Susanna A. Wood, Jonathan Puddick, Hugo Borges,
Daniel R. Dietrich, and David P. Hamilton

5 Potential effects of climate change on cyanobacterial toxin production

5.1 Introduction

Cyanobacteria are a group of ancient oxygenic photosynthetic prokaryotic organisms originating between three and four billion years ago. They have been reported in a wide range of environments, including oceans, lakes and rivers, as well as extreme habitats such as geothermal springs, desert soils and the Polar regions [1]. Cyanobacteria can exist as solitary, free-living cells or as colonies/filaments consisting of several to thousands of cells enclosed in mucilage. Whilst most cyanobacterial colonies/filaments and single cyanobacteria cells are microscopic, large populations become visible as mats, crusts and blooms. Populations may be planktonic (Fig. 5.1 (a, b)), suspended in the water column, or benthic, growing on bottom substrate and sometimes forming extensive mats (Fig. 5.1 (c, d))

Extensive blooms or benthic mats of cyanobacteria can be monocultures or more commonly consist of multiple species. They have been associated with increases in nutrient concentrations (i.e. eutrophication), which has been linked to human activities such as de-forestation, agriculture and urbanization. Blooms may be catalyzed by other factors which have been linked to climate change, including increased water temperature, variations in precipitation, extended droughts and increased carbon dioxide levels (see Chapter 7).

Fig. 5.1: Images of (a and b) planktonic cyanobacterial shoreline scums (*Microcystis* sp.) and (c and d) benthic *Phormidium* mats.

There are approximately 2000 cyanobacteria species described worldwide and more than 50 are known to have strains which produce natural compounds that are toxic (cyanotoxins). Cyanotoxins are a threat to humans when ingested (via water supplies or accidental swallowing) or from contact (dermal or inhalation). Cyanotoxins exhibit a wide range of toxicity mechanisms including: hepatotoxicity; nephrotoxicity; neurotoxicity; and dermatotoxicity [2]. Cyanotoxins can be divided into three broad groups based on chemical structures: cyclic peptides (microcystins and nodularins), alkaloids (cylindrospermopsins, saxitoxins and anatoxins) and lipopolysaccharides (LPS; Tab. 5.1).

Tab. 5.1: Cyanotoxins produced by freshwater cyanobacteria (Adapted from Tab. 3.1 of reference [2]).

Cyanotoxin	Primary target in mammals	Structural characteristics
Microcystins	Liver Protein phosphatase inhibitors	Cyclic heptapeptide
Nodularins	Liver Protein phosphatase inhibitors	Cyclic pentapeptide
Cylindrospermopsins	Liver Protein synthesis inhibitors	Tricyclic alkaloid with attached uracil
Saxitoxins	Nerve axon Blockage of voltage-gated sodium channels	Tricyclic alkaloid
Anatoxin-a	Nerve synapse Agonists of nicotinic acetylcholine receptors	Bicyclic secondary amine
Anatoxin-a(S)	Nerve synapse Acetylcholinesterase inhibitors	Organophosphate alkaloid
Lipopolysaccharides	Dermis Irritant of all exposed tissue	Lipopolysaccharide

5.1.1 Microcystins and nodularins

Globally, microcystins are the most frequently found cyanotoxins [2]. Microcystins are cyclic peptides (Fig. 5.2 (f)) and to date, more than 100 variants have been isolated and characterized [3]. Microcystins and nodularins both contain the unique beta-amino acid Adda, but whilst microcystins are heptapeptides, nodularins contain only five amino acids (Fig. 5.2 (e)). Many cyanobacteria genera produce microcystins [2]. In contrast, nodularins have only been reported to be produced by *Nodularia spumigena*, primarily a brackish-water species [2], as well as a *Nostoc* species that grows symbiotically with cycads [4].

Each microcystin variant differs with respect to the methyl groups and two amino acids within the cyclic peptide ring. This results in pronounced differences in the toxicity of variants. Microcystins and nodularins are hepatotoxins that inhibit protein phosphatases 1 and 2A in affected organisms [5, 6]. Numerous incidents of animal and human poisoning have been attributed to microcystins and nodularins [7]. One of the most severe cases occurred in Brazil in 1996, when water supply to a hospital was contaminated with microcystins. This resulted in 56 fatalities at a dialysis treatment clinic in the hospital [8].

