Phytoplankton dynamics in nearshore and offshore regions of the Great Lakes Erie, Malawi, Tanganyika, and Victoria

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Biology

Waterloo, Ontario, Canada, 2008

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

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Abstract

My doctoral thesis challenges the traditional paradigm of phosphorus (P) limitation of phytoplankton communities in freshwaters by suggesting colimitation of P, nitrogen (N), and iron (Fe) in Great Lakes. Oceanographers have recognized Fe, N and P colimitation, and biomass response to Fe is documented in freshwater lakes. I studied African and North American Great Lakes that are similar to large inland oceans. I discovered that Fe is a key nutrient that is often limiting in the offshore, and may explain the dominance of cyanobacteria in nutrient enriched lakes. I also discovered that the nearshore and offshore areas of these large lakes are very different, particularly when invasive dreissenid mussels are impacting the nearshore, as seen in the eastern basin of Lake Erie. As a result of the dreissenids, chlorophyll *a* (chl*a*) concentrations are significantly lower in the nearshore of Lake Erie, but higher in the nearshore in the three African Great Lakes, as well as pre-dreissenid Lake Erie. The objective of my thesis was to determine the limiting nutrient(s) to the phytoplankton of the Great Lakes Erie, Malawi, Tanganyika, and Victoria in both the nearshore and offshore by measuring the physiological status of the phytoplankton. I also examined how dreissenids affect the distribution of seston and nutrient concentrations between the nearshore and offshore of the eastern basin of Lake Erie. My study design included temporal and spatial surveys in the nearshore and offshore of the four lakes, in which I used a variety of nutrient limitation indicators for P (C:P, N:P, P debt, APA, F_v/F_m), N (C:N, NH_4 debt, NO_3 debt, F_v/F_m), and Fe (F_v/F_m), as well as photosynthetic efficiency (F_v/F_m) experiments. Nutrient enrichment experiments were also conducted in the nearshore and offshore of the eastern basin of Lake Erie which involved the addition and removal of Fe alone, as

well as in combination with P and/or N. Lake Erie nutrient enrichment experiments provided evidence for P, N and Fe colimitation where the addition of Fe with P relieved Fe and P limitation and allowed nitrate (NO_3^-) assimilation, alleviating N limitation. However, the offshore experiments indicated stronger Fe limitation than the nearshore experiment. Lower chl*a* concentrations in the post-dreissenid nearshore of the eastern basin of Lake Erie may not be due entirely to lower phytoplankton biomass, as photoacclimation of the phytoplankton may also be occurring. Dreissenid grazing effects can be seen in the distribution of dissolved nutrient concentrations between the nearshore and offshore of post-dreissenid Erie. The African Great Lakes are threatened by expanding human populations, resulting in increased nutrient runoff; the consequences of which will depend on the limiting nutrient(s). I found that the nearshore regions of Lakes Malawi and Tanganyika were colimited by N and P, while the offshore regions were colimited by N, P and Fe. The nearshore of Lake Victoria was colimited by light and N, while the offshore was colimited by N, P and Fe. Fe limitation only occurs in the offshore, and positive, significant relationships were found between total dissolved Fe concentrations and cyanobacteria. Continued P and Fe loading to the lakes will create a higher N demand that will result in a shift to N_2-fixing cyanobacteria, which has serious consequences to human and ecosystem health as they are a poor nutritive food source and some are potentially toxigenic. The majority of studies conducted on Great Lakes involve offshore sampling, however, the less understood nearshore is where human impacts and activities are concentrated. I discovered there are significant differences between the nearshore and offshore, which has implications for water quality monitoring.

Acknowledgements

This thesis is the result of six years of graduate work including three field seasons on Lake Erie, two sampling trips to Lake Malawi, one sampling trip to Lake Tanganyika and three sampling trips to Lake Victoria. The driving force behind all of these efforts was Stephanie Guildford. I would like to acknowledge Stephanie as my co-advisor, co-author and mentor. She introduced me to the African Great Lakes, and for that I will always be grateful. I would like to thank her for her energy, enthusiasm, unwavering support and companionship. I would also like to acknowledge my co-advisor and co-author, Ralph Smith for introducing me to limnology as an undergraduate student, and always being positive and supportive of my work. Michael Twiss has been a committee member and co-author on two of my publications and ran all of the Fe chemistry from the four lakes. He introduced me to the world of iron, and trained me in trace metal clean techniques; but most importantly, he did not laugh when I modified his methods for use in Africa and the clean room I created in a closet on campus. I would like to thank Kirsten Muller for serving as a committee member and a mentor, both personally and professionally. I would also like to thank my newest committee member, Bill Taylor for being so kind and supportive over the years.

All of my data chapters have benefited from the contributions of my co-authors, therefore, I would also like to acknowledge: Sonya Havens (chapter 2 and running the Fe chemistry from chapter 4), Hedy Kling (chapter 4), Dave Depew (chapter 3 and technical field and lab support), Murray Charlton (chapter 3 and coordination of field and lab support), and Luis León (chapter 3 and creation of figures 3.1-3.3). I would like to give a special thanks to Bob Hecky who was a co-author on chapter 3 and was instrumental to

all of my African work. Although Bob is not an official member of my committee, he has been involved and supportive of my work from the very beginning and has contributed through discussions and editing of all of my data chapters. I would also like to acknowledge the L&O reviewers, V.H. Smith and H.J.W. de Baar who greatly improved chapter 2.

I would like to acknowledge the institutes through which this research was conducted and the technical assistance provided including: SADC/GEF in Senga Bay, Malawi (M.J.G.S. Gondwe, M. Ngochera, R. Sipawe, T. Mhango, J. Sipawe, and M. Whayo), TAFIRI in Kigoma, Tanzania (Muhosa, Chale) and FIRRI in Jinja, Uganda (W. Okello, A. Aguzu). I would also like to acknowledge Rose Mugidde for her support of my work on Lake Victoria and Rashid Tamatamah for facilitating the work on Lake Tanganyika. The work on Lake Erie would not have been possible without the Captain and crew of the *C.C.G.S. Limnos* and Technical Operations of Environment Canada as well as the Ontario Ministry of Natural Resources (Port Dover) for allowing use of their boat and "lab" space. In addition, the use of the 1973 EPA data presented in chapter 3 was made possible by the efforts of Russell Kreis, David Rockwell, and Marc Tuchman.

Technical assistance for the work on Lake Erie was provided by Tara Nelson, Anna Desellas, Sunniva Ronzio, Andrea Lublin and Jackie Pridham. I also acknowledge the following analytical support: the Chemical Engineering Analytical Chemistry Services in the Department of Chemical Engineering at the University of Waterloo and Ralph Dickhout, the Analytical Laboratory at the Freshwater Institute, the Environmental Isotope Lab of the University of Waterloo and Richard Elgood, the National Laboratory

for Environmental Testing (NLET) at the Canada Centre for Inland Waters (CCIW), and M. Bridoux for the 2002 Fe chemistry.

This research was funded in part by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to Stephanie Guildford and Ralph Smith, and additional funding from the National Science Foundation (Chem Oce No. 0327730) to Michael Twiss. The 2004 work on Lakes Tanganyika and Victoria was funded by an IDRC Doctoral Research Award granted to me. I was supported by an Ontario Graduate Scholarship (1 year) and NSERC postgraduate scholarships (4 years) for the majority of my graduate work. I also received funding from the University of Waterloo Doctoral Thesis Completion Award, as well as Davis Memorial Scholarship in Ecology.

I would personally like to thank my boys; my husband, Jake Doran, who has been with me since before I knew what limnology was, and my son Isaak, who has a way of putting things in perspective. I would like to acknowledge my parents, Peter and Donna North, who have been supportive of me since my grade 8 science project on the Bay of Quinte; and my brothers, Ryan and Travis North. The support of my friends has been a constant throughout my life and I would like to acknowledge the Belleville girls, specifically Mandy Stone who is always there to listen, and the North A girls. Last, but not least, I would like to acknowledge all former and current members of the University of Waterloo Aquatic Ecology Group (UWAEG), specifically: Sairah Malkin, Scott Higgins, Paul Weidman, Brynn Upsdell, Reagan Johnson, Vicky Jackson, Greg Silsbe, Amanda Poste, Megan Puchniak, Sunita Sarkar, Mohamed Mohamed, Matt Patterson, Margaret Vogel, Jane Almond, Veronique Hiriart- Baer, and Ngan Diep.

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Chapter 1 General Introduction

The purpose of this thesis is to understand the factors controlling the growth of phytoplankton in different freshwater environments. The primary factors that regulate phytoplankton growth are light, nutrients (phosphorus (P), nitrogen (N), and iron (Fe)), and grazing. Four very different freshwater Great Lakes including Lakes Erie, Malawi, Tanganyika, and Victoria were used to represent different climatic environments and trophic status, as well as the nearshore and offshore regions within each lake. I define the nearshore as the region beginning at the shoreline of the lake and extending offshore to where the lower limit of the thermocline typically intersects with the lake bed during seasonal stratification, while offshore is defined as the region beyond the nearshore to the deepest point in the lake. The objective of my thesis is to determine the limiting nutrient(s) to the phytoplankton of the four Great Lakes in both the nearshore and offshore by measuring the physiological status of the phytoplankton. My approach was to use several nutrient limitation indicators in combination with nutrient enrichment experiments, while taking into consideration light and thermal stratification, in order to determine what controlled the growth of phytoplankton in the nearshore and offshore regions of each lake. Virtually all of the metabolic energy available for the growth and activities of organisms in large lakes is from photosynthesis by phytoplankton. Factors that affect the growth of phytoplankton will therefore affect the energy available to higher trophic levels, conceivably limiting the biomass or yield of fish that can be collected from the lake.

1.1 Factors controlling phytoplankton growth

1.1.1 Light

Light is electromagnetic energy that is composed of particles called photons, or quanta of light that are characterized by movement in wavelengths. Photosynthetically active radiation (PAR) occurs between 400 and 700 nm and accounts for approximately 50% of all the incoming solar energy (Kalff 2002). Light intensity and the spectral quality of the light decreases exponentially with increasing depth of water because of selective absorption and scattering by particles. The vertical light extinction coefficient (k_d) is used to indicate the extinction of PAR through the euphotic zone. A larger k_d indicates a higher probability that light will be absorbed (Kirk 2003). Phytoplankton use chlorophyll and accessory pigments to capture the light which is then converted into chemical energy via photosynthesis.

1.1.2 Nutrients

In order to photosynthesize, phytoplankton must have macronutrients such as carbon (C), N, P, silica (Si; for diatoms only), and micronutrients such as Fe. Different phytoplankton groups require different nutrients in different amounts. Thus, the growth rates of phytoplankton are often limited by several nutrients, not just one, and this is based on species composition and abundance (Brand 1991). One of the important theories involving phytoplankton growth is Liebig's Law of the Minimum, which states that organisms will become limited by whatever resource is in lowest supply compared to their needs (Von Liebig 1840).

Nutrient deficiency results in either morphological or physiological changes that reduce the overall performance of the phytoplankton as primary producers.

Phytoplankton respond to nutrient deficiency through increased uptake of additional nutrients (compensation), and the development of more efficient uptake systems for the limiting nutrient (acquisition). Severe nutrient deficiency will result in a complete shutdown of physiological processes; and cell death. Indicators of nutrient deficiency can be used to determine which nutrient or combination of nutrients is limiting to the phytoplankton communities. Elemental analysis cannot report adequately on the availability of nutrients (Hecky and Kilham 1988), therefore, we must use alternative technologies to assay nutrient bioavailability as sensed by the cells. Nutrient limitation indicators are based on the premise that the cellular constituents, nutrient uptake, and certain enzymatic activities will vary in predictable ways depending on the nutritional state of the phytoplankton cell (Healey and Hendzel 1979).

1.1.2.1 Phosphorus

Phosphorus is often considered to be the single limiting nutrient to temperate freshwater systems as 75% of freshwater lakes have been characterized as P-limited (Wetzel 2001). Total phosphorus (TP) concentrations have been positively correlated with the biomass of primary producers (Dillon and Rigler 1974), implying that algal biomass can be controlled by P concentrations, which was later confirmed by whole-lake experiments (Schindler 1977). In fact, the classification of the trophic status of lakes is often based on TP concentrations (Wetzel 2001). It is well known that P is a required macronutrient for phytoplankton growth. Primary producers require P for the synthesis of nucleotides such as RNA and DNA (Bjorkman and Karl 2003), sugar phosphates, structural components in cell membranes such as phospholipids, and cellular energetics by ATP-ADP-AMP interconversions (Wetzel 2001). P is widely distributed in the earth's crust, where it

comprises 0.1% by weight of the elements present (Horne and Goldman 1994). P can enter lake systems externally through direct atmospheric deposition on water surfaces, overland runoff, and tributaries. However, internal inputs of P are often predominant in freshwater systems arising from the regeneration of P within the water column and from the benthos through internal loading. Under stratified conditions, inputs of phosphate from the atmosphere, horizontal and vertical transport, and from larger organisms are small compared to planktonic regeneration (Hudson et al. 2000). Chemically, P can be complexed and made unavailable for phytoplankton uptake by adsorption and/or precipitation with inorganic compounds such as Fe, manganese (Mn), and clays. In addition, humic compounds can act as sequestering agents for phosphate ions, organophosphate compounds, and Fe, thereby reducing P and Fe bioavailability. P is also co-precipitated with and adsorbed to nucleating calcium carbonate ($CaCO_3$) therefore, the process of formation and sedimentation of $CaCO_3$ can markedly alter P availability to biota (Wetzel 2001). Greater than 90% of TP in freshwaters is not directly available for uptake by phytoplankton communities as it occurs as organic phosphates and cellular constituents in the biota, in addition to being adsorbed to inorganic and dead particulate organic materials. Dissolved inorganic P (DIP) is present in the form orthophosphate (PO_4^{3-}), polyphosphates, organic colloids, or P combined with adsorptive colloids and low-molecular weight phosphate esters (Wetzel 2001). Most P in the dissolved, soluble fraction is not PO_4^{3-} (the un-complexed ion), and not readily available for uptake by algae or other plants. Traditionally, soluble reactive P (SRP) was thought to be the biologically available component of DIP, or PO_4^{3-}, which is the only form of P directly taken up by

cells. However, it has been suggested that SRP does not measure PO_4^{3-} in true solution (Hudson and Taylor 1996; Hudson et al. 1999; Hudson et al. 2000).

The uptake of phosphate is a metabolically driven process that requires active uptake due to the ionic nature of PO_4^{3-} (Morel 1987). When PO_4^{3-} concentrations are low, phytoplankton can excrete extra-cellular enzymes called alkaline phosphatases that can cleave the chemical bond between PO_4^{3-} and organic molecules. Thus, phosphatases catalyze the liberation of orthophosphate from organic P complexes (Ammerman et al. 2003).

1.1.2.2 Nitrogen

Nitrogen cycling in aquatic ecosystems is very complex, as N exists in a variety of oxidized and reduced forms that allow it to serve as an electron donor and receiver (Kalff 2002). Sources or inputs of N to freshwater aquatic systems include atmospheric N deposition, lightning, precipitation directly onto the lake surface, N_2-fixation both in water and sediments, inputs from surface and groundwater drainage, tributaries, sewage treatment plants, and non-point sources such as agricultural runoff. N is removed from freshwater systems by denitrification, gaseous evasion, sedimentation and biomass harvest (Capone 2000). Phytoplankton require N for the production of nitrogenous compounds such as amino acids and proteins.

There are three major inorganic forms of N: nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+). In well-oxygenated epilimnia, the most abundant form of inorganic N is NO_3^-, which is its most oxidized form in solution. In order to utilize NO_3^- and NO_2^-, phytoplankton must first reduce them to NH_4^+ via the nitrate and nitrite reductase enzymes (Paerl and Zehr 2000). NH_4^+ contains N at the oxidation level of proteins

(Ward 2000), therefore, is the preferred form of N for uptake by phytoplankton. NH_4^+ does not exist long in aquatic environments as it is either assimilated by phytoplankton or readily oxidized to NO_3^- by nitrifying bacteria (Kalff 2002). NH_4^+ is produced upon death or decomposition of organisms by heterotrophic bacteria from the deamination of proteins, amino acids, urea, and other nitrogenous organic compounds, or excretion by animals including zooplankton (Lampert and Sommer 1997). After NH_4^+, the preferred form of inorganic N for uptake by phytoplankton and bacteria is NO_2^-, followed by NO_3^-. Only cyanobacteria can utilize N_2. This preference is based on the fact that less energy is required to assimilate NH_4^+ or NO_3^- than to synthesize nitrogenase and fix nitrogen (Howarth et al. 1988). The two main forms of organic N taken up by phytoplankton are amino acids and urea. Amino acids can be converted to NH_4^+ by the enzyme amino acid oxidase and urea can be taken up by the cell and converted into NH_4^+ by the enzyme urease or urea amidolase (Capone 2000).

Nitrification is the biological conversion of inorganic nitrogenous compounds from a reduced state to a more oxidized state. There are two main groups of nitrifying bacteria; ammonium oxidizers facilitate the conversion of NH_4^+ into NO_2^-, while nitrite oxidizers facilitate the oxidation of NO_2^- to NO_3^-. The highest nitrification rates occur at the oxycline between oxic and anoxic waters (Kalff 2002). Denitrification occurs in anaerobic environments like the hypolimnion and anoxic sediments, where oxidizable organic substrates are abundant (Wetzel 2001).

N_2-fixation is the reduction of N_2 gas to NH_4^+ for biosynthesis (Capone 2000). Phytoplankton that have the ability to fix atmospheric N_2 (diazotrophs) are prokaryotes, and include photoautotrophic cyanobacteria, aerobic and anaerobic heterotrophic, and

chemoautotrophic bacteria. Diazotrophs provide a source of utilizable N to the biosphere from the large pool of N_2 and balance losses of NO_3^- by denitrification (Capone 2000). The enzyme used to fix N_2 is nitrogenase, which has two protein components: the MoFe protein (dinitrogenase), and the Fe protein (dinitrogenase reductase). The most common adaptation to N limitation of phytoplankton is the production of heterocysts, which are biochemically and structurally differentiated oxygen devoid cells in which N_2-fixation occurs (Paerl and Zehr 2000). Studies have shown that there is a significant relationship between N_2-fixation rates and heterocyst abundance (Findlay et al. 1994; Gondwe et al. 2007). Thus, heterocyst abundance is often used to infer N_2-fixation rates. However, Zehr et al. (2001) reported that there are multiple strains or populations of unicellular, non-heterocystous N_2-fixing marine cyanobacterial groups that can equal or exceed the N_2-fixation contribution of the known heterocystous diazotrophs. This indicates that by calculating a N_2-fixation rate based on heterocyst counts alone, the actual N_2-fixing capacity is grossly underestimated.

1.1.2.3 Iron

Iron limitation of phytoplankton growth rates in oceanic high-nutrient, low-chlorophyll (HNLC) regions is well documented (Tsuda et al. 2003; Boyd et al. 2004) and has also been observed in coastal regions (Boyd et al. 1998; Hutchins et al. 1998; Hutchins and Bruland 1998). Fe plays a catalytic role in many biochemical reactions as a cofactor of enzymes and proteins involved in chlorophyll synthesis, detoxification of reactive oxygen species, electron transport, N assimilation, and is also essential to many energy obtaining and energy yielding processes in algal cells including photosynthesis and respiration (Falkowski et al. 1998). Inputs of Fe to lakes include tributaries, re-suspension of bottom

sediments, vertical mixing and upwelling of Fe-rich hypolimnetic water, and wet and dry deposition of atmospheric aerosols (Wells et al. 1995). Although the eolian transport of terrestrial dust particles is episodic and unpredictable, it supplies more dissolved Fe to lakes than the input from rivers (Price and Morel 2002). Recycled or regenerated Fe is often thought to be the dominant source of Fe to phytoplankton in aquatic systems as each individual Fe atom may be cycled through the biota an average of 169 times before being lost on sinking particles (Hutchins 1995). Mechanisms for removing Fe from surface waters include sorption and precipitation, biological assimilation, aggregation of inorganic or organic colloids, and sinking of mineral and biogenic particles (Wells et al. 1995). Due to the terrestrial nature of Fe, Fe limitation is a relatively unreported phenomenon in freshwater lakes, however it has recently been reported in Lake Superior (Sterner et al. 2004), Lake Erie (Twiss et al. 2000; Twiss et al. 2005) and Lakes Malawi and Victoria (Guildford et al. 2003).

In order for Fe to be biologically available, it must remain in the epilimnion, which is achieved by complexation by organic colloids and dissolved organic carbon (DOC). In fact, 99% of ferric Fe (Fe^{3+}) in natural lake systems is complexed to DOC (Sunda 2000). The stable oxidation state of Fe is Fe^{3+} in oxygenated water at circumneutral pH, however, the reduced form, ferrous Fe (Fe^{2+}), is significantly more soluble and thus is the form used for assimilation by phytoplankton. The two primary cell-mediated techniques to take up organically bound Fe are the use of siderophores (Wilhelm and Trick 1994; Wilhelm 1995) and the reduction of Fe^{3+} by membrane bound enzymes (Maldonado and Price 1999).

In situations of low Fe concentrations, many microorganisms have devised strategies to increase Fe accessibility. These strategies include reduction in cell size and growth rates (Sunda 2000), an increased density of Fe transport ligands (Hudson and Morel 1990), and the production of siderophores (Maldonado and Price 1999). Phytoplankton also have the ability to decrease the cellular metabolic requirements for Fe by altering metabolic pathways or by changing the metalloenzyme content of key pathways (Sunda 2000). For example, the non-ferrous electron carrier flavodoxin can be substituted for the Fe-sulfur protein ferredoxin (Fox 1976).

1.1.2.4 Nutrient Colimitation

Traditional paradigms of nutrient limitation identify N as the primary limiting nutrient in marine systems (Howarth and Marino 2006), and P in freshwater lakes (Schindler 1977; Hecky and Kilham 1988). In addition, tropical freshwaters were more frequently N limited than temperate ones (Downing et al. 1999a). However, recent work has begun to question these generalizations, calling attention to the equivalence of N and P limitation in lakes (Elser et al. 1990), and to frequent P limitation in the oceans (Downing et al. 1999b). As well, Elser et al. (2007) found little evidence for strong latitudinal variation in autotrophic nutrient limitation, contrary to Downing et al. (1999a).

There is historical evidence for multiple nutrient limitation in the Laurentian Great Lakes. In addition to the stimulatory effects of P alone on phytoplankton growth, interactions with N and trace metals or both were frequently observed (Schelske et al. 1978; Stoermer et al. 1978; Lin and Schelske 1981). Hartig and Wallen (1983), through nutrient enrichment experiments in the western basin of Lake Erie showed that the limiting nutrient was seasonally dependent. Si availablility limited phytoplankton growth

in early spring, while P-availability limited growth in summer. Elser et al. (2007) used a large-scale meta-analysis of experimental enrichments (653 freshwater and 243 marine) to show that P limitation was equally strong across freshwater and marine systems, and that N and P limitation were equivalent within freshwaters. Furthermore, simultaneous N and P enrichment produced similarly strong positive synergistic responses in both marine and freshwater systems resulting in higher responses than single nutrient additions. They concluded that freshwater and marine ecosystems are surprisingly similar in terms of N and P limitation of primary producers.

Most studies on multiple nutrient limitation focus on two elements (e.g., N and P) being simultaneously limiting, however, it is possible that three or more elements can be simultaneously limiting. Recent studies suggest that Fe and P colimit N_2-fixation in the N-limited eastern tropical North Atlantic (Mills et al. 2004). In fact, colimitation by Fe and light best describes the high nutrient low chlorophyll (HNLC) regions in 40% of the world oceans (de Baar et al. 2005). Although the concept of colimitation has recently been accepted in marine systems (de Baar et al. 2005), it has yet to be embraced by the freshwater community and the potential for colimiting nutrients and the different types of colimitation are often not discussed in the literature.

One reason for the ambiguity associated with the term colimitation is the fact that there are really several distinct types of colimitation. Arrigo (2005) distinguishes three types of nutrient colimiation. He states that multi-nutrient colimitation of growth rates can occur at the cellular level when two nutrients are below optimal concentrations for uptake and the simultaneous addition of both nutrients increases growth. He also defines cellular biochemical colimitation as a particular case of colimitation in which a trace

element facilitates the assimilation of a major nutrient. At the community level, colimitation can occur when one nutrient is below a threshold concentration for one species and another nutrient is below a threshold concentration for another species (Arrigo 2005). Perhaps the lack of identification of colimitation in freshwaters is a product of the traditional methods of detecting colimitation using bottle enrichment incubations. Bottle incubations have been powerful in demonstrating Fe limitation in the HNLC regions, however, they may not reliably detect colimitation due to experimental error, changes in community structure, or multiple limitations.

Global cycles of N and P have been amplified by c.100% and c.400% respectively, by post-industrial human activities (Falkowski et al. 2000). As well, Fe concentrations have increased in the Laurentian Great Lakes as a result of wastewater treatment practices (Medeiros and Molot 2006). Predicting and mitigating the effects of altered nutrient loading requires an understanding of if, where, and by how much these key nutrients limit production. Elser et al. (2007) recommends that instead of focusing intense scrutiny on the supply and cycling of a particular nutrient under a system-specific presumption that it is limiting, we would benefit from a more balanced view of the impacts of multiple key nutrients. The dual importance of N and P limitation indicates that effects of alterations of a particular nutrient may be manifested not simply via quantitative changes in ecosystem production, but also via qualitative shifts in the nature of nutrient limitation. This is likely to have impacts on competitive interactions among autotrophic species and on stoichiometric processing of primary production by consumers. Finally, their results clearly show that enrichment by either N or P can

increase autotroph production but that a simultaneous increase in both nutrients leads to dramatically higher levels of production in nearly all situations (Elser et al. 2007).

1.2 Study lakes

1.2.1 Lake Erie

Lake Erie is the 11th largest lake in the world by surface area (25 657 km^2; Herdendorf 1990) and is the shallowest of the Laurentian Great Lakes with a maximum depth of 64 m in the eastern basin. However, it is also the most heavily impacted of the Laurentian Great Lakes and although previously referred to as the "dead lake" (Sweeney 1993), its trophic status has reverted from highly eutrophic to meso-oligotropic (Charlton et al. 1993). An estimated one species per year has been introduced to the lake (Mills et al. 1993), including the exotic dreissenid mussels *Dreissena polymorpha* and *Dreissena bugensis.* Like all of the Laurentian Great Lakes, Lake Erie phytoplankton are most frequently characterized as P limited (Guildford et al. 2005).

1.2.2 Lake Malawi

Lake Malawi/Nyasa (hereafter referred to as Malawi), is the 7th largest lake in the world and the third largest by area in Africa (Herdendorf 1990). It is of considerable importance in terms of environmental quality and economic value to the surrounding countries of Malawi, Tanzania, and Mozambique, and of scientific interest to the world. It has more species of fish than any other lake in the world, and the fish nearly all evolved in the lake and are endemic to it (Arnegard et al. 1999; Bootsma and Hecky 1999). The lake supports a growing fishery, aquarium fish exports, and is estimated to have between 500-1500 species of fish. The lake remains in relatively pristine condition and is considered to be oligotrophic. Phytoplankton growth in Lake Malawi is rarely

controlled by light (Guildford et al. 2000), and has generally been described as seasonally N-limited. This is based on nutrient status indicators and nutrient enrichment studies (Guildford et al. 2000; Guildford et al. 2003; Guildford et al. 2007), low water column N:P ratios (Hecky et al. 1996; Guildford and Hecky 2000), and seasonal prevalence of heterocystous *Anabaena* filaments in the water column (Patterson and Kachinjika 1993). The N deficiency in the lake is imposed by the presence of a boundary layer that reduces internal N inputs from the hypolimnion (Hecky et al. 1996). Lake Malawi remains permanently stratified but mixing to 200 m once a year, leaving the 200-700 m of hypolimnion permanently anoxic (Guildford et al. 2000), resulting in accumulation of high nutrient concentrations in the slightly cooler and anoxic deep waters (Bootsma and Hecky 1993). However, there is some physiological evidence for codeficiency of N and P in the mixed layer (Guildford et al. 2000)

In Malawi, threats of eutrophication are present due to increased land use (Hecky et al. 1996), but the consequences of increased nutrient loading to the lake will depend on the degree to which the phytoplankton are nutrient limited and by which nutrient. Development in the riparian countries of Lake Malawi will increase nutrient runoff because of land clearing and subsequent erosion (Bootsma and Hecky 1993). Hecky et al. (2003) compared forested catchments with extensively cultivated catchments, and concluded that nutrient yields increased six to nine-fold over pre-disturbance yields. As well, biomass burning to clear land for agriculture mobilizes nutrients and transports them into the lake. Due to the large lake surface area available to receive dry fall, and the dominance of rain in the water budget, atmospheric deposition of P is an important external P source to Lake Malawi (Bootsma et al. 1999).

1.2.3 Lake Tanganyika

Lake Tanganyika is the 2nd deepest lake in the world (1470 m) and the second largest by area in Africa (Herdendorf 1990). The lake is permanently meromictic and anoxic below 150 m depth (Hecky 1991). As a result, the pelagic ecosystem is isolated from the deep nutrient-rich water and the production in the lake is dependent upon internal nutrient cycling (Hecky 1991). Periodic inputs of nutrients to the epilimnion from deeper waters are most important to the P and Si cycles within the lake (Hecky et al. 1991). Seasonal exchange of N between epilimnion and deeper water is likely less important because much N is lost from the lake by denitrification at the anoxic boundary. Denitrification lowers the N:P ratio of the deep water and creates a strong N demand when added to the mixed layer (Hecky and Kling 1981). N in the mixed layer is thought to be mostly supplied by fixation by cyanobacteria (although N_2-fixation has never been measured), and by import through rivers and rainfall on the lake. N_2-fixation may account for as much as 97% of total nitrogen input, while about 90% of P becomes available by vertical mixing (Hecky et al. 1991). Thus, owing to the hydrology and nutrient chemistry of the lake, the productivity of Lake Tanganyika has been suggested most likely to be N limited (Edmond et al. 1993). In 1995, nutrient enrichment bioassays in the offshore of Lake Tanganyika revealed that both N and P were potentially limiting phytoplankton production (Järvinen et al. 1999). Recent nutrient enrichment bioassays have demonstrated colimitation by N, P and Fe (de Wever et al. 2008).

1.2.4 Lake Victoria

Lake Victoria is the 2nd largest freshwater lake by surface area and the largest tropical lake in the world (Herdendorf 1990). It supports the largest freshwater commercial

fishery in the world (Simonit and Perrings 2005) and is a source of water and food for thirty million East Africans from the three riparian countries of Tanzania, Uganda, and Kenya. Lake Victoria is highly eutrophic due to increased inputs of P (Hecky 1993), and atmospheric deposition of P appears to be the dominant external source (Tamatamah et al. 2006). Phytoplankton growth and primary production is limited by light (Mugidde 1993; Silsbe et al. 2006). Evidence of N-deficiency in Lake Victoria was found in nutrient addition experiments (Guildford et al. 2003) and by the persistence and abundance of N_2-fixing cyanobacteria (Kling et al. 2001). In fact, the nearshore zone is dominated by N_2-fixers to the point that they are self-shading (Kling et al. 2001; Mugidde 2001).

1.3 Thesis overview

This thesis is divided into three independent data chapters (chapters 2, 3 and 4), that were written as manuscripts for publication in scientific journals. At the time of thesis submission, chapter 2 is published in Limnology and Oceanography: North, R.L., S.J. Guildford, R.E.H. Smith, S.M. Havens, and M.R. Twiss. 2007. Limnol. Oceanogr. **52**: 315-328. Chapter 4 has been accepted for publication in Verh. Internat. Verein. Limnol. **30** (2), April 2008; and chapter 3 is in preparation for submission to Limnology and Oceanography.

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Chapter 2

Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie

2.1 Introduction

Iron may be an important limiting nutrient in some freshwaters as well as in some areas of the oceans. Recent studies have reported that levels of dissolved Fe can decline to low concentrations in offshore waters of both Lake Erie (2 nmol L^{-1}; Twiss et al. 2005) and Lake Superior (1.1 nmol L^{-1}; Sterner et al. 2004). Also, the addition of Fe to lake water has been demonstrated at times to stimulate phytoplankton biomass (Twiss et al. 2000; Guildford et al. 2003). Iron limitation of phytoplankton growth rates in oceanic high-nutrient, low-chlorophyll (HNLC) regions is well documented (Tsuda et al. 2003). However, recent studies have shown that Fe is not the single limiting factor. Mills et al. (2004) suggest that Fe and P colimit N_2-fixation in the N-limited eastern tropical North Atlantic, while de Baar et al. (1990) postulated that light and large grazers are additionally major factors controlling phytoplankton in the Weddell and Scotia Seas. In fact, colimitation by Fe and light best describes the HNLC regions in 40% of the world oceans (de Baar et al. 2005).

