity), especially in riverine systems with large point source loads and low N retention rates.

This study represents the first test of the Cyano-Fe conceptual model in natural systems. This and other studies (Molot *et al.* 2010, 2014) support the model modified to include the impact of water temperature on cyanobacteria growth rate. The collective evidence from contrasting oligotrophic and meso-eutrophic freshwater systems suggests that warm temperatures, anoxia, and internal Fe^{2+} loading are critical preconditions for cyanobacteria dominance, and these preconditions along with high P are needed for relatively large, visible blooms to form.

The Cyano-Fe model could be useful for improved management of cyanobacteria blooms, but more work is needed to test validity of this conceptual model that couples the oxidation state of surficial sediments and geochemical cycles to cyanobacteria bloom formation under a range of real conditions, especially in polymictic and brackish waters. Paying close attention to the period prior to bloom onset will be critical to improving our mechanistic understanding of how blooms form. A warming climate will increase the risk of sediment anoxia and internal Fe^{2+} loading as well as increase water temperatures into a more optimal range for cyanobacteria growth in temperate regions. A warming climate, therefore, has implications for greater incidence of cyanobacteria dominance along a trophic gradient and bloom formation in P-rich waters.

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Chapter 3: Accumulation of acid volatile sulfides and acid extractable iron in lakes Erie and St. George

3.1 Abstract

Reductions in sulfur dioxide emissions may have indirectly contributed to increases in internal loading of ferrous iron by reducing the amount of sulfate available to form iron sulfide precipitates, which can act as a sink for ferrous iron: a microbial micronutrient. The paleolimnological records of acid volatile sulfides (AVS) and acid extractable iron in the central basin of Lake Erie and Lake St. George in southern Ontario were examined by sectioning and analysis of sediment cores. The results indicated that AVS and acid extractable iron declined in Lake Erie in a pattern generally consistent with a decline in historical sulfate concentrations, SO_2 deposition and productivity, with the exception of an anomaly which occurred around 1975. The pattern in Lake St. George was similar but levels of AVS and iron were an order of magnitude less than in Erie and more variable. Productivity in this lake has also declined, thus potentially contributing to variability and measurement limitations in the data. The large decline in AVS in central Erie under relatively constant anoxia suggests that internal ferrous loading may have increased since 1980. More research on the diagenesis of sulfide minerals and states of anoxia in these lakes, and investigating other systems may yield more conclusive results.

3.2 Introduction

Long-term datasets have revealed downward trends in sulfate (SO_4) anion levels after 1990 in acid-sensitive areas such as the Dorset lakes in central Ontario (Chapra *et al.* 2012). Similarly, reductions in total phosphorus (TP) concentrations also occurred in the Great Lakes beginning in the mid-1970s (Jeppesen *et al.* 2005). The latter trend reflects decades of research and active abatement strategies to reduce excessive TP loading and thus decrease lake productivity (Schindler 2012, Dolan 1993, Jeppesen *et al.* 2005). The former trend is a response to declines in acid precipitation following rigorous emission controls. A consequence of downward trends in both productivity and sulfate deposition may have been decreased sulfide production which is dependent on sulfate and organic matter, thus leading to more internal ferrous iron (Fe^{2+}) loading from anoxic sediments. Fe^{2+} is a readily available form of micronutrient to many algae, including cyanobacteria, which have a higher cellular requirement than eukaryotic algae (Wilhelm 1995). Fe^{2+} has been implicated as a contributing factor in the formation of nuisance algal blooms (Anderson & Morel 1982, Wilhelm 1995, Molot *et al.* 2010, 2014, Verschoor *et al.* 2017), some of which are toxic. Release of Fe^{2+} from sediments is promoted by microbial reduction processes under anoxic conditions at low nitrate but restricted by sulfide production (from sulfate reduction), which also occurs under anoxic conditions. Sulfide precipitates highly soluble Fe^{2+} into insoluble ferrous sulfides, thereby reducing overall lake iron availability and acting as a permanent sink of this element when they are buried in sediments (Hutchinson 1957; Carignan & Tessier 1988). Hence, lakes in which the sulfide formation rate is high enough to severely limit Fe^{2+} diffusion into overlying waters may not experience cyanobacteria blooms or bloom severity may be limited. Increases in Fe^{2+} due indirectly to decreased sulfate levels may be partially responsible for recent increases in bloom activity in Lake Erie and in inland lakes in Ontario and Quebec. Detailed biological, physical, and chemical records exist in sediments, revealing long-term trends via various proxies. Investigating sediment paleolimnology for sulfides and other biological and chemical markers may reveal supporting evidence for the decline of sulfide depositions and the co-occurrence of cyanobacterial blooms. An understanding of the biogeochemical characteristics of sediments and how they release nutrients, particularly under anoxic conditions, could eventually lead to improved methods of controlling cyanobacteria blooms through a robust, bottom-up approach.

3.3 Research Objectives

To perform paleo reconstruction of acid volatile sulfides (AVS) and acid extractable iron in order to determine if changes in these are related to SO_2 emission controls and increasing outbreaks of cyanobacteria blooms.

3.4 Materials and Methods

Initially, 2 m deep sediment cores were collected with a gravity corer aboard the CCGS Limnos during the summer of 2014 from the central basin in Lake Erie (Station 880, Figure 3.1), transported under refrigeration, then sectioned under a nitrogen atmosphere, with each section collected in glass pre-weighed jars, and promptly frozen for preservation.

Sediment cores were also collected from the west basin of Lake St. George: a small, mesotrophic inland kettle lake on the Oak Ridges Moraine in southern York Region north of the City of Toronto, on August 8, 2017 (surface area 10.3 ha, maximum depth 15.3 m; 43°57′20.71″N, 79°25′32.37″W; McQueen and Lean 1987, Bédard and Knowles 1997) (Fig. 3.1).

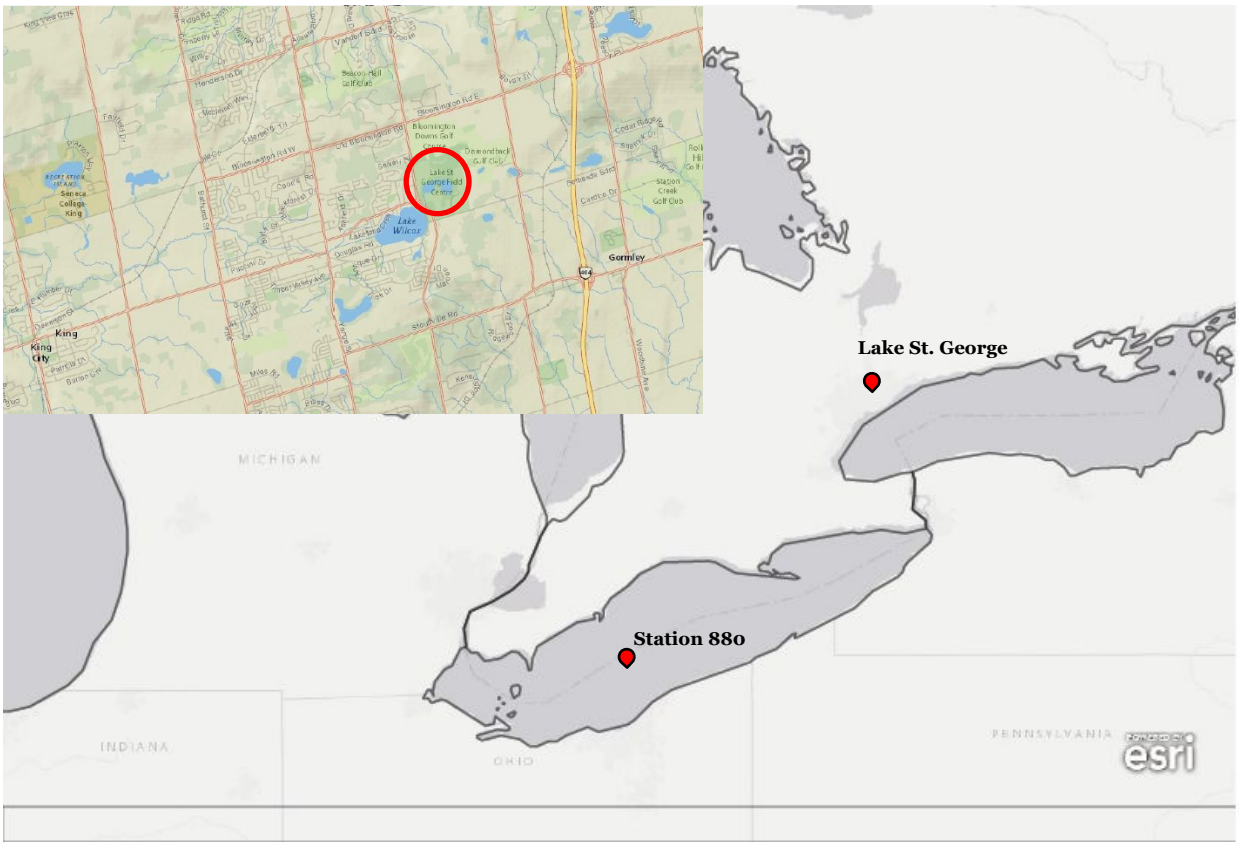

Figure 3.1 Sampling sites for sediment cores within Ontario. Inset details location of inland sampling site, Lake St. George (red circle). Images courtesy of NatGeo Mapmaker Interactive.

Dissolved oxygen (DO), temperature, pH, conductivity, chlorophyll fluorescence, and phycocyanin fluorescence profiles were measured at 1 m increments with a YSI 6500 sonde and 650 handheld display. Water samples were collected from the epilimnion (integrated 0-3 m), metalimnion (integrated 4-7 m) and hypolimnion (8, 10, 12, and 13 m) for total phosphorus (TP), chlorophyll a (GFC and GFF filters), nitrate, ammonium, total dissolved nitrogen (TDN), total Kjeldahl N, major cations, major anions, and dissolved trace metals. Samples were filtered on site with 0.45 um Minisart syringe filters with pre-filters (GF + cellulose acetate) and sent to the University of Alberta Biogeochemical Analytical Service Laboratory in Edmonton for analysis. Analysis was conducted on the frozen sediments using a 0.5 cm diameter brass punch to collect subsamples, which were then analysed for AVS and acid-extractable iron. The AVS of each section was determined using a modification of the method described by Brouwer and Murphy (1994), where approximately 0.5 g of frozen sediment subsample was deposited and weighed into a 5 mL inner glass vial, which was then placed inside an outer 20 mL glass scintillation vial containing 5.0 mL sulfide anti-oxidant buffer ("SAOB", which consisted of 2 M NaOH, 0.1 M ascorbic acid, and 0.1 M EDTA) trap solution. Nitrogen gas was then used to purge all headspaces, 2.0 mL 1 M HCl was pipetted onto the sediment, and the outer vial quickly sealed. All samples were then agitated on a rotary shaker at 150 rpm for 1 hour at room temperature. Trapped sulfide levels were determined with an Orion 9416BNWP ion selective electrode and Orion 3 Star digital millivolt meter after calibration with 10^{-1} to 10^{-6} M Na_2S in SAOB solutions.

Acid-extractable iron of each section was determined using a modification of the Psenner extraction method (Psenner and Pucsko 1988, Lukkari *et al.* 2007), where subsamples of approximately 1 g of frozen sediment were weighed into 50 mL centrifuge tubes, and sequential extraction commenced in 25 mL of bicarbonate-dithionite solution (0.11 M $NaHCO_3$ + 0.11 M $Na_2S_2O_4$) under nitrogen gas for 30 min at 40°C with manual shaking every 10 minutes, then in 25 mL of 0.1 M NaOH solution at room temperature for 16-20 hrs on an orbital shaker, and finally in 25 mL of 0.5 M HCl at 25°C for 1hr. At each extraction step, sediments were vigorously agitated in each tube with a vortex mixer to ensure adequate contact with reagents and rinsing solutions (2X 10 mL distilled water) and pelleted via centrifugation at 3500g for 10 min (Sorvall Legend RT). The supernatants were recovered into fresh 50 mL tubes, along with pellet rinses, and topped up to a total volume of 50 mL for each sample fraction. Each fraction was analysed for iron content via flame atomic absorption spectroscopy (PerkinElmer AAnalyst 200 and control software on a standard PC) with manual sampling and calibration with standard solutions derived from serial dilutions of a reference standard (SPEX CertiPrep Cat.# PLFE2-

2Y). The extractions prior to the HCl step served to remove other sources of iron from the sediments (e.g. humic bound) so that the HCl fraction, which was of primary interest, would more accurately reflect metals associated with sulfide deposits, including iron pyrites.

Gravimetric analysis of all sediment sections was conducted with approximately 1 g subsamples. Each subsample was deposited into a pre-weighed ceramic crucible and dried in an oven at 105°C for 24 hours, then cooled in a desiccator before re-weighing. The results from these data were used to convert wet weights of sediments to dry weights and calculate water composition. One core from each site was sectioned and sent in its entirety to the Canada Centre for Inland Waters in Burlington to be dated via the ^{210}Pb method.

3.5 Results

Lake Erie AVS declined steeply after 1980 with a 75% decline between 1980 and 1990, followed by a significant slowing of this decline post 1990 (Fig. 3.2). The acid-extractable Fe pattern was similar to the AVS pattern, though Fe levels were approximately an order of magnitude greater. The AVS and acid-extractable iron trends in Lake Erie seemed to closely follow the historical SO_2 emission trends of the region, except for sediments from the mid 1970's (Fig. 3.2). From 1960-1975, AVS and acid-extractable iron decreased and deviated from the historical pattern of emissions. The loss of AVS between 1960 and 1975 may be the result of diagenesis (where sulfides are degraded and solubilized by microorganisms), or may be the result of AVS degradation following a disturbance, such as a large storm, where exposure to oxygen will remove AVS deposits up to 10 cm deep when assisted by bioturbating benthic organisms (Howard & Evans 1993, Leonard *et al.* 1993, Eimers *et al.* 2006). Indeed, a very large regional storm occurred on November 10, 1975, with gusts in the area reported to almost 80 km/hr. Physical analysis of the sediments reveals higher porosity during this time period (Fig. 3.3), consistent with a physical disturbance of the sediments, and therefore the loss of AVS in this section of the core seems to be due to oxidation following physical disturbance. Comparison of AVS levels with known trends in lake sulfate levels (Fig. 3.4) also showed a close association post 1980, i.e., except for the c.1975 anomaly.

