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The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change

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Cyanobacteria are the most ancient phytoplankton on the planet and form harmful algal blooms in freshwater, estuarine, and marine ecosystems. Recent research suggests that eutrophication and climate change are two processes that may promote the proliferation and expansion of cyanobacterial harmful algal blooms. In this review, we specifically examine the relationships between eutrophication, climate change and representative cyanobacterial genera from freshwater (*Microcystis*, *Anabaena*, *Cylindrospermopsis*), estuarine (*Nodularia*, *Aphanizomenon*), and marine ecosystems (*Lyngbya*, *Synechococcus*, *Trichodesmium*). Commonalities among cyanobacterial genera include being highly competitive for low concentrations of inorganic P (DIP) and the ability to acquire organic P compounds. Both diazotrophic (= nitrogen (N_2) fixers) and non-diazotrophic cyanobacteria display great flexibility in the N sources they exploit to form blooms. Hence, while some cyanobacterial blooms are associated with eutrophication, several form blooms when concentrations of inorganic N and P are low. Cyanobacteria dominate phytoplankton assemblages under higher temperatures due to both physiological (e.g. more rapid growth) and physical factors (e.g. enhanced stratification), with individual species showing different temperature optima. Significantly less is known regarding how increasing carbon dioxide (CO_2) concentrations will affect cyanobacteria, although some evidence suggests several genera of cyanobacteria are well-suited to bloom under low concentrations of CO_2. While the interactive effects of future eutrophication and climate change on harmful cyanobacterial blooms are complex, much of the current knowledge suggests these processes are likely to enhance the magnitude and frequency of these events.

1. Introduction

While cyanobacterial harmful algal blooms have been reported in the scientific literature for more than 130 years (Francis, 1878), in recent decades, the incidence and intensity of these blooms, as well as economic loss associated with these events has increased in both fresh and marine waters (Chorus and Bartram, 1999; Carmichael, 2001, 2008; Hudnell, 2008; Heisler et al., 2008; Hoagland et al., 2002; Paerl, 2008; Paul, 2008; Paerl and Huisman, 2008). Recently, there have been discoveries of previously unidentified cyanobacterial toxins, such as amino b-methylamino-L-alanine (BMAA), and of new genera of cyanobacteria capable of producing previously described toxins (Cox et al., 2003, 2005, 2009; Cox, 2009; Brand, 2009; Kerbrat et al., 2011). To date, factors identified as contributing towards the global expansion of harmful cyanobacterial blooms have included increased nutrient inputs, the transport of cells or cysts via anthropogenic activities, and increased aquaculture production and/or overfishing that alters food webs and may permit harmful species to dominate algal communities (GEOHAB, 2001; HARRNESS, 2005; Heisler et al., 2008). It has also been shown that an increase in surface water temperatures due to changing global climate could play a role in the proliferation of cyanobacterial blooms (Peperzak, 2003; Paerl and Huisman, 2008; Paul, 2008). Importantly, there is consensus that harmful algal blooms are complex events, typically not caused by a single environmental driver but rather multiple factors occurring simultaneously (Heisler et al., 2008). Finally, an improved ability to detect and monitor harmful cyanobacterial blooms, and their toxins as well as increased scientific and public awareness of these events has also led to better documentation of these events (GEOHAB, 2001; HARRNESS, 2005; Sivonen and Bo¨rner, 2008).

There have been several reviews of the intensification and global expansion of harmful cyanobacterial blooms in terms of

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both abundance, geographic extent, and effects on ecosystem health, as well as factors that may be facilitating this expansion (Paerl, 1988, 1997; Paerl and Millie, 1996; Soranno, 1997; Carmichael, 2001; Saker and Griffiths, 2001; Landsberg, 2002; Codd et al., 2005a,b; Huisman and Hulot, 2005; see multiple papers in Hudnell, 2008). The purpose of this review is to: (1) Highlight important findings of the last decade of harmful cyanobacterial bloom research in fresh, estuarine and marine environments; and (2) Describe how factors associated with eutrophication and climate change affect some of the most widely studied harmful cyanobacterial bloom genera.

2. Background

Cyanobacteria are prokaryotes but have historically been grouped with eukaryotic "algae" and at varying times have been referred to as: blue–greens, blue–green algae, *Myxophyceae*, *Cyanophyceae* and *Cyanophyta* (Carmichael, 2008). More recently cyanobacteria that form harmful blooms have been termed "CyanoHABs" (Carmichael, 2001, 2008; Paerl, 2008) or "cyanobacterial blooms" (Hudnell et al., 2008).

2.1. Toxins

Many genera of cyanobacteria are known to produce a wide variety of toxins and bioactive compounds, which are secondary metabolites (i.e. compounds not essential to the cyanobacteria for growth or its own metabolism) (Sivonen and Jones, 1999). Toxins generally refer to compounds that cause animal and human poisonings or health risks, and bioactive compounds refer to compounds that can have antimicrobial and cytotoxic properties and are often of interest in pharmaceutical and as research tools (Codd et al., 2005a,b). While many of these compounds have recognized toxic effects, the impact and long term effects of many of these compounds is unknown (Tonk, 2007).

Hepatotoxins are globally the most prevalent cyanobacterial toxins followed by neurotoxins (Sivonen and Jones, 1999; Klisch and Ha¨ der, 2008; Sivonen and Bo¨ rner, 2008). Hepatotoxins include: (1) microcystins, (2) nodularins, and (3) cylindrospermopsins. The three most commonly produced types of cyanobacterial neurotoxins are: (1) anatoxin-a, (2) anatoxin-a (S), and (3) saxitoxins. As noted above, Cox et al. (2003, 2005) recently described the presence of the neurotoxic compound, BMAA in nearly all cyanobacteria they tested (Table 1). It has been hypothesized that BMAA may be a possible cause of the amyotrophic lateral sclerosis parkinsonism–dementia complex (ALS-PDC; Cox et al., 2003, 2009; Murch et al., 2004; Cox, 2009). As such, the discovery that this compound is potentially produced by a broad range of cyanobacteria greatly increases the potential for human exposure (Sivonen and Bo¨ rner, 2008; Brand, 2009). Indeed, in the Baltic Sea, an ecosystem whose primary production is dominated by cyanobacteria, BMAA has been measured in significant quantities in both fish and shelfish (Jonasson et al., 2010).