Fe limitation is not expected to occur in lakes due to the proximity of terrestrial influences, but several investigators have seen a response by phytoplankton to Fe in lakes of various sizes (Sakamoto 1971; Storch and Dunham 1986). The nearshore regions of lakes in particular are not expected to exhibit signs of Fe limitation because of the reductive dissolution of Fe oxides in the sediments that become remobilized and diffuse into the water column (Schoemann et al. 1998). In the well-mixed nearshore, this mechanism occurs together with elevated concentrations of Fe from fluvial inputs (Martin 1990). Nevertheless, coastal marine environments have also responded to Fe enrichment (Hutchins and Bruland 1998; Hutchins et al. 1998).

In diagnosing the nutrient factors controlling phytoplankton, I am specifically interested in limitation of growth rates (Blackman limitation). The variations in such limitation, and the

selective advantages and disadvantages thereby incurred among species, are among the fundamental controls on primary production and community composition (Tilman 1976). As defined by Arrigo (2005), multi-nutrient colimitation of growth rates can occur when two nutrients are below optimal concentrations for uptake and the simultaneous addition of both nutrients increases growth. Cellular biochemical colimitation is a particular case of colimitation in which a trace element facilitates the assimilation of a major nutrient, as in Fe-facilitated assimilation of NO_3^-. At the community level, colimitation can occur when one nutrient is below a threshold concentration for one species and another nutrient is below a threshold concentration for another species, as in the classic case of diatom competition for P and silica (Tilman 1976). We may therefore expect potentially complex patterns of nutrient limitation in nature, with simple control by a single nutrient being only one of several possibilities. Iron is particularly likely to exert its effects via colimitation.

Iron plays a catalytic role in many biochemical reactions as a cofactor of enzymes and proteins involved in chlorophyll synthesis, detoxification of reactive oxygen species, electron transport, and N assimilation. In order to utilize nitrite (NO_2^-) and NO_3^-, phytoplankton must first reduce them to NH_4^+, which utilizes the nitrate and nitrite reductase enzymes (Paerl and Zehr 2000). Iron limitation has been shown to decrease nitrate reductase activity in phytoplankton (Milligan and Harrison 2000) as Fe is a principal component of these enzymes. Due to the high energetic cost of NO_3^- reduction, $NH_4^+$is the preferred inorganic N source (Harrison et al. 1996). Iron is typically not limiting to biomass directly, yet NO_3^- uptake has high Fe requirements. Therefore, phytoplankton NO_3^- metabolism and thus growth rates may be reduced (Timmermans et al. 2004) by low bioavailable concentrations of Fe.

Historically, the simplest approach for demonstrating nutrient limitation in phytoplankton was to measure a biomass response to the addition of a single nutrient. Nutrient limitation can

also be investigated using indicators of the bioavailability of nutrients as sensed by the cells. Nutrient limitation indicators are based on the premise that the cellular constituents, nutrient uptake, and certain enzymatic activities will vary in predictable ways depending on the nutritional state of the phytoplankton cell (Healey and Hendzel 1979b).

Lake Erie is the 11th largest lake in the world by surface area, provides drinking water for 11 million people (EPA 2000), and is one of the largest and most productive freshwater fisheries in the world with annual landings of 21,900 MT (Nepszy 1999). The shallowest of the Laurentian Great Lakes with a maximum depth of 64 m in the eastern basin, it was once referred to as a "dead lake". However, its trophic status has reverted from eutrophic to meso-oligotrophic as a result of the implementation of the Great Lakes Water Quality agreement in 1972 that led to a decrease in total phosphorus (TP; Charlton et al. 1993). Lake Erie currently has low TP (0.2 μmol L^{-1}), high $NO_3^- + NO_2^-$ (13.2 μmol L^{-1}; Charlton and Milne 2004), and low NH_4^+ concentrations (0.37 μmol L^{-1}; this study). Lake Erie phytoplankton are most frequently characterized as P limited (Lean et al. 1983; Wilhelm et al. 2003; Guildford et al. 2005) although recently in the eastern basin, there has been evidence of multi-nutrient colimitation due to N limitation, as indicated by N bioreporters and N limitation indicators (Wilhelm et al. 2003; Guildford et al. 2005). This is surprising, as $NO_3^- + NO_2^-$ concentrations are already high and increasing, representing one of the largest anthropogenic changes detected in Lake Erie (Charlton and Milne 2004). Human activities have lead to major increases in global emissions of N to the atmosphere, nearly fourfold greater than before the industrial revolution, and total atmospheric deposition of reactive N is currently an order of magnitude greater than in pre-industrial times (Phoenix et al. 2006). Increased loading from Lake Erie agricultural tributaries has also been

documented, with increases in NO_3^- + NO_2^- concentrations from 0.71 to 10 μmol L^{-1} per year (Richards and Baker 1993).

The objective of this study was to examine the role of Fe in modifying the response to P and N of Lake Erie phytoplankton. I hypothesize that under certain situations P and N limitation detected in Lake Erie phytoplankton is the result of colimitation by P and Fe and the addition of Fe with P will relieve Fe and P limitation and allow NO_3^- assimilation, thereby alleviating N limitation. A corollary of this hypothesis is that ambient lake concentrations of P and NH_4^+ are at times too low to satisfy biological demand (multi-nutrient colimitation) and the NO_3^- levels, although high, represent an unavailable source of N due to low Fe bioavailability (biochemical colimitation). I suggest that low Fe concentrations in the offshore stratified regions of Lake Erie may prevent the uptake of NO_3^- that is present in excess during this period. I tested this hypothesis by measuring the biomass, nutrient concentrations and physiological nutrient limitation response of the phytoplankton community to P, N and Fe enrichment in both the nearshore and offshore regions of the lake. Three experiments were conducted over three years: two in the offshore area, and one in the nearshore zone. The in situ conditions were characterized through physical parameters, lake water chemistry, and nutrient limitation indicators, after which I conducted nutrient enrichment experiments using trace metal clean techniques in which specific combinations of nutrient treatments were applied, including the removal of Fe.

2.2 Materials and methods

2.2.1 Study area and field sampling

Three nutrient enrichment experiments were performed in the eastern basin of Lake Erie during the summer (July to September) from 2001 to 2003 (Table 2.1). The offshore station was located at 42°41.808'N; 79°56.650'W; while the nearshore station was located at 42°46.934'N;

79°59.045'W. A six station grid was also sampled monthly from June to September 2003 in order to determine the seasonal and spatial Fe and NH_4^+ concentrations in the eastern basin (Table 2.2). The grid contained three nearshore (Z_{max}=5 m) sites and three offshore (Z_{max}=20 m) sites. All water samples were taken from the epilimnion of nearshore and offshore sites. For the purposes of this paper, offshore represents stations $\geq$20m, and nearshore represents stations <20m.

Physical measurements on the lake included temperature, oxygen, conductivity, and pH profiles. For the 2001 experiment, these parameters were collected utilizing a SeaBird^{TM} SBE-19 profiler, and the 2002 and 2003 experiments employed a Hydrolab. In 2002 and 2003 pH was also measured using a hand-held pH meter. Stratification was assumed to have occurred when there was a vertical gradient of >1°C per meter. Vertical profiles of photosynthetically active radiation (PAR) were measured with a Li-Cor cosine underwater quantum sensor and a Li-Cor LI-1000 data logger (Lincoln, NB). The vertical attenuation coefficient for PAR: K_d, was determined from the linear regression of the natural logarithm of irradiance versus depth. The Z_{mix} and mean water column light intensity as a percentage of surface irradiance were calculated according to Guildford et al. (2000). Secchi disc depths were also recorded. The experimental water was collected using a tube sampler in 2001 and a hand pump with tubing in 2002 and 2003 using trace metal clean protocols (see below for details). The survey water (Table 2.2) was also collected using trace metal clean techniques (see below). All of the water was prescreened through a 200 micron nylon (Nitex) filter to remove macrozooplankton. The water samples were stored in containers kept in dark insulated boxes from the time of collection until processing.

2.2.2 Experimental design

Lake water was collected in 10 L low-density polyethylene trace clean level 3 containers (VWR) in triplicate, with the exception of the 2001 experiment which was conducted in duplicate. Prior to enrichment, samples were collected for all parameters herein referred to as collection samples. Chlorophyll *a* samples were taken prior to enrichment to ensure that the autotrophic biomass was equal among treatments.

Table 2.1 Experimental conditions for the three enrichment experiments conducted in the eastern basin of Lake Erie.

Date	Location	Depth of station (m)	Depth of sample (m)	Experimental treatment	Nutrient amendment concentrations (µmol L^{-1})	Days subsampled
Sept 2001	offshore	30	0 - 10	Control NO_3^- and P Fe NO_3^- and Fe and P DFB	$NaNO_3$ = 10 K_2HPO_4 = 1 $FeCl_6 \cdot H_2O$ = 0.5 DFB = 0.1	3, 6
Aug 2002	offshore	30	7.5	Control NO_3^- and P Fe NO_3^- and Fe and P DFB NO_3^- and Fe Fe and P	$NaNO_3$ = 27 K_2HPO_4 = 0.8 $FeCl_3 \cdot 6H_2O$ = 0.1 DFB = 0.08	2, 4, 6, 8
Jul 2003	nearshore	5	2.5	Control NO_3^- and P Fe NO_3^- and Fe and P	$NaNO_3$ = 27 K_2HPO_4 = 0.8 $FeCl_3 \cdot 6H_2O$ = 0.1 DFB = 0.08	4

The containers were enriched with a combination of the nutrients: Fe, P and N (Table 2.1). There was a change in the solute concentrations used in the treatments, and in the N:P supply ratios added between 2001 and the following experiments due to a restructuring of the

experimental design. A specific Fe chelator, desferrioxamine mesylate (desferal, DFB) was added as a treatment in the two offshore experiments to effectively remove all of the bioavailable Fe. Bioavailable Fe is difficult to quantify, and specific assays for Fe limitation were not used in these experiments. However, Fe removal using the fungal siderophore (DFB) allowed me to explore the effect of Fe limitation on phytoplankton biomass. After the additions were made, the containers were incubated for 2-8 days, after which time the biomass, water chemistry and nutrient limitation were assessed and compared with that of a control treatment to which nothing was added.

Containers were incubated in a growth chamber on a diel cycle (16/8 hour light/dark photocycle) under cool white fluorescent lights at the ambient light level and water temperature at the time and depth of collection.

Table 2.2 Iron and NH_4^+ concentrations shown for stations in the eastern basin of Lake Erie; June to September, 2003. NA = not available. BD = below detection (limit = 0.20 μmol L^{-1}). Offshore is designated as stations at a depth of ≥20 m where as nearshore is stations <20m. Data shown represents the range (minimum value – maximum value) of concentrations where *n* = 3.

Date	Station location	Total dissolved Fe (nmol L^{-1})	Particulate Fe (nmol L^{-1})	NH_4^+ (μmol L^{-1})
10 Jun 2003	nearshore	117.3 – 196.5	NA	0.35 – 0.53
	offshore	8.1 – 11.1	NA	0.22 – 0.25
23 Jul 2003	nearshore	22.3 – 108.1	270.1 – 341.6	0.36 – 0.71
	offshore	3.1 – 7.1	14.9 – 45.2	0.32 – 0.64
13 Aug 2003	nearshore	4.1 – 63.0	92.9 – 116.2	0.51 – 0.63
	offshore	2.9 – 37.7	7.5 – 50.6	BD – 0.30
16 Sep 2003	nearshore	25.4 – 31.1	104.4 – 496.5	BD – 0.88
	offshore	3.4 – 7.4	22.1 – 36.3	0.63 – 0.72

2.2.4 Trace metal clean protocols

The experimental water was collected using either a tube sampler in 2001 or a hand pump in 2002 and 2003. The survey work conducted in 2003 (Table 2.2) employed a Teflon™-coated, acid cleaned, Go-Flo bottle (General Oceanics, Miami, FL) suspended on a Kevlar™ line, or the hand pump at specified depths within the photic zone, or a surface grab was taken. For the surface grabs, a low-density polyethylene trace clean level 3 cubitainer (VWR) was held over the

side of the boat with gloved hands and filled from the surface of the lake while the boat was moving slowly. In the 2001 experiment, a tube sampler was utilized containing no metal parts and acid washed prior to use. The Guzzler (Rintoul's Hand Pumps) hand pump diaphragm was constructed of silicone, and the body was Delrin^{TM}; all interior metal screws were replaced with plastic screws. The tubing in 2002 consisted of a polyethylene-coated Teflon^{TM} tube and the tubing in 2003 was Masterflex silicone (platinum) suspended at depth using a Kevlar^{TM} line weighted with a plastic coated sand-filled weight. Once the water arrived back at the University of Waterloo (within 5 hours) it was taken directly to a clean room and the water was filtered for total dissolved Fe (TDFe) and particulate Fe via a Teflon^{TM} filter holder (Savillex Corp., Minnetonka, MN) employing a hand pump in a HEPA laminar flow hood. The P and N additions were pretreated by passing through an ion-exchange resin (Chelex-100; Bio-Rad, Hercules, CA) to remove any metallic impurities. All materials used in this study were acid cleaned to reduce the incidence of trace metal contamination and all manipulations of the experimental containers occurred in the laminar flow hood. The de-ionized, distilled water contained concentrations of TDFe that were below detection limits (see below) and thus was used for all solution preparations and washing purposes. The acid cleaning procedure consisted of a Citranox^{TM} detergent wash, followed by a rinse with de-ionized, distilled water, proceeded by an ethanol rinse, followed by a water rinse prior to soaking in 5% HCl (Trace Metal Grade, Fisher Scientific). Items were soaked for 24 hours and then rinsed seven-fold in de-ionized, distilled water and dried in a HEPA-filtered laminar flow hood in a clean room. Items were double-bagged for transport to the field.

2.2.5 Water chemistry

Particulate C and N samples were filtered (500 mLs) onto combusted (GFF:nominal pore-size 0.7 μm, 47 mm) filters which were placed in clean petri dishes and kept frozen until later analysis by the methods described by Stainton et al. (1977). For the 2001 enrichment experiment, the particulate C samples were measured at the Analytical Laboratory at the Freshwater Institute (Stainton et al. 1977). For the 2002 and 2003 enrichment experiments, the samples were processed at the University of Waterloo. The samples were removed from the freezer and placed in a drying oven set to 70^{o}C. The filters were allowed to dry for up to 24 hours or to a constant weight. The dried filters were placed in a desiccator containing hydrochloric acid and fumed for 24 hours and then analyzed on an Exeter Analytical Inc. CEC-440 (combustion 980^{o}C, reduction 700^{o}C) autoanalyzer. As the 2002 and 2003 particulate C filters were fumed with acid prior to analysis, they represent organic C only and thus any increase in particulate C can be regarded as an estimate of autocthonous production because the containers were all sealed for the duration of the incubations with no exogenous source of organic carbon.

Total dissolved Fe was not measured in 2001, however I believe the concentration to be slightly higher than found at the same station for 2002 based on the response to Fe removal. Samples for TDFe were filtered using acid cleaned 0.2 μm pore-size polycarbonate membrane filters and stored at 4°C until analyzed for dissolved Fe using a graphite furnace atomic absorption spectrophotometer (Perkin Elmer AAnalyst 600) at Clarkson University. Samples were acidified with trace metal clean HNO_3 (Baseline; Seastar) to pH 2. Sub-samples (20 μL) were analyzed in replicate by direct injection with 15 μg $Mg(NO_3)_2$ of matrix modifier. Accuracy was assured by SLRS-4 certified standard freshwater reference solution (National Research Council of Canada) appropriately diluted to be within expected range of Fe content.

Accuracy was always within the certified range. Particulate Fe was determined by filtering 100 mL lake water onto acid cleaned 0.2 μm polycarbonate membrane filters. Filters and retained seston were deposited in 8 mL Teflon jars and digested with 1 mL concentrated HNO_3 (Seastar) for 2 - 3 days at 20^{o}C. Iron concentration was subsequently determined using the same procedure as TDFe following appropriate dilution. Blank values have been subtracted from measured concentrations. The detection limit for the samples was 2.0 nmol L^{-1}.

Samples for TP and total dissolved P (TDP) were analyzed following preservation and analytical procedures of NLET (1994). Soluble reactive P (SRP) samples for all years were analyzed according to Stainton et al. (1977). Particulate P samples were filtered (500 mL) onto (GFF:nominal pore-size 0.7 μm, 47 mm) filters which were placed in clean petri dishes and kept frozen until later analysis. The 2001 and 2002 particulate P samples were analyzed using the muffle furnace digestion method according to Stainton et al. (1977). In 2003, particulate P was measured using the persulphate digestion method in an autoclave (Parsons et al. 1984).

Nitrate + NO_2^- from the 2001 collection container were analyzed following preservation and analytical procedures of NLET (1994). The 2002 and 2003 NO_3^- samples were analyzed on a Dionex DX500 chromatography system (ion chromatograph).

The NH_4^+ samples were analyzed using the indophenol blue method (Stainton et al. 1977) in 2001, and the fluorometric method in 2002 and 2003 (detection limit = 0.2 μmol L^{-1}) (Holmes et al. 1999).

The dissolved organic carbon (DOC) samples were filtered through combusted (GFF:nominal pore-size 0.7 μm, 47 mm) filters which were placed in pre-combusted glass vials with a Teflon^{TM}-lined lid. They were preserved with 50% phosphoric acid and refrigerated until

submission to the Environmental Isotope Lab of the University of Waterloo using a Rosemount Dohrmann DC-190 High-Temperature TOC analyzer (Hinton et al. 1997).

2.2.6 Chlorophyll *a* analysis

Sample water was filtered (500 mL) onto glass fiber (GFF:nominal pore-size 0.7 μm, 47 mm) filters that were kept in the dark and stored frozen (-20^{o}C) before passive extraction with 90% acetone. Picoplankton size fractionation was conducted by filtering 500 mLs of lake water through 2-μm pore-size polycarbonate membrane filters. The filtrate (< 2 μm) was then treated exactly as the sample water for the determination of picoplankton (0.2-2 μm) chl*a* concentrations. The extracts were quantified by fluorometry (Turner Designs 10-AU) that was calibrated annually with pure chlorophyll *a*. Regardless of the seston concentrations in the sample water, the same volume was used for picoplankton size fractionation, however, the 2-μm filters were changed frequently to prevent clogging.

2.2.7 Phytoplankton nutrient limitation indicators

Phosphorus and N limitation were determined by particulate C:P, N:P and C:N composition ratios, P and N debt assays (Healey and Hendzel 1979b) and alkaline phosphatase activity (APA; Healey and Hendzel 1979a; Table 2.3). The P debt assay measured the phosphate removed over a 24-hour period per unit of chlorophyll *a*. The determination of limitation was then assessed according to the criteria developed by Healey and Hendzel (1979b). In the P debt assay, KH_2PO_4 was added (final concentration 5 μmol L^{-1}) to an unfiltered water sample. The SRP concentrations were measured at the beginning and end of a 24 hour incubation in the dark at room temperature. The N debt assay follows the same methodology as P debt, except that it is the NH_4^+ removed over a 24 hr period. In the N debt assay, NH_4Cl was added (final concentration 5

μmol L^{-1}) to an unfiltered water sample. N debt was calculated as the N removed over a 24 hour period per unit of chlorophyll *a* (Healey and Hendzel 1979b).

Alkaline phosphatase is an enzyme localized on the cell surface of algal and bacterial cells that is produced when the organisms are P limited. This enzyme removes the phosphate molecules from dissolved organic P compounds, therefore, utilizing an otherwise unavailable source of P. APA was measured fluorometrically (Healey and Hendzel 1979a), using 5 μmol L^{-1} of o-methyl-fluorescein-phosphate as the substrate. Parallel determinations were made of total and soluble activities to distinguish between APA associated with particles and APA in solution, the soluble activity being that passing through 0.2-μm pore-size polycarbonate membrane filters. The difference was reported as particulate APA.

2.2.8 Statistical analyses and data presentation

One-way ANOVA was used to determine the difference between the experimental treatments for all variables and the survey data, and a Tukey-Kramer post hoc test was employed with a significance value of $p < 0.05$. The results shown in Figures 2.1 - 2.4 illustrate the mean and standard error for all of the days subsampled for that parameter (Table 2.1). One-way ANOVA determined that there was no significant difference in the chlorophyll *a* concentrations (used here as a proxy of photoautotrophic biomass) between the times subsampled in the two offshore experiments. Length of incubation had no significant effect on biomass.

Table 2.3 Nutrient limitation indicators. Values shown are indicative of presence or absence or degree of nutrient limitation for indicators used in this study. C = particulate carbon; N = particulate N; P = particulate P; Chl *a* = chlorophyll *a*; APA = alkaline phosphatase activity. Criteria for nutrient limitation are based on Healey and Hendzel (1979b) and adapted from Guildford et al. (2005).

Indicator	Nutrient	No limitation	Moderate limitation	Extreme limitation	Limited
C:Chl *a* (µmol C µg chl a^{-1})	N or P	<4.2	4.2 – 8.3	>8.3	
N:P (atomic ratio)	P	<22			>22
C:P (atomic ratio)	P	<129	129 - 258	>258	
P debt (µmol P µg chl a^{-1})	P	<0.075			>0.075
APA (µmol P µg chl a^{-1} h^{-1})	P	<0.003	0.003 – 0.005	>0.005	
C:N (atomic ratio)	N	<8.3	8.3 – 14.6	>14.6	
N debt (µmol NH_4^+ µg chl a^{-1})	N	<0.15			>0.15

2.3 Results

2.3.1 In situ conditions at the initiation of experiments

Comparing the in situ conditions at the offshore sampling location for the experiments conducted in 2001 and 2002, I found the water column was thermally stratified in both years. However, in 2002 the mixing depth was shallower (11m vs. 16m); the epilimnion had better light conditions for algal growth, but undetectable TDFe concentrations, and the lowest TP and NO_3^- of all three experiments (Table 2.4). Although phytoplankton communities were P limited according to all four P limitation indicators (C:P, N:P, APA and P debt) in both years' offshore experiments,

2002 was the most strongly P limited (Table 2.4). In 2002 and 2003, the C:N ratio was indicative of moderate N limitation, even though NO_3^- concentrations were high (Table 2.4). The well-mixed nearshore station sampled in 2003 also had low TP and NH_4^+ concentrations but the TDFe concentration was much higher (Table 2.4). The initial Fe conditions of the experiments correlate well with those found in both the nearshore and offshore environments of the eastern basin where there is significantly lower TDFe in the offshore than the nearshore (Table 2.2). Chlorophyll *a* was lowest (0.79 $\mu g\ L^{-1}$) at the nearshore station in 2003 but the phytoplankton community did not appear nutrient limited as indicators of P and N limitation were low and inconsistent (Table 2.4). Light was never low enough to be limiting at any of the stations and mean PAR was 25% or more of surface irradiance in all years (Table 2.4).

Table 2.4 Initial conditions for the three enrichment experiments. NA = not available; BD= below detection (limit = 2.0 nmol L^{-1}); K_d = attenuation coefficient for PAR; Chl *a* = chlorophyll *a*; C = particulate carbon; N = particulate N; P = particulate P; APA = alkaline phosphatase activity; Z_{max} = maximum depth of station; Z_{mix} = mixed layer depth; mean PAR (%) = mean water column light intensity as a percent of surface irradiance; DOC = dissolved organic carbon. The 2001 NO_3^- data includes NO_2^- concentrations. Bolded values indicate P or N limitation according to the nutrient limitation indicators (Table 2.3).

	Parameter	2001: Offshore	2002: Offshore	2003: Nearshore
	Date	**September**	**August**	**July**
Physical	Surface water temp (^oC)	21.6	22.6	20.5
	Z_{max} (m)	30	30	5
	Z_{mix} (m)	16	11	5
	K_d (m^{-1})	0.25	0.27	0.45
	pH	NA	8.19	7.82
	Secchi (m)	4.25	5.3	3
	Mean PAR (%)	25	32	40
Chemical	TDFe (nmol L^{-1})	NA	BD	38.5
	Particulate Fe (nmol L^{-1})	NA	NA	734
	DOC (mg L^{-1})	NA	2.5	2.8
	TP (µmol L^{-1})	0.35	0.19	0.27
	Particulate P (µmol L^{-1})	0.16	0.08	0.13
	Particulate N (µmol L^{-1})	4.0	3.7	2.4
	NO_3^- (µmol L^{-1})	9.14	3.8	13.04
	NH_4^+ (µmol L^{-1})	0.55	0.55	0.29
Biological	Chl *a* (µg L^{-1})	2.48	1.2	0.79
	Particulate C (µmol L^{-1})	30	32	20
	C:Chl *a* (µmol C µg chl a^{-1})	**12.1**	**26.1**	**25.3**
	N:P (atomic ratio)	**24.2**	**44.8**	18.9
	C:P (atomic ratio)	**186**	**387**	**160**
	APA (µmol P µg chl a^{-1} h^{-1})	**0.058**	**0.058**	0.002
	P Debt (µmol P µg chl a^{-1})	**0.31**	**0.14**	**0.09**
	C:N (atomic ratio)	7.1	**8.6**	**8.5**
	N Debt (µmol NH_4^+ µg chl a^{-1})	0	0	0

2.3.2 Response to enclosures

The control enclosures during the 2-8 day incubation experiments were reflective of the initial conditions with the understanding that enclosure isolates the phytoplankton communities from external sources of nutrients and recycling mechanisms normally present in lake systems. The chlorophyll *a* concentrations in the control enclosures decreased from the time of collection in all three experiments while the particulate C concentrations increased (Figure 2.1). The enclosure effect alone exacerbated P limitation in the offshore waters of both years (Figure 2.2). In the offshore water examined in 2001, the increase in P limitation was particularly pronounced and consistent. The offshore water collected in 2002 was already very P limited so the enclosure response was not as pronounced; however, the P limitation indicator values were highest in the control treatments. Enclosure of the nearshore water in 2003 stimulated APA and resulted in a higher C:P ratio (Figure 2.2). Enclosure resulted in slightly but consistently higher C:N ratios in all three experiments and detectable NH_4^+ uptake in two of the experiments (Figure 2.3) that suggests the phytoplankton in the enclosures were unable to use ambient NO_3^- and that the phytoplankton were on the cusp of N as well as P limitation.

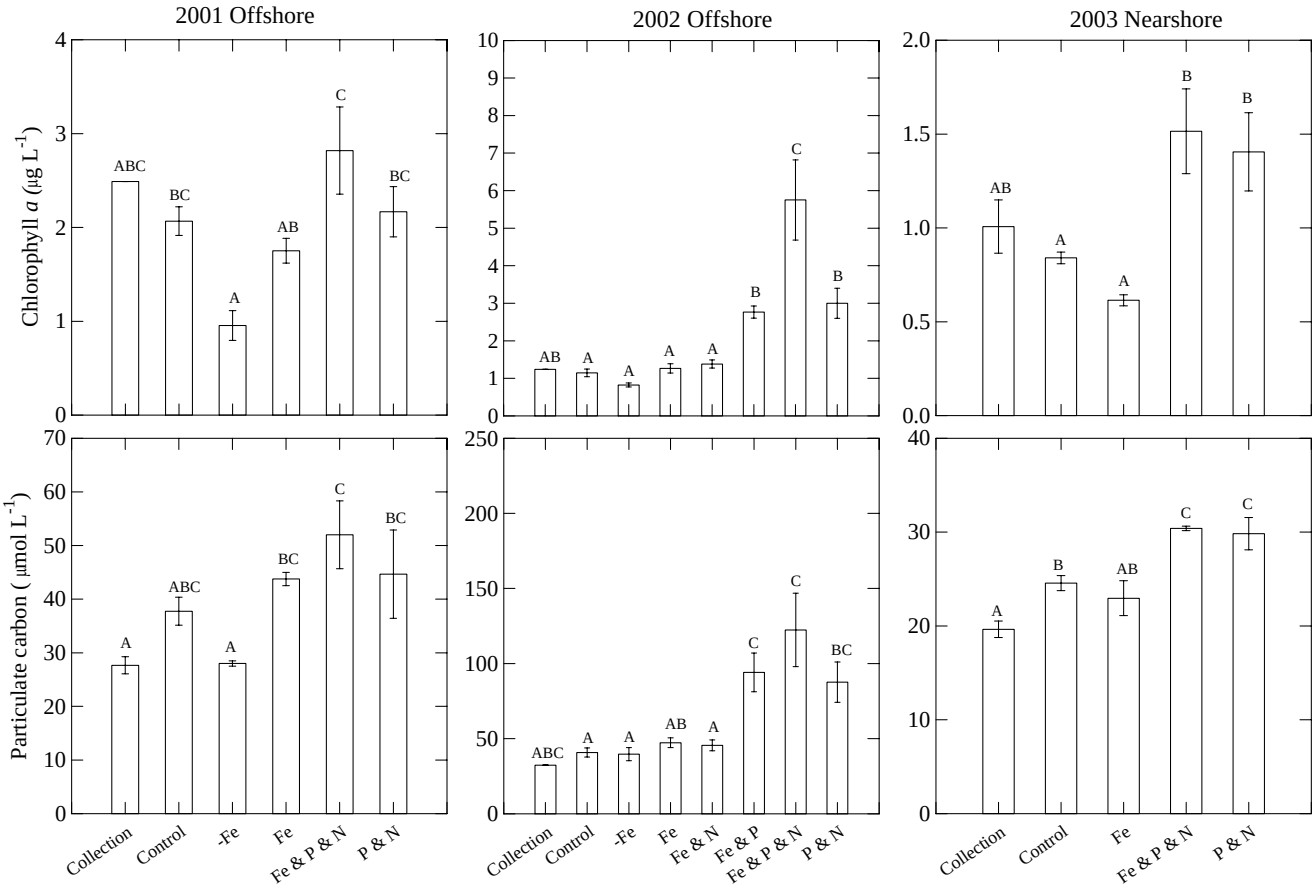

Figure 2.1 Biomass response of offshore and nearshore phytoplankton communities from incubation in enclosures under 5-8 enrichment treatments. Chlorophyll *a* and particulate C concentrations shown for the three experiments. The 2001 offshore experiment was subsampled for both parameters on days 3 and 6 (*n*= 4 per treatment). The 2002 offshore experiment was subsampled for both parameters on days 2, 4, 6 and 8 (*n*= 12 per treatment). The 2003 nearshore experiment was subsampled for both parameters on day 4 (*n*= 3 per treatment). For all graphs, the collection was sampled on day 0. The concentrations added for each treatment are listed in Table 2.1. Error bars represent the standard error of the mean. In this and the following figures, the letters above bars indicate statistical significance at a significance level of $p<0.05$. The relationship between identical letters is not statistically significant, whereas the relationship between different letters is significant. For example, in the 2001 offshore experiment, the relationship between

chlorophyll *a* for the collection and the control treatment is not statistically significant, while the relationship between the control and the –Fe treatment is statistically significant.

2.3.3 Response to Fe removal

The responses to Fe removal in the two offshore experiments were quite different and suggest that in 2002 Fe was already colimiting with P and N at the time of collection yet not in 2001. In 2001, Fe removal (indicated by –Fe) brought about by the addition of the strong Fe-binding ligand DFB resulted in significantly less chlorophyll *a* than the control (Figure 2.1), a reduction in P limitation (Figure 2.2), and stimulation of N limitation according to the C:N ratio (Figure 2.3). The muted response to Fe removal in 2002 suggests that Fe was already limiting the phytoplankton community as Fe removal had no effect on chlorophyll *a* concentrations (Figure 2.1). P limitation was reduced but not as dramatically as in 2001 (Figure 2.2) and N limitation increased according to the C:N ratio and NH_4^+ uptake was evident in the N debt assay (Figure 2.3). In addition, the NO_3^- concentration in the Fe removal enclosure actually increased over the course of the experiment (Figure 2.4), unlike in the other treatments where NO_3^- decreased or stayed the same.

