Chapter 3: Occurrence of Cyanobacterial Harmful Algal Blooms Workgroup Report

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Introduction

Freshwater cyanobacteria periodically accumulate, or bloom, in water bodies across the United States (US). These blooms, also known as cyanobacterial harmful algal blooms (CHAB), can lead to a reduction in the number of individuals who engage in recreational activities in lakes and reservoirs, degrade aquatic habitats and potentially impact human health. In 1998, Congress passed the 1998 Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA) to address CHABs that impacted living marine resources, fish and shellfish harvests and recreational and service industries along US coastal waters. In 2004, as part of its reauthorization, HABHRCA requires federal agencies to assess CHABs to include freshwater and estuarine environments and develop plans to reduce the likelihood of CHAB formation and to mitigate their damage (NOAA 2004). Many federal agencies recognize the potential impacts of CHABs and share risk management responsibilities; an interagency task force was established and charged to prepare a scientific assessment of the causes, occurrence, effects and economic costs of freshwater. The United States Environmental Protection Agency (EPA) has included "cyanobacteria (blue–green algae), other freshwater algae, and their toxins" in its Contaminant Candidate List (CCL) as one of the microbial drinking water contaminants targeted for additional study, but it does not specify which toxins should be targeted for study (EPA 2005b). Based on toxicological, epidemiology and occurrence studies, the EPA Office of Ground Water and Drinking Water has restricted its efforts to 3 of the over 80 variants of cyanotoxins reported, recommending that Microcystin (MC) congeners LR, YR, RR and LA, Anatoxin–a (AA) and Cylindrospermopsin (CY) be placed on the Unregulated Contaminant Monitoring Rule (UCMR) (EPA 2001). The EPA uses the UCMR program to collect data for contaminants suspected to be present in drinking water that do not have health–based standards set. This monitoring supplies information on the nature and size of populations exposed to cyanotoxins through tap water use.

Various federal agencies are mandated to address CHABs and their impacts, which commonly have been managed on a case–by–case, somewhat fragmented basis. A new national US plan, the Harmful Algal Research and Response National Environmental Science Strategy (HARNESS), is "designed to facilitate coordination by highlighting and justifying the needs and priorities of the research and management communities and by suggesting strategies or approaches to address them" (HARNESS 2005). CHABs cross all four critical areas identified for harmful algal research and response: bloom ecology and dynamics; toxins and their effects; food

webs and fisheries; and public health and socioeconomic impacts. HARNESS (2005) also noted that "research on freshwater harmful algal blooms [mostly formed by cyanobacteria] has lagged behind efforts to address marine harmful algal blooms [in general], and there is no comprehensive source of information on the occurrence and effects of freshwater harmful algal blooms in the US". This is ironic, considering that CHABs have been documented to be highly responsive to nutrient pollution (Glibert and Burkholder 2006), and toxic to livestock, wildlife and humans in the US and worldwide (Chorus and Bartram 1999).

This chapter will describe the occurrence of bloom–forming cyanobacteria and their toxins. Cyanobacteria water blooms are defined here as the visible coloration of a water body due to the presence of suspended cells, filaments and/or colonies and, in some cases, subsequent surface scums (surface accumulations of cells resembling clotted mats or paint–like slicks). They are a common occurrence in the US and throughout the world. Over 2000 species of cyanobacteria, many associated with nuisance blooms, are currently known (e.g. Komárek and Anagnostidis 1999, Komárek 2003). Freshwater HABs can assume many forms and while most are composed of prokaryotic cyanobacteria (blue–green algae), eukaryotic species may be present in blooms. While surface scums are commonly associated with blooms of Microcystis, blooms of other species such as Cylindrospermopsis in Arizona, Indiana and Iowa can be characterized by lower cell numbers events. Although not all blooms are characterized by high biomass events, high biomass blooms of toxic species/strains can have significant impacts on ecosystems by adding potentially large amounts of toxins (Glibert and Burkholder 2006). In addition, high–biomass blooms, whether of toxic or nontoxic species, also increase in oxygen demand during blooms and bloom decline, leading to localized hypoxia/anoxia and fish kills. These blooms can also cause habitat loss and food web changes. For example, surface blooms can block the light from reaching the benthos, leading to changes in attached plant communities and increased re–suspension of nutrients from the sediments. High–biomass blooms also commonly lead to economic loss through their negative impacts on recreation activities, and tourism and their production of substances that cause taste–and–odor problems and increased treatment costs in potable water supply plants. Blooms of all types can lead to a decreased quality of life by causing a negative perception of the state of health of the water body.

Multiple interacting physical, chemical and biological factors lead to the formation of CHABs. Planktonic cyanobacteria are a natural component of the phytoplankton in most surface waters of the world. Toxigenic spe-

cies (capable of producing toxins, although not all populations or strains do so) are all naturally occurring members of freshwater and sometimes brackish water phytoplankton. The toxins that they produce can be compared with other naturally produced toxins (Carmichael 1992). Since the first report of toxic cyanobacteria in the late 19th century, all continents except Antarctica have reported toxic blooms (Carmichael 1992). Toxic blooms can be especially problematic and are characterized by their production of hepatotoxins, neurotoxins and acute dermatotoxic compounds (Chorus and Bartram 1999). In the US, blooms of cyanobacteria have been associated with the death of both wildlife and domestic animals (Carmichael 1998). To date, no human fatalities in the US can unambiguously be attributed to cyanobacterial toxins, though in at least one instance, this connection has been made in the popular press (Behm 2003; Campbell and Sargent 2004). Human health effects within the US have primarily included gastrointestinal illness in Pennsylvania (Lippy and Erb 1976), swimmers itch in Florida and Hawaii (Williams et al. 2001) and skin rashes, nausea, and other gastrointestinal disorders in Nebraska (Walker 2005). In Brazil, a gastroenteritis epidemic was associated with the construction of the Itaparica Dam in Brazil (Teixeira et al. 1993), and the hospitalization of 140 children when supplied with drinking water from a reservoir containing a bloom of Cylindrospermopsis raciborskii in Australia (Chorus and Bartram 1999). The use of cyanotoxin–contaminated water in dialysis equipment in Brazil accounted for the only reported human fatalities (Carmichael et al. 2001a; Azevedo et al. 2002).

Distribution of CHABs across the US

Blooms in freshwater environments

Freshwater CHABs are ubiquitous throughout the US and Canada (Fig. 1). Cyanobacteria perform many roles that are vital for the health of ecosystems, especially as photosynthetic organisms, but they may also cause harm through either excessively dense growth or release of toxins or other harmful metabolites. CHABs span habitats ranging from smaller eutrophic prairie ponds to larger more oligotrophic regions of the Great Lakes. This broad distribution is expected given the considerable diversity in habitats occupied by cyanobacteria and the widespread distribution of potential toxicity in the more common genera.

Blooms in estuarine and marine environments

While toxigenic cyanobacteria are generally associated with freshwater blooms, there is increasing recognition that, in certain regions of the world, blooms in estuarine and marine environments may also have an important impact on human health. The Baltic Sea and the Gulf of Finland often sustain massive blooms of toxic *Nodularia spumigena* that are easily visible in satellite images of the region (Sivonen et al. 1989). These blooms affect the natural biota and can spoil recreational opportunities on the coastline. Similarly, large blooms of benthic cyanobacterium *Lyngbya majuscule* in Moreton Bay, Australia (Albert et al. 2005), and off the Hawaiian Islands (Moikeha and Chu 1971; Moikeha et al. 1971) have caused skin, eye and respiratory effects on recreational users of these waters. Not all detrimental effects are directly associated with toxins; for example, increased cyanobacterial biomass in Florida Bay and other estuaries has been associated with sea grass die–offs and the decline of the adjacent coral reef systems (Williams et al. 2001).

  Both *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii* have only limited tolerance to salinity (< 2–7 ppt) and this may limit the growth of these common toxic species in brackish waters environments (Barron et al. 2002). Increasing salinity in oligohaline and mesohaline estuaries results in increased osmotic stress and aggregation of cyanobacterial cells (Sellner et al. 1988). Many cyanobacterial species rapidly drop out from phytoplankton assemblages as salinity concentrations exceed their thresholds and thus are limited to freshwater tidal portions of these major rivers (Sellner et al. 1988). Nevertheless, increased freshwater inputs into rivers from increased runoff dilute the estuarine environment and can promote blooms of these toxic cyanobacterial (Sellner et al. 1988). For example, Lehman et al. (2005) recently documented a large bloom of *M. aeruginosa* in the upper San Francisco Bay Estuary. The bloom was widespread throughout 180 km of waterways; microcystins were detected at all stations sampled, and were also found in zooplankton and clam tissues. Other species of toxigenic cyanobacteria readily bloom in full salinity conditions and can dominate these environments. Non–toxic *Aphanizomenon flos–aquae* and toxic *Nodularia spumigena* form high biomass blooms in the Baltic Sea, and have increased in abundance (Sivonen et al. 1989; Finn et al. 2001). Anabaena (*Aphanizomenon*) *aphanizomenoides* occasionally has been reported to be abundant in the Neuse Estuary in North Carolina (Lung and Paerl 1988).

Changes in the Distribution of Toxic Cyanobacteria

In considering long–term trends of both the frequency of occurrence and distribution of cyanobacterial toxins in the US and Canada, it is important to recognize the remarkable advances that increased interest and awareness that has brought to the discipline over the last few decades. Both the number of known cyanobacterial toxins and toxigenic species has dramatically increased with the increased focus. While few, if any, states had monitoring programs for cyanobacterial toxins in the mid 1970's in most cases, toxic cyanobacteria were identified as being responsible for livestock or wildlife fatalities. By 2005, a number of states had monitoring programs in place, resulting in an increase in the number of CHABs reported. It is difficult to determine if this increasing number of reports represents an increased incidence of CHABS, or is simply a reflection of increasing awareness and monitoring efforts.

Several geographical regions have recently introduced "regional" surveys for the occurrence of cyanobacterial toxins. These regional studies have documented that the occurrence of cyanobacterial toxins has expanded outside of the midwestern prairie states (Fig. 3, see Color Plate 1). Studies in New York, Nebraska, New Hampshire and Florida have shown an increase in the abundance of cyanobacterial blooms. Sasner et al. studied 50 New Hampshire lakes and reported that all had detectable levels of microcystins (Sasner Jr. et al. 1981). Particulate concentrations of microcystins in the bloom spanned over four orders of magnitude and ranged from 0.8 to 31,470 ng/g^{-1} wet weight; concentrations were generally higher in the summer than in the spring. In a similar study of 180 New York lakes and rivers, over 50% of the samples contained easily detectable levels of particulate microcystins in the water column, ranging from 0 to over 1000 μg/L (Boyer et al. 2004). Results of a short–term occurrence study by Carmichael et al. (unpublished 2006) in the Pacific Northwest states of Washington, Oregon and Northern California (Fig. 4, see Color Plate 1), for the years 2002–2005, show that producers of microcystin and anatoxin–a occur on regular basis.

In warmer climates, both the intensity and duration of these bloom events increase. For example, cyanobacterial blooms are common in Florida lakes, rivers, streams and ponds; data has been collected on a limited basis since 1999. Approximately 20 bloom–forming cyanobacteria, including *Microcystis*, *Cylindrospermopsis*, *Anabaena*, *Aphanizomenon*, *Lyngbya* and *Planktothrix*, are found distributed throughout the state with cyanotoxins detected in surface waters and post–treated drinking water (Williams et al. 2001).

