Harmful Algae 54 (2016) 4–20

Contents lists available at ScienceDirect

Harmful Algae

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

Review

A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp.

Matthew J. Harke [a], Morgan M. Steffen [b,*], Christopher J. Gobler [c], Timothy G. Otten [d], Steven W. Wilhelm [e], Susanna A. Wood [f], Hans W. Paerl [g]

[a] Department of Earth and Environmental Sciences, Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, United States
[b] James Madison University, Department of Biology, 951 Carrier Dr., Harrisonburg, VA 22807, United States
[c] Stony Brook University, School of Marine and Atmospheric Sciences, 239 Montauk Hwy, Southampton, NY 11968, United States
[d] Oregon State University, Department of Microbiology, Nash Hall 226, Corvallis, OR 97331, United States
[e] University of Tennessee, Department of Microbiology, 1414 West Cumberland Ave., Knoxville, TN 37996, United States
[f] Cawthron Institute, Private Bag 2, Nelson, New Zealand and Environmental Research Institute, University of Waikato, Hamilton, New Zealand
[g] University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557, United States

ARTICLE INFO

Article history:
Received 21 August 2015
Accepted 22 December 2015

Keywords:
Microcystis
Cyanobacterial harmful algal blooms
Microcystin

ABSTRACT

This review summarizes the present state of knowledge regarding the toxic, bloom-forming cyanobacterium, *Microcystis*, with a specific focus on its geographic distribution, toxins, genomics, phylogeny, and ecology. A global analysis found documentation suggesting geographic expansion of *Microcystis*, with recorded blooms in at least 108 countries, 79 of which have also reported the hepatatoxin microcystin. The production of microcystins (originally "Fast-Death Factor") by *Microcystis* and factors that control synthesis of this toxin are reviewed, as well as the putative ecophysiological roles of this metabolite. Molecular biological analyses have provided significant insight into the ecology and physiology of *Microcystis*, as well as revealed the highly dynamic, and potentially unstable, nature of its genome. A genetic sequence analysis of 27 *Microcystis* species, including 15 complete/draft genomes are presented. Using the strictest biological definition of what constitutes a bacterial species, these analyses indicate that all *Microcystis* species warrant placement into the same species complex since the average nucleotide identity values were above 95%, 16S rRNA nucleotide identity scores exceeded 99%, and DNA–DNA hybridization was consistently greater than 70%. The review further provides evidence from around the globe for the key role that both nitrogen and phosphorus play in controlling *Microcystis* bloom dynamics, and the effect of elevated temperature on bloom intensification. Finally, highlighted is the ability of *Microcystis* assemblages to minimize their mortality losses by resisting grazing by zooplankton and bivalves, as well as viral lysis, and discuss factors facilitating assemblage resilience.

Contents

* Corresponding author.
E-mail address: steffemm@jmu.edu (M.M. Steffen).

http://dx.doi.org/10.1016/j.hal.2015.12.007
1568-9883/

1. Introduction

Blooms of toxic cyanobacteria have become a common occurrence in water bodies worldwide. One of the most pervasive bloom-forming cyanobacteria in freshwater ecosystems is *Microcystis*. In temperate systems, this organism overwinters in the benthos and during the summer rises to the epilimnion where it can accumulate to form blooms and scums on the water surface (Reynolds and Rogers, 1976; Ibelings et al., 1991). Blooms of *Microcystis* generally occur when water temperatures exceed 15 °C (Okino, 1974; Reynolds et al., 1981; Jacoby et al., 2000) and the occurrence of blooms has been linked to anthropogenic nutrient loading (Perovich et al., 2008; Dolman et al., 2012). Many *Microcystis* strains can produce the potent hepatotoxin microcystin, and thus persistent blooms pose a risk to those who use impaired water resources for drinking water supplies, recreational activities, and fisheries. Microcystins are the only cyanotoxins for which the World Health Organization has set drinking and recreational water standards and are typically the only cyanotoxins screened for by municipal management agencies (Chorus and Bartram, 1999; Hudnell et al., 2008). As global climate changes, the occurrence and intensity of *Microcystis* blooms is expected to increase (Paerl and Huisman, 2008; Michalak et al., 2013; Paerl and Otten, 2013).

This review synthesizes the current state of knowledge regarding *Microcystis*; focusing on its geographic distribution, toxin production, phylogeny, and structural genomics. How these factors influence the ecology of this globally significant cyanobacterium is discussed and a series of knowledge gaps are identified and a list of high priority research topics are provided.

2. Geographic distribution

The cosmopolitan cyanobacterium *Microcystis* has been reported to bloom on every continent except Antarctica (Zurawell et al., 2005). Over the past decade there has been an expansion in the awareness of toxic cyanobacterial blooms and reports of these events (O'Neil et al., 2012; Paerl and Paul, 2012). To provide an update on the global geographic distribution of *Microcystis* blooms, a literature search for records from 257 countries and territories was conducted. Reports of *Microcystis* blooms were found for 108 countries (Fig. 1, Table S1). Many of the countries without reported incidents were small island nations, such as those in the Pacific region. The number of reports per country varied markedly with North American, Australasian, and European countries having many hundreds of records, whereas accounts from developing countries were often scarce or from only a single study. Occurrence rate or specific sites of blooms within each country are not reported, as this is likely a representative function of the extent and intensity of monitoring and research programs (and their geographic locations) in each country rather than a true reflection of bloom prevalence. Where *Microcystis* blooms were identified, it was also investigated whether there were associated reports of toxins. Confirmation of microcystins associated with blooms was identified for 79 countries. In some cases, there was conclusive evidence that *Microcystis* was the producer, e.g., strains of *Microcystis* were isolated, cultured, and toxin production confirmed, or molecular techniques such as screening for microcystin synthetase (*mcy*) genes were used. In many instances, these steps were not undertaken and it is plausible that other cyanobacteria present in the blooms (e.g., *Planktothrix* or *Dolichospermum*/*Anabaena*) were the producers. These scenarios have not been differentiated in Fig. 1. In one instance (Niger), the evidence for microcystin production was based on symptoms in a mouse bioassay. In all other studies, chemical or biochemical methods were used to identify the toxins. The analysis suggests an expansion of *Microcystis*, as previous documentation noted less than 30 countries (Zurawell et al., 2005), demonstrating that *Microcystis* has proliferated and dominated phytoplankton communities in a wide range of freshwater ecosystems in both temperate and tropical climates.

3. Toxins

Many cyanobacterial species produce natural compounds that are toxic (cyanotoxins) to other organisms, including mammals. Cyanotoxins exhibit a wide range of toxicities, including hepatotoxicity, nephrotoxicity, neurotoxicity, and dermatotoxicity. *Microcystis* is most well-known for its ability to produce the hepatotoxin microcystin (Bishop et al., 1959) and has been studied globally for many decades. However, data on the production of other cyanotoxins by this genus are scarce or preliminary. Here, these other compounds are mentioned briefly and the remainder of this section focuses on microcystins.

There are few reports of *Microcystis* producing neurotoxins. For instance, there is a single report of four *Microcystis* strains isolated from three Japanese lakes producing the neurotoxic anatoxin-a (Park et al., 1993) with several of the strains also producing microcystin. Since this finding has not been replicated in the past two decades of intensive cyanobacterial research, it remains possible that the anatoxin-a measured in these cultures was derived from a co-cultured microbe. Similarly, an isolate of *Microcystis* from a lagoon in São Paulo (Brazil; SPC 777) was reported to produce a range of paralytic shellfish poison (PSP) neurotoxins (Sant'Anna et al., 2011). Upon sequencing the genome of the isolate however, no saxitoxin biosynthesis genes were identified casting significant doubt that *Microcystis* was truly the causative agent. Although the study of β-*N*-methylamino-L-alanine (BMAA), has become a somewhat contentious issue (Holtcamp, 2012; Otten and Paerl, 2015), studies also suggest that the majority of cyanobacteria, including *Microcystis*, may produce BMAA (Cox et al., 2005), whereas many other investigators have failed to identify this compound (Faassen, 2014). BMAA is a non-protein amino acid which that has been linked to neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's, and Alzheimer's Disease (Cox and Sacks, 2002; Bradley and Mash, 2009; Banack et al., 2010; Holtcamp, 2012). The exact mode of BMAA toxicity is still under investigation, with both acute and chronic mechanisms indicated (Lobner et al., 2007). Perhaps the most methodical investigation to date was conducted by Réveillon et al. (2014), who reanalyzed a number of cyanobacterial isolates, including *Microcystis aeruginosa* PCC 7806, that were reported as BMAA producers. Notably, using highly sensitive and specific hydrophilic interaction chromatography coupled to tandem mass spectrometry (HILIC-MS/MS), they failed to detect BMAA in any

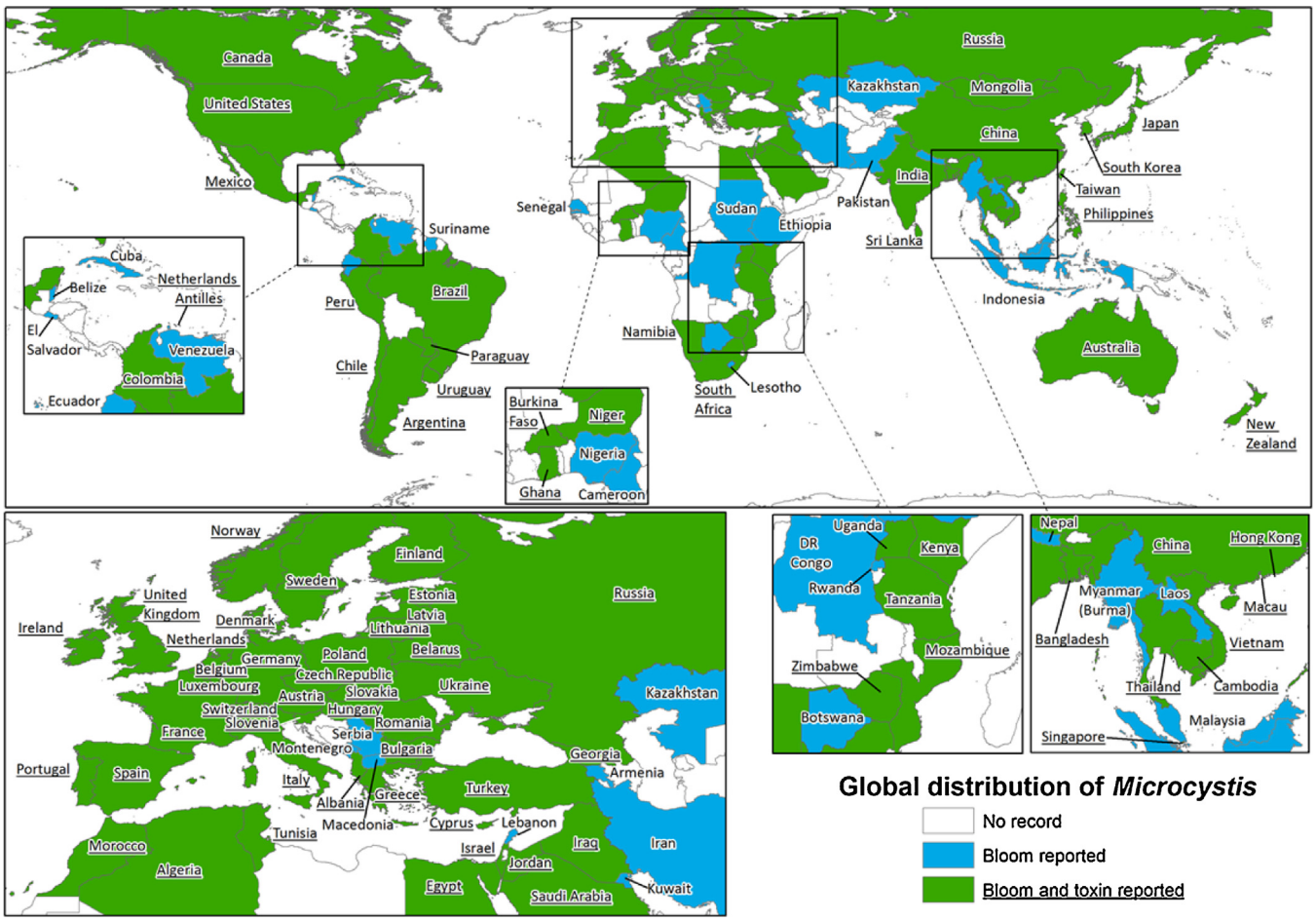

Fig. 1. The global occurrence of *Microcystis* blooms and microcystin as determined through literature searches for records of *Microcystis* blooms from 257 countries and territories. White indicate no record of bloom or microcystin, blue indicates a record was found for the occurrence of a bloom, and green indicates countries or territories where there was a record of both bloom and microcystin.

cultures. Both free and bound forms of the closely related isomer, 2,4-diaminobutyric acid (DAB) were detected in all cultures, and may be the compound identified as BMAA in previous studies using other analytical approaches. Beyond direct toxins, *Microcystis* has also been shown to produce compounds that act as endocrine disruptors; while not lethal to fish, these compounds regulate genetic elements associated with sexual maturity and differentiation (Rogers et al., 2011).

Microcystin was originally identified as Fast-Death Factor (Bishop et al., 1959), but was renamed microcystin a few years later (Konst et al., 1965). Microcystins are cyclic heptapeptides which contain a unique β-amino acid, Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; Rinehart et al., 1988; Tillett et al., 2000). Microcystins generally contain two conventional D-amino acids in positions one and six, a D-erythro-β-methylaspartic acid in position three, and position seven is often *N*-methyldehydroalanine (Tillett et al., 2000). To date, over 100 different microcystin congeners have been characterized (Puddick et al., 2014), mostly due to substitutions of variable L-amino acids in positions two and four, although modifications have been reported for all amino acids (Rinehart et al., 1994; Puddick et al., 2014). Microcystin congeners are named according to the single letter code of the amino acids incorporated at positions two and four; e.g., microcystin-LR contains leucine (L) in position two and arginine (R) in position four.

Different microcystin congeners vary in toxicity from essentially non-toxic (e.g., [(6*Z*)-Adda^5] microcystin-LR, $LD_{50} > 1200$ μg kg^{-1}) to highly toxic (e.g., microcystin-LR, $LD_{50} = 50$ μg kg^{-1}; Rinehart et al., 1994). Toxicity is manifested as an irreversible covalent bond formed between the toxin and protein phosphatases, especially in hepatocytes, which leads to subsequent cell structure damage (Goldberg et al., 1995; Maynes et al., 2006; Feurstein et al., 2009, 2010) and can result in liver disease as well as nephrotoxicity (Milutinović et al., 2003). This toxicity has led the World Health Organization (WHO) to propose a drinking water guideline of 1 μg l^{-1} for the common hydrophilic variant microcystin-LR (WHO, 2003).

Numerous fatalities and severe poisonings of livestock, pets and wildlife have been attributed to microcystin-containing *Microcystis* blooms (reviewed in Stewart et al. (2008)). Reports of human illness from microcystins are also well documented, with major exposure routes including direct consumption of drinking water and accidental ingestion of water or skin contact during recreational use of waterbodies (Ressom et al., 2004; Falconer, 2005). A less widely reported exposure route is via inhalation that may result from recreational activity in the vicinity of blooms (Wood and Dietrich, 2011). One of the most severe cases of human poisoning occurred in Brazil in 1996, when a bloom of *Microcystis* in a drinking reservoir contaminated the water supply of a dialysis treatment clinic with microcystins resulting in 56 fatalities (Azevedo et al., 2002). A promising potential therapy utilizing cholestyramine to competitively bind microcystins and facilitate their excretion was successfully used to treat a dog suffering cyanotoxicosis and is a promising avenue for future research (Rankin et al., 2013).

