Traditional Methods and New Fluorometric Methods to Determine Phytoplankton Nutrient Status for Freshwater Ecosystems, and Their Application in the Lower Laurentian Great Lakes

by

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AUTHOR'S DECLARATION

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

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Abstract

The Laurentian Great Lakes are the largest system of freshwater on earth containing 22% of the world's supply. Although part of a single system, each lake shows substantial variation regarding physical, chemical and biological parameters. The main goals of this thesis were to characterize the nutrient status of natural phytoplankton communities while comparing several commonly used measurements of nutrient status and Chlorophyll a (Chl a) fluorescence measurements. The study sites include the western basin (WB), west-central basin (WCB), and central basin (CB) of Lake Erie, the Bay of Quinte in Lake Ontario, and Colpoys Bay in Lake Huron. Independent measures of nutrient status were assessed by measurements of nitrogen (N) debt, phosphorus (P) debt, particulate C:N:P ratios, and alkaline phosphatase activity (APA). Variable fluorescence of chlorophyll *a* was measured by pulse amplitude modulated (PAM) fluorometry and fast repetition rate (FRR) fluorometry in parallel with the independent measures. In 2005, the phytoplankton communities in Lake Erie were generally N deficient in May, P deficient in June, and neither N nor P deficient in September. The maximum dark adapted quantum yield (F_v/F_m) measured by PAM or FRRF was lower in May and June, and maximal in September, while the functional absorption cross section of photosystem II (σ_{PSII}) was maximal in May and June, and minimal in September. Relationships between the variable fluorescence indicators and independent measures of nutrient status showed strong associations with N or P deficient sites having low F_v/F_m and high σ_{PSII}. In 2006, the electron transport rate (ETR) and the initial slope (α) derived from the PAM fluorescence rapid light-response curves (RLC) were compared to independent measures and F_v/F_m measurements in Lake Erie. Relationships between ETR, α, independent measures of nutrient status, and F_v/F_m

measurements revealed strong associations with nutrient status. Confirming previous reports, N deficiency was highest in the WB during isothermal conditions while P deficiency was highest in the CB during summer stratification. The fluorescence parameters generally decreased as the severity of N and P deficiency increased. N and P enrichment assays also revealed increased values of F_v/F_m, ETR, and α from N and P deficient samples over twenty-four hours. Additionally, spatial variability of P status was evaluated during summer stratification. Colpoys Bay, the most oligotrophic site, had the strongest P deficiency, and evidence for existence of P deficiency was weakest in the Bay of Quinte, the most eutrophic site. Nutrient enrichment assays revealed that all fluorescence parameters showed a positive response to P additions in oligotrophic sites, with no response in eutrophic sites. Community structure was also associated with nutrient status and Chl a fluorescence at all locations. In P deficient sites, nano-flagellates such as chrysophytes and cryptophytes were prevalent; cyanobacteria were dominant at sites that displayed N deficiency.

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Last but certainly not least, I would like to thank my family, Sterling and my "peeps" for their support and love.

Dedication

I dedicate my dissertation research to my parents, Jatinder Rattan and Surjit Rattan. Thank you for your unconditional love, support, and guidance. The gods only know how grateful I am to have you both in my life.

When you get to the end of your rope, tie a knot and hang on. – Franklin D. Roosevelt

Table of Contents

List of Figures

List of Tables

Examining Traditional Methods and New Fluorometric Methods for Detecting Phytoplankton Nutrient Status in Freshwater Ecosystems, and Their Application in the Lower Laurentian Great Lakes

1.1 Introduction

The ability to identify factors limiting algal growth is of considerable importance to our understanding of algal ecology and management for ecosystem health (Hecky and Kilham 1988). "Established" methods used to identify limiting nutrients include metabolic assays and particulate stoichiometric ratios and have yielded valuable information. However, these methods for estimating phytoplankton nutrient status all have potential problems of interpretation and some have limited spatial and temporal resolution since they require experimental incubations and/or are labor intensive. Also, these methods rely on nutrient stress to be sufficiently constant in time to induce gene expression for the activation of enzymes or compositional changes in nutrient content. This lack of sensitivity may cause an underestimation of nutrient stress and its effect on algal photosynthesis (Young and Beardall 2003).

Commercial fluorometers such as pulse amplitude modulated fluorometers (PAM) and fast rate repetitition fluorometers (FRRF) have been used to estimate the physiological status of phytoplankton based on the fluorescence of chlorophyll *a* (Chl *a*) in photosystem II (PS II) (Kolber et al. 1993, Falkowski et al. 1995, Beardall 2001*a* and Sylvan et al. 2007). The fluorescence parameters derived from PAM and FRRF may allow for quick, sensitive and non-invasive assessment of the nutrient status of phytoplankton, and were used here to provide detailed information about the physiological state of the phytoplankton communities in Great Lakes sites of varying trophic status (Lake Erie, Bay of Quinte (Lake Ontario) and Colpoys Bay (Lake Huron).

1.2 Background

1.2.1 Nutrient Limitation

One of the important and early definitions of nutrient limitation involving plant growth is Liebig's Law of the Minimum, which states that biomass will become limited by a single resource (Odum 1971). The law is applicable in steady state conditions (the inflow of energy and materials is equivalent to the outflow of energy and materials) in which essential nutrients available with the lowest ratio of supply: demand tends to be the limiting one. Liebig's law of the minimum is less applicable under transient state condition when the amounts of nutrients are rapidly changing.

Growth rates of phytoplankton are often limited by one or more nutrients and nutrient requirements differ between species (Hecky and Kilham 1988, Beardall et al. 2001*a*). The Law of Limiting Factors (Blackman 1905) assumes that when several factors such as nutrients are required for a metabolic process, the nutrient that is most limiting will determine the rate of process (Lampert and Somner 1997). In essence, Blackman models are used to determine the growth rate of an organism using the nutrients considered to be most limiting. However, other factors must be considered since suboptimal temperature and light conditions can change nutrient requirements for growth (Rhee and Gotham 1981a, b).

Nutrient deficiency results in morphological and physiological changes that can help phytoplankton cope with limiting nutrient supplies but it still reduces the overall performance of the phytoplankton as primary producers (Beardall et al. 2001*a*). Severe nutrient deficiency will result in a complete shutdown of physiological processes and eventually cell death (Beardall et al. 2001*a*). Nutrient limitation is presumed to lead to varying degrees of deficiency, with increased deficiency and restriction of growth occurring

as the nutrient supply:demand ratio decreases and nutrient uptake fails to match biosynthetic demand. In measuring nutrient deficiency, we are therefore making an indirect assessment of the variations of nutrient-limited growth rates, which in turn are hypothesized to regulate phytoplankton dynamics.

1.2.2 Chemical and Biological Assays for Nutrient status

Methods for identifying limiting nutrients include those which can be termed nutrient status assays. These include particulate stoichiometric ratios (Redfield ratio), the alkaline phosphate activity assay (APA), the phosphorus (P) and nitrogen (N) debt assays, the phosphorus deficiency index (PDI), and phosphate turnover time. They have been widely used for natural communities and validated to varying degrees in laboratory studies. In this study, P and N debt, APA assay, and elemental composition ratios were used to determine nutrient status for comparison with other methods. Table 1.1 shows the quantitative interpretation of these assays.

Table 1.1 Nutrient status indicators. Values either show an absence, presence or the degree of nutrient limitation for phytoplankton. Criteria for nutrient limitation are based on Healey and Hendzel (1979b) and adapted from Guildford et al. (2005)

Indicator	Nutrient	No deficiency	Moderate deficiency	Extreme deficiency	Deficient
C/N (atomic ratio)	N	<8.3	8.3-14.6	>14.6	
N debt (µmol N µg Chl a $^{-1}$)	N	<0.15			>0.15
C/P (atomic ratio)	P	<129	129-258	>258	
N/P (atomic ratio)	P	<22			>22
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	

A brief description of the principle assay and some of their known limitations are as follows:

1.2.2.1 Elemental Composition Ratios

Ideally, the elemental composition of algae reflects the macromolecular composition of cells, which in turn reflects the integration of the various processes involved in nutrient uptake and assimilation (Healey and Hendzel 1979*b*). For example, when P and N become limited in supply and growth is reduced after exhaustion of stored nutrients, phytoplankton can accumulate carbohydrates during photosynthesis. As a result, C:P and C:N values are considerably higher compared to nutrient sufficient conditions. Particulate ratios, however, are susceptible to interference from non-algal material such as bacteria, microzooplankton and detritus (Arrigo 2005) and are influenced by environmental factors other than nutrient limitation, such as light availability.

1.2.2.2. Nutrient uptake assays

In nutrient deficient conditions, phytoplankton are able to respond either by increased uptake of the limited nutrient(s) or produce a more efficient uptake systems for the limiting nutrient (Arrigo 2005). The uptake of PO_4^{3-} is a metabolically driven process that requires active transport. In addition, the utilization of NO_3^- requires phytoplankton to reduce NO_3^- to NH_4^+ via NO_3^- reductase. NH_4^+ is the preferred form for uptake by many phytoplankton because it is the most reduced of the commonly available combined inorganic nitrogen forms, NH_4^+ contains N at the oxidation level of proteins. In contrast, NO_3^- uptake involves assimilative nitrate reduction, a process in which energy that is captured photosynthetically is used to reduce oxidized NO_3^- to NH_4^+ Cyanobacteria have the ability to fix

atmospheric N_2 (diazotrophs). 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 (Kalff 2002). The enzyme used to fix N_2 is nitrogenase.

Severe nutrient deficiency can impede the physiological processes necessary for a cell's survival (Healey and Hendzel 1979*b*). The metabolic uptake assays developed by Healey and Hendzel (1979*b*) are based on controlled laboratory culture experiments showing that under growth rate limitation by N or P, phytoplankton increase their capacity for uptake of the limiting nutrient. This capacity can be measured when high (saturating) concentrations of the nutrient are added, and is expressed per unit chl *a*. This surge of uptake by N or P deficient algae is commonly known as N or P debt (Guildford et al. 2005). Disadvantages of debt assays are that the experiments require incubations, so they are relatively time consuming, and the assays are calculated as the N or P removed over a 24 hour period per unit of Chl *a.* Using Chl a as a proxy for algal biomass may introduce error because Chl *a* values can vary among species especially under low temperature and light conditions.

1.2.2.3 Alkaline phosphatase activity (APA) assay

Alkaline phosphatase (AP) activity has been used as an indicator of P deficiency because it is synthesized at low levels of P availability (Rose and Axler 1998). When P levels are low, the phosphatase enzyme hydrolyzes the ester bonds between P and dissolved organic molecules, such as polyphosphates making P available for assimilation. The APA assay has been used as an indicator for P status (Healey and Hendzel 1979, Smith and Kalff 1981, Pick 1987, Rose and Axler 1998, Sterner et al. 2004, Guildford et al. 2005, Sylvan et

al. 2007) however, bacteria and microzooplankton contribute to AP under P limited conditions (Taylor and Lean 1991) which can confuse attempts to determine the P status of the phytoplankton.

1.2.3 Phytoplankton Nutrient Status in the Great Lakes

P is the nutrient element most likely limiting algal production in the Great Lakes (Lean et al. 1983, Allen and Smith 2002, Guildford et al. 2005). Historically, excessive P loads to the Great Lakes promoted phytoplankton growth, which in turn degraded both water column and benthic habitats by inducing algal blooms, reduced water clarity and depleted oxygen in deep waters (Carrick 2004). The determination that P was the limiting nutrient in freshwater systems was the major factor behind the Great Lake Water Quality Agreement, which set limits for P loading into the lake. The reduction of P loads to Lake Erie since 1970 has been credited with a return of the lake to a meso-oligtophic condition (Charlton et al. 1993).

Lake Erie, Bay of Quinte (Lake Ontario) and Georgian Bay (Lake Huron) represent a range of physical and chemical environments that can be used to study changes along a trophic gradient of nutrient concentrations (Table 1.2).

Table 1.2 P and N deficiency measured by traditional methods in lower Great Lakes sites

System	Bioassay	Deficiency	Occurrence	Reference
Bay of Quinte	C:P	P (moderate)	July	S. Yakobowski (per. comm..)
L. Erie (West)	N/P debt, C:P, C:N, N:P, APA, Biosensor	N (moderate) P (moderate)	May June	Guildford et al. (2005), Wilhelm et al. (2003)
L. Erie (Central)	N/P debt, C:P, C:N, N:P, APA, ^{32} P (P uptake), (Phosphorus deficiency index) PDI	P (moderate)	July	Lean et. al. (1983), Allen and Smith (2002), Guildford et al. (2005)
Georgian Bay (West)	Phosphorus deficiency index (PDI)	P (severe)	July	Furgal et al (1998)

1.2.3.1 Georgian Bay (Lake Huron)

Based on studies of nutrient chemistry, the open basins of Lake Huron (Georgian Bay) are oligotrophic (Weiler 1988, Beeton and Saylor 1995). From 1978 to 1995, open-lake spring total phosphorus concentrations were stable and, with one minor exception, remained below the target level of P control of 5.0 $mg \cdot m^{-3}$ (Beeton and Saylor 1995). During the period prior to and subsequent to the reduction in phosphorus loadings, a number of studies classified all three basins of Lake Huron as oligotrophic (Beeton and Saylor 1995). Regarding nutrient status, little research has been conducted in Georgian Bay. For example, Furgal et al. (1997), used the phosphorus deficiency index (PDI) which is the ratio of light saturated C-uptake to P saturated P uptake. PDI values <10 denote extreme P deficiency,values between 10 and 100 indicate moderate to low deficiency, and values >100 denote P sufficiency (Lean and Pick 1981). The results of their study show that the western

portion of Georgian Bay can be strongly P deficient (PDI = 1.31) in summer stratified conditions.

1.2.3.2 Lake Erie

Lake Erie 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. In the early 20th century, early signs of eutrophication were observed. During the 1950's and 1960's, Lake Erie total phosphorus (TP) concentrations were reported as being high as 50 μg L^{-1} (Dobson et al. 1974) and toxic metals such as mercury are present due to atmospheric transport (Jackson 1998, Munawar et al. 2002). Also, populations of fish and invertebrates decreased and large blooms of cyanobacteria occurred, reducing both water-column transparency and hypolimnetic dissolved oxygen levels (Beeton 2002). By the 1970's, the Great Lakes Water Quality Agreement (GLWQA) set limits and measures on pollution and eutrophication, especially in Lake Erie. The ecosystem of Lake Erie rapidly responded to these abatement efforts and, overall, reduced P led to decreases in phytoplankton biomass and improved water quality in both western and central Lake Erie (Makarewicz and Bertram 1991, Makarewicz 1993). Studies regarding nutrient status in Lake Erie (Lean and Pick 1981, Guildford and Hecky 1994, Twiss et al. 2000, Wilhelm et al. 2003, Guildford et al. 2005, North et al. 2007) suggest that the phytoplankton populations are not as severely P deficient compared to smaller lakes found in the Canadian Shield or in other large lakes (Lake Superior).

1.2.3.3 Bay of Quinte (Lake Ontario)

Like Lake Erie, the Bay of Quinte has been strongly influenced by human settlement. After colonization in the 1800's, watershed deforestation, mining, and agricultural practices all resulted in increased nutrient inputs to the bay (Nicholls et al. 2001). In the 1900's, a growing population furthered eutrophication and algal blooms had already been reported twice in the Bay of Quinte by the 1930's (Minns 1995). In 1909, the Boundary Waters Treaty established the International Joint Commission in which the main goal of the Commission was to control pollution and toxic substances in the Great Lakes. In 1972, the first Great Lakes Water Quality Agreement between the governments of Canada and the United States was put into action to reduce the causes of eutrophication (controlling TP concentrations). In 1987 the governments signed a Protocol called the "Remedial Action Plan" which was implemented to examine the effects of eutrophication in areas of concern (including Bay of Quinte) (Nicholls et al. 2004).

Although measures were taken for the reduction of eutrophication and pollution, in the 1980's a new threat emerged that posed a risk to the integrity of the Bay of Quinte. The invasion of dreissenids quickly resulted in an undesirable species shift in which *Microcystis sp.* showed a significant increase, while other algal taxa remained the same or decreased (Nicholls et al. 2002). The dominant presence of *Microcystis sp.* may be a result of inefficient grazing (colonies are large), and the dreissenids' capability to differentiate between toxic and non-toxic forms of *Microcystis sp*. by selectively rejecting still viable toxic cells as pseudofeces, hence promoting harmful blooms (Vanderploeg et al. 2001). Another important factor is that dreissenids may indirectly promote the growth of

Microcystis sp. by altering the ratio of available TN:TP. This process occurs when nutrients are regenerated, as mussel excretion enhances P relative to N (excreted N:P ratios can be <20, Arnott and Vanni 1996). Regarding nutrient status of phytoplankton communities in the Bay of Quinte, research is needed to examine eutrophication and harmful algal bloom development. Studies have found a relationship between TP concentrations and *Microcystis* (Raikow et al. 2004, Nicholls et al. 2002). Although metabolic assays and particulate ratios of C:P and C:N have been useful in determining nutrient status of natural phytoplankton communities, more recent research, largely in marine environments, suggest that newer fluorometric methods could provide robust, sensitive and instantaneous measures of the physiological status in phytoplankton that would overcome some of the drawbacks experienced by traditional methods.

1.2.4 Chlorophyll a Fluorescence

The principle of Chl *a* fluorescence analysis is based on the assumption that when a pigment absorbs the energy of a photon and enters an excited electronic state, there are only three competing routes for dissipation of the excitation energy: i) it can be used to drive photosynthesis (photochemistry) ii) excess energy can be dissipated as heat, or iii) in can be re-emitted as fluorescence (Krause and Weiz 1991, Campbell 1998). By measuring fluorescence, information about heat dissipation and photochemistry can be obtained. Figure 1.1 depicts the Z scheme showing the pathway of electron transfer from water to NADPH.

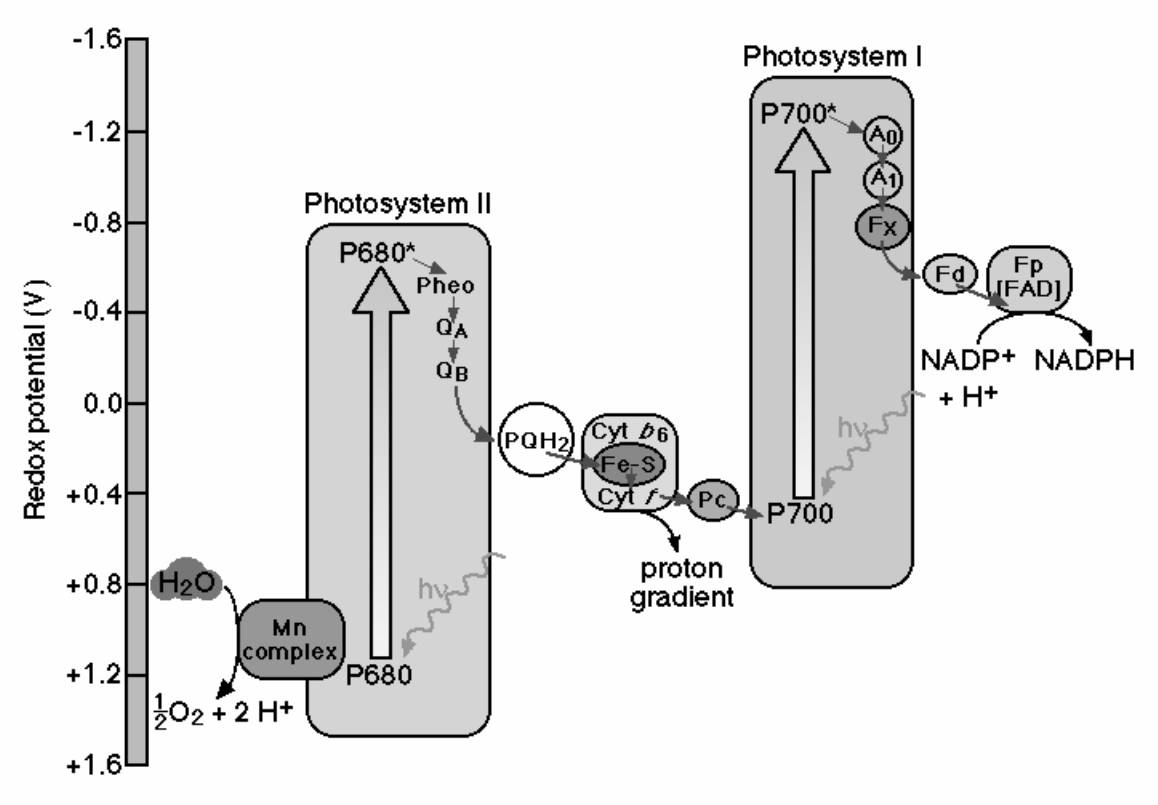


Figure 1.1 The Z scheme showing the pathway of electron transport from water to NADPH (http://bio/Courses/biochem2/Photosynthesis/Photosystem1.html)

For any pigment, the level of fluorescence emission depends on the pigment concentration, the excitation light intensity and the fluorescence yield or efficiency of florescence emission (Campbell 1998). When the light source is sufficiently low, or in the absence of solar irradiance, a low level of fluorescence emanating from the light harvesting antenna is measured. Fluorescence, reemission as light of excess energy, is one of the three possible fates that light energy absorbed by a cell can undergo, the other two being reemission as heat or utilization for photosynthesis/photochemistry. Most of the fluorescence signal at ambient temperature is emitted by photosystem (PS) II and is a

function of the state of reaction centers (RC) in the cells. When cells are exposed to light, RC of PSII get progressively reduced or "closed", increasing the fluorescence yield, with the rate of fluorescence emission (F) being proportional to the amount of absorbed light energy and divided by the rate constant of fluorescence k_F (Krause and Weis 1991) (Eq. 1):

$$F = k_F/(k_D + k_T + k_P) \quad \text{Eq. 1}$$

Where k_D is the rate constant for heat de-excitation, k_T is the rate constant for energy transfer to non-fluorescent pigments, and k_P is rate constant for photochemical reaction. Fluorescence yield is minimal (F_o) when all RC are active or "open", all primary quinine acceptor of (Q_A) of PSII are oxidized, and when k_P is >> $k_F + k_D + k_T$, maximum fluorescence (F_m) is achieved where all RC are closed and Q^-_A is fully reduced, impeding excitation of PSII. The potential yield of photochemistry of PSII is therefore given by the ratio of the maximum variable fluorescence (F_v) and the maximum total fluorescence (F_m) Eq. 2:

$$(F_m - F_0)/F_m = F_v / F_m \quad \text{Eq. 2}$$

Nutrient starved cells often show a decline in F_v/F_m suggesting damage to some PS II centres resulting in a reduced fraction of functional PS II centres. For example, N and P starved cells may be impaired due to the lack of nitrogen rich amino acids to produce vital proteins such as the D1 turnover protein located in PS II reaction centres, and reduced ATP regeneration that leads to an accumulation of non functional PSII centres (Lippenmier et al. 2001).

1.2.5 Fluorometric Methods for Assessing Phytoplankton Nutrient Status and Community Composition

The use of variable fluorescence involves two basically similar but significantly different ways of measuring Chl *a* fluorescence (Suggett et al. 2003). The two protocols have been incorporated in various ways in the designs of commercially available fluorometers such as the Pulse amplitude modulated (PAM) fluorometer and Fast Repetition Rate Fluorometer (FRRF).

The PAM fluorometer can be applied to detect fluorescence signals rapidly (Fig. 1.2). The fluorescence parameters have been used to determine the effect of environmental perturbations (metal toxicity, light shock and nutrient starvation) that may inhibit photosynthesis (Marwood et al. 2000, Lippemeier et al. 2001, Yentsch et al. 2004). In the presence of photosynthetic (actinic) irradiance the functional quantum yield of PS II can be measured and can provide estimates of photosynthetic electron transport rates through PS II (Genty 1989, Geider et al. 1993, Kobler et al. 1993, Ralph and Gademann 2005). The rapid fluorescence light response curves (RLC's) can be constructed and these curves can be fit with models to estimate the light harvesting efficiency (α) and the light saturated rate of maximum electron transport (ETR_{max}) (Kalff 2002, Sorousi and Beer 2007) (Fig. 1.3).

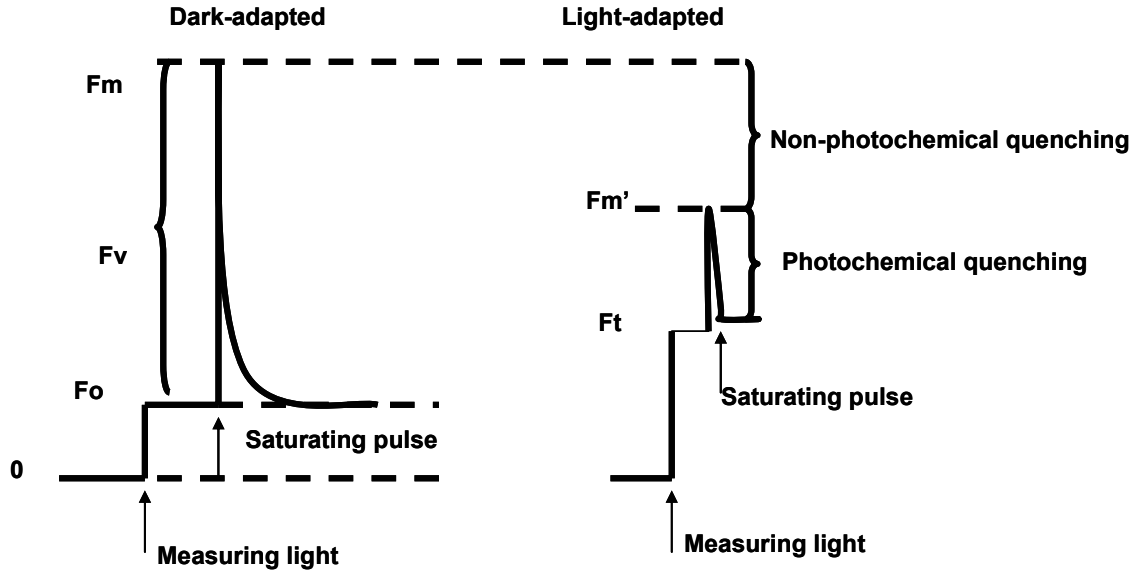


Figure 1.2 Principle of saturation pulse quenching method derived by Pulse Amplitude Modulated (PAM) fluorometry, adapted from Walz, (1998)

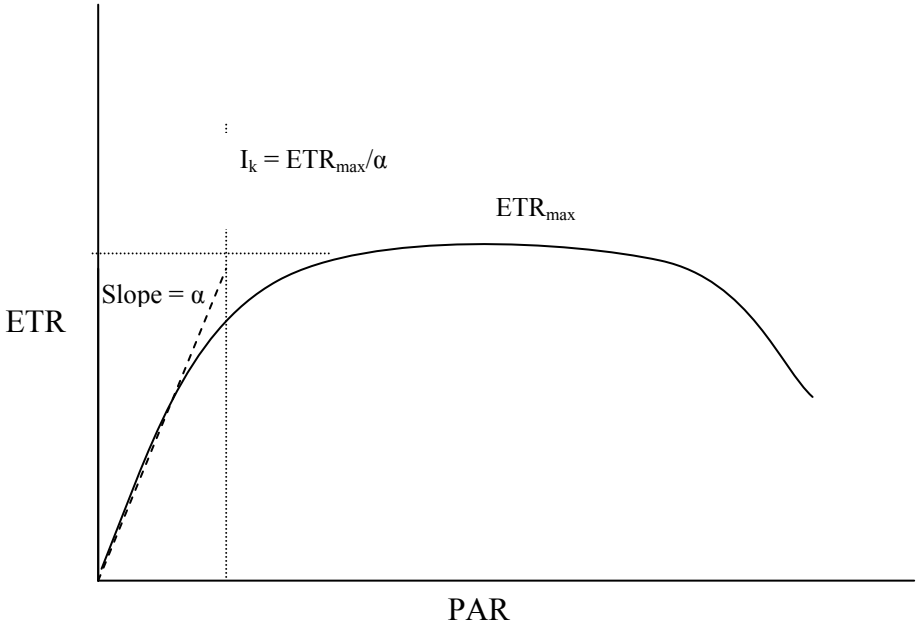


Figure 1.3 Generalized photosynthesis-irradiance curve. ETR = electron transport rate, PAR= Photosynthetic active radiation, α = light limiting slope, I_k = index of the onset of light saturated photosynthesis, ETR_{max} = the saturation rate of the electron transfer rate, adapted from Kalff, (2002)

The FRRF is quite sensitive for use in oligotrophic systems (Chl *a* concentrations ranging from <0.1 to 30 mg m^{-3}) (Suggett et al. 2003). The FRRF allows quick, non-invasive assessment of phytoplankton in vivo fluorescence signatures, including measurements of photosynthetic parameters such as F_v/F_m and the functional absorption cross section of PS II (σ PS II). The FRRF uses short flashlets to progressively reduce PS II and measure the fluorescence rise kinetics (Kromkamp and Forster 2003). By quantifying the kinetics of the fluorescence rise, the FRRF can estimate σ PS II. σ PS II describes the functional 'target area' of the light harvesting antenna that is energetically coupled to the O_2-evolving reaction centres (RCIIs) (Suggett et al. 2009). Therefore, the measurements of σ PS II can be valuable, since this variable is able to detect physiological responses to changes in environmental conditions (Suggett et al. 2009).

The two instruments differ in the duration of saturating light (flash) used to obtain F_m. The FRFF applies single turnover (ST) flashes that allows for only one charge separation during the flash and reduces only the primary acceptor Q_A, raising F to a level of F_m. The PAM applies multiple turnovers (MT) flashes, in which repeated charge separation is achieved until all electron acceptors of PS II are reduced. The longer flash induces a relaxation of quenching and thus raise the F to F_m. Application of these different approaches to phytoplankton communities may result in differing values of F_m. That is, the MT protocol may elicit higher F_v/F_m values, compared to the ST protocol (Kolber et al. 1998; Suggett et al. 2003).

1.3 Research Objectives

Measurements of nutrient concentrations can be used to indicate trophic status of aquatic systems and can also be used to indicate whether or not a particular nutrient is limiting. In north temperate lakes, the role of phosphorus (P) as a controlling factor for phytoplankton biomass has been widely accepted and total P (TP) concentrations adopted as an indicator of trophic status (Dillon and Rigler 1974, Carlson 1977, Schindler 1978, McQueen et al. 1986, Downing and McCauley 1992). We might therefore expect that P deficiency is generally the strongest in lakes with low Chl and TP concentrations and is the lowest or negligible in high TP conditions. However, there are certainly other factors besides P that can affect algal biomass. Other nutrients such as N can be limiting in nutrient rich lakes where the growth of filamentous cyanobacteria can lead to undesirable toxic consequences such as liver or neurological impairment for fish and humans. Also, measurements of P status may show that phytoplankton communities in P limited lakes are not as severely P limited due to biotic and abiotic factors such as light penetration, mixing depth, temperature, other nutrients and grazing. To address these issues, I examined the use of "established" nutrient status indicators and Chl *a* fluorescence to determine the nutrient status of phytoplankton communities in Lake Erie, Napanee (Bay of Quinte) and Colpoys Bay (Georgian Bay).

This thesis consists of 3 data chapters each written as separate, but related, studies. The first data chapter (chapter 2) lays the basis for the other two chapters. It compares previous nutrient status assessments of phytoplankton to the measurements of the photosynthetic efficiency (F_v/F_m and σ PS II) of the phytoplankton community when

measured in a dark acclimated state. The specific objective was to test that variable fluorescence, particularly F_v/F_m, was a reliable indicator of N and P deficiency in freshwater phytoplankton. The second objective was to examine if N and P deficiency in Lake Erie has a spatially and temporally limited occurrence. The third objective was to examine if nutrient status may be related to the taxonomic composition of the phytoplankton community, which may in turn influence the relationship between nutrient status and variable fluorescence properties.

In Chapter 3, the first objective was to confirm, with additional observations in a subsequent year, that previous indications of N and P deficiency in Lake Erie are reproducible. The second objective was to use additional methods (nutrient amendment experiments) to further test whether the previously-used assays were correct in their indications of N and P deficiency. The third objective was to examine the idea that variable fluorescence measured under excitation pressure (RLC's) may provide better a more sensitive measures of nutrient status indicators and even an indication of which nutrient might be limiting.

In Chapter 4, the specific objectives were to first determine whether the relationships between F_v/F_m, σ PSII, RLC parameters, and nutrient deficiency still apply to sites with a wide range in the severity of deficiency and taxonomic composition. The second objective was to further test the nutrient amendment /variable florescence assays as a tool for quantifying deficiency and identifying the limiting nutrient in each site. The third objective was to determine if P deficiency would apply in more oligotrophic and eutrophic sites in other lakes, and the last objective was to determine if the severity of summer P deficiency is systematically related to trophic status among a set of these Great Lakes sites. All chapters

were based predominately on field work in Lake Erie (western, west-central and central basin), Lake Ontario (Bay of Quinte) and Georgian Bay (Colpoys Bay-Lake Huron) in 2005 and 2006.

Chapter 2. Nutrient status of phytoplankton in an oligo-mesotrophic lake (Lake Erie): Evidence from new fluorescence methods

Overview

The variable fluorescence of chlorophyll *a* was measured by pulse amplitude modulated (PAM) and fast repetition rate (FRR) fluorometry in parallel with independent measures of nutrient status to investigate nutrient status and its relationship to community structure of phytoplankton in Lake Erie in May, June, and September, 2005. Nutrient status was assessed by measurements of nitrogen (N) debt, phosphorus (P) debt, particulate C:N:P ratios and alkaline phosphatase activity (APA). Sampling sites revealed P deficiency was most common in June, while N deficiency was most common in May; neither N nor P deficiency was common in September. The maximum quantum yield (F_v/F_m) measured by PAM or FRRF was generally lower in May and June and maximal in September, while the functional absorption cross section of photosystem II (σ_{PSII}) was maximal in May and June, and minimal in September. F_v/F_m and σ_{PSII} were correlated with nutrient status indicators, with N or P deficient sites having low F_v/F_m and high σ_{PSII}. Community structure was also associated with nutrient status. Cyanobacteria contributed a larger, often dominant, share of total biomass at sites that displayed N deficiency. Chrysophyte and cryptophyte flagellates were more important, and usually dominant, at P deficient stations in all basins. The occurrence of N deficiency is surprising in a lake with generally high inorganic N (as NO_3^-), but is supported by a variety of measures and by its association with cyanobacteria. The results indicate that nutrient deficiency helps to structure the phytoplankton community in this large lake, and that variable fluorescence measures can characterize the strength of nutrient deficiency.

2.1 Introduction

Total dissolved nutrient concentrations are strongly related to variations in phytoplankton biomass and composition among temperate lakes (e.g. Watson et al. 1997) and to seasonal and spatial variations within lakes (e.g. Teubner and Dokulil 2002). Aqueous nutrient concentrations were the earliest data used to estimate nutrient limitation (Ketchum 1939). However, the effect of nutrient limitation as assessed using static nutrient concentrations are varied and complex. For example, nutrient concentrations in surrounding water may not be analytically detectable, however phytoplankton communities if nutrient limited, may be able to maintain an adequate supply of available nutrients though rapid recycling. Nutrient concentration data are therefore difficult to interpret in terms of growth limitation. Indirect methods of determining the degree of physiological nutrient control ("nutrient status") can help provide a better understanding of the effects of nutrient limitation on growth and composition of phytoplankton.

A common index of nutrient status is the elemental composition (stoichiometry) of suspended particles (e.g. Guildford and Hecky 2000), which is assumed to reflect the macromolecular composition of cells and the balance between demands for synthetic processes and rates of nutrient uptake and assimilation. Nutrient addition bioassays (e.g., Moon and Carrick 2007) can reveal growth responses of phytoplankton and help identify limiting nutrients. Nutrient debt assays measure the capacity of phytoplankton for nutrient uptake, which tends to be higher in nutrient-deficient algae (Healey and Hendzel 1979b, 1980). Enzymatic assays include alkaline phosphatase activity (APA), which can be expressed when available phosphorus (P) concentrations are low (Healey and Hendzel

1979*b*, Smith and Kalff 1981, Rose and Axler 1998, Renefors et al. 2003). Such assays have provided useful information on variability of nutrient status (e.g. Guildford et al. 2005) but none is without drawbacks. Particle stoichiometry is the most practical for assessing variability in natural systems, where numerous measurements are typically needed, but it is under complex control and can include contributions from non algal particles (e.g. Teubner et al. 2003). Other methods for estimating phytoplankton nutrient stress typically have limited spatial and temporal resolution because they require experimental incubations and are not ideally suited to routine or extensive surveys.

Chlorophyll a (Chl *a)* variable fluorescence has been used to provide sensitive and rapid estimates of phytoplankton condition (Kolber and Falkowski 1993, Graziano et al 1996, Behrenfield et al. 2006, Sylvan et al 2007 and Juhl and Murrell 2008), primarily in marine environments, and offers potential to deal with the typical variability of natural systems. The quantum efficiency of electron transport in photosystem II (PS II) reaction centres can be assessed by the variable fluorescence ratio (F_v/F_m) of Chl *a*. Kolber et al. (1988) found that some species of unstressed diatoms, chlorophytes and chrysophytes are commonly assumed to manifest F_v/F_m close to 0.65, although this optimal value may be lower for some taxa (e.g. Suggett et al. 2009). Many stressors can lower the observed value**.** Pulse amplitude modulated (PAM) and fast repetition rate (FRR) fluorometers have been used to measure (F_v/F_m) (Kolber and Falkowski 1995, Beardall 2001 *a,* Young et al. 2005, Behrenfeld et al. 2006, Juhl and Murrell 2008). A potential advantage to FRRF is that it can additionally measure the efficiency of energy capture by PSII reaction centres (σ PSII).

These new technologies have had some success in evaluating nutrient status of marine phytoplankton. For example, Beardall et al. (2001 *a*) were able to assess P deficiency

in several groups of phytoplankton using F_v/F_m derived from PAM. Sylvan et al. (2007) used APA and nutrient addition experiments to document P limitation in the Mississippi River plume, and showed that variable fluorescence was diminished and σ PS II was increased under P limited conditions. However, the reliability of variable fluorescence as an indicator of growth rate or nutrient status has been questioned in other studies. Kruskopf and Flynn (2006) found no consistent relationships between nutrient status and F_v/F_m in cultures of several different phytoplankton taxa while Cullen et al. (1992) and Parkhill et al. (2001) have suggested that F_v/F_m responds to nutrient stress mainly in laboratory situations of extreme non-steady state deficiency. Suggett et al. (2009) have shown that taxonomic affiliation accounts for much variation of F_v/F_m, potentially masking signals induced by varying physiological condition. Additional studies, preferably of natural communities and particularly in freshwater systems, are needed to better characterize the relationship between variable fluorescence and physiological stresses such as nutrient deficiency in nature.

In this study, Lake Erie provided a relevant and useful system in which to study spatial and temporal variations of nutrient status, community composition and the corresponding variable fluorescence attributes of phytoplankton. Like many temperate lakes (Sommer et al. 1986, Currie 1990, Lean and Nalewajko 1979), Lake Erie exhibits a spring or early summer maximum of biomass and production followed by a summer period of low P availability, relatively rapid P cycling and signs of P deficiency in the phytoplankton (Lean et al. 1983, Allen and Smith 2002, Guildford et al. 2005). Lake Erie is also an example of a lake in which P loading controls have been used to constrain phytoplankton biomass (e.g. Makarewicz and Bertram 1991, Charlton et al. 1999) and therefore can be considered a "P-limited" lake. Nonetheless, at times there is evidence for N deficiency (Guildford et al.

2005) as well as limitation or co-limitation by both N and Fe (Twiss et al. 2000, North et al. 2007). There is a pronounced seasonal succession, leading from spring diatom dominance to summer dominance by chromophyte flagellates, chlorophytes and cyanobacteria, varying among different regions of the lake (Makarewicz 1993, Makarewicz et al. 1999, Barbiero and Tuchman 2001 and Moon and Carrick 2007). Nutrient loading and the yet-uncertain details of how nutrients regulate algal composition in the lake are vital in understanding and controlling problematic algal blooms (e.g. Budd et al. 2001) and hypolimnetic hypoxia (e.g. Carrick 2004). In addition to a pronounced temporal sequence of nutrient and phytoplankton development, Lake Erie also has a strong longitudinal gradient from meso-eutrophic in the west to oligotrophic in the east (Charlton 1999, Makarewicz et al. 2000, Barbiero and Tuchman 2004), and there are suggestions that the proximate nutrient controls vary along this spatial gradient (Guildford et al. 2005). This large and dynamic lake is a clear example of a situation where more efficient means of surveying the physiological state of phytoplankton are sorely needed.