Microcystin concentration was most often detected within the 0.1 to 10 μg L^{-1} range with a maximum concentration of 107 μg L^{-1}; detected in post–treated drinking water at a maximum concentration of ~10 μg L^{-1} (Burns 2005). Anatoxin–A was not detected in most samples collected, but a maximum concentration of 156 μg L^{-1} was detected in one sample; detected in post–treated drinking water at ~10 μg L^{-1}. Cylindrospermopsin was detected between 10 and 100 μg L^{-1} (Burns 2005). The maximum cylindrospermopsin concentration detected in surface waters was 202 μg L^{-1}. The maximum concentration of cylindrospermopsin detected in post–treated drinking water was ~100 μg L^{-1} (Burns 2005). The first report of *cylindrospermopsis* in North America is mapped in Fig. 5.

While there is some evidence for seasonal changes in CHABS occurrence (Graham et al. 2004), overall there is a paucity of data related to the temporal distribution of CHABs.

**<u>Do invasive species change the occurrence of toxic species?</u>**
Zebra mussels and Lake Erie (Vanderploeg et al. 2001)

The Great Lakes and especially Lake Erie have experienced many problems with invasive species over the past 100 years. The dreissenid zebra mussel, *Dreissena polymorpha* established itself in Lake Erie in the late 1980's and by 1995 blooms of the toxic cyanobacterium, *Microcystis aeruginosa* had become a problem. Studies done since the late 90's have led to the conclusion that zebra mussel selectively filtrate toxic *Microcystis aeruginosa,* promoting toxic *Microcystis* blooms. This has resulted in new efforts to understand and control CHABs in the Great Lakes.

Fig. 1. Distribution of Documented CHAB outbreaks in North America. Unpublished data (Carmichael 2006).
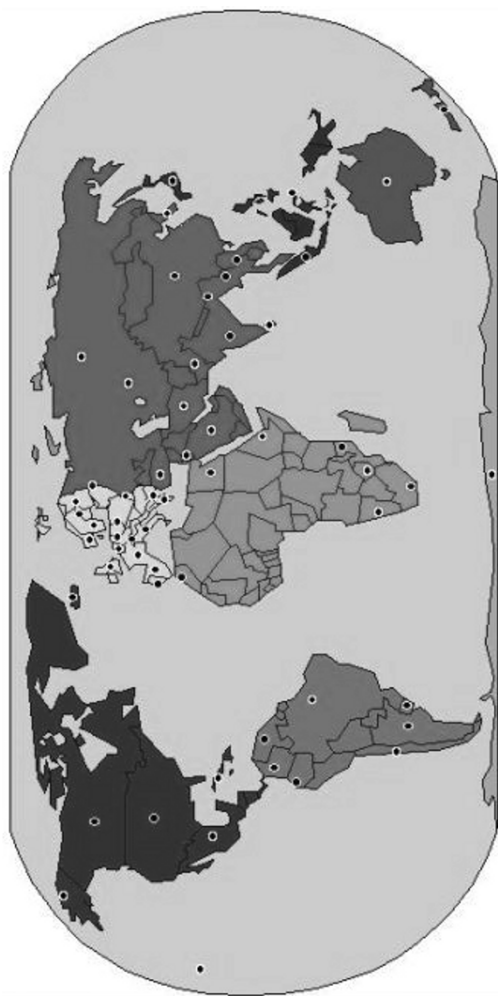
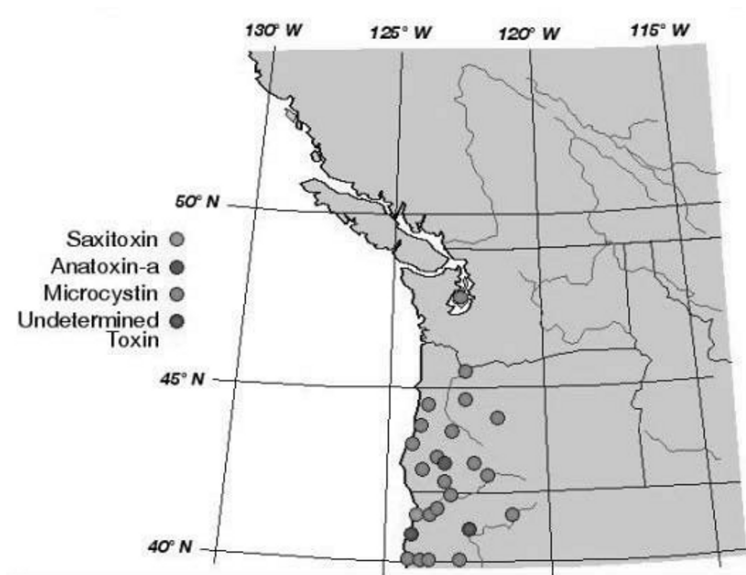**Fig. 2.** Countries Reporting One or More CHAB Outbreaks. Unpublished data (Carmichael 2006).
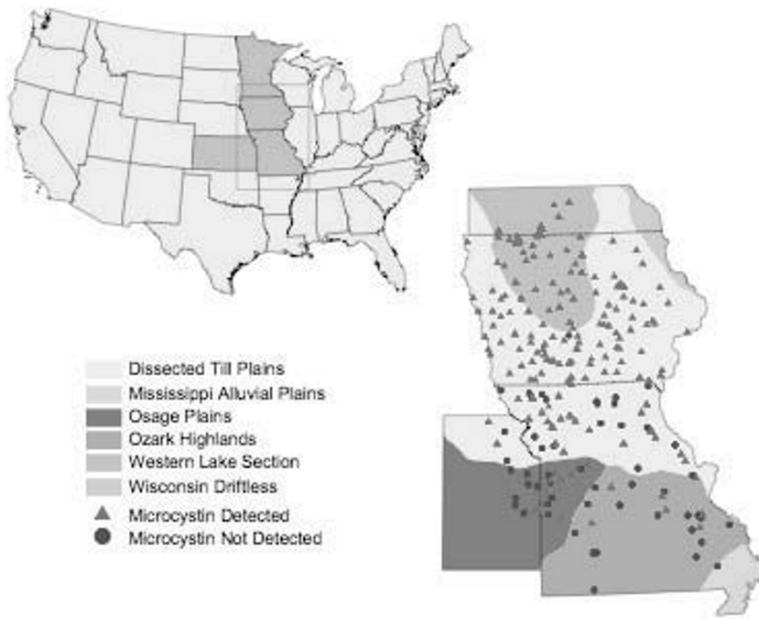
Fig. 3. Trends in Microcystin Occurrence in Midwestern Lakes. Taken from Graham et al. 2004. (See Color Plate 1).

Fig. 4. Cyanotoxin Events in the Pacific Northwest (2001–2005). Taken from Carmichael 2006. (See Color Plate 1).
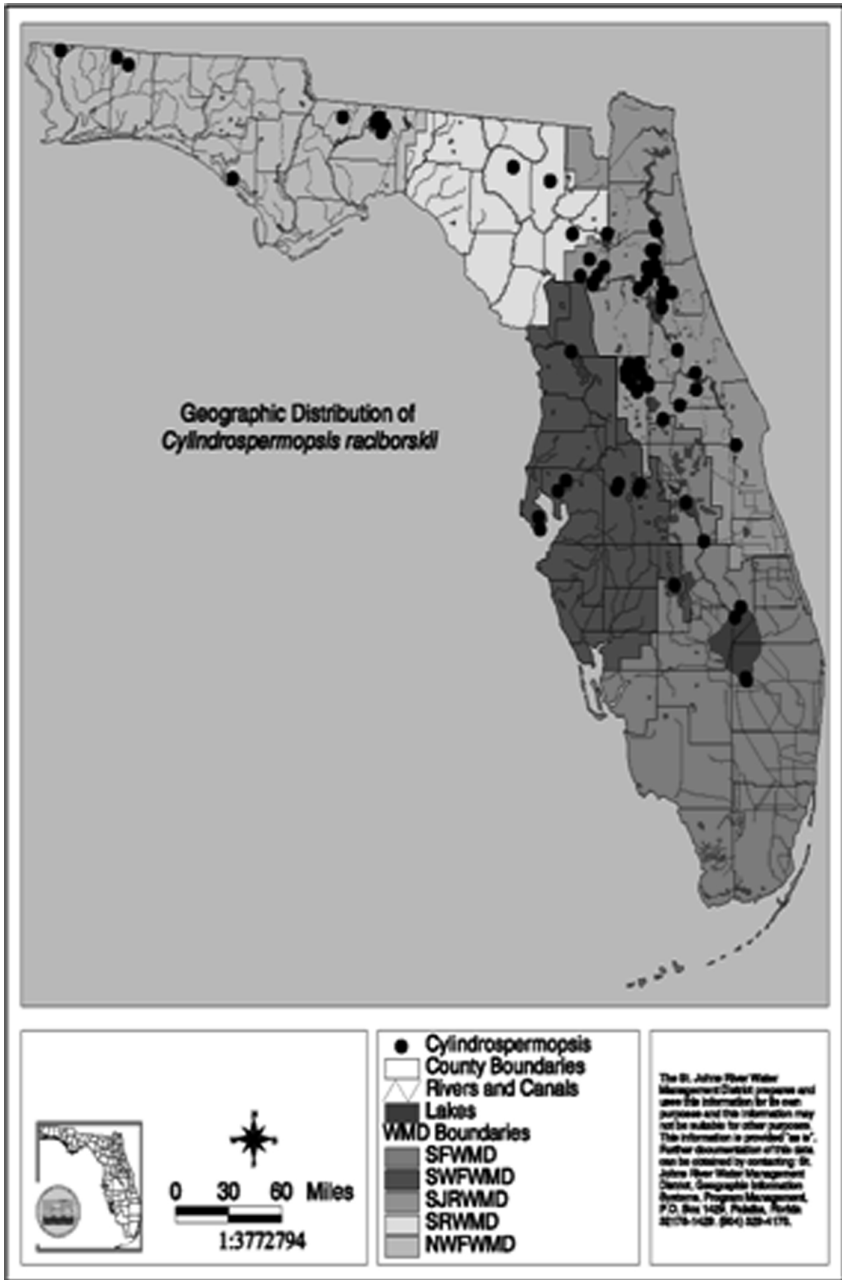
Fig. 5. Florida Distribution of *Cylindrospermopsin.* (Williams et al. 2001).

Cyanotoxin Production

The taxonomy of cyanobacteria, often imprecise, is currently under a state of revision. In general, cyanobacteria cells may be unicellular or filaments, existing singularly or in colonies. Morphological features can be distinct or variable, which has lead to considerable uncertainty when using the botanical taxonomic system that is based on morphological characteristics. For example, an early guide by Geitler (1932) and others listed more than 2000 species of cyanobacteria. Drouet and Daily, in a series of monograms published in the 1950s and 1960s (e.g. Drouet and Daily 1956; l957; Drouet l968), revised this flora using only the simplest of markers to lump everything together in only 62 species. Other taxonomists such as Stanier (1971), Castenholz (1992) and others proposed a taxonomic scheme for cyanobacteria based not on the botanical system, but using the provisions of the International Code of Nomenclature for Bacteria (Lapage et al. 1992). This system uses axenic clonal cultures and morphology as well as modern molecular and biochemical techniques to define the species. Despite the age of these proposals, only one taxon of cyanobacteria is currently described under the International Code of Nomenclature of Prokaryotes or ICNP (Euzeby 2004). Bergey's Manual of Systematic Bacteriology contains only a few of the many described cyanobacterial genera, and no species. Recently, Castenholz (2001) reversed his earlier position and stated "I believe that the current rules of Nomenclature (botanical and bacteriological) should be mostly disregarded" and indicated that he preferred a moratorium on naming any species or undertaking any taxonomic revisions for 50 years or more so that molecular data sets could accrue (Hoffmann 2005).