Microcystin concentrations have been determined in cultures and environmental samples using a variety of methods including

protein phosphatase inhibition assays, enzyme-linked immunosorbent assay (ELISA), chemical derivatization with gas chromatography–mass spectrometry analysis, and high performance liquid chromatography (HPLC) coupled to either ultra-violet, photodiode array detector or mass spectrometry detection (Spoof, 2005; Sangolkar et al., 2006). Choice of the most appropriate analytical method requires consideration regarding sensitivity, specificity, and associated consumables and equipment costs. For instance, ELISA broadly detects all microcystin congeners, but provides no information on which specific congeners are present, however the equipment required to perform the assay is minimal. Variations in detection methodology in concert with different starting materials (i.e., dried, filtered, wet samples) and methods of microcystin extraction make comparisons of toxin concentrations among waterbodies and between studies challenging. Nevertheless, it is clear that microcystin concentrations can reach extremely high levels during *Microcystis* blooms worldwide. For example, levels reached 7300 $\mu g\ g^{-1}$ dry weight (dw) in China (Zhang et al., 1991), 7100 $\mu g\ g^{-1}$ dw in Portugal (Vasconcelos et al., 1996), 4100 $\mu g\ g^{-1}$ dw in Australia (Jones et al., 1995), 19.5 $mg\ l^{-1}$ in Japan (Nagata et al., 1997) and 36 $mg\ l^{-1}$ in New Zealand (Wood et al., 2006).

Factors that regulate production of microcystin and the potential ecophysiological role of the toxin for *Microcystis* have been topics of intense scientific research in recent decades. Early studies focused on factors commonly associated with the formation and senescence of blooms such as temperature, nutrients, and light. Such studies primarily used laboratory cultures and only observed relatively minor (3- to 4-fold) shifts in microcystin production. Another popular hypothesis as discussed below (*Grazing* section) is that microcystins act as feeding deterrents for predators such as zooplankton and fish (Jang et al., 2003, 2004). However, phylogenetic analysis suggests that the genes responsible for microcystin synthesis pre-date the eukaryotic lineage (Rantala et al., 2004). More recently, factors such as chelation of metals (Humble et al., 1997; Sevilla et al., 2008), intraspecies communication (Schatz et al., 2007), colony formation (Gan et al., 2012), and protein-modulation (Zilliges et al., 2011) have been implicated as potential functions for microcystin. A brief review of studies in these areas is given below.

Culture-based studies have shown that microcystin concentrations are generally highest between 20 and 25 °C (van der Westhuizen and Eloff, 1985; Watanabe and Oishi, 1985; van der Westhuizen et al., 1986; Codd and Poon, 1998; Amé and Wunderlin, 2005). Dziallas and Grossart (2011) provided further evidence regarding the influence of temperature on microcystin production by incorporating gene expression assays. These authors found that the fraction of microcystin-producing *Microcystis aeruginosa* were significantly lower at 32 °C than at 20 and 26 °C, although microcystin concentrations increased at these higher temperature (26 and 32 °C). Temperature has also been shown to alter ratios of microcystin congeners. Using batch cultures and a natural population of *M. aeruginosa* kept at 20 °C, microcystin-LR was predominately produced, whereas at 28 °C the ratio of microcystin-LR and microcystin-RR remained constant (Amé and Wunderlin, 2005).

The availability of nutrients is a major factor controlling the proliferation of *Microcystis* (see Ecology – Nutrients section). However, their role in regulating microcystin production or whether microcystin may play a role in improving access to nutrients is less well defined. In batch cultures of axenic *Microcystis*, microcystin production decreased in proportion to cell division when the culture became nitrogen (N) limited, suggesting that microcystin production is controlled by environmental effects related to the rate of cell division (Orr and Jones, 1998). Similarly, using continuous cultures under either N (Long et al., 2001) or phosphorus (P) limitation (Oh et al., 2000), a linear relationship was also observed between microcystin production and growth rate. Downing et al. (2005) suggested that considering a single nutrient in isolation was an oversimplified approach and found microcystin quotas to be positively correlated with nitrate uptake and cellular N content, and negatively correlated with carbon fixation rate, phosphate uptake, and cellular P. They concluded that microcystin quotas were controlled by variables other than growth rate, with N having the most significant effect. In support of this, Harke and Gobler (2013) observed that under conditions of low inorganic N, many of the peptide synthesis genes in the microcystin synthetase cassette (*mcyABCDEF*) were down-regulated and microcystin content per cell decreased when cells were N limited. Furthermore, increases in exogenous N concentrations have been associated with increases in microcystin (Van de Waal et al., 2009, 2014; Horst et al., 2014; Scott et al., 2014). Increases in microcystin concentrations or expression of individual *mcy* genes during N limitation have also been observed (Ginn et al., 2010; Pimentel and Giani, 2014) suggesting a more complicated relationship or perhaps strain to strain variability.

Transcription of *mcy* genes in *Microcystis* is thought to be regulated via a bidirectional promoter that is located between the *mcyA* and *mcyD* genes. The promoter contains sequence motifs for both the DNA binding proteins Fur (ferric uptake regulator) and *ntcA* (global nitrogen regulator): observations that support the hypotheses that nitrogen and possibly iron may influence microcystin synthesis (Martin-Luna et al., 2006; Ginn et al., 2010; Neilan et al., 2013). Exploring the nitrogen link further, Kuniyoshi et al. (2011) observed that increased 2-oxoglutarate levels (a signal of the C to N balance in cells) increased the binding affinity of *ntcA* to these promoter regions.

Light has also been investigated as a factor controlling microcystin synthesis. For instance, Kaebernick et al. (2000) demonstrated that light intensity affects microcystin synthase expression, whereby increases in transcription occurred between dark and low light (16 $\mu mol\ of\ photons\ m^{-2}\ s^{-1}$) and between medium and high light (31 and 68 $\mu mol\ of\ photons\ m^{-2}\ s^{-1}$; respectively). Phelan and Downing (2011) found a strong correlation between microcystin concentration and growth rate under high light (37 $\mu mol\ photons\ m^{-2}\ s^{-1}$) conditions for *Microcystis aeruginosa* PCC 7806 and suggested a possible role for microcystin in protection against photo-oxidation. There is also some evidence to suggest microcystins may allow *Microcystis* to acclimate to high light and oxidative stress (Zilliges et al., 2011). Alexova et al. (2011b) showed that microcystins bind to proteins under high light and during periods of oxidative stress. Interestingly, oxidative stress is often brought about by supersaturated oxygen conditions produced by vigorously photosynthesizing surface blooms themselves. Paerl and Otten (2013) suggested that under these conditions, microcystins act as protectants against cellular damage during active surface bloom formation. Indeed, microcystin production is often highest during early nutrient-replete phases of the bloom (Davis et al., 2009), when photosynthetic oxygen production is maximal (Otten et al., 2012). Using a DNA microarray based on the genome of *M. aeruginosa* PCC 7806, Straub et al. (2011) demonstrated that the biosynthesis of microcystins occurred primarily during the light period, although this has been disputed by Penn et al. (2014) who found microcystins were produced throughout the day/night cycle in natural populations of *Microcystis*. One possible explanation for the often observed disconnect between microcystin concentration and *mcy* transcript abundance is that toxin may be bound to proteins and therefore not detectable by standard methods (Meissner et al., 2013).

There is also mounting evidence that microcystins may be involved in cell-to-cell signaling. For instance, Dittmann et al. (2001) identified a microcystin related protein (*mrpA*) that shares

similarities with proteins thought to be involved in quorum-sensing in *Rhizobium*. This protein was only present in a wild-type culture but not an inactivated mutant and was responsive to light, with a rapid decline of transcription under high light conditions. Kehr et al. (2006) provided further evidence for this process by demonstrating interactions between microcystin and the lectin microvirin (MVN) isolated from *Microcystis aeruginosa* PCC 7806. MVN is believed to be involved in the aggregation of single *Microcystis* cells into colonies (Kehr et al., 2006). Schatz et al. (2007) found that the release of microcystin from lysed cells into the extracellular environment induced a significant upregulation of *mcyB* and an accrual of microcystins in remaining *Microcystis* cells. Additionally, Gan et al. (2012) found that high concentrations of extracellular microcystins significantly enhanced *Microcystis* colony size, and that microcystins induced the production of extra-cellular polysaccharides. This contributed to cell colony formation and upregulated genes related to its synthesis. Using field-based studies (Wood et al., 2011) showed that *Microcystis* sp. can 'switch' microcystin production on and off. Field and experimentally induced 20-fold changes in microcystin quotas within a five-hour period were observed in concert with up to a 400-fold change in *mcyE* expression (Wood et al., 2011, 2012). In both studies the changes in microcystin quotas were associated with increased *Microcystis* densities (i.e., scum formation, in this case defined as a thin (ca. 3 mm) layer of cells of the lake surface) and were not caused by a shift in the relative abundance of toxic/non-toxic genotypes. Wood et al. (2012) suggest that this upregulation could either indicate a cell-to-cell signaling role for microcystins (although no increase in extracellular toxins was observed in their mesocosm study), or be a response to stress caused by rapid changes in other bloom-related variables (e.g., pH, light, oxidative stressors) that are mutually correlated with scum formation. Hypotheses regarding the role of microcystin in quorum sensing require that microcystin be exported from the cell. Using ^{14}C tracers to track the location and fate (either exported from the cell or metabolized) of microcystin under various light conditions, Rohrlack and Hyenstrand (2007) found no evidence of export or intracellular breakdown under these conditions, suggesting these theories need further investigation. Moreover, protein location prediction (Yu et al., 2010) of the single transporter gene (*mcyH*) in the microcystin synthetase gene cassette (Pearson et al., 2004) suggests microcystin is transported to the periplasmic space, not extracellularly.

Collectively these studies highlight the complexity in understanding the regulation and ecological role of microcystins in *Microcystis*. It seems plausible that there may be multiple triggers and the toxin could serve several functions for *Microcystis*, or that microcystin is a regulatory molecule linked to multiple cell processes (Wilhelm and Boyer, 2011). Culture-based studies using non-colony forming populations have provided the foundation for much of the current knowledge, however, often only one parameter is changed while others are maintained at optimal levels. There is a pressing need for results of laboratory-based studies to be validated in the field. Increased understanding of the regulation of microcystins in the environment may ultimately help in identifying the times of greatest toxicity and health risk.

4. Genomics and phylogeny

4.1. Genomics of Microcystis spp.

The first Microcystis genome was sequenced from the toxic isolate *Microcystis aeruginosa* NIES-843 (Kaneko et al., 2007), followed shortly by that of *M. aeruginosa* PCC 7806 (Frangeul et al., 2008). To our knowledge, as late as 2015, only one *M. aeruginosa* genomes had been closed, however, the number of draft genomes has subsequently increased, as strains isolated from diverse locations have been sequenced. To date, 15 draft or closed genomes are available, sequenced from strains isolated in Japan (Kaneko et al., 2007; Okano et al., 2015), the Netherlands (Frangeul et al., 2008), China (Yang et al., 2013, 2015), and Brazil (Fiore et al., 2013) accompany a collection of draft sequences from Humbert et al. (2013) for isolates from Canada, the Central African Republic, France, the United States, South Africa, Australia, and Thailand. Genomes range in size from 4.26 Mbp (*M. aeruginosa* PCC 9806) to 5.84 Mbp (*M. aeruginosa* NIES 843). Previous studies have highlighted genetic diversity between species of *Microcystis*, for example between the potentially toxic *M. aeruginosa* and nontoxic *Microcystis wesenbergii* (Harke et al., 2012), and sequencing of such species may reveal important insight into the divergent ecological strategies that may exist between strains, potentially driven by each strain's unique flexible genes.

The use of targeted genomics (e.g., PCR/QPCR, amplicon and shotgun sequencing) for detection, quantification, and phylogenetic analysis of *Microcystis* in the environment has rapidly expanded in recent years. The most frequent targets of these techniques include the microcystin synthetase gene operon, cyanobacterial and *Microcystis*-specific 16S rRNA or c-phycocyanin photopigment genes (*cpcBA*) (Ouellette and Wilhelm, 2003; Otten et al., 2015), and genes involved in nutrient transport and metabolism (Harke et al., 2012). Much of this work has centered on the characterization of toxic verses nontoxic populations that occur simultaneously or consecutively throughout the bloom season (Rinta-Kanto et al., 2005; Ha et al., 2009; Baxa et al., 2010; Wood et al., 2011). Similarly, these tools have bolstered the ability to identify organism(s) responsible for toxin production, even in mixed phytoplankton communities (Dittmann and Börner, 2005; Rinta-Kanto and Wilhelm, 2006; Gobler et al., 2007; Steffen et al., 2014b). Recent efforts have resulted in a better understanding of the factors that drive *Microcystis* growth and/or toxicity in the environment, including the role of macronutrients such as phosphorus and nitrogen (Davis et al., 2009; Rinta-Kanto et al., 2009; Sevilla et al., 2010; Harke et al., 2012), micronutrients such as iron (Sevilla et al., 2008; Alexova et al., 2011a), and rising global temperatures (Davis et al., 2009) and carbon dioxide (CO_2) concentrations (Van de Waal et al., 2011).

The combined impact of the availability of *Microcystis* genome information and the application of high-throughput sequencing and targeted genetic analyses has marked a transition to global genomic studies of *Microcystis* ecology and physiology, in both laboratory and field studies. To date, the number of studies employing the genomes of strains NIES-843 and PCC 7806 as type strains far exceeds the usage of the other 13, more recently sequenced genomes. *Microcystis aeruginosa* NIES-843 has been used for transcriptomic (Harke and Gobler, 2013; Penn et al., 2014; Steffen et al., 2014a, 2015), proteomic (Alexova et al., 2011b), and metabolomic studies (Steffen et al., 2014a), as has *M. aeruginosa* PCC 7806 (Straub et al., 2011; Penn et al., 2014; Makower et al., 2015; Meissner et al., 2015; Sandrini et al., 2015). These studies have provided insight into the nutritional ecology, responses to changing CO_2, and toxin production, among others factors.

As with many other currently sequenced genomes, a large number of the 12,000+ predicted genes across *Microcystis* strains remain uncharacterized. Probing these putative coding sequences with bioinformatic tools for protein prediction and functional/pathway analysis in targeted studies is needed to provide new insight into the genetic response of *Microcystis* to environmental parameters. For instance, Harke and Gobler (2013) identified a number of genes designated as hypothetical which were highly responsive to growth on high molecular weight organic matter, suggesting *Microcystis* may have unique capabilities to use organic compounds for nutrition. These findings emphasize the need for future genetic function studies such as insertional mutagenesis

(Alberts et al., 2002), as employed by Pearson et al. (2004) studying the function of the microcystin transporter *mcyH*, to characterize the role of these hypothetical genes.

The development and increasing availability of high-throughput sequencing technologies has made it possible to generate read libraries containing millions of sequences, well-beyond the scale of traditional clone libraries. The application of this technology to *Microcystis* blooms has thus far been limited to understanding the relationships between bacteria associated with bloom-forming organisms and environmental conditions (Tang et al., 2010; Wilhelm et al., 2011; Dziallas and Grossart, 2012; Parveen et al., 2013). Extension of these pursuits to functional gene libraries will provide new insights into how bloom communities transport and metabolize nutrients and interact with fluctuating environmental conditions, possibly even revealing the ecological mechanisms promoting bloom formation.

The use of both targeted and global approaches are useful tools for gaining insight as to why *Microcystis* dominates when and where it does and which factors may be most important in controlling toxin production. Advances in sequencing technology have allowed for higher resolution investigations into the unique genetic capability of this organism. Challenges remain due to the highly plastic and mosaic nature of the *Microcystis* genome and the large portion of predicted genes that remain uncharacterized. Further, methods employed have yet to be standardized leading to difficulty when comparing results. Future effort in this regard is needed, a strong focus should be given to understanding the purpose of microcystin production in *Microcystis*, a central debate due to its toxicity to humans and animals (see Section 3).

4.2. Phylogeny

The genus *Microcystis* is characterized morphologically by highly buoyant, unicellular, coccoid-shaped cells with a diameter ranging between 1 and 9 μm (Komárek and Komárková, 2002). Its defining feature, and primary basis for species delineation, is that it exhibits a variety of colonial morphologies consisting of dense aggregations of cells under natural environmental conditions (Fig. 2). There are over a dozen recognized *Microcystis* 'morpho-species'. The most commonly observed variants appear to be *M. aeruginosa*, *M. botrys*, *M. firma*, *M. flos-aquae*, *M. ichthyoblabe*, *M. natans*, *M. novacekii*, *M. panniformis*, *M. smithii*, *M. viridis*, and *M. wesenbergii* (Fig. 2; Komárek and Komárková, 2002). There is concern, however, that species designations have been over-prescribed and that single strains can exhibit multiple morphological characteristics in response to environmental or physiological stimuli (Yang et al., 2006). Based on the established standard that DNA–DNA hybridization (DDH) greater than 70% between two bacteria delineates them as likely belonging to the same species (Wayne et al., 1987), Otsuka et al. (2001) proposed the unification of five species of *Microcystis* (*aeruginosa*, *ichthyoblabe*, *novacekii*, *viridis*, and *wesenbergii*) under the formal name '*Microcystis aeruginosa* (Kützing) Lemmermann 1907', with isolate NIES-843 serving as the type strain for this species complex. In their study, DDH was greater than 70% for all species tested, with the two isolates classified as *M. aeruginosa* species actually displaying the lowest similarity (Table 1). Similarly, Kondo et al. (2000) used DDH to study nine different strains of *Microcystis* identified as *M. aeruginosa, M. viridis* and *M. wesenbergii* and all strains exhibited greater than 70% DNA relatedness, providing further evidence for the unification of these species.