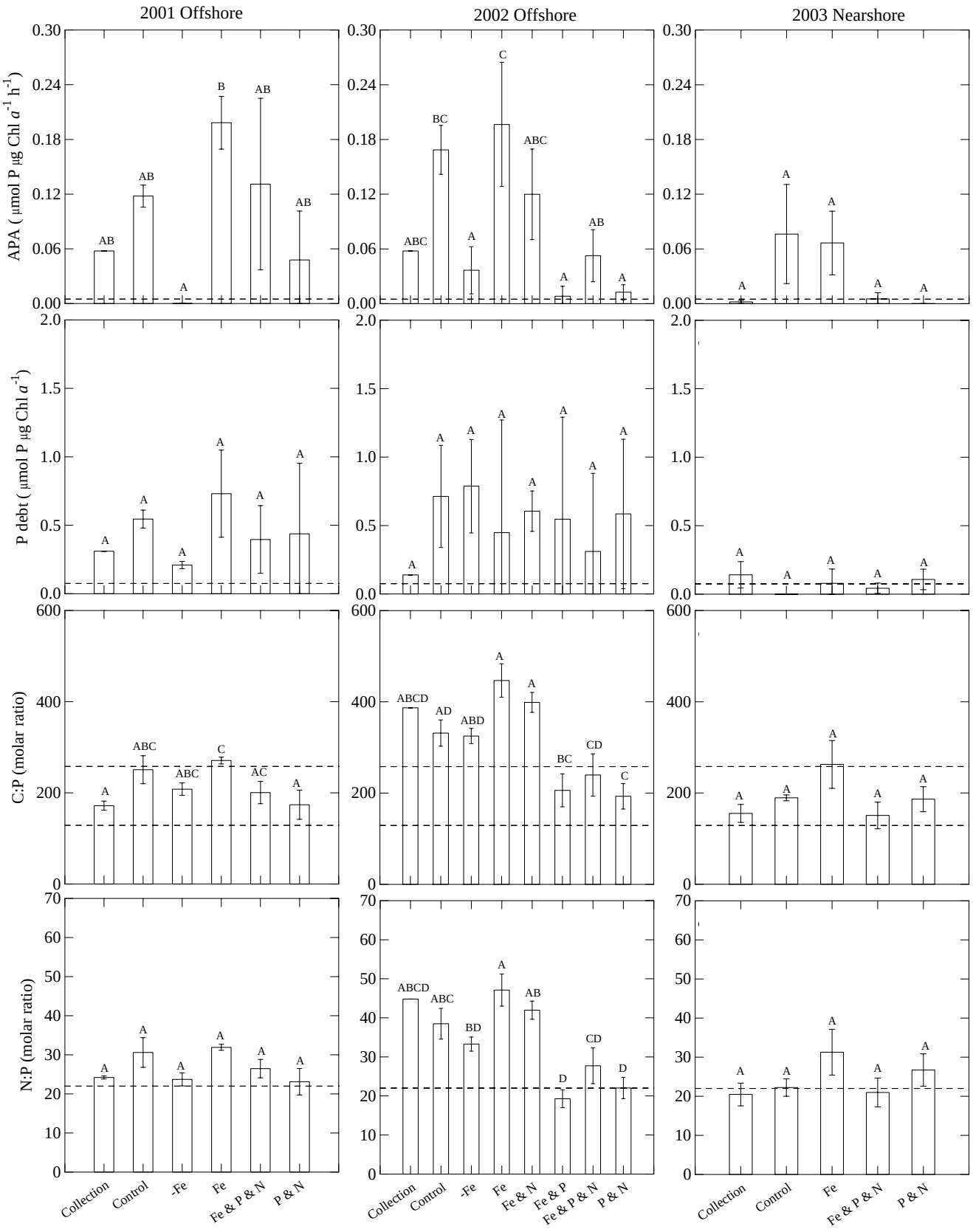

Figure 2.2 Indicators of P limitation in three enclosure experiments. Alkaline phosphatase activity (APA) normalized to chlorophyll *a*, P debt normalized to chlorophyll *a*, particulate

carbon : particulate P (C:P) and particulate N : particulate P (N:P) ratios shown for the three experiments. The 2001 offshore experiment was subsampled for APA and P debt on day 3 (*n*=2 per treatment), and on days 3 and 6 (*n*= 4 per treatment) for the C:P and N:P ratios. The 2002 offshore experiment was subsampled for APA on days 2, 6 and 8 (*n*=3 per treatment), on days 2 and 8 (*n*=2 per treatment) for P debt, and on days 2, 4, 6 and 8 (*n*= 12 per treatment) for the C:P and N:P ratios. The 2003 nearshore experiment was subsampled for all parameters on day 4 (*n*=3 per treatment). The dashed lines represent criteria for P limitation as defined by Healey and Hendzel (1979b). Values above the dashed lines are indicative of P limitation, while values below the dashed lines are not considered to be P limited. When two lines are present, values between the upper and lower dashed lines are indicative of moderate P limitation and values greater than the upper dashed lines are indicative of severe limitation. The concentrations added for each treatment are listed in Table 2.1. Error bars represent the standard error of the mean. Letters above bars indicate statistical significance.

2.3.4 Response to enrichment

The response of the offshore phytoplankton communities to multiple enrichment with Fe, P, and N indicated that all three nutrients were colimiting at the time of collection in 2002 and were close to colimiting in 2001. Fe was not colimiting in the nearshore phytoplankton community sampled in 2003. The addition of Fe, P and N stimulated phytoplankton biomass to a greater extent than P and N addition without Fe in the offshore experiments (Figure 2.1). In the nearshore waters, the addition of P and N with and without Fe stimulated phytoplankton biomass to the same degree (Figure 2.2). This was consistent with the relatively high TDFe concentration

of 38.5 nmol L^{-1} in the nearshore water, compared to the undetectable concentration in the offshore water in 2002 (Table 2.4).

Colimitation of the phytoplankton community was most clearly observed in the 2002 experiment because TDFe was undetectable (Table 2.4), and the largest variety of treatments was applied. Iron added alone or in combination with N did not stimulate biomass because P was also limiting. Phytoplankton in enclosures enriched with Fe, or Fe and N, remained P limited or became more strongly P limited according to all of the indicators for P limitation applied (Figure 2.2). I believe that the increased P limitation was not a result of the addition of Fe removing the bioavailable P through a chemical complexing event. There was no significant difference between P fractions (SRP, TDP, TP, particulate P), time or treatment at the end of the incubations (data not shown). Also, when I added Fe in all three experiments the SRP concentrations were higher than the control.

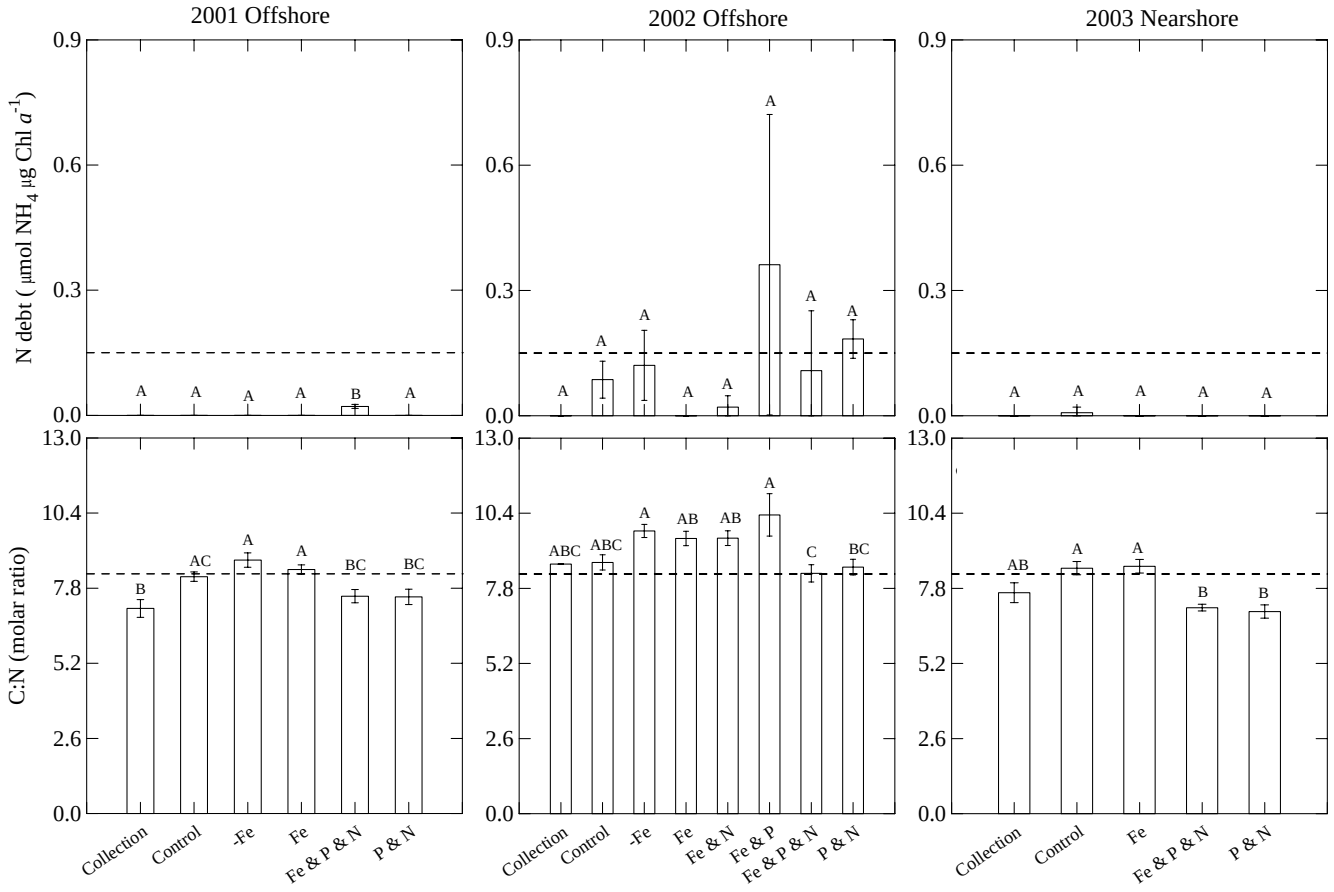

Figure 2.3 Indicators of N limitation in three enclosure experiments. N debt normalized to chlorophyll *a* and particulate carbon : particulate N (C:N) ratios are shown for the three experiments. The 2001 offshore experiment was subsampled for N debt on day 3 (*n*=2 per treatment), and on days 3 and 6 (*n*= 4 per treatment) for the C:N ratio. The 2002 offshore experiment was subsampled for N debt on days 2, 6 and 8 (*n*=3 per treatment), and on days 2, 4, 6 and 8 (*n*= 12 per treatment) for the C:N ratio. The 2003 nearshore experiment was subsampled for both parameters on day 4 (*n*=3 per treatment). The dashed line represents criteria for N limitation as defined by Healey and Hendzel (1979b). Values below the dashed line are considered not to be N limited, while values above the dashed line are considered to be N limited. The concentrations added for each treatment are listed in Table 2.1. Error bars represent the standard error of the mean. Letters above bars indicate statistical significance.

The addition of NO_3^- did not relieve the moderate N limitation indicated by the C:N ratio (Figure 2.3). However, in the enclosures enriched with Fe and P, but not N, a higher biomass was observed (Figure 2.1), indicating that the phytoplankton community was able to access ambient NO_3^- once Fe and P were available. In these enclosures, P limitation was relieved (Figure 2.2), N limitation became strongest (Figure 2.3), NO_3^- was reduced to undetectable concentrations, and particulate N increased (Figure 2.4). In the same experiment, P and N addition without Fe also stimulated biomass (Figure 2.1) and reduced indicators of both P and N limitation (Figures 2.2 and 2.3). Added NO_3^- was taken up by the extant phytoplankton communities, but not to the extent that it was in the Fe and P enriched container or the container enriched with all three nutrients (Figure 2.4). Particulate N concentrations reflected the changes in NO_3^- concentrations that may be viewed as uptake by the extant phytoplankton and heterotrophic communities. The addition of all three nutrients resulted in the highest particulate N concentrations relative to the other treatments (Figure 2.4).

Draw down of Fe added alone or in combination with P and/or N is shown for the 2002 experiment (Figure 2.5). Every treatment that was given an Fe addition drew it down on average by 88%, indicating again that there was essentially no excess of Fe relative to demand (Figure 2.5). The addition of Fe in combination with P and N resulted in the fastest draw down of TDFe, however, a large draw down in the Fe alone treatment was also observed (Figure 2.5).

In the 2002 experiment where P, N, and Fe were colimiting from the outset, I observed a shift in the size distribution of the phytoplankton community in response to the different treatments. In enclosures with no enrichment or only one available form of the three limiting nutrients, picoplankton (0.2 – 2 μm) comprised on average 28% of the total chlorophyll *a*. In

contrast, in enclosures able to access P, N, and Fe, picoplankton became less important (Figure 2.6).

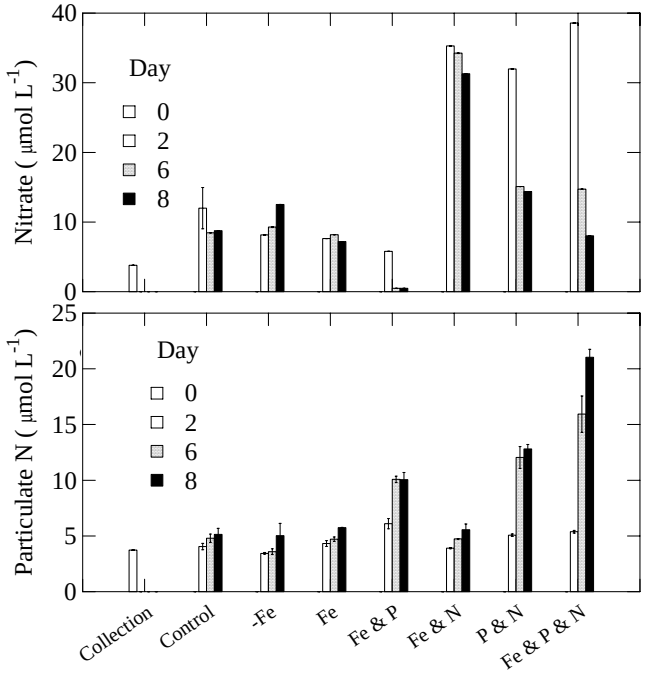

Figure 2.4 Nitrate and particulate N concentrations for the 2002 offshore experiment. The NO_3^- data were collected on days 2, 6 and 8 (*n*=1). The concentrations added for each treatment are listed in Table 2.1. The particulate N data were collected on days 2, 4, 6 and 8 (*n*= 12). Error bars represent the standard error of the mean.

Evidence for colimitation of P, N and Fe is not as clear in the offshore experiment conducted in 2001 as it is for 2002. However, the biomass and nutrient limitation measurements support the conclusion that once the water was enclosed, all three nutrients were unavailable and although sufficient ambient NO_3^- was present, it was not taken up until P and Fe were supplied. The addition of Fe alone did not stimulate biomass or relieve N limitation presumably because P

was limiting. The addition of P and N without Fe also stimulated biomass (Figure 2.1) and relieved both P and N limitation (Figures 2.2 and 2.3) but not to the same extent as P and N with Fe.

The nearshore water exhibited only slight P and N limitation at the time of collection (Figures 2.2 and 2.3) even though the ambient TP and NH_4^+ concentrations were in the same range as the more strongly P and N limited offshore stations (Table 2.4). The nutrient enrichments of the water collected from the nearshore did not demonstrate colimitation by Fe; however, the addition of Fe alone increased P limitation relative to the control. The P and N treatment generated the same biomass response and a similar release from P and N limitation as P and N with Fe.

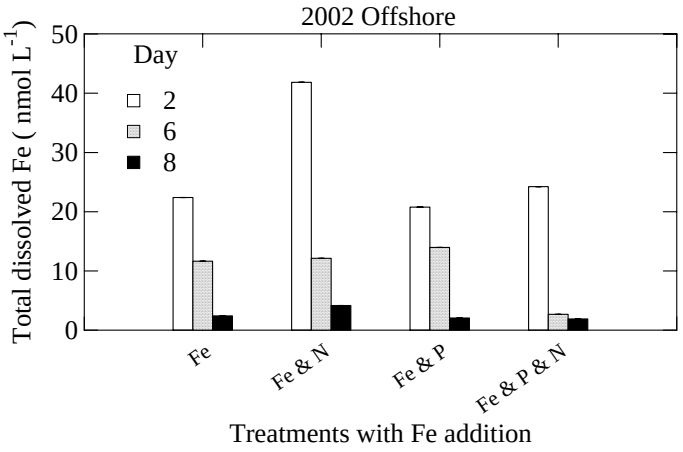

Figure 2.5 Total dissolved Fe concentrations over time for an offshore enclosure experiment. The bars represent the TDFe concentrations for the 2002 offshore experiment that was subsampled on days 2, 6 and 8 (*n*=1). Only treatments to which Fe was added are shown. Initially, Fe was added to all treatments shown at a concentration of 110 nmol L^{-1}.

2.4 Discussion

The Lake Erie phytoplankton community can be colimited by P, N and Fe in the offshore, stratified waters of the eastern basin. The reason that N can be limiting despite the abundance of NO_3^- is that the Fe that is required for NO_3^- assimilation is evidently not bioavailable. This phenomenon was clearly demonstrated in the 2002 experiment when the biomass of the P- and N-limited phytoplankton community was stimulated by the addition of Fe with P. Phytoplankton were able to access the ambient NO_3^- only following the supply of exogenous Fe and P. In the waters of Lake Erie where NO_3^- is the most abundant source of N due to low NH_4^+ concentrations, my enrichment experiments show that Fe can facilitate the assimilation of NO_3^-. Once the phytoplankton are no longer N limited they take up more P causing them to exhibit stronger indications of P limitation.

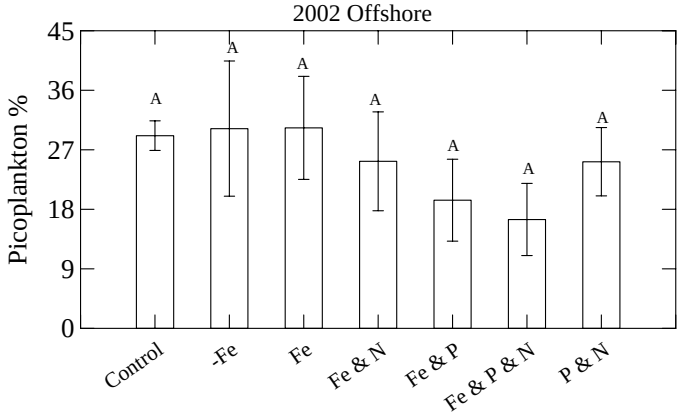

Figure 2.6 Picoplankton (0.2 – 2 μm) percentage of total chlorophyll *a* for an offshore enclosure experiment. The bars represent the picoplankton percentage of total chlorophyll *a* for the 2002 offshore experiment that was subsampled on days 2, 6 and 8 (*n*= 3 per treatment). The concentrations added for each treatment are listed in Table 2.1. Error bars

represent the standard error of the mean. Letters above bars indicate statistical significance.

Fe limitation has been documented in several small lake systems (Happey-Wood et al. 1981; Priscu et al. 1982). These previous studies were initiated because Fe was thought to be unavailable to the phytoplankton. In natural waters, complexation by dissolved organic compounds allows Fe to remain in the euphotic zone where it is accessible to the phytoplankton communities. The availability of these Fe complexes for assimilation by phytoplankton depends on many factors: the kinetic lability of the complex, rates of photoreduction and photooxidation, and the uptake strategies employed by phytoplankton, which vary among species (Wells et al. 1995). Therefore, it is not just the measurable concentration of Fe that is important but also the availability. The original Fe work on freshwater systems by Schelske (1962), Schelske et al. (1962) and Wetzel (1966, 1972) observed increased primary production as a result of Fe enrichment experiments in water from marl lakes. Whiting events, characteristic of marl lakes (Wetzel 1966) are caused by supersaturation of calcium carbonate ($CaCO_3$) and occurred regularly in summer and fall from 1972-1975 in Lake Erie. In 1969, Lange (1971) reported a stimulation of cell numbers with the addition of Fe in Lake Erie. Whiting events precipitate Fe, as $CaCO_3$ crystal formation can scavenge trace metals by co-precipitation and surface sorption (Strong and Eadie 1978). Therefore, Fe limitation detected in the past in Lake Erie, may be due to this scavenging. Recently, in the eastern basin of Lake Erie a decline in spring alkalinity combined with a decreased frequency of whiting events has been observed. Barbiero and Tuchman (2004) attribute the decline in marl events to Ca uptake by dreissenid populations. However, Fe limitation is still occasionally reported on Lake Erie (Twiss et al. 2000; this study).

Although most temperate, freshwater lakes are considered to be P limited (Schindler 1977), studies report that N and P limitation in lakes is not mutually exclusive and have

documented simultaneous limitation of N and P (Davies et al. 2004). In a survey of enrichment experiments conducted on sixty North American lakes, Elser et al. (1990) concluded that total algal biomass production was commonly limited by the availability of both N and P. It has also been reported that the combined addition of Fe with either N and/or P in nutrient enrichment experiments brought about a greater biomass response than either N and/or P (Schelske 1962; Sakamoto 1971). My study has provided an explanation wherein Fe is needed to be able to assimilate the NO_3^-.

In eastern Lake Erie, the nearshore region is different, and more variable compared to the offshore region in terms of chlorophyll *a* and nutrient concentrations (Hecky et al. 2004; Chapter 3). In my experiments, the chlorophyll *a* concentration was lower in the nearshore than the offshore likely due to the high density of exotic dreissenid mussels in this region that have been implicated in chlorophyll *a* reduction and changes in the nutrient cycling of the nearshore environment (Nicholls and Hopkins 1993; Hecky et al. 2004; Chapter 3). Dissolved Fe concentrations in Lake Erie are highly heterogeneous (Twiss et al. 2000; Porta et al. 2005; this study) and range from 2-404 nmol L^{-1} in the eastern basin during thermal stratification. Both the dissolved and particulate Fe concentrations were higher in the nearshore than the offshore regions of Lake Erie and Lake Superior (McKay et al. 2004, 2005). Through the application of an immunoblotting approach for flavodoxin accumulation in diatoms and a cyanobacterial Fe bioreporter, McKay et al. (2004) found Fe to be more available nearshore in Lake Superior. Iron is rapidly depleted by various scavenging mechanisms (Twiss and Campbell 1998) as water moves offshore. Also, the nearshore is typically not stratified, which allows mixing to the bottom, thereby enhancing the flux of Fe from sediments during early diagenesis. An indication of such an Fe source is the lower pH values in the water column as the pH tends to be lower within sediment pore waters (Schoemann et al. 1998). There are lower pH values at the

nearshore station than the offshore (Table 2.4); thus, reductive dissolution and subsequent mixing in the nearshore could explain the higher Fe concentrations in the nearshore.

In general, Lake Erie phytoplankton are considered to be limited by P (Lean et al. 1983; Guildford et al. 2005). However, N limitation has also been detected (Wilhelm et al. 2003; Guildford et al. 2005; North, unpublished data). Evidence for N and P colimitation in the eastern basin of Lake Erie was reported by DeBruyn et al. (2004) where P enrichment of water from an offshore stratified station induced N limitation. My experiments demonstrate that in offshore stratified waters where Fe is frequently low (Porta et al. 2005; this study), N was colimiting with P. Lange (1971) conducted Fe enrichment experiments at a nearshore site in the western basin of Lake Erie consisting of biweekly experiments during one growing season using filtered lake water inoculated singly with four algal cultures. He reported a stimulation of cell numbers with the addition of unchelated Fe in 22% of the experiments conducted, although chelated Fe yielded a higher growth response seen in 35% of the experiments. Storch and Dunham (1986) also found that chelated Fe yielded a higher cell yield compared to unchelated Fe which frequently inhibited algal growth in their experiments. They conducted eighteen experiments over four years using phytoplankton collected from the nearshore of eastern Lake Erie. At lower concentrations of chelated Fe additions, photosynthesis was enhanced in 67% of the experiments. There was also evidence of colimitation in these experiments as the addition of Fe in combination with N and P increased cell yield compared to Fe alone (Storch and Dunham 1986). Recent experiments by Twiss et al. (2000, 2005) showed that the addition of unchelated Fe caused an increase in biomass in only 5% of the twenty experiments conducted. However, one experiment conducted on the strongly stratified offshore waters of the eastern basin showed a dramatic 180 and 30% increase in biomass of picoplankton and nanoplankton respectively. In addition, Fe stressed phytoplankton exhibited draw down of Fe as a result of uptake (Twiss et al. 2000). They also

demonstrated a colimitation of P and Fe to phytoplankton growth (Twiss et al. 2000, 2005) as the addition of Fe and P combined yielded a higher biomass than the addition of P alone Iron bioreporter results also indicated Fe limitation in Lake Erie (Durham et al. 2002). In these previous experiments, primary production and algal biomass were the typical measured responses to nutrient additions and the pre-existing nutrient limitations of the in situ phytoplankton communities were often not considered. In this study I demonstrated that P and N limitation indicators were more sensitive than biomass to the addition of Fe and emphasize the importance of the interaction of Fe and phytoplankton P and N limitation.

Sterner et al. (2004) performed eight nutrient enrichment experiments in western Lake Superior from September 1999 to May 2001 and reported no stimulation of chlorophyll *a* with the addition of Fe. It is likely that the simultaneous P limitation of the phytoplankton made it impossible to observe a direct response to Fe in these experiments. Colimitation of P and Fe was observed as the addition of P resulted in increased growth rates and induced Fe limitation, and Fe additions increased APA, an indicator of P limitation. This colimitation was also evidenced as the simultaneous additions of P and Fe yielded the greatest biomass response (Sterner et al. 2004). In several experiments, the percentage of picoplankton present relative to total chlorophyll *a* was higher for the control and treatments where only Fe was added (Sterner et al. 2004). In the treatments where Fe and P, and P alone were added, the picoplankton percentage was smaller (Sterner et al. 2004), which is what I observed in my Lake Erie experiments where larger phytoplankton dominated when all of the colimiting nutrients were supplied. McKay et al. (2005) also investigated Fe limitation in Lake Superior through the use of a cyanobacterial Fe bioreporter. Results show that the Fe bioreporter gave an Fe-deficient response at offshore stations (McKay et al. 2005). In Lake Huron, Lin and Schelske (1981) conducted nutrient enrichment experiments monthly from April to December 1975. They found that the

simultaneous additions of P and Fe resulted in large increases in chlorophyll production and concluded that chelated Fe was an important secondary limiting nutrient after P, during the summer months (Lin and Schelske 1981).

Guildford et al. (2003) conducted Fe enrichment experiments in two N-limited African Great Lakes, Lakes Malawi and Victoria. In both lakes, the response to enrichment was assessed using chlorophyll *a*, photosynthesis, and nutrient limitation indicators. Three Fe enrichment experiments were conducted in the offshore of Lake Malawi from 1998 to 1999 during two different stratification regimes. Although the addition of Fe alone caused an increase in phytoplankton biomass in only one of the three experiments, when Fe was added with P and N the chlorophyll *a* response was four times the response to P and N alone. They also reported that rates of P uptake were higher in Fe amended samples, and that Fe additions stimulated N uptake. In Lake Victoria, two experiments were conducted in both the nearshore and offshore during the early stratified season of 1998. The addition of Fe did not result in an increase in chlorophyll *a* in either experiment, however, at the offshore station, Fe additions stimulated N uptake and at the nearshore station the addition of Fe stimulated N_2-fixation rates (Guildford et al. 2003).

Several examples of colimitation by Fe can be found in the marine literature in both coastal and open ocean environments (de Baar et al. 2005). The results of Fe enrichment experiments in the coastal California upwelling region vary from dramatically enhanced particulate C production, to no increase at all (Hutchins et al. 1998). However, Fe additions did stimulate NO_3^- draw down to almost complete depletion (Hutchins and Bruland 1998), and resulted in increased particulate N concentrations (Hutchins et al. 1998). In the open ocean, bottle incubations conducted by Timmermans et al. (1998) from HNLC waters showed that although the addition of Fe did not result in a change in chlorophyll *a* concentrations, a draw down of the total and dissolved Fe was observed. In addition, upon Fe enrichment, a

physiological N stimulation was observed as the addition of Fe increased the NO_3^- uptake rates by a factor of 1.06, although NH_4^+ uptake remained unaltered (Timmermans et al. 1998). Mills et al. (2004) conducted nutrient enrichment experiments at three stations in the eastern tropical North Atlantic. Response to P, N and Fe enrichment was assessed using chlorophyll *a*, C and N_2-fixation rates. Evidence of P, N and Fe colimitation was observed as the largest biomass increase was found when P, N and Fe were added simultaneously. Although community primary productivity was N-limited, N_2-fixation was colimited by Fe and P as the addition of P and Fe together resulted in a 2-3 fold enhancement of N_2-fixation rates at all three stations (Mills et al. 2004). In other HNLC regions, it was found that the addition of Fe increased the NO_3^- uptake rates by a factor of 5-7 (Martin and Fitzwater 1988), and 2 (Timmermans et al. 2004). In some cases, the addition of Fe caused a complete draw down of NO_3^- (Martin and Fitzwater 1988; Tsuda et al. 2003), and an increase in particulate N concentrations (Price et al. 1991).

Communities that are N limited are often colimited by Fe at low NH_4^+ concentrations (Price et al. 1991; de Baar et al. 2005). It should be possible to ameliorate this multiple nutrient limitation either by adding Fe, or an N source that does not require Fe for its uptake, such as NH_4^+, provided no additional colimitation prevents a response. Guildford et al. (2005) have pointed out that the eastern basin of Lake Erie is not as P limited as expected for such oligotrophic waters. Although moderate P limitation was detectable throughout the summer, it is hypothesized that phytoplankton may be controlled by factors additional to P. The present results point specifically to low available Fe and NH_4^+ as factors preventing development of strong P limitation in the eastern basin. Furthermore, N limitation in the east basin (Wilhelm et al. 2003; Guildford et al. 2005; North, unpublished data) is likely due more to the availability of Fe, than to N concentrations.

The three multi-response experiments conducted in the eastern basin of Lake Erie provide evidence that under conditions of strong stratification, the phytoplankton communities may become colimited by P, N and Fe, despite high NO_3^- concentrations. Phytoplankton need Fe when NO_3^- is the source of N for growth, thus the availability of Fe can limit the uptake of NO_3^-. By alleviating the N limitation, the phytoplankton communities thus become more P limited, leading to a greater response to changes in P concentrations. Therefore, by alleviating N limitation, Fe can affect P limitation in this Laurentian Great Lake. The data presented in this paper support the conclusion that the phytoplankton communities of the eastern basin of Lake Erie exhibit colimitation of P, N and Fe and suggest that this condition may be more widespread in large areas of the Laurentian Great Lakes.

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Chapter 3

Changes in the spatial distribution of seston and nutrient concentrations in the eastern basin of Lake Erie

3.1 Introduction

Lake Erie is the oldest, shallowest, most productive, and heavily impacted of the Laurentian Great Lakes of North America. Over the last three decades there have been many changes to Lake Erie including increased population growth in the watershed, nutrient controls, and the introduction of exotic invaders such as dreissenid mussels. All of these changes might be expected to impact the nearshore regions of the lake differently than the offshore.

Due to Lake Erie's highly eutrophic status (Beeton and Edmondson 1972), nutrient controls were initiated with the U.S.-Canada Great Lakes Water Quality Agreements (GLWQAs) of 1972 and 1978. The GLWAQs specified a 30 μmol L^{-1} total phosphorus (TP) effluent limit on municipal sewage treatment plants discharging in excess of 3,800 m^3 day^{-1} and full or partial bans on phosphorus (P) in detergents (Dolan and McGunagle 2005). It was predicted that reduced P load from municipal sources would lead to a reduction in the total algal biomass in the lake. These P controls did not show immediate results, but by the mid-1980s, declines in lake-wide P loadings were obvious (Dolan and McGunagle 2005), and decreased total phytoplankton biomass (Makarewicz 1993) suggested that Lake Erie water quality was improving. In the early 1990s, the TP target load set by the GLWQA of 11,000 metric tonnes $year^{-1}$ (MTA) was achieved (Dolan and McGunagle 2005). Since 1991, no statistically significant changes in the total load have been observed (Dolan and McGunagle 2005).

It is uncertain whether the observed changes in chlorophyll *a* (chl*a*) and TP concentrations can be explained solely by interannual changes in external P loading, or by the establishment of exotic dreissenid mussels in Lake Erie. The zebra mussel (*Dreissena polymorpha*) invaded Lake Erie in 1988 (Hebert et al. 1989) and first appeared in the eastern basin in August of 1989 (Mackie 1991), followed closely by the quagga mussel (*Dreissena*

bugensis; Mills et al. 1993). For the purposes of this chapter, both zebra and quagga mussels will be collectively referred to as dreissenids.