The botanical system for cyanobacteria has recently been revised using traditional morphological characters resulting in a major revision of species, especially for the non–heterocystous filamentous forms such as *Oscillatoria* (Komárek and Anagnostidis 1999, 2005). The revised system resurrects many of the older botanical names for genera. While cell structure and ultrastructure (thylakoids, cell wall formation, cellular dimensions) and molecular data have been congruent, sheath characteristics, which are critical in definition of both species and genera, are not always congruent with the molecular data. Unfortunately, ultrastructure, biochemical, and molecular studies, such as RAPD fingerprinting and 16S rRNA gene sequences are only available for a small portion of cyanobacterial taxa.

The ecological diversity observed in cyanobacteria is grossly underestimated if one looks only at those taxa in culture collections (the taxa which are the source of the molecular and biochemical data). In some cases, molecular sequencing produces similar phylogenies. However, it is also quite easy to generate quite distinct phylogenetic lineages, depending on the particular part of the genome or trait that was used to generate the phylogeny. Most field taxonomists still rely on the traditional morphology–based botanical approach because molecular data have not yet been available for many species (Burkholder 2002), although the use of molecular based techniques is rapidly increasing, especially in those species that lack or contain confusing morphological features. Fortunately, toxic cyanobacteria have received concentrated attention, and so the taxonomy of these taxa may be among the first to be revised based on polyphasic character sets.

Toxic cyanobacteria blooms are associated with a wide diversity of species and environments. Chorus and Bartram (1999) (1999), in their classic monogram for the World Health Organization, *Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring and Management,* reviewed which species of cyanobacteria have been associated with the production of toxins based on observations and reports since the mid 1980's. Since that time, there have numerous new observations, including new toxigenic species such as the unknown *Stigonematales* species responsible for avian vacuolar myelinopathy (AVM) and new toxins such as β–N–methylamino alanine (BMAA) produced by a wide number of species. *Phormidium* sp., which produces microcystin, has been found in shallows of reservoirs. Microcystin LA is reported to account for the protein phosphatase inhibitory activity of the terrestrial cyanobacterium, *Hapalosiphon hibernicus*. Other terrestrial isolates produce the toxin β–methylamino alanine (BMAA). With the recent report of microcystins from lichen–associated *Nostoc*, it becomes apparent that non–aquatic environments must also be considered when discussing cyanobacterial toxicity. Table 1 lists 22 genera and 42 species associated with toxin formation. Most of the data was obtained from planktonic environments, although there are a number of toxic benthic species. Where appropriate, we have updated the nomenclature to the taxonomic scheme of Komárek and Anagnostidis (Komárek and Anagnostidis 1999, 2005).

Table 1. Major Toxigenic Cyanobacteria Species, their Toxins and Distribution.

Organism	Toxin	Location	Reference
Anabaena spp.	microcystins	Denmark, Egypt, Finland, Germany, Ireland, Japan, US	
	anatoxin–a		
A. circinalis	microcystins	Finland, France, Australia	
	saxitoxins		
A. flos–aquae	microcystins	Canada, Finland, Norway, Canada	
	anatoxin–a		
A. lemmermannii	microcystins	Finland	
	saxitoxins		
A. planktonica	anatoxin–a	Italy	
A. variabilis	BMAA	US	
Aphanizomenon spp.	anatoxin–a	Finland, Germany	
A. (flos–aquae) issatschenkoi	saxitoxins	US	
A. flos–aquae	anatoxin–a(s)	Canada	
A. gracile	LPS endotoxins	US	
A. lemmermannii	saxitoxins	Portugal, Denmark	
	anatoxin–a(s)		
A. ovalisporum	cylindrospermopsin	Israel	
Arthrospira fusiformis	microcystins	Africa, Spain	
Anabaenopsis millerii	microcystins	Greece	

Organism	Toxin	Location	Reference
Cylindrospermum spp.	anatoxin–a	Finland	
Cylindrospermospis raciborskii, C. phil-lipinensis	saxitoxins cylindrospermopsin BMAA	Brazil, Australia, Hungary, US, Australia	
Haphalosiphon hibernicus	microcystins	US	
Lyngbya spp. *L. majuscula*	pahayokolide–a BMAA	US Africa, Australia, US, Pa-cific Is.	
L. wollei (*Plectonema wollei*) Farlowe ex Gomont	dermatotoxins saxitoxins	US	
Microcystis spp.	microcystins anatoxin–a BMAA	Worldwide	
M. aeruginosa *M. viridis* *M. botrys*	microcystins microcystins microcystins	Worldwide Japan Denmark	
Nodularia spumigena *N. spumigena*	nodularins nodularins	Canada Australia, New Zealand, Baltic Sea	
Nostoc spp.	microcystins	Finland, England and US	

Organism	Toxin	Location	Reference
Oscillatoria spp.	BMAA	Finland, Scotland, Ireland and US	
O. brevis (Phormidium breve) (Kützing ex Gomont) Agnostidis et Komárek	anatoxin–a LPS endotoxins	Switzerland	
O. limosa Agardh ex Gomont	microcystins	US	
O. nigroviridis (O. nigro-viridis) Twaites ex. Gomont sensu Parakutty	dermatotoxins	US	
O. tenuis	LPS endotoxins	US	
Phormidium spp.	microcystins BMAA saxitoxins	US and Italy	
Planktothrix	microcystins, anatoxin–a	US, Finland	
P. agardhii	Microcystins BMAA	Denmark, Finland, Norway, Ireland	
P. flavosum	anatoxin–a	France	
P. formosa*	homoanatoxin–a	Norway	
P. mougeotii* (P. isothrix) (Skuja) Komárek and Komarkova	microcystins	Denmark	
Plectonema sp.	BMAA	Unknown origin	
Prochlorococcus marinus	BMAA	Sargasso Sea	

Organism	Toxin	Location	Reference
Raphidiopsis spp.			
R. curvata	cylindrospermopsin anatoxin–a,	Japan	
R. mediterranea	homoanatoxin–a		
Schizothrix calcicola	aplysiatoxins LPS (nontoxic)	Pacific Islands and US	
Stigonematales sp.	avm	US	
Synechococcus sp.	BMAA	US	
Trichodesmium thiebautii	BMAA saxitoxin	Sargasso Sea	
Umezakia natans	cylindrospermopsin	Japan	

*denotes a species designation that is considered invalid using the current classification scheme

Cyanobacteria toxicity is very common in natural blooms with estimates of toxicity approaching as high as 50% (WHO 2003). In the late 1990s, in an attempt to systematically estimate the abundance of toxic cyanobacteria, approximately 200 strains of the Pasteur Collection of Cyanobacteria (PCC) were screened for microcystins using ELISA and HPLC–PDA techniques. G. Codd and R Rippka (unpublished) confirmed that more than 20 species produce cyanobacterial toxins, of which 11 strains of *Microcystis* and 1 strain of *Nostoc* produced concentrations of more than 100 µg microcystins per gram dry weight (Boyer 2006). Many of the strains in the PCC have also been screened for the genetic potential for microcystin production using PCR techniques (Dittmann and Borner 2005). Similar results were also obtained from the NIVA culture collection in Norway, where a large percentage of the nearly 500 strains of cyanobacteria in the collection appeared to produce toxins or other bioactive substances (Skulberg 1984). Microcystin and harmful hepatotoxic peptides were produced by many, but not all strains of the cyanobacteria belonging to the genera *Anabaena, Microcystis, Planktothrix* and *Nostoc* (Hisbergues et al. 2003).

Toxigenic cyanobacteria are generally defined as species that have toxic strains (populations), which are capable of producing neurotoxic, hepatotoxic or dermatotoxic compounds (Burkholder and Glibert 2006). This term is important to note, since within a given species of toxigenic cyanobacteria, there commonly occur both toxic and nontoxic strains (Chorus and Bartram 1999; Burkholder and Glibert 2006). If one expands the definition of toxins to include bioactive peptides with protease inhibition activity such as the micropeptins, cyanopeptolins, microviridins, oscillapeptins, oscillamides, nostopeptins, aeruginosins, aeuginopeptins anabaenopeptilides, anabaenopepins and the cytotoxic compounds from marine origin, then the list of toxin–producing cyanobacteria species becomes quite large. Recently, the PCC were screened for the presence of non–ribosomal peptide synthetase (NRPS) genes, those genes responsible for the synthesis of many bioactive peptides such as the microcystins, microveridins and anabaenapeptins (Christiansen et al. 2001). This biosynthetic activity is widespread throughout the cyanobacteria taxa, with NRPS activity found in over 75% of the 146 strains tested. While the presence of this gene activity does not directly correlate to toxicity, it indicates the potential for production of bioactive peptides. The non–ribosomal peptide synthetases used for microcystin toxin biosynthesis in cyanobacteria allow for considerable structural diversity (Christiansen et al. 2001). More than 400 individual peptides have been identified, mostly from *Anabaena*, *Lyngbya*, *Nostoc* and *Microcystis* species (Krishnamurthy et al. 1986;

Luesch et al. 2000; Pluotno and Carmeli 2005). Many of these peptides have protease inhibition activity and can lead to detrimental effects if consumed as part of the food web.

In addition to the production of toxins, cyanobacteria have often been associated with the production of taste and odor compounds in with drinking water; the two most are geosmin and 2–methylisoborneol (MIB), although a number of different hydroxyketones and β–ionone derivatives can also add to the off–flavors associated with freshwater cyanobacteria (Izaguirre and Taylor 2004). While these taste and odor compounds are not actually toxic, they are of concern to the consuming public when their drinking water has an "off" flavor, resulting in considerable monitoring and treatment costs to water supply providers. Taste and odor issues are complex and need not be associated solely with the presence of cyanobacteria. Many actinomycetes bacteria such as *Actinomyces* and *Streptomyces* species, aquatic fungi such as *Basidiobolus ranarum*, and myxobacteria such as *Nannocystis exedens* can also produce these compounds (Niemi et al. 1982). Table 2 illustrates the large number of species associated with the production of compounds that affect taste and odor. These species may or may not be the same species reported to produce toxins. The biosynthetic pathways for taste and odor compounds are separate and unrelated to the toxin biosynthetic pathways (Carmichael 2001).