With the advent of high-throughput DNA sequencing, it is now tenable to compare microbial genomes in silico. The average nucleotide identity (ANI) of conserved genes from two strains of bacteria has been demonstrated to be as robust as DDH for delineating species when using a cut-off for delineation of 95–96% identity or greater (Goris et al., 2007). This metric is also slowly replacing the use of 16S rRNA comparisons to infer phylogeny

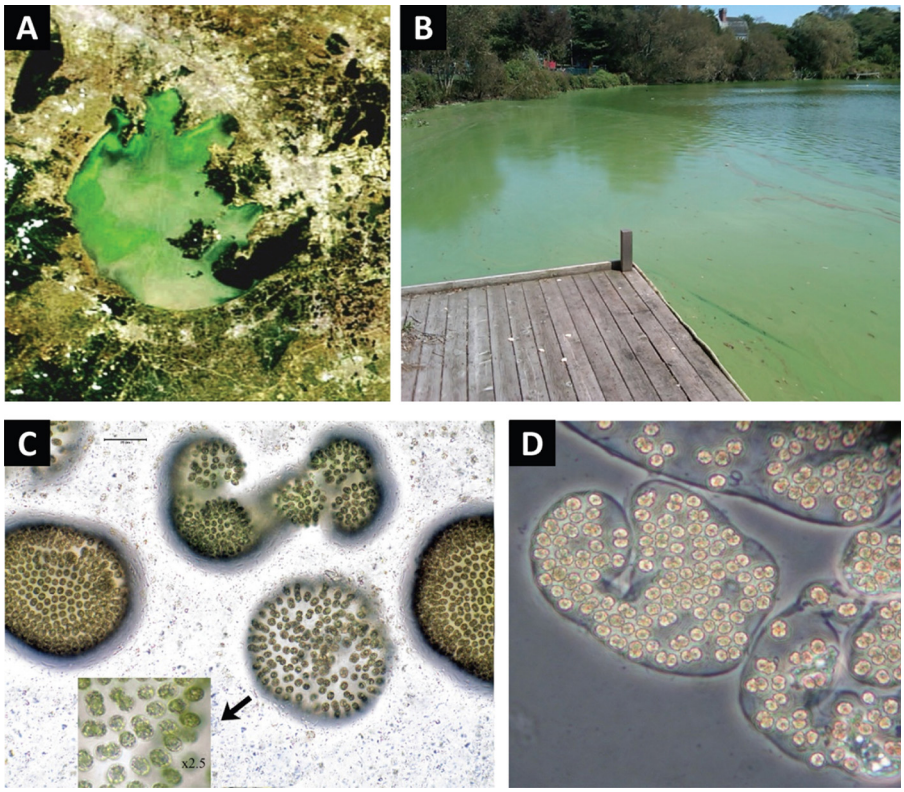

Fig. 2. (A) Satellite image of *Microcystis* bloom in Taihu, China. (B) Bloom of *Microcystis* in Lake Agawam, New York, USA, (C and D) light microscope images of diverse colony morphologies.

Table 1
Phylogenetic comparisons from a variety of *Microcystis* species exhibit too low genetic diversity to warrant their placement as separate species based on whole genome DNA–DNA hybridization (DDH) values greater than 70%, two-way average nucleotide identity (ANI) values greater than 95% or 16S rRNA sequence homology greater than 98.7%.

Genus species	Strain	GenBank Assembly or Accession #	16S Identity (%) NIES-843	Genome ANI (%) NIES-843	ANI Fragments	[b]DNA–DNA (%) NIES-843
Microcystis aeruginosa	NIES-843[a]	NC_010296.1	100.00	100.00	29,201	100.0
Microcystis aeruginosa	NIES-2549	CP011304.1	99.79	95.95	10,491	NA
Microcystis aeruginosa	NIES-44	GCA_000787675.1	99.60	96.35	10,695	NA
Microcystis aeruginosa	DIANCHI-905[a]	NZ_AOCI00000000.1	99.59	95.65	10,387	NA
Microcystis aeruginosa	PCC 7005	GCA_000599945.1	99.66	95.90	9915	NA
Microcystis aeruginosa	PCC 7806[a]	AM778844.1–AM778958.1	99.72	95.64	10,408	NA
Microcystis aeruginosa	PCC 7941[a]	GCA_000312205.1	99.58	95.95	10,912	NA
Microcystis aeruginosa	PCC 9432	GCA_000307995.2	99.73	95.96	10,685	NA
Microcystis aeruginosa	PCC 9443[a]	GCA_000312185.1	99.66	96.16	10,927	NA
Microcystis aeruginosa	PCC 9701	GCA_000312285.1	99.73	96.34	10,539	NA
Microcystis aeruginosa	PCC 9717[a]	GCA_000312165.1	99.80	97.28	12,947	NA
Microcystis aeruginosa	PCC 9806	GCA_000312725.1	99.66	96.18	10,872	NA
Microcystis aeruginosa	PCC 9807[a]	GCA_000312225.1	99.93	95.80	11,503	NA
Microcystis aeruginosa	PCC 9808[a]	GCA_000312245.1	99.73	95.97	10,814	NA
Microcystis aeruginosa	PCC 9809[a]	NZ_CAIO00000000.1	99.73	98.57	15,062	NA
Microcystis aeruginosa	SPC-777[a]	NZ_ASZQ00000000.1	99.66	96.11	10,914	NA
Microcystis aeruginosa	Taihu-98	ANKQ01000001.1	99.46	96.02	11,102	NA
Microcystis sp.	T1-4	NZ_CAIP00000000.1	99.72	95.95	10,594	NA
Microcystis aeruginosa	TAC86[a]	AB012333.1	99.59	NA	NA	75.0
Microcystis flos-aquae	UWOCC C2	AF139328.1	99.65	NA	NA	NA
Microcystis ichthyoblabe	TC24	AB035550.1	99.66	NA	NA	80.7
Microcystis novacekii	BC18	AB012336.1	99.93	NA	NA	74.0
Microcystis panniformis	VN425	AB666076.1	99.58	NA	NA	NA
Microcystis protocystis	VN111	AB666054.1	99.86	NA	NA	NA
Microcystis viridis	CC9	AB035552.1	99.73	NA	NA	91.7
Microcystis wesenbergii	TC7	AB035553.1	99.59	NA	NA	89.7
Microcystis wesenbergii	NIES-107[a]	DQ648028.1	99.72	NA	NA	NA
Aphanocapsa montana	BDHKU210001	NZ_JTJD00000000.1	88.06	80.36	17	NA
Cyanobium gracile	PCC-6307	NC_019675.1	87.62	81.97	19	NA
Gloeocapsa sp.	PCC-7428	GCA_000317555.1	90.18	76.79	52	NA
Gloeocapsa sp.	PCC-73106	GCA_000332035.1	89.74	74.90	95	NA
Synechococcus elongatus	PCC-6301	AP008231.1	89.53	81.33	25	NA

[a] Denotes microcystin producer; NA – not available.
[b] DNA–DNA hybridization data from Otsuka et al. (2001).

because it is based on a larger sample of genetic information. Recent studies now suggest that when using 16S rRNA gene sequences to infer phylogeny, the cut-off to distinguish one species from another should be raised from 97% to 98.7% or greater (Stackebrandt and Ebers, 2006; Kim et al., 2014). For this review, ANI alignments were performed on all *Microcystis* genomes sequenced to date using the following parameters: 700 bp minimum length, 70% minimum identity, 50% alignment minimum and fragment options were set to a window size of 1000 bp and step size of 200 bp. Comparisons of the 16S rRNA locus (1489 bp) were made with *Microcystis aeruginosa* NIES-843 as the type strain. Table 1 displays the ANI and 16S rRNA gene sequence similarity of all *Microcystis* genomes sequenced to date and their 16S rRNA gene identity relative to the first fully sequenced *M. aeruginosa* genome and type strain NIES-843, along with additional *Microcystis* strains which have been investigated although not fully sequenced. For comparison, the genomes of other unicellular, but non-*Microcystis* genera are provided. Based on these outlined assumptions, all *Microcystis* species whose genomes or 16S rRNAs have been sequenced to date warrant placement into the same species complex since all ANI values exceeded 95%, 16S identity scores always exceeded 99%, and DNA–DNA hybridization were consistently greater than 70% (Table 1).

An analogy can be drawn from the bacterial systematics used to characterize *Escherichia coli*. Whole genome sequencing of a number of *E. coli* isolates suggests that the core genome for this species is approximately 47% shared across all strains, and that specific pathovars, such as those inducing uropathogenic or enterohaemorrhagic symptoms, are due to laterally acquired genes/plasmids (Welch et al., 2002; Rasko et al., 2008). In this vain, it is likely that if all *E. coli* strains did not share similar morphological characteristics, then there would be far more species groups assigned to this genus. Similar to *E. coli*, a recent genomic comparison of 12 different strains of *Microcystis aeruginosa* indicated that only about half the genome of a given strain consists of a shared core set of genes (~2462 core genes, 5085 ± 749 total genes; Humbert et al., 2013). The remainder of each *M. aeruginosa* genome was comprised of genes shared among some but not all of the strains, including a variety of non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) such as those involved in microcystin biosynthesis (see Section 3) among other genes, and collectively the flexible pangenome appears to be very diverse, consisting of over 12,000 genes identified in only these 12 strains (Humbert et al., 2013). In this context, any two strains of *Microcystis* may exhibit vastly different morphological, physiological or ecological characteristics, likely due to accrued mutations or rearrangements in core genes or variation in the flexible genes they possess owing to widespread horizontal gene transfer. Yet fundamentally at their core, they share the same genes that ultimately identify *Microcystis* as distinct from other bacteria. Hence, they should be placed within the same species complex. The extent to which gene rearrangements and DNA methylation patterns may be influencing *Microcystis* strain ecology and function is unclear. Genome architecture (synteny) between strains may be considerably different, even if gene content is shared, owing to the diverse array (10% or more of the total genome) of transposable elements and insertion and repeat sequences (Kaneko et al., 2007; Frangeul et al., 2008). As previously mentioned (above), until recently, only one strain of *Microcystis* had been fully sequenced and its genome closed (NIES-843). Using Pac Bio RS II long read sequencing a second strain of *M. aeruginosa* (NIES-2549) has been recently sequenced and its genome closed (Yamaguchi et al., 2015). The completion of this second genome

enables, for the first time, a true assessment of genome synteny (i.e., gene order/arrangement) in *Microcystis*. Using the bioinformatics program Gepard (GEnome PAir – Rapid Dotter; Krumsiek et al., 2007) the genome synteny was assessed for these two strains, and for comparison, the genome synteny analysis originally provided by Novichkov et al. (2009) was recreated in order to illustrate the five different patterns of genome rearrangement presently recognized to occur in prokaryotes (Fig. 3). This assessment clearly demonstrates that *Microcystis* retains almost zero genome synteny, a finding in stark contrast to the synteny values of 68–86% reported elsewhere (Humbert et al., 2013) that relied only upon relatively short contiguous fragments of draft genomes. Considering that these two *Microcystis* isolates were both classified as *M. aeruginosa*, exhibited 99.66% 16S rRNA identity, shared 3342/4282 coding sequences (CDS), exhibited an ANI of 95.95%, and were both isolated from the same water body (Lake Kasumigaura, Japan), it is anticipated that all closed *Microcystis* genomes will exhibit a similar decay pattern in genome synteny. Furthermore, evidence for active genome rearrangement was recently observed for transposase genes that exhibit differential transcription patterns in response to nutrient availability in culture (Steffen et al., 2014a) and in environmental samples (Harke et al., 2015; Steffen et al., 2015). Indeed, these observations imply that regional heterogeneities in drivers of transposable element activity may lead to localized evolution of genetically similar populations due to rearrangements (or gains/losses) in genomic architecture: in some ways a microbial manifestation of the island theory of biogeography

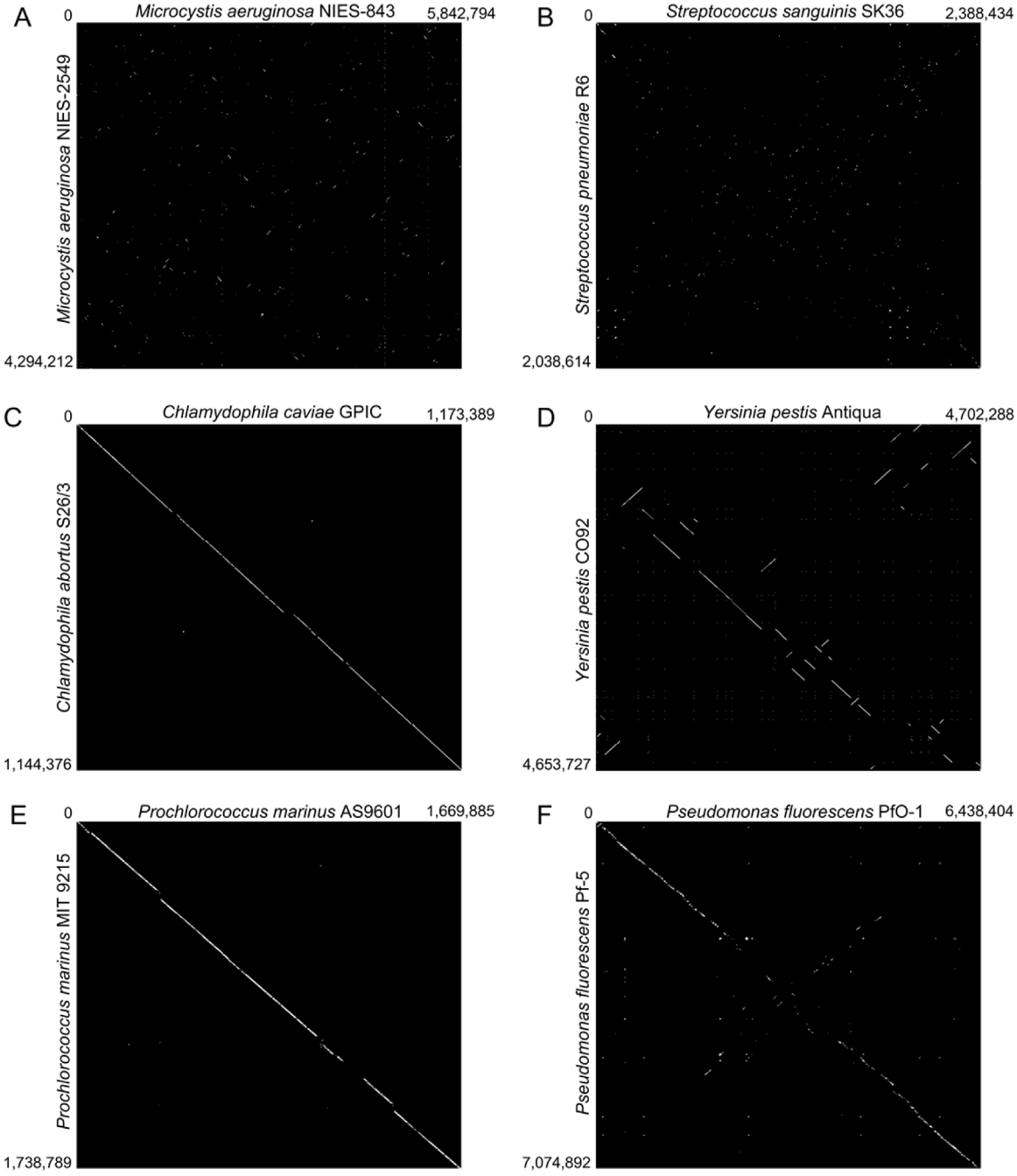

Fig. 3. Dot plot matrix comparing whole genome synteny for *Microcystis aeruginosa* (A) relative to five other genera of bacteria known to exhibit different genome rearrangement patterns as described by Novichkov et al. (2009). (B) Complete decay of genome synteny. (C) Absence of rearrangement. (D) Multiple inversions with limited transposition of genes. (E) Lack of inversions but hotspots for recombination, (F) Multiple inversions and transposition of genes/operons.

