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Hot and toxic: Temperature regulates microcystin release from cyanobacteria

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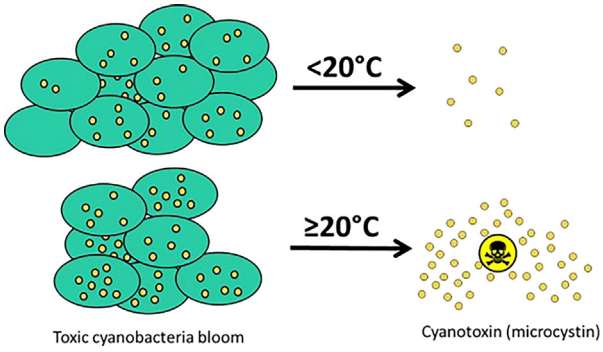
HIGHLIGHTS

- Toxin release from harmful cyanobacteria increases with warming.
- In-situ and laboratory studies showed elevated microcystin release between 20 and 25 °C.
- Elevated toxin release was coupled with a decline in cyanobacteria biomass.
- Water temperature could be used to forecast harmful algal bloom severity.

GRAPHICAL ABSTRACT



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ABSTRACT

The mechanisms regulating toxin release by cyanobacteria are poorly understood despite the threat cyanotoxins pose to water quality and human health globally. To determine the potential for temperature to regulate microcystin release by toxin-producing cyanobacteria, we evaluated seasonal patterns of water temperature, cyanobacteria biomass, and extracellular microcystin concentration in a eutrophic freshwater lake dominated by *Planktothrix agardhii*. We replicated seasonal variation in water temperature in a concurrent laboratory incubation experiment designed to evaluate cause-effect relationships between temperature and toxin release. Lake temperature ranged from 3 to 27 °C and cyanobacteria biomass increased with warming up to 18 °C, but declined rapidly thereafter with further increases in temperature. Extracellular microcystin concentration was tightly coupled with temperature and was most elevated between 20 and 25 °C, which was concurrent with the decline in cyanobacteria biomass. A similar trend was observed in laboratory incubations where productivity-specific microcystin release was most elevated between 20 and 25 °C and then declined sharply at 30 °C. We applied generalized linear mixed modeling to evaluate the strength of water temperature as a predictor of cyanobacteria abundance and microcystin release, and determined that warming ≥ 20 °C would result in a 36% increase in microcystin release when Chlorophyll *a* was ≤50 μg l^{-1}. These results show a temperature threshold for toxin release in *P. agardhii*, which demonstrates a potential to use water temperature to forecast bloom severity in eutrophic lakes where blooms can persist year-round with varying degrees of toxicity.

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1. Introduction

Blooms of toxin-producing cyanobacteria have increased in frequency with environmental degradation of freshwater ecosystems worldwide (Kaebernick and Neilan, 2001; Watson et al., 2015; Pick, 2016). In addition to compounds that are used for growth and development, most species of algae release a fraction of their photosynthetic products directly into the surrounding environment (Smith and Underwood, 2000; Bertilsson and Jones, 2003). These substances are typically complex and include a mixture of both small and large molecular weight compounds (Aaronson, 1971; McCarthy et al., 1996; Wyatt et al., 2014). In some species of cyanobacteria, the exudate pool can include a suite of toxic compounds that are harmful to human health (Carmichael, 1992; Welker and von Döhren, 2006; Kaplan et al., 2012). For example, microcystin is among the most commonly occurring toxin produced by cyanobacteria in natural waters (Babica et al., 2006; Rastogi et al., 2014), and can cause liver complications and damage to the nervous system if ingested (Falconer, 2005; Bláha et al., 2009). In an effort to manage health risks associated with toxin exposure, studies have aimed to forecast the occurrence of toxic cyanobacteria blooms (Srivastava et al., 2013), often using predictive models (Downing et al., 2001; Taranu et al., 2012; Beaulieu et al., 2013). Although our ability to predict bloom formation has been improved by these studies, the concentration of toxins does not always increase linearly with the abundance of cyanobacteria in surface waters (Beaulieu et al., 2013), suggesting that other factors may play a role in the release of toxins during bloom formation.

In the absence of nutrient limitation, temperature is often considered the most important determinant of growth and metabolism in freshwater algae, including cyanobacteria (Raven and Geider, 1988). This is due in part to the fact that many of the enzymatic reactions involved in photosynthesis and respiration are temperature dependent. The typical photosynthetic response of cyanobacteria to temperature is a progressive increase until the physiological optimum, followed by a rapid decline (Davison, 1991). Cyanobacterial dominance at elevated temperatures has been attributed to their higher temperature optimum growth rates (Butterwick et al., 2005; Helbling et al., 2015) and to their greater affinity for nutrients at elevated temperatures compared to eukaryotic algae (Xie et al., 2012). Temperature manipulation experiments have revealed that cyanobacteria toxin content also increases with temperature (Rapala et al., 1997; Brutemark et al., 2015) and elevated growth rates at warmer temperatures promote replication of toxic cells (Davis et al., 2009). Although toxins are typically contained within the cyanobacteria cell (i.e., cell-bound), they can be released into the water column upon cell lysis, death, or as extracellular release (Shi et al., 1995; Christoffersen et al., 2002; Pearson et al., 2004; Paerl and Otten, 2013). Despite the threat cyanotoxins pose to water quality and human health, the mechanisms regulating toxin release by cyanobacteria are poorly understood.

