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Urea as a Nitrogen Source for *Microcystis aeruginosa*

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To the Graduate Council:

I am submitting herewith a thesis written by Bernard Shafer Belisle entitled "Urea as a Nitrogen Source for *Microcystis aeruginosa*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Microbiology.

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Urea as a Nitrogen Source for *Microcystis aeruginosa*

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Abstract

Over the last decade, Lake Erie has experienced annual harmful algal blooms events dominated by the toxic cyanobacterium *Microcystis aeruginosa*. It is still unclear what causes *Microcystis* blooms to occur, but there is broad agreement that eutrophication of freshwater systems from anthropogenic sources (urban, industrial, etc.), has led to their proliferation. In particular, the organic compound urea has been implicated as an important source of anthropogenic nitrogen, due to its increased use in agricultural practices. Currently, urea constitutes more than 50% of the nitrogen used for agricultural fertilizer globally, and its usage has increased more than a 100-fold over the past four decades. To determine the effects of urea on Lake Erie phytoplankton, environmental surveys were conducted for urease enzymatic activity, phytoplankton biomass, nutrient concentrations, and phytoplankton community composition. 48-hour *in situ* microcosm experiments, spiked with various nitrogen species (nitrate, ammonium, and urea), were also performed to identify if the species of nitrogen influences phytoplankton biomass in Lake Erie. Results from this study confirm the presence of urea as a bioavailable form of nitrogen in Lake Erie, and indicated that as the bloom prolongs in duration the primary nutrient limiting phytoplankton biomass shifts from phosphorus to nitrogen. These results reinforce the importance of understanding the role of both nitrogen and phosphorous in driving harmful algal blooms in freshwater systems, and present the new idea that seasonal nitrogen limitation of plankton growth may be crucial in determining the size and extent of *Microcystis* blooms in Lake Erie.

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I. Introduction

Harmful Algal Blooms in Lake Erie

The Great Lakes basin consists of the Laurentian Great Lakes (Superior, Michigan, Huron, Erie, and Ontario) and the surrounding urban and agricultural areas that forms a large drainage basin that flows into the lake systems (Fuller *et al.* 2012). Currently, one-tenth of the population of the United States and one-quarter of the population of Canada reside in the Great Lakes basin. Additionally, 7% of the United States and 25% of Canadian total agricultural production occurs in the Great Lakes basin (Fuller *et al.* 2012). The presence of urban and agricultural lands in the Great Lakes watershed provides an input of point source and non-point source pollution, in the form of nitrogen and phosphorus, to the system. The excessive nutrient input leads to eutrophic conditions, resulting in annual increased algae biomass formation (Paerl *et al.* 2013).

Over the past several decades' large phytoplankton blooms have become an annual event for large freshwater systems around the world, such as Lake Erie and China's Lake Tai (or *Taihu*) (Rinta-Kanto *et al.* 2009, Qin *et al.* 2010, Steffen *et al.* 2014). The phytoplankton communities within these blooms consist of diverse phototrophic members of the plankton community, and include members such as diatoms, eukaryotic microalgae, and cyanobacteria (Oliver *et al.* 2012). Large phytoplankton blooms are often referred to as harmful algal blooms (HABs), because they have a negative impact on human activities or the environment (Zingone *et al.* 2000). The "harmful" aspects of these blooms can include oxygen

depletion (hypoxia and anoxia), deterioration of water quality and clarity, and/or the production of toxic metabolites, such as microcystin (Paerl *et al.* 2013).

Bloom-forming cyanobacteria tend to dominate freshwater systems because of physiological advantages, such as the ability to fix atmospheric nitrogen, regulate buoyance within the water column and the ability to form colonies and filaments (Oliver *et al.* 2012, Paerl *et al.* 2013). These physiological traits have allowed some cyanobacterial species to outcompete native phytoplankton species in aquatic system (Boyer 2006). The shift in phytoplankton community composition to cyanobacterial dominance can alter flow paths of energy and nutrients, thus altering the structure and trophic functionality of aquatic ecosystems (Oliver *et al.* 2012).

The greatest impacts of eutrophication from anthropogenic sources are observed in Lake Erie, compared to the other Great Lakes, due primarily to the physical characteristics and input of nutrients from waterways, such as the Maumee River (Koslow *et al.* 2013, Steffen *et al.* 2014). The Maumee River drains an area of 16,376 km^2, with 87% being used for agricultural purposes (Chaffin *et al.* 2013). Because of this, the Maumee River is considered the largest source of nutrient elements into Lake Erie (Koslow *et al.* 2013).

Lake Erie is the smallest of the Great Lakes by volume (26,000 km^2), with an average depth of 19 meters and a short retention time of 2.6 years (Fuller *et al.* 2012). It is geographically divided into three separate basins: eastern, central, and western (Chaffin *et al.* 2013). The eastern basin is the deepest of the three basins, with a max depth of 69 meters, and is generally oligotrophic, nutrient limited. The average depth of the central basin is 19 meters and can shift between oligotrophic

and mesotrophic conditions. The western basin is the shallowest, with 8 meters as an average depth, and is considered eutrophic to mesotrophic (Chaffin *et al.* 2013).

During the late 1960s, HABs emerged as a public concern for Lake Erie due to the decline in dissolved oxygen concentrations and water quality, forming large anoxic zones. Anoxic zones are regularly formed when dissolved oxygen is consumed in the degradation of algal biomass (Fuller *et al.* 2012). Conditions became so dire that, Lake Erie was described as being "dead" by the popular press in the early 1970s (Charlton 1980, Steffen *et al.* 2014). Scientists focused on identifying and managing limiting nutrients, in an attempt to reduce biomass in Lake Erie. Nutrient limitation is often considered the primary constraint on growth of phytoplankton and is an important driver of competition that can influence community composition in aquatic systems (Oliver *et al.* 2012). In freshwater ecosystems, phosphorus has been the conventional limiting nutrient for primary productivity and algal biomass (Vollenweider 1968, Schindler 1977, Sterner 2008).

The Great Lakes Water Quality Agreement (GLWQA) was implemented in 1972, partially due to pressure from the public and scientific communities(Fuller *et al.* 2012). The GLWQA was a joint effort by the Canadian and American government to reduce point source phosphorous loading into the great lakes (Morrison *et al.* 1986, Fuller *et al.* 2012). The U.S. Environmental Protection Agency (EPA) defines point source pollution as "any single identifiable source of pollution from which pollutants are discharged, such as a pipe, ditch, ship or factory smokestack" (Hill 1997). In the 1970s and 80s, Lake Erie HAB phytoplankton biomass began to decline in response to the GLWQA action to reduce point source phosphorous loading

(Fuller *et al.* 2012). By the 1980s, the observed increase of fish populations, reduction in algal biomass, and overall improvement of water quality suggested a recovery trend for the Lake Erie ecosystem.

Reemergence of Blooms in Lake Erie

Despite an apparent improvement in the overall health of the lake, in the mid-1990s a reemergence of cyanobacterial blooms began to occur in Lake Erie (Conroy *et al.* 2005). The cyanobacterial communities responsible for the mid-1990s blooms have shifted from the cyanobacterial species observed in blooms prior to GLWQA management (Conroy *et al.* 2005). During the 1960s, surveys of phytoplankton communities in Lake Erie identified that cyanobacterial biomass was dominated by nitrogen-fixing taxa such as *Aphanizomenon* spp. and *Anabaena* spp. (Conroy *et al.* 2005, Steffen *et al.* 2014). In contrast, the blooms occurring in the mid-1990s were dominated by, the non-nitrogen-fixing cyanobacterium, *Microcystis* spp. (Conroy *et al.* 2005), with other filamentous and N_2 fixing genera occurring in conjunction (e.g. *Anabaena* spp.) (Rinta-Kanto *et al.* 2006).

The switch to *Microcystis* spp., dominance suggests a shift in conditions that favored the competition between cyanobacterial taxa (Conroy *et al.* 2005). The need for nitrogen fixing cyanobacteria to meet nitrogen demands for the ecosystem has been reduced due to the increase loading of nitrogen into aquatic ecosystems. (Paerl *et al.* 2013). Because of the high energy requirements involved in nitrogen fixation, access to energetically favorable forms of nitrogen may provide a potential advantage for non-N_2 fixing cyanobacteria, over their N_2 fixing counterparts. This

may be an explanation for the success of *Microcystis* in Lake Erie, compared to the N_2 fixing species observed in the 1960s.

SUCCESS OF MICROCYSTIS IN LAKE ERIE

Morphology of *Microcystis*

The *Microcystis* genus consists of spherical cells that are irregularly grouped into colonies of various sizes and densities. *Microcystis* colonies may or may not have stratified, colorless mucilage that can differ by sizes ranging from micro- to macroscopic and morphologically depending on environmental conditions. (Šejnohová *et al.* 2012). This makes species level identification of *Microcystis* difficult, but a combination of morphological and molecular markers have divided *Microcystis spp.,* into three clusters or "morphospecies": small (S) cell-size groups such as *M. flos-aquae*, middle (M) cell-size groups represented by *M. aeruginosa*, and large (L) cell-sized group represented by *M. wesenbergii* (Šejnohová *et al.* 2012). The grouping of cells into colonies is generally associated with the increase in exudation of polysaccharides (Oliver *et al.* 2012). These morphospecies vary in their response to abiotic factors, such as wind and temperature.

Buoyancy Regulation

Microcystis has the ability to regulate its buoyancy in response to changing environmental conditions by the use of gas vesicles. These gas vesicles are comprised of gas-filled hollow structures with conical end caps (Šejnohová *et al.* 2012). In *Microcystis* spp., the gas vesicle membrane was shown to have three thin

layers, and comprised mainly of protein (Šejnohová *et al.* 2012). Gas vesicle membranes are impermeable to liquid due to a hydrophobic inner membrane, they are permeable to small gas molecules, such as H_2, N_2, O_2, CO_2, CO, CH_4, and Ar (Šejnohová *et al.* 2012). The internal gas compositions of these gas vesicles are equal to those of the surrounding environment. *Microcystis* is able to regulate its buoyance through changes in the development of gas vacuoles or by the balance of cellular constituents of different density such as proteins and carbohydrates (Oliver *et al.* 2012).

Buoyancy and regulation of depth in the water column allows *Microcystis* to bridge the vertical separation that develops in stratified waters between higher availability of nutrients (at depth) and access to greater illumination in surface waters (Oliver *et al.* 2012). Photosynthesis results in greater accumulation of cellular carbohydrate reserves, which decreases the buoyancy of the colony, and results in colonies from the surface layer becoming less buoyant during the day and more so at night (Šejnohová *et al.* 2012). The vertical movement in the water column regulates the amount of incident light available for photosynthetic activity. This access to both additional light and nutrients provides a clear competitive advantage of increased photosynthetic ability over other species that may be restricted to surface waters with limited nutrients or deep waters.

UV Adaptation

Phytoplankton, located in surface waters, are exposed to high levels of solar radiation (UV-A and UV-B), which can damage DNA and cause photo-oxidization of

the photosynthetic apparatus, under prolonged exposure (Hader *et al.* 2007). *Microcystis* has developed many strategies to cope with high solar radiation (specifically UV-B), including a screening mechanism by d-galacturonic acid, a main component of the mucilage layer surrounding the colony (Hader *et al.* 2007, Šejnohová *et al.* 2012). This suggests that the formation of large colonies allows for internal self-shading and is important in UV protection. Additionally, *M. aeruginosa* can cope with enhanced UV-B through: an increasing the synthesis of carotenoids to counteract reactive oxidants caused by exposure to UV-B, degrading phycocyanin and allophycocyanin to avoid further damage to DNA and reaction centers, and enhancing the repair of UV-B induced damage to the photosynthesis mechanism (Hader *et al.* 2007, Šejnohová *et al.* 2012).

The role of phosphorus as a nutrient in *Microcystis* growth

Phosphorus (P) is traditionally accepted as the primary nutrient controlling the development of cyanobacterial biomass in freshwater systems because of the extensive evidence that correlates growth rates to available concentrations of phosphorus (Schindler 1977, Dokulil *et al.* 2000, DeBruyn *et al.* 2004, Xu *et al.* 2010). Phosphorus is a required element for many integral cellular processes, including metabolism (adenosine phosphates) and the formation of new nucleic acids (sugar phosphates) (Correll 1999). An important source of phosphorus for assimilation into biomass for aquatic phytoplankton, including *Microcystis* spp., is in the form of orthophosphates, inorganic forms of phosphorous (Osmond *et al.* 1995, Šejnohová *et al.* 2012). Under phosphorus limiting conditions, cellular phosphorus

concentrations decline as the growth rate declines and phosphorus uptake potential increases (Oliver *et al.* 2012, Saxton *et al.* 2012). Additionally, *Microcystis* spp. can accumulate excess phosphorus in the form of polyphosphate granules, also known as the phosphate 'overplus' phenomenon (Saxton *et al.* 2012, Šejnohová *et al.* 2012). This provides a competitive advantage over other microalgae that are unable to store excess phosphorus, under P-limiting conditions.

In aquatic systems, phosphorus exists in the form of phosphates (PO_4^{3-}) that can vary in molecular formation (organic or inorganic), and are often categorized based upon the differences in particle size and chemical characterization. Phosphorus material categorized by size is referred to as "dissolved " (also known as soluble), material smaller than 0.2 μm, or "particulate", material larger 0.2 μm (Jiao *et al.* 2010). The terms "reactive" and "unreactive" are used to describe fractions of phosphorus that are in a form that is readably available for use in cellular processes and fractions that are unable for immediate use, respectively (Carlson *et al.* 1996).

Using these parameters, classifications of phosphorus in these systems are typically divided into three different components: soluble reactive phosphorus (SRP), soluble unreactive phosphorus (SUP), and particulate phosphorus (PP) (Carlson *et al.* 1996). The sum of the SRP and SUP fractions represents the soluble phosphorus (SP) portion, and the sum of all phosphorus components represents the total phosphorus (TP) measurement. The SRP fraction consists mostly of orthophosphates, which is bioavailable for use by bacteria, algae, and plants (Correll 1999). Conversely, the SUP fraction is comprised mostly of organic forms of

phosphorus and inorganic polyphosphates, and historically has not been considered immediately available for use in cellular processes.

The traditional "Phosphorus Limitation Paradigm" suggests that lake ecosystems will tap into atmospheric sources of carbon (C) and nitrogen (N) to overcome any limitation of these two elements; the same is not true for phosphorus (Schindler 1977, Sterner 2008). Based upon this rational, short-term deficiencies of C and N may exist, but phosphorus limitation is the primary controlling variable. Therefore, it was argued by the GLWQA that control of N input was unnecessary. The GLWQA successfully used this reasoning to target P loads to reduce annual Lake Erie HAB biomass in the early 1970s, while N inputs remained less strictly controlled. The mid 1990s reemergence of HABs in Lake Erie dominated by non-N_2 fixing cyanobacteria, primarily *Microcystis* sp., is in response to changes in nutrient loading dynamics, compared to blooms preceding GLWQA management. Nutrient loading from agricultural, urban, and industrial expansion have increased both N and P loading, into Lake Erie (Paerl *et al.* 2014).

