



Hot and toxic: Temperature regulates microcystin release from cyanobacteria



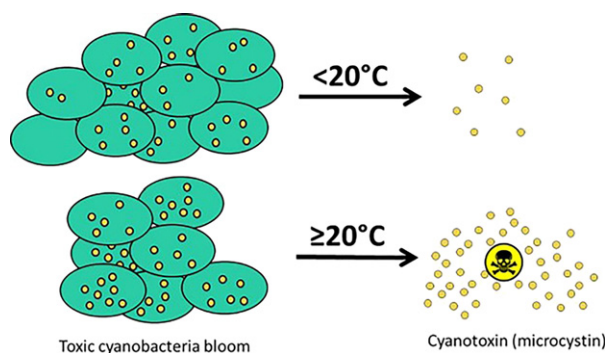
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HIGHLIGHTS

- 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



ARTICLE INFO

Article history:

Received 8 July 2017

Received in revised form 13 August 2017

Accepted 14 August 2017

Available online 18 August 2017

Editor: D. Barcelo

Keywords:

Cyanotoxin

Harmful algal bloom

Planktothrix agardhii

Toxicity

Warming

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 $\leq 50 \mu\text{g l}^{-1}$. 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 km². 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).

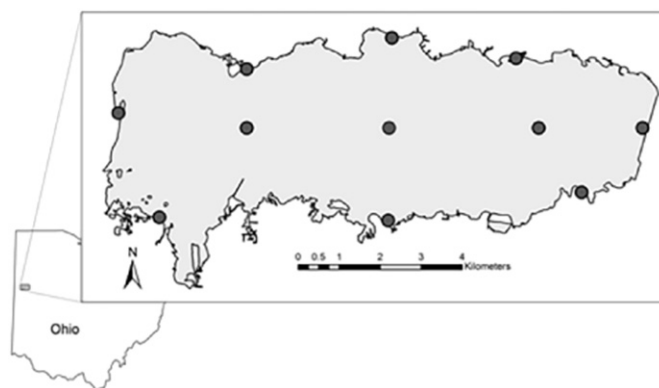


Fig. 1. Sampling locations within Grand Lake St. Marys, located in western OH, USA.

The occurrence of year-round toxic cyanobacteria blooms within this lake combined with single-species dominance provided a unique opportunity to evaluate temperature regulation of toxin release (in the absence of nutrient limitation).

2.2. Field sampling

To evaluate seasonal trends related to cyanobacteria abundance and toxin levels, we monitored physical and chemical parameters at 11 sampling locations within the lake for one year beginning May 2015. Sampling occurred monthly except for March, April, May, and November, which were sampled twice each month to capture transitional periods in water temperature. Sampling locations were chosen using a stratified sampling design to capture spatial variability and were accessed by boat (Fig. 1). Environmental parameters, including water temperature ($^{\circ}\text{C}$), dissolved oxygen (DO), specific conductivity, and pH, were measured with a calibrated Hydrolab sonde (Hach, Loveland, CO, USA) and water clarity was measured with a secchi disk (Wetzel and Likens, 2000). Dissolved organic carbon (DOC), total dissolved N (TDN), and soluble reactive P (SRP) were analyzed from 30 ml filtered water samples ($0.45\text{ }\mu\text{m}$ filter) collected from 5 cm below the surface at each location. Samples were stored in sterile polyethylene bottles on ice in a dark cooler and transported to the laboratory. Dissolved organic carbon was analyzed using a Shimadzu TOC analyzer (Shimadzu Corp., Columbia, MD, USA), and TDN and SRP were analyzed following persulfate digestion using the second-derivative UV spectroscopy and ascorbic acid methods, respectively (APHA, 1998).

To capture temporal variation in phytoplankton dynamics and toxin levels, we quantified phytoplankton biomass (Chlorophyll *a* and dry mass), cell density, taxonomic composition, algal nutrient content, and free microcystin concentration at each of the 11 locations within the study lake on each sampling date. Phytoplankton samples (120 ml) were collected from a 1 m plankton tow using a $10\text{ }\mu\text{m}$ nanoplankton net (Wildlife Supply Company, Yulee, FL, USA). Samples were placed on ice in a dark cooler and transported to the laboratory for analysis. A known volume of water from each sample was collected on a $0.7\text{ }\mu\text{m}$ glass fiber filter (GF/F; Whatman, Maidstone, UK) and analyzed for Chlorophyll *a* after ethanol extraction with an Agilent Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) following standard techniques (APHA, 1998). Phytoplankton dry mass was determined by drying a subsample at $105\text{ }^{\circ}\text{C}$ for 48 h in pre-weighed aluminum pans (APHA, 1998). A separate portion of each sample was preserved in a 2% formalin solution for phytoplankton cell abundance and taxonomic analysis. Cell abundance was quantified by counting and identifying a minimum of 300 natural units per sample to genus level at $400\times$ magnification using a Palmer-Maloney nanoplankton counting chamber (Charles et al., 2002). A separate subsample of water was filtered onto a GF/F filter for analysis of algal nitrogen (TN) and phosphorus (TP) content following oxidation with persulfate digestion. Samples were then analyzed for TN using the second-derivative UV spectroscopy method and SRP using the ascorbic acid method (APHA, 1998). Algal nutrient content was expressed per unit dry mass by dividing the mass of N and P by the dry mass. Free (i.e., water column) microcystin concentration was analyzed from a water sample collected from 5 cm below the surface at each location and $0.45\text{ }\mu\text{m}$ -filtered using low pressure to minimize cellular collapse. Microcystin concentration in each water sample was measured using an enzyme-linked immunosorbent assay (ELISA; $0.1\text{ }\mu\text{g l}^{-1}$ detection limit) and the colorimetric response at 450 nm was determined on a microtiter plate reader according to the manufacturer's protocol (Envirologix, Portland, MA, USA).

2.3. Laboratory incubation experiment

Seasonal variation in lake water temperature was replicated in a laboratory incubation experiment designed to evaluate the effects of

temperature on cyanobacteria production and toxin release while keeping all other variables constant. Water temperature was manipulated from 3 to $30\text{ }^{\circ}\text{C}$ at $5\text{ }^{\circ}\text{C}$ increments and from 15 to $20\text{ }^{\circ}\text{C}$ at $1\text{ }^{\circ}\text{C}$ increments using recirculating water baths plumbed to jacketed beakers (Ace Glass Inc., Vineland, NJ, USA) that circulated temperature-regulated water around mesocosms (500 ml glass flasks) placed within each beaker ($n = 3$ for each temperature treatment) (Fig. 2; Gu and Wyatt, 2016). Lake water containing toxin-producing *P. agardhii* was collected from the study lake for laboratory incubations on 15 July 2015. Each mesocosm was filled with 300 ml of $0.7\text{ }\mu\text{m}$ -filtered lake water and inoculated with a 50 ml aliquot of unfiltered lake water (containing natural abundances of cyanobacteria). Mesocosms were incubated at experimental temperatures for four days during which mesocosms were oxygenated using air stones and exposed to $500\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ of photosynthetically active radiation on a 12-h cycle.

