

# Factors affecting Growth of Cyanobacteria

With Special Emphasis on the Sacramento-San Joaquin Delta

Prepared for:

The Central Valley Regional Water Quality Control Board

And

The California Environmental Protection Agency

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## Executive Summary

A world-wide increase in the incidence of toxin-producing, harmful cyanobacterial blooms (cyanoHABs) over the last two decades has prompted a great deal of research into the triggers of their excessive growth. Massive surface blooms are known to decrease light penetration through the water, cause depletion of dissolved oxygen following bacterial mineralization of blooms, and cause mortality of aquatic life following ingestion of prey with high concentrations of toxins. Additionally, humans coming in contact with the water may develop digestive and skin diseases, and it may affect the drinking water supply.

The Central Valley Regional Water Quality Control Board (Water Board) is developing a science plan to scope the science needed to support decisions on policies governing nutrient management in the Delta. Blooms of cyanoHABs are one of three areas, identified by Water Board, that represent pathways of potential impairment that could be linked to nutrients. The Water Board commissioned a literature review of the factors that may be contributing to the presence of cyanoHABs in the Delta. The literature review had three major objectives:

- 1) Provide a basic review of biological and ecological factors that influence the prevalence of cyanobacteria and the production of cyanotoxins;
- 2) Summarize observations of cyanobacterial blooms and associated toxins in the Delta;
- 3) Synthesize literature to provide an understanding of what ecological factors, including nutrients, may be at play in promoting cyanobacterial blooms in the Delta.

This review had five major findings:

**#1. CyanoHABs appear to be a threat to Delta ecosystem services. However, a thorough characterization of the risks is greatly hindered by the fact that cyanoHAB prevalence and toxin concentrations are currently not routinely monitored. Based on existing data, the current risk to Delta ecosystem services appears to be on the lower end of the spectrum, though clearly not negligible.** This observation is based on: 1) phytoplankton biomass below benchmarks of concern, 2) cyanobacterial cell abundances that sometimes exceed the World Health Organisation (WHO) benchmark of low risk of health effects, but are consistently below that of moderate risk of health effects, and 3) toxin concentrations that are generally 16-80 times below the California Office of Environmental Health Hazard Assessment (OEHHA) action level, although occasionally exceeding both OEHHA action level and the WHO guidance level. Despite low risks to Delta ecosystem services, the risk of bioaccumulation in the food web is a potential factor that is generally not well quantified.

**#2. Five principal drivers emerged as important determinant of cyanobacterial blooms in a review of the global literature on factors influencing cyanobacteria blooms and toxins production. These include:** 1) Availability of N and P in non-limiting amounts; scientific consensus is lacking on the importance of N: P ratios and nutrient form (e.g. ammonium) as a driver for cyanoHABs; 2) high light

availability and water clarity; 3) Water temperatures; 4) Stratified water temperatures coupled with long residence times; and 5) salinity regime.

**#3. Comprehensive understanding of the role of nutrients vis-à-vis other environmental factors in influencing cyanoHAB presence in the Delta is severely hampered by the lack of a routine monitoring program.** Drawing on available information on the five factors influencing cyanoHABs, we can conclude the following:

- Temperature and irradiance appear to exert key roles in the regulation of the onset of blooms. Cyanobacteria require temperatures above 20°C for growth rates to be competitive with eukaryotic phytoplankton taxa, and above 25°C for growth rates to be competitive with diatoms. In addition, they require relatively high irradiances to grow at maximal growth rates.
- It appears that N and P are available in non-limiting amounts in the Delta; moreover, concentrations, or ratios, do not change sufficiently from year-to-year in order to explain year-to-year variation *Microcystis* biomass or occurrence. Therefore the initiation of *Microcystis* or other cyanoHAB blooms are probably not associated with changes in nutrient concentrations or their ratios in the Delta. However, as with all phytoplankton blooms, once initiated, cyanoHABs cannot persist without an ample supply of nutrients.
- Salinity is controlling the oceanward extent of cyanobacteria blooms in the Delta, but salinity gradients do not explain the spatial distribution of cyanoHABs in the Delta. Notably, salinity regime is not a barrier to toxin transport, as cyanotoxins have been detected in SF Bay.
- Turbidity, low temperatures, and higher flows during most of the year are likely restricting cyanobacteria blooms to the July-August time period.

**#4. Climate change and anthropogenic activity associated with land use changes have the potential to alter cyanoHAB prevalence in the future.** Climate change will likely result in warmer temperatures and increased drought, the latter of which could result in reduced flows, increased residence time and watercolumn stability leading to higher light availability in the Delta. Both temperature and reduced flows would presumably result in a greater prevalence of cyanoHABs. Increased nutrient loading on top of warmer temperatures and stratified water conditions could potentially increase the intensity (concentration) of cyanoHABs. It's noteworthy that phytoplankton biomass and primary productivity are depressed relative to available nutrients in the Delta, so it's unclear what the effect of modifying nutrient loads will have on frequency and intensity of cyanoHAB occurrence in the future.

Given these findings, two major science recommendations are proposed:

**R1: Implement Routine Monitoring of CyanoHABs.** DWR is currently conducting a monitoring program already exists which routinely samples many of the variables of interest known to influence cyanoHABs. Comprehensive cyanoHAB monitoring should be added as a component to this program. To begin, a work plan should be developed which specifically scopes the needed changes in the program to comprehensively monitor cyanoHABs. This report details specific components that should be considered in this workplan. The workplan should also consider monitoring needed to develop and calibrate an ecosystem model to further investigate controls on primary productivity and phytoplankton assemblage

(see R2 below). The workplan should be peer-reviewed by subject matter experts. After an initial period of 3-5 years, the monitoring data should be used to comprehensively report on the status and trends of cyanoHABs and the factors that favor bloom occurrence in the Delta.

**R2: Develop an Ecosystem Model of Phytoplankton Primary Productivity and HABs Occurrence to further Inform Future Risk and Hypotheses on Factors Controlling CyanoHABs.** Because nutrients are not currently limiting cyanobacterial blooms, it is critical that an improved understanding is gained of the factors that are controlling phytoplankton primary productivity in the Delta, since higher chlorophyll a could lead to increased risk of cyanoHAB blooms. To inform management action moving into the future, an ecosystem model of phytoplankton primary productivity and HABs occurrence should be developed. This model should have the capability to provide information on primary productivity and biomass as well as planktonic food quality and transfer of carbon to higher trophic levels. To step into model development, three actions should be taken: 1) examine existing models already available to determine suitability for this task, 2) utilize existing data to explore, to the extent possible, the relationships between chlorophyll a, phytoplankton composition, climate variables et al. factors. This analyses should inform hypotheses that can be tested through model development as well as potential future scenarios, and 3) a work plan should be developed that lays out the modeling strategy, model data requirements, and implementation strategy.

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## 1. Introduction, Purpose and Organization of the Review

### 1.1 Background and Context

The Sacramento–San Joaquin River Delta, is an inland river delta and estuary approximately 1300 square miles in size, found Northern California. Formed at the western edge of the Central Valley by the confluence of the Sacramento and San Joaquin Rivers, the Delta is a key component of the State’s water resource infrastructure and a region that is rapidly urbanizing, yet serves as critical habitat for fish, birds and wildlife. Water from the 45,000 square mile Delta watershed fuels both local and statewide economies, including important agricultural commodities. The Delta is widely recognized as in “crisis” because competing demands for the Delta’s resources. The consequences of these competing demands include point and non-point discharges, habitat fragmentation and loss, modified flow regimes, introduction of non-native species, all of which combine to threaten ecosystem health, including the continued decline of threatened and endangered species (Delta Plan 2013).

In 2009 the California legislature passed the Delta Reform Act creating the Delta Stewardship Council. The mission of the Council is to implement the coequal goals of the Reform Act and provide a more reliable water supply for California while protecting, restoring, and enhancing the Delta ecosystem. The Council wrote and adopted a Delta Plan in 2013 to implement these goals. Chapter 6 of the Delta Plan deals with water quality and contains recommendations to implement the coequal goals of the Delta Reform Act. Recommendation # 8 states, in part, “...the State Water Resources Control Board and the San Francisco Bay and Central Valley Regional Water Quality Control Boards (Water Board) should prepare and begin implementation of a study plan for the development of objectives for nutrients in the Delta ... by January 1, 2014. Studies needed for development of Delta... nutrient objectives should be completed by January 1, 2016. The Water Boards should adopt and begin implementation of nutrient objectives, either narrative or numeric, where appropriate, in the Delta... by January 1, 2018.

Potential nutrient related problems identified in the Delta Plan for evaluation are:

1. Decreases in algal abundance and shifts in algal species composition,
2. Increases in the abundance and distribution of macrophytes, including water hyacinth and Brazilian waterweed,
3. Increases in the magnitude and frequency of cyanobacterial blooms

To provide better scientific grounding for the study plan, the Water Board commissioned three literature reviews centered on these three potential areas of impairment. This document provides a synthesis of literature on cyanobacterial blooms in the Delta.

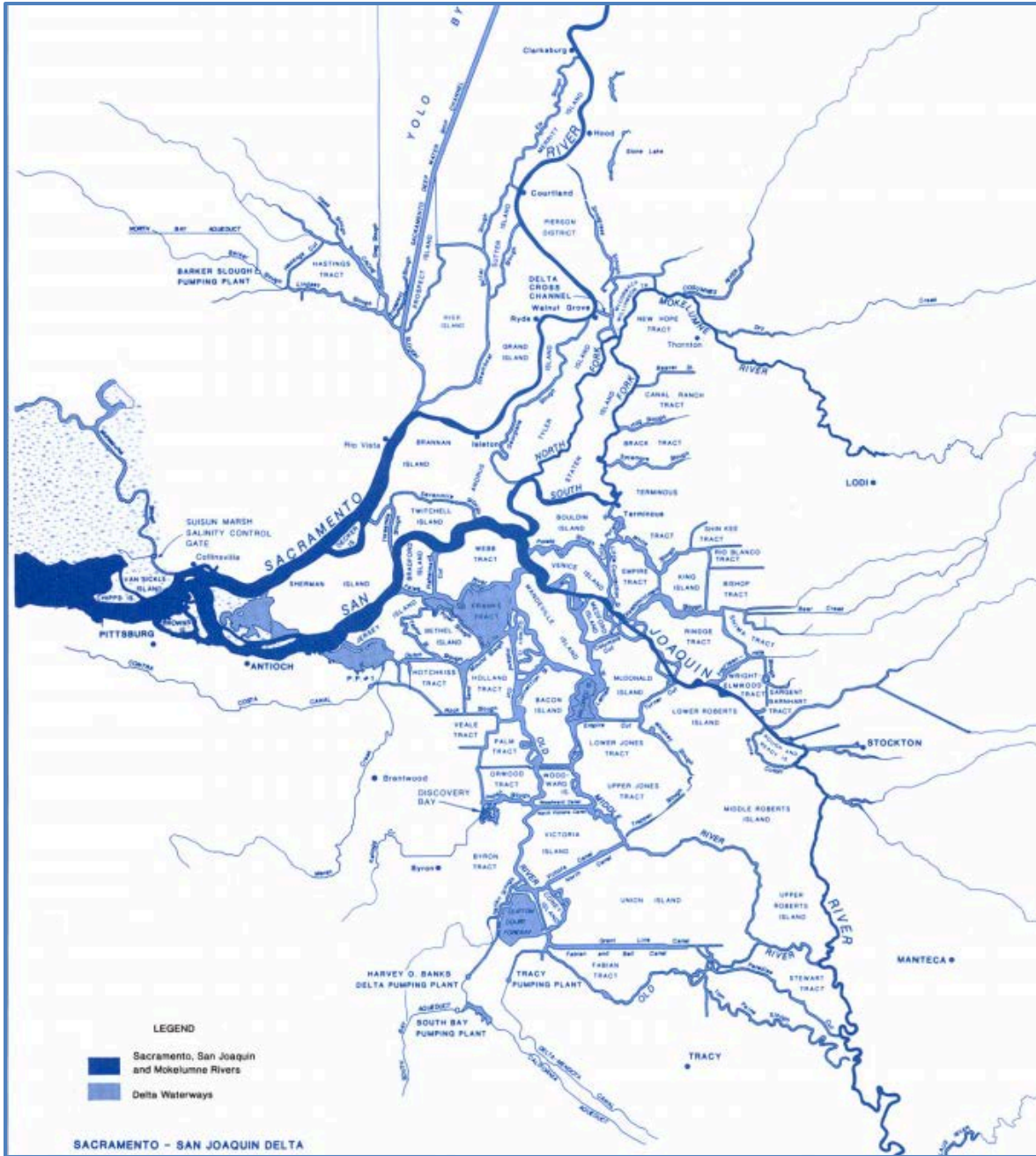


Figure 1.1 The Sacramento-San Joaquin Delta Region

## 1.2 Goal and Organization of Cyanobacterial Literature Review

The goal of the cyanobacterial literature review is to synthesize available information to provide insight into cyanobacterial blooms in the Delta. The review had three major objectives:

- 1) Provide a basic review of biological and ecological factors that influence the prevalence of cyanobacteria and production of cyanotoxins;
- 2) Summarize observations of cyanobacteria blooms and associated toxins in the Delta;
- 3) Synthesize literature to provide an understanding of what ecological factors, including nutrients, may be at play in promoting cyanobacteria blooms in the Delta.

This review, and the recommended next steps, will contribute to a science plan to determine whether or how to proceed with the development of nutrient objectives for the Delta. The document is organized as follows:

Section 1: Introduction, Purpose and Organization of the Review

Section 2: Basic Biology and Ecology of Cyanobacteria

Section 3: Factors Influencing Cyanobacterial Blooms and Toxin Production

Section 4: Effects of CyanoHABs on Ecosystem Services and Beneficial Uses in the Delta

Section 5: Synthesis of Factors Influencing CyanoHABs Presence and Toxin Production in the Delta

Section 6: Recommendations

Section 7: Literature Cited

## 2. Basic Biology and Ecology of Cyanobacteria

### 2.1 Overview

Cyanobacteria are a versatile group of bacteria that were the ancient colonizers of Earth and the photosynthetic ancestors of chloroplasts in eukaryotes such as plants and algae. As pioneers of photosynthesis, cyanobacteria were responsible for oxygenating Earth's atmosphere 2.5 billion years ago. In addition to being photosynthetic, cyanobacteria can differentiate into specialized cell types called heterocysts and fix nitrogen (N), exhibit gliding mobility, and tolerate a wide range of temperatures as evidenced by their ability to thrive in hot springs and ice-covered Antarctic lakes. Cyanobacteria also produce an array of bioactive compounds, some of which possess anti-microbial, anti-cancer and UV protectant properties. However, a subset of these bioactive compounds are highly toxic to humans and wildlife.

Blooms of cyanobacteria that produce these toxins, collectively known as harmful cyanobacterial algal blooms (cyanoHABs), has garnered a great deal of attention due to their increased occurrence in recent decades (Chorus and Bartram 1999, Carmichael 2008, Paerl and Huisman 2008, Hudnell 2010). The geographical distribution of these blooms has also increased with blooms appearing in areas previously unaffected (Lopez et al. 2008, Lehman et al. 2005). CyanoHABs can have major negative impacts on aquatic ecosystems. Toxins produced by cyanobacteria can lead to mortality in aquatic animals, waterfowl and domestic animals (Havens 2008, Miller et al. 2010). Moreover, toxins in drinking water supplies can pose a variety of adverse health effects and therefore require expensive treatment options such as filtration, disinfection, and adsorption with activated carbon (Cheung et al. 2013). In addition to the threat of toxins, oxygen depletion due to organic matter decomposition following the die-off of blooms can result in massive fish kills. CyanoHABs can also lead to revenue losses and impact local economies by reducing business in affected water bodies during the peak of tourism season. Considerable costs are associated with mitigation of blooms and lake restoration (Dodds et al. 2009).

The San Francisco Bay Delta is an area where cyanoHABs were previously undetected but have become commonplace since early 2000 (Lehman et al. 2005). In addition to providing a home for several species of pelagic fish and other wildlife, the Delta serves as a critical source of freshwater for irrigation of farms as well as drinking water to communities farther south including the Los Angeles Metropolitan Water District. In concert with the occurrence of cyanoHABs, concentrations of the toxins they produce have been detected in the water and in higher trophic levels including zooplankton and fish (Lehman et al. 2010). The purpose of the following sections summarizes the basic biology of cyanobacteria beginning with classification, light harvesting, carbon metabolism, buoyancy regulation, nitrogen metabolism, cellular N:P ratios and toxin production, in order to build fundamental concepts that are later utilized in the review.