2.2. Nutrients

Of all of the potential environmental drivers behind harmful algal and cyanobacterial blooms, the one that has received the most attention among the global scientific community has been anthropogenic nutrient pollution. Research indicates that cultural eutrophication associated with the increased global human population has stimulated the occurrences of harmful algal blooms (Anderson, 1989; Hallegraeff, 1993; Burkholder, 1998; Anderson et al., 2002; Glibert et al., 2005; Glibert and Burkholder, 2006; Heisler et al., 2008). As bodies of freshwater become enriched in nutrients, especially phosphorus (P), there is often a shift in the phytoplankton community towards dominance by cyanobacteria (Smith, 1986; Trimbee and Prepas, 1987; Watson et al., 1997; Paerl and Huisman, 2009). Examples of these changes are the dense blooms often found in newly eutrophied lakes, reservoirs, and rivers previously devoid of these events (Fogg, 1969; Reynolds and Walsby, 1975; Reynolds, 1987; Paerl, 1988, 1997). Empirical models predict that in temperate ecosystems, summer phytoplankton communities will be potentially dominated by cyanobacteria at total phosphorus (TP) concentrations of 100–1000 $\mu g\ L^{-1}$ (Trimbee and Prepas, 1987; Jensen et al., 1994; Watson et al., 1997; Downing et al., 2001).

One reason that P often controls the proliferation of freshwater ecosystems is that many cyanobacteria that bloom in warm waters have the ability to fix nitrogen (N; Paerl, 1988; Paerl et al., 2001). Since many of the bloom forming cyanobacteria genera are not diazotrophic and the proliferation of some blooms may be limited by N (Gobler et al., 2007; Davis et al., 2010), it has been hypothesized both N and P may control harmful cyanobacterial blooms (Paerl et al., 2008; Paerl and Huisman, 2009). While research on cyanobacterial blooms has traditionally considered inorganic N and P pools as being accessed by cyanobacteria or total N and P pools for understanding the trophic state of ecosystems, recent research has demonstrated that organic N and P may be important nutrient sources for cyanobacteria. Much of the soluble N and P pools in most aquatic environments are comprised of organic compounds (Franko and Heath, 1979; Seitzinger and Sanders, 1997; Kolowith et al., 2001) and many cyanobacteria can utilize various forms of dissolved and particulate organic N and P (Glibert and Bronk, 1994; Paerl, 1988; Paerl and Millie, 1996; Pinckney et al., 1997; Berman and Chava, 1999; Glibert and O'Neil, 1999; Davis et al., 2010). Since neither inorganic nutrient pools nor nutrients ratios typically are able to sufficiently explain the extended duration of dense cyanobacterial blooms (Heisler et al.,

Table 1
Major cyanobacterial bloom toxins.

Toxin group	Primary target organ in mammals	Cyanobactrial genera
Microcystins	Liver	*Microcystis*, *Anabaena*, *Planktothrix (Oscillatoria)*, *Nostoc*, *Hapalosiphon*, *Anabaenopsis*, *Trichodesmium*, *Synechococcus*, *Snowella*
Nodularian	Liver	*Nodularia*
Cylindrospermopsin	Liver	*Cylindrospermopsis*, *Umezakia*, *Aphanizomenon*, *Lyngbya*, *Raphidiopsis*, *Anabaena*
Anatoxin-a	Nerve synapse	*Anabaena*, *Planktothrix (Oscillatoria)*, *Aphanizomenon*, *Phormidium*, *Rhaphidiopsis*
Anatoxin-a(S)	Nerve synapse	*Anabaena*
Saxitoxins	Nerve axons	*Anabaena*, *Planktothrix (Oscillatoria)*, *Aphanizomenon*, *Lyngbya*, *Cylindrospermopsis*, *Scytonema*
Palytoxins	Nerve axons	*Trichodesmium*
Aplysiatoxins	Skin	*Lyngbya*, *Schizothrix*, *Planktothrix (Oscillatoria)*
Lyngbyatoxin-a	Skin, gatro-intestinal tract	*Lyngbya*
Lipopolysaccharides	Irritant; affects exposed tissue	All
BMAA	Nerve synapse	All

Sources: Chorus and Bartram (1999), Li et al. (2001a), Codd et al. (2005a,b), Humpage (2008), Klisch and Ha¨ der (2008), Smith et al. (2011).

2008; Paerl, 2008), research of these events must consider the impacts of all nutrient species, including micro-nutrients. Iron (Fe) has also been found to be an important micro-nutrient in determining cyanobacterial bloom abundance, especially for diazotrophs, given that the enzyme nitrogenase has a high Fe requirement (Kustka et al., 2003). The recent expansion of molecular investigations of cyanobacteria has permitted a clearer understanding of the manner in which harmful cyanobacterial bloom species respond to all nutrients at the cellular level. Importantly, there are diverse responses to nutrient sources and concentrations among cyanobacterial blooms species that will be highlighted in this review.

2.3. Climate change

The sum of research conducted regarding the evolutionary history, ecophysiology, and *in situ* dynamics of cyanobacteria suggests that they will thrive under the conditions predicted for global climate change (Paul, 2008; Paerl and Huisman, 2009). The details of how specific genera of cyanobacteria may respond to climate change, however, are less clear. This review will focus on the specific effects of temperature and concomitant changes in stratification, as well as the effect of CO_2 and pH on multiple freshwater, estuarine, and marine cyanobacteria genera.

