A Thesis

Entitled

Physiological Ecology of *Microcystis* Blooms in Turbid Waters of Western Lake Erie

By

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Submitted as partial fulfillment of the requirements for
The Master of Science degree in Biology

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An Abstract of

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Microcystis blooms are annual occurrences in western Lake Erie. Field measurements of *Microcystis* biovolumes from 2002- 2008 show that blooms are most dense in waters adjacent to the Maumee Bay. This suggests that conditions within these waters support rapid *Microcystis* growth. Field measurements and a laboratory experiment showed that the high turbidity of the bay and adjacent waters alleviated high-light stress which results in less photo-inhibition, while *Microcystis* in less turbid water had more photo-inhibition. Damage occurs to *Microcystis* during a surface bloom as a result of prolonged time in high-intensity light. Further, *Microcystis* from nearshore water had higher protein content then that of offshore which indicates greater cellular health. This is likely a function of turbidity and high soluble nutrients in turbid waters. Therefore, reduced sediment loading would presumably increase growth stress for *Microcystis* and would lessen the magnitude of blooms seen in western Lake Erie.

Dedication

This thesis is dedicated to my family for getting me interested in Lake Erie very early in my life.

Acknowledgements

I would like to thank my advisor Dr. Thomas Bridgeman for just the right amount of help and guidance with my research. Also, I would like to thank my committee- Dr. Scott Heckathorn for teaching photosynthesis physiology and use of his laboratory instruments and Dr. Ann Krause for statistical help with my data and office space in her laboratory on campus. Further I need to thank several other researchers, faculty, and students who have helped me tremendously: Dr. Sasmita Mirsha for her assistance with biochemistry techniques, Dr. Daryl Dwyer for help while I was the teacher's assistant for his biodiversity class, Dr. Cyndee Gruden and Olga Mileyeva-Biebesheimer for use of their lab's sonicator, Dr. Jonathan Franz for analyzing my samples for nutrient content via ICP-OES, Kristi Mock for training me how to use the CHN auto-analyzer, Dr. Mike McKay for giving me a phycocyanin protocol, and my team of undergraduates for field assistance: Jesse Filbrun, Jill Reighard, Steve Timmons, Catie Wukusick, and Janine Cannell. I would also like to thank the Lake Erie Center for use of their boat and the Plant Research Science Center for green-house space.

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Chapter One

Review of *Microcystis* Blooms in Western Lake Erie and Overview of Thesis

General background

History of Lake Erie Eutrophication

Lake Erie experienced eutrophication during the mid-1900s. Yearly average density of phytoplankton increased by 30 times from 1929 to 1963 (Davis 1964). During this period there was a shift in species composition that indicated oligotrophic conditions to a species composition that indicated eutrophic conditions. For example, the diatom *Asterionella* (previously *Melosira*) was dominant during spring in the first half of the century, then was replaced by *Fragilaria* and *Aulacoseira* in later years (Davis 1964). Diatoms such as *Asterionella, Synedra,* and *Aulacoseira* that were dominant during the autumn of earlier years were later replaced by *Fragilaria*, green algae, and cyanobacteria species (Davis 1964). Extensive eutrophication and poor overall water quality were observed in the western basin with the loss of the burrowing mayfly (*Hexagenia* spp.) in the mid 1950s (Britt 1955), fish kills due to anoxia (Matisoff and Ciborowski 2005), and heavy surface blooms of cyanobacteria in the 1960s and 1970s (Matisoff and Ciborowski 2005).

In response to eutrophication, the United States and Canada passed the Great Lakes Water Quality Agreement in 1972 with the goal of decreasing phosphorus loads

into the lake (DePinto et al. 1986). Following pollution control programs of the 1970s, lake phosphorus concentrations declined (DePinto et al. 1986; Matisoff and Ciborowski 2005), which resulted in a reduction of total phytoplankton biomass (Makarewicz 1993). Furthermore, cyanobacteria were a relatively small composition of the phytoplankton community (Makarewicz 1993). Since the mid 1990s, however, in spite of nutrient control programs, total phytoplankton biomass has increased (Conroy et al. 2005b) and cyanobacteria blooms have reoccurred in western Lake Erie (Brittain et al. 2000). Unlike cyanobacteria blooms prior to nutrient controls, which were dominated by *Anabaena* spp. and *Aphanizomenon* spp. (nitrogen fixers), *Microcystis aeruginosa* (non-nitrogen fixer) dominates the currents blooms of western Lake Erie (Brittain et al. 2000; Rinta-Kanto et al. 2005). The *Microcystis* bloom of 2003 was perhaps the most severe in recent years, but of a greater concern is that blooms have become yearly occurrences in western Lake Erie over the last decade (Chaffin et al. 2008). The reoccurrence of *Microcystis* blooms, presence of large algal mats of the cyanobacteria *Lyngbya wollei* (personal observation), and discovery of the non-native cyanobacteria *Cylindrospermopsis* (Conroy et al. 2007) suggest that Lake Erie may be moving towards a more eutrophic state.

Microcystis as a concern

Microcystis is able to completely exclude other phytoplankton via buoyancy regulation under light-limiting conditions (Reynolds et al. 1987; Downing et al. 2001) and allelopathic mechanisms (Sukenik et al. 2002), allowing it to reach a high density and forming surface blooms. During a bloom, the aesthetic value of lakes is dramatically reduced. These algal blooms wash ashore, resulting in foul-smelling rotting algal mats, decreasing the quality of recreational boating and beaches. *Microcystis* is capable of

producing microcystin, a hepatotoxin that has raised many human health concerns world-wide (Ouellette and Wilhelm 2003; Falconer 2007). Fish mortality can be high (Rodger et al. 1994), and livestock can be poisoned, during cyanobacteria blooms (Falconer 2007). The microcystin concentration limit of 1 μg L^{-1}, established by the World Health Organization, is often surpassed during blooms in western Lake Erie and may have an impact on drinking water for millions of people (Brittian et al. 2000; Rinta-Kanto et al. 2005; Dyble et al. 2008).

Microcystis annual cycle

Microcystis is found in many eutrophic lakes world-wide (Visser et al. 2005). During the winter and spring months, *Microcystis* cells overwinter on the surface of lake sediments (Preston et al. 1980). Summer *Microcystis* blooms may be attributed to benthic recruitment of colonies that re-inoculate the water column during the spring (Verspagen et al. 2005). *Microcystis* remains photochemically active on sediments throughout the year, but no difference in internal buoyancy was seen, suggesting that passive processes (wind suspension, bioturbation) release colonies into the water column (Verspagen et al. 2004). Increasing water clarity, which allows light to reach the sediments, may also play a role in *Microcystis* recruitment (Reynolds and Bellinger 1992). Once in the water column, and water temperature exceeds 15 °C (Reynolds 1973), *Microcystis* takes advantage of high nutrients (Steinberg and Hartmann1988; Downing et al. 2001) and water column stability (Reynolds et al. 1984, Huisman et al. 2004) to form scums on the surface of lakes. A wide range of maximum growths rates for *Microcystis* has been reported, ranging from less than 0.17 (Ibelings et al. 1994), 0.50 divisions day^{-1} (Reynolds et al. 1984), 0.67 (Baldia et al. 2007), to 1.6 divisions day^{-1} (Nalewajko and

Murphy 2001). In the fall, cooler water temperatures prevent *Microcystis* from metabolizing accumulated carbohydrate, preventing it from regaining positive buoyancy (Thomas and Walsby 1986; Visser et al. 1995). The rate that *Microcystis* colonies settle to the lake bottom is highest during and after blooms (Verspagen et al. 2005), where they overwinter until the following summer (Preston et al. 1980).

The re-occurrence of *Microcystis* blooms were first documented in western Lake Erie in 1995 (Brittain et al. 2000), and have been observed nearly every year since (Vincent et al. 2004; Chaffin et al. 2008). The timing of these blooms varies greatly among years; blooms may be first seen in early July or not until September (Chaffin et al. 2008). Chaffin et al. (2008) quantified the magnitude of the annual blooms in western Lake Erie from 2002 to 2008 and found that the *Microcystis* biomass is highly correlated with annual summer Maumee River discharge. It is suspected that the source of *Microcystis* is the lake sediments (Rinta-Kanto et al. 2009) or that tributaries are loading *Microcystis* into the lake (Conroy 2008). It has been observed that the spatial pattern of *Microcystis* blooms in western Lake Erie is very similar to the suspended sediment plume of the Maumee River (personal observation).

Environmental factors associated with a *Microcystis* bloom

Role of nutrients in *Microcystis* blooms

Nutrients, especially phosphorus, typically limit freshwater offshore productivity. Traditionally, low total nitrogen (TN) to total phosphorus (TP) ratio is a predictor of cyanobacteria (Smith 1983). However, high TP may be more sufficient in predicting cyanobacteria blooms. Phosphate added experimentally to water from all three basins of

Lake Erie resulted in an increase of all types of phytoplankton, indicting phosphorus limitation (Wilhelm et al. 2003). In nutrient-poor waters, *Microcystis* is generally out-competed for P, due to higher P uptake kinetics of green algae and diatoms (Baldia et al. 2007; Tilman et al. 1986). Cyanobacteria have a TP threshold of 10 µg L^{-1} (Steinberg and Hartmann 1988) and potential for bloom formation increases with increasing TP (Downing et al. 2001). The likelihood of a bloom plateaus at about 80% when lake TP reach 100 µg L^{-1} (Downing et al. 2001). Laboratory experiments show that *Microcystis* growth increases linearly with TP and plateaus at 220 µg L^{-1} TP (Baldia et al. 2007). Above this concentration, *Microcystis* accumulates enough P (Baldia et al. 2007) to sustain a constant rate of cell growth for five (Tsukada et al 2006) to nine days (Nalewajko and Murphy 2001).

Maumee Bay and western Lake Erie have very high phosphorus concentrations compared to other regions of the Great Lakes. The Maumee River watershed is predominantly agricultural, which results in a high amount of run-off that discharges a large TP loading into western Lake Erie (Baker and Richards 2002). TP in Maumee Bay and western Lake Erie often exceed 150 µg L^{-1}, and TP decreases with increasing distance from the mouth of the Maumee River (Moorhead et al. 2008). Annual phytoplankton biomass has increased in western Lake Erie, while total phosphorus loads have remained constant over the past 15 years (Conroy et al. 2005b). This increase in biomass could be a result of the soluble phosphorus fraction of the total load, which has been increasing in recent years (Baker et al. 2008). Or it could be from alternative phosphorus sources such as glyphosate (Ilikchyan et al. 2009), which is the active

ingredient in herbicides, or from internal phosphorus loading from benthic invertebrate bioturbation (Chaffin and Kane 2009).

Non-nitrogen-fixing algae (diatoms, green algae) and cyanobacteria (*Microcystis*, *Oscillatoria*) need inorganic nitrogen to thrive. *Anabaena* and *Aphanizomenon* are able to fix atmospheric nitrogen. Supplies of nitrogen tend to be depleted quicker than phosphorus at periods of high algal growth, which may result in nitrogen limitation (Kim et al. 2007). Thus, under nitrogen-limiting conditions, nitrogen-fixing cyanobacteria will have an advantage over *Microcystis* and other non-nitrogen fixers (Steinberg and Hartmann 1988). *Microcystis* favors NH_4 over NO_3 as an N source, and it would be out-competed for N at high NO_3 concentrations (Kim et al. 2007). Differences in NH_4 and NO_3 were observed in Steilacoom Lake, Washington, between years of *Microcystis* bloom and non-bloom years (Jacoby et al. 2000). During the bloom year, NO_3 was very low ($< 20\ \mu g\ L^{-1}$) and very high in the non-bloom year ($> 100 \mu g\ L^{-1}$) (Jacoby et al. 2000). Sufficient NH_4 when NO_3 is depleted would allow *Microcystis* to persist (Jacoby et al. 2000). Unlike P, *Microcystis* is unable to store N to maintain a constant growth rate (Baldia et al. 2007).

During the 1960s and 1970s, cyanobacteria blooms consisted of mostly *Anabaena* and *Aphanizomenon*, species able to fix atmospheric nitrogen. The GLWQA, which targeted phosphorus, was not passed until 1972. The nutrient load from the Maumee River would have had a lower TN:TP before the agreement as compared to after its passage. Rapid algal growth would have been stimulated by high TP that then would have depleted available nitrogen, favoring the nitrogen fixers. The current *Microcystis* blooms suggest that the current ratio of TN to TP loading would be greater than ratios

that lead to blooms of nitrogen-fixing species. Large blooms of *Microcystis* could give away to nitrogen-fixing species once nitrogen becomes limiting.

Role of turbidity and mixing in *Microcystis* blooms

Light may become limiting to algae in highly turbid waters. Light is attenuated exponentially with water depth and attenuation is greater in turbid or highly productive waters (Kirk 1994). The photic zone is the area of the water column that is illuminated by > 1% of surface light intensity. The depth of 1% light marks the approximate lower margin of the photic zone depth and is also commonly referred to as the compensation point between algal photosynthesis and respiration (Wetzel 2001). In clear and/or shallow waters where 1% of surface light is able to reach the lake bottom, phytoplankton and benthic algal growth is stimulated (Lowe and Pillsbury 1995). Further, light does not need to reach the lake bottom to achieve net phytoplankton photosynthesis because vertical mixing of the water column will continually mix phytoplankton in and out of the photic zone. However, light limitation may occur in highly turbid waters generated from river sediment plumes and/or resuspension of lake sediments. In general, light is considered limiting when the photic zone is less than 16% of the mixing depth (Alpine and Cloern 1988). However, the compensation point is species specific and the depth at which it occurs is called the 'critical depth' for each species (Huisman et al. 1999a,b,c). Phytoplankton that remain below their critical depth will experience losses (Huisman et al. 1999b,c; 2002). Phytoplankton are dependent on either vertical mixing or buoyancy regulation if the critical depth exceeds the lake depth (Huisman et al. 1999b,c; 2002; 2004).

Cyanobacteria are able to regulate buoyancy via colony size (Brooks et al. 2003), regulation of gas vacuole synthesis, turgor pressure applied to gas vacuoles, and the accumulation of carbohydrate (Reynolds et al 1987; Konpka et al. 1987; Kromkamp and Walsby 1990). In weakly mixed waters, *Microcystis* (and other buoyant phytoplankton) are able to escape vertical mixing once their upwards migration rate exceeds the turbulent diffusion rate (Huisman et al. 1999c, 2004). On the other hand, in strongly mixed waters, the turbulent diffusion rate exceeds *Microcystis*' upward migration rate, thus keeping *Microcystis* and other phytoplankton entrained in mixing (Huisman et al. 1999c, 2002, 2004). This usually occurs once wind speeds above the water's surface exceed 3 m s^{-1} (Webster and Hutchinson 1994; Visser et al. 2005). The rate of turbulent diffusion that is equal to the rate of *Microcystis* migration is termed 'critical turbulence' (Huisman et al. 1999a). If vertical mixing is less than the critical turbulence, *Microcystis* will float towards the surface accessing light needed for photosynthesis. If weak mixing continues, *Microcystis* will increase in biomass, developing surface scums that are capable of absorbing 90% of surface light after 1 cm (Ibelings 1996), which in turn further shades sinking species (Huisman et al. 2004). *Microcystis* surface scums can be very dynamic throughout the day. This gives *Microcystis* an ecological advantage over sinking species and is the reason why it is believed that warmer summers with less violent storms will result in more cyanobacteria blooms (Jöhnk et al. 2008; Paerl and Huisman 2008). Colonies may become negatively buoyant and descend after accumulating enough carbohydrate, while accessing nutrients at deeper depths (Kromkamp and Walsby 1990; Visser et al. 1997). Descending colonies will regain positive buoyancy at deeper depth

once accumulated carbohydrate is respired (Kromkamp and Walsby 1990; Visser et al. 1997).

Turbidity may play a role in promoting *Microcystis* blooms in western Lake Erie. The main source of turbidity into western Lake Erie is the Maumee River (Richards et al. 2008). Turbidity is usually highest in the bay (secchi depths > 30 cm) and water clarity increases with distance into the lake (Moorhead et al. 2008). The depth and turbidity gradients into the lake may favor buoyant *Microcystis* in the competition for light in waters that are still highly turbid but where depth has increased. In computer simulations Huisman et al. (1999c) found that background turbidity (*i.e.* suspended sediments) did not change the competitive outcome between buoyant and sinking phytoplankton. However, if the water column is weakly mixed, background turbidity is high, and depth is great enough so that light does not reach the lake bottom, heavily dense phytoplankton will sink out of the photic zone, and thus fail to grow (Huisman et al. 2002). These conditions would favor a buoyant phytoplankton species such as *Microcystis*. Further, suspended sediments may play a key role in fostering algal growth by reducing high light stress.

Role of *Dreissena* mussels in *Microcystis* blooms

Many recent studies have attributed cyanobacteria dominance to exotic dreissenid mussels. In Saginaw Bay (Lake Huron), total phytoplankton was directly proportional to TP and inversely proportional to dreissenid density, but cyanobacteria is directly proportional to both TP and dreissenid density (Bierman et al. 2005). Raikow et al. (2004) found a positive influence of *Dreissena* on *Microcystis* in lakes with < 25 µg L^{-1} TP, but no influence in lakes with > 25 µg L^{-1} TP. The reoccurrence of cyanobacteria

blooms in western Lake Erie was correlated with the arrival of dreissenid mussels in the 1990s. Dreissenids decreased the resilience and resistance of Lake Erie (Conroy and Culver 2005) and favor dominance of nitrogen-fixing cyanobacteria by excreting at low nitrogen to phosphorus ratios (Arnott and Vanni 1996; Conroy et al. 2005a), slowing sediment uptake of phosphorus (Bykova et al. 2006), and selective rejection of toxic species (Vanderploeg et al 2001). In western Lake Erie, however, *Microcystis* bloom intensity is highly variable among years (Chaffin et al. 2008). It is likely that dreissenid population fluctuations alone cannot explain the high variability of *Microcystis* bloom intensity among years.

Goals of this thesis

The goal of this research is to gain a better understanding of the environmental factors that promote *Microcystis* growth in western Lake Erie. Observations of *Microcystis* blooms in western Lake Erie over the past decade lead to the general hypothesis that the Maumee River has a large influence on bloom formation because the spatial pattern of the blooms correlates with the pattern of the sediment plume generated by the river. The Maumee River loads a high amount of nutrients into the lake that may fertilize *Microcystis* blooms and suspended sediments that might reduce light stress and/or reduce light to limiting levels in deeper waters of the lake. Calm light-limiting waters would favor buoyant phytoplankton. Therefore, the general hypothesis of this research is that *Microcystis* growth is greatest in the sediment plume of the nearshore waters because of the high concentration of nutrients and suspended sediments. If, as hypothesized, high nutrient and low-light conditions in river plumes are important in the

role of *Microcystis* blooms, the results of this research can help to inform efforts in preventing or lessening the intensity of blooms. Results of this study can inform those who develop recommendations to suppress sediment loading into the lake and shift timing of shipping channel dredging, therefore increasing water clarity. Because phosphorus is mostly bound to sediments, reducing sediments would also reduce the amount of nutrients entering the lake. Results of this study can also be applied by managers of other areas of the Great Lakes with frequent cyanobacteria blooms generated from favorable conditions from river discharge, *i.e*. Saginaw River of Lake Huron and Sandusky River of Lake Erie.

Overview of subsequent chapters in this thesis

Chapter 2. An Eco-physiological trade-off of *Microcystis*: Buoyancy results in photosynthetic damage.

Surface scums allow *Microcystis* to exclude sinking phytoplankton. However, these scums are exposed to high light intensities that can be damaging to photosynthesis. This chapter presents data that suggests that these surface scums are highly damaged by sunlight, but have the advantage of excluding other phytoplankton through shading.

In this chapter, chlorophyll fluorescence measurements were used to determine algal photosynthetic efficiency. Photosynthesis is an important part of algal metabolism that leads to carbon fixation and growth, and chlorophyll fluorescence is used to monitor net photosynthesis. A brief review of chlorophyll fluorescence is needed to acquaint unfamiliar readers to these measurements. Photosynthesis is greatly affected by light intensity and changes in photosynthetic efficiency will be reflected by changes in

chlorophyll fluorescence, which is easily measureable (Schreiber et al. 1994). Photons absorbed by chlorophyll have one of three fates. The majority of energy absorbed is used to drive photochemistry (photochemical quenching, PQ) or dissipated as heat (non-photochemical quenching, NPQ). Chlorophyll fluorescence is the small fraction of energy absorbed that is then re-emitted as light, and analysis of chlorophyll fluorescence can used to determine the state of photosynthesis physiology. For example, decreasing efficiency of electron transport, most notably photosystem II (PSII), results in a decrease of F_v/F_m ($(F_m-F_o)/F_m$; where F_m is the maximum fluorescence after a saturating light pulse, and F_o is basal fluorescence before the saturating light pulse; Schreiber et al. 1994). PSII is often the weak link of photosynthetic electron transport, as it is most vulnerable to light-induced damage, *i.e.* photoinhibition (Maxwell and Johnson 2000). PSII absorbs light energy, causing the donation of an electron to Q_A. PSII does not accept another photon until it passes one electron onto the subsequent electron acceptors of the electron transport chain (Maxwell and Johnson 2000). During this time, the reaction centers are 'closed,' and a greater percentage of closed reaction centers results in decreased photosynthetic efficiency and an increase in PSII fluorescence (Maxwell and Johnson 2000). Two measurements of chlorophyll fluorescence were used to determine PSII efficiency. Quantum yield of PSII electron transport (Φ_{et}) is a measure of the relative rate of electron transport of PSII, which is dependent on (1) the photosynthetic efficiency of open PSII reaction centers of light adapted samples to capture light energy and utilize it to drive photochemical reactions, and (2) the relative efficiency with which post PSII electron transfer keeps PSII open (Genty et al. 1989). Φ_{et} decreases with an increased proportion of closed PSII reaction centers (Genty et al. 1989) and Φ_{et} decreases

with increasing light intensity (Schreiber et al. 1994; Maxwell and Johnson 2000). Electron transport correlates well with carbon fixation at a given light intensity (Genty et al. 1989). Decreases in Φ_{et} indicate damage to, or protective down-regulation of electron transport (Krause 1988). Pre-adapting samples to dark conditions allows all reaction centers to open and the ratio of variable-to-maximum chlorophyll fluorescence (F_v/F_m) can be determined, which measures maximum PSII efficiency (or capacity) (Schreiber et al. 1994; Maxwell and Johnson 2000). Decreases in F_v/F_m indicate damage to PSII. In healthy higher plants and green algae, F_v/F_m typically ranges from 0.80 to 0.83 and less than 0.60 for other phytoplankton (Schreiber et al. 1994; Büchel and Wilhelm 1993), however 0.40 to 0.50 are typical values for phytoplankton collected from a lake (Marwood et al. 2000).

Chapter 3. Measurements of *Microcystis* cellular health in western Lake Erie during the 2008 bloom indicate the high turbidity and nutrients support rapid growth.

Microcystis is most abundant in waters adjacent to the Maumee Bay. These waters are high in nutrients and maybe become light-limited. Also, *Microcystis* from these waters have higher cellular health than in the offshore.

Chapter 4. Conclusion.

This summarizes the work of chapters two and three and offers recommendations to mitigate the intensity of the *Microcystis* blooms in western Lake Erie.

Chapter 5. Detailed Methods and Supplemental Data.

This chapter contains detailed descriptions of methodology that are more briefly summarized in chapters two and three. This chapter also describes trials and experiments

for determining algal pigment concentrations, including phycocyanin and chlorophyll *a*, and pigment results form western Lake Erie. Also, complete methods for the laboratory experiment and protein extractions are presented. Macro and micronutrients concentrations of *Microcystis* are given here.

Literature Cited

Alpine, A. E., and J. E. Cloern. 1988. Phytoplankton growth-rates in a light-limited environment, San Francisco bay. Marine Ecology-Progress Series **44:** 167-173.

Arnott, D.L., and Vanni, M.J. 1996. Nitrogen and phosphorus recycling by the zebra mussel (*Dreissena polymorpha*) in the western basin of Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **53**(3): 646-659.

Baker, D.B., Crumrine, J.P., Richards, R.P., and Kramer, J.W. 2008. Increasing trends in dissolved phosphorus loading to Lake Erie from Northwestern Ohio watersheds. *In* Declining water quality in the western Lake Erie basin: Increasing invasion of blue-green algae (Cyanobacteria), and increasing levels of soluble reactive phosphorus. Ohio Journal of Science. 108: A-9.

Baker, D.B., and Richards, R.P. 2002. Phosphorus budgets and riverine phosphorus export in northwestern Ohio watersheds. Journal of Environmental Quality **31**(1): 96-108.

Baldia, S.F., Evangelista, A.D., Aralar, E.V., and Santiago, A.E. 2007. Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines. Journal of Applied Ecology. **19**(6): 607-613.

Bierman, V.J., Kaur, J., DePinto, J.V., Feist, T.J., and Dilks, D.W. 2005. Modeling the role of zebra mussels in the proliferation of blue-green algae in Saginaw Bay, Lake Huron. Journal of Great Lakes Research **31**(1): 32-55.

Britt. N. W. 1955. Stratification in Western Lake Erie in Summer of 1953: Effects on the *Hexagenia* (Ephemeroptera) Population. Ecology 36: 239-244.

Brittain, S.M., Wang, J., Babcock-Jackson, L., Carmichael, W.W., Rinehart, K.L., and Culver, D.A. 2000. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. Journal of Great Lakes Research **26**(3): 241-249.

Brookes, J.D., Regel, R.H., and Ganf, G.G. 2003. Changes in the photo-chemistry of *Microcystis aeruginosa* in response to light and mixing. New Phytologist **158**(1): 151-164.

Büchel, C., and Wilhelm, C. 1993. In-vivo analysis of slow chlorophyll fluorescence induction kinetics in algae - progress, problems and perspectives. Photochemistry and Photobiology **58**: 137-148.

Bykova, O., Laursen, A., Bostan, V., Bautista, J., and McCarthy, L. 2006. Do zebra mussels (*Dreissena polymorpha*) alter lake water chemistry in a way that favours *Microcystis* growth? Science of the Total Environment **371**(1-3): 362-372.

Chaffin, J. D., Bridgeman, T. B. and Filbrun, J. F. 2008. Quantification of *Microcystis* sp. blooms in western Lake Erie (2002-2007) and relation to tributary flow. Conference presentation. International Association of Great Lakes Research. Petersburg Ontario, Canada.

Chaffin J. D. and D. D. Kane 2009. Burrowing mayfly (*Hexagenia* spp.) bioturbation and bioirrigation: A neglected source of internal phosphorus loads to lakes. Journal of Great Lakes Research. *In revision*

Conroy, J. D. 2008. Testing the algal loading hypothesis: the importance of Sandusky River phytoplankton inputs to offshore Lake Erie processes. PhD. Thesis. The Ohio State University.

Conroy, J.D., and Culver, D.A. 2005. Do dreissenid mussels affect Lake Erie ecosystem stability processes? American Midland Naturalist. **153**: 20-32.

Conroy, J.D., Edwards, W.J., Pontius, R.A., Kane, D.D., Zhang, H.Y., Shea, J.F., Richey, J.N., and Culver, D.A. 2005a. Soluble nitrogen and phosphorus excretion of exotic freshwater mussels (*Dreissena* spp.): potential impacts for nutrient remineralisation in western Lake Erie. Freshwater Biology **50**(7): 1146-1162.

Conroy, J.D., Kane, D.D., Dolan, D.M., Edwards, W.J., Charlton, M.N., and Culver, D.A. 2005b. Temporal trends in Lake Erie plankton biomass: Roles of external phosphorus loading and dreissenid mussels. Journal of Great Lakes Research **31**: 89-110.

Conroy, J.D., Quinlan, E.L., Kane, D.D., and Culver, D.A. 2007. *Cylindrospermopsis* in Lake Erie: Testing its association with other cyanobacterial genera and major linmological parameters. Journal of Great Lakes Research **33**(3): 519-535.

Depinto, J.V., Young, T.C., and McIlroy, L.M. 1986. Great-lakes water-quality improvement - The strategy of phosphorus discharge control is evaluated. Environmental Science & Technology **20**(8): 752-759.

Downing, J.A., Watson, S.B., and McCauley, E. 2001. Predicting Cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences **58**(10): 1905-1908.

Dyble, J., Fahnenstiel, G.L., Litaker, R.W., Millie, D.F., and Tester, P.A. 2008. Microcystin concentrations and genetic diversity of *Microcystis* in the lower Great Lakes. Environmental Toxicology **23**(4): 507-516.

Falconer, I.R. 2007. Cyanobacterial toxins present in *Microcystis aeruginosa* extracts - More than microcystins! Toxicon **50**(4): 585-588.

Genty, B., Briantais, J.M., and Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. Biochimica Et Biophysica Acta **990**(1): 87-92.

Huisman, J., Arrayas, M., Ebert, U., and Sommeijer, B. 2002. How do sinking Phytoplankton species manage to persist? American Naturalist **159**(3): 245-254.

Huisman, J., Jonker, R.R., Zonneveld, C., and Weissing, F.J. 1999a. Competition for light between phytoplankton species: Experimental tests of mechanistic theory. Ecology **80**(1): 211-222.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M.H., and Sommeijer, B. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology **85**(11): 2960-2970.

Huisman, J., van Oostveen, P., and Weissing, F.J. 1999b. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. Limnology and Oceanography **44**(7): 1781-1787.

Huisman, J., van Oostveen, P., and Weissing, F.J. 1999c. Species dynamics in phytoplankton blooms: Incomplete mixing and competition for light. American Naturalist **154**(1): 46-68.