This study used multiple indicators of N and P status to determine how nutrient limitation relates to phytoplankton community composition and variable fluorescence properties in Lake Erie. I sought to determine whether previous reports of the spatial and temporal incidence of N and P deficiency could be confirmed and whether variable fluorescence parameters at the community level would reflect nutrient limitation. By measuring species composition of the community, I also sought to determine whether nutrient limitation may be associated with community composition, and whether such associations might contribute to the variation in Chl *a* fluorescence properties. To my knowledge, this is the first time that relationships between nutrient status, Chl *a* fluorescence

and community composition have been simultaneously determined for any natural aquatic system. The specific objectives were, first, to test the hypothesis that variable fluorescence, particularly F_v/F_m, is a reliable indicator of N and P deficiency in freshwater phytoplankton. The second objective was to test the hypothesis that, even in this P-limited system, P deficiency has a spatially and temporally limited occurrence, and that N deficiency can be frequently observed in the spring. The third objective was to test the hypothesis that nutrient deficiency may be associated with the taxonomic structure of the phytoplankton in ways consistent with previous inter-lake comparisons (e.g. prevalence of flagellates from the cryptophyte and chrysophyte groups under P limitation (e.g., Graham and Wilcox 2000; Urabe et al. 2000). Finally, I sought to examine if some of the relationships between variable fluorescence properties and nutrient deficiency are due, at least in part, to the intrinsic variable fluorescence characteristics of different algal taxa.

2.2 Material and Methods

2.2.1 Study Area and Sampling Design

Lake Erie contains three morphometrically different basins (western (WB), central (CB) and east (EB), Fig. 1) with the shallow (mean depth 10 m) WB receiving most of the lake's external nutrient load from the Detroit and Maumee Rivers. Nutrient concentrations are typically highest in WB compared to the deeper CB (mean depth 20 m) and lowest in the relatively deep EB (mean depth 47 m; Charlton et al. 1999). There is often a particularly strong gradient in nutrients and plankton abundance from WB to CB, with the western part of CB often denoted as an additional sub-basin, the west central basin (WCB) due to its distinctive nutrient, plankton and circulation features (e.g. Leon et al. 2005). Sampling sites

for this study (Fig. 1) were distributed in WB, WCB and CB to obtain the best representation of the strong spatial variability in the western part of the lake.

Three cruises were conducted in 2005, in which water samples were obtained from the *CCGS* Limnos and the RV Lake Guardian in spring overturn (early May), early summer stratification (late June) and late summer stratification (mid- September) (Fig. 2.1). All data presented here are from samples collected at 2-m depth with a sampling CTD-Rosette (Seabird) equipped with 8-L Niskin bottles and immediately transferred to 20-L polyethylene carboys, darkened to prevent light shock. The ship's schedule dictated the sampling times, which explains the different sampling times among sites. I selected a subset of stations for further analysis of phytoplankton composition (Fig. 2.1-3). The stations were selected based on whether or not they manifested N or P deficiencies in the nutrient status assays described below. The threshold values used to determine if samples were N or P deficient or nutrient sufficient are found in Table 2.1. An effort was made to represent nutrient status categories equally based on months and basins. Stations classified as N deficient showed N deficiency as indicated by N debt and C:N but no P deficiency, and stations classified as P deficient showed P deficiency with all P status indicators.

Table 2.1 Nutrient status indicators. Values either show an absence, presence or the degree of nutrient limitation for phytoplankton. Criteria for nutrient limitation are based on Healey and Hendzel (1979b) and adapted from Guildford et al. (2005

Indicator	Nutrient	No deficiency	Moderate deficiency	Extreme deficiency	Deficient
C/N (atomic ratio)	N	<8.3	8.3-14.6	>14.6	
N debt (µmol N µg Chl a $^{-1}$)	N	<0.15			>0.15
C/P (atomic ratio)	P	<129	129-258	>258	
N/P (atomic ratio)	P	<22			>22
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	

Physical measurements important to this study were temperature profiles from the Seabird CTD and vertical profiles of photosynthetically active radiation (PAR) from a PAR sensor attached to the Fast Repetition Rate fluorometer (FRRF Fasttracka^{TM} Chelsea Instruments). The vertical attenuation coefficient (K_d) was estimated by linear regression of the natural logarithm of PAR versus depth. The mixing depth (Z_{mix}) and mean PAR of the surface mixed layer were calculated according to Hiriart et al. (2002).

2.2.2 Nutrient and Chlorophyll Analysis

Samples for ammonium (NH_4^+) analysis were passed through a (0.2 μm) polycarbonate filter and were measured with the orthophtaldialdehyde (OPA) method outlined in Holmes et al. (1999) using a Turner Designs (TD) fluorometer with a detection limit of 0.02 μmol L^{-1}. Filtered samples were measured for nitrate (NO_3^-), and nitrite (NO_2^-) on an Dionex ICS 2500 ion chromatograph. All samples were frozen in the field and analyzed immediately upon returning to the laboratory. Particulate C and N samples were analyzed using methods described by Stainton et al. (1977). The samples were collected on pre- combusted GF/F (pore size 0.7, 47 mm) filters then dried and placed in desiccators containing hydrochloric acid for 24 h. A CEC-440 elemental analyzer (Exeter Analytical Inc) was used to analyze the samples.

Samples for total phosphorus (TP) and total dissolved P (TDP) concentrations were analyzed following preservation and analytical procedures of NLET (1994). Soluble reactive P (SRP) was analyzed according to Stainton et al. (1977). Particulate P (PP) concentration was measured by the ascorbic acid method following persulphate digestion (Stainton et al. 1977, North et al 2007). Soluble reactive silicate (SrSi) concentration was analyzed within

48 h according to Stainton et al. (1977). Triplicate Chl *a* measurements were made by filtration onto glass fiber filters (GF/F pore size 0.7 μm, 47 mm) followed by extraction method outlined by Strickland and Parsons (1972) in 90% acetone, without mechanical dispersion, and analysis by a fluorometric method (North et al. 2007) using a Turner Designs 10-AU fluorometer.

2.2.3 Phytoplankton Nutrient Status Indicators

P and N limitations were assessed by elemental ratios of particulate C:P, N:P, C:N and by the results of nutrient debt and phosphatase enzyme assays (Healey and Hendzel 1979*b*). For the N debt assay, NH_4Cl was added (final concentration = 5 μmol L^{-1}) to sample water. The NH_4^+ concentrations were measured at the beginning and end of 24 h incubation in the dark at room temperature. The P debt assay followed the same procedure, except that KH_2PO_4 was added and SRP concentrations were measured at the beginning and end of incubation. N and P debt were calculated by the amount of N or P removed over a 24 h period per Chl *a* (μmol N/P μg Chl *a* L^{-1}) (Healey and Hendzel 1979*b*). Alkaline phosphatase activity (APA) assays used the fluorometric method of Healey and Hendzel (1979*a*), with 5 μmol L^{-1} of O-methyl-fluorescein-phosphate as the substrate (Table 1). Total and soluble forms of APA were measured, and particulate activity was determined by difference.

2.2.4 Chl a Variable Fluorescence Measurements

For PAM measurements, 1-L water samples were concentrated onto 24-mm glass fiber filters (GF/F, Whatman, Springfield Mill, U.K.) under low (<10 mm Hg) vacuum.

Samples were dark adapted for 30 min in covered Petri dishes to keep cells hydrated. A Diving PAM (Heinz Walz, Germany) was used to measure minimum (F_o) and maximum (F_m) fluorescence using white light with a measurement intensity of 0.15 μmol m^{-2} s^{-1} and a saturation pulse intensity of 18,000 μmol m^{-2} s^{-1} PAR (Walz 1993). The ratio of variable fluorescence to F_m (F_v/F_m), was calculated by instrument software. Fluorescence rise kinetics were inspected to verify that F_m was indeed obtained, that is, to verify that a clear plateau in fluorescence was observable within the period of the saturation pulse. Gain was adjusted as necessary and all measurements were corrected for background signal using a blank consisting of 24 mm glass fiber filters through which distilled water had been filtered (Walz 1998).

A fast repetition rate fluorometer (FRRF), FastTracka, Chelsea Instruments, UK, was used to measure F_v/F_m and σ_{PSII}. The FastTracka FRRF used blue excitation light centred at 470 nm. Dark-adapted, unfiltered samples (50 ml) were measured in a 100ml quartz cuvette using the dark chamber in benchtop (discrete acquisition) mode. For each sample, 50 successive acquisitions were collected, with each acquisition comprising 5 consecutive flash sequences, with each flash sequence in turn comprising 100-200 flashlets, each 1.5 μs in length and delivered at 2.8 μs intervals (Falkowski and Kolber 1995). While the PAM uses a relatively long saturation pulse to completely reduce PSII and plastiquinone (PQ) in a so-called multiple turnover protocol, the FRRF uses these very short flashlets to progressively reduce PSII and measure the fluorescence rise in a so-called single-turnover protocol (Kromkamp and Forster 2003). By quantifying the kinetics of the fluorescence rise, the FRRF can estimate the effective cross section of PSII, (σ_{PSII}). The variable fluorescence parameters from the raw FRRF fluorescence data were derived using Submersible FRR Data

Reduction software (FRS1 ver. 1.8 Chelsea Instruments Ltd.). Although this commercial software provides estimates of photosynthetic properties, Laney (2003) reports that the design of the measurement protocol and the numerical algorithm used to fit the physiological model to measure fluorescence yield transient F(*t*) can introduce error in derived properties such as estimates of connectivity of electron transfer *(p)* and the time constant for PS II reoxidation (τ). Also, FRS protocol may overestimate F_v/F_m by 20% on average (Laney 2003) and may fail to completely saturate F_m. However, I have inspected the fluorescence rise kinetics to verify that F_m was indeed acquired and that Fv/Fm was truly obtained. Lake water blanks were prepared by filtering 1 L of sample water through a 47-mm glass fiber filters (0.7 mm pore size, GF/F, Whatman, Springfield Mill, U.K.) and used to correct the FRRF for background fluorescence.

2.2.5 Phytoplankton Cell Counts

Phytoplankton composition was measured using samples from 2 m depth at the same station where nutrient status measurements were made. The samples were preserved with 1% Lugol's solution and 1% glutaraldehyde. Taxa were enumerated to the lowest level possible following Prescott (1975, 1978), Komarek and Anagnostidis (1986), Lee (1999), Carty (2003), Komarek (2003), and Nicholls and Wujek (2003). Phytoplankton were counted using the Utermohl method at 400x on an inverted phase contrast microscope (Axiovert 35, Zeiss). In total, at least 300 individuals were counted from randomly chosen microscope fields. For biovolume measurements, the dimensions of the algal cell were measured using ocular micrometers and cell dimensions were fit to geometrical shapes that portray the shape of the taxon (Wetzel 1991).

2.2.6 Statistical analyses

Pearson's correlation coefficients was used to evaluate statistical significant relationships between nutrient status indicators and variable fluorescence parameters (Systat ver. 10). Multivariate analysis (Canonical correspondence analysis) was used to further characterize relationships among nutrient status variables and variable fluorescence parameters in samples across the range of locations and dates. Multivariate analysis was also used to visualize relationships between environmental variables, Chl a fluorescence and nutrient status measurements. Detrended correspondence analysis (DCA) was preformed on the phytoplankton data to determine if unimodal or linear ordination techniques were most appropriate to analyze the data. The gradient length of the first axis was 2.090 and the gradient length of the second axis was 1.981. According to Pienitz et al. (1995), if the gradient length of the first axis is above 2 standard deviation units, unimodal ordination techniques should be employed. Canonical correspondence analysis (CCA) provides the power of regression methodology to ordination because CCA uses, as linear regression does, linear combination of environmental variables to explain optimally the species variables (Ter Braak and Verdonschot 1995). Additionally, the features of CCA are that the measure of fit is unconventional (weighted variance of species centroids) and that the data of many species are explained simultaneously. CCA has been widely used to evaluate phytoplankton communities in light of environmental data (Dixit et al. 1989; Enache and Prairie 2002). The ordination analysis was performed using PC-ORD version 4 (MjM Software, Oregon USA). The input data consisted of two files: one that contained

biovolumes of phytoplankton (for all months) and one that contained the nutrient status variables at each site over the same time periods.

The eigenvalues represent the proportion of variance in the phytoplankton data accounted for by the respective canonical variates. These canonical variates are interpreted by examining the weights or importance of each variable with respect to the canonical variates. These weights are called canonical weights and, in general, a larger weight indicates that a variable has a greater contribution to those canonical variables. Their values are standardized as correlations to 0 +/- 1, where 0 indicates no relationship (Mc Cune 1997).

In the CCA analyses, rare phytoplankton species were discarded (1% of total biomass) and the results were plotted as ordination diagrams to illustrate the relationships between environmental variables and species. The relationships between the nutrient status variables and the principal axes are depicted as arrows, and species and site scores depicted by points. In this study, the site scores have been shaded to emphasize the relationships between species and the environmental variables. The length of an arrow reflects the strength of the correlations between an environmental variable and the axes and is considered to be proportional to the relative importance of that environmental variable. The direction represents the nature of those correlations.

2.3 Results

2.3.1 Environmental conditions

Physical measurements (Tables 2.2 to 2.4) revealed a gradient from the WB to the CB. In May, there was no thermal stratification and mean surface temperatures ranged from 5.7 to 8.9 ^{o}C with highest values in west basin (WB). All three basins were thermally stratified in June but in September only the deeper central basin (CB) and west central basin (WCB) remained stratified. Surface temperatures in the WB were 2-3^{o}C higher than elsewhere. Mean PAR was highest during the summer stratification period in which the mixing depth were shallower and vertical attenuation coefficients (k_d) were smaller. In May, k_d was high due to the high concentrations of algal and non-algal material in the water column (Tables 2.2-4).

In all three basins, concentrations of TP and SRP were lowest (Tables 2.2-4) in early summer stratification (June) and highest in late stratification (September), although concentrations at spring overturn (May) were almost as high. TP and SRP concentrations were highest in WB in May and June, while the lowest concentrations were in CB or WCB. Spatial gradients of TP and SRP were weaker in September. NO_3^- and NH_4^+ concentrations were highest in WB , but the spatial trend from WB to CB, especially for NH_4^+, was weaker and more variable than for TP and SRP (Tables 2.2-4). Seasonally, concentrations were lowest in June. Unlike the pattern for N and P, the June survey showed the highest (by far) concentrations of soluble reactive silicate (SrSi) in CB. Otherwise, the temporal and spatial variations of SrSi concentrations were mostly similar to those for N or P, with relatively low values in June and higher values in WB than in other basins.

Mean Chl *a* concentrations (Tables 2.2-4) were higher in WCB and WB in September but differed little among basins in May. Over all basins, concentrations were higher in September than in other months (Fig. 2.4 a-c).

Table 2.2 Initial conditions (mean + standard deviation (number of samples)) of chemical, biological and physical parameters, Central Basin (Bold = values above the threshold of deficiency)

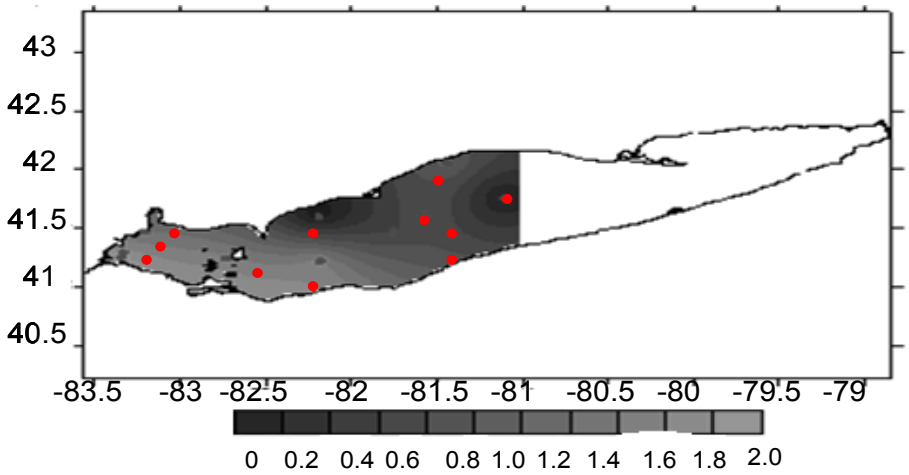
Chemical	Variables	May	June	September
	TP (µmol L^{-1})	0.55 ± 0.22 (7)	0.55 ± 0.09 (6)	0.74 ± 0.12 (6)
	SRP(µmol L^{-1})	0.09 ± 0.02 (7)	0.10 ± 0.09 (6)	0.15 ± 0.01 (6)
	NO_3^-(µmol L^{-1})	12.98 ± 5.43 (7)	13.81 ± 2.82 (6)	16.65 ± 2.33 (6)
	NH_4^+(µmol L^{-1})	1.73 ± 0.21 (7)	1.05 ± 0.33 (6)	1.65 ± 0.21 (6)
	SrSi (µmol L^{-1})	10.5 ± 5.62 (7)	26.5 + 12.2 (6)	8.63 + 1.23 (6)
Biological	**Chl (ug/L)**	2.93 ± 2.58 (7)	2.34 ± 1.65 (6)	5.45 ± 1.93 (6)
	CN (atomic ratio)	**10.72 ± 0.928 (7)**	7.62 ± 2.1 (6)	7.94 + 1.19 (6)
	N Debt (µmol N µg Chl a^{-1})	**0.16 ± 1.12 (7)**	0.14 ± 0.07 (6)	0.12 ± 0.04 (6)
	CP (atomic ratio)	**208.1 ± 152.2 (7)**	**239.1 ± 42.3 (6)**	**217.0 ± 96.6 (6)**
	NP (atomic ratio)	12.85 ± 1.22 (7)	18.91 ± 3.22 (6)	14.21 ± 1.45 (6)
	APA (µmol P µg Chl $a^{-1}h^{-1}$)	0.002 ± 0.001 (7)	**0.015 ± 0.003 (6)**	**0.004 ± 0.002 (6)**
	P Debt (µmol P µg Chl a^{-1})	0.07 ± 0.04 (7)	**0.18 ± 0.06 (6)**	0.072 ± 0.02 (6)
	Fv/Fm(PAM)	0.170 ± 0.112 (7)	0.086 ± 0.078 (6)	0.313 ± 0.112 (6)
	Fv/Fm(FRRF)	0.201 ± 0.212 (7)	0.140 ± 0.052 (6)	0.251 ± 0.154 (6)
	σ PS II	718.01 ± 270 (7)	768.3 ± 211.3 (6)	261.24 ± 114 (6)
Physical	**Surface Temp (^oC)**	6.4± 1.4 (7)	19.23 ± 0.9 (6)	21.6 ± 1.9 (6)
	Max Depth (m)	17.8 ± 6.0 (7)	17.3 ± 4.9 (6)	18.2 ± 5. (6)
	Mixing Depth (m)	17.8 ± 6.0 (7)	7.7 ± 2.9 (6)	9.6 ± 3.7 (6)
	kd (m^{-1})	0.38 ±0 .33 (7)	0.18 ± 0.04 (6)	0.34 ±.13 (6)
	Mean PAR (µmol m^{-2} s^{-1})	93.21 ± 3.67 (7)	216.97 ± 23.21 (6)	102.3± 10.34 (6)

Table 2.3 Initial conditions (mean + standard deviation (number of samples) of chemical, biological and physical parameters, Central – west Basin (Bold = values above the threshold of deficiency)

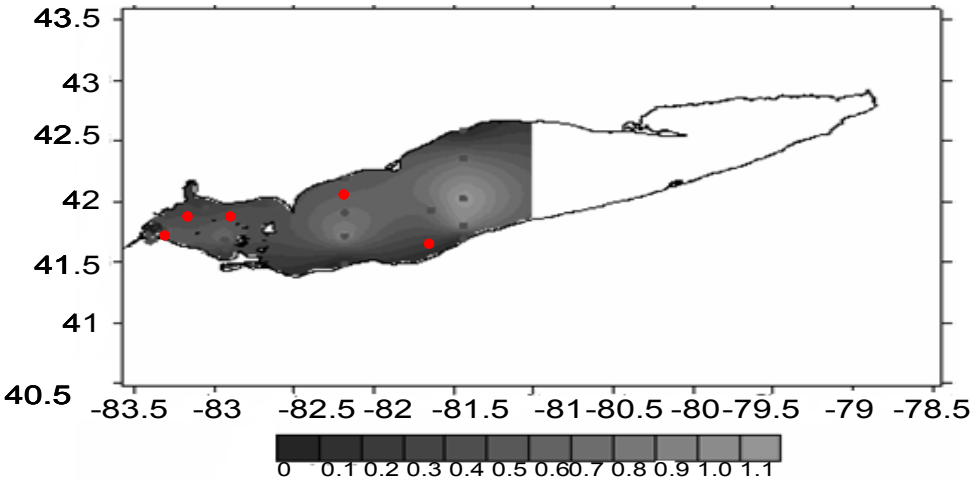
Chemical	Variables	May	June	September
	TP (µmol L^{-1})	0.68 ± 0.08 (6)	0.45 ± 0.01 (3)	0.64 ± 0.01 (3)
	SRP(µmol L^{-1})	0.09 ± 0.01 (6)	0.07 ± 0.09 (3)	0.13 ± 1.35 (2)
	NO_3^-(µmol L^{-1})	15.30 ± 2.33 (6	12.48 ± 2.33 (3)	19.74 ± 2.23 (3)
	NH_4^+(µmol L^{-1})	1.68 ± 0.09 (6)	0.74 ± 0.08 (3)	1.55 ± 0.01 (3)
	SrSi (µmol L^{-1})	6.84 ± 1.14 (6)	9.24 ± 0.22 (3)	4.87 ± 0.09 (3)
Biological	**Chl (ug/L)**	2.35 ± 1.39 (6)	2.26 ± 1.49 (3)	11.22 ± 1.48 (3)
	CN (atomic ratio)	**9.68 ± 1.23 (6)**	7.52 ± 1.43 (3)	8.02 ± 0.65 (3)
	N Debt (µmol N µg Chl a^{-1})	**0.16 ±0.29 (6)**	0.140 ± 0.04 (3)	0.14± 0.03 (3)
	CP (atomic ratio)	**158.7 ± 40.7 (6)**	**219.0 ± 138.9 (3)**	**132.2 ± 15.4 (3)**
	NP (atomic ratio)	18.64 ± 1.22 (6)	13.22 ± 1.31 (3)	20.22 ± 2.21 (3)
	APA (µmol P µg Chl $a^{-1}h^{-1}$)	0.001 ± 0.001 (6)	**0.08 ± 0.004 (3)**	0.002 ± 0.001 (3)
	P Debt (µmol P µg Chl a^{-1})	0.04 ± 0.02 (6)	**0.12 ± 0.06 (3)**	0.06 ± 0.02 (3)
	Fv/Fm(PAM)	0.194 ± 0.008 (6)	0.137 ± 0.035 (3)	0.466 ± 0.024 (3)
	Fv/Fm(FRRF)	0.154 ± 0.062 (6)	0.198 ± 0.062 (3)	0.512 ± 0.216 (3)
	σ PS II	745.41 ± 210.34 (6)	797.33 ± 122.10 (3)	186.33 ± 67.12 (3)
Physical	**Surface Temp (^oC)**	5.7 ± 0.5 (6)	19.9 ± 0.3 (3)	23.0 ±0.5 (3)
	Max Depth (m)	15.0 ± 4 (6)	16.0 ± 2.3 (3)	15.6 ± 5.1 (3)
	Mixing Depth (m)	15.0 ± 4.0 (6)	6.2 ± 3.5 (3)	6.8 ± 5.1 (3)
	kd (m^{-1})	.42 ± .22 (6)	.36 ± 0.18 (3)	.31 ± .12 (3)
	Mean PAR (µmol m^{-2} s^{-1})	99 ± 5.58 (6)	178.22 ± 3.51 (3)	127.43 ± 0.85 (3)

Table 2.4 Initial conditions (mean + standard deviation and number of samples)) of chemical, biological and physical parameters, West Basin. (Bold = values above the threshold of deficiency).

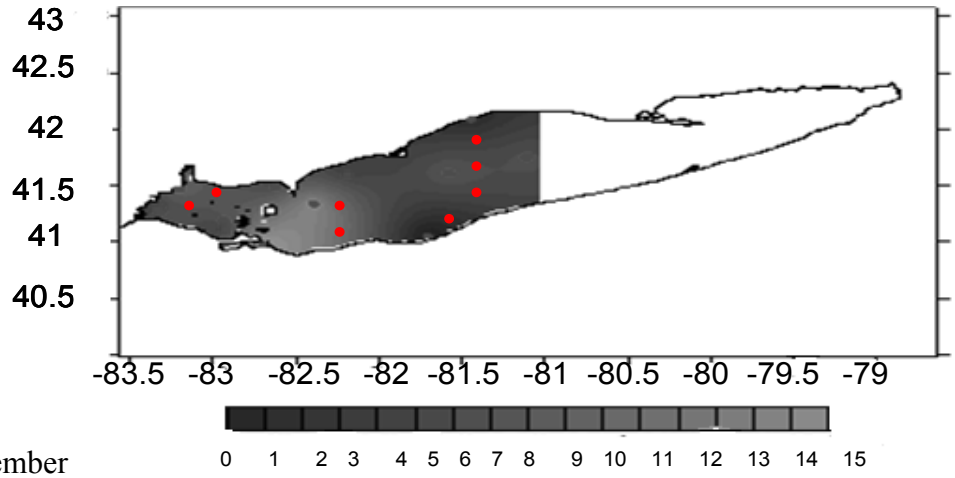
	Variables	May	June	September
Chemical	**TP (µmol L^{-1})**	0.75 ± 0.5 (9)	0.65 ± 0.09 (7)	0.75 ± .051(7)
	SRP(µmol L^{-1})	0.139 ± 0.5 (9)	0.13 ± 0.08 (7)	0.19 ± .41 (7)
	NO_3^-(µmol L^{-1})	16.45 ± 2.22 (9)	14.32 ± 1.22 (7)	22.81 ± 4.51 (7)
	NH_4^+(µmol L^{-1})	2.15 ± 1.2 (9)	0.94 ± 0.44 (7)	1.71 ± 1.44 (7)
	SrSi (µmol L^{-1})	28.66 ± 2.31 (9)	8.01 ± 2.2 (9)	13.05 ± 1.4 (7)
Biological	**Chl (ug/L)**	2.57 ± 1.01 (9)	6.67 ± 3.43 (7)	11.06 ± 6.43 (7)
	CN (atomic ratio)	**9.15 ± 1.53 (9)**	**8.36 ± 2.32 (7)**	7.54 ± 1.73 (7)
	N Debt (µmol N µg Chl a^{-1})	**0.19 ± 0.02 (9)**	0.14 ± 0.10 (7)	0.13 ± 0.03 (7)
	CP (atomic ratio)	**197.9 ± 43.3 (9)**	**166.5 ± 61.2 (7)**	**164.8 ± 14 (7)**
	NP (atomic ratio)	21.44± 1.22 (9)	12.22 ± 2.22 (7)	10.22 ± 2.12 (7)
	APA (µmol P µg Chl $a^{-1}h^{-1}$)	**0.004 ± 0.002 (9)**	**0.006 ± 0.002 (7)**	0.002 ± 0.001 (7)
	P Debt (µmol P µg Chl a^{-1})	0.07 ± 0.02 (9)	0.06 ± 0.01 (7)	0.04 ± 0.03 (7)
	Fv/Fm(PAM)	0.193 ± 0.127 (9)	0.335± 0.166 (7)	0.521 ± 0.077 (7)
	Fv/Fm(FRRF)	0.206 ± 0.141 (9)	0.376 ± 0.133 (7)	0.566 ± 0.042 (7)
	σ PS II	616.34 + 143.32 (9)	558.33 ± 102.11 (9)	112.2 ± 72.23 (7)
Physical	**Surface Temp (^oC)**	8.9 ± 0.8 (9)	21.7 ± 1.2 (7)	23.6 ± 0.6 (7)
	Max Depth (m)	8.1 ± 1.9 (9)	6.8 ± 2.1 (7)	7.1 ± 2.0 (7)
	Mixing Depth (m)	8.1 ± 1.9 (9)	3.8 ± 1.6 (7)	7.1 ± 2.0 (7)
	kd (m^{-1})	0.62 ± 0.13 (9)	0.54 ± 0.14 (7)	0.52 ± 0.22 (7)
	Mean PAR (µmol m^{-2} s^{-1})	87.00 ± 12.33 (9)	97.02± 13.58 (7)	111.21 ± 2.26 (7)



(a) May



(b) June



(c) September

Figure 2.1 ***(a-c)*** Chlorophyll distributions (µg L^{-1}) across WB, CWB and CB of Lake Erie (2005). Red dots = sampling stations

2.3.2 Phytoplankton Nutrient Status

In May, average values for both N status indicators (N debt and C:N) were above the threshold for inferring N deficiency in all three basins and numerous sites were classified as N deficient, especially in WB (Tables 2.1to 2.4). In other months, only one or neither indicator suggested N deficiency in any of the basins, except possibly June in WCB, when average C:N was near threshold, and fewer stations were classified as N deficient (Fig. 2.2 to 2.4). There was little evidence of N deficiency in September except in WCB where one of three stations had relatively high indicator values. In no case did the average C:N value for any basin or month indicate severe N deficiency.

For the P status indicators, the average particulate C:P ratios suggest moderate P deficiency in all months and basins except CB in June, when values corresponded to extreme deficiency (Tables 2.2 to 2.4). Average particulate N:P ratios were below the threshold of deficiency in every month in CB, June only in WCB, and May and June in WB. N:P was highest overall in June and lowest in September. Average P debt and APA values revealed similar P deficient patterns among basins and months, except in June and September where APA showed P deficiency in WB. The P debt assays did support the APA pattern of greatest overall deficiency in June and least in September. While N deficiency also appeared minimal in September, it was most prevalent in May rather than June in all months and basins. Average APA was above the deficiency threshold.

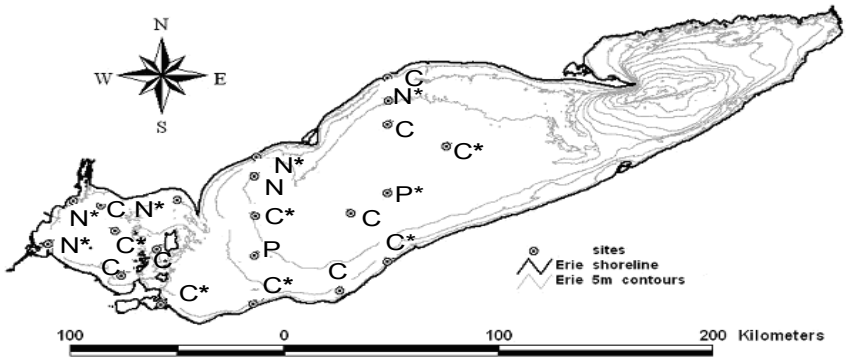


Figure 2.2.Distribution of nutrient status among sample sites in western, central-western and central basin of Lake Erie, May 2005. N = Nitrogen deficient, P= Phosphorus deficient, C= Nutrient sufficient. * denotes sites selected for phytoplankton taxonomic analysis

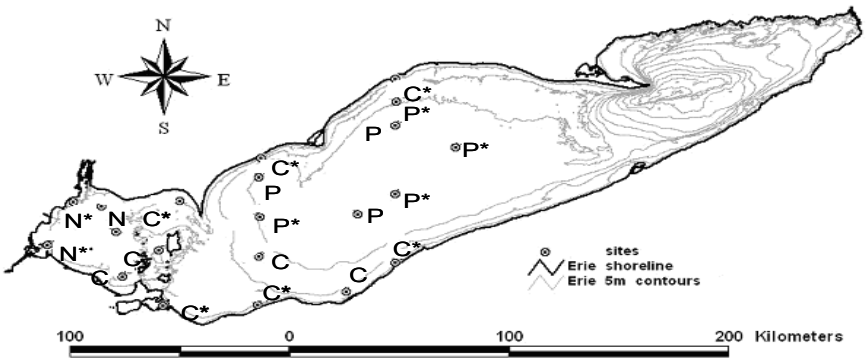


Figure 2.3 As Fig. 1 but for June 2005.

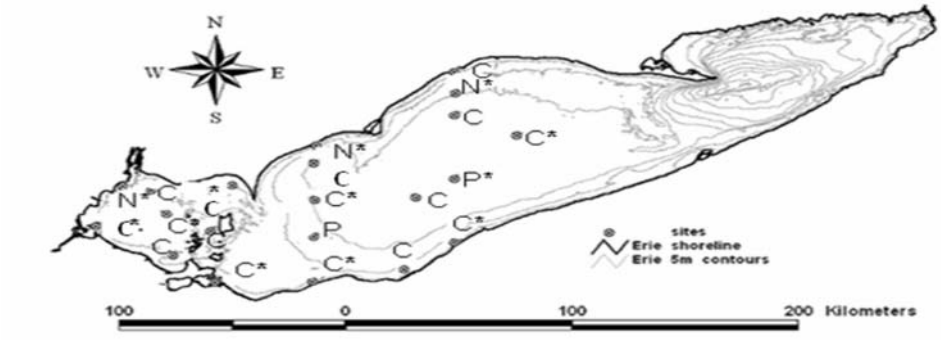


Figure 2.4 As Fig. 1 but for September, 2005.

2.3.3 Variable Fluorescence

F_v/F_m as measured by PAM or FRRF showed lowest measurements in June (CB, WCB) or May (WB) (Tables 2.2-4). The highest F_v/F_m, approaching the commonly accepted optimal value of 0.65 (Kolber et al. 1988) in WB, was observed in September. September was also the month of minimal N and P deficiency according to the nutrient status measurements. Over all months, F_v/F_m was lowest in CB and highest in WB.

Linear regression analysis indicated a high degree of correlation between PAM and FRRF measures of F_v/F_m ($y = 0.6200 + 0.99x$, $R^2 = 0.92$, n = 22, $p < 0.05$). Relationships between variable fluorescence, nutrient status, and environmental factors were examined with both the PAM and FRRF data and the results were quite similar. Therefore, I show only the results for the FRRF data here.

In assessing site-and date-specific relationships between F_v/F_m and nutrient status, data were screened to focus on the nutrient of interest. To examine relationships with N deficiency, for example, sites also showing P deficiency or SrSi depletion were excluded. Most of the sites with N debt below the deficiency threshold had relatively high F_v/F_m values and occurred in September (Fig. 2.5). Sites with N debt above the deficiency threshold had mostly low F_v/F_m values and occurred mainly in May. The pattern was similar but not as clear when the particulate C:N ratio was plotted against F_v/F_m (not shown). The relationship between N debt and F_v/F_m was strong (r = -0.85, n = 33, $p<0.05$), whereas the relationship between C:N ratio and F_v/F_m was weaker (r = -0.45, n = 33, $p < 0.05$).

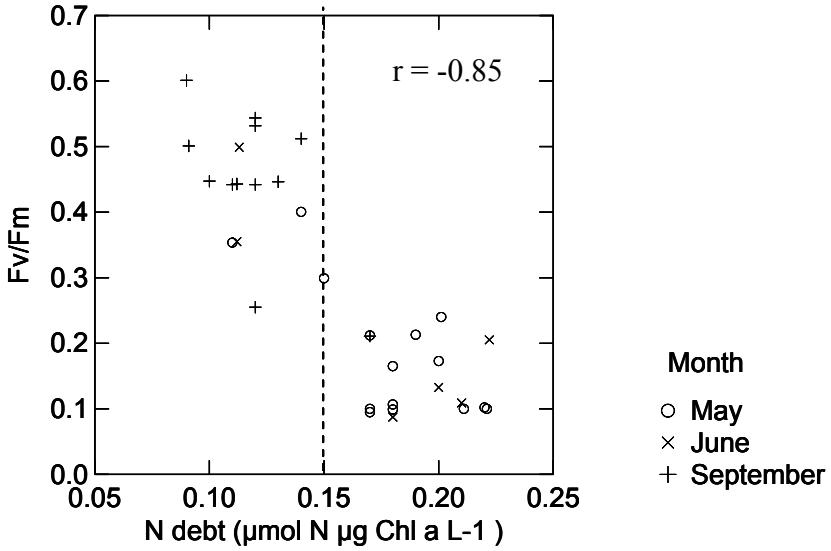
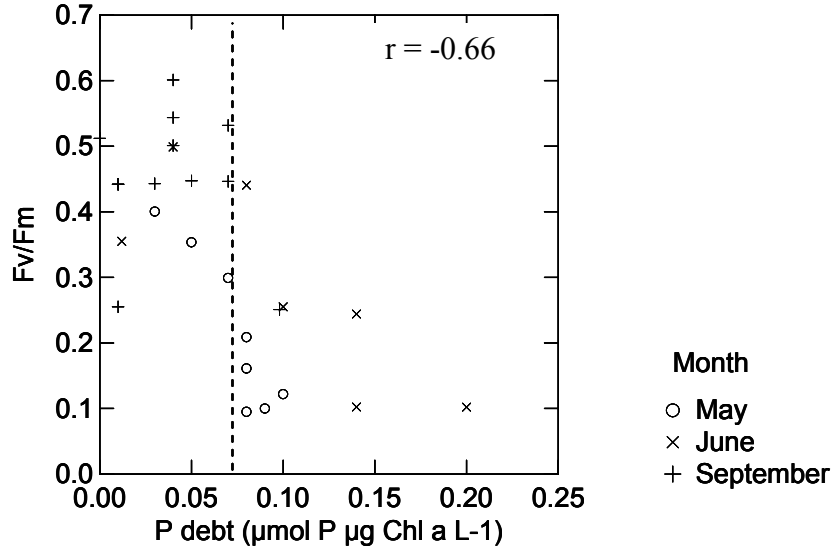


Figure 2.5. Scatter plot of Fv/Fm versus N debt. Each point represents sampling station. Dashed line = threshold of deficiency (N debt = 15 (Healey and Hendzel (1979b)).

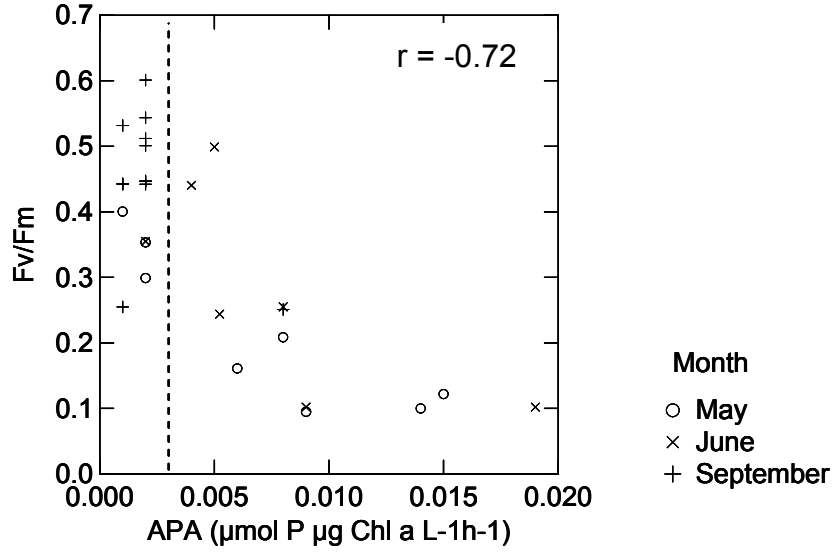
With N deficient and/or SrSi-depleted sites excluded, P debt and APA suggested a trend towards higher F_v/F_m at sites below the threshold of deficiency, and lower F_v/F_m values at sites above the threshold of deficiency (Fig. 2.6a and b). Most of the sites that were not P deficient were from September, while sites that were P deficient were mainly from June. Pearson correlation analysis revealed a strong relationship between P debt and F_v/F_m (r = -0.66 n = 27, $p<0.05$) as well as APA and F_v/F_m (r = -0.72, n = 27 $p<0.05$), while the F_v/F_m vs C:P relationship (not shown) was not as strong (r = -0.38, n = 27, $p<0.05$).