Productivity-specific toxin release was measured at each temperature interval by transferring cyanobacteria from incubation jars into biological oxygen demand (BOD) bottles filled with 300 ml of filtered lake water. Prior to productivity measurements, a water sample was collected from each BOD bottle for initial concentrations of free microcystin and dissolved nutrients (TDN and SRP). Nitrogen gas was bubbled into BOD bottles to reduce oxygen levels prior to the initiation of productivity measures to ensure that changes in DO were the result of photosynthesis and not temperature (i.e., to reduce O_2 saturation). Initial O_2 concentration was measured with a Hach model 40d multi-probe (Hach Company, Loveland, CO, USA), and bottles were immediately capped without trapping gas bubbles and incubated at experimental temperatures inside jacketed beakers (as described above). After 30 min, final O_2 concentration was measured, and a $20\text{ }\mu\text{l}$ water sample was collected for final free microcystin concentration. Samples for microcystin concentration filtered and analyzed as described above. Each BOD bottle was wrapped with aluminum foil and incubated for an additional 30 min in the dark. Initial and final DO measurements were used to estimate net productivity and respiration, respectively. We calculated gross primary production (GPP) following Wetzel and Likens (2000) and converted GPP values into carbon units based on a C:O molar ratio of 0.375 and a photosynthetic quotient of 1.2. Following productivity measurements, a 30 ml water sample was collected for final dissolved nutrient concentrations and cyanobacteria were dried for estimates of dry mass (APHA, 1998). We calculated toxin release as the difference between initial and final microcystin concentration. We expressed productivity rates as carbon evolution per unit dry mass and microcystin release as a fraction of GPP.

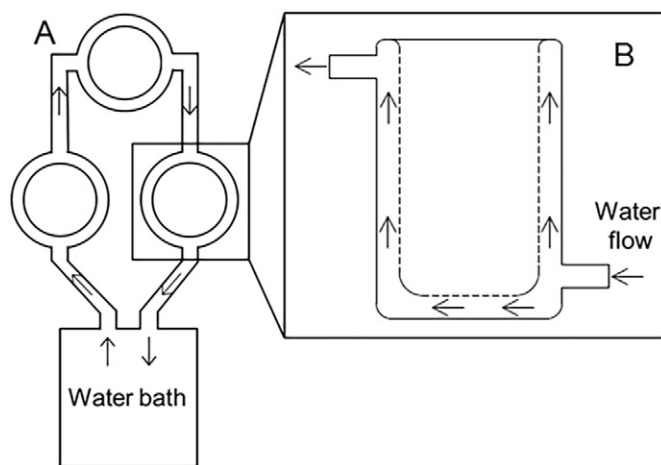


Fig. 2. Schematic of (A) circulating water bath during the laboratory temperature manipulation and (B) jacketed beakers (from Gu and Wyatt, 2016). An internal pump circulates temperature-regulated water around the inner walls of each beaker, maintaining constant temperature of water inside each incubation flask.

2.4. Statistical analyses

We analyzed differences among cyanobacteria abundance (biomass and cell density) and toxin concentration over time using repeated measures general linear models (RM-GLM) and Tukey's test for post hoc comparison of means tests. We used a generalized linear mixed model with a gamma error distribution to predict microcystin concentration as a function of temperature. A gamma error distribution was selected as only non-negative continuous variables were included in the model. The model included environmental factors as covariates, an interaction term (temperature $\times$ Chlorophyll *a*) that was treated as a fixed effect, and sampling site was treated as a random effect using the following equation:

$$g(\mu_{ism}) = \alpha + \beta X_i + \lambda^* T_i^* Chla_i + \delta_s$$

where $g(\mu_{ism})$ is the log link function, μ is the measured concentration of microcystin ($\mu\text{g l}^{-1}$), β is a vector of coefficients for the fixed effects, X_i is a matrix of covariates, λ is the coefficient for the interaction term, T_i is water temperature ($^{\circ}\text{C}$) of observation *i*, $Chla_i$ is the amount of Chlorophyll *a* measured at observation *i*, and δ_s is the random effect for site.

To improve the efficiency of estimating coefficients, all covariates were converted to z-scores using the following equation:

$$z\text{-score}(x_{ki}) = \frac{x_{ki} - \text{AVG}(x_k)}{\text{SD}(x_k)}$$

where x_k is covariate *k*, x_{ki} is covariate *k* for observation *i*, $\text{AVG}(x_k)$ is the average value of covariate *k*, and $\text{SD}(x_k)$ is the sample standard deviation for covariate *k*.

We evaluated all possible combinations of fixed effects and the most parsimonious model was selected using Akaike's Information Criteria corrected for small sample size (AICc). All models that had a change (Δ) in AICc of ≤ 2 were considered equally plausible. Highly correlated predictor variables were never used in the same model during the model-selection process. We used a model averaging technique to reduce all of the most plausible models to one global model used for prediction. The relative strength of each variable was assessed by an importance index. Importance was calculated by summing the AIC weights from all models that included the independent variable as a covariate. If a covariate was included in every model, the importance score would equal 1.00 indicating that the influence of the covariate on the relationship between microcystin and temperature was significant 100% of the time. Lower importance scores indicated that the covariate was not a strong predictor of microcystin release and was included in fewer models. Additionally, we used leave-one-out cross validation to evaluate the ability of the top models to predict microcystin concentration. All models that were selected based on a ΔAICc of ≤ 2 were fit using the original dataset with one observation removed and then averaged as above. The model averaged coefficients were then used to predict the removed value. This process was repeated for the original dataset with each observation removed. To evaluate model fit and predictive ability from the leave-one-out cross validation, we calculated the root mean squared error (RMSE). The RMSE is a measure of variability around the predicted regression line similar to standard deviation as a measure of variability around a mean. Thus, approximately 95% of the observed values fell within ± 2 RMSE units of the predicted value. The RMSE was calculated using the following equation:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}}$$

where $\hat{y}_i$ is the predicted value of the *i*th observation, y_i is the observed value of the *i*th observation, and *n* is the total number of observations.

All analyses were conducted using the R statistical programming environment version 3.3.2 (R Core Team, 2016). Mixed effects models

were fit using the lme4 package version 1.1–12 (Bates et al., 2015), and multimodel inference was conducted using the MuMIn package version 1.15–6 (Barton, 2016).

3. Results

3.1. Field survey

Year-round field sampling captured variation in environmental conditions that were used to predict cyanobacteria abundance and toxin levels (Fig. 3; Table 1). Water temperature (mean $\pm$ SD, *n* = 11) ranged from 1.5 ± 0.2 to 27.0 ± 0.4 $^{\circ}\text{C}$ during the study period. Dissolved oxygen levels (mean $\pm$ SD, *n* = 11) ranged from 9.1 ± 1.1 to 15.3 ± 0.5 mg l^{-1} and increased with decreasing water temperature. Specific conductivity ranged from 412.0 ± 2.0 to 491.4 ± 7.2 $\mu\text{S cm}^{-1}$, with higher readings recorded during springtime sampling. Lake pH was consistently alkaline but was at its minimum (8.87 ± 0.01) during periods of ice cover and increased to 11.59 ± 0.05 concurrent with periods of elevated algal biomass, likely owing to the influence of photosynthesis on the uptake of inorganic carbon from the water column. Secchi depth ranged from 18.0 ± 0.3 to 54.4 ± 2.5 cm, and decreased with increasing algal biomass. Dissolved nutrient concentrations (mean $\pm$ SD, *n* = 11) remained above growth-limiting levels throughout the study period, where mean TDN ranged from 431.2 ± 36.8 to 2315.2 ± 102.0 $\mu\text{g l}^{-1}$ and SRP ranged from 15.1 ± 0.87 to 52.3 ± 6.12 $\mu\text{g l}^{-1}$.