5.1.2 Cylindrospermopsins

Cylindrospermopsin is a tricyclic alkaloid (Fig. 5.2(c)). Two variants of cylindrospermopsin exist; 7-epicylindrospermopsin, a minor metabolite of the cyanobacterium *Aphanizomenon ovalisporum* [9] and deoxy-cylindrospermopsin [10], which is now thought to have a similar toxicity to cylindrospermopsin. Cylindrospermopsin is a potent protein synthesis inhibitor and causes extensive damage to the liver and kidney [11, 12]. Falconer and Humpage (2001) have also suggested that cylindrospermopsin may act directly as a tumor initiator [13]. Cylindrospermopsin was implicated in one of the most significant cases of human poisoning from exposure to a cyanobacterial toxin when 148 people were hospitalized in 1979 with symptoms of gastro-enteritis after a local water supply on Palm Island (Australia) was dosed with copper sulphate to control a dense *Cylindrospermopsis raciborskii* bloom [14–16].

5.1.3 Saxitoxins

Saxitoxins are alkaloids (Fig. 5.2(d)) and are fast-acting neurotoxins that inhibit nerve conduction by blocking sodium channels [17]. Saxitoxins are commonly produced by marine dinoflagellates under the name of paralytic shellfish toxins. Whilst saxitoxins from freshwater cyanobacteria have not been implicated in any cases of human intoxication [2], they have killed animals [18] and were identified in an extensive bloom of *Dolichospermum* (formally known as *Anabaena*) *circinalis* on the Murray Darling River (Australia); this resulted in the death of over 1600 sheep and cattle [19].

5.1.4 Anatoxin-a and homo-anatoxin-a

These alkaloid toxins (Fig. 5.2(a)) are powerful depolarizing neuromuscular blocking agents acting through the nicotinic acetylcholine receptor [20]. They are rapidly absorbed when ingested orally. In sufficiently exposed animals, these alkaloid toxins can induce convulsions, coma, rigors, cyanosis, limb twitching, hypersalivation

and death. Both have been linked with animal and wildfowl poisonings; however, there have been no sufficiently verified reports of human poisonings/fatalities from anatoxin-a [7]. Anatoxins are produced by both planktonic [2] and benthic species [21].

5.1.5 Anatoxin-a(S)

Anatoxin-a(S) (Fig. 5.2 (b)), which is structurally different from anatoxin-a, is around ten-fold more potent using the mouse bioassay [2]. It is a cholinesterase inhibitor that induces hypersalivation, diarrhoea, shaking and nasal mucus discharge in mammals [22, 23]. It is thought to be produced only by *Dolichospermum lemmermannii* [24] and *Dolichospermum flos-aquae* [23]. Because it is so rarely detected, very little research has been undertaken on this toxin and it is not discussed further in this chapter.

5.1.6 Lipopolysaccharides (LPS)

These are integral components of the cell wall of all gram-negative bacteria, including cyanobacteria. The LPS are found in the outer cell membrane and form complexes with proteins and phospholipids [2]. LPS can elicit irritant and allergenic responses in humans and animals tissue [25, 26]. Although comparatively poorly studied, LPS have been implicated in human health problems associated with exposure to cyanobacteria [7]. Because of the limited information on these toxins, they are not discussed further in this chapter.

Recent studies suggest that cyanobacteria will thrive under conditions of global climate change (see Chapter 7 or [27–31]). Cyanobacteria are considered the most important and dominant phototrophs in polar freshwater and terrestrial ecosystems, and recent research has shown that toxin production is more widespread than initially thought [32–35]. Climate change therefore has the potential to have a pronounced and dramatic effect on cyanobacterial composition and toxin production. In this chapter, we investigate how climate change may impact biomass. We also consider how climate change could impact cyanotoxin production through three key mechanisms: change in the concentration of toxin-producing species (including the expansion of species into new environments, e.g. from tropical to temperate regions); shifts in the relative abundance of toxic and non-toxic genotypes of bloom-forming species; and variations in the relative amount of toxin produced at a strain level. We review data from both planktonic and benthic species. We also consider a geographic spread including temperate, tropical and polar cyanobacteria.

Fig. 5.2: Chemical structures of several common freshwater cyanotoxins: (a) anatoxin-a, (b) anatoxin-a(S), (c) cylindrospermopsin, (d) saxitoxin, (e) nodularin-R and (f) microcystin-LR.

5.2 Effects of climate change on common toxin producing species

In this section, we review four of the most common freshwater planktonic bloom forming genera: *Microcystis*, *Cylindrospermopsis*, *Dolichospermum* (Fig. 5.3(a–c)), and *Planktothrix*. Benthic cyanobacteria have also become increasingly problematic in some countries [36]. We therefore use *Phormidium* (Fig. 5.3(d)) as a case study to explore how climate change might affect benthic blooms in river environments.