Importance of Nitrogen

Cyanobacteria are able to utilize a wide range of nitrogen sources, such as ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and urea ($CO(NH_2)_2$), each having specific energetic properties relative to nutrient limitation in lakes (Sterner 2008, Solomon *et al.* 2010, Oliver *et al.* 2012). Some cyanobacteria species, including *Anabaena, Aphanizomenon* and *Cylindrospermopsis,* species possess the nitrogenase enzyme, (Boyer 2006), which allows for the fixation of atmospheric nitrogen (N_2),

but is energetically costly (Stainer *et al.* 1977, Flores *et al.* 2005, Fuller *et al.* 2012). Generally freshwater systems that are phosphorus rich, relative to nitrogen (low N:P ratio), favor the growth of N_2-fixing cyanobacteria that are able to fix N for cellular demands (Paerl *et al.* 2014). The order of energetically favorable forms of inorganic nitrogen for cyanobacteria is $NH_4^+ > NO_3^- > NO_2^- > N_2$ (Oliver *et al.* 2012, Šejnohová *et al.* 2012). NH_4^+ is energetically favorable to NO_3 because the latter requires nine NAD(P)H or ferredoxin and one ATP to assimilate into the glutamate pathway, while NH_4^+ assimilation requires only one reductant and one ATP (Oliver *et al.* 2012). These energetic substrates for nitrogen assimilation are provided by the photosynthetic process and are also utilized by the carbon fixation pathway. Therefore, the form of nitrogen utilized by an organism influences other cellular processes, by diminishing the pool of energetic substrates (Stainer *et al.* 1977, Šejnohová *et al.* 2012).

Nitrogen Utilization

In cyanobacteria, the global nitrogen transcription regulator, NtcA, mediates nitrogen control by responding to environmental levels of nitrogen availability (Solomon *et al.* 2010). The acquisition of nitrate, nitrite, urea, and various amino acids are mediated by active transport using membrane permeases, a membrane transport protein (Herrero *et al.* 2001, Flores *et al.* 2005). After nitrogen is acquired, it is reduced to NH_4^+ and incorporated into the carbon skeleton of the cyanobacterium, *via* the glutamine synthase-glutamate synthase cycle (GS-GOGAT) (Flores *et al.* 2005, Oliver *et al.* 2012). This is done by the reduction of intracellular

NO_3^- to NO_2^- by nitrate reductase, and then NO_2^- is reduced to NH_4^+ by nitrite reductase (Herrero *et al.* 2001). The acquisition of NH_4^+ is mediated by membrane transporters that are permeable to NH_4^+ when extracellular concentrations are lower than 1 μM per liter (Herrero *et al.* 2001, Flores *et al.* 2005). When NH_4^+ is present, its uptake and utilization in the GS-GOGAT pathway represses the genes that are responsible for permeases and enzymes involved in the assimilation of less favorable nitrogen sources, a process known as 'nitrogen control' (Oliver *et al.* 2012). Excessive nitrogen can be stored intracellularly in the form of phycocyanin, a component pigment of the light harvesting antenna, and cyanophycin, a co-polymer of aspartate and arginine. The storage of nitrogen as cyanophycin is controlled by the N-acetylglutamate kinase (NAGK) enzyme, which produces sufficient quantities of arginine for by the cyanophycin synthase (Oliver *et al.* 2012). Under nitrogen limiting conditions, phycocyanin and cyanophycin are both at low concentrations, due to consumption and/or inability to secure sufficient nitrogen for storage. The limited availability of nitrogen inhibits the storage pathway by the accumulation of 2-oxo-glutarate, which binds to the photosystem II complex (PSII), and leads to the dissociation of the PSII-NAGK complex (Herrero *et al.* 2001, Oliver *et al.* 2012). The dissociation of the PSII-NAGK complex, under nitrogen limited conditions, restricts the production of arginine and therefore inhibits the nitrogen storage mechanism.

Urea as a Nitrogen Source

Urea ($CO(NH_2)_2$) is a stable form of organic nitrogen, often used in agricultural fertilizer, and is utilizable by cyanobacteria and phytoplankton in

freshwater and marine environments (Solomon *et al.* 2010). Urea currently constitutes more than 50% of the nitrogen used globally for agricultural fertilizer, more than a 100-fold increase in the past 4 decades (**Figure 1;** (Glibert *et al.* 2006)). Urea is a favorable source nitrogen for agricultural fertilizer because of its solubility in water, low cost, and reduced damage to root crops. In general, urea concentrations in aquatic ecosystems are less than those of NH_4^+ and NO_3^-, but may exceed these inorganic nitrogen sources for short periods of time, particularly when runoff from heavily fertilized areas occurs (Solomon *et al.* 2010).

The use of urea as a fertilizer is effective for agricultural practices, but resulting urea runoff can degrade the water quality of neighboring aquatic systems (Glibert *et al.* 2006, Finlay *et al.* 2010). In the last decade, bloom events in Lake Erie have increased in size and duration. The bloom in 2011 was the most extensive in recent history (Michalak *et al.* 2013). Recent published reports have linked heavy rainfall years (1998, 2003, 2008, 2011) and increased bloom size, while drought years (1999, 2001, 2012) have been associated with below average bloom events (Koslow *et al.* 2013). For Lake Erie, increased precipitation is believed to contribute to elevated levels of nutrient loading, which exacerbates the levels of cyanobacteria in the lake (Michalak *et al.* 2013).

The preferential use of urea over other inorganic nitrogen sources, high rates of urea uptake, and increased urease activity have been shown to occur in many phytoplankton species, some of which are associated with HAB forming genera (Fan *et al.* 2003, Glibert *et al.* 2006, Solomon *et al.* 2010). Furthermore, the presence of

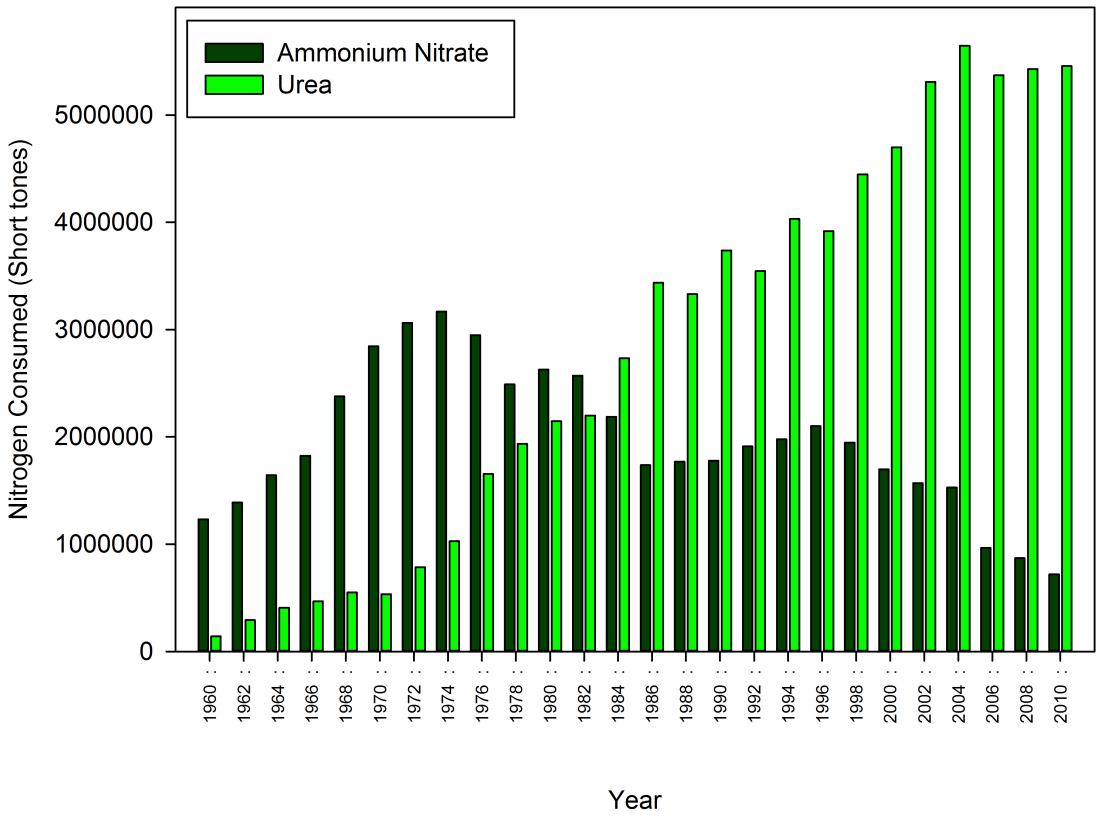

Figure 1: Ammonium Nitrate (Dark Green) and Urea (Light Green) usage as a nitrogen source for fertilizer in the United States, from 1960 to 2010. Source United States Department of Agriculture Economic Research Service (USDA-ERA 2013).

chemically reduced forms of nitrogen (NH_4^+, urea) have been shown to specifically suppress N_2-fixing cyanobacteria (*Aphanizomenon, Anabaena*) and stimulate growth in non-N_2-fixing cyanobacteria (*Microcystis, Planktothrix*) in pure culture (Finlay *et al.* 2010). This occurs because reduced forms of nitrogen are rapidly acquired by cyanobacteria increasing the intracellular concentrations of NH_4^+, which alters glutamate concentrations and represses transcription NtcA promoter activity, thus immediately suppressing N_2-fixation in heterocysts (Finlay *et al.* 2010).

Extracellular urea uptake utilizes the same mechanism of diffusion and permeases involved in the extracellular uptake of NH_4^+ (Fan *et al.* 2003, Finlay *et al.* 2010). The use of urea as a nitrogen source for cellular processes requires the urease enzyme (Solomon *et al.* 2010). The urease enzyme is a nickel (Ni) dependent enzyme that hydrolyzes urea into one CO_2 and two NH_4^+ molecules (Manunza *et al.* 1999, Fan *et al.* 2003). The fate of anthropogenic (agricultural) sources of urea can vary based on environmental factors such as temperature, timing of application, and pH. Hydrolysis of urea-based fertilizer can occur initially after application to the field by soil microbes containing the urease enzyme. Additionally, hydrolysis can occur by aquatic cyanobacteria and heterotrophs possessing the urease enzyme, when direct runoff of urea occurs (Glibert *et al.* 2006).

Agricultural practices, such as no-till farming and application of urease inhibitors with fertilizer, may exacerbate the amount of urea directly transferred to aquatic ecosystems (Glibert *et al.* 2006). Agrotain™ is an example of a commercial fertilizer additive that reduces urea loss. Agrotain™ reduces urea loss occurring via hydrolysis and NH_4^+ volatilization by soil microbes, by inhibiting urease activity

(Boyer 2013). Agrotain™ uses N-(n-butyl)-thiophosphoric triamide (NBPT) to inhibit urease activity via binding to the active site of the urease enzyme to form a stable complex (Manunza *et al.* 1999). Under normal conditions, urea is able to coordinate with the active site of the urease enzyme by the interaction of urea's oxygen (O) atom to one Ni atom in the active site. NBPT inhibits the urease enzyme by targeting both Ni atoms in the active site with its amide group and oxygen atom, to form a stable complex (NBPT-urease), which inhibits the ability of urea to interact with the Ni atoms of the active site (Manunza *et al.* 1999).

II. Research Objectives

The goal of this study was to confirm whether *Microcystis aeruginosa* is capable of utilizing urea as a nitrogen source in Lake Erie. To identify the role of urea in promoting *Microcystis* blooms occurring in Lake Erie, environmental measurements of urease enzymatic activity, phytoplankton biomass, nutrient concentrations, and phytoplankton community composition were measured during three sampling cruises (August 2012, May 2013, July 2013). 48-hour *in situ* microcosm experiments, spiked with various nitrogen species (urea, nitrate, ammonium, Agrotain (NBPT+Urea) and environmental control), were performed to determine if the species of nitrogen influences phytoplankton biomass in Lake Erie.

The specific hypotheses of this thesis are:

H1: Higher phytoplankton biomass, nutrient concentrations, toxin levels, and greater phytoplankton community diversity will be observed in the western basin of Lake Erie compared to the central and eastern basin, because of the high input from major water sources that empty into the western basin.

H2a: Urease activity in Lake Erie is correlated to phytoplankton biomass.

H2b: Urease activity in Lake Erie is correlated to urea concentrations.

H3a: Phytoplankton biomass and urease activity, of the microcosm experiment, will vary based on the nitrogen species provided.

H3b: Microcosm treatments that contain nitrogen and phosphorous will have increased phytoplankton biomass and urease activity, compared to treatments with nitrogen only, regardless of the nitrogen source.

III. Methods and Materials

Microcystis Culture Work

The ability of *Microcystis aeruginosa* to use ammonium (NH_4^+), nitrate (NO_3), and urea ($CO(NH_2)_2$) as a sole nitrogen source was examined by growing unialgal cultures in modified CT growth media (Watanabe *et al.* 1977). CT media was prepared for each species of nitrogen by replacing the standard source of nitrogen, (KNO_3 and $Ca(NO_3)_2$), with 180 μM N of NH_4^+, NO_3, or urea. This represents 10% of N contained in standard CT media, as seen in **Table 1**. Experiments were performed using two laboratory strains of *M. aeruginosa*: (1) PCC 7806, obtained from the Pasteur Culture Collection (PCC), and (2) NIES 843, from the National Institute for Environmental Studies (NIES) (Kaneko *et al.* 2007). PCC 7806 was selected because it is a strain used globally for laboratory work, and NIES 843 was chosen because it is the only strain of *Microcystis* with a fully sequenced genome (Kaneko *et al.* 2007).

Each strain was grown in 50 mL glass culture tubes (Kimble, Vineland, NJ) at 24°C with ~30 μmol photons m^{-2} s^{-1} illumination under a 12h-12h day-night light cycle. Prior to use, all culture tubes were soaked overnight in 1% HCl and rinsed thoroughly with Milli-Q® water (Millipore Corp., Billerica, MA, USA), prior to autoclaving. Samples were grown in triplicate under each form of nitrogen and transferred three times to a starting density of ~10^4 cells per mL, prior to data collection. This was done to ensure that cell cultures had exhausted any intracellular sources of nitrogen and only utilized the nitrogen source provided for cell growth. Cyanobacterial growth was monitored daily using a Turner Designs TD-700 fluorometer (Sunnyvale, CA, USA).

Table 1: *M. aeruginosa* strain NIES 843 and PCC 7806 growth rates on various chemical species of nitrogen. Samples were grown in triplicate at 24°C under a 12h-12h day-night light cycle, for each nitrogen treatment. Measurements of biomass accumulation were taken over a sixteen-day growth period, on the third transfer.

Organism	Treatment	Growth Media N concentration	Growth Rate (per day ± SD)
M. aeruginosa NIES 843	Positive Control	1.8 mM KNO_3 + $Ca(NO_3)_2$	0.256 ± 0.007
	Nitrate	180 µM NO_3	0.252 ± 0.008
	Urea	180 µM Urea	0.228 ± 0.011
	NBPT	180 µM NBPT	0.209 ± 0.011
	No N Control	0.0 µM-N	0.0 ± 0.0
M. aeruginosa PCC 7806	Positive Control	1.8 mM KNO_3 + $Ca(NO_3)_2$	0.247 ± 0.008
	Nitrate	180 µM NO_3	0.245 ± 0.010
	Urea	180 µM Urea	0.238 ± 0.006
	NBPT	180 µM NBPT	0.227 ± 0.014
	No N Control	0.0 µM-N	0.0 ± 0.0

Environmental sample collection

Field samples and *in situ* microcosm experiments were conducted aboard the CCGS *Limnos* during three Lake Erie environmental sampling cruises: August 13-17, 2012, May 27-31, 2013, and July 22-26, 2013. Surface water (1 meter depth) was collected from each station using 10 L Niskin sampling bottles and transferred to the ship's laboratory in 3 L acid-cleaned polycarbonate bottles. Prior to use, all polycarbonate bottles were soaked in 1% HCl and rinsed thoroughly with Milli-Q® water (Millipore Corp., Billerica, MA, USA). Environmental water samples from each station were then filtered through 0.2-µm nominal pore-size polycarbonate membrane filters (25 mm diam., Millipore) and analyzed for urease activity, chlorophyll *a* concentrations, urea concentrations, and nutrient measurements.