(MacArthur and Wilson, 1967). As in the previous example, the rise of long read sequencing (e.g., Pac Bio RS) that is becoming commonplace in bacterial genomics should help to further resolve these questions.

Whether or not all *Microcystis* morpho-species should be placed within a single *Microcystis aeruginosa* complex could be construed as a purely esoteric question, but doing so could provide additional applied benefits for the scientific, research, and managerial communities. For example, it would simplify the task of microscopic identification and enumeration for public health purposes and remove much of the subjectivity inherent to each taxonomist. More importantly, such a unification of *Microcystis* morpho-species would also counter the widespread belief that certain cyanobacterial species are universally toxic or nontoxic. For example, *Microcystis wesenbergii* and *Aphanizomenon flos-aquae* are frequently cited by water quality managers as being nontoxic species despite documented reports to the contrary (Yoshida et al., 2008).

5. Ecology

5.1. Nutrients

Traditionally, P input reductions have been the focus for controlling cyanobacterial blooms based on the premise that N supplies can be met by N_2 fixation (Schindler et al., 2008). An important distinction between the genus *Microcystis* and several other major bloom forming cyanobacterial genera (e.g., *Dolichospermum*/*Anabaeana*, *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia*) is that the former is incapable of supplying its N requirements via N_2 fixation, while the latter are capable of doing so (Carr and Whitton, 1982; Potts and Whitton, 2000). This distinction has important ecophysiological and nutrient management ramifications, because growth and proliferation of *Microcystis* are exclusively reliant on either external N sources generated by various human activities (whose natural occurrence can be markedly augmented), including agriculture, urbanization, and industrial pollution or internal regeneration of combined N forms (largely ammonium). While P input controls are still very much at the center of bloom management strategies, an increasing number of freshwater ecosystems are now experiencing expanding blooms of non-N_2 fixers like *Microcystis* and/or *Planktothrix*, despite having such controls in place. This suggests that anthropogenic N inputs play a role in the global proliferation of these organisms (Paerl et al., 2014a). Indeed, in numerous eutrophic systems experiencing both spatial and temporal expansions of *Microcystis* blooms (e.g., Lakes Taihu-China, Erie-USA/Canada, Okeechobee-Florida/USA, Ponchartrain – Louisiana), it has been shown that N enrichment plays a key role in bloom proliferation (Paerl and Huisman, 2009; Chaffin and Bridgeman, 2014; Paerl et al., 2014a, 2015). Overall, the world-wide proliferation of *Microcystis* appears closely linked to increases in both P and N loading from expanding human activities (Paerl, 2014). This conclusion confirms the changing nutrient limitation paradigm, where N and P co-limitation (and hence the need for N and P nutrient inputs controls) is much more common than previously thought, especially in eutrophic waters (Dodds et al., 1989; Elser et al., 2007; Lewis and Wurtsbaugh, 2008; Conley et al., 2009; Lewis et al., 2011; Paerl et al., 2014b).

Several studies have indicated that, among biologically available forms of N, reduced N (as ammonium) is generally preferred over oxidized N (nitrate/nitrite) by *Microcystis* as well as other bloom forming taxa (Blomqvist et al., 1994; Hyenstrand et al., 1998; Flores and Herrero, 2005). Therefore, eutrophic freshwater ecosystems that contain relatively high concentrations of reduced N may have a tendency to favor cyanobacterial blooms. This, combined with the fact that most eutrophic systems are highly turbid and potentially light-limited, will favor cyanobacterial blooms that can regulate their buoyancy and vertically migrate in order to access nutrient rich bottom waters (i.e., by sinking) and optimize utilization of radiant energy (by floating as buoyant surface blooms). *Microcystis* is particularly adept at using such a strategy, especially during thermally stratified summer bloom periods, when bottom waters will be relatively enriched with reduced N, while near-surface irradiance is maximal and reduced N inventories may be depleted.

The ability to migrate vertically also optimizes access to biologically available P. *Microcystis* is extremely effective in sequestering sources of P, even at low concentrations (Jacobson and Halmann, 1982; Kromkamp et al., 1989; Sbiyyaa et al., 1998; Baldia et al., 2007; Saxton et al., 2012). This strategy is particularly effective in eutrophic, turbid, shallow water systems in which *Microcystis* can rapidly migrate between P-rich bottom sediments and take advantage of periodic sediment resuspension due to wind-mixing. By rapidly adjusting its buoyancy depending on photosynthetic CO_2 fixation versus nutrient acquisition needs, *Microcystis* can maintain dominance. *Microcystis* is capable of intracellular storage of P (polyphosphate bodies), enabling it to survive during periods of P deprivation (Carr and Whitton, 1982) and it is also capable of collecting P on it exterior surface (Saxton et al., 2012). Moreover, *Microcystis* has been shown to upregulate genes to synthesize high-affinity phosphate transporters and alkaline phosphatases that allow it to persist under low P conditions (Harke et al., 2012; Harke and Gobler, 2013). In summary, *Microcystis* is exceptionally good at accessing both N and P via a variety of cellular mechanisms, including buoyancy regulation, cellular storage, high affinity transporters, and coloniality, which both enhances buoyancy and plays a pivotal role in developing close associations with other microbes, including heterotrophic bacteria, and a range of protozoans (Paerl, 1982).

Despite the fact that *Microcystis* is capable of extracting N and P over a wide range of ambient concentrations, members of this genus do exhibit periods of nutrient limitation, when ambient nutrient levels fall well below saturation. In highly eutrophic Taihu, China (Taihu means "large lake" in Mandarin), where *Microcystis* blooms can account for more than 80% of total phytoplankton community biomass, in situ microcosm and mesocosm bioassays indicate that the lake exhibits P limitation during early phases of the blooms, while N limitation characterizes summer bloom conditions (Xu et al., 2013). In most instances combined N and P additions provide the greatest biomass yields (Paerl et al., 2014b, 2015). This pattern appears to also be present in Lake Erie (Chaffin and Bridgeman, 2014) and Lake Okeechobee (Havens et al., 2001). These results strongly argue for dual nutrient (N and P) input reductions as a best overall bloom control strategy (Paerl et al., 2014a, 2015).

It has been proposed that reducing N inputs under elevated P conditions may lead to replacement of non-N_2 fixing cyanobacteria such as *Microcystis* with N_2 fixing cyanobacterial bloom species such as *Dolichospermum/Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia* (Schindler et al., 2008; Schindler, 2012). This possibility has recently been tested using in situ mesocosms in Lake Taihu (China) by enriching summer cyanobacterial bloom communities dominated by *Microcystis* with P (as PO_4^{3-}) without adding dissolved inorganic nitrogen (DIN; to enhance N limitation) while ensuring sufficient supplies of iron (Fe) and other trace metals. Incubations of up to a month under these conditions failed to induce succession of N_2 fixers over *Microcystis* and no significant increases in N_2 fixation were reported (Paerl et al., 2014b). In fact, net increases in *Microcystis* biomass were observed during the course of the experiment. This indicated that *Microcystis* was able to effectively compete with N_2-fixing taxa under conditions highly favorable for N_2 fixation (Paerl et al., 2014b). In summary, these findings argue for increased attention to dual nutrient input constraints to deplete the lake of previously loaded nutrients. Once

the overall phytoplankton biomass is reduced by these measures, it may be possible to shift to a more P-oriented control strategy, although in more eutrophied ecosystems this may take years to decades to accomplish (Paerl et al., 2014b).

5.2. Physical factors

Physical factors, including irradiance, temperature, turbulence, vertical mixing and hydrologic flushing have all been implicated in the potential control of *Microcystis*-dominated blooms (Paerl, 2014). Adequate irradiance is of fundamental importance for maintaining optimal rates of photosynthesis. *Microcystis* colonies exhibit physiological strategies aimed at optimizing photosynthetic production in the highly turbid systems that characterize eutrophic waters during bloom conditions. First and foremost is its ability to regulate buoyancy through the formation and collapse of intracellular gas vesicles (Walsby et al., 1997). When cells are depleted in photosynthate (i.e., following periods of darkness or poor irradiance conditions), cell turgor pressure decreases and gas vesicles can readily form, making cells buoyant. This enables colonies to rise to water surfaces, where photosynthetic rates can be optimized. *Microcystis* is also capable of producing carotenoid and other photoprotective pigments (Paerl et al., 1983), allowing for efficient access to light while minimizing photo-inhibition and photo-oxidation (Paerl et al., 1985). Once photosynthetic needs have been met, the buildup of cellular photosynthate (i.e., ballast) leads to increased cell turgor pressure, causing gas vesicles to collapse and decreasing buoyancy. Using these oscillating processes, cells can optimize photosynthetic production during the day, while accessing hypolimnetic nutrient pools at night (Walsby et al., 1997).

In many instances, buoyancy compensation by *Microcystis* can overcome light to moderate wind mixing, which enables it to remain in surface waters more readily than other bloom forming taxa that it may be competing with. For example, in Taihu, China, highly buoyant *Microcystis* colonies maintain strong dominance in surface waters during N-limited summer conditions, despite the fact that N_2-fixing genera (e.g., *Dolichospermum/Anabaena*, *Aphanizomenon*) are present during this period. This superior buoyancy and the ability to thrive on regenerated N may contribute to this dominance over radiant energy demanding diazotrophs during N-limitation (Paerl, 2014).

It is well known that vertical stability through stratification and long water replacement times favor cyanobacteria over eukaryotic phytoplankton (Reynolds et al., 1981; Reynolds, 2008); hence, disruption of these conditions can, under certain circumstances, modulate bloom dynamics. Vertical mixing devices, bubblers and other means of destratification have proven effective in controlling *Microcystis* blooms in relatively small lakes and ponds (Visser et al., 1996). However, these devices have limited applicability in large lake, estuarine and coastal waters, because they cannot exert their forces over large areas and volumes (Paerl, 2014).

Increasing flushing rates, i.e., decreasing water residence times or water ages, can also be effective in reducing or controlling bloom taxa; mainly because cyanobacteria exhibit relatively slow growth rates, relative to eukaryotes (Butterwick et al., 2005; Paerl and Otten, 2013). Horizontal flushing, by increasing the water flow, can reduce the time for cyanobacterial bloom development (Mitrovic et al., 2006). While this approach can suppress blooms, inducing these hydrologic changes can be quite expensive and depend on the availability of freshwater supplies for flushing purposes. Furthermore, water quality managers must ensure that the flushing water is relatively low in nutrient content, so as not to worsen the enrichment problem, especially in long residence time large water bodies, which can retain nutrients and hence have a long "memory" for nutrient inputs. For example, in hypereutrophic Taihu, efforts to reduce *Microcystis*-dominated blooms by flushing this large lake with nearby Yangtze River water reduced the overall residence time in the lake from approximately one year to 200 days, but have not had a significant impact on reducing bloom intensity or duration (Qin et al., 2010). Yangtze River water is exceedingly high in biologically available N and P compounds, making it a nutrient source for further eutrophication. The inflow pattern of Yangtze River water has altered the circulation pattern of Taihu, trapping blooms in the lake's northern bays, where they were most intense to begin with (Qin et al., 2010). Lastly, few catchments have the luxury of being able to use precious water resources normally reserved for drinking or irrigation for flushing purposes. This is especially true of regions susceptible to extensive droughts (e.g. Australia, Western USA).

Climatic changes, including rising global temperatures, increasing CO_2 levels, altered precipitation patterns, and resultant changes in freshwater discharge or flushing rates have synergistically influenced *Microcystis* bloom dynamics (Paerl and Paul, 2012). Warmer temperatures favor cyanobacterial blooms over eukaryotic phytoplankton taxa because growth rates of the former are optimized at relatively high temperatures (Jöhnk et al., 2008; Paerl and Huisman, 2008, 2009). In addition, warmer global temperatures and changes to precipitation patterns have led to the earlier onset of and longer lasting conditions favoring cyanobacterial blooms (Paerl and Huisman, 2008; Paul, 2008; Paerl and Huisman, 2009; Michalak et al., 2013). Intensification of vertical stratification (Paerl and Huisman, 2009) in combination with eutrophication also appears to be particularly favorable for development and persistence of *Microcystis* blooms (Jöhnk et al., 2008). With regards to CO_2 levels, *Microcystis* is known to have both high- and low-affinity bicarbonate uptake systems as well as two CO_2 uptake systems (Sandrini et al., 2014). At high pCO_2, *Microcystis* uses its low-affinity bicarbonate uptake systems and increases biomass as well as increasing cellular chlorophyll *a* and phycocyanin content, raising PSI/PSII ratios, and decreasing overall dry weight and carbohydrate content which may improve buoyancy (Sandrini et al., 2015). Steffen et al. (2015) showed that *Microcystis* transcribed its carbon concentration mechanism genes (ccm) at sites across the Western Basin of Lake Erie and proposed that conditions of dense algal biomass, with resultant high-pH and CO_2 limitation, further promote cyanobacterial dominance. *Microcystis* appears to be well adapted to high or low CO_2 concentrations (Sandrini et al., 2015), characteristics that likely permit it to continue to dominate blooms, even as CO_2 concentrations are drawn-down to low levels. Transitions of CO_2 in lakes today due to algal bloom formation and demise (Balmer and Downing, 2011), however, far exceeds anthropogenic changes that will be produced in the future from atmospheric CO_2. Further, the response of other freshwater phytoplankton to changing CO_2 levels has been poorly studied. As such, there remain significant unknowns regarding how rising levels of atmospheric CO_2 will affect future *Microcystis* blooms.

5.3. Grazing

The ability of any algal group to form blooms is related to its ability to outgrow competitors and avoid routes of mortality. In aquatic ecosystems, mortality is generally attributed to top-down ecological controls such as grazing and viral lysis (Sunda et al., 2006; Smayda, 2008). *Microcystis* has been shown to experience lower rates of mortality than other algae via grazing by zooplankton and bivalves (Vanderploeg et al., 2001; Wilson et al., 2006). Among the zooplankton, larger grazers including daphnids and copepods, are generally less capable of grazing *Microcystis* than smaller protozoan species (Fulton and Paerl, 1987; Gobler et al., 2007). While early studies predicted that grazer

inhibition may be related to synthesis of microcystin (Arnold, 1971; Fulton and Paerl, 1987; Rohrlack et al., 1999; DeMott et al., 2001), multiple lines of evidence demonstrate this is not the case. Rantala et al. (2008) found that the evolution of microcystin synthesis significantly predated that of metazoans and thus suggested the toxin did not evolve as a grazing deterrent. Meta-analyses of laboratory studies have concluded that while *Microcystis* reduces zooplankton population growth rates, the effects are typically not related to microcystin content of cultures (Wilson et al., 2006; Tillmanns et al., 2008; Chislock et al., 2013). Within an ecosystem setting, Davis and Gobler (2011) quantified grazing rates by multiple classes of zooplankton on toxic and non-toxic strains of *Microcystis* in two ecosystems and found that both microzooplankton and mesozooplankton were capable of grazing both toxic and nontoxic strains with similar frequencies and rates. Incongruence in culture grazing experiments may be due to differential production of other, non-microcystin, secondary metabolites that have not been considered in previous studies.

Beyond microcystin, there is evidence that *Microcystis* colony formation and synthesis of other potential secondary metabolites can act as grazing deterrents. Studies have reported that larger colonies of *Microcystis* are poorly grazed, particularly by smaller crustacean zooplankton (de Bernardi and Giussani, 1990; Wilson et al., 2006), and Yang et al. (2006) reported on a strain of *Microcystis* that transformed from uni-cellular to colonial in direct response to small, flagellated zooplankton grazers that could not consume the colonies. Many studies have concluded that *Microcystis* may be a nutritionally inadequate food source for zooplankton (Wilson et al., 2006) and the ability of *Microcystis* to synthesize protease inhibitors such as aeruginosin and cyanopeptolin may both prohibit digestion of cells and discourage zooplankton grazing (Agrawal et al., 2001, 2005).