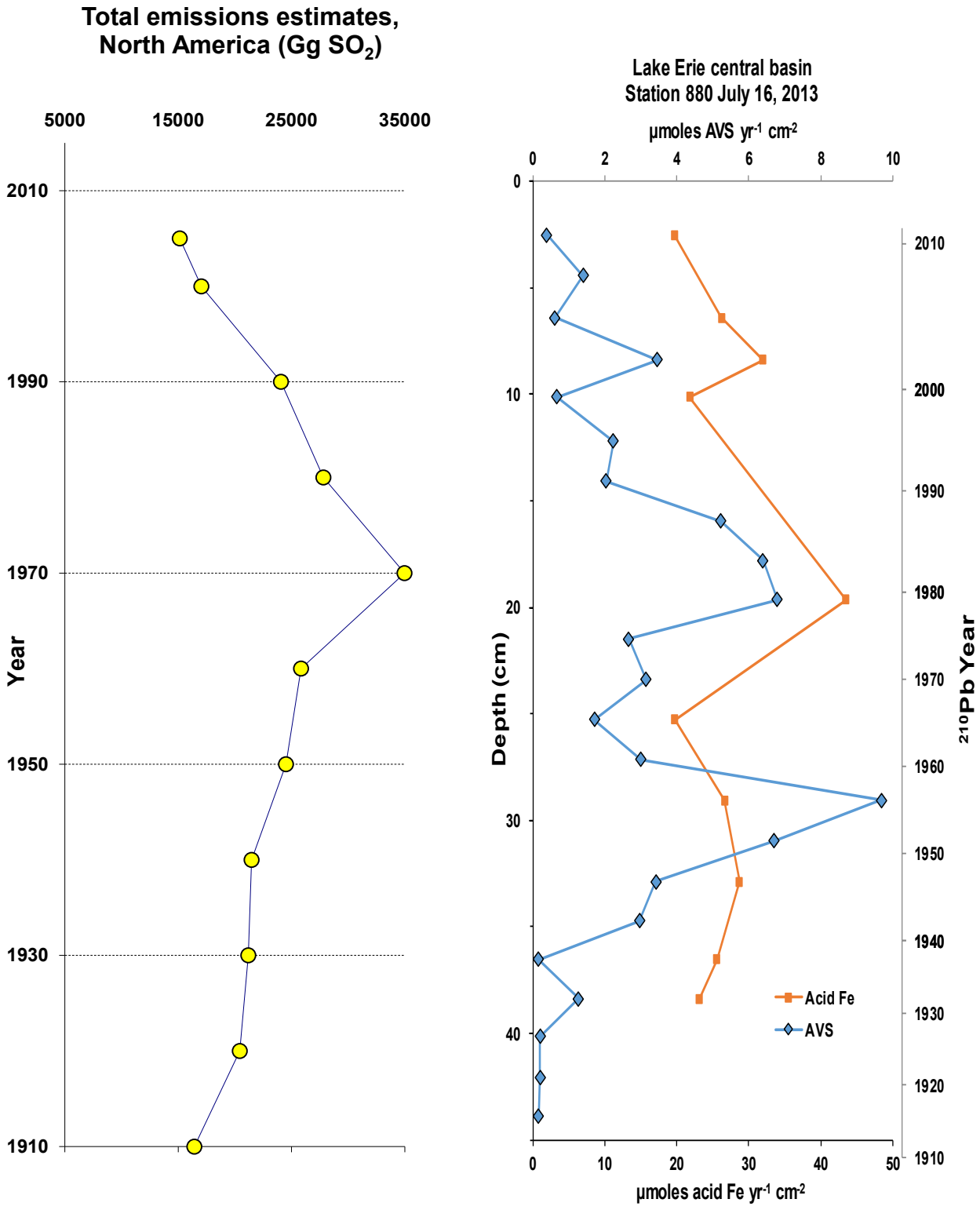

Figure 3.2 Acid-volatile sulfide (AVS) and acid-extractable iron levels in the 2013 wet sediments of Lake Erie in the central basin (Station 880) compared to historical emissions of sulfur dioxides (Gg SO_2) in the USA and Canada (historical data from Smith *et al.* 2011).

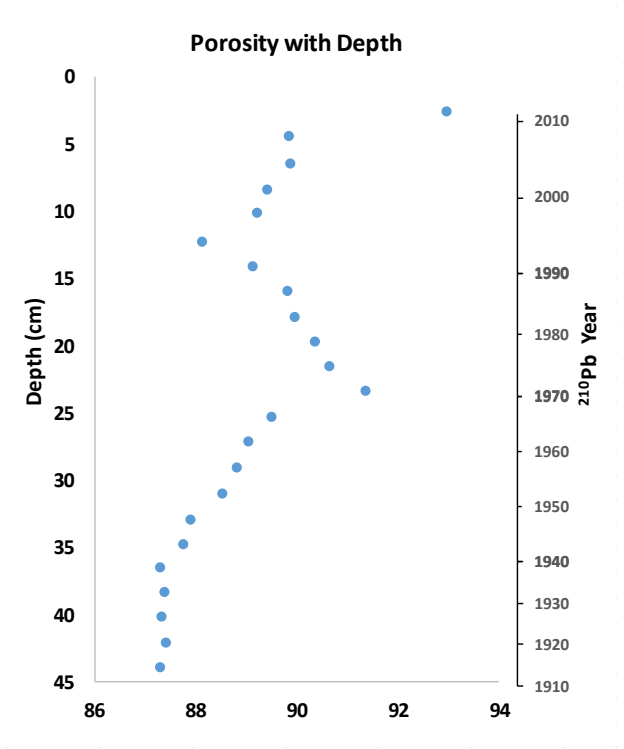

Figure 3.3 Water content (as porosity, percentage of volume) of Lake Erie sediments from cores taken at station 880 in 2013. An anomaly is evident in both plots, circa 1970, which likely led to oxidation and loss of AVS from the sediment.

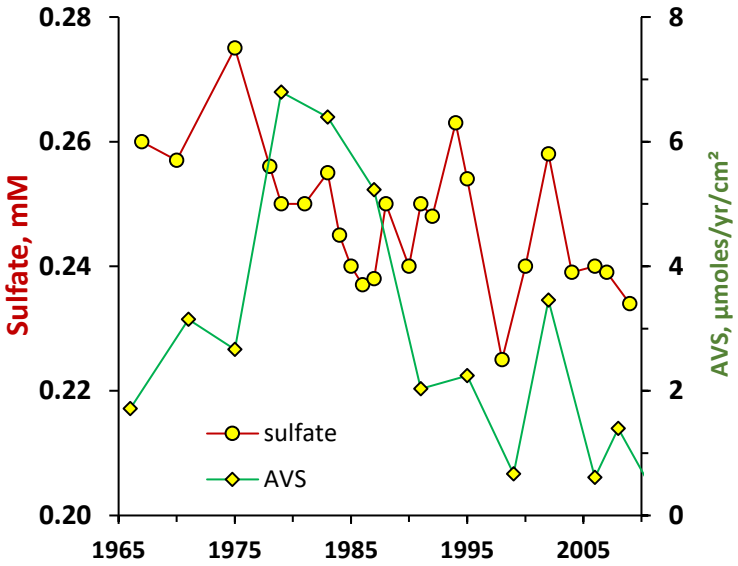

Figure 3.4 Acid-volatile sulfide (AVS) and sulfate levels in Lake Erie 1965-2010. AVS levels before 1979 are likely erroneous, due to physical disturbance. Otherwise, a downward trend may be observed for both parameters although the AVS decline is relatively larger (eastern Erie sulfate concentrations taken from Chapra *et al.* 2012).

The current mesotrophic status of Lake St. George from a formerly eutrophic state (Molot *et al.* 2003) was confirmed by relatively clear water (3.6 m Secchi depth) and water quality field data, as indicated by the YSI profile and water chemistry samples (TP, NH_3, Cl^-, SO_4^{2-}, and dissolved iron) at several depths (Fig. 3.5). Both a thermocline and oxycline were present and distinct, and there were peaks in chlorophyll, phycocyanin, and TP at the thermocline, suggesting that a significant metalimnetic population of phytoplankton (including cyanobacteria) exists (Fig. 3.5). TP concentrations did not exceed 25 μg L^{-1} at the surface but increased substantially with depth. Fe and ammonia also increased with depth, and anoxia (< 1 mg L^{-1} O_2) was established at 4.5 m, indicating that the lake experiences internal loading (Fig. 3.5). Nitrite and nitrate were undetectable at all depths, but chloride, sodium (15-54 mg L^{-1}), and calcium (56-73 mg L^{-1}) (last two not shown in figure) increased with depth, suggesting that road salts enter the lake, or the groundwater in this area leaches ancient salt deposits within the sedimentary rocks.

Lake St George sediments were found to be gelatinous and very high in organic matter and had to be sectioned in a cold room to avoid excessive expansion and water loss. Subsequent AVS and acid-extractable iron analysis (Fig. 3.6) indicated a pattern of sulfide deposition that was somewhat consistent with the Lake Erie data: there were increases in deposition during the period after the Industrial Revolution, the unusual loss of sulfide in the 1970s similar to the Lake Erie results (though the porosity and density data did not indicate anything unusual this time), and a peak in the 1980's (which differs from peak emissions c.1970), and a decline in AVS accumulation rate, though overall the changes were relatively subtle.

The acid-extractable iron accumulation rates appeared to be relatively constant, and closely coupled with the AVS data, though the molar ratio of acid-extractable iron was several times greater than sulfide, which was similar to the Lake Erie sample.

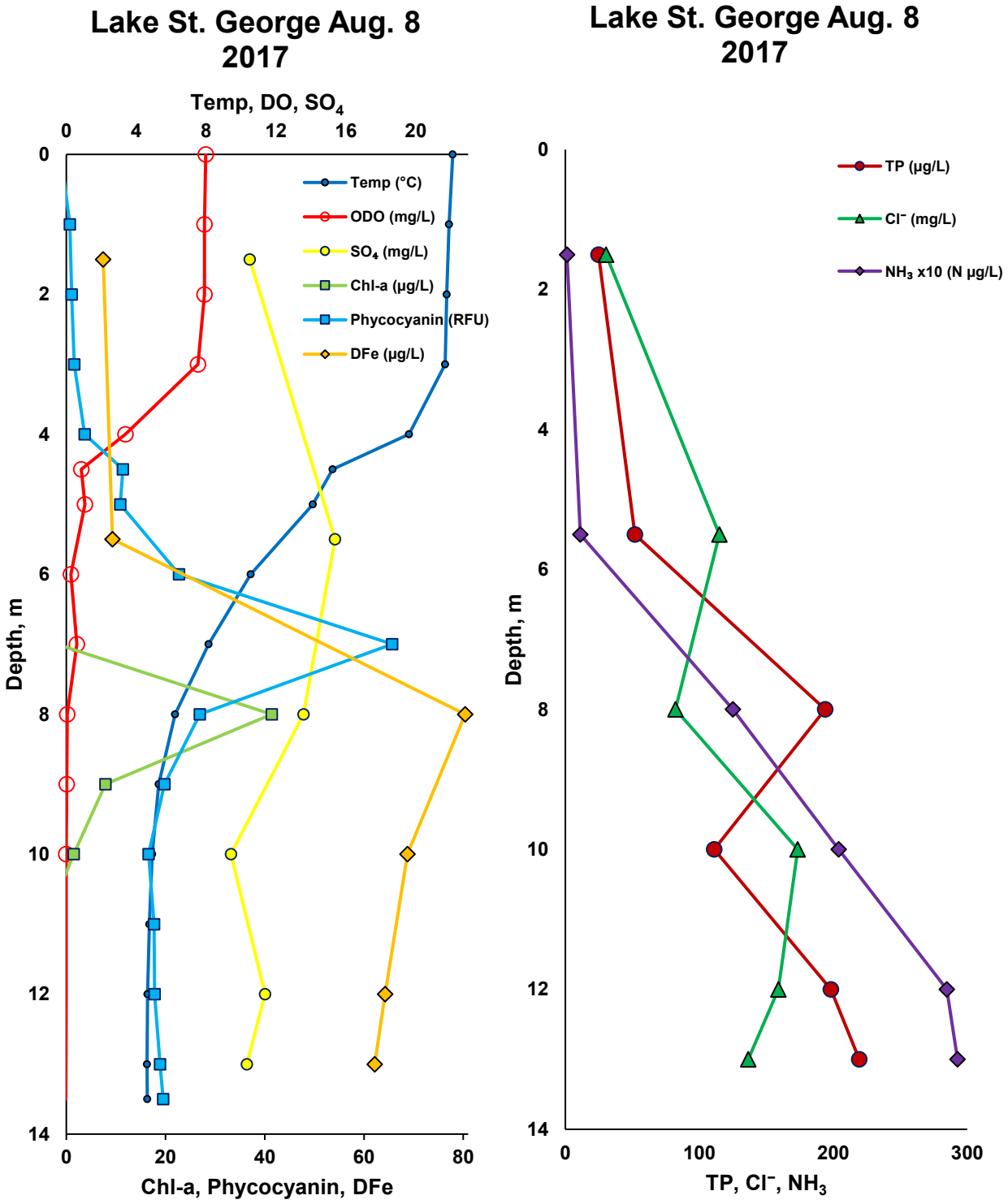

Figure 3.5 Physiochemical water profiles of Lake St. George. ODO = optical dissolved oxygen (YSI), Chl-a from optical YSI data, RFU = relative fluorescence units (YSI), DFe = dissolved iron.

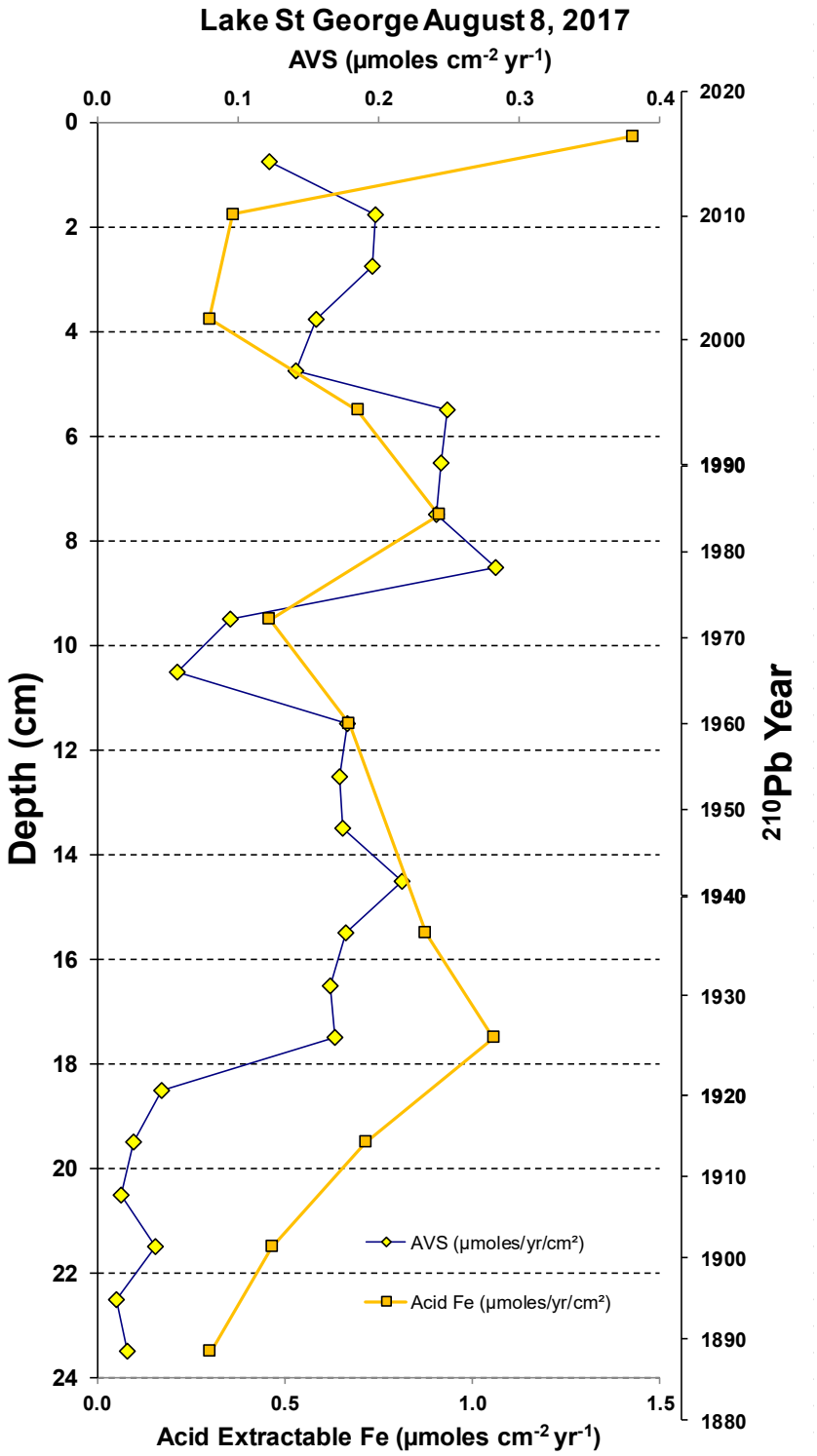

Figure 3.6 Sediment acid-volatile sulfide (AVS) and acid-extractable iron levels in the west basin of Lake St George.