Chlorophyll *a* measurements

Chlorophyll *a* (chl*a*) concentrations were determined using the non-acidification method (Welschmeyer 1994, Wetzel *et al.* 2000), and served as a proxy for total phytoplankton biomass. A 50 mL volume of surface water from each station was filtered through 0.2-µm nominal pore-size polycarbonate membrane filters (25 mm diam., Millipore) and deposited into a 2.0 mL cryovial (Corning, NY). The sample was then flash frozen in liquid nitrogen and transferred to -80°C, until analyzed. Chl*a* concentrations were then determined by placing the filter into 90% acetone overnight (24 hours) at -20°C and measured using a fluorometer (Turner Designs 10-AU; Sunnyvale, CA, USA).

Measuring urease activity

Urease activity was determined by measuring the accumulation of NH_4^+, which is a product of urea metabolism. A 50 mL volume of surface water from each station was filtered through pre-combusted (450°C for 4 h) 25 mm Whatman (Kent, UK) GF/F glass fiber filters. Urease activity was measured using the indophenol method optimized for aquatic environmental samples (Solomon *et al.* 2007). Each sample was performed in triplicate and analyzed using a BioMate 5 UV-Visible Spectrophotometer, at 640 nm. An external calibration was measured for each assay performed, using a range of NH_4^+ stock solutions (0, 1.0, 2.5, 5.0, 7.5, and 10.0 μM). The NH_4^+ solutions were prepared from a 10 mM NH_4Cl standard stock solution, 0.267 g NH_4Cl into 500 mL Milli-Q® water (Millipore Corp., Billerica, MA, USA).

Urea Measurements

Urea concentrations for field samples were measured using the 'Direct Method', a colormetric assay based on the reaction of urea with diacetylmonoxime, as outlined by Revilla *et al*., 2005. Whole water samples were collected from each station and filtered through pre-combusted 25 mm Glass Fiber Filters (GF/F) (Kent, UK). Filtrate was stored at -20°C until analyzed in a laboratory setting. Water samples were measured in triplicate and analyzed using a BioMate 5 UV-Visible Spectrophotometer at 520 nm (Revilla *et al.* 2005). Standard curves were performed for each experiment by using a range of urea stock solutions (0, 0.5, 2.0, 5.0, 7.5, and 10.0 μM).

Microscopic enumeration

Phytoplankton identification and enumeration of environmental samples was performed using Lugol's Iodine-preserved samples and microscopic morphometrics (Wehr *et al.* 2003, Auinger *et al.* 2008, Saxton *et al.* 2012). Samples (50 mL) were collected and preserved with 0.5 mL of Lugol's Iodine (100 g KI $liter^{-1}$ and 50 g I_2 $liter^{-1}$), and stored at 4°C (Auinger *et al.* 2008). Fixed samples were allowed to settle (3-5 min) in a 1 mL Sedgwick-Rafter counting slide (Chorus *et al.* 1999) then analyzed on a Micromaster light microscope (ThermoFisher). Each sample was analyzed by taking four horizontal transects, ten fields of view each, which encompassed the full height and width of the slide (Chorus *et al.,* 1999 and Saxton *et al*., 2012). The resulting 40 randomly selected fields of view were imaged and saved using Micron imaging software (Westover Scientific), for later identification using taxonomic features (Wehr *et al.* 2003, Guillard *et al.* 2005). Organisms observed for each sample were identified and counted from the 40 randomly selected fields of view.

Water Chemistry Analysis

Water samples for nutrient analysis was collected at each environmental station and analyzed for total dissolved nitrogen (TDN), total kjeldahl nitrogen (TKN), total dissolved phosphorous (TDP), soluble reactive phosphorus (SRP), NH_3-N (ammonia), NO_3NO_2 –N (nitrate/nitrite), and silicate (SiO_2). TDN is the measurement of all dissolved forms of nitrogen, such as nitrate, nitrite, and TKN (ammonia + organic nitrogen). The National Laboratory for Environmental Testing

(Environment Canada) performed chemical analyses of the environmental samples using their standard techniques (NLET 1994). Environment Canada provided pH and surface temperature at the time of sampling, for each station, attained from the ship's automated water column profiler (NLET 1994).

Microcosm Experiments

Nutrient enrichment microcosm experiments were conducted under *in situ* conditions during three *CCGS Limnos cruises*. Microcosm experiments were performed at two ecologically relevant stations, Station 885 and Station 973. Station 885 was chosen due to the diversity of cyanobacteria historically present at this station, and Station 973 was chosen due to its proximity to the entrance of the Maumee River into Lake Erie. Surface water (1 m) from each station was dispensed into acid washed 1.2 L polycarbonate flasks and enriched with one of four chemical species of nitrogen, as shown in **Table 2**. Treatments for each microcosm experiment included: Nitrogen only treatments (N-only) enriched with only 180 µM N for each nitrogen species, Nitrogen plus Phosphorous treatments (N+P) enriched with 180 µM N for each nitrogen species in addition to 1 µM phosphorus, Environmental Control (Control) of whole water with no nutrient enrichment, and Environmental Control plus Phosphorus (Control +P) of whole water with only with 1 µM phosphorus added (**Table 2**). Each treatment was performed in triplicate and incubated in a deck incubator for 72 hours under *in situ* conditions. After incubation, aliquots from each treatment was collected and analyzed for chl*a* concentrations, microscopic enumeration, and nutrient concentration, as previously described.

Table 2: Microcosm experimental treatments utilized for three Lake Erie sampling cruises, at Station 973 and 885. Each treatment was performed in triplicate and incubated in a deck incubator for 72 hours under *in situ* conditions. Whole water, from each station, was spiked to a final concentration of 180µM-N (Nitrogen only Treatments) and 180µM-N + 1µM Phosphorous (Nitrogen +PO_4 treatments), for each species of Nitrogen.

Treatment	Treatment Label	Added Nutrient concentration
Nitrogen Only	Environmental Control	No-N added
	NO_3	180 µM NO_3
	Urea	180 µM Urea
	NBPT	180 µM NBPT
	NH_4	180 µM NH_4
Nitrogen + Phosphorus	Environmental Control + P	No-N added; +1µM PO_4
	NO_3 + P	180 µM NO_3 + 1µM PO_4
	Urea +P	180 µM Urea + 1µM PO_4
	NBPT + P	180 µM NBPT + 1µM PO_4
	NH_4 + P	180 µM NH_4 + 1µM PO_4

IV. Results

Microcystis Laboratory Culture Work

Biomass accumulation for PCC 7806 and NIES 843, compared to the no nitrogen control, demonstrates the ability of *M. aeruginosa* to successfully utilize several different chemical forms of nitrogen for cellular processes, as shown in **Figure 2** and **Figure 3** respectively. Nitrogen-limited treatments exhibited similar patterns of overall biomass accumulation, for both strains of *M. aeruginosa*. Furthermore, strains of *M. aeruginosa* exhibited the highest biomass accumulation for the Positive Control treatment and lowest for the No N Control treatment. The absence of growth in the No N Control treatment ensures that the observed biomass accumulation was from the nitrogen species provided and not from contamination and/or other components of the CT growth media.

M. aeruginosa PCC 7806 growth rates ranged between 0.247 ± 0.008 to 0.227 ± 0.014 divisions per day and NIES 843 ranged between 0.256 ± 0.007 to 0.209 ± 0.011 divisions per day (**Table 1**). Growth rates were highest in the 1.8 mM N Positive Control treatments and lowest in the 180 μM N NBPT+Urea treatments for both strains. PCC 7806 growth rates for all treatments were statistically similar to each other ($p>0.05$). Growth rates for NIES 843 Positive Control and Nitrate treatments were statistically similar to each other and statistically different for all other treatments ($p<0.05$). Even though growth rates varied between *M. aeruginosa* strains, biomass was notably lower for the nitrogen-limited treatments (180 μM N), compared to the high N (1.8 mM N) Positive Control treatment.

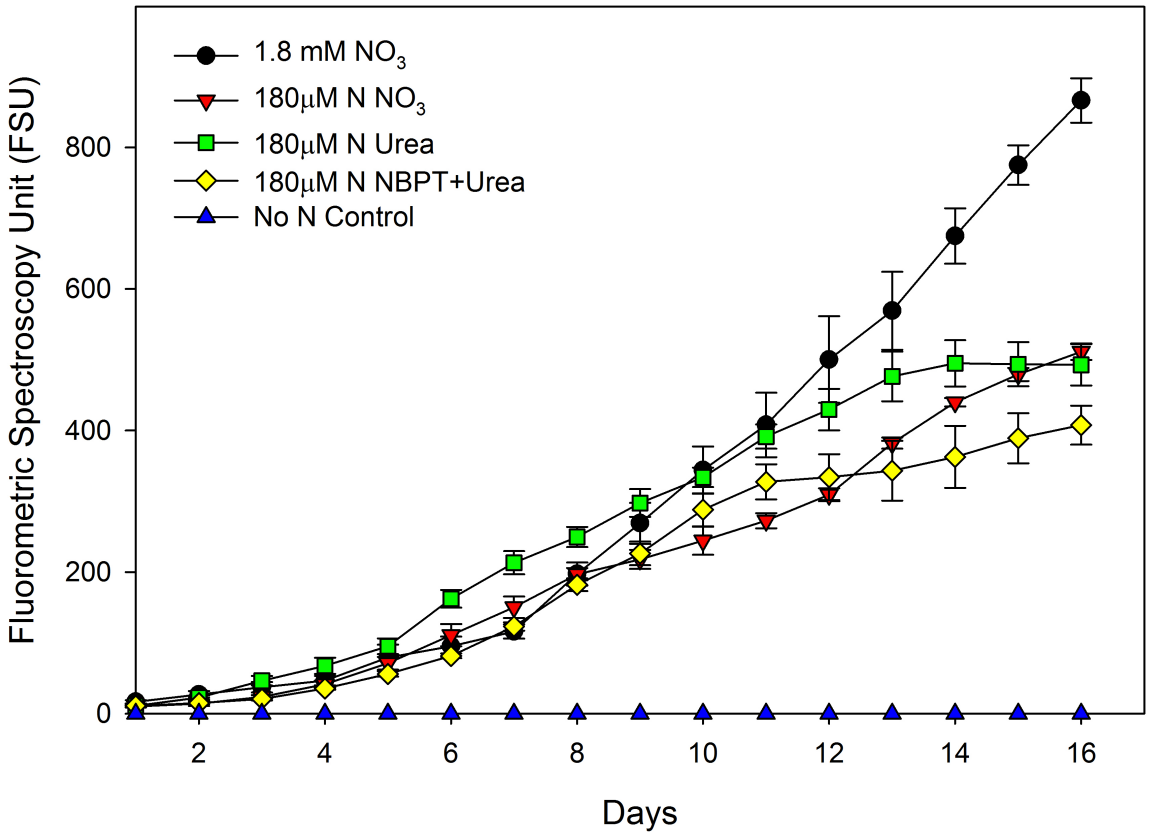

Figure 2: *M. aeruginosa* culture strain PCC 7806 growth over sixteen days on various chemical species of nitrogen, as the sole nitrogen source. Nitrogen limited treatments (180 µM N) for NO_3, Urea, and NBPT are represented by a red triangle, green square, and yellow diamond, respectively. Positive Control (1.8 mM KNO_3 and $Ca(NO_3)_2$) and No N Control treatments are represented by a black circle, and blue triangle, respectively.

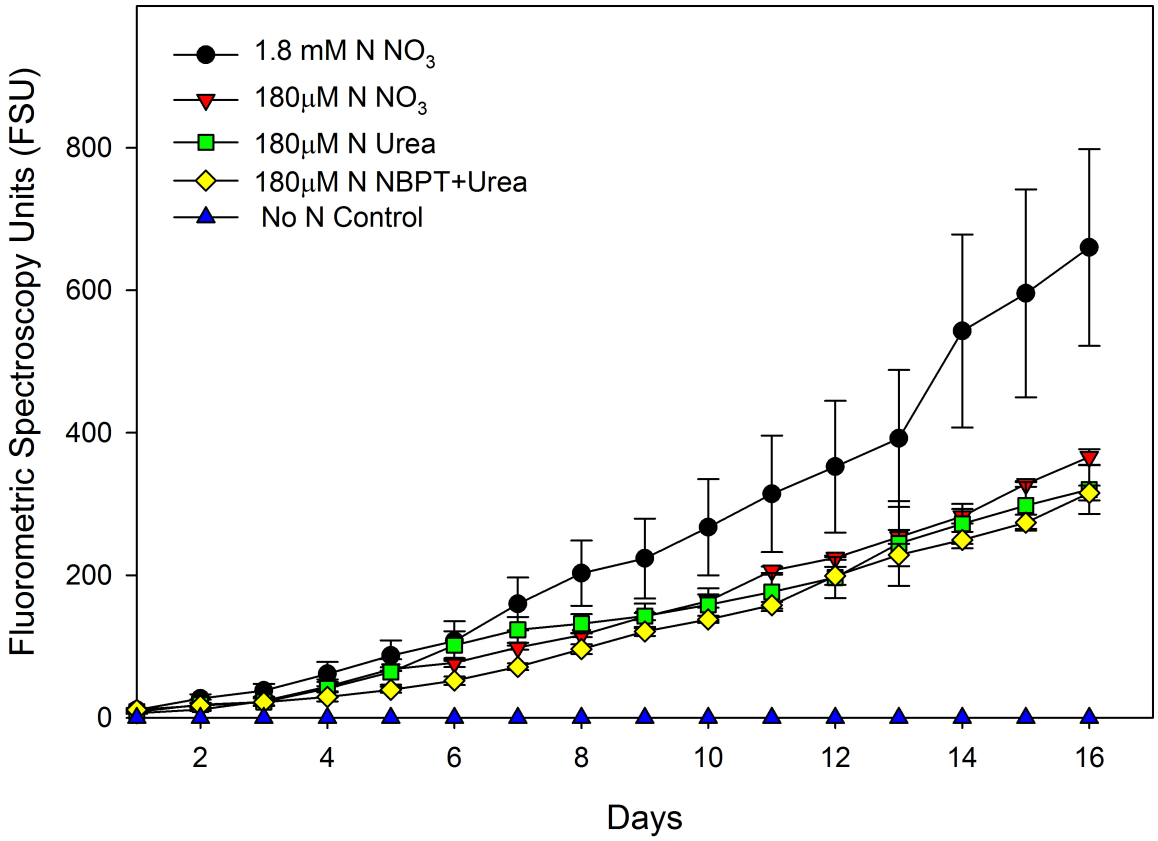

Figure 3: *M. aeruginosa* culture strain NIES 843 growth over sixteen days on various chemical species of nitrogen, as the sole nitrogen source. Nitrogen limited treatments (180 µM N) for NO_3, Urea, and NBPT are represented by a red triangle, green square, and yellow diamond, respectively. Positive Control (1.8 mM KNO_3 and $Ca(NO_3)_2$) and No N Control treatments are represented by a black circle, and blue triangle, respectively.