Fig. 5.3: Common bloom forming cyanobacteria species: (a) *Dolichospermum* sp., (b) *Cylindrospermopsis raciborskii*, (c) *Microcystis* sp. and (d) *Phormidium* sp.

5.2.1 *Microcystis*

Microcystis is a ubiquitous genus that has been identified on all continents except Antarctica. *Microcystis* sp. are well known as a dominant bloom-forming species and they are commonly associated with the production of microcystins. *Microcystis* cells lack individual sheaths, but under natural conditions are usually organized in colonies that can consist of many thousands of cells which are surrounded by a common mucilage [37]. *Microcystis* can regulate its position in the water column through gas vesicle production [38, 39] and negatively buoyant carbohydrate stores [40, 41]. Both toxic and non-toxic genotypes exist, and blooms are usually comprised of both; they are only distinguishable via molecular detection of genes in the microcystin synthetase gene operon [42].

Increased water temperature and density stratification induced by climate change [43] are likely to favor proliferation of *Microcystis* sp. Optimal temperatures for growth and photosynthesis are greater than 25 °C [44–46]. Intense stratification generally favors buoyancy-regulating cyanobacteria, and may also result in more extensive *Microcystis* blooms [31]. Davis et al. (2009) provided environmental and experimental data to suggest that predicted climatic changes in concert with eutrophication may promote the growth of toxic, rather than non-toxic *Microcystis* [47]. They conducted surveys at six study sites in temperate northeast United States of America and found that *Microcystis* became the dominant phytoplankton species at all sites as water temperature approached its annual maximum. They also showed that elevated temperatures resulted in significantly increased growth rates of toxic *Microcystis* strains in 83 % of experiments, whereas growth rates of non-toxic strains only increased in 33 %. Further evidence of shifts to toxic strains was provided by Dziallas and Grossart (2011) who incubated toxic and non-toxic strains of *M. aeruginosa* both with and without (axenic conditions) bacteria in the laboratory at three temperatures (20, 26 and 32 °C) [48]. They observed a shift toward toxic strains at the higher temperature. In contrast, Martins et al. (2011) found selection of non-toxic *Microcystis* over toxic genotypes under favorable environmental growth conditions (including increased temperature) in a temperate reservoir in Northern Portugal [49].

Recent research suggests that rising atmospheric CO₂ levels may also affect the composition of toxic and non-toxic genotypes. Van de Waal et al. (2011) conducted chemostat experiments using mixed cultures of toxic and non-toxic *M. aeruginosa* [50]. The toxic strain reduced dissolved CO₂ to lower concentrations than the non-toxic strain, and became dominant at low CO₂ levels. Conversely, the non-toxic strain could grow at lower light levels, and became dominant at high CO₂ levels, but only under low light conditions.

5.2.2 *Cylindrospermopsis*

Cylindrospermopsis raciborskii is a solitary, filamentous diazotrophic freshwater cyanobacterium that was originally isolated from the lakes of Java (Indonesia) [51]. It poses a significant threat to ecological and human health because it can form dense blooms and produces cylindrospermopsins and saxitoxins [52]. Initially, it was believed to be confined to tropical environments, but over the past few decades appears to have expanded its range to many temperate regions across the globe [53–55].

Several researchers have suggested that *C. raciborskii* will become increasingly dominant due to global warming [52, 53, 56]. Growth rates of *C. raciborskii* respond strongly to temperature between 20 and 35 °C, with maximum rates around 30 °C [57–59]. In tropical environments, *C. raciborskii* can bloom year-round, but it only dominates in temperate systems during periods of elevated temperatures (i.e. summer months) [54, 55]. In temperate environments it can form spore-like cells (akinetes) that allow it to overwinter and subsequently germinate under favorable temperatures (22 to 24 °C) [60].

Both toxic and non-toxic *C. raciborskii* strains exist, and among toxic genotypes the amount of toxin produced per cell can vary markedly [57]. The characterization of the cylindrospermopsin biosynthesis pathway [61] and subsequent development of specific QPCR assays [62] have enabled researchers to demonstrate that toxic and non-toxic genotypes co-occur in field populations [63]. To our knowledge, there are no published studies investigating how variables related to climate change will impact the relative abundance of toxin-producing vs. non-toxin producing genotypes, either in laboratory experiments or in the field. Although there is speculation that *C. raciborskii* blooms will expand and intensify with climate change, the paucity of knowledge on responses of specific toxic genotypes makes inferences regarding the potential toxicity blooms uncertain.