3.6 Discussion

AVS appears to be useful a useful paleo-indicator that integrates changes in anoxia, productivity, and sulfate controls. AVS declined in Lake Erie after 1980, with the rate of decline slowing after 1990. The decline was consistent with a decline in sulfate concentration in the water column which, in turn, was likely due to declining SO_2 deposition, and lower productivity following implementation of point source P controls in the 1970s. Changes in productivity would also have affected the extent of anoxia, which is a necessary condition for sulfate reduction. The decline in AVS between 1980 and 1990 (74%) was greater than the decline in sulfate (approximately 4%) suggesting other constraints such as organic matter influx and anoxia were more important. TP may have declined approximately 44% in the central basin between 1980 and 1995 based on two years of data (Dove and Chapra 2015) but has been increasing since then (Dove and Chapra 2015, Scavia *et al.* 2014).

The slowing of the decline in AVS accumulation after 1990 might have been due to increasing TP and relatively constant sulfate (Chapra *et al.* 2012) during this period, or it could be a climate signal indicating longer periods of anoxia. The AVS pattern is consistent with the pattern in the spatial extent of hypolimnetic hypoxia in the central basin of Erie, which declined between 1988 and 1992, remained relatively low for several years, and then increased between 2002 and 2012 (Zhou *et al.* 2015). Alternatively, there may have been increased shunting of pelagic materials (which would add sulfur and increase anoxia) to the benthic zone following dreissenid invasion (Kerks et al. 1996, Baustian et al. 2020).

Declining AVS in the presence of continuing anoxia has implications for sulfide sequestration of metals, with the possibility of increased internal loading of redox-sensitive metals such as Fe, after 1980. Upwelling of hypolimnetic water can subsequently transport metal and nutrient-rich anoxic waters into shallower regions (Jabbari et al. 2019) closer to the euphotic zone.

The AVS minimum between 1960 and 1975, lower sediment density and higher porosity are consistent with a major mixing event that disturbed and oxidized sediments deposited between circa 1960 and the surface at that time. The large regional storm of November 10, 1975 responsible for the sinking of the freighter Edmund Fitzgerald in Lake Superior is a likely candidate.

The changes observed in Lake St George sediments were likely buffered by its transition from eutropic to mesotropic states, and a land use pattern which was less anthopogenic: overall deposition of AVS and acid-extractable Fe were several fold less than in Lake Erie, thus making measured variations more difficult to detect. Neither Lake Erie nor Lake St George paleolimnological data demonstrate a clear historical relationship between acid extractable iron

accumulation and AVS levels, although the Fe and AVS patterns are similar. In both sites, extractable Fe was considerably higher than AVS, most likely due to other forms of iron, such as hydroxides and carbonates that were not removed in the previous two extraction steps (dithionite and NaOH) and were subsequently extracted in the final HCl step. A weaker acid and/or additional steps may be more selective for AVS. Further research on the diagenesis of sulfide and iron minerals in these lakes may help explain the results.

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Chapter 4: Sediment incubation experiments with sulfate and organic carbon

4.1 Abstract

The effects of sulfate (SO_4) and organic matter under anoxic conditions on the rate of internal phosphorus (P) and ferrous iron (Fe^{2+}) loading was investigated using homogenized surficial sediments from Hamilton Harbour, Lake Ontario and the central basin of Lake Erie. Sediments were incubated with various SO_4 and organic carbon additions, and redox, dissolved P, dissolved Fe^{2+}, and SO_4 were monitored. The results indicated that bacterial metabolism of organic matter in and upon sediments is the primary driver of internal P and Fe^{2+} loading, and at later stages of incubation, iron sulfide formation. Soluble iron loading was controlled by sulfide production only late in the incubations, and only at high sulfate typical of Hamilton Harbour today but much higher than historical concentrations in the open waters of Lake Erie. Historical sulfate concentrations are therefore unlikely to have controlled dissolved iron levels any better than modern concentrations. The results suggest that internal Fe^{2+} loading might have decreased in Lake Erie and Hamilton Harbour following improvements to municipal waste water treatment beginning in the 1970s, assuming constant anoxia and Fe inputs to Lake Erie, while declines in sulfide production might have offset the decrease to some extent.

4.2 Introduction

The ferrous iron (Fe^{2+}) concentration in low nitrate anoxic waters is governed by the reducible iron content in surface sediments, which can have a significant range in freshwaters (Loh *et al.* 2013, Powe *et al.* 2013) on one hand, and sulfide production (from sulfate) on the other, which limits Fe^{2+} diffusion rates from anoxic sediments due to the formation of insoluble iron sulfide precipitates (Hutchinson 1957, Leonov and Chicherina 2008). Sulfide can also dissolve ferrous phosphate (vivianite) (Murphy et al. 2001). Hence, net Fe^{2+} release is a function of microbial ferric iron (Fe^{3+}) reduction rates and sulfide production rates, both of which depend on the supply of organic matter. Fe^{2+} can combine with sulfide to form insoluble precipitates of iron monosulfides (FeS) and pyrites (FeS_2) in sediments, and with phosphate as vivianite ($Fe_3(PO_4)_2.8H_2O$), thereby potentially sequestering iron, reducing Fe^{2+} diffusion into the water column, and limiting its future availability to phytoplankton (Carignan & Tessier 1988). Hence, lakes in which the sulfide formation rate is high enough to prevent Fe^{2+} diffusion may prevent blooms of phytoplankton species dependent on supply of Fe^{2+} from anoxic sediments, particularly cyanobacteria (Molot *et al.* 2014, Verschoor *et al.* 2017).

Sulfide formation rates in sediments can be limited by the sulfate diffusion rate at concentrations < 3 mM in overlying water (which includes non-saline freshwater systems) (Boudreau & Westrich 1984). In Canada, sulfate concentrations vary 4 orders of magnitude from 65 mM in saline lakes in Alberta (Marino *et al.* 1990), to 0.06 mM in inland lakes on the Precambrian Shield in northwestern Ontario (Kelly and Rudd 1984). For comparison, the open ocean sulfate is 28 mM (Boudreau & Westrich 1984). Hence, the sulfate concentration in overlying water can limit sulfide formation in many freshwater lakes.

4.3 Research Objective

To explore how internal P and Fe^{2+} loading is affected by the availability of sulfate and organic matter under anoxic conditions in incubating sediment cores from two sites in the Great Lakes.

4.4 Materials and Methods

4.4.1 Study Sites

Hamilton Harbour, at the western tip of Lake Ontario, has been eutrophic for decades, with high inputs of sewage effluent and iron from nearby steel mills in the form of iron chloride pickling salts (until 2005) to precipitate phosphates in waste water treatment plants (WWTP), which are also known to discharge high levels of sulfate into the harbor (Medeiros and Molot 2006). Lake Erie has experienced extensive anthropogenic disturbance and massive algal blooms that continue despite more than a decade of improvements in water quality, owing chiefly to legacy P released from sediments (Paytan et al. 2016). Both sites therefore warrant further study; their surficial sediments were collected from the deepest zone in Hamilton Harbour (station 1001) and from within the central basin of Lake Erie (Environment Canada station 1062; Painter et al. 2001) with a box corer and deposited into buckets or storage totes.

4.4.2 Procedure

All sediments were sieved to 0.5 mm to remove bioturbating organisms and large particles, then mixed with sufficient distilled water to form a slurry (average density of 1.2 g cm^{-3}), in order to minimize variations in each core. The slurry was aerated continuously at 5°C and periodically mixed for at least one month to ensure complete oxidation of organic and inorganic material prior to commencement of incubations. Portions of slurry were deposited into 8 cm diameter UWITEC core tubes to form a settled depth of approximately 10 cm of sediment and then topped up with deionized water and stored at 5° C for one week to form a sediment-water interface (SWI). Sulfate levels in the aqueous layer were checked via the barium sulfate turbidity method of Rossum and Villarruz (1961), and if they exceeded 0 mg L^{-1}, the aqueous layer was drained and replaced with new deionized water, and the sediments mixed and re-settled. Washed and settled cores were then drained and re-filled with 20 cm of hardened deionized water to simulate the natural buffering and average 30 mg L^{-1} Ca concentrations found in Lake Ontario and Lake Erie. Hardened water was made in batches by deaerating deionized water with nitrogen gas and a glass frit wand, dissolving in $Ca(OH)_2$, then bubbling in CO_2 continuously until the solution became clear again. Sulfate was added to select Hamilton Harbour cores at specified concentrations (0, 20, 40, and 60 mg L^{-1} SO_4^{2-}) as $MgSO_4$, while some of the same cores also had organic carbon added to them. The amount of carbon added was targeted to be similar to levels typically found in the surficial layers of sediments, or to levels following algal blooms, but a pure insoluble carbohydrate that was easily degradable rather than algae was desired in order to determine the true sediment P, Fe, and S fluxes without introducing these

elements from algae or similar proxies. Starches were considered ideal candidates, and tapioca starch was listed as a very pure source of carbohydrate; more so than cornstarch, which was verified by in-house hot aqua regia digestion of 1 gram samples, followed by spectrophotmetric analysis of undiluted, neutralized samples for DP, DFe, and SO_4^{2-}: all samples were below the detection limits. The organic carbon sequestration (sediment burial) rates in large and small mesoeutrophic lakes is 1.8 to 7.8 moles C m^{-2} yr^{-1}, and much lower than the median burial rate for eutrophic impoundments (177 moles C m^{-2} yr^{-1}) (Downing et al. 2008). Han et al. (2015) added 5000 g wet wgt m^{-2} of algal scum from a eutrophic lake to experimental sediment incubations; similar to the amount of algae in the water column observed in the field during the algae blooming season. Since algae consists of approximately 90% water, and roughly 25% of the dry weight is carbon (Li *et al.* 2014), approximately 10 moles C m^{-2} had been added: the equivalent of starch added to experimental cores would weigh 1.5 g, which seemed excessive. In another study of the anaerobic mineralization of organic material and resultant inorganic fluxes, Hansen and Blackburn (1991) added 1.8 mol C m^{-2} of ground fresh macroalgae to their cores, which would be equivalent to 235 mg starch to experimental cores. Algal blooms and hypereutrophic systems are associated with very high chlorophyll *a* concentration, which can reach more than 100 μg L^{-1} (Carlson 1977). An algal biomass of such proportions would supply up to 0.2 mol C m^{-2} (Carlson 1991). Based on the above and practical considerations, it was decided that two ranges of carbon additions would be utilized for this study: 2 mol C m^{-2} and 0.2 mol C m^{-2}, with the higher addition to be tested on the Hamilton Harbour sediments first. 320 mg dry weight of tapioca starch was mixed with a portion of the core water, then poured into the overlying water to settle evenly upon the SWI. Lake Erie cores were later amended with combinations of 0 and 60 mg L^{-1} sulfate and 2 mol m^{-2} and 0.2 mol m^{-2} carbon. The initial redox and dissolved oxygen were measured with standard probes (protruding from a long plastic sleeve made watertight at the bottom with rings of silicone tubing) at 0.5 cm above the SWI. Pilot testing indicated that no significant changes occurred more than a few centimeters above the SWI (i.e. stratification occurs to a very significant degree even at the microcosm scale), so all subsequent measurements were confined to a narrow zone 0.5 cm above the SWI.

For chemistry sampling, the bung was replaced and Teflon capillary tubing was inserted into the central hole of the bung a few centimeters from the SWI, and dry nitrogen was gently bubbled for ten minutes to mix the aqueous layer and purge the headspace of air. Another length of capillary tubing was inserted 0.5 cm from the SWI to draw 20 mL of aqueous sample, which was then passed through a 0.2 μm syringe filter (Sartorius Minisart Cellulose Acetate with GF prefilter), discarding the first 5 mL, and three 5 mL subsamples were collected in separate 15 mL

centrifuge tubes for sulfate, dissolved reactive iron (DRFe), and dissolved phosphorus (DP) measurements. All cores were then left to incubate at 20°C. After three days, and at weekly intervals, the redox and dissolved oxygen were measured, and an aqueous sample was taken as described previously. DFe was measured spectrophotometrically with ferrozine and ascorbic acid as the reductant at 562 nm (Verschoor and Molot 2013) on an Ultrospec 3100pro spectrophotometer (Biochrom Ltd.) zeroed with distilled water in a 5 cm long narrow-walled glass cuvette. DP was measured similarly at 827 nm with the same equipment using the original molybdenum blue method of Murphy and Riley (1958), which uses heat for colour development (24 hours at 20°C, 30 minutes at 60°C) rather than the environmentally toxic potassium antimonyl tatrate method (which reduces the reaction time to ten minutes) developed by them later and currently the standard method (Murphy and Riley 1962): the absorbances attained are otherwise similar.

4.5 Results

Dissolved oxygen and redox dropped to very hypoxic levels (from 8 to 1 mg L^{-1} and from 400 to <100 mV respectively) 0.5 cm above the sediments in treatments without added organic carbon within three days after initialization in the Hamilton Harbour cores, followed by a gradual rise in DP over the remainder of the experiment (Fig. 4.1). Dissolved Fe levels remained low in all cores without added carbon, and sulfate levels remained constant. DP increased gradually throughout the incubation to about 300-550 μg L^{-1} in all sulfate treatments. There appears to be no significant difference in the behaviour of the cores among the differing sulfate concentration treatments, but the slightly increased flux of P in the 0 mg L^{-1} SO_4^{2-} core was associated with lower redox levels throughout the incubation. In cores with added carbon, redox levels quickly became negative, and both DP and dissolved Fe were released at levels many orders of magnitude greater than those cores without added carbon (Fig. 4.2). Developments in the core with 60 mg L^{-1} sulfate + organic carbon showed the most change: a black haze began to form at the sediment-water interface and extended upwards to approximately one centimeter in height just ten days after incubations began, and continued to expand upwards, darken, and deposit black iron sulfide precipitate onto the surface of the sediment thereafter (Fig. 4.3). Concentrations of both DP and dissolved Fe in both the 0 mg L^{-1} and 60 mg L^{-1} sulfate cores rose to similar levels, but dissolved iron levels began to fall after the second week of incubation in the 60 mg L^{-1} sulfate core. Concomitantly, sulfate levels in the 60 mg L^{-1} core dropped to half by the first week of incubation, and zero by the third week, indicating that both dissolved Fe and sulfate were consumed in the formation of the black precipitate.