Temporal variation in cyanobacteria abundance was related to changes in lake water temperature. Cyanobacteria biomass measured as Chlorophyll *a* ranged from 8.24 ± 0.73 to 83 ± 7.4 $\mu\text{g l}^{-1}$ throughout the study but was most elevated when lake temperature was $9\text{--}19$ $^{\circ}\text{C}$ (RM-GLM, $p < 0.0001$; Fig. 4a). At water temperatures > 19 $^{\circ}\text{C}$, Chlorophyll *a* declined nearly 4-fold, from 60.1 ± 3.68 to 16.4 ± 1.18 $\mu\text{g l}^{-1}$ (RM-GLM, $p < 0.0001$), resulting in Chlorophyll *a* values similar to those measured during periods of ice cover (RM-GLM, $p = 0.99$; Fig. 4a). Algal dry mass followed a similar pattern as Chlorophyll *a*, showing a sharp decline at water temperatures > 19 $^{\circ}\text{C}$ (RM-GLM, $p < 0.0001$; Fig. 4b). Both measures of biomass (Chlorophyll *a* and dry mass) recovered from the decline once water temperatures dropped below 19 $^{\circ}\text{C}$ (Fig. 4a,b). Cell density (10^6 cells l^{-1}) was most elevated at 24 $^{\circ}\text{C}$ (103.5 ± 8.0), but declined nearly six-fold with further increases in water temperature (RM-GLM, $p < 0.0001$; Fig. 4c). Cell density (10^6 cells l^{-1}) was dominated by *P. agardhii* which comprised $\geq 96\%$ of community composition throughout the study and ranged from 5.65 ± 1.22 (during ice cover) to 103.5 ± 8.0 (Fig. 4c). A total of 24 other algal genera were identified, but remained $< 5\%$ relative abundance. Algal tissue TN varied more than five-fold (51.2 ± 3.8 to 275.5 ± 20.1 $\mu\text{g l}^{-1}$ g^{-1} dry mass) throughout the study period (Table 1), and was unrelated to changes in biomass (Fig. 3). Conversely, algal tissue TP more closely followed changes in biomass and ranged from 54.0 ± 7.4 to 154.9 ± 11.4 $\mu\text{g g}^{-1}$ dry mass l^{-1} throughout the study period (Table 1).

Free microcystin concentration was influenced by both water temperature and cyanobacteria abundance (biomass and cell density), but in opposing directions. Free microcystin concentration tracked closely with water temperature throughout our study period and was greatest (1.10 ± 0.08 $\mu\text{g l}^{-1}$) at temperatures ≥ 20 $^{\circ}\text{C}$ and lowest (0.21 ± 0.07 $\mu\text{g l}^{-1}$) during periods of ice cover when water temperature was at its minimum (Fig. 4a–c). Free microcystin concentration was coupled with changes in cyanobacteria abundance at temperatures between 3 and 19 $^{\circ}\text{C}$ (Fig. 4a–c). However, microcystin concentration was inversely related to measures of cyanobacteria abundance at temperatures > 19 $^{\circ}\text{C}$, where there was a significant decline in cyanobacteria and a simultaneous two-fold increase in microcystin (RM-GLM, $p = 0.01$; Fig. 4).

The best model to predict free microcystin concentration included water temperature, Chlorophyll *a*, tissue TP, tissue TN, DOC, and the temperature $\times$ Chlorophyll *a* interaction term (Table 2). Based on their magnitude of coefficients and importance values, water

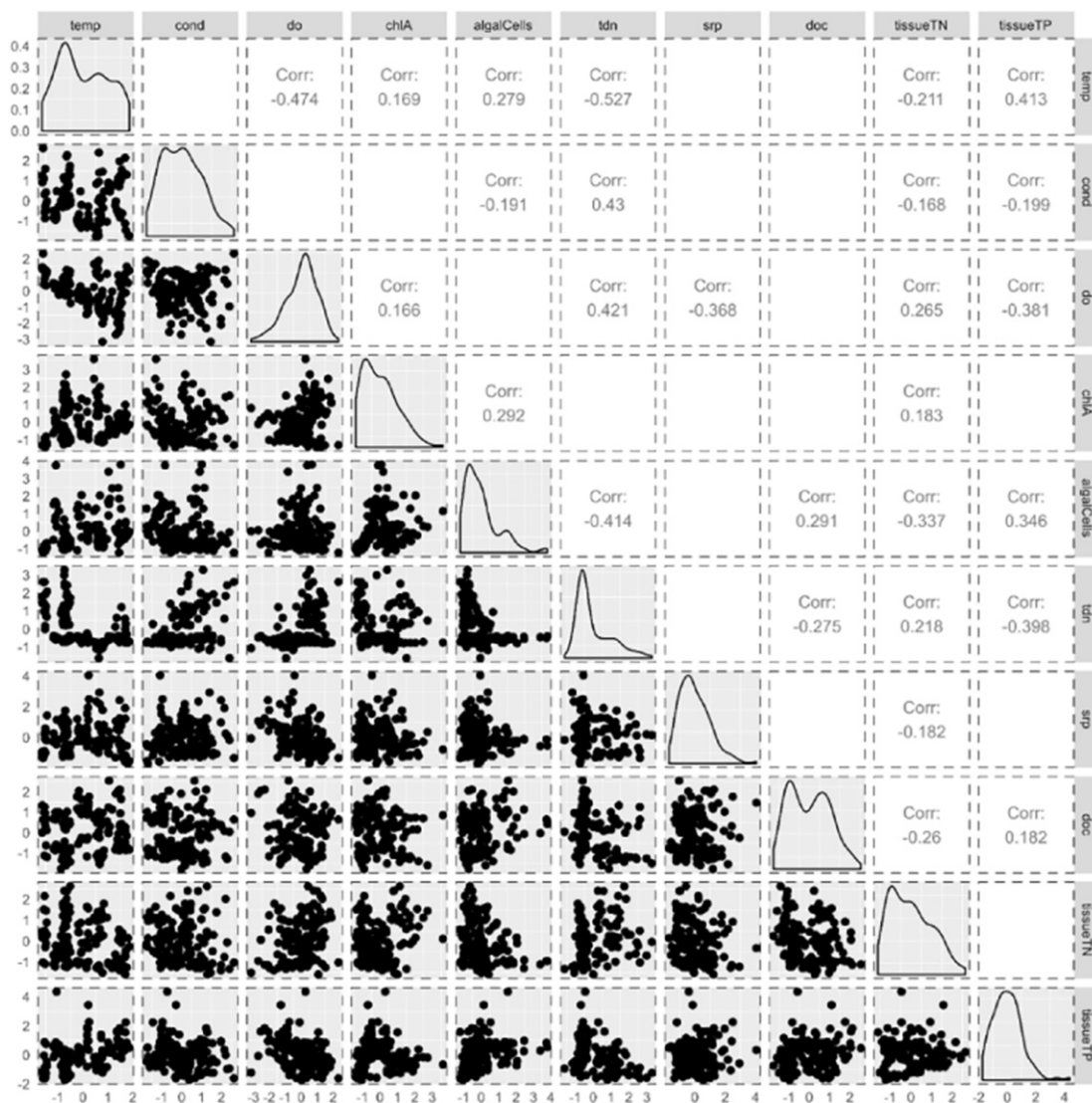


Fig. 3. Correlation matrix of predictor variables used in generalized linear mixed modeling. Only significant Pearson's correlations coefficients are shown. Temp = water temperature, cond = specific conductivity, DO = dissolved oxygen, chlA = Chlorophyll *a*, TDN = water column total dissolved nitrogen, SRP = water column soluble reactive phosphorus, DOC = dissolved organic carbon, tissue TN = tissue total nitrogen, tissue TP = tissue total phosphorus.

Table 1

Descriptive statistics of environmental conditions, algal parameters, and microcystin concentrations from year-long field sampling. Mean and standard deviation (SD) values are overall distribution of variables throughout the study period (all sampling locations and dates; $n = 165$). Minimum, median, and maximum values represent the range of conditions among sampling dates (mean; $n = 11$).