Hecky et al. (2004) proposed the "Nearshore Shunt" hypothesis, wherein dreissenids have re-engineered nutrient cycling through their filter-feeding behaviour that most strongly impacts the shallow, nearshore regions of large lakes. The nearshore shunt suggests that by intercepting, detaining, and redirecting both energy and nutrient flow, dreissenids have changed the ecological function of the nearshore and its relationship to the offshore. To date, there have been a limited number of studies examining the effects of P loading controls and dreissenids on the nearshore of the eastern basin of Lake Erie (Nicholls and Hopkins 1993; Howell et al. 1996; Nicholls et al. 1997; Nicholls et al. 1999a; Nicholls et al. 1999b; Depew et al. 2006).

The seston and nutrient concentrations reported for the nearshore and offshore regions of large lakes have historically been different. The nearshore regions of Lakes Erie, Ontario, and Michigan were more eutrophic than the offshore waters, as evidenced by higher nutrient concentrations and phytoplankton biomass in the nearshore, and a greater light transparency offshore (Beeton and Edmondson 1972). Lake Erie phytoplankton have been characterized as P limited in both pre-dreissenid (1979; Lean et al. 1983), and post-dreissenid (1997; Guildford et al. 2005) years. These studies were both conducted in the offshore of all three basins. To date, this is the first study conducted assessing phytoplankton P limitation in the nearshore of the eastern basin of Lake Erie using phytoplankton physiological indicators (as described in detail in Chapter 2). The nearshore of the eastern basin of Lake Erie is understudied relative to the offshore, especially considering that the nearshore comprises nearly 40% of the basin area (Haltuch et al. 2000). The nearshore is complex (Howell et al. 1996; Hecky et al. 2004) and difficult to sample, however, it is also where human impacts and activities are concentrated. The importance of understanding nearshore processes is critical, as current water quality issues

affecting human health (e.g. toxic algal blooms, taste and odour issues in drinking water, nuisance *Cladophora* blooms blocking water intake pipes and fouling the shorelines) have been linked to processes originating in the nearshore. The majority of studies conducted on Great Lakes involve offshore sampling, however, trophic level responses in the offshore may be a poor surrogate of similar changes in the nearshore.

The main objective of this study was to examine in detail the spatial and temporal distribution of seston and nutrient concentrations in the nearshore and offshore regions of the eastern basin of Lake Erie. Data collected from 2001-2003 were compared to data from previous studies including a major study conducted at similar locations in 1973. Nearshore and offshore measurements of chl*a*, light parameters, and nutrient chemistry (P, nitrogen (N), and silica (Si)) over three decades in the eastern basin of Lake Erie reveal that changes in the distribution of seston and nutrient concentrations have occurred since the implementation of P controls and invasion of dreissenid mussels.

3.2 Materials and methods

3.2.1 Study sites and surveys

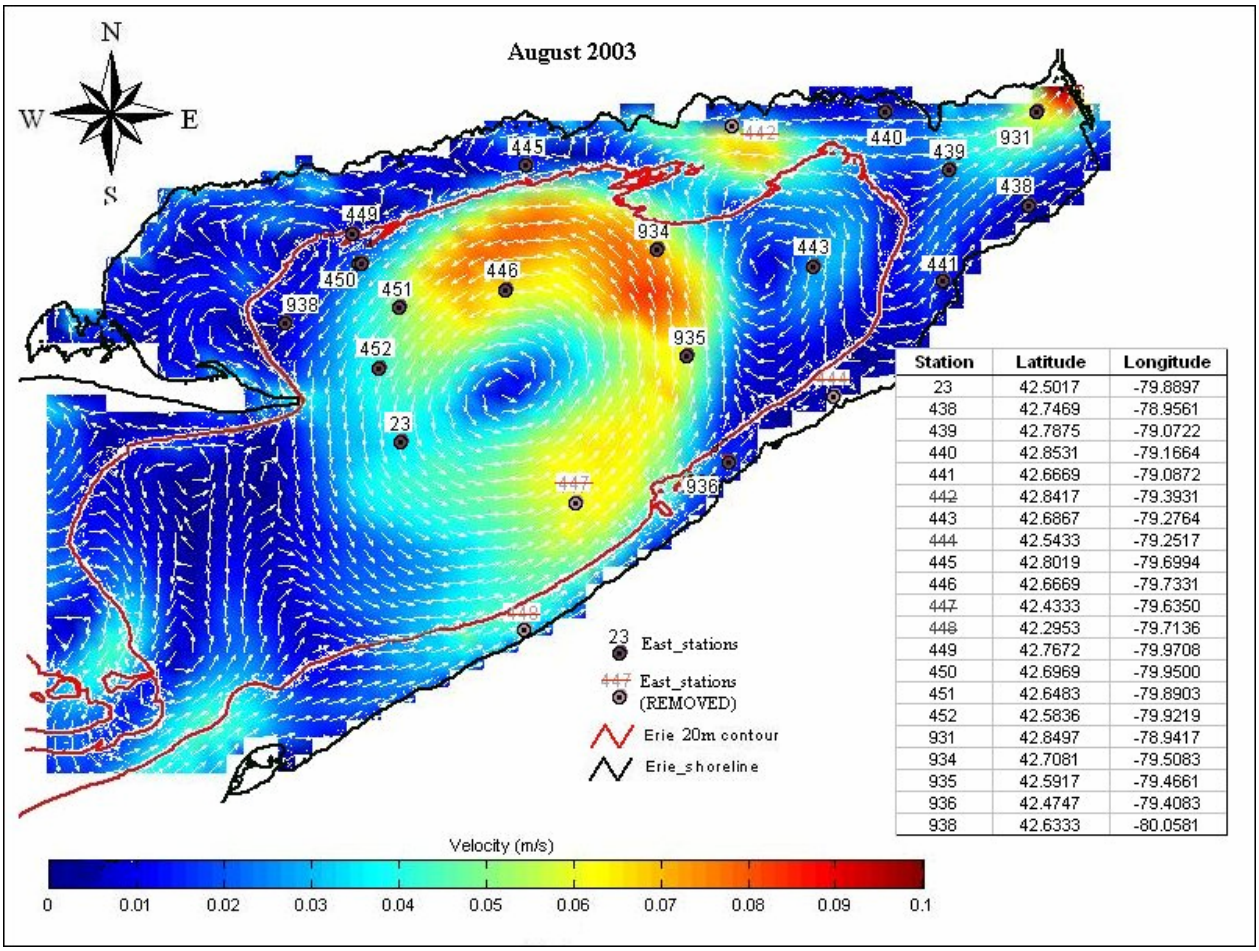
3.2.1.1 Basin-wide survey (2001-2003)

The study site for the basin-wide survey was the eastern basin of Lake Erie (Figure 3.1). The eastern basin is the deepest of the three basins (maximum depth of 64 m) and is considered to be oligotrophic (Charlton et al. 1999). Lake Erie receives most of its nutrient load in its shallow western basin (Dolan and McGunagle 2005), therefore, the east basin receives most of its nutrient load from the offshore waters of the central basin. Twenty-one stations were sampled with maximum depths (Z_{max}) ranging from 10 to 62 m (Figure 3.1). Seston and nutrient concentration sampling was conducted monthly from April to October in 2001, 2002 and 2003 aboard the *CCGS Limnos*.

3.2.1.2 Historical surveys

In 1973 the U.S. Environmental Protection Agency (EPA) conducted a survey of twenty-five stations in the nearshore and offshore of the eastern basin of Lake Erie from July to October (Figure 3.2; Great Lakes Laboratory 1974).

Environment Canada (Env. Can.) surveyed four offshore stations in the eastern basin of Lake Erie in 1979 (April to October), 1983-85 (June to September) and 1990 (June and July) (Figure 3.2). They changed their surveys to six nearshore and offshore stations (931, 936, 935, 934, 23, and 938) in 1994 (May, June, July, October), 1996 (June to September), 1997 (April to August, and October), and 1998-1999 (April to October; Figure 3.1). Not all stations were sampled in all months or all years. The Env. Can. historical data were obtained from files of the National Water Research Institute (NWRI) that were gathered by Environment Canada, Ontario Region, at the Canada Centre for Inland Waters (CCIW).



Station	Latitude	Longitude
23	42.5017	-79.8897
438	42.7469	-78.9561
439	42.7875	-79.0722
440	42.8531	-79.1664
441	42.6669	-79.0872
~~442~~	42.8417	-79.3931
443	42.6867	-79.2764
~~444~~	42.5433	-79.2517
445	42.8019	-79.6994
446	42.6669	-79.7331
~~447~~	42.4333	-79.6350
~~448~~	42.2953	-79.7136
449	42.7672	-79.9708
450	42.6969	-79.9500
451	42.6483	-79.8903
452	42.5836	-79.9219
931	42.8497	-78.9417
934	42.7081	-79.5083
935	42.5917	-79.4661
936	42.4747	-79.4083
938	42.6333	-80.0581

Figure 3.1 Vertically averaged circulation patterns for the eastern basin of Lake Erie in August 2003 showing the twenty-one basin-wide stations sampled by Environment Canada (1996-2003). Four stations were removed based on circulation patterns, STP discharges, and tributary influences as identified in section 3.2.

The Canadian Department of Fisheries and Oceans (DFO) conducted lower trophic level surveys at one offshore and two nearshore stations in the vicinity of Long Point Bay in 1993 (May to October; Dahl et al. 1995), 1994 (May to November; Graham et al. 1996) and 1998 (April to November; MacDougall et al. 2001).

3.2.1.3 North shore survey

To examine the differences between nearshore and offshore locations at a higher spatial resolution, surveys were conducted along the north shore of the eastern basin of Lake Erie in 2001, 2002 and 2003 (Figure 3.3). In 2001, a five station transect was sampled across increasing depths and distance from the coast (stations 500, 501, 502, 503 and 504). In 2002 and 2003, a six-station grid composed of three 5 m stations (503, 507 and 508) and three 20 m stations (501, 505, and 506) was used to increase replication of nearshore and offshore stations (Figure 3.3). Sampling was conducted monthly from May to October in 2001, 2002 and 2003.

3.2.2 Spatial selection

3.2.2.1 Basin-wide survey (2001-2003)

Of the twenty-one stations sampled in 2001-2003, four stations were removed from analyses due to evidence challenging their nearshore/offshore classification or evidence of significant tributary inputs. Nearshore or coastal waters were defined as <20m and offshore waters as $\geq$20m. This classification criterion was selected to remain consistent with previous studies (Depew et al. 2006), and the 20 m depth contour was chosen as a typical depth for the mid-summer thermocline and is the lower limit of the upper mixed layer. Over bottoms greater than 20 m depth, it was assumed that phytoplankton populations were isolated vertically from dreissenid influence.

Horizontal water circulation patterns and tributary and sewage treatment plant (STP) influences were examined for exceptions to the nearshore/offshore classification based on water circulation patterns (Figure 3.1). Physical transport processes are often the dominant factor in mediating geochemical and biological processes in the coastal environment. The coastal regions are not isolated but are coupled to a greater or lesser degree by exchanges with midlake waters involving transport of materials, momentum and energy (Rao and Schwab 2007). Monthly

vertically averaged water circulation patterns for May to October 2001, 2002, and 2003 were examined for the twenty-one stations in the eastern basin of Lake Erie to determine the relative influence of offshore transport to nearshore stations (Figure 3.1). August 2003 is illustrated to represent the typical circulation patterns present when the seston and nutrient concentrations were measured (June to September). The most obvious circulation features during August are the gyres present in the centre of the basin (Figure 3.1). Gyres, or rotary motions, are one of the important mechanisms for nearshore-offshore transport in the Great Lakes (Rao and Schwab 2007). Based on the water circulation patterns, three stations were identified that did not fit the arbitrary classification of nearshore or offshore based on station depth. Station 447 is 40 m deep and originally was classified as an offshore station. However, the circulation patterns show that it is influenced heavily by nearshore water. Similarly, station 444 is a nearshore station that can be heavily influenced by offshore waters due to a small gyre that forms in the late summer/early fall (Figure 3.1). Upon examination of monthly chloride (Cl^-) patterns in 2001 and 2002 (data not shown), it appears that there are high Cl^- concentrations in the spring at 444, decreasing steadily into the fall. This seasonal Cl^- pattern could be the result of the Fredonia and Dunkirk, NY STPs that discharge close to the station with existing flows of 3 and 6 millions of gallons day^{-1}, respectively (CWNS 2000). Station 448 is another nearshore station influenced heavily by offshore waters, particularly by central basin waters transported into the eastern basin. Station 448 also displayed seasonal Cl^- patterns (data not shown) indicative of STP effluent. Three major STPs discharge close to station 448, including: Erie City and Northeast Boro STP's both in Pennsylvania, and Ripley, NY SD/STP with existing flows of 69, 1.4, and 0.3 millions of gallons day^{-1}, respectively (CWNS 2000).

The Grand River provides the major inflow to the eastern basin of Lake Erie and is the largest river system entering the north shore (He et al. 2006). The river mouth is directly

upstream of station 442 which displays seasonal Cl^- patterns indicating influence by the Grand River (data not shown). The Grand River plume has been identified as one of the main sources affecting the water quality of the surrounding area (Rao and Schwab 2007), and Nicholls et al. (1983) demonstrated an impact of the Grand River on north shore nutrients and phytoplankton. Therefore, on the basis of horizontal water circulation patterns, tributary and STP influences, four stations (447, 444, 448 and 442) were removed from all further analyses.

The assessment of circulation patterns did not account for wind resulting in internal seiches and upwelling, which can occasionally occur in the east basin. Inspection of the 2008 temperature and current data from the Great Lakes Coastal Forecasting System (GLCFS) revealed that winds blowing surface waters from the central basin to the southeast corner of the east basin, cause hypolimnetic water from the offshore to surge into the nearshore (internal seiches), particularly on the north shore of the east basin (GLCFS 2008).

3.2.2.3 Historical surveys

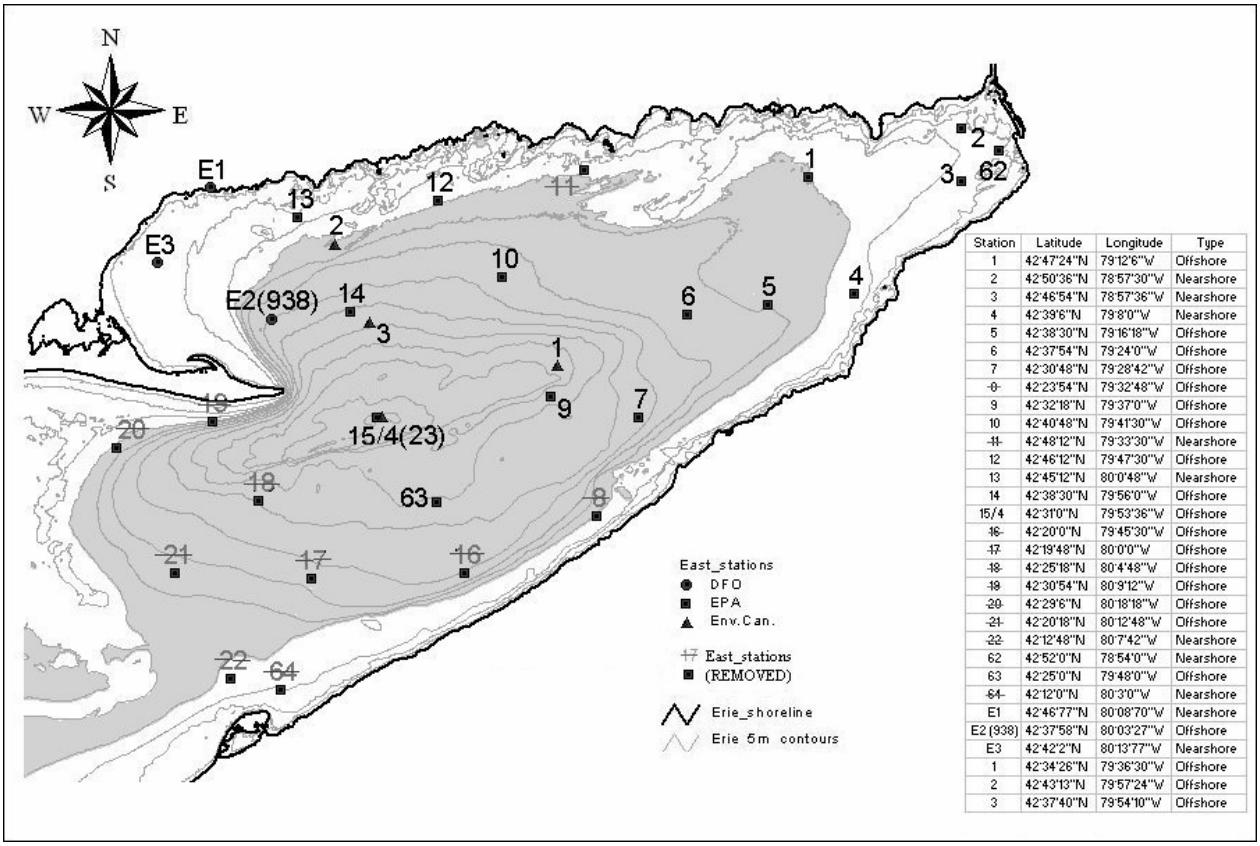
Select EPA, Env. Can., and DFO stations were also removed from the analyses using the same criteria as applied to the basin-wide 2001-2003 surveys for reasons of consistency and under the assumption that the major circulation patterns in the eastern basin (Figure 3.1) have not changed in the last 35 years. Ten historical stations sampled by the EPA in 1973 were removed from the analyses. Station 11 was removed due to its vicinity to the Grand River discharge, stations 8 and 16 were removed as offshore stations due to nearshore influences and stations 17, 18, 19, 20, 21, 22, and 64 were removed due to their proximity and influence by central basin waters (Figure 3.2).

For the Env. Can. surveys in 1994, 1996-1999, the same stations as those sampled in 2001-2003 were chosen to represent the eastern basin for these years for consistency and to facilitate comparison with the basin-wide survey data (Figure 3.1).

3.2.3 Temporal sample selection

For all of the surveys, only the data from June, July, August and September were used in the analyses due to complex early and late-season circulation dynamics such as thermal bars. The summer months were also selected with reference to the surface water temperature (data not shown) as the season when dreissenids would have the most impact due to their optimal consumption temperature range (8-25 ^{o}C; Stanczykowska et al. 1975). The results shown in Figures 3.4, 3.6-3.11 illustrate the mean and standard error for the June-September data for each year. As the objective of this study was to examine spatial patterns over three decades, and seasonal data was not available for all of the historical surveys, seasonal data are not presented.

For the north shore survey, a two-way ANOVA revealed that the year-to-year (2001-2003) variability (p=0.022) in chl*a* concentrations was less than the spatial (nearshore/offshore) variability (p=0.000) with no significant interaction between the two factors (p=0.953). It then seemed reasonable to use the 2001-2003 means to look for nearshore/offshore differences.



Station	Latitude	Longitude	Type
1	42°47'24"N	79°12'6"W	Offshore
2	42°50'36"N	78°57'30"W	Nearshore
3	42°46'54"N	78°57'36"W	Nearshore
4	42°39'6"N	79°8'0"W	Nearshore
5	42°38'30"N	79°16'18"W	Offshore
6	42°37'54"N	79°24'0"W	Offshore
7	42°30'48"N	79°28'42"W	Offshore
~~8~~	42°23'54"N	79°32'48"W	Offshore
9	42°32'18"N	79°37'0"W	Offshore
10	42°40'48"N	79°41'30"W	Offshore
~~11~~	42°48'12"N	79°33'30"W	Nearshore
12	42°46'12"N	79°47'30"W	Offshore
13	42°45'12"N	80°0'48"W	Nearshore
14	42°38'30"N	79°56'0"W	Offshore
15/4	42°31'0"N	79°53'36"W	Offshore
~~16~~	42°20'0"N	79°45'30"W	Offshore
~~17~~	42°19'48"N	80°0'0"W	Offshore
~~18~~	42°25'18"N	80°4'48"W	Offshore
~~19~~	42°30'54"N	80°9'12"W	Offshore
~~20~~	42°29'6"N	80°18'18"W	Offshore
~~21~~	42°20'18"N	80°12'48"W	Offshore
~~22~~	42°12'48"N	80°7'42"W	Nearshore
62	42°52'0"N	78°54'0"W	Nearshore
63	42°25'0"N	79°48'0"W	Offshore
~~64~~	42°12'0"N	80°3'0"W	Nearshore
E1	42°46'77"N	80°08'70"W	Nearshore
E2 (938)	42°37'58"N	80°03'27"W	Offshore
E3	42°42'2"N	80°13'77"W	Nearshore
1	42°34'26"N	79°36'30"W	Offshore
2	42°43'13"N	79°57'24"W	Offshore
3	42°37'40"N	79°54'10"W	Offshore

Figure 3.2 Historical basin-wide survey stations sampled by the EPA (1973), Environment Canada (1979-1990) and the DFO (1993, 1994, 1998). Ten stations were removed based on circulation patterns as identified in section 3.2.

3.2.4 Physical Conditions

3.2.4.1 Basin-wide survey (2001-2003)

The physical properties of the water column (conductivity, temperature, and depth) were measured with an Electronic Bathy Thermograph (EBTT) profiler. Vertical profiles of photosynthetically active radiation (PAR) were measured with a Li-Cor cosine underwater quantum sensor and a Li-Cor LI-1000 data logger. The vertical attenuation coefficient for PAR (k_d), was determined from the linear regression of the natural logarithm of irradiance versus depth (Kirk 1994). Where light profiles were not collected, k_d was estimated from the linear

relationship between k_d and %T (percent transmission measured by the EBTT profiler). The Z_{mix} and mean water column light intensity as a percentage of surface irradiance (mean PAR) were calculated according to Guildford et al. (2000). Secchi disc depths were also recorded. Epilimnetic whole water samples were collected with an integrating sampler from the surface to 1 m above the bottom (if isothermal) to a maximum depth of 20 m, or to the top of the thermocline, where the thermocline was defined by a change in water temperature gradient $>1^oC\ m^{-1}$. After collection, water was transferred to darkened 20 L polyethylene carboys prior to subsampling, which was initiated within 2 hours.

3.2.4.2 Historical surveys

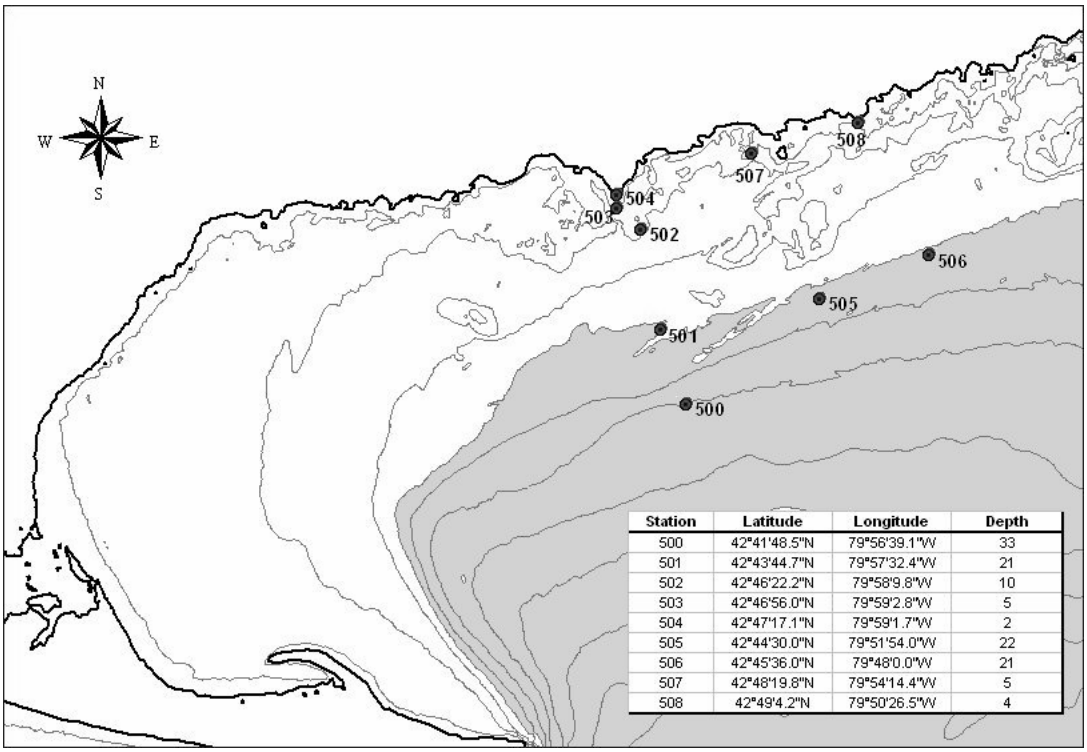
The EPA survey in 1973 employed a YSI model 54 dissolved oxygen-temperature profiler and collected discrete water samples from 1 m depths at all stations (Great Lakes Laboratory 1974).

The Env. Can. surveys employed an EBTT profiler and Secchi disc. Vertical extinction coefficients were measured at select stations from 1979-1990. Otherwise, all historical Env. Can. k_d values were estimated from %T. Epilimnetic whole water samples were collected from either discrete depths within the epilimnion (ideally twice the Secchi depth) or with an integrating sampler as in the basin-wide surveys.

The DFO surveys in 1993, 1994 and 1998 employed a Hydrolab H2O profiling system that was used to determine Z_{mix} (Dahl et al. 1995). Secchi depths were measured directly and a Licor Li-192S underwater quantum sensor was used to determine k_d as outlined previously. During isothermal conditions, water samples were taken from 4 to 5 equally spaced depths from 2 m above the bottom to within 1 m of the surface. Under thermally stratified conditions, water was collected from 1 m below the surface to 1 m above Z_{mix} using a diaphragm pump in 1993 (Dahl et al. 1995) and 1994 (Graham et al. 1996), and a water column integrator at deep stations in 1998 (MacDougall et al. 2001).

3.2.4.3 North shore survey

The water column was profiled with either a SeaBird^{TM} SBE-19 profiler or a Hydrolab, and k_d was determined from vertical profiles of PAR. Epilimnetic whole water samples were collected with an integrating tube sampler from surface to 1 m above the bottom (if isothermal) to a maximum depth of 10 m, or to the top of the thermocline. After collection, water was transferred to darkened 20 L polyethylene carboys prior to subsampling, which was initiated within 12 hours at the University of Waterloo, Ontario.



Station	Latitude	Longitude	Depth
500	42°41'48.5"N	79°56'39.1"W	33
501	42°43'44.7"N	79°57'32.4"W	21
502	42°46'22.2"N	79°58'9.8"W	10
503	42°46'56.0"N	79°59'2.8"W	5
504	42°47'17.1"N	79°59'1.7"W	2
505	42°44'30.0"N	79°51'54.0"W	22
506	42°45'36.0"N	79°48'0.0"W	21
507	42°48'19.8"N	79°54'14.4"W	5
508	42°49'4.2"N	79°50'26.5"W	4

Figure 3.3 North shore survey stations sampled in 2001, 2002 and 2003 on the north shore of the eastern basin of Lake Erie. Contour lines are shown in 5 m increments with the 20 m contour identified by shading.

3.2.5 Water Chemistry

3.2.5.1 Basin-wide and north shore surveys (2001-2003)

TP, total dissolved P (TDP) and soluble reactive P (SRP) samples were analyzed following preservation and analytical procedures of NLET (1994). The 2001 and 2002 particulate P samples were analyzed using the muffle furnace digestion method according to Stainton et al. (1977). In 2003, particulate P was measured using the persulphate digestion method in an autoclave (Parsons et al. 1984; Chapter 2).

Nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) concentrations were analyzed following preservation and analytical procedures of NLET (1994). In 2002 and 2003, the NH_4^+ samples were analyzed following a fluorometric method (Holmes et al. 1999).

The dissolved reactive silica (DRSi) and Cl^- samples were analyzed following preservation and analytical procedures of NLET (1994). The particulate Si samples were analyzed at the DFO Freshwater Institute (FWI) in Winnipeg, Manitoba, according to Stainton et al. (1977; Chapter 2).

Particulate carbon (C) and N samples were analyzed by the methods described by Stainton et al. (1977). In 2001, the particulate C and N samples were measured at the Analytical Laboratory at the FWI (Stainton et al. 1977). In 2002 and 2003, the samples were processed at the University of Waterloo. The dried GFF filters were placed in a desiccator containing hydrochloric acid and fumed for 24 hours and then analyzed on an Exeter Analytical Inc. CEC-440 (combustion 980^{o}C, reduction 700^{o}C) autoanalyzer (Stainton et al. 1977). The picoplankton

particulate C samples were filtered through a 2-μm pore-size polycarbonate membrane filter (Chapter 2) and the filtrate was treated exactly as the sample water.

3.2.5.2 Historical surveys

All of the 1973 water chemistry was measured according to USEPA methods (Anon 1971). The particulate C samples were measured on GFC filters to which 20% HCl solution was added and were then combusted in a Coleman Carbon-Hydrogen Analyzer (Great Lakes Laboratory 1974). The particulate P concentrations were calculated as the difference between TP and TDP.

The Env. Can. and DFO samples were analyzed following preservation and analytical procedures of NLET (1994). The particulate P concentrations were also calculated as the difference between TP and TDP with the exception of the particulate P samples collected in 1997 (Guildford et al. 2005) which were analyzed at the FWI in addition to the particulate C and Si samples (Stainton et al. 1977).

3.2.6 Chl*a* analysis

3.2.6.1 Basin-wide and north shore surveys (2001-2003)

Sample water was filtered onto glass fiber (GFF: nominal pore-size 0.7 μm, 47 mm) filters that were kept in the dark and stored frozen before passive extraction with 90% acetone. Sample water was also filtered through a 2-μm pore-size polycarbonate membrane filter for the determination of picoplankton chl*a* concentrations (Chapter 2). The filtrate was then treated exactly as the sample water. The extracts were quantified by fluorometry (Turner Designs 10-AU) and corrected for phaeophytins.

3.2.6.2 Historical surveys

All of the historical surveys followed the same protocol for chl*a* determinations. Sample water was filtered onto glass fiber (GFC: nominal pore-size 1.2 μm) filters that were ground in 90%

acetone and the extracts were quantified spectrophotometrically at the appropriate wavelengths (Strickland and Parsons 1968).

The basin-wide and historical survey methods for chl*a* determination were different, therefore, a comparison was conducted between the two methodologies for select samples from 2001-2003. A regression of basin-wide chl*a* concentrations and NLET phaeophytin-corrected chl*a* concentrations (R^2=0.544, *p*= 0.000, *n*=61) was compared with a regression of basin-wide chl*a* concentrations and NLET uncorrected chl*a* concentrations (R^2=0.587, *p*= 0.000, *n*=89). Due to the fact that the NLET uncorrected chl*a* concentrations were more comparable to the basin-wide chl*a* concentrations, and that there was more uncorrected chl*a* historical data available, all of the historical chl*a* concentrations reported were uncorrected for phaeophytins.

3.2.7 Phosphorus limitation indicators

Phosphorus limitation was determined by particulate C:P and N:P composition ratios (Healey 1973), and alkaline phosphatase activity (APA; Healey and Hendzel 1979a). The determination of P limitation was assessed according to the criteria developed by Healey and Hendzel (1979b). The C:P (molar ratio) indicator is used to indicate no P limitation (<129), moderate P limitation (129-258) and extreme P limitation (>258). APA is an enzymatic assay used to determine P limitation. APA is activated when inorganic sources of P are scarce. Alkaline phosphatase is an enzyme localized on the cell surface of algal and bacterial cells that is produced when the algae are P limited. This enzyme removes the phosphate molecules from dissolved organic P compounds, therefore, utilizing an otherwise unavailable source of P (Ammerman et al. 2003). APA was measured fluorometrically (Healey and Hendzel 1979a), using 5 μmol L^{-1} of o-methyl-fluorescein-phosphate as the substrate. Parallel determinations were made of total and soluble activities to distinguish between APA associated with particles and APA in solution, the soluble

activity being that passing through 0.2 μm pore-size filters. The difference was reported as particulate activity. APA (μmol P μg $chla^{-1}$ h^{-1}) indicator is used to indicate no P limitation (<0.003), moderate P limitation (0.003-0.005), and extreme P limitation (>0.005). The 1997 C:P and APA data is from Guildford et al. (2005) and followed the same methodology.

3.2.8 Statistical analyses

3.2.8.1 Basin-wide and historical surveys

Two-way analysis of variances (ANOVA) were used to determine the differences between nearshore and offshore, and 1973 versus post-dreissenid (1990-2003) years. These analyses were followed by one-way ANOVAs to determine the difference between nearshore and offshore for 1973. Two-way ANOVAs were then performed on the post-dreissenid (1990-2003) years to determine the difference between year and nearshore and offshore. Finally, one-way ANOVAs were performed on the offshore data to determine the differences between pre-dreissenid (1973-1985) versus post-dreissenid (1990-2003) years.