FRRF-derived σ_{PSII} values were highest in May (WB, WCB) or June (CB), and lowest in September (Fig. 2.7 *a-b*, Table 2.2 to 2.4). There was an inverse relationship with F_v/F_m. With sites screened for P or Si limitation as for the inter-site analysis of F_v/F_m, σ_{PSII} varied positively with N debt (Fig. 2.7 *a*) and C:N (not shown). The relationship with C:N was weak but significant (r = 0.32 n = 33 $p<0.05$) but the relationship with N debt was

highly significant (r = 0.73 n = 33 p<0.05). With N and Si deficient sites excluded, σ_{PSII} was significantly and positively correlated with P debt (Fig. 2.7 *b*) and APA (not shown) (r = 0.38, 0.42 n = 27, p<0.05) although relationships were weaker than with N debt.

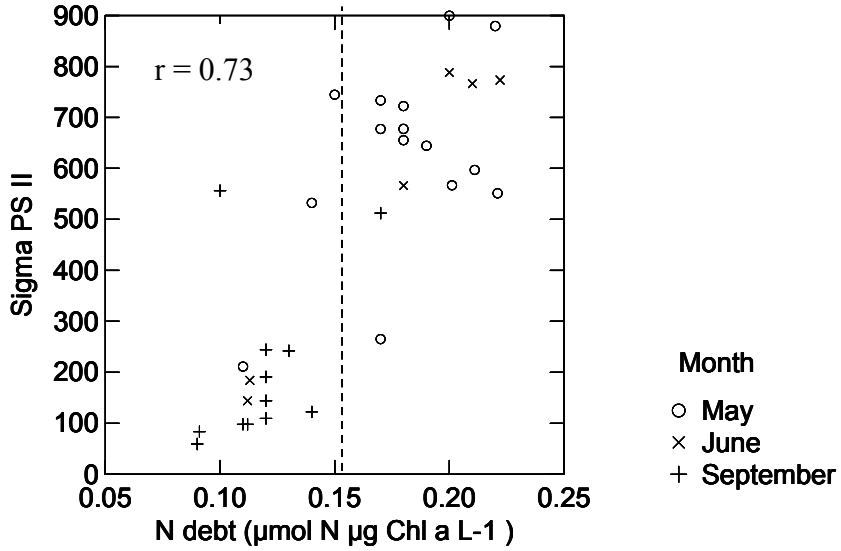


(a)

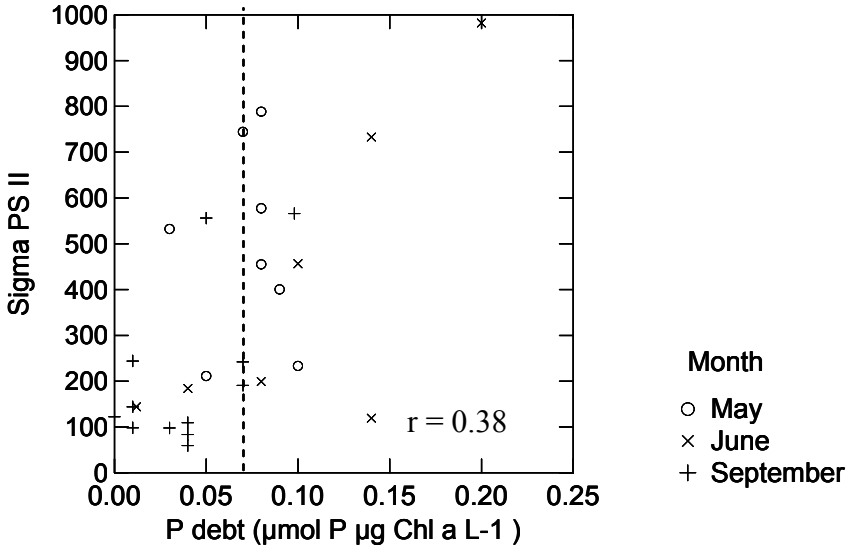


(b)

Figure 2.6 Scatter plot of Fv/Fm vs. P debt (b) Fv/Fm vs. APA. Each point represents sampling station. Dashed line = threshold of deficiency (P debt = 0.075, APA = 0.003 (Healey and Hendzel (1975, 1979b)).



(a)



(b)

Figure 2.7. Scatter plot of (a) Sigma PSII vs. N debt and (b) Sigma PS II vs. P debt. Each point represents sampling station. Dashed line = threshold of deficiency.

2.3.4 Phytoplankton Composition and Nutrient Status

Selected samples from May, June and August were enumerated to the lowest possible taxonomic denomination. In total, 158 phytoplankton taxa, belonging to six major groups, were identified.

For each month, 18 stations of varying nutrient status were analyzed to assess relationships with phytoplankton biomass and nutrient status. In spring, all basins supported populations of diatoms such as *Asterionella formosa, Aulacoseira islandica, Stephanodiscus binderanus*, and *Cyclotella ocellata*. Summer phytoplankton communities had more cyanobacteria, cryptophytes, chyrysophytes and pyrrophytes compared to diatoms (Figure 2.8 a-c) but seasonal and inter-basin patterns were significantly affected by variations associated with nutrient status. For P deficient stations, cryptophytes generally appeared in extensive populations usually represented by members of *Rhodomonas* and *Cryptomonas*. Chrysophytes were also prevalent and were often represented by *Dinobryon divergens, Dinobryon boregii, Rhizochromonas endoricata* and *Rhizochrysis spp.* Overall, P deficient stations tended towards dominance by flagellates and small or medium sized taxa.

In all basins and months, N deficient stations had a higher proportion of cyanobacteria than P deficient or nutrient sufficient sites. In spring, major taxa included *Synechococcus spp., Chroococcus sonoresis, Cyanothece spp. and Aphanothece clathrata*. During summer stratification and the onset of autumn turnover, species from larger-sized taxa such as *Microcystis aeruginosa, Cylindrospermopsis spp., Limnothrix spp., and Anabaena spp.* were important.

For nutrient sufficient stations, pyrrophytes and cyanobacteria were dominant in summer stratified conditions (Fig. 2.8 a-c). *Cylindrospermopsis spp*. (cyanobacteria), and

Peridinium spp. (Pyrrophyta) were often seen in the WB, CWB and CB. In spring, the major contributors to the biovolume of phytoplankton were from bacillariophytes and pyrrophytes.

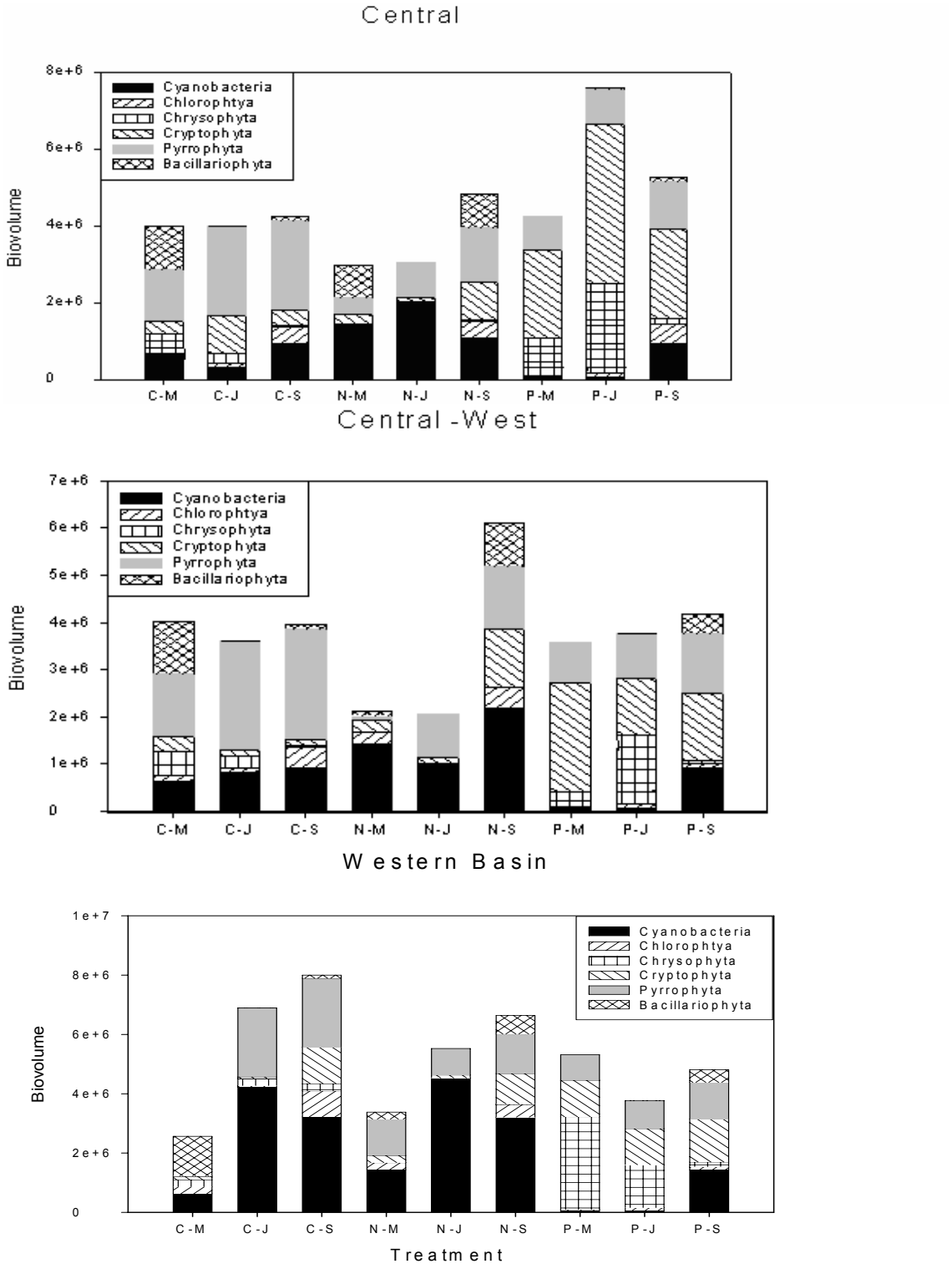


Figure 8 (a-c) .Average Biovolume (cubic (CU)µm.mL) of phytoplankton groups in (a) Central, (b) West-Central and (c) West basins of Lake Erie 2005. Samples were collected in May (M), June (J) and September (S). C = nutrient sufficient stations, P = P deficient stations, and N = N deficient stations

2.3.5 Multivariate Analyses

The highest correlations among canonical variates were seen with F_v/F_m (PAM) and F_v/F_m (FRRF) (Table 2.5). CCA was able to capture 45% and 37% of the variance in the phytoplankton data on the first two canonical variates (Table 2.6). All variables except N debt, C:N, C:P, P debt and σ PS II showed high correlations (>0.5) with axis 1, and all variables except for C:P and C:N ,showed high correlations with axis 2 (Table 2.5)**.**

Table 2.5 Weighted correlations of the selected environmental variables/fluorescence for Lake Erie 2005 (Bold = significant correlation between variables (p>0.05)

Weighted correlation matrix (weight = sample total)									
	Chl a	N debt	C:N Ratio	P debt	APA	C:P	Fv/Fm (PAM)	Fv/Fm (FRRF)	σ PS II
Chl a	1.00								
N debt	-0.26	1.00							
C:N Ratio	-0.25	**0.70**	1.00						
P debt	-0.19	-0.13	-0.05	1.00					
APA	-0.13	-0.28	-0.25	**0.72**	1.00				
C:P	-0.12	-0.19	-0.08	**0.61**	**0.70**	1.00			
Fv/Fm (PAM)	**0.79**	**-0.83**	**-0.71**	-0.22	-0.33	-0.31	1.00		
Fv/Fm (FRRF)	**0.72**	**-0.81**	**-0.72**	-0.41	-0.25	-0.24	**0.87**	1.00	
σ PS II	**-0.51**	0.24	0.14	**0.52**	**0.62**	**0.43**	**-0.84**	**-0.88**	1.00

Table 2.6 Interset correlations between first two axes and environmental variables/fluorescence

Variable	Axis 1	Axis 2
Chl a	-0.64	-0.55
N debt	-0.32	0.52
C:N Ratio	-0.24	0.34
P debt	0.22	-0.45
APA	0.23	-0.62
C:P	0.66	-0.22
Fv/Fm (PAM)	-0.56	-0.55
Fv/Fm (FRRF)	-0.53	-0.51
σ PS II	0.22	-0.72

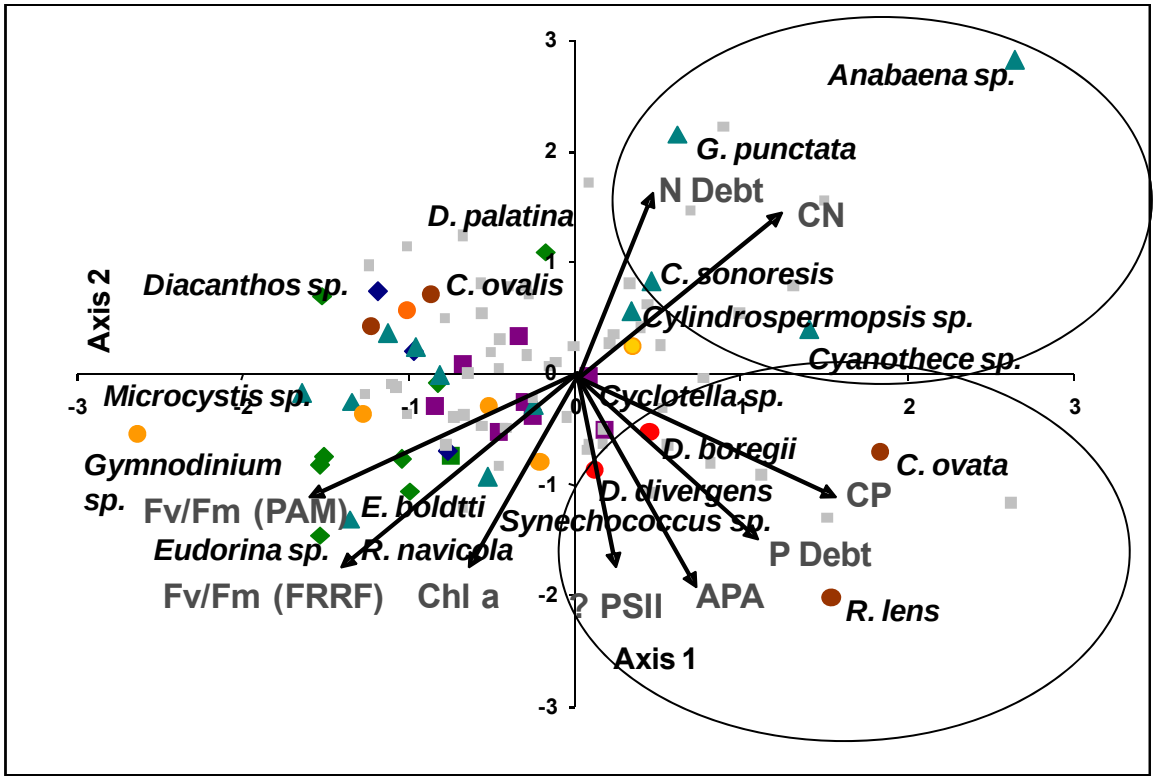


Figure 2.9 CCA plot of phytoplankton taxa, variable fluorescence parameters and nutrient status variables. Brown circles = cryptophytes, orange circles = pyrrophytes, red circles = chrysophytes, teal triangles = cyanobacteria, purple squares = diatoms and green diamonds = chlorophytes. Phytoplankton species enclosed in the upper right-hand circle were dominant in the western basin, and phytoplankton species enclosed in the lower right-hand circle were dominant in the central basin

Species of cyanobacteria were positioned to the right of the second axis, and were positively related to N debt and C:N and negatively related to F_v/F_m and Chl *a* (Fig. 2.9). Chl *a* and F_v/F_m (PAM and FRRF) were located opposite planes from the N deficiency variables, indicating a negative relationship between the two groups of variables. That is, high N debt and C: N samples were associated with populations displaying low F_v/F_m and Chl *a* values. P deficiency vectors (APA, P debt, C:P) were orthogonal to those for N deficiency variables and had weak associations with species from the cyanobacteria group.

In contrast, flagellate groups (chrysophytes and cryptophytes) were positioned to the lower right of the first axis and had strong positive relations with P debt, APA, C:P and σ PSII (Fig 2.9). *Rhodomonas, Cryptomonas, Dinobryon and Rhizochromonas* were were prominently associated with elevated values of the P deficiency indicators.

Species within the dinoflagellate group were spread along the first axis and second axis (Fig 2.9). This group was more weakly associated with nutrient status although some taxa could show associations with N or P deficiency (e.g. *Gymnodinium punctata* with N deficiency). Chlorophytes were strongly associated with higher Chl *a* and fluorescence parameters in all seasons and basins, and negatively associated with N deficiency. Diatoms plotted primarily to the left of axis 2 but not far from the origin. They did not show a strong association with N or P status variables and were more associated with moderate to higher values of Chl *a* and fluorescence parameters.

2.4 Discussion

The present results confirmed previous findings (Guildford et al. 2005) that phytoplankton communities in Lake Erie can display both P and N deficiency according to a

suite of commonly-used indicators. In isothermal conditions when nutrient concentrations are relatively high, nutrient deficiency would not normally be expected (Lean et al. 1983, Millard et al. 1996), but the present study supports Guildford et al. (2005) in finding indications of moderate N deficiency under such conditions, particularly in the WB. The prevalence of P deficiency in summer-stratified conditions observed here was consistent with many previous reports (Lean et al. 1983, Allen and Smith 2002, Guildford et al. 2005, Moon and Carrick 2007) and would be expected in a lake such as Erie which, through its ecosystem-level responses to altered P loading, would appear to be a P-limited system displaying low concentrations of SRP (Makarewicz and Bertram 1993, Charlton et al 1993). The current results additionally show an association between nutrient deficiency symptoms (both N and P) and photosynthetic physiology as inferred from variable fluorescence, strengthening the evidence that both N and P deficiency occur and advancing the case for believing that variable fluorescence can help assess nutrient status in natural communities. However, the results also indicate associations between nutrient status, variable fluorescence, and phytoplankton community composition, notably a tendency for heightened importance of cyanobacteria in samples showing N deficiency and depressed quantum yield (F_v/F_m). These additional associations suggest that selective changes in community composition may be driven in part by variations in nutrient status while contributing to the apparent effects of nutrient deficiency on variable fluorescence.

In temperate lakes, N limitation of phytoplankton is expected to be more common in eutrophic than oligotrophic lakes (e.g. Guildford and Hecky 2000) and at least short-term N limitation can be quite common in lakes of intermediate trophic status (Elser et al. 2007, Elser 2008). As discussed in Moon and Carrick (2007), the eutrophication of Lakes Erie and

Ontario in the 1960's and 1970's was associated with development of N limitation in some areas (e.g., Murphy 1980). P loading controls subsequently implemented through the Great Lakes Water Quality Agreement (GLWQA) were believed to strengthen P limitation and eliminate N limitation. Since the mid-1990's, and consequent to colonization by dreissenid mussels in the late 1980's, a renewed upward trend in total P extending at least into the early 2000's was noted (Charlton 2001, Moon and Carrick 2007). Unrecognized P loading and nutrient recycling by dreissenid mussels could both be agents of renewed N limitation (Moon and Carrick 2007, Boegman et al. 2008) in the early to mid-2000's. In the present results, N deficiency was most prevalent in May, particularly in the WB where both N indicators revealed N deficiency (Table 2-4). Although they are consistent with Guildford et al. (2005) and may have been favored by the recent (since 1995) nutrient changes in the lake, these observations are still surprising in the timing and apparent primacy of N deficiency that they suggest. There is evidence from enrichment experiments performed in the early 2000's for N, P, and Fe co-limitation (but not N limitation) of phytoplankton in eastern Lake Erie in summer (North et al. 2007). However, the nitrate and ammonium concentrations observed here (and in Guildford et al. 2005) were much higher, especially for NO_3^-. Enrichment experiments in the CB also showed N limitation in Lake Erie in the early 2000's, but again in later seasons (summer-fall) and only secondary to P limitation (DeBruyn et al. 2004, Moon and Carrick 2007).

It is possible that my N status indicators were influenced by factors other than actual physiological deficiency of the phytoplankton and gave misleading results. Particulate C: N ratios, for example, could be affected by inclusion of non-algal material, particularly resuspended and/or riverine sediments that would be especially abundant in the shallow west

basin and during the spring mixing period. The N debt assay could in theory reflect non-algal N dissimilatory processes such as nitrification as well as actual algal assimilation. Despite these uncertainties, both of the N status indicators (and especially N debt) were correlated with F_v/F_m and σ PS II, suggesting that algal physiology really was altered and that N deficiency actually did occur.

Iron (Fe) can at times be a limiting nutrient for phytoplankton growth in the Great Lakes (Sterner et al. 2004, Twiss et al. 2000, 2005) and can limit NO_3^- utilization, resulting in Fe-N co-limitation (North et al. 2007). Although Fe was not measured in the current study, it seems unlikely that low measurements of Fe would be responsible for appearance of N deficiency in spring, particularly in the shallow, well-mixed and strongly river-influenced WB. Sterner et al. (2004) reported higher Fe values in May (18 nmol L^{-1}) compared to summer stratified conditions in July (1.5 nmol L^{-1}) in western Lake Superior. Twiss et al. (2005) characterized trace metal limitation of Lake Erie phytoplankton as occurring infrequently and primarily in summer stratified conditions. While direct investigations of Fe availability in the spring season have not been done to my knowledge, Fe limitation or co-limitation would seem unlikely.

The seasonal incidence of N deficiency suggests that the lower light and/or temperatures in May might be critical in promoting N deficiency. The N requirements of phytoplankton have been shown to increase as temperature decreases in the sub-optimal range, so that higher N assimilation rates are required to maintain N-sufficient growth (e.g. Rhee and Gotham 1981). Acclimation to low irradiance is also associated with increased N requirements and N:P ratios (e.g. Wynne and Rhee 1986, Leonardos and Geider 2004, Finkel et al. 2006). The observed NO_3^- concentrations in May were mostly in the range 13

to 18 μmol/L. Although relatively high, such concentrations would still limit nitrate uptake to approximately 70 to 90% of the maximum rate given the range of half-saturation constants for growth (approximately 1.5 to 5.0 μmol/L) considered typical of freshwater phytoplankton in culture (Lehman 1975). Ammonium (NH_4^+) uptake from the typical May concentrations of 1.5 to 3.0 μmol/L would also be limited to 80% or less of maximum rates for most taxa (including some relatively efficient marine species). Utilization of the more abundant NO_3^- could be additionally impeded by the effect of the lower mean irradiance in May on NO_3^- reductase activity (Falkowski and Raven 2007) and through competitive inhibition by NH_4^+ (Dortch 1990). The situation in May could be a state of temperature, light and N co-limitation, in which the elevated need for N cannot quite be met despite fairly high available N concentrations. In this situation, I would not expect N deficiency to be severe and indeed the N status indicators indicated only a mild or moderate degree of limitation.

In contrast to N deficiency, P deficiency as indicated by P debt and APA was prevalent under summer stratified conditions (June). Previous studies in the CB showed that phosphate turnover times became shorter and indicative of P limitation in summer, but were longer and less suggestive of limitation in spring (Lean et al. 1983, Allen and Smith 2002, Smith unpub. data), while enrichment bioassays have shown consistent primary limitation by P in summer and fall, with maximum response in summer (Moon and Carrick 2007). Using the same P status indicators as those used here, Guildford et al. (2005) showed that P deficiency was largely absent in spring and fall and maximal in summer, with greatest deficiency in CB and EB. Studies from the late 1970's (Lean et al. 1983), mid-to-late 1990's (Allen and Smith 2002, Guildford et al. 2005) and the early to mid-2000's (Moon

and Carrick 2007, present results), these studies suggest a consistent summer condition of P deficiency over much of the lake. However, all studies of turnover times, P debt and APA have suggested that deficiency is typically mild or moderate, with only isolated episodes of severe P deficiency. Comparative lake studies, including measurements in Lake Ontario, suggest that large lakes such as Erie tend to have less severe P deficiency than smaller lakes (Guildford et al. 1994, Millard et al. 1996). Stronger mixing together with lower temperatures and mean irradiance in large lakes can contribute to this difference but in Lake Erie a relatively efficient P regeneration cycle may also be involved (Guildford et al. 2005).

The stoichiometry of C, N and P has been used in biogeochemical models to link phytoplankton production and the carbon cycle to the nitrogen and phosphorus cycles (Finkel et al. 2006) and to infer the identity of limiting nutrients (Healey and Hendzel 1979b, Guildford and Hecky 2000, Elser et al. 2008). In the present study, the average C:P ratio was above the nominal threshold for P deficiency in every month and basin, while the average N:P ratio was below. The disparate results probably reflect the plasticity of stoichiometry for balanced growth both within and among taxa (e.g. Finkel et al. 2006, Sterner et al. 2008), which can make fixed thresholds such as those used here misleading. At least in May, it may also be that the same cold, well-mixed and comparatively low-irradiance conditions that could promote higher N:P ratios in the phytoplankton could also favour re-suspension of sedimentary and detrital particles with relatively high C:P, thus inflating the estimate of the planktonic C:P ratio. Sediment re-suspension could also, in opposition to the expected effects on phytoplankton physiology, depress N:P ratios (Niemistö et al. 2008). The stoichiometry of the seston may furthermore respond on different time scales from more physiological measures such as P debt and APA and thus

suggest different patterns, depending on the recent history of conditions at any given site. The C:P ratios (and C:N) did have weaker among-site correlations with F_v/F_m and σ PS II in this study than did the nutrient debt or APA measurements but the relationships were nonetheless statistically significant. At least C:P and C:N, if not N:P, therefore appeared to have some relationship with physiological nutrient deficiency in the phytoplankton.

The significant relationships between site-specific values of F_v/F_m and the indicators N debt and C:N are the most extensive evidence to date that N deficiency in natural communities, as measured by independent assays of algal condition, can produce consistent, quantitative decreases in F_v/F_m and diminished quantum efficiency in PSII across a range of community composition and environmental conditions. Although the physiological mechanisms were not resolved here, the results were consistent with suggestions that N deficiency, among its other effects, specifically limits the supply of proteins essential to the function of PSII, notably the D1 protein (Falkowski and Raven 2007, Shelley et al. 2007). The relationships between N debt, C:N and σ PS II are likewise the most direct and extensive demonstration for natural communities that the effective cross section of PSII is increased by N deficiency. These results for F_v/F_m and σ PS II were consistent with a number of laboratory studies of algal cultures grown under N limitation (e.g. Kolber et al. 1988, Geider et al. 1993).

P status indicators (P debt and APA) also revealed strong negative associations with F_v/F_m and positive associations with σ PS II. There is considerable laboratory evidence for decreases of F_v/F_m under P limitation (e.g. Geider et al. 1993, Beardall et al. 2001*a*, Shelley et al. 2005) but similar evidence for increases of σ under P deficiency is limited to one study of a marine diatom (Geider et al. 1993). The response of σ to P deficiency did appear

weaker than that of F_v/F_m, however, which may reflect differences in the physiological mechanisms by which N and P deficiency affect photosynthesis. While N deficiency may specifically and differentially impede synthesis of key reaction centre proteins relative to PSII light harvesting components (Kolber et al. 1988, Falkowski et al. 1989) the effect of P deficiency may be less specific. Nucleic acids, and especially RNA, comprise a large part of the total cellular P content (Geider et al. 1993, Arrigo 2005, Falkowski and Raven 2007). P limitation will constrain synthesis of proteins, including those in the reaction centre of PSII, but the most severe effects will not necessarily be concentrated in PSII. The primary effects could include, for example, diminished activity of RUBISCO due either to reduced protein synthesis capacity or diminished rates of substrate phosphorylation (Falkowski and Raven 2007). Comparatively little work appears to have been done on the mechanisms by which P limitation regulates photosynthesis and variable fluorescence in microalgae, but the present results point to some possibly distinctive differences in response to these two macronutrients.

The results in this study show promise for application of F_v/F_m and σ PS II in determining nutrient status in freshwater phytoplankton communities, but some reports suggest otherwise. Some laboratory studies using cultures of marine phytoplankton have concluded that F_v/F_m is not a reliable indicator of nutrient status (Cullen et al. 1992, Parkhill et al. 2001, and Kruskopf and Flynn 2006). For example, Kruskopf and Flynn (2006) examined the relationship of F_v/F_m to both N- and P-limited growth rate in continuous cultures of four different phytoplankton species. They failed to find consistent relationships between F_v/F_m and the degree of nutrient stress, as quantified by the growth rate. Under nutrient replete conditions, F_v/F_m can vary widely among algal taxa, confounding the effects

of physiological variations (Parkhill et al. 2001, Suggett et al. 2009). Diatoms and chlorophytes, for example, have been observed to have higher F_v/F_m values compared to most species of cyanobacteria and rhodophytes. According to these studies, a common maximum value for F_v/F_m cannot be assigned for all algal groups (Suggett et al. 2009) and deviations from it are not necessarily interpretable in terms of nutrient deficiency. This also opens the possibility that correlations between F_v/F_m and nutrient status in natural communities may actually reflect underlying shifts in community structure.

The selection of samples for analysis of phytoplankton community composition in this study was representative of samples from nutrient sufficient, P-deficient and N-deficient sites. It was not intended as a systematic survey of community composition in the lake, which limits the scope for comparison to previous studies. The total number of phytoplankton taxa observed in the WB, WCB, and CB, for example, was 158 whereas from 1983 to 1993, 286-288 taxa were reported from offshore stations of Lake Erie (Makarewicz et al. 1999 and Barbiero and Tuchman 2001). Here, the more limited sampling effort (including absence of samples from EB) in the present study likely played a role.

Cyanobacteria were important in most samples from nutrient-sufficient and N-deficient sites (Fig. 8 a-c), particularly in WB and especially in June, when they dominated the biomass. The general tendency towards a higher proportion of cyanobacteria in WB than CB, and in summer (June) compared to spring (May) was consistent with descriptions from the late 1990s (Barbiero and Tuchman 2001, Carrick 2004) but the overall importance of cyanobacteria was greater. This could reflect differences in sampling designs, but it is consistent with indications that cyanobacterial biomass increased from the mid- to late-1990's to the early 2000's (Conroy et al. 2005). Cyanobacterial importance was greater at

sites showing N deficiency, and less at those showing P deficiency, compared to nutrient sufficient sites. This was apparent in both May and June despite differences in the species composition of the cyanobacteria and of the basin-average degree of N deficiency between the two months. It appears that N deficient conditions can offer an advantage to cyanobacteria of diverse size and morphology in Lake Erie.

The small cyanobacteria that predominated in spring are mostly not known to fix atmospheric nitrogen but may be good competitors where light is limited and small size confers an advantage in taking up NH_4^+ (Murphy 1980, Morel et al. 1991, Stolte and Reigman 1996, Roberts and Howarth 2006). Compared to the green alga *Scenedesmus,* for example, the unicellular form of *Microcystis* can be an effective competitor for ammonium (Yoshida et al. 2007) while *Synechococcus* can be successful at low concentrations of both NO_3^- and NH_4^+ (Hyenstrand et al. 2000). Another possible advantage to cyanobacteria, both large and small, is the ability to mobilize nitrogen from their phycobilisomes in times of shortage (Stolte and Riegman 1996, Campbell et al. 1998. In summer, larger filamentous and colonial taxa became important. Some of these (e.g. *Anabaena sp.* and *Cylindrospermopsis sp*.) have heterocysts, suggesting nitrogen fixation activity. Others, such as *Microcystis sp.*, are not known to fix nitrogen. However, *Microcystis sp.* was also an example of the diversity evident within the major phytoplankton groups. Although important in June, it violated the general pattern for cyanobacteria in having little association with either N or P deficiency according to the CCA (Fig. 2.9).

The increased importance of cyanobacteria at N deficient sites would likely contribute to the observed inter-site correlations between F_v/F_m, σ PS II and N deficiency. Both F_v/F_m and σ PS II measurements have been reported for cyanobacterial species,

particularly those containing a considerable amount of phycocyanin (Campbell et al. 1998). The cellular phycobiliprotein content influences the F_o level fluorescence, particularly when phycobiliprotein levels are high. As a result, higher values of phycobiliprotein may contribute to a lower value of $F_v/F_{m,}$ and σ PS II values may appear to be lower than other taxa due to the blue excitation waveband employed by the FRRF. Since PS II absorption is dominated by phycobilisomes, the fluorescence excitation spectrum in PS II for cyanobacteria is much lower in blue than orange light (Campbell et al. 1998).

Previous reports of F_v/F_m for cyanobacteria cultures in nutrient replete conditions were usually in the range of 0.3-0.6 (Suggett et al. 2009). In this study, F_v/F_m values were observed at N deficient sites ranging from 0.17-0.2 much lower than the observed laboratory values. Also, evidence from traditional nutrient status indicators suggests N deficiency, primarily in the WB of Lake Erie. Furthermore, another possibility of low F_v/F_m values at N deficient sites could be that the phytoplankton biomass was not exclusively cyanobacterial, and other groups often contributed half or more of the total. While controlled studies on many of the important cyanobacterial (and other) phytoplankton taxa that were important in our samples are lacking, it appears that variable fluorescence values in this study could track changes in taxonomy, it could also be an indication on changes in nutrient status. Further experimental work is needed to address this challenge. For example, nutrient amendment experiments, in conjunction with studies like the present one may have potential for assessing this possibility.

Flagellates from the chrysophyte and cryptophyte groups were generally more important in CB than WB, and in June and September than in May. Their increased prevalence in summer was consistent with the findings of Barbiero and Tuchman (2001) but

their importance in WB was less then other basins. This could again reflect differences in sampling design or a real difference between the algal communities in the late 1990s and 2005. These algal groups showed the most pronounced association with nutrient status, being greatly elevated in importance under P deficiency in all basins and sampling months (Fig. 2.8). Some species within these groups are known to be nutritionally opportunistic, switching between autotrophy and heterotrophy depending upon cellular and environmental conditions (Watson et al. 1997). One can consider mixotrophic capabilities a successful strategy when resources are limited, particularly where P is limiting and bacterioplankton, with their typically low C:P ratios, are available. Experiments with cultured mixotrophic flagellates (Nygaard and Tobiesen 1993 and Olrik et al. 2007) support the view that phagotrophic activity can be induced under conditions of limited inorganic nutrient availability. The apparent selection for chrysophyte flagellates under P deficiency observed here echoes the strong association of such phytoplankton with very low P lakes (e.g. Watson et al. 1997).

At P deficient sites much lower F_v/F_m values were observed compared to nutrient sufficient sites (Table 2.2 to 2.4). In this study, F_v/F_m values ranged from 0.9 to 0.2. These values show a striking contrast to known laboratory values for nutrient replete flagellates. Suggett et al. (2009) have showed that nutrient replete flagellates often have high values of F_v/F_m ranging from 0.5-0.7. Based on these measurements, it seems that taxonomic shifts may not be the main cause of variation in the fluorescence indicators. Also, based on the traditional P status assays, evidence of physiological deficiency was observed when F_v/F_m measurements were low.

While changes in taxonomic composition seem unlikely to fully explain the correspondence between F_v/F_m and nutrient status observed in this study, it remains unclear why such relationships are evident in this study and some others on natural communities (e.g. Sylvan et al. 2007), when more controlled measurements on algal cultures are less encouraging (e.g. Parkhill et al. 2001, Kruskopf and Flynn 2006). One possibility is that relatively ideal laboratory steady state conditions involving highly predictable environmental conditions may not be as applicable in natural environments, where variations of temperature, light, mixing energy, grazing and nutrient inputs could affect the uptake rates of nutrients to optimize nutrient utilization in phytoplankton. The costs of accommodating the additional stressors and variable (but often suboptimal) environmental conditions in nature may prevent phytoplankton from fully maintaining the physiology of PSII.

The CCA biplot (Fig. 2.9) gave a somewhat different view of relationships among nutrient status and variable fluorescence, compared to the bi-variate analyses (Fig. 2.5 and 2.6). For example, the demonstrated bi-variate relationships between F_v/F_m and P deficiency, and between σ PS II and N deficiency, were scarcely evident and it would appear that N deficiency was much the strongest influence on F_v/F_m. In part this could be a result of compressing the multi-dimensional relationships into just two dimensions (Table 2.5 and 2.6). However, it also reflects the greater observed frequency of low F_v/F_m and N deficiency, compared to low F_v/F_m and P deficiency, and the strong association between N deficiency and community composition. This surprising result for the "P-limited" Lake Erie is an example of how the long-term biogeochemical limitation (in this case, P) can nonetheless be accompanied by significant occurrence of other limitations (Elser et al. 2007, Sterner 2008).

The CCA plot also confirmed and helped further visualize the strong associations between nutrient status and community composition. In addition to the associations already noted, it shows that dinoflagellates such as *Gymnodinium* were particularly associated with nutrient sufficient conditions, as were some diatoms.

This study was the first to combine determinations of variable fluorescence, independent measures of nutrient status, and analysis of phytoplankton species composition to assess their inter-relationships in natural communities. The fluorescence parameters revealed strong responses to nutrient deficiency, despite changes in community composition. Two distinct patterns of nutrient status were observed: the highest F_v/F_m and lowest σ PS II values were reported in nutrient sufficient sites, and the lowest F_v/F_m and highest σ PS II values were reported in N and P deficient sites (Fig. 2.10).

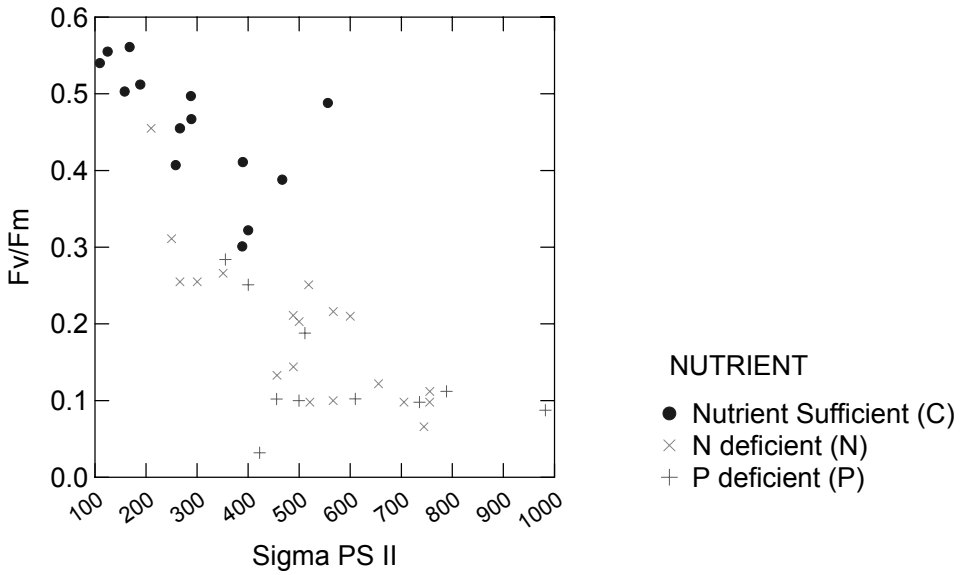


Figure 2.10 Scatter plot of Fv/Fm vs. Sigma (σ) PS II. Points represent samples/station

Despite some indictions that F_v/F_m and σ PS II differed in their responses to N and P deficiency, as noted above, the two measures were unfortunately not able to distinguish between N and P deficiency in this sample set. Additional work would be desirable to

further assess the significance of the apparent incidence of both N and P deficiency in lakes such as Erie, and to further fortify the conclusion that both types of limitation really occur. Such work could include additional sampling in locations where P deficiency is likely to be strong (e.g. some small inland lakes) or weak (nutrient rich lakes) and to determine if the incidence of N deficiency would follow the expected pattern of greater frequency in more eutrophic habitats. Simple extensions of the experimental protocols described here, notably experimental amendments with N and/or P, also have promise for further assessing the basis for, and implications of, variable patterns of N and P deficiency in lakes.