## 2.2 General Characteristics

### 2.2.1 Classification and Distribution

Traditionally, morphological traits have been used to subdivide the cyanobacteria into five sub-groups (Rippka et al. 1979). The major division is between cyanobacteria that are single celled and/or colonial and those that grow filaments (Table 2.1). Each category contains a mixture of marine and freshwater species. In the former category are the Group I Crocococcales including the freshwater *Microcystis* and *Synechocystis*, and the marine *Synechococcus* and *Prochlorococcus*. Group II Pleurocapsales include *Pleurocapsa* and *Xenococcus* (Table 2.1). The filamentous algae, Groups III, IV, and V, are further subdivided into the Oscillatoriales that produce only vegetative cells, including the freshwater benthic species *Oscillatoria* and *Lyngbya* as well as the marine *Trichodesmium* sp. (Table 2.1). Group IV, the Nostocales, contain filamentous algae that differentiate into heterocysts and fix  $N_2$ . This group includes *Aphanizomenon*, *Anabaena*, *Nostoc* and *Cylindrospermopsis* (Table 2.1). Group V, the Stigonematales include species with filaments that grow in complex branching patterns.

**Table 2.1 Cyanobacterial groupings based on morphological traits. Adapted from Rippka et al. (1979)**

<b>Crocococcales</b> Unicellular, reproduce by binary fission		<b>GROUP 1</b>	<i>Gloeotheca (N)</i>	<i>Synechococcus</i>
			<i>Microcystis</i>	<i>Synechocystis</i>
			<i>Prochlorococcus</i>	
			<i>Prochloron</i>	
<b>Pleurocapsales</b> Unicellular, reproduce by multiple fission		<b>GROUP 2</b>	<i>Pleurocapsa</i>	
			<i>Staniera (N)</i>	
			<i>Xenococcus (N)</i>	
Filamentous chain (trichome) forming; reproduce by random trichome breakage, hormogonia, germination of akinetes	Trichome composed of vegetative cells	<b>Oscillatoriales</b> 1 plane division <b>GROUP 3</b>	<i>Lyngbya (N)</i>	
			<i>Oscillatoria (N)</i>	
			<i>Phormidium</i>	
			<i>Prochlorothrix</i>	
			<i>Trichodesmium (N)</i>	
	In the absence of fixed $N_2$ , trichome contains heterocysts; some produce akinetes	<b>Nostocales</b> 1 plane division <b>GROUP 4</b>	<i>Aphanizomenon</i>	
			<i>Anabaena</i>	
			<i>Cylindrospermum</i>	
			<i>Nodularia</i>	
			<i>Nostoc</i>	
		<b>Stigonematales</b> Division in more than 1 plane <b>GROUP 5</b>	<i>Chlorogleopsis</i>	
			<i>Fisherella</i>	

It was originally thought that  $N_2$  fixation primarily existed in the Nostocales which had the ability to differentiate into heterocyst cells. More recent investigations tracking the *nifD* and *nifH* gene diversity has uncovered that  $N_2$  fixation occurs in a range of unicellular, non-filamentous cyanobacteria dispersed throughout the five original groups first proposed by Rippka et al. (1979). These species are indicated by an (N) after their name in Table 2.1. Depending on which functionality of the cyanobacteria is emphasized, recent gene-based groupings of cyanobacteria have created as many as ten different sub-

categories (Turner et al. 1999, Tomatini et al. 2006). However, there appears to exist no general consensus over the best manner in which to categorize the cyanobacteria based on functionality and marker genes.

Most cyanobacteria are planktonic and are dispersed throughout the five groups. The benthic cyanobacteria are found mainly in the Oscillatoriales subgroup. The toxic cyanoHAB-forming cyanobacteria are all freshwater planktonic species dispersed throughout groups 1, 3 and 5 and include the N<sub>2</sub> fixing genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, and *Nodularia*; the benthic N<sub>2</sub> fixing genera *Lyngbya* and some *Oscillatoria*; and the non-N<sub>2</sub> fixing genera *Microcystis* and *Planktothrix* (Paerl and Paul 2012).

### 2.2.2 Light Harvesting, Photosynthesis and Carbon fixation

Cyanobacteria are distinct from all other algae in that most of them possess two light harvesting systems (as opposed to one). Maintaining two light harvesting system is costly in terms of protein and N requirement and manifests strongly in their cell biology. For example, the extra protein requirement means that cyanobacteria have a high tissue nitrogen:phosphorus (N:P) ratio and a high N requirement for growth (discussed below). Despite this, light harvesting is necessary in photosynthetic organisms to 1) collect light energy from the sun and 2) convert it to chemical energy in the form of electrons and ATP that can be used to power carbon fixation.

#### Light harvesting pigments and photosynthesis

Light harvesting is performed by chlorophyll *a* (Chl *a*) pigment molecules that are associated with two photosystems (PSI and PSII) that comprise the centers of the photosynthetic process which starts with the liberation of an electron from the splitting of water and ends with the production of ATP. Sitting in each of the photosystems is a specialized Chl *a* molecule that initiates the flow of electrons through the electron transport chain that eventually powers ATP synthesis. The other Chl *a* molecules, 40 and 90, together with 12 and 22 carotenoid pigment molecules, in PSI and PSII respectively, funnel light energy to the reaction core. This complex of Chl *a* and carotenoid pigment molecules, coordinated by a large number of proteins, is very similar in its structure to the light-harvesting complex (LHC) embedded into the thylakoid membranes of vascular plants and eukaryotic phytoplankton (Fromme et al. 2001, 2002).

What makes the cyanobacteria unique is that they have a second light harvesting antenna complex peripheral to the thylakoid membrane that is water soluble (e.g. not membrane bound). This pigment complex, comprised of pigmented proteins arranged in rods fanning out from a core attached to the thylakoid membrane, called the phycobilisome (PBS), is what gives cyanobacteria their name (Grossman et al. 1993, Grossman 2003). Similar to the carotenoid pigments mentioned above, the PBS chromophores absorb light inbetween the Chl *a* absorption peaks of 440nm and 670nm (Grossman et al. 1993). Interestingly, the PBS proteins are not exclusive to cyanobacteria; they also occur in photosynthetic eukaryotes.

Up to 50% of cyanobacterial cellular protein content is bound in the PBS complex taking a large proportion of the cell's resources, particularly its nitrogen (N) allocation. Therefore, under stress

condition such as N starvation, the entire PBS can be degraded within a few hours and the N can become reused within the cell (Sauer et al. 1999). When conditions improve, the PBS will be re-synthesized and re-assembled (Collier and Grossman 1994, Grossman et al. 2001).

### Carbon fixation

The ATP produced and the electrons liberated during photosynthesis are used to power the fixation of carbon into sugars in the Calvin Cycle. They are also used to reduce oxidized sources of N to ammonia during N assimilation (discussed below). The primary and rate-limiting enzyme in carbon fixation is Rubisco which catalyzes the first step in the Calvin Cycle. To deal with the rate-limiting nature of Rubisco, cyanobacteria have evolved specialized structures called carboxysomes. In addition to housing Rubisco, the carboxysomes contain a number of other enzymes that help concentrate CO<sub>2</sub> its vicinity to speed its reaction rate (Kaplan and Reinhold 1999). Cyanobacteria fix carbon to provide the skeletons needed to assimilate N into amino acids and build protein and cellular biomass; fixed carbon can also be used to accumulate carbohydrate storage products (carbohydrate ballasting) in order to make the cell heavier during buoyancy regulation.

#### 2.2.3 Buoyancy Regulation

One distinct advantage of many cyanobacterial genera such as *Microcystis*, *Planktothrix* and *Aphenizomenon* is their ability to regulate their buoyancy by a combination of producing gas vesicles and carbohydrate storage products. The former renders them positively buoyant whereas the latter does the opposite (Walsby 1994, 2005). The carbohydrate storage products are derived from C-fixation and the amount produced varies depending on the species and on irradiance (Howard et al. 1996, Visser et al. 1997, Wallace and Hamilton 1999). At an irradiance that is specific to each species and strain, the amount of carbohydrate storage product will perfectly balance the upward lift created by the gas vesicles and the cyanobacteria will become neutrally buoyant (Walsby et al. 2004). In addition to producing and storing the carbohydrates, cyanobacteria also consume the storage products to produce energy.

By regulating the amount of carbohydrate storage products consumed, cyanobacteria control their vertical position in the water column (Thomas and Walsby 1985, Konopka et al. 1987, Wallace and Hamilton 1999). Models demonstrate that filamentous cyanobacteria can sink or float at speeds up to 0.3 m per day in order to position them at a depth where irradiance is such that it maximizes their growth potential (Walsby 2005). These speeds are only achievable for filaments of a certain size and weight; picocyanobacteria and small filaments do not have enough momentum to respond by vertical repositioning to changes in irradiance (Walsby 2005). Of course, carbohydrate production, therefore buoyancy regulation, is affected by nutrient availability; nitrogen starved cells have excess carbohydrate stores and tend to lose buoyancy more easily than nutrient sufficient cells (Brookes and Ganf 2001).

#### 2.2.4 Nitrogen Metabolism

Cyanobacteria use a wide variety of N sources for growth including ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), urea, amino acids, cyanate, and several species are also capable of dinitrogen gas (N<sub>2</sub>)



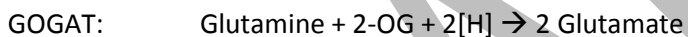
fixation to satisfy their cellular N demand. Below we discuss the pathways of N transport, metabolism and assimilation, and their regulation.

#### [Ammonium transport and assimilation of N into amino acids](#)

Being a charged molecule,  $\text{NH}_4^+$  cannot diffuse freely into the cell and has to be transported via active transport. Transport of  $\text{NH}_4^+$  into cyanobacteria (as well as in eukaryotic algae) occurs via the Amt family of transporters. These transporters are either expressed constitutively or differentially depending on external N concentrations. At environmental concentrations, most of the  $\text{NH}_4^+$  is transported into the cell via the high-affinity transporter Amt1 encoded by the gene *amt1* (Muro-Pastor et al. 2005).

Before it can be assimilated, all N sources, whether  $\text{N}_2$ ,  $\text{NO}_3^-$  or organic N containing molecules, first have to be converted to  $\text{NH}_4^+$ . The  $\text{NH}_4^+$  is then assimilated into amino nitrogen through the GS/GOGAT pathway. The primary  $\text{NH}_4^+$  assimilating enzymes in cyanobacteria (as well as in vascular plants and eukaryotic algae) are glutamine synthetase (GS) and glutamate synthase (also called glutamine-2-oxoglutarate-amido transferase, GOGAT) acting in concert to aminate 2-oxoglutarate (2-OG).

Photosystem I (PSI)-reduced ferredoxin ( $\text{Fd}_{\text{red}}$ ) is typically used as a reductant in this reaction:



An alternate route of  $\text{NH}_4^+$  assimilation involves the enzyme glutamate dehydrogenase (GDH) but it's postulated that this occurs only during select conditions such as stationary growth:



In all photosynthetic cells the link between the carbon (C) and N cycles in the cell occurs at the GS/GOGAT reactions because the two key ingredients in N assimilation is 1) 2-OG derived from carbon fixation, and 2)  $\text{Fd}_{\text{red}}$  derived from PSI. GOGAT (and also GDH) will not proceed without their presence, which avoids wasteful consumption of glutamine, and ensures that even in the presence of excess N, assimilation will not proceed unless an adequate supply of C skeletons is available (Flores and Herrero 2005, Muro-Pastor et al. 2005).

#### [Nitrate transport and reduction to \$\text{NH}\_4^+\$](#)

As  $\text{NO}_3^-$  is also a charged molecule it's transported into the cell via active transport. Cyanobacteria use two different transport systems. Most freshwater species, including *Anabaena*, *Synechocystis* and *Gloeobacter*, use the high affinity ATP-binding cassette (ABC) transporter NrtABCD (Flores et al. 2005). Most marine species (*Synechococcus* and others) take up  $\text{NO}_3^-$  and  $\text{NO}_2^-$  via the major facilitator superfamily transporter NrtP, also a high-affinity transporter (Flores et al. 2005). Some species also have a  $\text{NO}_2^-$ -specific transporter NIT (Maeda et al. 1998). Nitrate uptake is tightly regulated by the concentration of  $\text{NH}_4^+$ ; when  $\text{NH}_4^+$  becomes available, cells cease  $\text{NO}_3^-$  uptake and switch to use  $\text{NH}_4^+$

which is preferred. This process is regulated at the level of  $\text{NO}_3^-$  uptake (Flores and Herrero 1994). In addition,  $\text{CO}_2$ -fixation is required to maintain active  $\text{NO}_3^-$  uptake, a regulatory link that ensures that the product of  $\text{NO}_3^-$  reduction (ammonium) can be incorporated into carbon skeletons (Luque and Forchhammer 2008).

Reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  is a two-step process catalyzed by the enzymes nitrate reductase (NR) and nitrite reductase (NiR). The power for the reduction reaction, in the form of 2 electrons for NR and 6 electrons for NiR, is provided by  $\text{Fd}_{\text{red}}$  via PSI providing a strong link between the light reactions and  $\text{NO}_3^-$  use by the cell (Flores et al. 2005).

In cyanobacteria, the genes encoding NR, narB, and Nir, nirA, and the  $\text{NO}_3^-$  transporter NrtP, are typically clustered in the same operon. An operon is a unit that tells the cells to transcribe a sequence of genes simultaneously. In cyanobacteria, the transcription of operons associated with N metabolism is tightly regulated by the transcription factor NtcA (discussed below).

The only cyanobacteria discovered to date that is not able to use  $\text{NO}_3^-$  is *Prochlorococcus* which lives in the open ocean. While it was initially thought that some species could assimilate  $\text{NO}_2^-$ , sequencing of their genomes have demonstrates that they all lack the *nirA* genes and therefore cannot reduce  $\text{NO}_2^-$  (Garcia-Fernandez et al. 2004).

#### [Urea transport and metabolism](#)

Many, but not all, cyanobacteria can use urea as a source of N for growth. Because urea is not a charged molecule it diffuses freely into the cell; however, environmental concentration are not such that diffusion can supply the needed concentration of urea for the urease enzyme (based on its  $K_m$ ). Both in freshwater and marine cyanobacteria, an ABC-type active transport system specific for urea has been identified (Valladares et al. 2002). The subunits of this transporter are encoded by the five genes *urtA-E*. In *Anabaena*, the urea transporter genes are in the same NtcA-activated promoter and subject to metabolic repression by  $\text{NH}_4^+$  (Valladares et al. 2002).

Urea is metabolized to two molecules of  $\text{NH}_3$  and  $\text{CO}_2$  by the enzyme urease, also called urea amidohydrolase (Mobley et al. 1995). The urease enzyme is well-conserved throughout the bacteria and eukaryotic organisms and consists of two small and one large subunit encoded by at least seven genes, three which encode the structural subunits (*ureA*, *ureB*, *ureC*) and the other four (*ureD*, *ureE*, *ureF*, *ureG*) encoding accessory polypeptides required for the assembly of the nickel metallocenter (Collier et al. 1999, Palinska et al. 2000).

#### [Amino Acid transport](#)

All cyanobacteria tested to date have at least one transport system for amino acids. These transporters appear to have broad specificity (i.e. they can transport more than one type of amino acid) and different species have different combinations of transporters (Herrero and Flores 1990, Montesinos et al. 1997). For example, freshwater *Synechocystis* sp. has four different amino acid transporters, including the ABC transporter Nat for glutamine and histidine, the ABC transporter Bgt for basic amino acids, and two

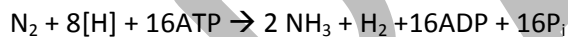
glutamate-specific transporters GHS and Gtr (Quintero et al. 2001). Once in the cell, cyanobacteria possess a variety of deaminase enzymes that can deaminate the amino acids to  $\text{NH}_3$  which then enters the GS/GOGAT pathway.

### Cyanate transport and metabolism

Cyanobacteria, including freshwater and marine species, can use cyanate (a toxin) as a N source for growth since they have the genes encoding a transporter (*cynA*, *cynB*, *cynC*) and the gene encoding the cyanase enzyme (*cynS*) which hydrolyzes cyanate to  $\text{NH}_3$  and  $\text{CO}_2$  (Kamennaya and Post 2011). In freshwater cyanobacteria, these genes are repressible by  $\text{NH}_4^+$  suggesting that they are under NtcA regulation.

### Nitrogen fixation

Arguably the most expensive (energetically speaking) source of N for cyanobacteria is molecular dinitrogen gas ( $\text{N}_2$ ). Nitrogen fixation, the process of reducing  $\text{N}_2$  to  $\text{NH}_3$ , is catalyzed by the nitrogenase enzyme. The nitrogenase has two subunits. The first is the dinitrogenase subunit which catalyzes the reduction of  $\text{N}_2$  to  $\text{NH}_4^+$ , composed of the NifD and NifK polypeptides encoded by the *nifD* and *nifK* genes. The dinitrogenase contains an iron-molybdate active site and two iron-sulfur clusters. The second is the dinitrogenase reductase subunit (NifH polypeptide encoded by the *nifH* gene) which contains a central iron-sulfur cluster whose function it is to donate electrons derived from ferredoxin to dinitrogenase. Reduction of  $\text{N}_2$  to  $\text{NH}_3$  requires 8 electrons and 15 molecules of ATP in the following reaction:



It was recently discovered that under conditions of molybdate limitation, some *Anabaena* species express an alternative nitrogenase containing a vanadium-iron cofactor instead of the molybdate-iron cofactor (Thiel 1993, Boison et al. 2006). Both these variants require iron cofactors to function and  $\text{N}_2$  fixation cannot proceed under iron-limiting conditions.