The low ammonium (180 µM-NH_4) treatment failed to exhibit growth through multiple transfers, in spite of repeated attempts.

Urease activity for PCC 7806 strain was significantly higher for the low Nitrate and Urea treatments compared to Positive Control treatment (**Figure 4**). *M. aeruginosa* NIES 843 urease activity was also highest in the low Urea treatment, but significantly lower for all other treatments compared to the Positive Control treatment ($p < 0.05$). For both PCC 7806 and NIES 843, urease activity was lowest for the low NBPT+Urea treatment, suggesting inhibition of the urease enzyme by NBPT.

Field surveys and experiments

Lake Conditions. Environmental conditions were surveyed during August 2012, May 2013, and July 2013 by collecting water samples at 21 stations (**Figure 5**) across Lake Erie. Chlorophyll *a* measurements for August 2012, May 2013 and July 2013 ranged from 1.56 - 25.4 µg/L, 0.726 – 37.35 µg/L, and 1.0 – 72.45 µg/L respectively (**Table 3**). Station 1163 exhibited the highest chlorophyll biomass for May 2013 and July 2013, with only Station 973 being higher for August 2012 measurements. Water samples for nutrient analysis was taken for each station and measured for: total kjeldahl nitrogen (TKN), total nitrogen (TN), total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate/nitrite (NO_3/NO_2), ammonia (NH_3), and silica (SiO_2), as shown in **Table 4**. August 2012 TN, TP, and SRP measurements

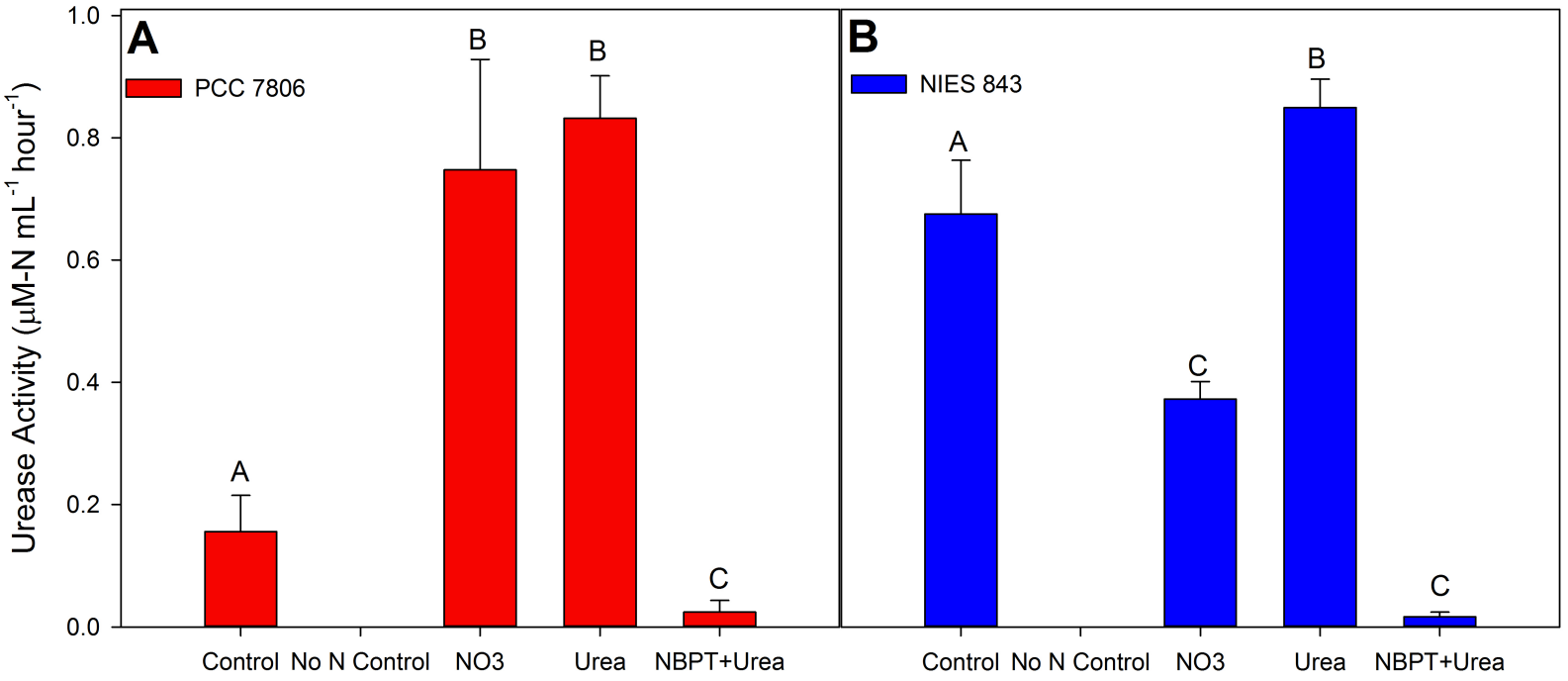

Figure 4: Urease activity for *M. aeruginosa* strains PCC 7806 (A) and NIES 843 (B) grown on various species of nitrogen as the sole nitrogen source. Letters (A/B/C) compare the nitrogen treatment to the 'Positive Control' treatment. Identical letters indicate no statistical difference ($p > 0.05$); different letters represent statistical difference ($p < 0.05$).

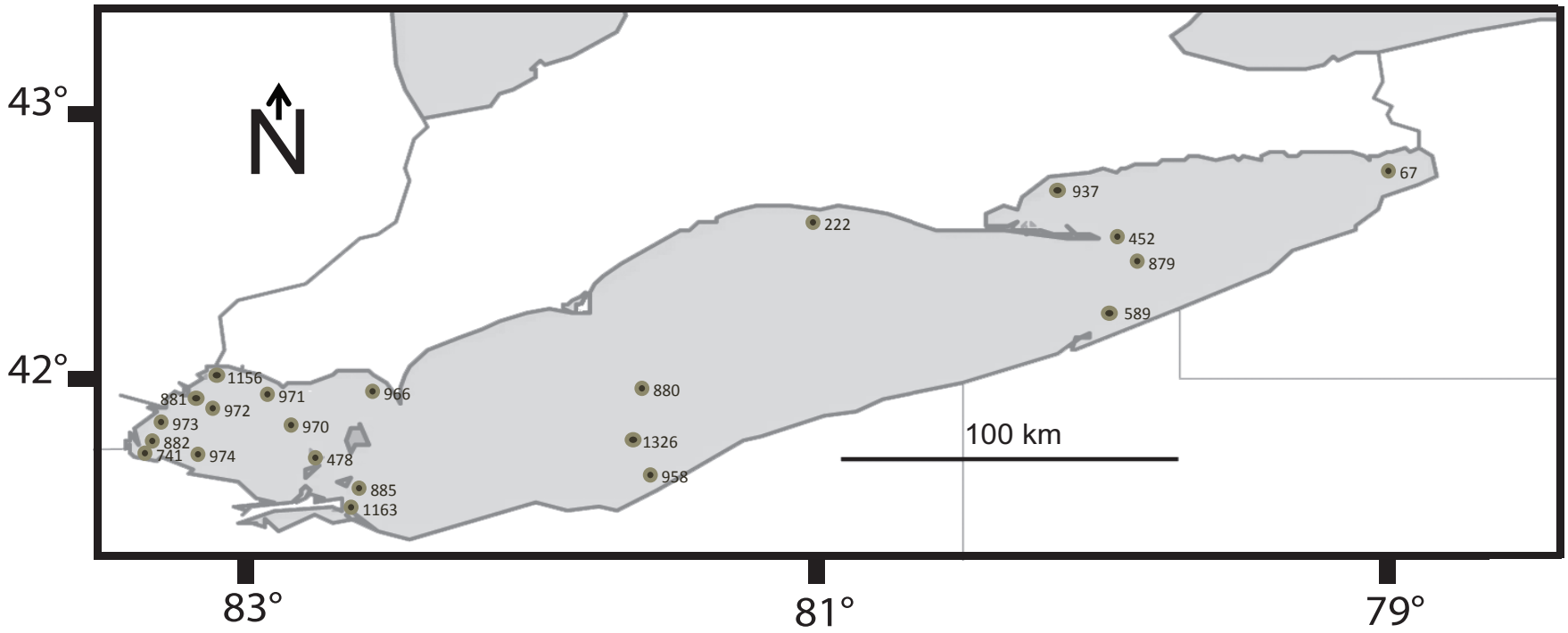

Figure 5: Lake Erie map of environmental sampling stations visited during the August 2012, May 2013, and July 2013 sampling cruises, aboard the CCGS *Limnos*.

Table 3: Lake Erie station measurements for the August 2012, May 2013, and July 2013 sampling cruises were collected from a 1-meter depth and analyzed for Chlorophyll *a* concentration, Urea concentration, and Urease activity aboard the C.C.G.S. *Limnos*. Environmental measurements of Microcystin concentration, Dissolved oxygen, Temperature, Transmission, and Specific conductance were also taken at the time of sampling by Environment Canada (NLET 1994); (N.D.) indicates samples that were not determined.

Station Number	Sample Date	Chlorophyll *a* Conc. (µg/L)	Urea Conc. (µg/L)	Urease Activity (µM-N per hour)	Microcystin Conc. (µg/L)	Dissolved O_2 (mg/L)	Temp. (°C)	Transmission (%)	Specific Conductance (µS/cm)
August 2012 Cruise									
879	8/16/12	2.305	1.225	0.0182	N.D.	9.41	23.2	1	74
478	8/15/12	6.615	1.544	0.4147	0.017	8.75	23.9	1	44
589	8/16/12	9.43	1.624	0.0125	0.042	10.1	23.9	1	41
741	8/15/12	14.9	6.203	0.2976	0.048	5.31	23.7	1	1
880	8/14/12	3.08	1.065	0.0073	0.006	8.72	23.9	1	74
882	8/15/12	16.05	0.932	0.4406	0.8	10.1	23.4	1	35
885	8/14/12	12.85	0.932	0.0228	0.14	10.3	24.5	1	25
937	8/16/12	1.56	1.225	0.0242	N.D.	9.71	22.5	1	72
966	8/13/12	5.65	1.735	0.0824	0.03	8.85	24.5	1	44
970	8/13/12	4.985	1.708	0.0688	>0.05	9.51	24.1	1	56
971	8/13/12	2.055	2.536	0.1327	0.025	9.22	23.2	1	52
972	8/15/12	7.405	1.735	0.0154	0.007	10.7	23.3	1	59
973	8/15/12	25.4	2.269	0.4863	>0.06	10.9	23.7	1	30
974	8/15/12	9.015	1.495	0.2145	0.09	N.D.	22.8	1	N.D.
1163	8/14/12	15.6	1.495	0.0248	0.59	8.72	23.9	1	74
1326	8/14/12	3.16	5.605	0.2985	0.026	N.D.	23.9	1	N.D.
May 2013 Cruise									
67	5/29/13	8.865	0.823	0.6562	<0.02	9.52	14.9	1	485
222	5/28/13	3.4	0.415	0.7659	<0.00	11.5	11.2	63	280
478	5/30/13	6.555	0.431	N.D.	<0.00	10.1	17.2	57	274
879	5/29/13	0.726	0.183	0.8698	<0.00	11.9	10.4	72	282
880	5/30/13	3.8	0.375	N.D.	<0.00	12.5	10.7	63	273
881	5/27/13	13	0.383	1.7239	<0.01	9.6	14.9	33	263
885	5/28/13	5.43	0.534	1.0238	<0.01	9.9	15.17	32	291
937	5/29/13	1.45	0.175	0.9851	<0.00	10.3	11.42	70	282
958	5/28/13	4.31	0.606	1.5911	<0.01	9.88	15.58	27	290

Table 3: Continued

Station Number	Sample Date	Chlorophyll *a* Conc. (µg/L)	Urea Conc. (µg/L)	Urease Activity (µM-N per hour)	Microcystin Conc. (µg/L)	Dissolved O_2 (mg/L)	Temp. (°C)	Transmission (%)	Specific Conductance (µS/cm)
966	5/28/13	2.79	0.327	1.5716	<0.00	9.62	14.95	55	258
970	5/30/13	3.3	0.431	N.D.	<0.00	3.68	17.26	34	294
971	5/30/13	1.55	0.447	N.D.	<0.01	10.4	16.79	38	245
973	5/27/13	2.605	0.574	1.5589	<0.03	8.51	16.79	3	334
1156	5/27/13	5.35	0.327	0.9481	<0.01	N.D.	N.D.	N.D.	N.D.
1163	5/28/13	37.35	0.510	1.3949	0.418	N.D.	N.D	N.D.	N.D.
1326	5/28/13	3.33	0.877	1.9628	<0.00	11.8	11.42	59	282
July 2013 Cruise									
452	7/25/13	1.3855	0.734	0.1118	<0.00	9.78	21.25	74	280
478	7/23/13	4.795	1.244	0.1152	0.03	8.84	26.45	59	260
741	7/24/13	18	3.333	0.4944	<0.03	6.05	26.89	2	411
880	7/25/13	1.445	1.069	0.1493	<0.00	9.28	23.61	84	275
882	7/24/13	6.535	1.427	0.1061	0.13	7.39	25.15	52	325
885	7/23/13	21	0.710	0.2253	0.235	9.05	25.67	29	294
937	7/22/13	4.455	1.148	0.0540	<0.00	10.2	19.82	77	286
958	7/24/13	1.06	1.364	0.1041	0.04	8.77	25.52	59	301
970	7/23/13	3.465	0.893	0.0487	0.03	7.33	25.49	73	259
971	7/23/13	4.24	1.643	0.1726	<0.00	8.02	25.85	54	231
973	7/24/13	3.44	2.113	0.1569	0.07	7.25	25.19	27	339
1163	7/23/13	72.45	2.041	0.1996	0.11	9.07	26.37	1	330
1326	7/24/13	3.04	1.388	0.1873	<0.00	9.77	23.62	81	274

Table 4: Lake Erie station nutrient measurements for Total Kjeldahl Nitrogen (TKN), Total Nitrogen (TN), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Nitrate/Nitrite (NO_3NO_2), Ammonia (NH_3), Silicon Dioxide (SiO_2) for the August 2012, May 2013, and July 2013 sampling cruises. All environmental water samples were collected from a 1-meter depth and chemically analyzed by the National Laboratory for Environmental Testing (NLET 1994); (N.D.) indicates samples that were not determined.