Chapter 3. Nutrient deficiency and the irradiance response of pulse-amplitude modulated (PAM) fluorescence in Lake Erie phytoplankton.

Overview

Phytoplankton in Lake Erie have displayed seasonally- and spatially varying patterns of P and N deficiency in previous studies. To further assess these patterns and examine more informative measures of nutrient status, I used PAM fluorescence to measure the maximum quantum efficiency (F_v/F_m) of photosystem II (PSII) and rapid light-response curves (RLC) of natural communities in 2006. Parallel measurements of nutrient status, using chemical and physiological assays as well as enrichment experiments, provided independent indices of nutrient status. Confirming previous reports, F_v/F_m was generally maximal in the more eutrophic west basin (WB) and minimal in the central basin (CB), while P deficiency was generally highest in the CB during summer stratification and N deficiency was highest in the WB during isothermal conditions. Like F_v/F_m, the electron transport rate at light saturation (ETR_{max}) and the initial slope of the ETR vs. irradiance curve (α) decreased as the severity of N and P deficiency increased. N and P enrichment for 24 hours stimulated increased F_v/F_m, ETR_{max} and α in N and P deficient samples respectively. The enrichment results were consistent with the view that the N and P deficiency assays, and corresponding variations of variable fluorescence parameters, were valid indicators of widely variable N and P deficiency in the phytoplankton. Contrary to my hopes, it did not appear that RLC responses provided more sensitive measures of deficiency than F_v/F_m alone, or a reliable discrimination between N and P deficiency. Identification of the most limiting nutrient still demanded additional information beyond the variable fluorescence measurements.

3.1 Introduction

In freshwater ecosystems, nitrogen (N) and phosphorus (P) frequently appear to be the two most important nutrients limiting phytoplankton growth (Lean et al. 1981, Hecky and Kilham 1988, Dodds et al. 1993) and can result in manifestation of deficiency symptoms in phytoplankton (e.g. Healey and Hendzel 1980). The severity of nutrient limitation is variable and is thought to help shape phytoplankton community dynamics (e.g. Teubner and Dokulil 2002, Teubner et al. 2003). Measures of the nutrient status of phytoplankton, based on their physiological response to deficiency, can help identify the patterns of nutrient limitation and elucidate how nutrients determine seasonal and spatial patterns in phytoplankton communities (Healey and Hendzel 1980). As explained in chapter 2, effective indices of, and assays for, nutrient status of phytoplankton have been developed but none is without its limitations. A particular need is for methods that are rapid to use, specific to phytoplankton, unambiguous in their diagnosis and, ideally, capable of discriminating which inorganic nutrient is most limiting at the time.

The variable fluorescence of chlorophyll *a* ($F_v/F_{m)}$ has for some time been considered a promising tool for detecting nutrient deficiency (e.g. Kolber and Falkowski 1993) that can meet at least some of the aforementioned criteria. It can be measured using Pulse Amplitude Modulated (PAM) and Fast Repetition Rate (FRR) fluorometers that are commercially available and convenient to use even on natural phytoplankton samples with low concentrations of chlorophyll *a* (Chl *a*). Chapter 2 described some of the disparate results that other researchers have obtained with F_v/F_m, including evidence (mainly from laboratory cultures) that constitutive differences between taxonomic groups, and widely variable

physiological responsiveness of F_v/F_m within some taxa, might confound its use as a nutrient status indicator. Despite this, chapter 2 showed that F_v/F_m correlated well with independent indices of N and P deficiency in Lake Erie, and encouraging results have been obtained in other field applications (e.g., Sylvan et al. 2007). Although taxonomic composition in Lake Erie also varied with F_v/F_m, the range of variation in fluorescence and its excellent correlation with other nutrient status assays showed that it was most likely reflecting physiological variations of nutrient deficiency and not just taxon-based constitutive differences. The purpose of this chapter is to verify and strengthen the conclusions from Chapter 2 with evidence from an additional type of assay, nutrient amendments, and to explore the potential of some of the additional capabilities of PAM fluorometry. Of particular interest was the variable fluorescence expressed under photosynthetically-useful irradiance (also known as actinic irradiance).

Dark adapted F_v/F_m values reflect the maximum quantum yield of PS II, which is sensitive to many environmental factors that alter the algal physiological state (Kolber et al. 1988, Falkowski and Raven 2007). In the presence of photosynthetic irradiance, the functional quantum yield (denoted $\Delta F/F_m'$) of photosystem II (PS II) is measured and can provide estimates of photosynthetic electron transport rates (ETR) through PS II (Genty 1989, Geider et al. 1993, Kobler et al. 1993, Ralph and Gademann 2005). Commercially-available PAM fluorometers make it quite easy to measure the functional relationship between photosynthetic irradiance and $\Delta F/F_m'$ (and by inference ETR). The resulting ETR vs irradiance relationships, termed rapid light curves or RLCs, have been proposed as useful tools for determining physiological changes in algae (Ralph and Gademann 2005). Insofar as nitrogen, iron and phosphorus deficiency affects the irradiance dependence and maximum

values of photosynthesis in many types of algae (e.g. Senft 1978, Osborne and Geider 1986, Cleveland and Perry 1987, Greene et al. 1991), RLCs may be a more robust indicator of nutrient stress than F_v/F_m.

ETR measurements can have a variable relationship to oxygen evolution or inorganic carbon fixation (Gilbert et al. 2000 and Beer and Axelsson 2004), in part because of the existence of multiple pathways for electron flow (Prasil et al. 1996, Kana et al. 2002). Such alternative pathways (i.e., alternative to a linear electron flow to carbon fixation) may allow cells to dissipate excitation energy and maintain high $\Delta F/F_m$' even if linear electron flow is inhibited. The Mehler reaction, for example, can maintain electron flow through PSII without any net production of oxygen or fixation of carbon (Falkowski and Raven 2007). Nonetheless, Kolber et al. (1988) found that nitrogen deficiency increased the susceptibility of the ETR to photoinhibition in some marine phytoplankton taxa, while Shelley et al. (2007) demonstrated that N deficiency decreased the light-saturated ETR in the green algal *Chlorella emersonii*. P starvation causes re-allocation of excitation energy and changes in light-saturated PSII activity in some algae (Wykoff et al. 1998), while Hiriart- Baer et al. (2008) showed that high C:P ratios (indicative of P deficiency) in benthic macroalgae in Lake Ontario were associated with decreased F_v/F_m and ETR.

In addition to allowing an estimate of light-saturated ETR, the initial slope of an RLC provides a measure of the efficiency of the phytoplankton in translating light into electron transport at limiting irradiance levels. This offers another metric of photosynthetic performance that may be less influenced by alternate electron pathways. Not only the Mehler reaction but also some other alternate pathways (e.g. plastoquinone terminal oxidase, Mackey et al. 2008) are thought to be less important under limiting than saturating

irradiance. There may also be potential for discriminating between different types of nutrient deficiency (e.g. N versus P) by comparison of light-saturated ETR and the initial slope of the RLC. N deficiency tends to limit production of many cell proteins, but chloroplast-encoded proteins essential to PSII functioning appear to suffer more relative to nuclear-encoded proteins associated with light harvesting chlorophyll complexes in at least some algae (Kolber et al. 1988, Falkowski et al. 1989). This tends to depress F_v/F_m (and $\Delta F/F_m'$) while elevating the PSII-specific light harvesting efficiency (functional cross section). This effect may be relatively specific to N deficiency, which is proposed to limit amino acid synthesis and favour translation of the nuclear-encoded proteins (Falkowski and Raven 2007). The same mechanisms might not be specifically induced by P limitation, which might rather be expected to constrain overall protein synthesis rates (Arrigo 2005) and the availability of phosphorylated substrates for carbon fixation (ATP production and Calvin – Benson cycle intermediates). Specific predictions are difficult to make, and it is important to recognize that the functional cross section of PSII is a different quantity from the initial slope of an RLC. It nonetheless seems possible that N and P deficiency might be manifested in differential effects on maximum ETR versus initial slope, but no evidence on this appears to have been published.

Another useful extension of variable fluorescence methods involves measuring responses to nutrient additions. Such assays have potential to provide a more convincing demonstration that any impairment of variable fluorescence is truly due to nutrient deficiency and to identify the nutrient responsible. The nutrient induced fluorescence transient (NIFT) bioassay examines short term (minutes) changes in F_v/F_m (Turpin and Wegner 1988). The response can include a transient drop in fluorescence and a subsequent

increase (Wood and Oliver 1995), which can complicate interpretation, but NIFTs have been studied in laboratory cultures to characterize responses to phosphate, nitrate and ammonia amendments (Turpin and Weger 1988, Wood and Oliver 1995, Shelley et al. 2007). Longer term (hours) responses to the addition of a limiting nutrient should normally reveal an increase in F_v/F_m, as shown by Sylvan et al. (2007) for P-deficient marine phytoplankton communities.

Lake Erie has been shown to display variable degrees of both N and P deficiency (Guildford et al. 2005, Chapter 2 of this thesis) associated with shifts in community structure, suggesting an important structuring effect of both nutrients in this lake. However, there are still aspects of the results for Lake Erie that present challenges to my understanding. Notably, the occurrence of N deficiency in such a high nitrate lake is unexpected and still not well-explained, raising the possibility that the nutrient assays used to date are somehow misleading. There was also frequent disagreement between some of the stoichiometric indicators (N:P and C:P), metabolic bioassays (N/P debt and APA) and F_v/F_m measurements used to infer nutrient status. A more direct confirmation that F_v/F_m truly reflects nutrient status in this lake, and progress towards better means of identifying the limiting nutrient, would be highly desirable.

The first objective of this study was to confirm, with additional observations in a subsequent year (2005), that previous indications of N and P deficiency in Lake Erie are reproducible. The second objective was to use amendment experiments to further test whether the previously-used assays are correct in their indications of N and P deficiency. The third objective was to test the idea that variable fluorescence measured under excitation

pressure (RLC's) may provide a more sensitive measure of nutrient status and even an indication of which nutrient might be limiting.

3.2 Methods
3.2.1 Study Site and Design

Water samples were collected in early May and late June of 2006 in the western basin (WB), western-central basin (WCB) and central basin (CB) of Lake Erie aboard the *CGGS Limnos*. There is often a particularly strong gradient in nutrients and plankton abundance from WB to CB, with the western part of CB often denoted as an additional sub-basin (west central basin, or WCB) due to its distinctive nutrient, plankton and circulation features (e.g. Leon et al. 2005). Sampling sites for this study (Fig. 1) were distributed in WB, WCB and CB to obtain the best representation of the strong spatial variability in the western part of the lake.

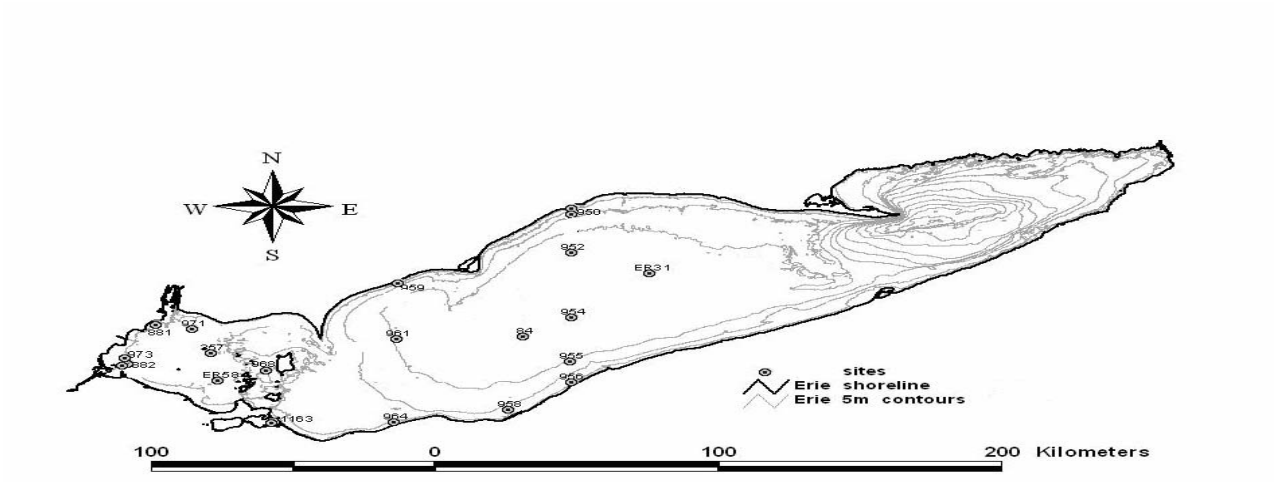


Figure 3.1 Distribution of sampling stations in the western, central-west and central basin of Lake Erie 2006.

Water samples were collected using 8 L Niskin bottles mounted on a Rosette with a CTD (Seabird TM) sampler. Immediately after collection, water was transferred to 20 L polyethylene carboys covered in black plastic bags to prevent light shock.

The physical variables measured in this study were temperature profiles from the Seabird CTD and vertical profiles of photosynthetically active radiation (PAR) from a PAR sensor attached to the Fast Repetition Rate fluorometer (FRRF Fasttracka ^{TM} Chelsea Instruments). The vertical attenuation coefficient (Kd) was estimated by linear regression of the natural logarithm of PAR vs. depth. The mixing depth (Z_{mix}) and mean PAR of the surface mixed layer were calculated according to Hiriart et al. (2002).

3.2.2. Nutrient and Chl a Analysis

Samples for NH_4^+ were first run through a polycarbonate filter (0.2 μm pore size) and were measured with the orthophtaldialdehyde (OPA) method outlined in Holmes et al. (1999). Filtered samples were also analyzed for NO_3^-, and nitrite (NO_2^-), using a Dionex Ion Chromatograph (ICS 2500). All samples were frozen in the field and analyzed immediately upon returning to the laboratory.

Particulate C and N samples were analyzed by the methods described by Strickland and Parsons (1972). The filters were dried and placed in desiccators containing hydrochloric acid for 24 h. C/N filters were autoclaved for 5 hours at 980^{o}C. A C/N autoanalyzer (Exeter Analytical Inc. CEC-440) was used to measure particulate C and N.

TP and SRP analysis followed the same procedure outlined in Chapter 2 and Chl a measurements were done in triplicate using GFF glass fiber filters (0.7 μm, 47 mm) using the method outlined by Strickland and Parsons (1972).

3.2.3 Phytoplankton Nutrient Status Indicators

P and N limitation were assessed by stoichiometric ratios (particulate C:P and C:N) and metabolic assays (N/P debt and APA) (Healey and Hendzel 1979*b*). The analysis of N debt, P debt and APA followed the procedure outlined in Chapter 2. Sites were only classified as N deficient when N status indicators (N debt and C:N) were both above the threshold of deficiency (Table 3.1), and as P deficient when P debt, C:P and APA were above the threshold of deficiency.

3.2.4 Chl a Fluorescence

Phytoplankton samples (1 L) obtained from the sampling sites, were concentrated onto 24-mm glass fiber filters (GF/F, Whatman, Springfield Mill, U.K.) under low (<10mmHg) vacuum. Phytoplankton were dark adapted for 30 min on filtered lake water in covered Petri dishes to keep cells hydrated. A pulse amplitude modulated (PAM) fluorometer (Diving PAM, Heinz Walz, Germany) was used to measure Chl *a* fluorescence. The quantum efficiency of PS II (F_v/F_m) was calculated by instrument software (Walz 1993) and fluorescence rise kinetics were inspected to verify that maximum fluorescence (F_m) was obtained (i.e. to verify that a clear plateau in fluorescence was observable within the period of the saturation pulse). Gain was adjusted and all measurements were corrected for background signal using a blank consisting of distilled water filtered onto 24-mm glass fiber filters (Walz 1993).

Rapid light curves (RLCs) were constructed by exposing the sample to 9 progressively-increasing actinic (photosynthetic) light levels. Maximum actinic light was

1150 μmol $m^{-2}s^{-1}$ and the exposure period per level was 20 s. The instrument measures the effective quantum yield of PS II (F_v'/F_m') and calculates the relative ETR as the product of F_v'/F_m' and actinic light flux. The RLCs were fitted to a model that allows for photoinhibition at high irradiance (Platt et al. 1982):

$$P^B = p^B_s [1 - \exp (-\alpha l / P^B_s) \exp (-\beta I / P^B_s) \quad \text{Eq. 3.1}$$

Where P^B in the original application is the carbon fixation rate normalized to the chlorophyll biomass but here is ETR (which is intrinsically normalized to chlorophyll). p^B_s [mg C (mg Chl a) $^{-1}$ h $^{-1}$] is thus the maximum ETR of the experimental population if there were no photoinhibition, α[(mg C mg Chl a) $^{-1}$ h^{-1} (W m^{-2}) $^{-1}$] is the initial slope of the RLC, β describes the strength of the photoinhibition and I is the actinic light level. If there is no photoinhibition then β is not significantly different from zero. In such cases, a simpler model was used:

$$P^B = p^B_s [1 - \exp (-\alpha l / P^B_s) \quad \text{Eq. 3.2}$$

Amendment experiments were used to assess responses to potentially limiting nutrients. From each site, water samples were collected into three 1L acid washed carboys. Two of the carboys contained 5 μM aliquots of either ammonia (NH_4Cl) or phosphate ($KH_2PO_4^{3-}$). The third carboy was the control and received no additions. All three carboys were incubated in temperature controlled and dark adapted environments with a maximum deviation of site temperature of $\pm 5^oC$. After 24 h, F_v/F_m and RLC's were measured as

described above. Two-way Analysis of Variance (ANOVA) was used to test for differences between lake sites (basins), treatments, and their interactions on Chl a fluorescence parameters (Fv/Fm, α and ETR_{max}). When significant effects were detected, the Holm-Sidak multiple comparison test was used to test for differences between site/treatment means and Chl a fluorescence values. This was chosen over the more commonly applied Tukey's test and Boneferroni tests because it is considered to be more for independent comparisons (Shaw 2003).

3.3 Results

3.3.1 Environmental Conditions

In May, there was no thermal stratification and mean surface temperatures ranged from 6.01 to 9.10 ^{o}C, highest in the WB. All three basins were thermally stratified in June and temperature values ranged from 22.30 to 25.60^{o}C with the highest values in WB. Mean PAR was highest in June, as the mixing depth became shallower and vertical attenuation coefficients (Kd) were smaller (Tables 3.2 and 3.3).

Spatially, TP and SRP concentrations were highest in WB in May and June while the lowest concentrations were reported in CB. NO_3^- and NH_4^+ concentrations were also high in May (WB) and low in June. However, the spatial trend from WB to CB was weak compared to TP and SRP (Tables 3.2 and 3.3). In contrast, soluble reactive silicate (SrSi) values were highest in the CB for both May and June. Mean Chl *a* concentrations (Tables 3.2 and 3.3) were higher in the WB for both sampling months, with June concentrations being much higher due to the presence of *Microcystis sp*. blooms.

Table 3.1 Nutrient status indicators. Values either show an absence, presence or the degree of nutrient limitation for phytoplankton. Criteria for nutrient limitation are based on Healey and Hendzel (1979b) and adapted from Guildford et al. (2005)

Indicator	Nutrient	No deficiency	Moderate deficiency	Extreme deficiency	Deficient
C/N (atomic ratio)	N	<8.3	8.3-14.6	>14.6	
N debt (µmol N µg Chl a $^{-1}$)	N	<0.15			>0.15
C/P (atomic ratio)	P	<129	129-258	>258	
N/P (atomic ratio)	P	<22			>22
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	

3.3.2 Phytoplankton Nutrient Status Assays

The average values of N debt and C:N in May were both above the threshold of deficiency (Table 3.1) in the WCB and WB, however the highest values were found in the WB. In June there was no evidence of N deficiency. For the P status indicators, the average particulate C:P ratios in May suggested moderate P deficiency in the WCB and CB, and in June in the WCB and CB (Tables 3.2 and 3.3). In May, average APA values were above the deficiency threshold in CB, while in June the values were highest in WCB and CB. Values were highest overall in June and lowest in May. Average P debt values had a similar pattern among basins and months as APA except that May values in WCB were in the non-deficient range. The P debt values did support the APA pattern of greatest overall deficiency in June and least in May.

Table 3.2 Concentrations of chemical, biological and physical data, May 2006 Lake Erie. (Bold = values above the threshold of deficiency)

Chl a Fluorescence	Western Basin	Central-west Basin	Central Basin
Chl a (µg L^{-1})	2.01 ± 0.62 (8)	1.85 ± 0.74 (3)	1.78 ± 1.34 (8)
Fv/Fm	0.154 ± 0.081 (8)	0.205 ± 0.066 (3)	0.125 ± 0.062 (8)
Fv/Fm C 24	0.144 ± 0.006 (8)	0.293 ± 0.037 (3)	0.288 ± 0.037 (8)
Fv/Fm N 24	0.401 ±0.147 (8)	0.312 ± 0.088 (3)	0.287 ± 0.051 (8)
Fv/Fm P 24	0.276± 0.043(8)	0.211 ± 0.114 (3)	0.289 ± 0.040 (8)
Ik	210.12± 9.30 (8)	157.20 ± 18.08 (3)	200.90 ± 11.73 (8)
Ik C 24	222.30± 9.60 (8)	187.30 ± 17.54 (3)	192.22 ± 10.43 (8)
Ik N 24	173.20 ± 39.15 (8)	222.40 ± 15.90 (3)	212.22 ± 1.41(8)
Ik P 24	232.22 ± 18.1 (8)	198.40 ± 16.45 (3)	202.41 ± 17.75 (8)
ETRmax	25.20 ± 5.02 (8)	22.20 ± 4.87 (3)	18.73 ± 4.34 (8)
ETRmax C 24	39.44 ± 1.10 (8)	27.40 ± 4.54 (3)	35.30 ± 8.15(8)
ETRmax N 24	61.22 ± 1.14 (8)	61.10 ± 5.01 (3)	51.22 ± 7.07 (8)
ETRmax P 24	50.11± 2.81 (8)	51.20 ± 2.45 (3)	48.50 ± 2.81 (8)
α	0.12 ± 0.04 (8)	0.14 ± 0.87 (3)	0.09 ± 0.06 (8)
α C 24	0.19± 0.06 (8)	0.12 ± 15.06 (3)	0.15 ± 0.12 (8)
α N 24	0.34 ±0 .01 (8)	0.26 ± 0.02 (3)	0.19 ± 0.14 (8)
α P 24	0.20 ± 0.11(8)	0.21 ± 0.06 (3)	0.13 ± 0.16 (8)
Physiological Assays			
CN (atomic ratio)	**13.22 ± 2.61 (8)**	**10.72 ± 2.29 (3)**	7.30 ± 2.36 (8)
N Debt (µmol N µg Chl a^{-1})	**0.21 ± .02 (8)**	**0.18 ± 0.12 (3)**	0.14 ± 0.06 (8)
CP (atomic ratio)	111.5 ± 152.2 (8)	**144.2 ± 150.2 (3)**	**155.4 ± 12.3 (8)**
APA (µmol P µg Chl $a^{-1}h^{-1}$)	0.001 ± 0.002 (8)	0.002 ± 0.002 (3)	0.002 ± 0.001 (8)
P Debt (µmol P µg Chl a^{-1})	0.05 ± 0.01 (8)	0.04 ± 0.01 (3)	0.07± 0.02 (8)
Physical			
Surface Temp (^{0}C)	9.10 ± 2.10 (8)	6.33 + 1.21 (3)	6.01 + 1.32 (8)
Max Depth (m)	6.65 ± 0.91 (8)	13.20 ± 0.60 (3)	14.65 + 2.20(8)
Mixing Depth (m)	6.65 ±0 .91(8)	13.20± 0.60 (3)	14.65 + 2.20 (8)
kd (m^{-1})	0.52 ± 0.33 (8)	0.47 ± 0.63 (3)	0.35 ± 0.30 (8)
Mean PAR (µmol m^{-2} s^{-1})	83.57 ± 12.75 (8)	79.54 ± 27.03 (3)	83.25 ± 13.01 (8)
Chemical			
TP (µmol L^{-1})	0.69 ± 0.09 (8)	0.52± 0.05 (3)	0.38 ± 0.05 (8)
SRP(µmol L^{-1})	0.16 ± 0.00 (8)	0.11 ± 0.03 (3)	0.07 ± 0.03 (8)
NO_3^-(µmol L^{-1})	13.53 + 2.44 (8)	12.07 ± 3.22 (3)	12.04 ±1.79 (8)
NH_4^+(µmol L^{-1})	1.58 ± 0.17 (8)	0.85 ± 0.04(3)	0.78 ± 0.06 (8)
SrSi (µmol L-1)	1.42 ± 0.91 (8)	0.94 ± 0.03 (3)	2.32 ± 0.53 (8)

Table 3.3 Concentrations of chemical, biological and physical data, June 2006 Lake Erie. (Bold = values above the threshold of deficiency)

Chl a Fluorescence	Western Basin	Central-west Basin	Central Basin
Chl a (µg L^{-1})	16.01 ± 4.62 (8)	4.85 ± 1.74 (3)	0.87 ± 0.34 (8)
Fv/Fm	0.454 ± 0.181 (8)	0.105 ± 0.166 (3)	0.089 ± 0.052 (8)
Fv/Fm C 24	0.444 ± 0.212 (8)	0.193 ± 0.137 (3)	0.158 ± 0.137 (8)
Fv/Fm N 24	0.401 ±0.221 (8)	0.282 ± 0.258 (3)	0.187 ± 0.151 (8)
Fv/Fm P 24	0.386± 0.103(8)	0.311 ± 0.214 (3)	0.519 ± 0.240 (8)
Ik	121.12± 6.30 (8)	125.20 ± 18.08 (3)	146.90 ± 1.73(8)
Ik C 24	167.30± 5.60 (8)	145.30 ± 17.54 (3)	180.30 ± 19.46 (8)
Ik N 24	182.20 ± 19.15 (8)	173.40 ± 13.90 (3)	172.22 ± 9.43 (8)
Ik P 24	182.22 ± 18.63 (8)	178.40 ± 166.45 (3)	180.41 ± 11.75 (8)
ETRmax	34.20 ± 5.02 (8)	32.20 ± 4.87 (3)	24.73 ± 8.34 (8)
ETRmax C 24	39.44 ± 4.27 (8)	37.40 ± 6.50 (3)	40.30 ± 1.21 (8)
ETRmax N 24	51.22 ± 4.15 (8)	49.20 ± 5.02 (3)	41.22 ± 7.07 (8)
ETRmax P 24	50.11± 7.84 (8)	54.20 ± 8.65 (3)	65.50 ± 2.82 (8)
α	0.28 ± .04 (8)	0.26 ± 0.87 (3)	0.12 ± 0.06 (8)
α C 24	0.24± .06 (8)	0.22 ± 15.06 (3)	0.25 ± 0.12 (8)
α N 24	0.28 ± .01 (8)	0.21 ± 0.02 (3)	0.24 ± 0.14 (8)
α P 24	0.28 ± .11 (8)	0.25 ± 0.06 (3)	0.36 ± 0.16 (8)
Biological			
CN (atomic ratio)	5.24 ± 2.01 (8)	5.72 ± 2.29 (3)	7.30 ± 2.36 (8)
N Debt (µmol N µg Chl a^{-1})	0.12 ± .02 (8)	0.14 ± 0.12 (3)	0.12 ± 0.06 (8)
CP (atomic ratio)	111.5 ± 152.20 (8)	**144.2 ± 150.20 (3)**	**201.2 ± 52.30 (8)**
APA (µmol P µg Chl $a^{-1}h^{-1}$)	0.001 ± .002 (8)	**0.008 ± 0.002 (3)**	**0.015 ± 0.009 (8)**
P Debt (µmol P µg Chl a^{-1})	0.05 ± .05 (8)	**0.08 ± 0.04 (3)**	**0.11 ± 0.04 (8)**
Physical			
Surface Temp (^{o}C)	25.60 ± 0.90 (8)	22.3 ± 0.60 (3)	23.2 + 2.20 (8)
Max Depth (m)	6.65 ±0.90 (8)	13.2 ± 0.60 (3)	14.65 + 2.20 (8)
Mixing Depth (m)	4.80 ± 2.30 (8)	6.01 ± 1.10 (3)	4.68± 0.11 (8)
kd (m^{-1})	0.45 ± 0.33 (8)	0.42 ± 0.63 (3)	0.35 ± 0.30 (8)
Mean PAR (µmol m^{-2} s^{-1})	123.5 ± 12.75 (8)	167.4 ± 27.03 (3)	219.29 ± 33.04 (8)
Chemical			
TP (µmol L^{-1})	0.59 ± 0.09 (8)	0.52± 0.05 (3)	0.48 ± 0.05 (8)
SRP(µmol L^{-1})	0.12 ± 0.00 (8)	0.10 ± 0.03 (3)	0.07 ± 0.03 (8)
NO_3^-(µmol L^{-1})	15.53 + 2.44 (8)	11.03 ± 1.12 (3)	12.44 ±1.79 (8)
NH_4^+(µmol L^{-1})	1.08 ± 0.17 (8)	0.95 ± 0.24(3)	1.78 ± 0.06 (8)
SrSi (µmol L-1)	0.94 ± 0.03 (8)	0.54 ± 0.01(3)	1.12 ± 0.13 (8)

3.3.3 Variable Fluorescence

In May, the mean values for F_v/F_m ranged from 0.125 to 0.154, where the lowest values were in CB. In June, the average value for F_v/F_m was high in WB and low in CB. Overall, the mean values of F_v/F_m were lowest in CB and highest in WB.

To examine site and date specific relationships between F_v/F_m and nutrient status were examined in which the data were screened to focus on the nutrient of interest. To examine relationships with N deficiency, sites that were P and Si deficient were excluded. Most of the sites located in the WCB and WB in June, had high F_v/F_m values and these values were well below the threshold of deficiency (Fig. 3.2, Table 3.2 and 3.3).

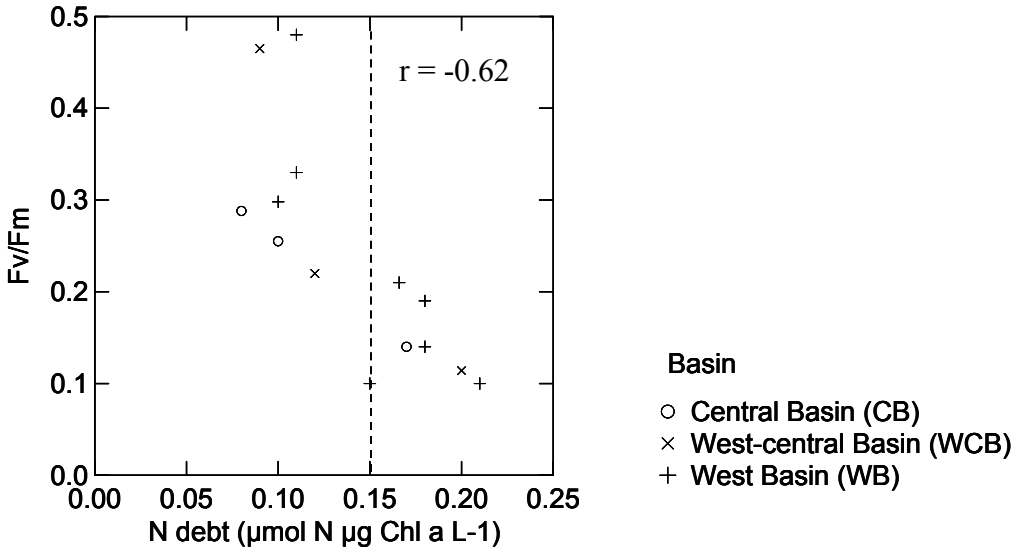


Figure 3.2 Scatter plot of Fv/Fm vs. N debt. Each point represents sampling station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b)

Sites with N debt above the deficiency threshold had mostly low F_v/F_m values and occurred mainly in May. The pattern was similar when the particulate C:N ratio was plotted against F_v/F_m (not shown). Pearson's correlation analysis was used to analyze the relationships between the nutrient status variables and F_v/F_m. The relationship between N debt and F_v/F_m was significant ($r = -0.62$, $n = 14$, $p<0.05$), whereas the relationship between C:N ratio and F_v/F_m was weaker (not shown) ($r= -0.38$, $n = 14$, $p <0.05$).

Excluding N and Si deficient sites, P debt and APA values were highest and above the threshold of deficiency when F_v/F_m values were low. The reverse was seen when high F_v/F_m values were observed when P status indicator values were below the threshold of deficiency (Fig. 3.3). Most of the recordings of P deficient sites occurred in June. Correlation analysis revealed a strong relationship between P debt and F_v/F_m ($r = -0.69$, $n = 17$ $p<0.05$) as shown in Fig. 3.3, as well as APA and F_v/F_m (not shown) ($r = -0.66$, $n =17$, $p<0.05$), though not as strong as for F_v/F_m vs C:P (not shown) ($r = -0.29$, $n = 17$, $p <0.05$).

The fitted parameters of the RLC were used to examine relationships with N debt and P debt assays. I chose N and P debt assays instead of particulate ratios to examine the associations since the strongest relationships were reported between F_v/F_m and the metabolic assays. Excluding P and Si deficient sites, α values were higher when N debt values were low or below the threshold of deficiency (Fig. 3.4). Correlation analysis showed strong relationships between α and N debt ($r = -0.59$, $n = 17$, $p<0.05$). However, weak associations were witnessed between C:N and α ($r = - 0.28$, $n = 17$, $p <0.05$). The same trend was seen

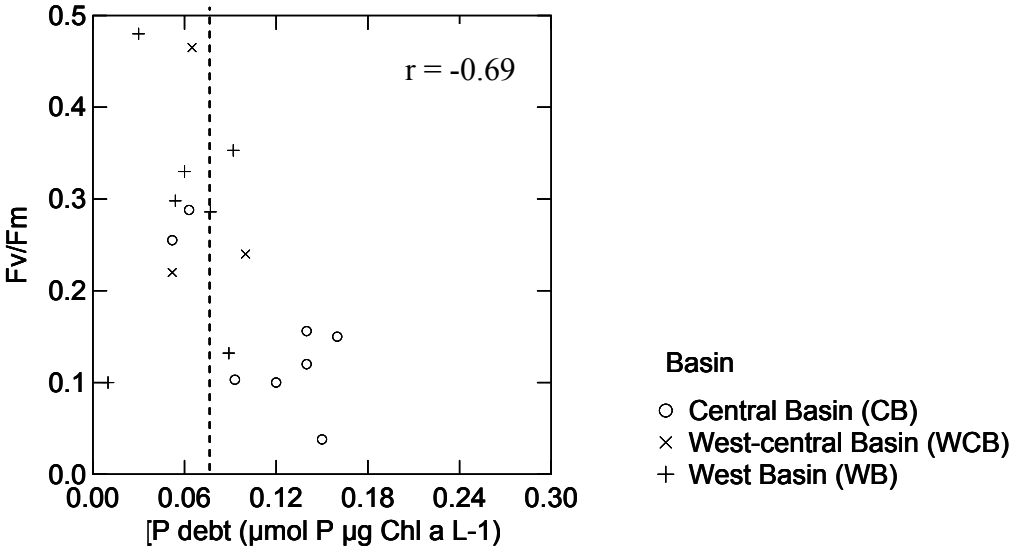


Figure 3.3 Scatter plot of quantum yield (F_v/F_m versus P debt. Points represent site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b)

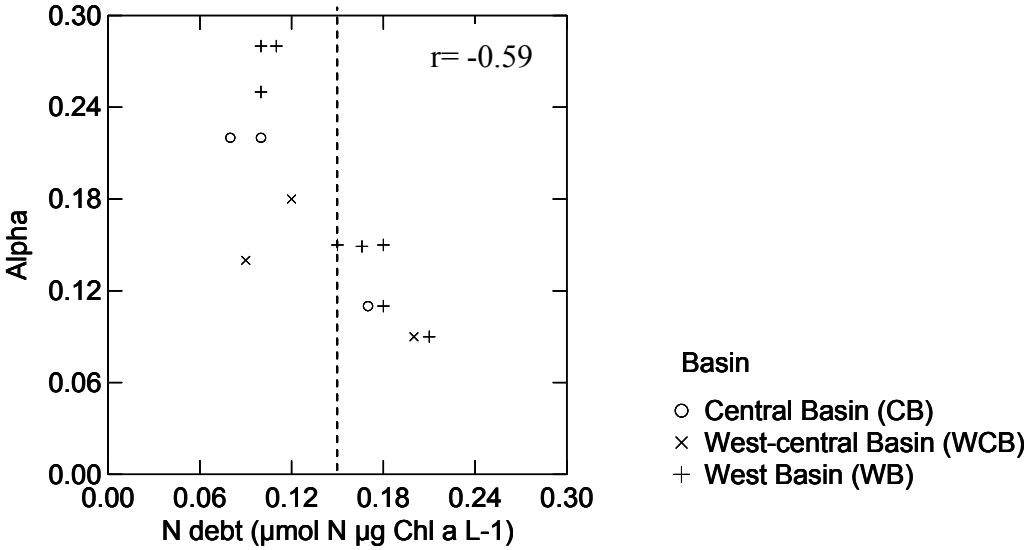


Figure 3.4 Scatter plot of alpha (α) versus N debt. Points represent site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b)

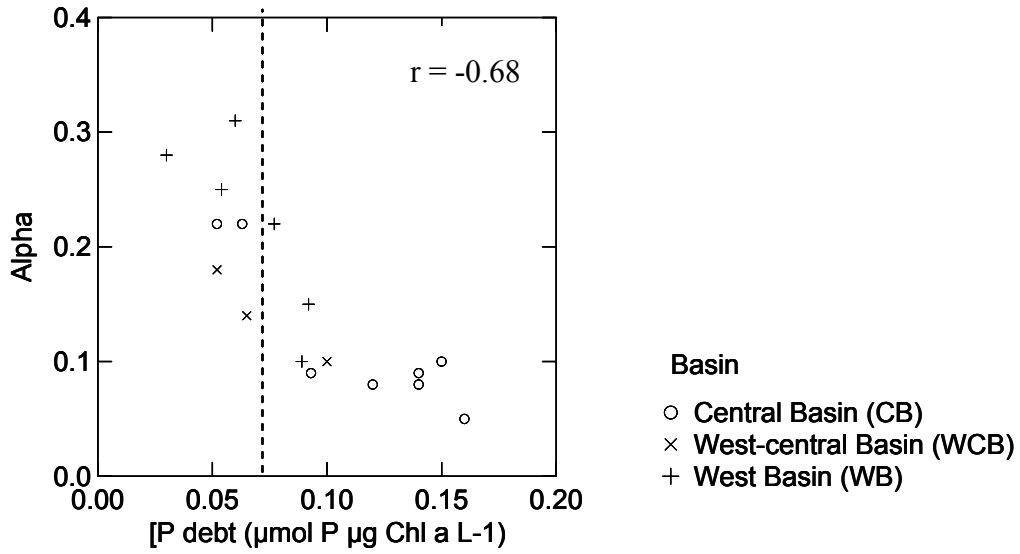


Figure 3.5 Scatter plot of α versus P debt. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).

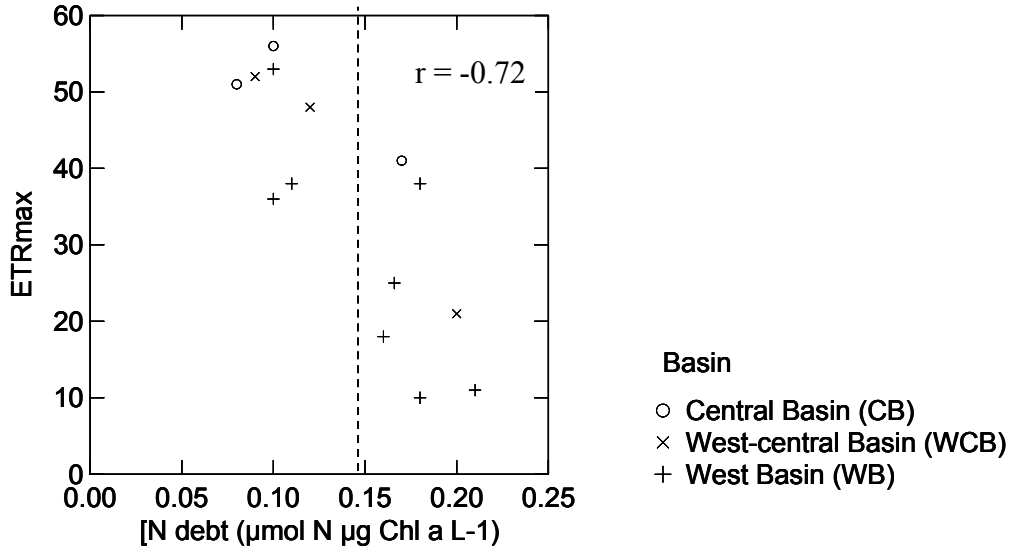


Figure 3.6 Scatter plot of ETR versus N debt. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).