5.2.3 *Dolichospermum*

Dolichospermum (formally known as *Anabaena*) is a ubiquitous filamentous diazotrophic genus found worldwide. Species within this genus have been reported to produce a suite of cyanotoxins including: microcystins; anatoxin-a; anatoxin-a(S); cylindrospermopsin and saxitoxin [2]. The two most commonly reported bloom-forming species are *D. circinalis*, *D. flos-aquae* and *D. lemmermannii*.

Dolichospermum most commonly blooms in bodies of water where there is strong temperature stratification. Both water temperature and stratification are predicted to increase with rising air temperature from climate change [56]. *Dolichospermum* may be selectively advantaged over other species in stratified systems; it can regulate buoyancy to allow for access to nutrients at depth or to regulate light dosage [64, 65]. It can

also fix atmospheric nitrogen with heterocytes under conditions of low availability of dissolved inorganic nitrogen [66, 67].

Using molecular techniques, researchers have shown that field populations of *Dolichospermum* comprise both toxic and non-toxic strains [68]. However, there are no published studies that explore how the relative abundance of toxic and non-toxic genotypes will shift with climate change.

5.2.4 *Planktothrix*

Planktothrix is filamentous genus that is usually planktonic, forms solitary trichomes and has gas vacuoles. Two species, *P. agardhii* (green coloured due to presence of phycocyanins) and *P. rubescens* (red coloured due to phycoerythrins), are common in the Northern Hemisphere where they form blooms. Some strains produce microcystins [69]. *Planktothrix agardhii* prefers lakes with low light availability [70]. In contrast, *P. rubescens* usually blooms in deep oligotrophic lakes, in the low light conditions of the metalimnion [71].

Dokulil and Teubner (2012) analysed a long-term dataset from Lake Mondsee (Austria) to explore the possible impacts of climate change on *P. rubescens* [72]. From this analysis, they suggest that *P. rubescens* will only benefit from climate warming early in the year, during late spring overturn and early summer. They also suggest that longer periods of summer stratification are unlikely to favor increasing biomass. The potential impacts of climate change on *P. agardhii* are still unclear. Bonilla et al. (2012) assembled a large, global dataset for this species [70] and showed that although it was only observed in temperate and subtropical lakes, it could also bloom at a range of temperatures from $< 2^{\circ}\text{C}$ [73] to 29°C [74, 75].

Both *Planktothrix* species can co-exist in water bodies and several studies have investigated the selective advantage of each under field conditions. *Planktothrix rubescens* is adapted to low light conditions, whereas *P. agardhii* is more tolerant of higher light intensities [76]. Oberhaus et al. (2007) demonstrated that the combined effects of temperature and light quality and quantity can impact the relative abundance of both species [77]. *Planktothrix rubescens* tended to be more competitive at lower temperatures (15°C) and low intensities of green light, whereas *P. agardhii* was more competitive at higher temperatures (25°C) and generally less specialized to light quality.

Briand et al. (2008a) investigated the effects of temperature, light intensity and nitrate concentrations on several microcystin-producing and non-microcystin-producing *P. agardhii* strains in monoculture and competition experiments [78]. They observed an advantage of toxic over non-toxic *P. agardhii* strains under growth limiting conditions (i.e. low light, low temperature or nitrogen limiting conditions). The reverse trend was observed under non-growth-limiting conditions, suggesting that toxic strains may dominate if water temperature rises. Briand et al. (2008b) tracked changes

in toxic and non-toxic genotypes in a shallow artificial lake in Paris [79]. They found that the abundance of the toxic genotype appeared to be inversely correlated with changes in the density of *P. agardhii* cells and to a lesser extent, with the availability of certain nutrients and the abundance of cladocerans. These data demonstrate the complexities of predicting the impact of climate change on natural populations.

5.2.5 *Phormidium*

The filamentous cyanobacterial genus *Phormidium* is found worldwide in diverse habitats ranging from intertidal marshes to Arctic and Antarctic lakes [80]. Under favorable conditions, some *Phormidium* species form benthic mats. In rivers in some countries, e.g. France and New Zealand, there has been an apparent increase in the prevalence of benthic *Phormidium* mats [21, 81–83]. When hydrological and environmental conditions are suitable, *Phormidium* can form thick cohesive black/brown mats that cover extensive areas of the river substrate (Fig. 5.1 (c, d)).