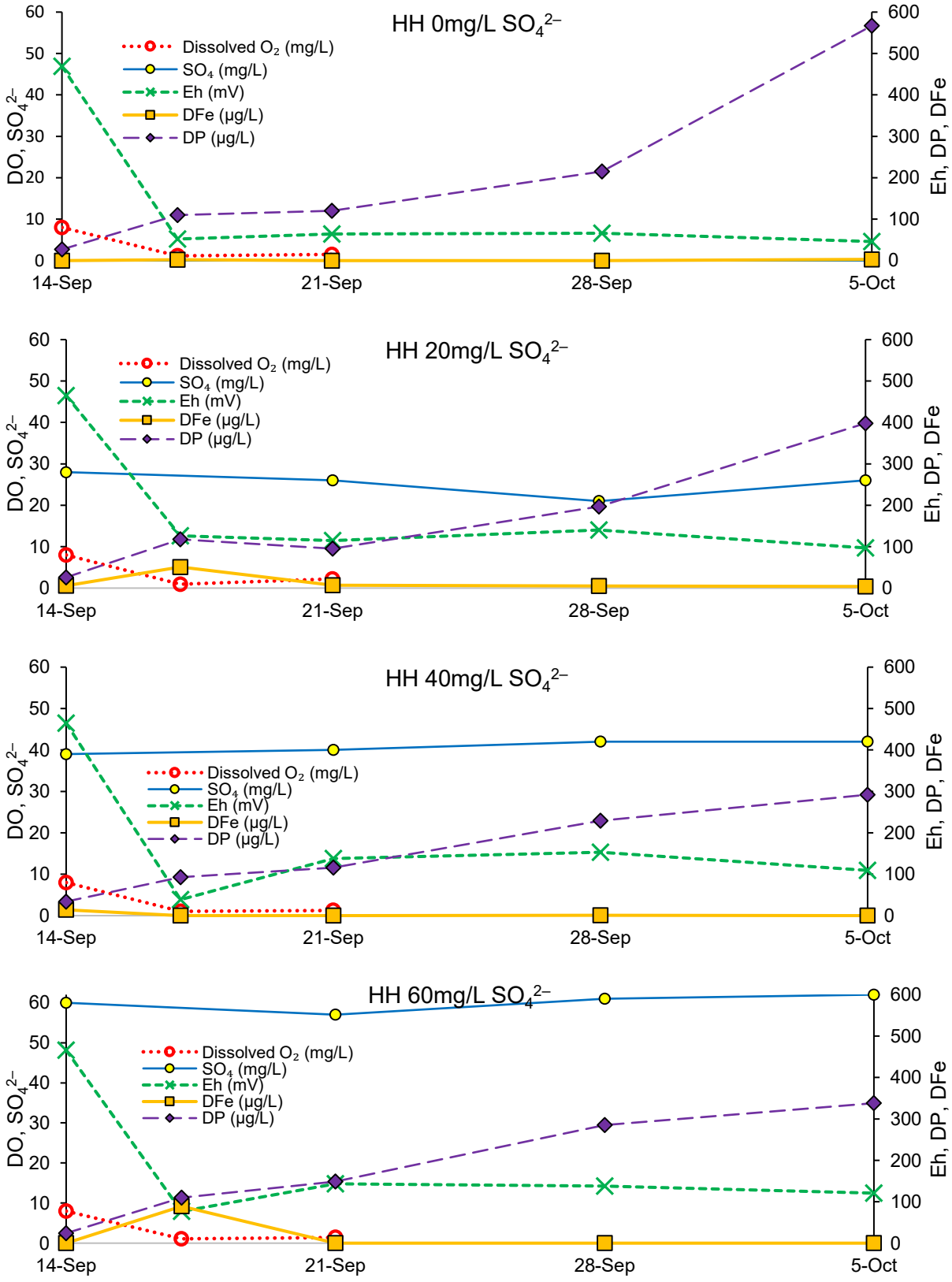


Figure 4.1 Response of incubating cores of processed Hamilton Harbour surficial sediments incubated at 20°C with varying sulfate additions and no added organic carbon. Sulfate levels remained constant.

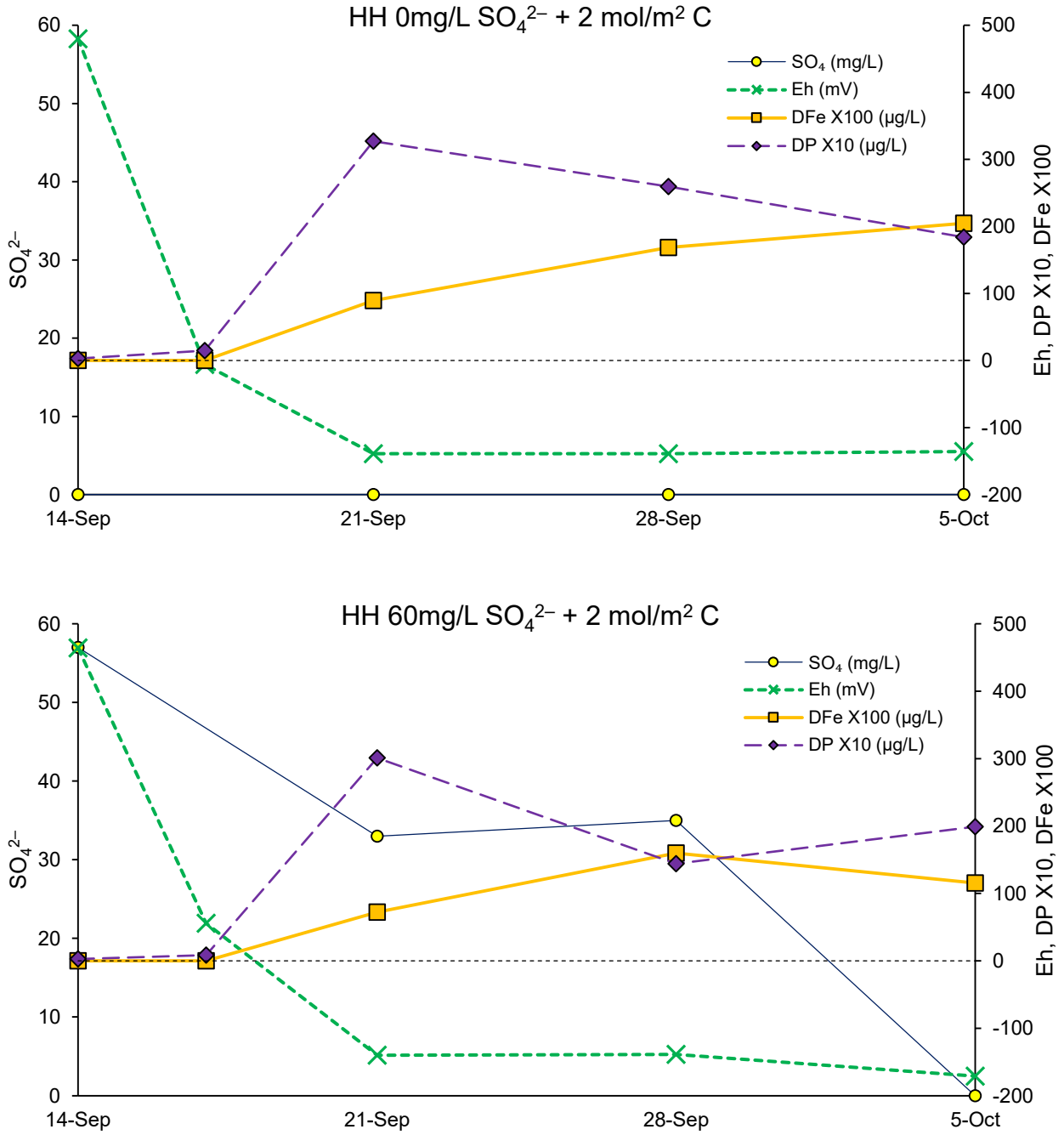


Figure 4.2 Response of incubating cores of processed Hamilton Harbour surficial sediments incubated at 20°C with 0 and 60 mg L^{-1} sulfate and organic carbon. Sulfate levels in the core with 60 mg L^{-1} dropped to half by the first week, and zero by the third week, co-incident with a drop in dissolved Fe levels.

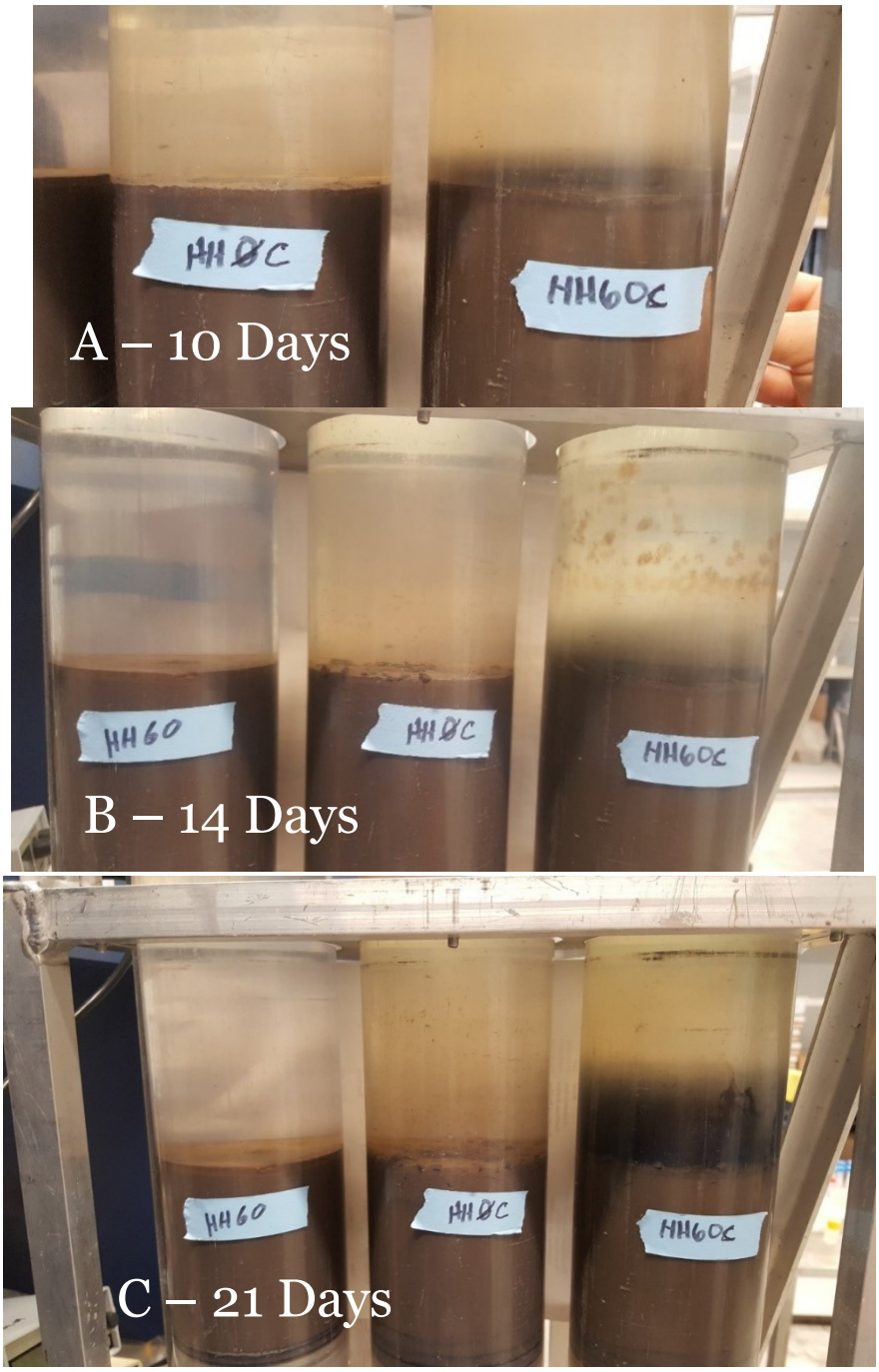


Figure 4.3 Photographs of Hamilton Harbour cores with added organic carbon and no (labelled "HH0C") or 60 mg L^{-1} (labelled "HH60C") sulfate additions and incubated at 20°C. (A) shows the initial formation of black ferrous sulfide just above the sediment of the 60 mg L^{-1} sulfate and carbohydrate-supplemented core after 10 days of incubation, (B) shows clear differences between the core treated with sulfate and no added carbohydrate (leftmost, labelled "HH60"), the core treated with carbohydrate but no sulfate (middle, labelled "HH0C"), and the core treated with both sulfate and carbohydrate (rightmost, labelled "HH60C") after 14 days of incubation. (C) contrasts apparently little activity in the carbon-poor yet sulfate-rich core "HH60" with expanding ferrous sulfide deposition in the core with both high carbon and high sulfate "HH60C" after 3 weeks of incubation.

For the Lake Erie cores, the experiment was amended to include intermediate levels of carbon and sulfate (0.2 mol m^{-2} and 30 mg L^{-1} respectively) in order to determine the effects of biogeochemical parameters typically encountered within the lake. Fig. 4.4 indicates dynamics within the Lake Erie cores similar to those of Hamilton Harbour, but the redox did not drop as rapidly or as far after initialization in cores without added carbon. As a consequence, dissolved Fe was not released to any significant degree, and DP showed only modest increases; no visual changes were apparent (Fig. 4.6).

In those cores with intermediate carbon additions (0.2 mol m^{-2}), the redox dropped further, but gradually, followed by a gradual increase in redox after two weeks (Fig. 4.4). DP and dissolved Fe gradually rose after the redox dropped below 100 mV, then gradually fell again as the redox increased after the two-week point. Sulfate concentrations fell by about 10 mg L^{-1} in both cores with carbon added, yet only the core with intermediate sulfate levels showed black precipitate (first visible by the second week), indicating ferrous sulfide formation (Fig. 4.6); the other core very gradually precipitated orange ferric oxyhydroxides, and in both cores, the dissolved Fe concentrations fell to nearly zero after one month.

In the cores with high levels of added carbon (2 mol m^{-2}), redox levels quickly declined and remained low for the duration of the experiment, although the high sulfate core exhibited a significantly more gradual redox change, which seemed to cause a delay in iron release, but not phosphorus (Fig. 4.5). Dissolved iron concentrations rose to an order of magnitude above cores with intermediate carbon levels added (0.2 mol m^{-2}), and DP concentrations increased several times greater than the intermediate cores. Of particular note was a marked drop in sulfate and dissolved Fe concentrations in the high sulfate core as the incubation proceeded. The appearance of black precipitate was observed in this core by the second week of incubation as well, and increased up to several centimeters thick, by the end of the experiment (Fig. 4.6), one month later. Sulfate levels became undetectable by the end of the fourth week while iron levels were lowered more than in the core without added sulfate, indicating that both dissolved Fe and sulfate were consumed in the formation of the black ferrous sulfide precipitate.