Table 2. Major Cyanobacterial Taste and Odor Producing Species.

| Organism	Compound
Anabaena spp.	Geosmin
A. circinalis	
A. laxa	
A. macrospora	
A. scheremetievi	
A. spiroides	
A. viguieri	
Aphanizomenon flos–aquae	Geosmin
Calothrix sp. (*C. parietina*)	Ketones, Ionones
Fischerella muscicola	Geosmin
Jaaginema geminatum	MIB
(*Oscillatoria geminata*) (Menehgnin ex Gomont Komárek and Anagnostidis)	
Lyngbya spp.	
L. aestuarii (*Lyngbya aestuarii*) Liebman ex Gomont	MIB
L. cryptovaginata (*Planktothrix cryptovaginalt*) (*Škorbatov*) *Anagnostidis et Komárek*	Geosmin
Oscillatoria spp.	
O. chalybea (*Phormidium chalybeum*) (*Mertens ex Gomont*) *Anagnostidis*	MIB
O. raciborskii (*Planktothrix raciborskii*) (*Woloszynska*) *Anagnostidis et Komárek et Komárek*	
O. agardii (*Planktothrix agardhii*) (*Gomont*) *Anagnostidis et Komárek*	Geosmin
O. amoena (*Phormidium amoenum*) (*Kützing 1843*) *Anagnostidis et Komárek*	
O. amphibia (*Geitlerinema amphibium*) (*Agardh ex Gomont*) *Anagnostidis*	
O. bornetii (*Tychonema bornetii*) (*Zuka*) *Anagnostidis et Komárek*	

Organism	Compound
O. brevis (Phormidium breve) (Kützing ex Gomont) Anagnostidis et Komárek	
O. variabilis (Oscillatoria variabilis) (Rao)	
O. prolifica (Planktothrix prolifica ([Grevelle] Gomont) Anagnostidis et Komárek	
O. simplicissima (Phormidium simplicissimum) (Gomont) Anagnostidis et Komárek	
O. splendida (Geitlerinema splendidum) (Greville ex Gomont) Anagnostidis	
O. cortiana (Phormidium cortiamum) (Meneghini ex Gomont) Anagnostidis	
et Komárek	Geosmin, MIB
O. curviceps Agardh ex Gomont	
O. limosa Agardh ex Gomont	
O. tenuis Agardh ex Gomont	
Phormidium sp.	
P. autumnale (Phormidium autumnale) [Agardh] Trevisan ex Gomont	Geosmin
P. calcicola (Phormidium calcicola) Gardner	Geosmin, MIB
P. tenue (Phormidium tergestinum) (Kützing) Anagnostidis et Komárek	MIB
Plectonema sp.	Ketones, Ionones
Pseudanabaena catenata	
P. notatum (Leptolyngbya notata) (Schmidle) Anagnostidis et Komárek	
P. catenata	MIB
P. limnetica (Oscillatoria limnetica) (Lemmermann) Komárek and Anagnostidis	
Rivularia spp.	Ketones, Ionones
Schizothrix muelleri	Geosmin
(Symplocastrum muelleri) (Nägeli ex Gomont) Anagnostidis	
Symploca muscorum *Gomont ex Gomont*	Geosmin
Tolypothrix distorta	Ketones, Ionones

Cyanotoxin Occurrence and Distribution

Cyanobacterial toxins fall into several diverse categories and are usually divided based on their biological activity (hepatotoxins versus neurotoxins) or chemical structure (peptide toxins versus alkaloids) (Table 3). This division is becoming more complicated as new toxins are being identified. For example, in 1988, there were 10 reported microcystins; as of 1998, there are over 60 different chemically identified microcystins (Chorus and Bartram 1999). In the sections below, we summarize the basic classes of cyanobacterial toxins, their source organisms and their biological effects.

Microcystins

As shown in Table 1, microcystins have been reported in *Microcystis*, *Anabaena*, *Oscillatoria*, *Planktothrix*, *Nostoc*, *Hapalosiphon* and *Anabaenopsis* with new sources appearing yearly (Huisman and Hulot 2005). Microcystins (Fig. 6) contain 7 amino acids in their cyclic peptide ring system, are closely related to nodularin (Fig. 7) or nodulapeptins, which have 5 amino acids in the ring system, as well as anabaenopeptins, aeruginopeptin and other bioactive peptides (Sivonen and Rapala 1998; Harada 2004). Most of these peptides are hepatotoxins, though their LD–50 depends on the specific amino acids present.
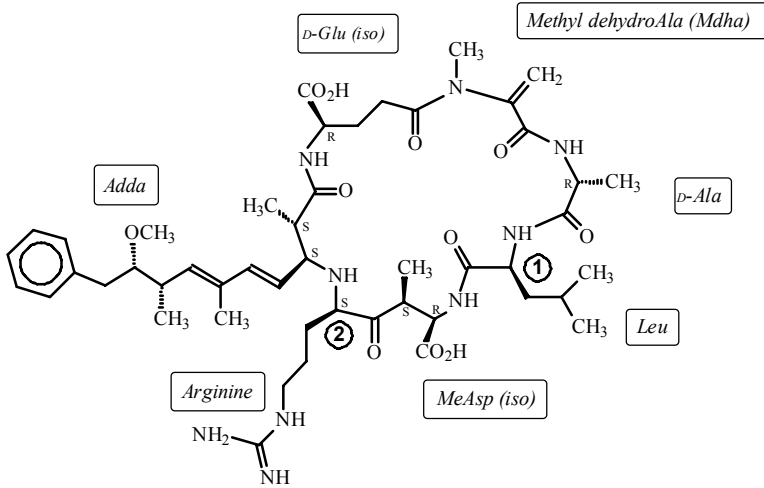
Fig. 6. The generic structure of a microcystin.

Variations occur primarily at positions 1 and 2. For example, micro-cystin–LR contains the amino acids leucine (L) and arginine (R) at positions 1 and 2 respectively; microcystin–RR has arginine at both positions. Nodularins are similar with the five amino acids Adda–γGlu–Mdhb–βMeAsp–Arg making up the core ring system (Harada et al. 1996).

Table 3. Commonly observed cyanotoxins in US fresh, estuarine and marine waters. (adapted from Codd et al. 2005).

Toxin	Type and (# congeners)	Mode of Action
Hepatotoxins		
Microcystins	Cyclic heptapaptides (>70)	Protein phosphatase inhibitor, tumor promoter
Nodularins	Cyclic penta–peptide (9)	Protein phosphatase inhibitor,
Cylindrospermopsin	Guanidine alkaloid (3)	Protein synthesis inhibitor, genotoxic and necrotic injury to liver and other organelles.
Neurotoxins		
Anatoxin–a	azobicyclic alkaloid (5)	Postsynaptic neuromuscular blocking agent and Acetylcholinesterase agonist
Anatoxin–a(S)	Guanidine organophosphate (1)	Acetylcholinesterase inhibitor
Saxitoxin	Carbamate alkaloid (>20)	Sodium channel blocker
B–methylamino alanine	Modified amino acid	Neurodegenerative agent
Dermatotoxins		
Lyngbyatoxin–a	Indole Alkaloid (1)	Inflammatory agent and Protein Kinase C activator
Aplysiatoxin	Polyacetate Alkaloid (2)	Inflammatory agent and Protein Kinase C activator
Endotoxins		
Lipopolysaccharides	Lipopolysaccharides	Gastrointestinal irritants
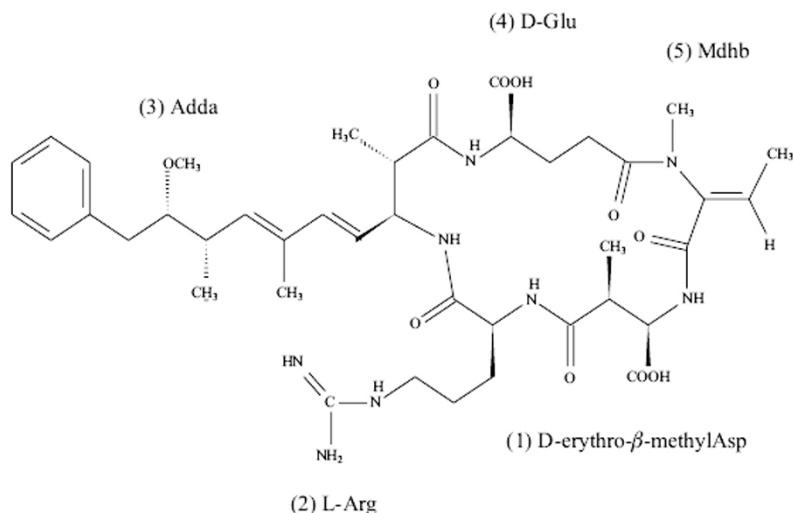
Fig. 7. The generic structure of a Nodularin.

Cylindrospermopsin

Separate from the hepatotoxic cyclic peptides is the hepatotoxic alkaloid cylindrospermopsin (Fig. 8a). This toxin is usually produced by *Cylindrospermopsis raciborskii* and an outbreak of this organism in the drinking water supply on Palm Island, Queensland Australia led to a severe outbreak of hepatoenteritis among the inhabitants of the island (WHO 2002). Cylindrospermopsin has also been reported from *Umezakia natans* in Japan, *Aphanizomenon ovalisporum* in Israel (Sivonen and Jones 1999), and *Raphidiopsis Curuata* in China (Li et al., 2001). It was generally assumed that the toxigenic cylindrospermopsin–forming species only bloomed in tropical and arid environments and *Cylindrospermopsis raciborskii* blooms are commonplace in the warmer Florida drinking water reservoirs. Increasing reports of *C. raciborskii* occurring in temperate zones of Europe and the U.S have been documented (Padisak 1997). Cylindrospermopsin–producing blooms can be both high and low–biomass events, leading to difficulties in identifying the source of this toxin.

Anatoxin–a

The neurotoxic cyanobacterial toxins consist of anatoxin, anatoxin–a(S), saxitoxin and related analogs. The most important of these compounds

from an environmental health aspect is probably anatoxin–a (Fig. 8b) (Carmichael et al. 1975). Anatoxin–a was originally isolated from *Anabaena flos–aquae* (Devlin et al. 1977), but is also reported in *A. planktonica*, *Oscillatoria* spp., *Planktothrix* spp., and *Cylindrosperum*. Homoanatoxin–a, a toxic homologue with a propyl group replacing the acetyl group, was isolated from *Phormidium* (*Oscillatoria*) *formosa*. Both are potent nicotinic agonists and act as neuromuscular blocking agents.

Anatoxin–a(S)

Anatoxin–a(S) (Fig. 8c) is a naturally occurring organophosphate, with no reported structural variants, produced by *Anabaena flos–aquae* strain NRC 525–17 and more recently by *Anabaena lemmermannii* (Henriksen et al. 1997; Onodera et al. 1997). It is distinct from the neurotoxic anatoxin–a in both its chemical structure and biological mode of action (Mahmood and Carmichael 1986b). Anatoxin–a(S) acts as an acetyl–cholinesterase inhibitor and its name was derived from the fact that the intoxicated animals often suffered from extreme salivation (Matsunaga et al. 1989). Anatoxin–a(S) is commonly reported in the prairie states of the US (Kenefick et al. 1992); this may lead to an underestimate of its occurrence since, lacking a clear cyanobacterial source, death of livestock or pets from anatoxin–a(S) would likely be attributed to pesticide intoxication.

