Harmful Algae 78 (2018) 118–128

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Environmental factors influencing the quantitative distribution of microcystin and common potentially toxigenic cyanobacteria in U.S. lakes and reservoirs

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ARTICLE INFO

Keywords:
National Lakes Assessment
Ecoregion
Water quality
Phytoplankton
Harmful algal blooms
Canonical correlation analysis

ABSTRACT

Many species of cyanobacteria are capable of producing toxins and causing nuisance blooms, however response to environmental conditions is likely taxon-specific. Environmental factors influencing cyanobacterial composition and toxin production in lakes have been examined in many studies; yet are often confined to individual water bodies, or to a small number of systems within the same region. Here, data from the 2012 USEPA National Lakes Assessment are used to examine relationships between biovolume of common potentially-toxigenic cyanobacteria (*Aphanizomenon* spp., *Cylindrospermopsis* spp., *Dolichospermum* spp., *Microcystis* spp. and *Planktothrix* spp.) and environmental variables across the entire conterminous United States, and results are compared across nine distinct ecoregions. Total phosphorus and water clarity were identified as the most influential environmental factors correlated with phytoplankton community composition. The Northern, Southern and Temperate Plains ecoregions displayed the highest biovolumes of potentially toxigenic taxa on average, as well as highest mean concentrations of microcystin. In those three ecoregions, samples with microcystin concentrations greater than 1 ppb were primarily dominated by *Planktothrix* spp. while in all other ecoregions *Dolichospermum* spp. was the dominant genus. Canonical Correlation Analysis revealed a strong association between high microcystin concentrations and high nutrient concentrations (total nitrogen and total phosphorus), and between high microcystin concentrations and low percentage of watershed forest cover. Results from this study indicate that the likely occurrence of potentially toxigenic taxa in lakes and reservoirs is predictable on a biogeographical basis, depending on morphological and water quality characteristics. Data from this study may be useful to regional managers attempting to prevent or mitigate nuisance cyanobacterial blooms.

1. Introduction

Phytoplankton community composition in lakes and reservoirs (hereinafter, lakes) is strongly influenced both spatially and temporally by variability in water quality factors such as nutrient concentrations, hydrodynamics and water temperature (Tillman et al., 1982; Watson et al., 1997; de Souza Cardoso and da Motta Marques, 2009; Beaver et al., 2012, 2013). Generally, phytoplankton communities tend to be dominated by cyanobacteria in systems with high concentrations of nutrients, low water clarity and warmer surface temperatures – conditions that are characteristic of eutrophic water bodies (Smith, 1986; Downing et al., 2001; Kosten et al., 2012). Planktonic species of cyanobacteria can produce toxins as secondary metabolites, which can negatively impact lake ecosystems at higher trophic levels and cause adverse effects on human health and recreation. Both climate warming and eutrophication are predicted to increase the frequency and severity of toxic cyanobacterial blooms worldwide (Heisler et al., 2008; Paerl and Huisman, 2008; O'Neil et al., 2012; Paerl, 2017). Thus, determining which environmental conditions are most likely to lead to cyanobacterial blooms (and subsequent toxin production) is of great interest to lake managers and natural resource professionals (Paerl, 2017).

Most studies that monitor phytoplankton community structure and occurrence of toxins over space and time focus on a single lake or a small region, as large-scale and prolonged sampling can be difficult and costly. Consequently, conflicting results have been reported regarding which factors are most likely to lead to toxic bloom events. It is also likely that toxin production in specific species and strains of cyanobacteria is triggered by different environmental conditions (Marmen et al., 2016; Wood et al., 2017). The USEPA's National Lakes

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https://doi.org/10.1016/j.hal.2018.08.004
Received 18 April 2018; Received in revised form 1 August 2018; Accepted 8 August 2018

Assessment (NLA), which surveys over 1000 randomly-selected lakes across the U.S. every five years is a relevant public resource for broad-scale limnological research (Pollard et al., 2018) and provides a unique opportunity to examine cyanobacterial communities over multiple ecoregions and a gradient of water quality conditions.

The current study seeks to determine which water quality variables are related to phytoplankton community composition and what environmental conditions promote the prevalence of potentially-toxigenic cyanobacteria in lakes in the conterminous U.S. Analysis of water quality and land use data from the 2007 NLA (Beaver et al., 2014) showed that microcystin concentrations > 1 ppb (above the World Health Organization recommended threshold for safe drinking water) were primarily located in the upper Midwest region of the U.S., and were positively associated with agricultural land-use practices, high total nitrogen (TN) and high dissolved organic carbon (DOC). The current study utilizes data from the 2012 NLA and extends upon previous analyses to determine whether microcystin concentrations in those regions and elsewhere in the U.S. are associated with particular cyanobacterial taxa, and how those taxa are related to specific water quality variables. Based on the finding from Beaver et al. (2014) that similar land-use practices in different regions of the country did not yield similar toxicity, it is expected that high microcystin concentrations are correlated to high biovolumes of toxigenic taxa, and that those taxa are strongly influenced by water quality parameters associated with agriculture-dominated watersheds (high nutrients and DOC). Recent studies have used data from the NLA to examine the relationship between water quality variables and total cyanobacterial abundance (Beaver et al., 2014) or biomass (Beaulieu et al., 2013). This study expands on previous records by utilizing individually derived biovolumes to examine relationships between specific taxa and microcystin concentrations on a national scale.