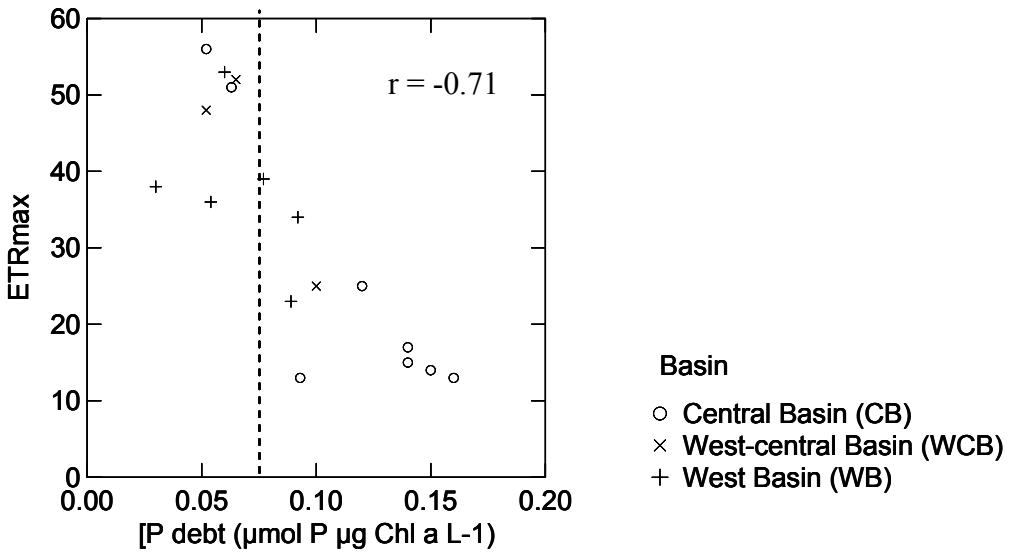


Figure 3.7 Scatter plot of ETR versus P debt. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).

when α was regressed with P debt where r= -0.68 (n = 17, $p<0.05$) (Fig. 3.5). Like C:N, C:P values regressed with α had a weaker relationship (r = -0.38 n = 17, $p<0.05$). ETR_{max} values regressed with N and P debt showed significant and strong associations (r = -0.72, -0.71, n =17, $p<0.05$), where low ETR_{max} values revealed N and P debt values above the threshold of deficiency (Fig. 3.6 and 3.7). When C:P and C:N were regressed with ETR_{max}, like α, the relationships were weak (r = -0.28, -0.31, n = 17, $p<0.05$).

The response to the amendment assays were visualized by the shapes of the RLC fitted to the chosen P-I model for the treatment in question (Fig. 3.8 and 3.9). These curves provided interesting results regarding the use of ETR_{max} as an indicator of nutrient status. N deficient samples (Fig. 3.8) showed strong photoinhibition of ETR and low values of ETR_{max}. After 24 hours in darkness but without added nutrient (Control, or C-24 treatment)

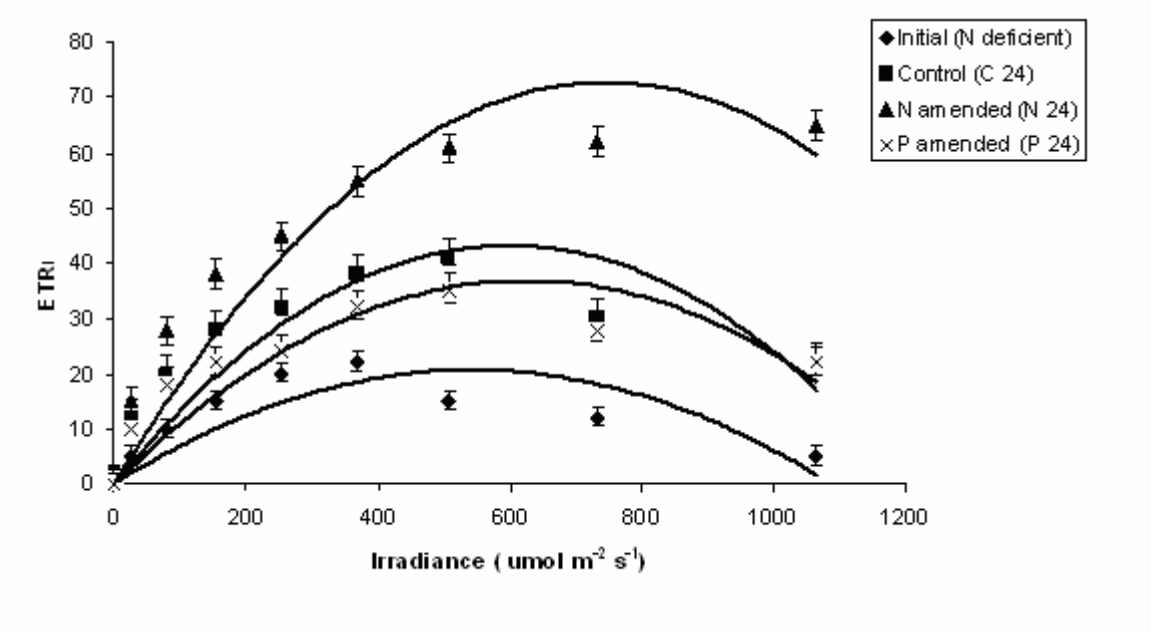


Figure 3.8 Relationships between ETR and irradiance for N deficient samples. RLC's were labeled as Initial (N deficient), control (non-amended dark adapted for 24 h), N amended (dark adapted for 24 h) and P amended (dark adapted for 24 h). Error bars show 1 + 0.5 SEM

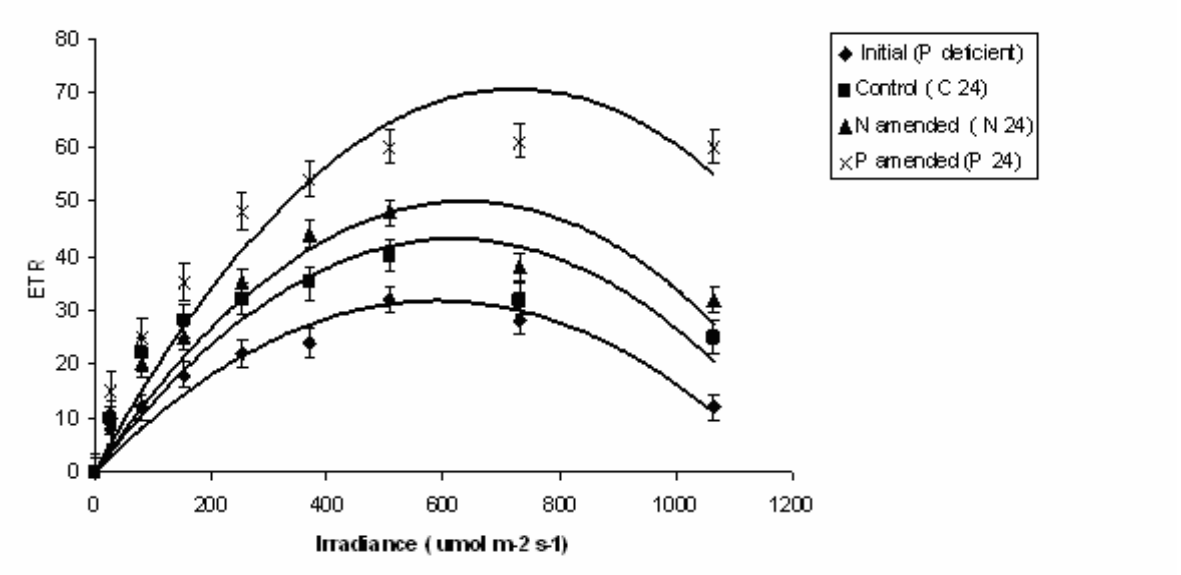


Figure 3.9 Relationships between ETR and irradiance for P deficient samples. RLC's were labeled as Initial (P deficient), control (non-amended dark adapted for 24 h), N amended (dark adapted for 24 h) and P amended (dark adapted for 24 h). Error bars show 1 + 0.5 SEM

higher ETR_{max} and initial slopes were observed but photoinhibition was still apparent. Addition of P (P-amended, or P-24, Fig. 3.8) produced results similar to the no-nutrient treatment. Addition of N (N-amended or N-24 treatment) mostly abolished photoinhibition and produced the highest ETR_{max} and initial slopes.

Results were similar for P-deficient samples (Fig. 3.9) in showing strong photoinhibition and low ETR_{max} in the nutrient deficient state (Initial, or P deficient treatment). Holding samples in darkness (C-24) or adding the putative non-limiting nutrient (in this case, N) produced similar improvements in ETR_{max} but photoinhibition was still observed. Adding P mostly abolished photoinhibition and resulted in high ETR_{max} and initial slope.

One of the objectives of my study was to evaluate if ETR_{max} and/or α was more specific in determining nutrient deficiency compared to F_v/F_m. Amendment assays were also used to evaluate the response to N and P addition using F_v/F_m. N deficient phytoplankton indeed revealed a positive response to N additions in which average F_v/F_m values increased from 0.144 to 0.460 (Fig. 3.10 *a*). Holding the samples in darkness (C 24) produced only a small increase of F_v/F_m in N deficient samples. P addition produced more of an increase in F_v/F_m, but still much less observed with N addition (Fig. 3.10 *a*). Chl a fluorescence parameter, F_v/F_m, were analyzed using 2- way analysis of variance (ANOVA) to determine if significant differences were seen between sites and nutrient addition experiments. Samples were screened based upon N or P deficiency. For N deficient samples, F_v/F_m values showed significant variation, between sites, treatments, and the interaction between sites and treatments (Table 3.4 and 3.5). Post-hoc tests revealed significant differences ($p< 0.05$) between initial N deficient and N 24 treatments in all three basins. Also, N 24 and P 24

treatments differed significantly in all three basins. Initial N deficient and C 24 samples did not differ significantly, however initial N deficient and P 24 samples did differ significantly in the CB.

P deficient phytoplankton likewise showed a strong positive response to P addition, with average F_v/F_m values increasing from 0.105 to 0.485 (Fig. 3. 10*b*). Based on 2-way ANOVA, post-hoc tests revealed significant differences ($p<0.05$) between initial P deficient and P 24 treatments in the CB and WCB. No significant differences ($p>0.05$) between initial and P 24 treatments were observed in the WB. N 24 and P 24 treatments differed significantly, however C 24 and N 24 treatments had comparatively little effect on F_v/F_m of the P deficient samples, and showed no statistically significant difference ($p> 0.05$) between initial P deficient with C 24 and N 24 treatments in all lake sites.

In comparison, average α and ETR_{max} measurements were used to evaluate the effects of N and P additions on N/P deficient and C 24 sites (Fig. 3.11 *a, b* and 3.12 *a, b*). N deficient phytoplankton revealed a strong positive response to N addition in which average α values increased from 0.16 to 0.31 and average ETR_{max} values increased from 23 to 59 (3. 12 *a*). P addition samples did not produce a strong response however, increases in α and ETR_{max} were more pronounced in P addition samples compared to C 24. The RLC parameters ETR_{max} and a were analyzed using 2- way analysis of variance (ANOVA) to determine if significant differences were seen between sites and nutrient addition experiments. Samples were also screened based upon N or P deficiency. ETR_{max} measurements based on post-hoc tests revealed significant differences ($p<0.05$) between initial N deficient, C 24 and N 24 treatments in the CB, WCB, and CB. Initial N deficient and P 24 treatments also were significantly different as well as C 24 and P 24 treatments.

Likewise, α measurements revealed statistical significant differences between initial N deficient, C 24 and N addition treatments for all basins. Initial N deficient and C 24 treatments did not differ significantly for ETR and α parameters, however initial N deficient and P 24 treatments differed significantly in the CB.

Initial P deficient treatments showed a strong positive response to P addition, with average α values increasing from 0.12 – 0.30 (3. 11 *b*) and average ETR_{max} values increasing from 20-58 (Fig. 3. 12*b*). N amended treatments produced moderate increases in α and $ETR_{max,}$ however C 24 treatments also had a modest effect on α and ETR_{max} of the P deficient samples. ETR_{max} and α measurements based on post-hoc tests revealed statistically significant differences between initial P deficient, C 24, N 24 and P 24 treatments in all basins. Initial P deficient and C 24 treatments did not differ significantly.

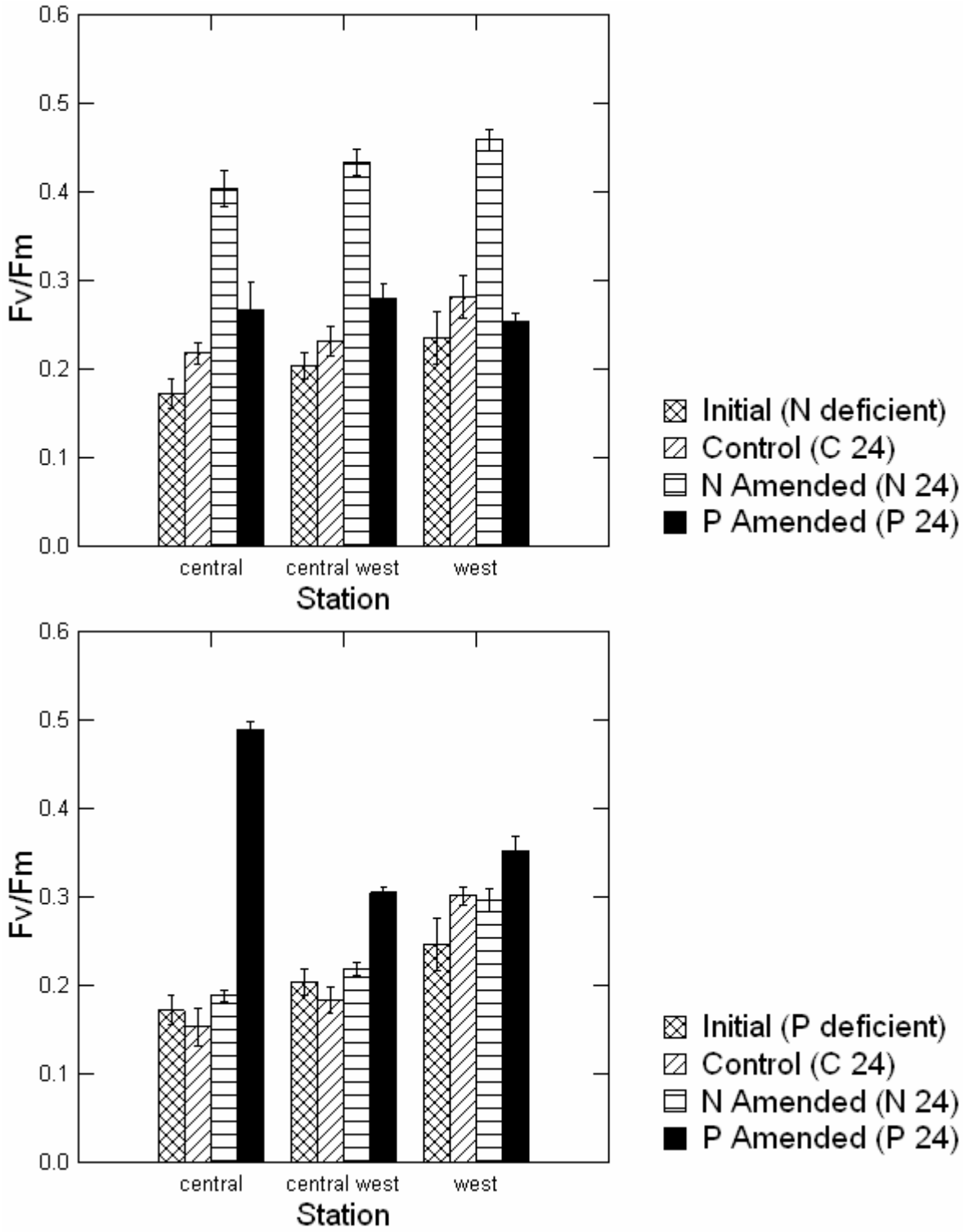


Figure 3.10 *a* and *b*. Amendment experiments using F_v/F_m as a response to nutrient status change in phytoplankton. The nutrient status data were categorized based on conditions of Initial (N deficient), control (non- amended dark adapted for 24 h), N amended (N 24) and P amended (P 24). Error bars show 1 + 0.5 SEM

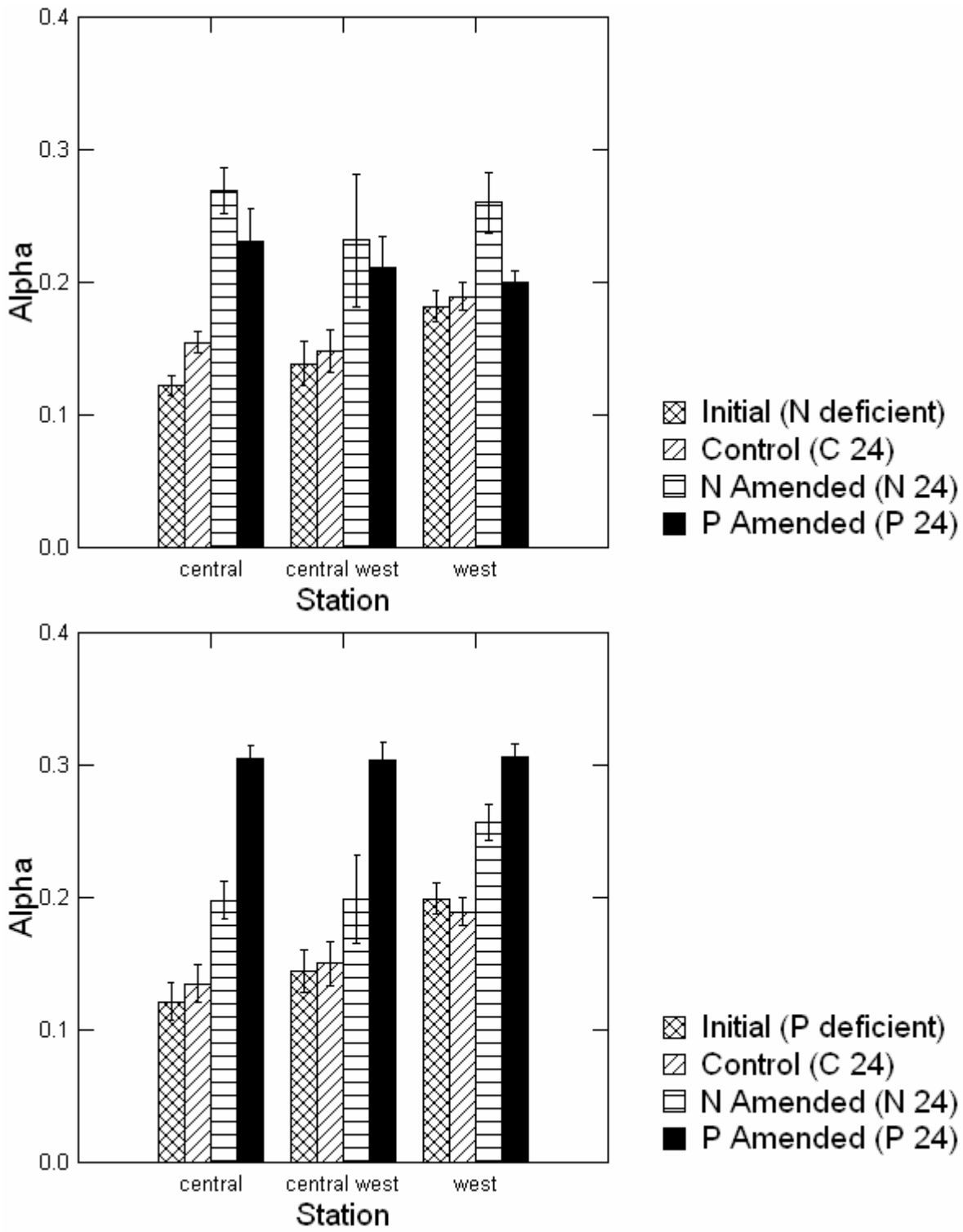


Figure 3.11 a and b. Amendment experiments using Fv/Fm as a response to nutrient status change in phytoplankton. The nutrient status data were categorized based on conditions of Initial (N deficient), control (non- amended dark adapted for 24 h), N amended (N 24) and P amended (P 24). Error bars show 1 + 0.5 SEM

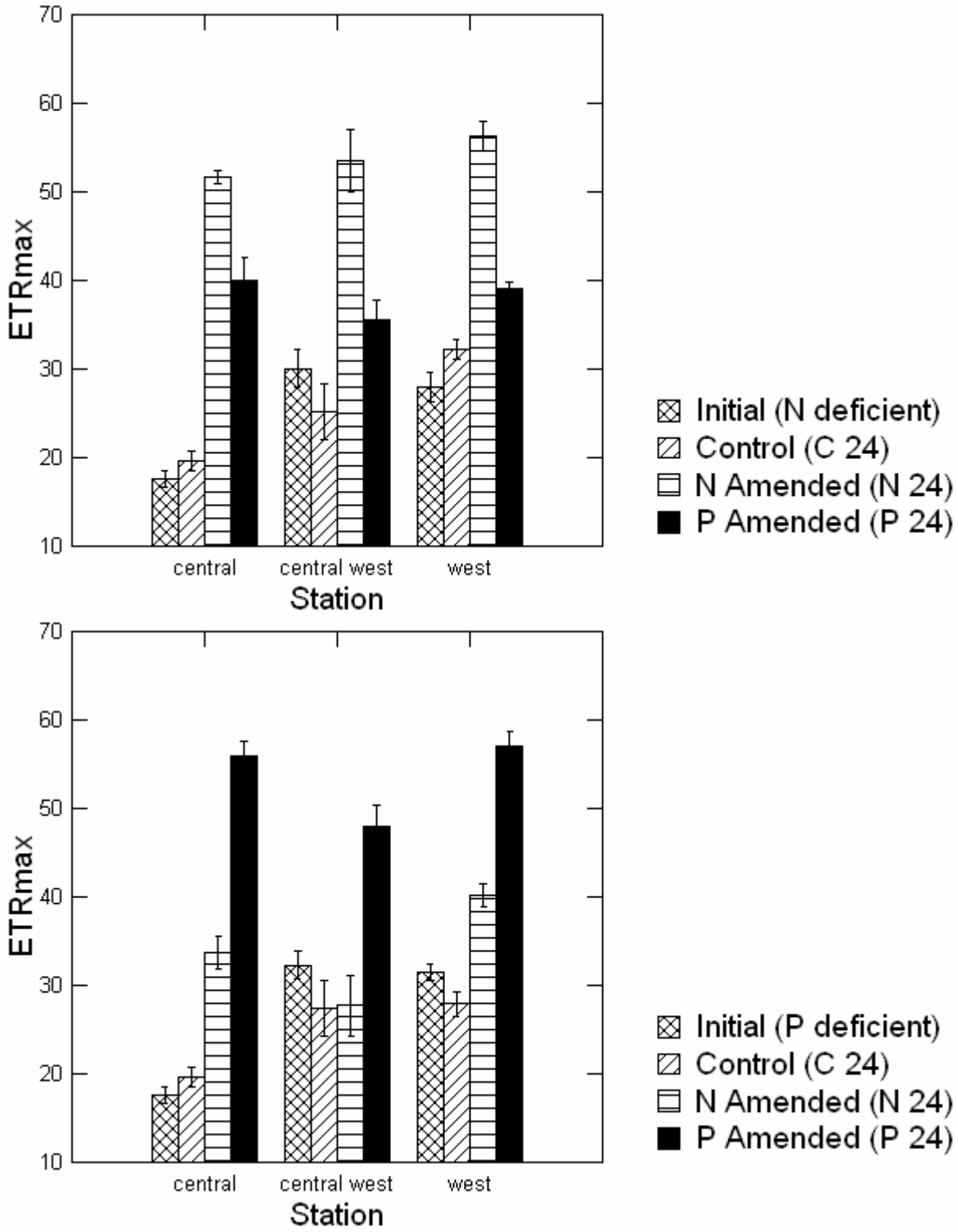


Figure 3.12 a and b Amendment experiments using ETR_{max} as a response to nutrient status change in phytoplankton. The nutrient status data were categorized based on conditions of Initial (P deficient), control (non- amended dark adapted for 24 h), N amended (N 24) and P amended (P 24). Error bars show 1 + 0.5 SEM

Table 3.4 2-way ANOVA revealing significant differences (p <0.05) for Chl a fluorescence parameters using lake sites (basins) and nutrient addition treatments as factors.

Comparison	F-statistic	df	*p* value
F_v/F_m			
Site	45.25	13	0.011
Treatment	55.65	3	<0.001
Interaction	124.22	39	<0.001
ETR_{max}			
Site	22.30	13	0.031
Treatment	113.00	3	<0.001
Interaction	102.40	39	0.036
α			
Site	42.12	13	0.011
Treatment	92.31	3	<0.001
Interaction	99.68	39	<0.001

Table 3.5 2-way ANOVA revealing significant differences (p <0.05) for Chl a fluorescence parameters using lake sites (basins) and nutrient addition treatments as factors.

Comparison	F-statistic	df	*p* value
F_v/F_m			
Site	50.25	16	0.021
Treatment	85.20	3	<0.001
Interaction	102.21	48	<0.001
ETR_{max}			
Site	20.12	16	0.012
Treatment	98.51	3	<0.001
Interaction	113.25	48	0.003
α			
Site	38.65	16	0.002
Treatment	95.41	3	<0.001
Interaction	112.54	48	<0.001

The photosynthesis-irradiance curves of nutrient sufficient sites were compared to those observed in nutrient deficient samples after amendment with the limiting nutrient (N or P as appropriate; Fig. 3.13). Despite their initially deficient condition, the amended samples appeared to display physiological status comparable to that of nutrient sufficient sites after the 24 hour amendment period.

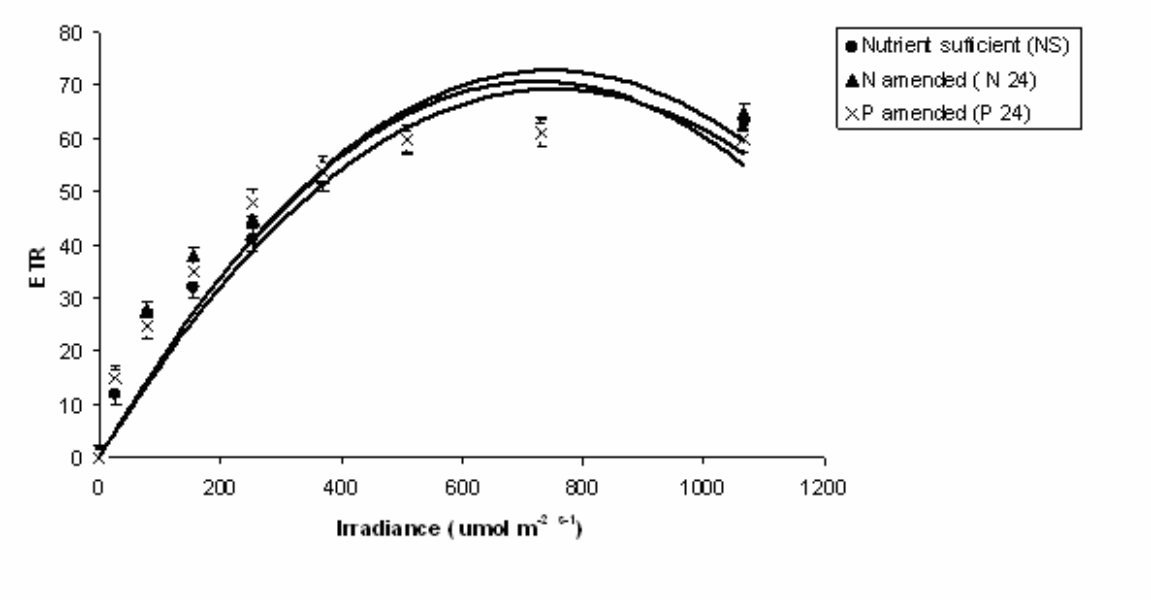


Figure 3.13 Relationships between ETR and irradiance. RLC's were labeled as Nutrient sufficient, N amended (dark adapted for 24 h) and P amended (dark adapted for 24 h). Error bars show 1 + 0.5 SEM

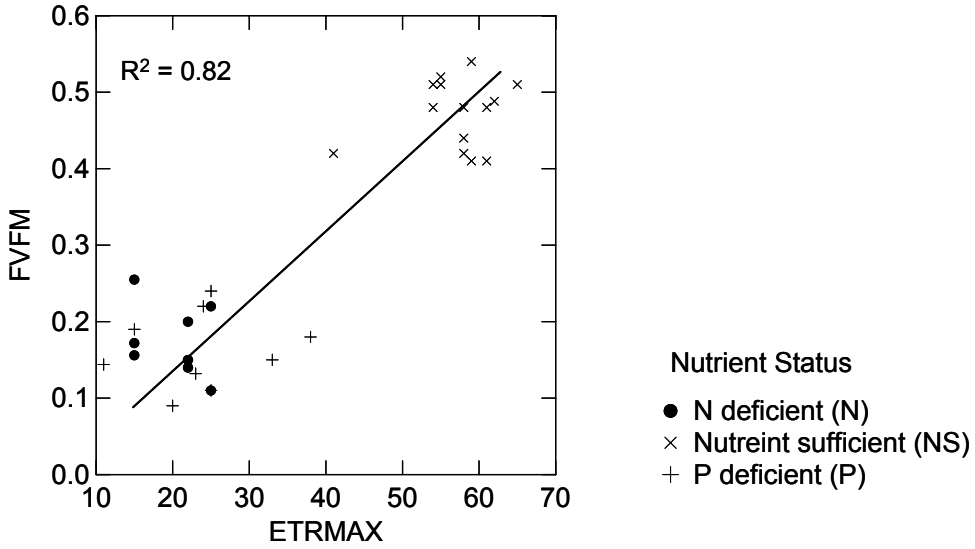


Figure 3.14 Scatter plot of F_v/F_m versus ETR_{max} for N deficient (N), nutrient sufficient (NS) and P deficient (P) sites.

Linear regression analyses demonstrated strong associations (R^2 = 0.82, n = 30, p <0.05) between F_v/F_m, and ETR_{max} (Fig. 3. 14). The same trend was observed when F_v/F_m was regressed with α where R^2= 0.79 (n = 30, p<0.05) (Figure not shown). One of the objectives was to evaluate if ETR_{max} and α could provide a more sensitive measure of nutrient status compared to F_v/F_m and also reveal which nutrient might be limiting. The scatter plot actually revealed two distinct clusters, one of nutrient sufficient sites (high F_v/F_m and ETR_{max}), and another of nutrient deficient sites (low F_v/F_m and ETR_{max}). In this sample set, the N-deficient and P-deficient sites clustered together and could not be distinguished on the basis of F_v/F_m and ETR_{max}. A similar pattern was observed with F_v/F_m vs α.

3.4 Discussion

The results in my study corroborated previous studies (Chapter 2, Guildford et al. 2005) that phytoplankton can display N limitation in isothermal conditions and P limitation in summer stratified conditions. In chapter 2, I discussed possible explanations for the

occurrence of N deficiency under the relatively high NO_3^- conditions in the WB of Lake Erie. The occurrence of P deficiency in summer-stratified conditions was also consistent with previous reports (Lean et al. 1983, Allen and Smith 2002, Guildford et al. 2005 Chapter 2) where P deficiency was mainly detected in the CB. The maximum quantum yield of fluorescence (F_v/F_m) showed strong associations between N and P metabolic deficiency indicators, similar to the results found in Chapter 2. Here, I have additionally shown that the RLC derived parameters ETR_{max} and α, revealed strong associations with N and P deficiency indicators and that amendment with the putative limiting nutrient had specific effects on variable fluorescence. These results provide further evidence that both N and P deficiency truly occur in Lake Erie, and that variable fluorescence can be used to assess nutrient status in natural communities.

Previous studies have shown N limitation can occur due to co-limitation, mediated by Fe limitation during periods of strong thermal stratification (North et al.2007), while NO_3^- assimilation may be impeded in low PAR, low temperature conditions, even as algal N requirements are increased (Falkowski and Raven 2007). In Chapter 2, I suggested that each of these factors could be involved in Lake Erie but that light and temperature effects, together with a limited supply of ammonium, were likely the most important, at least in spring. In this study, the metabolic indicator, N debt again showed a strong negative association with F_v/F_m, consistent with some previous studies of cultures (Kolber et al. 1988, Falkowski and Kolber 1995) and natural communities Chapter 2). However, changes in F_v/F_m could also be determined by the taxonomic signature of phytoplankton groups (Suggett et al. 2009). In chapter 2, phytoplankton communities were shown to display different patterns in algal dominance depending on the nature of nutrient deficiency. In N

deficient sites, cyanobacterial species were often dominant, possibly due to competitive advantages such as N_2 fixation and utilization of internal N sources (phycobilisomes). Cyanobacteria contain phycobilisomes as light harvesting antennae for PSII. The phycobilin pigment phycocyanin can lower F_v/F_m values due to interference of the fluorescence emission bands of phycocyanin and Chl *a* (Campbell et al. 1998). In this study, I did not enumerate phytoplankton communities; however in Chapter 2, cyanobacteria were prevalent in N deficient sites, and might manifest lower F_v/F_m independent of their N status. Not all cyanobacteria have constitutively low F_v/F_m. *Microcystis sp.* for example, was associated with high values of F_v/F_m (0.454; chapter 2) and displays values close to those for many eukaryotes in culture (R. Smith, pers. comm.). I concluded in chapter 2 that taxonomic selection could contribute to the observed relationships between variable fluorescence and N status but was unlikely to be the main factor. More evidence to support the actual occurrence of N deficiency would nonetheless be desirable; especially as the occurrence of N deficiency in Lake Erie is not entirely expected or easy to explain with current information.

Historically, the lower Laurentian Great Lakes have been demonstrated to be P limited at least in offshore regions, although secondary N limitation has been documented and recent (since 1995) changes to the lake may have increased the incidence of N limitation (Moon and Carrick 2007). Lean et al. (1983), Guildford et al. (2005) and Moon and Carrick (2007) have examined patterns of nutrient status of phytoplankton in Lake Erie and revealed a common occurrence of P deficiency of phytoplankton in offshore regions (CB) in the summer stratified season (June-August). In Chapter 2, I have also confirmed a similar trend in P deficiency using metabolic indicators (P debt, APA). In 2005, I found the highest

occurrence of P deficiency was at low TP concentrations and during summer-stratified conditions. In 2006, my results revealed a similar trend in P status of phytoplankton with the highest values of deficiency in June.

Measurements of F_v/F_m revealed strong associations with P debt and APA values. Like N deficient sites, P deficiency in phytoplankton resulted in consistently lower F_v/F_m measurements compared to nutrient sufficient sites. However, changes in F_v/F_m may also be caused by different kinds of phytoplankton that predominate under P deficiency. In chapter 2, flagellates such as cryptophytes and chrysophytes were shown to be dominant in P deficient sites. The ecological advantage of flagellates is that they are nutritionally opportunistic (Watson et al. 1997). Experiments with cultured mixotrophic flagellates support this statement (Nygaard and Tobiesen 1993 and Olrik et al. 2007), which suggests that an inverse relationship should be expected between dissolved inorganic nutrient concentrations and the phagotrophic activity of mixotrophs. Eukaryotes such as diatoms, dinoflagellates and some species of cryptophytes contain chlorophyll –xanthophyll based light harvesting complexes (Chl *a/b*, Chl *a/c* and Chl *a*/ peridinin) that may induce higher F_v/F_m values compared to cyanobacteria for which blue light from the FRRF can be an efficient excitation source (Suggett et al. 2004). F_v/F_m reflects the maximum quantum yield of PSII in its dark adapted state, and the effects of taxonomic and cell size variations are clearly important influences on its variations among phytoplankton species and communities. Measurements of the functional quantum yield of PSII under photosynthetic illumination, by contrast, can provide estimates of photosynthetic electron transport rates through PS II (Genty et al. 1989) and may reveal more of the physiological influences of nutrient deficiency.

Photosynthesis-irradiance curves (RLC's) and the derived parameters, ETR_{max} and α were used here to additionally evaluate nutrient stress. In nutrient deficient conditions, impairments of PS II functional reaction centres can cause impairment of the electron flow. However nutrient limitation may cause an impairment of the electron flow downstream of PS II due to limitation of RUBISCO activity, inhibition of phosphorylation reactions, and other effects. If such downstream limitation is the dominant effect of nutrient limitation, it may not be detectable for F_v/F_m because the cells can use the dark adaptation period to gradually re-oxidize the photosynthetic electron chain and PS II. As a result, values of F_v/F_m may appear to reflect conditions in which cells are generally stress free. However, in light saturating conditions, cells cannot keep up with the electron flow and the functional yields will be lower than in nutrient sufficient algae. In this case, ETR could be a more sensitive detector than F_v/F_m.

Regarding light interception and optimization, phytoplankton have a range of mechanisms to change actual photosynthesis activity and maximize photosynthetic capacity in response to prevailing light intensity conditions. These mechanisms include non-photochemical quenching mechanisms which serve to protect phytoplankton by down-regulating the yield of PS II chemistry without causing photo-damage and also protect the photosynthetic apparatus during summer when photon flux is high (Ralph et al. 1998). Also, alternate pathways for dissipating excitation energy and reduction of O_2 can occur at various points downstream of PS II. In the Mehler reaction, O_2 is reduced at the acceptor side of PS I, where O_2 is generated by the oxidation of water is reduced, and eventually leads to the production of water by the ascorbate peroxidase activity (Falkowski and Raven 2007). Another pathway involves the plastoquinol terminal oxidase (PTOX), which uses electrons

from the plastoquinone (PQ) pool to reduce O_2 (Mackey et al. 2008). The third possibility is that for both N and P deficiency, the primary effect includes changes to PS II (increased prevalence of non-functional reaction centres) that can decrease its maximum quantum efficiency. These alternative pathways may allow cells to dissipate excitation energy and maintain high functional yields even if linear electron flow is inhibited.

The results of this study suggest that impairment of the maximum quantum yield of PSII, and possibly the operation of alternative pathways, are important in nutrient deficient phytoplankton in Lake Erie because neither ETR_{max} nor α seemed to be more sensitive to nutrient deficiency than F_v/F_m. F_v/F_m, ETR_{max} and α were similarly sensitive to N and P deficiency as judged by nutrient status assays, and responded similarly to nutrient amendments. However, the results also showed nutrient deficiency can cause inhibition of ETR at high nutrient limitation in both N and P deficient samples, revealing effects of nutrient deficiency beyond the decrease of maximum quantum efficiency in PSII, and the relative electron transport rates were diminished under nutrient deficiency. Alternate electron pathways do not abolish the effects of nutrient deficiency, however, these pathways are able to moderate the effects of deficiency. The occurrence of photoinhibition was consistent with the occurrence of downstream limitation of electron transport, and would suggest that the combination of nutrient limitation and high irradiance could be more damaging than either factor on its own.