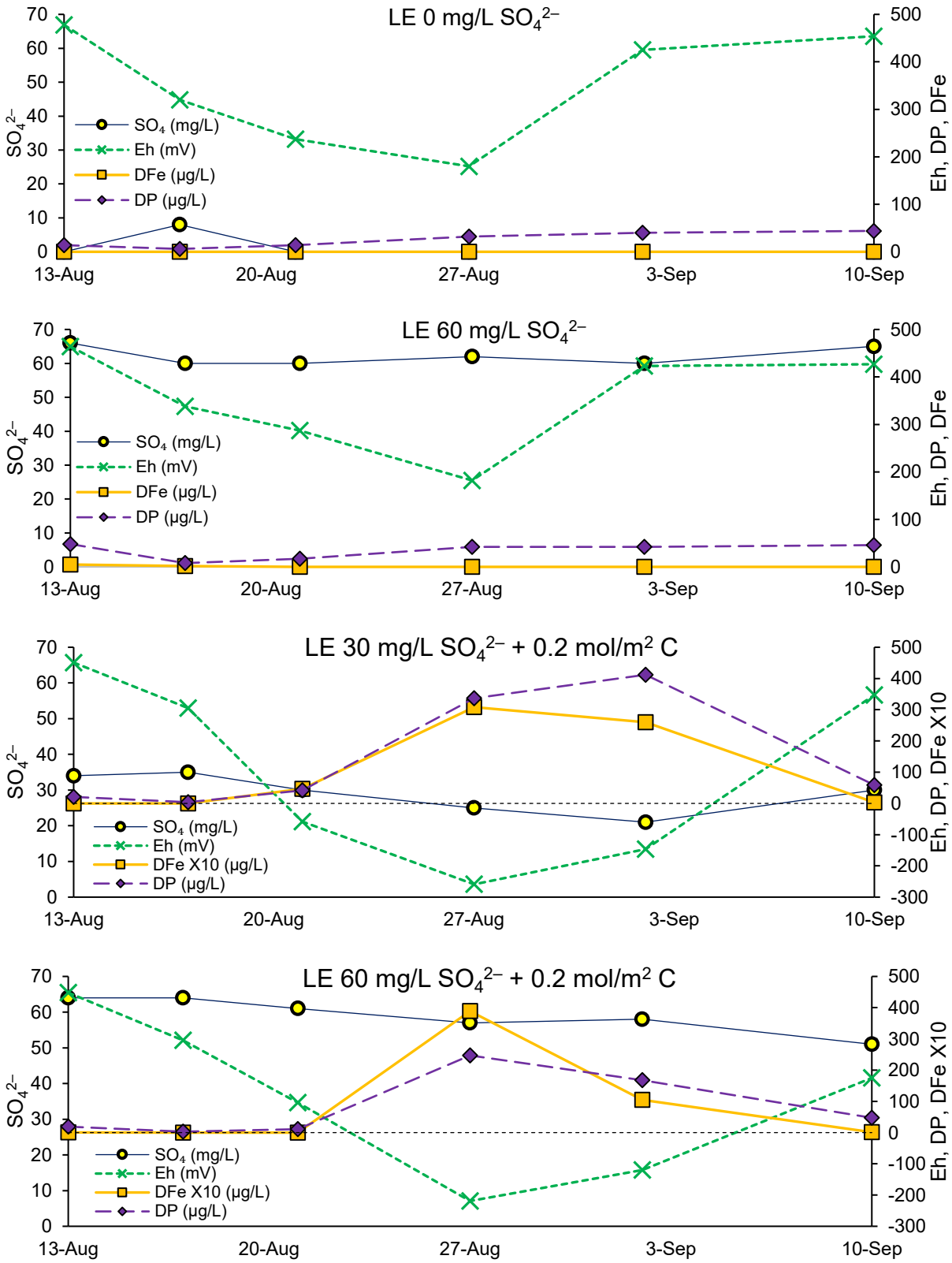


Figure 4.4 Lake Erie processed surficial sediments with no added organic carbon and 0.2 mol m^{-2} added carbon and varying sulfate, incubated at 20°C.

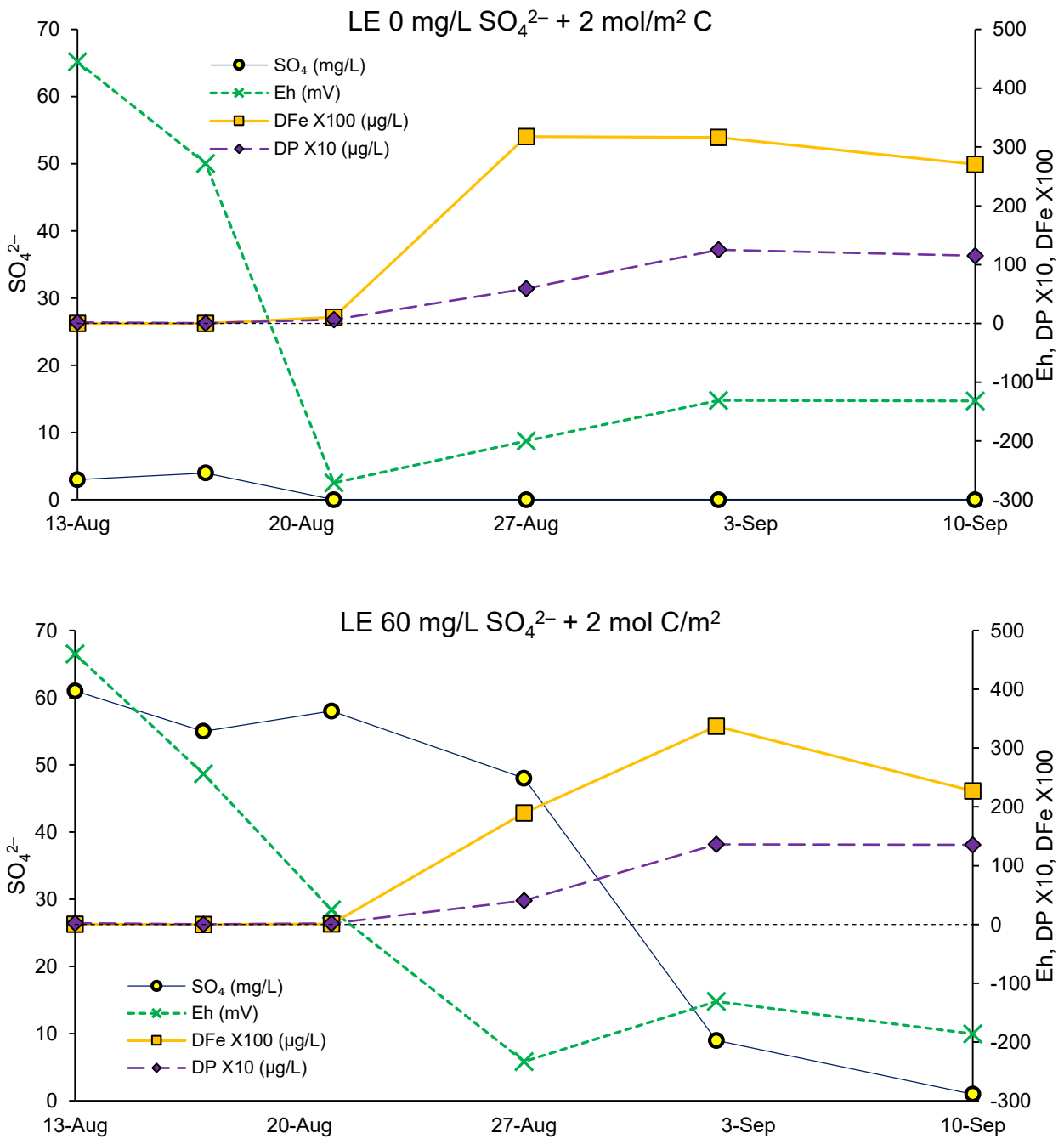
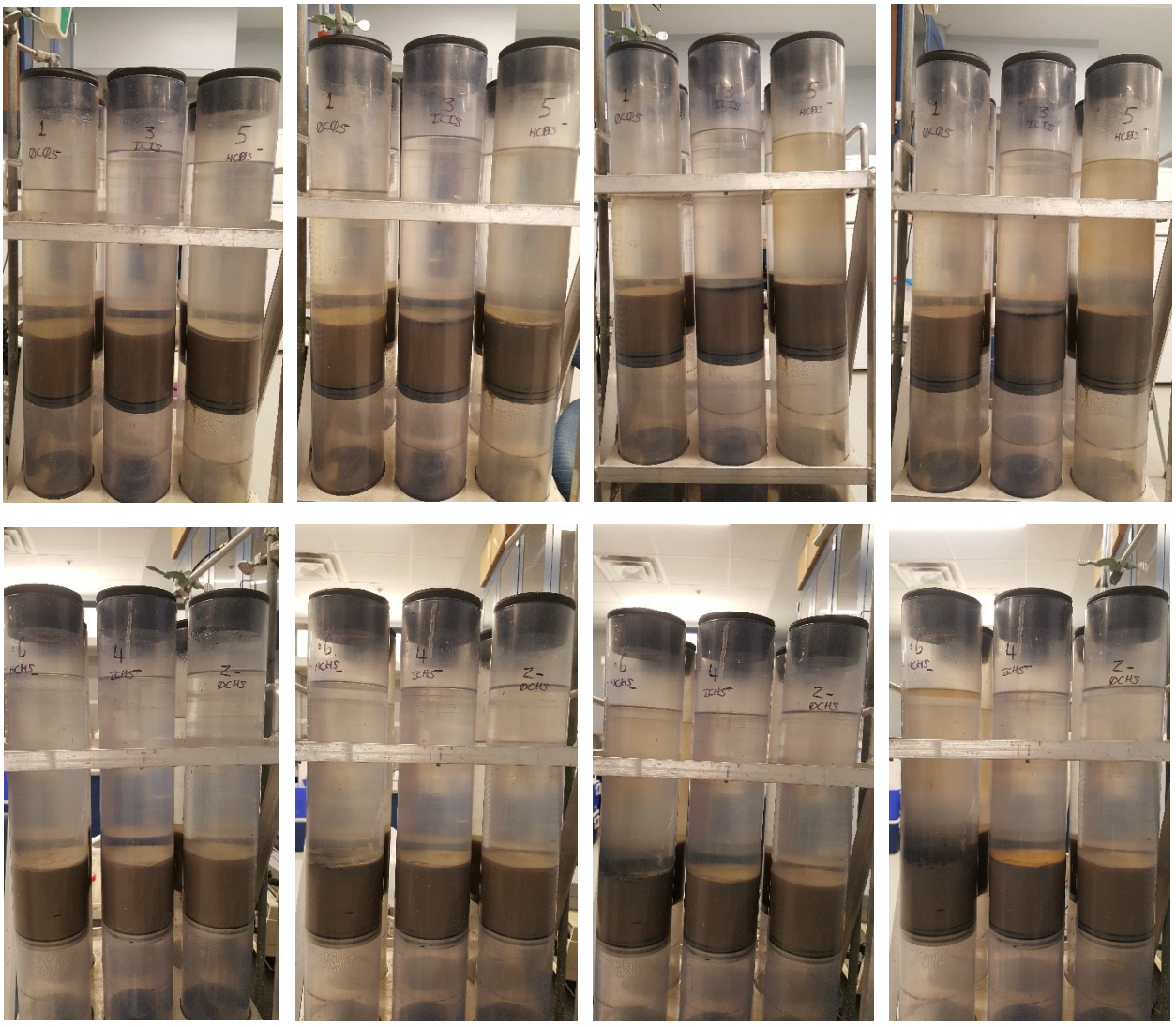


Figure 4.5 Lake Erie processed surficial sediments, with additions of 0 or 60 mg L^{-1} sulfate and 2 mol m^{-2} carbon, incubated at 20°C.



Week 1 – Aug 21 **Week 2 – Aug 27** **Week 3 – Sept 2** **Week 4 – Sept 10**

Figure 4.6 Photographs of Lake Erie processed surficial sediments with added organic carbon (to 2 mol m^{-2}, labelled "HC" or 0.2 mol m^{-2}, labelled "IC"), and/or sulfate additions (to 30 mg L^{-1}, labelled "IS" or 60 mg L^{-1}, labelled "HS") and incubated at 20°C. Hence, treatments were from top left to top right: #1 0C0S = no additions, #3 ICIS = 0.2 mol m^{-2} C + 30 mg L^{-1} sulfate, #5 HC0S = 2 mol m^{-2} C + no sulfate; bottom left to bottom right: #6 HCHS = 2 mol m^{-2} C + 60 mg L^{-1} sulfate, #4 ICHS = 0.2 mol m^{-2} C + 60 mg L^{-1} sulfate, and #2 0CHS = no carbon + 60 mg L^{-1} sulfate.

Table 4.1 summarizes the incubations carried out in this study in terms of the maximum release and consumption rates (fluxes) of the geochemical species of interest in mg m^{-2} day^{-1}: a unit found in most internal loading studies. DP release was negligible in Lake Erie cores without added carbon and averaged 5 mg m^{-2} day^{-1} in cores treated with moderate amounts of added carbon (0.2 mol m^{-2}). The flux of P in Hamilton Harbour cores without added carbon was similar, with varying sulfate concentrations having no apparent effect. In cores with high loadings of added carbon (2 mol m^{-2}), release rates for Lake Erie sediments averaged 12 mg m^{-2} day^{-1} and for Hamilton Harbour 90 mg m^{-2} day^{-1}. Iron release rates were much more dependent on strong redox conditions and were associated only with cores amended with carbon. In such cases, iron release rates in Hamilton Harbour were roughly half those of Lake Erie, with no apparent relation to P release rates. High sulfate slowed the flux of Fe slightly compared to the controls. Likewise, sulfate did not show significant changes without high levels of added carbon.

Table 4.1 Maximum fluxes (mg m^{-2} day^{-1}) of sediment cores incubated for 28 days at 20°C with various sulfate and carbon additions. HH = Hamilton Harbour, LE = Lake Erie surficial sediments.

Sediment source & treatment:	HH 0	HH 20	HH 40	HH 60	HH 0+C	HH 60+C	LE 0CoS	LE 0CHS	LE ICIS	LE ICHS	LE HCoS	LE HCHS
$[SO_4^{2-}]$ (mg L^{-1})	0	20	40	60	0	60	0	60	30	60	0	60
C (mol m^{-2})	0	0	0	0	2	2	0	0	0.2	0.2	2	2
DP	5.5	6.1	3.9	5.7	92	85	0.3	0	4.5	3.3	12	13
DFe	0.1	0	-0.9	0	256	228	0	-0.2	44	55	454	337
SO_4	0	-0.1	0	0	0	-0.7	0	-0.1	-0.1	-0.1	0	-0.5

4.6 Discussion

The intent of the sediment processing protocol employed in this study allowed close control of sulfate and organic matter content in incubating cores: the two primary drivers being examined. Sediment processing undoubtedly affected other components, but clearly, sufficient P and Fe remained. Taken together, the data from both Hamilton Harbour (HH) and Lake Erie (LE) core incubations revealed aspects of internal loading that were expected, but also other aspects particular to each site. P did not appear above the SWI until after the overlying water redox was less than 120 mV in HH, while the LE cores remained inert (at 180 mV) until C was added, at which point both P and Fe were released due to the very low redox attained with even "moderate" amounts of carbon. It is therefore surmised that P release occurs around 100 mV, though LE cores amended with less C or sampled more frequently may have given a better estimate. In general, Fe was not released until a redox below 0 mV was reached, which is consistent with the known redox potentials of Fe^{+2} described above in the Introduction. Fe^{+2} concentrations also appear to have an inverse relationship with redox down to at least -250 mV,

where sulfide generation is prevalent. Likewise, sulfate did not show definitive declines until the redox fell below -140 mV or more for most cores, also consistent with the known -100 to -300 mV potential of sulfate reduction to sulfide, though again more frequent sampling may have been able to determine a more accurate estimation.