Variable	Mean	SD	Minimum	Median	Maximum
Water temperature ($^{\circ}\text{C}$)	14.2	8.19	1.50	13.20	27.0
Dissolved oxygen (mg l^{-1})	11.9	2.50	9.14	11.6	15.3
Specific conductivity ($\mu\text{S cm}^{-1}$)	451.5	27.5	412.0	452.7	491.4
pH	10.0	0.71	8.87	10.1	11.6
Secchi disk depth (cm)	27.9	10.8	18.0	23.3	54.4
DOC (mg l^{-1})	7.13	1.06	5.90	7.12	8.07
TDN ($\mu\text{g l}^{-1}$)	1160.0	622.3	431.2	841.3	2315.2
SRP ($\mu\text{g l}^{-1}$)	33.2	15.9	15.1	35.4	52.3
Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	41.9	27.1	8.24	40.9	83.4
Dry mass (mg l^{-1})	5.89	2.70	2.84	5.50	9.85
10^6 algal cells l^{-1}	37.7	30.7	5.65	29.7	103.5
Tissue TN ($\mu\text{g N g}^{-1}$ algae l^{-1})	159.7	87.0	51.2	143.0	275.5
Tissue TP ($\mu\text{g P g}^{-1}$ algae l^{-1})	99.6	40.6	54.0	84.4	154.9
Microcystin ($\mu\text{g l}^{-1}$)	0.61	0.36	0.27	0.49	1.10

Note: DOC = dissolved organic carbon, TDN = total dissolved nitrogen, SRP = soluble reactive phosphorus, TN = total nitrogen, TP = total phosphorus.

temperature, Chlorophyll *a*, tissue TP, and temperature $\times$ Chlorophyll *a* were the strongest predictors of free microcystin within the model (Table 3). There was a significant relationship between free microcystin concentration and the temperature $\times$ Chlorophyll *a* interaction, indicating that the rate of microcystin release, as a function of temperature, increases as Chlorophyll *a* decreases (Fig. 5). Specifically, at low temperatures (e.g., 5°C), the model predicted free microcystin concentration to be 0.40, 0.46, and $0.54 \mu\text{g l}^{-1}$ when Chlorophyll *a* was 1, 50, and $100 \mu\text{g l}^{-1}$, respectively (Fig. 5). In contrast, at temperatures above 20°C , the model predicted free microcystin concentration to be 1.26, 0.72, and $0.42 \mu\text{g l}^{-1}$ when Chlorophyll *a* was 1, 50 and $100 \mu\text{g l}^{-1}$, respectively (Fig. 5). Predicted microcystin values from the leave-one-out cross-validation (RMSE = 0.28) were within $\pm 0.56 \mu\text{g l}^{-1}$ of the observed values and were highest during the warmest months and lowest during the coldest months (Fig. 6). Model predictions also indicated that free microcystin concentration was expected to increase 36% when tissue TP increased from 100 to $200 \mu\text{g P g}^{-1}$ algae l^{-1} (estimates calculated with all other covariates held at their average value; Fig. S1). However, there was greater uncertainty in estimates at the high range of tissue TP because of limited observations ($n = 5$) above $175 \mu\text{g P g}^{-1}$ algae l^{-1} . The remaining covariates (tissue

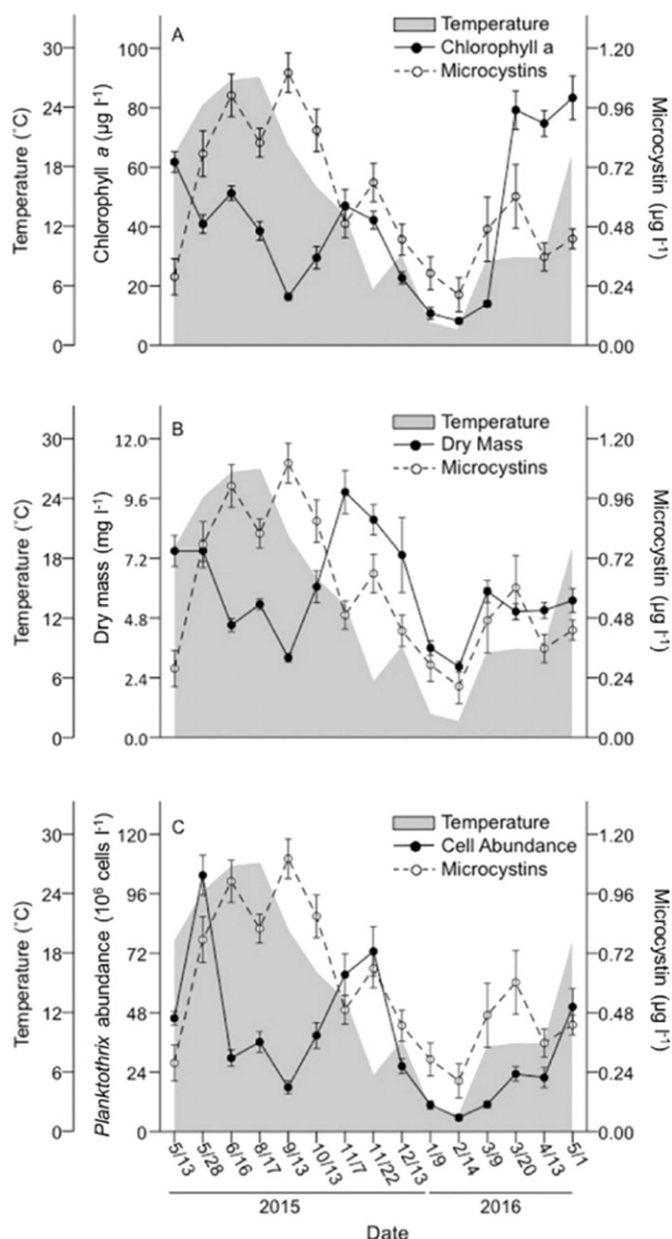


Fig. 4. The relationship between water temperature ($^{\circ}\text{C}$) (grey shading), free microcystin concentration ($\mu\text{g l}^{-1}$) (dotted lines with open circles), and cyanobacteria abundance (solid lines with closed circles) measured as (A) Chlorophyll *a* ($\mu\text{g l}^{-1}$), (B) dry mass ($\mu\text{g l}^{-1}$), and (C) *Planktothrix* cell density (10^6 cells l^{-1}) during year-long sampling. Values are mean $\pm$ 1 SE.

Table 3

Model-averaged coefficients and relative variable importance. Coefficients are sorted by importance. Importance is the sum of AIC_c weights across all models in which the term is included.

Coefficient	Estimate	Standard error	Importance
Intercept	−0.535	0.068	1.00
Water temperature	0.222	0.059	1.00
Tissue TP	0.125	0.052	1.00
Chlorophyll <i>a</i>	−0.095	0.055	0.93
Water temperature $\times$ Chlorophyll <i>a</i>	−0.153	0.074	0.93
Dissolved organic carbon	−0.057	0.059	0.65
Tissue TN	−0.034	0.051	0.45
Soluble reactive phosphorus	0.017	0.037	0.30
Conductivity	−0.002	0.015	0.07
Dissolved oxygen	−0.007	0.029	0.07

TN, SRP, DOC, algal dry mass, specific conductivity, and DO) predicted $\leq 1\%$ change in microcystin concentration (Fig. S1).

3.2. Laboratory incubation experiment

Water temperature had a significant influence on microcystin release in *P. agardhii* during our laboratory incubation ($F_{10,22} = 7.24$, $p < 0.001$; Fig. 7). Mean GPP-specific microcystin release remained at reduced levels (below $0.10 \mu\text{g microcystin mg}^{-1} \text{C h}^{-1} \text{g}^{-1}$ dry mass) and similar among treatments up to 19°C ($p \geq 0.14$; Fig. 7). Mean GPP-specific microcystin release increased 3–4-fold at 20°C and remained at elevated levels to 25°C ($p \leq 0.02$; Fig. 7). Toxin release declined at 30°C to levels that were not significantly different from 3 to 19°C temperature treatments ($p = 0.15$; Fig. 7). Dissolved nutrient concentrations remained above growth-limiting levels throughout the incubation experiment beginning at $803.3 \pm 42.7 \mu\text{g l}^{-1}$ TDN and $77.1 \pm 1.69 \mu\text{g l}^{-1}$ SRP and declined to $665.5 \pm 30.0 \mu\text{g l}^{-1}$ TDN and $23.5 \pm 2.67 \mu\text{g l}^{-1}$ SRP by the end of the incubation.