Saxitoxins and related analogs

Saxitoxins (STX) (Fig. 8d) are representative of a large toxin family referred to as the Paralytic Shellfish Poisoning (PSP) toxins. These include the N–1 hydroxysaxitoxin or neosaxitoxin (NeoSTX), 11–sulfate analogs of STX and NeoSTX called gonyautoxins (GTX) and 6 N21 sulfo derivatives of STX, neoSTX and GTX toxins B1, B2, C1 to C4 (Mahmood and Carmichael 1986a; Chorus and Bartram 1999). Not all of these 18 PSP analogs have been identified in marine dinoflagellates and shellfish. These toxins are identical to those produced by some toxigenic marine dinoflagellates that accumulate in shellfish that feed on those algae (Anderson 1994). STX, neoSTX, C1, C2 and GTX have all been reported in freshwater cyanobacteria including *Aphanizomenon* spp., (Pereira et al. 2004, Li and Carmichael, 2003, Li et al. 2000) *Anabaena circinalis, Lyngbya wollei* and a Brazilian isolate of *Cylindrospermopsis raciborskii* (Lagos et al. 1999).
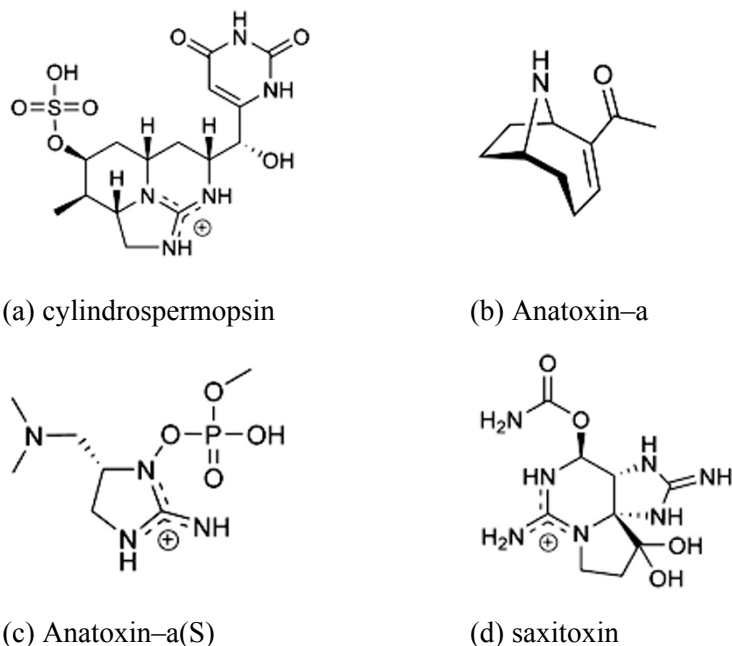
(a) cylindrospermopsin

(b) Anatoxin–a

(c) Anatoxin–a(S)

(d) saxitoxin

Fig. 8a–d. Structures of the alkaloid cyanobacterial toxins.

β–methylamino alanine (BMAA)

A recently discovered cyanobacterial neurotoxin is non–protein amino acid β–N–methylamino–L–alanine (BMAA). BMAA, originally isolated from a Guam cycad, *Cycas circinalis*, has been implicated as the causative agent of amyotrophic lateral sclerosis or Parkinsonism dementia (Murch et al. 2004b). Symbiotic *Nostoc* species associated with the roots of cycads produce BMAA and it accumulates in the seeds of the plant. The indigenous Chamorro people of Guam feed on the flying foxes, which consume these seeds, and are subject to a classic biomagnification of the toxin up through the higher trophic levels (Cox et al. 2005). BMAA may also be incorporated into peptides or proteins, both increasing its biomagnification and acting as a reservoir to slowly releasing toxins upon peptide hydrolysis (Murch et al. 2004a).

Cox et al. (2005) surveyed 30 different strains of cyanobacteria obtained from culture collections that spanned the entire spectrum of cyanobacterial morphology and taxonomy for the presence of BMAA. Although BMAA is most common in *Nostoc* species, with 7 out of 10 isolates con-

taining high levels of either the free or protein–bound amino acid, it is ubiquitous in its distribution, being found in freshwater, marine, brackish and terrestrial environments. Only 1 of the 3 species tested, a freshwater *Synechocystis* species, lacked BMAA. Algal strains in culture often produce much lower levels of toxins than found *in situ* suggesting that there is potential for widespread human exposure to this toxin (Cox et al. 2005). BMAA was recently detected in the brains of nine Canadian Alzheimer patients, but was not found in the brains of 14 other Canadians that died from causes unrelated to neuro–degeneration (Murch et al. 2004a, 2004b). Since cycads are not part of the normal Canadian flora, cyanobacteria may be the source of BMAA in these patients. Drinking water contaminated by cyanobacterial blooms has been suggested as a potential pathway for human exposure to BMAA and a topic of future data needs.

Lyngbyatoxins, Aplysiatoxin and Debromaplysiatoxin.

Lyngbyatoxins, contact irritants, are mainly marine water toxins but their occurrence in freshwaters has not been fully assessed. *Lyngbya*, an aggressive genus capable of widespread growth, has been found in freshwater supplies in the US. *Lyngbya* blooms are common in Florida, forming mats in freshwater springs and overgrowing some coastal reefs, where low levels of *Lyngbya*–toxin–A, debromoaplysiatoxin and saxitoxin have been detected (Williams et al. 2001). More data are needed to determine its health effects and occurrence in freshwater supplies. The inflammatory activity of *Lyngbya* is caused by aplysiatoxin and debromoaplysiatoxin, which are tumor promoters and protein kinase–C activators (Berry et al. 2004). Debromoaplysiatoxin along with other toxic compounds has also been isolated from other members of the family Oscillatoriaceae such as *Schizothrix calcicola* and *Oscillatoria nigroviridis* (Chorus and Bartram 1999).

Other Bioactive Compounds

Many cyanobacteria including *Microcystis*, *Planktothrix* and *Anabaena* species have the ability to produce cyclic and linear peptide protease inhibitors that are potentially toxic (Harada 2004). Examples of these compounds include the micropeptins, cyanopeptolins, microviridin, oscillapeptin, oscillamide, nostopeptin, aeruginosins, aeruginopeptins, anabaenaopeptilides, anabaenopeptins and circinamide (papain inhibitor). Two such compounds isolated from *Microcystis aeruginosa*, aeruginopept-

ins and cyanopeptolins, are shown in Fig. 9. The effects of these compounds on the toxicity of cyanotoxins is not well known.
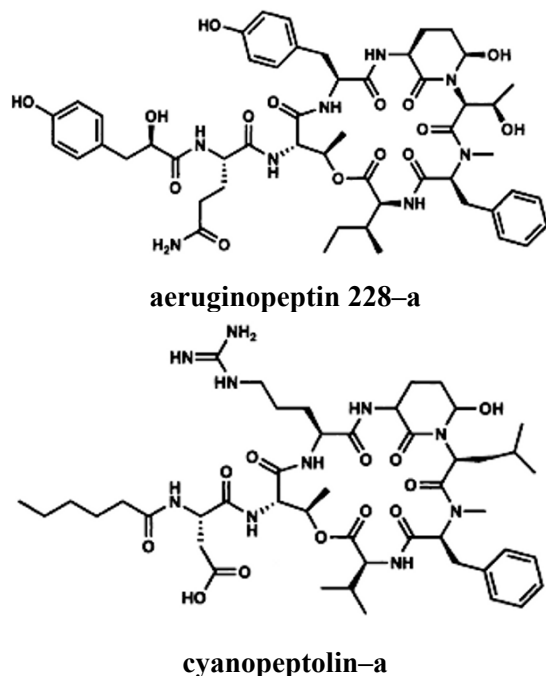

aeruginopeptin 228–a

cyanopeptolin–a

Fig. 9. Structures of two bioactive peptides isolated from Microcystis aeruginosa.

Impacts on Waterbody Health and Ecosystem Viability

The factors that lead to CHAB formation and induce toxin production are not well known (Chorus and Bartram 1999). Although multiple studies have examined the empirical relationship between environmental variables and toxin concentrations, few have measured the many different variables necessary to compile a comprehensive data set for analysis, (Kneale and Howard 1997). Even fewer have analyzed the data based on sound statistical methods recommended by EPA and other agencies (EPA 2003). Temporal and spatial patterns of CHAB formation are often complex, and the mechanisms of bloom progression are largely unexplained. Additionally, CHABs can include both toxic and non–toxic strains, and many strains can be present during CHABs in specific lakes (Vezie et al. 1998; Jacoby et al. 2000; Pawlik–Skowronska et al. 2004). A major source of variation in toxin concentration appears to be the strain–dependant influence of envi-

ronmental factors on toxin release (Rapala and Sivonen 1998; Vezie et al. 2002) and successive replacement of toxic and non–toxic species (Kardinal and Visser 2005). No single modeling study has attempted to predict the dynamics of toxin releases and/or toxin levels, reflecting a limited understanding of factors influencing toxin release.

Several recent reviews (Paerl 1996; Soranno 1997; Carmichael et al. 2001b; Saker and Griffiths 2001; Landsberg 2002; Codd et al. 2005; Huisman and Hulot 2005). provide a comprehensive discussion of individual environmental factors that may be responsible for CHABs and their correlated effects on ecosystem health. Physical factors include temperature, light availability and meteorological conditions. Hydrologic factors include alteration of water flow, turbidity and vertical mixing. Chemical factors include nutrient loading (principally nitrogen [N] and phosphorous [P]), pH changes and trace metals, such as copper, iron and zinc.

Freshwater Habitats

Most of the world's population relies on surface freshwaters as their primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect public health. Recent reports suggest that CHABs are an emerging issue in the US because of increased source–water nutrient pollution causing eutrophication. Although CHABs historically have been strongly correlated with nutrient over–enrichment in many freshwater lakes with low abiotic turbidity (e.g Schindler 1987, Whitton and Potts 2000), flowing waters can also develop planktonic CHABs in slowly flowing segments (e.g Pinckney et al. 1997). Intermediate systems – run–of–river impoundments or reservoirs – generally have faster flushing and higher abiotic turbidity from suspended sediment loads than natural lakes (Wetzel 2001). In these systems light, rather than nutrients, often is the most important resource limiting cyanobacteria growth (Cuker et al. 1990). In addition, because of the surface characteristics of their cells, cyanobacteria can be especially susceptible to being attracted to sediment particles (Avnimelech et al. 1982, Søballe and Threlkeld 1988, Burkholder 1992). When that occurs, they become too heavy to remain in the water column and settle out of the water column (Burkholder and Cuker 1991). Nevertheless, during intervals between episodic sediment loading events from land erosion and runoff, dense cyanobacteria blooms can develop in reservoir systems, and nutrients – both nitrogen and phosphorus – can become important in controlling their production (Touchette et al. (submitted), Cuker et al. 1990, Burkholder and Cuker 1991); Fig. 10.

Many eutrophic lakes and impoundments (reservoirs) have documented CHAB problems, for example: The Metropolitan Water District of Southern California (MWDSC) has a long history of algal problems, in the form of planktonic blooms and benthic proliferations (Izaguirre 2005). The main concern is taste and odor, specifically the compounds geosmin and MIB. All of the reservoirs listed below have experienced algal blooms of one kind or another, including in some cases known toxigenic species. In addition, Lakes Mathews, Skinner, Perris and Diamond Valley have developed benthic mats that have resulted in severe off–flavor problems (Izaguirre 2005). MWDSC, is composed of 26 member agencies and supplies drinking water to about 18 million people in six counties in the coastal plain of southern California; its two sources of water are the Colorado River and water from northern California delivered through the California aqueduct feeding into three reservoirs in Riverside County: Lake Mathews, Lake Skinner and Diamond Valley Lake (Izaguirre 2005). In North Carolina, a large, previously undetected, sewer spill that occurred over several months was positively correlated with *Lyngbya* blooms (Frazier 2006).