Attention may be drawn to the delayed release of Fe and P in most of the cores as the redox declined to levels where flux begins, usually several days after initiating the experiments, and it has been argued that perhaps flux calculations should be calculated from that time, rather than from the initiation date (Loh et al. 2013, Matisoff et al. 2016). For this study, the maximum flux rates were calculated from the initiation date, which is standard practice, rather than from the date of detectable flux, in the interests of obtaining comparable data.

Sediment release rates (flux) for P in natural systems range from 0-10 mg m^{-2} day^{-1} in most mesotropic systems, 5-20 mg m^{-2} day^{-1} in most eutrophic systems, and 15-30 mg m^{-2} day^{-1} in most hypereutrophic systems (Nürnberg 1994). The results reported here (~5 mg m^{-2} day^{-1} for untreated HH cores and LE cores treated with 0.2 mol C m^{-2} and ~12 mg m^{-2} day^{-1} with 2 mol C m^{-2}) fall within the range of results typically reported for sediment incubations (Kelton and Chow-Fraser 2005) and agree with anoxic/hypolimnetic fluxes reported elsewhere for the central basin of Lake Erie (averages of 7.4 - Burns and Ross 1972, and 6.6 - Matisoff et al. 2016) and for Hamilton Harbour (2-6.5 mg m^{-2} day^{-1} - Markovic et al. 2019).

P flux was negligible in Lake Erie cores without added carbon, whereas Hamilton Harbour sediments released P at levels comparable to Lake Erie sediments with moderate amounts of added carbon (0.2 mol m^{-2}), which suggests that either Hamilton Harbour sediments differ in their organic carbon, or P content, or both. Hamilton Harbour sediments may have a much higher organic carbon content, or perhaps of more recalcitrant forms (such as humic acids or hydrocarbons) that survived the aeration stage of processing, although if recalcitrant organic matter remained, their degradation rates during the experiment would have been low. Alternatively, the P content may be much more labile. When incubated at 2 mol C m^{-2}, Hamilton Harbour sediments released P at rates almost eight times greater than Lake Erie sediments, and greater than in the experiment by Han et al. (2015) that used far more C as raw algae - calculated as 30 mg m^{-2} day^{-1}. This also supports the case for a very high and/or very labile P content, and is consistent with recent work that detected a high P release rate in Hamilton Harbour and attributed that to a reactive Fe content insufficient to bind all the P released during diagenesis (Markovic et al. 2019).

Flux data for Fe in natural systems is less available, but one study reported a flux of 21.6 mg m^{-2} day^{-1} for Lake Erie central basin using peepers (Adams et al. 1982) and from another study

of the same area, I calculated a flux of up 44 mg m^{-2} day^{-1} derived from hypolimnetic sampling data (Burns and Ross 1972). This compares somewhat favorably to the maximum fluxes of the Lake Erie 0.2 mol C m^{-2} incubations (44-55 mg m^{-2} day^{-1}), but these rates are eclipsed by those seen after application of 2 mol m^{-2} carbon, which were 6-10 times higher and had no precedent in the literature. Even more unusual, these fluxes were almost twice those of Hamilton Harbour, which may reflect inhibition of Fe^{+2} release by an overwhelmingly high P content in the Harbour, as noted earlier. Incubation of Hamilton Harbour sediments with 0.2 mol m^{-2} (or less) carbon amendments may yield Fe fluxes that are more representative of normal conditions, and thus may help resolve the differences between these sites as well as corroborate these findings with that of others. The value of the high carbon input experiments may lie in their ability to highlight and specify the consequences of large bloom events: similar data following the effects of algal blooms with unmodified, natural cores is not likely to be attainable without careful preparation or fortuitous circumstances.

Overall, these results demonstrate that internal P and Fe^{2+} loading rates were highly dependent on organic carbon availability and that even 60 mg L^{-1} sulfate was not high enough to prevent internal Fe^{2+} loading at 2 mol m^{-2} carbon in Erie and Hamilton Harbour sediments. This implies that sulfate levels are not high enough in the Great Lakes to suppress cyanobacteria blooms, nor are they high enough to delay bloom onset, based on the response of Fe^{2+} release to varying levels of sulfate when organic C was added.

The incubations were intended to simulate conditions in which a phytoplankton bloom is large enough to lead to anoxic conditions. The first two weeks after organic carbon additions, therefore, represent the initial period where organic matter availability is enough to drive the redox downwards, generating anoxic conditions. However, the incubations differ from *in situ* conditions because the cores are closed systems, cut off from continued inputs of organic matter and sulfate, whereas endogenous sediments would continually receive inputs. While sulfate and organic carbon were fully depleted in some of the incubating cores (e.g., organic carbon was depleted in the 0.2 mol C m^{-2} cores in Erie judging by the rebound in redox in weeks 3 and 4 (Figure 4.4); and 60 mg L^{-1} sulfate dropped to 0 mg L^{-1} in the Erie core with high organic carbon (Figure 4.5)), *in situ* sediments would receive continued inputs as sulfate diffused downwards from the water column and fresh detritus settled to the bottom. Therefore, *in situ* sulfate and iron reduction rates would probably not cease entirely but would, instead, be limited by supply rates if reducing conditions prevailed.

The experimental results confirm that heterotrophic bacterial metabolism of organic materials in and on sediments is the primary driver of redox-associated events such as sulfate reduction,

microbial reduction of ferric hydroxides with release of dissolved P and Fe, and sulfide deposition. Given that the zone of sulfide deposition continued to expand upwards and dissolved Fe levels declined to the end of the experiment in the high sulfate and high organic carbon treatment, the sequence of biogeochemical events witnessed suggest that in eutrophic systems with high depositions of organic material (i.e. high productivity), low redox conditions are quickly established, and sulfide generation rapidly commences. However, there may not be enough sulfate present to sequester all the iron previously released, and the timing of sequestration (due to the very low redox state required) may be too late to prevent the promotion of cyanobacterial blooms, because ferrous iron is available long before it is removed by sulfide in most cases.

The reaction rates in Lake Erie sediments were somewhat slower than the Hamilton Harbour sediments, but similar maximum levels of iron and phosphorus were released, and may reflect the slightly longer mineralization period of Lake Erie sediments during storage and aeration prior to use, which would likely reduce the amount of native organic material, or they may reflect differences in the total amount and type (humic, recalcitrant, etc.) of organic material.

It may be argued that the sieving and mixing of the sediments disrupted the microbial community, and therefore did not represent, nor give enough time to establish, functional redox layers as would occur in "aged" or *in situ* cores, and therefore the results do not reflect natural conditions and are therefore of limited value. Yet the results indicate that most of the biogeochemical activity occurs at the surface-water interface, driven by organic matter, which is consistent with well-accepted paradigms; essentially, the cores functioned as expected.

Given the inability of even relatively high levels of sulfate to sequester soluble iron until late in the incubations, and only then if adequate levels of organic carbon are present, it seems unlikely that ferrous iron will be controlled by sulfide production in natural systems during the course of one season. This is borne out from field observations of collected cores, which often have black surficial precipitates only late in the season, usually after one or more significant algal bloom events have already occurred. However, some of the precipitated sulfide may become buried and thereby sequestered during the following season if relatively large quantities are formed following a large bloom, then settle to the deep anoxic zones where they can mineralize further to pyrites. In this way, the lake system may be able to indirectly or temporally affect iron levels (and thereby cyanobacteria blooms) in a type of feedback loop.

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Chapter 5: A simple and sensitive method for determining *in situ* ferrous iron levels in lakes

5.1 Abstract

The direct measurement of ferrous iron (Fe^{+2}) in natural water bodies, particularly in shallow and polymictic systems, is difficult because it is easily oxidized. Yet Fe^{+2} may be transiently available in calmer conditions or close to the surface-water interface (SWI), thus contributing to cyanobacteria bloom formation and maintenance; thus, a detection method under *in situ* conditions is desirable. A simple diffusion device was developed using dialysis tubing and ferrozine solutions, where Fe^{+2} is captured by the chromophoric chelator *in situ* from its surroundings for a period of time, and later detected by spectrophotometry. The devices were deployed in a shallow marina and in the hypolimnion of two stratified lakes, and were able to detect traces of Fe^{+2} at the SWI in the marina and in hypoxic zones of up to 1.0 mg/L oxygen. In the anoxic hypolimnia of lakes, the devices may be used semi-quantitatively to estimate ambient concentrations of Fe^{+2}, but the amount of time *in situ* must be limited and consistent, and they do not obey open-water Fickian diffusion kinetics: the effective diffusion coefficient for the membrane must be determined for the temperature ranges expected at the sites of interest.

5.2 Introduction

The direct measurement of ferrous iron (Fe^{+2}) in natural water bodies can be useful as an indicator of the redox state of sediments and may serve as a warning proxy to predict when cyanobacterial blooms are likely to occur (Verschoor et al. 2017). Detection of Fe^{+2} at the sediment-water interface (SWI) in shallow zones in thermally stratified lakes and in polymictic systems is particularly desirable, because these are biologically active areas and can contribute to bloom formation and maintenance. Since these same systems are often well oxygenated within a short distance of the sediment, direct Fe^{+2} detection can be difficult due to very low concentrations; but in calmer conditions, close to the SWI in the boundary layer, Fe^{+2} probably accumulates to high levels, thus enabling detectability. Regardless, direct measurement, even in anoxic waters, is technically difficult (Verschoor and Molot 2013).

To overcome this difficulty in natural waters, I devised a method that involved incubating dialysis tubing filled with the colourimetric Fe reagent ferrozine for several days to capture Fe^{+2} diffusing inwards. Ferrozine is a very sensitive and stable chelator that is a useful and practical tool for direct measurements Fe^{+2}, but must be used under reducing or slightly acidic conditions: rapid oxidation occurs above pH 6 (unpublished data), because Fe^{+2} is easily oxidized to ferric iron (Fe^{+3}), which forms insoluble and inert ferric oxyhydroxides and ferric phosphates (Stumm and Lee 1960). Once formed however, the ferrozine-Fe^{+2} complex is large (3 ferrozine: 1 Fe^{+2} at ~1500 Da) and very stable, even under oxygenated conditions (Stookey 1970). This novel method delivers ferrozine to the SWI under natural conditions that would allow formation and containment of the complex without oxidation losses or interference from humic acids (Verschoor and Molot 2013). I surmised that if the complex could be contained while allowing free diffusion of Fe^{+2} ion, the detection of even trace amounts of Fe^{+2} could be made with long enough exposure time via accumulation of complex. Dialysis tubing is a readily available and practical means of achieving the above requirements, and careful placement of the tubing would therefore permit detection and measurement of *in situ* Fe^{+2} in any area desired. The following describes a simple *in situ* method which utilizes dialysis tubing and ferrozine to detect and measure trace and hypolimnetic levels of Fe^{+2}.

5.3 Materials and Methods

5.3.1 Materials

- Ferrozine (MW 492.46 g mol) (Sigma-Aldrich 82950; max absorbance 562 nm)
- 500 Da MWCO Spectro/Por Biotech Cellulose Ester (CE) dialysis tubing, 31 mm flat width (Spectrum Laboratories #131 060)
- Dialysis closures, universal, various colours (Spectrum Laboratories, 50 mm width)
- Ferrous sulfate (molecular weight of ferrous sulfate heptahydrate is 277.9)
- Hydrochloric acid, trace metal grade

5.3.2 Construction of devices

Strips of 31 mm dialysis tubing were cut to 8 cm lengths, then rinsed and soaked in deionized water overnight to remove the sodium azide preservative. One end of the tubing was then clamped shut (with some overhang) using a dialysis clip. 5 mL of a 1 mM ferrozine solution (pH 6) was added to each dialysis tube, and the other end clamped shut, ensuring no air bubbles were trapped inside. The finished pillow-like devices (Fig. 5.1) were stored in containers filled with deionized water adjusted to pH 6 with hydrochloric acid, which provides counter-ions to facilitate Fe^{+2} diffusion and permits handling and deployment in aerated environments. The oxidation of Fe^{+2} entering the aerobic ferrozine solution would thereby be delayed long enough to form complexes reflecting the equilibrium concentration of the internal solution (i.e. maximum detection limit) without excessively acidifying the surrounding lake water, which might otherwise release Fe^{+2} bound to natural DOC (Shapiro 1966, McMahon 1969).

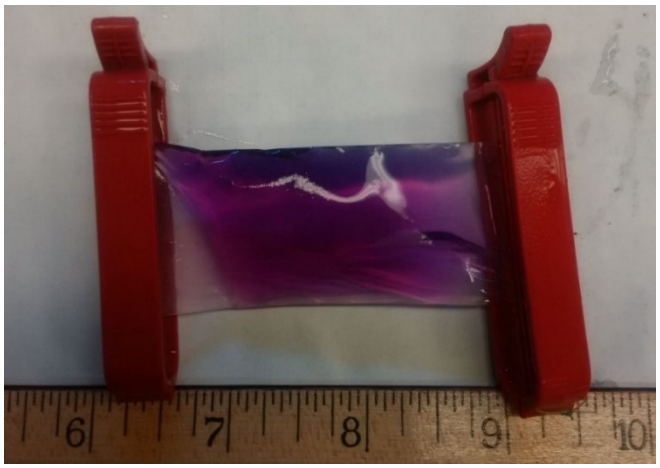

Figure 5.1 Configuration and approximate dimensions of ferrozine pillow devices used to detect and measure ferrous iron *in situ*. This device is shown with ferrous iron captured on the sediment-water interface within a shaded boat dock at 0.5 m after deployment for 48 hours.

5.3.3 Deployment and measurement

During the 2015 field season, several experiments were conducted to detect and measure Fe^{+2} in natural water bodies. Detection was tested at the sediment-water interface (SWI) in a quiet (to minimize turbulence oxidation), shallow (0.5 m, to test oxygen penetration), shaded (to reduce photoreduction effects), area inside the covered boat docks of the Sturgeon Lake Marina, in the Kawartha lakes region. Ferrozine "pillows" were deployed within, upon, 2 cm above, and 4 cm above the SWI using nylon line attached to stakes driven into the bottom, and left in place for 48 hours. Similar ferrozine pillows were deployed throughout the hypolimnia of the deepest sites in both Sturgeon Bay and Deep Bay (Georgian Bay region) by sewing floating-clip devices onto nylon rope at fixed intervals with nylon line, including *in situ* blanks containing deionized water only. The intervals and anchor points were determined on site to correspond to fixed depths below the surface for each sampling site and trip in order to accurately track seasonal fluctuations in Fe^{+2}. Simultaneously, profiles of temperature, dissolved oxygen, and other parameters were obtained with a YSI 6600 sonde and 650 console and used to determine the depths of the layer boundaries between the epilimnion, metalimnion, and hypolimnion, defined by the intersection of straight lines drawn through the temperature–depth profile of adjacent layers. Water samples were collected using a horizontal sampler without metal parts at 2 m intervals to a depth of 0.5 m above the sediment. Equal sample volumes from discrete depths were pooled to create "integrated" epilimnetic and metalimnetic samples. To minimize oxidation, hypolimnetic samples were transferred directly from the sampler to an acid-washed syringe via a T-fitting with an attached one-way valve and a Luer-lock, which held filter cartridges. This configuration allowed air to be removed and the syringe to be reloaded multiple times to collect metals samples, which were filtered through 0.2 μm syringe-tip filters (Sartorius cellulose acetate with glass fibre prefilter) into acid-washed 50 mL snap cap vials after discarding the first 5 mL to avoid contamination and adsorption errors. Total dissolved Fe (DFe) was measured at the Water Quality Centre at Trent University in Peterborough, Ontario, with ICP-MS (Agilent 8800 Triple Quadrupole).