The amendment assays were intended to further test whether nutrient status assays and variable fluorescence variations were truly indicating nutrient deficiency and correctly identifying the limiting nutrient, particularly in the case of the rather surprising N deficient samples. Unlike the more studied NIFT approach, the amendment protocol used here was

intended not to detect the short-term changes in electron flow induced by addition of the limiting nutrient but rather to reveal the longer-term beneficial effects of restoring the limiting nutrient. NIFT experiments examine very short term (minutes) changes in fluorescence and have been used in laboratory cultures to diagnose and study PO_4^{3-}, NO_3^- and NH_4^+ limitation (Turpin and Weger 1988, Wood and Oliver 1995, Young et al. 1999). The NIFT approach is powerful, not least because it offers a potentially fast assessment, but proper interpretation of the response kinetics is still an object of research. Wood and Oliver (1999) for example, used additions of NH_4^+, NO_3^- and PO_4^{3-} in laboratory cultures of *Microcystis aeruginosa* to test fluorescence responses to nutrient enrichment. Their values of F_v/F_m were maximal within the first 2 minutes after spiking, with variable fluorescence returning to pre-enrichment steady-state levels generally within 10 minutes but the kinetics are likely to be variable and make a standard protocol hard to design. In this study, amendment assays were simply dark incubated for 24 hours to provide time for nutrient restoration and the (presumed) resumption of protein synthesis it allows to permit repair and replacement of damaged PSII and other photosynthetic electron transport components. This type of amendment protocol has enjoyed apparent success in other applications (Sylvan et al. 2007) but there still is some question of whether 24 hours in darkness can provide enough time (and energy) for effective repair.

In this study, the amendment treatments did seem to be successful in restoring a high level of photosynthetic function to nutrient deficient phytoplankton. Simply holding nutrient deficient samples in darkness (C 24 treatments) did improve F_v/F_m and RLC parameters, but the samples still displayed lower average light saturation values (410 μmol m^{-2} s^{-1}) F_v/F_m, α and ETR_{max} compared to nutrient sufficient samples or nutrient deficient

samples amended with the putative limiting nutrient. These results showed that dark adaptation of 24 h was not sufficient to restore the quantum efficiency of PSII or remove the downstream limitation suggested by the occurrence of photoinhibition. By contrast, amendment with the putative limiting nutrient restored all measures of photosynthetic function to those typical of nutrient sufficient samples.

Jimenez del Rio et al. (1995) and Longstaff et al. (2002) also reported strong positive responses to NO_3^- and NH_4^+ enrichments in the marine alga *Ulva rigida.* Both studies demonstrated that NH_4^+ availability controlled the level of RUBISCO activity and pigment-protein synthesis, contributing to the known influence of N limitation on net photosynthetic rates (Osborne and Geider 1986). In N deficient samples of this study, ETR_{max} occurred at a lower saturating irradiance (315 μmol m^{-2} s^{-1}) compared to N amended samples or those from nutrient sufficient sites (750 μmol m^{-2} s^{-1}). These results suggested that under N limitation the relative abundance of RUBISCO, Chl *a,* and accessory pigments decreased compared to nutrient sufficient phytoplankton at different saturating irradiances. As a result, the reduction of cell pigment concentration can be considered as a down-regulation of the photosynthetic apparatus that brings light harvesting in closer balance with the energy demands for growth and maintenance (Geider et al. 1993).

For P deficient samples, ETR_{max} and α were considerably lower compared to the P amended samples or nutrient sufficient sites (Table 3.2 and 3.3 Fig. 3.8 and 3.9). Also, ETR for P deficient samples had low light saturated irradiance (414 μmol m^{-2} s^{-1}) compared to P amended samples (760 μmol m^{-2} s^{-1}). These results, like those for N deficiency, suggest that under P deficiency, photoinhibition could occur due to an increased demand of P for growth (Geider et al. 1998). Nutrient deficiency may result in a decreased rate of photochemistry

through down-regulation of carbon fixation enzymes and loss of PS II reaction centres. Healey (1979) reported suppression of net oxygen evolution in P-starved *S. quadricauda* and Wykoff et al (1998) reported that P starvation in *C. reinhardtii* resulted in a 75% decrease of maximal O_2 evolution. Their results suggest that P deprivation may cause a decline in the levels of reductive pentose phosphate cycle intermediates, and diminished regeneration of intermediates in the Calvin-Benson cycle, thus inhibiting the ability to use higher irradiance fluxes for linear electron transport.

This study revealed strong associations between nutrient status (N/P debt, APA), Fv/Fm, ETR_{max} and α. The association between photoinhibition of ETR and nutrient deficiency provided further evidence that nutrient stress, and not only constitutive taxon-specific characteristics, were responsible for the observed patterns. The amendment assays appeared to provide very strong support for this interpretation, showing strong and highly specific response to the putative limiting nutrient, including N. These results make it hard to deny that N deficiency really does occur frequently even in this "P-limited" lake**.** Future work should aim to elucidate the biogeochemical processes responsible for such limitation, and the consequences of both N and P limitation for phenomena such as the resurgence of *Microcystis* in Lake Erie (Vanderploeg et al.2001) and elsewhere (Raikow et al. 2004).

The results did not reveal the identity of the limiting nutrient solely by variable fluorescence, even with the additional information from the RLCs. F_v/F_m and ETR_{max} measurements were correlated and the results revealed strong associations (R^2 = 0.82, n = 31, $p<0.05$; Fig. 3.13) although the pattern was more one of nutrient-sufficient vs. nutrient-deficient samples rather than a continuous relationship. F_v/F_m and α were likewise correlated (R^2 = 0.75 n = 31, $p<0.05$, plot not shown). When F_v/F_m values were high (or

low), ETR_{max} and α values were also high (or low). Both N and P limitation can affect cell physiology through processes that involve synthesis of proteins and nucleic acids. For example, N is needed for the synthesis of proteins, and chlorophylls. P is used for RNA, DNA, phospholipids and polyphosphates synthesis (Geider et al. 1998). Hence, both nutrients are key components for a cell's survival and Figure 3.14 illustrates the importance of N and P. One might expect some differences in the effects of N vs. P deficiency on these different measures of photosynthesis (F_v/F_m, ETR_{max}, α) and the nutrient amendment results did hint at some differences. However, the differences were not significant, and Fig. 3.14 exemplifies the essential similarity of response in both N and P deficient samples. Overall, this study showed that variable fluorescence in conjunction with metabolic, enzymatic assays may provide additional information pertaining to photosynthetic cellular processes such as nutrient and light acquisition in determining the degree of nutrient status under dynamic environmental conditions.

Chapter 4 . Comparative analyses of physiological assays and Chl *a* fluorescence parameters: Investigating the importance of phosphorus availability in oligotrophic and eutrophic systems

Overview

Diverse measurements of nutrient status indicators were used in this study to test how the severity of physiological P limitation of phytoplankton varies among lake systems ranging from oligotrophic to eutrophic, based on P and Chl *a* concentrations. Metabolic assays and particulate ratios of C:N, C:P and N:P were used to estimate nutrient status at sites located in Lake Erie, Lake Ontario and Lake Huron. Variable fluorescence ratios ($F_v/F_{m)}$, relative electron transport rates (ETR) and their response to irradiance, and the effective cross section of photosystem II (σ PS II) were measured by Pulse Amplitude Modulated (PAM) and/or Fast Repetition Rate (FRR) fluorometers. Community structure was also associated with nutrient status, where chrysophytes and cryptophytes were more important in P deficient sites and cyanobacteria, pyrrophytes and diatoms were prevalent in nutrient rich sites. Under the summer stratified conditions studied here, P deficiency was strongest in the most oligotrophic site and least in the most eutrophic site. N status indicators and variable fluorescence revealed no N deficiency. In a series of nutrient amendment assays, P additions showed a positive effect on F_v/F_m, and RLC parameters at P deficient sites and little or no effect on the least deficient site. N additions revealed a modest positive effect on F_v/F_m and RLC parameters compared to P additions, in the most oligotrophic sites. The results confirmed that F_v/F_m, σ PS II and RLC parameters can reveal P deficiency and indicate its severity among the range of sites sampled.

4.1 Introduction

Research on phosphorus (P) dynamics in lakes has been a major focus for limnologists, largely due to problems of cultural eutrophication. There is ample evidence that phytoplankton biomass, primary production and community composition are correlated with total phosphorus (TP) among many north temperate lakes (Schindler 1977, Guildford et al. 1994, Sterner et al. 1997, Guildford and Hecky 2000, Jeppessen et al. 2005, Sterner 2008). Also, it has been predicted that an increase of P can lead to eutrophic conditions and undesirable characteristics such as the formation of potentially harmful algal blooms (Schindler 1977, Smith 1983, Watson et al. 1997, Ouellette et al. 2006). For example, Schindler et al. (2008) observed changes in the phytoplankton community at the Experimental Lakes Area (ELA) when concentrations of P were increased. The outcome of the experiment was an increase of surface blooms of N_2- fixing cyanobacteria. Therefore, to reduce the impact of eutrophication, the focus of management must be on decreasing inputs of P.

There is nonetheless evidence that phytoplankton in P-limited lakes may be limited or co-limited by other nutrients such as N and Si (Guildford et al. 2005, North et al. 2007, Moon and Carrick 2007 and Sterner 2008). N is often considered as a secondary limiting nutrient, but considerable evidence suggests it may often be co-limiting with P or even be the primary limiting nutrient, at least on within-season time scales (Sterner 2008, Lewis and Wurtsbaugh 2008). Enrichment experiments with N and P reveal that phytoplankton can exhibit a strong synergistic response to combined N and P additions compared to the addition of either N or P alone (Elser et al 2007, Moon and Carrick 2007, Sterner 2008). Understanding how P regulates biomass, composition and production in plankton

communities thus requires an improved understanding of how P and other nutrients may limit growth rates on various times scales. Direct measurements of growth rates of nutrient limited natural populations are relatively difficult, however, while inferences from inorganic nutrient concentrations in the water can be problematic as measurements may not reflect the actual bioavailable concentrations. A particular problem for P is that the standard spectrophotometric analysis of SRP can seriously overestimate phosphate concentrations (Hudson et al. 2000).

Indirect measures have been developed that are intended to reflect the degree of physiological deficiency that the phytoplankton experience and, by inference, the nutrient-limited growth rates. For example, the P debt assay is based on the work by Healey and Hendzel (1979b) who demonstrated that several different groups of algae could take up PO_4^{3-} when P deficient. The particulate stoichiometric ratios of C:P and N:P have been the most widely-used as indicators of P status. When P or N become limited in supply, cell division will be reduced after exhaustion of stored nutrients but the phytoplankton can still store excess photosynthetic carbon. C:P and C:N ratios are then expected to be considerably higher compared to the ratios under nutrient sufficient conditions (Leonardos and Geider 2004).

The use of such indicators as well as additional measurements of nutrient concentrations and cycling rates, have already shown that the phytoplankton of P limited lakes do not always appear to be P deficient. This likely reflects, in part, a degree of balance between supply and demand for P, mediated by efficient grazing and nutrient recycling (Capblanq 1990, Graziano et al. 1996, Hudson et al. 2000, Dodds et al. 2003, Lewis and Wurtsbaugh 2008) as well as periodic or recurrent deficiencies of other nutrients.

Knowledge of patterns in the severity of limitation is currently limited, however, due to limitations in the existing assays and indices of nutrient stress, as explained in chapters 2 and 3. There are issues of practicality and of interpretation, but also uncertainties around response times and sensitivity. Most methods require nutrient deficiency to be sufficiently persistent in time to induce activation of nutrient uptake enzymes or compositional changes in nutrient content or taxonomic composition. This lack of sensitivity to short-term variations of phytoplankton nutrient stress may cause us to underestimate the frequency of nutrient stress and its effects on algal photosynthesis (Moore et al. 2005, Suggett et al. 2004, 2009).

In chapter 2 and 3, variable fluorescence parameters appeared to offer a more sensitive alternative method for assessing nutrient status of phytoplankton in Lake Erie. The parameters explored in those chapters included $F_v/F_{m,}$, a measure of the maximum quantum efficiency of photosystem II (PS II). When nutrient availability diminishes relative to the cellular demand, a decline in F_v/F_m in response to stress may infer a decline in the proportion of reaction centres in PS II that are functional (Geider et al. 1994). The functional absorption cross section of PS II (σ PS II) has been shown to increase under P and N limitation (Kolber et al. 1988, Geider et al. 1993, Falkowski and Kolber 1995, Sylvan et al. 2007, Suggett et al. 2009). This response can reflect the sharing of excitation energy among a smaller number of functional reaction centres when cells are nutrient deficient (Falkowski and Kolber 1995, Kolber et al. 1998). Therefore, σ PS II may provide additional information on the physiological status of nutrient limited phytoplankton.

In the presence of photosynthetic (actinic) irradiance, the functional quantum yield of photosystem II (PS II) is measured and can provide estimates of photosynthetic electron

transport rates (ETR) through PS II. ETR measurements can have a variable relationship to oxygen evolution or inorganic carbon fixation (Gilbert et al. 2000 and Beer and Axelsson 2004) but do provide an index of photosynthetic competence at a range of irradiance levels. When the physiological apparatus of a cell is impaired due to a lack of nutrients, photosynthetic down regulation of carbon fixation is observed and can be apparent in ETR and its relationship to irradiance (chapter 3).

In this study, five distinct lake sites with varying nutrient, light and Chl *a* concentrations were examined to study spatial variations of nutrient status, Chl *a* fluorescence and community composition. I wanted to see if results from this study corroborate with previous results (Chapter 2 and 3) that increased nutrient deficiency could effect variable fluorescence measurements and in turn reflect changes in phytoplankton composition.

The specific objectives of this study were first to determine whether the relationships between F_v/F_m, σ PSII, RLC parameters, and nutrient deficiency shown to exist among sites in Lake Erie (Chapter 2 and 3), still apply to sites with a wide range in the severity of deficiency and taxonomic composition. The second objective was to further test the nutrient amendment variable fluorescence assays as a tool for quantifying deficiency and identifying the limiting nutrient in each site. The third objective was to determine if the pattern of mainly P (as opposed to N) deficiency in summer conditions noted in Lake Erie (Chapters 2 and 3) would apply in more oligotrophic as well as more eutrophic sites in other lakes, and the fourth objective was to determine if the severity of summer P deficiency is systematically related to trophic status among a set of these Great Lakes sites.

4.2 Method and Materials

4.2.1 Study Area

Water samples were collected in late June of 2006 from the western basin (WB), west-central basin (WCB), central basin (CB) of Lake Erie (41°20'N, 83°33'W). On July 6th of 2006, water samples were collected from Napanee (Bay of Quinte- Lake Ontario) (44°50'N, -77°05'W) and on July 15th of 2006, water samples were collected from Colpoys Bay (Georgian Bay- Lake Huron) (44°50′N, 81°02′W). Stratification was assumed to have occurred when there was a vertical gradient of >1°C per meter (Guildford et al. 2000). Lake Erie, Bay of Quinte and Colpoys Bay sites were chosen based on their differences in nutrient, light, and Chl *a* concentrations.

Nutrient concentrations in the Bay of Quinte have been well documented (Millard and Sager 1994; Nicholls et al. 1977, 1999, 2002, 2004) and reports have indicated TP and Chl *a* concentrations to be as high as 70 μg L^{-1} and 29.3 μg L^{-1} before P loading control and 46 μg L^{-1} and 19.8 μg L^{-1} after P loading control (Robinson 1986). In the current study, the Bay of Quinte represented the most eutrophic (nutrient rich) site. Lake Erie contains 3 morphometrically different basins based on the conditions of the water column. The WB receives most of its nutrient load from the Detroit and Maumee Rivers and typically contains higher nutrient concentrations compared to the eastern basin (Charlton et al. 1999). WB in this study is classified as meso-eutrophic. The WCB and CB concentrations of Chl *a* are usually lower than the WB, and in this study, these sites were classified as meso-oligotrophic. Regarding Colpoys Bay, only a few studies have examined the water chemistry

chemistry and nutrient status of phytoplankton. From these studies TP and Chl *a* concentrations have been reported to be as low as 4.7 μg L^{-1} and 0.19 μg L^{-1} (Weiler 1988, Furgal and Smith 1997). In this study, Colpoys Bay represented the most oligotrophic (nutrient poor) site.

In Lake Erie, water samples at 2 m depth, were collected using 8L Niskin bottles mounted on a Rosette (CTD Seabird TM) sampler. Immediately after collection, water was transferred to 20L polyethylene carboys covered in black plastic bags to prevent excess light shock. In the Bay of Quinte water samples were taken at 2m depth using a 5L Van Dorn sampler and transferred to acid-cleaned and covered polyethylene carboys. Samples collected in Colpoys Bay were taken at 5m depth with a 5L Van Dorn sampler and also transferred to covered polyethylene carboys. All water samples were stored in a cooler during transport to University of Waterloo for analysis of nutrient chemistry.

The physical variables measured in this study were temperature profiles from the Seabird CTD and vertical profiles of photosynthetically active radiation (PAR) from a PAR sensor attached to the Fast Repetition Rate fluorometer (FRRF Fasttracka ^{TM} Chelsea Instruments). The vertical attenuation coefficient (K_d) was estimated by linear regression of the natural logarithm of PAR vs. depth. The mixing depth (Z_{mix}) and mean PAR of the surface mixed layer were calculated according to Hiriart et al. (2002).

4.2.2 Nutrient Analysis

Samples for soluble reactive phosphorus (SRP) and soluble reactive silicate analysis were filtered through 0.4-μm pore size polycarbonate filters. SRP was measured with the molybdate–ascorbate assay, and silicate concentration with the molybdate method

(Strickland and Parsons 1972). Samples for TP were analyzed following the same procedure outlined in Chapter 2 and 3. For particulate P samples were filtered (GFF:nominal pore-size 0.7 μm, 47 mm) filters and placed in clean petri dishes where samples were kept frozen until later analysis. Samples were then measured using the persulphate digestion method (Parsons et al. 1984).

Samples for NH_4^+ analysis were first run through a 0.2 μm polycarbonate filter and were measured with the orthophtaldialdehyde (OPA) method outlined in Holmes et al. (1999). The filtered samples were also analysed for NO_3^-, and nitrite (NO_2^-) on a Ion Chromatograph Dionex ICS 2500. Particulate C and N samples were analyzed by the methods described by Strickland and Parsons (1972). C/N filters were dried and placed in desiccators containing hydrochloric acid for 24 h. An C/N autoanalyzer (Exeter Analytical Inc. CEC-440) was used to measure particulate C and N after samples were placed in a muffle furnace for 5 h at 980^0C.

For Chl a analysis, water samples water were filtered onto glass fiber filters (GF/F:nominal pore-size 0.7 μm, 47 mm) that were kept in the dark and stored frozen (-20 ^{o}C) before passive extraction with 90% acetone (North et al. 2007). Chl *a* concentrations were then determined fluorometrically using a Turner Designs model 10AU fluorometer calibrated against pure chlorophyll *a* (North et al. 2007).

4.2.3 Nutrient Status Analysis

P and N limitation were assessed by particulate ratios of C:P, N:P and C:N, and by metabolic assays (nutrient debt and alkaline phosphatase enzyme assays (Healey and Hendzel 1979*b*). The analysis of N debt, P debt and APA follow the same procedures in

Chapter 2 and 3. Lake sites were classified as N (or P) deficient only when all N (or P) status indicators revealed values above the threshold of deficiency (Table 4.1).

Table 4.1 Nutrient status indicators. Values either show an absence, presence or the degree of nutrient limitation for phytoplankton. Criteria for nutrient limitation are based on Healey and Hendzel (1979b) and adapted from Guildford et al. (2005)

Indicator	Nutrient	No deficiency	Moderate deficiency	Extreme deficiency	Deficient
C/N (atomic ratio)	N	<8.3	8.3-14.6	>14.6	
N debt (µmol N µg Chl a $^{-1}$)	N	<0.15			>0.15
C/P (atomic ratio)	P	<129	129-258	>258	
N/P (atomic ratio)	P	<22			>22
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	

4 .2.4 Phytoplankton Cell Counts

Water samples were collected at 2 m depth (WC, WCB, CB, Napanee) and 5 m depth (Colpoys Bay) for phytoplankton enumeration. Samples were preserved with 1% Lugol's solution and 1% glutaraldehyde. Taxa were enumerated to the lowest level possible following Prescott (1975, 1978), Komarek and Anagnostidis (1986), Lee (2002), Carty (2003), Komarek (2003), and Nicholls and Wujek (2003). Phytoplankton were counted using the Utermohl method at 400x on an inverted phase contrast microscope (Axiovert 35, Zeiss). In total, at least 300 individuals were counted from randomly chosen microscope fields. For biovolume measurements, the dimensions of the algal cell were measured using ocular micrometers and cell dimensions were fit to geometrical shapes that portray the shape of the taxon (Wetzel 1991).

4.2.5 Chl a Fluorescence Analyses

1L phytoplankton samples were concentrated onto 24-mm glass fiber filters (GF/F, Whatman, Springfield Mill, U.K.) under low (<10 mm Hg) vacuum. Phytoplankton were dark adapted for 30 min on filtered lake water in covered Petri dishes to keep cells hydrated. A pulse amplitude modulated (PAM) fluorometer (Diving PAM, Heinz Walz, Germany) was used to measure the quantum efficiency of PS II on dark adapted cells (F_v/F_m). Measurements of F_v/F_m measured by PAM were the initial (ambient) values. For each measurement, a blank or correction filter was applied. The blank consisted of distilled water filtered onto 24-mm glass fiber filters (Walz 1993).

The RLC's were constructed by exposing the sample to 9 actinic (photosynthetic) light levels. Maximum actinic light level attained was 1150 μmol $m^{-2}s^{-1}$ under the light exposure period per level at 20 s. For each level of actinic light, the electron transport rates (ETR) and effective quantum yield of PS II (Fv'/Fm') were measured. The light response curve was characterized by fitting an exponential model (Platt et al. 1982). Each RLC was fitted to a double-exponential decay function in order to quantify the characteristic parameters, α and ETR (Platt 1982). The initial slope of the RLC (α) is a measure of the light harvesting efficiency and the asymptote of the curve, the maximum electron transport rate at light saturation, ETR_{max}, is a measure of the capacity of the photosystems to utilize the absorbed light energy (Soroussi and Beer 2007).

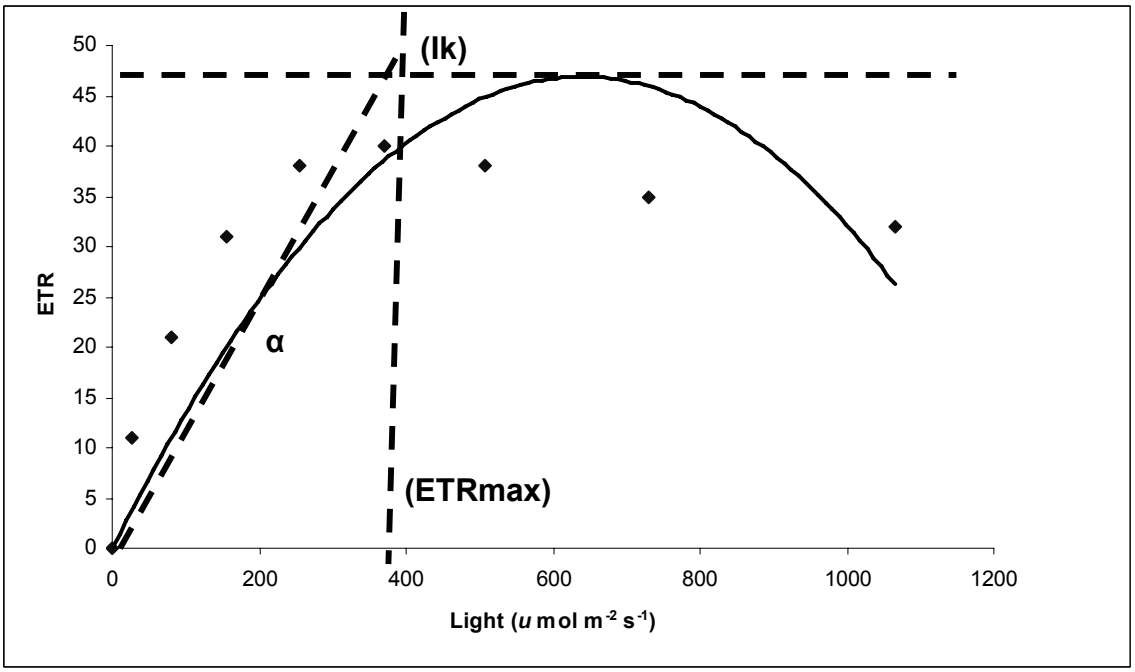


Figure 4.1 Rapid light curve (RLC) parameters derived from PAM fluorometry. α = initial slope and function of light intensity, ETRmax = maximal ETR at light saturation, Ik= light saturation

The fast repetition rate fluorometer (FRRF, FastTracka, Chelsea, UK) was used to measure F_v/F_m and σ PS II. The FastTracka FRRF used excited light at 470 nm (blue light) and samples were analyzed in a 100 ml quartz cuvette using the dark chamber in benchtop mode (discrete acquisition model). 50 mL aliquots of lake water were placed in the cuvette for 50 acquisitions. Acquisitions were determined using five consecutive flash sequences (100-200 flashlets, each 1.5 µs in length) at 2.8 µs intervals (Falkowski and Kolber 1995). The variable fluorescence parameters from the raw fluorescence data were derived using Submersible FRR Data Reduction (FRS1 ver. 1.8 Chelsea Instruments Ltd.) to deliver the calculated F_v/F_m and σ PSII values. For each measurement, a blank or correction filter of lake water was applied to correct for any background noise.

Samples were amended with P and N to assess nutrient sufficiency or deficiency in the selected sites. From each site, water samples were collected into three 1L acid washed carboys. Two of the carboys contained 5 μM aliquots of either ammonia (NH_4Cl) or phosphate (KH_2PO_4). The third carboy was the control and did not receive any nutrient amendment. All three carboys were incubated at temperature controlled environments with a maximum deviation of site temperature of $\pm$ 5°C. After 24 h, dark adapted samples were examined for changes in Chl *a* fluorescence parameters (F_v/F_m, σ PSII, ETR and α).

Two-way Analysis of Variance (ANOVA) was used to test for differences between lake sites, treatments, and their interactions on Chl a fluorescence parameters (Fv/Fm, α, σ PS II and ETR_{max}). When significant effects were detected, the Holm-Sidak multiple comparison test was used to test for differences between site/treatment means and Chl a fluorescence values. This was chosen over the more commonly applied Tukey's test and Boneferroni tests because it is considered to be more for independent comparisons (Shaw 2003).

4.3 Results

4.3.1 Environmental Conditions and Trends in Water Quality Parameters

During seasonal thermal stratification, surface water temperatures varied over the spatial scale among sites. Average values from each site ranged from 22.3°C to 24.5°C in which the highest temperature was observed in the Bay of Quinte, at Napanee and the lowest in Colpoys Bay (Table 4.2). Mean PAR values measured during this study period ranged from 69.9 to 241.06 μmol m^{-2} s^{-1}, with highest values measured in Colpoys Bay and lowest values measured in the Napanee station. Measurements of the light extinction coefficient (k_d)

variable showed an opposite trend to that of irradiance, where k_d decreased from 0.79 m^{-1} in Napanee to 0.11 m^{-1} in Colpoys Bay.

Average TP, SRP and NH_4^+ concentrations were highest in the Napanee station and lowest in Colpoys Bay (Table 4.1). NO_3^- concentrations showed a similar trend to that of P; however, the highest concentrations were reported in the WB of Lake Erie, not in the Napanee station. Chl *a* concentrations also revealed the same trend as NO_3^-, where the highest values were reported in the WB and the lowest in Colpoys Bay.

Table 4.2 Initial concentrations of chemical, biological and physical data (mean + standard deviation (number of samples)) for Napanee (Bay of Quinte), West Basin (Lake Erie), West-Central Basin (WCB), Central Basin (CB) and Colpoys Bay (Georgian Bay) Bold = Values above threshold of deficiency

Parameters	Napanee	WB	WCB	CB	Colpoys Bay
Chemical					
TP (μmol L^{-1})	1.27 ± .02 (3)	0.49 ± 0.09 (5)	0.32± 0.05 (4)	0.32 ± 0.05 (5)	0.16 ± 0.08 (4)
SRP(μmol L^{-1})	0.12 ± 0.10 (3)	0.10 ± 0.00 (5)	0.08 ± 0.03 (4)	0.07 ± 0.03 (5)	0.05 ± 0.02 (4)
NO_3^-(μmol L^{-1})	23.03 ± 2.53 (3)	33.53 + 2.44 (5)	26.07 ± 3.22 (4)	15.44 ±1.79 (5)	15.23 ± 2.45 (4)
NH_4^+(μmol L^{-1})	1.93 ± .08 (3)	1.68 ± 0.17 (5)	1.55 ± 0.94(4)	1.02 ± 0.06 (5)	0.91 ± 0.09 (4)
Biological					
Chl (μg L^{-1})	7.56± 2.36 (3)	10.62 ± 8.62 (5)	2.27 ± 1.72(4)	2.29 ± 2.19 (5)	0.61 ± 0.05 (4)
Algal biovolume (CUμm. mL)	7.41 x 10^{+6}	5.61 x 10^{+6}	3.46 x 10^{+6}	1.08 x 10^{+6}	9.58 x 10^{+5}
α	0.25 ± 0.11 (6)	0.18 ± 0.07 (5)	0.11 ± 0.02 (4)	0.08 ± 0.03 (5)	0.05± 0.00 (4)
ETRmax	48.85± 14.31 (3)	28.20 ± 5.02 (5)	22.20 ± 4.87 (4)	18.73 ± 4.34 (6)	5.0± 0.00 (4)
Fv/Fm(FRRF)	0.483 ± 0.028 (3)	0.289± .154 (5)	0.187 ± .004(4)	0.121 ± 0.052 (5)	0.102 ± 0.017 (4)
σ PSII	148.32 ±38.14 (3)	362.94 ± 22.59 (5)	530.01 ± 84.06 (4)	899.60 ± 246.37 (5)	914.94 ± 210.05 (4)
N debt	0.03 ± 0.00 (3)	0.12 ± 0.00 (5)	0.11± 0.09 (4)	0.14 ± 0.01 (5)	0.14 ± 0.02 (4)
C:N (atomic ratio)	7.41 ± 0.11	7.22 ± 0.21	8.01 ± 0.08	7.58 ± 0.15	7.95 ± 0.21
N:P (atomic ratio)	12 ± 1.14	18 ± 0.39	16 ± 0.86	15 ± 0.25	18 ± 0.05
C:P (atomic ratio)	**133 ± 12.52**	**154.12 ± 0.24**	**184.33 ± 14.65**	**218.01 ± 14.20**	**255.85 ± 12.65**
P debt	0.04 ± 0.00 (3)	0.05 ± 0.00 (5)	**0.08 ± 0.01(4)**	**0.13 ± 0.02 (5)**	**0.32 ± 0.03 (4)**
APA	0.002 ± 0.002 (3)	0.002 ± 0.001 (6)	**0.003± 0.001 (4)**	**0.008 ± 0.002 (5)**	**0.021 ± 0.001 (4)**
Physical					
Surface Temp (^oC)	24.9 ± .60 (3)	23.1 ± .90 (5)	22.3 ± 0.60 (4)	18.9 ± 0.80 (5)	22.3 ± 0.90 (4)
Max Depth (m)	5.3 ± 0.00 (3)	9.1 ± 1.32 (5)	14.3 ± 3.80 (4)	14.6 ± 3.9 (5)	50.0± 0.00(4)
Mixing Depth (m)	5.3 ± 0.00 (3)	4.8 ± 2.01 (5)	6.03 ± 1.16 (4)	4.6 ± 1.10 (5)	19.3 ±2.70 (4)
kd (m^{-1})	0.79 ± .18 (3)	0.52 ± 0.13 (5)	0.47 ± 0.23 (4)	0.36 ± 0.10 (5)	0.11 ± 0.00 (4)
Mean PAR (μmol m^{-2} s^{-1})	69.9 ±0.04 (3)	83.57 ± 12.75 (5)	79.54 ± 27.03 (4)	93.25 ± 13.01 (5)	241.06 ± 24.12 (4)

4.3.2 Independent Measures of Nutrient Status

The average values for both C:N and N debt were below the threshold of deficiency for all 5 study areas (Table 4.2). Spatial variations for P debt and AP revealed a gradient in P deficiency. The lowest values were observed in the WCB and the highest in Colpoys Bay. Average values of P debt and C:P were below the threshold value of deficiency in the Napanee station and WB, and P deficiency was not observed at these sites. P deficiency measured by C:P and N:P ratios gave different accounts of the relative degree of deficiency;

C:P measurements showed P deficiency in all study sites and N:P ratios showed P deficiency in none.

4.3.3 Variable Chl *a* Fluorescence

The maximum quantum efficiency of PSII (F_v/F_m) on dark adapted samples was measured using PAM and FRRF as a general indicator of nutrient status. Linear regression analysis (Fig. 4.2) indicated a high degree of association between PAM and FRRF ($y = 0.58 + 0.98x$, $R^2 = 0.92$, n = 22, $p < 0.05$). Therefore, for this study I chose F_v/F_m values measured by the FRRF to illustrated relationships between metabolic assays and stoichiometric ratios.

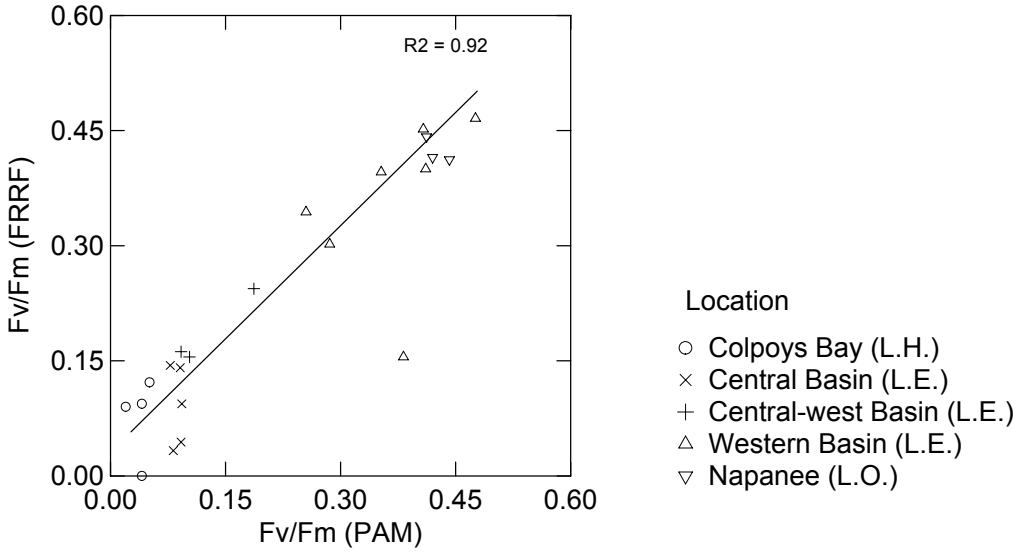
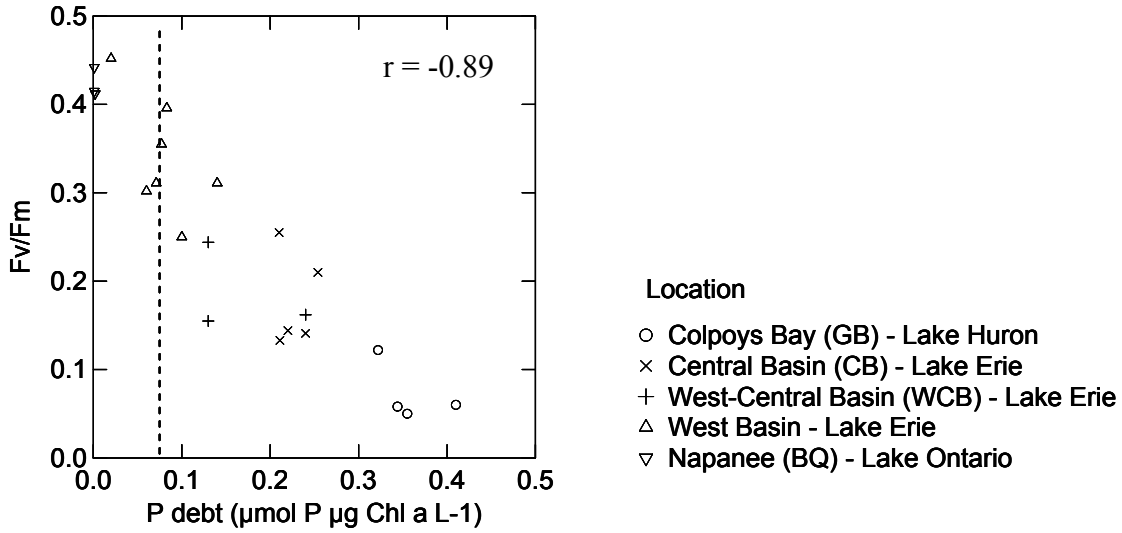


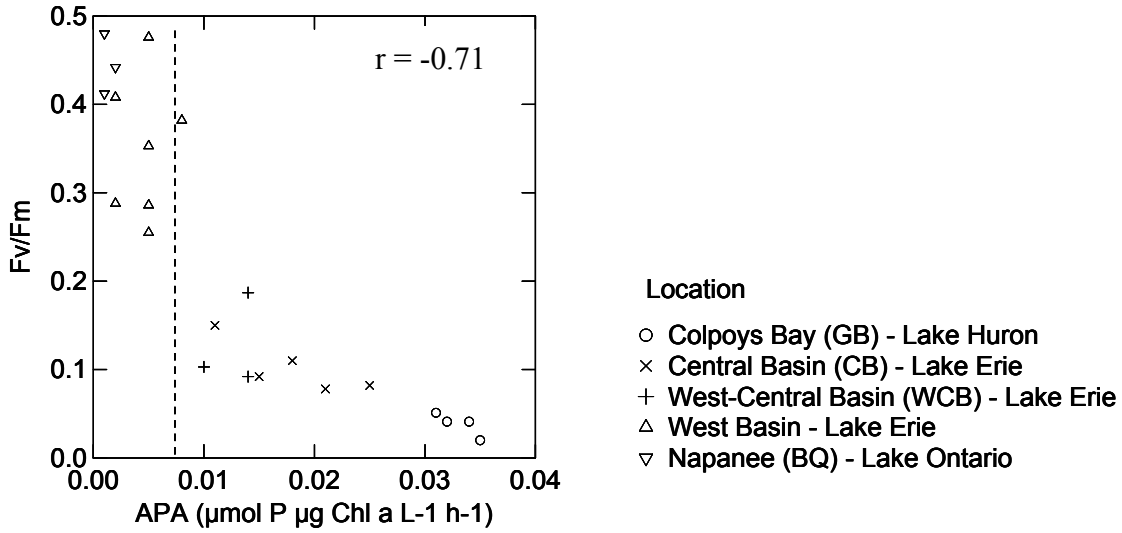
Figure 4.2 Scatter plot of quantum yield of PS II (F_v/F_m) values measured from commercial fluorometers (PAM and FRRF)

Average values of F_v/F_m were highest in the Napanee station and lowest in Colpoys Bay (Table 4.2). P debt and APA values suggested a trend towards higher F_v/F_m values at sites below the threshold of deficiency, and lower F_v/F_m values at sites above the threshold of deficiency (Fig.4.3 *a* and *b*). Pearson's correlation analysis revealed a strong relationship between P debt APA and F_v/F_m r= -0.89, and -0.71 ($n = 18$, $p<0.05$). C:P and N:P values were also regressed with F_v/F_m, however the relationships were weaker. (C:P and F_v/F_m ($r = -0.41$, $n = 18$, $p<0.05$, N:P and F_v/F_m ($r = -0.29$, $n = 18$, $p<0.05$)). N status indicators were not regressed with F_v/F_m due to a lack of N deficiency reported (Table 4.2).

σ PSII values derived from the FRRF revealed an opposite trend compared to F_v/F_m. The highest average values were reported in Colpoys Bay, and the lowest values were observed in the Napanee station (Table 4.2). Like the inter-site analysis of F_v/F_m, σ_{PSII} varied positively with P debt (Fig. 4.4 a, $r = 0.74$ $n = 18$, $p<0.05$) and APA (Fig. 4.3b, $r = 0.71$, $n = 18$, $p <0.05$).