2.3.1. Temperature

The burning of fossil fuels and subsequent rise in atmospheric carbon dioxide has caused the earth's surface temperature to increase by approximately 1 °C during the 20th century, with most of the increase having occurred during the last 40 years (IPCC, 2007). In the current century, global temperatures are expected to increase an additional 1.5–5 °C (Houghton et al., 2001; IPCC, 2007). Natural communities of phytoplankton have been and will continue to be influenced by these increases in temperature as algal growth rates are strongly, but differentially, temperature dependent (Eppley, 1972; Goldman and Carpenter, 1974; Raven and Geider, 1988). As temperatures approach and exceed 20 °C, the growth rates of freshwater eukaryotic phytoplankton generally stabilize or decrease while growth rates of many cyanobacteria increase, providing a competitive advantage (Canale and Vogel, 1974; Peperzak, 2003; Paerl and Huisman, 2009).

Beyond the direct effects on cyanobacterial growth rates, rising temperatures will change many of the physical characteristics of aquatic environments in ways that may be favorable for cyanobacteria. For instance, higher temperatures will decrease surface water viscosity and increase nutrient diffusion towards the cell surface, an important process when competition for nutrients between species occurs (Vogel, 1996; Peperzak, 2003). Secondly, since many cyanobacteria can regulate buoyancy to offset their sedimentation, a decrease in viscosity will preferentially promote the sinking of larger, non-motile phytoplankton with weak buoyancy regulation mechanisms (e.g. diatoms) giving cyanobacteria a further advantage in these systems (Wagner and Adrian, 2009; Paerl and Huisman, 2009). Thirdly, insular heating will increase the frequency, strength, and duration of stratification. This process will generally reduce the availability of nutrients in surface waters favoring cyanobacteria that regulate buoyancy to obtain nutrients from deeper water, or that are diazotrophic. Consistent with the sum of these observations, cyanobacteria tend to dominate phytoplankton assemblages in eutrophic, freshwater environments during the warmest periods of the year, particularly in temperate ecosystems (Paerl, 1988; Paerl et al., 2001; Paerl and Huisman, 2008; Paul, 2008; Liu et al., 2011). For all of these reasons, it has generally been concluded that cyanobacterial blooms may increase in distribution, duration and intensity, as global temperatures rise (Paerl and Huisman, 2009; Paul, 2008). The precise response of individual cyanobacterial taxa to rising temperatures will be diverse and has not been reviewed in detail to date.

2.3.2. Carbon dioxide and pH

The combustion of fossil fuels during the past two centuries has significantly increased concentrations of atmospheric carbon dioxide (CO_2), a trend that is projected to continue in the coming decades (IPCC, 2007). Atmospheric CO_2 concentrations that had previously increased at a rate of 1% per year in the 20th century are now increasing 3% per year and may exceed 800 ppm by the end of this century (IPCC, 2007; Fu¨ ssel, 2009). Aquatic chemistry will be strongly altered by this rising CO_2 as levels of both pH and carbonate ions will decline (Cao and Caldeira, 2008).

The pH of aquatic water bodies is intimately linked to the speciation of dissolved inorganic carbon (DIC) (e.g. CO_2; carbonic acid H_2CO_3; bicarbonate HCO_3; or carbonate CO_3^2) and the pH of most systems (7.5–8.1) maintains inorganic carbon primarily in the form of HCO_3 . The buffering capacity of marine ecosystems maintains the pH and speciation of inorganic DIC in a smaller range than those typically observed in freshwaters. Many lakes are supersaturated with CO_2 (Cole et al., 1994; Maberly, 1996) due to terrestrial C inputs and sediment respiration (Cole et al., 1994). The pH and speciation of inorganic carbon in lakes can vary widely on a scale from daily (diel), to episodic, to seasonal (Maberly, 1996; Qui and Gao, 2002) with diel variations in productive lakes as high as 2 pH units and 60 μmol DIC L^{-1} (Maberly, 1996). The large, diel drawdown in DIC associated with algal blooms in eutrophic lakes may cause phytoplankton to become ephemerally C-limited. It has been hypothesized that surface-dwelling cyanobacteria may have an advantage over other phytoplankton due to their closer proximity to atmospheric CO_2 that may rapidly diffuse into surface waters and promote their growth when water column CO_2 concentrations are drawn down by dense blooms (Paerl and Huisman, 2009). Alternatively, there is evidence that low DIC environments may favor cyanobacteria. Several studies have reported that cyanobacteria out-compete eukaryotic algae under high pH and low CO_2 conditions (Shapiro and Wright, 1990; Oliver and Ganf, 2000; Qui and Gao, 2002). Furthermore, some cyanobacteria decrease cell division rates in response to lower pH conditions (Shapiro and Wright, 1990; Whitton and Potts, 2000; Czerny et al., 2009). However, laboratory and field studies have demonstrated that other cyanobacteria respond to increased CO_2 with increased cell division rates, carbon fixation, or both (Hein and Sand-Jensen, 1997; Burkhardt et al., 1999; Hinga, 2002; Yang and Gao, 2003; Riebesell, 2004; Barcelos e Ramos et al., 2007; Fu et al., 2007, 2008; Hutchins et al., 2007; Levitan et al., 2007; Riebesell et al., 2007; Kranz et al., 2009).

There are a number of phylogenetically distinct ways phytoplankton take up, transport, or convert CO_2 and HCO_3 (Raven, 1997; Kaplan and Reinhold, 1999; Beardall and Giordano, 2002; Badger and Price, 2003; Reinfelder, 2011). Nearly all, eukaryotic algae and all cyanobacteria possess carbon-concentrating mechanisms (CCMs; Giordano et al., 2005). Cyanobacteria have evolved pathways for the active inorganic carbon uptake and partition their ribulose *bis*phosphate carboxylase-oxygenase (Rubisco) into micro-compartments known as carboxysomes that generate a high concentration of CO_2 around the Rubisco enzyme (Badger et al., 2002). It has been demonstrated that CCMs in cyanobacteria are more efficient than other algae or higher plants at low CO_2 concentrations (Badger and Price, 2003; Badger et al., 2006) and that this heightened efficiency may facilitate their dominance under low CO_2 conditions (Price et al., 2008). Considered in the context of climate change, increases in atmospheric concentrations of CO_2 could have a more beneficial impact on species that, unlike cyanobacteria, possess inferior CCMs, do not contain any CCMs, and/or rely primarily on CO_2 transport (Fu et al., 2007). While this