The devices were retrieved after 40-90 hours (in order to find the optimal incubation time, and as logistics permitted) and placed in storage containers filled with epilimnetic lake water and refrigerated until the absorbance was measured. Devices were weighed to obtain final volumes prior to opening and decanting the samples into a 5 cm glass cuvette. Samples were read at 562 nm and corrected with *in situ* blanks consisting of deionized water only, and compared to Fe^{+2} standards (0 to 10 mM) using acidified ferrous sulfate solutions (pH 4.8) and 1 mM ferrozine solution, allowing 10 minutes for colour development.

5.3.4 Calculating Fe^{+2} concentrations and diffusion coefficients from flux

Indirect estimation of Fe^{+2} levels outside the pillows utilizes Ficks first law of diffusion:

$$J = D\frac{\varphi}{x}$$

where J is the diffusion flux in mol $cm^{-2}\,s^{-1}$, D is the diffusion coefficient in $cm^{-2}\,s^{-1}$, φ is the external concentration in mol L^{-1}, and x the thickness of the membrane (0.008 cm). Because the membrane physically and electrostatically causes ions to travel in non-linear paths, D will not be the same as the simple ionic diffusion coefficient of Fe^{+2}. Technically, diffusion is through a porous body, but since the porosity of the dialysis membrane is not a published parameter, a more appropriate equation cannot be utilized, and thus an "effective diffusion coefficient", D_{eff} was determined. All DFe sample concentrations below the 1 mg/L DO boundary were assumed to be Fe^{+2}, after correction for epilimnetic DFe which was subtracted to estimate the concentration of Fe^{+2} outside the membrane, φ, for each sample depth. The accumulation rate J (or flux) was calculated from the device's internal Fe^{+2} concentration, multiplied by the device volume and divided by the product of the deployment time and device surface area.

5.4 Results

At the shallow, shaded site in Sturgeon Lake, the devices that were partially buried or laid upon the SWI accumulated significant levels of Fe^{+2} (Fig. 5.1), whereas neither device at 4 cm nor 2 cm above the SWI showed any appreciable colour changes.

From the profiles obtained from Deep Bay and Sturgeon Bay (Figs. 5.2 & 5.3), it is clear that the oxycline was more useful than the thermocline for our purposes: it is more distinct and has more biogeochemical relevance. Devices deployed in the hypolimnion of both Sturgeon Bay and Deep Bay for example, confirmed the presence of Fe^{+2} below the 1 mg L^{-1} oxygen "boundary". Like the DFe samples collected simultaneously, there was an exponential rise in Fe^{+2} levels with increasing depth.

In Deep Bay, the temperature and dissolved oxygen profiles remained relatively constant throughout the season, as did DFe concentrations (Fig. 5.2). There was more variability in Fe^{+2} concentrations, which initially tailed, then overtook DFe concentrations as the season progressed. On the last sampling date, both DFe and Fe^{+2} concentrations were significantly lower than expected: they would not typically be lower than the previous sampling date if the hypolimnion remained anoxic. The constant Fe^{+2} concentrations below 10 m on September 14 suggest that the amount of ferrozine added was insufficient and samples had become fully saturated with Fe^{+2}.

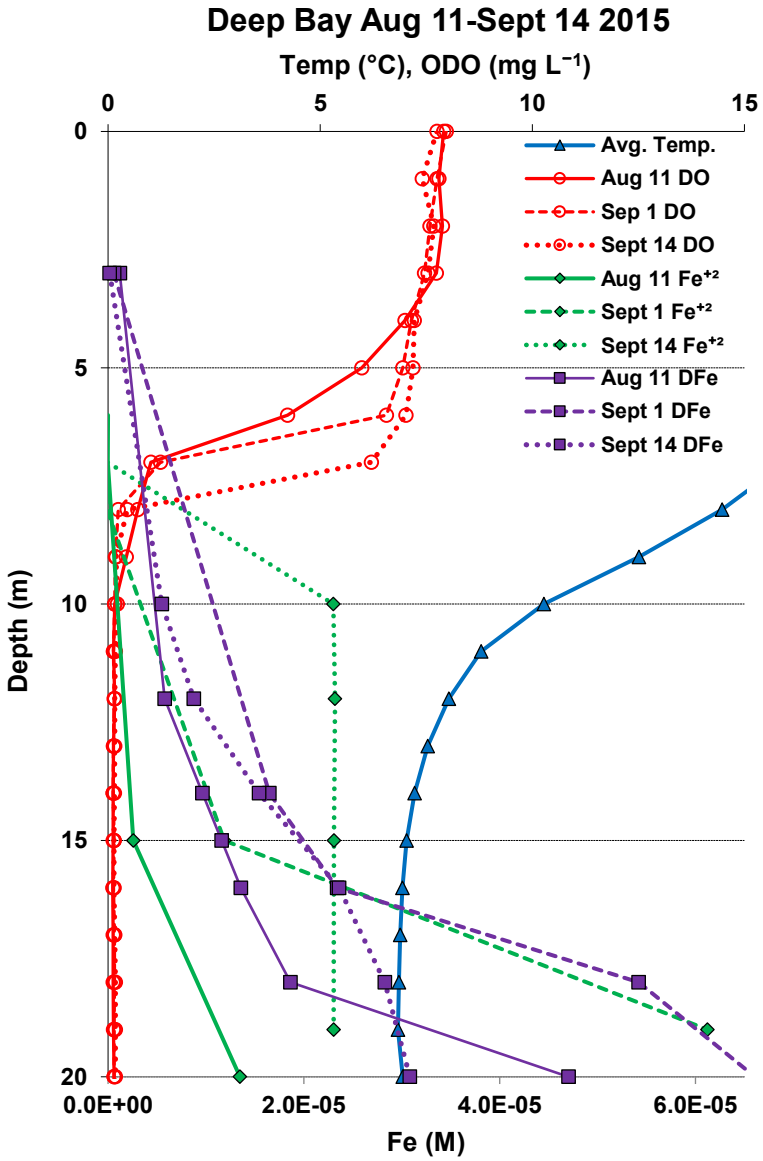

Figure 5.2 Seasonal changes in temperature, optical dissolved oxygen (ODO), dialyzed Fe^{+2}, and total dissolved iron (DFe) profiles in Deep Bay, 2015.

In Sturgeon Bay, experimentation with the devices began earlier. Oxygenation and the location of the metalimnion became increasingly deeper as the season progressed. Hypolimnetic concentrations of DFe and Fe^{+2} first accumulated at 12 m in mid-July, peaked from mid to late August, then rapidly declined, concomitant with the migration of the 1 mg L^{-1} oxygen boundary downwards (Fig. 5.3). Fe^{+2} was detected at 9 m on August 10 and September 1 when DO was approximately 2 and 5 mg L^{-1}, respectively.

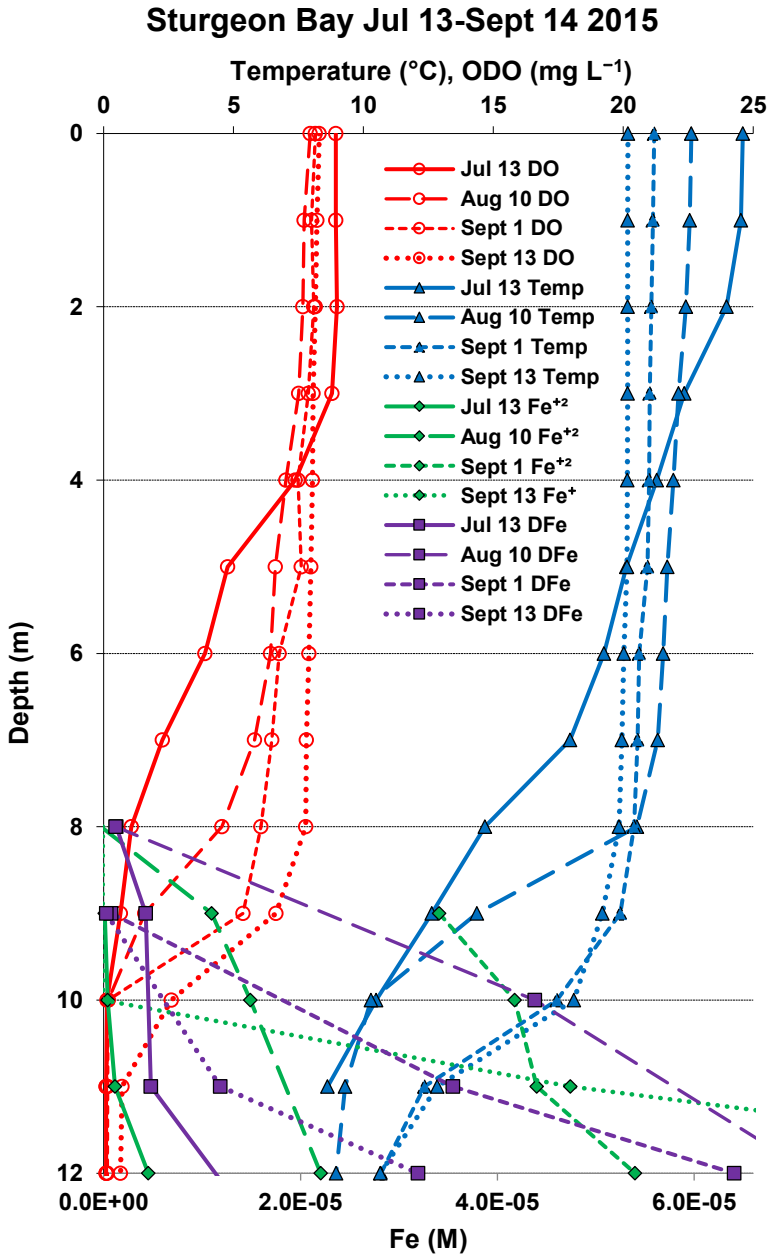

Figure 5.3 Seasonal changes in dissolved oxygen, Fe^{+2}, and total dissolved iron (DFe) profiles in Sturgeon Bay, 2015.

The data from both sites represented above were processed to determine the relationships between temperature and hypolymnetic DFe concentrations on the concentration of Fe^{+2} captured within the ferrozine devices. Temperature did not seem to influence the final concentration during the incubation times tested (Fig. 5.4 (A), Pearson correlation of -0.068, $p = 3.47 \times 10^{-10}$), but DFe concentrations appeared to have a positive relationship (Fig. 5.4 (B) Pearson correlation of 0.577, p = 0.038).

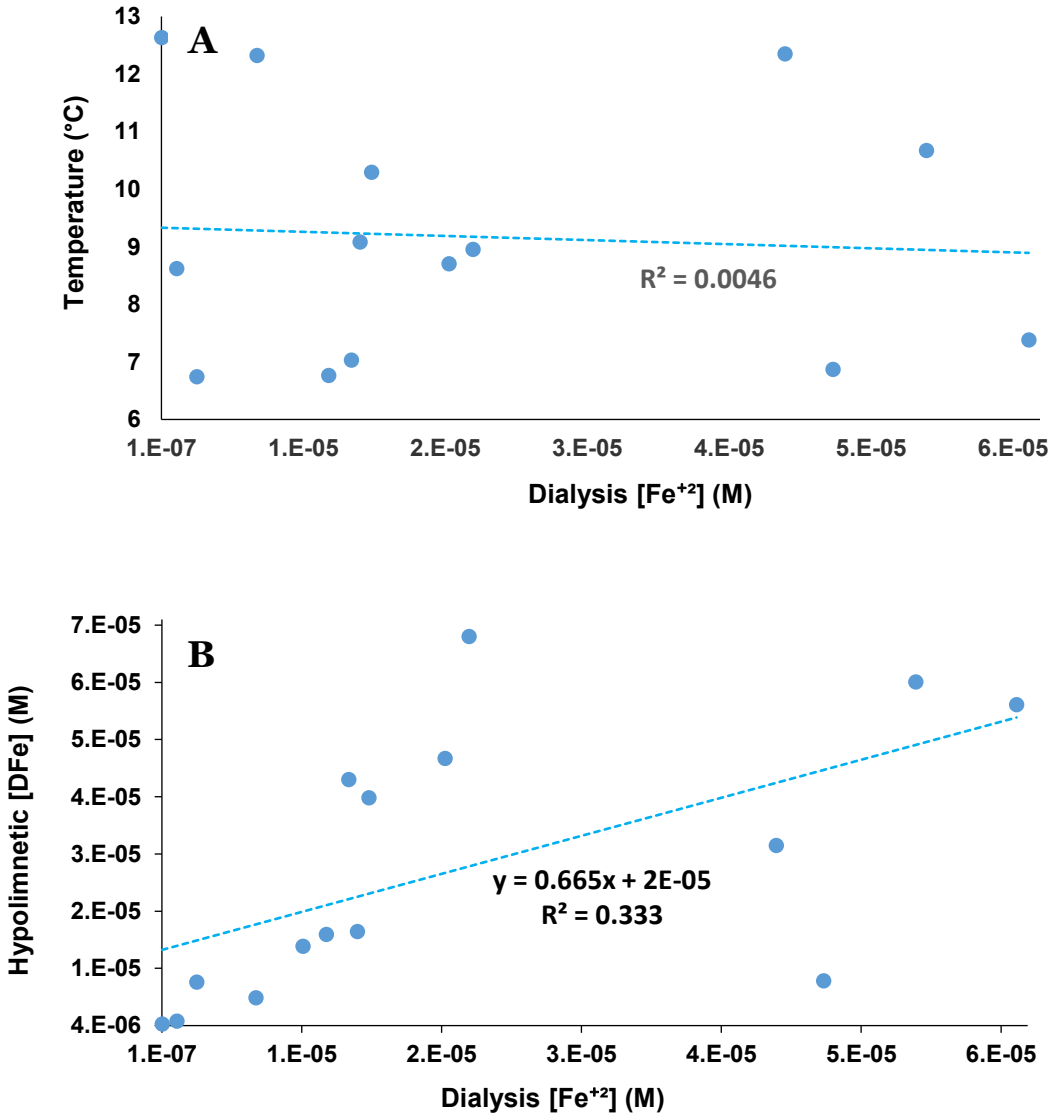

Figure 5.4 Relationship of temperature (A) and dissolved iron concentration (DFe) (B) on the final concentration of ferrous iron (Fe^{+2}) captured by ferrozine in natural lake waters. Linear correlations are shown on graphs.

The results were used to obtain the effective Ficks diffusion coefficient D_{eff} (Fig. 5.5), yielding a mean value of $4.88 \times 10^{-9} \pm 1.88 \times 10^{-9}$ $cm^{-2}\,s^{-1}$. Hypolimnetic temperature during the incubation positively influenced the coefficient (Fig. 5.5 (A), Pearson correlation of 0.56, p = 2.13×10^{-10}), but DFe concentrations appeared to have negligible influence on the coefficient (Fig. 5.5 (B), Pearson correlation of -0.0089, $p = 1.39 \times 10^{-5}$).