Heath et al. (2011) demonstrated that water temperature and river flow were the two main factors driving the presence of *Phormidium* mats [84]. They observed a marked increase in mats when river flows decreased below one-half of the yearly average and at water temperature $> 14^{\circ}\text{C}$. Using an in-stream habitat assessment method, Heath et al. (2013) showed that decreases in summer minimum low flows exhibited negligible change to available *Phormidium* habitat [85]. The authors concluded that the frequency of flushing flows (a flow that removes the *Phormidium* from the river substrate), and not flow alone, was critical in determining the presence of *Phormidium*.

Climate change is predicted to cause shifts in precipitation through: (a) strengthening of existing precipitation patterns, i.e. wet regions become wetter and dry regions become drier; and (b) changing storm tracks, which should move away from the equator and toward the poles as atmospheric circulation changes [86]. In France and New Zealand, rivers in drier areas are likely to have prolonged low-flow periods that would be conducive to promoting *Phormidium* mats.

Field and laboratory studies have demonstrated that *Phormidium* mats are commonly a mixture of toxic and non-toxic genotypes. Among toxic genotypes, anatoxin concentrations can vary up to 100-fold [87, 88]. Heath et al. (2011) undertook a one-year study at eight sites on two rivers and observed that anatoxin-a and homoanatoxin-a occurrence was restricted to where water temperature exceeded 13.4°C [84], and suggested that the toxin-producing strains may 'out-compete' non-toxic *Phormidium* strains under these conditions. More recent research has shown no correlation between water temperature and toxin production [89]. Further culture and laboratory-based studies are required to explore how temperature and other variables indirectly associated with climate change (e.g. river flow, rainfall) may impact toxin concentrations within the mats.

5.3 Effects of climate change on toxin regulation

Environmental variables regulating cyanotoxin production have been a topic of intense interest over recent decades; however, the research has been strongly biased towards microcystin production. Most studies have used laboratory cultures to demonstrate relatively small (three to four-fold) changes in toxin production in response to changes in a number of different variables (e.g. light, nutrients or temperature). More recently, researchers have used field studies to examine the factors regulating toxin regulation [90, 91]. Here we review research on toxin regulation related to variables that are predicted to be altered by climate change: temperature, light, dissolved carbon dioxide concentration and cell density (as a result of increased severity of cyanobacterial blooms).

5.3.1 Microcystins

Although the biosynthetic pathway for microcystins is now known [42, 92], there is still uncertainty surrounding the transcriptional and post-translational regulation of the microcystin synthase. Microcystins were initially thought to be feeding deterrents for predators, and studies showing increased microcystin content in cyanobacteria exposed to predators supported this theory [93, 94]. However, the genes responsible for microcystin synthesis pre-date the eukaryotic lineage [95].

Many early studies focused on the effect of temperature on microcystin production and showed that toxin concentrations were maximal around 20 to 25 °C and decreased at higher and lower temperatures [96–102]. More recent research incorporating gene expression has corroborated these results. For example, Dziallas et al. (2011) showed that microcystin transcripts were significantly lower at 32 °C than at 20 and 26 °C in axenic *Microcystis* cultures [48].

There are strong indications that light may play a regulatory role in microcystin production. Kaebernick et al. (2000) demonstrated that light quality specifically affects microcystin synthase expression, which is initiated at certain threshold intensities [103]. However, transcriptional changes in genes coding for microcystin production changes due to light did not correlate with observed variations in toxin production. Phelan and Downing (2011) showed a strong correlation between microcystin concentration and growth rate under high-light conditions in *Microcystis aeruginosa* PCC7806 [104]. However, no change was observed at optimal or low-light conditions, and media composition had no significant effect on the relationship between toxins and survival at high-light conditions. The authors therefore suggested a possible role for microcystin was in protection against photo-oxidation [104]. Recently, researchers have provided evidence to support an intracellular function of microcystin in acclimation of *Microcystis* to high light and oxidative stress [105]. Sevilla et al. (2012) used quantitative reverse transcriptase PCR to monitor changes in the

levels of transcripts encoding the microcystin-D-synthetase gene (*mcyD*) in *M. aeruginosa* PCC7806 subjected to oxidative agents and under different light intensities [106]. The study identified a link between microcystin synthesis and photosynthesis, and indicated that oxidative stress decreased microcystin synthesis. Using a DNA microarray based on the genome of *M. aeruginosa* PCC 7806, Straub et al. (2011) studied the dynamics of gene expression during the light/dark cycle [107]. They showed that the biosynthesis of secondary metabolites, including microcystins, occurred mostly during the light period, suggesting that microcystins synthesis is connected with photosystem II.