4. Discussion

Toxic cyanobacteria commonly occur in eutrophic ecosystems, making nutrients the primary focus of harmful algal bloom (HAB) ecology and management (Downing et al., 2001; Beaulieu et al., 2013; Deng et al., 2014; Lv et al., 2014). However, nutrient enrichment alone does not facilitate cyanobacteria dominance over other types of eukaryotic algae (Schindler, 1977; Jensen et al., 1994; Beaulieu et al., 2013), nor have nutrients been found to regulate toxin synthesis or gene expression in cyanobacteria (Briand et al., 2005; Davis et al., 2009; Joung et al., 2011; Neilan et al., 2012). On the other hand, the synergistic effects of elevated water temperature and nutrient enrichment have been shown to favor toxic cyanobacteria (Posch et al., 2012), and the frequency and duration of toxic cyanobacteria blooms are expected to increase in eutrophic ecosystems with rising water temperatures (Paerl and Otten, 2013). Since the production and subsequent release of cyanotoxins into the water column during bloom events pose a threat

Table 2

Predictive models for free microcystin concentration based on data collected during year-long field sampling. Model selection results include the intercept and random effects for sampling location and date. Predictors include TTP = tissue total phosphorus, DOC = dissolved organic carbon, TTN = tissue total nitrogen, water column SRP = soluble reactive phosphorus, and DO = dissolved oxygen. The stronger models are those with the highest AIC_c weight.

Model	df	ΔAIC_c	AIC_c weight
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , DOC, TTN	9	0.00	0.168
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i>	7	0.31	0.144
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , DOC	8	0.34	0.141
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , TTN	8	0.88	0.108
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , DOC, SRP	9	0.94	0.105
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , DOC, SRP, TTN	10	1.00	0.102
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , SRP	8	1.09	0.097
Water temperature, TTP, Chlorophyll <i>a</i> , water temperature $\times$ Chlorophyll <i>a</i> , conductivity, DOC, TTN	10	0.82	0.068
Water temperature, TTP, DO, DOC	7	1.85	0.067

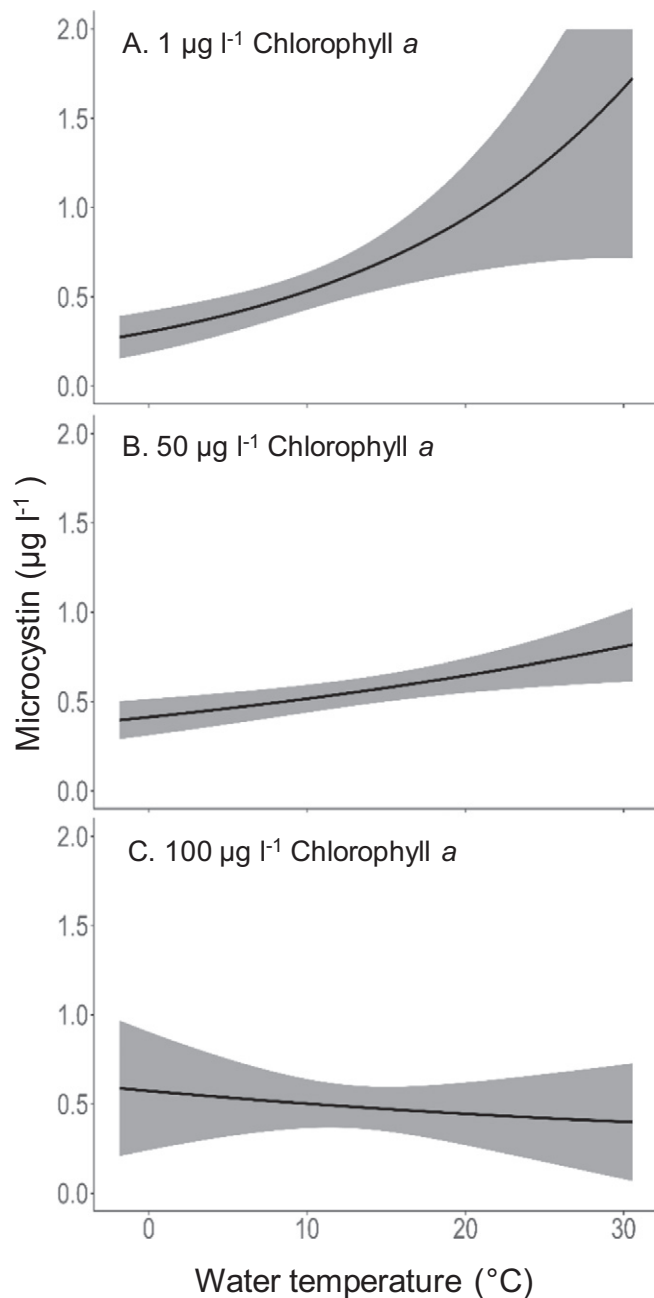


Fig. 5. Model averaged predictions of free microcystin concentration ($\mu\text{g l}^{-1}$) as a function of water temperature ($^{\circ}\text{C}$) with Chlorophyll *a* fixed at (A) $1 \mu\text{g l}^{-1}$, (B) $50 \mu\text{g l}^{-1}$, and (C) $100 \mu\text{g l}^{-1}$ and other covariates held at their mean value. Solid lines represent the mean predicted microcystin concentration and grey ribbons represent the 95% confidence intervals approximated as $\pm 2 \times$ prediction standard error.

to water quality and human health (Carmichael, 1992), identifying factors that promote toxin release is imperative to minimize risk of exposure. In this study, we took advantage of an ecosystem with year-round blooms (2009–present) of *P. agardhii* to evaluate the role of temperature on toxin release without the confounding influence of nutrient limitation. Our results indicate that the biomass and productivity of toxin-producing *P. agardhii*, both in-situ and in the laboratory, decline at elevated water temperatures. Further, toxin release varied with temperature-mediated changes in productivity, pointing to the importance of temperature as a key factor regulating toxin release.

The strong influence of temperature on cyanobacteria abundance observed during this study (in the absence of nutrient limitation) is consistent with research across marine and freshwater ecosystems

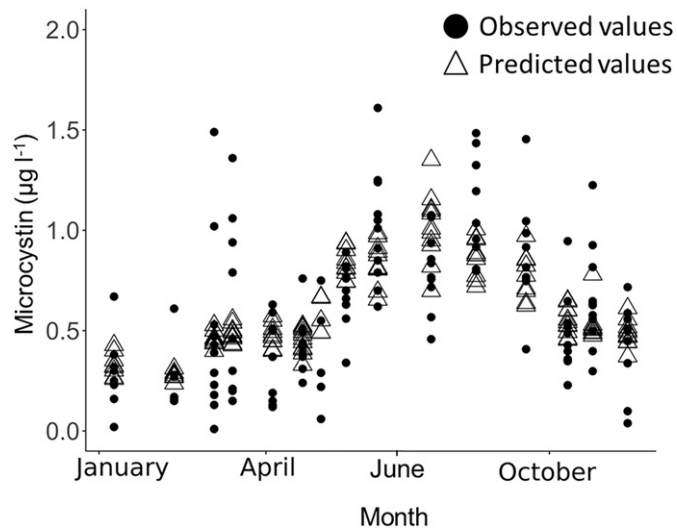


Fig. 6. Time series of observed free microcystin concentration and leave-one-out cross-validation predicted free microcystin concentration.