As research progresses, new toxins from cyanobacteria in freshwater systems are being discovered (Codd et al. 2001; Huisman and Hulot 2005), as well as new potential roles in their impacts on food webs. For example, avian vacuolar myelopathy (AVM), a neurological disease in waterfowl and their predators, was first described in the mid–1990s, with unknown cause (Thomas et al. 1998). AVM causes characteristic lesions in the myelin of the brain and spinal cord. Affected birds have abnormal flying and swimming because their muscle coordination is compromised. The causative agent of AVM is associated with submersed aquatic vegetation such as the aquatic weed hydrilla (Birrenkott et al. 2004). At present, the prime "suspect" is a toxin producing, yet unnamed cyanobacterium within the order Stigonematales that colonizes the leaves of hydrilla and other submersed plants (Wilde et al. 2005). Thus, the working hypothesis being examined is that waterfowl such as coots consume submersed vegetation covered with the toxic cyanobacteria, bioaccumulate the toxin(s), and are impaired in their ability to escape from predators. The affected waterfowl are caught and consumed by higher predators such as bald eagles, which then also develop AVM.
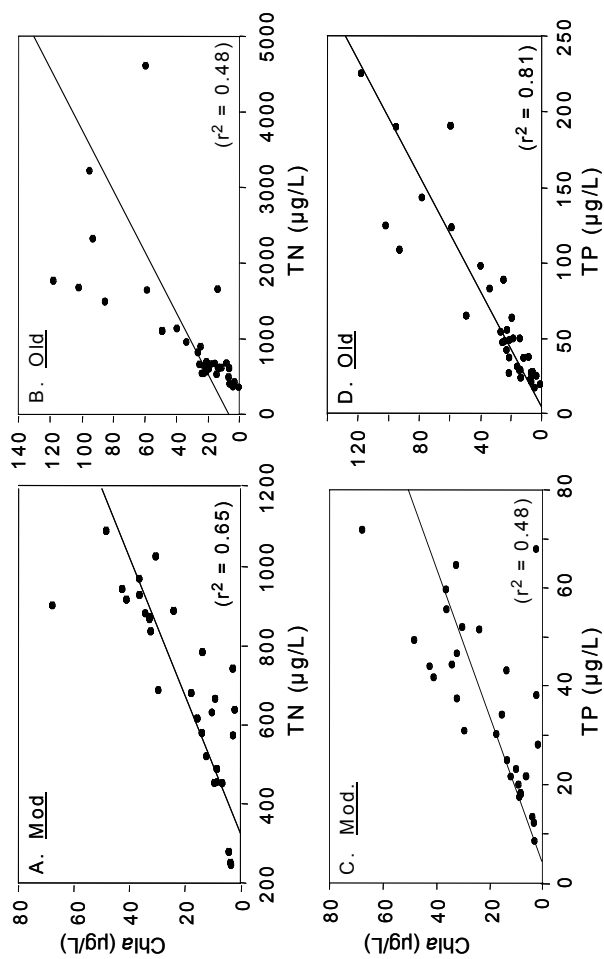
Fig. 10. Relationship between chlorophyll a (chla) and total nitrogen (TN, upper panels) or total phosphorus (TP, lower panels). *(A, C) moderate-age reservoirs (mod., Group 1, 20–30 years post–fill, n = 5) and (B, D) old reservoirs (Group 2, 60–85 years post–fill, n = 6) during summer months when cyanobacteria were dominant* (Touchette et al. (submitted)).

Estuarine Habitats

Estuaries are comprised of high population densities of microbes, plankton, benthic flora and fauna. These organisms tend to be highly vulnerable to human activities and a wide array of human impacts that can compromise their ecological integrity. Many of the factors responsible for CHABS as well as the CHABs themselves affect estuarine habitat health. Anthropogenic stress in the form of increased nutrient input significantly impacts estuaries as well as other water bodies. Modeling studies suggest the importance of nutrient loading to water quality and CHAB probability and severity. Nitrogen (N) and phosphorus (P) enrichment are considered cyanobacteria bloom precursors. Many cyanobacteria are nitrogen–fixers, making N a limiting factor in competition over other phytoplankton species (Burkholder 2002). Although nutrient influences are widely studied factor linked to stimulation of CHABs, the nutrient(s) that are most important in limiting cyanobacteria growth are still in debate, and likely depend upon the habitat and history of nutrition (e.g. Lenes et al. 2001; Glibert et al. 2005, 2006).

Cyanobacteria blooms were noted in the Potomac River and the upper Chesapeake Bay during the 1950s and 1960s coincident with the invasion of water milfoil. Since 1985, cyanobacteria blooms have been documented in the tidal tributaries of Chesapeake Bay almost annually during summer months by the Maryland Department of Natural Resources (MD–DNR) long–term comprehensive water–quality monitoring program. During September 2000, an extensive late summer bloom of *Microcystis* on the Sassafras River, however, was among the first blooms tested for cyanotoxins in Maryland and results were positive for elevated concentrations of microcystin. The microcystin levels (591.4–1041 μg g^{-1} dry wt) led to the Kent County Health Department closing a public beach in the bloom area for the remainder of the year, the first beach closure in the history of the state due to detected levels of cyanotoxins (Tango et al. 2005). Dr. Harold Marshall, a renowned phytoplankton specialist in the Chesapeake Bay area, has also documented significant increases in cyanobacteria biomass, mostly as *Microcystis aeruginosa*, from long–term datasets in tributaries of lower Chesapeake Bay (Fig. 11).
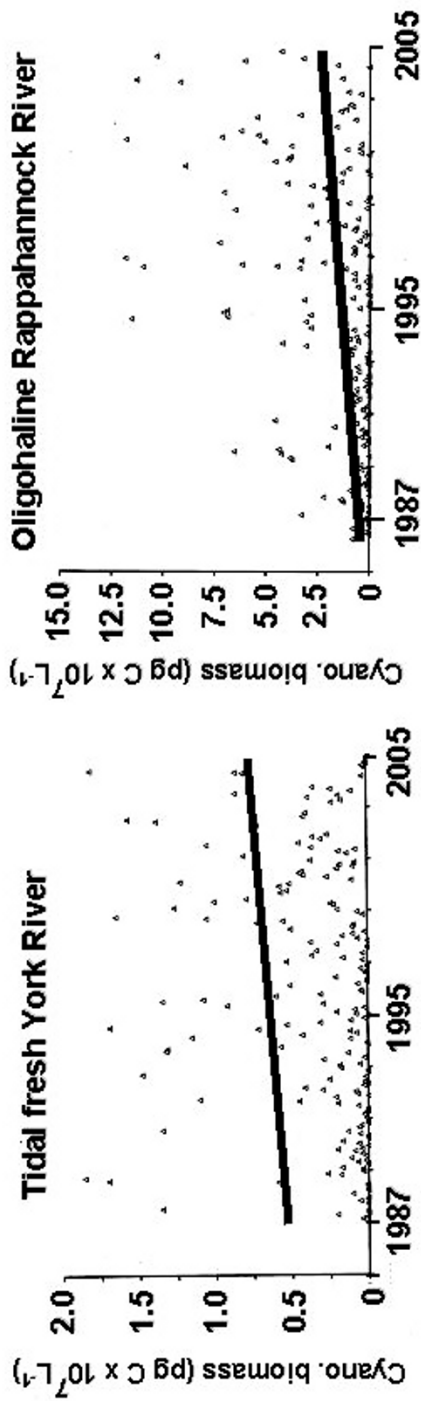
Fig. 11. Long–term datasets (1987–2005) show increasing cyanobacteria biomass in tributaries of lower Chesapeake Bay.

Marine Habitats

In comparison to fresh and estuarine waters, relatively little is known about harmful cyanobacteria in marine ecosystems, except for their negative effects on coral reefs. For example, the toxigenic, benthic cyanobacterium *Lyngbya majuscula* proliferates in warm marine waters enriched with nutrients (especially phosphorus or iron) (e.g., Watkinson et al. 2005). Nicknamed "stinging seaweed" (Sims and Zandee Van Rilland 1981), *L. majuscula* produces an array of toxins that adversely affect human health (Osborne et al. 2001) and have been implicated as causes of tumors and other diseases in marine fauna (e.g. Arthur et al. 2006). It also has caused serious ecological impacts in smothering coral reefs and seagrass meadows. For example, a recently described bloom expanded to 8 km^2 of coverage within 55 days, and its dense accumulation (average biomass 210 grams dry weight · m^{-2}) led to declines in beneficial seagrasses (Watkinson et al. 2005). As another example, the cyanobacterium *Phormidium corallyticum* is a member of a microbial consortium that causes black band disease and death of corals (Richardson 2005).

Although cyanobacteria in many saltwater ecosystems have not been rigorously examined for toxicity, limited available evidence suggests that cryptic toxigenic species can be abundant in these habitats. For example, a survey of the landlocked, brackish to hypersaline, Salton Sea revealed that 85% of all water samples tested from areas with high incidence of grebe deaths contained low but detectable levels of microcystins produced by picoplanktonic *Synechococcus* and benthic *Oscillatoria* (Carmichael and Li 2006). Moreover, the livers of affected grebes contained microcystins at potentially lethal levels.

> Possible interactions between cyanobacteria and marine harmful algal blooms: *Trichodesmum* spp. are marine cyanobacteria that fix nitrogen gas into inorganic nitrogen forms that are used to make proteins not only by the cyanobacteria, but by other organisms as nitrogen leaks from the cyanobacteria cells. The impacts are currently being explored and are not necessarily bad, but the added available nitrogen has been hypothesized to help support blooms of harmful algae such as *Karenia brevis*, the toxic 'red tide' organism of the Gulf of Mexico (Lenes et al. 2001, Glibert et al. 2006). This hypothesis is presently under debate (Hu et al. 2006).

Potential Chronic, Insidious Impacts on Human Health

Present understanding about the effects of toxigenic cyanobacteria on human health are mostly limited to obvious, acute impacts, and thus do not include chronic, subtle or insidious impacts that are happening but not yet detected, and potential impacts where hazards exist in remote areas where health impacts have not yet been sustained. It is expected that the risk from cyanobacterial CHABs to drinking and recreational waters will increase with increased stress and demand on those systems (Glibert and Burkholder 2006) (e.g. Fig. 12, see Color Plate 2 and Fig. 13, see Color Plate 2). The magnitude of the threat will depend on many variables including the increase in population and its exposure to toxins, the requirement for new drinking water and recreational sources and the speed and extent with which effective management strategies are implemented.

It is expected that the demand for drinking water in the US will increase dramatically in the future. Although many utilities in the US use underground water supplies, many also use surface water and that proportion is expected to increase in many areas (e.g. Martin 2001). Increasing population has placed increased demand on surface water supplies, either through their anthropogenic inputs or through their increased demand for drinking water. Conventional water treatment appears to be ineffective in removing cyanotoxins from drinking water (Hoeger et al. 2004, 2005); cyanobacterial cells lyse during filtration and chlorine does not appear to readily oxidize the resulting MC (Wannemacher Jr. et al. 1993; Hitzfeld et al. 2000). To control HABS, algaecides, especially copper sulfate, are commonly added to surface drinking water sources (Chorus and Bartram 1999), but this leads to cell lysis and a substantial release of cyanotoxins into the water, as well as potential copper toxicity (Kenefick et al. 1993). Powdered and granular activated carbon adsorption, with reported removal efficiencies of greater than 90%, removes toxins as well as intact cells and appears to be a more effective and reliable methods for removing cyanotoxins than Cl (Hamann et al. 1990; Donati et al. 1994). A similar increased in risk is also expected for recreational waters where, for humans, the primary route of exposure is oral from accidental or deliberate ingestion of recreational water, inhalation of aerosolized cells or from dermal contact.

OCCURRENCE OF CYANOBACTERIAL BLOOMS IN THE LAKE PONTCHARTRAIN ESTUARY, LOUISIANA

Toxic cyanobacterial blooms have been reported in Lake Pontchartrain, an oligohaline lake bordering New Orleans, Louisiana. Lake Pontchartrain is a tidally influenced estuary that connects to the Mississippi River via the Bonnet Carré Spillway and to the Gulf of Mexico by the Rigolets strait, Chef Menteur Pass, and Lake Borgne. It represents the second largest salt–water lake in the US (1630 km^2), after the Great Salt Lake in Utah, and the largest lake in Louisiana.