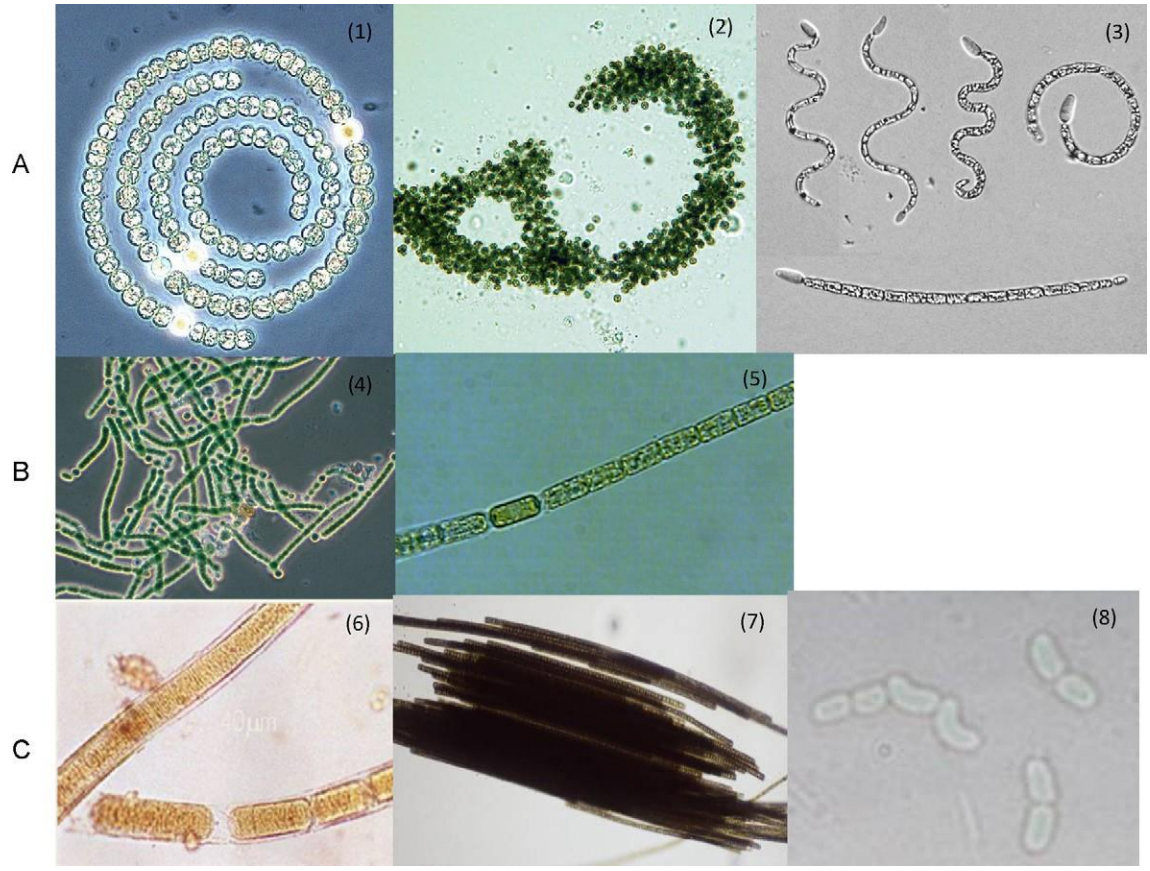

Fig. 1. Major CHAB genera from (A) freshwater: (1) *Anabaena* (photo: Michele Burford); (2) *Microcystis* (photo: Glenn MacGegor); (3) *Cylindrospermopsis* (photo: Glenn MacGregor); (B) estuarine: (4) *Nodularia* (photo: Hans Paerl); (5) *Aphanizomena* (photo: Christina Esplund-Lindquist); (C) marine enivronments: (6) *Lynbya* (photo: Judy O'Neil); (7) *Trichodesmium* (photo: Judy O'Neil); and (8) *Synechococcus* (photo: Florida Fish & Wildlife Institute-FWRI).

might suggest that globally rising CO_2 may diminish the intensity of cyanobacterial blooms, little is known regarding how increases in the concentration of CO_2 will impact cell physiology and growth rates of individual cyanobacteria genera. Changing CO_2 conditions may also effect the strain composition within a cyanobacterial community. One study addressed this question using competition experiments with toxic versus non-toxic strains of cyanobacteria at high CO_2 availability; which resulted in a competitive advantage of the non-toxic strain (Van de Waal et al., 2011). Below we will discuss what is known in regard to potential climate change effects for each of the major harmful cyanobacterial bloom genera across the fresh to marine spectrum.

2.3.3. Salinity

Climate change may also affect salinity in estuaries and freshwater systems due to rising sea-level: an increase in drought frequency and duration in some regions and concommittant increase in dessication; or in other areas, increases in precipitation due to storms. This may cause shifts in phytoplankton species composition (Ahmed et al., 1985; Moisander et al., 2002; Bordalo and Vieira, 2005). Although many eukaryotic phytoplankton cannot tolerate changes in salinity, a number of cyanobacterial species have very euryhaline tolerances. Therefore changes in salinity may affect both community composition as well as potential toxin concentrations and distribution (Laamanen et al., 2002; Orr et al., 2004; Tonk et al., 2007).

3. Freshwater environments

As noted above, freshwater harmful algal blooms are predominantly caused by pelagic cyanobacteria (Carmichael, 2001, 2008). As such, this review will focus on the role of eutrophication and climate change in the occurrence of three of the most prevalent pelagic cyanobacterial bloom forming genera in this environment, *Anabaena*, *Microcystis*, and *Cylindrospermopsis* (Fig. 1A).