Ibelings, B.W. 1996. Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms. Journal of Phycology **32**(4): 549-557.

Ilikchyan, I.N., McKay, R.M.L., Zehr, J.P., Dyhrman, S.T., and Bullerjahn, G.S. 2009. Detection and expression of the phosphonate transporter gene phnD in marine and freshwater picocyanobacteria. Environmental Microbiology **11**(5): 1314-1324.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J., and Crayton, M. 2000. Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. Canadian Journal of Fisheries and Aquatic Sciences **57**(1): 231-240.

Jöhnk, K.D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P.M., and Stroom, J.M. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biology **14**(3): 495-512.

Kim, H.S., Hwang, S.J., Shin, J.K., An, K.G., and Yoon, C.G. 2007. Effects of limiting nutrients and N : P ratios on the phytoplankton growth in a shallow hypertrophic reservoir. Hydrobiologia **581**: 255-267.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems: second edition. Cambridge University Press.

Konopka, A., Kromkamp, J., and Mur, L.R. 1987. Regulation of gas vesicle content and buoyancy in light-limited or phosphate-limited cultures of *Aphanizomenon flos-aquae* (cyanophyta). Journal of Phycology **23**(1): 70-78.

Kromkamp, J., and Walsby, A.E. 1990. A computer-model of buoyancy and vertical migration in cyanobacteria. Journal of Plankton Research **12**(1): 161-183.

Lowe, R.L., and Pillsbury, R.W. 1995. Shifts in benthic algal community structure and function following the appearance of zebra mussels (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron. Journal of Great Lakes Research **21**(4): 558-566.

Makarewicz, J.C. 1993. Phytoplankton biomass and species composition in Lake Erie, 1970 to 1987. Journal of Great Lakes Research **19**(2): 258-274.

Marwood, C.A., Smith, R.E.H., Furgal, J.A., Charlton, M.N., Solomon, K.R., and Greenberg, B.M. 2000. Photoinhibition of natural phytoplankton assemblages in Lake Erie exposed to solar ultraviolet radiation. Canadian Journal of Fisheries and Aquatic Sciences **57**(2): 371-379.

Matisoff, G., and Ciborowski, J.J.H. 2005. Lake Erie trophic status collaborative study. Journal of Great Lakes Research **31**: 1-10.

Maxwell, K., and Johnson, G.N. 2000. Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany **51**(345): 659-668.

Moorhead, D., T. B. Bridgeman, and J. Morris. 2008. Changes in water quality of Maumee Bay 1928 – 2003. *In*: Checking the pulse of Lake Erie. Editors Munawar, M and Heath, R. Goodword Books.

Nalewajko, C. and T. P. Murphy. 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan: an experimental approach. Limnology **2**:45-48.

Ouellette, A.J.A., and Wilhelm, S.W. 2003. Toxic cyanobacteria: the evolving molecular toolbox. Frontiers in Ecology and the Environment **1**(7): 359-366.

Paerl, H.W., and Huisman, J. 2008. Climate - Blooms like it hot. Science **320**(5872): 57-58.

Preston, T., Stewart, W.D.P., and Reynolds, C.S. 1980. Bloom-forming cyanobacterium *Microcystis aeruginosa* overwinters on sediment surface. Nature **288**(5789): 365-367.

Raikow, D.F., Sarnelle, O., Wilson, A.E., and Hamilton, S.K. 2004. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. Limnology and Oceanography **49**(2): 482-487.

Reynolds, C.S., and Bellinger, E.G. 1992. Patterns of abundance and dominance of the phytoplankton of Rostherne Mere, England - evidence from an 18-year data set. Aquatic Sciences **54**(1): 10-36.

Reynolds, C.S., Oliver, R.L., and Walsby, A.E. 1987. Cyanobacterial dominance - The role of buoyancy regulation in dynamic lake environments. New Zealand Journal of Marine and Freshwater Research **21**(3): 379-390.

Reynolds, C.S., Wiseman, S.W., and Clarke, M.J.O. 1984. Growth-rate and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential

application to the control of planktonic algal biomass. Journal of Applied Ecology **21**(1): 11-39.

Richards, R.P., Baker, D.B., Crumirine, J.P., Kramer, J.W., Ewing, D.E., and Merryfield, B.J. 2008. Thirty-year trends in suspended sediment in seven Lake Erie tributaries. Journal of Environmental Quality **37**(5): 1894-1908.

Rinta-Kanto, J.M., Ouellette, A.J.A., Boyer, G.L., Twiss, M.R., Bridgeman, T.B., and Wilhelm, S.W. 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environmental Science & Technology **39**(11): 4198-4205.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. Science **221**(4611): 669-671.

Steinberg, C.E.W., and Hartmann, H.M. 1988. Planktonic bloom-forming cyanobacteria and the eutrophication of lakes and rivers. Freshwater Biology **20**(2): 279-287.

Sukenik, A., Eshkol, R., Livne, A., Hadas, O., Rom, M., Tchernov, D., Vardi, A., and Kaplan, A. 2002. Inhibition of growth and photosynthesis of the dinoflagellate Peridinium gatunense by *Microcystis* sp (cyanobacteria): A novel allelopathic mechanism. Limnology and Oceanography **47**(6): 1656-1663.

Thomas, R.H., and Walsby, A.E. 1986. The effect of temperature on recovery of buoyancy by *Microcystis*. Journal of General Microbiology **132**: 1665-1672.

Tilman, D., Kiesling, R., Sterner, R., Kilham, S.S., and Johnson, F.A. 1986. Green, bluegreen and diatom algae - Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archiv Fur Hydrobiologie **106**(4): 473-485.

Tsukada, H., Tsujimura, S., and Nakahara, H. 2006. Effect of nutrient availability on the C, N, and P elemental ratios in the cyanobacterium *Microcystis aeruginosa*. Limnology **7**(3): 185-192.

Verspagen, J.M.H., Snelder, E., Visser, P.M., Huisman, J., Mur, L.R., and Ibelings, B.W. 2004. Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: Internal buoyancy changes or resuspension? Journal of Phycology **40**(2): 260-270.

Verspagen, J.M.H., Snelder, E., Visser, P.M., Johnk, K.D., Ibelings, B.W., Mur, L.R., and Huisman, J. 2005. Benthic-pelagic coupling in the population dynamics of the harmful cyanobacterium *Microcystis*. Freshwater Biology **50**(5): 854-867.

Vincent, R.K., Qin, X.M., McKay, R.M.L., Miner, J., Czajkowski, K., Savino, J., and Bridgeman, T. 2004. Phycocyanin detection from LANDSAT TM data for mapping cyanobacterial blooms in Lake Erie. Remote Sensing of Environment **89**(3): 381-392.

Visser, P. M., Ibelings, B. W., Mur, L. R., and Walsby, A. E. 2005. Ecophysiology of *Microcystis*. *In* Harmful Cyanobacteria. Editors Huisman, J., Matthijs, H. C. P., and Visser, P. M. Springer Press. Netherlands

Visser, P.M., Ibelings, B.W., and Mur, L.R. 1995. Autumnal sedimentation of *Microcystis* spp as result of an increase in carbohydrate ballast at reduced temperature. Journal of Plankton Research **17**(5): 919-933.

Visser, P.M., Passarge, J., and Mur, L.R. 1997. Modelling vertical migration of the cyanobacterium *Microcystis*. Hydrobiologia. **349**: 99-109.

Webster, I.T., and Hutchinson, P.A. 1994. Effect of wind on the distribution of phytoplankton cells in lakes revisited. Limnology and Oceanography **39**(2): 365-373.

Wetzel R. G. 2001. Limnology Lake and River Ecosystems: Third Edition. Academic Press. San Diego, California, USA.

Wilhelm, S.W., DeBruyn, J.M., Gillor, O., Twiss, M.R., Livingston, K., Bourbonniere, R.A., Pickell, L.D., Trick, C.G., Dean, A.L., and McKay, R.M.L. 2003. Effect of phosphorus amendments on present day plankton communities in pelagic Lake Erie. Aquatic Microbial Ecology **32**(3): 275-285.

Chapter Two.

An Eco-physiological trade-off of *Microcystis*: Buoyancy results in photosynthetic damage.

Abstract

Microcystis is generally predicted to have an ecological advantage over sinking phytoplankton in calm turbid waters because buoyancy regulation allows access to more light and surface scums further shade non-buoyant phytoplankton. However, exposure to full sunlight may have negative effects on *Microcystis* physiology, particularly photosynthesis. Field sampling of the 2008 bloom in western Lake Erie and laboratory experiments were used to study the photosynthetic status of *Microcystis* in relation to turbidity, vertical mixing, and nutrients. Photosynthetic efficiency (quantum yield of photosystem II electron transport) (Φ_{et}) of *Microcystis* from the lake was measured throughout the summer. Φ_{et} was greatest under vertical mixing conditions and lower in calm waters. Under both mixing and calm conditions, Φ_{et} increased with turbidity at all depths. Light-response curves revealed that strong vertical mixing combined with high turbidity offered *Microcystis* more protection from photosynthetic damage than did high turbidity alone. Because turbidity often co-varies with nutrients in Lake Erie, laboratory experiments were used to separate the effects of turbidity and nutrients (P, N). Φ_{et} was highest in the mixed turbid treatment and lowest in the calm clear treatment. A range of nutrient concentrations typical of western Lake Erie did not affect Φ_{et}. Thus, an eco-

physiological trade-off exists between being highly competitive for light and decreased photosynthetic efficiency in calm turbid waters.

Introduction

Dense *Microcystis* blooms are an annual occurrence in western Lake Erie (Chaffin et al. 2008). Western Lake Erie is generally highly turbid and nutrient-rich because of suspended sediment and nutrient loading from the Maumee River (Richards et al. 2008; Baker and Richards 2002). Sediment resuspension from the lake bottom is also a likely source of turbidity and nutrients because of the shallowness (Søndergaard et al. 2003). *Microcystis* blooms appear to originate in the sediment plume, which suggests that conditions in the turbidity support rapid growth. The effect of high nutrients on the development of *Microcystis* blooms has been studied in depth, however the effect that the sediments have on *Microcystis* photosynthesis has been neglected. This study uses an eco-physiological approach to study how *Microcystis* photosynthetic efficiency is affected by turbidity.

High turbidity may cause light limitation in phytoplankton due to the rapid attenuation of light by suspended particles (Alpine and Cloern 1988); however, very shallow bays may not be light limited despite the high turbidity (Conroy 2008). If water depth increases while high turbidity is maintained, this may bring about light limitation as algal cells spend an increasing proportion of the time at low light levels. These conditions would favor buoyant species that can remain in the photic zone, such as the highly-buoyant cyanobacterium *Microcystis*. Cyanobacteria are able to regulate their buoyancy via colony size (Brookes et al. 2003), gas vacuole synthesis, turgor pressure, and

accumulation/ depletion of carbohydrate produced by photosynthesis (Reynolds et al. 1987; Konpka et al. 1987; Kromkamp and Walsby 1990). *Microcystis* will accumulate carbohydrate while in the light near the surface, then become negatively buoyant and sink (Kromkamp and Walsby 1990; Visser et al. 1997). In deeper darker depths, *Microcystis* will respire accumulated carbohydrate becoming less dense, and float up towards the surface (Kromkamp and Walsby 1990; Visser et al. 1997). Thus, in light-limited waters, buoyancy gives *Microcystis* an advantage in the competition for light.

Water column mixing plays a large role in determining the outcome of the competition for light among buoyant and sinking phytoplankton (Huisman et al 1999b; 2004). Buoyancy only allows *Microcystis* to gain an advantage in relatively stable water columns. *Microcystis* will be able to migrate towards the surface if the turbulent mixing of the water column is less than the upward migration rate of *Microcystis* (Huisman et al. 1999a). Dense surface blooms absorb a high amount of light (Ibelings 1996) that will further decrease light intensities for sinking phytoplankton (Huisman et al. 2004). Sinking phytoplankton species depend on vertical mixing of the water column for access to light in light-limited waters (Huisman et al. 2002). Wind speeds of greater than 3 m s^{-1} are needed to break up surface blooms (Webster and Hutchinson 1994; Visser et al. 2005) and would also circulate sinking phytoplankton species up into the photic zone, thus negating the advantage of buoyancy regulating species (Huisman et al. 2002, 2004). After mixing, *Microcystis* is able to return to the surface.

Positive buoyancy of *Microcystis* near the surface, however, may result in exposure to very high light intensities that can be damaging to photosynthetic machinery. High light is a stressor to many photosynthetic organisms. Phytoplankton, in general, are

low-light adapted. *Microcystis*, on the other hand, is relatively high-light adapted compared to other phytoplankton. *Microcystis* has a high light-saturation point (I_k), and wide ranges of I_k have been reported for this species [135-323 (Ganf 1975); 253 ± 82 (Köhler 1992); 357- 487 µmol m^{-2} s^{-1} (Wu and Song 2008)]. Diatoms and green algae normally have an I_k of less than 100 µmol m^{-2} s^{-1} (Kirk 1994). Further, *Microcystis* has a relatively high amount of photo-protective carotenoids ,which are involved in non-photochemical quenching, compared to green algae and diatoms (Paerl et al. 1983; Ibelings et al. 1994). *Microcystis* also increases production of superoxide dismutase (SOD) enzymes when grown under higher-intensity light (Canini et al. 2001). Carotenoids and SOD aid in protection from photooxidation by absorbing free radicals and oxide anions (Gotz et al. 1999; Scandalios 1993), which are produced by photosynthesis in high intensity light (Hopkins 1999). Despite these adaptations, *Microcystis* photosynthesis physiology may be damaged at the surface of calm waters where it is exposed to the extremely high light intensity of full sunlight, which exceeds 2000 µmol m^{-2} s^{-1}.

Light is exponentially attenuated with depth in lakes and greater turbidity increases the rate of attenuation (Kirk 1994). Photosynthetic efficiency, measured here as the quantum yield of photosystem II (PSII) electron transport (Φ_{et}), generally increases with decreasing light intensity (Schreiber et al. 1994). Φ_{et} is proportional to carbon fixation at a given light level (Genty et al. 1989), and decreases in Φ_{et} indicate either damage to PSII or post PSII electron transport, or photo-protective down-regulation of electron transport (Krause 1988). Thus, phytoplankton cells at lower light intensities have greater efficiency than those in higher light. High turbidity, due to a high

concentration of suspended sediments that absorb and reflect light, may mitigate high-light stress in algae. Phytoplankton in Lake Erie's western basin had a greater photosynthetic efficiency than the central and eastern basins because of higher turbidity in the west (Marwood et al. 2000). River-generated sediment plumes increased Lake Michigan phytoplankton primary production (Johengen et al. 2008). Others studies have shown that mixing provides phytoplankton relief from high-light intensities by circulating phytoplankton vertically, thereby lowing the average light exposure (Brookes et al. 2003; Ibelings et al. 1994). However, the interaction between turbidity and mixing at various depths of the water column is poorly understood.

In addition to affecting the photosynthetic efficiency of phytoplankton, turbidity may also influence the production of photosynthetic pigments and proteins. Low light induces an increase of phycobilins in cyanobacteria relative to chlorophyll *a* (Raps et al 1985; Post 1986) and increases the number of photosynthetic units per cell (Raps et al. 1983). Excess light reduces total chlorophyll (Post 1986; Ibelings et al. 1994) and phycobilins (Post 1986; Bhandari and Sharma 2006) and increases protective carotenoids (Paerl et al. 1983; Bhandari and Sharma 2006). Light also affects the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco). Rubisco is the enzyme that catalyzes the carboxylation of ribulose-1,5-bisphosphate with carbon dioxide during the first step of the Calvin cycle. Rubisco is highly abundant in photosynthetic cells and its activity decreases at low light (Hopkins 1999).

Another widely used measurement of general cellular stress is level of heat shock proteins (Hsps). Hsps are ubiquitous in both prokaryotes and eukaryotes and function as chaperones to protect proteins from unfolding or from forming an improper conformation

or facilitate repair of degradation of damaged proteins (Schlesinger 1990). Certain small Hsps (15-30 kDa; sHsps) are associated with the chloroplasts of higher plants and algae (Kloppstech et al. 1985; Preczewski et al. 2000; Heckathorn et al. 2002), and also associate with phycobiliproteins of cyanobacteria (Nakamoto and Honma 2006). Production of sHsps is increased in response to stress to protect PSII and light harvesting structures (Preczewski et al. 2000; Heckathorn et al. 2002; Nakamoto and Honma 2006).

The combination of being highly competitive for light and reduced light stress in highly turbid waters may explain why *Microcystis* is able to reach high biomasses in western Lake Erie and other highly turbid lakes. I hypothesized that *Microcystis* will experience less light stress in turbid waters. Therefore, I expected that the photosynthetic efficiency of phytoplankton will increase with depth and turbidity because of the decreased light intensities, and that strong mixing of the water may provide surface phytoplankton protection from the high-light intensities by reducing the length of time exposed to the high-light intensity of the sun. A laboratory experiment was performed to isolate effects that often covary in lakes; *i.e*. turbidity and nutrient concentration. Additionally, I hypothesized that *Microcystis* collected from western Lake Erie in turbid waters will be able to adjust photosynthetic pigment and protein content, thus, chlorophyll *a* and phycocyanin content will be greater while carotenoids and rubsico will be less in turbid water than *Microcystis* in clear water. Further, I hypothesize that sHsps will be greatest in the most photo-inhibited samples.

Methods

Limnological Measurements

Collections and measurements were made aboard the Lake Erie Center boat at six sites along an approximately 80 km route in western Lake Erie and in Maumee Bay (Fig. 2-1) during the summer of 2008. At each sampling site, temperature, dissolved oxygen, and chlorophyll (chl) *a* fluorescence were recorded using a YSI #6600 (Yellow Springs Instruments) multi-probe at two-meter intervals from surface to bottom. The multi-probe was calibrated before each sampling trip. Wind speed and direction were measured (Kestrel #1000). The water column was classified as "strongly mixed" if wind speeds exceeded 3 m s^{-1} (Webster and Hutchinson 1994; Visser et al. 2005). This classification was supported by correlations of wind speed with vertical profiles of chl *a*, temperature, and dissolved oxygen that indicated that the water column was uniformly mixed above 3 m s^{-1}. Wind speeds below 3 m s^{-1} allow *Microcystis* to escape the turbulent motion of the water column (Webster and Hutchinson 1994; Huisman et al. 1999b) and were classified in my study as "weakly mixed." This was associated with stratification of chlorophyll profiles and presence of surface scums. Underwater profiles of photosynthetically active radiation (PAR, Li-Cor # LI-188B) were recorded and used to calculate the light extinction coefficient (K_{PAR}), which was used as an index of turbidity. *Microcystis* biomass was estimated by colonies retained in quantitative plankton tows (Chaffin et al. 2008). All collections and measurements were recorded between 10:00 am to 3:00 pm on full sun days.

For laboratory measurements of photosynthesis, *Microcystis* was collected from the entire water column from five sites in western Lake Erie, using a 64 μm mesh plankton net on six dates between July 24 and September 25, 2008 (Fig. 2-1). An additional site (MB20) was sampled for the *in situ* photosynthetic efficiency study, but

Microcystis at this site was not abundant enough to provide physiological measurements. *Microcystis* colonies retained in the net were concentrated and stored in dark polyethylene bottles during transportation back to the laboratory. Depending on sample location, two to six hours passed between collection on the lake and laboratory analysis. Upon arriving at the laboratory, the *Microcystis* samples were added to 1L imhoff cones and diluted to 1,000 ml with tap water. This allowed the *Microcystis* colonies to separate from the sinking diatoms and green algae via floatation (Chaffin et al. 2008). After 30 minutes, the settled phytoplankton consisting mainly of diatoms was drawn off through the bottom of the cone and discarded. The sample was then diluted again to 1,000 ml with tap water and process repeated. At this time, *Microcystis* was sucked off the surface for light-response curves and to determine the ratio of variable-to-maximum fluorescence. After light-response curves were complete, *Microcystis* colonies were drawn out of the bottom of the cone and concentrated on a 35 μm mesh, transferred to 1.5 ml tubes, and stored at -80 ° C until further analysis. Samples were also checked for the presence of other cyanobacteria by microscopy. *Anabaena* was very sparse relative to *Microcystis* colonies on July 24 and August 6, and *Aphanizomenon* was not seen in samples.

Photosynthetic Efficiency

To determine the effects of turbidity and mixing on the *in situ* photosynthetic efficiency of *Microcystis* present in the lake, the quantum yield of photosystem II (PSII) electron transport (Φ_{et}) was recorded for surface and 1 m for all sites, and 3 m and 5 m for the deeper sites 8M, 7M, GR1, and 4P, respectively. Water was collected using a von-dorn bottle, transferred to dark polyethylene bottles, and immediately filtered

through Whatman GF/C filters or Fisher Brand G4 filters (1.2 µm pore sizes) (Marwood et al. 2000). This larger pore size allowed water to pass through the filter more quickly than using GF/F filter, giving a more accurate measurement of *in situ* conditions. Approximately 20 to 50 ml of water was used per filter. Filtering and measuring of Φ_{et} took place in the boat's cabin to avoid direct sun light. The Φ_{et} of algae was determined within 60 seconds from collection using an OS1-FL Opti-Sciences modulated fluorometer. Briefly, steady-state fluorescence of these light-adapted samples (F_s') was read with a low-intensity (<0.1µmol m^{-2} s^{-1}) far-red light. Maximum fluorescence of the light-adapted sample (F_m') was recorded following a high-intensity (> 5,000 µmol m^{-2} s^{-1}) saturating white-light pulse with a duration of 0.8 seconds. Φ_{et} is calculated as follows (Genty et al. 1989):

Equation 1. $\Phi_{et} = (F_m' - F_s')/F_m'$

Water was also collected from these depths to determine phycocyanin concentration, which is used to gauge the amount of *Microcystis* present for each Φ_{et} measurement (see method below).

In order to determine if damage occurred to PSII, light-response curves and the ratio of variable to maximum fluorescence (F_v/F_m) were determined on *Microcystis* collected from the lake on the same dates as the *in situ* sampling. After *Microcystis* was separated from other phytoplankton, a small amount of colonies was filtered onto a GF/C or G4 filter. The filters were kept moist with lake water to prevent desiccation. Photosynthetic light-response were generated using a Pulse Amplitude Modulation (PAM) fluorometer measuring Φ_{et} (equation 1) at nine light intensities from 20 to 1640 µmol m^{-2} s^{-1}, at two minute intervals. F_v/F_m was determined on separate samples which

had been dark adapted for 30 minutes. F_v/F_m is calculated as follows (Schreiber et al. 1994);

Equation 2: $F_v/F_m = (F_m - F_o)/F_m$

where F_m is the maximum fluorescence of dark-adapted samples and F_o is the minimum fluorescence of dark-adapted samples. Light-response and F_v/F_m was measured using a Walz fluorometer (model PAM 101/103) and light pulse provided by a Schott flash lamp (model KL1500) as in Heckathorn et al. (2002).

Pigment Concentration

For the *in situ* photosynthetic-efficiency measurement, it was not possible to separate *Microcystis* from other phytoplankton species; therefore, in order to determine if *Microcystis* was present in samples of the *in situ* Φ_{et} study, water from each sample was brought back to the laboratory to determine the concentration of phycocyanin (PC), the accessory light-harvesting pigment of *Microcystis*. Water was stored in polyethylene bottles and stored on ice while transported back to the laboratory. Between 20 ml and 300 ml (depending on amount of sediments and plankton) were filtered through GF/F (0.45 um) filters and stored on silica gel at -20° C (within six hours of collection). The following day, silica gel was replaced with fresh gel and stored at -80° C until pigment content was determined. PC was extracted in 0.1 M phosphate buffer pH 6.8 (Furuki et al. 2003; Sampath-Wiley and Neefus. 2007). Filters were placed in plastic tubes with 5 ml of buffer and cells were broken by sonication (Bransonic #1510) for 15 minutes. Samples were then filled to 10 ml, then incubated at 4° C for 60 minutes. Samples were then centrifuged for 10 minutes at 3,800 rpm. PC fluorescence was recorded in a 10-AU

Turner Design fluorometer with P/N 103-80 filters. PC was quantified using a standard curve of C-PC standards.

Photosynthetic pigments were extracted from *Microcystis* separated in the imhoff cones to determine if *Microcystis* is able to adjust pigment content over a range of turbidity. Chlorophyll (chl) *a* and carotenoids were extracted from approximately 0.2 g of fresh weight tissue in 100% dimethylsulphoxide (DMSO). Tissue was added to 5 ml DMSO, then heated to 70° C for 45 minutes. One ml was taken from the sample and centrifuged at 21,000 *g* for 10 minutes. An absorption spectrum (400 – 700 nm at 1 nm intervals) was determined using the UV-1650 PC Shimadzu spectrophotometer and concentrations of chl *a* and total carotenoids were calculated from the spectra using equations shown in Table 2-1 (Wellburn 1994). Negative values calculated for chl *b* for all samples indicate that samples did not contain green algae. PC was extracted from approximately 0.05 g of fresh tissue by sonication as above. Pigment content was then corrected for dry weight determined by drying tissue until a constant weight at 70° C. Dry weight was constant after 24 hours.

Protein Content

To determine the effects turbidity had on rubisco and sHsps, *Microcystis* was ground to a powder in liquid nitrogen using a mortar and pestle, then transfered to a protein extraction buffer containing 0.1 M Tris buffer (pH = 8.0), SDS detergent, glycerol, bromophenol-blue, sucrose, protease inhibitors, a phenolic inhibitor, and reductants (Mishra et al. 2008). Samples were then centrifuged at 15,000 *g* for 10 minutes at 4 ° C. Supernatant containing soluble proteins was collected and total protein content was determined using the method of Peterson (1977), and quantified using a

standard curve of Bovine Serum Albumin. Samples in extraction buffer were kept at -80° C until further analysises.

To prepare protein for gel separation and quantification of rubisco and sHsp, proteins were precipitated in 10x volume acetone for 60 minutes at -20° C, centrifuged at 15,000 *g* at 4° C, then resuspended overnight at 4° C in a sample buffer containing 1% SDS, 100 mM Tris buffer (pH = 6.8), 10% glycerol, 0.1% bromophenol-blue, and 0.4% β-mercaptoethanol. Protein samples were boiled for 4 minutes to denature protein then centrifuged at 21,000 *g* for 30 seconds to remove debris. Then proteins were separated in 12.5% SDS-PAGE, using 30 µg of protein in each well. For rubisco, gels were stained using Coomassie blue R-250. After de-staining, the gel was scanned and the relative content of rubisco large subunit band (52 kDa) was quantified by color densitometry. For sHsps, proteins were transferred to nitro-cellulose membranes by electroblotting, and sHsps were detected using protein specific antibodies (Heckathorn et al. 2002). Also, *Microcystis* grown in culture was heat-shocked at 40°C for 45 minutes to induce expression of sHsp and used as a positive control. A heat-shocked tomato plant was also used as a positive control.

Laboratory Experiment

A 2x2x2x2 factorial experiment was used to test the effects of nutrient concentration (low and high nutrients), turbidity (low and high), mixing (mixed or non-mixing), and sample depth (surface and at depth) on photosynthetic efficiency and pigments. All eight treatments combination (nutrient x turbidity x mixing) were randomized between trials. Experiment was replicated by three independent trials, with each treatment combination in each trial. Experimental tanks were constructed out of

61x76x90 cm (228 L) polyethylene (PE) bins. Bins were divided into six 61x9x90 cm (36.5 L) chambers using black sheets of Acrylonitrile-Butadiene-Styrene (ABS) board with PE foam inserted along the edges of the ABS board to fill the space created by the expanding walls upon adding water (Fig. 2-2). Experiments were conducted in a greenhouse and exposed to natural sunlight (up to 1,500 µmol m^{-2} s^{-1}) at ambient temperature (25 – 28 °C).

Mixing of the chamber was achieved using powerhead pumps (Aquagardens #601), so that the intake hose was placed at the bottom of the chamber and outflow just beneath the surface (Fig. 2-2). Sieved Lake Erie sediments (400 µm) were added to create turbidity. High turbidity was achieved by adding 0.55 ml L^{-1} sediments (~30 NTU) and 0.02 ml L^{-1} for low turbidity (~1 NTU). High nutrient level was 3.0 mg L^{-1} nitrogen (N) and 0.15 mg L^{-1} P, and low level was 0.6 mg L^{-1} N and 0.03 mg L^{-1} P. The N:P was 45 in both levels and all other nutrient concentration were the same in each level. All other nutrients were half concentration of the WC media (Guillard and Lorenzen 1972). Twiss et al. (2005) enriched Lake Erie water with micro-nutrients to a final concentration similar to WC growth media and observed no additional phytoplankton growth. Equal amounts of *Chlamydomonas* and *Microcystis* were used in this experiment, based on content of chl *a*. *Chlamydomonas* and *Microcystis* were added so that they each had a initial chl *a* of 2.5 µg L^{-1}, making the total initial concentration 5.0 µg L^{-1}. *Chlamydomonas* and *Microcystis* that were intended for the experiment were grown in separate liquid cultures with the nutrient concentration of the low treatment level for two weeks before used in the experiment to insure that internal phosphorus storage did not take place.