A linear regression was performed on the Log_{10} Chl*a* versus Log_{10} TP data, in addition to an ANCOVA with nearshore/offshore as the factor. For all of the above analyses, a significance value of $p<0.050$ was applied.

3.2.8.2 North shore survey

Two-way ANOVAs were used to determine the difference between nearshore and offshore, and the years 2001-2003 with a significance value of $p<0.050$.

3.3 Results

3.3.1 Basin-wide and historical surveys

3.3.1.1. Chla – TP relationships

There was a significant decrease in chl*a* and TP concentrations from 1973 to the years after P loading controls and the dreissenid invasion. A two-way ANOVA revealed a significant interaction (p=0.000) between 1973 and post-dreissenid (1993-2003) years, and nearshore versus offshore. Chl*a* concentrations were significantly higher in the eastern basin of Lake Erie in 1973 (mean=3.3 μg L^{-1}) compared to post-dreissenid years (1993-2003; mean=1.7 μg L^{-1}). In the offshore, pre-dreissenid (1973-1985) chl*a* concentrations were also significantly higher than post-dreissenid (1990-2003) years. In 1973, nearshore chl*a* concentrations were significantly higher than offshore. In contrast, post-dreissenid years (1993-2003) show nearshore chl*a* concentrations significantly lower than offshore (Figure 3.4).

Post-dreissenid (2001-2002) picoplankton (0.2 – 2 μm) concentrations determined by size-fractionated chl*a* concentrations were also significantly lower in the nearshore (n=35, mean=0.50 μg L^{-1}) than the offshore (n=48, mean=0.70 μg L^{-1}; data not shown). The percentage of the total chl*a* that is represented by picoplankton is similar between the nearshore (39%) and the offshore (41%).

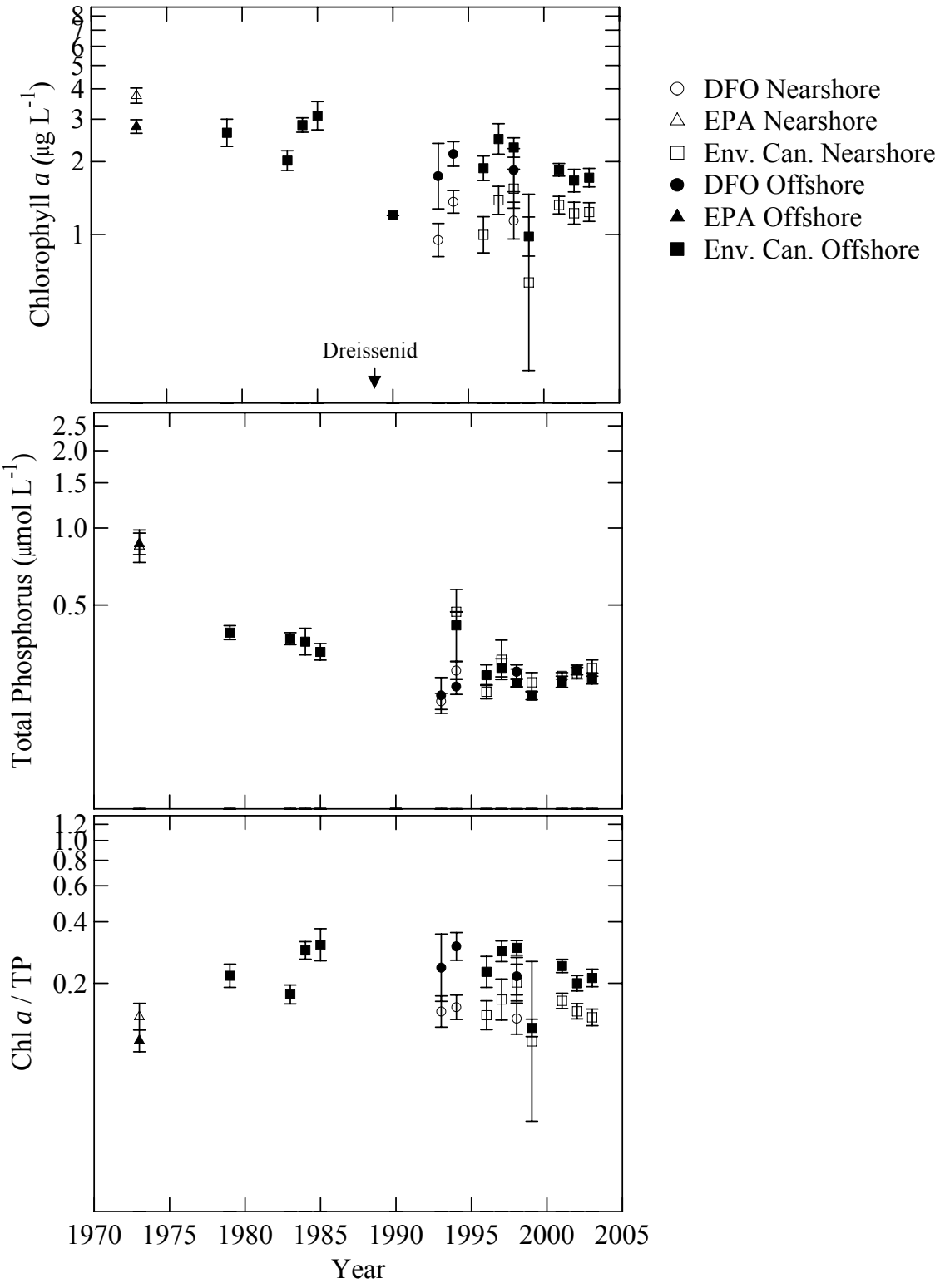
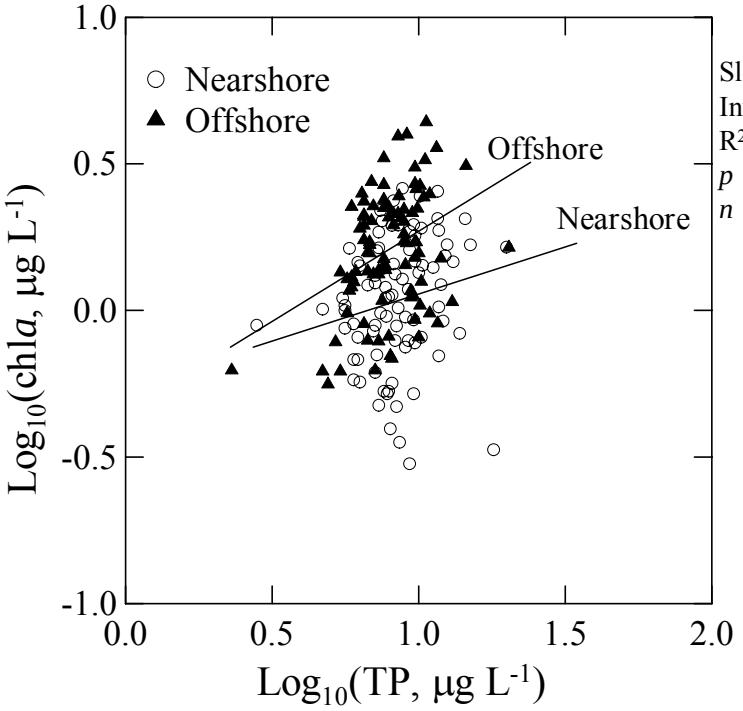

Chlorophyll a (µg L-1)
Dreissenid
Total Phosphorus (µmol L-1)
Chl a / TP
Year
1970
1975
1980
1985
1990
1995
2000
2005
DFO Nearshore
EPA Nearshore
Env. Can. Nearshore
DFO Offshore
EPA Offshore
Env. Can. Offshore

Figure 3.4 Comparison of basin-wide nearshore and offshore historical trends in mean annual chl*a* and TP concentrations. The chl*a* concentrations (*n*=358), TP concentrations (*n*=404) and the chl*a*:TP ratio (*n*=350) are shown on log axes. In this and the following figures, the values shown are from data collected in June, July, August and September only and stations identified in Figures 3.1 and 3.2 have been removed. Error bars represent the standard error of the mean and the arrows indicate the year of dreissenid invasion to the eastern basin.

TP concentrations at both nearshore and offshore stations were significantly higher in 1973 (mean=1.1 μmol L^{-1}) compared to post-dreissenid years (1993-2003; mean=0.28 μmol L^{-1}). Pre-dreissenid (1973-1985) offshore stations had significantly higher TP concentrations compared to the post-dreissenid (1993-2003) offshore stations. The nearshore/offshore differences in TP have not changed over the three decades, as there were no significant differences between nearshore and offshore TP concentrations in either 1973 or post-dreissenid years (1993-2003; Figure 3.4).

Coincident with the historical changes in chl*a* and TP concentrations in the lake, there was also a difference in the proportion of chl*a* relative to TP. A two-way ANOVA revealed a significant interaction (p=0.000) between 1973 and post-dreissenid (1993-2003) years, and nearshore versus offshore. The chl*a*/TP ratio has changed since 1973 (mean=0.15), and was significantly higher post-dreissenids (1990-2003; mean=0.22). The offshore, post-dreissenid (1993-2003) ratio was also significantly higher than the offshore, pre-dreissenid (1973-1985) ratio. There was no significant difference between nearshore and offshore ratios in 1973, however, there was significantly more chl*a* per unit of TP offshore compared to nearshore in the post-dreissenid years (1993-2003; Figure 3.4). This post-dreissenid (2001-2003) relationship was

examined further via a regression of $\log_{10}$ chl*a* on $\log_{10}$TP for both nearshore and offshore stations (Figure 3.5). The chl*a*/TP relationship was not strong in the nearshore or the offshore, and only the offshore regression was statistically significant. An ANCOVA revealed a highly significant difference between the nearshore and offshore regressions (p=0.000; Figure 3.5).



	Offshore	Nearshore
Slope	0.629	0.300
Intercept	-0.366	-0.247
R^2	0.137	0.032
p	0.000	0.103
n	91	85

Figure 3.5 Basin-wide chl*a*-TP regressions (log_{10}-transformed data) from 2001-2003 during June to September for nearshore and offshore stations.

3.3.1.2 Light conditions

There were no significant differences at the offshore stations between Secchi depths for pre-dreissenid years (1979-1985) and post-dreissenid years (1990-2003; data not shown). The k_d values in the pre-dreissenid (1979-1985) offshore were significantly higher than in the post-dreissenid (1993-2003) offshore (Figure 3.6), but there was no significant differences between nearshore and offshore in the post-dreissenid years (1993-2003; Figure 3.6). However, both of these light parameters were influenced by shallow mixing depths. The parameter mean PAR, is a function of k_d and the mixing depth of the epilimnion (Z_{mix}). Nearshore and offshore mean PAR values expressed as a percentage of surface light, reveal that there is an improved light climate in the nearshore post-dreissenids (1993-2003), as the nearshore mean PAR was significantly higher than the offshore (Figure 3.6).

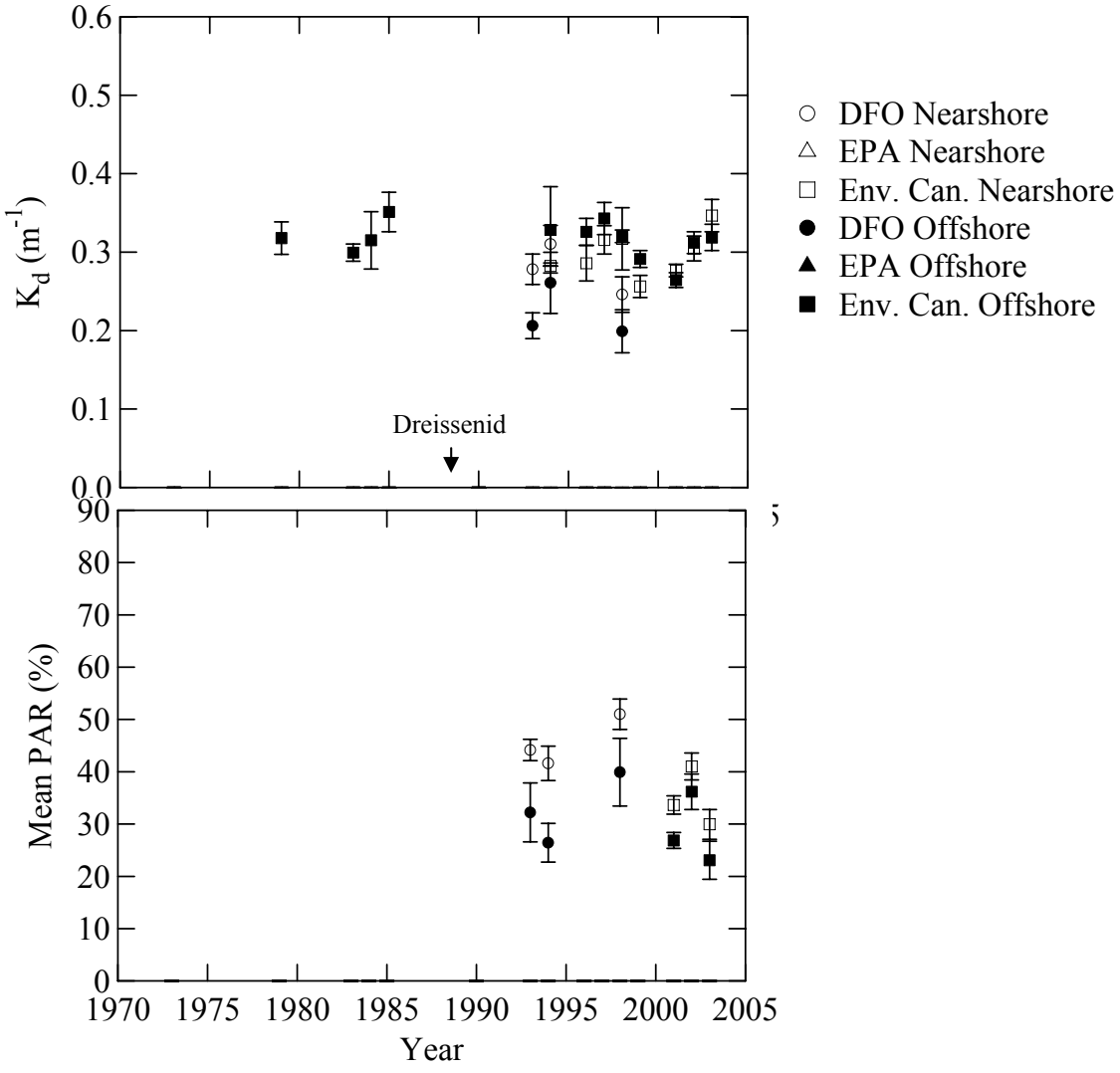


Figure 3.6 Comparison of basin-wide nearshore and offshore historical trends in mean annual light parameters. Means are shown for the attenuation coefficient for PAR (k_d; *n*=392), and the mean water column light intensity as a percent of surface irradiance (mean PAR; *n*=230). Error bars represent the standard error of the mean.

3.3.1.3 Nutrient chemistry

Analysis of the P water chemistry shows that 1973 TDP concentrations (mean=0.11 μmol L^{-1}) were significantly lower than post-dreissenid (1993-2003) concentrations (mean=0.15 μmol L^{-1});

nevertheless TDP was a small component of TP in 1973 (Figures 3.4 and 3.7). As well, offshore TDP concentrations in pre-dreissenid years (1973-1985) were significantly higher than post-dreissenid (1993-2003) offshore concentrations (Figure 3.7). In 1973, there were no significant differences between nearshore and offshore TDP concentrations, while in the post-dreissenid years (1993-2003), nearshore concentrations were significantly higher than they were offshore (Figure 3.7). Pre-dreissenid (1979-1985) offshore SRP concentrations were significantly higher than offshore post-dreissenid (1993-2003) concentrations. In the post-dreissenid years (1993-2003), SRP concentrations were significantly higher in the nearshore than the offshore (Figure 3.7). The 1973 particulate P concentrations (mean=0.96 μmol L^{-1}) were significantly higher than the post-dreissenid (1993-2003) concentrations (mean=0.14 μmol L^{-1}; Figure 3.7), as were the offshore particulate P concentrations in pre-dreissenid (1973-1985) versus post-dreissenid (1993-2003) years. In 1973 and the post-dreissenid years (1993-2003), there were no significant differences between nearshore and offshore particulate P concentrations.

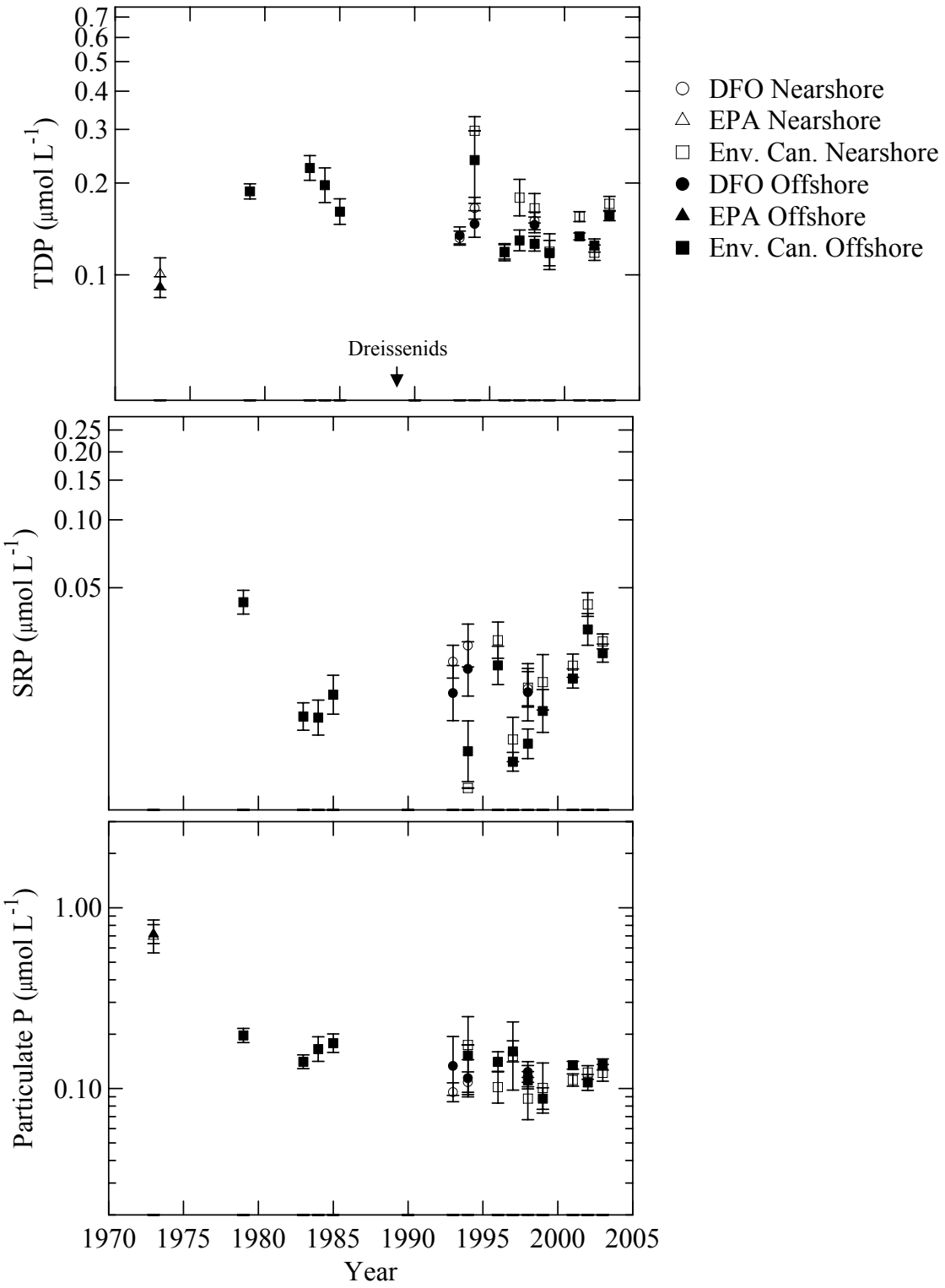

TDP (µmol L-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Dreissenids
SRP (µmol L-1)
0.25
0.20
0.15
0.10
0.05
Particulate P (µmol L-1)
1.00
0.10
1970
1975
1980
1985
1990
1995
2000
2005
Year
DFO Nearshore
EPA Nearshore
Env. Can. Nearshore
DFO Offshore
EPA Offshore
Env. Can. Offshore

Figure 3.7 Comparison of nearshore and offshore basin-wide trends in mean annual P chemistry. The TDP (*n*=405), SRP (*n*=381), and particulate P (*n*=342) concentrations are shown on log axes. Error bars represent the standard error of the mean.

Examination of the N water chemistry shows that while 1973 NO_3^- concentrations (mean=6.9 μmol L^{-1}) were significantly lower than post-dreissenid (1993-2003) concentrations (mean=15.5 μmol L^{-1}), 1973 NH_4^+ concentrations (mean=4.8 μmol L^{-1}) were significantly higher than post-dreissenid (1993-2003) concentrations (mean=1.2 μmol L^{-1}; Figure 3.8). NO_3^- concentrations have more than doubled since 1973 and comparison of offshore concentrations also show significantly lower pre-dreissenid (1973-1985) concentrations compared to post-dreissenid (1993-2003) concentrations. The 1973 NH_4^+ concentrations appear to be unusually high, and removal of the 1973 data reveals no significant difference between offshore pre-dreissenid (1979-1985) and post-dreissenid (1993-2003) NH_4^+ concentrations. In 1973, there was no significant difference between nearshore and offshore dissolved N (NO_3^-, NH_4^+) concentrations. In the post-dreissenid years (1993-2003) the NO_3^- concentrations were significantly higher in the nearshore than the offshore, and there was no significant difference between nearshore and offshore NH_4^+ concentrations (Figure 3.8). There were no significant differences in particulate N concentrations between pre-dreissenid (1979-1985) and post-dreissenid (1993-2003) years or between nearshore and offshore in the post-dreissenid (1993-2003) years (Figure 3.8).

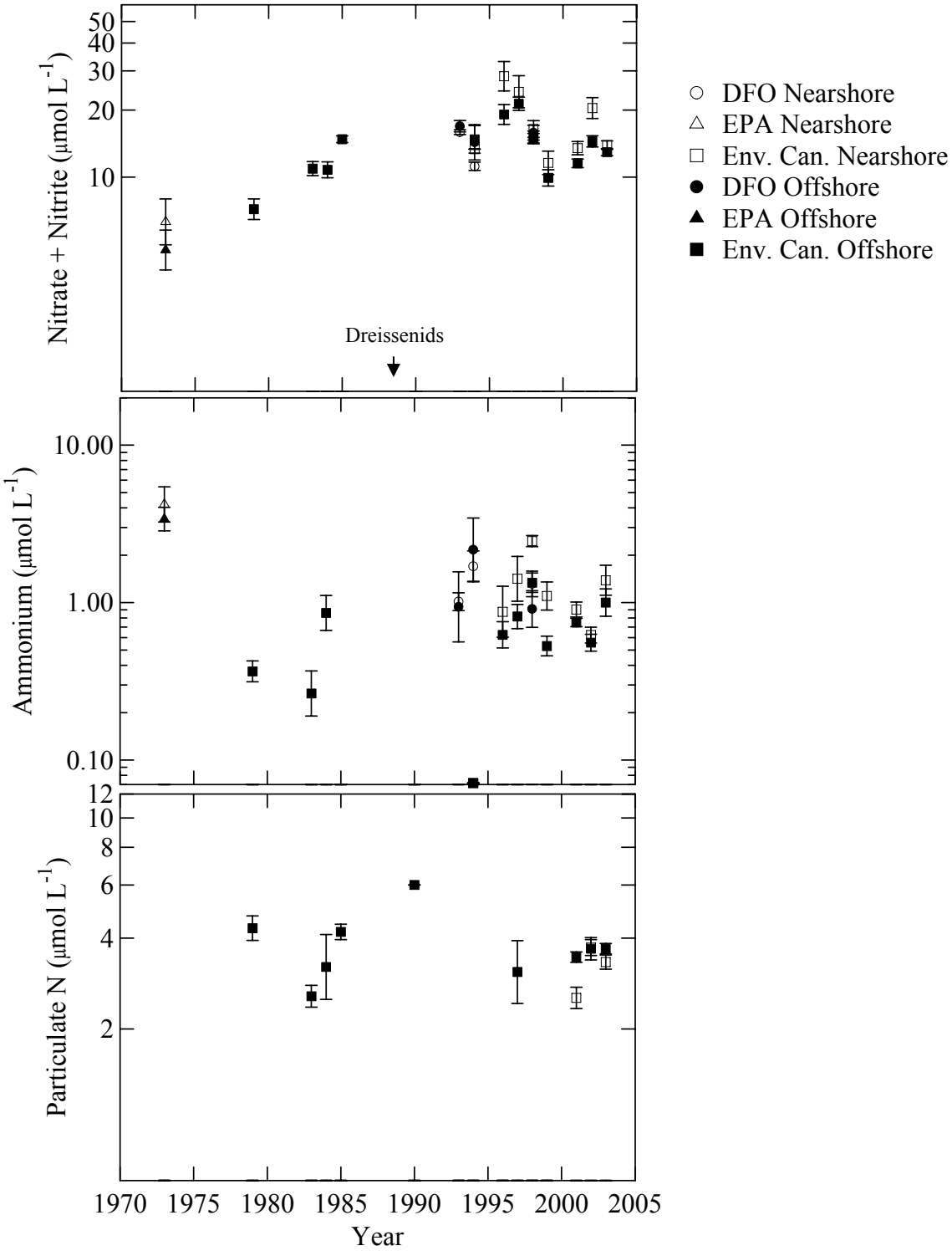


Figure 3.8 Comparison of nearshore and offshore basin-wide trends in mean annual N chemistry. NO_3^- (*n*=337), NH_4^+ (*n*=342) and particulate N (*n*=182) concentrations are

shown on log axes. Note that the 1973 NO_3^- concentration estimates do not include NO_2^-. Error bars represent the standard error of the mean.

Analysis of Si water chemistry shows that there were no significant differences in offshore dissolved reactive Si (DRSi) concentrations between 1985 and post-dreissenid years (1993-2003; Figure 3.9). However, within the post-dreissenid years (1993-2003), DRSi concentrations were significantly higher in the nearshore compared to the offshore. There were no significant differences between the nearshore and offshore for post-dreissenid (2001-2003) particulate Si concentrations (Figure 3.9).

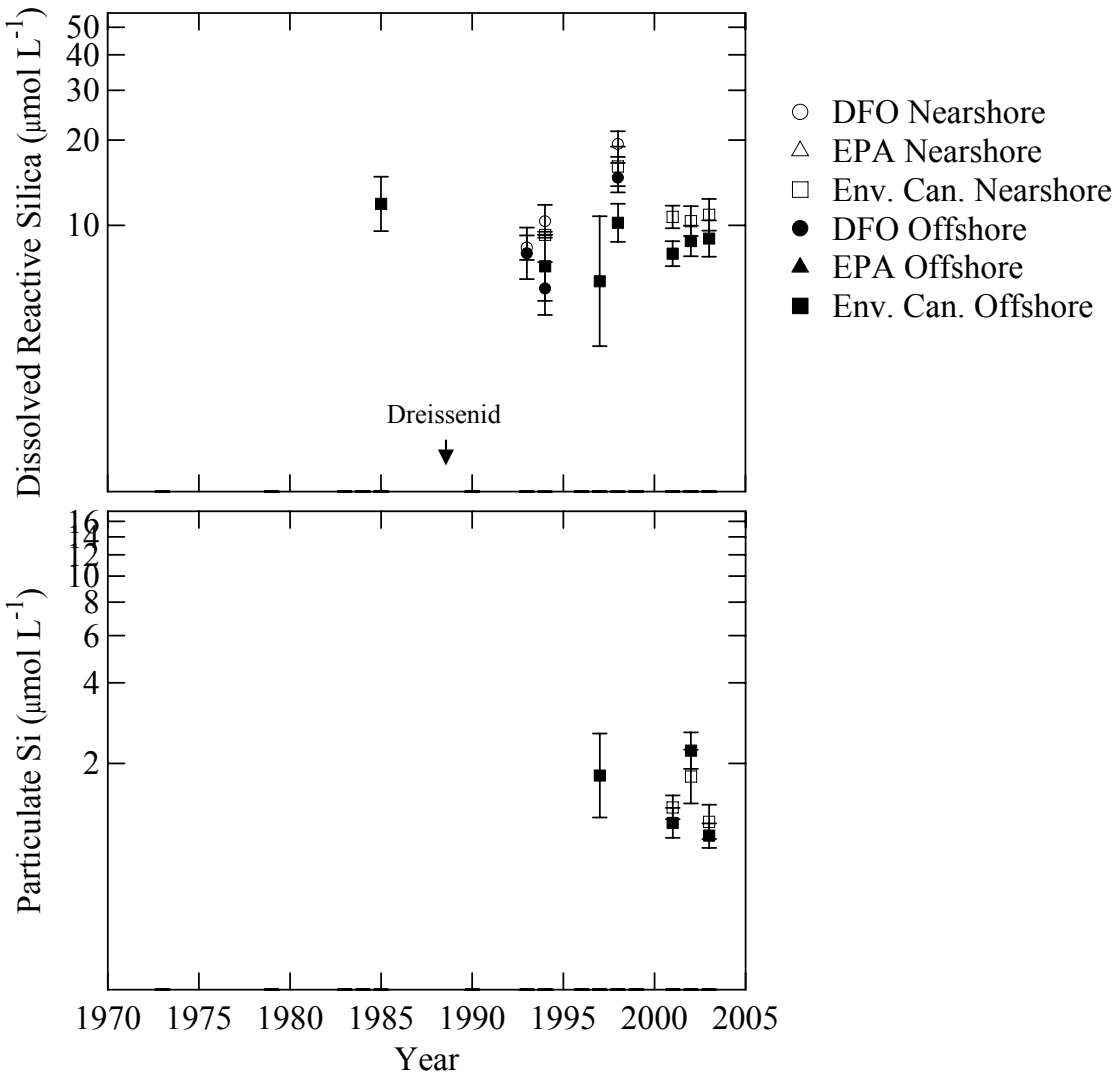


Figure 3.9 Comparison of nearshore and offshore basin-wide trends in mean annual Si chemistry. The DRSi (*n*=269) and particulate Si (*n*=109) concentrations are shown on log axes. Error bars represent the standard error of the mean.

Post-dreissenid (1993-2003) particulate C concentrations were significantly lower than the 1973 concentrations. As well, the offshore post-dreissenid (1993-2003) particulate C concentrations were significantly lower than the offshore pre-dreissenid (1973-1985) concentrations. There were no nearshore/offshore differences in 1973 particulate C concentrations, however, within the post-dreissenid years (1993-2003), the particulate C was significantly lower nearshore than offshore (data not shown). Size fractioned (0.2 – 2 μm) particulate C concentrations, and the percentage of particulate C that are represented by picoplankton were similar between the nearshore (mean=13.01 μmol L^{-1}; 50%) and offshore (mean=13.90 μmol L^{-1}; 48%), respectively.

The C:Chl*a* ratios in 1973 were not significantly different than post-dreissenid (1993-2003) ratios, although, pre-dreissenid (1973-1985) offshore ratios were significantly lower than post-dreissenid (1993-2003) offshore ratios. The difference between nearshore and offshore 1973 ratios were not significant, while the post-dreissenid (1993-2003) ratios were significantly lower in the offshore compared to the nearshore (Figure 3.10).