Outbreaks of *Microcystis* blooms in some lakes in the United States appear to be stimulated in part by the arrival of recently established zebra mussel (*Dreissena* sp.) populations (Vanderploeg et al., 2001; Raikow et al., 2004) and this correlation may be linked to the trophic status of lakes (Sarnelle et al., 2005). While zebra mussel invasions of new ecosystems typically result in significant reductions in all plankton biomass due to intense filter feeding (Caraco et al., 1997), *Microcystis* cells consumed by zebra mussels are typically rejected as pseudofeces from which cells can emerge and regrow (Vanderploeg et al., 2001). Given the ability of zebra mussels to consume both phytoplankton and zooplankton (Jack and Thorp, 2000; Higgins and Zanden, 2010; Kissman et al., 2010), *Dreissena* invasions also effectively eliminate competitors and predators of *Microcystis*. Further, zebra mussels may alter ambient nutrient regimes to favor *Microcystis*. Zebra mussels can increase concentrations of dissolved organic phosphorus (DOP; (Heath et al., 1995), and under low P loads zebra mussels may promote *Microcystis* blooms (Sarnelle et al., 2005; Bykova et al., 2006), perhaps via regeneration of organic P given *Microcystis* has the ability to grow efficiently on DOP using alkaline phosphatase (Harke et al., 2012).

Although ecological and evolutionary processes are traditionally assumed to occupy different timescales, a wave of recent studies has demonstrated overlap and reciprocal interplay of these processes (Thompson, 1998; Carroll et al., 2007; Hendry et al., 2007; Post and Palkovacs, 2009). For example, multiple studies have found that a diverse array of zooplankton that are regularly exposed to dense *Microcystis* blooms are generally more adept to grazing on and growing during blooms than naïve populations that do not encounter *Microcystis*. This suggests a genetic shift occurs in wild zooplankton populations toward populations able to graze *Microcystis* (Hairston et al., 1999; Sarnelle et al., 2005; Davis and Gobler, 2011; Chislock et al., 2013). While filter feeding bivalves may ultimately also be capable of such adaptation (Bricelj et al., 2005), this possibility has yet to be explored.

5.4. Microbial interactions

As a largely colonial bloom forming genus, *Microcystis* has numerous complex interactions with both bacteria and protists (protozoans, microalgae, fungi; Paerl, 1982; Paerl and Millie, 1996; Shen et al., 2011; Shao et al., 2014). These interactions can be both intimate, such as is the case of microbes attached to or existing within *Microcystis* colonies, or more diffuse for microbes co-occurring in time and space. Regarding bacteria associated with colonies, during the decline of a *Microcystis* bloom, Parveen et al. (2013) found colonies to be depleted in *Actinobacteria*, but enriched in *Gammaproteobacteria* and changes in temperature may shape associated bacterial communities (Dziallas and Grossart, 2012). While many of the functional roles of *Microcystis*-bacterial associations remain unknown, it is clear that such associations can be both mutually beneficial as well as antagonistic with regard to their effects on growth potentials, viability and mortality of *Microcystis* and associated microbes. It has been noted that photosynthetic performance and growth rates of epiphytized *Microcystis* cells and colonies are often higher than bacteria-free or axenic cultures (Paerl, 1982; Paerl and Millie, 1996), indicating a mutualistic, if not symbiotic properties of such associations. Paerl and Millie (1996) speculated that while heterotrophic bacteria associated with bloom-forming cyanobacteria (e.g., *Dolichospermum/Anabaena*, *Microcystis*) clearly benefitted from the organic matter produced by the cyanobacteria, the cyanobacteria benefitted from organic matter decomposition, CO_2 production, and nutrient (N, P, Fe and trace metals) regeneration provided by associated heterotrophs, which can include bacteria and protozoans. Amoeboid protozoans have also been found actively grazing *Microcystis* cells inside colonies (Paerl, 1982). While grazed *Microcystis* cells clearly result in a loss of cyanobacterial biomass, ungrazed cells in these colonies displayed higher rates of photosynthetic growth than cells in colonies that were not grazed by the protozoans (Paerl and Millie, 1996). This suggested that nutrient recycling associated with grazers may have benefitted those cells that escaped grazing, indicating a positive feedback of grazers on "host" colonies (Paerl and Millie, 1996; Paerl and Pinckney, 1996). The extent to which microcystins and other secondary metabolites produced by host *Microcystis* colonies play a role in establishing and mediating such mutually-beneficial associations remains unknown, but this is an important area for research into biotic factors mediating cyanobacterial blooms in aquatic ecosystems.

Over the last two decades, several groups have demonstrated the ability of heterotrophic bacteria to degrade microcystins (Bourne et al., 1996; Cousins et al., 1996; Park et al., 2001). Since the initial characterization of this process (Bourne et al., 1996, 2001), organisms capable of microcystin degradation have been identified in blooms worldwide, including lakes in North America (Mou et al., 2013), Asia (Park et al., 2001; Saito et al., 2003; Zhu et al., 2014), Oceania (Bourne et al., 2001; Somdee et al., 2013), South America (Valeria et al., 2006), and Europe (Berg et al., 2008). This relatively recent discovery may have important implications for biological management of toxic blooms in freshwater systems (Ho et al., 2006, 2007).

5.5. Interactions with viruses

The presence of viruses in environmental samples dates to the initial observations and independent discovery of bacteriophage by Twort (1915) and d'Herelle (1917). Since that time there have been recurring observations of the potential role of viruses as mortality factors for different populations including a variety of freshwater microbial populations (Wommack and Colwell, 2000; Wilhelm and Matteson, 2008).

Indeed, viruses that may constrain cyanobacterial blooms have long been a "holy grail" for microbial ecologists (Safferman and Morris, 1963, 1964). Indeed much of the early work in virus ecology was dedicated to the idea that bacteriophage might be used to mitigate or even control harmful cyanobacterial bloom populations in the environment (Safferman and Morris, 1964). Chief amongst these efforts, the study of two virus types (LPP-1 and SM-1) were of interest, especially as the later was reported to include the bloom-producer *Microcystis aeruginosa* NRC-1 amongst putative hosts (Safferman and Morris, 1967).

One of the major conclusions of early microbial-viral research was that there was rapid selection for resistant phenotypes of cyanobacteria in the environment. This rapid selection has been considered one of several models of the ongoing evolutionary race between viruses and their hosts: a concept described by the "Red Queen Theory" (Van Valen, 1973) where hosts continually evolve to become resistant to infection and viruses must continue to adapt to infect the population. Like the character of the Red Queen in *Through the looking-glass and what Alice found there* (Carroll, 1917) who states "*it takes all the running you can do, to keep in the same place*", viruses and hosts continue to be selected for in a manner that makes their applied use for bloom control, at best, difficult. However, recent efforts point to components of viruses (e.g., lysins) as future targets for the biological control of *Microcystis* blooms, although such efforts will require significant research before they can be realized.

Studies on the potential impact of viruses on *Microcystis* remained at an effective stand-still until a virus particle infecting multiple strains of *Microcystis* was described (Tucker and Pollard, 2005; Yoshida et al., 2006). As part of this effort, Yoshida et al. (2006) sequenced the genome of a virus (Ma-LMM-01), and subsequently (along with other research groups) designed PCR and qPCR primers to study the viruses in various natural systems (Takashima et al., 2007a, 2007b; Yoshida et al., 2007, 2008, 2010; Rozon and Short, 2013). Although distributed at high abundances (e.g., over 250,000 per ml) in the Bay of Quinte (Lake Ontario; Rozon and Short, 2013) and consistently detected in the presence of blooms, the virus does not appear to cause senescence of dense blooms. This is also apparent from recent metatrascriptomic studies. Steffen et al. (2015) demonstrated ongoing phage infections of *Microcystis* (based on the presence of virus-specific gene transcripts) in the face of relatively dense *Microcystis* populations, whereas Harke et al. (2015) observed upregulation of phage defense genes in *Microcystis* populations in Lake Erie, USA, in response to P-loading. In a recent survey of more than 1000 genomes, *Microcystis* was found to contain 80% more defense genes than *Cyanothece* PCC 8802 or *Roseiflexus* RS-1 (the next highest) with 29% of its genome assigned to defense islands (Makarova et al., 2011). Furthermore, the presence of a large diverse number of CRISPR (clustered regularly interspaced short palindromic repeats) spaces within the *Microcystis* NIES-843 genome suggests this cyanobacterium is frequently exposed to viruses (Kuno et al., 2012).

6. Conclusions

Toxic blooms of *Microcystis* continue to plague eutrophic waters worldwide, and despite decades of research, many questions remain. The occurrence of toxic blooms of *Microcystis* appears to be expanding, with 108 countries or territories around the world having documented toxic blooms, whereas previous documentation identified fewer than 30 countries (Zurawell et al., 2005). This may be due to increased monitoring efforts, but also illustrates a need for further efforts to curb eutrophication of freshwater resources. This review highlights the great diversity of microcystins produced by *Microcystis*. Despite several decades of research, the physiological basis for microcystin production in *Microcystis*, and the variables that regulate its biosynthesis, remains a contentious and debated question. Collectively, the studies reviewed herein suggest microcystins might be regulated by multiple variables. They also indicate that the toxin could have several ecological functions for *Microcystis*, or that microcystin may be a regulatory molecule linked to many cellular processes. To date, most studies have been undertaken in the laboratory providing essential knowledge, however, often only one parameter was changed while others were maintained at optimal levels. There is a pressing need for results of laboratory-based studies to be validated in the field and for more multi-parameter investigations. The advent of omics provides an exciting new avenue to explore the genetic basis of toxin synthesis in complex environmental samples. Increased understanding of the regulation of microcystins in the environment may ultimately help in identifying the times of greatest toxicity and health risk.

Evidence is presented suggesting that all *Microcystis* warrant placement into the same species complex as ANI values were above 95%, 16S rRNA identity scores exceeded 99%, and DNA–DNA hybridization was consistently greater than 70%. Genomic analyses of *Microcystis* has provided significant insight into the ecology, physiology and factors influencing toxin production and have revealed the highly dynamic nature of its genome due to the great number of transposons. Challenges still remain due to the highly plastic nature of the *Microcystis* genome and the large portion of predicted genes that remain uncharacterized. Further, targeted and global genomics approaches employed have yet to be standardized leading to difficulty when comparing results. Nutrient loading is regarded as the primary driver of bloom formation. The precise nutrient remediation strategy to limit bloom formation remains the subject of considerable debate. Increasingly, dual (N and P) reduction strategies are being prescribed for eutropic systems suffering from chronic blooms problems. This review provides evidence from across the globe of the important role that both N and P have in controlling the dynamics of *Microcystis* blooms, as well as the ability of elevated temperatures to promote these events. This review also highlights the ability of *Microcystis* to minimize mortality losses during blooms due to zooplankton, bivalve grazing, and viral lysis and discusses some of the factors facilitating these trends. Studies on the potential impact of viruses on *Microcystis*, however, remain at an effective stand-still and future efforts at bloom control with viruses or virus components will require significant research before they can be realized.

Acknowledgements

S.A.W. thanks the New Zealand Ministry of Business, Innovation and Employment (UOWX0505; Lake Biodiversity Restoration), and the Marsden Fund of the Royal Society of New Zealand (12-UOW-087). Lisa Peacock (Cawthron) is thanked for assistance with Fig. 1. HWP was supported by the US National Science Foundation (CBET 0826819, 1230543, and DEB 1240851). SWW thanks the National Science Foundation (DEB 1240870, CBET 1230543 and IOS 1451528) and the NOAA Center for Sponsored Coastal Ocean Research Prevention, Control and Mitigation of Harmful Algal Blooms Program for award NA11NOS4780021. T.G.O. was supported by the US Geological Survey (G12AP20157). C.J.G. and M.J.H. were supported by The New Tamarind Foundation and the NOAA-ECOHAB program being funded by the National Oceanic and Atmospheric Center for Sponsored Coastal Ocean Research under award no. NA10NOS4780140 to Stony Brook University. [SS]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hal.2015.12.007.

References

Agrawal, M., Bagchi, D., Bagchi, S., 2001. Acute inhibition of protease and suppression of growth in zooplankter, *Moina macrocopa*, by *Microcystis* blooms collected in Central India. Hydrobiologia 464 (1–3), 37–44.

Agrawal, M.K., Zitt, A., Bagchi, D., Weckesser, J., Bagchi, S.N., von Elert, E., 2005. Characterization of proteases in guts of *Daphnia magna* and their inhibition by *Microcystis aeruginosa* PCC 7806. Environ. Toxicol. 20 (3), 314–322.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Studying Gene Expression and Function, Molecular Biology of the Cell. Garland Science, New York.

Alexova, R., Fujii, M., Birch, D., Cheng, J., Waite, T.D., Ferrari, B.C., Neilan, B.A., 2011a. Iron uptake and toxin synthesis in the bloom-forming *Microcystis aeruginosa* under iron limitation. Environ. Microbiol. 13 (4), 1064–1077.

Alexova, R., Haynes, P.A., Ferrari, B.C., Neilan, B.A., 2011b. Comparative protein expression in different strains of the bloom-forming cyanobacterium *Microcystis aeruginosa*. Mol. Cell. Proteomics 10 (9) .

Amé, M., Wunderlin, D., 2005. Effects of iron, ammonium and temperature on microcystin content by a natural concentrated *Microcystis aeruginosa* population. Water Air Soil Pollut. 168 (1–4), 235–248.

Arnold, D.E., 1971. Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue-green algae. Limnol. Oceanogr. 16 (6), 906–920.

Azevedo, S.M.F.O., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R., Eaglesham, G.K., 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru—Brazil. Toxicology 181–182 (0), 441–446.

Baldia, S.F., Evangelista, A.D., Aralar, E.V., Santiago, A.E., 2007. Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines. J. Appl. Phycol. 19 (6), 607–613.

Balmer, M., Downing, J., 2011. Carbon dioxide concentrations in eutrophic lakes: undersaturation implies atmospheric uptake. Inland Waters 1 (2), 125–132.

Banack, S.A., Caller, T.A., Stommel, E.W., 2010. The cyanobacteria derived toxin beta-N-methylamino-L-alanine and amyotrophic lateral sclerosis. Toxins 2 (12), 2837–2850.

Baxa, D.V., Kurobe, T., Ger, K.A., Lehman, P.W., Teh, S.J., 2010. Estimating the abundance of toxic *Microcystis* in the San Francisco Estuary using quantitative real-time PCR. Harmful Algae 9 (3), 342–349.

Berg, K.A., Lyra, C., Sivonen, K., Paulin, L., Suomalainen, S., Tuomi, P., Rapala, J., 2008. High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. ISME J. 3 (3), 314–325.

Bishop, C.T., Anet, E.F.L.J., Gorham, P.R., 1959. Isolation and identification of the fast-death factor in *Microcystis aeruginosa* NRC-1. Can. J. Biochem. Physiol. 37 (1), 453–471.

Blomqvist, P., Petterson, A., Hyenstrand, P., 1994. Ammonium-nitrogen: a key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. Arch. Hydrobiol. 132 (2,), 141–164.

Bourne, D.G., Jones, G.J., Blakeley, R.L., Jones, A., Negri, A.P., Riddles, P., 1996. Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR. Appl. Environ. Microbiol. 62 (11), 4086–4094.

Bourne, D.G., Riddles, P., Jones, G.J., Smith, W., Blakeley, R.L., 2001. Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environ. Toxicol. 16 (6), 523–534.

Bradley, W.G., Mash, D.C., 2009. Beyond Guam: the cyanobacteria/BMAA hypothesis of the cause of ALS and other neurodegenerative diseases. Amyotroph. Lateral Scler. 10 (s2), 7–20.

Bricelj, V.M., Connell, L., Konoki, K., Macquarrie, S.P., Scheuer, T., Catterall, W.A., Trainer, V.L., 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434 (7034), 763–767.

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.

Bykova, O., Laursen, A., Bostan, V., Bautista, J., McCarthy, L., 2006. Do zebra mussels (*Dreissena polymorpha*) alter lake water chemistry in a way that favours *Microcystis* growth? Sci. Total Environ. 371 (1–3), 362–372.

Caraco, N.F., Cole, J.J., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S.E.G., Fischer, D.T., 1997. Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. Ecology 78 (2), 588–602.

Carr, N.G., Whitton, B.A., 1982. The Biology of Cyanobacteria. Blackwell Scientific Publications, Oxford.