3.1. Anabaena

Anabaena is a ubiquitous freshwater genus found throughout the world, but typically prevalent in lentic waterbodies such as lakes, reservoirs, cease-to-flow rivers and weir pools. *Anabaena* is a filamentous, akinete-forming diazotroph in the order Nostocales. Some species of this genera produce the toxins microcystins (MCYs), anatoxin-a and anatoxin-a(S) and cylindrospermopsin (CYN), while others, principally *Anabaena circinalis*, produces a saxitoxin (STX). The gene cluster responsible for anatoxin biosynthesis has recently been described for *Anabaena* (Rantala-Yilnen et al., 2011), the characterization of the gene clusters responsible for saxitoxin biosynthesis (*stx*; Mihali et al., 2009) and microcystin biosynthesis (*mcyA – I*; Rouhiainen et al., 2004) in *Anabaena* have allowed for the distinction between strains that can and cannot produce STX (Al-Tebrineh et al., 2010) and MCY (Rouhiainen et al., 2004). As a diazotroph, *Anabaena* has been functionally classified as tolerant of low nitrogen conditions, but sensitive to mixing and low light, utilizing buoyancy regulation to counteract this sensitivity (Reynolds et al., 2002). Like a number of other cyanobacterial genera, it is tolerant of low CO_2 concentrations, as it relies on the enzyme, carbonic anhydrase, to access bicarbonate (Shiraiwa and Miyachi, 1985). There are two main HAB-forming species typically reported in the scientific literature – *A. circinalis* and *A. flos-aquae*. Recent studies of *Anabaena* have focused principally on two main aspects: the life cycle; and the role

of physical conditions, namely light, stratification, salinity and water flow regimes in promoting growth. There have also been advances in the understanding of the life cycle of *Anabaena*, particularly focused on factors causing akinete formation and germination (Tsujimura and Okubo, 2003; Karlsson-Elfgren and Brunberg, 2004; Faithfull and Burns, 2006; Thompson et al., 2009). The role of nutrients, and the interaction with physical conditions, has also received some attention.

3.1.1. Potential nutrient effects

Anabaena is diazotrophic under low dissolved inorganic nitrogen conditions (Fogg, 1942). Many papers have examined this capacity in both field and laboratory studies, and demonstrated that this physiological ability permits *Anabaena* to outcompete non-nitrogen fixers in N depauperate waters (e.g. Kangatharalingam et al., 1991; Chan et al., 2004; Wood et al., 2010) and even other diazotrophs such as *Aphanizomenon* (DeNobel et al., 1997). Since *Anabaena* is a diazotroph, P appears to be a key limiting nutrient for surface blooms of this genus. Limitation by P may also promote akinete production, a strategy for ensuring that populations can recover when P becomes available again (Olli et al., 2005). Recently an agent-based model of the life cycle of *Anabaena* determined that soon after germination, populations get most of their nutrients from the sediment bed (Hellweger et al., 2008). This may give *Anabaena* a competitive advantage over other non-akinete forming genera, at least in the early stages of bloom formation, until P becomes depleted or cells move into surface waters. Furthermore, Rapala et al. (1997) found that both growth rate and intracellular MCY concentrations of two *Anabaena* isolates increased with increasing P concentrations. However, increases in DIN (e.g. nitrate) did not yield a significant increase in growth rate and had differing effects on the production of various microcystin congeners. An additional strategy available to *Anabaena* (and other cyanobacterial species) is the ability to utilize organic forms of N and P. Recently genes putatively encoding alkaline phosphatase analogs have been identified in *Anabaena* (Luo et al., 2010).

3.1.2. Potential climate change effects

It has been proposed that increasing temperature will benefit cyanobacteria, both directly and indirectly by increasing thermal stratification (Paerl and Huisman, 2008) and there is evidence these processes will specifically promote *Anabaena*. Strong stratification that minimizes the availability of remineralized nutrients in surface waters should favor diazotrophs such as *Anabaena* and also specifically favors *Anabaena* physiology due to its ability to control buoyancy in the water column (Oliver, 1994). Consistent with this concept, Brookes et al. (1999) reported that *Anabaena* forms blooms under thermally stratified conditions due to the ability to regulate its buoyancy, and access sufficient light for growth and McCausland et al. (2005) specifically demonstrated that stable conditions indicative of diurnal stratification promote growth of *A. circinalis*. A recent study in a German lake showed that *Anabaena* may benefit from increased thermal stratification as a result of temperature increases, although, this appeared to be linked to their ability to regulate their buoyancy and access nutrients in the hypolimnion rather than a direct temperature effect (Wagner and Adrian, 2009). Studies of *A. circinalis* populations in the lower Murray River, Australia, exposed to persistent stratification were shown to grow faster than under diurnally stratified or mixed conditions (Westwood and Ganf, 2004a). Additionally, Westwood and Ganf (2004b) found that blooms were unlikely to form when periods of diurnal stratification were less than 1 week. Finally, temperature will also have differential physiological impacts on phytoplankton and a recent laboratory study found that increasing water temperatures from 18 to 23 °C increased *Anabaena* photosynthetic performance in comparison with the cyanobacteria *Microcystis* and *Arthrospira* (Giordanino et al., 2011).

Flow conditions affect stratification in cease-to-flow river systems, and therefore changes in rainfall patterns, and hence runoff to rivers, will have an impact on this. Studies have identified critical discharges to control *A. circinalis* blooms in the Barwon-Darling River, Australia (Mitrovic et al., 2003, 2006). Researchers found that there was a 12% probability of *A. circinalis* blooms exceeding 15,000 cells mL $^{-1}$ under typical flow conditions in the Murray River and threshold flow rates were described to reduce probability of blooms (Maier et al., 2004). Modeling studies of a thermally stratified reservoir with regular blooms of *A. circinalis* have shown that they can be controlled by the use of aerators or surface mixers (Lewis et al., 2004). *Anabaena* is purported to grow in both fresh- and brackish waters and a recent study of *Anabaena* in field experiments demonstrated that both growth and toxin production were higher at lower salinity (Engström-Öst and Mikkonen, 2011). As such, higher flow rates within river systems connected to estuaries may move *Anabaena* blooms into the brackish portions of estuaries.