The nitrogenase enzyme is very sensitive to oxygen ( $\text{O}_2$ ), and  $\text{O}_2$  is evolved as a byproduct of the water-splitting reactions at photosystem II (PSII), requiring the nitrogenase enzyme to be kept separate from PSII. Accordingly, freshwater cyanobacteria have evolved heterocysts (Wolk et al. 1994). These are specialized cells where PSII is inactivated, the PBS antenna proteins are degraded, and energy to power the cell is derived from cyclic electron flow around PSI. Rates of respiration in these cells are also high to scavenge any  $\text{O}_2$ . The ATP and reductant needed for  $\text{N}_2$  reduction is generated by carbohydrate metabolism inside the heterocyst. The carbohydrate is synthesized in the non-heterocyst, vegetative cells flanking the heterocyst and transported inside. In turn,  $\text{NH}_3$  produced inside the heterocyst is exported to the vegetative cells in the form of amino acids (Wolk et al. 1994). However, many species of cyanobacteria that fix  $\text{N}_2$  do not form heterocysts; these species either separate  $\text{N}_2$  fixation from photosynthesis in time (e.g. by fixing  $\text{N}_2$  at night such as *Lyngbya aestuarii* and *Crocospaera watsonii*) or in different regions of filaments as is hypothesized to be the case for *Trichodesmium* sp. (Frederiksson and Bergman 1997).

Because nitrogen fixation is such an energy expensive process, from the formation of the heterocysts to the reduction of  $N_2$ , it is tightly regulated by NtcA and is only induced under N starvation and in the absence of any other fixed N source (Herrero et al. 2004).

### Regulation of Nitrogen Metabolism

As evident from the preceding sections, the transcription factor NtcA (encoded by the gene *ntcA*) regulates most of the cyanobacterial genes associated with nitrogen uptake and assimilation, and is therefore considered the master regulator of N metabolism (Herrero et al 2004). NtcA binds to and activates the operons for heterocyst differentiation,  $N_2$  fixation,  $NO_3^-$  uptake and reduction, urea uptake and hydrolysis, and glutamine synthetase to mention a few. In other words, none of the genes related to N metabolism are transcribed and their enzymes synthesized unless NtcA binds to their promoter in the genome (Wei et al. 1994, Luque et al. 1994, Forchammer 2004, Luque and Forchammer 2008). The exception to this rule are some  $NH_4^+$  transport proteins which are not under NtcA control and are transcribed constitutively, i.e. always “on” (Herrero et al. 2001). NtcA also controls signaling proteins that fine-tune cellular activities in response to fluctuating C/N conditions (Herrero et al. 2001).

NtcA is under negative control by  $NH_4^+$ , meaning that when  $NH_4^+$  is detectable by the cell, *ntcA* gene transcription is repressed (Herrero et al. 2001, Lindell and Post 2001). There is an inverse relationship between  $NH_4^+$  concentration and *ntcA* expression in all cyanobacteria tested to date, with basal levels of *ntcA* expression observed in the presence of high external  $NH_4^+$  concentrations and maximal levels of *ntcA* expression observed under N starvation (Frias et al. 1994, Lee et al. 1999, Sauer et al. 1999, Lindell and Post, 2001, Lindell et al. 1998). Ammonium regulates expression of *ntcA* via 2-OG which is synthesized in the Calvin cycle and consumed in the GS/GOGAT cycle. Thus 2-OG is at the crossroads between C and N metabolism and is ideally suited to “sense”  $NH_4^+$  concentrations (Vazquez-Bermudez et al. 2002, Tanigawa et al. 2002, Forchammer 2004).

The repression of *ntcA* expression by  $NH_4^+$  places  $NH_4^+$  at the top of the hierarchy of N substrates utilized and assimilated by cyanobacteria. The order in which N substrates other than  $NH_4^+$  is assimilated differs depending on species. For example, in  $N_2$  fixing cyanobacteria,  $NH_4^+$  represses both  $N_2$  fixation and  $NO_3^-$  assimilation. Nitrate, in turn, represses  $N_2$  fixation. Therefore  $N_2$  fixation is at the bottom of the hierarchy in some cyanobacteria (Ramasubramanian et al. 1994). But in others such as marine *Trichodesmium* sp.,  $NO_3^-$  does not repress  $N_2$  fixation genes and the process of  $N_2$  fixation is on a more even footing with  $NO_3^-$  assimilation (Post et al. 2012).

### 2.2.5 Cellular Nitrogen:Phosphorus (N:P) Requirement

In 1958 Redfield published his discovery that phytoplankton particulate matter was composed of N and P in a molar ratio of 16, similar to the ratio of dissolved N:P in the water (Redfield 1958). Redfield suggested that the ratio of dissolved N:P in the ocean was driven by the remineralization of phytoplankton particulate matter, a theory which has since taken hold (Falkowski 2000, Geider and LaRoche 2002). Given that the average optimal N:P ratio was discovered to be 16 in phytoplankton, it was deduced that phytoplankton would become limited by N at dissolved N:P less than 16 and limited by P at dissolved N:P ratios greater than 16.

Shortly after Redfield's discovery of the universality of the N:P ratio of 16, investigators turned to phytoplankton cultures to examine how closely phytoplankton cellular N:P ratios varied around 16. Parsons et al. (1961) published the first investigation demonstrating variability in cellular N:P ratios depending on the phytoplankton species. Following investigations noted that diatoms and dinoflagellates tended to have cellular N:P ratios below 16 whereas chlorophytes and cyanobacteria typically had ratios above 25 (Geider and LaRoche 2002, Ho et al. 2003, Quigg et al. 2003, Klausmeier et al. 2004, Hillebrand et al. 2013, Figure 2.1). This difference among the taxa stems from slight variations in macromolecular composition of the phytoplankton, principally in their ratio of protein, the largest store of N in the cell, to nucleic acids, the largest store of P in the cell (Fuhs 1969, Terry et al. 1985, Falkowski 2000, Geider and LaRoche 2002, Elser et al. 2000). As mentioned above in section 2.2.2, cyanobacteria have two light-harvesting complexes requiring a greater association of proteins with the light-harvesting pigments compared with eukaryotic cells which only have one light harvesting complex (Raven 1984, Geider and LaRoche 2002). The "excess" protein associated with the peripheral phycobilisomes substantially increase the cellular N:P ratios of cyanobacteria. Once it was realized that there were significant departures in the cellular N:P ratio depending on taxa, it also became clear that the ratio of N:P uptake differed with respect to taxa and that this was a major basis of resource-based competition among taxa (Rhee 1978). That phytoplankton take up N:P in proportion to their tissue composition was subsequently confirmed in culture experiments (Droop 1974, Elrifi and Turpin 1985, Tett et al. 1985, Quigg et al. 2003, Leonardis and Geider 2004). In other words, phytoplankton do not take up nutrients according to the ratio that occurs in water, but rather the ratio dictated by the macromolecular composition of their tissues.

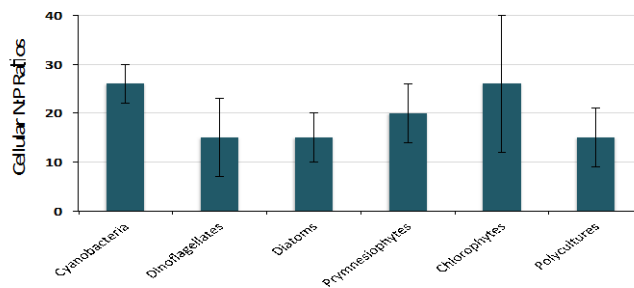


Figure 2.1 Cellular N:P ratios in different phytoplankton taxa. Data from Hillebrand et al. 2013.

Tissue N:P composition is not a fixed trait and phytoplankton are able to adjust it, within certain limits, in order to keep growing when environmental conditions change for the suboptimal. When limited for a nutrient, uptake of the non-limiting nutrient can proceed for a while skewing cellular ratios. But, severe limitation by one nutrient will eventually prevent the uptake of the other, non-limiting nutrient, even when the other is present in excess. This quirk of nature constrains the extent to which cellular ratios

vary (Droop 1974, Tett et al. 1985, Leonardis and Geider 2004, Hillebrand et al. 2013). For example, a summary of nearly 50 phytoplankton studies demonstrates that the N:P ratio of P-limited phytoplankton converge around 28 and the N:P ratio of N-limited phytoplankton converges around 16 (Hillebrand et al. 2013).

Irradiance may also change the cellular N:P ratio through its influence on the cellular protein content (LaRoche and Geider 2002). Pigments (Chl *a* and light harvesting antenna pigments) are bound in pigment-protein complexes rich in N that increase as irradiance decreases, and decrease under high light as cells reduce the size of the light harvesting complex to avoid photodamage (Falkowski and LaRoche 1991, Wynne and Rhee 1986, Nielsen 1992, Leonardis and Geider 2004). The irradiance-dependent change in N:P ratios is even more pronounced among cyanobacteria due to the greater association of protein with the phycobilisome than in the eukaryotic light harvesting complex (Raven 1984, Geider and LaRoche 2002).

In contrast with limiting nutrient concentrations or changes in irradiance, changes in the medium N:P ratio when nutrient concentrations are in excess of demand was found not to affect cellular N:P ratios in phytoplankton in early experiments (i.e. Tett et al. 1985) and has not been pursued by the scientific community.

### 2.2.6 Toxin Production

Cyanobacteria produce a large variety of toxins with a number of different actions in animals and humans leading to significant health risks and drinking water issues globally (c.f. Chorus and Batram 1999, Chamichael 2008, Cheung et al. 2013). The toxin-producing cyanobacteria, and the suite of different toxins that each species produces, is discussed below.

#### Toxin-producing taxa

The cyanobacterial toxins were named according to the species that they were originally discovered in and isolated from. For example, microcystin was discovered in *Microcystis aeruginosa* and anatoxin was originally isolated from *Anabaena*. However, most cyanobacteria produce several different types of toxins, with the exception of nodularin which is only produced by *Nodularia spumigena*.

The toxin most widely produced by different cyanobacterial taxa is the recently discovered neurotoxin Beta-N-methylamino-L-alanine (BMAA, Cox et al. 2005). This is followed by the microcystins which are produced by nine different taxa (Table 2.2). Chief among the microcystin producing taxa are *Microcystis* (the toxin was originally isolated from *Microcystis aeruginosa*), followed by *Planktothrix* and *Anabaena*. Another widely distributed toxin is anatoxin-a, which is produced by eight different cyanobacterial taxa, principally *Anabaena*, the genus from which the toxin was originally isolated.

**Table 2.2 Toxins produced by cyanobacteria. Based on data from Sivonen and Borner 2008, Cox et al. 2005, Cheung et al. 2013.**

	Microcystin	Nodularin	Cylindro-spermopsin	Anatoxin-a	Anatoxina(S)	Saxitoxin	Dermatotoxin	BMAA
<i>Microcystis</i>	X							X
<i>Planktothrix</i>	X			X		X		X
<i>Anabaena</i>	X		X	X	X	X		X
<i>Nostoc</i>	X							X
<i>Anabaenopsis</i>	X							
<i>Radiocystis</i>	X							X
<i>Synechococcus</i>	X							X
<i>Phormidium</i>	X			X				X
<i>Oscillatoria limosa</i>	X			X				
<i>Oscillatoria</i>				X			X	
<i>Nodularia</i>		X						X
<i>Cylindro-spermopsis</i>			X			X		X
<i>Aphanizomenon</i>			X	X		X		X
<i>Raphidiopsis</i>			X	X				X
<i>Cylindro-spermum</i>				X				X
<i>Lyngbya</i>						X	X	X
<i>Shizothrix</i>							X	
<i>Umezakia natans</i>			X					

*Anabaena* species, including *flos-aquae/ lemmermannii/ circinalis*, may be the most toxically versatile of all the cyanobacteria as they can produce all the toxins, including BMAA, microcystins, cylindrospermopsin, anatoxin-a, anatoxin-a(S) and saxitoxins, save nodularin (Table 2.2). Nodularin is only produced by *Nodularia spumigena*. Another versatile toxin producer is *Aphanizomenon flos-aquae* which produces BMAA, cylindrospermopsin, anatoxin-a and saxitoxins (Table 2.2). *Planktothrix* also produces four different toxins including BMAA, microcystins, anatoxin-a and saxitoxins. The cyanobacteria *Cylindrospermopsis raciborskii* from whence cylindrospermopsin was originally isolated also produces saxitoxins (Table 2.2).

Benthic cyanobacteria are also versatile when it comes to toxin production. For example, *Oscillatoria limosa* can produce microcystins as well as anatoxin-a while *Lyngbya wollei* can produce saxitoxins and dermatotoxins (Table 2.2).

### [Toxin types and their biosynthetic pathways](#)

The toxins produced by cyanobacteria can be divided into three main groups: hepatotoxins that damage the liver of the organisms ingesting them, neurotoxins that cause respiratory arrest, and dermatotoxins that cause rashes and inflammations. Each are discussed separately below.

**Hepatotoxins.** The most well-known hepatotoxins are microcystins and nodularin which are serine/threonine protein phosphatase inhibitors (Table 2.3). A large variety of different microcystins (close to 80) have been identified, with the most toxic being microcystin-LR. These cyclic heptapeptides contain seven amino acids, including a unique beta amino acid ADDA (MacKintosh et al. 1990, Yoshizawa et al. 1990). In contrast with microcystins, only a few varieties of nodularin have been identified (Yoshizawa et al. 1990). The toxicity of cyanobacterial toxins is typically measured by injecting them into mice and calculating the lethal dosage to half the population ( $LD_{50}$ ) (Table 2.3).

Biosynthesis of the microcystin and nodularin peptides occurs by non-ribosomal peptide synthases (NRPS) and polyketide synthases (PKS) found mainly in bacteria (Welker and von Dohren 2006). Both of these enzyme classes are needed for both the microcystin and nodularin biosynthesis pathways which have been sequenced from a number of cyanobacterial species including *Microcystis*, *Planktothrix* and *Anabaena* (Borner and Dittman 2005). For example, the *mcyA*, *mcyB* and *mcyC* genes encode the NRPS that synthesize the pentapeptide portion of microcystins. The *mcyD*, *mcyE*, *mcyF* genes encode the PKS which synthesize the ADDA amino acid unique to microcystins. Finally, the *mcyF*, *mcyG*, *mcyH*, *mcyI*, *mcyJ* genes encode the proteins that tailor and transport specific microcystins (Table 2.3). Similarly, the *nda* gene cluster specific to nodularin encode the NRPS and PKS synthases as well as the tailoring and transport proteins (Table 2.3). Although not verified through functional investigations, the cylindrospermopsin gene cluster, encoding the genes *cyrA*, *cyrB*, *cyrC*, has recently been characterized in *Aphanizomenon flos-aquae* (Stuken and Jakobsen 2010).



Table 2.3 Common cyanobacterial toxins. ND: Not determined.

Toxin	Chemical Class	Action	Effect	LD <sub>50</sub>	Reference	Gene Name	Gene Reference
<b>Microcystins</b>	Cyclic heptapeptides; 80 variants; microcystin-LR is most toxic	Serine/threonine protein phosphatase (1 and 2A) inhibitors	Hepatotoxin; damages liver	50 µg kg <sup>-1</sup>	MacKintosh et al. 1990, Yoshizawa et al. 1990	<i>mcyA-I</i>	Tillett et al. 2000, Christiansen et al. 2003
<b>Nodularin</b>	Cyclic pentapeptide; only a few variants identified	Serine/threonine protein phosphatase 1 and 2A inhibitor	Hepatotoxin; damages liver	50 µg kg <sup>-1</sup>	Yoshizawa et al. 1990	<i>ndaA-I</i>	Moffitt and Neilan 2004
<b>Cylindrospermopsin</b>	Cyclic guanidine alkaloid	Protein synthesis inhibitor	Hepatotoxin/Cytotoxin; affects liver as well as kidney, spleen, thymus and heart	200 µg kg <sup>-1</sup> at 6 days 2000 µg kg <sup>-1</sup> at 24 hrs	Runnegar et al. 1994, Terao et al. 1994, Ohtani et al. 1992	<i>cyrA-C</i>	Stuken and Jakobsen 2010
<b>Anatoxin-a</b>	Alkaloid	Competitive inhibitor of acetyl choline	Neurotoxins: causes death by respiratory arrest	200-250 µg kg <sup>-1</sup>	Devlin et al. 1977, Carmichael et al. 1990, Skulberg et al. 1992	<i>ana</i>	Mejean et al. (2010)
<b>Anatoxin-a(S)</b>	Phosphate ester of cyclic N-hydroxyguanine	Anticholinesterase	Neurotoxins: causes death by respiratory arrest	20 µg kg <sup>-1</sup>	Carmichael et al. 1990	<i>ana</i>	Mejean et al. (2010)
<b>Saxitoxins</b>	Carbamate alkaloids; the most potent are saxitoxins and neosaxitoxins	Sodium channels blocker	Neurotoxin	10 µg kg <sup>-1</sup>	Sivonen and Jones 1999	<i>stxA-Z</i>	Kellmann et al. 2008
<b>BMAA</b>	Non-protein amino acid		Neurotoxin: linked with neurodegenerative diseases (e.g. Parkinson's Dementia Complex)	ND	Cox et al. 2005	ND	
<b>Dermatoxins</b>	Aplysiatoxins	Protein kinase C activators	Dermatotoxin: tumor promoters; dermatitis and oral/gastrointestinal inflammations	ND	Mynderse et al. 1977, Fujiki et al. 1990	ND	

**Neurotoxins.** By far the most potent toxins are the neurotoxin saxitoxin that causes paralytic shellfish poisoning (PSP) syndrome and respiratory arrest in humans and animals. This neurotoxin is produced both by cyanobacteria and dinoflagellates and is an alkaloid that acts as a sodium channel blocker. Another alkaloid neurotoxin, anatoxin-a, competitively inhibits acetyl choline, and a variant, anatoxin-a(S), acts as an anti-cholinesterase (Devlin et al. 1977, Myderse et al. 1977, Carmichael et al. 1990, Sivonen and Jones 1999). The LD<sub>50</sub> of these toxins vary from 200-250 µg kg<sup>-1</sup> in the case of anatoxin-a, 20 µg kg<sup>-1</sup> in the case of anatoxin-a(S), to 10µg kg<sup>-1</sup> in the case of saxitoxins (Table 3). The gene clusters encoding the saxitoxin biosynthesis and anatoxin biosynthesis pathways were very recently elucidated via functional homology and each contains 20 or more genes (Kellmann et al. 2008, Mejean et al. 2010). The recently discovered neurotoxin BMAA, a non-protein amino acid that is potentially linked to neurodegenerative diseases such as Parkinson Dementia Complex (PDC), is produced in almost all cyanobacteria tested to date (Cox et al. 2005).