Toxic cyanobacterial blooms (e.g., *Microcystis & Anabaena*) have occurred on Lake Pontchartrain following the discharge of water from the Mississippi River to the estuary via the Bonnet Carré Bonnet Spillway. The spillway was constructed to divert floodwater through the lake before it reaches the city of New Orleans. The floodgate between the spillway and the river is only opened during severe flood events, but water can seep into the spillway through the closed gate (Lane et al. 2001). The seeping river water leaks brown clouds of sediment and nutrients into Lake Pontchartrain. When the floodgates have been opened in the past, the influx of fresh, nutrient–rich water has been followed by fish kills and toxic cyanobacterial blooms.

The 1997 opening of the Bonnet Carré Spillway is one of the better–documented events where toxic cyanobacterial booms occurred on the lake following the release of water from the river to the lake (Dortch et al. 1998). The algal bloom became apparent over large parts of the lake during May and produced high levels of hepatotoxins, prompting health advisories by the Louisiana Department of Health and Hospitals.

Fig. 12. Anabaena bloom on Lake Pontchartrain.

Fig. 12. (cont.) Anabaena bloom on Lake Pontchartrain (Photo courtesy of John Burns. See Color Plate 2).
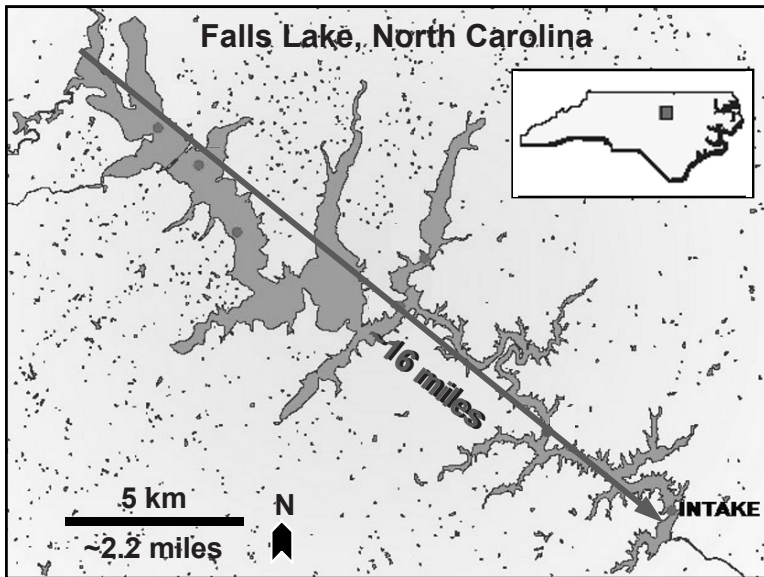
Fig. 13. Map of Falls Lake, NC, indicating six sampling stations and the location of the intake for the water treatment plant of the capital city, Raleigh. *The watershed is sustaining rapid human population growth and associated increased nutrient runoff into receiving waters. From Burkholder (2006b).* (See Color Plate 2).
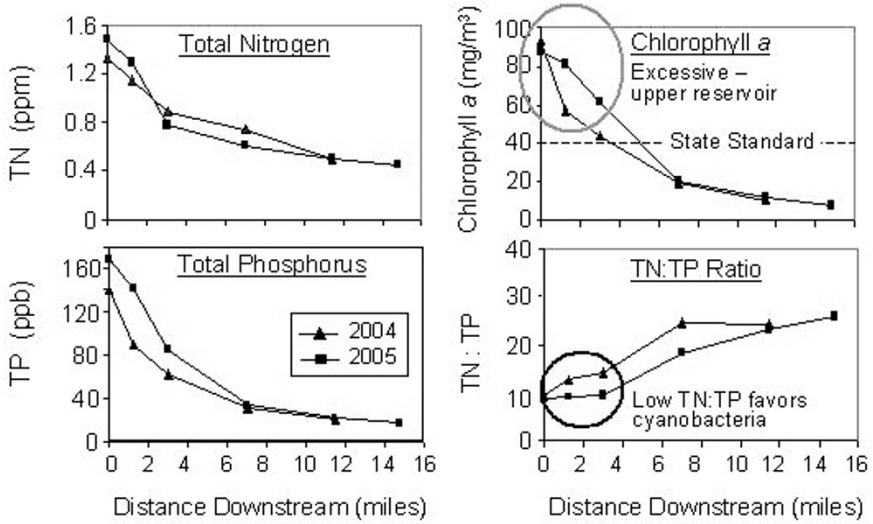
Fig. 14. Total N, total P, chlorophyll a (indicator of algal/cyanobacterial biomass), and total N : total P ratios in Falls Lake plotted against distance downstream from the headwaters to the dam.

The last station (intake) is the location of the intake for the City of Raleigh water treatment plant. About 75% of the phytoplankton cells in this reservoir during summer months are cyanobacteria, including various toxigenic species based on data from 2002–2005 (Burkholder 2006). The lower end of the reservoir where the water treatment plant is located presently has good water quality, but there is concern that as nutrient loading to the reservoir continues to increase, water quality will become more degraded throughout the reservoir and cyanobacteria will be further stimulated.

Frequency and Severity Ranking.

Both our exposure and our knowledge follow the hierarchy of frequency and severity *freshwater > estuarine (brackish) > marine*. While adverse impacts of cyanobacteria in eutrophic freshwaters and (less so) estuaries are known worldwide (Whitton and Potts 2000), impacts on marine ecosystems have only recently begun to be recognized. Thus, the indicated rankings may change as additional information becomes available.

Data Needs

To improve our ability to measure the incidence and predict the occurrence of CHABS, we need to make full use of existing information that is currently available through a variety of sources. State and regional investigators have conducted a number of surveys of cyanotoxins that needs to be compiled into a readily accessible database. Information that has been collected on the factors related to bloom occurrence should be compiled so that it can be related to toxin occurrence. This includes land use data, nutrient concentration in water, and weather conditions. Information on robust analytical methods for cyanobacteria and toxin quantification in environmental waters needs also needs to be compiled.

Short—term Objectives

Research Needs

There is currently a lack of agreement on species level taxonomy for cyanobacteria. Because of this lack of agreement, cyanobacteria can only be identified easily to the genus level, which means that information may be lost in surveys when only genera are identified. Additionally, changing taxonomy can be a confounding factor for older surveys. Modern molecular techniques offer one solution to this problem but even these techniques require a consensus on the definition of toxigenic cyanobacterial species.

Considerable information on cyanobacterial species and their toxins is also available in non–traditional literature sources. A comprehensive literature survey, including the grey literature, is needed. Both planktonic and benthic cyanobacteria should be included in this survey. These and newer references need to be reviewed and summarized for occurrence of toxigenic species. A working bibliography on cyanobacteria compiled by the EPA can be found at http://www.epa.gov/safewater/ucmr/ucmr1/docs/meetings_umcr1_cyanobacteria_references.doc.

An improved understanding is needed of the environmental factors that are responsible for cyanobacterial growth and bloom formation. Additionally, a better understanding is needed of when cyanobacteria do and do not produce toxins, and factors responsible for production of toxins. This information can be obtained from both field studies and supporting laboratory experiments. Perhaps the best information currently available is for *Microcystis aeruginosa*; the genes involved in toxin production have been identified, and various environmental factors including light, nitrogen,

phosphorus, and iron nutrient supplies have been shown to influence toxin production (Zurawell et al. 2005).

Research is needed to determine the importance of very small (picoplanktonic) cyanobacterial species in the production of cyanobacterial toxins. Similar research is needed for many benthic species. Among the many questions that remain unanswered are: how significant are levels of toxins produced by planktonic and benthic species? How does their toxin production vary over time as compared to historically recognized bloom species? What kinds of toxins are produced by these cyanobacteria?

BMAA originating from cyanobacteria on the roots of cycads has been reported to be associated with Alzheimer's disease in patients who have been exposed to BMAA from the food chain. Additional studies have detected BMAA in cyanobacteria from a variety of geographical locations (Cox et al. 2005). Because of the seriousness of Alzheimer's disease, plus the apparent widespread distribution of BMAA, research is needed to determine the occurrence of BMAA in water in the US, including the fate of this toxin during water treatment for drinking water production.

The conventional wisdom for cyanobacteria is that cylindrospermopsin is released extracellularly from healthy cells, whereas other commonly occurring cyanotoxins are contained in healthy cells, and only released if cells are damaged or dying. Recent evidence suggests that this generalization may not hold in some situations; thus, research is needed to verify that how toxins typically are distributed in commonly occurring species. These studies should use more than one strain of each species, because as mentioned, within a given species of cyanobacteria are many nontoxic as well as toxic strains (Burkholder and Glibert 2006).

Research is also needed to determine whether cyanotoxins are accumulated in the tissues of fish and shellfish. These studies should be done for the most important cyanotoxins which fish and shellfish are likely to be exposed to in both natural bodies of water as well as in aquaculture (e.g. nodularin in some estuarine/marine environments). The exposure of people to cyanotoxins originating from spray or aerosols should be investigated.

Analytical Needs

There is an immediate need for rapid techniques for both screening and confirmatory methods for the cyanotoxin analyses, including simple, inexpensive methods for rapid screening of samples during sampling of blooms. Different types of methods are needed for different applications and each must be targeted towards its end users. Several ELISA screening

methods are available for microcystins, but none are currently manufactured for cylindrospermopsin and other toxins anatoxin–a. On–line detectors would be useful for water utilities for continuous monitoring; instrumental methods are needed for use where quantitation and specificity are important. Methods including LC/MS and LC/MS/MS have been developed, but none have been accepted as validated US EPA methods or consensus organization methods. There is a need for standardized instrumental methods that can be run by commercial labs. Many of the instrumental methods for cyanotoxins are dependent on sample cleanup methods that remove interfering substances. To be successful, these methods must perform in a wide variety of water types, including those with high levels of total organic carbon.

In addition to identifying the toxins, there is a need for additional tools targeted towards identifying a potentially toxigenic organism. Species–specific and toxin–specific molecular probes, microarrays and other technology are needed for studies to determine the occurrence and distribution of potentially toxigenic cyanobacteria. To be most useful, these detection methods should be cost–effective, rapid and reliable.

Standardized methods for quantifying cells and estimating biomass are also needed. These methods should be developed in conjunction with taxonomic identification methods, since identification of species may depend on characteristics that may be altered in some cell quantification schemes. When developed, these methods should be validated. Since cell count methods using microscopy generally are tedious and labor–intensive, alternate, accurate methods to determine cell density or biomass are needed, such as improved techniques to quantify photosynthetic pigments, total suspended solids or particulate organic carbon. Unfortunately, these methods at present all fall short of accurately estimating cyanobacteria biomass, and cannot provide information about toxicity or the dominant taxa involved. Improvements in technology are needed to rapidly track toxigenic species and formation of blooms. These technologies include remote sensing, remote–operated vehicles (ROVs) and automated sampling stations located on vulnerable bodies of water (Glasgow et al. 2004).

Monitoring Needs

There is an immediate need for a nation–wide survey(s) on the occurrence and distribution of toxigenic cyanobacteria and their toxins throughout the US. Some surveys of cyanobacterial toxins have been conducted; however, information on cyanotoxin occurrence is still fragmented and limited. Any information of cyanotoxin occurrence is useful, but in particular, national

surveys, consistently conducted, are needed to assess cyanotoxin occurrence on a larger scale.