Carroll, L., 1917. Through the Looking Glass: And what Alice Found There. Rand, McNally, London, England.

Carroll, S.P., Hendry, A.P., Reznick, D.N., Fox, C.W., 2007. Evolution on ecological time-scales. Funct. Ecol. 21 (3), 387–393.

Chaffin, J., Bridgeman, T., 2014. Organic and inorganic nitrogen utilization by nitrogen-stressed cyanobacteria during bloom conditions. J. Appl. Phycol. 26 (1), 299–309.

Chislock, M.F., Sarnelle, O., Jernigan, L.M., Wilson, A.E., 2013. Do high concentrations of microcystin prevent *Daphnia* control of phytoplankton? Water Res. 47 (6), 1961–1970.

Chorus, I., Bartram, J., 1999. Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. E & FN Spon, London.

Codd, G., Poon, G., 1998. Cyanobacterial toxins. In: Gallon, J., Rogers, L. (Eds.), Proceedings of the Phytochemical Society of Europe, Oxford, pp. 283–296.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 323 (5917), 1014–1015.

Cousins, I.T., Bealing, D.J., James, H.A., Sutton, A., 1996. Biodegradation of microcystin-LR by indigenous mixed bacterial populations. Water Res. 30 (2), 481–485.

Cox, P.A., Banack, S.A., Murch, S.J., Rasmussen, U., Tien, G., Bidigare, R.R., Metcalf, J.S., Morrison, L.F., Codd, G.A., Bergman, B., 2005. Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. Proc. Natl. Acad. Sci. U. S. A. 102 (14), 5074–5078.

Cox, P.A., Sacks, O.W., 2002. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease in Guam. Neurology 58 (6), 956–959.

d'Herelle, F., 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. CR Acad. Sci. Paris 165, 373–375.

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 (5), 715–725.

Davis, T.W., Gobler, C.J., 2011. Grazing by mesozooplankton and microzooplankton on toxic and non-toxic strains of *Microcystis* in the Transquaking River, a tributary of Chesapeake Bay. J. Plankton Res. 33 (3), 415–430.

de Bernardi, R., Giussani, G., 1990. Are blue-green algae a suitable food for zooplankton? An overview. In: Gulati, R., Lammens, E.R.R., Meijer, M.-L., van Donk, E. (Eds.), Biomanipulation Tool for Water Management. Springer, Netherlands, pp. 29–41.

DeMott, W.R., Gulati, R.D., Donk, E.V., 2001. Effects of dietary phosphorus deficiency on the abundance, phosphorus balance, and growth of *Daphnia cucullata* in three hypereutrophic Dutch lakes. Limnol. Oceanogr. 46 (8), 1871–1880.

Dittmann, E., Börner, T., 2005. Genetic contributions to the risk assessment of microcystin in the environment. Toxicol. Appl. Pharmacol. 203 (3), 192–200.

Dittmann, E., Erhard, M., Kaebernick, M., Scheler, C., Neilan, B.A., von Döhren, H., Börner, T., 2001. Altered expression of two light-dependent genes in a microcystin-lacking mutant of *Microcystis aeruginosa* PCC 7806. Microbiology 147 (11), 3113–3119.

Dodds, W.K., Johnson, K.R., Priscu, J.C., 1989. Simultaneous nitrogen and phosphorus deficiency in natural phytoplankton assemblages: theory, empirical evidence, and implications for lake management. Lake Reserv. Manag. 5 (1), 21–26.

Dolman, A.M., Rücker, J., Pick, F.R., Fastner, J., Rohrlack, T., Mischke, U., Wiedner, C., 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLoS ONE 7 (6), e38757.

Downing, T.G., Meyer, C., Gehringer, M.M., van de Venter, M., 2005. Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. Environ. Toxicol. 20 (3), 257–262.

Dziallas, C., Grossart, H.-P., 2011. Increasing oxygen radicals and water temperature select for toxic *Microcystis* sp. PLoS ONE 6 (9), e25569.

Dziallas, C., Grossart, H.-P., 2012. Microbial interactions with the cyanobacterium *Microcystis aeruginosa* and their dependence on temperature. Mar. Biol. 159 (11), 2389–2398.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10 (12), 1135–1142.

Faassen, E.J., 2014. Presence of the neurotoxin BMAA in aquatic ecosystems: what do we really know? Toxins 6 (3), 1109–1138.

Falconer, I.R., 2005. Is there a human health hazard from microcystins in the drinking water supply? Acta Hydroch. Hydrob. 33 (1), 64–71.

Feurstein, D., Holst, K., Fischer, A., Dietrich, D.R., 2009. Oatp-associated uptake and toxicity of microcystins in primary murine whole brain cells. Toxicol. Appl. Pharmacol. 234 (2), 247–255.

Feurstein, D., Kleinteich, J., Stemmer, K., Dietrich, D., 2010. Organic anion transporting polypeptides expressed in primary murine neuronal cells mediate microcystin congener-dependent uptake. Environ. Health Perspect. 118 (10), 1370–1375.

Fiore, M.F., Alvarenga, D.O., Varani, A.M., Hoff-Risseti, C., Crespim, E., Ramos, R.T.J., Silva, A., Schaker, P.D.C., Heck, K., Rigonato, J., Schneider, M.P.C., 2013. Draft genome sequence of the Brazilian toxic bloom-forming cyanobacterium *Microcystis aeruginosa* strain SPC777. Genome Announc. 1 (4) .

Flores, E., Herrero, A., 2005. Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem. Soc. Trans. 33 (1), 164–167.

Frangeul, L., Quillardet, P., Castets, A.-M., Humbert, J.-F., Matthijs, H., Cortez, D., Tolonen, A., Zhang, C.-C., Gribaldo, S., Kehr, J.-C., Zilliges, Y., Ziemert, N., Becker, S., Talla, E., Latifi, A., Billault, A., Lepelletier, A., Dittmann, E., Bouchier, C., Tandeau de Marsac, N., 2008. Highly plastic genome of *Microcystis aeruginosa* PCC 7806, a ubiquitous toxic freshwater cyanobacterium. BMC Genomics 9 (1), 274.

Fulton, R.S., Paerl, H.W., 1987. Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton. J. Plankton Res. 9 (5), 837–855.

Gan, N., Xiao, Y., Zhu, L., Wu, Z., Liu, J., Hu, C., Song, L., 2012. The role of microcystins in maintaining colonies of bloom-forming *Microcystis* spp. Environ. Microbiol. 14 (3), 730–742.

Ginn, H.P., Pearson, L.A., Neilan, B.A., 2010. NtcA from *Microcystis aeruginosa* PCC 7806 is autoregulatory and binds to the microcystin promoter. Appl. Environ. Microbiol. 76 (13), 4362–4368.

Gobler, C.J., Davis, T.W., Coyne, K.J., Boyer, G.L., 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. Harmful Algae 6 (1), 119–133.

Goldberg, J., Huang, H.-b., Kwon, Y.-g., Greengard, P., Nairn, A.C., Kuriyan, J., 1995. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. Nature 376 (6543), 745–753.

Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M., 2007. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57 (1), 81–91.

Ha, J.H., Hidaka, T., Tsuno, H., 2009. Quantification of toxic *Microcystis* and evaluation of its dominance ratio in blooms using real-time PCR. Environ. Sci. Technol. 43 (3), 812–818.

Hairston, N.G., Lampert, W., Caceres, C.E., Holtmeier, C.L., Weider, L.J., Gaedke, U., Fischer, J.M., Fox, J.A., Post, D.M., 1999. Lake ecosystems: rapid evolution revealed by dormant eggs. Nature 401 (6752), 446.

Harke, M., Davis, T., Watson, S., Gobler, C.J., 2015. Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. Environ. Sci. Technol., http://dx.doi.org/10.1021/acs.est.5b03931 (in press).

Harke, M.J., Berry, D.L., Ammerman, J.W., Gobler, C.J., 2012. Molecular response of the bloom-forming cyanobacterium, *Microcystis aeruginosa*, to phosphorus limitation. Microb. Ecol. 63 (1), 188–198.

Harke, M.J., Gobler, C.J., 2013. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. PLoS ONE 8 (7), e69834.

Havens, K.E., Fukushima, T., Xie, P., Iwakuma, T., James, R.T., Takamura, N., Hanazato, T., Yamamoto, T., 2001. Nutrient dynamics and the eutrophication of shallow lakes Kasumigaura (Japan), Donghu (PR China), and Okeechobee (USA). Environ. Pollut. 111 (2), 263–272.

Heath, R.T., Fahnenstiel, G.L., Gardner, W.S., Cavaletto, J.F., Hwang, S.-J., 1995. Ecosystem-level effects of zebra mussels (*Dreissena polymorpha*): an enclosure experiment in Saginaw Bay, Lake Huron. J. Great Lakes Res. 21 (4), 501–516.

Hendry, A.P., Nosil, P., Rieseberg, L.H., 2007. The speed of ecological speciation. Funct. Ecol. 21 (3), 455–464.

Higgins, S.N., Zanden, M.J.V., 2010. What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. Ecol. Monogr. 80 (2), 179–196.

Ho, L., Gaudieux, A.-L., Fanok, S., Newcombe, G., Humpage, A.R., 2007. Bacterial degradation of microcystin toxins in drinking water eliminates their toxicity. Toxicon 50 (3), 438–441.

Ho, L., Meyn, T., Keegan, A., Hoefel, D., Brookes, J., Saint, C.P., Newcombe, G., 2006. Bacterial degradation of microcystin toxins within a biologically active sand filter. Water Res. 40 (4), 768–774.

Holtcamp, W., 2012. The emerging science of BMAA: do cyanobacteria contribute to neurodegenerative disease? Environ. Health Perspect. 120 (1), a110–a116.

Horst, G.P., Sarnelle, O., White, J.D., Hamilton, S.K., Kaul, R.B., Bressie, J.D., 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Res. 54, 188–198.

Hudnell, H.K., Dortch, Q., Zenick, H., 2008. An overview of the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB): advancing the scientific understanding of freshwater harmful algal blooms, cyanobacterial harmful algal blooms: state of the science and research needs. In: Hudnell, K.H. (Ed.), Volume 619 of Advances in Experimental Medicine and Biology. Springer, Amsterdam, pp. 1–16.

Humbert, J.-F., Barbe, V., Latifi, A., Gugger, M., Calteau, A., Coursin, T., Lajus, A., Castelli, V., Oztas, S., Samson, G., Longin, C., Medigue, C., de Marsac, N.T., 2013. A tribute to disorder in the genome of the bloom-forming freshwater cyanobacterium *Microcystis aeruginosa*. PLoS ONE 8 (8), e70747.

Humble, A.V., Gadd, G.M., Codd, G.A., 1997. Binding of copper and zinc to three cyanobacterial microcystins quantified by differential pulse polarography. Water Res. 31 (7), 1679–1686.

Hyenstrand, P., Nyvall, P., Pettersson, A., Blomqvist, P., 1998. Regulation of non-nitrogen-fixing cyanobacteria by inorganic nitrogen sources-experiments from Lake Erken. Arch. Hydrobiol. Spec. Adv. Limnol. 51, 29–40.

Ibelings, B.W., Mur, L.R., Walsby, A.E., 1991. Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in two shallow lakes. J. Plankton Res. 13 (2), 419–436.

Jack, J.D., Thorp, J.H., 2000. Effects of the benthic suspension feeder *Dreissena polymorpha* on zooplankton in a large river. Freshw. Biol. 44 (4), 569–579.

Jacobson, L., Halmann, M., 1982. Polyphosphate metabolism in the blue-green alga *Microcystis aeru-ginosa*. J. Plankton Res. 4 (3), 481–488.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J., Crayton, M., 2000. Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. Can. J. Fish. Aquat. Sci. 57 (1), 231–240.

Jang, M.-H., Ha, K., Joo, G.-J., Takamura, N., 2003. Toxin production of cyanobacteria is increased by exposure to zooplankton. Freshw. Biol. 48 (9), 1540–1550.

Jang, M.-H., Ha, K., Lucas, M.C., Joo, G.-J., Takamura, N., 2004. Changes in microcystin production by *Microcystis aeruginosa* exposed to phytoplanktivorous and omnivorous fish. Aquat. Toxicol. 68 (1), 51–59.

Jöhnk, K.D., Huisman, J.E.F., Sharples, J., Sommeijer, B.E.N., Visser, P.M., Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biol. 14 (3), 495–512.

Jones, G.J., Falconer, I.R., Wilkins, R.M., 1995. Persistence of cyclic peptide toxins in dried *Microcystis aeruginosa* crusts from Lake Mokoan, Australia. Environ. Toxicol. Water Q. 10 (1), 19–24.

Kaebernick, M., Neilan, B.A., Börner, T., Dittmann, E., 2000. Light and the transcriptional response of the microcystin biosynthesis gene cluster. Appl. Environ. Microbiol. 66 (8), 3387–3392.

Kaneko, T., Nakajima, N., Okamoto, S., Suzuki, I., Tanabe, Y., Tamaoki, M., Nakamura, Y., Kasai, F., Watanabe, A., Kawashima, K., Kishida, Y., Ono, A., Shimizu, Y., Takahashi, C., Minami, C., Fujishiro, T., Kohara, M., Katoh, M., Nakazaki, N., Nakayama, S., Yamada, M., Tabata, S., Watanabe, M.M., 2007. Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. DNA Res. 14 (6), 247–256.

Kehr, J.-C., Zilliges, Y., Springer, A., Disney, M.D., Ratner, D.D., Bouchier, C., Seeberger, P.H., De Marsac, N.T., Dittmann, E., 2006. A mannan binding lectin is involved in cell–cell attachment in a toxic strain of *Microcystis aeruginosa*. Mol. Microbiol. 59 (3), 893–906.

Kim, M., Oh, H.-S., Park, S.-C., Chun, J., 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int. J. Syst. Evol. Microbiol. 64 (Pt 2), 346–351.

Kissman, C.E.H., Knoll, L.B., Sarnelleb, O., 2010. Dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) reduce microzooplankton and macrozooplankton biomass in thermally stratified lakes. Limnol. Oceanogr. 55 (5), 1851–1859.

Komárek, J., Komárková, J., 2002. Review of the European *Microcystis*-morphospecies (Cyanoprokaryotes) from nature. Czech Phycol. Olomouc 2, 1–24.

Kondo, R., Yoshida, T., Yuki, Y., Hiroishi, S., 2000. DNA–DNA reassociation among a bloom-forming cyanobacterial genus, *Microcystis*. Int. J. Syst. Evol. Microbiol. 50 (2), 767–770.

Konst, H., McKercher, P.D., Gorham, P.R., Robertson, A., Howell, J., 1965. Symptoms and pathology produced by toxic *Microcystis aeruginosa* NRC-1 in laboratory and domestic animals. Can. J. Comp. Med. Vet. Sci. 29 (9), 221–228.

Kromkamp, J., van den Heuvel, A., Mur, L.R., 1989. Phosphorus uptake and photosynthesis by phosphate-limited cultures of the cyanobacterium *Microcystis aeruginosa*. Br. Phycol. J. 24 (4), 347–355.

Krumsiek, J., Arnold, R., Rattei, T., 2007. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. Bioinformatics 23 (8), 1026–1028.

Kuniyoshi, T.M., Gonzalez, A., Lopez-Gomollon, S., Valladares, A., Bes, M.T., Fillat, M.F., Peleato, M.L., 2011. 2-Oxoglutarate enhances NtcA binding activity to promoter regions of the microcystin synthesis gene cluster. FEBS Lett. 585 (24), 3921–3926.

Kuno, S., Yoshida, T., Kaneko, T., Sako, Y., 2012. Intricate interactions between the bloom-forming cyanobacterium *Microcystis aeruginosa* and foreign genetic elements, revealed by diversified clustered regularly interspaced short palindromic repeat (CRISPR) signatures. Appl. Environ. Microbiol. 78 (15), 5353–5360.

Lewis, W.M., Wurtsbaugh, W.A., 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. Int. Rev. Hydrobiol. 93 (4–5), 446–465.

Lewis, W.M., Wurtsbaugh, W.A., Paerl, H.W., 2011. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. Environ. Sci. Technol. 45 (24), 10300–10305.

Lobner, D., Piana, P.M.T., Salous, A.K., Peoples, R.W., 2007. β-N-methylamino-L-alanine enhances neurotoxicity through multiple mechanisms. Neurobiol. Dis. 25 (2), 360–366.