**Dermatotoxins.** Benthic cyanobacteria, including *Lyngbya*, *Oscillatoria* and *Schizothrix*, produce a number of different toxins including aplysiatoxins, debromoaplysiatoxins and lyngbyatoxin-a. These toxins are protein kinase C activators that cause dermatitis and oral and gastrointestinal inflammations, and can also promote tumor formation (Mynderse et al. 1977, Cardellina et al. 1979, Fujiki et al. 1990). The pathways and genes involved with the production of the dermatotoxins have yet to be elucidated.

#### Potential functions of toxin production

Interestingly, researchers have not been able to determine the purpose of toxin production in cyanobacteria, or under what conditions toxins are most likely to be produced (Sivonen and Borner 2008). Moreover, under environmental conditions cyanobacteria that produce toxins co-exist with cyanobacteria of the same genus that do not produce toxins; it's unclear whether the possession of, or lack of, the toxins confers an ecological advantage (Sivonen and Borner 2008, Baxa et al. 2010).

Despite these complications, several explanations for the potential function of toxin production exist. Originally it was thought that cyanotoxins acted as allelochemicals and that their secretion into the surrounding water would suppress the growth of competitors (Keating 1977, Flores and Wolk 1986, Klein et al. 1995, for a detailed discussion see Babica et al. 2006). However, when distribution of toxins, such as microcystins, was compared between cells and the surrounding medium using immunodetection combined with electron microscopy, most of the toxin was found to be cell-bound (Rapala et al. 1997, Wiedner et al. 2003, Youg et al. 2005, Tonk et al. 2005, Gerbersdorf 2006). Therefore, recent research does not indicate that live (i.e. non-lysed) cyanobacteria secrete the toxins they produce.

One explanation that is gaining ground is that the primary role of toxins is probably not to be toxic (Llewellyn 2006). Rather, investigators are hypothesizing that toxins may be produced to protect the cells from abiotic stresses. For example, microcystins are produced during all phases of growth but the greatest accumulation typically occurs under conditions that support optimal growth, including growing under optimal light levels (Sivonen and Jones 1999, Wiedner et al. 2003). Several lines of evidence point towards increases in irradiance as being a trigger for microcystin production. These include accumulation of intracellular microcystin-LR with increased irradiance, the association of intracellular

microcystins with the thylakoid membranes, and increased microcystin gene expression with increased irradiance (Kaebernick et al. 2000, Tonk et al. 2005, Borner and Dittman 2005, Gerbersdorf 2006). As such, it makes sense that microcystins are produced across a number of cyanobacterial taxa, such as *Microcystis*, *Anabaena*, and *Planktothrix*, that grow well in high-light environments (Paerl and Paul 2012). Microcystins may also be implicated in preventing iron-stress by acting as siderophores, an idea supported by the discovery that the iron-regulator factor Fur binds to the genes that produce microcystins in cyanobacteria (Utkilen and Gjølme 1995, Martin-Luna et al. 2006).

Another potential role for cyanotoxins is to act as a grazing deterrent (Burns 1987, Gilbert 1996). However, recent research using *Microcystis aeruginosa*, has demonstrated that it's not the toxic microcystins that deters *Daphnia* from grazing *M. aeruginosa* but other substances it produces. In other words, the substances causing toxicity and deterrence are not identical and the non-toxic substances may be much important in terms of grazing deterrence (Rohrlack et al. 1999, Rohrlack et al. 2003).

While the toxic substances are by far the most well-known, there are hundreds of other, secondary metabolites similar in structure to the toxins that are produced by cyanobacteria. Just as the toxins, these cyclic or linear peptides may not be needed for growth but may serve protective functions. For example, the grazing deterrents discussed above belong to a class of depsipeptides called microviridins (originally isolated from *Microcystis viridis*) and has since their isolation been found in a range of cyanobacteria (Rohrlack et al. 2003). These secondary metabolites may also have important pharmacological applications. An alkaloid produced by *Nostoc*, called nostocarboline, is a cholinesterase inhibitor which has an effect comparable to galanthamine, a drug approved for Alzheimer's disease (Becher et al. 2005). Also isolated from *Nostoc* is a compound called cyanovirin-N which has antiviral activity and is under development as an antiviral agent against HIV (Boyd et al. 1997, Bolmstedt et al. 2001).

### 3. Factors Influencing Cyanobacterial Blooms and Toxin Production

The world-wide increase in the incidence of cyanoHABs such as the  $N_2$  fixing genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, and *Nodularia*; the benthic  $N_2$  fixing genera *Lyngbya* and some *Oscillatoria*; and the non- $N_2$  fixing genera *Microcystis* and *Planktothrix* has prompted a great deal of research into the conditions that favor the growth of these species (Chorus and Bartram 1999, Carmichael 2008, Paerl and Huisman 2008, Hudnell 2010, O'Neill et al. 2012, Paerl and Paul 2012). These conditions typically include favorable salinity, ample supply of nutrients, calm water and stratified conditions, plenty of irradiance and warm water temperatures (Figure 3.1). In contrast, the most successful strategies to mitigate blooms of cyanoHABs include reducing the supply of nutrients, increasing the flow of water to promote mixing and destratify the water column (Figure 3.1). In the following sections, we will focus on the conditions that are favorable for the growth of the cyanoHAB genera.

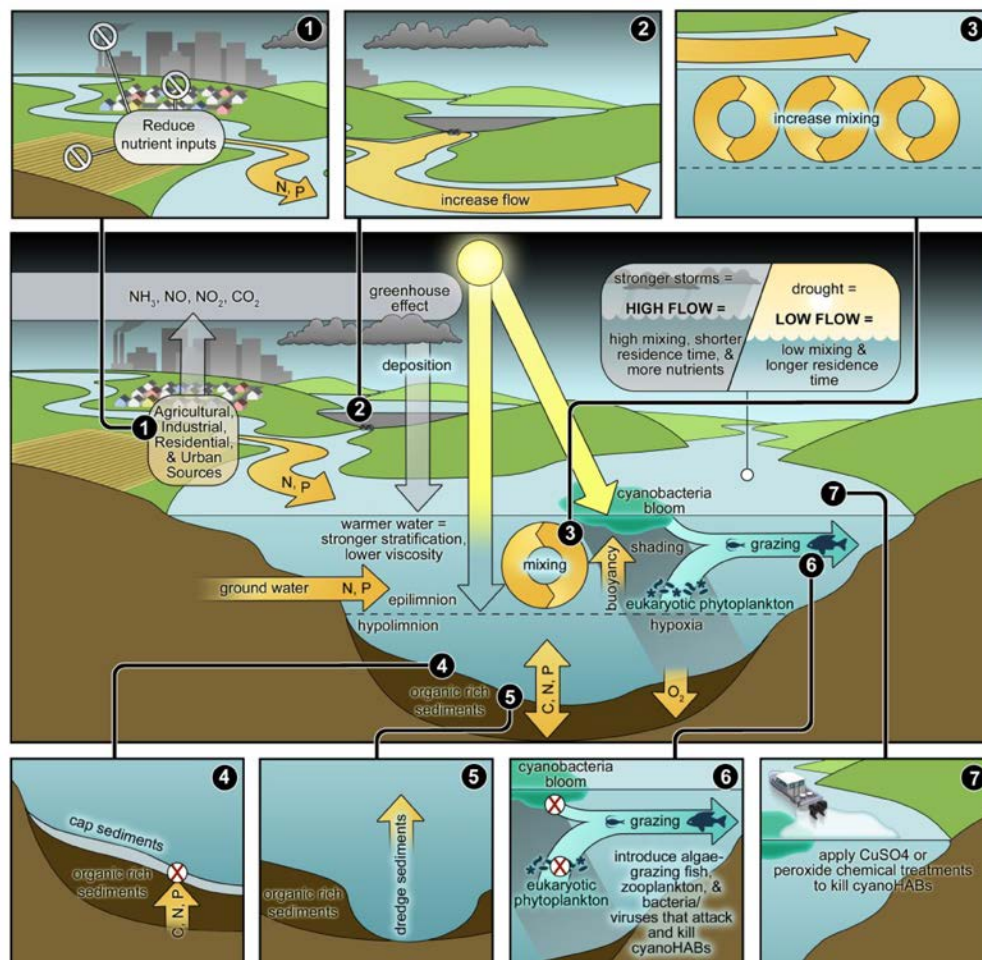


Figure 3.1 Conceptual model of factors affecting cyanobacteria blooms and mitigation strategies (1=reduce nutrient inputs, 2=increase water flow, 3=increase mixing, 4=cap organic-rich sediments, 5=dredge organic-rich sediments, 6=introduce grazers, 7=apply algicides) from Paerl 2014.

### 3.1 Salinity

Most harmful algal bloom-forming and toxin-producing cyanobacteria (cyanoHABs) are freshwater species. In contrast, marine cyanobacteria such as *Prochlorococcus*, *Synechococcus* sp. and *Trichodesmium* sp. are not toxic and do not form cyanoHABs. However, laboratory investigations of freshwater cyanoHAB species demonstrate that these have quite wide salinity tolerance ranges. For example, the least tolerant, *Cylindrospermopsis* only thrives up to 2.5 ppt salinity, but the most tolerant, *Anabaenopsis* and *Nodularia* spp., thrive at salinities from 5-20 ppt (Moisander 2002). *Microcystis aeruginosa* tolerates up to 10 ppt salinity without a change in its growth rate compared to that on freshwater (Tonk et al. 2007). What these studies suggest is that given optimal growth conditions, these species could also bloom in brackish-water regions. Indeed, recent decades has witnessed a spread in the geographical extent of these species into the mesohaline (5-15 ppt) reaches of coastal systems (Paerl and Paul 2012). For example, blooms of *Microcystis aeruginosa* have occurred in the Baltic Sea (Maestrini et al. 1999) and the San Francisco Estuary (Lehman et al. 2013) suggesting 1) that factors other than salinity are regulating their geographical distribution and that 2) those factors are currently changing to allow cyanoHAB growth to occur in regions where they previously did not exist. In summary, salinity may not be the strongest “barrier” in terms of restricting the occurrence and geographical distribution of toxic cyanoHABs.

### 3.2 Nutrient Concentrations and Ratios

As with other photosynthetic phytoplankton, given adequate irradiance cyanobacterial biomass accumulation is directly proportional to the amount of nutrients (N and P) available in the water column. Therefore, strategies to reduce the accumulation of cyanoHAB biomass and severity of their blooms frequently focus on reductions of nutrient concentrations (Paerl 2008).

#### 3.2.1 Influence of N and P Loadings and Concentrations in Stimulating Cyanobacterial Growth

Cyanobacterial growth in freshwater systems (rivers and lakes), which tend to become limited by P sooner than by N, is frequently linked with excessive P loading (Likens 1972, Schindler 1975, Edmondson and Lehman 1981, Elmgren and Larsson 2002, Paerl 2008, Schindler et al. 2008). In contrast with freshwater systems, estuarine and marine systems tend to be more sensitive to N loading, and eutrophication due to cyanobacterial growth is frequently linked with excessive N loading (Ryther and Dunstan 1971, Nixon 1986, Elmgren and Larsson 2001, Suikkanen et al. 2007, Paerl 2008, Conley et al. 2009, Ahn et al. 2011).

However, both non-point and point source nutrient contributions, such as agriculture and wastewater effluent, tend to increase N and P concentrations simultaneously (Paerl and Paul 2012). For example, human population growth-induced intensification of wastewater discharge and agriculture has led to hypereutrophication of China’s third largest lake, Taihu (Quin et al. 2007). Increased nutrient loads, combined with low water column depth and increased water temperatures, has led to an explosive growth of cyanobacteria and a change in total phytoplankton community composition from being mainly diatom-dominated to being dominated by *Microcystis aeruginosa* (Quin et al. 2010, Paerl et al. 2014). Bioassay experiments during summer months when cyanobacterial biomass is at its maximum,

and nutrient concentrations at a minimum, demonstrate that N and P exert equal control over biomass accumulation in this system (Paerl et al. 2014).

In general, dominance of both N<sub>2</sub>-fixing and non-N<sub>2</sub> fixing cyanobacteria such as *Aphenizomenon flos aquae*, *Nodularia spumigena*, *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*, have increased world-wide in concert with increased loads of both N and P (Chapman and Schelske 1997, Jacoby et al. 2000, Gobler et al. 2007, O'Neill et al. 2012, Burford et al. 2006, Burford and O'Donahue 2006, Hong et al. 2006, Suikkanen et al. 2007).

### 3.2.2 Influence of changes in N:P ratios on stimulation or limitation of cyanobacterial growth

At low and intermediate nutrient loadings, reduction in only N or P may be sufficient to control blooms of cyanobacteria. But with elevated loadings of both N and P, reduction of only one type of nutrient can lead to an imbalance in the N:P ratio of the water column potentially leading to a worsening of the cyanoHAB problem, or even lead to a eukaryotic HAB problem (Smith 1983, Paerl 2008, Pearl et al. 2011, Pearl et al. 2014).

Pioneering studies by Smith (1983, 1990) predicted that phytoplankton community composition would be dominated by cyanobacteria when N:P ratios were < 15, and by eukaryotic phytoplankton when N:P ratios > 20. This was because many nuisance freshwater cyanobacteria can fix N<sub>2</sub>, and can thrive at very low ambient concentrations of fixed N, therefore at N:P < 15. In comparison, growth rates of eukaryotic phytoplankton that depend on fixed N would slow down at limiting N concentrations, and low N:P ratios, resulting in eukaryotic species becoming outcompeted. At N:P > 20, growth rates of eukaryotic phytoplankton would not be limited by N and therefore they could dominate phytoplankton community composition (Smith 1983, 1990). These results suggest that one could control growth of cyanobacteria by increasing the dissolved N:P ratio above 20. More recent investigations have found that this simple tenet is not widely applicable for a number of reasons discussed below.

**Cellular N:P composition:** As discussed above (Section 2.2.5), the cellular N:P requirement of cyanobacteria is greater than any other eukaryotic group due to the large protein demand of the peripheral light harvesting antennae. At N-limiting conditions, cyanobacteria would need to provide most, if not all, of their N demand by N<sub>2</sub> fixation in order to meet their high tissue N demand. This would lead to a sharp divide in the distribution of genera that fix N<sub>2</sub> from those that do not; the latter group would be much better suited to dominate high N:P ratio (>25) than low N:P ratio environments. On the flip side, many genera of eukaryotic phytoplankton, such as diatoms and dinoflagellates, have relatively high tissue P requirements and have cellular N:P ratios <16 (Geider and LaRoche 2002, Quigg et al. 2003, Hillebrand et al. 2013) rendering them better suited for environments with N:P <16 (Arrigo et al. 1998, Mills et al. ). Based on their cellular N:P ratios, cyanobacteria are better suited to dominate high N:P ratio systems (>25) and some eukaryotes low N:P ratio systems (<16) which is opposite of the conclusions reached by Smith (1983).

**Ratios at non-limiting nutrient concentrations:** In order for changes in nutrient ratios to affect phytoplankton growth, nutrient concentrations must be low so that either P or N will eventually limit growth rates. In the last decades, both N and P loadings have increased to the point that they exceed

the assimilative capacity of the resident phytoplankton in many systems (Paerl 2008, Paerl et al. 2011, Dolman et al. 2012, Paerl et al. 2014). Therefore, changes in the N:P ratio have little effect on the growth of any of the phytoplankton taxa present in the water column (Paerl 2008, Davidson et al. 2012). However, a recent hypothesis challenges the assumption that changes in the N:P ratio at non-limiting nutrient concentrations does not affect growth. According to this new hypothesis, increased N:P ratios may negatively affect phytoplankton, specifically diatom growth, even at non-limiting concentrations (Glibert 2010). This hypothesis rests on the assumption that phytoplankton take up nutrients in the ratio they occur in the water column rather than according to their tissue composition. Moreover, the proposed negative effect of skewed phytoplankton tissue N:P composition is thought to translate up to the next trophic level as growth of herbivores is also proposed to be affected negatively by prey N:P ratios skewed away from the optimal (Glibert et al. 2013).