2. Methods

2.1. Study sites

1038 lakes were comprehensively sampled throughout the conterminous U.S. from May through September of 2012 as part of the U.S. Environmental Protection Agency's 2012 National Lakes Assessment (USEPA, 2016). Lakes greater than 1 ha in area and 1 m in depth, with at least a quarter-acre of open water area were selected, using unbiased stratified random sampling, from the USGS/USEPA National Hydrography Dataset (NHDPlus) version 2 (see Simley and Carswell, 2009). Both natural and man-made lakes were included in the survey; however the Laurentian Great Lakes, the Great Salt Lake, commercial treatment/disposal ponds, brackish lakes and ephemeral lakes were excluded. A subset of lakes were sampled twice within the study period, and each sampling event was treated as an independent sample.

2.2. Sample collection

Each lake was sampled for water quality, biological condition, habitat conditions, and recreational suitability, however collection methodology described here applies only to the variables included in this study. Phytoplankton, microcystin and water quality samples were taken from an index site in each lake. The index site was considered an open water area up to 50 m deep or at the mid-point in reservoirs. Lake surface area was also recorded, and a ratio of area:depth was calculated using a modified version the equation for dynamic ratio (i.e. potential for disturbance) described in Håkansan (1982):

$$\sqrt{\text{surface area}}\ (\text{km}^2) \div \text{index site depth}\ (\text{m})$$

Vertical temperature profiles were conducted at the index site; however in this study only mean water temperatures from the upper 5 m of the water column were evaluated. Secchi depth was recorded using a standard Secchi disk on the shady side of the boat. An integrated sampler was used to collect whole water grab samples from within the euphotic zone, generally within the top 2 m of the water column. Water was transferred from the sampler into a rinsed 4 L cubitainer and this process was repeated until the cubitainer was filled. Subsamples were then taken from the cubitainer for nutrients, phytoplankton and microcystin. A 500 ml subsample for microcystin was immediately frozen following collection. A 1000 ml subsample for phytoplankton was preserved with Lugol's iodine solution. Microcystin and phytoplankton samples were then shipped to BSA Environmental Services, Inc. (Beachwood, OH) for analysis. A 250 ml subsample for nutrient analysis was acidified and shipped overnight to processing labs. Microcystin and phytoplankton were also sampled in the littoral zone of all lakes, however in this study only open-water samples were analyzed in order to be consistent with water quality variables. For more details on sample collection, see USEPA (2012a).

2.3. Laboratory analyses

Total nitrogen (mg L^{-1}) and total phosphorus (μg L^{-1}) were determined using automated colorimetric analysis following persulfate digestion. Dissolved organic carbon (mg L^{-1}) was determined using UV promoted persulfate oxidation to CO_2 with infrared detection. Total microcystin concentrations (ppb) were determined using a Microcystins/Nodularins enzyme-linked immunosorbent assay (Abraxis, detection limit 0.1 μg L^{-1}, -ADDA specific) following three freeze-thaw cycles to lyse cyanobacteria cells (Graham et al., 2010). Phytoplankton were examined under inverted light microscopes (Leica DMLB) at 400X using pre-concentrated Utermöhl sedimentation chambers that had been allowed to settle for a minimum of 8 h. Taxonomists identified organisms to the lowest possible taxonomic level, usually species, and enumerated at least 400 natural algal units (colonies, filaments and unicells). Up to 10 individual cells for each taxon were measured, and biovolume (μm^3 L^{-1}) was calculated using formulae for geometric shapes closely resembling specific taxa (Hillebrand et al., 1999). Total biovolume for each taxon was calculated by multiplying mean individual biovolume by cell abundance (# L^{-1}). More information on laboratory analyses can be found in USEPA (2012b).

Five cyanobacterial genera including *Aphanizomenon* Morren, *Cylindrospermopsis* (Woloszynska) Seenayya and Subba Raju, *Dolichospermum* (Ralfs ex Bornet et Flahault), *Microcystis* Lemmermann, and *Planktothrix* (Gomont) constitute the focus of this study. *Aphanizomenon, Cylindrospermopsis, Dolichospermum* and *Planktothrix* were chosen because they were the most frequent potentially toxigenic taxa observed in the 2012 NLA survey. *Microcystis* was also included due to the extensive degree of previous scientific study regarding its occurrence and toxicity. While all five genera contain species and strains capable of cyanotoxin production, only *Dolichospermum*, *Microcystis*, and *Planktothrix* are capable of producing microcystins (Carmichael, 2001). As such, only those three genera are analyzed in the context of microcystin production. All species originally identified under the genus *Anabaena* are referred to and treated as *Dolichospermum* in this report, following recent nomenclatural changes that occurred between the time of initial identification and present data analysis (Komárek, 2016). Specimens identified as *Planktothrix* spp. may have been previously classified as *Oscillatoria* (Anagnostidis and Komárek, 1988).

2.4. Mapping, land use and trophic state determination

Using the latitude and longitude for all sample sites and the absolute value for each criterion (individual species biovolume, microcystin concentration), the data were plotted onto a map using Arc GIS® software. Quantitative data were overlaid onto a background map detailing 9 distinct ecoregions. For the purposes of the National Lakes Assessment and other components of the EPA's National Aquatic Resource Surveys