Once treatments were set up and phytoplankton added, 96 hours were allowed for growth. This duration was decided upon following an initial trial ran for 168 hours using twice as much initial algae, which resulted in light levels that were too similar between the high and low turbidity treatments. Following the 96 hours, samples were collected at the surface and at a depth of 70 cm. At 70 cm, light levels in the low-turbidity treatment were 20% of surface light. In the high-turbidity treatment, light levels at 70 cm were < 0.5% of the surface irradiance. At the end of the incubation period, phytoplankton (100 ml) was filtered onto GF/F filters and Φ_{et} was measured within 60 seconds after collection. Separate samples were dark-adapted for 30 minutes and F_v/F_m was determined. Φ_{et} and F_v/F_m were determined in triplicate (three samples from each treatment of each trial) using an OS1-FL Opti-Sciences modulated fluorometer. Additional samples of algae for the determination of chl *a* and total carotenoids concentration were filtered and stored on silica gel at -80 ° C. Pigments were extracted in DMSO and calculated with published equations (Table 2-1). Chambers 1 and 6 were not analyzed because the white outside wall resulted in higher light intensity than chambers 2 through 5, which had black walls. Light levels in the tanks were recorded after all samples were collected. Photosynthetic measurements and light levels were recorded between 12:00 pm and 2:00 pm on sunny days.

Data Analysis

In situ Φ_{et} data were analyzed for samples that had PC greater than 0.5 µg L^{-1} (n = 78). A two-factor ANCOVA [depth (0 m, 1 m, pooled 3 m and 5 m) and mixing (strongly mixed or weakly mixed)] was performed using PROC REG of the statistical software SAS (v. 9.1) using K_{PAR} as a covariate and dummy variables were used for the

depth and mixing effects. Test for parallel slopes showed that these slopes where parallel to each other ($F_{(2,68)} = 3.14$, $F^* = 0.315$; $p = 0.7309$).

Repeated measure ANOVA was done for light-response curves with light intensity as the repeated variable and with site and mixing as fixed effects. Compound symmetry was assumed because unstructured and spatial power failed to meet requirements. To test for the effect that turbidity had on Φ_{et} within light intensities that approximate *Microcystis*' light saturation (I_k) (261 µmol $m^{-2} s^{-1}$), 2x I_k (522 µmol $m^{-2} s^{-1}$) and 4x I_k (about half full sunlight; 1044 µmol $m^{-2} s^{-1}$), ANCOVAs were used with K_{PAR} as covariate and dummy variables were used for the mixing effects. Tests for parallel slopes were then performed for all light intensities. Because many of the slopes between mixing and calm dates were not parallel, regressions were performed on each treatment separately. Linear regressions were used for weakly mixed dates, while non-linear regressions were used for strongly mixed dates and the maximum Φ_{et} ($\Phi_{et\ max}$) value and half-saturation coefficient (K_m) for $\Phi_{et\ max}$ at each light intensity were calculated in Sigma Plot. PROC MIXED of SAS was used for repeated measures, while PROC REG was used for ANCOVAs and regressions.

ANCOVA tests were done to test for the effects of turbidity and site on the pigment and protein content of *Microcystis*. K_{PAR} was used as the covariate. Because ANCOVA assumes all treatments (the five sites) have a similar distribution on the covariate, and scatter plots of data suggest that this may not be the case, a single factor ANOVA (Quinn and Keough 2002) test was done on the centered K_{PAR} values by site. This showed that there was no significant difference among sites ($p = 0.30$) and, thus appropriate for the ANCOVA. Dummy variables were used for the five sites.

Differences among sites were tested for with a reduced model that excluded dummy variables. This showed that site had no significant effect on pigment content (see results); therefore regressions by K_{PAR} were done to see the effect of turbidity on pigment content. PROC REG of SAS was used for ANCOVAs and regressions, while PROC ANOVA was used for ANOVA.

Four-way ANOVA was performed to test for the effect of mixing (mixed or calm), turbidity (high or low), nutrients (high or low), and sample depth (surface and at depth) on Φ_{et}, F_v/F_m, and pigment composition. Tukey test was performed for multiple comparisons. PROC GLM of SAS was used.

Results

Photosynthetic Efficiency

In situ photosynthetic efficiency was greatest in higher turbidity and at depth (Fig. 2-3). Φ_{et} increased with turbidity ($F_{(1,71)} = 3.98$; F*= 156.6; $p < 0.0001$) and with depth ($F_{(2,71)} = 3.13$; F* = 15.87; $p < 0.0001$) (Fig. 2-3). Mixing did not affect Φ_{et} ($F_{(1,71)} = 3.98$; F* = 0.0828; $p = 0.7754$). For surface samples, mixing increased Φ_{et} at higher K_{PAR} values, in contrast to 3 and 5 m where mixing decreased Φ_{et} at higher K_{PAR} (Fig. 2-3). The regression lines for mixing and calm at one meter were identical and values were intermediate between those from the surface and 3 or 5 m depth (Fig. 2-3). There was no interaction between factors ($F_{(2,71)} = 3.13$; F* = 0.750; $p = 0.476$).

Light-response curves of *Microcystis* showed the typical response of any photosynthetic organism of PSII Φ_{et} with increasing light intensities. The light-response curves also show that *Microcystis* becomes damaged on calm dates. Across all light

intensities, Φ_{et} was greater on vertically strongly mixed days than weakly mixed days (p = 0.0335; Fig. 2-4a). F_v/F_m was also greater on strongly mixed days (t-test, p = 0.0021; Fig. 2-4b). For every light-response curve, F'_s increased with increasing light intensity. F'_m was more or less stable over all light intensities for *Microcystis* collected from weakly mixed water, while F'_m decreased with light intensity for strongly mixed dates, indicating greater non-photochemical quenching. Collection location did not affect the light-response curve (p = 0.9743). To analyze the effect turbidity had on photosynthetic efficiency at I_k, 2x I_k, and 4x I_k, and F_v/F_m, ANCOVA's were performed within each light intensity using K_{PAR} as the covariate. For these light intensities, the ANCOVA slopes for weakly and strongly mixed conditions were not parallel (Table 2-2). Because of non-parallel slopes, separate regressions were performed to analyze the effect of turbidity on Φ_{et}. Under weakly mixed conditions, Φ_{et} was unaffected by turbidity (a non-significant slope) (Table 2-2; Fig. 2-5). For strongly mixed conditions, non-linear regressions were used because of the hyperbolic function, and the maximum Φ_{et} ($\Phi_{et\ max}$) value and half-saturation coefficient (K_m) for $\Phi_{et\ max}$ at each light intensity were calculated (Table 2-2; Figs. 2-5,6). At low light levels, K_m was very low and increased at greater light intensities (Fig. 2-6). For example, at I_k (261 µmol m^{-2} s^{-1}), K_m was 0.91, and at 4x I_k (1044 µmol m^{-2} s^{-1}), K_m was equal to 4.66. Slopes for F_v/F_m were parallel between strong and weak mixing, and non-significant when regressed against K_{PAR} (Table 2-2).

Pigments

Chlorophyll (chl) *a* and phycocyanin (PC), the light-harvesting pigments in *Microcystis*, had higher content in turbid water as compared to less turbid water. Reduced ANCOVA models excluding site effect variables were not significantly

different from full models that included the site effects (Table 2-3); therefore, site alone does not have an effect on pigment content. Chl *a* content of *Microcystis* increased over the sampling season from 4.84 ± 0.20 mg g^{-1} (dry weight) (mean ± SE) on August 6 to 6.71 ± 0.44 on September 1. Further, the difference between the minimum (4.35 mg g^{-1}) and maximum (7.90 mg g^{-1}) values nearly doubled over the sampling period. When pigment content was regressed *vs*. K_{PAR}, Chl *a* content was found to increase linearly with turbidity (Table 2-3; Fig. 2-7a). Total carotenoids content also increased with turbidity; however, more importantly, the total carotenoids/ chl *a* ratio decreased with increasing K_{PAR} (Table 2-3; Fig. 2-7b). PC content of *Microcystis* also increased with turbidity (Table 2-3; Fig. 2-7c), but to a much greater extent than chl *a*. PC was 20.24 ± 2.52 mg g^{-1} August 6 and PC more than doubled to 48.35 ± 6.22 mg g^{-1} on August 21. Further, there was nearly a six-fold increase between the minimum (10.83 mg g^{-1}) and maximum (62. 33 mg g^{-1}) values. The PC/ chl *a* ratio increased significantly with increasing K_{PAR} (Table 2-3; Fig. 2-7d).

Protein Content

Total proteins separated by SDS-PAGE are shown in figure 2-8. Relative density of rubisco large subunit (52 kDa) was not affected by turbidity, but rather it increased with increasing average PAR (Table 2-3; Fig. 2-9). Small Hsp were only detected from samples collected on August 21 and were found at all sites in same quantity.

Laboratory Experiment

The laboratory photosynthetic efficiency experiment gave similar results as the *in situ* study. Φ_{et} ranged from 0.334 to 0.568 (Fig. 2-10a), and was significantly affected by the depth*mixing interaction ($F^* = 38.80$; $p < 0.001$) and turbidity ($F^* = 26.49$; $p <$

0.001) (Table 2-4). Nutrients did not have a significant effect on Φ_{et} ($F^* = 0.2238$; $p = 0.2238$). Turbidity increased Φ_{et} for each treatment combination of mixing and depth. Φ_{et} was statistically (Tukey test, $p < 0.05$) greater at depth than surface for the calm treatment among both turbidity levels. Within the mixed treatment, Φ_{et} was greater at depth than at the surface, but this difference was not statistically significant (Tukey test, $p > 0.05$).

The ratio of variable to maximum fluorescence (F_v/F_m) ranged from 0.567 to 0.604, and was only significantly affected by turbidity ($F^* = 15.65$; $p = 0.0004$). F_v/F_m was greatest in the high turbidity treatment (Fig. 2-10b). F_v/F_m was greater at 70 cm, but this was not significant ($F^* = 3.21$; $p = 0.08$). Neither mixing, nutrients, nor any interactions significantly affected F_v/F_m (Table 2-4).

Only total carotenoids/ chl *a* was significantly affected ($p = 0.0007$) by turbidity and was greater in the low turbidity treatment (Fig. 2-11). Total carotenoids/ chl *a* was not affected by any other factors or interactions (Table 2-4).

Discussion

Most research on *Microcystis* bloom-development is focused on nutrient effects. This study of photosynthetic efficiency and pigment content of *Microcystis* provides insights on how turbidity and vertical mixing can decrease high light stress. The *in situ* Φ_{et} lake study (Fig. 2-3) and laboratory experiments (Fig. 2-10a) showed that Φ_{et} increased with both increasing turbidity and depth. Mixing added an interesting effect to Φ_{et}. The lack of mixing allowed phytoplankton to remain at a constant light level, where it either became photo-inhibited if at the surface exposed to full sunlight, or became adapted to low light if it were sitting at a deeper depth in turbid water. The average light

exposure would have been very high for surface *Microcystis*, while average light was very low at depth. Mixing would transport surface algae downward providing relief from high-intensities light, while upward-mixing exposes algae that were adapted to low light levels at depth to high light intensities near the surface. The difference between the photosynthetic efficiency of phytoplankton collected at the surface and at depth under mixing conditions indicates that phytoplankton were able to recover/photo-inhibit between upward/downward transportation. In lakes, turbidity and nutrient concentration co-vary. Nutrient concentration did not affect Φ_{et} in the laboratory experiment. Very low nutrient concentration would most certainly decrease Φ_{et} (Stehfest et al. 2005), but nutrients in the low treatment were still high enough to prevent decreases in Φ_{et}. This indicates that the photo-inhibition observed in the lake in clear water were not due to low nutrients, but from lack of protective suspended sediments.

Microcystis becomes damaged on weakly mixed dates as evidence of lower values of Φ_{et} across the light-response curve and lower F_v/F_m (Fig. 2-4). Further, the stable F'_m of the light-response curve of the weakly mixed samples indicates less photo-protection of PSII via non-photochemical quenching (Schofield et al. 1998). *Microcystis* on weakly mixed dates floats near the surface where it is exposed to high light intensities for extended lengths of time that results in damage to the photosynthetic machinery and induction of photo-protective mechanisms. Damage to PSII was not repaired during transportation back to laboratory, which was 4 to 6 hours from collection time to time of analysis. Marwood et al. (2000) exposed Lake Erie phytoplankton to similar light intensities as surface light for 30 minutes and saw complete recovery of F_v/F_m following a two-hour recovery period. *Microcystis* collected from western Lake Erie in weakly

mixed water would have been exposed to full sunlight for several hours before I sampled. Köhler (1992) suggested that it takes *Microcystis* two days to adjust to high light intensities. On the other hand, strong mixing circulates *Microcystis* through out the water column which decreases exposure to high light intensities, preventing damage to the photosynthetic machinery, but *Microcystis* growth stops in mixed waters (Reynolds et al. 1984; Köhler 1992) and the competitive edge is shifted towards sinking phytoplankton (Huisman et al. 2004).

Microcystis itself can bring about photo-inhibitory conditions that result in damage, because surface blooms can increase the lake surface temperatures by 5 °C through non-photochemical quenching (Ibelings et al. 2003). High light intensities and high temperatures act synergistically together to damage cyanobacteria photosynthetic machinery (Ibelings 1996). However, the addition of 5 °C would still have been in the tolerable temperature range for *Microcystis* (lake surface temperature < 25 °C, Fig. 2-12) (Ibelings 1996); likewise, sHsps were not detected in photo-inhibited *Microcystis*. Therefore, damage seen in this study must have been a result of high light intensities rather than heat or a combination of the two.

Turbidity offered no protection for *Microcystis* on weakly mixed dates. F_v/F_m and Φ_{et} at I_k, 2x I_k and 4x I_k were not affected by water column turbidity in which the *Microcystis* was collected when the water column was weakly mixed. *Microcystis* floats on top of the water on extremely calm dates, therefore on top of any suspended sediment, which would offer protection. During strong mixing, however, *Microcystis'* photosynthetic efficiency benefited from increased turbidity because photosynthetic efficiency at high light intensities was greater with higher turbidity (Fig. 2-6). This

indicates that turbidity has a larger role in photo-protection at greater light intensities, such as those seen near the surface. However, turbidity increased photosynthetic efficiency in the *in situ* study, even at the surface of weakly mixed days (Fig. 2-3). This may suggest that there are additional intra-cellular protective mechanisms present *in situ* that are not showing up in the laboratory light-response curves.

Damage was seen in *Microcystis* collected from the lake; however, very little damage was seen in the laboratory experiment (Fig. 2-10b). In the laboratory experiment, the slight decreases F_v/F_m indicate that the large decreases in Φ_{et} were due to photo-protective down-regulation of electron transport through non-photochemical quenching rather than damage to PSII. While the large decreases in F_v/F_m of *Microcystis* collected from the lake indicate that the decreases in Φ_{et} were due to damage to PSII. This could due to differences in light levels. In the lake, *Microcystis* would be exposed to full sunlight that exceeded 2,000 μmol m^{-2} s^{-1} on the lake, while the maximum light intensity of the laboratory experiment was around 1,500 μmol m^{-2} s^{-1}. This suggests that *Microcystis* becomes light damaged at intensities between 1,500 and 2,000 μmol m^{-2} s^{-1}.

Carotenoids have a several function acting as light-harvesting pigments and also are photo-protective molecules that absorb free radicals and are involved in non-photochemical quenching (Gotz et al. 1999; Bhandari and Sharma 2006). The total carotenoids/ chl *a* ratio was highest in less turbid water for both *Microcystis* collected from the lake and in the laboratory experiment (Figs. 2-7b; 2-11). Paerl et al. (1983) observed a steady increase of total carotenoids/ chl *a* of *Microcystis* over a summer. I found that *Microcystis*' carotenoids/ chl *a* decreased through out the summer of 2008. This is because *Microcystis* first appeared in clear water, which resulted in the need for

more of the photo-protective pigments. Then, as the lake became more turbid, the carotenoids/ chl *a* ratio dropped. The increase in total carotenoids during my sampling is most likely a function of increasing turbidity to aid in light harvest that suggests they are having more of a role in light harvest than photo-protection in turbid water. If carotenoids role was solely photo-protective, then it would be expected that the high total carotenoids/ chl *a* seen on August 6 and 12 in clear water would have been maintained as the lake became turbid.

Buoyancy gives *Microcystis* an advantage in weakly mixed waters for competition of light (Huisman et al. 2004; Jöhnk et al. 2008). However, my research indicates that this ecological advantage comes at a physiological price, because *Microcystis* collected on weakly mixed dates had more damage to photosynthetic machinery than did *Microcystis* collected on strongly mixed dates. *In situ* photosynthetic efficiency and carotenoids/ chl *a* data suggests that turbidity did alleviate this stress but could not compensate for damage due to very high light intensities. The ideal situation for *Microcystis* would be to regulate buoyancy to maintain an ideal position in the water column through out the day, preventing photo-inhibition and accessing nutrients while maintaining a net photosynthesis and a surface scum (Kromkamp and Walsby 1990; Visser et al. 1997). This may not be the case in western Lake Erie, because of the very large size of *Microcystis* colonies, which often exceed 2 cm in length (personal observation). Large *Microcystis* colonies are more prone to photo-inhibition because of increased buoyancy (Brookes et al. 2003). Further, *Microcystis* is unable to accumulate carbohydrate needed to become negatively buoyant at very high light intensities (Visser et al. 1997). The highest light intensity of the light-response curve I studied was 1680

μmol m^{-2} s^{-1} (84% of surface light), and samples collected on weakly mixed dates had an efficiency of 0.0014, while samples from mixed dates had an efficiency of 0.014 at the highest light intensity (Fig 2-4a). This indicates that *Microcystis* at the surface on calm dates has very low efficiency in the high intensity as compared to strongly mixed days. Large colonies of western Lake Erie *Microcystis* would become damaged when at the surface, resulting in the loss of the ability to produce carbohydrate needed to become negatively buoyant, thus the colonies become trapped on the surface. For that reason, there is an eco-physiological trade-off between being highly competitive for light under low-light in weakly mixed conditions and being highly prone to photosynthetic damage.

Damage that occurs at the surface would explain why *Microcystis* appears to increase in biomass at the surface throughout a calm day. *Microcystis* will float to the surface where it becomes damaged in the high intensity sunlight and unable to become negatively buoyant. Colonies will continually float upwards throughout the day, becoming damaged. Therefore *Microcystis* accumulates at the surface throughout the day. I have observed that *Microcystis* biomass continues to increase over time despite very low Φ_{et}. This suggests that *Microcystis* must be recovering during the night, or that *Microcystis* on the underside of the surface scum is protected and remains healthy.

Microcystis had the highest photosynthetic efficiency in waters that were turbid and strongly mixed. The bloom of 2008 first appeared in relatively clear water (Fig. 2-12). During the clear water phase, low biovolumes of *Microcystis* were recorded. *Microcystis* photosynthetic efficiency was low and total carotenoids/ chl *a* was high during this time. This would indicate stressful conditions and explain (in part) low biomass. Large biovolumes of *Microcystis* were seen after August 21 once the lake

became highly turbid. Photosynthetic efficiency was highest and carotenoids decreased during this time. The turbid waters resulted in increased Φ_{et}, which is proportional to carbon fixation (Genty et al. 1989). Highly turbid water provides a more favorable growth condition for *Microcystis* than clearer water. Thus, if nutrient loading is held constant but suspended sediment loading is reduced, *Microcystis* biomass would be reduced because of higher light stress and no competitive advantage.

Chl *a* content of *Microcystis* nearly doubled with increasing turbidity (Fig. 2-7a). Most photosynthetic organisms increase the relative amount of chl when grown under low light conditions to catch more photons (Kirk 1994; Hopkins 1999). Cyanobacteria increase chl *a* content by producing more light-harvesting complex with constant chl *a* per unit, rather than increasing chl *a* per complex (Raps et al. 1983). Phycocyanin (PC) is the major light-harvesting pigment in the antennae of cyanobacteria (Adir 2005) and its content increased nearly six-fold as the lake transitioned from very clear to highly turbid (Fig. 2-7c). Rubisco was unaffected by K_{PAR}, but did significantly increase with average PAR (Table 2-3). This would be expected because Rubisco activity decreases with less light (Hopkins 1999) and the calculation of average PAR factors in lake depth. Average PAR can be quite high in turbid, shallow water. Although significant, these regressions of pigment *vs*. K_{PAR} were not very tight (Table 2-3). This may be due to the high to low turbidity gradient from the mouth of the Maumee River into the lake. When transitioned from low to high light, chl *a* and PC content of cyanobacteria reached a steady state after 250 hours (Post 1986), while pigments reached a steady state after only 50-60 hours when transitioned from high to low light (Post 1986). Small Hsp were only detected from samples collected on Aug. 21. Small Hsp have been shown to protect PSII and

phycobilins during stressful conditions (Heckathorn et al. 2002; Nakamoto and Honma 2006). It is likely that sHsp where associated with PC rather than PSII, because Φ_{et} values were high (non photo-inhibited) and PC content was greatest on this day.

These field findings of dramatic shifts in pigment content of *Microcystis* would have implications for remote sensing of blooms. Researchers rely on satellites to detect concentrations of chl *a* or PC to map the spatial coverage of these blooms (Vincent et al. 2004; Simis et al. 2007; Wynne et al. 2008). Further, concentrations of these pigments in lake water are used to gauge the intensity of these blooms. Flawed conclusions might be made when comparing PC or chl *a* concentration of clear water to highly turbid water. For example, if a researcher observes a lake wide PC of 5 μg L^{-1}, this may lead to the conclusion that *Microcystis* biomass is constant through out the lake. However, if there is a strong turbidity gradient from bay to offshore (which is usually the case in western Lake Erie), the *Microcystis* biomass of the bay would most likely be much less than that of the offshore. The low biomass in the turbid bay would need to produce more pigments in order to capture light. The scenario would be similar when comparing pigments between data collected on two different dates that had different turbidity. Chl *a* would give remote sensing researchers a more accurate estimate of algal biomass rather then PC, because chl *a* content of *Microcystis* only double over the range of turbidity while PC content increased nearly six-fold.

In conclusion, surface *Microcystis* had the lowest photosynthetic efficiencies in calm water as a result of damage due to long-term exposure to full sunlight. Increased turbidity did not mitigate damage but did increase *in situ* photosynthetic efficiency. Less damage was observed when the water column was vertically mixed. However,

Microcystis requires stable water to allow it to competitively exclude other phytoplankton through the formation of surface scums that shade diatoms and green algae as they settle out of the photic zone. Thus, there is an eco-physiological trade-off in calm waters between being highly competitive for light but also being more susceptible to damage to photosynthetic machinery.

Literature Cited

Adir, N. 2005. Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant. Photosynthesis Research. **85**(1): 15-32.

Alpine, A. E., and J. E. Cloern. 1988. Phytoplankton growth-rates in a light-limited environment, San Francisco bay. Marine Ecology-Progress Series **44:** 167-173.

Baker, D.B., and Richards, R.P. 2002. Phosphorus budgets and riverine phosphorus export in northwestern Ohio watersheds. Journal of Environmental Quality **31**(1): 96-108.

Bhandari, R., and Sharma, P.K. 2006. High-light-induced changes on photosynthesis, pigments, sugars, lipids and antioxidant enzymes in freshwater (*Nostoc spongiaeforme*) and marine (*Phormidium corium*) cyanobacteria. Photochemistry and Photobiology **82**(3): 702-710.

Brookes, J.D., Regel, R.H., and Ganf, G.G. 2003. Changes in the photo-chemistry of *Microcystis aeruginosa* in response to light and mixing. New Phytologist **158**(1): 151-164.

Canini, A., Leonardi, D., and Caiola, M.G. 2001. Superoxide dismutase activity in the cyanobacterium *Microcystis aeruginosa* after surface bloom formation. New Phytologist **152**(1): 107-116.

Chaffin, J. D., Bridgeman, T. B. and Filbrun, J. F. 2008. Quantification of *Microcystis* sp. blooms in western Lake Erie (2002-2007) and relation to tributary flow. Conference presentation. International Association of Great Lakes Research. Petersburg Ontario, Canada.

Conroy, J. D. 2008. Testing the algal loading hypothesis: the importance of Sandusky River phytoplankton inputs to offshore Lake Erie processes. PhD. Thesis. The Ohio State University.

Furuki, T., Maeda, S., Imajo, S., Hiroi, T., Amaya, T., Hirokawa, T., Ito, K., and Nozawa, H. 2003. Rapid and selective extraction of phycocyanin from *Spirulina platensis* with ultrasonic cell disruption. Journal of Applied Phycology **15**(4): 319-324.

Ganf. G. G. 1975. Photosynthetic production and irriadiance-photosynthesis relationships of the phytoplankton from a shallow equatorial lake (Lake George, Uganda). Oecologia. **18**:165-183.

Genty, B., Briantais, J.M., and Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. Biochimica Et Biophysica Acta **990**(1): 87-92.

Gotz, T., Windhovel, U., Boger, P., and Sandmann, G. 1999. Protection of photosynthesis against ultraviolet-B radiation by carotenoids in transformants of the cyanobacterium *Synechococcus* PCC7942. Plant Physiology **120**(2): 599-604.

Guillard, R. R. L. and Lorenzen, C. J. 1972. Yellow-green algae with chlorophyllide *c*. Journal of Phycology. **8**:10-14

Heckathorn, S.A., Ryan, S.L., Baylis, J.A., Wang, D.F., Hamilton, E.W., Cundiff, L., and Luthe, D.S. 2002. In vivo evidence from an *Agrostis stolonifera* selection genotype that chloroplast small heat-shock proteins can protect photosystem II during heat stress. Functional Plant Biology **29**(8): 933-944.

Hopkins, G. 1999. Introduction to Plant Physiology: 2nd Edition. Wiley Publishers. New York, New York.

Huisman, J., Arrayas, M., Ebert, U., and Sommeijer, B. 2002. How do sinking Phytoplankton species manage to persist? American Naturalist **159**(3): 245-254.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M.H., and Sommeijer, B. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology **85**(11): 2960-2970.

Huisman, J., van Oostveen, P., and Weissing, F.J. 1999a. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. Limnology and Oceanography **44**(7): 1781-1787.

Huisman, J., van Oostveen, P., and Weissing, F.J. 1999b. Species dynamics in phytoplankton blooms: Incomplete mixing and competition for light. American Naturalist **154**(1): 46-68.

Ibelings, B.W., Vonk, M., Los, H.F.J., van der Molen, D.T., and Mooij, W.M. 2003. Fuzzy modeling of cyanobacterial surface waterblooms: Validation with NOAA-AVHRR satellite images. Ecological Applications **13**(5): 1456-1472.

Ibelings, B.W. 1996. Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms. Journal of Phycology **32**(4): 549-557.

Ibelings, B.W., Kroon, B.M.A., and Mur, L.R. 1994. Acclimation of photosystem II in a cyanobacterium and a eukaryotic green-alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes. New Phytologist **128**(3): 407-424.

Johengen, T.H., Biddanda, B.A., and Cotner, J.B. 2008. Stimulation of Lake Michigan plankton metabolism by sediment resuspension and river runoff. Journal of Great Lakes Research **34**(2): 213-227.

Jöhnk, K.D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P.M., and Stroom, J.M. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biology **14**(3): 495-512.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems: second edition. Cambridge University Press.

Kloppstech, K., Meyer, G., Schuster, G., and Ohad, I. 1985. Synthesis, transport and localization of a nuclear coded 22-kd heat-shock protein in the chloroplast membranes of peas and *Chlamydomonas-reinhardi*. Embo Journal **4**(8): 1901-1909.

Köhler, J. 1992. Influence of turbulent mixing on growth and primary production of *Microcystis aeruginosa* in the hypertrophic Bautzen Reservoir. Archiv Fur Hydrobiologie **123**(4): 413-429

Konopka, A., Kromkamp, J., and Mur, L.R. 1987. Regulation of gas vesicle content and buoyancy in light-limited or phosphate-limited cultures of *Aphanizomenon flos-aquae* (cyanophyta). Journal of Phycology **23**(1): 70-78.

Krause, G.H. 1988. Photoinhibition of photosynthesis - an evaluation of damaging and protective mechanisms. Physiologia Plantarum **74**(3): 566-574.

Kromkamp, J., and Walsby, A.E. 1990. A computer-model of buoyancy and vertical migration in cyanobacteria. Journal of Plankton Research **12**(1): 161-183.

Marwood, C.A., Smith, R.E.H., Furgal, J.A., Charlton, M.N., Solomon, K.R., and Greenberg, B.M. 2000. Photoinhibition of natural phytoplankton assemblages in Lake Erie exposed to solar ultraviolet radiation. Canadian Journal of Fisheries and Aquatic Sciences **57**(2): 371-379.

Mishra, S., Heckathorn, S.A., Barua, D., Wang, D., Joshi, P., Hamilton, E.W., and Frantz, J. 2008. Interactive Effects of Elevated CO2 and Ozone on Leaf Thermotolerance in Field-Grown *Glycine max*. Journal of Integrative Plant Biology **50**(11): 1396-1405.

Moorhead, D., T. B. Bridgeman, and J. Morris. 2008. Changes in water quality of Maumee Bay 1928 – 2003. *In*: Checking the pulse of Lake Erie. Editors Munawar, M and Heath, R. Goodword Books.

Nalewajko, C. and T. P. Murphy. 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan: an experimental approach. Limnology **2**:45-48.

Paerl, H.W., Tucker, J., and Bland, P.T. 1983. Carotenoid enhancement and its role in maintaining blue-green-algal (*Microcystis aeruginosa*) surface blooms. Limnology and Oceanography **28**(5): 847-857.

Peterson, G.L. 1977. A simplification of the protein assay of Lowry *et al*. which is generally applicable. Analytical Biochemistry. **83**(2): 346-356.

Post. A.F. 1986. Transient state characteristics of adaptation to changes in light conditions for the cyanobacterium *Oscillatoria agardhii*. Arch Microbiol. 145:353-357

Preczewski, P.J., Heckathorn, S.A., Downs, C.A., and Coleman, J.S. 2000. Photosynthetic thermotolerance is quantitatively and positively correlated with

production of specific heat-shock proteins among nine genotypes of *Lycopersicon* (tomato). Photosynthetica **38**(1): 127-134.