**Confounding factors:** Because the height of a phytoplankton bloom, including blooms of N<sub>2</sub> fixers, frequently coincides with a depletion in N and N:P <15, it is often assumed that the major control on the cyanobacteria is the nutrient ratio, rather than the other way around. Blooms of N<sub>2</sub> fixers also coincide with a warm, stratified water column coupled with adequate or high irradiance. Because all these parameters (warm water, high irradiance, stratification, depletion of N, overall increase in Chl *a*) occur in concert, it's difficult to separate out the impact of nutrients from other co-occurring environmental variables in order to quantify the most important effect on increases in cyanobacterial biomass. Investigations that do find that factors such as changes in absolute concentrations of nutrients or changes in Chl *a* biomass are more strongly related to changes in cyanobacterial biomass than changes in the ratio of N:P (Trimbee and Prepas 1987, Downing et al. 2001, Dolman et al. 2012).

**The prevalence of N<sub>2</sub> fixation:** Lastly, an assumption that must be met in order that N<sub>2</sub> fixing cyanobacteria dominate the community at low N:P ratios (and N limiting conditions) is that they mostly use N<sub>2</sub> gas rather than fixed N for growth. However, investigations demonstrate that the proportion of the N demand of N<sub>2</sub> fixers that is met by N<sub>2</sub>-fixation is typically less than 25% (Levine and Lewis 1987, Findlay et al. 1994, Laamanen and Kuosa 2005). For example, in Baltic Sea phytoplankton communities dominated by the N<sub>2</sub> fixers *Aphanizomenon flos aquae* and *Nodularia spumigena*, less than 20% of N utilization is due to N<sub>2</sub> fixation under N-limiting conditions (Sorensson and Sahlsten 1987, Berg et al. 2001, 2003, Laamanen and Kuosa 2005). As mentioned in section 2.2.4, N<sub>2</sub> fixation is repressed in the presence of NH<sub>4</sub><sup>+</sup>; culture studies of the N<sub>2</sub> fixing cyanobacterium *Cylindrospermopsis raciborskii* demonstrate that N<sub>2</sub> fixation is shut down in the presence of NH<sub>4</sub><sup>+</sup> and that it's competitive for fixed N (Sprosser et al. 2004, Moisander et al. 2008). Based on a wide range of investigations, the assumption that most of the N demand of cyanobacteria is met by N<sub>2</sub> fixation does not hold.

**Meta analyses of recent Lake Data:** Consistent with the problems of assigning shifts in phytoplankton community composition to changes in N:P ratios described above, Downing et al. (2001) demonstrated that changes in cyanobacterial biomass was more strongly associated with changes in the absolute concentrations of N and P than with changes in the dissolved N:P ratio in 99 different freshwater systems. In a study of 102 lakes in Germany, Dolman et al. (2012) found that the more enriched in both N and P the lakes were, the greater was their total cyanobacterial biomass. The cyanobacterial taxa that responded most to nutrient enrichment included *Planktothrix agardhii*, *Microcystis* and *Anabaenopsis*.

Moreover, differences between cyanobacterial taxa were not consistent with the hypothesis that N fixing taxa were favored in low N:P conditions as the greatest biomass of *Aphaenizomenon* and *Cylindrospermopsis raciborskii* were found lakes with the greatest N:P ratios (Dolman et al. 2012).

### 3.2.3 Influence of Type of N on Growth of Cyanobacteria

As previously mentioned, NtcA is central in cyanobacterial N regulation and is under negative control by  $\text{NH}_4^+$  (Section 2.2.4). Other than  $\text{NH}_4^+$ -transporters, transcription of all N related enzymes requires binding of the NtcA transcription factor in order to be transcribed. Therefore, uptake and metabolism of sources other than  $\text{NH}_4^+$  does not take place unless  $\text{NH}_4^+$  is at limiting concentrations (Lindell and Post 2001, Lindell et al. 2005). In contrast,  $\text{NH}_4^+$  transporters are constitutively expressed, or always “on”, regardless of external concentration of  $\text{NH}_4^+$  (Berg et al. 2011). In addition, the *amt1*  $\text{NH}_4^+$  transporter gene is one of the most highly expressed in cyanobacterial genomes. In the marine cyanobacteria *Synechococcus* and *Prochlorococcus*, *amt1* is expressed on par with, or at a greater level, respectively, than the gene encoding the C-fixation enzyme Rubisco (Berg et al. 2011). Considering the countless other critical processes happening within cells, it is noteworthy that the protein responsible for  $\text{NH}_4^+$  uptake is one of the most abundant proteins in cyanobacteria.

Given that  $\text{NH}_4^+$  exerts such a strong control over the use of other N sources in cyanobacteria, is the preference for  $\text{NH}_4^+$  reflected in different rates of growth on different N sources? There is no clear answer to this question. From a theoretical perspective it should not be the case because the magnitude of reductant and ATP needed for carbon fixation dwarfs the energetic costs of N assimilation, even assimilation of “expensive” sources such as  $\text{NO}_3^-$  or  $\text{N}_2$  gas (Turpin 1991). The type of N should not affect the rate of growth other than under conditions of very low irradiance where assimilation of  $\text{NO}_3^-$  may compete with carbon fixation for reductant and ATP, thereby lowering the growth rate (Turpin 1991). Culture investigations appear to bear this out as faster rates of growth are typically not observed when cyanobacteria are grown on  $\text{NH}_4^+$  versus  $\text{NO}_3^-$  (i.e. Berman and Chava 1999, Hawkins et al. 2001, Post et al. 2001, Saker and Neilan 2001, Solomon et al. 2010). Differences in growth rates when growing on  $\text{NO}_3^-$  versus on  $\text{NH}_4^+$  are frequently detected for individual strains (i.e. Hawkins et al. 2001, Saker and Neilan 2001), but there is no pattern that can be generalized with respect to cyanobacteria as a whole. Even within the same species, some strains may be growing faster on  $\text{NH}_4^+$  and some on  $\text{NO}_3^-$ , but the difference with N source in most cases is smaller than the difference in growth rate among different strains (Figure 3.2). Therefore, observations of fast growth of cyanobacteria using  $\text{NH}_4^+$  in the field are most likely due to 1) factors that promote fast growth of cyanobacteria generally (i.e. high temperature and high irradiance) combined with 2) high enough availability of  $\text{NH}_4^+$  such that NtcA is repressed and only  $\text{NH}_4^+$  is taken up and utilized by the cell.



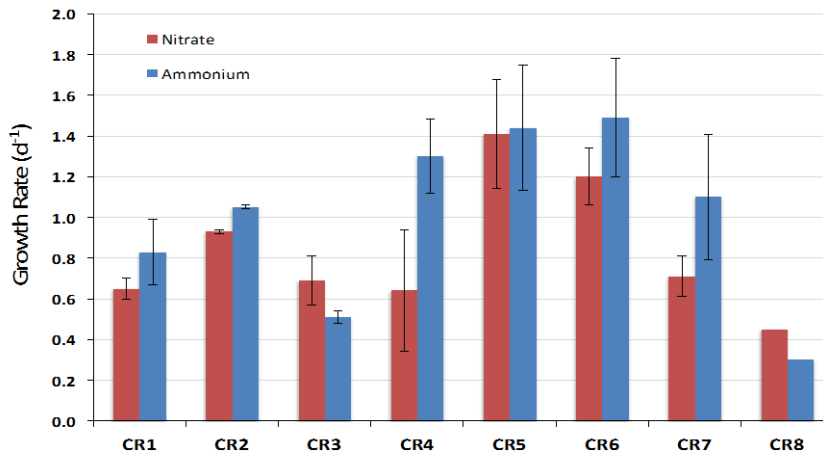


Figure 3.2 Difference in growth rates of *Cylindrospermopsis raciborskii* when growth on  $\text{NO}_3^-$  (red bars) versus  $\text{NH}_4^+$  (blue bars) for eight different strains. Data from Saker and Neilan 2001 and Stucken et al. 2014.

### 3.3 Irradiance and Water Clarity

Cyanobacteria have a distinct advantage with respect to other photosynthetic organisms in the amount of carotenoid pigments per cell volume (Section 2.2.2). These pigments serve a photoprotective function by dissipating excess light energy when required allowing cyanobacteria to be exposed to high irradiances without experiencing photoinhibition (Paerl et al. 1983). Recent investigations also demonstrate that the toxic peptides produced by cyanoHAB species accumulate in the thylakoid membranes potentially serving a role in photoprotection of the cells (Kaebernick et al. 2000, Borner and Dittman 2005, Gerbersdorf 2006). Interestingly, cyanobacteria such as *Microcystis* are not strong competitors for light in a well-mixed environment due to their poor light absorption efficiency (Huisman et al. 1999, Reynolds et al. 2006). The upshot of these traits is that cyanobacteria grow ineffectively at low and mixed light, but very effectively when exposed to high light, particularly the toxic peptide-producing varieties (Huisman 1999, Reynolds 2006, Carey 2012).

Aided by their positive buoyancy, cyanobacteria such as *M. aeruginosa*, can grow very close to the surface by tolerating irradiance levels that are inhibitory to other members of the phytoplankton community. As a result, these cyanobacteria can increase their cell densities past the point where they would ordinarily become light-limited by self-shading. Growing close to the surface can also help cyanobacteria avoid light limitation if there is a high concentration of suspended sediment matter in the water. In contrast, phytoplankton that are not positively buoyant can become shaded by the cyanobacteria growing at the surface (Carey et al. 2012).

Other species, such as *Cylindrospermopsis raciborskii* and *Planktothrix* sp. are good competitors at low light. Cultures of *C. Raciborskii* can grow at optimal rates at very low irradiances (Shafik et al. 2001, Briand et al. 2004, Dyble et al. 2006) and it grows well in deep water columns where it's exposed to fluctuating light levels as it mixes from the surface to the bottom (McGregor and Fabbro 2000, Burford and Donohue 2006, O'Brien et al. 2009).

### 3.4 Factors Impacting Toxin Production

Just as there is substantial discussion surrounding the purpose of toxin production in cyanobacteria, the conditions under which toxin production is enhanced is also vigorously debated. Previous studies have concluded that the greatest intracellular toxin concentrations are detected under favorable growth conditions, including high irradiance as discussed above, with maximal toxin production occurring at maximal rates of cell division and in late log phase (Watanabe and Oishi 1985, Orr and Jones 1998, Sivonen and Jones 1999, Van Der Westhuizen and Eloff 1985).

Investigations specifically focused on changes in nutrient concentrations and ratios, demonstrate that microcystin content reaches a maximum under maximum growth rates, regardless of medium N:P ratio, but that the microcystin content of the cells correlates with total cellular N and protein content (Lee et al. 2000, Vezie et al. 2002, Downing et al. 2005). These results make sense as the toxins, being peptides, require ample N in order to be synthesized. Consistent with this, total toxin production per cell decreases at N-limiting concentrations (Tonk et al. 2008).

Not only does toxin concentration per cell vary in strains that produce toxins (i.e. are toxigenic), but natural populations are typically comprised of a mix of toxigenic and non-toxigenic strains of the same species. It is also of interest to know whether the proportion of toxigenic:non-toxigenic strains within a population changes with nutrient concentrations or ratios. Laboratory culture investigations comparing growth of toxigenic and non-toxigenic strains of *Microcystis* demonstrated that toxigenic strains of *Microcystis* grew faster than non-toxigenic strains at N concentrations of 6000  $\mu\text{moles L}^{-1}$  and at N:P ratios  $\gg 200$  (Vezie et al. 2002). The reason for this is not clear, but could include microcystin conferring protection from  $\text{NO}_3^-$  toxicity in the toxin-producing strains at such unnaturally high concentrations of  $\text{NO}_3^-$ .

While results obtained with unnaturally high nutrient concentrations and ratios do not easily translate to natural systems, a nutrient enrichment bioassay investigation has demonstrated that toxigenic strains within a *Microcystis* population were promoted to a greater degree with N (and P) additions than non-toxigenic strains (Davis et al. 2010). However, the pattern of selective stimulation of toxigenic strains with increased nutrient concentrations is not evident in natural communities which typically exhibit a high degree of variability across small spatial scales in the proportion of toxigenic:non-toxigenic strains within a population. This variability appears not to be related to nutrient concentrations or ratios which do not exhibit the same spatial variability (Vezie et al. 1998, Baxa et al. 2010, Mbedi et al. 2005, Dolman et al. 2012).

### 3.5 Temperature

Perhaps one of the most important factors in controlling the growth rate of cyanobacteria is temperature (Robarts and Zohary 1987, Butterwick et al. 2005, Watkinson et al. 2005, Reynolds 2006, Paerl and Huisman 2009). Cyanobacteria isolated from temperate latitudes (i.e. excluding polar regions) typically have temperature growth optima between 25 and 35°C (Reynolds 2006, Lurling et al. 2013). For example, in a survey of eight cyanobacteria the growth optima of two *Microcystis aeruginosa* strains were 30-32.5°C and that of *Aphanizomenon gracile* was 32.5°C. Lower growth temperature optima were observed in *Cylindrospermopsis raciborskii* and *Planktothrix agardhii*, both at 27.5°C while *Anabaena* sp had an optimum of 25°C (Lurling et al. 2013). The optima of these freshwater HAB-forming cyanobacteria are greater than for marine cyanobacteria which typically have growth temperature optima ranging from 20-27.5°C (Breitbarth and LaRoche 2005, Boyd et al. 2013).

Compared with other phytoplankton taxa, cyanobacteria typically demonstrate lower growth rates at colder temperatures and higher growth rates at higher temperatures. For example, diatoms typically have a 6-fold higher growth rate at 15°C, 3-fold higher growth rate at 20°C and a similar growth rate at 25°C, compared with cyanobacteria (Figure 3.3). Growth rates of dinoflagellates typically peak at 25°C. Above 25°C both chlorophytes and cyanobacteria have faster growth rates than diatoms and dinoflagellates (Figure 3.3). The difference in the optimum growth temperatures of the various phytoplankton taxa is hypothesized to become increasingly important in determining phytoplankton community composition as global temperatures continue to increase above 20°C (Lehman 2005, Paerl and Huisman 2008, 2009). For example, the acceleration of growth rate with a 10°C increase in temperature ( $Q_{10}$ ) commonly varies from 1-4 for cyanobacteria and 1-3 for chlorophytes (Reynolds 2006). However, it varies from 4-9 for *M. aeruginosa*, the highest recorded for any phytoplankton (prokaryotic or eukaryotic) species (Reynolds 2006). These data suggest that in a mixed phytoplankton assemblage, all else being equal, cyanobacteria will be able to grow faster and outcompete other phytoplankton taxa as the temperature increases.

With continued climate change and global warming, there's an increased risk that cyanoHABs will become increasingly competitive vis-à-vis diatoms which often dominate community composition in temperate regions.

### 3.6 Stratification and residence time

CyanoHAB blooms tend to occur during times of calm, stratified water columns. The degree of stratification and water column stability increases with increased temperature, therefore stratification and temperature are closely linked. The reasons that stratified conditions promote blooms of cyanobacteria are at least three-fold. First, growth rates will increase as a result of the increase in the temperature in the top layer of the water column. Second, cyanobacteria will remain in the top layer of the water column where irradiance is greater, and not become mixed down to the bottom and into lower light, allowing them to maintain higher growth rates.