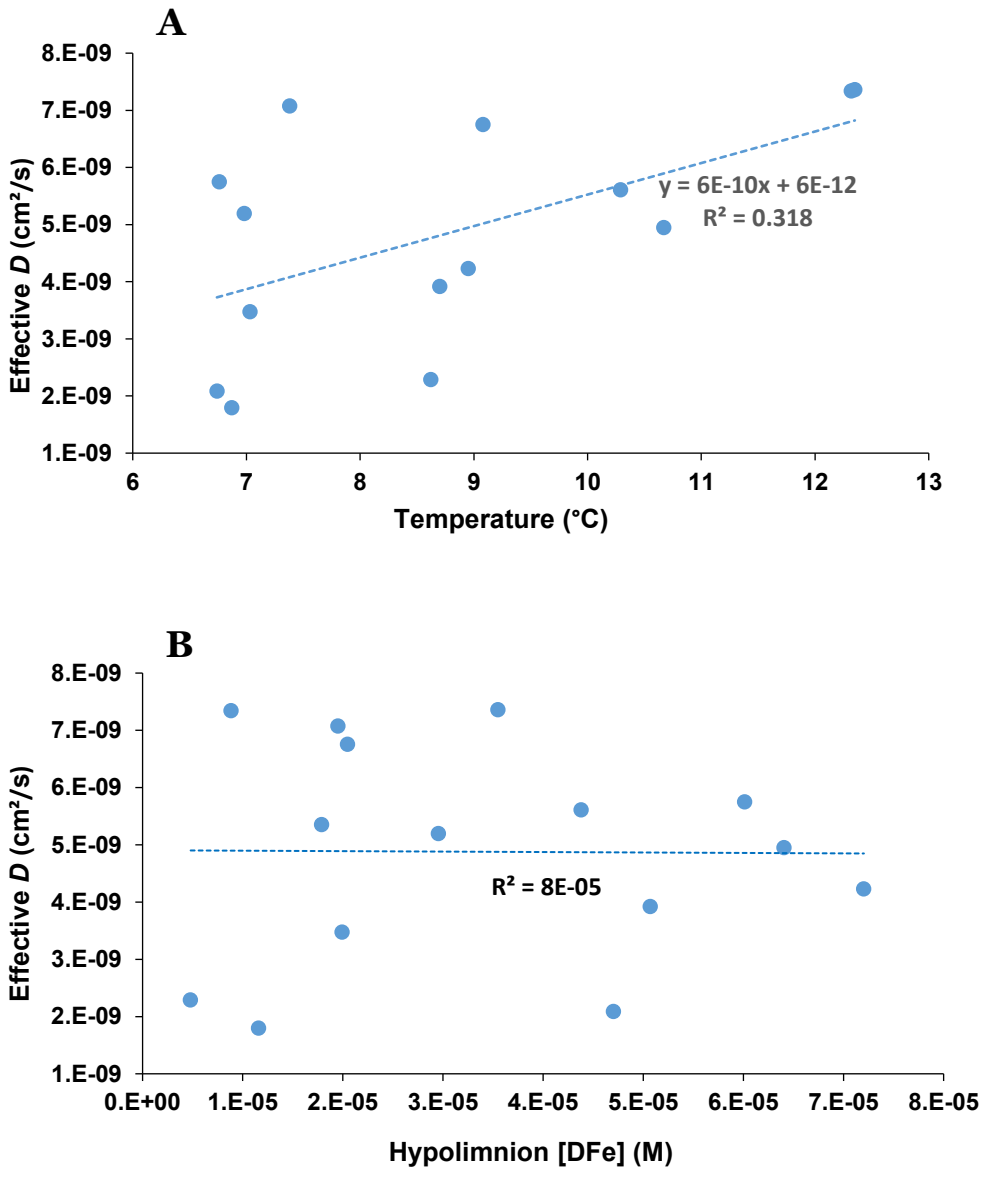

Figure 5.5 Relationship of temperature (A) and dissolved Fe concentration (DFe) (B) on the effective diffusion coefficient, D_{eff}, of ferrous iron (Fe^{+2}) captured by ferrozine in natural lake waters. Linear correlations are shown on graphs.

5.5 Discussion

At the shallow, shaded site in Sturgeon Lake, Fe^{+2} was detected at the SWI, but not in the control pillows a few centimetres up, which indicates that shallow oxygenated zones have anoxic boundary layers during calm periods and can supply concentrated sources of nutrients such as Fe^{+2} to phytoplankton like hypolimnetic anoxic zones do. The hypolimnetic devices in thermally stratified Sturgeon Bay and Deep Bay mirrored the exponential rise in Fe^{+2} concentrations with depth that was seen in DFe samples (Figure 5.4), which indicates that the devices were functioning as expected, and also that Fe^{+2} was a significant component of DFe. Concentrations of Fe^{+2} in the devices may not necessarily reflect ambient DFe because the former are dependent on the exposure time and can be cumulative, whereas DFe values represent snapshots in time.

With the Deep Bay site having both temperature and dissolved oxygen profiles relatively constant (owing to its depth and sheltered location), the DFe concentration became the primary variable, and gradually increased as internal loading proceeded. On the last sampling date, Fe^{+2} concentrations in the devices exceeded DFe levels and seemed to reach a limit. This was likely an artifact of the unusually long incubation period of 90 hours (for logistical reasons), as opposed to the 70 hours of the previous two samples: the samples needed much dilution during analysis, and probably the ferrozine was entirely saturated. This demonstrates that the devices will continue to accumulate iron long after equilibrium with ambient DFe concentration is reached, and therefore incubation times must consider the hypolimnetic iron concentrations, or at least be standardized. The decline in both DFe and Fe^{+2} on the last sampling day relative to the previous samplings is more difficult to explain, but may be due to oxidation losses of Fe from a sampling error or unrecorded disturbance, or from dilution by an intrusion of fresher water, such as from a large rainfall event, seiche, groundwater seep, or from the adjacent outlet, though the conductivity did not change significantly between the last two sampling dates.

The Sturgeon Bay system was much more dynamic regarding temperature and oxygen profiles due to the combined effects of shallower depth and longer fetch: the site often experiences heavy wave action. The deepening oxycline and thermocline likely caused a rapid oxidation of hypolimnetic iron after mid-August, probably leading to the decline in levels of DFe. Alternatively, the decline may reflect oxidative losses of filtered samples if Fe^{+2} is more sensitive than expected, and comprised the majority of iron. Indeed, the ferrozine devices indicated that Fe^{+2} levels were constantly increasing, except where oxygen levels exceeded ~2 mg L^{-1} (Fig. 5.3). On the other hand, the incubation times for each sampling trip increased by

~24 hours each month for logistical reasons, so it is difficult to separate concentration from time effects (i.e. exceeding ambient Fe^{+2} levels due to the tendency of ferrozine to accumulate stable iron chromogen).

A preliminary analysis of the pooled data from both sites indicated that temperature did not seem to influence the final Fe^{+2} concentration during the incubations, whereas DFe concentrations did. Lower temperatures were expected to slow the rate of diffusion, but apparently concentration effects are far more dominant, as predicted by the Fickian diffusion equation. When normalized for the DFe concentration and time incubated in calculating the flux rate, the apparent diffusion coefficent, D_{eff} was found to be independent of concentration, but did vary somewhat with temperature. Temperature, viscocity, and particle size are all factors that change the diffusion coefficient, as they influence particle velocity, the primary driver of diffusion kinetics (Atkins and De Paula 2011).

In dilute solutions, the diffusion coefficients of most ions at room temperature range from 0.6×10^{-9} to 2×10^{-9} $m^{-2}\,s^{-1}$ (Ibid.): the values obtained here average 4.88×10^{-13} $m^{-2}\,s^{-1}$, which is considerably slower, due in part to lower temperatures, but most likely due to the impedance of diffusion across the porous membrane. Ideally, the molecular weight cutoff (MWCO) of dialysis membranes should be half the molecular weight of the solute to be retained, but 10-50 times greater than the solutes to be passed, which should be less than 1/10 the membrane MWCO for rapid dialysis (Thermo Scientific 2013). The size ratio for ferrozine versus Fe^{+2} is less than 10, and furthermore, a solute's permeability is also dependent upon molecular shape, degree of hydration, ionic charge, and polarity (Interchim 2018), so these and the aforementioned factors combined likely contributed to the high D_{eff}. Overall, MWCO selection was not ideal, but it reflected the best practical compromise to affect separation and retention of reactants; and it was functional. A smaller MWCO did not seem to be commercially available.

The method developed here is effective in detecting trace amounts of Fe^{+2} even when transiently available, owing to the ability of ferrozine to stably accumulate and “fix” Fe^{+2} when contained within a semipermeable membrane, such as dialysis tubing. Use of these devices to infer Fe^{+2} concentrations within the hypolimnion must be treated with caution, as factors such as water temperature should be known and incubation times controlled to avoid saturating ferrozine reagent with Fe^{+2} or requiring too much dilution to obtain accurate colourimetric readings. Determination of D_{eff} from laboratory-controlled experiments simulating the hypolimnia of lakes (anoxic and bicarbonate-buffered) with known concentrations of Fe^{+2} would likely be required before these devices could be used to infer natural Fe^{+2} concentrations with a greater degree of confidence.

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Chapter 6: Conclusions

The initial objective of this research was to examine the relationship between the release of Fe^{2+} during internal loading, and its influence on the formation of cyanobacterial blooms. Two years of sampling mesotrophic and oligotrophic embayments along Georgian Bay supported the hypothesis that anoxia followed by internal Fe^{2+} loading was necessary for cyanobacteria to dominate, but it also became apparent that warm temperatures played an important and expected role: the contrast between the 2012 and 2014 sampling seasons on Sturgeon Bay provided a graphic example of this effect. This study clarified that although anoxic sediments and subsequent Fe^{2+} release provides an advantage to cyanobacteria and allows them to dominate, high P is required for relatively large, visible blooms to form, consistent with prevailing lake management paradigms. These findings could have useful management and monitoring applications, but more work is needed to test the model under a larger range of natural systems and indicates that global warming will likely increase the incidence of cyanobacteria dominance and blooms, if it has not already.

Since Fe^{2+} was shown to be a specific promoter of cyanobacterial blooms, consideration then was given to the various ways it could be sequestered. Natural sinks of iron exist as carbonates, phosphates, and sulfides. Under the anoxic conditions prevailing during cyanobacterial blooms, sulfides become the dominant Fe sink, and the role of sulfates in that capacity therefore demanded some investigation. Specifically, it was hypothesized that recent increases in cyanobacterial blooms might be attributed in part to less sulfate in the lakes due to mitigation of acid rain deposition in the 1990s. As a first step, if trends were found in sulfide deposition in the sedimentary record that matched historical data showing declines in atmospheric sulfur oxide emissions and/or lake sulfate levels, further investigations could be justified. Sectioning and analysis of sediment cores from Lake Erie indicated that AVS and acid extractable iron declined in a pattern consistent with a decline in historical sulfate concentrations and SO_2 deposition, but there was an unusual loss of iron and sulfide at the expected peak, which created more questions than provided answers. The patterns in the Lake St. George cores were not very consistent with the historical data, and levels of AVS and iron were an order of magnitude less than in Lake Erie, thus contributing to variability and uncertainties in the data. More research on the diagenesis of sulfide minerals in these lakes, investigating other causes of sulfide loss, or sampling a meromictic lake may be required before more definitive conclusions can be drawn at this time, while the direct measurement of pyrites may also have yielded useful information.

While collecting sediment cores at many sites, it was noticed that towards the end of the season, a thick layer of black precipitate had accumulated at the sediment surface along with what appeared to be organic matter; this phenomenon was not encountered at other times of the year, though hypolimnetic waters would often have a sulfurous odour and leave a black residue on syringe filters weeks before the precipitate was present. This suggested that settling organic matter could greatly accelerate the generation of (primarily iron) sulfides, and lead to the burial and sequestering of at least a portion of AVS, which would otherwise be oxidized during the fall turnover. In creating such strongly reducing conditions, organic loading would also accelerate the release of P and Fe^{2+}, and the question remained if Fe^{2+} release into the hypolimnion could actually be supressed by sulfide generation, as proposed in the previous paleolimnological study, which was inconclusive. To test this, a controlled experiment simulating expected historical conditions was conducted by incubating sediment cores from two sites in the Great Lakes under anoxic conditions with varying sulfate and organic carbon additions. The results confirmed that bacterial metabolism of organic materials in and upon sediments is the primary driver of internal loading, and at later stages, sulfide deposition. But Fe^{2+} loading was not controlled by sulfide production until late in the incubations, even using relatively high sulfate concentrations comparable to concentrations in Hamilton Harbour, and therefore sulfide precipitation is unlikely to control cyanobacteria biomass until late in the season in most natural freshwater systems. However, if large enough amounts of organic matter are deposited following a large bloom or other carbon influx, iron availability may be reduced in subsequent seasons though burial of a relatively large mass of AVS, or conversion of AVS to pyrites (especially during prolonged anoxia), which are more resistant to oxidation. Historical sulfate concentrations are therefore unlikely to have controlled dissolved iron levels any better than modern concentrations, but have apparently produced significant quantities of AVS, which show up in the sedimentary record, most likely after periods of extensive anoxia.

The investigations in this thesis have given evidence supporting the role of ferrous iron and increased temperatures in promoting shifts in phytoplankton composition towards cyanobacteria dominance and the formation of harmful algal blooms. A larger array of lake systems showing similar results would be preferable, but there is an element of chance in the timing and selection of natural systems which produce blooms during a given field season, and there may be logistical and financial barriers to an extensive sampling program. Intuitively, controlled phytoplankton culture or mesocosm studies would appear to yield more direct evidence, but have been criticized as not reflecting natural conditions, and would require very

careful design in order to exclude confounding variables and include relevant ones; though ironically, natural conditions often have far more variables to contend with.

The role of sulfate as an indirect control of cyanobacteria blooms through Fe^{2+} sequestration was not conclusive from the sedimentary record, nor supported by sediment incubation experiments. The incubations did, however, demonstrate that organic matter is the primary driver leading to anoxic sediments and subsequent internal loading phenomenon, including formation of insoluble metal sulfides, which sequester a portion of the available iron and record anoxic events in the sedimentary record. Nevertheless, the measurement of AVS and/or pyrites might be useful proxies of the historical extent of anoxia, and might correspond to historical bloom events, which could be detected as stable pigment concentrations in the sediments.

The evidence from this investigation suggests that in order to prevent cyanobacteria blooms, either the amount of organic materials that exhaust dissolved oxygen must be lowered to levels that maintain surface sediments in an oxidized state and prevent Fe^{2+} release, or systemic lake temperatures must be lowered. Both tasks would seem impractical on large lake systems, though they have been done without too much difficulty on smaller lakes by simple physical means such as dredging, hypolimnetic withdrawal, aeration, and by increasing shading by submerged, floating, and emergent macrophytes, and increasing riparian vegetation along inlets. Reducing primary productivity through P controls has been the established means of reducing blooms, even in large systems like Lake Erie, but this does not always work due to legacy P released from sediments during internal loading and resuspension events. Additionally, non-point sources such as agricultural runoff and urban siltation inputs, which may not be readily acknowledged, but are evident by observing satellite images, appear to be the main sources of P and organic materials now contributing to eutrophication. These sources must be mitigated before we can expect to see improvements, which will be delayed by the water residence (flushing) time. Phytoplankton biomass therefore perpetuates the cycle by adding organic carbon sources required to lower the redox state of sediments. The Fe^{2+} which soon follows could be monitored as a sensitive indicator of the redox state of sediments, thus providing guidance for lake management efforts, and may even serve as an early warning proxy to predict when cyanobacterial blooms are more likely.

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