Quinn, G.P. and Keough, M.J. 2002. Experimental design and data analysis for biologists. Cambridge University Press.

Raps, S., Kycia, J.H., Ledbetter, M.C., and Siegelman, H.W. 1985. Light-intensity adaptation and phycobilisome composition of *Microcystis aeruginosa*. Plant Physiology **79**(4): 983-987.

Raps, S., Wyman, K., Siegelman, H.W., and Falkowski, P.G. 1983. Adaptation of the cyanobacterium *Microcystis aeruginosa* to light-intensity. Plant Physiology **72**(3): 829-832.

Reynolds, C.S., Oliver, R.L., and Walsby, A.E. 1987. Cyanobacterial dominance - The role of buoyancy regulation in dynamic lake environments. New Zealand Journal of Marine and Freshwater Research **21**(3): 379-390.

Reynolds, C.S., Wiseman, S.W., and Clarke, M.J.O. 1984. Growth-rate and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. Journal of Applied Ecology **21**(1): 11-39.

Richards, R.P., Baker, D.B., Crumirine, J.P., Kramer, J.W., Ewing, D.E., and Merryfield, B.J. 2008. Thirty-year trends in suspended sediment in seven Lake Erie tributaries. Journal of Environmental Quality **37**(5): 1894-1908.

Sampath-Wiley, P., and Neefus, C.D. 2007. An improved method for estimating R-phycoerythrin and R-phycocyanin contents from crude aqueous extracts of *Porphyra* (Bangiales, Rhodophyta). Journal of Applied Phycology **19**(2): 123-129.

Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases. Plant Physiology **101**(1): 7-12.

Schlesinger, M.J. 1990. Heat-shock proteins. Journal of Biological Chemistry **265**(21): 12111-12114.

Schreiber, U., Bilger, W., and Neubauer, C. 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment on in vivo photosynthesis. *In*. Ecophysiology of photosynthesis. Editors: Schulze, E. and Caldwell, M.M. Springer-Verlag Press

Schofield, O., Evens, T.J., and Millie, D.F. 1998. Photosystem II quantum yields and Xanthophyll-cycle pigments of the macroalga *Sargassum natans* (Phaeophyceae): Responses under natural sunlight. Journal of Phycology **34**(1): 104-112.

Simis, S.G.H., Ruiz-Verdu, A., Dominguez-Gomez, J.A., Pena-Martinez, R., Peters, S.W.M., and Gons, H.J. 2007. Influence of phytoplankton pigment composition on remote sensing of cyanobacterial biomass. Remote Sensing of Environment **106**(4): 414-427.

Søndergaard, M., Jensen, J.P., and Jeppesen, E. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia **506**(1-3): 135-145.

Stehfest, K., Toepel, J., and Wilhelm, C. 2005. The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiology and Biochemistry **43**(7): 717-726.

Twiss, M.R., Gouvea, S.P., Bourbonniere, R.A., McKay, R.M.L., and Wilhelm, S.W. 2005. Field investigations of trace metal effects on Lake Erie phytoplankton productivity. Journal of Great Lakes Research **31**: 168-179.

Vincent, R.K., Qin, X.M., McKay, R.M.L., Miner, J., Czajkowski, K., Savino, J., and Bridgeman, T. 2004. Phycocyanin detection from LANDSAT TM data for mapping cyanobacterial blooms in Lake Erie. Remote Sensing of Environment **89**(3): 381-392.

Visser, P. M., Ibelings, B. W., Mur, L. R., and Walsby, A. E. 2005. Ecophysiology of *Microcystis*. *In* Harmful Cyanobacteria. Editors Huisman, J., Matthijs, H. C. P., and Visser, P. M. Springer Press. Netherlands

Visser, P.M., Passarge, J., and Mur, L.R. 1997. Modelling vertical migration of the cyanobacterium *Microcystis*. Hydrobiologia. **349**: 99-109.

Webster, I.T., and Hutchinson, P.A. 1994. Effect of wind on the distribution of phytoplankton cells in lakes revisited. Limnology and Oceanography **39**(2): 365-373.

Wellburn, A.R. 1994. The spectral determination of chlorophyll *a* and chlorophyll *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology **144**(3): 307-313.

Wu, Z.X., and Song, L.R. 2008. Physiological comparison between colonial and unicellular forms of *Microcystis aeruginosa* Kutz. (Cyanobacteria). Phycologia **47**(1): 98-104.

Wynne, T.T., Stumpf, R.P., Tomlinson, M.C., Warner, R.A., Tester, P.A., Dyble, J., and Fahnenstiel, G.L. 2008. Relating spectral shape to cyanobacterial blooms in the Laurentian Great Lakes. International Journal of Remote Sensing **29**(12): 3665-3672.

Tables

Table 2-1. Equations used to calculate chlorophyll *a* and *b* and total caroteniods in DMSO from filtered lake water or *Microcystis* (Wellburn 1994). X is L for filtered water for pigment per volume or g for *Microcystis* pigment per mass. v is the volume (ml) of DMSO used for extraction.

Pigment	Unit	Equation
Chlorophyll *a*	$\mu g\ X^{-1}$	$[(12.47*A_{666})-(3.62*A_{649})] \times v / X$
Chlorophyll *b*	$\mu g\ X^{-1}$	$[(25.06*A_{649})-(6.5*A_{665})] \times v / X$
Total Carotenoids	$\mu g\ X^{-1}$	$\{[(1000*A_{480})-(1.29*_{Chl\ a})-(53.78*_{Chl\ b})]/220\} \times v / X$

Table 2-2. ANCOVA and regression table of *Microcystis* Φ_{et} at light saturation (I_k), 2x I_k, and 4x I_k and the light intensities used to represent these reading (μmol m^{-2} s^{-1} PAR). Because slopes are not parallel ($\alpha = 0.05$; $F_{(1,25)} = 4.26$), regressions were used to test the effect turbidity had on Φ_{et}. *P* values, slope and r^2 are from linear or non-linear regressions of K_{PAR} *vs.* Φ_{et} in a given light intensity. The half-saturation coefficient (K_m) and maximum Φ_{et} ($\Phi_{et\ max}$) for the K_{PAR}-Φ_{et} curve are given for non-linear function.

	Light	ANCOVA Slope Test							
Sample	Intensity	F*	Result	Mixing	*p* value	Function	r^2	K_m	$\Phi_{et\ max}$
	Dark			Calm	0.5544	Linear	0.0275	NA	NA
F_v/F_m	Adapted	0.05	Parallel	Mixed	0.1121	Linear	0.1967	NA	NA
				Calm	0.2356	Linear	0.1063	NA	NA
Ik	261	9.45	Not Parallel	Mixed	0.0006	Non-linear	0.6361	0.9126	0.2999
				Calm	0.2662	Linear	0.0942	NA	NA
2x Ik	521	12.98	Not Parallel	Mixed	<.0001	Non-linear	0.7413	1.4979	0.2417
				Calm	0.7701	Linear	0.0068	NA	NA
4x Ik	1044	11.3	Not Parallel	Mixed	0.0003	Non-linear	0.6757	4.6623	0.1808

Table 2-3. *Microcystis* pigment and Rubisco ANOVA tables. A). ANCOVA results for error DF and error SS for the reduced and full models that contain site effect. This indicates that site had no significant ($\alpha = 0.05$; $F_{(4,14)} = 3.36$) on pigment content. B). Regression table for pigments content as a function of K_{PAR}. Rubisco* regressed against average PAR.

A). ANCOVA

Pigment	DF_{Full}	SS_{Full}	$DF_{Red.}$	$SS_{Red.}$	F*	*p* value
Chl *a*	14	9.678	18	10.744	0.385	0.816
Carot./ Chl *a*	14	0.009	18	0.012	1.423	0.278
PC	14	1071.747	18	1744.130	2.196	0.122
PC/ Chl *a*	14	25.953	18	38.373	1.675	0.211
Rubisco	14	224.885	18	611.598	6.02	0.005
Rubisco*	14	247.879	18	312.009	0.91	0.487

B). Regressions

p value	Slope	r^2
0.0003	0.523	0.5336
0.0025	-0.014	0.4059
0.0003	6.568	0.5260
0.0096	0.632	0.3181
0.2035	NS	0.0882
0.0002	0.0187	0.5348

Table 2-4. *P* values for the quantum yield of PSII electron transport (Φ_{et}), the ratio of variable to maximum fluorescence (F_v/F_m), and total carotenoids/ chl *a*. Bold values indicate significance at $\alpha = 0.05$ after bonferroni adjustment.

Source	Φ_{et}	F_v/F_m	Carot./ Chl *a*
Depth (D)	**<.0001**	0.0825	0.5864
Turbidity (T)	**<.0001**	**0.0004**	**0.0007**
Mixing (M)	0.0838	0.1790	0.4071
Nutrients (N)	0.2238	0.1333	0.9778
D*T	0.7584	0.9958	0.9726
D*M	**<.0001**	0.6323	0.4182
D*N	0.9042	0.4484	0.5149
T*M	0.6557	0.3282	0.4427
T*N	0.6878	0.7415	0.4922
M*N	0.8796	0.9279	0.7746
D*T*M	0.5749	0.8522	0.9700
D*T*N	0.7698	0.3519	0.7031
D*M*N	0.8263	0.6211	0.8353
T*M*N	0.7569	0.8066	0.9543
D*T*M*N	0.6971	0.0679	0.4182

Figures

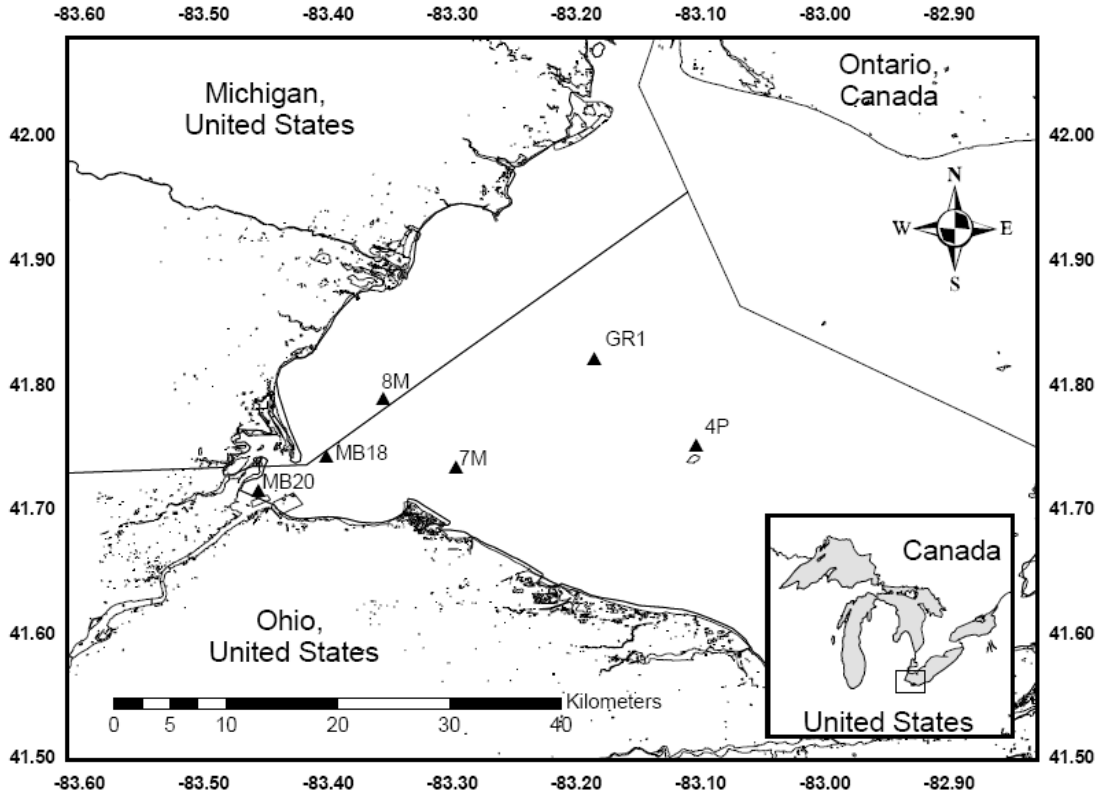

Figure 2-1. Six sample sites in western Lake Erie

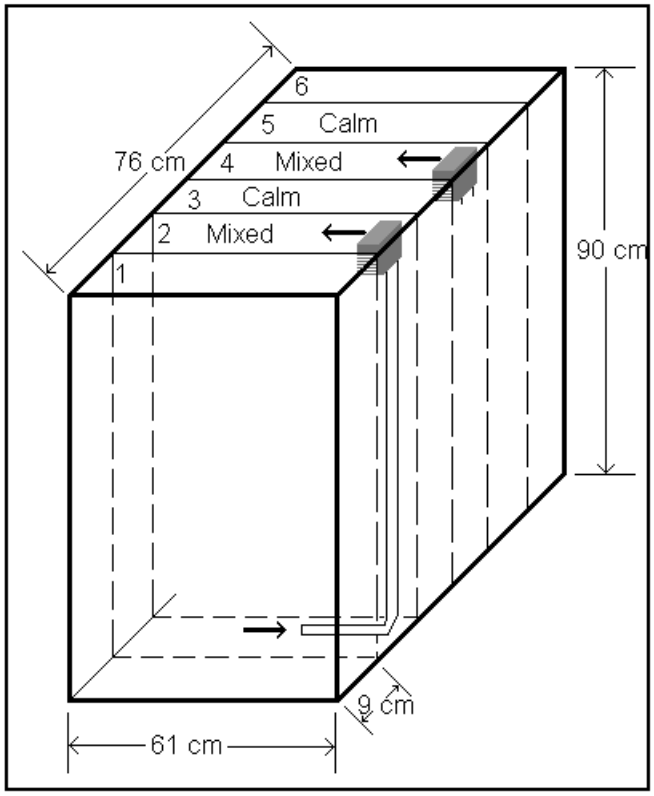

Figure 2-2. Diagram of experimental chambers of the 2x2x2x2 PSII efficiency experiment. Arrows depict the flow of circulating water of mixed chambers.

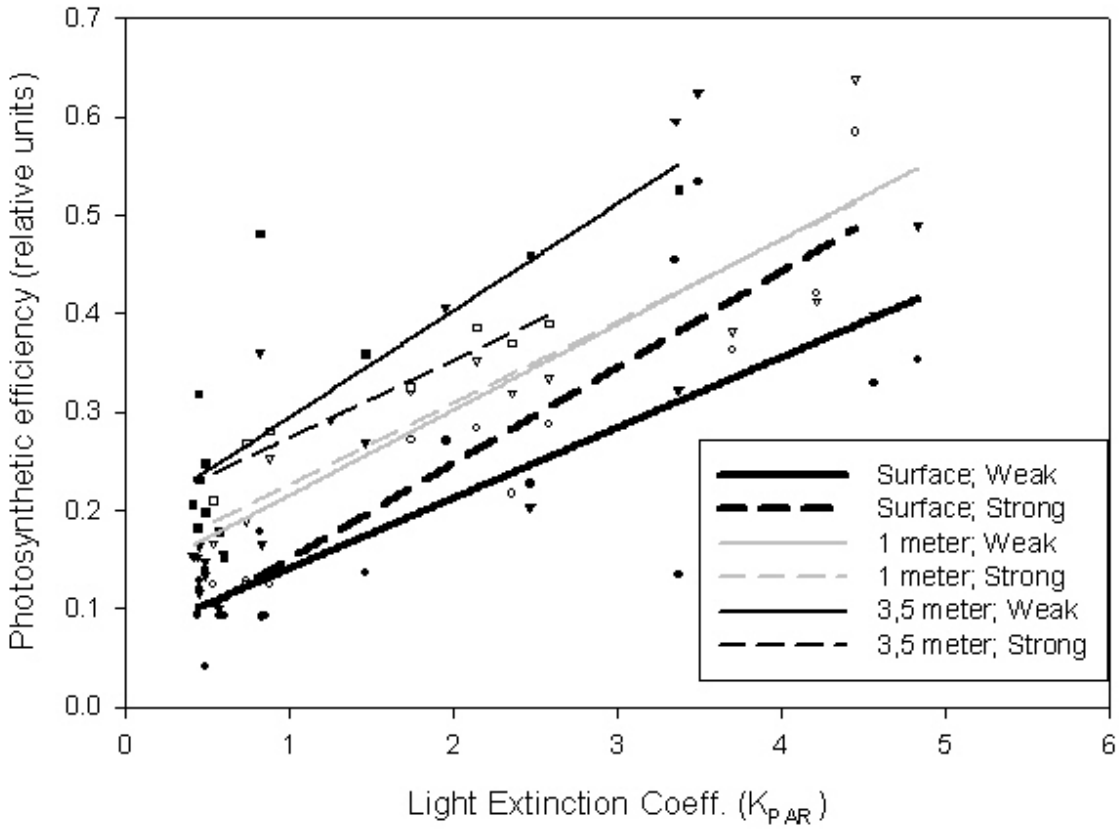

Figure 2-3. *In situ* quantum yield of photosystem II electron transport for samples with a phycocyanin concentration of greater than 0.5 μg L^{-1} from western Lake Erie measured at the surface (bold black lines; circles), 1 meter (gray lines; triangles) and 3 and 5 meters (thin black lines; squares) as a function of turbidity, as $K_{PAR.}$ Filled symbols and solid lines are weakly mixed dates. Open symbols and dashed lines are strong mixing dates.

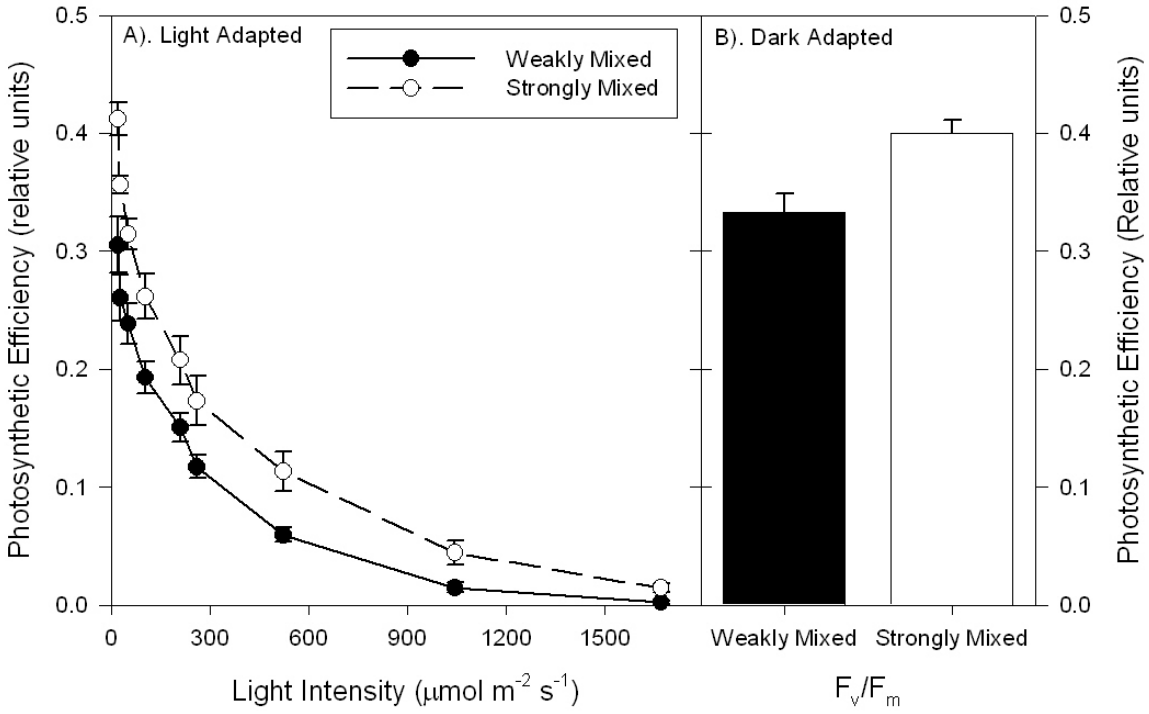

Figure 2-4. Light-response curves (A) and F_v/F_m (B) of *Microcystis* collected in western Lake Erie on weakly mixed dates (filled symbols; solid lines) and strongly mixed dates (open symbols; dashed lines). Values are mean ± SE of n = 15 for both mixing levels.

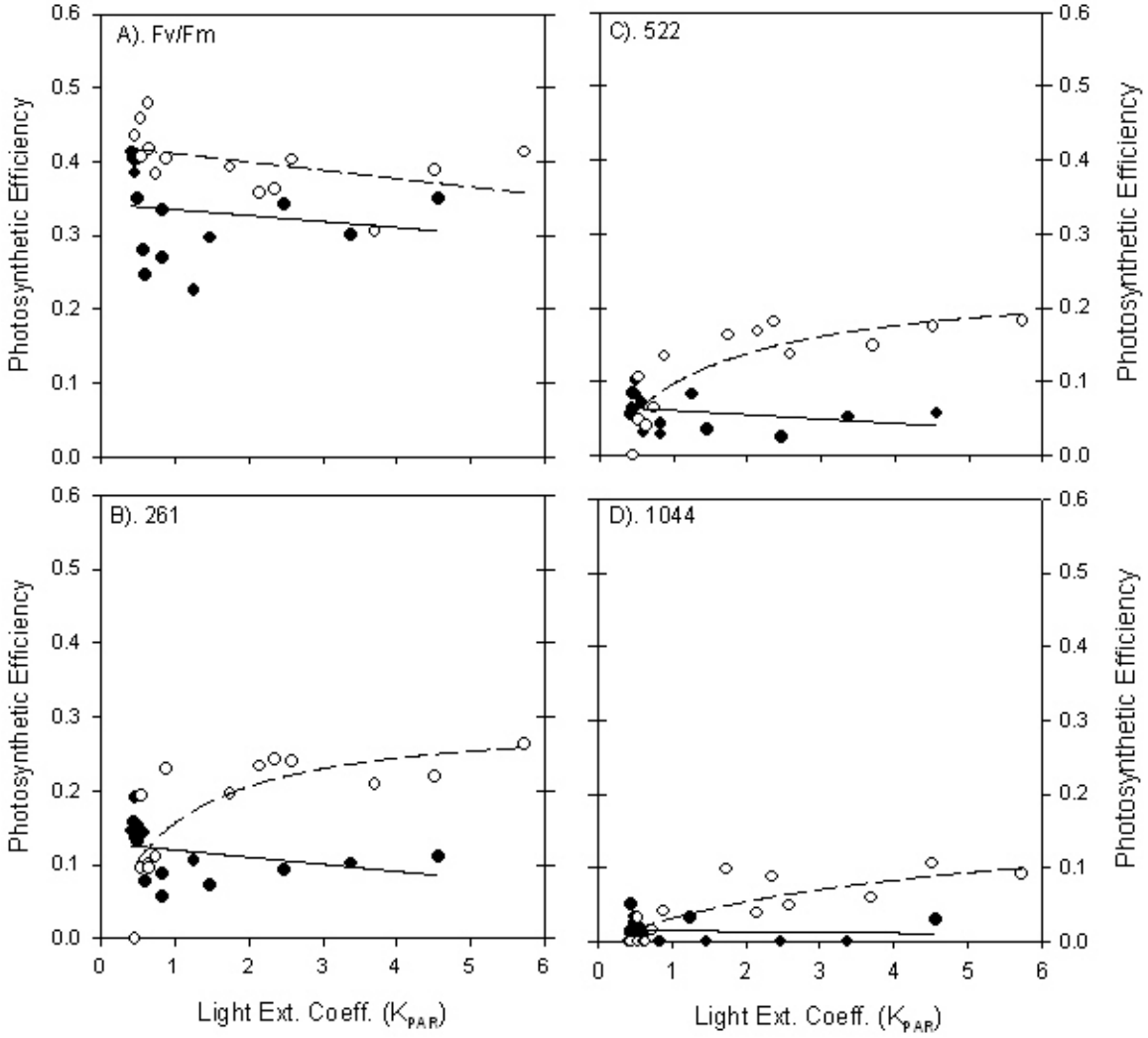

Figure 2-5. Photosynthetic efficiency of *Microcystis* from western Lake Erie at a given light intensity of the light-response curve as a function of K_{PAR}. Weakly mixed dates – filled circles and solid lines; Strongly mixed dates – open circles and dashed lines. A). F_v/F_m (dark adapted); B). 261 µmol $m^{-2}s^{-1}$ (light saturation; I_k); C). 522 µmol $m^{-2}s^{-1}$ (2x I_k); D). 1044 µmol $m^{-2}s^{-1}$ (4x I_k; half full sunlight).

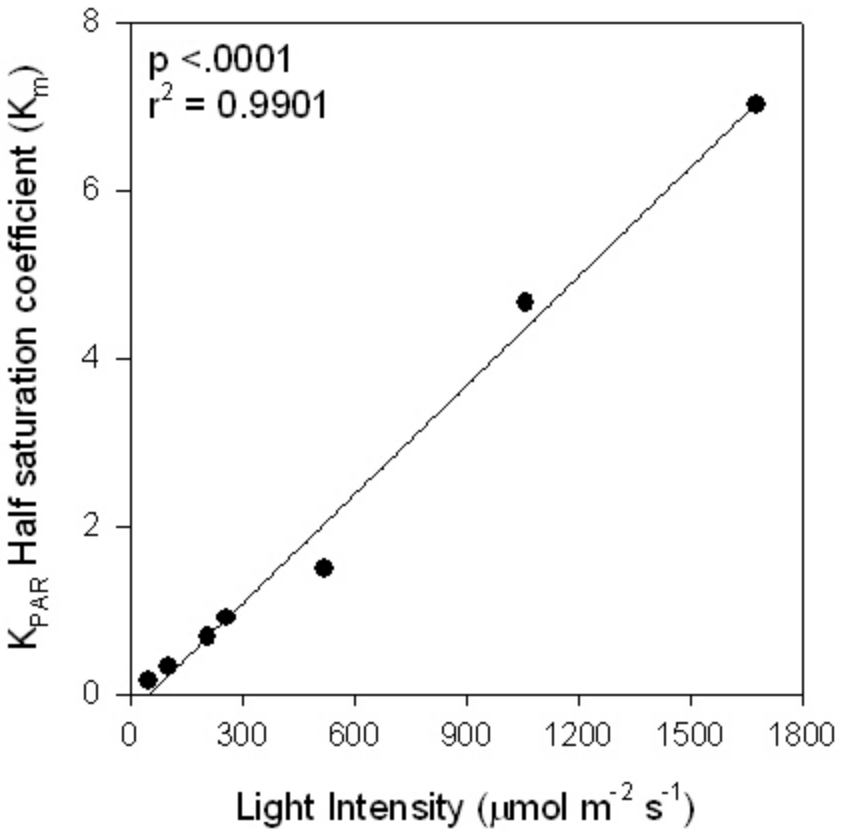

Figure 2-6. K_{PAR} half-saturation coefficient (K_m) of the light-response curves for *Microcystis* collected on strongly mixed days increases at higher light intensity, which indicates turbidity has a larger effect on Φ_{et} at high light levels.

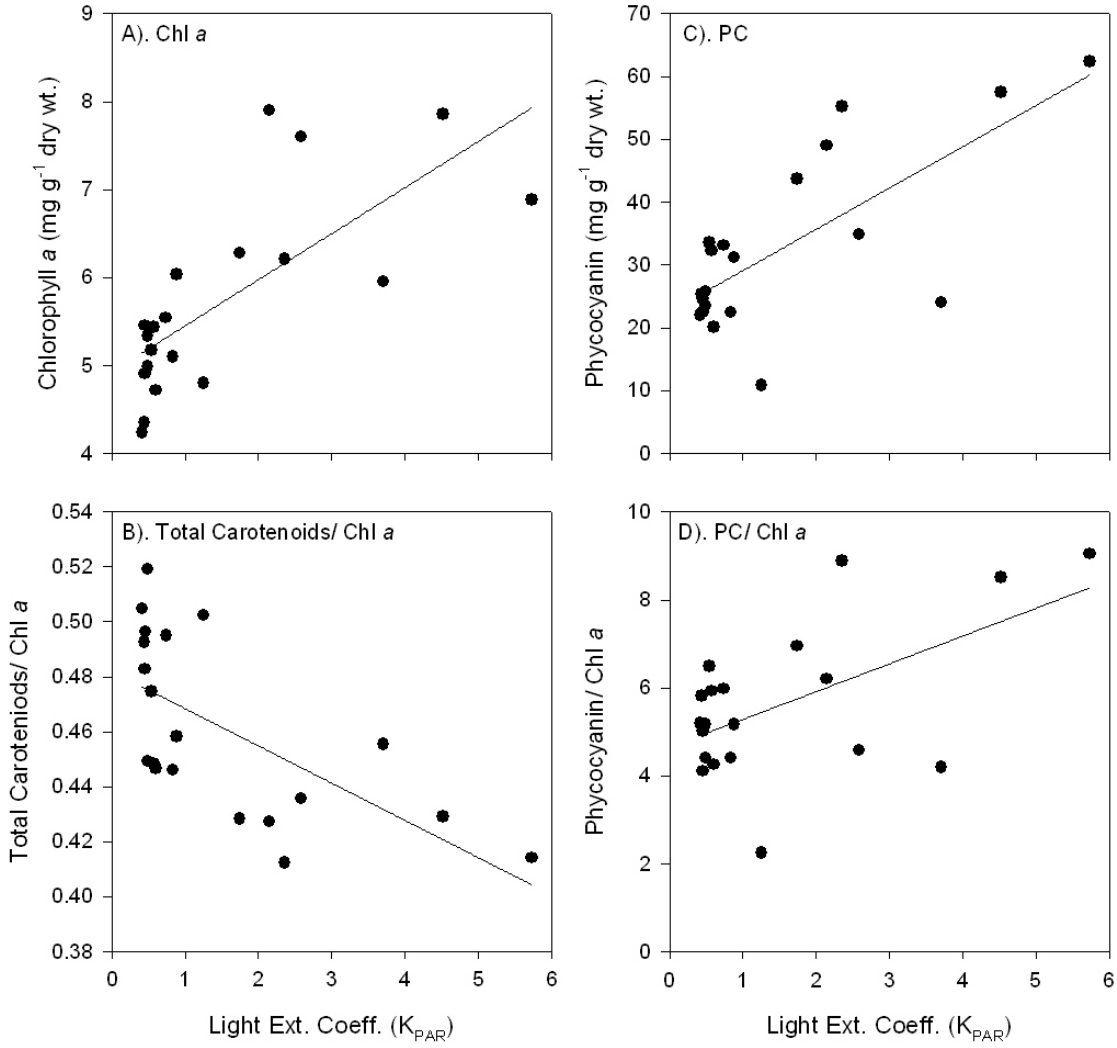

Figure 2-7. Pigment content of *Microcystis* collected from western Lake Erie as a function of K_{PAR}. A). Chlorophyll (chl) *a*; B). Phycocyanin; C). Total Caroteniods/ Chl *a*; D). Phycocyanin / Chl *a*.