(Ibelings, 1996; O'Neil et al., 2012). Although, cyanobacteria were present at bloom levels ($\geq 20,000$ cyanobacterial cells ml^{-1} or $10 \mu\text{g l}^{-1}$ Chlorophyll *a*; Bartram and Chorus, 1999; WHO, 2003) across a wide range of temperatures ($3\text{--}27^{\circ}\text{C}$), biomass increased with warming and was greatest at temperatures ranging from 9 to 19°C . Given that *P. agardhii* comprised $\geq 96\%$ of phytoplankton community composition throughout the year, we anticipated maximum growth rates at temperatures within their optimal range ($10\text{--}20^{\circ}\text{C}$; Dokulil and Teubner, 2000). However, there was a significant decline in biomass at temperatures $\geq 19^{\circ}\text{C}$, suggesting that elevated water temperatures had exceeded tolerable conditions. This finding is noteworthy because, unlike low-temperature thresholds that tend to limit cyanobacteria accumulation and associated toxins (Roberts and Zohary, 1987; Liu et al., 2011), the reduction in biomass at elevated temperatures was coupled with a marked increase in extracellular (i.e., free) microcystin concentration. The decoupling of *P. agardhii* biomass and extracellular microcystin concentration at elevated temperatures is consistent with research showing an inverse relationship between *P. agardhii* biomass and the proportion of microcystin-producing genotypes at

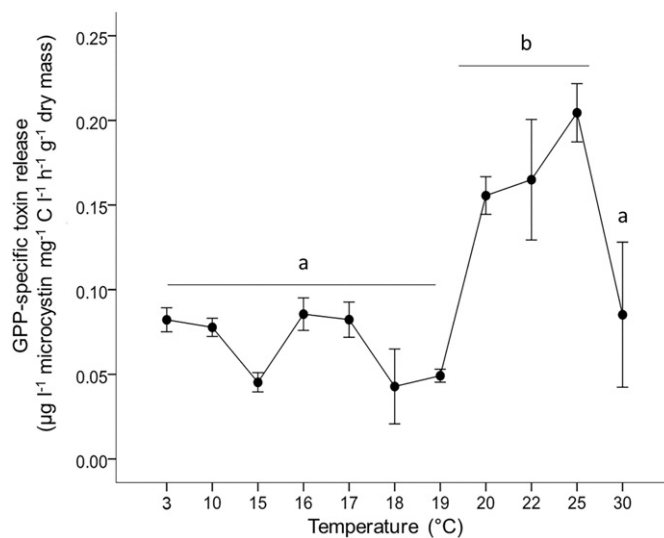


Fig. 7. Mean ± 1 SE gross primary productivity-specific microcystin release among experimental temperature treatments. Points with the same letter are not significantly different among treatments at the $\alpha = 0.05$ significance level.

temperatures > 19 °C (Briand et al., 2008). Consequently, our findings add to a growing body of literature demonstrating that toxin concentration does not always increase linearly with cyanobacteria abundance (Briand et al., 2008; Davis et al., 2009; Srivastava et al., 2013), but instead, is at its maximum when optimal environmental conditions for cyanobacteria are exceeded.

The close association between temperature and toxin release was confirmed by our laboratory incubation experiment. During incubations, microcystin release was lower from cells with elevated photosynthetic capacity (at temperatures between 3 and 19 °C), compared to cyanobacteria with a reduced photosynthetic capacity (at temperatures between 20 and 25 °C). Microcystin is a secondary metabolite and its production tends to increase with temperature, owing to the influence of warming on photosynthesis (Dziallas and Grossart, 2011; Neilan et al., 2012; Brutemark et al., 2015). The rise in extracellular microcystin at elevated temperatures is consistent with previous research showing release of secondary metabolites can prevent damage to the cell when high photosynthetic output is coupled with unfavorable growth conditions (Smith and Underwood, 2000). Cyanotoxins in particular have been shown to be released in response to oxidative stress and can signal for enhanced toxin production by the remaining intact cells (Schatz et al., 2007; Zilliges et al., 2011; Holland and Kinnear, 2013). Given that elevated photosynthetic rates at higher temperatures can cause oxidative stress (Brutemark et al., 2015), the decline in production and rise in extracellular microcystin concentration measured at temperatures above optimal conditions during our study may have been due to temperature-induced oxidative stress. Although we are not able to rule out light intensity as a possible stress inducing factor (Kaebernick et al., 2000; Wiedner et al., 2003), a similar response was observed in-situ under variable light conditions and in the laboratory with controlled light levels, suggesting that light was not the primary factor contributing to microcystin release in this study.

In an effort to improve on our ability to predict the frequency or severity of toxic cyanobacteria blooms in areas of concern (Gilbert et al., 2005; Bernard et al., 2014; Wells et al., 2015), we integrated our findings into a model that predicted free microcystin concentration as a function of water temperature. Specifically, our model predicted that temperatures ≥ 20 °C would result in a 36% ($0.26 \mu\text{g l}^{-1}$) increase in extracellular microcystin concentration when Chlorophyll *a* was $\leq 50 \mu\text{g l}^{-1}$. Model predictions also indicated that free microcystin concentration would be expected to increase with intracellular P content, suggesting that P indirectly influences toxin release through its effect on algal biomass (Davis et al., 2009; Kosten et al., 2012; Beaulieu et al., 2013). Similar to other studies (Briand et al., 2008; Davis et al., 2009; Beaulieu et al., 2013), we found that measures of cyanobacteria abundance alone were poor predictors of toxin concentration and their use as metrics to determine risk will likely underestimate toxicity. Although there are a number of toxin-producing cyanobacteria species, each with varying temperature optima (Dokulil and Teubner, 2000), particularly those originating from tropical habitats (e.g., *Cylindrospermopsis raciborskii*; Wiedner et al., 2007), the results of this study demonstrate a potential to use water temperature as a metric to forecast bloom severity in eutrophic lakes. Given the extent of publically accessible long-term temperature records in freshwater ecosystems (e.g., National Lakes Assessment; USEPA, 2009), we anticipate that our model could be integrated into existing frameworks to improve efforts to minimize health risks associated with toxin exposure. Such tools may be especially relevant for eutrophic lakes where blooms can persist year-round with varying degrees of toxicity.

Although the role of temperature has been widely recognized as a factor contributing to toxic cyanobacteria growth (Robarts and Zohary, 1987; Paerl and Huisman, 2009) and toxin production (Wiedner et al., 2007; Brutemark et al., 2015), our study provides new insight into the influence of temperature on extracellular toxin release. Specifically, our results show that toxin release from harmful cyanobacteria (i.e., *P. agardhii*) increases significantly at temperatures above optimal growth

conditions. Although toxicity has frequently been reported to be a result of warmer temperatures supporting higher growth rates of toxic cells (Rapala et al., 1997; Paerl and Huisman, 2008; Paerl and Paul, 2012), we found that free microcystin concentration was highest when *P. agardhii* biomass and cell density were reduced. This inverse relationship occurred at temperatures between 20 and 25 °C, pointing to a possible temperature threshold where *P. agardhii* release a greater proportion of their toxins into the water column. This finding is notable since instances of water temperatures exceeding 20 °C during warm summer months have become increasingly common with ongoing climate change (Wiedner et al., 2007; Kosten et al., 2012). As a consequence, there is an increased likelihood that warming will generate a positive feedback on the frequency and severity of harmful blooms by promoting toxic cyanobacteria over non-toxic strains (Briand et al., 2008; Kleinteich et al., 2012), stimulating intracellular (i.e., cell-bound) toxin synthesis (Davis et al., 2009), and subsequently triggering toxin release. Though it is not possible to rule out the potential for thermophilic cyanobacteria species to competitively exclude those stressed at elevated temperatures, such as the *P. agardhii* observed in this study. Nonetheless, there is growing evidence that climate change has caused rapid rates of warming and facilitated the expansion of toxic cyanobacteria into ecosystems that were previously undisturbed (Winter et al., 2011; Carey et al., 2012; Kleinteich et al., 2012; Taranu et al., 2015). The results of our study point to the importance of monitoring temperature changes in these ecosystems, which may aid in forecasting the likelihood for warming to catalyze significant lake change at larger scales.