Light intensity has also been shown to alter ratios of microcystin variants. A strain of *Planktothrix agardhii* which produced [Asp³] congeners of both microcystin-LR and microcystin-RR exhibited increased levels of microcystin-LR at elevated irradiance levels [108]. The implication of this finding is that microcystin-LR is an order of magnitude more toxic than microcystin-RR, so the resulting phenotype from increased light intensity was more toxic. Another study using batch culture and a natural population of *M. aeruginosa* found that at a moderate temperature (20 °C) microcystin content would switch from being predominantly microcystin-LR to a less toxic phenotype that was predominantly microcystin-RR. At a higher temperature (28 °C), this did not occur and the levels of microcystin-LR and microcystin-RR production remained constant [101]. A similar observation was reported by Song et al. (1998) using *Microcystis viridis*, with higher proportions of microcystin-RR at lower temperatures (15–20 °C) [109].

There is very limited data on the impact of pH on toxin production. Van der Westhuizen and Eloff (1983) suggested *Microcystis* cells were more toxic at high and low pH values corresponding to slower rates of growth [110]. Jahnichen et al. (2007) studied the impact of inorganic carbon availability on microcystin production and suggested that microcystins may be involved in enhancing the efficiency of the adaptation of the photosynthetic apparatus to fluctuating inorganic carbon concentrations [111]. The potential effects of varying carbon dioxide levels on microcystin production from *Microcystis* are not well known. Van de Waal et al. (2009) showed that an excess supply of both nitrogen and carbon yielded high cellular nitrogen : carbon (N : C) ratios, accompanied by high cellular contents of total microcystin and the nitrogen-rich variant microcystin-RR [112]. They found comparable patterns in *Microcystis*-dominated lakes, where the relative microcystin-RR content increased with the seston N : C ratio.

Using field-based studies Wood et al. (2011) showed that *Microcystis* sp. can “switch” microcystin production on and off [90]. They also observed and experimentally induced 20-fold changes in microcystin quotas within a five-hour period and measured up to a 400-fold change in microcystin-E-synthetase gene (*mcyE*) expression [90, 91]. Both of these studies and a recent laboratory study [113] draw a correlation between microcystin production and cyanobacteria cell densities. This suggests that toxin production per cell may increase if blooms and scum formations intensify as is predicted under future climate change scenarios [56].

5.3.2 Nodularins

Little work has been performed on the environmental parameters that affect nodularin production. Lehtimäki et al. (1997) demonstrated that nodularin production was generally highest under conditions that promoted growth [114]. Intracellular nodularin concentrations increased at higher temperature (25 – 28 °C), higher irradiance (45–155 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and higher phosphate concentrations (200–5500 $\text{P } \mu\text{g l}^{-1}$).

5.3.3 Cylindrospermopsins

A study exploring the effect of temperature (20–35 °C) on growth and cylindrospermopsin content of *C. raciborskii* grown in batch culture showed a strong negative correlation between toxin content and temperature. When grown at 35 °C, none of the study strains produced any detectable cylindrospermopsin, but when transferred back to lower temperatures, cylindrospermopsin production was restored [57].

Preußel et al. (2009) highlighted how toxin production can vary among strains of the same species [115]. They investigated the influence of light and temperature on cylindrospermopsin production of two *Aphanizomenon flos-aquae* strains using semi-continuous cultures. A light gradient from 10–60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, in combination with temperatures of 16, 20, and 25 °C, was assessed. Cylindrospermopsin concentrations showed a significant decrease with increasing temperature in one strain, whilst there was no clear relationship with temperature in the other. They also noted that cylindrospermopsin production at different light intensities varied at the three temperatures. In both strains, cylindrospermopsin concentrations increased significantly at 20 °C for light intensities from 10 to 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, whereas they decreased significantly at 25 °C in the same light gradient. They also suggested that conditions which constitute physiological stress (i.e. low light and temperature) triggered active release of the toxin into the medium.

Dyble et al. (2006) investigated the role of light intensity on growth and cylindrospermopsin production in *C. raciborskii* cultures [116]. Maximum growth rates occurred at 75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, whereas maximum intracellular and extracellular toxin concentrations occurred in cultures grown under the highest light intensity (140 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The authors speculated that the highest intracellular toxin concentrations in the field are likely to occur in actively growing *C. raciborskii* populations exposed to light intensities of 75–150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for more than two weeks.