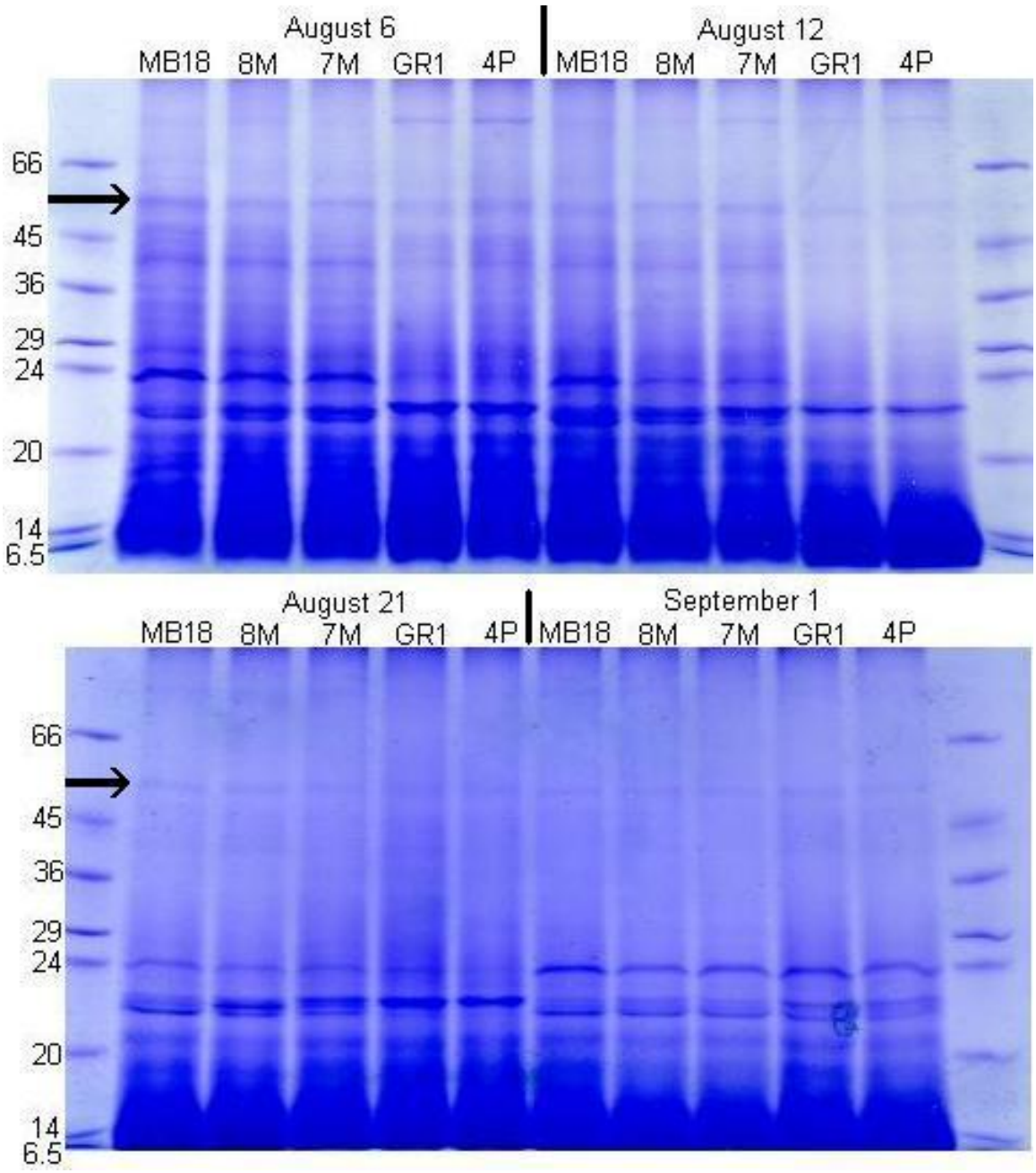

Figure 2-8. Total protein profile of *Microcystis* that had been separated from other phytoplankton collected in western Lake Erie from five sample sites and on four dates during 2008. Rubisco large sub-unit (52 kDa) is marked with an arrow.

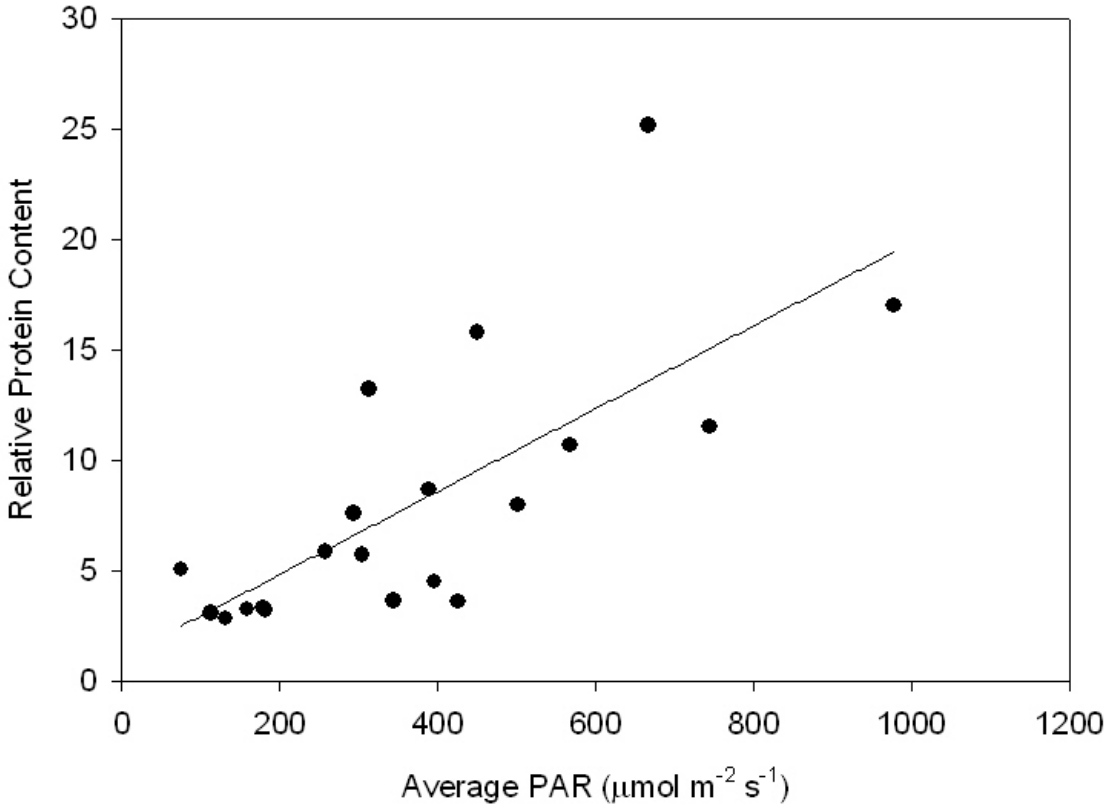

Figure 2-9. Rubisco content of *Microcystis* increases with average PAR over the water column.

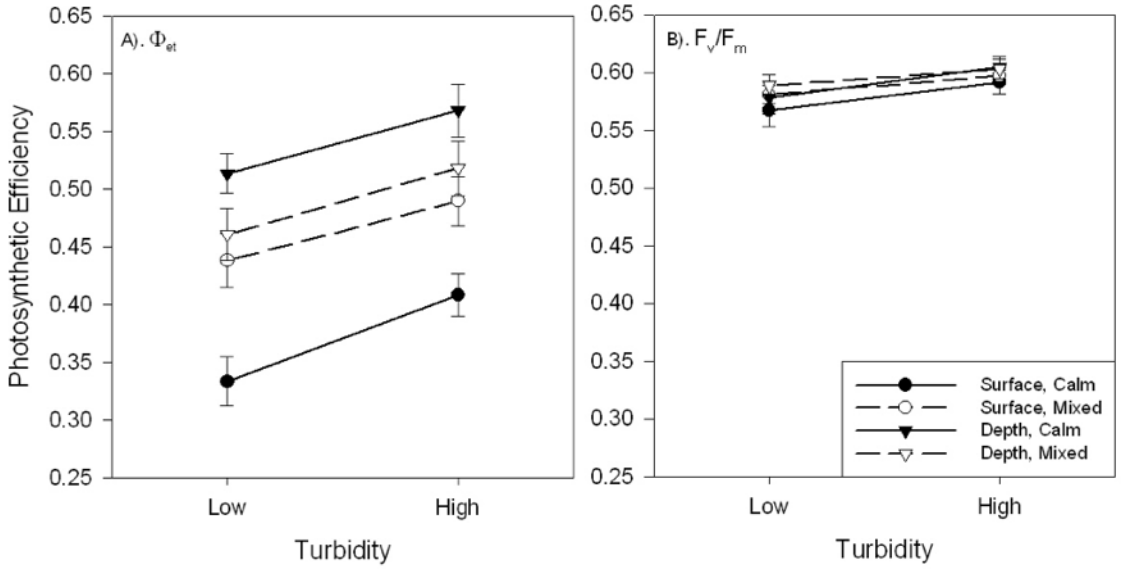

Figure 2-10. *In situ* quantum yield of photosystem II electron transport (Φ_{et}) (A) and the ratio of variable to maximum fluorescence (F_v/F_m) of Lake Erie phytoplankton grown in laboratory conditions under natural sunlight intensities, high or low turbidity, and mixing or calm water.

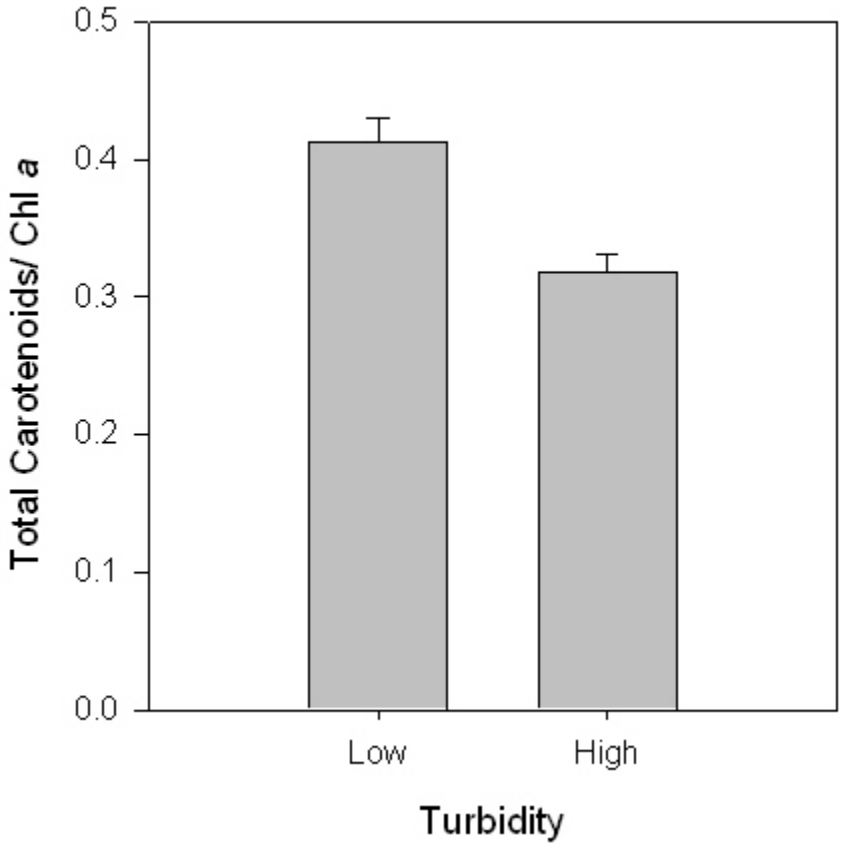

Figure 2-11. Total carotenoids/ Chl *a* of Lake Erie phytoplankton grown in the laboratory experiment in low or high turbidity.

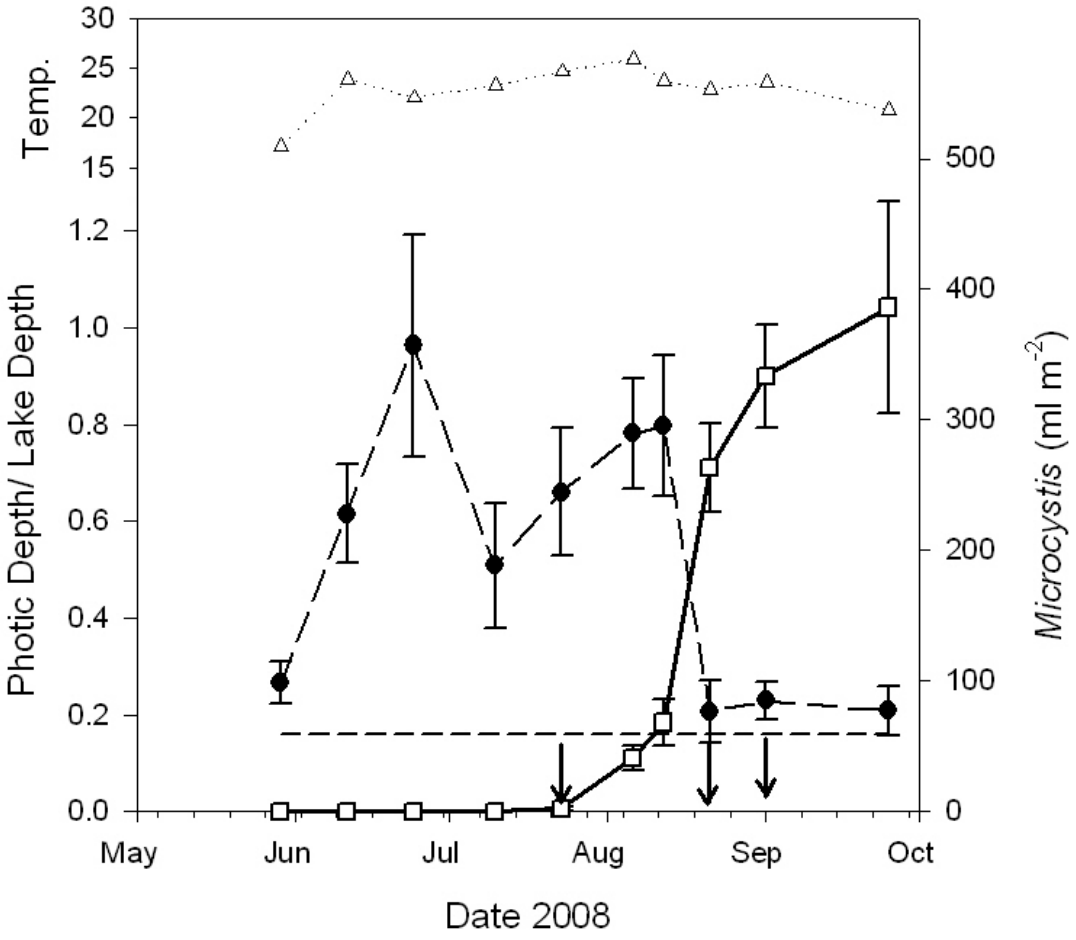

Figure 2-12. Turbidity (dashed line; filled circles), temperature (dotted line; open triangles) and *Microcystis* biovolume (bold line; open squares) in western Lake Erie during 2008. Arrows on represents dates sample that were strongly mixed. Dashed line with no symbols corresponds to 0.16, the value that indicated light limitation (Alpine and Cloern 1988). Values are the mean (± SE) of six sites.

Chapter Three

Measurements of *Microcystis* cellular health in western Lake Erie during the 2008 bloom indicate the high turbidity and nutrients support rapid growth.

Abstract

Measurements of *Microcystis* biovolume collected from 2002 through 2008 show that blooms are most dense in waters just outside of the Maumee Bay. The waters adjacent to the bay are generally high in nutrients and suspended sediments. Light-limitation may occur in these waters due to the combination of high turbidity and increasing depth, providing a potential ecological advantage for buoyant phytoplankton species, such as *Microcystis* over non-buoyant species. *Microcystis* was collected from western Lake Erie during the 2008 bloom for measurements of total protein content and nutrient content to determine physiological health. The results of lake sampling indicates that *Microcystis* cellular health is greater in the highly turbid, light-limiting conditions adjacent to Maumee Bay waters compared to clearer offshore waters. Nutrient content of *Microcystis* suggest that *Microcystis* was nitrogen replete and phosphorus limited, but not extremely deficient. Further, in a laboratory experiment, *Microcystis* growth was greatest in the high nutrient, high turbidity treatment. Other combinations of nutrients and turbidity resulted in less *Microcystis* growth. The combination of high nutrients and high turbidity of the waters adjacent of the Maumee Bay provide *Microcystis* not only an

ecological advantage in light competition, but also, offers condition that favors rapid growth and cellular health.

Introduction

Microcystis blooms were a relatively small proportion of the Lake Erie phytoplankton community prior to the 1990s (Makarewicz 1993). Since the mid 1990s, total phytoplankton biomass has increased and *Microcystis* blooms have returned to western Lake Erie, despite an increase in annual nutrient loading (Conroy et al. 2005; Brittian et al. 2000). *Microcystis* blooms in western Lake Erie are a concern because of their ability to produce a hepatotoxin (Microcystin) in concentrations that frequently surpasses the World Health Organization's safety limit of 1 $\mu g\ L^{-1}$ in western Lake Erie (Brittian et al. 2000; Rinta-Kanto et al. 2005; Dyble et al. 2008).

Western Lake Erie is an ideal location for *Microcystis* growth because of high nutrients and high turbidity (Moorhead et al. 2008). Total phosphorus concentration of Maumee Bay is usually greater than 0.100 $mg\ L^{-1}$ and concentration decreases with distance into the lake (Moorhead et al. 2008). The potential for bloom formation increases with increasing TP and plateaus at about 80% once TP reaches 0.100 $mg\ L^{-1}$ (Downing et al. 2001). High concentrations of suspended sediments may bring about phytoplankton light-limitation (Alpine and Cloern 1988) favoring buoyant *Microcystis* in light-limited calm waters (Huisman et al. 2004). The Maumee River is a source of nutrients and suspended sediments to Maumee Bay and western Lake Erie because of its highly agricultural watershed (Baker and Richards 2002; Richards et al. 2008). Shallow lakes allow sediments and nutrients to be resuspended from the lake bottom by wind-

driven currents (Søndergaard et al. 2003). Thus, due to the shallow depth of Maumee Bay and western Lake Erie, internal loads of sediments may also generate turbidity. Field sampling of previous *Microcystis* blooms in western Lake Erie lead to observations that suggest that the spatial extent of a bloom follows the spatial pattern of the suspended sediment plume. Conditions in the sediment plume include high nutrients that fertilize *Microcystis* and high turbidity that protects *Microcystis* from high light and light-limit the water column (see Chapter 2), while waters outside the sediment plume have lower nutrient concentrations and less turbidity. Based on nutrient and light conditions, I hypothesized that *Microcystis* in the sediment plume would have a better cellular health compared to *Microcystis* in waters outside of the sediment plume.

Cyanobacteria are poor competitors for nutrients compared to green algae and diatoms (Tilman et al. 1986). Thus, *Microcystis* should be out-competed for nutrients in nutrient-limited lakes. However, *Microcystis* is able to store excess phosphorus in waters that have total phosphorus concentrations greater than 0.220 mg L^{-1} (Baldia et al. 2007), to sustain a constant rate of cell growth for five (Tsukada et al. 2006) to nine days (Nalewajko and Murphy 2001). Likewise, eukaryotic algae are also capable of luxury uptake in nutrient rich waters and phosphorus can make up 11% of total dry weight (Ducobu et al. 1998). If nitrogen is depleted in waters, cyanobacteria are able to break down phycobiliproteins and allocate nitrogen to other compounds (Post 2005); however, *Microcystis* can only sustain growth for one day following nitrogen deprivation (Baldia et al. 2007). In the case of western Lake Erie, I hypothesized that *Microcystis* accumulates phosphorus in the nutrient rich bay before currents carry the cells into the relatively nutrient-poor offshore water. This hypothesis would be supported if *Microcystis* is found

to have a similar internal phosphorus content in the nearshore *vs*. offshore despite large differences in ambient dissolved phosphorus concentrations. I further hypothesized that *Microcystis'* inability to store nitrogen would result in lower internal nitrogen content in the relatively low total nitrogen offshore region.

Plankton samples were collected from six sites in western Lake Erie as a part of an on-going monitoring program established during 2002 at the Lake Erie Center (University of Toledo, Toledo, Ohio, USA). *Microcystis* colonies retained in those samples were measured to determine if there was a spatial pattern to *Microcystis* blooms in western Lake Erie. During the summer of 2008, I sampled of the annual *Microcystis* bloom to measure physiological parameters (*i.e.* nutrient content, and total protein content) that would help explain any spatial pattern observed in the seven years of sampling. If any spatial pattern of *Microcystis* density was observed, my hypothesis was that *Microcystis* collected during 2008 would have higher cellular health in regions that *Microcystis* is most abundant. The null hypothesis would be that cellular health parameters would not show differences among sites.

Further, this chapter presents a factorial *Microcystis* growth laboratory experiment with four treatments and two levels in each treatment. Turbidity and nutrients (high and low) were used to study the effect of suspended sediments and nutrient concentrations typical of the bay and offshore. Mixing (mixed or calm) and sample depth (surface or at depth) were used to study the effect of water column turbulence on the buoyancy capabilities of *Microcystis*. It was hypothesized that *Microcystis* biomass would be greatest at the surface in the high nutrient and high turbidity treatment, while *Microcystis* biomass would be lowest in the clear, low nutrients, and mixed treatment.

Methods

Spatial Pattern of *Microcystis*

To determine if there was a spatial pattern of *Microcystis*, plankton tow samples were analyzed that were collected from 2002 to 2008 from six sites in western Lake Erie (Fig. 3-1). *Microcystis* and other plankton were collected using a 112 μm mesh plankton net over the entire water column. Plankton retained in the net was preserved in buffered-sugar formalin. *Microcystis* was separated from other phytoplankton using Imhoff cones and biovolume of *Microcystis* was used as an estimate of biomass (as in Chaffin et al. 2008).

Health Status of *Microcystis*

For physiological measurements to be made in the laboratory, *Microcystis* was collected over the entire water column from five sites in western Lake Erie using a 64 μm mesh plankton net on four dates between August 6 and September 1 2008 (Fig. 3-1). During these dates, *Microcystis* biomass steadily increased. An additional site (MB20) was sampled for nutrients, but *Microcystis* was not abundant enough there to collect for physiological measurements. Water from all six sites was collected at 1 m to determine nutrient concentration of lake water. Water was filtered through a 0.45 μm Millipore filter for soluble nutrients (nitrate, nitrite, ammonium, soluble reactive phosphorus) while unfiltered water was used for total phosphorus and total kjeldahl nitrogen analyses. Filtered and unfiltered samples were sent to the National Center for Water Quality Research at Heidelberg University for analysis. *Microcystis* colonies retained in the net were concentrated and stored in dark polyethylene bottles on ice during transportation back to the laboratory. Upon arrival at the laboratory, the *Microcystis* samples were

separated from other phytoplankton using Imhoff cones and stored at -80 °C as in chapter two of this thesis.

Total protein content is an indicator of cellular health. Protein of *Microcystis* was extracted by grinding approximately 1 g of fresh weight tissue to a powder in liquid nitrogen using mortar and pestle, then transfering to a protein extraction buffer containing 0.1 M Tris buffer (pH = 8.0), SDS detergent, glycerol, bromophenol-blue, sucrose, protease inhibitors, a phenolic inhibitor, and reductants (Mishra et al. 2008). Samples were then centrifuged at 15,000 *g* for 10 minutes at 4 ° C. Supernatant containing soluble proteins was collected and total protein content was determined using the method of Peterson (1977), and quantified using a standard curve of Bovine Serum Albumin and corrected for dry mass.

Carbon (C) and nitrogen (N) content was determined on 3.1- 3.7 mg of dried *Microcystis* tissue. Tissue was placed in foil and percentage of C and N were measured by gas-chromatography following combustion (HCNO/S analyzer Perkin-Elmer 2400 series II). Acetanilide was used as a standard, with errors of -0.27% and 0.01 % for C and N respectively. Phosphorus (P) and other macronutrients and micronutrients content were determined by ICP-OES on 100 to 200 mg of dried tissue. Nutrient deficiencies were indicated by atomic ratios shown in Table 3-1 (Healey and Hendzel 1975).

Laboratory Experiment

A 2x2x2x2 factorial experiment was used to test the effects of nutrient concentration [low (0.6 mg L^{-1} N, 0.03 mg L^{-1} P) and high (3.0 mg L^{-1} N, 0.15 mg L^{-1} P)], turbidity [low (~ 1 NTU) and high (~30 NTU)], mixing (vertically mixed or non-mixing) and sample depth (surface and at depth) on *Microcystis* growth. Mixing of the chamber

was achieved using powerhead pumps (Aquagardens #601) so that the intake hose was placed at the bottom of the chamber and outflow just beneath the surface (Fig. 3-2). Sieved Lake Erie sediments (400 µm) were added to create turbidity. All other nutrients were half concentration of the WC media (Guillard and Lorenzen 1972). Twiss et al. (2005) enriched Lake Erie water with micronutrients to a final concentration similar of the WC growth media and observed no additional phytoplankton growth.

Chlamydomonas (a common unicellular green alga) and *Microcystis* were grown separately in WC liquid medium (Guillard and Lorenzen 1972) under illumination of ~50 µmol m^{-2} s^{-1} on 12:12 hour light/dark cycles. *Chlamydomonas* was aerated while *Microcystis* was not. *Chlamydomonas* and *Microcystis* were initially collected from Lake Erie. Equal amounts of *Chlamydomonas* and *Microcystis* were used in this experiment, based on content of chl *a*. *Chlamydomonas* and *Microcystis* were added so that they each had an initial level chlorophyll (chl) *a* of 2.5 µg L^{-1}, making the total initial concentration 5.0 µg L^{-1}. Phytoplankton intended for experiment was grown in liquid culture with the nutrient concentration that of the low treatment level for two weeks before use in the experiment, to insure that internal phosphorus storage did not occur.

Once treatments were set up and phytoplankton added, 96 hours were allowed for growth. Following the 96 hours, samples were collected at the surface and 70 cm. The 70 cm sample represents the 20% of surface light in the low turbidity level and < 0.5 % in the highly turbid treatment. Phytoplankton (100 ml) was filtered onto GF/F filters and stored on silica gel at -80 °C until pigment concentration was measured. Chl *a* was used as a surrogate for total algal biomass, phycocyanin (PC) as an index of *Microcystis*

abundance, and chl *b* for green algae. Chl *a* and *b* were extracted in DMSO and PC extracted by sonication following methods in chapter two.

Data Analysis

Total protein and nutrient content were analyzed using repeated measures ANOVA with PROC MIXED of SAS with sample date as repeated variable and sample site as fixed effect. Tukey test was performed for difference among sites. A four-way ANOVA was performed for the laboratory experiment. PROC GLM of SAS was used and Tukey was used to compare differences between treatments.

Results

Spatial Pattern of *Microcystis*

Microcystis biovolume was highly temporally and spatially variable between 2002 and 2008 (Table 3-2). On the temporal scale, lowest biovolumes were recorded during 2002 and highest average biovolumes during 2008, while the greatest value was recorded during 2003 at 7M. Spatially, across all years, MB20 had the lowest biovolume, while 7M and 8M had the highest. 4P had more *Microcystis* biovolume than GR1, and 4P biovolumes are correlated with 7M.

Lake Nutrient Concentration

Total phosphorus (TP) ranged from 0.50 to 0.90 mg L^{-1} and decreased with distance from the river (Fig. 3-3a,b). Soluble reactive phosphorus (SRP) concentrations were 13% to 33% of the TP concentration and show similar patterns over space and time as did TP. Nitrate (NO_3-N) was high during May through early July (lake mean about 2.0 mg L^{-1}), then was nearly depleted by the end of the sampling season and also showed the

same spatial pattern of decreasing with increasing distance from river mouth (Fig. 3-3c,d). Ammonium (NH_4-N) was more or less constant throughout the season (0.025 mg L^{-1}) and did not have a spatial pattern (Fig. 3-3c,d). The ratio of total N: total P (TN:TP) was around 35 (mass) during the first half of the season then dropped as NO_3 became depleted during August (Fig. 3-3e,). TN:TP was similar at all sites (Fig. 3-3f).

