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Urea in Lake Erie: Organic nutrient sources as potentially important drivers of phytoplankton biomass

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ABSTRACT

Significant evidence shows that nitrogen (N) supply may influence microbial community structure and, in some cases, the rate of primary productivity in fresh waters. To date, however, most focus has been on dissolved inorganic N (*i.e.*, ammonia and nitrate), or dinitrogen gas. Far less is known about the effects of dissolved organic N such as urea on plankton activity, although this compound is both produced by in-lake processes and is a significant component of external loading. We evaluated the urea distribution and the activity of the major enzyme responsible for its assimilation (urease) in Lake Erie, which has a significant history of eutrophication. During 2012 and 2013, lake-wide surveys estimated surface urea concentrations and urease activity, along with phytoplankton composition and biomass, cyanobacterial toxins (microcystin), major nutrients and other physico-chemical parameters. In parallel, *in situ* 48-h microcosm experiments were executed to test whether different chemical forms of dissolved N could stimulate phytoplankton biomass. Results confirmed urea was a bioavailable form of N with *in situ* urea turnover times ranging from hours (for summer, *i.e.*, Aug. 2012 and July 2013) to days (May 2013). Furthermore, we observed a positive correlation between urease activity and both microcystin concentrations and cyanobacterial dominance. Results also indicated a potential seasonal shift in the nutrient limiting phytoplankton biomass from phosphorus (P) to N. Our results reinforce the importance of both N and P in promoting phytoplankton growth and highlighted the need to consider organic nutrient sources as potentially important drivers of cyanobacterial blooms and toxin production.

Introduction

Phytoplankton blooms have become annual events in large freshwater systems, including Lake Erie and China's Lake Tai (or *Taihu*) (Qin et al., 2010; Rinta-Kanto et al., 2009b). The phytoplankton communities within these blooms consist of a diverse array of phototrophs, including diatoms, eukaryotic microalgae and cyanobacteria (Oliver et al., 2012). While phytoplankton are the base of the food web and drive processes and ecosystem services ranging from water quality to fisheries, large phytoplankton blooms are commonly referred to as harmful algal blooms (HABs) because they have a negative impact on human activities or the environment (Ho and Michalak, 2015; Zingone and Enevoldsen, 2000).

In the North American (Laurentian) Great Lakes, severe lake-wide blooms of diatoms and N-fixing cyanobacteria and associated deterioration in water quality and hypolimnetic anoxia became so dire in the mid- to late-1960s that Lake Erie was described as "dead" by the popular press (Ashworth, 1986; Charlton, 1980). Nutrient (phosphorus, P) abatement efforts led to a short-lived (1980s through to the mid-1990s) recovery in the overall health of the lake, but by the mid-1990s a reemergence of cyanobacterial blooms dominated by different (relative to the 1960s), non-N-fixing cyanobacteria species began to occur in Lake Erie (Conroy et al., 2005). Recent biomolecular evidence suggests that *Microcystis* blooms in Lake Erie during August 2012 actively transported major nutrients (various N and/or P-containing compounds) as well as inorganic carbon (Steffen et al., 2015). The co-expression of N and P transporters by the cells was interpreted to mean that *Microcystis* spp. were actively scavenging both elements, which *de facto* implies that neither element was in excess. The expression of genes associated with inorganic carbon concentrating mechanisms was further suggestive of a limited availability of CO_2 to this population (McGinn et al., 2003). This shift in phytoplankton community composition to cyanobacterial dominance can alter flow paths of

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energy and nutrients, reshaping the structure and trophic functionality of entire aquatic ecosystems (Oliver et al., 2012). This shift has also left researchers looking more closely at environmental drivers of community composition.

The observed historical transitions from a system dominated by N-fixing cyanobacteria (*Aphanizomenon flos-aquae* and *Anabaena spiroides*) to a "recovered" Lake Erie and on now to one where *Microcystis* spp. is a more dominant component have been linked to changes in nutrient flux and chemistry (Steffen et al., 2014a). Changes in nutrient loading (specifically P-reductions) suggest a shift in conditions that determined the outcome of competition between cyanobacterial taxa, since the genetic richness (*i.e.*, which species are present) of the cyanobacterial community has remained fairly constant over the last 40 years (Rinta-Kanto et al., 2009b). Of particular interest are members of the genus *Microcystis*, spherical cells that are irregularly grouped into colonies of various sizes and densities, which may have a stratified, colorless mucilage (Šejnohová and Maršálek, 2012). While some cyanobacteria, including *Dolichospermum* (syn. *Anabaena*), *Aphanizomenon* and *Cylindrospermopsis*, have the capacity to produce the enzyme nitrogenase to fix atmospheric nitrogen gas (N_2), *Microcystis* does not possess the complete set of genes required for this process (Frangeul et al., 2008; Kaneko et al., 2007; Steffen et al., 2014a) and must assimilate N from other sources.

Concurrent with the increasing dominance of *Microcystis* as a bloom-forming taxon in the mid-1990s (Brittain et al., 2000; Conroy et al., 2005), agricultural practices in North America have also experienced a major shift. Use of ammonium nitrate as the preferred N-fertilizer in North America has waned in favor of urea-based fertilizers (USDA-ERS, 2013). Urea ($CO(NH_2)_2$) currently accounts for more than 50% of the N used globally for agricultural fertilizer, constituting more than a 100-fold increase in the past 4 decades (Glibert et al., 2006). It is a preferred N source for agricultural fertilizer because of its solubility in water, low cost of application, and the minimal damage it causes to root crops. Urea leaving the land and entering the aquatic system has been shown to be assimilated as an N source by some cyanobacteria and phytoplankton in both freshwater and marine environments (Donald et al., 2011; Finlay et al., 2010; Solomon et al., 2010).