Few studies have investigated changes in cylindrospermopsin production in benthic cyanobacteria. Bormans et al. (2014) explored cylindrospermopsin production in *Oscillatoria* sp. PCC 6506 and demonstrated that the total cylindrospermopsin content was highest during the exponential growth phase at intermediate light levels (10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and during the stationary growth phase under lower and higher light levels.

5.3.4 Saxitoxins

Only a small number of studies have explored changes in saxitoxin synthesis in relation to potential climate change. In a study on *C. raciborskii* (strain T3), saxitoxin synthesis was up-regulated at a light intensity $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and not at 50 and $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ [117]. This study also showed that saxitoxin production exhibited a circadian rhythm under the three light intensities tested. A study on a different *C. raciborskii* strain (C10) found contrasting results [118]. In this study, the extracellular and intracellular saxitoxin content of this strain was analyzed at two different temperatures; 19 and 25 °C. At 25 °C an additional variant (dcSTX) was detected, but there was no significant change in toxin concentration. Comparing these data to other studies illustrates how responses to environmental parameters may differ among species. For example, Dias et al. (2002) studied *Aphanizomenon* sp. (LMECYA 31) and showed enhanced saxitoxin production at a high temperature (28 °C) compared to a lower temperature (22 °C) which was typical of *in situ* conditions [119].

5.3.5 Anatoxins

Rapala et al. (1993) studied *Dolichospermum* and *Aphanizomenon* and demonstrated that high temperature decreased the amount of the toxin produced regardless of growth rates [120]. Growth-limiting, low-light conditions and growth-inhibiting, high-light conditions decreased the amount of anatoxin in *Dolichospermum* cells. In contrast, the highest light flux of the study did not limit the growth or decrease the level of the toxin in the cells of *Aphanizomenon*. Rapala and Sivonen (1998) monitored anatoxin production in *Dolichospermum* strains and observed that anatoxin-a production was slightly higher under sub-optimal temperature and light levels [96]. Also, under low light, considerable amounts of extracellular anatoxin-a were detected. Araoz et al. (2005) studied *Ocillatoria* sp. (PCC6506), a benthic anatoxin-producing cyanobacteria, and noted shifts in the relative production of variants at different temperatures and when different carbon sources were utilized [121]. They observed that synthesis of anatoxin-a occurred at 22 °C when there was no carbon dioxide enrichment of the growth medium, while homanatoxin-a was produced when the medium was at 25 °C and supplemented with 10 mM sodium hydrogen carbonate to maintain carbon dioxide enrichment.

5.4 Climate change and its effect on cyanobacteria and toxin production in Polar environments

In Antarctica, cyanobacteria are considered the most important and dominant phototrophs in freshwater and terrestrial ecosystems [122]. Although less well studied, cyanobacteria are also abundant in the Arctic [123]. In both regions (where there is available water), they commonly form benthic or floating microbial mat communities which can be several centimeters thick (Fig. 5.4). Nostocales and oscillatoriales are usually dominant within these mats, and these taxa have adapted to tolerate harsh conditions, including high salinity, intense ultraviolet radiation and repeated cycles of desiccation/hydration [124, 125].

One of the fastest rates of climate warming has been documented in the Arctic and maritime Antarctic [126], with mean annual temperatures over the Antarctic Peninsula having increased by 0.5 °C per decade [127]. Freshwater habitats may be more severely impacted than terrestrial habitats as the relative increase in water temperature has been reported as two to three-fold higher than the corresponding change in local air temperature [128]. A rise in temperature in ice-free areas is expected to increase the availability of liquid water (from glacial and permafrost melting) [129]. Such changes could dramatically increase water availability, possibly facilitating increased cyanobacterial growth. Wood et al. (2008) found high abundance of “dormant” cyanobacteria in Dry Valley soils [130]. As water availability increases, extensive macroscopic growth could occur within a relatively short time frame. Experiments using cloches (small covers that provided protection from wind and increased temperature and water availability) positioned on arid Dry Valley soils provided evidence to support the suggestion that increased water availability will favor cyanobacteria, and showed rapid growth of moss and cyanobacterial biomass (personal communication with D. D. Wynn-Williams as cited in [129]).

Recent reports suggest that there is widespread microcystin production in Antarctic microbial mats including from the McMurdo Ice Shelf, Bratina Island [131, 132], McMurdo Dry Valleys [32] and the Antarctic Peninsula [35]. Although the microcystin producing species have not been definitively identified, Wood et al. (2008) used molecular techniques to provide compelling evidence that *Nostoc* sp. was the producer in at least some of the microcystin-positive samples [32]. Despite the high abundance of cyanobacteria in the Arctic [133], there is only one report of microcystins in cyanobacterial mats [33] and one report in cyanobacteria from lichens in this region [134].