Health Status of *Microcystis*

Total protein content (TPC) was used as a measure of cellular health. TPC was significantly affected by sample site ($F_{(4,12)} = 3.26$; $F^* = 5.01$, $p = 0.0131$) and sample date ($F_{(3,12)} = 3.49$; $F^* = 4.66$, $p = 0.0222$). *Microcystis* collected from 8M and 7M had significantly greater TPC than *Microcystis* from GR1 and 4P (Fig. 3-4). The bay site had mean protein content slightly less than the middle sites, but Tukey test indicated that MB18 was similar to all other sites (Fig. 3-4). The significant date effect can be attributed to the variation observed in the bay and two middle sites, and resulted in a general increase of TPC over the summer. There was while little variation in TPC in the offshore waters over the summer sampling.

Carbon (C) content of *Microcystis* ranged from 45.65% to 49.91% and nitrogen (N) ranged from 6.31% to 9.16%. Phosphorus (P) was more variable ranging from 0.52% to 1.25%. The atomic ratios of C to N (C:N) and C to P (C:P) were used indicate nutrient deficiencies. Sample date had a statistically significant effect on both C:N and C:P ($F_{(3,12)} = 3.49$; $F^* = 8.29$, 3.15, respectively; $p = 0.0030$, 0.0494, respectively), while sample location did not have an effect ($F_{(4,12)} = 3.26$; $F^* = 2.22$, 0.88, respectively; $p = 0.1285$, 0.5033, respectively). Figure 3-5 shows the plot of C:N *vs*. C:P. The thin dashed lines represent the boundary between no deficiency and moderate deficiency, while the

bold lines indicate the boundary between moderate and extreme deficiency. In terms of N, 19 of 20 samples were N-replete, only once did *Microcystis* have a slight moderate N-deficiency (August 12 at 8M). Moderate P-deficiency was measured in a majority of the *Microcystis* samples. Further, *Microcystis* collected from the middle sites (8M and 7M) had the lowest C:P on most sampling trip (Fig. 3-6).

Laboratory Experiment

Chl *a* in the laboratory experiments ranged from 15.58 to 33.22 µg L^{-1} (Table 3-3). Chl *a* was significantly affected by the three-way interaction of depth*mixing*nutrients ($F^* = 5.09$; $p = 0.0311$) and by turbidity ($F^* = 4.88$; $p = 0.0344$) (Table 3-4). PC ranged from 0.49 to 9.89 µg L^{-1} (Table 3-3) and was significantly affected by depth*mixing ($F^* = 41.23$; $p < .0001$) and nutrients ($F^* = 4.61$; $p = 0.0395$) (Table 3-4). The interaction between turbidity and nutrients was nearly statistically significant ($F^* = 3.99$; $p = 0.0543$). Chl *b* varied little among treatments (Table 3-3) and no factors had significant effects (Table 3-4).

Mixing had the greatest effect on chl *a* and PC. At the surface of the calm treatment, chl *a* and PC was statistically greater than all other combinations of depth and mixing (Tukey test, $p < 0.0001$ for all pair-wise comparisons of surface*calm to other combinations). In the calm treatments, the concentrations of chl *a* and PC were much higher at the surface than at depth, as result of *Microcystis* concentrating at the surface (Table 3-3). Within the calm*surface treatment, chl *a* was greater in the high nutrient treatment (Tukey test, $p = 0.0053$). All other combinations of comparison (excluding calm*surface) were nearly identical (Tukey test, $p > 0.89$). In the mixing treatment, the surface and 70 cm sample had nearly identical concentrations of chl *a* and PC (Table 3-

3). The mixed treatments had much less PC and more chl *b*, and chl *b* was greater in the low turbidity treatment than the high turbidity treatment across turbidity levels of the mixed treatments (Table 3-3).

The interaction between turbidity and nutrients is evident within the calm treatment, at the surface (although not statistically significant when considering the mixed treatments and depth samples). At low turbidity, nutrients did not have an effect on either chl *a* or PC (Fig. 3-7). At high turbidity, concentrations of chl *a* and PC were greater in the high nutrient treatment (Fig. 3-7).

Discussion

Microcystis biomass was highly variable among the seven years of data collection (Table 3-2). *Microcystis* did show a distinct spatial pattern in western Lake Erie, with low biomass of *Microcystis* found in the bay, highest biomass at the middle sites and slightly less or similar biomass in the offshore (Table 3-2). This pattern can be explained by several observations. First, the region that is intermediate between Maumee Bay and the open waters of the western basin of Lake Erie (designated here as "Middle"), often has the lowest photic depth to lake depth ratio. In the Middle region, *Microcystis* would have an advantage in the competition for light over sinking phytoplankton (Huisman et al. 2004). The shallow bay would have a higher photic depth to lake depth ratio; also shallow waters are more likely to be mixed, which prevent *Microcystis* blooms (Huisman et al. 1999). However, *Microcystis* might actually have very rapid growth in the bay sites, but the short retention time of the bay may be simply pushing *Microcystis* to the offshore before high biomasses can be accumulated.

Total protein content (TPC) was greatest at the two middle sites (Fig. 3-4), which suggests that *Microcystis* from these sites have a higher overall cellular health compared to offshore waters. The trend of higher TPC in the bay and middle waters can be explained by two factors. First, nutrient concentration is very high in the bay and adjacent waters and decreases with distance into the lake (Fig 3-3; Moorhead et al. 2008). High concentrations of soluble nutrients in the bay and middle region would result in healthier *Microcystis* and more growth. Secondly, *Microcystis* from turbid waters has less light stress compared to *Microcystis* in clear water (chapter 2). The combination of high nutrient and high turbidity in the bay and adjacent waters would have provided favorable conditions for *Microcystis* growth. There was also a general increasing of TPC, which resulted in the statistically significant date effect. *Microcystis* from the second half of sampling dates was collected when the water column was strongly mixed. In an effort to try to maintain position in the upper layer of the mixed water, *Microcystis* will produce more gas vacuoles as a response to lower average light intensities (Walsby 1994). When *Anabaena* was grown at low light, gas vacuoles proteins can make up nearly 10% of all cellular proteins (Oliver and Walsby 1984).

No N-deficiency was seen in *Microcystis* during 2008, despite the low TN:TP ratios. The NO_3 supply decreased nearly 50-fold throughout the summer, but NH_4 remained more or less stable and was above 0.025 mg L^{-1} for the entire summer. This would suggest that NH_4 of 0.025 mg L^{-1} would be adequate to support an intense *Microcystis* bloom. This would follow the observations of Jacoby et al. (2000) who reported that NH_4 of 0.007 mg L^{-1} was able to support a *Microcystis* bloom in Steilacoom Lake (Washington, USA), while NO_3 had became depleted. *Microcystis* is not a nitrogen

fixer. Therefore, according to traditional TN:TP models, *Microcystis* should not be able to persist at low TN:TP because N-fixing cyanobacteria would out-compete non-N fixers (Smith 1983). During 2008, the NH_4 proportion was 2.4% to 4.7% of the TN. Thus, TN:TP ratios may not apply in lakes with adequate NH_4, or because a high propitiation of the TP might actually be stored in the algae.

Microcystis was generally moderately P-deficient (Fig. 3-5). However, *Microcystis* from the middle sites was less P-deficient than the offshore sites on each sample date (Fig. 3-6), although this was not statistically supported. Lake total phosphorus concentrations decreased with distance from the river mouth and increased from July to September (Fig. 3-3a,b). *Microcystis* is capable of luxury uptake when grown in phosphorus-rich waters (Baldia et al. 2007; Tsukada et al. 2006; Nalewajko and Murphy 2001). Because P was higher at the middle sites than the offshore, this did not support my hypothesis that phosphorus accumulated in the nearshore sustains *Microcystis* growth in the lower nutrient, offshore waters. *Microcystis* growth is linear with phosphorus concentrations up to 220 $\mu g\ L^{-1}$ (Baldia et al. 2007), which is greater than phosphorus concentrations of the bay. Therefore, *Microcystis* remains phosphorus limited in western Lake Erie and reduction in phosphorus loading would further increase the phosphorus deficiency and slow growth.

In the laboratory experiment, mixing of the chambers resulted in much less *Microcystis* and more green algae (Table 3-3). Highly turbid, mixed treatments had less chl *a* than did low turbidity, mixed treatments. This would indicate light limitation in the mixed and turbid treatments. In the high turbidity treatments, the lack of mixing allowed *Microcystis* to float to the surface access light and preventing light-limitation. These

findings agree with the modeling work of Huisman et al. (1999, 2004) who showed that buoyant phytoplankton will dominate calm waters, while mixed waters will result in less of the buoyant species and more of the sinking species. *Microcystis* growth is prevented in mixing waters (Reynolds et al. 1984).

Chl *a* and PC were greatest at the surface of the calm treatments. This would indicate a *Microcystis* surface bloom. Within these surface blooms, *Microcystis* was most abundant in the high turbidity and high nutrient treatments (Fig. 3-7). While other combinations of nutrients and turbidity resulted in lower *Microcystis* growth. This would suggest that turbidity and nutrients are equally important in determining the magnitude of a *Microcystis* surface bloom, because high nutrients alone did not increase *Microcystis*. The high turbidity in the calm treatments allowed *Microcystis* to float to the surface to access light, while the green algae would have remained light-limited. *Microcystis* is a poor competitor for nutrients, and therefore would be expected to be out-competed for limiting nutrients (Tilman et al. 1986). *Microcystis* compensates for this by being highly competitive for light in calm light-limited waters. In this experiment, *Microcystis* was able to use the nutrients in the high turbidity treatment because green algal growth was prevented.

In western Lake Erie, the combination of high nutrients and high turbidity allows *Microcystis* to reach great biomasses. *Microcystis* is most abundant at middle sites where the ratio of photic depth to lake depth is lowest. Here, buoyant *Microcystis* has an advantage in the competition for light when the water column is calm. The hypothesis that the Middle region is best-suited for *Microcystis* growth is supported by results indicating that *Microcystis* in the Middle region had greater total protein content and less

phosphorus deficiency than *Microcystis* in the offshore, and that high turbidity in the Middle region is photo-protective (Chapter 2). These measurements show that *Microcystis* at the middle sites are healthier than that of the offshore and explain why *Microcystis* is most dense in these regions of western Lake Erie.

Management efforts that seek to suppress *Microcystis* blooms could potentially take the approach of changing the conditions of the middle regions of western Lake Erie need to be more like that of the offshore, where light and nutrient stress is the greatest. Best-management land-use practices for agriculture in the Maumee River watershed can target the loading of suspended sediments and nutrients for terrestrial sources. These would include no-tillage and conservation tillage, buffer strips along ditches, and remediation of wetlands. Although these practices will be beneficial in the long term for reducing *Microcystis* blooms, there may be a lag-time before improvements are observed because there may be a high rate of internal loading of suspended sediments and nutrients from the lake sediments (Visser et al. 2005).

The bloom of 2008 started in clear water during late July (Chapter 2). TP concentrations in western Lake Erie were high in early July (Fig. 3-3), while the water column was still clear. *Microcystis* biomass increased with turbidity (Chapter 2) that also corresponded to an increase of TP (Fig. 3-3). Therefore, because *Microcystis* first appeared in clear water with high TP, then TP might be a better predictor to when a bloom will occur. The magnitude of the bloom might be best predicted based on the combination of turbidity and TP, because in the laboratory experiment, Microcystis biomass was greatest in the high turbidity and high nutrient treatment. Hence, if sediment loads are reduced, *Microcystis* may not be able to take advantage of the high

concentration of nutrients because sinking phytoplankton would have access to light in clear water and out-compete *Microcystis* for phosphorus.

Literature Cited

Alpine, A. E., and J. E. Cloern. 1988. Phytoplankton growth-rates in a light-limited environment, San Francisco bay. Marine Ecology-Progress Series **44:** 167-173.

Baker, D.B., and Richards, R.P. 2002. Phosphorus budgets and riverine phosphorus export in northwestern Ohio watersheds. Journal of Environmental Quality **31**(1): 96-108.

Baldia, S.F., Evangelista, A.D., Aralar, E.V., and Santiago, A.E. 2007. Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines. Journal of Applied Ecology. **19**(6): 607-613.

Brittain, S.M., Wang, J., Babcock-Jackson, L., Carmichael, W.W., Rinehart, K.L., and Culver, D.A. 2000. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. Journal of Great Lakes Research **26**(3): 241-249.

Chaffin, J. D., Bridgeman, T. B. and Filbrun, J. E. 2008. Quantification of *Microcystis* sp. blooms in western Lake Erie (2002-2007) and relation to tributary flow. Conference presentation. International Association of Great Lakes Research. Petersburg Ontario, Canada.

Conroy, J.D., Kane, D.D., Dolan, D.M., Edwards, W.J., Charlton, M.N., and Culver, D.A. 2005. Temporal trends in Lake Erie plankton biomass: Roles of external phosphorus loading and dreissenid mussels. Journal of Great Lakes Research **31**: 89-110.

Downing, J.A., Watson, S.B., and McCauley, E. 2001. Predicting Cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences **58**(10): 1905-1908.

Ducobu, H., Huisman, J., Jonker, R.R., and Mur, L.R. 1998. Competition between a prochlorophyte and a cyanobacterium under various phosphorus regimes: Comparison with the Droop model. Journal of Phycology **34**(3): 467-476.

Dyble, J., Fahnenstiel, G.L., Litaker, R.W., Millie, D.F., and Tester, P.A. 2008. Microcystin concentrations and genetic diversity of Microcystis in the lower Great Lakes. Environmental Toxicology **23**(4): 507-516.

Guillard, R. R. L. and Lorenzen, C. J. 1972. Yellow-green algae with chlorophyllide *c*. Journal of Phycology. **8**:10-14

Healey, F.P. and Hendzel, L.L. 1975. Indicators of phosphorus and nitrogen deficiency in five algae cultures. Canadian Journal of Fisheries and Aquatic Sciences **36**: 1364-1369.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M.H., and Sommeijer, B. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology **85**(11): 2960-2970.

Huisman, J., van Oostveen, P., and Weissing, F.J. 1999. Species dynamics in phytoplankton blooms: Incomplete mixing and competition for light. American Naturalist **154**(1): 46-68.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J., and Crayton, M. 2000. Environmental factors associated with a toxic bloom of Microcystis aeruginosa. Canadian Journal of Fisheries and Aquatic Sciences **57**(1): 231-240.

Makarewicz, J.C. 1993. Phytoplankton biomass and species composition in Lake Erie, 1970 to 1987. Journal of Great Lakes Research **19**(2): 258-274.

Mishra, S., Heckathorn, S.A., Barua, D., Wang, D., Joshi, P., Hamilton, E.W., and Frantz, J. 2008. Interactive Effects of Elevated CO2 and Ozone on Leaf Thermotolerance in Field-Grown Glycine max. Journal of Integrative Plant Biology **50**(11): 1396-1405.

Moorhead, D., T. B. Bridgeman, and J. Morris. 2008. Changes in water quality of Maumee Bay 1928 – 2003. *In*: Checking the pulse of Lake Erie. Editors Munawar, M and Heath, R. Goodword Books.

Nalewajko, C. and T. P. Murphy. 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan: an experimental approach. Limnology **2**:45-48.

Oliver, R.L., and Walsby, A.E. 1984. Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena flos-aqua* (Cyanobacteria). Limnology and Oceanography **29**(4): 879-886.

Peterson, G.L. 1977. A simplification of the protein assay of Lowry *et al*. which is generally applicable. Analytical Biochemistry. **83**(2): 346-356.

Post. A. F. 2005. Nitrogen limitation in cyanobacteria. *In* Harmful Cyanobacteria. Editors Huisman, J., Matthijs, H. C. P., and Visser, P. M. Springer Press. Netherlands.

Reynolds, C.S., Wiseman, S.W., and Clarke, M.J.O. 1984. Growth-rate and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. Journal of Applied Ecology **21**(1): 11-39.

Richards, R.P., Baker, D.B., Crumirine, J.P., Kramer, J.W., Ewing, D.E., and Merryfield, B.J. 2008. Thirty-year trends in suspended sediment in seven Lake Erie tributaries. Journal of Environmental Quality **37**(5): 1894-1908.

Rinta-Kanto, J.M., Ouellette, A.J.A., Boyer, G.L., Twiss, M.R., Bridgeman, T.B., and Wilhelm, S.W. 2005. Quantification of toxic Microcystis spp. during the 2003 and

2004 blooms in western Lake Erie using quantitative real-time PCR. Environmental Science & Technology **39**(11): 4198-4205.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. Science **221**(4611): 669-671.

Søndergaard, M., Jensen, J.P., and Jeppesen, E. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia **506**(1-3): 135-145.

Tilman, D., Kiesling, R., Sterner, R., Kilham, S.S., and Johnson, F.A. 1986. Green, bluegreen and diatom algae - Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archiv Fur Hydrobiologie **106**(4): 473-485.

Tsukada, H., Tsujimura, S., and Nakahara, H. 2006. Effect of nutrient availability on the C, N, and P elemental ratios in the cyanobacterium *Microcystis aeruginosa*. Limnology **7**(3): 185-192.

Twiss, M.R., Gouvea, S.P., Bourbonniere, R.A., McKay, R.M.L., and Wilhelm, S.W. 2005. Field investigations of trace metal effects on Lake Erie phytoplankton productivity. Journal of Great Lakes Research **31**: 168-179.

Visser, P. M., Ibelings, B. W., Mur, L. R., and Walsby, A. E. 2005. Ecophysiology of *Microcystis*. *In* Harmful Cyanobacteria. Editors Huisman, J., Matthijs, H. C. P., and Visser, P. M. Springer Press. Netherlands

Walsby, A.E. 1994. Gas vesicles. Microbiological Reviews **58**(1): 94-144.

Tables

Table 3-1. Criterion for algal nutrient deficiency based on atomic ratios (Healey and Hendzel 1975).

Nutrient	Atomic Ratio	No Deficiency	Moderate Deficiency	Extreme Deficiency
Nitrogen	C:N	< 8.3	8.3-14.6	> 14.6
Phosphorus	C:P	< 129	129-258	> 258

Table 3-2. Average and maximum values of *Microcystis* biovolume (ml m^{-2}) recorded at six sites in western Lake Erie from 2002- 2008. Number of plankton samples collected from July 1 to October 15 is in parenthesis next to the mean. * "Too much *Microcystis* to collect" was recorded on data sheets for 8M during 2003.

Year	Site	**MB20**	**MB18**	**8M**	**7M**	**GR1**	**4P**
2002	Mean	1.5 (1)	10.7 (3)	16.6 (4)	28.6 (7)	6.3 (4)	9.0 (6)
	Max.	1.5	16.6	63.3	67.3	19.1	20.4
2003	Mean	20.4 (1)	0.0 (2)	58.7 (3)	218.8 (7)	53.8 (5)	185.4 (7)
	Max.	20.4	0.0	173.5*	892.9	119.9	632.7
2004	Mean	10.4 (7)	35.2 (7)	177.9 (7)	126.5 (7)	49.5 (7)	22.8 (7)
	Max.	23.0	58.7	332.1	311.2	105.9	74.0
2005	Mean	4.7 (6)	11.4 (6)	87.5 (5)	59.2 (7)	9.1 (5)	6.4 (7)
	Max.	10.2	25.5	324.0	188.8	21.7	20.4
2006	Mean	3.2 (5)	17.6 (5)	57.3 (5)	42.6 (5)	54.0 (5)	63.5 (5)
	Max.	7.7	40.8	149.2	125.0	118.6	176.0
2007	Mean	12.6 (5)	38.1 (5)	99.0 (5)	17.9 (5)	26.4 (5)	11.2 (6)
	Max.	51.8	135.2	267.9	42.1	89.3	16.6
2008	Mean	104.1 (6)	122.2 (7)	224.6 (7)	206.2 (7)	157.9 (7)	252.3 (7)
	Max.	295.9	229.6	591.8	413.3	359.7	665.8

Table 3-3. Pigment concentration (μg L^{-1}) of the experiment. Values are mean (± SE) n=3.

Depth	Mixing	Turbidity	Nutrients	Chlorophyll *a*	Phycocyanin	Chlorophyll *b*
Surface	No	High	High	33.22 (2.43)	9.89 (0.60)	3.73 (1.98)
Surface	No	High	Low	23.46 (1.62)	4.55 (2.67)	4.10 (0.99)
Surface	No	Low	High	27.81 (0.15)	5.97 (0.29)	3.08 (0.48)
Surface	No	Low	Low	26.17 (3.63)	5.48 (1.03)	2.90 (0.70)
Surface	Yes	High	High	15.42 (0.12)	1.47 (0.55)	3.68 (0.49)
Surface	Yes	High	Low	15.56 (0.71)	1.04 (0.36)	3.61 (0.38)
Surface	Yes	Low	High	17.76 (1.18)	1.19 (0.30)	3.87 (0.23)
Surface	Yes	Low	Low	17.18 (0.68)	1.37 (0.40)	3.88 (0.34)
70 cm	No	High	High	16.12 (0.76)	0.73 (0.19)	2.80 (1.29)
70 cm	No	High	Low	16.97 (0.88)	0.41 (0.07)	3.13 (0.54)
70 cm	No	Low	High	18.92 (0.96)	1.27 (0.61)	3.85 (0.72)
70 cm	No	Low	Low	19.08 (0.77)	1.38 (0.56)	3.83 (0.71)
70 cm	Yes	High	High	16.52 (0.46)	1.01 (0.37)	2.72 (0.93)
70 cm	Yes	High	Low	15.58 (0.77)	0.49 (0.04)	2.56 (0.95)
70 cm	Yes	Low	High	18.85 (0.99)	0.99 (0.15)	3.63 (0.33)
70 cm	Yes	Low	Low	19.30 (1.33)	0.96 (0.10)	4.10 (0.82)

Table 3-4. *P* values for pigment concentration. Bold values indicate significance at α = 0.05.

Source	Chl *a*	PC	Chl *b*
Depth (D)	**<.0001**	**<.0001**	0.5174
Turbidity (T)	**0.0344**	0.7613	0.4137
Mixing (M)	**<.0001**	**<.0001**	0.8518
Nutrients (N)	**0.0488**	**0.0395**	0.8216
D*T	0.0885	0.1338	0.1125
D*M	**<.0001**	**<.0001**	0.6000
D*N	**0.0324**	0.1051	0.8853
T*M	0.1660	0.5369	0.3839
T*N	0.1525	0.0543	0.9606
M*N	0.0966	0.1089	0.9421
D*T*M	0.3237	0.2073	0.6380
D*T*N	0.2333	0.1644	0.8277
D*M*N	**0.0311**	0.0739	0.9375
T*M*N	0.2293	0.1988	0.6421
D*T*M*N	0.0568	0.1872	0.9160

Figures

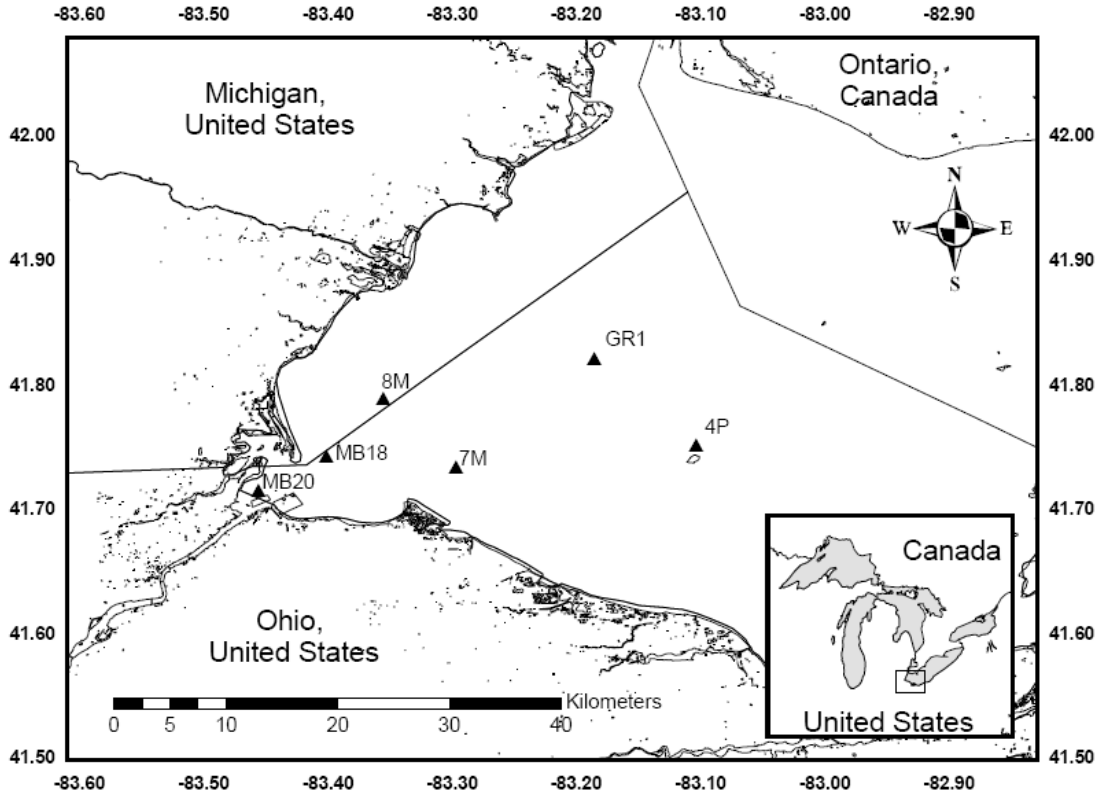

Figure 3-1. Six sample sites in western Lake Erie

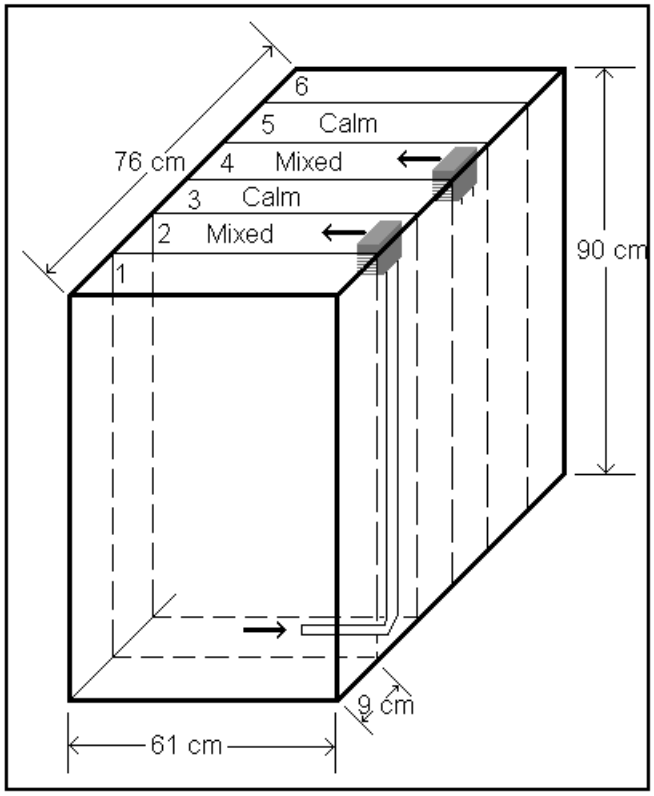

Figure 3-2. Diagram of experimental chambers of the 2x2x2x2 *Microcystis* growth experiment. Arrows depict the flow of circulating water of mixed chambers.