There are a variety of natural biological sources of urea in lakes, including phytoplankton, bacteria, zooplankton and fish (Bogard et al., 2012). Previous estimates suggest urea concentrations in aquatic ecosystems are consistently less than those of NH_4^+ and NO_3^-, although urea may temporarily exceed these inorganic N-sources for short periods or even for prolonged period when runoff from either non-point or sustained point inputs of N occurs (Glibert et al., 2014; Solomon et al., 2010). Indeed recent estimates from Lake Tai (*Taihu*) in China indicate urea concentrations were higher in riverine inputs than in open lake waters: an observation that suggests urea is rapidly consumed in aquatic systems (Glibert et al., 2014; Han et al., 2014). In total, contributions, studies have suggested urea may provide 10–50% of the bioavailable N in lake and river surface waters (Bogard et al., 2012; Wiegner et al., 2006).

Despite the clear importance of urea in aquatic systems, no studies have evaluated its occurrence and few its potential for biological assimilation in Lake Erie (Chaffin and Bridgeman, 2014; Davis et al., 2015; Davis et al., 2010). The goal of this study was to address this knowledge gap. Most microorganisms use urease to assimilate urea; an enzyme that catalyzes the conversion of urea into carbon dioxide and ammonia. We therefore measured urease activity as a proxy for the ability of planktonic communities to assimilate this compound as an N source. We also investigated whether urea could act as an N-source for Lake Erie phytoplankton over the growing season. Measurements of urease enzymatic activity, nutrient concentrations and phytoplankton biomass were made during three lake-wide surveys (August 2012, May 2013, July 2013). This work was complemented by *in situ* microcosm experiments, where lake water was amended with various N species (urea, nitrate, ammonium) to determine if different chemical forms of N influenced the accumulation of phytoplankton biomass in a manner consistent with other recent studies (Chaffin and Bridgeman, 2014; Chaffin et al., 2013; Finlay et al., 2010). Our results contribute to the growing body of work implicating urea as an important N source in large freshwater systems.

Methods and materials

Environmental survey sample collection

Water for field samples surveys and microcosm experiments were collected from Lake Erie during three cruises of opportunity that occurred in August 13–17, 2012, May 27–31, 2013, and July 22–26, 2013 aboard the CCGS *Limnos*. Surface water (1 m depth) was collected from each station (21 different sites in total, although due to operational limitations, not all stations were visited on all trips) using 10-L Niskin sampling bottles and transferred to the ship's laboratory in 4-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). Statistical relationships within the field data were determined using R version 3.02 (http://cran.r-project.org/). Principal components analysis was done using Primer v7.0.9 (Primer-E Ltd., Ivybridge, UK). Missing environmental data were estimated using an expectation maximum likelihood algorithm with 1000 iterations. Prior to PCA, variables were normalized (the mean was subtracted from each value and the resultant then divided by the standard deviation) to account for the fact that they are measured on different scales.

Microcosm experiments

Nutrient enrichment experiments were conducted in microcosms maintained in on-deck incubators that simulated *in situ* water column conditions during three *CCGS Limnos* cruises. Microcosm experiments were performed at two ecologically relevant and historically well-studied stations, Station 885 (41°31′08″ N, 82°38′29″ W) and Station 973 (41°47′30″ N, 83°20′00″ W) (Fig. 1). Station 885 was chosen because of the diversity of cyanobacteria historically present at this site, while Station 973 was chosen because of its proximity to the inflow of the Maumee River into Lake Erie, and a long history of *Microcystis*-dominated phytoplankton populations in the region (*e.g.*, Rinta-Kanto et al., 2005, 2009b). Surface water (1 m) from each station was dispensed into acid-washed 1.2-L polycarbonate bottles and enriched with different nutrient amendments. Along with control (no nutrients added) treatments, a matrix of individual N additions (180 μM final concentration added N, from either ammonium, nitrate or urea (*i.e.*, 90 μM urea added)) or P (final concentration 1 μM-PO_4, added from a mixture of 1:4, KH_2PO_4:K_2HPO_4) was deployed in triplicate. These concentrations were chosen to be consistent with those used in previous lab (Steffen et al., 2014b) and field (Davis et al., 2015) experiments, and provided non-limiting, but also non-toxic, concentrations of nutrients. Sealed bottles were incubated in the deck incubator for 48 h at ~37% incident radiation (approximating light at 1 m in depth, DeBruyn et al., 2004; Wilhelm et al., 2003). Following incubation, aliquots from each microcosm treatment were removed and analyzed for chlorophyll a (chl*a*) concentrations as well as the microscopic enumeration of phytoplankton as described below.

Biological parameters measured

Chl*a* concentrations were estimated using the non-acidification method (Welschmeyer, 1994), and served as a proxy for total phytoplankton biomass. For both surface waters and microcosms, 50 mL of water was filtered through 0.2-μm nominal pore-size polycarbonate membrane filters (47 mm diam., Millipore) and stored in a 2.0 mL cryovials (Corning, NY) at −20 °C until analysis. Filters were extracted

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