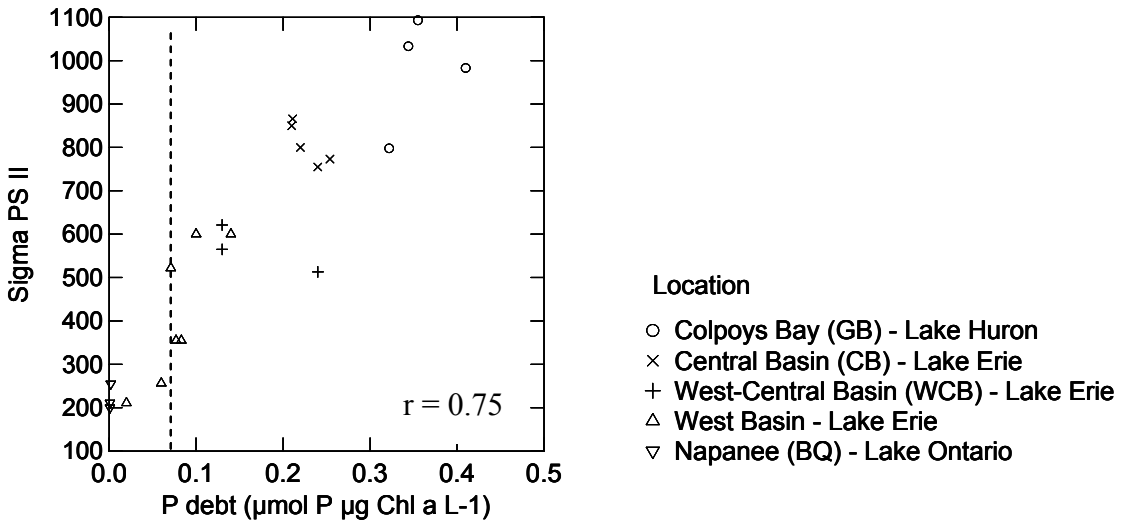


(a)

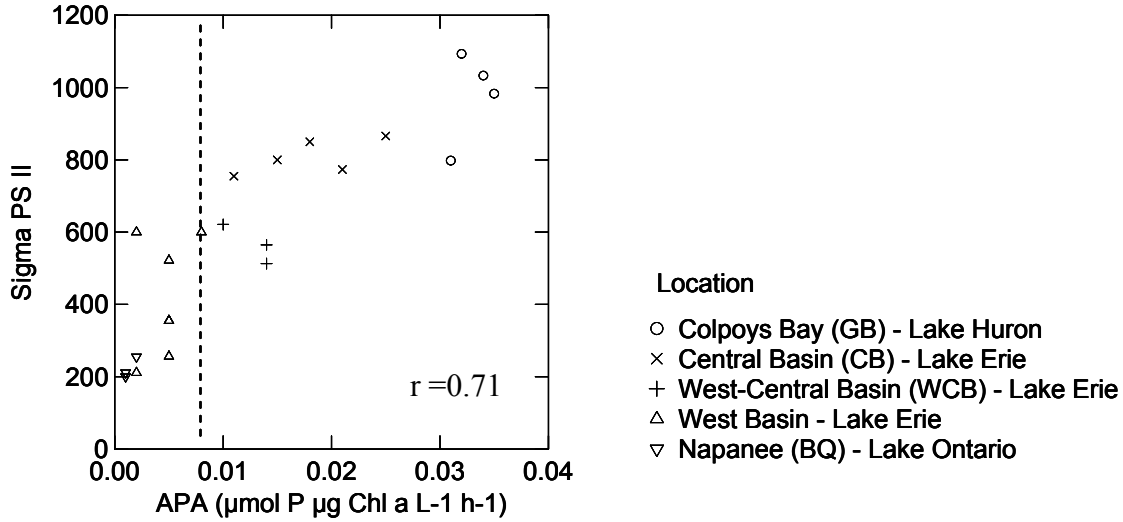


(b)

Figure 4.3 Scatter plots of F_v/F_m versus (a) P debt and (b) APA. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).

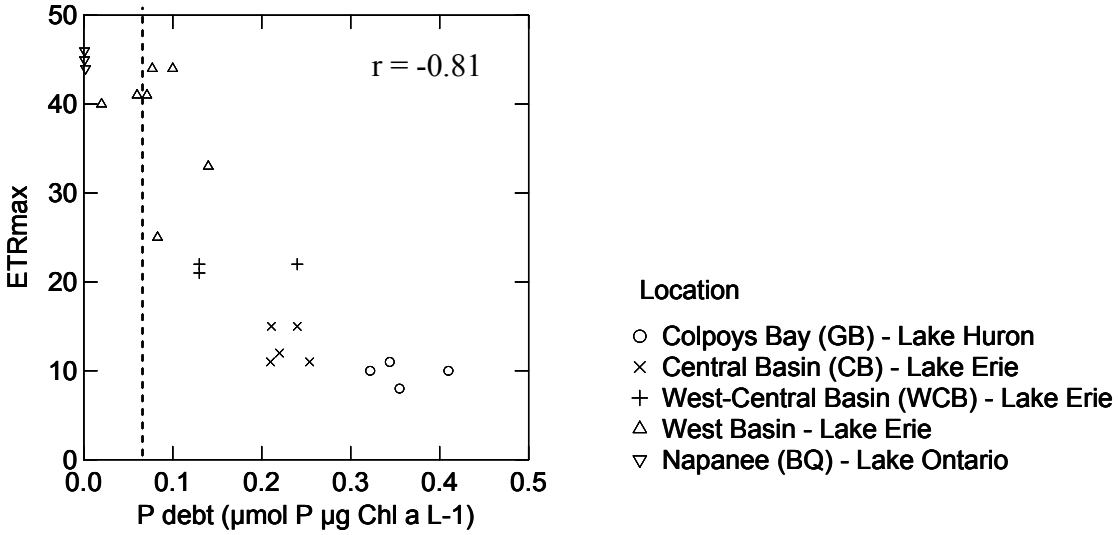


(a)

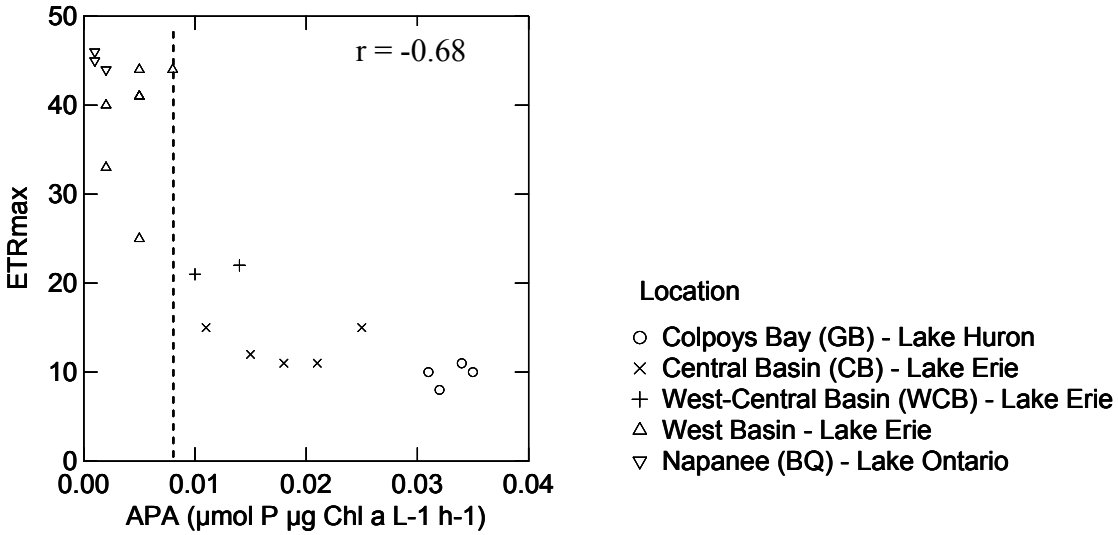


(b)

Figure 4.4 Scatter plots of σ PS II versus (a) P debt (b) and APA. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).

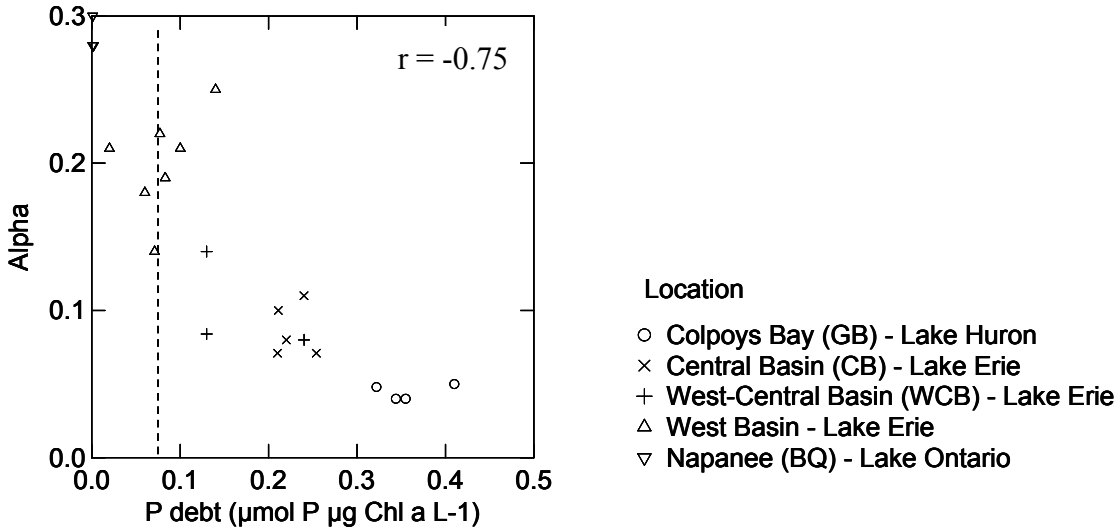


(a)

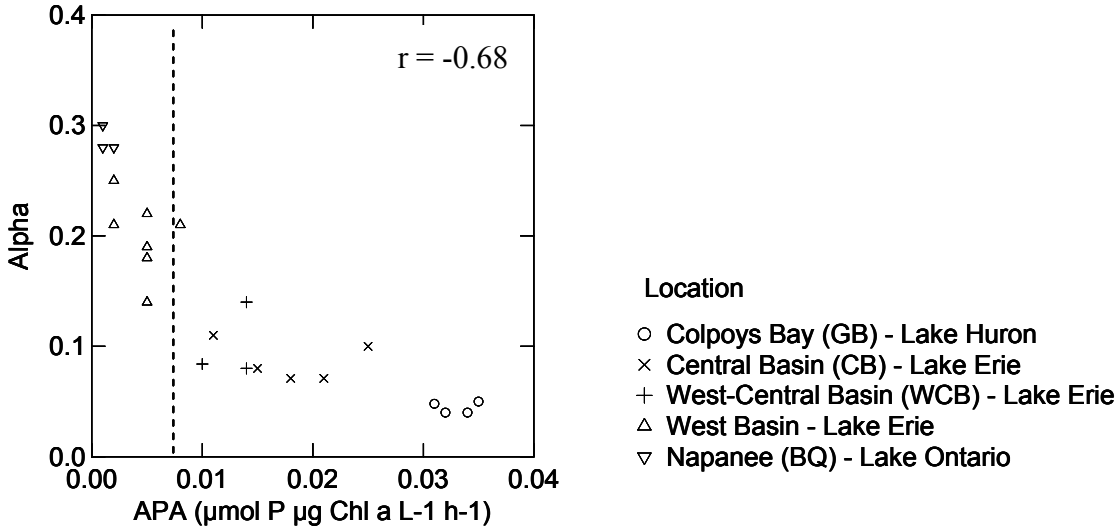


(b)

Figure 4.5 Scatter plots of ETR versus (a) P debt (b) APA. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).



(a)



(b)

Figure 4.6 Scatter plots of α versus (a) P debt and (b) APA. Each point represents site/station. Dashed line = threshold of deficiency (Healey and Hendzel 1979b).

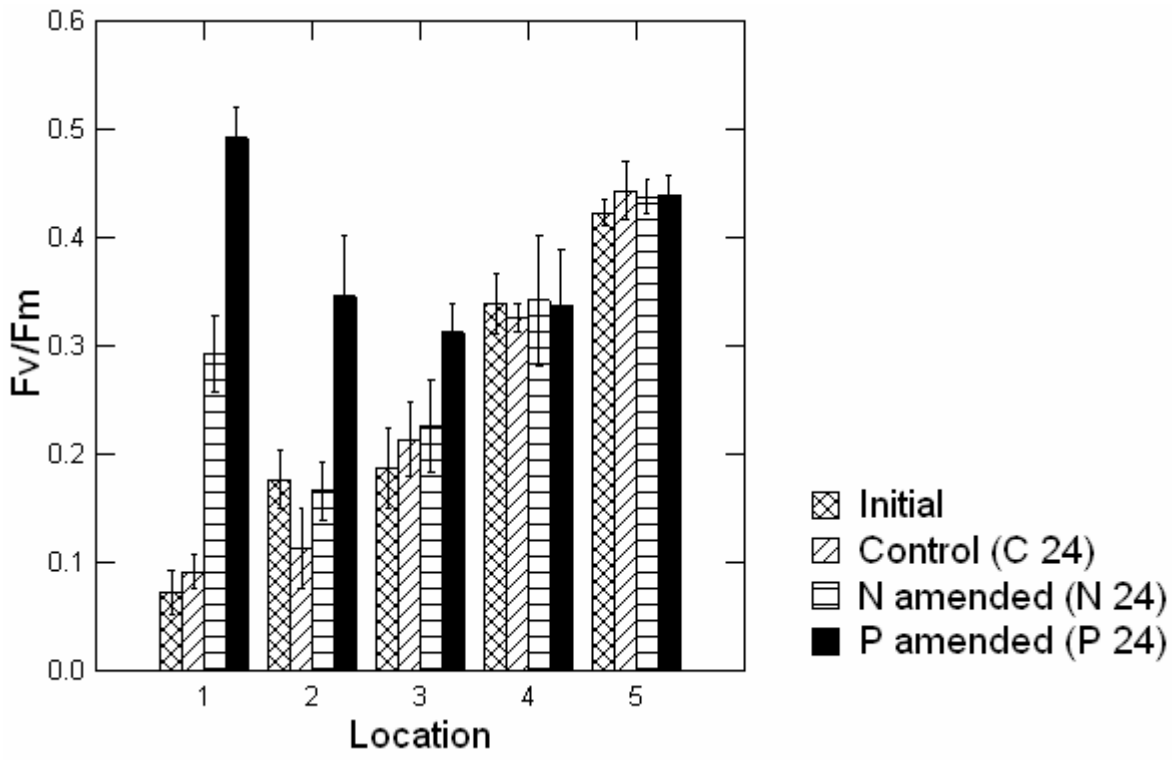
The fitted parameters of RLC's were used to examine relationships with P debt and APA. I chose P debt and APA instead of particulate ratios to examine the associations between α and ETR since the strongest relationships were observed with F_v/F_m and the metabolic assays. ETR values were highest when P debt values were low or below the threshold of deficiency (Fig 4.5*a*). Correlation analysis showed strong relationships between ETR and P debt ($r = -0.81$, $n = 18$, $p<0.05$). Also, regression analysis revealed strong relationships between APA and ETR (Fig4.4*b*. r=- 0.71, n =18, $p<0.05$). Likewise, α values regressed with P debt and APA showed strong associations ($r = -0.75$, $r = -0.68$, $n =18$, $p<0.05$) where α values were highest when P debt and APA values were low (Fig. 4.6 *a* and *b*).

Amendment experiments were also used to evaluate the response to N and P addition using $F_v/F_{m,}$ ETR_{max} and α (Table 4.3). Initial Chl a fluorescence indices of P deficient phytoplankton indeed showed a positive response to P additions in Colpoys Bay, CB, WCB and in some sites located in WB (Fig. 4.7 a-d). Interestingly, initial Chl a fluorescence measurements of sites from Colpoys Bay and CB revealed that phytoplankton were N deficient and the Chl a fluorescence indicators responded positively to N additions; however, P additions produced more increase in $F_v/F_{m,}$ ETR_{max} and α in the oligotrophic sites. Initial Chl a fluorescence values in the Napanee station and some sites in WB did not show any response to N and P additions. Chl a fluorescence measurements were analyzed using 2-way analysis of variance (ANOVA) to determine any significant differences between sites and nutrient addition experiments (Table 4.4). Chl a fluorescence values showed significant variation between sites, treatments, and the interaction between sites and treatments. Based on post-hoc tests, the

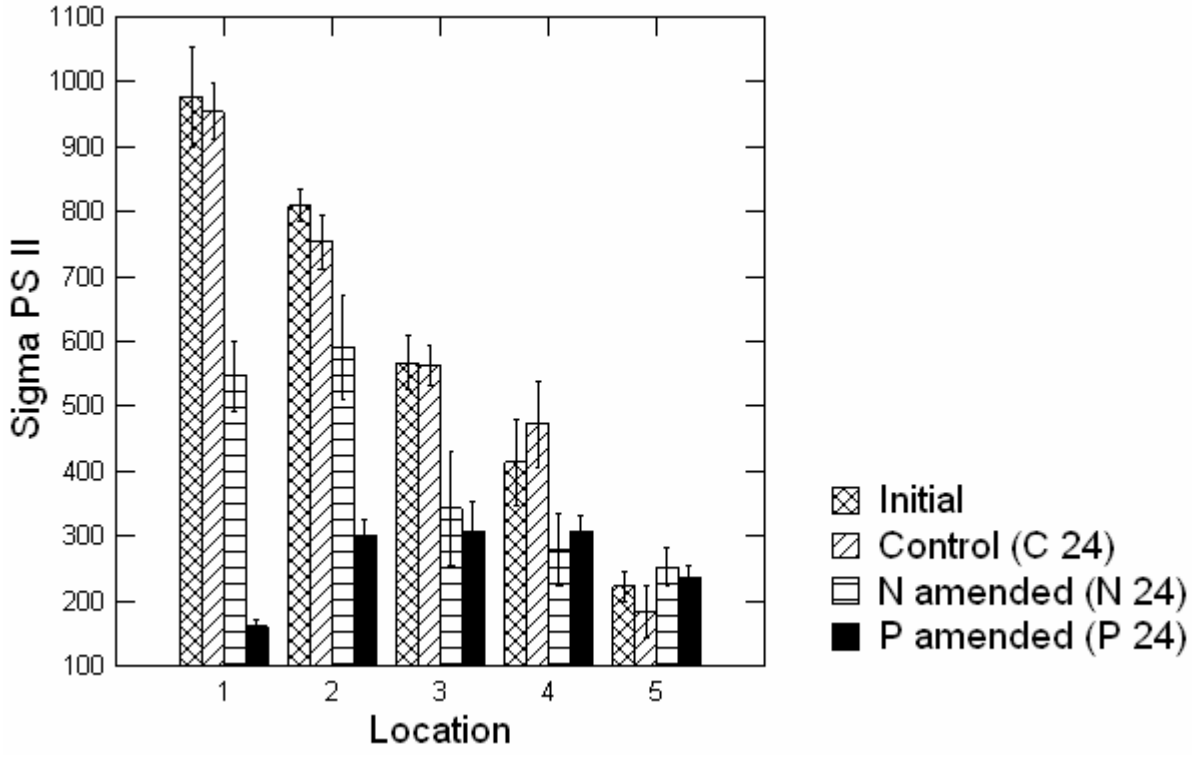
N addition treatments showed statistically significant differences (p<0.05) in all Chl a fluorescence parameters between initial and N 24, and C24 and N 24 treatments in Colpoys Bay, CB and WCB. Only ETR_{max}, α and σ PS II showed statistically significant differences between initial, C 24 and N 24 treatments in WB. In all Chl a fluorescence parameters, statistically significant differences were revealed between initial, C24 and P 24 treatments in Colpoys Bay, CB and WCB. Only statistically significant differences between initial, C 24 and P 24 treatments based on α and σ PS II were revealed in WB. No statistical significant differences were observed between the initial and C 24 treatments for all Chl a fluorescence parameters and C 24 treatments did not differ significantly from N 24 or P 24 treatments in Napanee. Detailed lists of the comparisons are presented in Appendix A3 respectively.

Table 4.3 Nutrient amendment assays (N and P) for variable fluorescence parameters (mean + standard deviation (number of samples)) for Napanee (Bay of Quinte), West Basin (Lake Erie), West-central basin (WCB), Central Basin (CB) and Colpoys Bay (Georgian Bay) * denotes significant difference ($p<0.05$) between C 24 and nutrient amended samples

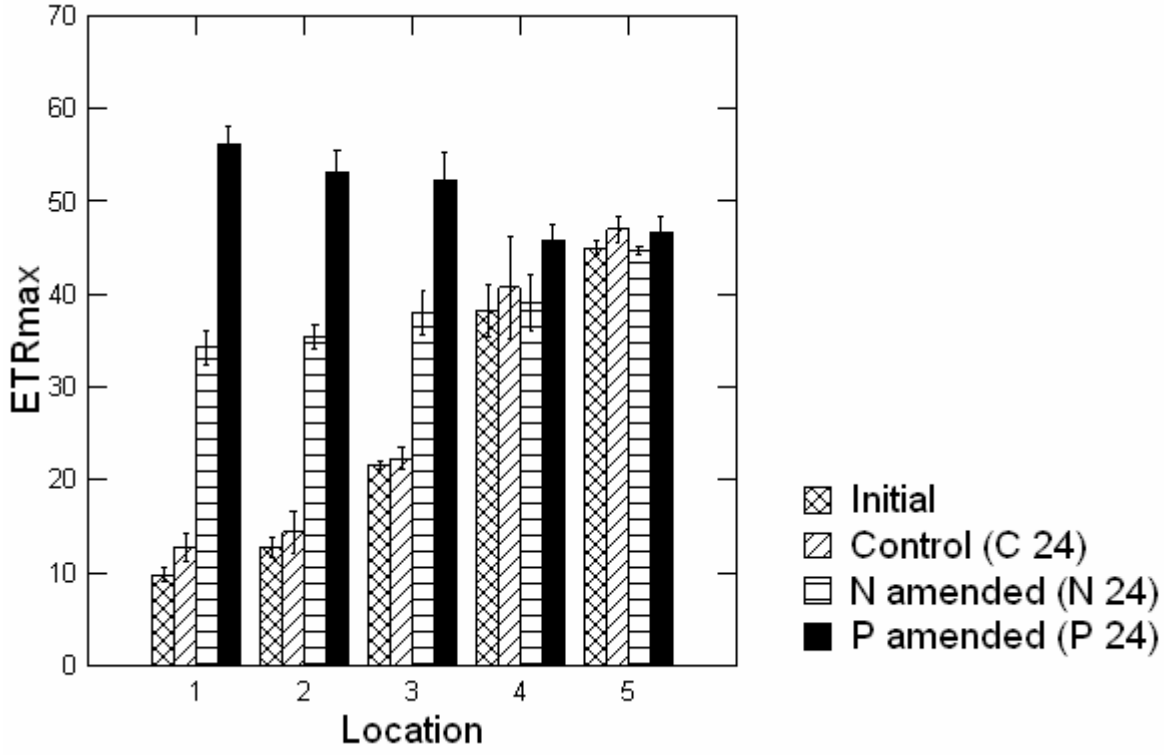
Parameters	Napanee	WB	WCB	CB	Colpoys Bay
ETR C 24	50.03 ± 3.26 (3)	39.44 ± 2.45 (5)	22.10 ± 3.54 (4)	15.22 ± 4.51 (5)	8.79 ± 0.05 (4)
ETR N 24	48.01 ± 1.23 (3)	32.22 ± 1.14 (5)	35.20 ± 5.21 (4)*	31.22 ± 8.15 (5)*	32.71 ± 0.09 (4)*
ETR P 24	52.02 ± 3.22 (3)	41.11 ± 9 .81 (5)	35.20 ± 2.45 (4)*	50.50 ± 3.28 (5)*	59.03 ± 4.01 (5)*
α C 24	0.28 ± 0.04 (3)	0.22 ± 0.01 (5)	0.14 ± 0.01 (4)	0.08 ± 0.04 (5)	0.09 ± 0.01 (4)
α N 24	0.31 ± 0.01 (3)	0.25 ± 0.08 (5)	0.23 ± 0.02 (4)*	0.19 ± 0.04 (5)*	0.19 ± 0.03 (4)*
α P24	0.29 ± 0.01 (3)	0.25 ± 0.01 (5)	0.25 ±0.05 (4)*	0.28 ± 0.06 (5)*	0.29 ± 0.03 (4)*
F_v/F_m C 24	0.403 ± 0.01 (3)	0.351 ± 0.06 (5)	0.216 ± 0.02 (4)	0.174 ± 0.14 (5)	0.144 ± 0.08 (4)
F_v/F_m N 24	0.437 ± 0.02 (3)	0.411 ± 0.03 (5)	0.253 ± 0.04 (4)	0.255 ± 0.01 (5)*	0.292 ± 0.10 (4)*
F_v/F_m P 24	0.439 ± 0.01 (3)	0.372 ± 0.02 (5)	0.312 ± 0.102 (4) *	0.389 ± 0.10 (5)*	0.533 ± 0.09 (4)*
σ PS II C 24	187.05 ± 6.33 (3)	311.21 ± 19.25 (5)	561.40 ± 14.23 (4)	714.30 ± 75.21 (5)	814.25 ± 25.64 (4)
σ PS II N 24	198.02 ±10.22 (3)	280.49 ± 22.31 (5)	310.33± 12.31 (4)*	565.25 ± 21.38 (5)*	502.22 ± 25.25 (4)*
σ PS II P 24	210.33 ± 5.22 (3)	200.30 ± 21.23 (5)	280.22 ± 25.31 (4)*	280.22 ± 15.61 (5)*	154.03 ± 14.02 (4)*

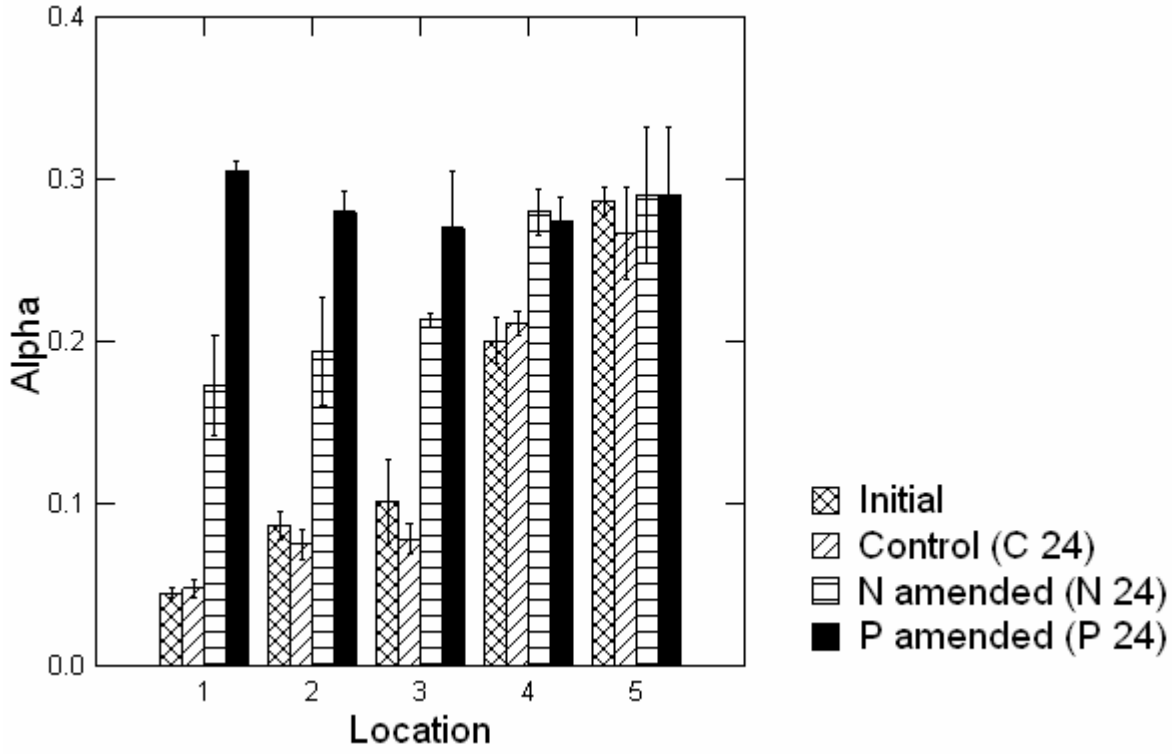

Fv/Fm
0.0
0.1
0.2
0.3
0.4
0.5
0.6
1
2
3
4
5
Location
Initial
Control (C 24)
N amended (N 24)
P amended (P 24)

(a)


Sigma PS II
100
200
300
400
500
600
700
800
900
1000
1100
1
2
3
4
5
Location
Initial
Control (C 24)
N amended (N 24)
P amended (P 24)

(b)





(d)

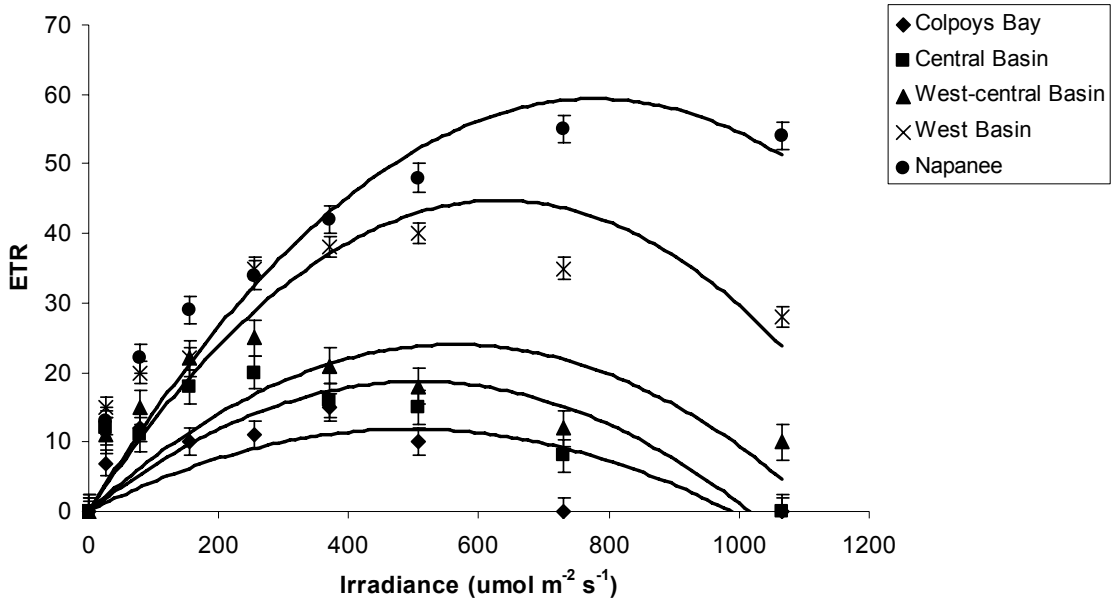
Figure 4.7 (a-d) Amendment experiments of N and P additions in Colpoys Bay (1), central basin of Lake Erie (2), west-central basin of Lake Erie (3), western basin (4) and Napanee (5).

Table 4.4 2-way ANOVA revealing significant differences (p <0.05) for Chl a fluorescence parameters using lake sites (basins) and nutrient addition treatments as factors.

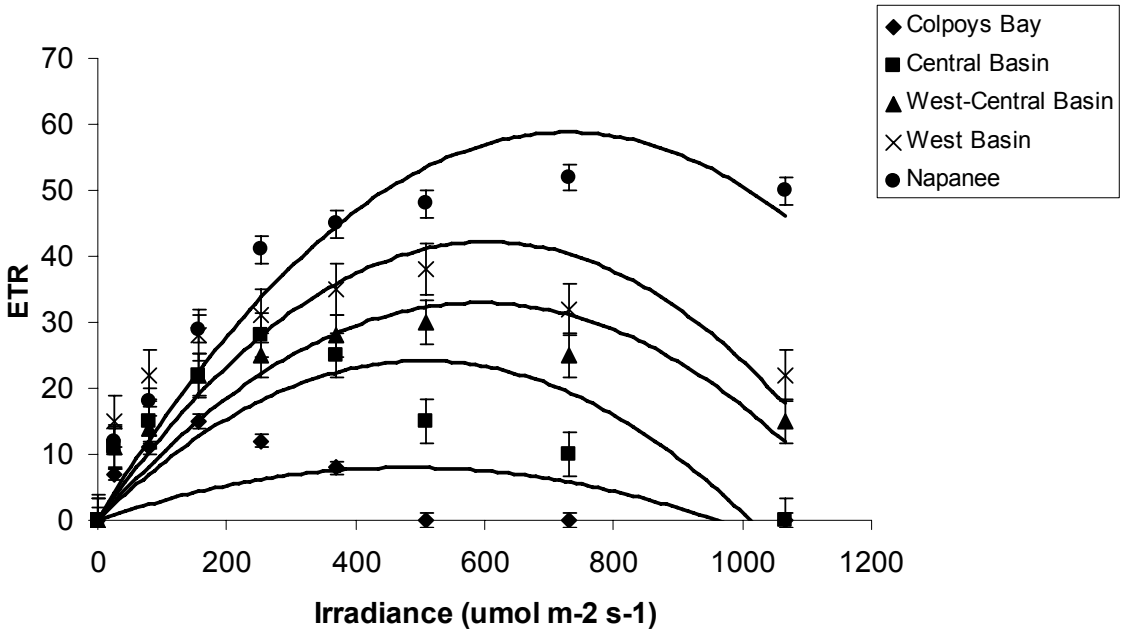
Comparison	F-statistic	df	*p* value
Fv/Fm			
Site	23.51	20	0.031
Treatment	102.61	3	<0.001
Interaction	93.67	60	0.035
σ PS II			
Site	46.84	20	0.035
Treatment	92.36	3	<0.001
Interaction	166.72	60	<0.001
ETR_{max}			
Site	59.34	20	0.022
Treatment	154.31	3	<0.001
Interaction	133.58	60	<0.001
α			
Site	10.25	20	0.034
Treatment	155.98	3	<0.001
Interaction	198.36	60	0.041

The response of ETR to irradiance and to nutrient amendments at the different sites can be visualized by the shapes of the RLCs as fitted to the chosen P-I model (Fig. 4.8). Measurements taken on dark-adapted samples represent the initial condition and showed a wide range among sites (Fig. 4.8 *a*). The lowest values of ETR were in Colpoys Bay, with an ETR_{max} of only 8.71, with severe inhibition at higher irradiance. Sites with higher TP and Chl *a* showed progressively higher ambient ETR and less inhibition at high irradiances, with Napanee showing no photoinhibition. Holding samples in darkness for 24 hours (C 24, the control treatment for the amendment experiments) had little

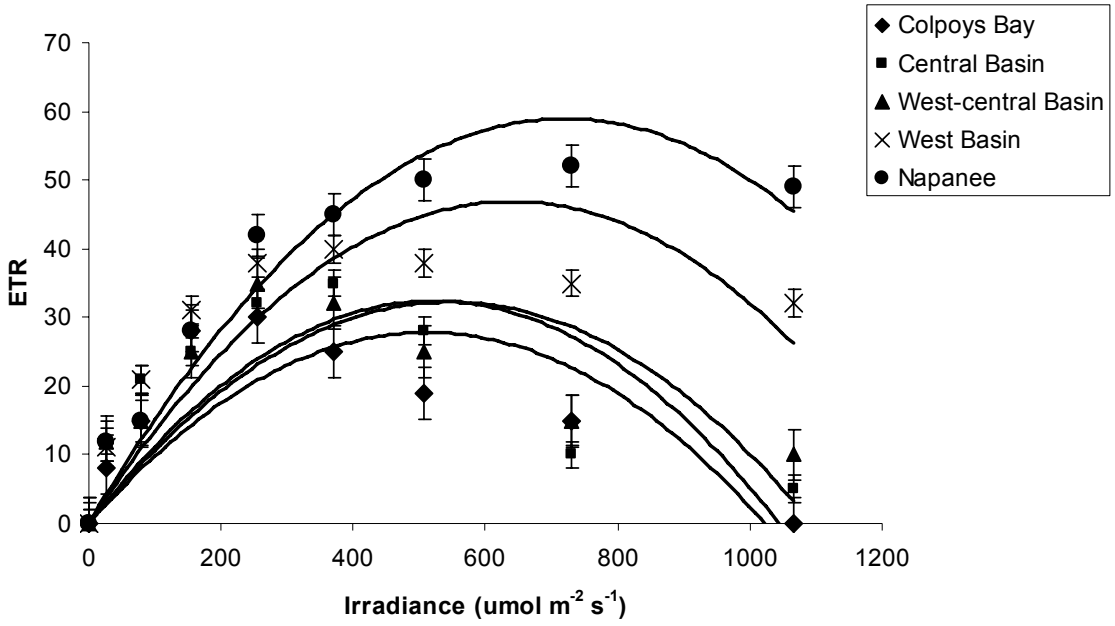
apparent effect on the shape of the RLC curves compared to the initial measurements (Fig. 4.8 *b*). Samples receiving nitrogen (N 24 samples, Fig. 4.8 *c*) compared to ambient samples had elevated ETR at some sites, especially in Colpoys Bay (ETR_{max} increased to 28.33) but also in CB. The saturating irradiance was higher in N 24 than C 24 or initial for Colpoys Bay and CB and the onset of severe photoinhibition was displaced to higher irradiance values (Fig. 4.8 *c*). Samples


Colpoys Bay
Central Basin
West-central Basin
West Basin
Napanee
ETR
Irradiance (umol m-2 s-1)
0
10
20
30
40
50
60
70
0
200
400
600
800
1000
1200

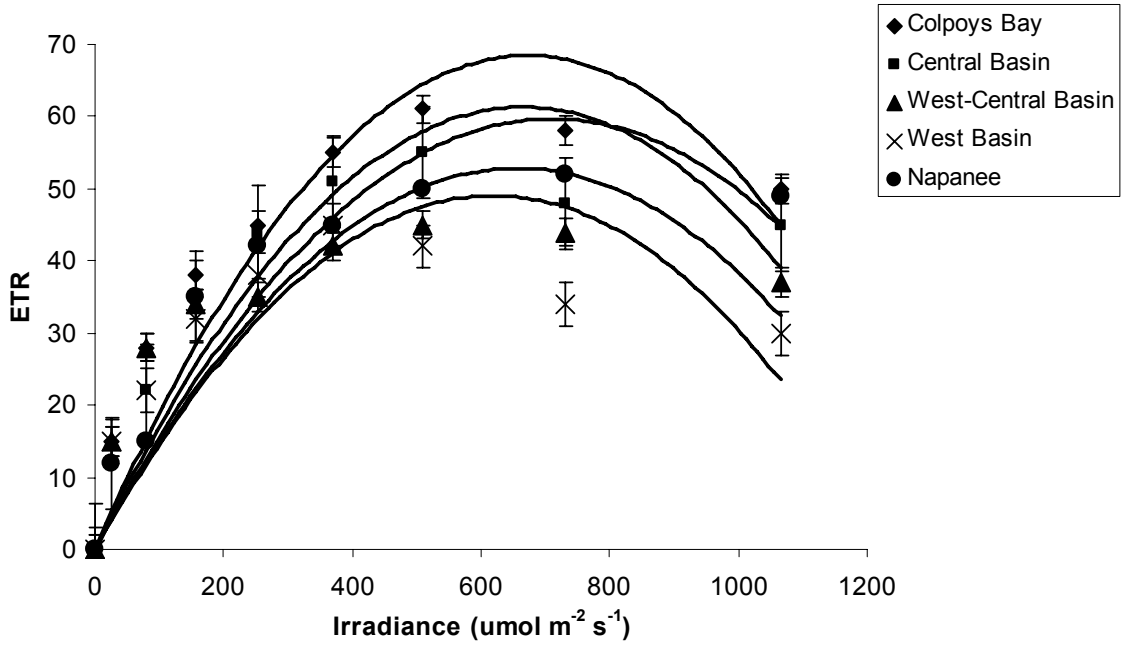
(a)


Colpoys Bay
Central Basin
West-Central Basin
West Basin
Napanee
ETR
Irradiance (umol m-2 s-1)
0
10
20
30
40
50
60
70
0
200
400
600
800
1000
1200

(b)



(c)



(d)

Figure 4.8 ETR versus Irradiance (PFD) curves. (a) Inital measurements, (b) Control 24 h (C 24) treatments (c) N amended 24 h (N 24) treatments and (d) P amended 24 h (P 24) treatments

treatments (Table 4.4). Multiple comparison tests were conducted to investigate where the differences existed in the data. In receiving P additions (P 24) showed the greatest changes relative to initial or C 24 treatments (Fig. 4.8 *d*). Particularly large increases in ETR values for CB (ETR_{max} 50.50) and Colpoys Bay (59.0) were observed, with higher values of saturating irradiance and far less photoinhibition. Table 4.3 summarizes the RLC parameters α and ETR_{max} for the sites and nutrient amendment treatments, while Table 4.2 presents initial values.