These studies highlight the potential effect of climate change driven effects on rainfall patterns, and hence flow regimes. In southeast Australia, the combination of a predicted decrease in rainfall coupled with increases in air temperature and evaporation is modeled to give rise to measurable increases in *Anabaena* bloom occurrence and duration (Viney et al., 2007).

3.2. Microcystis

Microcystis is one of the most common bloom formers in freshwater systems on every continent except Antarctica (Fristachi and Sinclair, 2008). This genus can produce a suite of potentially harmful compounds including MCYs, anatoxin-(a), and BMAA (Fristachi and Sinclair, 2008). Not all *Microcystis* cells produce MCY as bloom populations of *Microcystis* are typically comprised of MCY-producing (MCY+) and non-MCY producing (MCY) strains that are distinguishable only via molecular quantification of the MCY synthetase gene operon (*mcyA – J*; Tillett et al., 2000) and a molecular marker for the total *Microcystis* population, such as the 16S rRNA gene (Kurmayer and Kutzenberger, 2003; Davis et al., 2009). This method of distinguishing between these sub-populations has been used in laboratory and field studies during the past decade (e.g. Rinta-Kanto et al., 2005; Davis et al., 2010; Van de Waal et al., 2011; Wood et al., 2011). There is evidence that indicates that global change to aquatic ecosystems such as rising temperatures, nutrient loads, and CO_2 concentrations will affect the dominance and toxicity of *Microcystis*.

3.2.1. Potential nutrient effects

Historically, P has been considered the primary limiting nutrient in freshwater ecosystems (Likens, 1972; Schindler, 1977; Wetzel, 2001; Kalff, 2002; Paerl, 2008). There is evidence to suggest, however, that N may be equally or more important than P in the occurrence of toxic, non-diazotrophic cyanobacteria blooms, such as *Microcystis*. Laboratory studies have shown that increasing N concentrations will generally increase the growth and toxicity of *Microcystis* (Watanabe and Oishi, 1985; Codd and Poon, 1988; Orr and Jones, 1998). Furthermore, experiments have established positive relationships between DIN supply, MCY production, and MCY content in toxic strains of *Microcystis* (Utkilen and Gjølme, 1995; Orr and Jones, 1998; Long et al., 2001). Field studies of *Microcystis* have also found that blooms are often associated with high levels of N (Jacoby et al., 2000; Gobler et al., 2007; Davis et al., 2010; Liu et al., 2011; Te and Gin, 2011; Paerl et al., 2011).

Nutrients can also differentially affect the relative abundance of MCY+ and MCY− *Microcystis* strains. Laboratory experiments have shown that MCY− strains of *Microcystis* require lower nutrient concentrations to achieve maximal growth rates compared to MCY+ strains whereas MCY+ strains yield higher growth rates than MCY− strains at high N concentrations (Ve´zie et al., 2002). Consistent with this trend, field studies have shown that bloom populations of *Microcystis* shifted from dominance of MCY+ strains to MCY− strains as inorganic N concentrations declined through the summer (Davis et al., 2010). Several other studies have observed a similar seasonal succession of *Microcystis* populations (Briand et al., 2004; Fastner et al., 2001; Welker et al., 2007) or have noted the dominance of MCY− strains during the peak of *Microcystis* bloom event (Welker et al., 2003, 2007; Kardinaal et al., 2007). Since inorganic nutrient levels are generally reduced when algal blooms occur (Sunda et al., 2006), the predominance of MCY− strains during this period may be a function of their ability to outcompete MCY+ strains when nutrient levels are lower (Ve´zie et al., 2002). Consistent with this hypothesis, during field-based, incubation experiments, MCY+ strains were more frequently stimulated by higher concentrations of N than their MCY− counterparts (Davis et al., 2009, 2010). Microcystin is a N-rich compound (10 N atoms per molecule) and studies have found that microcystin can represent up to 2% of cellular dry weight of *Microcystis* (Nagata et al., 1997). Additionally, toxic *Microcystis* strains have N requirements associated with the enzymes involved in the synthesis of MCY (Tillett et al., 2000) as well as with additional light-harvesting pigments they may possess (Hesse and Kohl, 2001). Although the precise mechanism is unclear, toxic *Microcystis* cells seem to have a higher N requirement than non-toxic cells (Ve´zie et al., 2002; Davis et al., 2010).

Studies have shown that some forms of DON can be utilized by *Microcystis* blooms. Field studies conducted by Takamura et al. (1987) and Pre´sing et al. (2008) using ^{15}N-labeled nitrogenous compounds demonstrated that *Microcystis* was able to take up nitrate, ammonium, and urea. During a study of a New York lake where *Microcystis* represented more than 98% of the >20 μm phytoplankton population, this size-fraction displayed flexibility in N assimilation, obtaining the majority of its N from nitrate, ammonium or urea on different occasions, as well as some of its N from glutamic acid (Davis, 2009). Uptake rates of ammonium and urea by the >20 μm size plankton community were significantly correlated with ambient concentrations of these nutrients ($P < 0.05$) suggesting that N utilization by *Microcystis* was dependent on nutrient availability. The >20 μm phytoplankton group also obtained significantly more of its total N from organic compounds than did smaller plankton (<20 μm), emphasizing the importance of organic N as a source of nutrition for *Microcystis*. In support of this hypothesis, Berman and Chava (1999) found that non-axenic cultured *Microcystis aeruginosa* consistently grew best using urea as a N source. Additionally, Dai et al. (2009) found that a Chinese strain of *M. aeruginosa* was able to utilize amino acids, such as alanine, leucine, and arginine to support growth and toxin production. Furthermore, genes associated with the uptake and utilization of urea and amino acids have been identified in *M. aeruginosa* (Kaneko et al., 2007; Frangeul et al., 2008). Given that *Microcystis* can efficiently utilize both organic and inorganic species of N, successful bloom mitigation strategies will need to target reductions in both N sources.