Station Number	Sampling Date	TKN (mg/L)	TN (mg/L)	TP (mg/L)	SRP (mg/L)	NO_3NO_2 (mg/L)	NH_3 (mg/L)	SiO_2 (mg/L)
August 2012 Cruise								
879	8/16/12	0.209	0.356	0.0044	< 0.0002	0.078	0.009	0.38
478	8/15/12	0.174	0.203	0.016	0.008	0.006	< 0.005	1.79
589	8/16/12	N.D.	N.D.	0.0126	N.D.	N.D.	N.D.	1.77
741	8/15/12	1.03	1.63	0.153	0.14	0.683	0.249	1.81
880	8/14/12	0.198	0.216	0.0055	0.0008	0.006	0.007	0.34
882	8/15/12	0.298	0.343	0.0107	0.0004	0.006	< 0.005	0.95
885	8/14/12	0.211	0.233	0.0092	0.0004	0.005	0.007	1.16
937	8/16/12	N.D.	N.D.	N.D.	N.D	N.D.	N.D.	0.22
966	8/13/12	0.16	0.234	0.005	0.0002	0.045	0.005	2.29
970	8/13/12	N.D.	N.D.	N.D	N.D.	N.D.	N.D.	N.D.
971	8/13/12	0.139	0.339	0.0035	0.0003	0.146	< 0.005	1.56
972	8/15/12	0.178	0.464	0.0045	0.0004	0.258	0.023	1.64
973	8/15/12	0.346	0.375	0.0113	0.0003	0.01	0.017	1.73
974	8/15/12	0.218	0.245	0.0155	0.0087	0.005	< 0.005	0.79
1163	8/14/12	0.281	0.294	0.0189	0.0088	0.005	< 0.005	2.6
1326	8/14/12	0.209	0.288	0.006	0.0003	0.03	0.025	0.75
May 2013 Cruise								
67	5/29/13	0.485	N.D.	N.D.	0.0199	1.3855	0.07	N.D.
222	5/28/13	0.247	N.D.	N.D.	< 0.0002	0.0875	0.022	N.D.
478	5/30/13	0.189	N.D.	N.D.	0.0021	0.726	0.011	N.D.
879	5/29/13	0.21	N.D.	N.D.	0.0009	0.159	0.011	N.D.
880	5/30/13	0.2615	N.D.	N.D.	0.0034	0.0685	0.012	N.D.
881	5/27/13	0.349	N.D.	N.D.	< 0.0002	0.75	0.1155	N.D.
885	5/28/13	0.2755	N.D.	N.D.	0.0018	0.681	0.018	N.D.
937	5/29/13	0.263	N.D.	N.D.	< 0.0002	0.0755	0.0215	N.D.

Table 4: Continued

Station Number	Sampling Date	TKN (mg/L)	TN (mg/L)	TP (mg/L)	SRP (mg/L)	NO3NO2 (mg/L)	NH3 (mg/L)	SiO2 (mg/L)
	May 2013 Cruise cont.							
958	5/28/13	0.217	N.D.	N.D.	0.0056	0.4985	0.0245	N.D.
966	5/28/13	0.1905	N.D.	N.D.	< 0.0002	0.571	0.014	N.D.
970	5/30/13	0.253	N.D.	N.D.	0.0004	0.736	0.011	N.D.
971	5/30/13	0.15	N.D.	N.D.	0.0002	0.398	0.018	N.D.
973	5/27/13	0.359	N.D.	N.D.	N.D.	0.441	0.021	N.D.
1156	5/27/13	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1163	5/28/13	N.D.	N.D.	N.D.	0.0007	N.D.	N.D.	N.D.
1326	5/28/13	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	July 2013 Cruise							
452	7/25/13	N.D.	N.D.	N.D.	N.D	N.D.	N.D.	N.D.
478	7/23/13	N.D.	0.674	0.0791	0.0006	0.414	< 0.005	N.D.
741	7/24/13	N.D.	3.65	0.154	0.0827	2.88	0.163	N.D.
880	7/25/13	N.D.	0.444	0.051	0.0002	0.164	0.007	N.D.
882	7/24/13	N.D.	1.92	0.0682	0.0012	1.73	0.014	N.D.
885	7/23/13	N.D.	0.902	0.053	0.0005	0.567	0.012	N.D.
937	7/22/13	N.D.	0.369	0.0627	0.0004	0.124	0.018	N.D.
958	7/24/13	N.D.	0.678	0.0575	0.0003	0.412	< 0.005	N.D.
970	7/23/13	N.D.	0.754	0.0479	0.0006	0.532	0.006	N.D.
971	7/23/13	N.D.	0.55	0.0504	0.0004	0.352	< 0.005	N.D.
973	7/24/13	N.D.	2.13	0.0706	0.0081	1.93	0.009	N.D.
1163	7/23/13	N.D.	1.83	0.0747	0.0005	1.53	< 0.005	N.D.
1326	7/24/13	N.D.	0.349	0.0528	< 0.0002	0.126	< 0.005	N.D.

ranged from 0.203 – 1.63 mg/L, 0.0035 – 0.153 mg/L, and 0.0003 – 0.14 mg/L respectively, with the Station 741 exhibiting the highest measurements for all three. Similarly, July 2013 measurements for TN, TP, and SRP were highest at Station 741 and ranged from 0.349 – 3.65 mg/L, 0.0479 – 0.154 mg/L, and 0.0002 – 0.0827 mg/L respectively. May 2013 station measurements for SRP ranged from 0.0002 – 0.0199 mg/L. Unfortunately, May 2013 measurements for TN and TP were unavailable and Station 741 was not sampled for this cruise.

A significant correlation between urease activity and chlorophyll biomass was observed only for the August 2012 cruise (**Figure 6;** $p = 0.0343$), and not for the May 2013 (**Figure 7;** $p = 0.403$) or July 2013 cruises (**Figure 8;** $p = 0.312$). Notably higher urease activity was observed during the May 2013 cruise, compared to August 2012 July 2013 data, ranging from 0.656 - 1.963 μM-N per hour. August 2012 and July 2013 urease activity ranged from 0.007 - 0.441 μM-N per hour and 0.049 - 0.225 μM-N per hour, respectively.

Station measurements of urease activity vs. urea concentration for August 2012, May 2013, and July 2013 are show in **Figure 9**. A significant correlation between urease activity and urea concentration was observed only for the July 2013 cruise ($p = 0.003$), and not for the August 2012 ($p = 0.181$) or May 2013 cruises ($p = 0.285$). Additionally, no correlation ($p > 0.05$) was observed between chlorophyll biomass and urea concentration, for any of the three cruises (**Figure 10**).

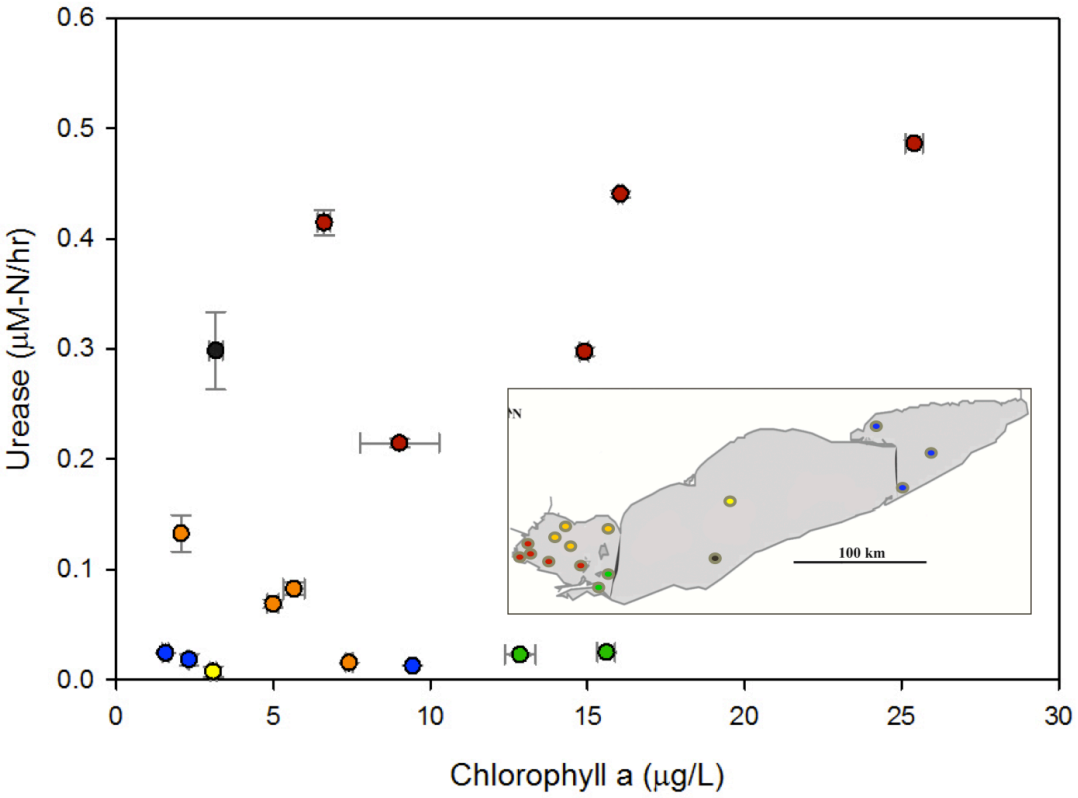

Figure 6: August 2012 Lake Erie station measurements of chlorophyll a and urease activity. Color correlates to sample station location, as indicated by the map insert. The station color categories are: Northwestern basin (Orange), Southwestern basin (Red), Central basin (Yellow), Eastern basin (Blue), Stations in proximity to Sandusky bay (Green), Stations in proximity to Cleveland, OH (Black). P-value = 0.0343

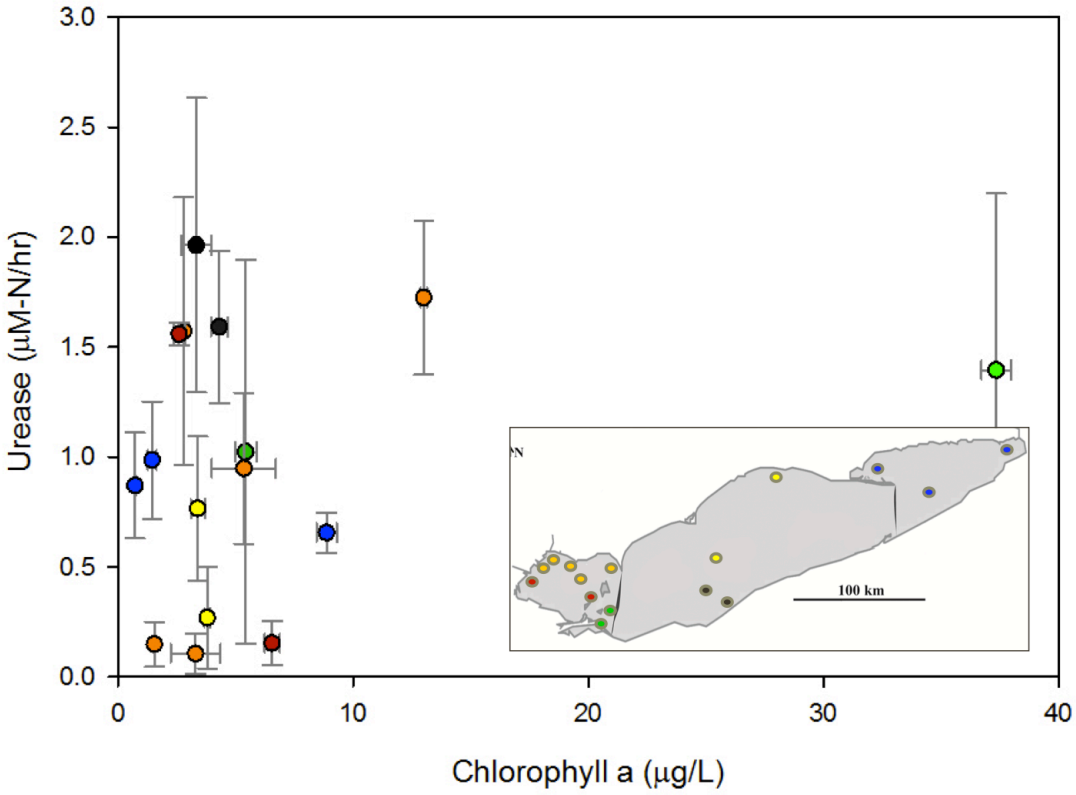

Figure 7: May 2013 Lake Erie station measurements of chlorophyll a and urease activity. Color correlates to sample station location, as indicated by the map insert. Color schemes are the same as those previously described in Figure 6. P-value = 0.4029

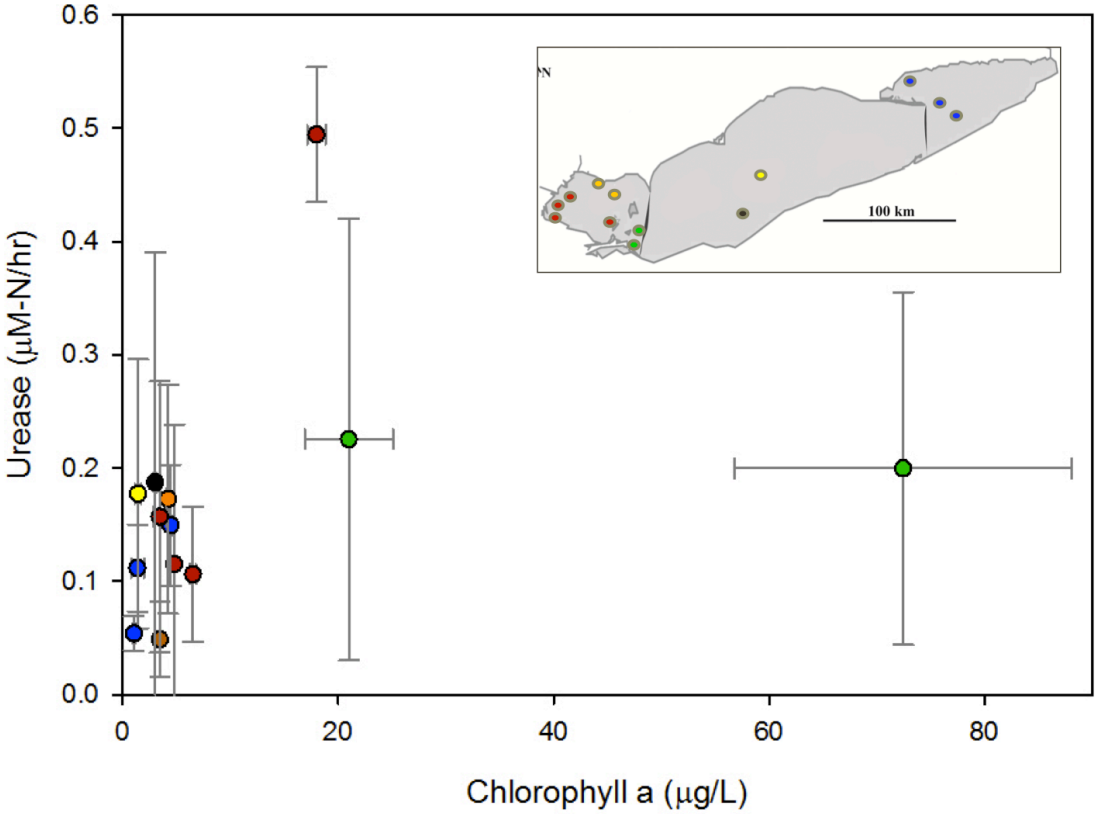

Figure 8: July 2013 Lake Erie station measurements of chlorophyll a and urease activity. Color correlates to sample station location, as indicated by the map insert. Color schemes are the same as those previously described in Figure 6. P-value = 0.3115