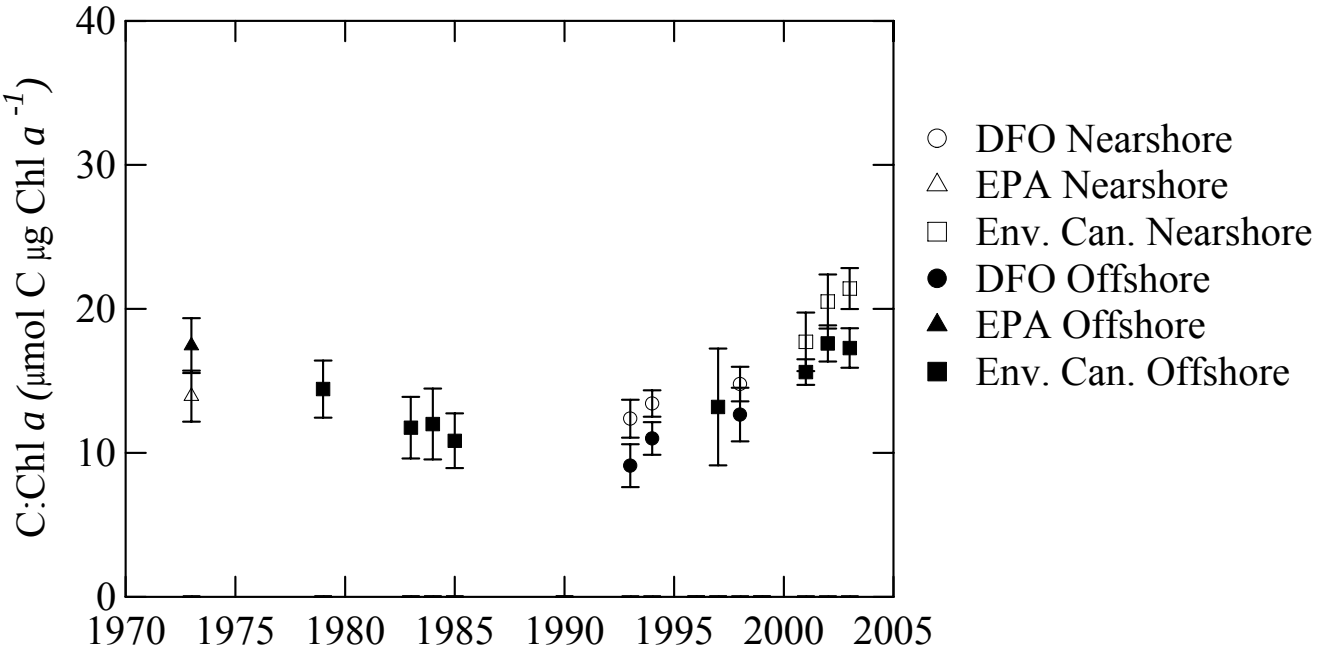


Figure 3.10 Comparison of nearshore and offshore basin-wide trends in mean annual C:Chl*a* ratios (*n*=214). Error bars represent the standard error of the mean.

3.3.1.4 P limitation

Lake Erie is becoming more P limited according to the C:P stoichiometry of the seston. The 1973 C:P molar ratio (mean=62) is significantly lower than the post-dreissenid (1990-2003) ratio (mean=203). As well, the offshore pre-dreissenid (1973-1985) ratio is significantly lower than the offshore post-dreissenid (1993-2003) ratio. In 1973, the phytoplankton were considered not P limited (Figure 3.11), while in 2002 the offshore phytoplankton reached a level of extreme P limitation (Figure 3.11). In 1973 and the post-dreissenid years (1993-2003), there were no significant differences in P limitation (C:P ratios) between nearshore and offshore stations (Figure 3.11).

The physiological indicator APA, showed no significant differences between the offshore post-dreissenid years (1997, 2001, 2002), which were all considered to be moderately to

extremely P limited. APA indicated significantly stronger P limitation in the offshore than the nearshore for both 2001 and 2002 (Figure 3.11).

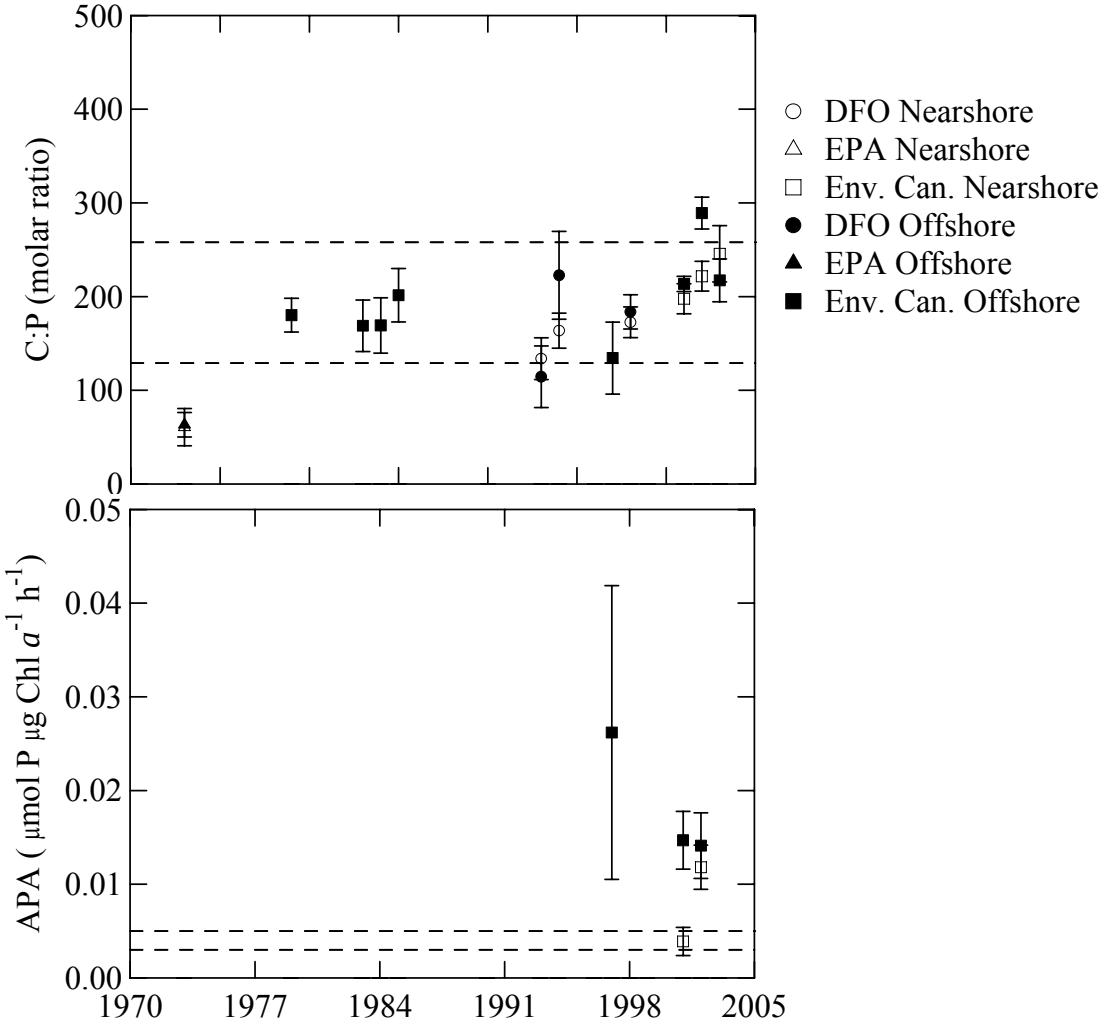


Figure 3.11 Basin-wide historical trends in mean annual indicators of P limitation. P limitation indicators illustrated are C:P ratios (*n*=193), and APA normalized to chl*a* (*n*=34). The dashed lines represent criteria for P limitation as defined by Healey and Hendzel (1979b). Values above the upper dashed lines are indicative of severe P limitation, while values between the upper and lower dashed lines are indicative of moderate P limitation,

and values below the dashed lines are not considered to be P limited. Error bars represent the standard error of the mean. The 1997 data are from Guildford et al. (2005).

3.3.2 North shore survey

3.3.2.1 Chla - TP relationships

Mean chl*a* concentrations for the post-dreissenid years 2001-2003 show that nearshore chl*a* concentrations were significantly lower than offshore (Figure 3.12). Picoplankton (0.2 – 2 μm) concentrations determined by size-fractionated chl*a* were also significantly lower in the nearshore (n=35, mean=0.35 μg L^{-1}) than the offshore (n=31, mean=0.51 μg L^{-1}; data not shown). The percentage of total chl*a* that is represented by picoplankton is similar between the nearshore (40%) and the offshore (39%). Conversely, the TP concentrations were significantly higher in the nearshore relative to the offshore, and a comparison of the chl*a*:TP ratio revealed a significantly lower ratio in the nearshore (Figure 3.12).

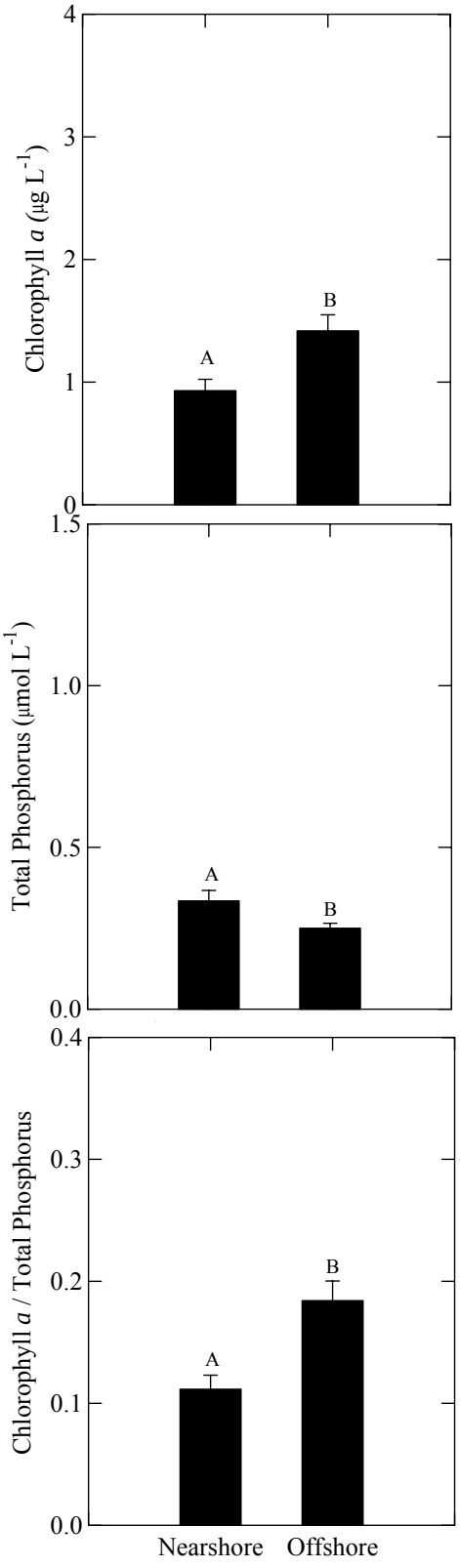

Chlorophyll a (µg L-1)
4
3
2
1
0
A
B
Total Phosphorus (µmol L-1)
1.5
1.0
0.5
0.0
A
B
Chlorophyll a / Total Phosphorus
0.4
0.3
0.2
0.1
0.0
A
B
Nearshore
Offshore

Figure 3.12 Comparison of nearshore and offshore north shore survey chl*a* and TP concentrations. The mean chl*a* (*n*=66) and TP concentrations (*n*=65), and the chl*a*:TP ratio (*n*=62) from 2001-2003 are shown. Error bars represent the standard error of the mean. In this and following figures, the letters above bars indicate statistical significance at a significance level of *p*<0.05. The relationship between identical letters is not statistically significant, whereas the relationship between different letters is significant.

3.3.2.2 Light conditions

Post-dreissenid k_d was significantly higher in the nearshore relative to the offshore (Figure 3.13). As observed in the basin-wide survey, mean PAR integrates Z_{mix} and k_d and demonstrates a significantly better light climate in the nearshore (Figure 3.13).

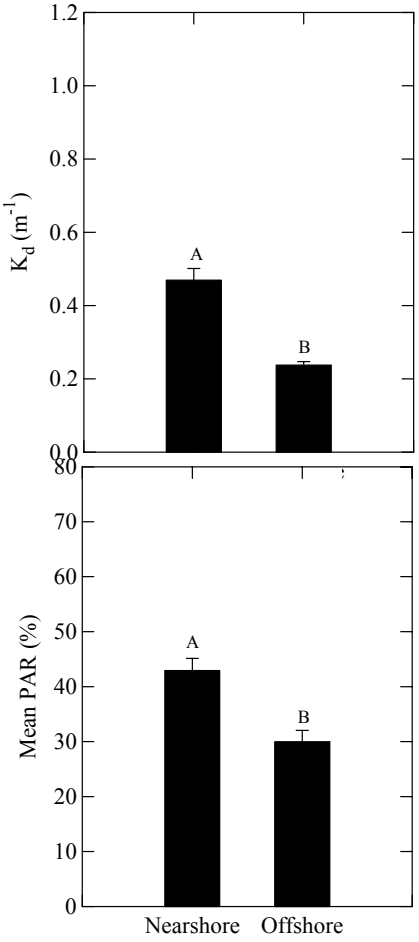


Figure 3.13 Comparison of nearshore and offshore north shore survey light parameters from 2001-2003. Bars represent the mean attenuation coefficient for PAR (k_d; *n*=70) and the mean water column light intensity as a percent of surface irradiance (Mean PAR; *n*=70). Error bars represent the standard error of the mean and letters above bars indicate statistical significance.

3.3.2.3 Nutrient chemistry

There were no significant differences between nearshore and offshore TDP or SRP concentrations, while the particulate P concentrations were significantly higher in the nearshore (Figure 3.14). There were also no significant differences between the nearshore and offshore N chemistry (NO_3^- + NO_2^-, particulate N), with the exception of the NH_4^+ concentrations that were significantly higher in the nearshore (Figure 3.14). There were also no significant differences between the nearshore and offshore DRSi concentrations. However, particulate Si was significantly higher in the nearshore than in the offshore (Figure 3.14). There were no significant differences between nearshore and offshore particulate C concentrations (data not shown). Size-fractioned (0.2 – 2 μm) particulate C concentrations and the percentage of particulate C that are represented by picoplankton were similar between the nearshore (15.1 μmol L^{-1}; 58%) and offshore (14.4 μmol L^{-1}; 57%), respectively (data not shown).

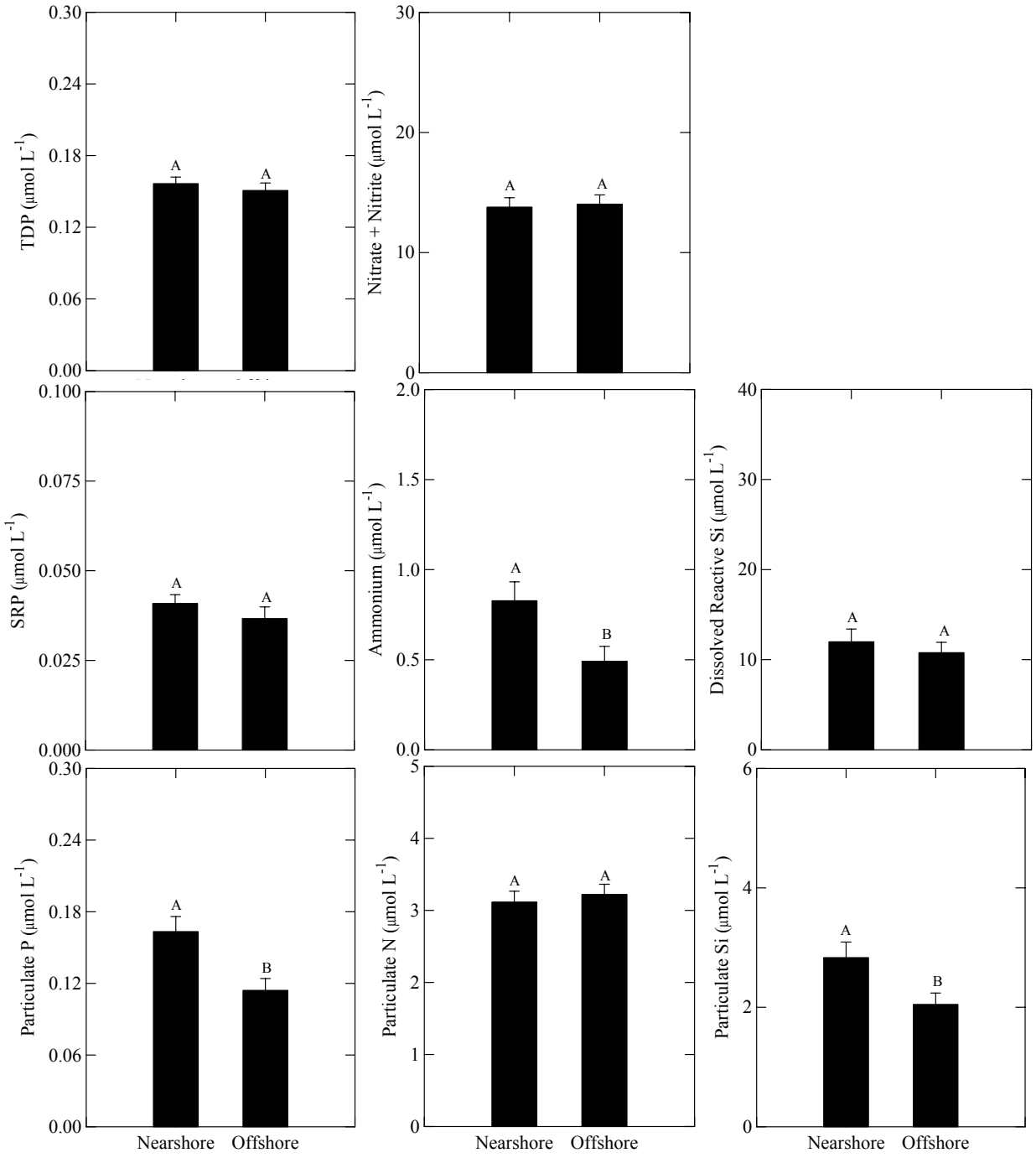


Figure 3.14 Comparison of nearshore and offshore north shore survey nutrient water chemistry from 2001-2003. Bars represent mean TDP (*n*=65), SRP (*n*=65), particulate P (*n*=62), NO_3^- + NO_2^- (*n*=70), NH_4^+ (*n*=70), particulate N (*n*=66), DRSi (*n*=70), and

particulate Si (*n*=68) concentrations. Error bars represent the standard error of the mean and letters above bars indicate statistical significance.

3.3.2.4 P limitation

The C:P molar ratio indicates significantly higher P limitation in the offshore (borderline extreme P limitation) than the nearshore (moderately P limited; Figure 3.15). The physiological indicator APA showed no significant differences between the nearshore and the offshore although the nearshore phytoplankton were classified borderline moderately to extremely P limited, while the offshore phytoplankton were extremely P limited (Figure 3.15).

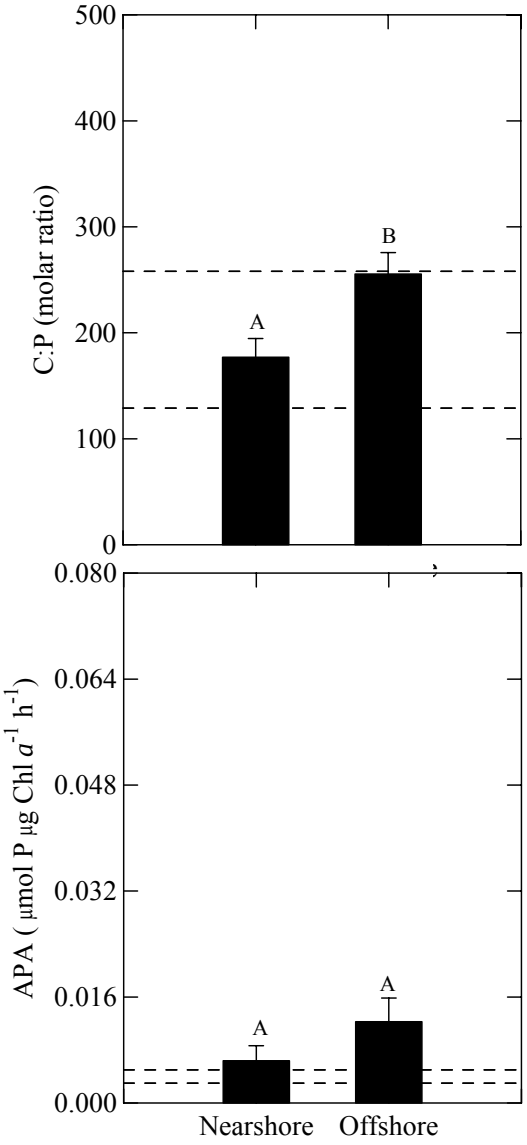


Figure 3.15 Indicators of P limitation for north shore surveys from 2001-2003. C:P ratios (*n*=57), and APA normalized to chl*a* (*n*=62) are shown. The dashed lines represent criteria for P limitation as defined by Healey and Hendzel (1979b). Values above the upper dashed lines are indicative of severe P limitation, while values between the upper and lower dashed lines are indicative of moderate P limitation, and values below the dashed lines are not considered to be P limited. Error bars represent the standard error of the mean and letters above bars indicate statistical significance.

3.4 Discussion

Post-dreissenid chl*a* concentrations were significantly lower nearshore than offshore, as were the chl*a*:TP ratios, with a higher mean PAR occurring in the nearshore. The majority of dissolved nutrients (TDP, SRP, $NO_3^- + NO_2^-$, NH_4^+, DRSi) had higher concentrations in the nearshore than the offshore, while the particulate nutrients (P, N, Si, C) distribution was not as apparent. The particulate nutrient concentrations varied between the nearshore and offshore, depending on the survey.

The data show that the absolute concentrations of both TP and chl*a* have decreased over the decades of nutrient control. Lake Erie receives most of its nutrient load in its shallow western basin (Dolan and McGunagle 2005); consequently, the east basin receives most of its nutrient load from the offshore waters of the central basin. The application of mass balance modeling to the central basin has revealed that total lake loadings are capable of explaining the current offshore TP concentrations (Rockwell et al. 2005). It appears that the TP concentrations in the eastern basin of Lake Erie are reflective of P controls, and these P reductions should be coupled to reductions in chl*a* as predicted by Dillon and Rigler (1974). Smith et al. (2005) showed that the chl*a*:TP ratio did not change significantly consequent to dreissenid colonization in the offshore of Lake Erie, and that TP was a highly significant predictor of chl*a* (R^2= 83%) in all three basins. In 2002, Dolan and McGunagle (2005) estimated that non-point source loading is now larger (61%) than point source loading (20%) to Lake Erie. In addition, the un-monitored contribution to non-point loadings has increased from 15-20% in the early 1980s and is now greater than 50%. Currently, non-point sources constitute the dominant external loading to the east basin (Dolan and McGunagle 2005). Therefore, it might be expected that the eastern basin nearshore would have higher TP concentrations relative to the offshore, however, this was not apparent in my study.

Confounding the issue in the nearshore is the presence of the dreissenid mussels. Dreissenids have been present throughout the lake from 1989 to present day and in 2002, densities of 9 480 m^{-2} were reported in the east basin (Patterson et al. 2005). All of the dreissenids in the eastern basin from 2001-2004 were quagga mussels (Barton et al. 2005; Patterson et al. 2005). Diminished chl*a*/TP ratios can be expected where dreissenid grazing rates are high enough to cause the combined phytoplankton loss rates to exceed replacement rates (Padilla et al. 1996). In the eastern basin nearshore of Lake Erie, Nicholls et al. (1999a) reported that pre-dreissenid (1976-1989) chl*a*/TP ratios were 2-6 times higher than post-dreissenid years (1991-1995). Their pre-dreissenid ratios are similar to the 1973 nearshore ratios reported here, but their post-dreissenid ratios are 10 times lower than what I am reporting for the nearshore in 2001-2003. They also found that pre-dreissenid logarithmically transformed chl*a* and TP data were highly correlated, with 75% of the chl*a* variance explained by TP. While their post-dreissenid regression was significant, only 14% of the variance in the chl*a* data could be explained by TP. This is very similar to my post-dreissenid nearshore regression where only 3.2% of the variance in chl*a* could be explained by TP. They also reported a lower chl*a* at the same TP in the post-dreissenid years, and a decline in the slope of 35% relative to pre-dreissenid years. I observed a 48% decline in the nearshore slope relative to the offshore and Nicholls et al. (1999a) reported a similar slope for their post-dreissenid nearshore (0.465) to what I reported for the nearshore of the eastern basin post-dreissenids (0.300).

Post-dreissenid decoupling of the chl*a*-TP relationship has also been observed in western Lake Erie and Lake St. Clair (Mellina et al. 1995). In Green Bay, Qualls et al. (2007) observed that the pre-dreissenid R^2 value (0.35), slope (0.73), and intercept (5.13) of the regression of chl*a* on TP decreased post-dreissenids (R^2=0.01, slope=0.11, intercept =2.26). They concluded that chl*a* was no longer dependent on TP post-dreissenids as the slope was not significantly different

from zero. I observed the same dreissenid impact wherein the R^2 value and slope of the regression of chl*a* on TP decreased in the nearshore (Figure 3.5). In addition, the chl*a*:TP ratios were historically higher in the nearshore and are now significantly lower in the nearshore than the offshore. In post-dreissenid Lake Ontario, Hall et al. (2003) also found evidence of decoupling of chl*a*-TP relationship in the nearshore resulting in a lower yield of chl*a* per unit TP. However, no evidence of this was found in the offshore. In dreissenid-impacted mesocosms, Wilson (2003) reported lower chl*a* at the same TP and a decoupling of the chl*a*/TP relationship relative to the controls. Similar experiments by Mellina et al. (1995) also showed that with increasing dreissenid densities the chl*a*-TP relationship became decoupled. Decoupling of the chl*a*-TP relationship in the nearshore of Lake Erie appears to be a result of lower chl*a* concentrations and not changes in TP.

3.4.1 Changes in seston concentrations

The most significant and obvious result of this study is that post-dreissenid chl*a* concentrations were lower nearshore than offshore, while pre-dreissenid concentrations were higher in the nearshore. Numerous studies of dreissenid effects on chl*a* can be found in the literature. In mesocosms in Saginaw Bay, chl*a* concentrations rapidly declined in enclosures containing dreissenids (Heath et al. 1995). Researchers have reported reductions in chl*a* from pre-driessenid to post-dreissenid years in many aquatic systems. Examples of these studies include the western basin of Lake Erie (Leach 1993; Mellina et al. 1995), Green Bay (Qualls et al. 2007), Oneida Lake (Mellina et al. 1995; Idrisi et al. 2001), Lake St. Clair (Mellina et al. 1995) and even rivers such as the Seneca River (Effler et al. 1996). Lower chl*a* concentrations post-dreissenids in the offshore of eastern basin Lake Erie have also been shown (Barbiero et al. 2006a). Examination of a long-term nearshore/offshore data set from Lake Ontario by Millard et al. (2003) detected decreases in chl*a* post-dreissenids, particularly in the nearshore. Looking more closely at the

northern nearshore of Lake Erie, a decrease in chl*a* concentrations from 3 μg L^{-1} to 1 μg L^{-1} was reported by Howell et al. (1996). Nicholls and Hopkins (1993) and Nicholls et al. (1997) reported a dramatic decline in chl*a* (>90%) in all basins post-dreissenids. The studies conducted by Nicholls and Hopkins (1993), and Nicholls et al. (1997, 1999a, 1999b) may not be representative of the northern nearshore of the east basin of Lake Erie due to the fact that they sampled the nearshore water at a 6 m depth through a 500 m long municipal water intake pipe at the Dunnville site. This sampling technique may over-estimate the impact of dreissenids on the nearshore as dreissenids located within the intake pipe were estimated to have reduced the phytoplankton density by an additional 50% at the sampling point (Nicholls and Hopkins 1993). As well, their Dunnville site is located at the mouth of the Grand River; a location I removed from the analysis due to the influences of the Grand River and mis-representation of a typical northern nearshore site. However, these studies were the only ones located in the northern nearshore of the eastern basin where my study was conducted and as they assessed similar parameters to my study, it is appropriate to include their studies in my discussion of the data.

Dreissenid impacts on chl*a* have also been examined in dreissenid-impacted sites such as bays or the nearshore of large lakes compared to non-dreissenid impacted sites such as the offshore environments of large lakes. In Saginaw Bay at dreissenid-impacted inner bay sites, post-dreissenid chl*a* concentrations decreased by 66% from pre-dreissenid concentrations, whereas for the same time periods no significant differences were found at non-impacted offshore Lake Huron sites (Fahnenstiel et al. 1995). Surveys of shallow habitats in western Lake Erie indicated that chl*a* concentrations were strongly depleted above mussel beds (MacIsaac et al. 1999). In nearshore versus offshore and pre-dreissenid versus post-dreissenid comparisons in Lakes Michigan (Carrick et al. 2001) and Ontario (Hall et al. 2003), the nearshore sites no longer

had higher chl*a* concentrations than the offshore sites and the concentrations in both the nearshore and offshore decreased post-dreissenids, with strong dreissenid-impact detected at nearshore sites. The Lake Michigan study was conducted in 1994-1995 (Carrick et al. 2001), and is interesting because they do not attribute any changes in the nearshore to dreissenids. However, there were high densities of *Dreissena polymorpha* present in the nearshore of the southern basin in 1993 (1,929 m^{-2}; Nalepa et al. 2001). Therefore, for the purposes of this study, I will assume that the nearshore of Lake Michigan in 1994-1995 was impacted by dreissenids.

Additional evidence for dreissenid impacts on seston relate to the size-selectivity of dreissenid grazing. Dreissenids filter a broad size spectrum of particles (MacIsaac 1996) ranging from as small as 0.36 μm (Cotner et al. 1995) to as high as 750 μm, although well-fed individuals have been shown to exhibit positive selection within the mantle cavity and/or stomach for particles from 15 to 45 μm (Ten Winkel and Davids 1982). As a result, picoplankton cells (0.2 – 2 μm) are not captured by dreissenids due to their small size and have been documented to contribute substantially to phytoplankton biomass in dreissenid-impacted areas as Bec et al. (2005) showed in extensively cultured aquaculture embayments. In post-dreissenid Oneida Lake, there was an increase in the number of small algal cells (<10 μm) relative to pre-dreissenid years (Mellina et al. 1995). This evidence was collaborated by Naddafi et al. (2007) who reported that phytoplankton ≤7 μm were not preferred by dreissenids and were rejected as pseudofaeces. However, they reported that the smaller cells in the pseudofaeces were still viable, resulting in a similar size distribution pre- and post-dreissenids. There is evidence of this occurring in the nearshore of the eastern basin of Lake Erie in my study as the picoplankton percentage of both chl*a* and particulate C were similar to the offshore in post-dreissenid years (2001-2003).

There is sufficient evidence that dreissenid grazing results in lowered chl*a* concentrations, however, do chl*a* concentrations approximate phytoplankton biomass? Chl*a* was not significantly correlated to the particulate fractions (C, N, P, Si; Guildford et al. 2005), nor phytoplankton wet biomass (Conroy et al. 2005b) in the offshore of Lake Erie. In fact, while chl*a* concentrations are decreasing post-dreissenids, phytoplankton wet biomass appears to be increasing (Conroy et al. 2005b). In the Bay of Quinte, post-dreissenid decreases in chl*a* at nearshore stations corresponded with no change in phytoplankton biomass (Nicholls et al. 2002). Similarly, the post-dreissenid nearshore of Lake Michigan had lower chl*a* concentrations, but no differences were found between nearshore and offshore phytoplankton biomass (Carrick et al. 2001). The only record of post-dreissenid phytoplankton biomass data for the nearshore of east basin Lake Erie is Nicholls and Hopkins (1993) at the Dunnville site. Their data shows that although there was a decrease in both chl*a* and phytoplankton biomass from pre-dreissenid years (1986-1987) to post-dreissenid years (1989-1990), the decrease in biomass was greater than the decrease in chl*a*. As well, they report the first significant impact of dreissenids at the Dunnville sampling site in the summer of 1990, which brings their post-dreissenid (1989-1990) data into question.

Particulate C concentrations can also be used as an indicator of phytoplankton biomass and my data showed significantly higher pre-dreissenid concentrations than post-dreissenid concentrations, as well as significantly lower concentrations in the nearshore of post-dreissenid Erie. However, there is concern that particulate C concentrations are not representative of living biomass and contain resuspended and detrital C. If this were true of my data, I would expect higher particulate C concentrations and C:P ratios in the nearshore, but in fact, the opposite is true (Figures 3.11 and 3.15). Upon further examination, I can determine if the particulate C is isotopically different in the offshore relative to the nearshore and associated tributaries. In the

summer of 2002, Upsdell (2005) determined that the $\delta^{13}C$ signatures of the east basin tributaries (Grand River, Nanticoke Creek and Sandusk Creek) were significantly lower than the nearshore of the eastern basin (including stations 504, 503, 502, 449; Figures 3.1 and 3.3) which suggests that my nearshore stations were representative of the lake, rather than allocthonous material. Additionally, there were no significant difference between the $\delta^{13}C$ signatures for nearshore versus offshore stations (represented by 23, 451, 443; Figure 3.1, and two additional stations located at maximum depths of 62 and 44 m). It appears that the nearshore and offshore particulate C are not different, and the nearshore particulate C is not heavily influenced by the tributaries. As well, the C:N ratios are similar across the nearshore, offshore, and tributaries, indicating that the suspended particulate matter is mainly derived from autochthonous sources, particularly plankton (Upsdell 2005). Therefore, it appears that the particulate C concentrations I measured approximate phytoplankton biomass in both the nearshore and offshore.