Kleinteich et al. (2012) demonstrated that microcystin production and species diversity increased in laboratory-cultured Arctic and Antarctic cyanobacterial mats when they were exposed to elevated temperatures (8–16 °C compared to 4 °C) [33]. It was suggested that this could be the result of increased toxin production per cell due to more favorable conditions. These conditions might include higher temperatures favoring the growth of toxin-producing species and allowing them to become dominant

(a)

(b)

(c)

Fig. 5.4: Antarctic cyanobacterial mats: (a) extensive mats in a meltwater pond in Miers Valley, (b) small pinnacles dominated by *Leptolyngbya* sp. and (c) a large colony of *Nostoc* sp.

during the experiment, or higher species diversity/abundance that could intensify inter-species competition at higher temperatures. In the case of the latter hypothesis, increased toxin concentrations could serve as a means for quorum-sensing, intra-species communication or as allelopathic compounds. In addition to the potential ecosystem effects of increased microcystin production, Kleinteich et al. (2012) warned that changes in cyanobacterial diversity could lead to instability of the mat ecosystems, allowing the establishment of non-polar mesophilic species [33].

Although less well studied, several other cyanotoxins have been detected in the Polar regions. Kleinteich et al. (2014) confirmed the presence of cylindrospermopsin and deoxycylindrospermopsin in several cyanobacterial mats collected around Rothera (Antarctic Peninsula), although the concentrations measured were low compared with benthic species of warmer climatic zones [35]. Saxitoxin was detected for the first time in a cyanobacterial mat collected from northern Baffin Island in the vicinity of Cape Hatt in the Arctic [34]. To date, the identity of the toxin producer/s has not been determined. Anatoxins have not been identified in Polar samples, however this may be due to a lack of systematic surveys for this toxin, as several known producers are widespread in these regions (e.g. *Phormidium autumnale*). Although no data exist on how climate change will impact the abundance and diversity of toxins produced by cyanobacteria in Polar regions, rates of metabolism, nitrogen fixation and photosynthesis of Antarctic cyanobacteria are thought to be optimal around 15 °C [135]. Kleinteich et al. (2012) theorized that production of secondary metabolites may also be optimal at this temperature [33]. It is therefore plausible that climate change could yield increases in the concentration of these toxins, with as-yet unknown impacts on these fragile ecosystems.

5.5 Conclusions

There is increasing evidence that regional and global climatic change will benefit many species of harmful bloom-forming cyanobacteria (see Chapter 7 and [27–30]). It is predicted that there will be increases in cyanobacteria growth rates, leading to greater longevity and severity of bloom events, and shifts in geographic distributions. Research indicates that many of the key bloom forming genera, i.e. *Microcystis*, *Cylindrospermopsis* *Dolichospermum*, will benefit under predicted climatic change scenarios such as rising temperatures, enhanced water column vertical stratification and shifts in seasonal weather patterns (e.g. droughts).

It is extremely challenging to make generalizations about how climate change will impact cyanotoxin production. The studies to date have been undertaken using different conditions (e.g. growth media, batch vs. continuous culture), different methods for determining toxin concentrations (e.g. enzyme-linked immunosorbent assay, liquid chromatography-mass spectrometry) and have utilized different strategies for normalizing data to biomass (e.g. cell concentration, dry weight, optical density). Comparable studies have also shown contrasting responses between species (e.g. microcystin-producing *Microcystis* and *Planktothrix*), and even among strains of the same species (e.g. saxitoxin-producing *C. raciborskii*).

We have only reviewed studies that have investigated variables closely associated with climate change. Many other variables may be indirectly related to climate change (i.e. nutrients, metal availability) and have also been shown to impact toxin production. Changes in these variables may have an equal or greater impact on toxin production, or act synergistically with the variables discussed. Studies that have undertaken multi-stressor experiments, for example, investigating the effect on toxin production of temperature at a range of light intensities, have shown complex interactions and relationships, reinforcing how difficult it will be to predict the impacts of climate change in the natural environment.

Finally, it should be noted that the majority of studies have been undertaken in the laboratory. Whilst these have provided essential and underpinning knowledge on toxin synthesis, and enabled detailed studies of single stressors, important natural variables and interactions that may regulate toxin production are removed. There is an urgent need for further field-based investigations that can better mimic the complex interactions and multiple stressors that influence toxin production in cyanobacteria.

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