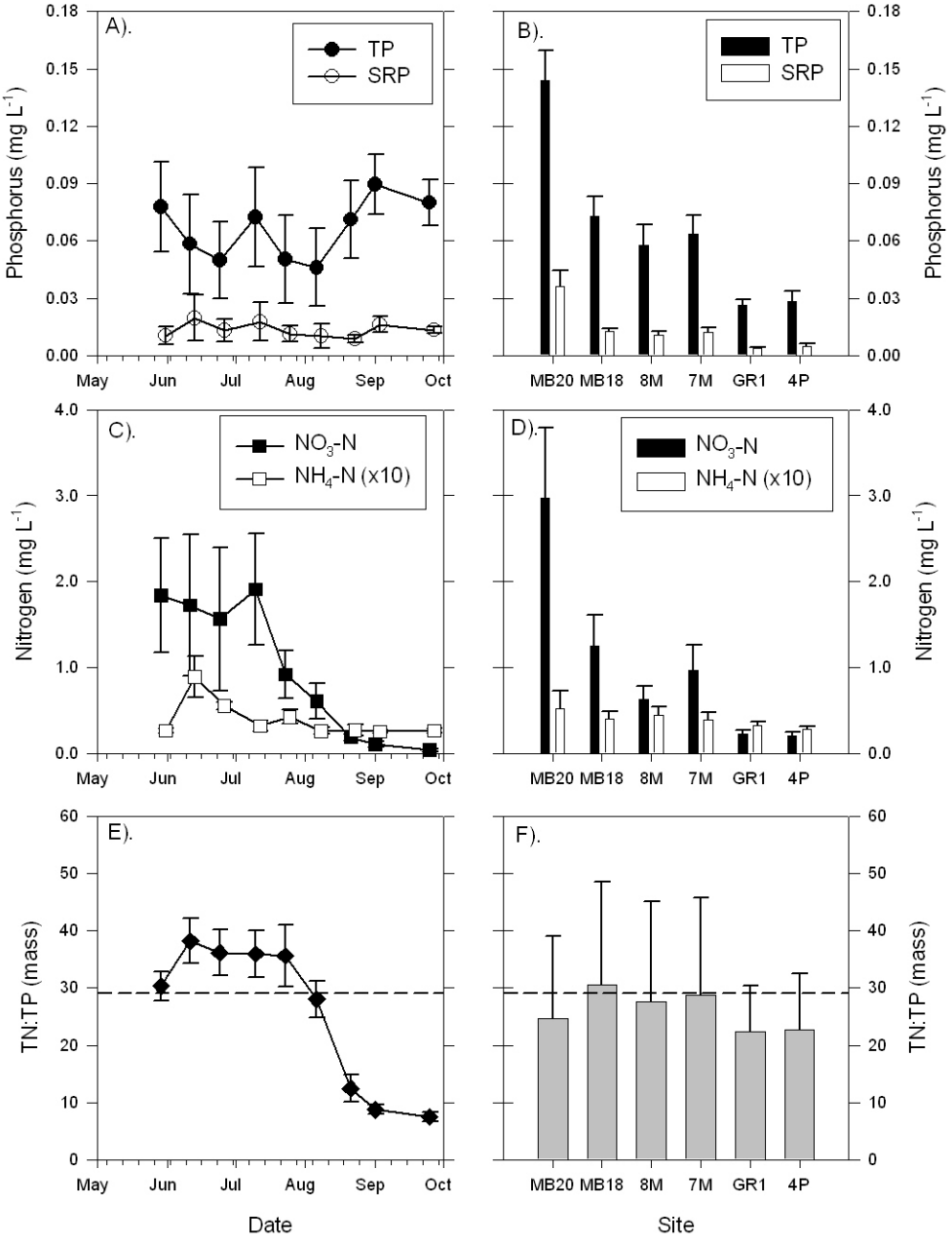

Figure 3-3. Phosphorus and nitrogen concentrations of western Lake Erie during 2008. A, C, and E are mean ± s.e for date from all sample sites. B, D, and F are sample site mean ± s.e for all sampling dates during 2008. Error bars of A and C are off-set for ease in viewing. A & B) Total Phosphorus (TP; filled circles, filled bars) and soluble reactive phosphorus (SRP; open circle, open bars). C & D) Nitrate (NO_3-N; filled squares, filled

bars) and Ammonium (NH_4-N; open triangles, open bars). Note NH_4-N values on D are x10. E & F) Total nitrogen: Total phosphorus ratio (by mass), dashed line represents 29, the value below which nitrogen-fixing species become favored (Smith 1983).

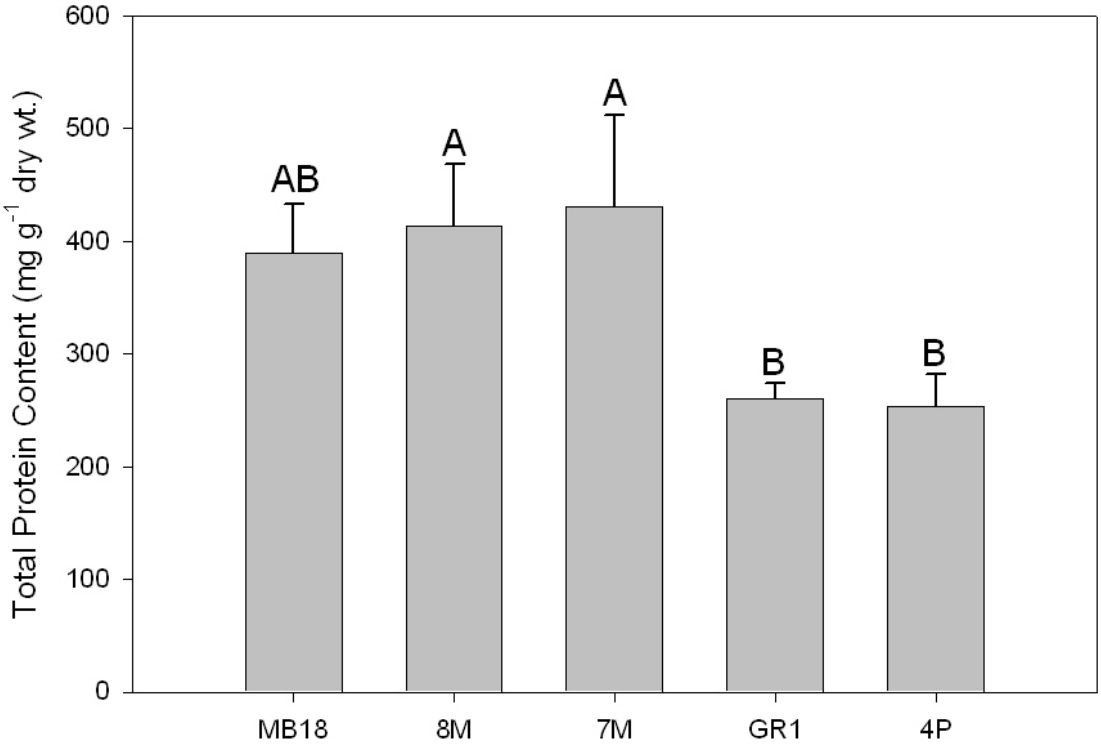

Figure 3-4. Higher total protein content (dry mass basis) of *Microcystis* collected from nearshore waters of western Lake Erie indicates greater cellular health than the offshore. Letters represent groups of significant difference based on Tukey test ($\alpha = 0.05$). Sites are arranged with increasing distance from the Maumee River.

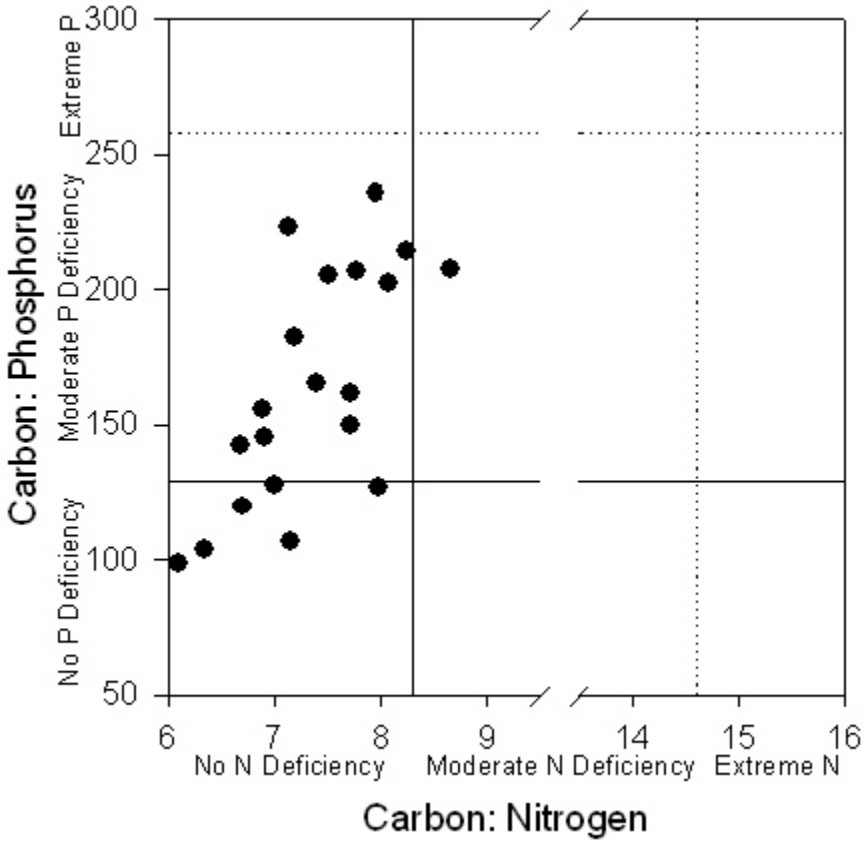

Figure 3-5. Nutrient content of *Microcystis* as atomic ratios of carbon to nitrogen and carbon to phosphorus. Bold line corresponds to the boundary of no deficiency and moderate deficiency and dotted lines the boundary of moderate and extreme deficiency. Vertical lines represent nitrogen and horizontal lines for phosphorus. Only one *Microcystis* sample was slightly nitrogen deficient, while phosphorus deficiency was seen frequently. Criterion for deficiency is given in Table 3-1.

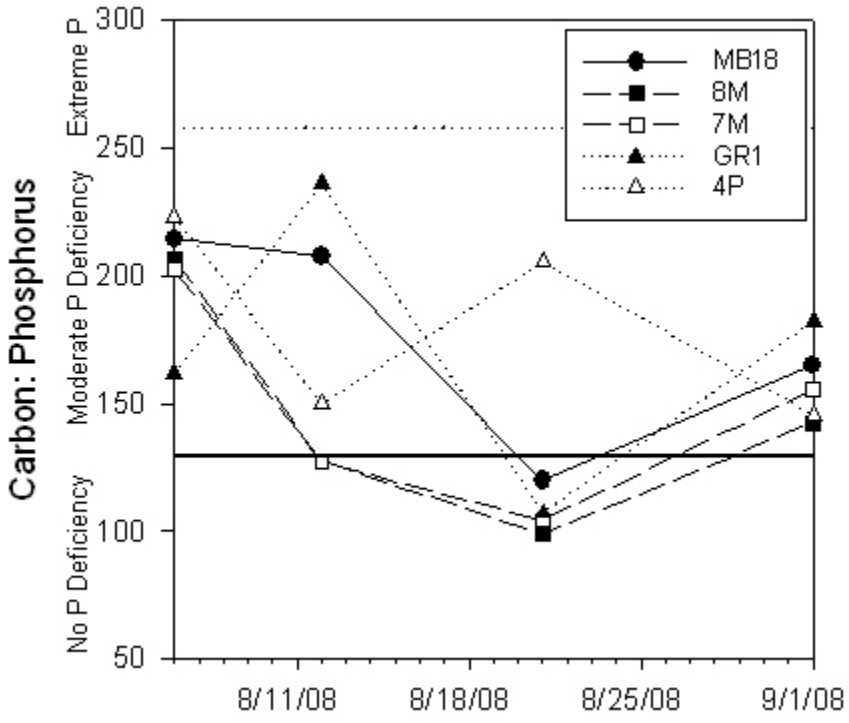

Figure 3-6. Phosphorus deficiency in *Microcystis* in western Lake Erie.

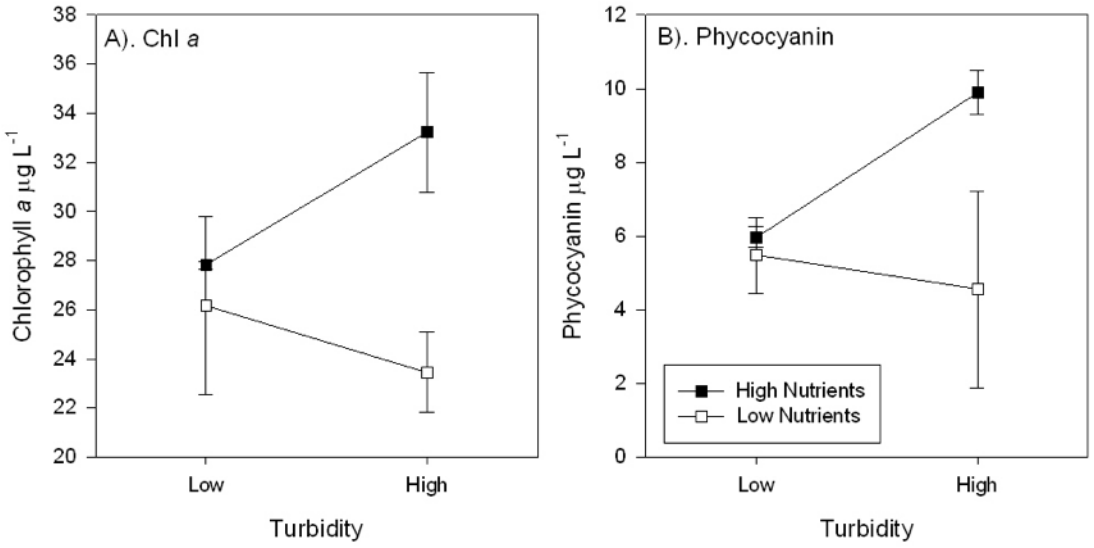

Figure 3-7. Chlorophyll *a* (A) and phycocyanin (B) concentration at the surface of the calm treatment. At low turbidity, nutrient concentration did not have an effect, but high turbidity increased *Microcystis* growth.

Chapter Four

Conclusions

Buoyancy gives *Microcystis* an ecological advantage in calm turbid water because it is able to access light and shade sinking phytoplankton. However, this ecological advantage comes at a physiological price because *Microcystis* collected from calm water had more photosynthetic damage than *Microcystis* collected from mixed water. Thus, there is an eco-physiological trade-off for being highly competitive for light and photosynthetic damage. My field measurements suggest that sediment plumes, generated by rivers or lake bottom resuspension, mitigate photo-inhibitation. Further, *Microcystis* also had a lower content of carotenoids in turbid water, which further supports that turbidity creates a favorable light environment. *Microcystis* was also able to adjust light harvesting pigments in turbid water.

Microcystis blooms of western Lake Erie are an annual occurrence. The spatial pattern of these blooms closely follows the extant of the sediment plume of the Maumee Bay and adjacent waters. This suggests that conditions with in these plumes favors rapid *Microcystis* growth. Total protein content was highest in regions of the lake that are often in the sediment plume and indicates greater health in these turbid waters. Turbidity of the plume gives *Microcystis* a competitive edge over sinking phytoplankton and increases the photosynthetic efficiency. Also, nutrient concentrations of the sediment

plumes are very high. No nitrogen deficiency was observed in *Microcystis* while frequent phosphorus deficiencies were measured.

Goal of management should be to lessen the magnitude of *Microcystis* blooms. Efforts to reduce sediment loading into lakes would increase light stress for *Microcystis* and slow its growth. In the laboratory experiment, *Microcystis* growth was similar among nutrient concentrations at low turbidity- *Microcystis* only took advantage of high nutrients in high turbidity treatment. Further reducing sediment loading would concomitantly reduce nutrient loading and would increase the severity of phosphorus deficiency of *Microcystis*. Best-management land-use practices and lessening summer dredging of shipping channels could be tools used to reach this goal.

Chapter Five

Detailed Methods and Supplemental Data

Experiment to Determine Photosynthetic Pigment Concentration

Introduction

There is no standard method for extraction of pigments from phytoplankton. For phycocyanin (PC), most methods rely on mechanical grinding with a mortar and pestle (Sampath-Wiley and Neefus 2007; Simis et al. 2007), sonication (Furuki et al. 2003; Patel et al. 2004; Downes and Hall. 1998), or freeze/ thaw cycles (Simis et al. 2007) using either tris or phosphate buffer. For chlorophyll, the method of Lorenzen (1967) uses grinding in acetone and reading absorbance at 665 nm. Other methods use strong organic solvents such as dimethylsulphoxide (DMSO) (Wellburn 1994; Seely et al. 1972) or N-N dimethylformamide (DMF) (Speziale et al. 1984). After extraction, then absorbance spectra are read and chlorophyll concentration is calculated from equations that use several wavelengths, or chlorophyll fluorescence is read and concentration calculated using a standard curve. Absorbance allows for calculation of all chlorophylls and carotenoids from one extraction, while fluorescence only allows for calculation of one pigment at a time. Because of the ambiguities for phycocyanin extractions and potential differences between the chlorophyll quantification, I examined different methods to quantify phycocyanin and compared our laboratory's method of chlorophyll

quantification which uses DMF and fluorescence (Speziale et al. 1984) to a method that uses DMSO and absorbance.

Phycocyanin

There is a wide variety of published methods for the extraction of PC from cyanobacteria and red algae. Most methods rely on freeze/thaw cycles, grinding with mortar and pestle, or sonication in Tris or Sodium phosphate buffers. Michael McKay of Bowling Green State University provided me with their lab protocol that uses sonication to break cells walls. However, there is uncertainty as to the duration needed to sonicate samples. One study (Furuki et al. 2003) stated that more PC is extracted with increased length of sonication, while another (Downes and Hall 1998) showed that PC is damaged by prolonged sonication. To address this, an experiment using cultured *Microcystis* was done using two buffers (0.05 M Tris pH 7.0 or 0.1 M Sodium phosphate pH 6.8) and two types of extraction (mortar and pestle or sonication of several lengths (1, 5, 10, 15, 20, and 40 minutes)) to determine the best protocol for extracting PC from my samples. *Microcystis* was grown in WC liquid medium (Guillard and Lorenzen 1972) under ~50 µmol m^{-2} s^{-1} on 12:12 hour light/dark cycles in 2 L jars. One culture jar was selected for this experiment. The jar was capped and *Microcystis* mixed thoroughly by turning it over several times before each aliquot was taken. 100 ml were filtered onto 25 cm GF/F filters. This process would insure that all filters contained the same amount of *Microcystis* and therefore PC. Filters were then stored at -80° C as above. Half of the samples would be used for Tris buffer and the other half for phosphate. Each buffer x sonication length combination length was replicated with 3 separate filters. For grinding with mortar and pestle, 12 filters were ground in one of the two buffers, transferred to plastic screw-top

tubes, and filled to 10 ml with buffer and incubated at 4° C for 60 minutes. Samples where then centrifuged for 10 minutes at 3,800 rpm and PC fluorescence was recorded in a 10-AU Turner Design fluorometer with P/N 103-80 filters. For sonication, filters were placed in 5 ml of buffer in polystyrene screw-top tubes and sonicated for the length of time. After sonication, samples were then filled to 10 ml and incubated at 4° C for 60 minutes (Michael McKay, BGSU). Samples were then centrifuged for 10 minutes at 3,800 rpm and then PC fluorescence was recorded. Further, the absorbance spectrum was read for these samples to determine pureness of extract.

Grinding filtered sample by mortar and pestle gave higher fluorescence values than sonication (Fig. 5-1), but this was a result of the cloudiness of the solution. Therefore, grinding was eliminated as a choice for PC extraction. For sonication, Tris gave higher fluorescence readings (Fig. 5-2), but calculated concentration of PC was similar between the two types of buffer. PC fluorescence increased with sonication length up to 10 minutes and was similar at 15 and 20 minutes, and then fluorescence was less at 40 minutes (Fig. 5-2). Because fluorescence was constant between 10 and 20 minutes, 15 minutes was selected. The absorbance spectra for Tris had peaks at 619 nm (PC) and 665 nm (Chl *a*), whilst phosphate buffer only had the 619 nm peak (Fig. 5-3). This indicates that phosphate buffer had a high purity of PC than Tris. Both buffers contained caroteniods. The phosphate buffer was selected because of the PC purity and because the 10 mg ml^{-1} C-PC standard was in a solution of sodium phosphate. Standard curve of known concentration *vs*. PC fluorescence was linear through the range of the machine.

Chlorophyll *a*

Because chl *a* is extracted in N-N dimethylformamide (DMF) as a part of an on-going lake monitoring program, I compared DMF to DMSO extractions (as above chapters; Wellburn 1994) for 60 samples. Same method of collecting, filtering, and storage of was used between the two extraction types. Filters were placed in polyethylene screw-top tubes with 4 ml of DMF and stored at -20° C overnight. The following day, samples were heated to 70° C for 15 minutes then filled to 10 ml (Speziale et al. 1984). Samples were then centrifuged at 2,000 rpm for 10 minutes (Speziale et al. 1984). Chl *a* fluorescence was read using the Turner Design fluorometer with P/N 10-037R. An acid adjustment was used with two drops of 2 N HCl and fluorescence read. Chl *a* was quantified using a standard curve.

Chl *a* extractions from 60 samples were compared by using a DMSO with absorbance and DMF with fluorescence measured. No difference was seen between the two methods for extracting chl *a* from phytoplankton on filters (Fig. 5-4). The two methods were highly correlated when concentrations measured are plotted *vs*. each other ($r^2 = 0.93$).

In this experiment, I determined the best method of extraction of phycocyanin from *Microcystis* collected from Lake Erie. This method relies on sonication that is less labor-intensive than mechanical grinding with mortar and pestle, also sonication is less time consuming as compared to freeze/ thaw cycles. This method was used to extract PC from *Microcystis* taken from Lake Erie. For chl *a*, the two methods gave very similar results. I used the DMSO method because it allows for determination of several pigments from one extraction. The DMF method was an establish protocol as part of an

on-going monitoring method. Therefore, the similar between the two methods gives confidence that the chl *a* values obtained from DMSO are correct.

Phytoplankton Community of Western Lake Erie 2007-2008

The 2007 sampling season began in early July during a *Microcystis* bloom. Chl *a* and PC were 18.73 and 51.93 µg L^{-1} respectively at site 8M surface. *Microcystis* and other phytoplankton decreased throughout the summer. August and September had low chl a and low PC. A second *Microcystis* bloom was seen in October. Chl *a* and PC were 101.8 and 178.7 µg L^{-1} respectively at site 8M surface. The left side of Figure 5-5 gives chl *a* isopleths for sites 8M and MB20 during 2007. PC isopleths for sites 8M and MB20 are shown in Figure 5-6.

2008 sampling started with high chl *a* concentration on May 29 at sites MB20 (107.9 µg L^{-1}) and 7M (32.2 µg L^{-1}). Chl *c* to chl *b* ratios greater than 7.0 would indicate that this algal bloom was diatoms (*Aulacoseira* sp). By mid June, for all sites except MB20, chl *a* dropped to less than 12 µg L^{-1}, and further dropped to values less 4.0 µg L^{-1}on June 24. Phycocyanin (PC) values were less than 0.1 µg L^{-1} through June. A second *Aulacoseira* bloom was seen on July 10 with highest chl *a* at 7M (47.7 µg L^{-1}). Sparse *Microcystis* was first collected in plankton tow on July 24, but PC was low ranging from 0.07 to 0.38 µg L^{-1}. Diatoms were still most abundant taxon but chl *a* decreased to less than 10 µg L^{-1}. *Microcystis* was present in the lake and it was concentrated at the surface on August 6 and 12. Greatest surface values of chl *a* and PC were 112.5 and 245.6 µg L^{-1}, respectively, during these two dates. *Microcystis* biomass collected in plankton tows was very high on August 21 and remained high for the rest of sampling season, but strong

mixing of water column evenly distributed *Microcystis*. Strong mixing resulted in much lower concentrations of chl *a* and PC at the surface. Highest chl *a* and PC at the surface was 35.7 and 33.2 µg L^{-1}, respectively, during strong mixing. Calmness was restored September 25 and *Microcystis* concentrated on the surface. Highest chl *a* was 1100.9 µg $L^{-1}$and PC was 418.2 µg L^{-1}on the surface at 8M. The right half of Figure 5-5 show the chl *a* isopleths for sites 8M and MB20 for 2008. Phycocyanin isopleths for sites 8M and MB20 are shown in Figure 5-6 respectively.

Complete Methods for Laboratory Experiment

Chlamydomonas (a common unicellular green alga) and *Microcystis* were grown separately in WC liquid medium (Guillard and Lorenzen 1972) under ~50 µmol m^{-2} s^{-1} on 12:12 hour light/dark cycles. *Chlamydomonas* was aerated while *Microcystis* was not. *Chlamydomonas* and *Microcystis* were initially collected from Lake Erie. Equal amounts of *Chlamydomonas* and *Microcystis* were used in this experiment, based on content of chl *a*. *Chlamydomonas* and *Microcystis* were added so that they each had a initial chlorophyll chl *a* of 2.5 µg L^{-1}, making the total initial concentration 5.0 µg L^{-1} (determined in DMSO). Phytoplankton intended for the experiment was grown in liquid culture with the nutrient concentration of the low treatment level for two weeks before used in the experiment to insure that internal phosphorus (P) storage was not accumulated.

A 2x2x2x2 factorial experiment was used to test the effects of nutrient concentration (low and high nutrients), turbidity (low and high), mixing (mixed or non-mixing) and sample depth (surface and at depth) on photosynthetic efficiency and pigments. Experimental tanks were constructed out of 61x76x90 cm (228 L)

polyethylene (PE) bins. Bins were divided into six 61x9x90 cm (36.5 L) chambers using black sheets of Acrylonitrile-Butadiene-Styrene (ABS) board (Fig. 3-1). PE foam was inserted along the edges of the ABS board to fill the space created by the expanding walls upon adding water. Outside walls of the PE bins were supported in a wooden frame to minimize expansion. Experiments were conducted in a greenhouse and exposed to natural sunlight (~1,500 µmol m^{-2} s^{-1}) at ambient temperature (25 – 28 °C).

Tap water enriched with nutrient was used to replicate lake water. Because tap water P concentration was greater than what was intended for the low nutrient level, excess P was precipitated with 30 mg L^{-1} Alum (Aluminum sulfate, Toledo Pools, Toledo, Ohio). Water intended for experimental use was held in 121 L bins. Water was aerated for at least 24 hours then alum was added and aeration was turned off to allow precipitation. The following day, flock was siphoned from the bottom of the bins. Treatment of tap water with alum decreased P to 0.01 mg L^{-1}. Alum treated water and non-treated water grew algae equally in culture.

Mixing of the chamber was achieved using powerhead pumps (Aquagardens #601) so that the intake hose was placed at the bottom of the chamber and outflow just beneath the surface (Fig. 1). Sieved Lake Erie sediments (400 µm) were added to create turbidity. High turbidity was achieved by adding 0.55 ml L^{-1} sediments (~30 NTU) and 0.02 ml L^{-1} for low turbidity (~1 NTU). High nutrient level included 3.0 mg L^{-1} nitrogen (N) and 0.15 mg L^{-1} P, and low level was 0.6 mg L^{-1} N and 0.03 mg L^{-1} P. The N:P was 45 in both levels and all other nutrient concentration were the same in each level. All other nutrients were half concentration of the WC media. Twiss et al. (2005) enriched

Lake Erie water with micro-nutrients to a final concentration similar of the WC growth media and observed no additional phytoplankton growth.

Once treatments were set up and phytoplankton added, 96 hours were allowed for growth. (An initial trial ran for 168 hours and twice as much initial alga resulted in similar light levels between the high and low turbidity treatments.) Following the 96 hours, samples were collected at the surface and 70 cm. The 70 cm sample represents the 20% of surface light in the low turbidity level and < 0.5 % in the highly turbid treatment. Phytoplankton (100ml) was filtered onto GF/F filters and Φ_{et} was measured. Samples were dark adapted for 30 minutes then used to determine F_v/F_m. Φ_{et} and F_v/F_m were determined using an OS1-FL Opti-Sciences modulated fluorometer in triplicate. Filters were stored on silica gel at -80 ° C for pigment concentration analysizes. Chambers 1 and 6 were not analysized because of white outside wall resulted in higher light intensity than chambers 2 through 5 that had black walls. Light was recorded after all samples were collected. Photosynthetic measurements and light was recorded between 12:00 pm and 2:00 pm on sunny days.

Chrolophyll (chl) *a* was used as a surrogate for total algal biomass. Chls *a* and *b* and carotenoids were extracted in DMSO following methods in chapter 2 and concentration of pigments calculated using Wellburn 1994. Phycocyanin (PC) was extracted in sodium phosphate buffer using sonication and fluorescence following the methods of chapter 2.

Four-way ANOVA was performed to test for the effect of mixing (mixed or calm), turbidity (high or low), nutrients (high or low), and sample depth (surface and at depth) on Φ_{et}, F_v/F_m, and pigment composition. PROC ANOVA of SAS was used.

Complete Methods for Protein Extraction and Quantification

Collection and Separation

Microcystis was collected from five sites over the entire water column from five sites in western Lake Erie using a 64 μm mesh plankton net in western Lake Erie during July through September 2008 (Fig. 2-1). An additional site (MB20) was sampled for nutrients but *Microcystis* was not abundant enough to collect for physiological measurements. *Microcystis* colonies retained in the net were concentrated and stored in dark polyethylene bottles during transportation back to the laboratory. Depending on sample location, two to six hours passed between collection on the lake and laboratory analysis. Upon arriving to the laboratory, the *Microcystis* sample was added to a 1L imhoff cone and diluted to 1,000 ml with tap water. This allows the *Microcystis* colonies to separate from the sinking diatoms and green algae via floatation (Chaffin et al. 2008). After 30 minutes, the settled phytoplankton was drawn out through the bottom of the cone and discarded. The sample was then diluted again to 1,000 ml with tap water and process repeated. *Microcystis* colonies were drawn out of the bottom of the cone and concentrated on a 35 μm mesh, transferred to 1.5 ml tubes and stored at -80 ° C until further analysis. Samples were also checked for the presence of other cyanobacteria by microscopy. *Anabaena* was very sparse relative to *Microcystis* colonies on July 24 and Aug. 6, and *Aphanizomenon* was not seen in samples.

Biochemistry

Proteins of *Microcystis* were extracted by grinding approximately 1 g of fresh weight tissue to a powder in liquid nitrogen using mortar and pestle then transfer to an extraction buffer containing 1% sodium dodecyl sulfate (SDS) detergent, 0.1 M Tris

buffer (pH = 8.0), 10% glycerol, 0.1% bromophenol-blue, 1% sucrose, protease inhibitors (1 mM pheynlmethylsulfonylfluoride (PMSF), 1 mM EDTA, 1 mM benzamidine, 10 mM leupeptin), a phenolic inhibitor (0.5% polyvinylpolypyrrolidone (PVPP)), and reductants (10 mM dithiothreitol (DTT), 0.1 M ascorbic acid) (Mishra et al. 2008). Sample was then centrifuged at 15,000 *g* for 10 minutes at 4 ° C. Supernatant containing soluble proteins was collected and total protein content was determined using the method of Peterson (1977). Breifly, proteins from a 20µl sample were precipitated with 20µl chilled trichloroacetic acid and incubated for 60 minutes at 4°. Sample was then centrifuged at 12,000 *g* for 10 minutes at 4° then supernatant decanted. 150µl of BioRads reagent A' was added to the protein pellet, votrexed and left for 5 minutes. Then 1,200µl of reagent B was mixed and incubated in the dark for 15 minutes at room temperature. Absorbance was measured at 750nm using the Shimadzu spectrophotometer and quantified using a standard curve of Bovine Serum Albumin and corrected for dry mass. Samples in extraction buffer were kept at -80° C until further analysises.