4.3.4 Phytoplankton Composition

Water samples for phytoplankton enumeration were collected in summer stratified conditions from the Napanee station, WC, WCB, CB and Colpoys Bay. In total, 112 phytoplankton taxa, belonging to six major groups, were identified (Figure 4.9). Colpoys Bay and CB summer phytoplankton communities had a prevalence of cryptophytes and chrysophytes. These results are similar to those found in Chapter 2 where in P deficient stations, cryptophytes generally were prevalent and usually represented by members of *Rhodomonas* and *Cryptomonas*. Chrysophytes were also prevalent and were often represented by *Dinobryon divergens, Rhizochromonas endoricata* and *Rhizochrysis spp.* Overall, Colpoys Bay and CB tended towards dominance by flagellates and small or medium sized taxa.

The Napanee station and WB sites had a prevalence of cyanobacteria and pyyrophytes. For example, in Napanee, all sites were considered nutrient sufficient and the phytoplankton community was mainly from the cyanobacteria group; however non N-

fixing species such as *Microcystis aeruginosa., Microcystis weisenbergii and Chroococcus sp*. composed 50-60% of the total algal biomass. N_2-fixing species such as *Anabaena sp.* and *Anabaena flos-aquae* were also present, however, their contribution to biomass was considerably less.

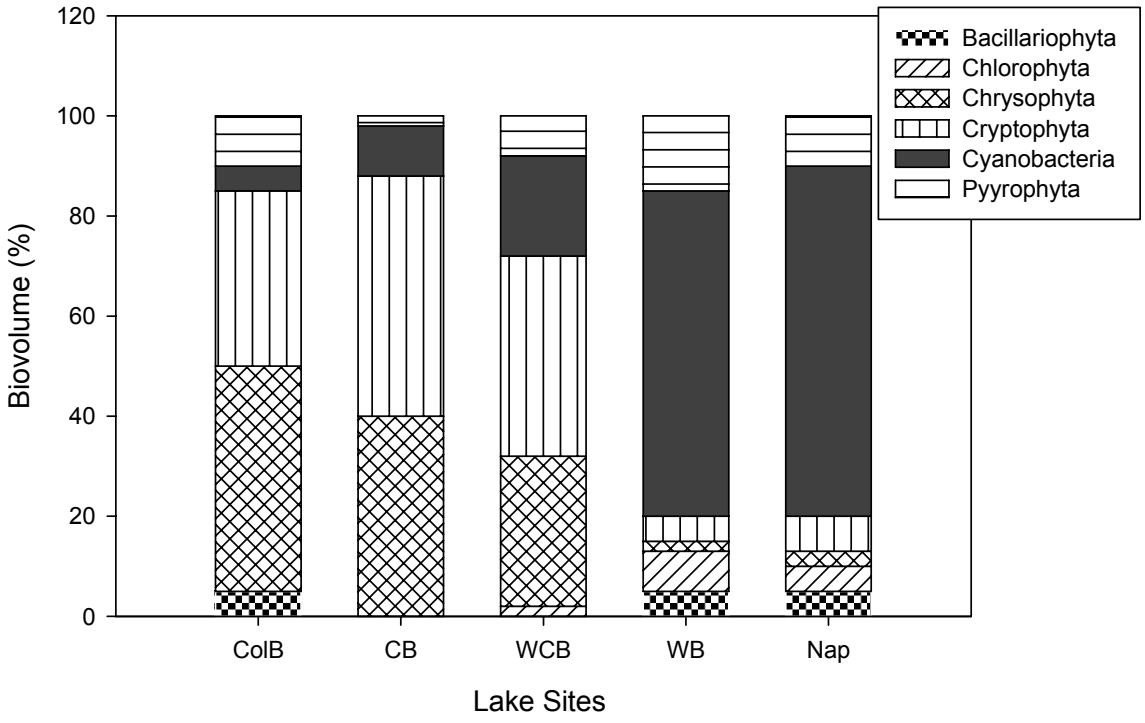


Figure 4.9 Average Biovolume (μm^3. mL) (expressed as %) of phytoplankton groups in Colpoys Bay (Col B), CB, WCB, WB and Napanee (Nap).

4.4 Discussion

In this study, the results from independent measures of nutrient status, variable fluorescence, and nutrient addition assays revealed that natural phytoplankton communities in summer stratified conditions were mainly limited by P availability. Independent measures of nutrient status and variable fluorescence parameters indicated

severe P limitation in the most oligotrophic sites (Colpoys Bay and CB), while P limitation was negligible in the most eutrophic sites (WB and Napanee). The nutrient amendment assays showed positive effects of N and P additions on all variable fluorescence measures in Colpoys Bay and CB, however P additions exerted a stronger positive effect compared to N additions. Nutrient additions in the eutrophic sites did not reveal any effects on variable fluorescence measurements. These results confirm that a trend in the severity of P limitation was seen across a trophic gradient of TP and Chl concentrations, and that Chl fluorescence (F_v/F_m, σ PSII, and RLC parameters) measurements were able to detect this trend.

The concentrations of NO_3^-, NH_4^+ and Chl *a* were highest in Napanee and WB compared to Colpoys Bay (Table 4.2). N debt and C:N values for all lake sites were generally below the threshold of deficiency. The N additions showed positive effects on ETR_{max}, α and σ PS II in Colpoys Bay, CB and WCB sites (Table 4.3). For example, initial average values of ETR_{max} from Colpoys Bay was 5.0 compared to average N amended values (ETR_{max} = 32.7). However, the N amended treatments on the initial samples in all three sites did not show the same effect on the maximum quantum efficiency (F_v/F_m). Based on these results, the N amended treatment tended to exert effects on fluorescence indices related to efficiency of light harvest for photosynthesis.

To maximize light harvesting and trapping of excitation energy, the synthesis of specific proteins are required. Under N limitation, a decrease in the fluorescence yield indicates a reduction in the photochemical energy conversion efficiency of PS II (Falkowski and Raven 2007). The decreased photochemical efficiency in PS II under N limited conditions could relate to non-radiative dissipation of absorbed excitation energy

in the pigment bed, in which changes correspond to the reduction in the relative abundance of D1 and CP47 proteins (Falkowski and Raven 2007). As a result, high irradiance levels in N limited conditions could increase the turnover rate of D1 protein. However, in the absence of sufficient amino acids to sustain a constant pool, high turnover rates could lead to a reduction in proteins. Under these conditions, fewer electron equivalents per unit Chl can be produced by a saturating flash, with the result being that excess excitation energy is dissipated (Kolber and Falkowski, 1988; Falkoswski and Raven 2007). The addition of NH_4^+ to N limited phytoplankton can lead to the recovery of maximal photosynthetic energy conversion in which a decrease in σ PSII and increases in α and ETR_{max} are observed (Kolber and Falkowski 1988, Chapter 2, 3).

Previous reports on phytoplankton nutrient status in Georgian Bay have primarily focused on P and Si limitation (Furgal and Smith 1997, Furgal et al. 1998) because NO_3^- and light were usually high during summer stratification. My results also revealed high NO_3^- and light measurements. However, average values of NH_4^+ were lower compared to the eutrophic sites. An explanation of N deficiency in an otherwise high NO_3^- site, may be due to co-limitation. Iron (Fe) limitation has been suggested to be common in temperate meso-oligotrophic lakes, such as the Laurentian Great Lakes (Twiss et al. 2000, Sterner et al. 2004, North et al. 2007). Fe is involved in many biochemical reactions as a cofactor of enzymes and proteins involved in chlorophyll synthesis, electron transport, and NO_3^- /NO_2^- assimilation (Paerl and Zehr 2000). For example, North et al. (2007) used enrichment experiments involving the addition of Fe, P, and N in the offshore and nearshore waters of the eastern basin of Lake Erie. Initial measurements

of Fe were below the detection level (2.0 nmol L^{-1}) while N and P limitation were evident in these sites. Their results suggest that a combination of Fe, P and N revealed a significant increase in biomass compared to measurements of only Fe, N or P enrichment. In this study I did not measure Fe concentrations, however previous work cited above suggests Fe availability can be limiting in summer stratified conditions.

In this study, the results of N status measured by the independent measures, Chl fluorescence and amendment treatments for all lake sites, showed variable effects of N addition. According to the N status indicators, no sites had values above the threshold of deficiency. The initial Chl a fluorescence measurements were relatively low in Colpoys Bay and CB, however the greatest response to N additions, was observed with the RLC parameters and σ PS II (Fig. 4.8 *a-d*). Although Chl *a* fluorescence indices showed strong and significant relationships with N debt (Chapter 2 and 3), the fluorescence indices suggest that the light harvesting efficiency and photosynthetic capacity of PS II could be affected by decreases in N concentrations.

In chapters 2 and 3, all Chl *a* fluorescence indices and metabolic assays were highly correlated. In fact, the lowest F_v/F_m and RLC parameters were observed when Lake Erie phytoplankton were generally P deficient in the CB during summer stratification. My studies corroborate with these results (eg. Lean et al. 1983, Guildford et al. 2005) and suggest that Lake Erie phytoplankton exhibited moderate P deficiency. In this study, my results also revealed strong associations between metabolic and Chl *a* fluorescence parameters in Lake Erie, however the sites in Colpoys Bay were more P deficient compared to Lake Erie. The initial measurements of fluorescence parameters in the oligotrophic sites suggest that P limited conditions can affect photosynthetic energy

conversion (Falkowski and Raven 2007). In contrast, high TP, SRP and Chl *a* concentrations were highest in Napanee and WB. The nutrient status results based on P debt, APA and Chl *a* fluorescence suggested little or no P deficiency in these sites.

The P amendment treatments (Table 4.3) showed positive effects on all Chl *a* fluorescence indicators in Colpoys Bay, CB, WCB and some WB sites. The RLC's also revealed changes in the efficiency of electron transport and light harvesting when the initial samples were amended with P (Fig 4.8 *a* and *d*). Both N and P amendment treatments showed the same positive effect on most oligotrophic sites, though the response to P additions in Colpoys Bay and CB revealed changes on the order of 40-70% for F_v/F_m, σ PSII and RLC parameters. Thus, it appears that P was the nutrient more limiting in summer stratification.

Oligotrophic lakes have been of special interest in the diagnosis of nutrient limitation. Their plankton dynamics depend mostly on internal mechanisms which act to recycle the limiting nutrient many times over within the surface waters. Hence, internal P recycling may alleviate P deficiency. As a result, the efficiency of nutrient regeneration should increase as P sources become depleted (Capblanq 1990). However, there are some cases in which the efficiency of P regeneration may not vary with TP. Hudson et al. (1999, 2000) measured P regeneration in lakes of varying TP and Chl a using a new bioassay developed to directly assess PO_4^{3-} concentrations. The bioassay was developed to estimate phosphate (PO_4^{3-}) concentrations in conditions where concentrations of PO_4^{3-} were too low to be estimated using chemical measurements and where the turnover of PO_4^{3-} is rapid and the uptake of PO_4^{3-} and regeneration are equal (Dodds 1993). Using this method, Hudson et al. (2000) found that P regeneration rates expressed per unit of

particulate P (turnover rate), did not vary with TP status. In fact the efficiency of P turnover rates, were generally the same in P limited and P-rich lakes. Thus, lake phytoplankton were able to utilize P within plankton communities, rather than from external loading. In this study, I've used the colorimetric analysis of SRP in conjunction with TP, PP, P debt and APA measurements. In contrast to Hudson et al. (2000) study, I found that decreasing TP concentrations was related to the trophic status among the Great Lakes sites. Perhaps additional factors such as light climate, lake size and mixing depth could play an important role in determining the extent of P deficiency in these sites.

The trophic gradient from Colpoys Bay to Napanee, was accompanied by a gradient in algal biomass (Table 4.2). Based on TP and SRP measurements, Napanee and WB sites were more eutrophic with average Chl values from 7.56-10.62 μg L^{-1} (Table 4.2). According to the nutrient status and Chl a fluorescence indicators, P deficiency was negligible or less severe compared to sites with low TP and Chl a (Colpoys Bay). Also, mean PAR measurements were lower (69-83 μmol m^{-2} s^{-1}) in Napanee and WB. Therefore, a combination of shallow depth, light, constant vertical mixing, high loading of nutrients and re-suspension may be the cause for eutrophic conditions. Trophic conditions in the mesotrophic sites (WCB) were probably influenced by the water exchange between the WB and CB. Due to low P, Chl *a* and high light conditions, the oligotrophic sites showed more P deficiency compared to the other sites. Therefore, nutrients and light climate can contribute to the gradient of P deficiency observed. Millard et al. (1996) reported that a gradient of P deficiency was observed when the seasonal transition from light to P limitation was driven by the change in the vertical mixing. Guildford et al. (1994) examined the effects of lake size and phytoplankton

nutrient status in seven Canadian Shield lakes, Lake Superior and Lake Nipigon. During summer stratification, all lakes exhibited P deficiency, however differences in the severity were noticeable when larger lakes (Lake Superior) were compared to smaller lakes (Canadian Shield lakes). For example, the deep turbulent mixed layers in larger lakes resulted in a more efficient retention of P in the epilimnion compare to the smaller lakes. I compared my results to those from Guildford et al. (1994), and found that the severity of P deficiency in Colpoys Bay was comparable to Lake Superior and Lake Nipigon. These results were interesting since irradiance values in Colpoys Bay were greater than Lake Superior, although TP measurements were similar (0.15 umol L^{-1}, Lake Superior; 0.16 umol L^{-1}, Colpoys Bay). Therefore, it seems that TP values have a greater influence on trophic status.

The results of this study provided evidence of P limitation and its effect on algal biomass. The relationship between P deficiency and trophic status showed that varying P deficiency corresponded well with the independent measures of nutrient status and Chl *a* fluorescence, despite influences of taxonomic effects. In chapter 2, phytoplankton composition was used to examine the effects of nutrient deficiency and Chl *a* fluorescence. The samples were categorized based on nutrient status and the results displayed interesting patterns of behavior in phytoplankton communities. In summer stratified conditions, larger filamentous species such as *Anabaena sp.* were able to produced heterocysts, suggesting N fixation activity (Chapter 2). In relation to Chl *a* fluorescence, the increased importance of cyanobacteria was probably due to cellular phycobiliprotein content. The cellular phycobiliprotein content influences the F_o level fluorescence, particularly when phycobiliprotein levels are high. As a result, higher

values of phycobiliprotein may contribute to a lower value of F_v/F_m (Campbell et al. 1998). Most sites sampled in this study were not found to be N deficient, however the phytoplankton communities in Napanee and WB mainly (65%) consisted of non-N_2 fixing species such as *Microcystis aeruginosa, Microcystis weisenbergii and Chroococcus sp*. Pyrrophyta were also present and comprised of 10% of the total phytoplankton community (Fig. 4.8). These results were consistent with nutrient sufficient sites in Lake Erie (Chapter 2) where pyrrophytes and cyanobacteria were dominant in summer stratified conditions. Even though, Fig. 4.8 illustrates that fluorescence properties were not responsive to nutrient amendments experiments at the more cyanobacteria dominated sites such as Napanee and WB, unlike Chapter 2, these particular sites did not show N or P deficiency but relatively high values of F_v/F_m, α, ETR_{max} and low σ PS II. Therefore, Fig. 4.8 provides evidence that cyanobacteria dominance does not necessarily lead to variable fluorescence properties typical of nutrient stressed phytoplankton.

In chapter 2, flagellates from the chrysophyte and cryptophyte groups were generally more important in CB than WB during summer stratification. Some species within these groups are known to be nutritionally opportunistic, switching between autotrophy and heterotrophy depending upon cellular and environmental conditions (Watson et al. 1997) For example, experiments with cultured mixotrophic flagellates (Olrik et al. 2007) support the view that phagotrophic activity can be induced under conditions of limited inorganic nutrient availability. In relation to variable fluorescence, cell size may be a contributing factor for decreases in F_v/F_m and increases in σ PS II. Values of F_v/F_m are highest in well-mixed systems dominated by large diatom cells and

lowest in summer stratified conditions where small flagellates (nanoflagellates) are prevalent. In this study, low F_v/F_m values in P deficient sites may reflect this seasonal change. Most sites were P deficient and phytoplankton communities in Colpoys Bay and CB consisted largely of chrysophytes (35-45%), cryptophytes (35-45%), and to a smaller extent dinoflagellates (10-12 %). In comparison, Smith and Maly (1993) have reported in Colpoys Bay a mixed community of diatoms, chrysophytes and chlorophytes during summer stratification in where species of the chrysophyte group dominated in the epilimnion and cryptophytes were prevalent in the metalimnion. These results were also consistent with P deficient sites in Lake Erie where in summer stratified conditions chrysophytes and cryptophytes were prevalent.

In conclusion, the outcome of this comparative study validates that algal productivity is strongly dependent on P availability in these systems. Based on the P and Chl *a* concentrations, metabolic assays and variable fluorescence; initial measurements of nutrient status in the oligotrophic sites depicted values suggesting an imbalance between P availability and demand. Also, the addition of PO_4^{3-} caused the phytoplankton to positively respond as shown by the increase in photosynthetic efficiency. The eutrophic sites revealed no P deficiency and amendment experiments also confirmed that the phytoplankton communities did not respond to PO_4^{3-} additions. Although this study has focused on providing evidence that the severity of P deficiency increases as P concentrations decrease, Chl *a* fluorescence parameters were able to predict this same trend among the range of sites sampled.

Chapter 5. Summary and Conclusions

5.1 Thesis summary

In this thesis, I have used a suite of nutrient status indicators and Chl *a* fluorescence parameters to explore that freshwater phytoplankton can become limited by the availability of nutrients. Pertinent issues discussed in this thesis include the consistency of nutrient status measurements, the use of Chl *a* fluorescence as a sensitive measure of the physiological condition of phytoplankton, and predicting which nutrient has the greatest effect in natural phytoplankton communities.

To examine spatial and temporal incidences of nutrient deficiency in Lake Erie phytoplankton, the nutrient status indicators, Chl *a* fluorescence, and the taxonomic structure of the phytoplankton communities were applied (Chapter 2). The occurrence of N limitation was confirmed by the N debt assay, and generally occurred in isothermal conditions. The occurrence of N deficiency is likely related to suboptimal temperature and light conditions that may increase NH_4^+ requirements of phytoplankton. The occurrence of P status was confirmed using P debt and APA assays, and P deficiency was generally observed more often in June than in May or September. These results for P were comparable to previous reports (Lean et al. 1983, Allen and Smith 2002, Guildford et al. 2005, Moon and Carrick 2007). The quantum yield of PS II (F_v/F_m) and the functional absorption cross section of PS II (σ PSII) correlated well with N and P debt and APA. C:N, C:P and N:P correlated weakly with the metabolic assays and Chl a fluorescence, suggesting that these ratios may respond on different time scales or be less sensitive compared to the metabolic assays. Also, re-suspension of sedimentary and

detrital particles can increase C:P and C:N ratios, resulting in an over-estimate of nutrient deficiency. The phytoplankton community structures in WB, WCB and CB of Lake Erie was analyzed and associated with nutrient status and Chl *a* fluorescence measurements. The occurrence of cyanobacterial species dominance in N deficient sites could be explained by adaptation (N fixation and utilizing N reserves from phycobilisomes) to extreme environmental conditions. Research on nutrient replete cyanobacteria cultures suggests that their optimal F_v/F_m values are between 0.3-0.4 (Suggett et al. 2009), lower than the suggest optimal value of 0.65 (Kolber et al. 1988). Based on these results I would expected that in N deficient sites dominated by cyanobacteria, I might obtain similar F_v/F_m values. However, I obtained, since my F_v/F_m values were well below 0.3 (0.1-0.2) and σ PSII values that were extremely high (800-1000). Since the phytoplankton biovolume at N deficient sites was not exclusively cyanobacterial (other groups contributed 30-40% of the total biovolume), influences from other groups may have contributed to these very extreme values. The apparent selection for chrysophyte and cryptophyte flagellates under P deficiency may be due to their mixotrophic capabilities (Urabe et al. 2000). Phagotrophic activity is known to be induced under conditions of limited inorganic nutrient availability. At P deficient sites, much lower F_v/F_m values were observed compared to nutrient sufficient sites. Cell size may also be a contributing factor for decreases in F_v/F_m and increases in σ PS II.

Chapter 3 introduces ETR vs. Irradiance relationships (termed as rapid light curves or RLC's), as a useful tool for determining physiological changes in natural phytoplankton communities. The specific goals of Chapter 3 were: to confirm with additional observations in a subsequent year that previous indications of N and P

deficiency in Lake Erie are reproducible, to examine the use of amendment experiments to further test whether the previously-used assays are correct in their indications of N and P deficiency, and to test the idea that variable fluorescence measured under excitation pressure (RLC's) may provide a more sensitive measure of nutrient status and even an indication of which nutrient might be limiting.

The nutrient deficiency patterns in Lake Erie observed in previous years (Lean et al. 1983, Allen and Smith 2002, Guildford et al. 2005) were similar to my findings in Chapter 2 and in Chapter 3. N deficiency occurred in isothermal conditions and P deficiency generally occurred in summer stratification. Like F_v/F_m, ETR_{max} and α values decreased as the severity of N and P deficiency increased. These results suggest that F_v/F_m is important in detecting nutrient status in phytoplankton because neither ETR_{max} nor α seemed to be more sensitive to nutrient deficiency than F_v/F_m. However, the results also show that N or P deficiency can cause inhibition of ETR at high nutrient limitation, revealing effects of nutrient deficiency beyond the decrease of maximum quantum efficiency in PSII. The amendment treatments were used to evaluate the response of N and P additions using F_v/F_m, ETR and α. The treatments were successful in restoring a high level of photosynthetic function to nutrient deficient phytoplankton. Also the treatments showed a strong and highly specific response to the limiting nutrient. However, measurements based solely on Chl *a* fluorescence parameters were not able to diagnose the identity of the limiting nutrient. This result can be explained by examining the effect of both N and P limitation on cell physiology. For example, N is needed for the synthesis of RNA, DNA, proteins, and chlorophylls. P is required for RNA, DNA, phospholipids and polyphosphates synthesis (Geider et al. 1993 a).

The main objectives of the works reported in Chapter 4, were to examine the diagnostic capabilities of Chl *a* fluorescence indicators at more widely varying Great Lake sites, evaluating the response of N and P additions on the variable fluorescence indicators as a tool for quantifying deficiency, and to determine if the severity of summer P deficiency is systematically related to trophic status among a set of these Great Lakes sites. P debt and APA values suggested that the severity of P deficiency increased when TP values decreased. The phytoplankton communities were generally not N deficient. Initial variable fluorescence measurements were strongly associated with N and P debt and APA assays. The amendment experiments revealed significant differences between initial and P amended samples in Colpoys Bay, CB, WCB and some sites in WB, and significant differences ($p<0.05$) were observed between initial and N amended samples in Colpoys Bay and CB. The amendment experiments provided evidence that P deficiency was the strongest in Colpoys Bay and weakest at Napanee. Even though N deficiency was detected in the most oligotrophic sites, the addition of P provoked more of a response (50%) in the Chl *a* fluorescence indicators. As in the study reported in Chapter 2, P deficient sites were dominated by nanoflagellates such as cryptophytes and chrysophytes, and nutrient sufficient sites had more larger cyanobacterial species (*Microcystis sp*.), dinoflagellates and diatoms.

5.2 Future Directions and Recommendations

The spatial and temporal scales in this study were relevant in order to address the issue of what nutrient limits phytoplankton communities in these lakes. In Chapter 2, I examined sites in three basins of Lake Erie in different seasons. The sum of evidence

from each nutrient status indicator and variable fluorescence supported the notion that N deficiency mainly occurred in conditions where light could be limiting, and P deficiency occurs in summer stratified conditions. The same study was conducted in the following year and concurred with the results in Chapter 2 of spatial and temporal patterns of N and P deficiency. In Chapter 4, I added two more lake sites (Colpoys Bay and Bay of Quinte at Napanee) to broaden the scale of nutrient status. The evidence revealed that P generally controlled phytoplankton growth and production in summer stratified conditions. Based on these results, I feel that time and space analyses on nutrient status should include multiple sampling or increased sampling frequency during the time period where N deficiency could occur. Also, including more temperate lake sites could be beneficial in order to examine the severity of P deficiency.

Ammonium (NH_4^+) deficiency occurring in the Great Lakes seem rather puzzling, however in both sampling years, the results proved that N deficiency existed in isothermal conditions. Previous work on NH_4^+ and NO_3^- uptake rates (Lehman et al. 1975) in freshwater phytoplankton have focused on several different phytoplankton cultures, however most of the phytoplankton species examined were not found in the Great Lakes. Future work on examining the patterns of N deficiency should include single nutrient (N) addition experiments to test the uptake capacity of different forms of N on freshwater phytoplankton. In addition to the enrichment experiments, I also suggest that manipulating other factors such as light, temperature and other nutrients could provide more answers on what certain environmental conditions could prevent N uptake in phytoplankton.

In Chapters 2 and 4, the characterization of natural phytoplankton communities in Lake Erie, Napanee, and Colpoys Bay provided fascinating results on how phytoplankton communities existed in differing seasons, trophic status and F_v/F_m. The main phytoplankton groups included cyanobacteria, chlorophytes, cryptophytes, chrysophytes, bacillariophytes and pyrrophytes. F_v/F_m values for nutrient replete or sufficient sites were between 0.34 -0.5. Contrary to Kolber et al. (1993), my results show that a common value of 0.65 for nutrient replete phytoplankton was not applicable since nutrient replete cultures of different groups of phytoplankton show a wide range of F_v/F_m values from 0.3-0.8 (Suggett et al. 2009). Future work on key taxa found in various nutrient status conditions will continue to aid in the interpretation of Chl *a* fluorescence data; however large scale environmental assessments should be considered. Based on these results, I suggest for future fluorometry work to utilize additional instrumentation for examining phytoplankton community structure and fluorescence indices simultaneously. The PHYTO-PAM (Walz) phytoplankton analyzer which allows for sensitive readings (Chl a detection limit = 0.1 $\mu g\ L^{-1}$), is able to differentiate between different pigment groups of algae (green algae, diatoms and cyanobacteria), and F_v/F_m of various types of phytoplankton. Another suggestion is using the video plankton recorder (VPR).The VPR is an underwater video system with magnifying optics that images and identifies plankton. It has been routinely used in marine systems and recently this system has been used to map mesoscale variations of plankton in the Sargasso Sea (McGillcuddy et al. 2007). Using the VPR along with physiological measurements including F_v/F_m, could help differentiate different phytoplankton groups, nutrient status and F_v/F_m instantaneously.

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Appendices
Appendix A1: Appendices of all pairwise multiple comparisons for N deficient samples

Table A1.1 Holm-Sidak matrices of all pairwise multiple comparison procedure of sites (basins) and treatments (N deficient only) for Chl a fluorescence parameters

F_v/F_m				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
CB vs. WCB	11.200	5.231	0.002	Yes
CB vs. WB	12.300	4.222	0.003	Yes
WCB vs. WB	11.000	3.540	0.005	Yes
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
N 24 vs. Initial	0.318	8.412	0.000	Yes
N 24 vs. C 24	0.289	6.554	0.001	Yes
N 24 vs.P 24	0.266	5.548	0.001	Yes
P 24 vs. Initial	0.112	4.672	0.312	No
P 24 vs. C 24	0.095	1.220	0.230	No
C 24 vs. Initial	0.040	0.876	0.122	No

ETR_{max}				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
CB vs. WCB	9.200	7.100	0.002	Yes
CB vs. WB	5.600	3.300	0.002	Yes
WCB vs. WB	10.000	4.100	0.001	Yes
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
N 24 vs. Initial	17.550	12.351	0.000	Yes
N 24 vs. C 24	16.540	11.554	0.000	Yes
N 24 vs. P 24	15.468	9.645	0.000	Yes
P 24 vs. Initial	13.564	8.466	0.000	Yes
P 24 vs. C 24	12.323	6.554	0.004	Yes
C 24 vs. Initial	1.000	0.614	0.504	No

Table A1.1 Continued

Alpha (α)				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
CB vs. WCB	6.900	10.200	0.002	Yes
CB vs. WB	9.200	7.320	0.003	Yes
WCB vs. WB	5.300	8.010	0.003	Yes
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
N 24 vs. Initial	0.310	21.139	0.000	Yes
N 24 vs. C 24	0.266	20.148	0.000	Yes
P 24 vs. N 24	0.201	15.193	0.000	Yes
P 24 vs. Initial	0.122	2.113	0.090	No
P 24 vs. C 24	0.060	0.920	0.060	No
C 24 vs. Initial	0.010	0.991	0.360	No

Appendix A2: Pair wise multiple comparison tests for P deficient samples

Table A2.1 Holm-Sidak matrices of all pairwise multiple comparison procedure of sites (basins) and treatments (P deficient only) for Chl a fluorescence parameters

F_v/F_m				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
CB vs. WCB	11.200	5.231	0.002	Yes
CB vs. WB	12.300	4.222	0.003	Yes
WCB vs. WB	11.000	3.540	0.005	Yes
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.382	8.412	0.000	Yes
P 24 vs. C 24	0.294	6.554	0.001	Yes
N 24 vs. Initial	0.122	2.212	0.561	No
N 24 vs. C 24	0.100	1.232	0.522	No
N 24 vs. P 24	0.089	0.856	0.511	No
C 24 vs. Initial	0.056	0.745	0.452	No

ETR_{max}				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
CB vs. WCB	9.200	7.100	0.002	Yes
CB vs. WB	5.600	3.300	0.002	Yes
WCB vs. WB	10.000	4.100	0.001	Yes
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	18.000	11.139	0.000	Yes
P 24 vs. C 24	17.000	10.520	0.000	Yes
N 24 vs. Initial	15.000	9.283	0.000	Yes
N 24 vs. C 24	14.000	8.664	0.000	Yes
P 24 vs. N 24	12.362	7.854	0.621	Yes
C 24 vs. Initial	1.000	0.619	0.559	No

Table A2.1 Continued

Alpha (α)				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
CB vs. WCB	6.900	10.200	0.002	Yes
CB vs. WB	9.200	7.320	0.003	Yes
WCB vs. WB	5.300	8.010	0.003	Yes
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.386	20.020	0.000	Yes
P 24 vs. C 24	0.301	18.220	0.000	Yes
N 24 vs. P 24	0.211	14.252	0.000	Yes
N 24 vs. C 24	0.100	1.233	0.133	No
N 24 vs. Initial	0.080	0.920	0.132	No
C 24 vs. Initial	0.010	0.991	0.360	No

Appendix A3 Pairwise multiple comparisons for all data sets

Table A3.1 Holm-Sidak matrices of all pairwise multiple comparison procedure of sites and treatments for Chl a fluorescence parameters

F_v/F_m

All Pairwise Multiple Comparison Procedures:

Comparisons for factor: Sites

Comparison	Difference of Means	t	Unadjusted P	Significant?
Col. Bay vs. Nap.	0.430	3.499	0.005	Yes
CB vs. Nap.	0.410	3.500	0.002	Yes
WCB vs. Nap.	0.410	4.100	0.001	Yes
Col. Bay vs. WB	0.408	5.120	0.001	Yes
Col. Bay vs. WCE	0.405	3.900	0.010	Yes
CB vs. WB	0.390	4.560	0.001	Yes
Col. Bay vs. CB	0.070	0.560	0.584	No
WB vs. Nap.	0.060	2.360	0.281	No
WCB vs. WB	0.056	0.460	0.661	No
CB vs. WCB	0.014	0.113	0.912	No

All Pairwise Multiple Comparison Procedures:

Comparisons for factor: Treatments

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	0.560	5.121	0.000	Yes
P 24 vs. C 24	0.540	4.986	0.000	Yes
N 24 vs. Initial	0.480	4.363	0.001	Yes
N 24 vs. C 24	0.440	4.228	0.001	Yes
P 24 vs. N 24	0.080	0.759	0.463	No
C 24 vs. Initial	0.015	0.135	0.895	No

Comparisons for factor: Treatments within Col. Bay

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	0.422	5.200	0.001	Yes
P 24 vs. C 24	0.410	5.010	0.001	Yes
N 24 vs. Initial	0.302	4.750	0.001	Yes
N 24 vs. C 24	0.288	4.220	0.002	Yes

Comparisons for factor: Treatments within CB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	0.401	6.010	0.001	Yes
P 24 vs. C 24	0.388	5.770	0.002	Yes
N 24 vs. Initial	0.301	5.550	0.001	Yes
N 24 vs. C 24	0.254	4.740	0.003	Yes

Comparisons for factor: Treatments within WCB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	0.440	4.893	0.010	Yes
P 24 vs. C 24	0.401	4.810	0.001	Yes
N 24 vs. Initial	0.321	4.520	0.001	Yes
N 24 vs. C 24	0.290	4.221	0.001	Yes

Table A3.1 Continued

Sigma (σ) PS II

All Pairwise Multiple Comparison Procedures:
Comparisons for factor: Sites

Comparison	Difference of Means	t	Unadjusted P	Significant?
Col. Bay vs. Nap.	419.320	10.200	0.002	Yes
CB vs. Nap.	401.560	7.840	0.001	Yes
WCB vs. Nap.	352.610	7.500	0.001	Yes
Col. Bay vs. WB	302.500	6.510	0.010	Yes
Col. Bay vs. WCB	301.400	5.210	0.001	Yes
CB vs. WB	298.650	4.540	0.002	Yes
Col. Bay vs. CB	102.500	0.920	0.410	No
WB vs. Nap.	84.520	0.590	0.661	No
WCB vs. WB	70.200	0.320	0.784	No
CB vs. WCB	45.580	0.120	0.844	No

All Pairwise Multiple Comparison Procedures:
Comparisons for factor: Treatments

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	679.000	10.667	0.000	Yes
P 24 vs. C 24	649.000	10.196	0.000	Yes
N 24 vs. Initial	590.333	9.274	0.000	Yes
N 24 vs. C 24	560.333	8.803	0.000	Yes
P 24 vs. N 24	88.667	1.393	0.213	No
C 24 vs. Initial	30.000	0.471	0.654	No

Comparisons for factor: Treatments within Col. Bay

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	699.500	6.210	0.010	Yes
P 24 vs. C 24	601.550	6.020	0.010	Yes
N 24 vs. Initial	522.200	5.220	0.020	Yes
N 24 vs. C 24	302.600	5.190	0.001	Yes

Comparisons for factor: Treatments within CB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	622.360	5.010	0.001	Yes
P 24 vs. C 24	603.510	5.220	0.002	Yes
N 24 vs. Initial	579.550	4.030	0.010	Yes
N 24 vs. C 24	501.240	3.790	0.010	Yes

Comparisons for factor: Treatments within WCB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	599.210	6.220	0.001	Yes
P 24 vs. C 24	501.520	6.010	0.001	Yes
N 24 vs. Initial	479.250	5.220	0.002	Yes
N 24 vs. C 24	401.650	5.650	0.001	Yes

Comparisons for factor: Treatments within WB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	501.250	4.650	0.001	Yes
P 24 vs. C 24	479.850	4.100	0.001	Yes

Table A3.1 Continued

ETR_{max}

All Pairwise Multiple Comparison Procedures:

Comparisons for factor: Sites

Comparison	Difference of Means	t	Unadjusted P	Significant?
Col. Bay vs. Nap.	10.500	3.900	0.002	Yes
CB vs. Nap.	10.010	3.714	0.003	Yes
WCB vs. Nap.	9.750	3.621	0.004	Yes
Col. Bay vs. WB	9.010	3.343	0.006	Yes
Col. Bay vs. WCE	8.520	3.210	0.002	Yes
CB vs. WB	7.990	3.170	0.002	Yes
Col. Bay vs. CB	0.750	0.279	0.785	No
WB vs. Nap.	0.750	0.279	0.785	No
WCB vs. WB	0.500	0.186	0.856	No
CB vs. WCB	0.250	0.093	0.928	No

All Pairwise Multiple Comparison Procedures:

Comparisons for factor: Treatments

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	16.200	6.727	0.000	Yes
P 24 vs. C 24	14.600	6.062	0.000	Yes
N 24 vs. Initial	13.400	5.564	0.000	Yes
N 24 vs. C 24	11.800	4.900	0.000	Yes
P 24 vs. N 24	2.800	1.163	0.268	No
C 24 vs. Initial	1.600	0.664	0.519	No

Comparisons for factor: Treatments within Col. Bay

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	20.210	5.620	0.002	Yes
P 24 vs. C 24	16.220	5.610	0.002	Yes
N 24 vs. Initial	15.220	5.010	0.000	Yes
N 24 vs. C 24	10.260	4.780	0.030	Yes

Comparisons for factor: Treatments within CB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	21.200	6.780	0.003	Yes
P 24 vs. C 24	20.100	6.540	0.020	Yes
N 24 vs. Initial	14.510	6.020	0.001	Yes
N 24 vs. C 24	13.020	5.740	0.001	Yes

Comparisons for factor: Treatments within WCB

Comparison	Difference of Means	t	Unadjusted P	Significant?
P 24 vs. Initial	22.610	6.110	0.003	Yes
P 24 vs. C 24	20.010	5.480	0.001	Yes
N 24 vs. Initial	14.560	4.660	0.001	Yes
N 24 vs. C 24	13.020	4.020	0.001	Yes

Comparisons for factor: Treatments within WB

Comparison	Difference of Means	t	Unadjusted P	Significant?
N 24 vs. Initial	14.520	6.320	0.003	Yes
N 24 vs. C 24	13.010	6.140	0.001	Yes

Table A3.1 Continued

Alpha (α)				
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Sites				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
Col. Bay vs. Nap.	0.190	4.210	0.004	Yes
CB vs. Nap.	0.160	4.010	0.002	Yes
WCB vs. Nap.	0.150	3.200	0.001	Yes
Col. Bay vs. WB	0.110	2.740	0.001	Yes
Col. Bay vs. WCB	0.110	2.160	0.001	Yes
CB vs. WB	0.120	2.010	0.010	Yes
Col. Bay vs. CB	0.060	0.680	0.500	No
WB vs. Nap.	0.070	0.570	0.620	No
WCB vs. WB	0.050	0.338	0.740	No
CB vs. WCB	0.050	0.050	0.930	No
All Pairwise Multiple Comparison Procedures:				
Comparisons for factor: Treatments				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.186	7.032	0.000	Yes
P 24 vs. C 24	0.184	6.956	0.000	Yes
N 24 vs. Initial	0.136	5.142	0.000	Yes
N 24 vs. C 24	0.134	5.066	0.000	Yes
P 24 vs. N 24	0.050	1.890	0.083	No
C 24 vs. Initial	0.002	0.076	0.941	No
Comparisons for factor: Treatments within Col. Bay				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.150	6.250	0.002	Yes
P 24 vs. C 24	0.140	6.010	0.002	Yes
N 24 vs. Initial	0.133	5.220	0.001	Yes
N 24 vs. C 24	0.140	5.160	0.002	Yes
Comparisons for factor: Treatments within CB				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.160	5.260	0.001	Yes
P 24 vs. C 24	0.140	5.030	0.002	Yes
N 24 vs. Initial	0.150	4.780	0.001	Yes
N 24 vs. C 24	0.120	4.010	0.001	Yes
Comparisons for factor: Treatments within WCB				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.160	6.220	0.001	Yes
P 24 vs. C 24	0.160	6.130	0.010	Yes
N 24 vs. Initial	0.210	5.240	0.010	Yes
N 24 vs. C 24	0.110	5.030	0.001	Yes
Comparisons for factor: Treatments within WB				
Comparison	**Difference of Means**	**t**	**Unadjusted P**	**Significant?**
P 24 vs. Initial	0.150	5.690	0.002	Yes
P 24 vs. C 24	0.160	5.030	0.001	Yes
N 24 vs. Initial	0.110	4.990	0.001	Yes
N 24 vs. C 24	0.130	4.780	0.001	Yes