5. Conclusions

Overall, the results of our study show that temperature regulates microcystin release from toxin-producing cyanobacteria. In particular, we found that *P. agardhii* can be present at bloom levels across a wide range of temperatures (3–27 °C), but declines at temperatures above those optimal for growth (≥ 19 °C). Extracellular microcystin was most elevated (on a biomass basis) at temperatures between 20 and 25 °C. The reduction in productivity and simultaneous increase in free toxin concentrations at elevated water temperatures (in both laboratory and in-situ sampling), supports our hypothesis that temperature regulates the production of harmful cyanobacteria and the release of toxins. By incorporating our results into a model that uses temperature to predict toxin release, this study will provide resources managers with a tool to minimize health risks associated with toxin exposure.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.08.149>.

Acknowledgements

This research was supported by a Grant-in-Aid of Research from the Phycological Society of America, the Sigma Xi Scientific Research Society, and an ASPIRE grant from BSU awarded to JTW. Support for algal identification was provided A PSA Croasdale Fellowship awarded to JTW.

References

- Aaronson, S., 1971. The synthesis of extracellular macromolecules and membranes by a population of the phytoflagellate *Ochromonas Danica*. *Limnol. Oceanogr.* 16, 1–9.
- American Public Health Association (APHA), 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. American Public Health Association, Washington, DC.
- Babica, P., Bláha, L., Maršálek, B., 2006. Exploring the natural role of microcystins – a review of effects on photoautotrophic organisms. *J. Phycol.* 42, 9–20.
- Barton, K., 2016. MuMIn: Multi-model Inference. R Package Version. 1.15:p. 6 Available from: <https://CRAN.R-project.org/package=MuMIn>.
- Bartram, J., Chorus, I., 1999. Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring, and Management. World Health Organization E and FN Spon Publishers, London.

- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67 (1), 1–48.
- Beaulieu, M., Pick, F., Gregory-Eaves, I., 2013. Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes data set. *Limnol. Oceanogr.* 58 (5), 1736–1746.
- Bernard, S., Kudela, R., Velo-Suarez, L., 2014. Developing Global Capabilities for the Observation and Prediction of Harmful Algal Blooms. In: Djavidnia, S., Cheung, V., Ott, M., Seevave, S. (Eds.), *Oceans and Society, Blue Planet*. Cambridge Scholars Publishing, UK, pp. 46–52.
- Bertilsson, S., Jones, J.B., 2003. Supply of dissolved organic matter to aquatic ecosystems: autochthonous sources. In: Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*. Academic Press, San Diego, CA, pp. 3–24.
- Bláha, L., Babica, P., Maršálek, B., 2009. Toxins produced in cyanobacterial water blooms – toxicity and risks. *Interdiscip. Toxicol.* 2, 36–41.
- Briand, J.F., Jacquet, S., Flinois, C., Avois-Jacquet, C., Maisonneuve, C., Leberre, B., Humbert, J.F., 2005. Variations in the microcystin production of *Planktothrix rubescens* (cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. *Microb. Ecol.* 50, 418–428.
- Briand, E., Yepremian, C., Humbert, J.F., Quiblier, C., 2008. Competition between microcystin- and non-microcystin producing *Planktothrix agardhii* (cyanobacteria) strains under different environmental conditions. *Environ. Microbiol.* 10 (12), 3337–3348.
- Brutemark, A., Engström-Öst, J., Vehmaa, A., Gorokhova, E., 2015. Growth, toxicity and oxidative stress of a cultured cyanobacterium (*Dolichospermum* sp.) under different CO₂/pH and temperature conditions. *Phycol. Res.* 63, 56–63.
- Butterwick, C., Heaney, S.I., Talling, J.F., 2005. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshw. Biol.* 50 (2), 291–300.
- Carey, C.C., Ewing, H.A., Cottingham, K.L., Weathers, K.C., Thomas, R.Q., Haney, J.F., 2012. Occurrence and toxicity of the cyanobacterium *Gloeotrichia echinulate* in low-nutrient lakes in the northeastern United States. *Aquat. Ecol.* 46, 395–409.
- Carmichael, W.W., 1992. Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* 72, 445–459.
- Charles, D.F., Knowles, C., Davis, R.S., 2002. Protocols for the Analysis of Algal Samples Collected as Part of the U.S. Geological Survey National Water-quality Assessment Program. Report No. 02–06. Academy of Natural Sciences, Philadelphia, Pennsylvania Available from: <http://water.usgs.gov/nawqa/protocols/algprotocol>.
- Christoffersen, K., Lyck, S., Winding, A., 2002. Microbial activity and bacterial community structure during degradation of microcystins. *Aquat. Microb. Ecol.* 27, 125–136.
- Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J., 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae* 8, 715–725.
- Davison, I.R., 1991. Environmental effects on algal photosynthesis: temperature. *J. Phycol.* 27, 2–8.
- Deng, J., Qin, B., Paerl, H.W., Zhang, Y., Wu, P., Ma, J., Chen, Y., 2014. Effects of nutrients, temperature, and their interactions on Spring phytoplankton community succession in Lake Taihu, China. *PLoS One* 9 (12), e113960.
- Dokulil, M.T., Teubner, K., 2000. Cyanobacterial dominance in lakes. *Hydrobiology* 438, 1–12.
- Downing, J.A., Watson, S.B., McCauley, E., 2001. Predicting cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* 58, 1905–1908.
- Dumouchelle, D.H., Stelzer, E.A., 2014. Chemical and biological quality of water in Grand Lake St. Marys, Ohio, 2011–2012, with emphasis on cyanobacteria. U.S. Geological Survey Scientific Investigations Report 2014–5210:p. 51 <http://dx.doi.org/10.3133/sir20145210>.
- Dziallas, C., Grossart, H., 2011. Increasing oxygen radicals and water temperature select for toxic *Microcystis* sp. *PLoS One* 6 (9), e25569.
- Falconer, I.R., 2005. Microcystin toxicity. *Cyanobacterial Toxins of Drinking Water Supplies*. CRC Press, Boca Raton, FL, pp. 109–140.
- Gilbert, P.M., Anderson, D.M., Gentien, P., Granéli, E., Sellner, K.G., 2005. The global complex phenomena of harmful algal blooms. *Oceanography* 18 (2), 136–147.
- Gu, L.Y., Wyatt, K.H., 2016. Light availability regulates the response of algae and heterotrophic bacteria to elevated nutrient levels and warming in a northern boreal peatland. *Freshw. Biol.* 61, 1441–1453.
- Helbling, E.W., Banaszak, A.T., Villafañe, V.E., 2015. Global change feed-back inhibits cyanobacterial photosynthesis. *Nat. Sci. Rep.* 5:14514. <http://dx.doi.org/10.1038/srep14514>.
- Holland, A., Kinnear, S., 2013. Interpreting the possible ecological role(s) of cyanotoxins: compounds for competitive advantage and/or physiological aide? *Mar. Drugs* 11, 2239–2258.
- Ibelings, B.W., 1996. Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms. *J. Phycol.* 32, 549–557.
- Jensen, J.P., Jeppesen, E., Olrik, K., Kristensen, P., 1994. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Can. J. Fish. Aquat. Sci.* 51 (8), 1692–1699.
- Joung, S.H., Oh, H.M., Ko, S.R., Ahn, C.Y., 2011. Correlations between environmental factors and toxic and non-toxic *Microcystis* dynamics during bloom in Daechung Reservoir, Korea. *Harmful Algae* 10, 188–193.
- Kaebnick, M., Neilan, B.A., 2001. Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol. Ecol.* 35, 1–9.
- Kaebnick, M., Neilan, B.A., Boerner, T., Dittmann, E., 2000. Light and the transcriptional response of the microcystin biosynthetic gene cluster. *Appl. Environ. Microbiol.* 66, 3387–3392.
- Kaplan, A., Harel, M., Kaplan-Levy, R.N., Hadas, O., Sukenik, A., Dittmann, E., 2012. The languages spoken in the water body (or the biological role of cyanobacterial toxins). *Front. Microbiol.* 3 (138). <http://dx.doi.org/10.3389/fmicb.2012.00138>.
- Kleinteich, J., Wood, S.A., Küpper, F.C., Camacho, A., Quesada, A., Frickey, T., Dietrich, D.R., 2012. Temperature-related changes in polar cyanobacteria mat diversity and toxin production. *Nat. Clim. Chang.* <http://dx.doi.org/10.1038/NCLIMATE1418>.
- Kosten, S., Huszar, V.L.M., Bécáres, E., Costa, L.S., van Donk, E., Hansson, L.A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., De Meester, L., Moss, B., Lurling, M., Noges, T., Romo, S., Scheffer, M., 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. *Glob. Chang. Biol.* 18 (1), 118–126.
- Liu, X., Lu, X., Chen, Y., 2011. The effects of temperature and nutrient ratios on *Microcystis* blooms in Lake Taihu, China: an 11-year investigation. *Harmful Algae* 10, 337–343.
- Lv, H., Yang, J., Liu, L., Yu, X., Yu, Z., Chiang, P., 2014. Temperature and nutrients are significant drivers of seasonal shift in phytoplankton community from a drinking water reservoir, subtropical China. *Environ. Sci. Pollut. Res. Int.* 21 (9), 5917–5928.
- McCarthy, M., Hedges, J., Benner, R., 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. *Mar. Chem.* 55, 281–297.
- Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E., 2012. Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environ. Microbiol.* 15 (5), 1239–1253.
- O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. *Harmful Algae* 14, 313–334.
- Paerl, H.W., Huisman, J., 2008. Blooms like it hot. *Science* 320, 57–58.
- Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* 1 (1), 27–37.
- Paerl, H.W., Millie, D.F., 1996. Physiological ecology of toxic aquatic cyanobacteria. *Phycologia* 35, 160–167.
- Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. *Microb. Ecol.* 65 (4), 995–1010.
- Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. *Water Res.* 46, 1349–1363.
- Pearson, L.A., Lisbergues, M., Börner, T., Dittmann, E., Neilan, B.A., 2004. Inactivation of an ABC transporter gene, *mcvH*, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. *Appl. Environ. Microbiol.* 70 (11), 6370–6378.
- Pick, F.R., 2016. Blooming algae: a Canadian perspective on the rise of toxic cyanobacteria. *Can. J. Fish. Aquat. Sci.* 73, 1149–1158.
- Posch, T., Köster, O., Salcher, M.M., Pernthaler, J., 2012. Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. *Nat. Clim. Chang.* <http://dx.doi.org/10.1038/NCLIMATE1581>.
- R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rapala, J., Sivonen, K., Lyra, C., Niemela, S.I., 1997. Variation of microcystin, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimulation. *Appl. Environ. Microbiol.* 63, 2206–2212.
- Rastogi, R.P., Sinha, R.P., Incharoensakdi, A., 2014. The cyanotoxin-microcystins: a current overview. *Rev. Environ. Sci. Biotechnol.* 13, 215–249.
- Raven, J.A., Geider, R.J., 1988. Temperature and algal growth. *New Phytol.* 110, 441–461.
- Roberts, R.D., Zohary, T., 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N. Z. J. Mar. Freshw. Res.* 21 (3), 391–399.
- Schatz, D., Keren, Y., Vardi, A., Sukenik, A., Carmeli, S., Börner, T., Dittmann, E., Kaplan, A., 2007. Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. *Environ. Microbiol.* 9 (4), 965–970.
- Schindler, D.W., 1977. Evolution of phosphorus limitation in lakes. *Science* 195 (4275), 260–262.
- Shi, L., Carmichael, W.W., Miller, I., 1995. Immuno-gold localization of hepatotoxins in cyanobacterial cells. *Arch. Microbiol.* 163, 7–15.
- Smith, D.J., Underwood, G.J.C., 2000. The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. *J. Phycol.* 36, 321–333.
- Srivastava, A., Singh, S., Ahn, C., Oh, H., Asthana, R.K., 2013. Monitoring approaches for a toxic cyanobacterial bloom. *Environ. Sci. Technol.* 47, 8999–9013.
- Taranu, Z.E., Zurawell, R.W., Pick, F., Gregory-Eaves, I., 2012. Predicting cyanobacterial dynamics in the face of global change: the importance of scale and environmental context. *Glob. Chang. Biol.* 18, 3477–3490.
- Taranu, Z.E., Gregory-Eaves, I., Leavitt, P.R., Bunting, L., Buchaca, T., Catalan, J., Domaizon, I., Guilizzoni, P., Lami, A., McGowan, S., Moorhouse, H., Morabito, G., Pick, F.R., Stevenson, M.A., Thompson, P.L., Vinebrooke, R.D., 2015. Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. *Ecol. Lett.* 18, 375–398.
- U.S. Environmental Protection Agency (EPA), 2009. National Lakes Assessment: A Collaborative Survey of the Nation's Lakes. EPA 841-R-09-001. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, D.C.
- U.S. Environmental Protection Agency (EPA), 2016. Ohio Algal Toxin Results from Lake Erie, State Park Beaches, Inland Lakes, and Public Water Supplies - 2010 to Present. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, D.C.
- Watson, S.B., Whitton, B.A., Higgins, S.N., Paerl, H.W., Brooks, B.W., Wehr, J.D., 2015. Harmful algal blooms. In: Wehr, J.D., Sheath, R.G., Kociolek, J.P. (Eds.), *Freshwater Algae of North America*, 2nd edition Academic Press, San Diego, CA, pp. 873–920.
- Welker, M., von Döhren, H., 2006. Cyanobacterial peptides – nature's own combinatorial biosynthesis. *FEMS Microbiol. Rev.* 30, 530–563.
- Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., Cochlan, W.P., 2015. Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93.

- Wetzel, R.G., Likens, G.E., 2000. *Limnological Analyses*. Springer-Verlag, New York, NY.
- Wiedner, C., Visser, P.M., Fastner, J., Metcalf, J.S., Codd, G.A., Mur, L.R., 2003. Effects of light on the microcystin content of *Microcystis* strain PCC 7806. *Appl. Environ. Microbiol.* 69, 1475–1481.
- Wiedner, C., Rucker, J., Bruggemann, R., Nixdorf, B., 2007. Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. *Oecologia* 152, 473–484.
- Winter, J.G., DeSellas, A.M., Fletcher, R., Heintsch, L., Morley, A., Nakamoto, L., Utsumi, K., 2011. Algal blooms in Ontario, Canada: increases in reports since 1994. *Lake Reservoir Manage.* 27, 107–114.
- World Health Organization (WHO), 2003. *Algae and cyanobacteria in fresh water*. World Health Organization's Guidelines for Safe Recreational Water Environments. Coastal and Fresh Waters 1, pp. 136–158.
- Wyatt, K.H., Rober, A.R., Schmidt, N., Davison, I.R., 2014. Effects of desiccation and rewetting on the release and decomposition of dissolved organic carbon from benthic macroalgae. *Freshw. Biol.* 59, 407–416.
- Xie, L., Rediske, R.R., Hong, Y., O'Keefe, N.D., Dyble, J., Steinman, A.D., 2012. The role of environmental parameters in the structure of phytoplankton assemblages and cyanobacteria toxins in two hypereutrophic lakes. *Hydrobiology* 691, 255–268.
- Zilliges, Y., Kehr, J.C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, M., Kaplan, A., Börner, T., Dittmann, E., 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. *PLoS ONE* 6 (3), e17615.