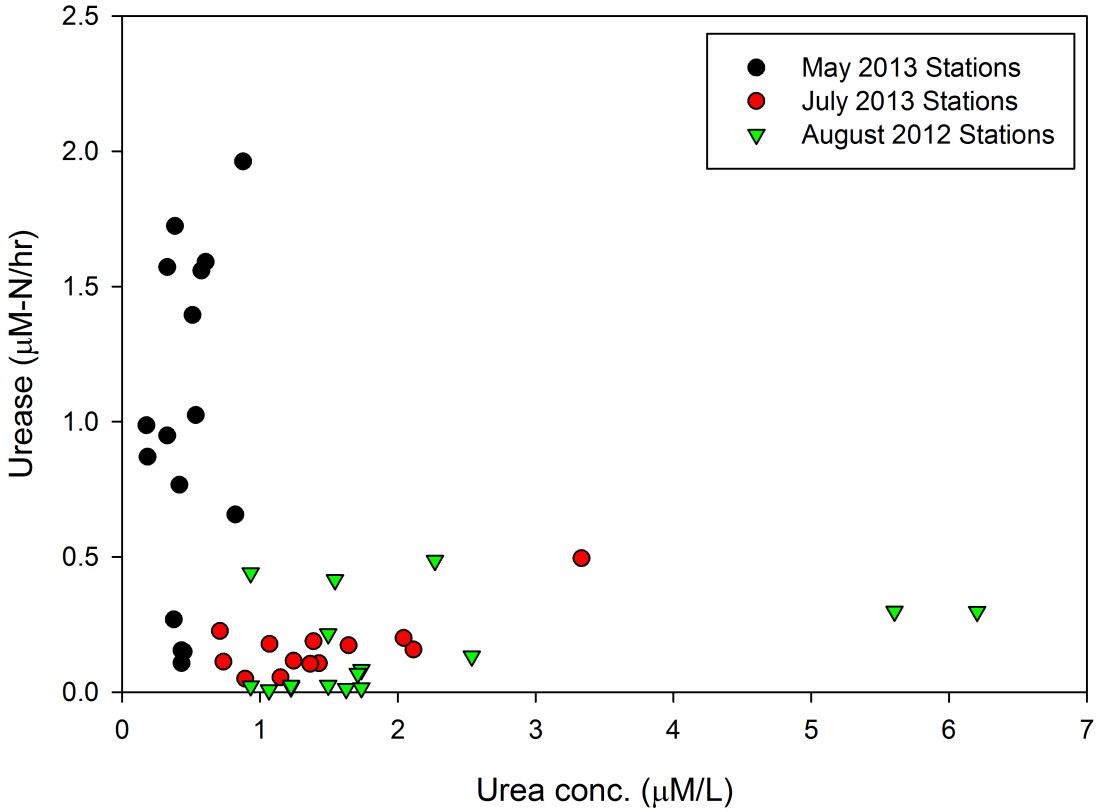

Figure 9: Lake Erie station measurements of urease activity and urea concentration, for three sampling cruises (August '12, May '13, July '13). Symbol color discerns between each sampling cruise: August 2012 stations (Green), May 2013 stations (Black), July 2013 stations (Red). P-values for May 2013, July 2013, and August 2012 are 0.285, 0.003, and 0.181, respectively.

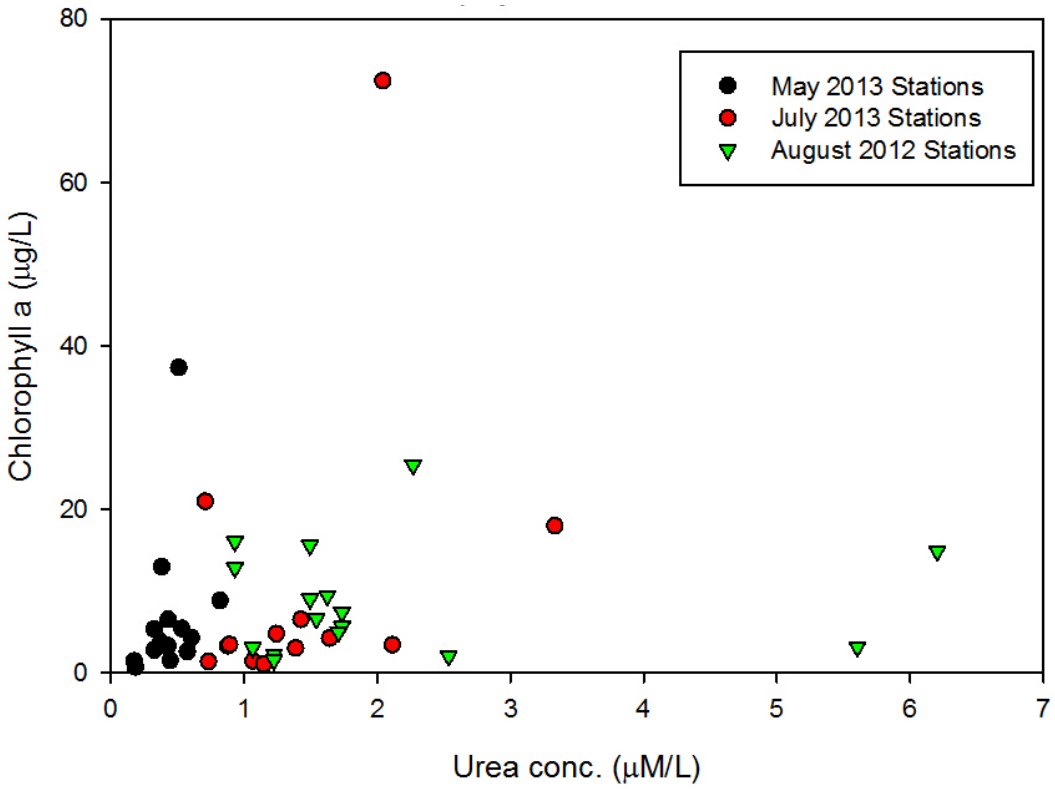

Figure 10: Lake Erie station measurements of chlorophyll a and urea concentrations, for three sampling cruises (August '12, May '13, July '13). Symbol color discerns between each sampling cruise, as previously described in Figure 9. P-values for May 2013, July 2013, and August 2012 are 0.5647, 0.286, and 0.819, respectively.

Microcosm Experiments

August 2012 microcosm measurements of chlorophyll *a* biomass for the ambient whole water "Control" treatment and the "Control + 1µM Phosphorous" treatment were statistically similar for Stations 973 and 885 (**Figure 11 A, D**). These results suggest that the addition of phosphorus did not stimulate biomass at the time of sampling for either station. The addition of 180µM-N NH_4, NO_3, Urea, and NBPT stimulated biomass accumulation in all "Nitrogen only" treatments, compared to the "Control" treatment, for both Stations 973 and 885 (p-values shown in **Table 6**). August 2012 microcosm chlorophyll *a* measurements for the "Nitrogen only" treatments ranged from 23.7 µg/L, for the "Control" treatment, and 36.0 µg/L, for the NO_3 treatment at Station 973, and 10.6 µg/L, for the "control" treatment, and 30.65 µg/L, for the NO_3 treatment at Station 885. Similar results were observed for the all "Nitrogen +PO_4" treatments at Station 885, but only NH_4 + 1µM PO_4 and NO_3+1µM PO_4 were statistically different for Station 973, compared to the Control +PO_4 treatment (**Table 6**). Chlorophyll *a* measurements for the "Nitrogen +PO_4" treatments ranged from 25.7 µg/L, for the "Control +P" treatment, and 46.57 µg/L, for the NO_3+P treatment at Station 973, and 13.37 µg/L, for the "Control +P" treatment, and 38.7 µg/L, for the Urea +P treatment at station 885.

For all nitrogen treatments at Stations 973 and 885, *Microcystis* cell densities were statistically similar to their respective control treatments (**Figure 11 B, E**). Similar results were observed for *Anabaena* cell counts for Station 885 (**Figure 11 F**) and Station 973 (**Figure 11 C**), with the NO_3 and Urea Nitrogen only

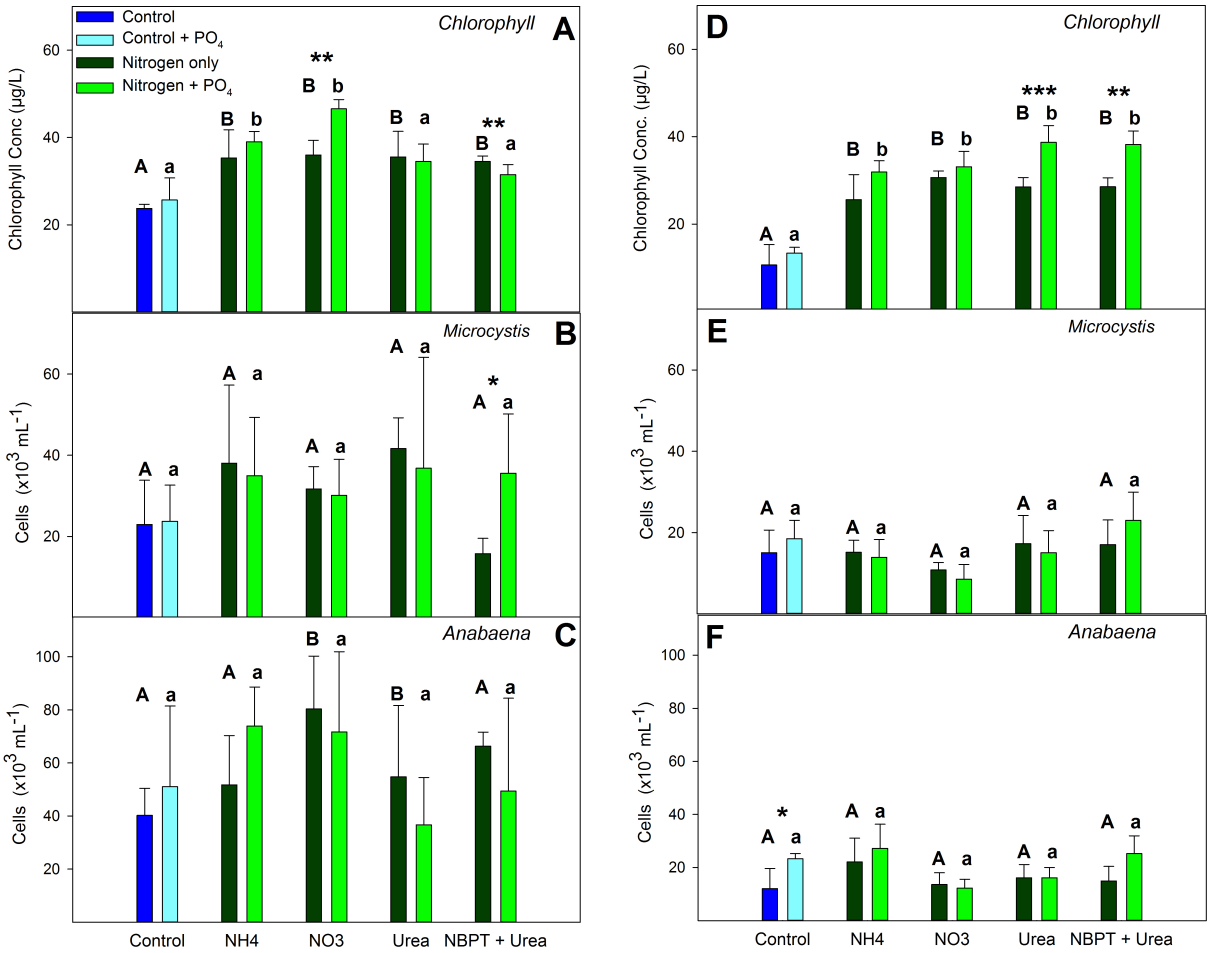

Figure 11: Station 973 (A, B, C) and Station 885 (D, E, F) August 2012 Microcosm measurements of Chlorophyll *a* conc., *Microcystis* cell counts, and *Anabaena* cell counts, after 48 h of incubation. Whole water from each station was spiked with 180µM-Nitroegen (Nitrogen only Treatments) and 180µM-N + 1µM Phosphorous (Nitrogen +PO_4 treatments), for each chemical form of Nitrogen. Letters above each bar represent levels of statistical significance compared to the control for each treatment. Letters (A/B) compare the Nitrogen only treatment to the nitrogen only 'Control' (Dark Blue). Letters (a/b) compare the Nitrogen +PO_4 treatments to the 'Control + PO_4' (Light Blue). Identical letters indicate no statistical difference ($p > 0.05$); different letters represent statistical difference ($p < 0.05$).
Asterisks represent comparison of the Nitrogen only and Nitrogen + P treatment, for each species of nitrogen (e.g. NH_4 only treatment compared to the NH_4 +PO_4 treatment). (*), (**), (***) represent the p-value (0.1-0.05), (0.05-0.01), (<0.01) respectively.

Table 5: August 2012 microcosm measurements of Chlorophyll *a* (Chl a), *Microcystis* cell counts, and *Anabaena* cell counts, after 48 h of incubation, for Station 973 and Station 885. Whole water from each station was spiked with 180µM-N (Nitrogen only Treatments) and 180µM-N + 1µM Phosphorous (Nitrogen +PO_4 treatments), for each species of nitrogen.

Stations and Treatments	Nutrient Addition	Chl a conc. (µg per L)	*Microcystis* (cells per mL)	*Anabaena* (cells per mL)
Station 973				
Nitrogen Only Treatments	NO_3	36.00	31686	80368
	Urea	35.57	41615	54735
	NBPT + Urea	34.50	15754	66311
	NH_4	35.33	38044	51721
	Control	23.73	22922	40247
Station 973				
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P	46.57	30090	71757
	Urea +1µM P	34.50	36828	36701
	NBPT+1µM P	31.53	35561	49340
	NH_4 + 1µM P	39.03	34954	73960
	Control +1µM P	25.70	23733	51012
Station 885				
Nitrogen Only Treatments	NO_3	30.65	10790	13601
	Urea	28.52	17274	16058
	NBPT + Urea	28.53	17046	14944
	NH_4	25.58	15146	22087
	Control	10.65	15045	12031
Station 885				
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P	33.12	8535	12234
	Urea +1µM P	38.70	15070	16160
	NBPT+1µM P	38.23	22998	25227
	NH_4 + 1µM P	31.90	13905	27178
	Control +1µM P	13.37	18490	23302

Table 6: August 2012 microcosm p-values of Chlorophyll *a* measurements, *Microcystis* cell counts, and *Anabaena* cell counts, after 48 h of incubation, for Station 973 and Station 885. (A/a) indicate no statistical difference (p> 0.05) from the control; (B/b) represent statistical difference (p < 0.05) from the control. Uppercase letters (A; B) compare the Nitrogen only treatment to the nitrogen only 'Control' and lowercase letters (a/b) compare the Nitrogen +PO_4 treatments to the 'Control + PO_4'. Asterisks represent comparison of the Nitrogen only and Nitrogen + P treatment, for each species of nitrogen (e.g. NH_4 only treatment compared to the NH_4 +PO_4 treatment). (*), (**), (***) represent the p-value (0.1-0.05), (0.05-0.01), (<0.01) respectively; (N.A.) is used when no symbol is applicable.