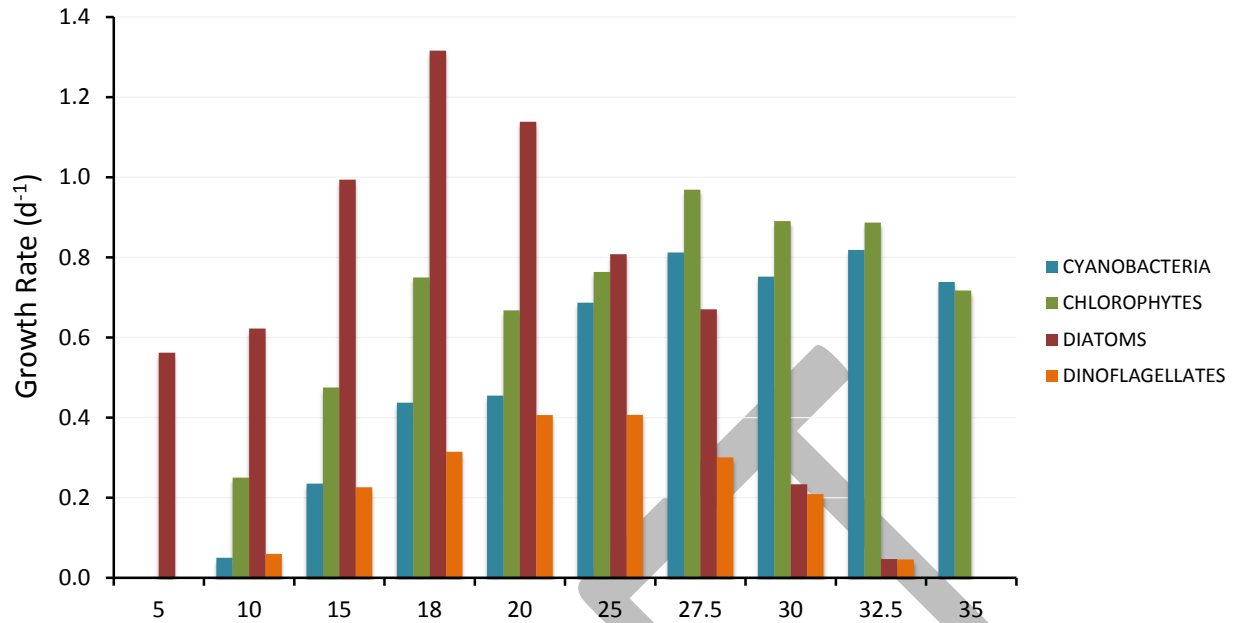


Figure 3.3 Changes in growth rate with temperature for diatoms (red  $\pm 0.35 \text{ d}^{-1}$ ,  $T_{opt}=20 \pm 1.8 \text{ }^\circ\text{C}$ ), Chlorophytes (green  $\pm 0.21 \text{ d}^{-1}$ ,  $T_{opt}=29 \pm 3.8$ ), Cyanobacteria (cyan  $\pm 0.13 \text{ d}^{-1}$ ,  $T_{opt}=29 \pm 4.5$ ) and dinoflagellates (orange  $\pm 0.1 \text{ d}^{-1}$ ,  $T_{opt}=21 \pm 2.8$ ). Data from Boyd et al. 2013, Butterwick et al. 2005, Kudo et al. 2000, Lurling et al. 2013, Yamamoto and Nakahara 2005.

Third, stratification may be a sign of increased residence times (reduced flushing rates), which minimizes loss of cyanobacterial biomass from the system and allows cyanobacteria to use all the nutrients available in the water column (Jeppesen et al. 2007). In other words, it's likely that stratification does not directly promote cyanobacterial blooms, but rather it promotes blooms indirectly through increased temperatures, irradiance and reduced loss rates (Jeppesen et al. 2009, 2011).

## 4. Prevalence of CyanoHABs and Potential for Effects on Ecosystem Services in the Delta

The Sacramento-San Joaquin Delta (hereafter Delta) is formed at the intersection of two of California's largest rivers, the Sacramento and the San Joaquin Rivers, and contains 700 miles of sloughs and waterways that drain 47% of the runoff in the State of California (Figure 1.1). The land surrounding the waterways is composed of 57 leveed island tracts, many of which provide wildlife habitat. In the Delta, freshwater from the rivers mix with saltwater from the San Francisco Bay; together the Bay and the Delta form the West Coast's largest estuary.

### 4.1 Ecosystem Services

The Delta region has many ecosystem services including agriculture, drinking water supplies, and wildlife habitat, all of which translate directly to the beneficial uses designated in the Water Board Basin Plan (Appendix 1). The population surrounding the Delta region, numbering 500,000 people, is principally engaged in agriculture and produce crops that bring in revenues exceeding \$500 million annually. While there is some local demand on the water from the Delta, most of the water is distributed via the State Water Project and Federal Central Valley Projects to the Central Valley to irrigate farmland and to provide drinking water to Southern California (<http://www.water.ca.gov/swp/delta.cfm>). According to the California Department of Water Resources, about two thirds of Californians and millions of acres of irrigated farmland rely on the Delta for their water. Besides acting as a source of drinking water, the Delta is a popular recreation spot and many people use it for sport fishing.

In addition to the human demand, the Delta supplies critical habitat to a large wildlife ecosystem and intersects migration paths for several fish species, including salmon, traveling between the Pacific Ocean and the Sacramento River and beyond. This habitat is in a fragile state with close to 20 of its endemic species listed as endangered. A recent and unexpected decline in four pelagic fish species including the endangered Delta Smelt and the Longfin Smelt, as well as Striped Bass and Threadfin Shad, has caused concern among resource managers and renewed calls for conservation of the fragile Delta ecosystem (Sommer et al. 1997).

Set against this backdrop of competing resource use by human populations and wildlife, a new threat to Delta ecosystem services and designated beneficial uses is emerging in the form of toxic cyanoHABs. High densities of cyanoHABs that produce toxins can result in mortality to aquatic animals such as sea otters, birds and seals that have ingested shellfish with accumulated toxins (Jessup et al. 2009, Miller et al. 2010). Humans coming in contact with the water may develop digestive and skin diseases (Section 2.2.6) and it may affect the drinking water supplies (Cheung et al. 2013). In addition, high densities of cyanoHABs increase the turbidity of the watercolumn to the point where light penetration is severely restricted and the growth of other phytoplankton, macrophytes, and benthic microalgae is restricted (Jeppesen et al. 2007, Paerl and Paul 2012). CyanoHABs also can cause night-time dissolved oxygen depletion via bacterial decomposition and respiration of dense blooms which results in fish kills and loss of benthic fauna (Paerl 2004, Paerl and Fulton 2006).

The impact of toxic cyanobacteria on the aquatic ecosystem differs widely depending on whether their density is low or high. At low concentrations, they are not dense enough to affect light penetration or dissolved O<sub>2</sub> concentration, therefore they do not affect the growth of other members of the aquatic community. However, even at low concentrations, toxins released (upon death and cell lysis, or by grazing) can bioaccumulate in higher trophic levels (Lehman et al. 2010). At high densities, cyanoHABs increase the turbidity of the watercolumn to the point where light penetration is severely restricted suppressing the growth of other phytoplankton, macrophytes, and benthic microalgae (Jeppesen et al. 2007, Paerl and Paul 2012). CyanoHABs also can cause night-time dissolved oxygen depletion via bacterial decomposition and respiration of dense blooms which results in fish kills and loss of benthic fauna (Paerl 2004, Paerl and Fulton 2006). At dense concentrations, mortality to aquatic animals such as sea otters, birds and seals may result from liver failure following ingestion of prey with high concentrations of toxin, or coming into physical contact with the toxin (Jessup et al. 2009, Miller et al. 2010). Humans coming in contact with the water may develop digestive and skin diseases (Section 2.2.6) and it may affect the drinking water supplies (Cheung et al. 2013).

However, cyanobacteria are a natural component of aquatic ecosystems; adverse effects of toxic cyanobacteria on the aquatic ecosystem differs widely depending on whether their density is low or high. At low concentrations, they are not dense enough to affect light penetration or dissolved O<sub>2</sub> concentrations, therefore they do not affect the growth of other members of the aquatic community. However, even at low concentrations, toxins released (upon death and cell lysis, or by grazing) can bioaccumulate in higher trophic levels (Lehman et al. 2010). In the following sections, cyanoHAB abundance and toxin levels in the Delta vis-à-vis published guidance on alert levels are summarized in order to place the threat of cyanoHABs in the Delta into context.

#### 4.2 Prevalence and Trends of CyanoHABs in the Delta

Since 1999 blooms of the toxin producing cyanobacteria *Microcystis aeruginosa* in the Delta have been observed by the Department of Water Resources (DWR), and have been reported in the scientific literature. In the beginning, only blooms of *Microcystis* were observed; these were documented visually appearing as little flakes of lettuce in the water (Lehman and Waller 2003). Later investigations employing microscopic enumeration and molecular characterizations have documented blooms comprised of a mix of *Aphanizomenon* sp. and *Microcystis*, with *Anabaena* sp. also present in much smaller densities (Lehman et al. 2010, Mioni et al. 2012).

While environmental indicators such as salinity, turbidity, temperature, total phytoplankton biomass (as Chl *a*), and phytoplankton species composition are monitored on a monthly basis by DWR, surface concentrations of phytoplankton, which requires special sampling, are not routinely monitored. Accordingly, the information on the chronology of cyanoHAB occurrences presented here is taken from a handful of publications and reports, and varies somewhat in geographical extent according to where the authors sampled. Additionally, because *Aphanizomenon* and *Anabaena* densities have only been documented for two time points, the following sections will focus on *Microcystis* biomass and microcystin toxin concentrations.

#### 4.2.1 Spatial Distribution of *Microcystis* throughout the Delta

The Central Delta, between Antioch and Mildred Island, is typically the region with the highest surface *Microcystis* and *Aphanizomenon* concentrations. In 2003, the stations with the greatest recorded abundance of Chl *a* due to *Microcystis* (as determined by horizontal surface tows with a 75- $\mu\text{m}$  mesh plankton net) were Jersey Point (D16), Mokelumne River Mouth and Navigation Marker 13 in the San Joaquin River, followed by San Mound Slough, Mildred Island, (D29) and Rancho del Rio (D28) in Old River (Figure 4). In following years, greatest abundance of *Microcystis* has repeatedly occurred in the same areas in the San Joaquin and Old Rivers (Lehman et al. 2008, Mioni et al. 2012, Lehman et al. 2013). In 2012, abundant *Microcystis* colonies were also observed in the South-East Delta region in the Turning Basin of the Stockton Shipping Channel (Spier et al. 2013). Moving west from Antioch into Suisun Bay, *Microcystis* abundance decreases substantially to almost non-detectable by Chipps Island (Lehman et al. 2005, 2008, 2010). The same holds true when moving north where abundances detected at Antioch decline to almost zero by Collinsville at the entrance of the Sacramento River (Figure 4).

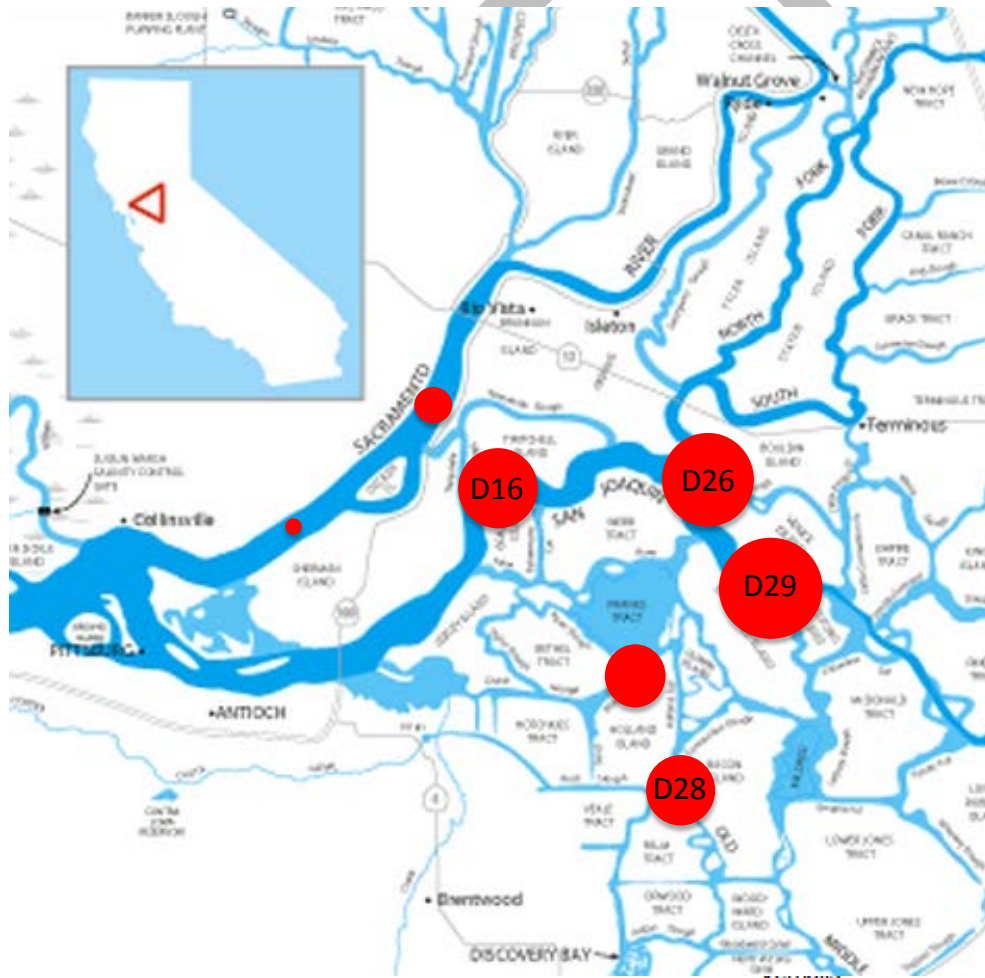


Figure 4.1. The Sacramento-San Joaquin Delta Region, source: Metropolitan Water District of Southern California. Red bubbles mark locations with greatest *Microcystis*-associated surface Chl *a* concentrations. Size of bubble is proportional to amount of Chl *a* (largest bubble= $0.55 \mu\text{g Chl } a \text{ L}^{-1}$ ). Data from Lehman et al. 2005.

#### 4.2.2 Interannual variability in *Microcystis* biomass in the Delta

Since 2003, *Microcystis* cell abundance in surface waters has varied from  $4\text{-}40\times 10^3$  cells  $\text{mL}^{-1}$  in the Delta (Lehman et al. 2008), at times higher than the threshold of low risk of health effects ( $20\times 10^3$  cells  $\text{mL}^{-1}$ ) but generally lower than the threshold for moderate risk health effects ( $100\times 10^3$  cells  $\text{mL}^{-1}$ ) as outlined by the World Health Organization, WHO (Table 4.1). The biomass (as surface Chl *a*) has also varied approximately 10-fold (Figure 4.2). Not only is *Microcystis* biomass patchy between years, its distribution in the years that it blooms is also variable. Even within a station, the distribution of *Microcystis* colonies is patchy, as evidenced by the low concentration of surface Chl *a*, sampled with horizontal net-tows normalized to total towed volume, which to date has not been above  $0.6\ \mu\text{g Chl } a\ \text{L}^{-1}$  (Figure 4.2). In the years following 2008, *Microcystis* was also present in the phytoplankton community together with *Aphanizomenon flos-aqua*, and to a lesser extent *Anabaena* sp. (Mioni et al. 2012).

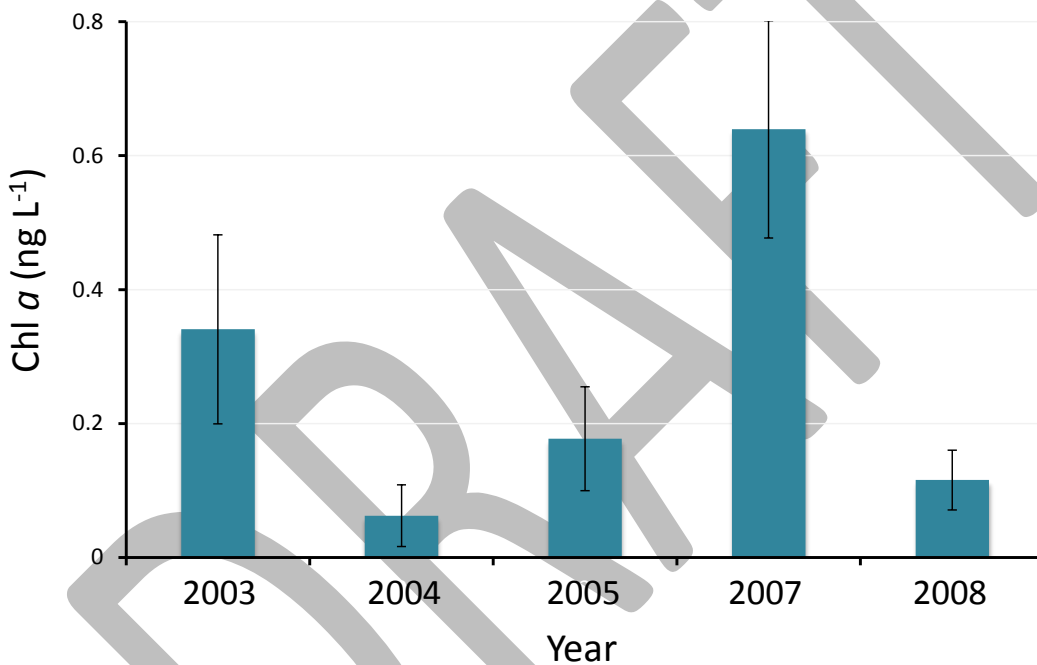


Figure 4.2. Interannual changes in surface Chl *a* due to abundance of *Microcystis* colonies. Means and standard deviations of 9 different stations in the San Joaquin River (Antioch (D12), Jersey Point (D16), Frank's Tract (D19), Potato Point (D26), Prisoners Point (D29), San Joaquin River at Turner Cut, Sand Mound Slough, Mildred Island, and Old River at Rancho del Rio (D28)). Data from Lehman et al. 2005, 2013.