Phosphorus loading can favor the dominance of cyanobacteria within phytoplankton communities (Fogg, 1969; Smith, 1986; Downing et al., 2001) and may also specifically promote the density and/or toxicity of *Microcystis*. For example, Utkilen and Gjølme (1995) found that an increase in P concentrations can lead to an increase in MCY content of *Microcystis* cells. Until recently, most field work conducted relating the toxicity of cyanobacteria blooms to P have been correlative field studies which found MCY to be both positively and negatively correlated with various P pools (Wicks and Thiel, 1990; Kotak et al., 1995; Lahti et al., 1997; Rinta-Kanto et al., 2009). Recent field studies in North America examining MCY+ and MCY− strains of *Microcystis* suggest that MCY+ strains of *Microcystis* dominated the community during times of elevated inorganic P (DIP) concentrations whereas MCY− strains became more abundant when DIP concentrations were depleted (Davis et al., 2010). Consistent with this trend, MCY+ strains were enhanced by experimental P loading more frequently than MCY− strains (Davis et al., 2009, 2010). These findings parallel the work of Ve´zie et al. (2002) who reported that the growth rates of MCY+ *Microcystis* cultures exceeded MCY− strains under high orthophosphate concentrations. Since some MCY+ have more light-harvesting pigments than MCY− strains (Hesse and Kohl, 2001), the RNA and DNA required for the synthesis of both light-harvesting pigments and microcystin by MCY+ strains may represent a significant P requirement not present in MCY− strains.

Recent genomic sequencing of two strains of *Microcystis* (Kaneko et al., 2007; Frangeul et al., 2008) has revealed an array of genes involved in the utilization of P including two high affinity phosphate binding proteins (*pstS* and *sphX*) and a putative alkaline phosphatase (*phoX*). Subsequent sequence analyses among 10 clones of *M. aeruginosa* has demonstrated that these genes are present and conserved within the species and are strongly up-regulated (50–400-fold) by low DIP conditions (<2 μM) but not by organic P sources (Harke et al., 2011). Since *Microcystis* dominates phytoplankton assemblages in summer when levels of DIP are often low (Bertram, 1993; Wilhelm et al., 2003) and/or dominate lakes with low DIP and high organic P (Heath et al., 1995; Vanderploeg et al., 2001; Raikow et al., 2004), this species may rely on *pstS*, *sphX*, and *phoX* to efficiently transport DIP and exploit organic sources of P to form blooms.

3.2.2. Potential climate change effects

Microcystis grows and photosynthesizes optimally at, or above, 25 °C (Konopka and Brock, 1978; Takamura et al., 1985; Robarts and Zohary, 1987; Reynolds, 2006; Jo¨hnk et al., 2008; Paerl and Huisman, 2008, 2009) and within an ecosystem setting, *Microcystis* has been shown to out-compete species of eukaryotic algae at even higher temperatures (≥30 °C; Fujimoto et al., 1997). Temperature effects on stratification may further promote this genus. For example, like many bloom-forming cyanobacteria, *Microcystis* can alter its position in the water column by regulating gas vesicle production (Walsby, 1975; Walsby et al., 1997) and negatively buoyant carbohydrate stores (Kromkamp and Walsby, 1990; Visser et al., 1995, 1997). Since strong stratification generally favors the proliferation of buoyancy regulating cyanobacteria (Kanoshina et al., 2003; Jacquet et al., 2005; Fernald et al., 2007; Jo¨hnk et al., 2008), increasing water temperatures that simultaneously increase stratification will further promote the dominance of cyanobacteria such as *Microcystis* (Paerl and Huisman, 2009). Beyond stratification, warmer temperatures also decrease water viscosity, a change that may increase the sedimentation rate of eukaryotic algae and further strengthen the competitive advantage of *Microcystis*.

Although theoretical studies have predicted that *Microcystis* and other bloom forming cyanobacteria will dominate under higher temperatures, information regarding how subpopulations of *Microcystis* will be affected by changes in water temperature has been scarce. Davis et al. (2009) conducted surveys and temperature manipulation experiments in multiple ecosystems across the temperate northeast USA and found that *Microcystis* became the dominant phytoplankton species present at all six study sites as temperatures reached their annual maximum. During field-based experiments, a ≥4 °C increase in temperatures yielded significantly

higher growth rates for the MCY+ cells in most experiments, while the growth rates of MCY cells were enhanced by higher temperature in only a third of experiments conducted (Davis et al., 2009). Consistent with these trends, Kim et al. (2005) found that toxic *Microcystis* strains cultured at 25 8C had more *mcyB* transcripts than cultures reared at 20 8C. Collectively these studies suggest that higher temperatures not only promote *Microcystis* blooms but may favor the proliferation of MCY+ strains, and/or strains with more MCY synthetase gene operons.

Changes in salinities due to changes in drought/storm cycles may affect *Microcystis* distribution and toxin production, since toxin production can increase with salinity. For instance, *Microcystis* PCC 7806 has high salt tolerance compared to most other freshwater phytoplankton (Tonk et al., 2007). This suggests that in freshwater ecosystems exposed to increasing salinity *Microcystis* may gain an advantage over other phytoplankton species with lower salt tolerances and may become more toxic (Robson and Hamilton, 2003).

The impacts of rising CO_2 concentrations on cyanobacterial blooms is an area of research that has not, to date, been explored in great detail and as described above, their precise response to these conditions is uncertain. A recent study investigating the impacts of increased CO_2 concentrations on competition between MCY+ and MCY strains of *Microcystis* found MCY+ strains dominated at low CO_2 concentrations, whereas MCY strains were more abundant under elevated CO_2 concentrations (Van de Waal et al., 2011). The authors note that prior studies have found that MCYs could play a role in the acquisition of CO_2 at low concentrations (Jähnichen et al., 2001, 2007). Furthermore, another study found elevated concentrations of MCYs in the carboxysomes of cyanobacteria (Gerbersdorf, 2006). Given that previous research has demonstrated that elevated temperature favors MCY+ *Microcystis* strains (Davis et al., 2009) the response of this harmful cyanobacterial bloom species to future climate change scenarios that include temperature *and* CO_2 concentrations is difficult to predict. Further research into the response of *Microcystis* to changes in CO_2 concentrations alone, and in conjunction with other global change parameters, is needed to better understand these interactions.