Pairwise T-test Statistical Comparison	Treatments Compared	Chl a P-Value	Symbol	*Microcystis* P-Value	Symbol	*Anabaena* P-Value	Symbol
Station 973							
Treatments With and Without Phosphorus	NO_3 and NO_3 + 1µM P	0.0100	**	0.8054	N.A.	0.7009	N.A.
	Urea and Urea + 1µM P	0.8071	N.A.	0.7846	N.A.	0.3886	N.A.
	NBPT and NBPT + 1µM P	0.0116	**	0.0858	*	0.4536	N.A.
	NH_4 and NH_4 + 1µM P	0.4027	N.A.	0.8346	N.A.	0.1789	N.A.
	Control and Control +1µM P	0.5438	N.A.	0.9253	N.A.	0.5927	N.A.
Station 973							
Nitrogen Only Treatments	NO_3 and Control	0.0037	**B**	0.2821	**A**	0.0358	**B**
	Urea and Control	0.0259	**B**	0.0717	**A**	0.4332	**A**
	NBPT and Control	0.0003	**B**	0.3425	**A**	0.0170	**B**
	NH_4 and Control	0.0367	**B**	0.3019	**A**	0.4017	**A**
Station 973							
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P and Control +P	0.0027	**b**	0.4315	**a**	0.4492	**a**
	Urea +1µM P and Control +P	0.0770	**a**	0.474	**a**	0.5212	**a**
	NBPT+1µM P and Control +P	0.1412	**a**	0.2977	**a**	0.9533	**a**
	NH_4 + 1µM P and Control +P	0.0143	**b**	0.3144	**a**	0.3048	**a**
Station 885							
Treatments With and Without Phosphorus	NO_3 and NO_3 + 1µM P	0.4509	N.A.	0.3919	N.A.	0.6907	N.A.
	Urea and Urea + 1µM P	0.0024	***	0.6871	N.A.	0.9793	N.A.
	NBPT and NBPT + 1µM P	0.0151	**	0.326	N.A.	0.1074	N.A.
	NH_4 and NH_4 + 1µM P	0.1767	N.A.	0.7063	N.A.	0.5281	N.A.

Table 6: Continued

Pairwise T-test Statistical Comparison	Treatments Compared	Chl a P-Value	Symbol	Microcystis P-Value	Symbol	Anabaena P-Value	Symbol
	Control and Control +1µM P	0.1705	N.A.	0.4515	N.A.	0.0658	*
Station 885							
Nitrogen Only Treatments	NO_3 and Control	0.0009	**B**	0.2752	**A**	0.7705	**A**
	Urea and Control	0.0012	**B**	0.6868	**A**	0.4845	**A**
	NBPT and Control	0.0025	**B**	0.6948	**A**	0.6156	**A**
	NH_4 and Control	0.0014	**B**	0.9791	**A**	0.2109	**A**
Station 885							
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P and Control +P	0.0008	**b**	0.0413	**b**	0.0078	**b**
	Urea +1µM P and Control +P	3.0 e-5	**b**	0.4478	**a**	0.0456	**b**
	NBPT+1µM P and Control +P	3.31 e-5	**b**	0.3996	**a**	0.6565	**a**
	NH_4 + 1µM P and Control +P	0.0002	**b**	0.2775	**a**	0.5107	**a**

treatments being the only treatments that were statically different from the Control, for the latter.

May 2013 and July 2013 microcosm chlorophyll *a* measurements (**Figure 12; Table 7**) were notably higher during July 2013, compared to May 2013, for both Stations 973 and 885. Chlorophyll biomass for May 2013 was statistically similar to their respective controls for all treatments at Station 973 (**Figure 12 A**) and for all treatments, except NBPT+Urea Nitrogen only treatment, for Station 885 (**Figure 12 C**), p-values are reported in **Table 8**. Chlorophyll biomass for July 2013 was statistically similar to their respective controls for all treatments at Station 973 (**Figure 12 B**) and for all treatments, except NH_4 + 1µM PO_4 treatment, for Station 885 (**Figure 12 D**). Interestingly, the addition of 1µM PO_4 showed a statistical increase in chlorophyll biomass for all nitrogen treatments for July 2013, at Station 885, and for the NH_4 treatment, at Station 973 (**Table 8**).

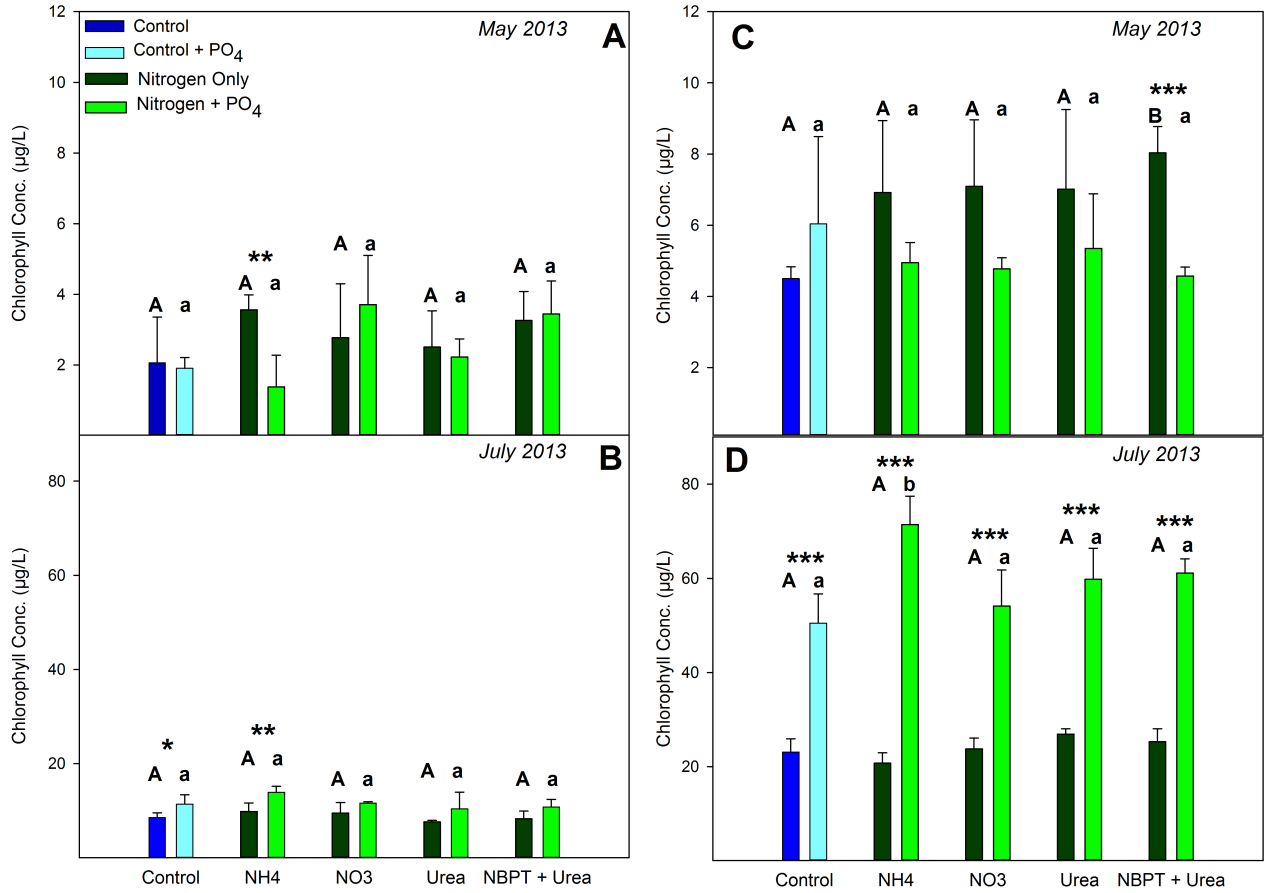

Figure 12: Station 973 (A, B) and Station 885 (C, D) microcosm measurements of chlorophyll *a* concentrations after 48 h of incubation, during May and July 2013 sampling cruises. Whole water, from each station, was spiked with 180µM of nitrogen (Nitrogen only Treatments) and 180µM-N + 1µM Phosphorous (Nitrogen +PO_4 treatments), for each species of Nitrogen. Letters above each bar represent levels of statistical significance compared to the control for each treatment, as previously described in Figure 11.

Table 7: May 2013 and July 2013 microcosm measurements for Chlorophyll *a* (Chl a), Total Nitrogen (TN), and Total Phosphorus (TP), for Stations 973 and 885.

Stations and Treatments	Nutrient Addition	May 2013 Chl a (µg/L)	May 2013 TP (mg/L)	May 2013 TN (mg/L)	July 2013 Chl a (µg/L)	July 2013 TN (mg/L)	July 2013 TP (mg/L)
Station 973							
Nitrogen Only Treatments	NO_3	2.77	4.337	0.024	9.49	4.91	0.092
	Urea	2.51	4.443	0.017	7.61	5.0	0.067
	NBPT + Urea	3.27	4.350	0.020	8.31	4.79	0.041
	NH_4	3.56	4.457	0.014	9.84	5.03	0.043
	Control	2.06	2.057	0.015	8.54	2.11	0.051
Station 973							
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P	3.71	4.42	31.30	11.60	4.78	34.33
	Urea +1µM P	2.22	4.01	30.40	10.36	4.81	33.97
	NBPT+1µM P	3.45	3.91	30.55	10.79	4.72	34.2
	NH_4 + 1µM P	1.38	4.69	31.00	13.90	4.76	33.4
	Control +1µM P	1.91	1.64	29.20	11.39	2.12	34.5
Station 885							
Nitrogen Only Treatments	NO_3	7.09	3.54	0.009	23.80	3.42	0.729
	Urea	7.01	3.46	0.010	26.97	3.36	0.549
	NBPT + Urea	8.04	3.42	0.011	25.33	3.13	0.026
	NH_4	6.92	3.40	0.019	20.77	3.26	0.042
	Control	4.50	1.20	0.012	23.10	0.66	0.055
Station 885							
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P	4.77	3.55	29.40	54.13	3.02	33.97
	Urea +1µM P	5.35	3.40	29.35	59.80	2.92	34.57
	NBPT+1µM P	4.58	3.39	31.35	61.13	2.67	34.5
	NH_4 + 1µM P	4.49	3.49	30.05	71.40	2.85	41.3
	Control +1µM P	6.04	1.20	29.70	50.50	0.34	34.93

Table 8: May 2013 and July 2013 microcosm P-values for Chlorophyll *a* (Chl a), at Station 973 and Station 885. (A/a) indicate no statistical difference (p> 0.05) from the control; (B/b) represent statistical difference (p < 0.05) from the control. Uppercase letters (A; B) compare the Nitrogen only treatment to the nitrogen only 'Control' and lowercase letters (a/b) compare the Nitrogen +PO_4 treatments to the 'Control + PO_4'. Asterisks represent comparison of the Nitrogen only and Nitrogen + P treatment, for each species of nitrogen (e.g. NH_4 only treatment compared to the NH_4 +PO_4 treatment). (*), (**), (***) represent the p-value (0.1-0.05), (0.05-0.01), (<0.01) respectively. (N.A.) is used when no symbol is applicable.

Pairwise T-test Statistical Comparison	Treatments Compared	May 2013 Chl a P-Value	Symbol	July 2013 Chl a P-Value	Symbol
Station 973					
Treatments With and Without Phosphorus	NO_3 and NO_3 + 1µM P	0.477	N.A.	0.182	N.A.
	Urea and Urea + 1µM P	0.692	N.A.	0.247	N.A.
	NBPT and NBPT + 1µM P	0.812	N.A.	0.131	N.A.
	NH_4 and NH_4 + 1µM P	0.019	**	0.033	**
	Control and Control +1µM P	0.850	N.A.	0.094	*
Station 973					
Nitrogen Only Treatments	NO_3 and Control	0.571	**A**	0.538	**A**
	Urea and Control	0.665	**A**	0.205	**A**
	NBPT and Control	0.245	**A**	0.843	**A**
	NH_4 and Control	0.130	**A**	0.335	**A**
Station 973					
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P and Control +P	0.094	**a**	0.867	**a**
	Urea +1µM P and Control +P	0.410	**a**	0.698	**a**
	NBPT+1µM P and Control +P	0.052	**a**	0.708	**a**
	NH_4 + 1µM P and Control +P	0.390	**a**	0.141	**a**
Station 885					
Treatments With and Without Phosphorus	NO_3 and NO_3 + 1µM P	0.102	N.A.	0.003	***
	Urea and Urea + 1µM P	0.347	N.A.	0.001	***
	NBPT and NBPT + 1µM P	0.009	***	0.0001	***
	NH_4 and NH_4 + 1µM P	0.178	N.A.	0.0002	***
	Control and Control +1µM P	0.343	N.A.	0.002	***

Table 8: Continued

Pairwise T-test Statistical Comparison	Treatments Compared	May 2013 Chl a P-Value	Symbol	July 2013 Chl a P-Value	Symbol
Station 885					
Nitrogen Only Treatments	NO_3 and Control	0.077	**A**	0.759	**A**
	Urea and Control	0.127	**A**	0.094	**A**
	NBPT and Control	0.002	**B**	0.387	**A**
	NH_4 and Control	0.110	**A**	0.323	**A**
Station 885					
Nitrogen + 1µM Phosphorous Treatments	NO_3 +1µM P and Control +P	0.425	**a**	0.559	**a**
	Urea +1µM P and Control +P	0.700	**a**	0.150	**a**
	NBPT+1µM P and Control +P	0.483	**a**	0.057	**a**
	NH_4 + 1µM P and Control +P	0.495	**a**	0.014	**b**

V. Discussion

Microcystis Growth on Various Chemical Forms of Nitrogen

Urea represents a significant source of anthropogenic nitrogen due to its increased usage as a form of nitrogen for agricultural fertilizers; therefore, the ability of *M. aeruginosa* to use urea as a nitrogen source is ecologically relevant for freshwater systems that receive large loads of anthropogenic pollution, such as Lake Erie. Laboratory strains of *M. aeruginosa* PCC 7806 and NIES 843 have demonstrated the ability to use urea as a sole nitrogen source, similar to other species of nitrogen (**Figure 2** and **Figure 3**). Likewise, observed growth rates for both PCC 7806 and NIES 843 (**Table 1**) were equivalent to other published growth rates of unialgal cultures of *M. aeruginosa* grown on nitrate and urea as the sole nitrogen substrate (Solomon *et al.* 2010).

Solomon *et al*., (2010) reported that not all phytoplankton possess the ability use urea as a source of nitrogen, because they may lack enzymes essential for the uptake or catabolism of urea. From this, it has been suggested that the ability to use urea as a nitrogen source is a reason for promoting the success of bloom forming cyanobacterial species, such as *M. aeruginosa* (Huang *et al.* 2014). This concept is supported by previous field studies from phosphorus rich freshwater lakes that have demonstrated that the addition of urea suppressed N_2-fixing cyanobacteria, such as *Anabaena* and *Aphanizomenon*, and stimulated the growth of non-N_2 fixing cyanobacteria, such as *Microcystis* and *Planktothrix* (Flores *et al.* 2005, Finlay *et al.* 2010, Solomon *et al.* 2010). Consequently, the increased practice of using urea for

agricultural purposes and these advantages that favor growth of HAB forming species of cyanobacteria, over native phytoplankton species, may result in increased occurrences of *M. aeruginosa* blooms in freshwater systems.

Urease Activity of PCC 7806 and NIES 843

The urease enzyme converts urea and water into NH_4^+ and CO_2, which is subsequently used in biochemical processes within the cell (Solomon *et al.* 2007). Urease activity was measured for both PCC 7806 and NIES 843 (**Figure 4**) during mid-exponential phase using the indophenol method (Solomon *et al.* 2007). These results provide the first reported measurements of urease activity for *M. aeruginosa* strains PCC 7806 and NIES 843. Urease activity was highest, for both strains, when urea was the sole nitrogen source provided, and lowest for the NBPT+Urea treatments. These results suggest that exposure to urea as a nitrogen source may promote increased urease activity and demonstrated that NBPT successfully inhibited urease activity for our experiments. These observations are consistent with previous urease studies of phytoplankton, that have shown that basal levels of urease activity are always detectable, but the level of urease activity varies with the nitrogen source provided (Solomon *et al.* 2008). This supports the rational that urease activity is regulated by external and/or internal factors.