In addition to a high degree of horizontal variability, *Microcystis* cell densities and biomass also varies vertically in the water column, decreasing from the surface to almost zero at 1 m depth. The density of *Microcystis* in surface waters at the Central Delta Stations does not affect phytoplankton community composition in a measurable way. For example, at four stations where *Microcystis* dominated abundance of phytoplankton at the surface, the communities at 1m depth was a variable mix of different species of phytoplankton that was equally variable at stations containing no *Microcystis* in the



surface. Rather than decreasing, the biomass of other phytoplankton taxa increased in tandem with increasing *Microcystis* biomass (Lehman et al. 2010).

**Table 4.1. Cyanobacterial abundance thresholds associated with risks from human exposure to cyanobacterial blooms in recreational or drinking waters. From WHO (1996).**

Risk Level	Cyanobacterial Cell Counts	Expected Toxin Concentration	Chl-a ( $\mu\text{g L}^{-1}$ )
<b>Low probability of health effect</b>	20,000 cells $\text{ml}^{-1}$	2-4 $\mu\text{g L}^{-1}$	< 10
<b>Moderate probability of health effect</b>	100,000 cells $\text{ml}^{-1}$	50 $\mu\text{g L}^{-1}$	<50

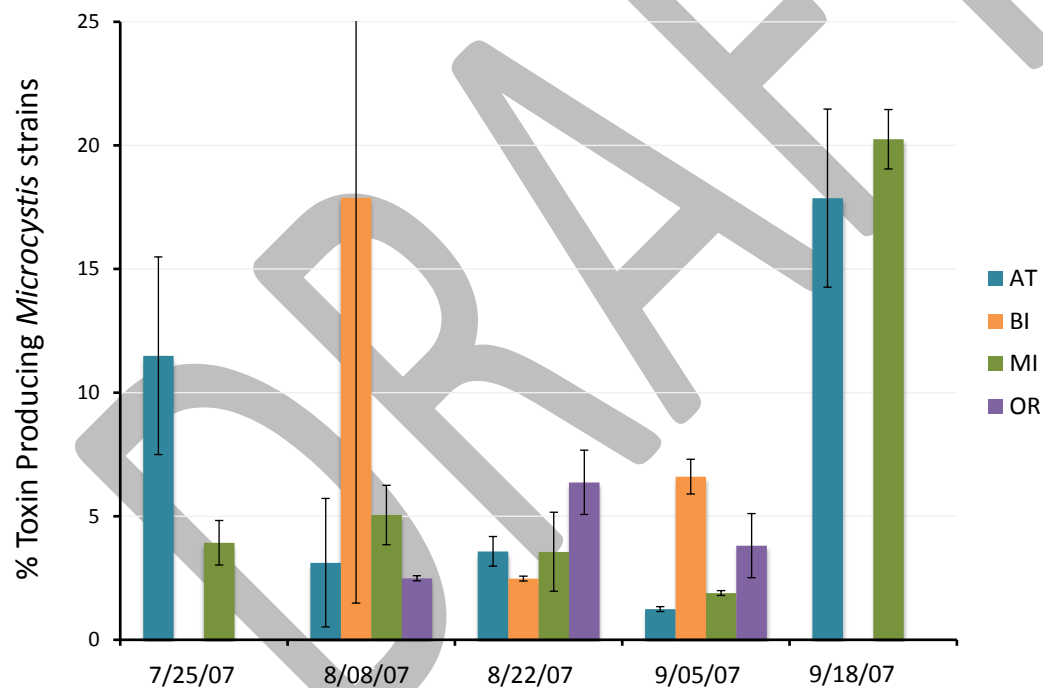
#### 4.2.3 *Microcystin* toxin concentrations in the Delta and San Francisco Bay

*Microcystis* produces approximately 100-400 ng microcystin per  $\mu\text{g}$  Chl *a* in toxin producing strains (Sivonen 1999). Just as with other regions where *Microcystis* occurs, the strains that occur in the Delta are a mix of toxigenic and non-toxigenic strains (Baxa et al. 2010). Toxigenic strains generally comprise 2-20% of the total number of *Microcystis* strains present. This variation in the proportion of toxigenic strains is observed everywhere (i.e. at every station) and at all times. No single station stands out as consistently producing a greater proportion of toxigenic strains compared with other stations (Figure 4.3). Accordingly, total microcystin concentrations reflect *Microcystis* cell abundance, typically varying from 10-50  $\text{ng L}^{-1}$  (Figure 4.4). These levels are 16-80 times lower than the Office of Environmental Health Hazard Assessment (OEHHA) Action Level (Table 4.2). However, in 2012 concentrations approaching 2000  $\text{ng L}^{-1}$  were detected in the Stockton shipping channel during a *Microcystis* event (Spier et al. 2013), exceeding both the OEHHA Action level and the WHO guideline of 1000  $\text{ng L}^{-1}$  (Table 4.2). Using the relationship 115 ng microcystin  $\mu\text{g}$  surface Chl *a*<sup>-1</sup> (Figure 4.4), *Microcystis*-associated surface Chl *a* concentration of 7  $\mu\text{g L}^{-1}$  (sampled using a horizontal net tow) would produce enough microcystin (800  $\text{ng L}^{-1}$ ) to reach the OEHHA Action Level, and constitute an action level for the Delta.

Outside the Delta, intermediate concentrations of total microcystins have been detected at a station close to Rio Vista (Brannan Island) where *Microcystis* cell abundance is low to non-detectable (Lehman et al. 2008, 2010). This station is connected via a channel to the San Joaquin River and the Frank's Tract area. Physical mixing of water directly from the San Joaquin River with brackish water at this station situated at the entrance to the Sacramento River may bring toxins but establishment of *Microcystis* populations may be prevented by the conditions in the Sacramento River including colder water, much greater flow rates, mixing down to the bottom, and lower water clarity (Lehman et al. 2008).

**Table 4.2** Action levels developed by OEHHA for exposure to cyanotoxins compared with the WHO guidance level for microcystins.

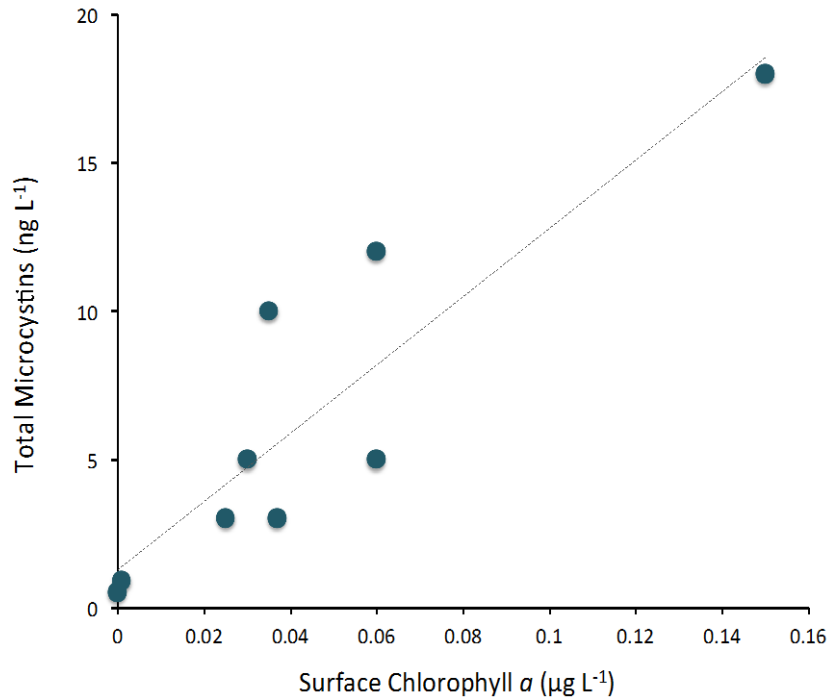
Toxin	OEHHA Recreational Use ( $\mu\text{g/L}$ water)	OEHHA Consumption Level ( $\text{ng/g}$ fish)	WHO recreational Use ( $\mu\text{g/L}$ water)
Microcystins	0.8	10	1.0
Cylindrospermopsin	4	70	
Anatoxin-a	90	5000	



**Figure 4.3.** Percent toxin-producing strains in *Microcystis* assemblage at stations AT, Antioch (D12); BI, Brannan Island (D23); MI, Mildred Island; and OR, Old River at Rancho del Rio (D28). Data from Baxa et al. 2010.

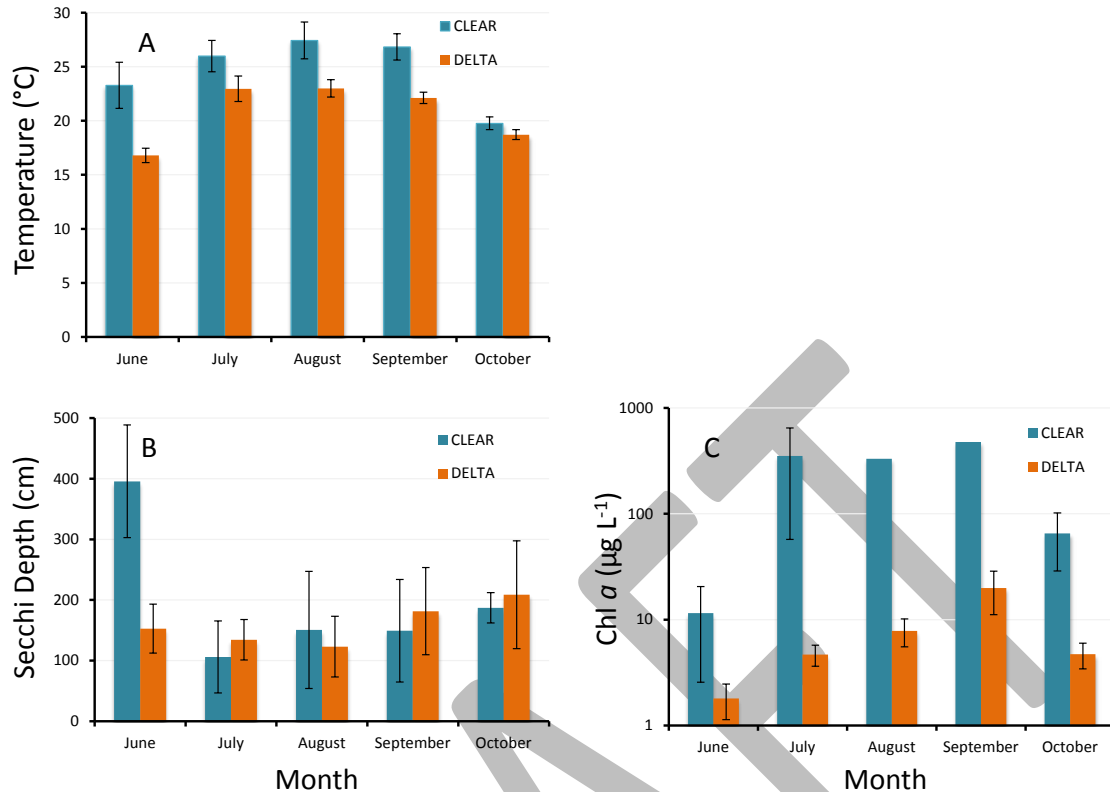
*Microcystin* toxin has also been detected at low concentrations throughout San Francisco Estuary using the SPATT technique, which integrates over one to two month-long time spans (acting more like a bivalve), suggesting that the risk of trophic transfer of the toxin is high (Kudela pers. com). For example, microcystin concentrations reached  $100,000 \text{ ng L}^{-1}$  during a 2007 *Microcystis* bloom in Pinto Lake (Miller et al. 2010), exceeding both the OEHHA and WHO Action Levels by a 100-fold. Transport of microcystin

from Pinto Lake downstream to Monterey Bay has resulted in poisoning and mortality of sea otters for a number of years (Miller et al. 2010).



**Figure 4.4.** Relationship between total microcystins and surface Chl *a* ( $y=115x+1.3$ ,  $r^2=0.8$ ). Data from Lehman et al. 2008.

While the risk of toxin bioaccumulation is present in the Delta, concentrations of *Microcystis* and toxins observed here are low compared to surrounding lakes in the regions (i.e. Richerson 1994, Jacoby and Kann 2007, Mioni et al. 2009, Miller et al. 2010). For example, in Clear Lake, spring and early summer Chl *a* concentrations average  $11.5 \pm 8 \mu\text{g Chl } a \text{ L}^{-1}$  but increase to  $352 \pm 295 \mu\text{g Chl } a \text{ L}^{-1}$  in the summer once *Microcystis* starts to bloom (Figure 4.5). In summer, *Microcystis*-associated Chl *a* concentration is a factor of 100 to 1000 greater in Clear Lake than it is in the Delta. One important caveat with respect to determining surface Chl *a* concentrations is that there is a 100-fold difference between performing an integrated measurement of *Microcystis* biomass using a surface net tow (akin to what is used in Lehman et al. 2013) compared with taking a grab sample in the middle of a patch (akin to Mioni et al. 2012). The difference between using the former and latter methods turns out to be 100-fold, i.e.  $0.2 \mu\text{g Chl } a \text{ L}^{-1}$  versus  $20 \mu\text{g Chl } a \text{ L}^{-1}$ , respectively. This suggests that the “coverage” of *Microcystis* colonies in surface waters of the Central Delta is around 1%, in sharp contrast with Clear Lake where surface Chl *a* is uniformly high (above  $150 \mu\text{g Chl } a \text{ L}^{-1}$ ) at all stations during a bloom (Richerson 1994, Mioni et al. 2012).



**Figure 4.5.** Comparison of environmental variables and Chl a in Clear Lake (Cyan) and the Delta (orange) using in-patch grab samples during the summer months of 2011. (A) Temperature, (B) Secchi disk depth, (C) Chl a. Data from Mioni et al. (2012).

#### 4.2.4 Summary of Potential for adverse effects on Delta beneficial uses

A thorough characterization of the risks for adverse effects on Delta beneficial uses is hindered by the fact that cyanoHAB prevalence and toxin concentrations are currently not routinely monitored in the Delta. However, based on existing data, the current risk to Delta ecosystem services appears to be on the lower end of the spectrum, though clearly not negligible. This observation is based on:

- Microcystis cell abundances that sometimes exceed WHO benchmark of low risk of health effects ( $40 \times 10^3$  cells  $\text{ml}^{-1}$ ), but are consistently below that of moderate risk of health effects ( $100 \times 10^3$  cells  $\text{ml}^{-1}$ ).
- Toxin concentrations that are generally 16-80 times below OEHHA action level, although occasionally exceeding both the OEHHA action level and the WHO guideline of  $1000 \text{ ng L}^{-1}$ .
- Shading of other phytoplankton, benthic diatoms, and other aquatic macrophytes does not appear to be an issue (because bloom densities are low).
- Bloom densities are not sufficient to cause oxygen depletion

Despite the low risks, the risk of bioaccumulation of toxins in the food web is a potential factor, but is generally not well quantified.

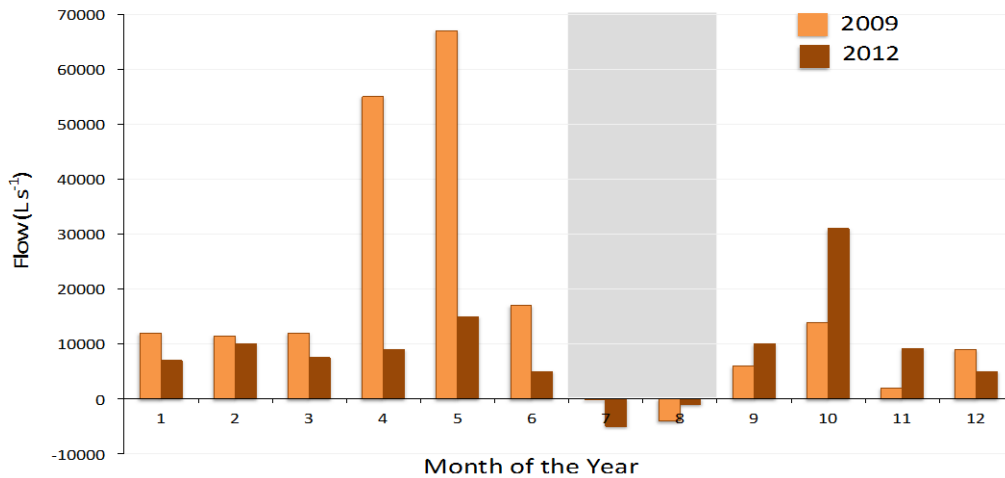
## 5.0 Synthesis of Factors Influencing CyanoHABs presence and Toxin Production in the Delta

The charge of the cyanobacterial workgroup, as outlined in the Delta Nutrient Management Charter, is to “assess whether observed increases in the magnitude and frequency of cyanobacterial blooms in the Delta is the result of long-term changes in nutrient concentrations and whether management of nutrient loads can remedy the problems associated with cyanobacteria.” The best way to characterize the relationship between the extent and frequency of bloom occurrence and nutrient concentrations, is by regression analysis. Ideally, this type of analysis ought to be performed in multiple locations for longer time scales. Given that temperature, irradiance and water column clarity are such powerful triggers of blooms, stepwise multiple regression analysis to test the influence of several environmental indicators simultaneously on cyanoHAB cell densities would be even more useful in order to ascertain key triggers of the blooms in the Delta region.