Another possibility for differences in chl*a* concentrations between nearshore and offshore could be a change in phytoplankton species composition to species with inherently lower chl*a* content in the nearshore. It is known that the cellular quota of chl*a* varies between species (Geider et al. 1997). Chl*a* cellular content also changes in response to changing light conditions for different phytoplankton groups (MacIntyre et al. 2002). Photoacclimation refers to the phenotypic adjustments that arise in response to variations in environmental factors. It is a physiological response that manifests as a reduction in photosynthetic pigment content in response to increased irradiance (MacIntyre et al. 2002). Examination of the differences in the basin-wide C:Chl*a* ratio between the nearshore and offshore of pre- and post-dreissenid years allowed me to assess photoacclimation as indicated by a large C:Chl*a* ratio (Figure 3.10; MacIntyre et al. 2002). It appears that the increasing post-dreissenid C:Chl*a* ratios are a reflection of decreasing chl*a* concentrations, as the particulate C concentrations are not increasing

in the eastern basin (data not shown). Evidence for dreissenid-induced photoacclimation has been reported in the post-dreissenid western basin of Lake Erie as evidenced by high C:Chl*a* ratios (15.9-32.2 μmol C μg chla^{-1}; Arnott and Vanni 1996).

In order for photoacclimation to occur, there has to be an improvement in light conditions. Although my data show no evidence of a post-dreissenid change in Secchi depths (data not shown), Secchi may not be the best indicator of light availability in nearshore areas due to shallow Z_{mix}. Measurements of k_d are an improved indication of light availability. Variability in k_d values increase at shallower depths (data not shown), perhaps due to increased potential for sediment resuspension. This may account for the lack of significant differences in k_d values between nearshore and offshore in post-dreissenid years. However, my data do show a significant decrease in offshore k_d values from pre-dreissenid years to post-dreissenid years. The k_d values that I measured were comparable to values reported by Smith et al. (2005) in 1997 in the offshore of the eastern basin. Evidence of an higher post-dreissenid light environment nearshore can be found in the mean PAR values. As there were no significant differences in k_d values between nearshore and offshore, mean PAR appears to be responding to changes in Z_{mix}.

There is sufficient evidence in the literature to conclude that dreissenid filtering results in improved light conditions. In Oneida Lake, k_d values were significantly higher pre-dreissenids compared to post-dreissenid years (Idrisi et al. 2001). The k_d values from a nearshore station in Lake Ontario also exhibited a 40% decline from pre-dreissenid to post-dreissenid years (Millard et al. 2003). In post-dreissenid Saginaw Bay, the dreissenid-impacted inner bay k_d values decreased by 35%, accompanied by a doubling in Secchi depth, while the outer bay (control) stations showed no significant differences in pre-dreissenid versus post-dreissenid years (Fahnenstiel et al. 1995). However, an alternative explanation for improved light conditions is

the uptake of calcium by dreissenids and the subsequent reduction in summer calcite precipitation events (whiting) as documented in Lake Ontario (Barbiero et al. 2006b). This is evidence that over time, Lake Erie phytoplankton may be responding to improving light conditions in the nearshore and photoacclimating by lowering their chl*a* content per cell.

Another possible exaplanation for lower chl*a* concenrations in the nearshore of the eastern basin is internal seiches or upwelling described section 3.2.2.1, which could potentially cause the north shore nearshore stations to experience periodic inputs of offshore hypolimnetic waters containing low chl*a* concentrations.

Grazing in the nearshore by zooplankton could also explain the lower seston concentrations. There is evidence that *Daphnia* grazing is more important than dreissenid grazing in the western basin of Lake Erie (Wu and Culver 1991). Wu and Culver (1991) found strong correlations between *Daphnia* grazing, edible algal dynamics and Secchi transparency that were similar pre- and post-dreissenids and proposed that a longer clear-water period would have occurred if increased transparency had been caused by dreissenids. Contradictory conclusions were reached by MacIsaac et al. (1992) after an examination of the filtration potentials of dreissenids and western basin Lake Erie zooplankton. MacIsaac et al. (1999) concluded that it is highly unlikely that vertical patterns in chl*a* in western Lake Erie resulted from zooplankton grazing, as the spatial distribution of zooplankton was opposite to that expected. Nicholls and Hopkins (1993) concluded that most of the chl*a* and phytoplankton declines in Lake Erie were attributed to dreissenids, not zooplankton, as evidenced by a dramatic decline in total phytoplankton in periods of typically low cladoceran grazing impacts. Reductions in chl*a* below expected levels are characteristic of north temperate lakes dominated by large *Daphnia*, which are generally believed to be more efficient grazers of algae than small zooplankton species (Mazumder 1994). If there are differences in herbivorous zooplankton biomass and composition

between the nearshore and the offshore of the eastern basin, it could explain the lower chl*a* concentrations in the nearshore. In Lake Ontario, the overall areal zooplankton biomass was highest in the offshore relative to the nearshore (Hall et al. 2003). In Oneida Lake, although the total zooplankton biomass did not change significantly pre- and post-dreissenids, the daphnid population clearance rates were considerably lower than dreissenid clearance rates (Idrisi et al. 2001). In the eastern basin of Lake Erie, zooplankton densities were higher in the nearshore than the offshore in 1993, 1994 and 1998, although this relationship was not significant (MacDougall et al. 2001). The western basin of Lake Erie had the highest grazing pressure on phytoplankton populations because its zooplankton community was dominated by cladocerans. In situ grazing experiments revealed that cladoceran species contributed >85% of zooplankton community grazing rate (Wu and Culver 1991). In the eastern basin of Lake Erie, the cladoceran densities were lower in the nearshore than the offshore; significantly so for *Daphnia* species. In fact, cladocerans formed a small percentage of the total density of zooplankton in the nearshore (15%) and offshore (36%) of the eastern basin of Lake Erie and composed a significantly lower percentage of the zooplankton population in the nearshore (MacDougall et al. 2001). Therefore, zooplankton grazing does not explain the lower chl*a* concentrations in the nearshore of the eastern basin of Lake Erie.

3.4.2 Changes in nutrient concentrations

If dreissenid grazing is lowering the phytoplankton biomass in the nearshore, then there should be a decline in particulate nutrients (P, N, Si), and possibly an increase in dissolved nutrients due to decreased uptake by phytoplankton. My particulate nutrient concentrations give a varied and non-significant difference depending on the survey. However, there is evidence of lower particulate nutrient concentations as a result of dreissenid grazing in Oneida Lake (Idrisi et al.

2001), Saginaw Bay (Johengen et al. 1995), the western basin of Lake Erie (Makarewicz et al. 2000) and mesocosm experiments (Heath et al. 1995; Wilson 2003).

Dissolved nutrient concentrations are higher in the nearshore post-dreissenids, which could be attributed to the internal seiches or upwelling described in section 3.2.2.1. This would cause the north shore nearshore stations to experience periodic inputs of offshore hypolimnetic waters containing higher dissolved nutrient concentrations, which could have potentially contributed to the higher nearshore dissolved nutrient concentrations. However, upon examination of hypolimnetic nutrient concentrations collected in combination with the epilimnetic nutrient concentrations in 2001-2003, I observed that most often, the dissolved nutrient concentrations in the hypolimnion were lower than the epilimnetic concentrations (data not shown).

The higher dissolved nutrient concentrations in the nearshore could also be a symptom of reduced phytoplankton biomass caused by dreissenid grazing. If this is true, I would predict that decreased dissolved nutrient uptake by phytoplankton would have the biggest impact on the most limiting nutrient to phytoplankton growth. Through the application of the P limitation indicators, I determined that the eastern basin of Lake Erie is becoming more P limited. This was evidenced by a significant increase in P limitation with C:P ratios significantly lower in pre-dreissenid compared to post-dreissenid years. This increasing trend is also apparent using N:P ratios (data not shown) and has also been reported for the western basin of Lake Erie (Makarewicz et al. 2000). There is only one year of pre-dreissenid measurements of P limitation using physiological indicators with which to compare to post-dreissenid indices. In 1979, Lean et al. (1983) evaluated the P status of three offshore stations in the eastern basin of Lake Erie. They found that the average phosphate turnover time (PO_4TT) was 120 minutes for the summer months, which is not considered to be P limited (Lean et al. 1983). The PO_4TT was also measured in 1997 at four

stations in the offshore eastern basin by Guildford et al. (2005). The average PO_4TT was 51 minutes for the summer months and was considered moderately P limited. The nearshore of Lake Erie also showed signs of becoming more P limited post-dreissenids. Nicholls et al. (1999b) showed that TN:TP ratios were higher at nearshore sites in the eastern basin of Lake Erie post-dreissenids (1990-1994) than pre-dreissenids (1984-1987). It is expected that post-dreissenid Erie would experience more P limitation than pre-dreissenid Erie, given the P reductions and subsequent decrease in TP concentrations.

In order to assess the impact of dreissenids on P limitation of the phytoplankton, a comparison of regions with and without dreissenids is necessary. Experimental studies indicate that dreissenids decrease the P limitation of the phytoplankton. Arnott and Vanni (1986) measured dreissenid excretion rates in the western basin of Lake Erie and found that mussels excreted N:P at levels below 16:1. Heath et al. (1995) reported that phytoplankton subjected to dreissenid grazing in mesocosms in Saginaw Bay had more P available to them per cell and measured this release from P limitation as a decline in the rate of phosphate uptake to less than 1% of the controls. My data also show that the offshore consistently experiences stronger P limitation than the nearshore. This confirms what I found in Chapter 2 in the east basin of Lake Erie where P limitation was stronger in the offshore than the nearshore. This also refutes the theory that the higher C:Chl*a* ratios in the post-dreissenid Erie nearshore are a reflection of higher nutrient deficiency.

Arnott and Vanni (1986) found that dreissenids excreted N:P at levels below 16:1, which may decrease P limitation but increase N limitation of the phytoplankton. The only evidence of dreissenids lowering dissolved N concentrations occurred in Oneida Lake, where post-dreissenid NO_3^- concentrations were slightly lower relative to pre-dreissenid years (Idrisi et al. 2001). In the nearshore of the eastern basin of Lake Erie, Nicholls et al. (1997) reported that the NH_4^+

concentrations were 74% lower in post-dreissenid years (1990-1993) relative to pre-dreissenid years (1984-1987). They concluded that the apparent reduction in NH_4^+ was caused by the cyclic supply of NH_4^+ from the watershed (Nicholls et al. 1999b). My data also showed that post-dreissenid NH_4^+ concentrations were significantly lower than the pre-dreissenid concentrations of 1973. However, the offshore NH_4^+ concentrations reported by Env. Can. in 1979, 1983 and 1984 were significantly lower than the offshore concentrations reported in 1973, bringing the 1973 data into question. It is possible that the high 1973 NH_4^+ concentrations are due to contamination during sampling and processing, however, they are comparable with concentrations reported for the east basin from 1967/1968 (5.0 μmol L^{-1}; Chawla 1971). My dissolved N concentrations in the nearshore were higher than they were in the offshore. In addition, in Chapter 2, I applied physiological indicators of N limitation to show that the nearshore of the eastern basin of Lake Erie was slightly less N limited than the offshore.

The only evidence of lower dissolved Si concentrations in post-dreissenid years coincided with the reduction in dissolved N concentrations in Lake Erie (Nicholls et al. 1997) and Oneida Lake (Idrisi et al. 2001).

Dissolved Fe concentrations were reported to be higher in the nearshore than the offshore of post-dreissenid Lake Erie (McKay et al. 2004). During the north shore survey in 2003 (Figure 3.3), I measured total dissolved Fe concentrations and found significantly higher concentrations in the nearshore; which was reflected in enrichment experiments where Fe limitation was detected in the offshore and not the nearshore (Chapter 2).

It appears that the Lake Erie offshore phytoplankton were more nutrient limited than the nearshore phytoplankton, and that the most limiting nutrient to phytoplankton growth is P. Therefore, greater differences between nearshore and offshore dissolved P concentrations would

be expected as a result of decreased dissolved nutrient uptake by phytoplankton subject to dreissenid grazing. I found that the largest differences in dissolved nutrients between nearshore and offshore for both surveys was NH_4^+ (39% higher nearshore), followed by SRP (28.5% higher nearshore), DRSi (19.5% higher nearshore) and TDP (14.5% higher nearshore). My data show that the difference in NH_4^+ concentrations between the nearshore and the offshore post-dreissenids is the largest of all of the dissolved nutrients, including dissolved P (TDP & SRP). The lack of post-dreissenid increases in dissolved P concentrations in the nearshore may be due to its high demand by bacteria, phytoplankton, and benthic algae. C:P ratios and APA show that the nearshore phytoplankton were less P limited than the offshore phytoplankton, therefore, the dreissenid-regenerated SRP could have been immediately removed by bacteria and phytoplankton as evidenced by experiments in Saginaw Bay (Gardner et al. 1995; Heath et al. 1995). In addition, the higher mean PAR in the nearshore facilitates the growth of benthic algae such as *Cladophora*, that exhibit P limited growth (Higgins et al. 2005). Therefore, the benthic algal drawdown of P in the nearshore may explain the lack of increase in dissolved P concentrations in the water column.

The excretion of NH_4^+(Quigley et al. 1993; Gardner et al. 1995; Heath et al. 1995; Arnott and Vanni 1996; James et al. 1997; Wilson 2003; Conroy et al. 2005a), and SRP (Quigley et al. 1993; Arnott and Vanni 1996; James et al. 1997) by dreissenids is well documented. There is also evidence of excretion of Si by mussel beds dominated by *Mytilus edulis* (Dame et al. 1991). Therefore, mussel excretion should result in higher concentrations of these nutrients in the nearshore relative to the offshore. In the eastern basin of Lake Erie, Upsdell (2005) reported that in 2002, the nearshore north shore survey stations (Figure 3.3) were on average, enriched in ^{15}N relative to the offshore signatures, which could be the result of dreissenid excretion of NH_4^+, which is ^{15}N enriched compared to NO_3^- (McCusker et al. 1999). Furthermore, the nearshore

stations had similar $\delta^{13}C$ and $\delta^{15}N$ signatures to dreissenid biodeposits collected from stations 504, 503, and 502 in 2002 (Figure 3.3; Szabo 2004; Upsdell 2005). This is evidence that dreissenid excrement is incorporated into particulate C and N at the nearshore stations.

Although higher DRSi concentrations in the nearshore could be due to reduced uptake by diatoms, it could also be caused by mussel excretion of Si (Dame et al. 1991). Post-dreissenid Si increases have been documented for Lake Ontario (Millard et al. 2003), western Lake Erie (Holland et al. 1995; Guildford et al. 2005)**,** eastern Lake Erie (Guildford et al. 2005; Barbiero et al. 2006a) and Saginaw Bay (Johengen et al. 1995). Johengen et al. (1995) also found higher Si concentrations at dreissenid-impacted inner bay sites relative to outer bay control sites. Carrick et al. (2001) reported no difference in dissolved Si concentrations between nearshore and offshore post-dreissenid Lake Michigan. However, this was different than pre-dreissenid years when dissolved Si was lower in the nearshore. The general trend for Si release from mussel beds is probably the result of phytoplankton cells breaking down as they are metabolized by mussels (Dame et al. 1991). Unfortunately, I cannot address whether there was a change in species composition of phytoplankton in the nearshore as diatoms are the only phytoplankton with Si requirements.

Contrary to Arnott and Vanni (1996), Dame et al. (1991) found that estuary mussel beds appear to have their greatest influence (as judged by short turnover time) on chl*a* and NH_4^+. This was collaborated by experiments conducted by Conroy et al. (2005a) that showed that *Dreissena bugensis* excrete at a higher N:P ratio than *Dreissena polymorpha*. As noted previously, in 2002, all of the dreissenids in the eastern basin were *Dreissena bugensis* (Patterson et al. 2005). Conroy et al. (2005a) reported that for size class 15-20 (the most abundant size class found in the eastern basin of Lake Erie in 2002 (Patterson et al. 2005)), *D. polymorpha* had excretion rates of 3.32 μg NH_4-N mg dry weight^{-1}day^{-1} and 0.420 μg PO_4-P mg dry weight^{-1}day^{-1} while *D. bugensis*

had excretion rates of 3.42 μg NH_4-N mg dry weight^{-1}day^{-1} and 0.168 μg PO_4-P mg dry weight^{-1}day^{-1}. In Lake Erie, shifting from dominance of *D. polymorpha* in 1998 to dominance by *D. bugensis* in 2003, caused a 9% increase in NH_4^+ and a 14% decrease in P excretion (Conroy et al. 2005a).

An additional explanation for the high dissolved N:P ratios in the nearshore could be increases in the ratio of N:P entering the eastern basin from the watershed and specifically, the Grand River. At the Dunnville monitoring station on the Grand River, NO_3^- concentrations have increased significantly (1980-1998), coinciding with a declining trend (1973-1997) in TP concentration and load (Environmental Monitoring and Reporting Branch 1998). My lake data reflect these trends and show a significant increase in $NO_3^- + NO_2^-$ and a significant decrease in TP concentrations from 1973-2003 in both the nearshore and the offshore of the eastern basin.

3.4.3 Conclusions

Dreissenid grazing is changing the seston and nutrient concentrations in the nearshore of the eastern basin of Lake Erie. This is evidenced by lower chla and particulate C concentrations, which are most likely due to a combination of photoacclimation by the phytoplankton and dreissenid grazing. Dissolved nutrient concentrations are higher in the nearshore post-dreissenids, with a higher dissolved N:P ratio, suggesting a combination of nutrient excretion by dreissenids as well as diminished nutrient demand by phytoplankton, which is counterbalanced by the nutritional needs of benthic macrophytes and periphyton.

The conceptual differences between the nearshore and offshore are best illustrated diagrammatically (Figure 3.16). Without direct evidence, I assume that phytoplankton biomass is the same nearshore and offshore. I also assume that dreissenid biomass is the same nearshore and offshore, however dreissenids will have a greater impact in the non-stratified shallow nearshore

regions and are isolated from the epilimnion by the thermocline during the summer months in the offshore. The combination of high dissolved nutrient concentrations and mean PAR in the nearshore, results in the excessive growth of benthic algae such as *Cladophora*. Cladoceran (*Daphnia*) densities are higher in the offshore, contributing to nutrient cycling. Exchanges of nutrients and epilimnetic biota are always occurring between the nearshore and offshore as evidenced by the water circulation patterns in the eastern basin.

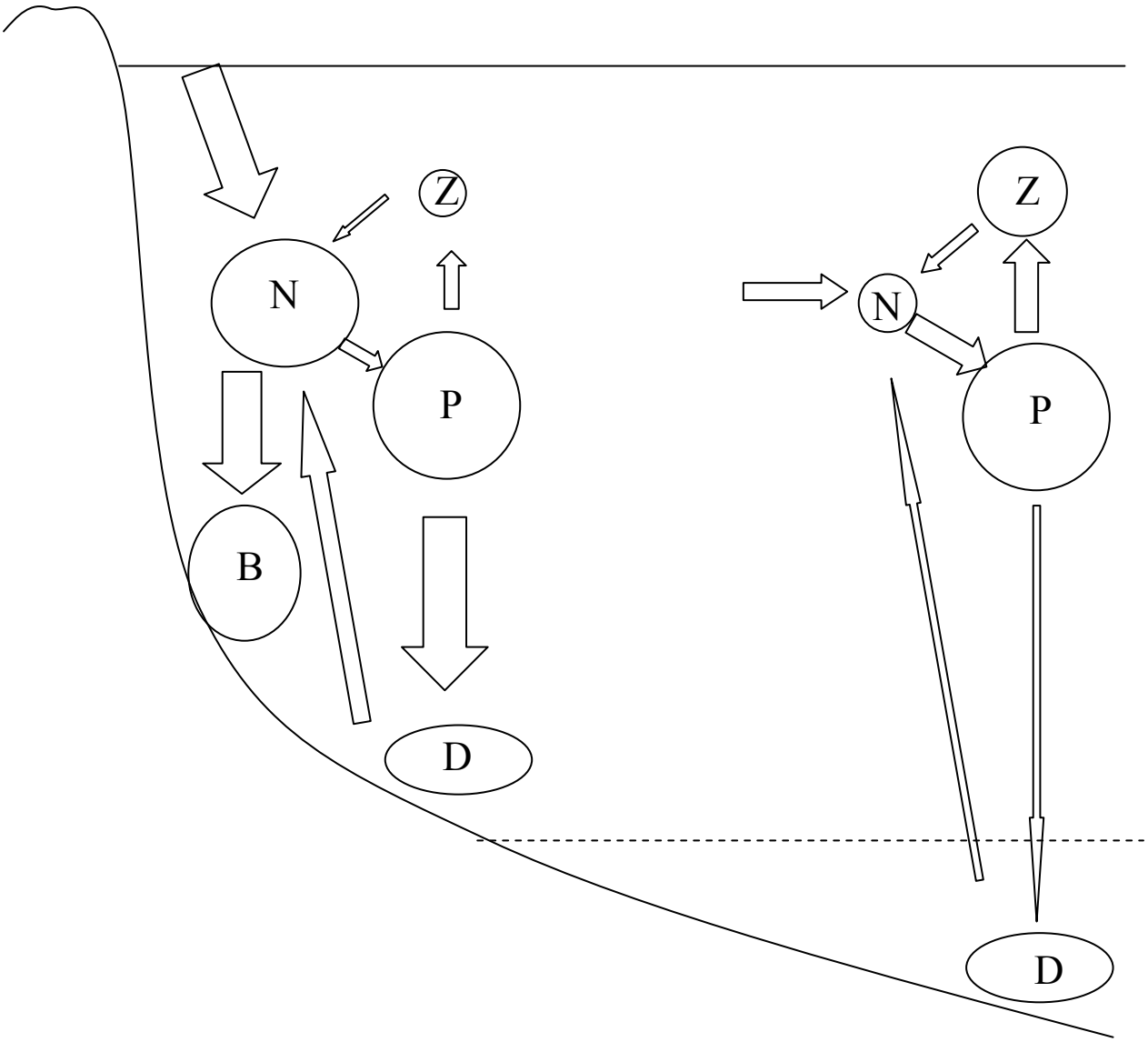


Figure 3.16 Diagram of conceptual differences between the nearshore and offshore regions of the eastern basin of Lake Erie. Circles represent major compartments for nutrients (P=phytoplankton, Z=zooplankton (representing cladoceran densities), D=dreissenids, B= benthic algae (dominated by *Cladophora*), N= dissolved nutrients (P, N, Si)). Arrows represent fluxes into and out of these compartments and dotted line represents the thermocline during stratified summer months.

I have documented and rationalized the differences in seston and nutrient concentrations between the nearshore and the offshore of the eastern basin of Lake Erie. I will now examine if these differences relate to changes in primary productivity. Smith et al. (2005) showed that offshore waters of post-dreissenid Lake Erie exhibited high primary production rates relative to TP when compared with dreissenid-free Great Lakes locations. A companion study to mine was conducted by Depew et al. (2006) who used a smaller subset of the basin-wide stations in 2001 and 2002. They reported significantly lower areal rates of primary production on a daily and seasonal basis in the nearshore relative to the offshore. They confirmed that dreissenids have the ability to depress primary production and influence the distribution of production between nearshore and offshore. However, their work was limited to the epilimnion of the nearshore and did not include the benthos. Davies and Hecky (2005) measured post-dreissenid (1997-1998) benthic photosynthesis rates in the nearshore of the eastern basin close to station 504 (Figure 3.3). They reported some of the highest photosynthetic rates ever measured in freshwaters and determined that areal benthic photosynthetic rates would greatly exceed areal pelagic photosynthesis in depths shallower than 5 m in the eastern basin. A key finding of their study was that algal-dreissenid mixed benthic assemblages can have greater gross photosynthetic rates than comparable algal communities without dreissenids and that benthic photosynthesis can quantitatively replace or even dominate over pelagic photosynthesis. With increasing light

transparency in the nearshore, benthic algae offset the loss of pelagic production in terms of the flow of trophic energy to consumers (Davies and Hecky 2005). Lowe and Pillsbury (1995) also concluded that the dreissenid occupancy of Saginaw Bay led to an increase in benthic primary production.

There is no basis for relaxation of P controls as suggested by Ryan et al. (1999) who expressed concerns regarding the decreasing TP concentrations on fish production. In Oneida Lake, despite the increases in dreissenid grazing rates and associated decreases in phytoplankton biomass, production at the primary, secondary, and tertiary levels did not decline in association with dreissenids; and no significant dreissenid effects were found on biomass, growth, or production of young-of-the-year yellow perch (Idrisi et al. 2001). Post-dreissenid Lake Erie remains highly efficient at translating TP into primary production, which is still higher than comparable oligotrophic lakes (Smith et al. 2005). As well, the presence of dreissenids in the nearshore may increase benthic primary production (Davies and Hecky 2005; Higgins et al. 2005) that may be beneficial in these nursery habitats of young fish. Although P limitation of phytoplankton is increasing in Lake Erie, Lake Erie is not that P limited relative to other systems (Guildford et al. 2005), thus any relaxation in P controls would not have a large impact on phytoplankton and may in fact lead to increases in N limitation. This may result in excessive algal growth due to the high NO_3^- concentrations in the lake, assuming sufficient quantities of bioavailable Fe are present (Chapter 2).

However, dreissenid effects may be diminishing in Lake Erie. Dreissenid densities have decreased significantly in the east basin in 2004 relative to 2001-2002 following a large increase in round goby (*Neogobius melanastomus*) abundance (Barton et al. 2005). In addition, the shift in dreissenid species from *D. polymorpha* to *D. bugensis* (Patterson et al. 2005), may reduce dreissenid effects as *D. polymorpha* have greater nutrient excretion rates than *D. bugensis*

(Conroy et al. 2005a). These changes may portend a decreased impact of dreissenids in future years as seen in freshwater systems of the former Soviet Union and eastern Europe (Karatayev et al. 1997), where dreissenids are well established. In these long-term studies, the initial populations of dreissenids grow to very high densities, but eventually overwhelm the carrying capacity of the ecosystem resulting in a decline in dreissenids as the populations stabilize (Karatayev et al. 1997). However, it is not clear whether diminished dreissenid abundance would necessarily reverse the changes associated with the nearshore shunt (Hecky et al. 2004).

3.5 Literature cited

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Chapter 4

Nitrogen, phosphorus, and iron colimitation of phytoplankton communities in the nearshore and offshore regions of the African Great Lakes

4.1 Introduction

Lake Victoria, Lake Tanganyika and Lake Malawi/Nyasa (hereafter referred to as Malawi) are the three largest lakes in Africa. These Great Lakes are threatened by expanding human populations, resulting in increased nutrient runoff due to land clearing and subsequent erosion (Bootsma and Hecky 1993). The consequences of increased nutrient loading to the lakes will depend partly on which nutrient(s) limit the phytoplankton, and the extent of the nutrient limitation.

Oligotrophic Lake Malawi is permanently stratified and mixes annually only to 200 m, resulting in a permanently anoxic hypolimnion with high nutrient concentrations (Bootsma and Hecky 1993). Denitrification at the oxycline is thought to cause extremely low nitrate (NO_3^-) and low total nitrogen (N) concentrations in the epilimnion (Hecky et al. 1996). As a result, Lake Malawi has been described as N-limited (Hecky et al. 1996, Guildford et al. 2007). Recently, there has been physiological evidence for co-deficiency of N and phosphorus (P) in the epilimnion (Guildford et al. 2000), and nutrient enrichment experiments have shown that iron (Fe) may be a potentially limiting nutrient in Lake Malawi as it stimulated N uptake (Guildford et al. 2003).

Lake Tanganyika is also oligotrophic and meromictic with anoxia occurring below 150 m depth, resulting in high concentrations of hypolimnetic nutrients and high denitrification rates. As a result, Lake Tanganyika is most likely to be N-limited (Edmond et al. 1993), although N and P colimitation has also been suggested (Järvinen et al. 1999).

The nearshore of eutrophic Lake Victoria is dominated by N_2-fixing cyanobacteria (Kling et al. 2001) to the point that they are self-shading and thus light

limited (Mugidde et al. 2003). Increased light levels in nutrient enrichment experiments resulted in N deficiency (Guildford et al. 2003), which others have also concluded is the most limiting nutrient in Lake Victoria (Lehman and Branstrator 1993).

Nitrogen and P colimitation (*sensu* Arrigo 2005) has been found in several freshwater lakes (Elser et al. 1990). Iron limitation of phytoplankton growth rates in vast oceanic regions characterized by high nutrient concentrations yet low chlorophyll concentrations is well documented (Tsuda et al. 2003). Recently, investigators have seen a response to Fe in freshwater African (Guildford et al. 2003) and North American Great Lakes (Twiss et al. 2000, Chapter 2). Iron is typically not limiting to biomass directly, yet NO_3^- assimilation has high Fe and energy requirements, thus ammonium (NH_4^+) is usually the preferred inorganic N source. Where NH_4^+ is scarce, phytoplankton NO_3^- metabolism and thus growth rates may be reduced by low bioavailable concentrations of Fe (Maldonado and Price 1996). Mills et al. (2004) suggested that Fe and P colimit N_2-fixation in the N limited eastern tropical North Atlantic. In chapter 2, I provided evidence for P, N and Fe colimitation in Lake Erie where the addition of Fe with P relieved Fe and P limitation and allowed NO_3^- assimilation, thereby alleviating N limitation as well.

The objective of this study was to determine the limiting nutrient(s) to the phytoplankton communities of the African Great Lakes Malawi, Tanganyika and Victoria in both the nearshore and offshore regions by measuring the physiological status of phytoplankton communities with respect to Fe.

4.2 Materials and methods

Lake Malawi was sampled at six nearshore (2-24 m) and two offshore (150-180 m) stations in 2001 and 2002. Lake Tanganyika was only sampled in 2004 at one nearshore (12 m) and two offshore (300-1100 m) stations. Lake Victoria was sampled at seven nearshore (8-26 m) and one offshore (60 m) station in 2001, 2002 and 2004. All three lakes were sampled during the thermally stratified season (September to December). The vertical attenuation coefficient (K_d; m^{-1}) for photosynthetically active radiation (PAR) was determined from the linear regression of the natural logarithm of irradiance versus depth. The mixing depth (Z_{mix}; m) and mean water column light intensity as a percentage of surface irradiance were calculated according to Guildford et al. (2000). Epilimnetic water was collected using either a Niskin bottle or a hand pump with tubing using trace metal clean techniques (detailed in Chapter 2). All sub-sampling occurred in a HEPA laminar flow hood under trace metal clean conditions.

Phosphorus and N limitation were determined by particulate C:P, N:P and C:N ratios, P and NH_4^+ debt assays (Table 4.1; Healey and Hendzel 1979b), alkaline phosphatase activity (APA; Healey and Hendzel 1979a) and a NO_3^- debt assay (detailed in Chapter 2). APA was measured according to the methods of Healey and Hendzel (1979a), with the exception that only total activity was reported. The NO_3^- debt assay follows the same methodology as P and NH_4^+ debt assays (Healey and Hendzel 1979b), except that $NaNO_3$ was added.

The photosynthetic efficiency of photosystem II can be determined by measuring the ratio of variable fluorescence to maximum fluorescence (F_v/F_m). This technique allows assessment of the physiological status of the phytoplankton and can be used as an

indicator of Fe stress (Behrenfeld et al. 1996), N limitation (Berges et al. 1996) and P limitation (Shelly et al. 2005). F_v/F_m was measured using a fluorometer (Turner 10-AU) by applying 3-(3,4-dichlorophenyl)-1,1-dimethylurea, a metabolic inhibitor of photosystem II, following the method of Neale et al. (1989). Photosynthetic efficiency experiments using measurements of F_v/F_m followed the addition of nutrients (NH_4^+, NO_3^-, P and Fe), and were used as an index of nutrient deficiency (Beardall et al. 2001).

Table 4.1 Nutrient limitation indicators. Values shown are indicative of presence or absence or degree of nutrient limitation for indicators used in this study. Criteria for nutrient limitation are based on Healey and Hendzel (1979b) and adapted from Guildford et al. (2007). * Criteria for APA are based on particulate activity (total – dissolved).