Long, B.M., Jones, G.J., Orr, P.T., 2001. Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. Appl. Environ. Microbiol. 67 (1), 278–283.

MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, NJ.

Makarova, K.S., Wolf, Y.I., Snir, S., Koonin, E.V., 2011. Defense islands in bacterial and archaeal genomes and prediction of novel defense systems. J. Bacteriol. 193 (21), 6039–6056.

Makower, A.K., Schuurmans, J.M., Groth, D., Zilliges, Y., Matthijs, H.C.P., Dittmann, E., 2015. Transcriptomics-aided dissection of the intracellular and extracellular roles of microcystin in *Microcystis aeruginosa* PCC 7806. Appl. Environ. Microbiol. 81 (2), 544–554.

Martin-Luna, B., Sevilla, E., Hernandez, J.A., Bes, M.T., Fillat, M.F., Peleato, M.L., 2006. Fur from *Microcystis aeruginosa* binds *in vitro* promoter regions of the microcystin biosynthesis gene cluster. Phytochemistry 67 (9), 876–881.

Maynes, J.T., Luu, H.A., Cherney, M.M., Andersen, R.J., Williams, D., Holmes, C.F.B., James, M.N.G., 2006. Crystal structures of protein phosphatase-1 bound to motuporin and dihydromicrocystin-LA: elucidation of the mechanism of enzyme inhibition by cyanobacterial toxins. J. Mol. Biol. 356 (1), 111–120.

Meissner, S., Fastner, J., Dittmann, E., 2013. Microcystin production revisited: conjugate formation makes a major contribution. Environ. Microbiol. 15 (6), 1810–1820.

Meissner, S., Steinhauser, D., Dittmann, E., 2015. Metabolomic analysis indicates a pivotal role of the hepatotoxin microcystin in high light adaptation of *Microcystis*. Environ. Microbiol. 17 (5), 1497–1509.

Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., Chaffin, J.D., Cho, K., Confesor, R., Daloğlu, I., DePinto, J.V., Evans, M.A., Fahnenstiel, G.L., He, L., Ho, J.C., Jenkins, L., Johengen, T.H., Kuo, K.C., LaPorte, E., Liu, X., McWilliams, M.R., Moore, M.R., Posselt, D.J., Richards, R.P., Scavia, D., Steiner, A.L., Verhamme, E., Wright, D.M., Zagorski, M.A., 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proc. Natl. Acad. Sci. U. S. A. 110 (16), 6448–6452.

Milutinović, A., Živin, M., Zorc-Pleskovič, R., Sedmak, B., Šuput, D., 2003. Nephrotoxic effects of chronic administration of microcystins -LR and -YR. Toxicon 42 (3), 281–288.

Mitrovic, S.M., Chessman, B.C., Bowling, L.C., Cooke, R.H., 2006. Modelling suppression of cyanobacterial blooms by flow management in a lowland river. River Res. Appl. 22 (1), 109–114.

Mou, X., Lu, X., Jacob, J., Sun, S., Heath, R., 2013. Metagenomic identification of bacterioplankton taxa and pathways involved in microcystin degradation in Lake Erie. PLoS ONE 8 (4), e61890.

Nagata, S., Tsutsumi, T., Hasegawa, A., Yoshida, F., Ueno, Y., Watanabe, M.F., 1997. Enzyme immunoassay for direct determination of microcystins in environmental water. J. AOAC Int. 80 (2), 408–417.

Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E., 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environ. Microbiol. 15 (5), 1239–1253.

Novichkov, P.S., Wolf, Y.I., Dubchak, I., Koonin, E.V., 2009. Trends in prokaryotic evolution revealed by comparison of closely related bacterial and archaeal genomes. J. Bacteriol. 191 (1), 65–73.

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.

Oh, H.-M., Lee, S.J., Jang, M.-H., Yoon, B.-D., 2000. Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. Appl. Environ. Microbiol. 66 (1), 176–179.

Okano, K., Miyata, N., Ozaki, Y., 2015. Whole genome sequence of the non-microcystin-producing *Microcystis aeruginosa* Strain NIES-44. Genome Announc. 3 (2.) .

Okino, T., 1974. Studies on the blooming of *Microcystis aeruginosa*. II: rapid accumulation of phosphate by *Microcystis aeruginosa*. J. Fac. Sci. Shinsu Univ. 8, 135–145.

Orr, P.T., Jones, G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnol. Oceanogr. 43 (7), 1604–1614.

Otsuka, S., Suda, S., Shibata, S., Oyaizu, H., Matsumoto, S., Watanabe, M.M., 2001. A proposal for the unification of five species of the cyanobacterial genus *Microcystis* Kützing ex Lemmermann 1907 under the rules of the Bacteriological Code. Int. J. Syst. Evol. Microbiol. 51 (3), 873–879.

Otten, T., Paerl, H., 2015. Health effects of toxic cyanobacteria in U.S. drinking and recreational waters: our current understanding and proposed direction. Curr. Environ. Health Rep. 2 (1), 75–84.

Otten, T.G., Crosswell, J.R., Mackey, S., Dreher, T.W., 2015. Application of molecular tools for microbial source tracking and public health risk assessment of a *Microcystis* bloom traversing 300 km of the Klamath River. Harmful Algae 46, 71–81.

Otten, T.G., Xu, H., Qin, B., Zhu, G., Paerl, H.W., 2012. Spatiotemporal patterns and ecophysiology of toxigenic *Microcystis* blooms in Lake Taihu, China: implications for water quality management. Environ. Sci. Technol. 46 (6), 3480–3488.

Ouellette, A.J.A., Wilhelm, S.W., 2003. Toxic cyanobacteria: the evolving molecular toolbox. Front. Ecol. Environ. 7, 359–366.

Paerl, H., 1982. Interactions with bacteria. In: Carr, N.G., B.A.W., (Eds.), The Biology of Cyanobacteria. University of California Press, Berkely and Los Angeles, pp. 441–461.

Paerl, H., Otten, T., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65 (4), 995–1010.

Paerl, H.W., 2014. Mitigating harmful cyanobacterial blooms in a human-and climatically-impacted world. Life 4 (4), 988–1012.

Paerl, H.W., Bland, P.T., Bowles, N.D., Haibach, M.E., 1985. Adaptation to high-intensity, low-wavelength light among surface blooms of the cyanobacterium *Microcystis aeruginosa*. Appl. Environ. Microbiol. 49 (5), 1046–1052.

Paerl, H.W., Gardner, W.S., McCarthy, M.J., Peierls, B.L., Wilhelm, S.W., 2014a. Algal blooms: noteworthy nitrogen. Science 346 (6206), 175.

Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science 320 (5872), 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 (6S), 160–167.

Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46 (5), 1349–1363.

Paerl, H.W., Pinckney, J.L., 1996. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. Microb. Ecol. 31 (3), 225–247.

Paerl, H.W., Tucker, J., Bland, P.T., 1983. Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aeruginosa*) surface blooms. Limnol. Oceanogr. 28 (5), 847–857.

Paerl, H.W., Xu, H., Hall, N.S., Rossignol, K.L., Joyner, A.R., Zhu, G., Qin, B., 2015. Nutrient limitation dynamics examined on a multi-annual scale in Lake Taihu, China: implications for controlling eutrophication and harmful algal blooms. J. Freshw. Ecol. 30 (1), 5–24.

Paerl, H.W., Xu, H., Hall, N.S., Zhu, G., Qin, B., Wu, Y., Rossignol, K.L., Dong, L., McCarthy, M.J., Joyner, A.R., 2014b. Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: will nitrogen reductions cause replacement of non-N_2 fixing by N_2 fixing taxa? PLOS ONE 9 (11), e113123.

Park, H.-D., Sasaki, Y., Maruyama, T., Yanagisawa, E., Hiraishi, A., Kato, K., 2001. Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environ. Toxicol. 16 (4), 337–343.

Park, H.-D., Watanabe, M.F., Harada, K.-I., Nagai, H., Suzuki, M., Watanabe, M., Hayashi, H., 1993. Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. Nat. Toxins 1 (6), 353–360.

Parveen, B., Ravet, V., Djediat, C., Mary, I., Quiblier, C., Debroas, D., Humbert, J.-F., 2013. Bacterial communities associated with *Microcystis* colonies differ from free-living communities living in the same ecosystem. Environ. Microbiol. Rep. 5 (5), 716–724.

Paul, V.J., 2008. Global warming and cyanobacterial harmful algal blooms, cyanobacterial harmful algal blooms: state of the science and research needs. In: Hudnell, K.H. (Ed.), Volume 619 of Advances in Experimental Medicine and Biology. Springer, Amsterdam, pp. 239–257.

Pearson, L.A., Hisbergues, M., Börner, T., Dittmann, E., Neilan, B.A., 2004. Inactivation of an ABC transporter gene, *mcyH*, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. Appl. Environ. Microbiol. 70 (11), 6370–6378.

Penn, K., Wang, J., Fernando, S.C., Thompson, J.R., 2014. Secondary metabolite gene expression and interplay of bacterial functions in a tropical freshwater cyanobacterial bloom. ISME J. 8 (9), 1866–1878.

Perovich, G., Dortch, Q., Goodrich, J., Berger, P.S., Brooks, J., Evens, T.J., Gobler, C.J., Graham, J., Hyde, J., Karner, D., 2008. Causes, prevention, and mitigation workgroup report, cyanobacterial harmful algal blooms: state of the science and research needs. In: Hudnell, K.H. (Ed.), Volume 619 of Advances in Experimental Medicine and Biology. Springer, Amsterdam, pp. 185–215.

Phelan, R.R., Downing, T.G., 2011. A growth advantage for microcystin production by *Microcystis* pcc7806 under high light. J. Phycol. 47 (6), 1241–1246.

Pimentel, J.S.M., Giani, A., 2014. Microcystin production and regulation under nutrient stress conditions in toxic *Microcystis* strains. Appl. Environ. Microbiol. 80 (18), 5836–5843.

Post, D.M., Palkovacs, E.P., 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. Philos. Trans. R. Soc. B: Biol. Sci. 364 (1523), 1629–1640.

Potts, M., Whitton, B., 2000. The Ecology of Cyanobacteria. Blackwell Scientific Publications, Oxford.

Puddick, J., Prinsep, M.R., Wood, S.A., Kaufononga, S.A., Cary, S.C., Hamilton, D.P., 2014. High levels of structural diversity observed in microcystins from *Microcystis* CAWBG11 and characterization of six new microcystin congeners. Marine Drugs 12 (11), 5372–5395.

Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H., Carmichael, W., 2010. A drinking water crisis in Lake Taihu, China: linkage to climatic variability and lake management. Environ. Manage. 45 (1), 105–112.

Raikow, D.F., Sarnelle, O., Wilson, A.E., Hamilton, S.K., 2004. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. Limnol. Oceanogr. 49 (2), 482–487.

Rankin, K., Alroy, K., Kudela, R., Oates, S., Murray, M., Miller, M., 2013. Treatment of cyanobacterial (microcystin) toxicosis using oral cholestyramine: case report of a dog from Montana. Toxins 5 (6), 1051–1063.

Rantala, A., Fewer, D.P., Hisbergues, M., Rouhiainen, L., Vaitomaa, J., Börner, T., Sivonen, K., 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. Proc. Natl. Acad. Sci. U. S. A. 101 (2), 568–573.

Rantala, A., Rizzi, E., Castiglioni, B., De Bellis, G., Sivonen, K., 2008. Identification of hepatotoxin-producing cyanobacteria by DNA-chip. Environ. Microbiol. 10 (3), 653–664.

Rasko, D.A., Rosovitz, M.J., Myers, G.S.A., Mongodin, E.F., Fricke, W.F., Gajer, P., Crabtree, J., Sebaihia, M., Thomson, N.R., Chaudhuri, R., Henderson, I.R., Sperandio, V., Ravel, J., 2008. The pangenome structure of *Escherichia coli*: comparative genomic analysis of *E. coli* commensal and pathogenic isolates. J. Bacteriol. 190 (20), 6881–6893.

Ressom, R., San Soong, F., Fitzgerald, J., Turczynowicz, L., El Saadi, O., Roder, D., Maynard, T., Falconer, I., 2004. Health Effects of Toxic Cyanobacteria (blue-green algae). National Health and Research Council, Canberra, pp. 108.

Réveillon, D., Abadie, E., Séchet, V., Brient, L., Savar, V., Bardouil, M., Hess, P., Amzil, Z., 2014. Beta-N-methylamino-L-alanine: LC-MS/MS optimization, screening of cyanobacterial strains and occurrence in shellfish from Thau, a French Mediterranean lagoon. Marine Drugs 12 (11), 5441–5467.

Reynolds, C.S., 2008. The ecology of phytoplankton. In: Hudnell, K.H. (Ed.), Volume 619 of Advances in Experimental Medicine and Biology. Cambridge University Press, Stewart.

Reynolds, C.S., Jaworski, G.H.M., Cmiech, H.A., Leedale, G.F., 1981. On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kutz. Emend. Elenkin. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 293 (1068), 419–477.

Reynolds, C.S., Rogers, D.A., 1976. Seasonal variations in the vertical distribution and buoyancy of *Microcystis aeruginosa* Kütz. emend. Elenkin in Rostherne Mere, England. Hydrobiologia 48 (1), 17–23.

Rinehart, K., Namikoshi, M., Choi, B., 1994. Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). J. Appl. Phycol. 6 (2), 159–176.

Rinehart, K.L., Harada, K., Namikoshi, M., Chen, C., Harvis, C.A., Munro, M.H.G., Blunt, J.W., Mulligan, P.E., Beasley, V.R., et al., 1988. Nodularin, microcystin, and the configuration of Adda. J. Am. Chem. Soc. 110 (25), 8557–8558.

Rinta-Kanto, J.M., Konopka, E.A., DeBruyn, J.M., Bourbonniere, R.A., Boyer, G.L., Wilhelm, S.W., 2009. Lake Erie *Microcystis*: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. Harmful Algae 8, 665–673.

Rinta-Kanto, J.M., Ouellette, A.J.A., Boyer, G.L., Twiss, M.R., Bridgeman, T.B., Wilhelm, S.W., 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environ. Sci. Technol. 39 (11), 4198–4205.

Rinta-Kanto, J.M., Wilhelm, S.W., 2006. Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. Appl. Environ. Microbiol. 72 (7), 5083–5085.

Rogers, E.D., Henry, T.B., Twiner, M.J., Gouffon, J.S., McPherson, J.T., Boyer, G.L., Sayler, G.S., Wilhelm, S.W., 2011. Global gene expression profiling in larval zebrafish exposed to microcystin-LR and *Microcystis* reveals endocrine disrupting effects of Cyanobacteria. Environ. Sci. Technol. 45 (5), 1962–1969.

Rohrlack, T., Dittmann, E., Henning, M., Börner, T., Kohl, J.-G., 1999. Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. Appl. Environ. Microbiol. 65 (2), 737–739.

Rohrlack, T., Hyenstrand, P., 2007. Fate of intracellular microcystins in the cyanobacterium *Microcystis aeruginosa* (Chroococcales, Cyanophyceae). Phycologia 46 (3), 277–283.

Rozon, R.M., Short, S.M., 2013. Complex seasonality observed amongst diverse phytoplankton viruses in the Bay of Quinte, an embayment of Lake Ontario. Freshw. Biol. 58 (12), 2648–2663.

Safferman, R.S., Morris, M.-E., 1963. Algal virus: isolation. Science 140 (3567), 679–680.

Safferman, R.S., Morris, M.-E., 1964. Control of algae with viruses. J. Am. Water Works Assoc. 56 (9), 1217–1224.

Safferman, R.S., Morris, M.E., 1967. Observations on the occurrence, distribution, and seasonal incidence of blue-green algal viruses. Appl. Microbiol. 15 (5), 1219–1222.

Saito, T., Okano, K., Park, H.-D., Itayama, T., Inamori, Y., Neilan, B.A., Burns, B.P., Sugiura, N., 2003. Detection and sequencing of the microcystin LR-degrading gene, mlrA, from new bacteria isolated from Japanese lakes. FEMS Microbiol. Lett. 229 (2), 271–276.

Sandrini, G., Cunsolo, S., Schuurmans, M., Matthijs, H., Huisman, J., 2015. Changes in gene expression, cell physiology and toxicity of the harmful cyanobacterium *Microcystis aeruginosa* at elevated CO_2. Front. Microbiol. 6 .