To prepare protein for gel separation and quantification of rubisco and sHsp, proteins were precipitated in 10x volume acetone for 60 minutes at -20° C, centrifuged at 15,000 g at 4° C, then resuspended over night at 4° C in a sample buffer containing 1% SDS, 100 mM Tris buffer (pH = 6.8), 10% glycerol, 0.1% bromophenol-blue, and 0.4% β-mercaptoethanol. Protein samples were boiled for 4 minutes to denature protein then centrifuged at 21,000 *g* for 30 seconds to remove debris. Then proteins were separated in 12.5% SDS-PAGE using 30 µg of protein in each well. For rubisco, gel was stained using Coomassie blue R-250. After de-staining, the gel was scanned and relative content of rubisco large subunit band (52 kDa) was quantified by color density determined using

computer software (Hewlett Packer Scanjet 8200). For sHsps, proteins were transferred to nitro-cellulose membranes by electroblotting, and sHsp were detected using protein specific antibodies (Heckathorn et al. 2002) following the protocol in Dumbroff and Gepstein (1993) then relative content was quantified. Also, *Microcystis* grown in culture was heat-shocked at 40°C for 45 minutes to induce expression of sHsp and used a positive control. A heat-shocked tomato plant was also used as a positive control.

Literature Cited

Chaffin, J. D., Bridgeman, T. B. and Filbrun, J. F. 2008. Quantification of *Microcystis* sp. blooms in western Lake Erie (2002-2007) and relation to tributary flow. Conference presentation. International Association of Great Lakes Research. Petersburg Ontario, Canada.

Downes, M.T., and Hall, J.A. 1998. A sensitive fluorometric technique for the measurement of phycobilin pigments and its application to the study of marine and freshwater picophytoplankton in oligotrophic environments. Journal of Applied Phycology **10**(4): 357-363.

Dumbroff, E.B. and Gepstein. S. 1993. Immunological methods for assessing protein expression in plants. *In*: Methods in plant molecular biology and biotechnology. Editors Glick, B.R. and Thompson, J.E. CRC Press.

Furuki, T., Maeda, S., Imajo, S., Hiroi, T., Amaya, T., Hirokawa, T., Ito, K., and Nozawa, H. 2003. Rapid and selective extraction of phycocyanin from *Spirulina*

platensis with ultrasonic cell disruption. Journal of Applied Phycology **15**(4): 319-324.

Guillard, R. R. L. and Lorenzen, C. J. 1972. Yellow-green algae with chlorophyllide *c*. Journal of Phycology. **8**:10-14

Heckathorn, S.A., Ryan, S.L., Baylis, J.A., Wang, D.F., Hamilton, E.W., Cundiff, L., and Luthe, D.S. 2002. In vivo evidence from an *Agrostis stolonifera* selection genotype that chloroplast small heat-shock proteins can protect photosystem II during heat stress. Functional Plant Biology **29**(8): 933-944.

Lorenzen. C. J. 1967. Determination of chlorophyll and pheo-pigments: Spectrophotometric equation. Limnology and Oceanography. **12**(2): 343-346

Mishra, S., Heckathorn, S.A., Barua, D., Wang, D., Joshi, P., Hamilton, E.W., and Frantz, J. 2008. Interactive Effects of Elevated CO2 and Ozone on Leaf Thermotolerance in Field-Grown *Glycine max*. Journal of Integrative Plant Biology **50**(11): 1396-1405.

Patel, A., Mishra, S., Pawar, R., and Ghosh, P.K. 2005. Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Expression and Purification **40**(2): 248-255.

Peterson, G.L. 1977. A simplification of the protein assay of Lowry *et al*. which is generally applicable. Analytical Biochemistry. **83**(2): 346-356.

Sampath-Wiley, P., and Neefus, C.D. 2007. An improved method for estimating R-phycoerythrin and R-phycocyanin contents from crude aqueous extracts of *Porphyra* (Bangiales, Rhodophyta). Journal of Applied Phycology **19**(2): 123-129.

Seely, G.R., Ducan, M.J., and Vidaver, W.E. 1972. Preparation and analytical extraction of pigments from brown algae with dimethyl sulfoxide. Marine Biology **12**(2): 184-188

Simis, S.G.H., Ruiz-Verdu, A., Dominguez-Gomez, J.A., Pena-Martinez, R., Peters, S.W.M., and Gons, H.J. 2007. Influence of phytoplankton pigment composition on remote sensing of cyanobacterial biomass. Remote Sensing of Environment **106**(4): 414-427.

Speziale, B.J., Schreiner, S.P., Giammatteo, P.A., and Schindler, J.E. 1984. Comparison of n,n-dimethylformamide, dimethylsulfoxide, and acetone for extraction of phytoplankton chlorophyll. Canadian Journal of Fisheries and Aquatic Sciences **41**(10): 1519-1522.

Twiss, M.R., Gouvea, S.P., Bourbonniere, R.A., McKay, R.M.L., and Wilhelm, S.W. 2005. Field investigations of trace metal effects on Lake Erie phytoplankton productivity. Journal of Great Lakes Research **31**: 168-179.

Wellburn, A.R. 1994. The spectral determination of chlorophyll *a* and chlorophyll *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology **144**(3): 307-313.

Tables

Table 5-1. Complete results of CHN auto-analyzer and ICP-OES for macronutrients of *Microcystis*. X is no sample. Units are mmol kg^{-1} dry weight.

Site	Date	C	H	N	P	K	Ca	Mg	S
MB18	8/6/08	40199.83	64484.13	4875.089	187.3762	204.0086	180.6329	96.8559	125.8127
8M	8/6/08	39400.50	65277.78	5074.946	190.5069	X	X	X	X
7M	8/6/08	40116.57	65674.60	4967.880	198.0701	187.9484	165.2320	87.2759	121.0384
GR1	8/6/08	38676.10	64781.75	5010.707	239.0595	207.2756	221.9695	98.6896	132.6011
4P	8/6/08	38193.17	64980.16	5353.319	171.1334	X	X	X	X
MB18	8/12/08	38984.18	64384.92	4503.926	187.6604	207.6130	209.4622	92.6090	127.7757
8M	8/12/08	38351.37	64087.30	5481.799	300.8221	223.8157	230.5105	115.5598	140.2096
7M	8/12/08	37993.34	64682.54	4760.885	298.8731	214.4505	192.5611	115.7123	125.6625
GR1	8/12/08	39883.43	66865.08	5017.844	169.0337	189.4191	230.6634	80.0826	132.3651
4P	8/12/08	40599.50	67658.73	5267.666	271.1472	203.9010	233.8579	107.0743	128.7941
MB18	8/21/08	39258.95	65376.98	5852.962	327.4039	251.3395	303.9398	143.3159	159.5722
8M	8/21/08	39883.43	67559.52	6538.187	403.5042	243.9975	297.4818	167.7147	164.2322
7M	8/21/08	38009.99	63392.86	5988.580	364.7349	239.2672	214.0160	143.7085	156.5344
GR1	8/21/08	40316.40	67857.14	5638.829	375.9265	262.6589	258.0593	150.6054	151.1651
4P	8/21/08	39641.97	67162.70	5274.804	192.9084	193.9238	216.2289	83.7546	128.5794
MB18	9/1/08	41557.04	67658.73	5617.416	251.5390	219.4427	168.4701	105.9121	139.3146
8M	9/1/08	41024.15	68055.56	6145.610	287.7663	218.0435	193.2873	121.9224	155.5820
7M	9/1/08	41407.16	68154.76	6009.993	266.3152	200.9868	177.2617	112.4765	149.7937
GR1	9/1/08	41290.59	67757.94	5738.758	226.4551	196.7534	196.4399	92.5325	143.1167
4P	9/1/08	40807.66	68353.17	5910.064	280.4537	214.3098	209.1951	123.2805	159.3954

Table 5-2. Complete results of ICP-OES for micronutrients of *Microcystis*. X is no sample, ND is not detected. Units are mmol kg^{-1} dry weight.

Site	Date	B	Cu	Fe	Mn	Zn	Mo	Ni	Si	Na
MB18	8/6/08	ND	0.182929	2.47391	ND	0.395610	0.012415	0.221543	8.41615	41.73772
8M	8/6/08	X	X	X	X	X	X	X	X	X
7M	8/6/08	ND	0.125030	2.83011	0.208328	0.230040	ND	0.186089	3.71091	X
GR1	8/6/08	ND	0.202007	4.92874	0.237495	1.089595	ND	0.292144	5.84379	X
4P	8/6/08	X	X	X	X	X	ND	X	X	X
MB18	8/12/08	0.133766	0.220538	3.24832	ND	0.433680	0.022608	0.239666	6.98909	29.15047
8M	8/12/08	0.166700	0.194015	4.04506	0.243409	1.275844	ND	0.203547	5.88170	X
7M	8/12/08	ND	0.213488	3.03084	0.249253	0.609931	ND	0.212730	3.41703	X
GR1	8/12/08	ND	0.251381	3.74728	0.244321	0.765566	ND	0.408157	3.30462	X
4P	8/12/08	ND	0.164166	3.62222	0.208883	0.443173	ND	0.311783	4.03143	X
MB18	8/21/08	ND	0.184300	4.29485	ND	0.362618	0.008952	0.260672	3.96385	38.96957
8M	8/21/08	ND	0.220981	6.16410	0.250055	0.458386	ND	0.300731	4.64075	X
7M	8/21/08	ND	0.168644	4.12658	0.252041	0.467108	ND	0.304513	4.05507	X
GR1	8/21/08	ND	0.171286	3.15061	0.062023	1.003227	0.006906	0.326477	7.67274	51.38244
4P	8/21/08	ND	0.227733	3.72437	0.278918	0.729182	0.014789	0.487375	5.41819	X
MB18	9/1/08	ND	0.166154	3.40311	ND	0.273489	0.005896	0.261749	4.03424	30.19428
8M	9/1/08	ND	0.205270	5.19271	0.251204	0.385313	ND	0.305917	6.59578	X
7M	9/1/08	ND	0.184323	3.81245	0.211490	0.438517	ND	0.267902	3.73625	X
GR1	9/1/08	ND	0.182473	3.50946	0.157873	0.514071	ND	0.283427	4.75584	X
4P	9/1/08	ND	0.317674	4.35632	0.191492	0.532758	ND	0.396330	5.26706	X

Figures

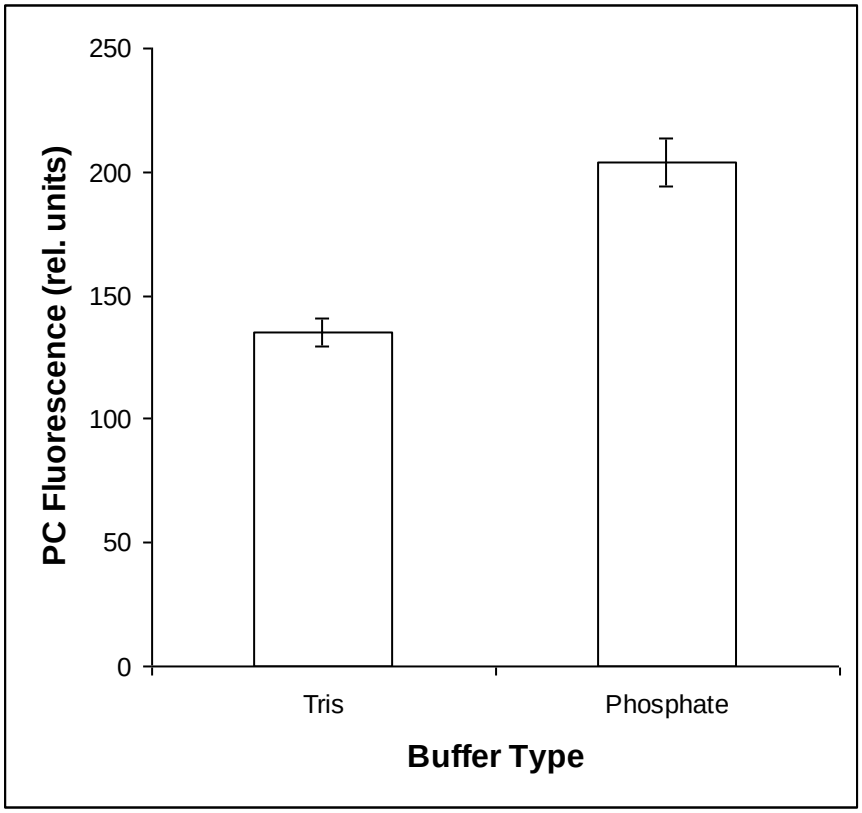

Figure 5-1. Comparison of 0.05 M Tris buffer pH 7.0 to 0.1M Sodium phosphate buffer pH 6.8 after grinding a filter by mortar and pestle. High fluorescence values are due to cloudiness of sample. Values are average of six filters ± SE.

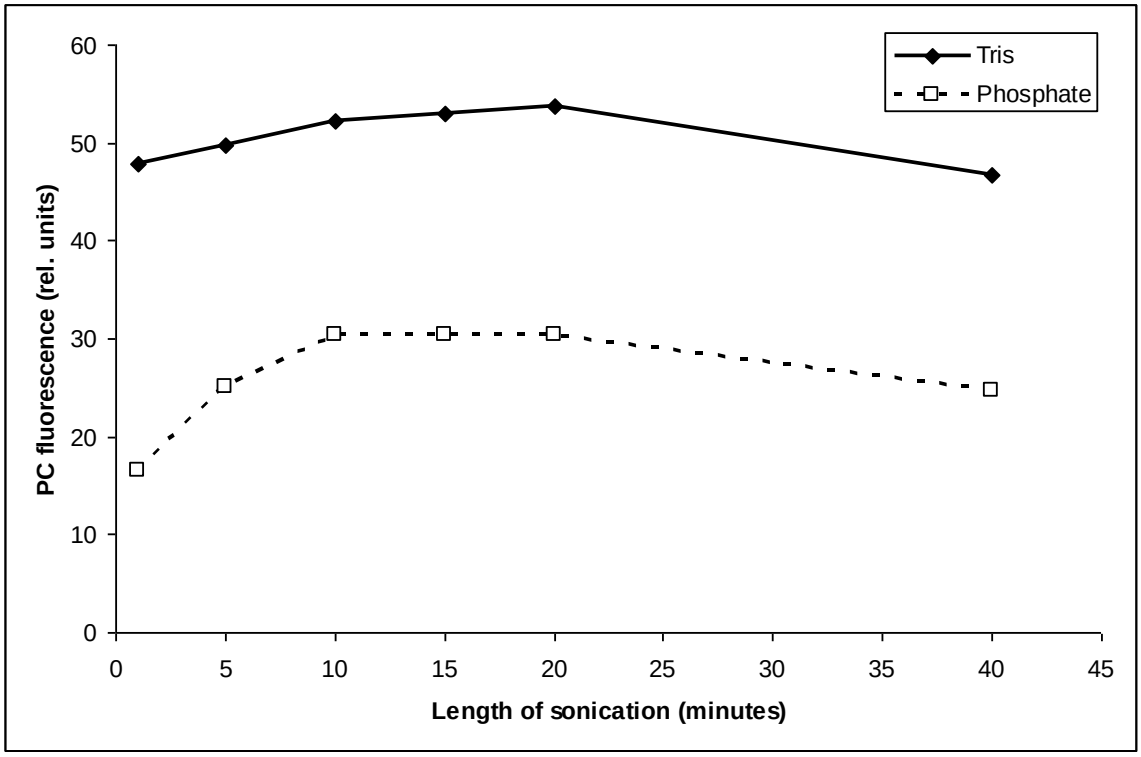

Figure 5-2. Comparison of phycocyanin (PC) fluorescence after sonication of various lengths in 0.05 M Tris buffer pH 7.0 and 0.1 M Sodium phosphate pH 6.8. Values are the average of three filters.

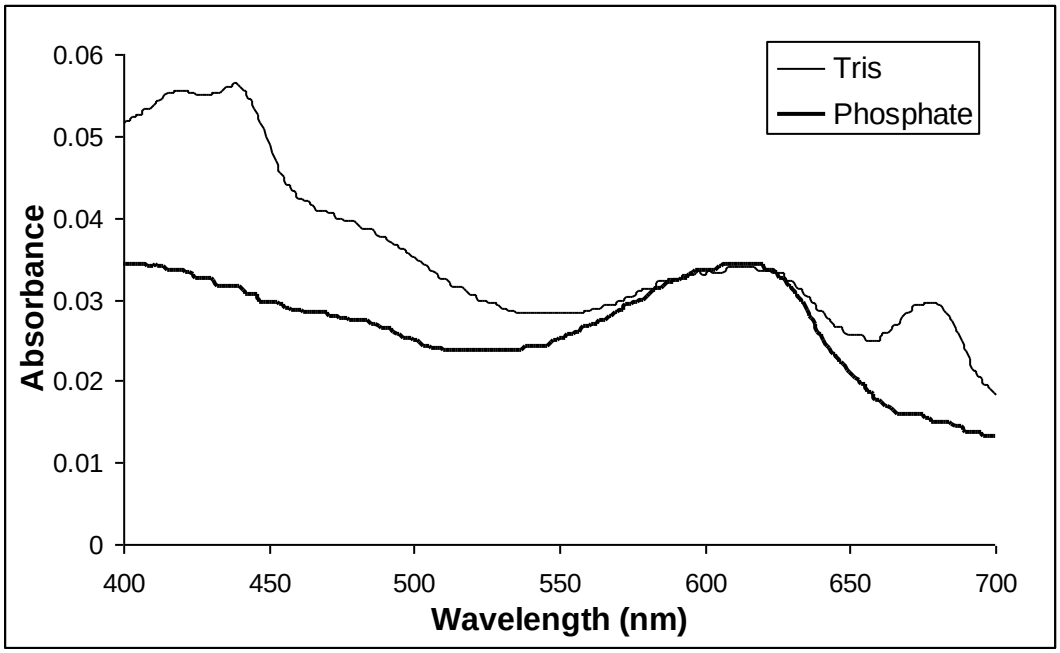

Figure 5-3. Absorbance spectra of *Microcystis* filtered and PC extracted by sonication for 15 minutes in 0.05 M Tris buffer pH 7.0 and 0.1 M Sodium phosphate pH 6.8.

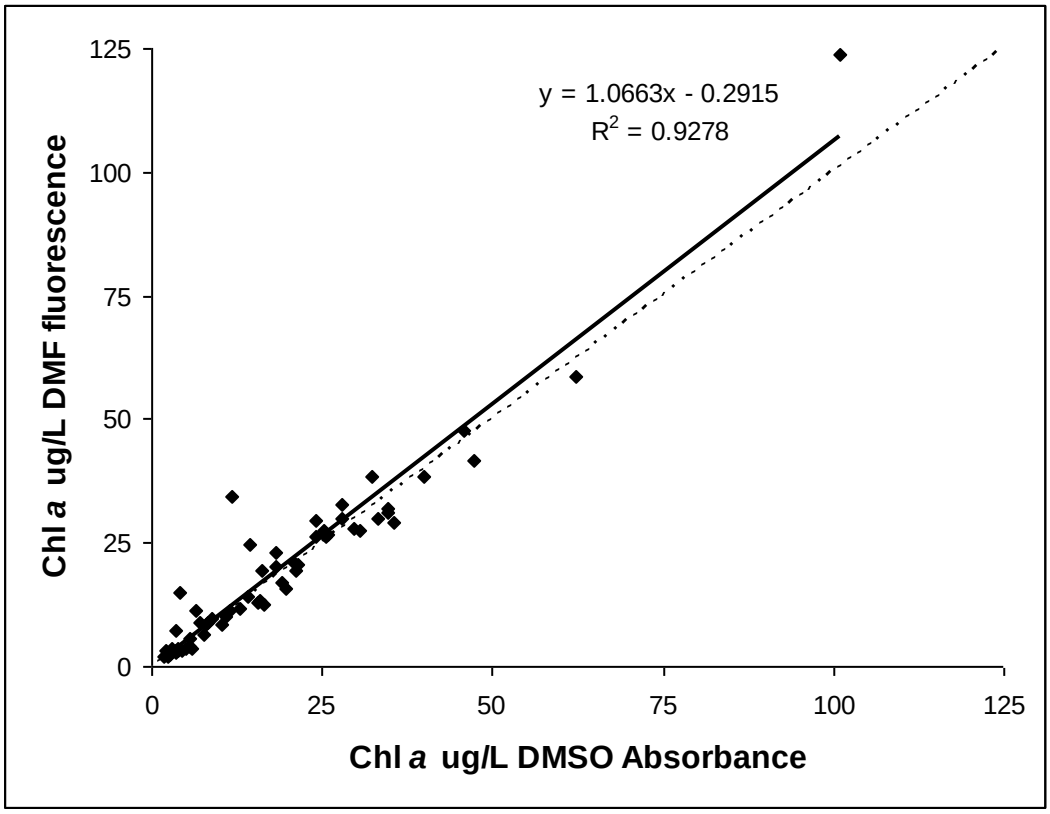

Figure 5-4. Comparison of chl *a* extractions and quantification using DMSO with absorbance quantification and DMF with fluorescence quantification. Bold line is regression between the two methods and dashed line is a one to one line.

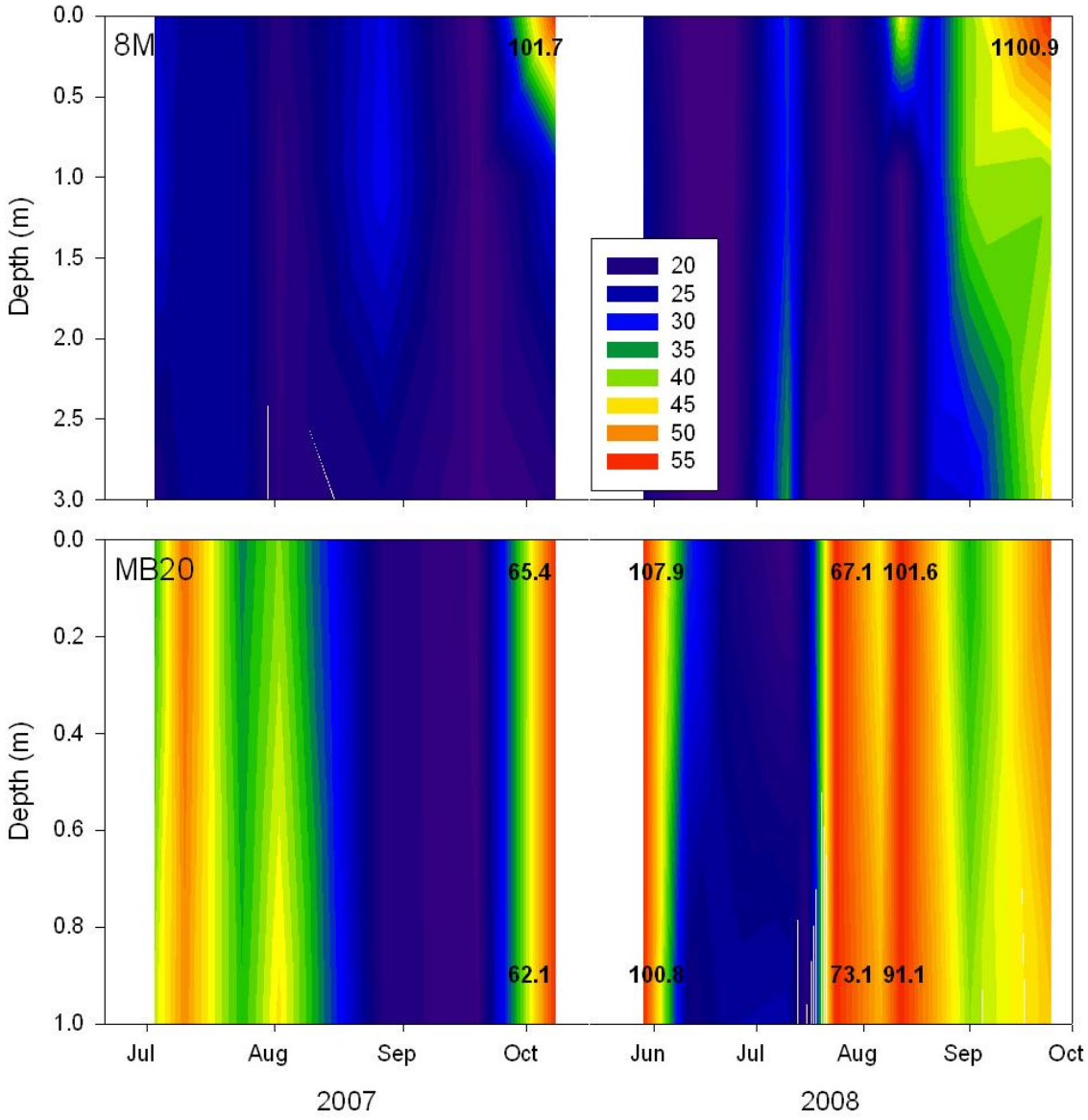

Figure 5-5. Chlorophyll *a* (μg L^{-1}) isopleths for sites 8M (top) and MB20 (bottom) for years 2007 (left panel) and 2008 (right panel). Values greater than the contour scale are written on the figure.

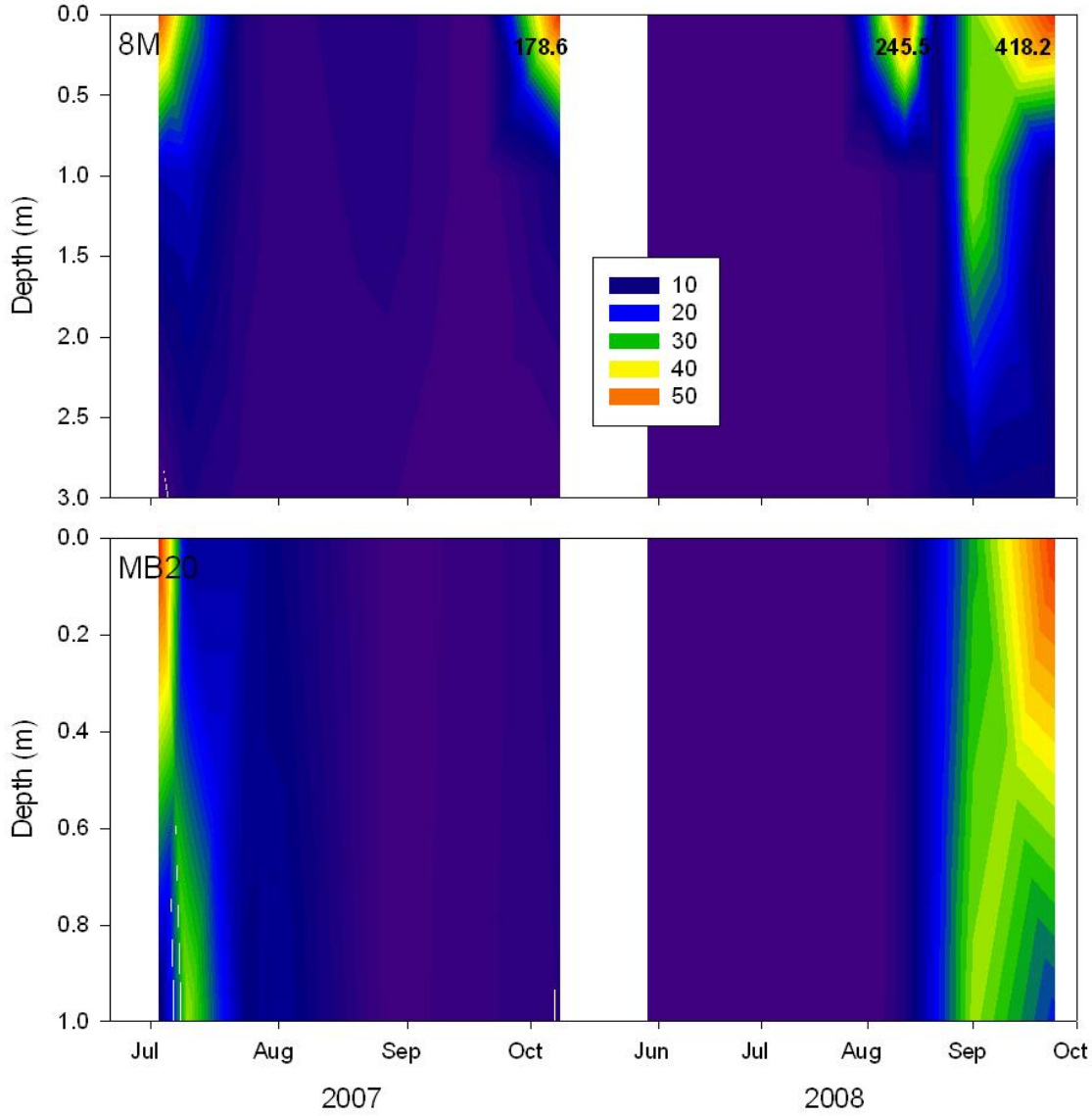

Figure 5-6. Phycocyanin (µg L^{-1}) isopleths for sites 8M (top) and MB20 (bottom) for years 2007 (left panel) and 2008 (right panel). Values greater than the contour scale are written on the figure.