The control of urease activity, based upon the chemical species of nitrogen present, is most apparent with available concentrations of NH_4^+ (Flores *et al.* 2005, Finlay *et al.* 2010, Solomon *et al.* 2010). When insufficient NH_4^+ is available to meet the nitrogen demands for the cell, urease activity may increase in response to the

limitation of inorganic nitrogen (Glibert *et al.* 2006). Conversely, high concentrations of NH_4^+ results in increased 2-oxoglutarate concentrations, via the glutamate synthase incorporation of nitrogen into new amino acids, allowing for the regulation of nitrogen assimilation and repression of urease activity in cyanobacteria (Solomon *et al.* 2008).

Environmental Samples

Station measurements of chlorophyll *a* and urease activity was highest for stations located in the western basin of Lake Erie, compared to central and eastern basin stations, for the August 2012, May 2013, and July 2013 sampling cruises. These results most likely reflect the influence of major waterways, such as the Maumee River, that influx point and nonpoint sources of nutrients into the western basin of Lake Erie. These results provide the first reported measurements of environmental levels of urease activity in Lake Erie.

August 2012 measurements of urease activity and chlorophyll *a* concentration, a proxy for biomass, exhibited a significant correlation (**Figure 6**; $p = 0.034$). However, no significant correlation was observed for May and July 2013 station samples (**Figure 7** and **8** respectively). In previous field measurements of urease activity with diverse phytoplankton assemblages, similar to Lake Erie, researchers have had difficulty in distinguishing which phytoplankton species contributes the largest percentage of community urease activity (Solomon 2006). The lack of a correlation between urease activity and chlorophyll *a* biomass may indicate that non-photosynthetic members of the community (e.g. heterotrophic

bacteria) may be responsible for the observed urease activity. Conversely, results from published *in vivo* experiments found that measurements of urease activity decreased as chlorophyll biomass increased (Solomon *et al.* 2007). The inability to identify the degree to which species are contributing to the observed community urease activity indicates the need for additional research to address to this problem.

Significantly higher urease activity was observed for the May 2013 cruise compared to the August 2012 and July 2013 cruises (**Figure 9**). These trends are similar to published field rates of urease activity that found a 5-fold higher urease activity in the summer months, compared to fall months (Solomon 2006). This seasonal pattern could be influenced by direct or indirect effects of phytoplankton community, temperature, and/or urea concentration (Solomon *et al.* 2010).

Urea concentrations measured across Lake Erie appeared to follow an inverse relationship to observed measurements of urease activity. Urea concentrations were lowest during sampling cruises conducted during the early summer months (May/June), and highest during warmer sampling cruises occurring in the late summer and fall months (July/August). Previously published reports have shown that higher temperatures, during summer months, can increase levels of heterotrophic bacteria activity and results in increased urea production from purines, compared to other seasons (Berg *et al.* 2006). It has been suggested that phytoplankton taxa that proliferate during the summer months and can utilize urea as a nitrogen source, such as cyanobacteria and dinoflagellates, may be able to use this increased supply of urea to their advantage (Solomon *et al.* 2010). Our

measured concentrations of urea in Lake Erie appeared to follow this trend of increasing as water temperature increases; however, we did not observe an increase in urease activity with the increase in water temperature.

Lake Erie Microcosm Experiments

Chlorophyll measurements for the August 2012 microcosm experiments (**Figure 11**) indicated that there was no statistical difference between the whole water "Control" and the "Control + 1µM Phosphorous" for Stations 973 and 885, suggesting that the addition of phosphorus did not increase biomass during this sampling period. However, there was a significant increase in biomass for all "Nitrogen only" treatments compared to the Control, indicating that water conditions were nitrogen limited. The stimulation of biomass by all chemical species of nitrogen demonstrates the ability of cyanobacteria to utilize multiple bioavailable forms of nitrogen (nitrate, urea, and ammonia), to support growth. Therefore, any future nitrogen management practices in Lake Erie should consider constraining all bioavailable from of nitrogen that promote eutrophic water conditions.

Microscopic analysis of all treatments from the August 2012 microcosm indicated no statistical difference in the phytoplankton communities (**Figure 11**; **Table 6**). Phytoplankton communities, at Stations 973 and 885, were comprised primarily of the cyanobacteria *Microcystis* and *Anabaena*. The overall stimulation of biomass and similar phytoplankton communities observed for all nitrogen treatments suggests no preference in the chemical form of nitrogen under nitrogen limiting conditions. The similar community composition may be due to the limited

48-hour incubation, which may not allow enough time to observe a shift in the phytoplankton community. Future microcosm experiments that examine the community composition would benefit from a longer incubation period under *in situ* conditions, if sampling constraints allow.

Chlorophyll measurements for the July 2013 microcosm experiment were notably higher than the May 2013 measurements, for both Stations 973 and 885 (**Figure 12**). These results are not surprising because Lake Erie HABs occur annually in late May/ early June and often last until October, with temperature being an important abiotic factor that regulates bloom duration (Šejnohová *et al.* 2012). Therefore, May 2013 sampling was conducted at an early stage of the bloom formation, while the July 2013 sampling cruise was conducted as the bloom increased in intensity. Satellite images from the Moderate Resolution Imaging Spectroradiometer (MODIS) system displays the difference bloom conditions of Lake Erie during the August 2012 (**Figure 13**), May 2013 (**Figure 14**), and July 2013 (**Figure 15**) sampling cruises. The MODIS images represent the volume of cyanobacteria present, with cooler colors (blue, purple) indicating low volumes cyanobacteria and warmer colors (orange, red, yellow) indicating high volumes of cyanobacteria.

Measurements of chlorophyll biomass for the May 2013 microcosm was significantly similar for all treatments relative to the Control treatment at Station 973 (**Figure 12 A**) and for all treatments, except NBPT+Urea "Nitrogen only" treatment, for Station 885 (**Figure 12 C**). Because the addition of both N and P did

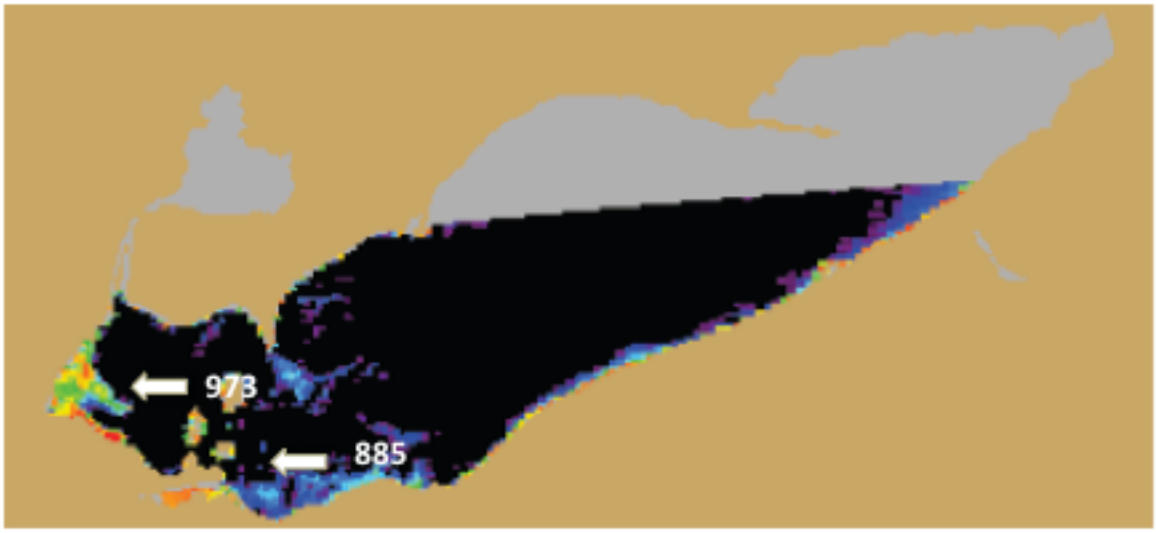

Figure 13: Moderate Resolution Imaging Spectroradiometer (MODIS) Cyanobacterial index of Lake Erie, for August 18th 2012. Lighter colors represent high concentrations of cyanobacteria, and dark blues and black represent low to no activity respectively. Gray areas indicate no data available. Source: NOAA; www.glerl.noaa.gov

Figure 14: Moderate Resolution Imaging Spectroradiometer (MODIS) Cyanobacterial index of Lake Erie, for May 31st 2013. → Lighter Colors represent high concentrations of cyanobacteria, and dark blues and black represent low to no activity respectively. Source: NOAA; www.glerl.noaa.gov

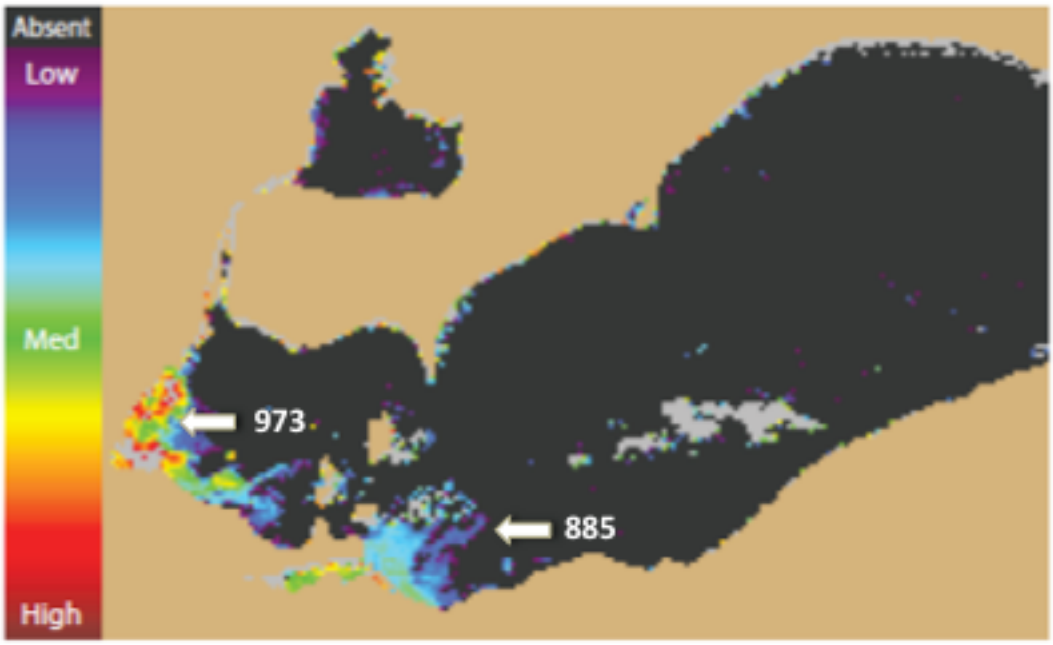

Figure 15: Moderate Resolution Imaging Spectroradiometer (MODIS) Cyanobacterial index of Lake Erie, for July 30st 2013. → Lighter Colors represent high concentrations of cyanobacteria, and dark blues and black represent low to no activity respectively. Source: NOAA; www.glerl.noaa.gov

not stimulate biomass, these results suggest that neither nitrogen nor phosphorus was limiting at these sampling locations. These results may be due to the low amount of biomass observed in Lake Erie at the time of sampling. As shown in **Figure 14**, the concentrations of cyanobacteria were very low in all basins of Lake Erie, with observable amounts of cyanobacteria only occurring in the near shore/shallow regions of the western basin. The low amount of biomass may be a result of other abiotic factors such as temperature. Average water temperatures for sampling station for the May 2013 cruise was 14.2°C (**Table 3**), below the 19-25°C preferred growth range of *Microcystis* (Varis 1993, Šejnohová *et al.* 2012). Conversely, measurements of chlorophyll biomass for July 2013 were much higher compared to May 2013 measurements, with the average station water temperature being 24.4°C. As shown in **Figure 15**, the bloom at this time was extensive throughout the western basin, with highest concentrations of cyanobacteria being near the Maumee River entrance into Lake Erie. Higher temperatures favor buoyant cyanobacteria, such as *Microcystis*, because it increases the stability of the water column and reduces the vertical turbulent mixing (Šejnohová *et al.* 2012).

July 2013 chlorophyll measurements were statistically similar to their relative controls for all treatments at Station 973 (**Figure 12 B**) and for all treatments, except NH_4 + 1µM PO_4 treatment, for Station 885 (**Figure 12 D**). The addition of 1µM PO_4 resulted in a significant increase of chlorophyll biomass for all nitrogen treatments for July 2013, at Station 885, and for the NH_4 treatment, at Station 973. These results suggest that water conditions were phosphorus limited during this time. This was confirmed by the low TP and SRP concentrations

observed for the July 2013 stations at the time of sampling (**Table 4**). The phosphorus-limited conditions of the lake at the time of sampling were due to its drawdown via the incorporation of phosphorus into biomass by phytoplankton species. *Microcystis* has developed the ability to adjust to these conditions by increasing its phosphorus uptake potential during phosphorus-limited conditions and store surplus phosphorus, in the form of polyphosphates (Oliver *et al.* 2012). This ability to maintain growth during periods of nutrient scarcity provides another advantage for *M. aeruginosa* to outcompete other phytoplankton in freshwater ecosystems.

The results from the July 2013 microcosm experiment presented here supports the GLWQA's nutrient management practices, that phosphorous is the limiting nutrient for phytoplankton biomass in freshwater systems. Therefore, by reducing phosphorus loads to eutrophic systems will result in reduced algal biomass accumulation (Schindler 1977, Schindler *et al.* 2008). However, lake conditions have been shown to become nitrogen limited in Lake Erie, as exhibited by the August 2012 microcosm experiment.

Conclusions

There is growing support for the idea that algal biomass is controlled by Phosphorus availability during the spring, and that the duration and magnitude of the bloom may be determined by nitrogen availability, during the summer and fall (Paerl *et al.* 2011). Results from the sampling cruises support this idea and indicate that during early bloom conditions neither element was limiting. Lake conditions

were found to be phosphorus limited when phytoplankton biomass increased, and conditions became nitrogen limited as the extent of the bloom progressed from summer into fall, prior to collapse. From these observations, we present the new idea that seasonal nitrogen limitation of phytoplankton growth may be a crucial in determining the extent and community composition of late season harmful algal blooms in Lake Erie. With this information, we are closer to understanding the impact that nitrogen and phosphorous have on *Microcystis* blooms in Lake Erie.

The continued reduction of phosphorus into eutrophic systems is important for future management practices, however failure to address point and nonpoint sources of nitrogen pollution may result in the continued proliferation of cyanobacterial blooms in freshwater eutrophic systems. The ability of cyanobacteria to use urea as a nitrogen source in Lake Erie and the 100-fold increase of the use of urea as a nitrogen source for agricultural fertilizer (Glibert *et al.* 2006), supports the ecological importance for comprehending the linkages between urea fertilization and its transport to downstream aquatic systems. There is hope that the continued research of nitrogen utilization by cyanobacteria and a better understanding of the factors involved in eutrophication of freshwater systems will contribute to future mitigation of harmful algal bloom events.

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