3.3. Cylindrospermopsis

The cyanobacterium *Cylindrospermopsis* is a solitary, filamentous diazotroph. It was once thought to be a strictly tropical/subtropical species being first identified in Java in 1912 (Komárková, 1998). In the past decade there has been a substantial expansion in its geographical range across every continent, except Antarctica: Australia/Oceania (Hawkins et al., 1985; Wood and Stirling, 2003), North America (Chapman and Schelske, 1997; Hamilton et al., 2005; Hong et al., 2006), South America (Branco and Senna, 1996; Bouvy et al., 2006; Figueredo and Giani, 2009), Europe (Fastner et al., 2003; Saker et al., 2003; Briand et al., 2004; Monteiro et al., 2011), Africa (Dufour et al., 2006; Mohamed, 2007) and Asia (Chonudomkul et al., 2004). *Cylindrospermopsis* was first deemed a harmful bloom species after a toxic bloom event in 1979 caused acute hepato-enteritis and renal damage among more than 150 people on Palm Island, off the coast of North Queensland, Australia (Hawkins et al., 1985; Carmichael, 2001). The structure of cylindrospermosin (CYN), the toxin responsible, was determined in 1992 (Ohtani et al., 1992), when the mystery of the so-called "Palm Island disease" was resolved (Griffiths and Saker, 2003). Subsequently, it has been shown that other cyanobacteria including *Umezakia natans* (Harada et al., 1994), *Aphanizomenon ovalisporum* (Shaw et al., 1999; Carmichael, 2001), *Lyngbya wollei* (Seifert et al., 2007), *Raphidiopsis mediterraena* (McGregor et al., 2011), and *Anabaena lapponica* (Spoof et al., 2006) also are capable of producing CYN.

Although, *Cylindrospermopsis raciborskii* can be found on almost every continent, like most cyanobacterial bloom species the ability to produce CYN is not universal. *C. raciborskii* has CYN producing (CYN+) and non-CYN producing (CYN) strains identifiable by the presence or absence of the CYN biosynthesis gene cluster (*cyrA – cyrO*; Mihali et al., 2008). Lagos et al. (1999) found that Brazilian strains of *C. raciborskii* do not produce CYN although some strains do produce the neurotoxin, saxitoxin. Also, previous studies have found that European and Asian *C. raciborskii* strains can be toxic to mice but do not contain any of the known cyanotoxins (Fastner et al., 2003; Saker et al., 2003). While there have been accounts of CYN being associated with systems containing *Cylindrospermopsis* in North America (Burns, 2008) and Italy (Messineo et al., 2010), no North American or European strain has been found to produce CYN or contain the CYN synthesis genes (Neilan et al., 2003; Kellmann et al., 2006; Yilmaz et al., 2008). Therefore, only Australian (Hawkins et al., 1985; Ohtani et al., 1992), New Zealand (Wood and Stirling, 2003) and some Asian (Li et al., 2001b; Chonudomkul et al., 2004) strains of *C. raciborskii* have been found to produce CYN with Australian and New Zealand strains also producing the CYN analogue, deoxy-cylindrospermopsin (Norris et al., 1999; Wood and Stirling, 2003).

3.3.1. Potential nutrient effects

C. raciborskii is a diazotroph, but low DIN conditions are not a prerequisite for blooms. A microcosm experiment examined the competition between *C. raciborskii* and another diazotroph, *Anabaena* spp. found that *C. raciborskii* was a stronger competitor for DIN than *Anabaena* (Moisander et al., 2008). Studies have shown that under DIN replete conditions, DIN uptake rates were higher than N fixation rates for *C. raciborskii*-dominated waters (Preísing et al., 1996; Burford et al., 2006). Since diazotrophy is an energetically costly biochemical process, it is not surprising that ammonium is preferentially used, when available. Laboratory studies have confirmed that *C. raciborskii* growth rates were fastest when N was supplied as ammonium, followed by nitrate, then urea (Saker et al., 1999; Hawkins et al., 2001; Saker and Neilan, 2001). It has been proposed that activation of N_2-fixation was dependent on the N content of the cells (Sprößer et al., 2003). Therefore, *C. raciborskii* seems to display a flexible N strategy: when DIN concentrations are sufficient, this source is used, and during periods of depletion, N_2-fixation is employed.

Little is known about the effect of N on CYN production. Several studies have investigated the impact of different sources of DIN on CYN content of Australian isolates of *C. raciborskii* and found that the highest intracellular CYN content (reported as % of freeze-dried weight) were in the cultures devoid of a fixed N source and lowest in cultures grown with saturating concentrations of ammonium (Saker et al., 1999; Saker, 2000; Saker and Neilan, 2001). This contrasts with patterns of growth rates that were highest in the presence of ammonium and the lowest in the absence of a fixed N source (Saker et al., 1999; Saker and Neilan, 2001). Mihali et al. (2008) hypothesized that increased intracellular CYN content in the absence of fixed N was due to the flanking of the CYN biosynthesis gene cluster in the *C. raciborskii* genome by *hyp* gene homologs associated with the maturation of hydrogenases. Since the *hyp* gene cluster is controlled by the global N regulator (*ntcA*; activates the transcription of the N assimilation genes) in another cyanobacterium, *Nostoc* sp. strain PCC73102, it is plausible that the *hyp* genes and, therefore, the CYN biosynthesis gene cluster are under the same regulation in *C. raciborskii* (Mihali et al., 2008).

Phosphorus appears to play an important role in the dominance of, and CYN production by, *C. raciborskii*. This species blooms in reservoirs and lakes when phosphate concentrations are below detection limits (Padisák and Istvanovics, 1997; Burford and O'Donohue, 2006). Istvánovics et al. (2000) showed that a