Indicator	Nutrient	No limitation	Moderate limitation	Extreme limitation	Limited
C:Chl *a* (µmol C µg chl a^{-1})	N or P	<4.2	4.2–8.3	>8.3	
N:P (atomic ratio)	P	<22			>22
C:P (atomic ratio)	P	<129	129-258	>258	
P debt (µmol P µg chl a^{-1})	P	<0.075			>0.075
APA* (µmol P µg chl a^{-1} h^{-1})	P	<0.003	0.003-0.005	>0.005	
C:N (atomic ratio)	N	<8.3	8.3–14.6	>14.6	
N debt (µmol NH_4^+ µg chla^{-1})	N	<0.15			>0.15

4.3 Results

The nearshore of Lake Malawi was colimited by P and N. Relative to the offshore, the light climate was better, and there were higher concentrations of nutrients and chlorophyll *a* (Table 4.2). The P debt, APA, C:P and C:N ratios indicated moderate P and N limitation. The nearshore NO_3^- debt was significantly lower than offshore, corresponding to the high observed total dissolved (<0.2 μm) Fe (TDFe) concentrations (Table 4.2). The offshore region of Lake Malawi was colimited by N, P and Fe. It was not as P deficient as the nearshore and was more N deficient. The significantly higher NO_3^- debt corresponded with the low TDFe concentrations (Table 4.2). Examination of one sample showed that 45.7 % of the offshore phytoplankton were cyanophytes with 4,000 heterocysts L^{-1}. The presence of heterocysts indicates that N_2-fixation was occurring in order to alleviate the N deficiency.

Table 4.2 Means and standard error in parentheses for measurements made at nearshore and offshore stations in Lakes Malawi, Tanganyika and Victoria from 2001-2004. NA = not available; BD= below detection (NH_4^+ detection limit (DL) =0.2 μmol L^{-1}, NO_3^- DL =0.36 μmol L^{-1}, SRP DL = 0.01 μmol L^{-1}); Bolded values indicate P or N limitation according to the nutrient limitation indicators (Table 4.1). Results of ANOVA between nearshore and offshore indicated by * for significant differences at $p<0.05$.

Parameter	Malawi		Tanganyika		Victoria	
	NS (*n*=11)	**OS (*n*=3)**	**NS (*n*=1)**	**OS (*n*=8)**	**NS (*n*=12)**	**OS (*n*=2)**
Z_{max} (m)	9* (2)	157* (7)	12	875 (149)	13* (2)	60* (0)
Z_{mix} (m)	3* (1)	28* (5)	NA	17 (4)	11* (2)	39* (21)
K_d (m^{-1})	0.38 (0.08)	0.11 (0.01)	0.21	0.17 (0.01)	1.42* (0.08)	0.47* (0.01)
pH	8.01 (0.09)	8.18	8.79	8.72 (0.06)	8.85* (0.08)	8.42* (0.10)
Secchi (m)	6.8* (1.4)	17.7* (1.5)	7.3	9.8 (0.4)	0.91* (0.05)	3.3* (0.4)
Mean PAR (%)	58 (17)	33 (6)	NA	35 (7)	10 (4)	8 (4)
TDFe (nmol L^{-1})	77.1 (30.4)	3.9	7.7	4.4 (1.0)	117.4 (25.3)	3.3 (0.8)
Particulate Fe (nmol L^{-1})	NA	NA	34.5	14.3 (5.4)	112.0 (29)	15.8 (12.4)
Particulate P (µmol L^{-1})	0.23 (0.07)	0.14 (0.04)	0.11	0.07 (0.01)	1.32* (0.18)	0.32* (0.02)
SRP (µmol L^{-1})	0.33 (0.11)	0.12 (0.01)	BD	0.04 (0.03)	0.18* (0.04)	1.65* (0.10)
Particulate N (µmol L^{-1})	2.6 (0.3)	1.4 (0.1)	2.0	1.7 (0.4)	21.6 (5.0)	5.6 (3.1)
NO_3^- (µmol L^{-1})	1.37 (0.91)	BD	BD	6.18 (3.20)	1.40 (0.58)	BD
NH_4^+ (µmol L^{-1})	2.70 (1.77)	0.25 (0.09)	BD	BD	BD	BD
Chl *a* (µg L^{-1})	1.19 (0.35)	0.50 (0.01)	2.42	1.48 (0.21)	52.90* (3.06)	10.42* (0.69)
Particulate C (µmol L^{-1})	24.36 (3.58)	14.63 (1.85)	23	20 (4)	148.07 (30.86)	52.24 (22.94)
C:Chl *a* (µmolC µg chl a^{-1})	**41.3** (12.2)	**29.4** (3.8)	**9.4**	**14.0** (1.9)	2.8 (0.6)	**4.9** (1.9)
N:P (atomic ratio)	12.4 (0.8)	12.0 (3.5)	17.6	**28.9** (10.3)	13.5 (2.6)	17.1 (8.9)
C:P (atomic ratio)	**134** (15)	127 (38)	**201**	**330** (92)	97 (14)	**161** (63)
APA (µmolP µg chla^{-1} h^{-1})	**0.058** (0.026)	**0.024**	**0.022**	**0.107** (0.041)	0.002* (0.000)	**0.010*** (0.001)
P Debt (µmol P µg chl a^{-1})	**0.25** (0.10)	**0.43** (0.09)	0.05	**0.58** (0.46)	0.02 (0.01)	0.01 (0.01)
C:N (atomic ratio)	**9.4** (0.5)	**10.4** (0.4)	**11.4**	**13.7** (1.5)	**8.4** (1.1)	**10.4** (1.7)
NH_4 Debt (µmol NH_4^+ µg chl a^{-1})	0.00 (0.00)	**0.62** (0.62)	0.04	0.12 (0.04)	0.04 (0.02)	0.04 (0.04)
NO_3 Debt (µmol NO_3^- µg chl a^{-1})	0.42* (0.21)	5.31*	2.26	2.79 (1.38)	0.03 (0.01)	0.00 (0.00)
F_v/F_m	NA	NA	0.53	0.46 (0.02)	0.56 (0.06)	0.57 (0.05)

The nearshore of Lake Tanganyika was colimited by N and P. The nearshore and the offshore were similar, both with good light climate and low nutrient concentrations, although higher chlorophyll *a* concentrations were found in the nearshore. The APA, C:P and C:N ratios indicated moderate P and N deficiency in the nearshore. The nearshore NO_3^- debt was lower, and the TDFe concentrations were slightly higher than the offshore (Table 4.2). Photosynthetic efficiency (F_v/F_m) was correlated with TDFe concentrations for all three lakes (R^2=0.892, p=0.000), suggesting an important influence of Fe availability. In the nearshore of Lake Tanganyika the photosynthetic efficiency was close to the theoretical limit of 0.65 (Table 4.2) and F_v/F_m did not respond to nutrient additions (data not shown). The offshore of Lake Tanganyika was colimited by P, N and Fe. The C:N ratios indicated moderate N limitation, and all P limitation indicators were higher than nearshore (Table 4.2). Examination of three samples of offshore phytoplankton showed that 25.1 % were cyanophytes with 9,400 heterocysts L^{-1}, which indicate N_2-fixation was occurring. Evidence of Fe limitation included lower TDFe and particulate Fe concentrations and lower photosynthetic efficiency relative to the nearshore (Table 4.2). N, P and Fe additions increased the F_v/F_m from the initial measure by 8.5%, with Fe alone resulting in a smaller stimulation (Figure 4.1). N and P alone had no significant effect, consistent with Fe, N and P colimitation.

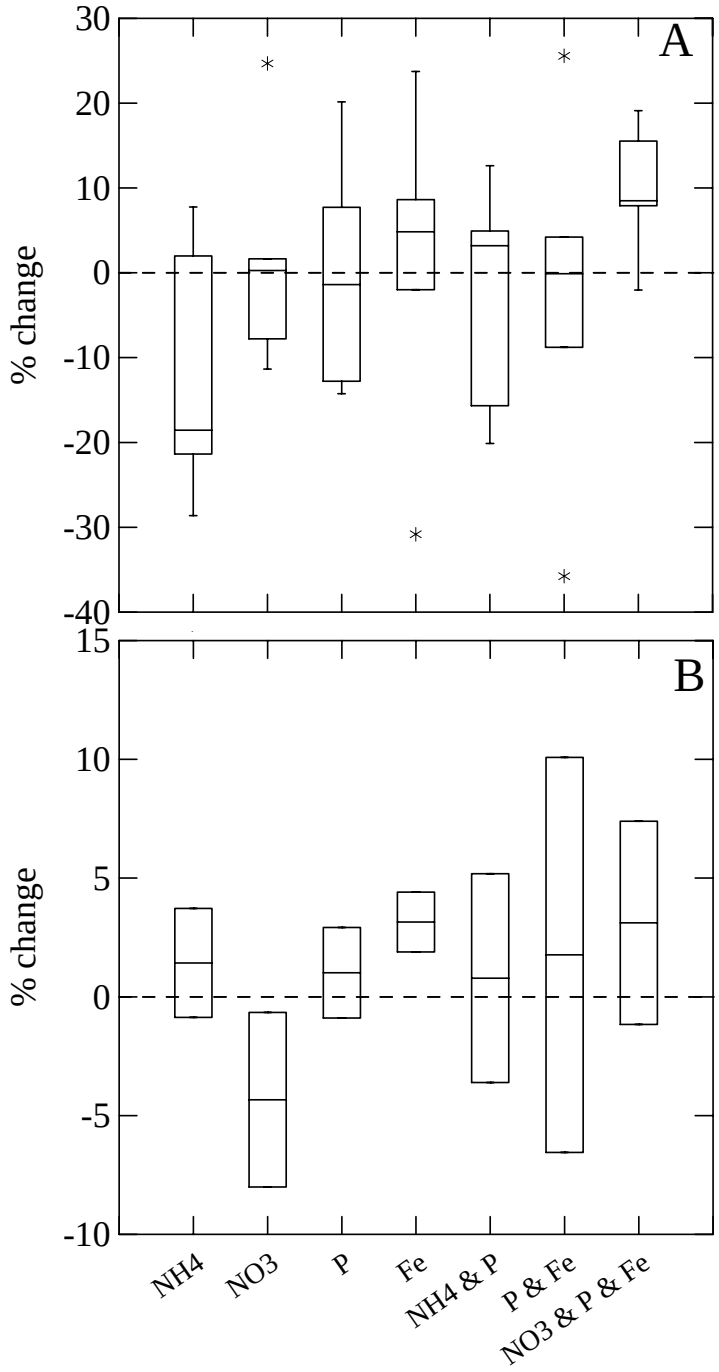
A
B
% change
% change
30
20
10
0
-10
-20
-30
-40
15
10
5
0
-5
-10
NH4
NO3
P
Fe
NH4 & P
P & Fe
NO3 & P & Fe

Figure 4.1 Box plots with median and interquartile range for photosynthetic efficiency experiments showing percent change in F_v/F_m from initial values. A= Offshore Lake Tanganyika (*n*=5), B=Offshore Lake Victoria (*n*=2).

The nearshore of Lake Victoria was limited by N only. It had a poor light climate with a significantly higher K_d and a significantly smaller Secchi depth than the offshore. It had low nutrient concentrations and significantly higher chlorophyll *a* concentrations than the offshore station. The C:N ratios indicated moderate N limitation and no evidence of P limitation was found (Table 4.2). The NO_3^- debt was low in both the nearshore and the offshore (Table 4.2), and examination of two samples of the nearshore phytoplankton found that 86.6% of the phytoplankton were cyanophytes with 6,700,000 heterocysts L^{-1}; the highest value in all three lakes. There was no evidence of Fe limitation in the nearshore due to the higher TDFe concentrations, higher particulate Fe concentrations, and similarly high F_v/F_m observed at nearshore stations relative to the offshore stations (Table 4.2). F_v/F_m increased for every combination of nutrients added, with no significant differences between additions (data not shown). It is concluded that the offshore of Lake Victoria was colimited by N, P and Fe. The C:N ratios indicated moderate N limitation and stronger P limitation than the nearshore according to C:P ratios and a significantly higher APA, although the SRP concentrations in the offshore were significantly higher (Table 4.2). A sample of the offshore phytoplankton showed that 93.1 % of the phytoplankton were cyanophytes with 96,000 heterocysts L^{-1}, further evidence for N limitation in the offshore region. Evidence of Fe limitation included lower TDFe and particulate Fe concentrations than the nearshore (Table 4.2). N, P and Fe colimitation was reflected in the response of F_v/F_m to nutrient additions (Figure 4.1).

The F_v/F_m response was positive for the addition of NH_4^+, and was negative for the addition of NO_3^-; further suggesting an Fe interaction (Figure 4.1).

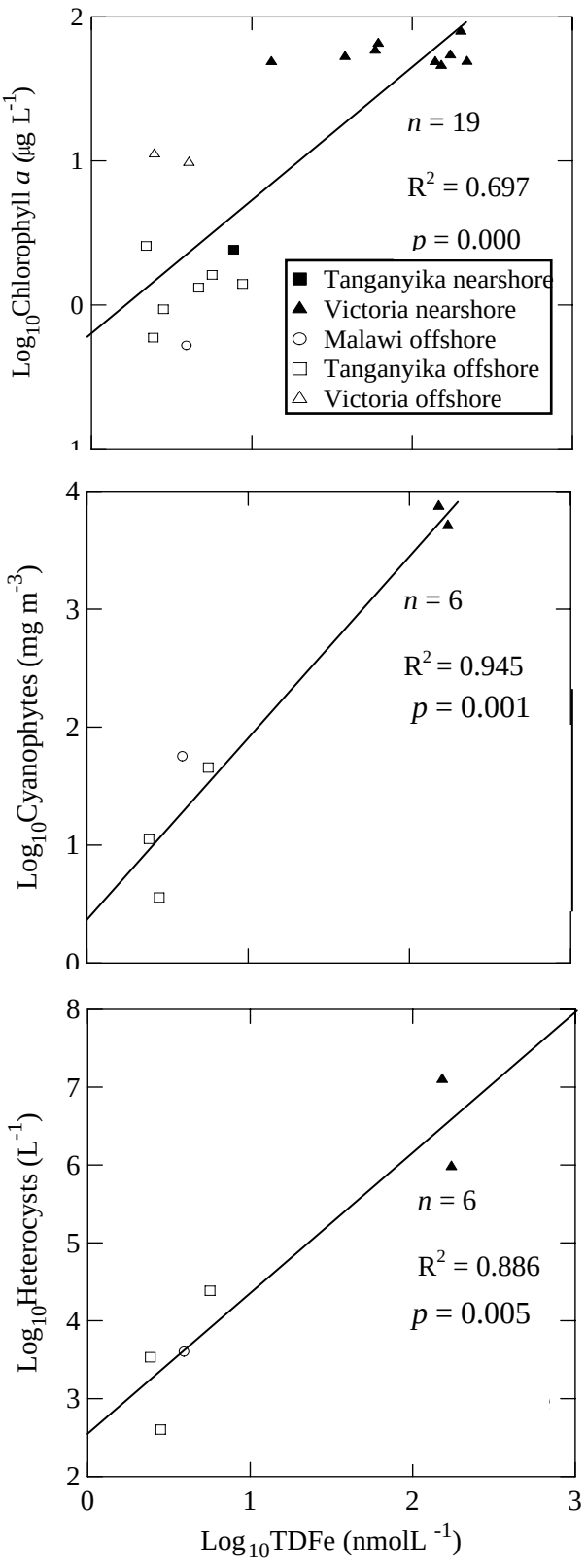
Log10Chlorophyll a (µg L-1)
n = 19
R2 = 0.697
p = 0.000
Tanganyika nearshore
Victoria nearshore
Malawi offshore
Tanganyika offshore
Victoria offshore
Log10Cyanophytes (mg m-3)
n = 6
R2 = 0.945
p = 0.001
Log10Heterocysts (L-1)
n = 6
R2 = 0.886
p = 0.005
Log10TDFe (nmolL -1)

Figure 4.2 Relationship of phytoplankton biomass proxies to total dissolved iron (TDFe). Chlorophyll a-TDFe, cyanophytes-TDFe and heterocysts-TDFe relationships (log10-transformed data) for the nearshore and offshore of Lakes Malawi, Tanganyika and Victoria.

Chlorophyll *a* concentrations, cyanophyte biomass, and number of heterocyst cells L^{-1} increased significantly with TDFe across all sites (Figure 4.2). Chlorophyll *a* concentrations were not significantly correlated with SRP (n=19, R^2=0.050, p= 0.359) or NH_4^+ (n=13, R^2=0.167, p=0.166). However, chlorophyll *a* concentrations were significantly negatively correlated with NO_3^- concentrations (n=8, R^2=0.707, p=0.009), suggesting Fe limitation of NO_3^- assimilation.

4.4 Discussion

Differences in phytoplankton biomass, composition, and nutrient limitation were found between and within the African Great Lakes Malawi, Tanganyika, and Victoria. In the offshore of Lake Malawi, my nutrient limitation indicators agreed with Guildford et al. (2007), and support for colimitation by N, P and Fe was provided by Guildford et al. (2003). Gondwe et al. (2007) showed that rates of N_2-fixation in Lake Malawi were generally higher nearshore than offshore. The results of this study suggest that higher Fe bioavailability in the nearshore contributes to these high N_2-fixation rates. My conclusion of P and N colimitation in Lake Tanganyika is supported by Järvinen et al. (1999) who examined the nutrient limitation of phytoplankton in the offshore. The results of nutrient enrichment experiments conducted in both the nearshore and offshore of Lake Victoria support my conclusions that the nearshore is primarily limited by N, while the offshore exhibits signs of N, P and Fe colimitation. In offshore Lake Victoria,

Lehman and Branstrator (1993) reported that the addition of N alone or in combination with P and sulfur in enrichment experiments increased algal biomass relative to control treatments. They also concluded that N was the most limiting nutrient to the phytoplankton in the nearshore. Guildford et al. (2003) reported more N limitation nearshore and more P limitation offshore. They also found evidence for Fe limitation as Fe additions stimulated N uptake and N_2-fixation in both the nearshore and offshore of Lake Victoria. My offshore Lake Victoria photosynthetic efficiency experiments showed that the addition of NO_3^- caused the phytoplankton to become Fe limited, as shown by the decrease in photosynthetic efficiency. Evidence of high N_2-fixation in the nearshore was provided by Mugidde et al. (2003) who found that rates of annual areal N_2-fixation were twice as high inshore as offshore. Rates of N_2-fixation observed in Lake Victoria were ~50 and 38 times higher inshore and offshore respectively than those in Lake Malawi (Gondwe et al. 2007).

My data show that Fe limitation only occurs in the offshore. This is consistent with greater Fe availability to the nearshore area due to proximity to terrestrial nutrient sources (fluvial inputs, erosion, vertical mixing of the water column contiguous with bottom sediments). An Fe limitation continuum appears to exist that ranges from Fe limited to no detectable Fe limitation from the offshore of Lakes Malawi and Tanganyika, followed by nearshore Tanganyika, offshore Victoria, and nearshore Victoria. Low TDFe leads to low biomass, but high TDFe enables high concentrations, implying a limiting role for Fe. Given that TDFe is a better predictor of algal biomass than SRP or NH_4^+ in these lakes (data not shown), the strong correlation between Fe and chlorophyll *a* illustrates the key nutritive role of Fe in these lakes (Figure 4.2). However, this

relationship should be interpreted with caution as the relationships between chlorophyll *a* and P and N total dissolved forms (TDP, TDN) were not tested. The significant negative relationship between chlorophyll *a* and NO_3^- (data not shown) is supportive of my conclusion regarding the importance of Fe to phytoplankton for the uptake of NO_3^- under conditions of N limitation. Dominance of cyanophytes in aquatic systems is undesirable as they are a poor nutritive food source to zooplankton, and some are potentially toxigenic. Due to the strong dependence of N_2-fixing cyanobacteria on Fe, the correlation between the two parameters is expected (Figure 4.2). Although this relationship is well understood in the oceans (Mills et al. 2004), it is currently understudied in freshwaters.

I predict that continuing environmental degradation in the African Lakes catchment areas will result in increased P and Fe loading to the lakes, creating a higher N demand that will result in a continued shift in the species composition to more N_2-fixing filamentous cyanobacteria, an inferior food source to higher trophic levels.

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Chapter 5 Overall Conclusions and Synthesis

5.1 Conclusions

The overall conclusion of my thesis is that phytoplankton are more nutrient limited in the offshore of large lakes, irrespective of the fact that there are dreissenids impacting the nearshore. As well, nutrient colimitation of nitrogen (N), phosphorus (P) and iron (Fe) is more common in the offshore of Great Lakes than a single limiting nutrient as previously thought, and Fe is an important modifier of N and P uptake.

I feel that these three data chapters are significant contributions to aquatic science and are representative of the broad spectrum of my Ph.D. work which encompasses nutrient colimitation in Lake Erie and the African Great Lakes, to the impact of dreissenids on water quality parameters in the eastern basin of Lake Erie. I have examined real research questions that have long reaching consequences to aquatic ecosystem health worldwide.

5.1.1 Chapter 2 summary and significance

This chapter is important because it is the first study to examine the coordinated role P, N and Fe play in the colimitation of Lake Erie phytoplankton communities. One of the main messages of this chapter was that colimitation is present in both freshwaters and the oceans, contrary to the traditional paradigm of N limitation in the oceans and P limitation in freshwaters. The application of nutrient limitation indicators to Fe enrichment experiments is a relatively new approach to looking at Fe as a limiting nutrient. To my knowledge, this chapter is one of the first studies to use nutrient limitation indicators to assess the use of Fe for nitrate (NO_3^-) acquisition and assimilation in freshwaters. These results have global implications as NO_3^- levels in the Laurentian Great Lakes are

experiencing an unprecedented increase in recorded history of the lakes. The impact of this work is the introduction and provision of evidence for P, N and Fe colimitation of phytoplankton in a freshwater lake. Acknowledging that colimitation does exist will significantly impact the interpretation and practice of nutrient limitation studies in aquatic sciences.

5.1.2 Chapter 3 summary and significance

This work is important because it is the first to examine the changes in seston and nutrient concentrations in the eastern basin of Lake Erie using pre- and post-dreissenid data for both the nearshore coastal zones and offshore regions of the lake. Temporal and spatial surveys concluded that the nearshore-offshore relationships have changed significantly since P reductions and the invasion of dreissenids. Dreissenid grazing is changing the seston and nutrient concentrations in the nearshore of the eastern basin of Lake Erie. This is evidenced by lower chl*a* and particulate C concentrations, which are most likely due to a combination of photoacclimation by the phytoplankton and dreissenid grazing. Dissolved nutrient concentrations are higher in the nearshore post-dreissenids, with a higher dissolved N:P ratio, suggesting a combination of nutrient excretion by dreissenids as well as diminished nutrient demand by phytoplankton, which is counterbalanced by the nutritional needs of benthic macrophytes and periphyton.

5.1.3 Chapter 4 summary and significance

This research is important in two ways. Firstly, it supports the understudied concept of the role of Fe as a limiting nutrient in freshwaters, and in particular, in N limited systems such as the African Great Lakes. Trace metal work, specifically Fe, is novel research for the African Great Lakes. In this chapter, I reported the total dissolved Fe (TDFe)

concentrations in Lakes Malawi, Tanganyika and Victoria for the first time using trace metal clean techniques. Secondly, I identified the limiting nutrient(s) to the phytoplankton communities of the African Great Lakes Malawi, Tanganyika and Victoria in both the nearshore and offshore regions by measuring the physiological status of phytoplankton communities. This chapter introduces the novel concept of N, P and Fe colimitation in African Great Lakes. My data shows that Fe limitation only occurs in the offshore and demonstrates a strong correlation between total dissolved Fe concentrations and chlorophyll *a* that speaks to the nutritive role of Fe in these lakes. I also show a significant correlation between cyanobacteria, heterocysts, and TDFe. Dominance of cyanobacteria in aquatic systems is undesirable as they are a poor nutritive food source to zooplankton, and some are potentially toxigenic.

5.2 Evaluation of nutrient limitation indicators

Every nutrient limitation indicator or methodology has its drawbacks, and the utilization of multiple indicators at multiple ecosystem levels is ideal in order to properly assess nutrient limitation of phytoplankton. I applied a suite of nutrient limitation indicators throughout my thesis to determine the availability of specific nutrients that are undetectable using chemical analyses. Hameed et al. (1999) concluded that nutrient limitation indices added little to conclusions that could be drawn from water chemistry alone. They also reported that the use of any single indicator could give a different conclusion from use of another during particular seasons, and concluded that nutrient limitation indices did not always accord with one another, though were not severely misleading (Hameed et al. 1999). They recommended that despite the subtleties and fluctuating response suggested by the physiological indicators, the overall conclusions

were correct (Hameed et al. 1999). I had the opportunity to apply the indicators under different nutrient regimes and the following is a summary of which ones were best under specific situations.

5.2.1 Phosphorus limitation indicators

Stoichiometric or seston composition ratios are simply the ratio of C:P or N:P that can be used to assess the relative concentration of each nutrient in the water column based on the elemental origin of nutrients (Sterner and Elser 2002). They represent a long-term view of nutrient status relative to physiological indicators such as alkaline phosphatase activity (APA) or P debt. I found the C:P and N:P ratios always correlated well with each other, indicating they are representative of the phytoplankton biomass, and most often correlated well with APA. Similar conclusions were reached by Hameed et al. (1999) who found that conclusions drawn from seston ratios were consistent with the results of physiological indicators.

The two physiological P indicators applied throughout my thesis were APA and P debt. I found that when APA was applied to Lake Erie waters, it provided an accurate representation of P limitation. However, the use of APA in the African Great Lakes Malawi, Tanganyika and Victoria revealed no P limitation, even under situations of high P debt. Most often the particulate activity typically reported (Healey and Hendzel 1979) was a negative number, as a result of the dissolved activity being higher than the total activity. The fact that such high dissolved APA occurred in all of the African Lakes representing very different nutrient regimes, and not in Lake Erie was very curious. The APA assay is experimental, and the APA measured values represent maximum values under optimum conditions of temperature and substrate concentration and thus reflect

potential rather than real *in situ* rates (Pick 1987). It is also doubtful that phosphatases have the same affinity for artificial substrates as they have for natural ones (Jansson et al. 1988). When assessing natural populations, it should be noted that some microorganisms lack a phosphatase inducible by P deficiency (Healey and Hendzel 1979). Additionally, alkaline phosphatases can be induced by starvation for pyrimidines or for guanine and not just by lowering the internal PO_4^{3-} pool (Jansson et al. 1988). Alkaline phosphatases are not the only enzymes produced that can cleave phosphate from the APA substrate. The enzyme 5'-nucleotidase can also catalyze the hydrolysis of phosphoryl groups from 5'-nucleotides, such as ATP, from the dissolved organic P pool in natural waters (Bjorkman and Karl 2003). Thus, nucleotidase activity may be more important to P regeneration in oligotrophic habitats than phosphatase activity (Cotner and Wetzel 1992).

An additional issue with the use of APA as an indicator of P deficiency is the variability and abundance of dissolved phosphatase enzymes, as I discovered in the African Great Lakes. Phosphatases are located on the cell surface or in cell membranes, however, the release of extra-cellular phosphatases in cultures is frequently reported (Jansson et al. 1988). This could simply be a result of cell breakage through filtration (Bentzen and Taylor 1991), as hypothesized by Healey and Hendzel (1979) who found that the filamentous, delicate diatom samples averaged 24% soluble activity while the single-celled blue-greens averaged only 8%. However, Kobori and Taga (1979) reported release of extra cellular phosphatases from actively growing marine bacteria. Dissolved phosphatase activities vary from zero (Berman 1970) to 100% (this author, unpublished results) of total activity. Diurnal variations in dissolved alkaline phosphatase enzyme production have been presented as an explanation for the variability (Jansson et al. 1988).

Seasonal variations also exist as Pettersson (1980) found that dissolved APA as a percent of total changed seasonally. Rai and Jacobsen (1993) found that dissolved APA activity was responsible for the majority of total activity before the spring turnover and after the fall turnover and accounted for 25-50% of total activity during stratification. Phosphatases also fluctuate spatially; Pettersson (1980) found that the dissolved activity was generally below 35% of the total activity, but at 19 m depth the contribution from dissolved phosphatases occasionally rose to 100%. This is contradictory to the results published by Healey and Hendzel (1979) that showed no trend with season or change of species composition. Reichardt et al. (1967) suggested that the decrease in the ratio of total to soluble APA corresponded to the region of maximum mineralization of algal remains.

Alkaline phosphatases may also be related to the food quality of particles ingested by zooplankton (Wynne and Gophen 1981). Phosphatase activities in zooplankton increase upon starvation (Jansson 1976; Wynne and Gophen 1981). Thus, a biological feedback mechanism exists whereby starving zooplankters produce and excrete phosphatases, which may in turn accelerate phytoplankton growth by supplying orthophosphate from suitable organic esters (Jansson 1976; Wynne and Gophen 1981; Boavida and Heath 1984). Characterization of phosphatases excreted by zooplankton using gel filtration (Jansson 1976), gel electrophoresis (Wynne and Gophen 1981) and anion exchange chromatography (Boavida and Heath 1984) indicates that enzymes released from zooplankton are indeed produced by the animals themselves and not by components of their food. In fact, Jansson (1976) found that 50% of the dissolved phosphatase activity was identical with phosphatases released by zooplankton. He was

also able to show different AP activities among different zooplankton species. Further evidence has been shown by Boavida and Heath (1984) wherein the enzyme activity released by *Daphnia magna* exceeded the amount of enzyme that could have been released from the small amount of algal cells present by three orders of magnitude. As with nucleotidase activity, phosphatases excreted by zooplankton seem to be less inhibited by phosphate than algal phosphatases (Jansson 1976; Boavida and Heath 1984; Jansson et al. 1988). In fact, Pick (1987) reported that most authors find no relationship between APA and ambient SRP concentrations and suggests that phosphate levels that inhibit APA are all higher than maximum SRP measurements, contrary to Smith and Kalff (1981). No relationship was found in a comparative study in the Broads and Meres of England between APA (total, specific, dissolved) and P concentrations in the water, however, the total and dissolved APA followed each other closely (Hameed et al. 1999).

I also applied the P debt assay developed by Healey (1978) to all four Great Lakes. Healey (1978) showed that a variety of algae take up more of a limiting nutrient when nutrient deficient, than when nutrient sufficient. The P debt assay measures the phosphate removed over a 24-hour period per unit of chlorophyll *a* (Healey 1978) and involves the addition of phosphate to whole lake water. Although this assay has been applied to a variety of aquatic systems and had been used as a good comparison tool to compare algal P nutrition in a variety of lakes (Guildford et al. 1994), I found that it did not correlate well with APA or C:P, N:P ratios in Lake Erie (Chapter 3; Guildford et al. 2005). I believe this may be due to a chemical complexing event involving the natural chelators present in the lake, particularly in the nearshore, that remove the phosphate from solution. Regressions between P debt and specific APA in the Broads and Meres of

England showed significant relationships, but there were significant relationships between P debt and TP only for one system (Hameed et al. 1999).

5.2.2 Nitrogen limitation indicators

The C:N seston ratio was combined with the physiological N limitation indicators NH_4^+ debt and NO_3^- debt, in order to assess N limitation in all four Great Lakes. I found the C:N and NH_4^+ debt indicators correlated well and gave positive responses to N limitation. A comparative study in the Broads and Meres of England revealed that NH_4^+ debt tended to follow the pattern of the dark NH_4^+ uptake rate (Hameed et al. 1999). I also developed an additional N debt assay that involved the addition of NO_3^-, instead of NH_4^+ in order to be representative of the Great Lakes where NO_3^- is the most abundant form of inorganic N. The NO_3^- debt assay also reflected the Fe-N interactions in the lakes, although there was no existing deficiency criterion. The NO_3^- debt assay worked well in the African lakes where NO_3^- concentrations were low, but was not effective in Lake Erie where NO_3^- concentrations exceeded the demand by phytoplankton.

5.2.3 Iron limitation indicators

The photosynthetic efficiency of photosystem II can be determined by measuring the ratio of variable fluorescence to maximum fluorescence (F_v/F_m). This technique allows assessment of the physiological status of the phytoplankton and can be used as an indicator of Fe stress (Behrenfeld et al. 1996), N limitation (Berges et al. 1996), and P limitation (Shelly et al. 2005). Kolber and Falkowski (1993) have shown that F_v/F_m is a rapid indicator of Fe stress and is usually one of the first parameters to respond to changes in Fe concentrations. However, decreases in F_v/F_m have been observed during N-, P-, or Fe-starvation in a range of algal taxa ((La Roche et al. 1993; Berges et al. 1996;

McKay et al. 1997), therefore, it is difficult to determine which nutrient is limiting as changes in F_v/F_m occur. I was able to rectify this situation through the application of photosynthetic efficiency experiments in Lakes Tanganyika and Victoria in 2004. Measurements of photosynthetic efficiency (F_v/F_m) following addition of nutrients (NH_4^+, NO_3^-, P and Fe) were used as an index of nutrient deficiency as F_v/F_m recovers following re-supply of the limiting nutrient (Geider et al. 1993). Thus, measurements of F_v/F_m following re-supply of nutrients are an important index of nutrient status (Beardall et al. 2001).

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