Given its influence on bloom formation and toxicity, temperature may also be a key environmental factor regulating toxin release. The production of secondary metabolites (e.g., microcystin) is often coupled with photosynthesis, and therefore mediated by changes in temperature (Paerl and Millie, 1996; Welker and von Döhren, 2006; Neilan et al., 2012). Consequently, toxin concentrations in a wide range of cyanobacteria species tend to be most elevated at temperatures that are also optimal for growth (15–25 °C), with reduced toxin levels measured at higher or lower temperatures (Kaebernick and Neilan, 2001). However, when high photosynthetic output (i.e., algal bloom) is combined with unfavorable growth conditions, the release of toxins can indicate damage to the cell (Schatz et al., 2007; Zilliges et al., 2011; Holland and Kinnear, 2013). Although cyanobacteria are able to tolerate a wide range of temperature conditions, cases of surface-water temperature exceeding optimal conditions during warm summer months are increasing (Kosten et al., 2012; Paerl and Otten, 2013). Elevated photosynthetic rates at warm temperatures can cause oxidative stress by increasing the number of reactive oxygen species in the cell, which inhibit repair to the photosynthetic apparatus (Brutemark et al., 2015). As a consequence, the stress caused by rising temperatures may induce the release of toxins into the water column (Paerl and Millie, 1996; Christoffersen et al., 2002), though too few studies exist to support this hypothesis.

In this study, we used a combination of field and laboratory approaches to evaluate temperature regulation of microcystin release by toxin-producing cyanobacteria. Long-term monitoring and field-based studies have provided valuable multivariate datasets, but often lack detail on the mechanisms controlling spatial and temporal trends on toxin release (Taranu et al., 2012; Wells et al., 2015). Conversely, laboratory investigations of toxin regulation mechanisms can be difficult to extrapolate to the environment (Kaebernick and Neilan, 2001; Kosten et al., 2012). We conducted this current study in a eutrophic freshwater lake in western Ohio, USA where toxin-producing cyanobacteria (i.e., *Planktothrix agardhii*) occur at elevated levels throughout the year, including beneath ice during winter months. This set of conditions allowed us to capture temporal variation in water temperature, cyanobacteria biomass, and free microcystin concentration across a wide range of temperatures (3–27 °C) at a single location. To evaluate cause-effect relationships between temperature and toxin release, we replicated seasonal variation in temperature in the laboratory using temperature-regulated recirculating water baths containing water and toxin-producing cyanobacteria from the study lake. We used data from these field and laboratory approaches to test the hypothesis that temperature regulates cyanobacteria production and the release of microcystin during photosynthesis. We incorporated our results into a model that uses temperature to predict toxin release with the aim of providing resource managers with a tool to minimize health risks associated with toxic cyanobacteria blooms.

2. Materials and methods

2.1. Study site

The field portion of our study was conducted in a eutrophic temperate lake located in western OH, USA (Grand Lake St. Marys; Latitude: 40.53°N; Longitude: 84.50°W; Fig. 1). Grand Lake St. Marys is a shallow (2 m average depth) lake with a surface area of 59 km^2. Approximately 90% of the surrounding watershed is agricultural land use, which has resulted in water column dissolved nutrient concentrations consistently >1000 μg l^{-1} total nitrogen (TN) and 100 μg l^{-1} total phosphorus (TP) (Dumouchelle and Stelzer, 2014). Since 2009, the lake has experienced perennial blooms of a microcystin-producing cyanobacterium *P. agardhii* containing the *mcyE* toxin-producing gene (Dumouchelle and Stelzer, 2014). As a consequence, total microcystin concentrations in the lake are regularly above the exposure limit for recreational use (>20 μg l^{-1}), resulting in lake closures to public access (USEPA, 2016).

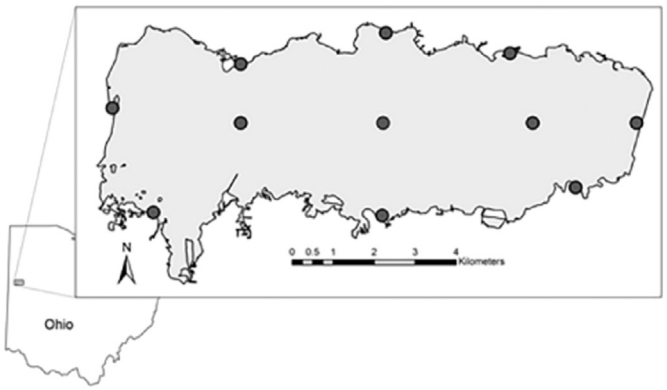

Fig. 1. Sampling locations within Grand Lake St. Marys, located in western OH, USA.