The US EPA is planning to conduct a national survey of drinking water for cyanotoxins, under its Unregulated Contaminant Monitoring Rule (UCMR) when the suite of analytical methods needed becomes available. Monitoring will occur for one year at approximately 2,800 large public water systems (PWS) and a representative sample of 800 (out of 66,000) small PWS. Transient water systems are not required to monitor nor are systems that purchase 100% of their water (EPA 2005a). The monitoring results from these systems will be used to form an initial nationally representative occurrence assessment of the most commonly occurring cyanotoxins in the US. A similar survey is needed for ambient water sources, including recreational and agricultural water. Currently, no program exists for conducting this kind of survey, which should include both randomly selected locations as well as targeted locations where blooms historically have been reported, or are reported to be in progress. This design would assure that both typical occurrences and unusual bloom events would be considered and could be used to determine risk to exposed people or animals.

In many locations in the US, cyanobacterial blooms are common events. Nevertheless, they can be sporadic in occurrence and difficult to predict. Even targeting locations that recently have experienced blooms may not mean that blooms will occur in these areas in the future; on the other hand, in developing watersheds blooms may begin to develop in previously "bloom–free" locations. Undetected or missed blooms would result in an underestimate of the risk of exposure. A central reporting website needs to be established to assist in documenting blooms. This website needs to be publicized to utility operators, state department of environmental quality officials and others who would be aware of blooms as they occur. These reports would allow researchers who have the ability to analyze water samples for cyanotoxins to sample these events while they are occurring.

Long—term Objectives

Research Needs

As a longer–term project, an easily accessed database (i.e. website) on the historic occurrence of toxigenic strains is critically needed, including user–friendly summaries for the general citizenry. This could be incorporated into a national database of toxin occurrence data that can be routinely updated. Currently, there is no central location for cyanotoxin data produced

by states or other organizations. Long–term monitoring needs to be done to determine how cyanotoxins occur over time. This monitoring should also be done over a wide geographical area to determine how cyanotoxins are distributed spatially and to establish baseline information for assessing how blooms and cyanotoxin distribution are changing over time. This research would clarify how commonly blooms resulting in high concentrations of cyanotoxins occur, and how this is related to geographical location. It is well known that not all cyanotoxins have been described, so the distribution of known cyanotoxins may be greater presently understood. Thus, studies are also needed to identify new cyanotoxins.

Analytical Needs

For continual monitoring and development of research tools, there is a long–term need for a stable supply of toxic organisms and toxins. Toxic strains of many species commonly have lost their ability to make toxins over time in the highly artificial conditions represented by laboratory cultures. To avoid the loss of these sources of toxins, culture collections are needed which can supply a continuing source of toxin–producing strains of cyanobacteria recently isolated from natural habitats. Similarly, the development of analytical methods and other studies have been hampered by lack of availability of analytical chemistry standards for cyanotoxins. Information on concentration or purity of available standards is unavailable or ambiguous. Manufacturers need to produce larger supplies of at least the more common toxins, and to certify their purity. These toxins should be made commercially available.

Monitoring Needs

For long–term priorities, the same types of surveys are needed that were described for short–term needs. These surveys would include sampling at locations with typical levels of cyanobacteria as well as targeted surveys that sample blooms in progress. To respond to the appearance of cyanobacterial toxins in a manner that will minimize public health risk, rapid analyses are needed which will permit a response before drinking water consumers or recreational water users are exposed to the toxins. Rapid assays or screening methods are needed for this purpose. Data on long–term occurrence of cyanotoxins and cyanobacteria that produce the toxins are needed to assess how commonly cyanotoxins occur, at what levels, and whether there are any long–term changes in their occurrence.

Summary

Distribution of CHABs across the US

- Freshwater blooms are ubiquitous across the US and Canada.

- Most cyanobacterial blooms are in freshwater, but some are found in estuarine or marine waters.

- More detections of cyanotoxins have been reported recently, however, increased awareness had led to more monitoring programs.

- Blooms of cyanobacteria tend to be greater and last longer in warmer climates.

- Detections in ambient waters have been observed to vary from no detection to 31,470 µg/L for microcystin, no detection to 156 µg/L for anatoxin–a, and no detection to 202 µg/L for cylindrospermopsin. Detections occurred in treated water but concentrations were lower.

- Few data are available on temporal changes in cyanotoxin occurrence.

Cyanotoxin production

- Determination of taxonomic groups which produce toxins has been hampered by uncertainty in the taxonomic definitions for cyanobacteria.

- Table 1 lists 22 genera and 42 species of cyanobacteria associated with toxin formation.

- Table 2 lists taste and odor–producing species of cyanobacteria.

- Table 3 lists the commonly produced types of cyanotoxins in freshwater.

- Microcystin has been reported to be produced by *Microcystis, Anabaena, Oscillatoria (Planktothrix), Nostoc, Hapalosiphon* and *Anabaenopsis*.

- Cylindrospermopsin is produced by *Cylindrospermopsis raciborskii, Aphanizomenon ovalisporum,* and *Uzmekia natans*.

- Anatoxin–a is produced by *Anabaena flos–aquae, A. planktonica, Oscillatoria* species, and *Cylindrosperum*. Homoanatoxin–a, was isolated from *Phormidium Formosa.*

- Anatoxin–a(s) was reported from A. flos–aquae strain NRC 525–17 and more recently by *A. lemmermannii.*

- Saxitoxin and related toxins are made by *Aphanizomenon flos–aquae, Anabaena circinalis, Lyngbya wollei* and a Brazilian isolate of *Cylindro-spermopsis raciborskii.*

- BMAA is produced by *Nostoc* species and many others.

- Lyngbyatoxins aplysiatoxin and debromoaplysiatoxin are made by *Lyngbya*, Oscillatoriaceae such as *Schizothrix calcicola* and *Oscillatoria nigroviridis*.

- Cyanobacteria also produce other bioactive compounds.

Impacts on waterbody health and ecosystem viability

- The factors that lead to bloom formation and toxin production are not well understood. Light, temperature, hydrological and chemical factors may be involved.

- In freshwater, blooms in Metropolitan Water District's reservoirs have led to taste and odor problems. A sewage spill in North Carolina led to a *Lyngbya* bloom.

- In estuarine habitats including the upper Chesapeake Bay, cyano-bacterial blooms occurred with an invasion of water milfoil. Cyano-bacterial blooms with fish kills have occurred in brackish Lake Pon-chartrain, Louisana from freshwater or nutrient influxes.

- Ecological effects have occurred as a result of cyanobacterial blooms that are caused by effects other than toxin production.

Data Needs

- A consensus is needed for species level taxonomy for cyanobacteria.

- Existing information needs to be compiled into an accessible form.

- New surveys should be conducted (including UCMR monitoring of drinking water), especially for newly emerging toxins in raw water and drinking water.

- A national monitoring program is needed for recreational water.

- Analytical methods are needed including screening methods, instrumental methods, and online and remote detection. Sample preparation methods and analytical chemistry standards are needed to support analytical methods.

- A better understanding is needed of what factors lead to bloom formation and cyanotoxin production.

- The relation between toxin retention and release for different toxins needs to be clarified.

- Accumulation of cyanotoxins in the tissues of fish and shellfish needs study.

- A website is needed for reporting of cyanotoxin occurrence information.

Glossary

Akinete – thick–walled, often enlarged cell that can survive adverse conditions and form a new filament; often refractive under light microscopy because it is filled with food reserve (cyanophycean starch); also often orangish in color from abundant, protective carotenoid pigments. This specialized cell is found in some members of the Order Nostocales.

Anaerobic – Without oxygen.

Anoxic – Referring to habitats with little or no oxygen, such as the bottom waters of many eutrophic lakes in summer

Avian vacuolar myelinopathy [AVM] – a recently discovered neurological disease affecting water birds and their predators, primarily American coots and bald eagles, in the southern US. AVM causes characteristic lesions in the myelin of the brain and spinal cord.

Benthic – Living in or on the bottom of a body of water, or growing attached to various substrata including other organisms (e.g. the noxious aquatic weed, hydrilla), but not free–floating in the water column. Note: Many benthic cyanobacteria can be moved into the plankton by wind/waves or other disturbance, and can thrive in the water column.

Brackish – Somewhat salty, as in brackish (estuarine) water.

Buoyant – Floats easily, and is less dense than water.

Chlorophyll *a* – The "universal plant pigment", found in all organisms that undergo plant–like photosynthesis with production of oxygen. The concentration of this pigment is often used as an indicator or surrogate measure for algal biomass or production.

Cyanobacteria – Formerly (and sometimes still) called blue–green algae, cyanophytes or mixophytes (means "slime plants"). In present–day taxonomy, considered a type of bacteria ("blue bacteria") because of their primitive cell structure and other features, although they have the "universal plant pigment" that is directly involved in "higher plant" photosynthesis, chlorophyll *a*.

Estuary – A semi–enclosed body of water where saltwater mixes with freshwater.

Eutrophic – Referring to aquatic freshwater and/or estuarine/coastal ecosystems that are nutrient–enriched and highly productive.

Filamentous – Group of cells aligned in a row; very thin, threadlike.

Freshwater – Aquatic ecosystem with salinity lower than 0.1 ppt (see below).

Genus (plural: genera) – A taxonomic group below family and above species

Habitats – The places where plants and animals live.

Harmful algal bloom – A proliferation of microscopic or macroscopic algae that are capable of causing disease or death of humans or beneficial aquatic life through production of toxins, or that adversely affect aquatic ecosystems through other mechanisms (e.g. by depleting oxygen needed to sustain fish, or by reducing light that is essential for growth of beneficial submersed plants).

Heterocyte (formerly heterocyst) – thick–walled cell, usually pale in color compared to the other cells, used for nitrogen fixation in some cyanobacteria. This specialized cell is found in some members of the Order Nostocales.

Heterotroph – An organism that depends on consumption of other organisms and organic material for energy.

Hypersaline – referring to water with salinity higher than average seawater salinity.

Indicator species – A species whose status provides information on the overall health of the ecosystem and of other species in that ecosystem. Indicator species reflect the quality and changes in environmental conditions as well as aspects of community composition.

Macrophyte – Usually referring to a rooted submersed aquatic plant.

Mesohaline – Moderately salty water (salinity range 5–18 ppt).

Mixotroph – An organism that uses both photosynthesis and particulate or dissolved organic substances made by other organisms for its energy. Many cyanobacteria are mixotrophs.

Oligohaline – Low salinity (0.5–5 ppt).

Phytoplankton– Microscopic algae that live and reproduce suspended in the water column of freshwater, estuarine, and marine ecosystems. Phytoplankton form the base of the food web in many aquatic ecosystems.

Picoplankton – Very small phytoplankton in aquatic ecosystems, including cyanobacteria (diameter 0.2 – 2 μm).

Polyhaline – Referring to waters that are variable in salinity, often highly brackish water with a salinity of 18–30 ppt.

Ppt – Parts per thousand, a measure of salinity. For example, the salinity of the open ocean is generally about 35 ppt; freshwaters are < 0.1 ppt.

Reservoir (impoundment) – an artificial (man–made) lake, formed by damming a river.

Specialized cell – akinete, heterocyte (see above).

Strain – A population within a given species of cyanobacteria or microscopic algae.

Terrestrial – Living or growing on land or in the soil.

Tidal freshwater – Freshwater (0–0.5 ppt) that is tidally influenced.

Toxigenic – potentially toxic; referring to a species of cyanobacteria that is known to have some toxic strains. (Note: toxigenic algae have both nontoxic and toxic strains.)

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