While environmental indicators such as salinity, turbidity, temperature, total phytoplankton biomass (as Chl  $\alpha$ ), and phytoplankton species composition are monitored on a monthly basis by DWR, surface concentrations of phytoplankton, which requires special sampling, are not routinely monitored. Therefore, the statistical analyses needed to answer the charge of the cyanobacterial working group cannot be performed at this time. Instead, this section focuses on summarizing factors known to favor cyanobacterial prevalence (from Chapter 2) and synthesizing available literature on the extent to which those factors may also be at play in the Delta.

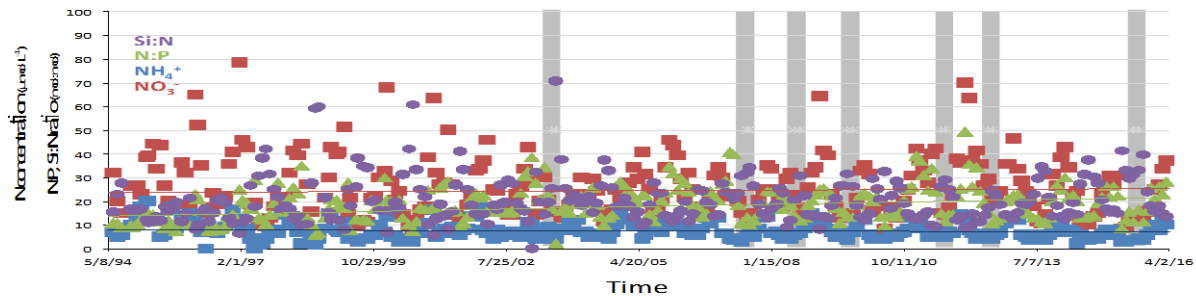
### 5.1 Factors associated with cyanoHAB prevalence in the Delta

The general growth characteristics outlined for cyanoHAB growth in freshwater bodies around the world also play key roles in regulating growth of cyanoHABs in the Delta (Table 5.1). For example, Lehman et al. (2013) noted that increased abundance of *Microcystis* is associated with up to a 50% reduction in flow of water in the San Joaquin River. In 2004, *Microcystis* only appeared in the Central Delta when stream flow was 1-35 m<sup>3</sup> s<sup>-1</sup> (Lehman et al. 2008). In turn, this reduction in flow is accompanied by a 50% reduction in turbidity and in volatile suspended solids. Decreased flow also leads to increased water temperatures. Conditions of decreased flow occur more predictably in dry years (Lehman et al. 2013). Within the summer season, reduced flows typically occur in the July-August time frame (Figure 5.1). In turn, reduced flows set the stage for the two factors necessary for bloom initiation, including increased water column temperature and water column clarity (decreased turbidity).



**Figure 5.1.** Variation in flow at Brandt Bridge in the Delta (years 2009 and 2012) illustrating the low- and reverse-flow window in July-August (shaded grey). Data and plot from Spier et al. 2013.

Nutrient concentrations are typically non-limiting to cyanoHAB growth in the Central Delta (Lehman et al. 2008). Moreover, concentrations, or ratios, do not change sufficiently from year-to-year in order to explain year-to-year variation *Microcystis* biomass or occurrence (Figure 5.1). Therefore the initiation of *Microcystis* or other cyanoHAB blooms are probably not associated with changes in nutrient concentrations or their ratios in the Delta. However, as with all phytoplankton blooms, once initiated, cyanoHABs cannot persist without an ample supply of nutrients. As such, blooms of cyanoHABs can be controlled by reducing the supply of nutrients in the water column in addition to hydrological or temperature controls.



**Figure 5.2.** Changes in  $\text{NO}_3^-$  (red squares),  $\text{NH}_4^+$  (blue squares), N:P ratio (green triangles) and Si:N ratio (purple circles) over time at station D26 in the Delta. Grey shaded areas denote occurrence of *Microcystis* blooms. Blue line denotes regression of  $\text{NH}_4^+$  concentration versus time ( $\text{NH}_4^+ = -0.0001(\text{time}) + 11.4$ ,  $r^2 = 0.0007$ ); green line denotes regression of N:P ratio versus time ( $\text{N:P} = 0.001(\text{time}) - 18.9$ ,  $r^2 = 0.16$ ); red line denotes regression of  $\text{NO}_3^-$  versus time ( $\text{NO}_3^- = 0.0002(\text{time}) + 17$ ,  $r^2 = 0.006$ ). Data collected by California DWR and accessed from the Interagency Ecological Program website (<http://www.water.ca.gov/iep/products/data.cfm>).

It is important to understand that while nutrient reductions may not limit the onset or frequency of bloom occurrence, it will limit their duration, their intensity and possibly also their geographical extent.

## 5.2 Factors that could allow an increase in cyanoHAB blooms in the Delta

The Delta is a region where 40-75% of the phytoplankton community is comprised of diatoms, followed by chlorophytes (15-30%), cyanobacteria (15-40%), cryptophytes (5-10%) and flagellates (0-10%), including dinoflagellates (Lehman 2007). Since 1999, surface blooms of colonial and filamentous cyanobacteria such as *Microcystis* and *Aphanizomenon* have become more common and it's of great interest to determine whether these blooms are on the rise, and whether they will someday displace the species that currently dominate phytoplankton community composition, as they have in so many other systems.

In order for cyanoHABs to displace diatoms as the dominant member of the phytoplankton community, they have to be able to accelerate their growth rates to grow faster than diatoms. Data from Figure 2 suggests that at 18-20°C, the average summertime temperature in the Delta, cyanoHABs would have to accelerate their growth rates 2-3- fold in order to compete with diatoms. Alternatively, temperatures would have to increase to the point where cyanoHAB growth rates become competitive with diatom growth rates. Data from Figure 2 indicates that a doubling in cyanobacterial growth rates occurs with an increase in temperature from 20-27°C; diatom growth rates decrease over the same temperature range.

Therefore, a rise in temperature is a scenario under which cyanobacteria are able to outcompete diatoms.

This scenario is consistent with differences in temperature between a system, such as Clear Lake, where cyanHABs dominate community composition, and the Delta. Comparing the 2011 environmental variables from Clear Lake and the Central Delta, two pre-bloom (June) differences become immediately clear. One is that the water temperature in Clear Lake is 7°C degrees warmer than the Delta (Fig. 4.5). The other is that the Secchi disk depth is 2.6-fold greater in Clear Lake compared with the Delta (Fig. 4.5). This difference in water clarity disappears in July when the *Microcystis* bloom takes off in Clear Lake, increasing Chl *a* 35-fold and decreasing the water clarity (Fig. 4.5). Lehman et al. (2013) also predicted that the two factors that potentially would make the greatest impact on accelerating the growth of *Microcystis*, and increase the frequency and duration of blooms in the Delta, would be increased water temperatures and increased water column clarity. The earlier in the growth season that these increases would occur the greater the window of opportunity for growth would become.

If water temperatures did not increase above the summer-time average of 18-20°C, could there be an acceleration in cyanobacterial growth rates with changes in N source, or with N:P ratio, that would enable them to outcompete diatoms and become dominant? As just mentioned, the magnitude of change in growth rates as a result of a change in nutrient concentrations or ratios would have to be on the order of 2-3 fold. Comparing the ratios of dissolved N:P between the Delta and Clear Lake,  $3.6 \pm 0.6$  and  $2.9 \pm 0.8$ , respectively, it's clear that these are essentially the same (Figure 4.5). Moreover, they do not vary from pre-bloom to bloom in the Delta indicating that nutrients are in excess of phytoplankton demand for the entire summer season. In Clear Lake, Both N and P are drawn down to below detection once *Microcystis* blooms making it impossible to calculate pre-bloom ratios. Because culture investigations also demonstrate that there is no significant, or consistent, change in growth rates with change in N source, or N:P ratios, at nutrient concentrations in excess of demand, nutrients are unlikely to play a role in the onset or frequency of bloom occurrence in the Delta.

### 5.3 Summary

In the review of the global literature on factors influencing cyanobacterial blooms and toxin production, five principal drivers emerged as important determinants:

- 1) Water temperatures above 19-20°C
- 2) High irradiance and water clarity
- 3) Availability of N and P in non-limiting amounts; scientific consensus is lacking on the importance of N:P ratios and nutrient forms (e.g. ammonium) as a driver for cyanoHABs
- 4) Long residence times and stratified water column
- 5) Low salinity (<10 ppt) waters

Comprehensive understanding of the role of nutrients vis-à-vis other environmental factors in influencing cyanoHAB presence in the Delta is severely hampered by the lack of a routine monitoring program. The DWR monitoring program currently measures many of the environmental factors of



interest, except cyanobacterial abundance and toxin concentration, which require a different approach than that used in standard phytoplankton monitoring.

Drawing on the five factors influencing cyanoHABs, we can conclude the following:

- Because of the large effects of temperature and irradiance on accelerating, and decelerating, the growth rates of cyanoHABs, these two factors appear to exert key roles in the regulation of the onset of blooms. Cyanobacteria require temperatures above 20°C for growth rates to be competitive with eukaryotic phytoplankton taxa, and above 25°C for growth rates to be competitive with diatoms (Table 5.1). In addition, they require relatively high irradiance to grow at maximal growth rates. This is in contrast with diatoms that are able to keep near-maximal growth rates at irradiances limiting to cyanoHABs in the Delta, e.g. 50  $\mu\text{mol phot m}^{-2} \text{s}^{-1}$  (Table 5.1).
- It appears that N and P are available in non-limiting amounts in the Delta; moreover concentrations, or ratios, do not change sufficiently from year-to-year to explain year-to-year variation in *Microcystis* biomass or occurrence. Therefore, the initiation of *Microcystis* blooms and other cyanoHABs are probably not associated with changes in nutrient concentrations or their ratios in the Delta. However, as with all phytoplankton blooms, once initiated, cyanoHABs cannot persist without an ample supply of nutrients. As long as temperatures and irradiance remains favorable for growth, the size of the nutrient pool will determine the magnitude and extent of cyanoHAB blooms.
- Salinity is controlling the oceanward extent of cyanobacterial blooms in the Delta, but salinity gradients do not explain the spatial distribution of cyanoHABs in the Delta (Table 5.1). Notably, salinity regime is not a barrier to toxin transport, as cyanotoxins have been detected in San Francisco Bay.
- Higher flows, turbidity and lower temperatures during most of the year are likely restricting cyanobacterial blooms to the July-August time period.

Climate change and anthropogenic activity associated with land use changes have the potential to alter cyanoHAB prevalence in the future. Climate change will likely result in warmer temperatures and increased drought, the latter of which could result in reduced flows, increased residence time and watercolumn stability leading to higher light availability in the Delta. Both temperature and reduced flows would presumably result in a greater prevalence of cyanoHABs. Increased nutrient loading on top of warmer temperatures and stratified water conditions could potentially increase the intensity (concentration) of cyanoHABs. It's noteworthy that phytoplankton biomass and primary productivity are depressed relative to available nutrients in the Delta, so it's unclear what the effect of modifying nutrient loads will have on frequency and intensity of cyanoHAB occurrence in the future.

**Table 5.1. Summary of general physiological drivers of cyanobacterial growth, how they are manifested in population growth and competition with diatoms, and how they compare with environmental drivers observed to be operating in the Delta**

Physiological Driver	Population Driver	Observations in the Delta
<b>Growth significantly slower below 20°C, and greater above 25°C, compared with eukaryotic phytoplankton taxa</b>	Requires temperatures above 20°C for growth rates to be competitive with eukaryotic phytoplankton taxa, and above 25°C for growth rates to be competitive with diatoms	Not observed at temperatures <19°C; temperatures above 25°C rarely occur.
<b>Cyanobacteria have greater cellular N:P ratios than diatoms due to two light harvesting systems and peptide toxin production</b>	At non-limiting nutrient concentrations, changes in ratios of nitrogen substrates or N:P does not affect competition among species or taxa	Nutrient concentrations, nitrogen speciation, and dissolved N:P ratios have not changed in the Delta over the last 25 years
<b>Production of bioactive peptide compounds (toxic and non-toxic) results in high N demand of cells</b>	Toxin production per cell is greatest at maximal growth rates; linked with external N concentrations and decrease at N limiting conditions; the affect of toxin production on other taxa is not known but cyanoHABs do not secrete toxin	Inorganic N and P concentrations are at non-limiting concentrations for growth and toxin production; Variation in toxin produced per cell or in number of toxigenic vs non-toxigenic strains in a population is not related to any specific environmental condition
<b>Inefficient photosynthesis, low alpha; efficient at dissipating excess light energy via high concentration of carotenoid pigments in photosystems (<i>Microcystis</i>, <i>Anabaena</i> and <i>Aphanizomenon</i>)</b>	CyanoHABs ( <i>Microcystis</i> , <i>Anabaena</i> and <i>Aphanizomenon</i> ) require high irradiance to grow; growth slowed significantly when irradiance decreases; diatoms able to keep near-maximal growth rates at irradiances limiting to cyanoHABs (e.g. 50 $\mu\text{mol phot m}^{-2} \text{s}^{-1}$ )	High rate of water flow and mixing results in relatively high turbidity most of the growing season, restricting blooms to low-flow periods (July-August), when turbidity is < 50 NTU, flow is <30 $\text{m}^3 \text{s}^{-1}$ and irradiance > 50 $\mu\text{mol phot m}^{-2} \text{s}^{-1}$
<b>Growth optimal at salinities &lt;10 ppt for most cyanoHAB species</b>	CyanoHABs generally restricted to freshwater habitats and estuaries with salinities <10 ppt (Baltic Sea, San Francisco Delta, North Carolina)	Does not proliferate outside the Delta in the Sacramento River (freshwater) or Suisun Bay (mesohaline) suggesting that the primary agent restricting its spread is not salinity

## 6.0 Recommendations

The goal of this review is to synthesize available information to provide insight into cyanobacterial bloom occurrence in the Delta. The review has three major objectives:

1. Provide a basic review of biological and ecological factors that influence the prevalence of cyanobacteria and the production of cyanotoxins;
2. Summarize observations of cyanobacterial blooms and associated toxins in the Delta;
3. Synthesize literature to provide an understanding of what ecological factors, including nutrients, may be at play in promoting cyanobacterial blooms in the Delta.

This review found that the lack of a routine monitoring of cyanoHAB occurrence in the Delta greatly hindered our ability to summarize, with confidence, the status and trends of cyanoHABs in the Delta (Objective 2), and to what extent nutrients versus other factors were controlling their occurrence (Objective 3). Given this finding, our recommendations are focused on two principal actions:

1. Strengthening routine monitoring; and
2. Development and use of an ecosystem model, coupled with routine monitoring and special studies, to understand controls on primary productivity and phytoplankton assemblage in the Delta.

### R1: Implement Routine Monitoring of CyanoHABs

DWR is currently conducting a monitoring program already exists which routinely samples many of the variables of interest known to influence cyanoHABs. Comprehensive cyanoHAB monitoring should be added as a component to this program.

To begin, a work plan should be developed which specifically scopes the needed changes in the program to comprehensively monitor cyanoHABs. Monitoring should include enumeration of the cell counts of major cyanobacteria species (e.g. *Microcystis*, *Aphanizomenon* and *Anabaena*). Sampling of toxins should include water column particulates as well as tissue concentrations in mussels or other important taxa that represent potential for bioaccumulation in the food web. Analyses of toxin concentrations should be expanded to include the six major cyanotoxins of concern identified in the OEHHA guidance. The workplan should also consider monitoring needed to develop and calibrate an ecosystem model to further investigate controls on primary productivity and phytoplankton assemblage (see R2 below).

After an initial period of 3-5 years, the monitoring data should be used to comprehensively report on the status and trends of cyanoHABs and the factors that favor bloom occurrence in the Delta.

### R2: Develop an Ecosystem Model of Phytoplankton Primary Productivity and HAB Occurrences to further Inform Future Risk and Hypotheses on Factors Controlling CyanoHABs

The Delta is at an advantage with respect to management of cyanoHABs in that naturally occurring high rates of flow and turbulence act to keep cyanobacteria in check. Despite this, future increases in temperature and residence time associated with climate change, increasing the degree and duration of

stratification events, may substantially degrade the effectiveness of the Delta's breaking mechanism and increase the risk of cyanoHAB occurrences. Because nutrients are not currently limiting cyanobacterial blooms, it is critical that an improved understanding is gained of the factors that are controlling phytoplankton primary productivity in the Delta, since higher chlorophyll a could lead to increased risk of cyanoHAB blooms.

To inform management action moving into the future, an ecosystem model of phytoplankton primary productivity and HAB occurrences should be developed. This model should have the capability to provide information on primary productivity and biomass as well as planktonic food quality and transfer of carbon to higher trophic levels. To step into model development, three steps should be taken: 1) examine existing models already available to determine suitability for this task, 2) utilize existing data to explore, to the extent possible, the relationships between chlorophyll a, phytoplankton composition, climate variables and other factors. This analyses should inform hypotheses that can be tested through model development as well as potential future scenarios, and 3) a work plan should be developed that lays out the modeling strategy, model data requirements, and implementation strategy.

DRAFT

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