Sandrini, G., Matthijs, H.C., Verspagen, J.M., Muyzer, G., Huisman, J., 2014. Genetic diversity of inorganic carbon uptake systems causes variation in CO_2 response of the cyanobacterium *Microcystis*. ISME J. 8 (3), 589–600.

Sangolkar, L.N., Maske, S.S., Chakrabarti, T., 2006. Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria. Water Res. 40 (19), 3485–3496.

Sant'Anna, C.L., de Carvalho, L.R., Fiore, M.F., Silva-Stenico, M.E., Lorenzi, A.S., Rios, F.R., Konno, K., Garcia, C., Lagos, N., 2011. Highly toxic *Microcystis aeruginosa* strain, isolated from Sao Paulo-Brazil, produce hepatotoxins and paralytic shellfish poison neurotoxins. Neurotox. Res. 19 (3), 389–402.

Sarnelle, O., Wilson, A.E., Hamilton, S.K., Knoll, L.B., Raikow, D.F., 2005. Complex interactions between the zebra mussel, *Dreissena polymorpha*, and the harmful phytoplankter, *Microcystis aeruginosa*. Limnol. Oceanogr. 50 (3), 896–904.

Saxton, M.A., Arnold, R.J., Bourbonniere, R.A., McKay, R.M.L., Wilhelm, S.W., 2012. Plasticity of total and intracellular phosphorus quotas in *Microcystis aeruginosa* cultures and Lake Erie algal assemblages. Front. Microbiol. 3 (3), http://dx.doi.org/10.3389/fmicb.2012.00003.

Sbiyyaa, B., Loudiki, M., Oudra, B., 1998. Nitrogen and phosphorus intracellular capacity in storage by *Microcystis aeruginosa* Kuetz and *Synechocystis* sp.: toxic cyanobacteria occasionally forming blooms in Marrakesch area (Morocco). Annal. Limnol. 247–257.

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., 2012. The dilemma of controlling cultural eutrophication of lakes. Proc. R. Soc. Lond. B: Biol. Sci. 279 (1746), 4322–4333.

Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., Kasian, S.E.M., 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc. Natl. Acad. Sci. U. S. A. 105 (32), 11254–11258.

Scott, L.L., Downing, S., Phelan, R.R., Downing, T.G., 2014. Environmental modulation of microcystin and β-N-methylamino-L-alanine as a function of nitrogen availability. Toxicon 87, 1–5.

Sevilla, E., Martin-Luna, B., Vela, L., Bes, M.T., Fillat, M.F., Peleato, M.L., 2008. Iron availability affects *mcyD* expression and microcystin-LR synthesis in *Microcystis aeruginosa* PCC7806. Environ. Microbiol. 10 (10), 2476–2483.

Sevilla, E., Martin-Luna, B., Vela, L., Teresa Bes, M., Luisa Peleato, M., Fillat, M., 2010. Microcystin-LR synthesis as response to nitrogen: transcriptional analysis of the mcyD gene in *Microcystis aeruginosa* PCC7806. Ecotoxicology 19 (7,), 1167–1173.

Shao, J., Jiang, Y., Wang, Z., Peng, L., Luo, S., Gu, J., Li, R., 2014. Interactions between algicidal bacteria and the cyanobacterium *Microcystis aeruginosa*: lytic characteristics and physiological responses in the cyanobacteria. Int. J. Environ. Sci. Technol. 11 (2), 469–476.

Shen, H., Niu, Y., Xie, P., Tao, M.I.N., Yang, X.I., 2011. Morphological and physiological changes in *Microcystis aeruginosa* as a result of interactions with heterotrophic bacteria. Freshw. Biol. 56 (6), 1065–1080.

Smayda, T.J., 2008. Complexity in the eutrophication–harmful algal bloom relationship, with comment on the importance of grazing. Harmful Algae 8 (1), 140–151.

Somdee, T., Thunders, M., Ruck, J., Lys, I., Allison, M., Page, R., 2013. Degradation of [Dha^7]MC-LR by a Microcystin Degrading Bacterium Isolated from Lake Rotoiti, New Zealand. ISRN Microbiol. 2013, 8.

Spoof, L., 2005. Cyanobacterial monitoring and cyanotoxin analysis. In: Meriluoto, J., Codd, G.A. (Eds.), Acta Academiae Aboensis. pp. 1–145.

Stackebrandt, E., Ebers, J., 2006. Taxonomic parameters revisited: tarnished gold standards. Microbiol. Today 33 (4), 152.

Steffen, M.M., Belisle, B.S., Watson, S.B., Boyer, G.L., Bourbonniere, R.A., Wilhelm, S.W., 2015. Metatranscriptomic evidence for co-occurring top-down and bottom-up controls on toxic cyanobacterial communities. Appl. Environ. Microbiol. 81 (9), 3268–3276.

Steffen, M.M., Dearth, S.P., Dill, B.D., Li, Z., Larsen, K.M., Campagna, S.R., Wilhelm, S.W., 2014a. Nutrients drive transcriptional changes that maintain metabolic homeostasis but alter genome architecture in *Microcystis*. ISME J. 8 (10), 2080–2092.

Steffen, M.M., Zhu, Z., McKay, R.M.L., Wilhelm, S.W., Bullerjahn, G.S., 2014b. Taxonomic assessment of a toxic cyanobacteria shift in hypereutrophic Grand Lake St. Marys (Ohio, USA). Harmful Algae 33, 12–18.

Stewart, I., Seawright, A.A., Shaw, G.R., 2008. Cyanobacterial Poisoning in Livestock, Wild Mammals and Birds – An Overview, Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer, , pp. 613–637.

Straub, C., Quillardet, P., Vergalli, J., de Marsac, N.T., Humbert, J.-F., 2011. A day in the life of *Microcystis aeruginosa* strain PCC 7806 as revealed by a transcriptomic analysis. PLoS ONE 6 (1), e16208.

Sunda, W.G., Graneli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. J. Phycol. 42 (5), 963–974.

Takashima, Y., Yoshida, T., Kashima, A., Hiroishi, S., Nagasaki, K., 2007a. Cryopreservation of a myovirus infecting the toxin-producing cyanobacterium *Microcystis aeruginosa*. Microbes Environ. 22 (3), 297–299.

Takashima, Y., Yoshida, T., Yoshida, M., Shirai, Y., Tomaru, Y., Takao, Y., Hiroishi, S., Nagasaki, K., 2007b. Development and application of quantitative detection of cyanophages phylogenetically related to cyanophage Ma-LMM01 infecting *Microcystis aeruginosa* in fresh water. Microbes Environ. 22 (3), 207–213.

Tang, X., Gao, G., Chao, J., Wang, X., Zhu, G., Qin, B., 2010. Dynamics of organic-aggregate-associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in China. Limnol. Oceanogr. 55 (2), 469–480.

Thompson, J.N., 1998. Rapid evolution as an ecological process. Trends Ecol. Evol. 13 (8), 329–332.

Tillett, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T., Neilan, B.A., 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide–polyketide synthetase system. Chem. Biol. 7 (10), 753–764.

Tillmanns, A.R., Wilson, A.E., Pick, F.R., Sarnelle, O., 2008. Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. Fundam. Appl. Limnol./Arch. Hydrobiol. 171 (4), 285–295.

Tucker, S., Pollard, P., 2005. Identification of cyanophage Ma-LBP and infection of the cyanobacterium *Microcystis aeruginosa* from an Australian subtropical lake by the virus. Appl. Environ. Microbiol. 71 (2), 629–635.

Twort, F.W., 1915. An investigation on the nature of ultra-microscopic viruses. Lancet 186 (4814), 1241–1243.

Valeria, A., Ricardo, E., Stephan, P., Alberto, W., 2006. Degradation of microcystin-RR by *Sphingomonas* sp. CBA4 isolated from San Roque reservoir (Córdoba – Argentina). Biodegradation 17 (5), 447–455.

Van de Waal, D.B., Smith, V.H., Declerck, S.A.J., Stam, E.C.M., Elser, J.J., 2014. Stoichiometric regulation of phytoplankton toxins. Ecol. Lett. 17 (6), 736–742.

Van de Waal, D.B., Verspagen, J.M.H., Finke, J.F., Vournazou, V., Immers, A.K., Kardinaal, W.E.A., Tonk, L., Becker, S., Van Donk, E., Visser, P.M., Huisman, J., 2011. Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO_2. ISME J. 5 (9), 1438–1450.

Van de Waal, D.B., Verspagen, J.M.H., Lürling, M., Van Donk, E., Visser, P.M., Huisman, J., 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. Ecol. Lett. 12 (12), 1326–1335.

van der Westhuizen, A., Eloff, J., Kruger, G., 1986. Effect of temperature and light (fluence rate) on the composition of the toxin of the cyanobacterium Microcystis aeruginosa (UV-006). Arch. Hydrobiol. 108 (2), 145–154.

van der Westhuizen, A.J., Eloff, J.N., 1985. Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). Planta 163 (1), 55–59.

Van Valen, L., 1973. A new evolutionary law. Evol. Theory 1, 1–30.

Vanderploeg, H.A., Liebig, J.R., Carmichael, W.W., Agy, M.A., Johengen, T.H., Fahnenstiel, G.L., Nalepa, T.F., 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Can. J. Fish. Aquat. Sci. 58 (6), 1208–1221.

Vasconcelos, V.M., Sivonen, K., Evans, W.R., Carmichael, W.W., Namikoshi, M., 1996. Hepatotoxic microcystin diversity in cyanobacterial blooms collected in Portuguese freshwaters. Water Res. 30 (10), 2377–2384.

Visser, P., Ibelings, B.A.S., Van Der Veer, B., Koedood, J.A.N., Mur, R., 1996. Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, the Netherlands. Freshw. Biol. 36 (2), 435–450.

Walsby, A.E., Hayes, P.K., Boje, R., Stal, L.J., 1997. The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea. New Phytol. 136 (3), 407–417.

Watanabe, M.F., Oishi, S., 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. Appl. Environ. Microbiol. 49 (5), 1342–1344.

Wayne, L., Brenner, D., Colwell, R., Grimont, P., Kandler, O., Krichevsky, M., Moore, L., Moore, E., Murray, R., Stackebrandt, E., Starr, M., Truper, H., 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol. 37 (4), 463–464.

Welch, R.A., Burland, V., Plunkett, G., Redford, P., Roesch, P., Rasko, D., Buckles, E.L., Liou, S.-R., Boutin, A., Hackett, J., Stroud, D., Mayhew, G.F., Rose, D.J., Zhou, S.,

Schwartz, D.C., Perna, N.T., Mobley, H.L.T., Donnenberg, M.S., Blattner, F.R., 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. 99 (26), 17020–17024.

WHO, 2003. Cyanobacterial toxins: microcystin-LR in drinking water. In: Organization W.H. (Eds.), Background Document for Preparation of WHO Guidelines for Drinking-water Quality. World Health Organization, Geneva.

Wilhelm, S.W., Boyer, G.L., 2011. Healthy competition. Nat. Clim. Change 1, 300–301.

Wilhelm, S.W., Farnsley, S.E., LeCleir, G.R., Layton, A.C., Satchwell, M.F., DeBruyn, J.M., Boyer, G.L., Zhu, G., Paerl, H.W., 2011. The relationships between nutrients, cyanobacterial toxins and the microbial community in Taihu (Lake Tai), China. Harmful Algae 10 (2), 207–215.

Wilhelm, S.W., Matteson, A.R., 2008. Freshwater and marine virioplankton: a brief overview of commonalities and differences. Freshw. Biol. 53 (6), 1076–1089.

Wilson, A.E., Sarnelle, O., Tillmanns, A.R., 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: meta-analyses of laboratory experiments. Limnol. Oceanogr. 51 (4), 1915–1924.

Wommack, K.E., Colwell, R.R., 2000. Virioplankton: Viruses in aquatic ecosystems. Microbiol. Mol. Biol. Rev. 64 (1), 69–114.

Wood, S.A., Dietrich, D.R., 2011. Quantitative assessment of aerosolized cyanobacterial toxins at two New Zealand lakes. J. Environ. Monit. 13 (6), 1617–1624.

Wood, S.A., Dietrich, D.R., Cary, S.C., Hamilton, D.P., 2012. Increasing *Microcystis* cell density enhances microcystin synthesis: a mesocosm study. Inland Waters 2 (1), 17–22.

Wood, S.A., Holland, P.T., Stirling, D.J., Briggs, L.R., Sprosen, J., Ruck, J.G., Wear, R.G., 2006. Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. N. Z. J. Mar. Freshw. Res. 40 (4), 585–597.

Wood, S.A., Rueckert, A., Hamilton, D.P., Cary, S.C., Dietrich, D.R., 2011. Switching toxin production on and off: intermittent microcystin synthesis in a *Microcystis* bloom. Environ. Microbiol. Rep. 3 (1), 118–124.

Xu, H., Zhu, G., Qin, B., Paerl, H., 2013. Growth response of *Microcystis* spp. to iron enrichment in different regions of Lake Taihu, China. Hydrobiologia 700 (1), 187–202.

Yamaguchi, H., Suzuki, S., Tanabe, Y., Osana, Y., Shimura, Y., Ishida, K., Kawachi, M., 2015. Complete Genome Sequence of *Microcystis aeruginosa* NIES-2549, a Bloom-Forming Cyanobacterium from Lake Kasumigaura, Japan. Genome Announc. 3 (3) .

Yang, C., Lin, F., Li, Q., Li, T., Zhao, J., 2015. Comparative genomics reveals diversified CRISPR-Cas systems of globally distributed *Microcystis aeruginosa*, a freshwater bloom-forming cyanobacterium. Front. Microbiol. 6 .

Yang, C., Zhang, W., Ren, M., Song, L., Li, T., Zhao, J., 2013. Whole-genome sequence of *Microcystis aeruginosa* TAIHU98, a nontoxic bloom-forming strain isolated from Taihu Lake, China. Genome Announc. 1 (3.) .

Yang, Z., Kong, F., Shi, X., Cao, H., 2006. Morphological response of *Microcystis aeruginosa* to grazing by different sorts of zooplankton. Hydrobiologia 563 (1), 225–230.

Yoshida, M., Yoshida, T., Satomi, M., Takashima, Y., Hosoda, N., Hiroishi, S., 2008. Intra-specific phenotypic and genotypic variation in toxic cyanobacterial *Microcystis* strains. J. Appl. Microbiol. 105 (2), 407–415.

Yoshida, M., Yoshida, T., Yoshida-Takashima, Y., Kashima, A., Hiroishi, S., 2010. Real-Time PCR detection of host-mediated cyanophage gene transcripts during infection of a natural *Microcystis aeruginosa* population. Microbes Environ. 25 (3), 211–215.

Yoshida, T., Takashima, Y., Tomaru, Y., Shirai, Y., Takao, Y., Hiroishi, S., Nagasaki, K., 2006. Isolation and characterization of a cyanophage infecting the toxic cyanobacterium *Microcystis aeruginosa*. Appl. Environ. Microbiol. 72 (2), 1239–1247.

Yoshida, T., Yoshida, M., Takashima, Y., Hiroishi, S., Nagasaki, K., 2007. Monitoring of a toxic cyanobacterium *Microcystis aeruginosa* and its infectious cyanophage. Nippon Suisan Gakkaishi 73 (2), 302–305.

Yu, N.Y., Wagner, J.R., Laird, M.R., Melli, G., Rey, S., Lo, R., Dao, P., Sahinalp, S.C., Ester, M., Foster, L.J., Brinkman, F.S., 2010. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics 26 (13), 1608–1615.

Zhang, Q.-X., Yu, M.-J., Li, S.-H., Carmichael, W.W., 1991. Cyclic peptide hepatotoxins from freshwater cyanobacterial (blue-green algae) waterblooms collected in Central China. Environ. Toxicol. Chem. 10 (3), 313–321.

Zhu, L., Wu, Y., Song, L., Gan, N., 2014. Ecological dynamics of toxic *Microcystis* spp. and microcystin-degrading bacteria in Dianchi Lake, China. Appl. Environ. Microbiol. 80 (6), 1874–1881.

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.

Zurawell, R.W., Chen, H., Burke, J.M., Prepas, E.E., 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J. Toxicol. Environ. Health B 8 (1), 1–37.