Spatial and Temporal Dynamics of Cyanobacteria and Microcystins in Freshwater Systems: Implications for the Management of Water Resources

Som Cit Si Nang

B.Sc, M.Eng, Universiti Teknologi Malaysia

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Abstract

Excessive growth of cyanobacteria, commonly known as cyanobacterial blooms, appear to be increasing in magnitude and frequency worldwide, thus posing a serious threat to the safety and security of water resources. With the increasing global water stress, there is a need for effective management of cyanobacterial blooms in water bodies. So far, it has been a great challenge to mitigate and assess public health risks associated with cyanobacterial blooms due to difficulty in predicting the level of cyanobacterial biomass and microcystin concentrations in water bodies. Moreover, the effectiveness of the current bloom prevention and risk assessment strategies depend upon the understanding of the dynamics of cyanobacteria and microcystin under natural conditions. Thus, this research aims to: i) assess the variability of the relationship between cyanobacterial biomass and microcystin concentration, which is currently used to assess the risk to human and ecosystems health; ii) determine the environmental drivers of the dynamics of cyanobacterial dominance and microcystin concentration and assess site specificity of the environmental drivers; and iii) investigate how changes in the structure of phytoplankton community and cyanobacterial composition in response to nutrient concentration affect the dynamics of microcystin concentration.

The results contained in this thesis revealed that the biomass-toxin relationship is a function of spatiotemporal patterns that affect cyanobacterial and microcystin dynamics. The correlation between the biomass and toxin is weak and site-specific, and large changes in total microcystin concentrations occur even at stable cyanobacterial biomass concentrations. This could pose a significant threat to the risk assessment associated with microcystin contamination in water bodies.

In relation to the environmental drivers of the dynamics of cyanobacterial dominance and microcystin concentration, the results revealed the significant role of phosphorus and iron concentrations in the water column. Low phosphorus and iron concentrations in the water column trigger the dominance of cyanobacterial biomass in the phytoplankton community. Specifically with regard to bloom toxicity, high phosphorus and iron concentrations in the water column trigger high microcystin concentration. Furthermore, different concentrations of phosphorus, nitrogen and iron species explained the succession of different cyanobacterial genera at the cyanobacterial community level. Nevertheless, the correlations between the dynamics of cyanobacterial dominance and microcystin concentration and environmental factors are site-specific. This might potentially be related to the effect of spatial heterogeneity of the local nutrient concentrations and cyanobacterial community present in the systems.

In addition to nutrients, changes in the structure of phytoplankton community are also significantly correlated to the dynamics of microcystin concentration. Under high nutrient concentrations, other phytoplankton groups are capable of growing faster and gain dominance over cyanobacteria. However, this study shows that under this scenario, cyanobacteria produce more toxins. This supports the hypothesis of allelopathic interaction in cyanobacteria.

The results presented in this thesis will improve the fundamental understanding of the dynamics of toxic cyanobacterial blooms. Additionally, these results can be used to supplement the existing strategies for the prevention of cyanobacterial blooms and assessment of risk associated with cyanobacterial toxins to humans and the environment.

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List of Abbreviations

°C	: Degree celsius
AHD	: Australian Height Datum (*Geodetic datum for altitude measurement in Australia, where the mean sea level for 1966-1968 was assigned the value of zero on the Australian Height Datum at thirty tide gauges around the coast of the Australian continent)*
AN	: *Anabaena* spp.
ANCOVA	: Analysis of covariance
ANP	: *Anabaenopsis* spp.
ANOVA	: Analysis of variance
CB	: Cyanobacteria
CBf	: Cyanobacterial fraction
Chl-*a*	: Chlorophyll-*a*
Chloro	: Chlorophytes
cMC	: Cellular microcystin concentration
Crypto	: Cryptophytes
Cyano	: Cyanobacteria
DO	: Dissolved oxygen
eMCf	: Extracellular microcystin fraction
ha	: Hectare
HPLC	: High-performance liquid chromatography
HPLC-PDA	: High-performance liquid chromatography-photodiode array
LC-MS	: Liquid chromatography-mass spectrometry
LSD	: Least significant difference
MC	: Microcystin
MI	: *Microcystis* spp.
N	: Number of samples
NH_4	: Ammonium
P	: Probability
PL	: *Planktothrix* spp.
R	: Correlation coefficient
R^2	: Coefficient of determination
RDA	: Redundancy analysis
rpm	: Round per minute
SPE	: Solid phase extraction
spp.	: Species
TDFe	: Total dissolved iron
TDN	: Total dissolved nitrogen
TDP	: Total dissolved phosphorus
TFA	: Trifluoroacetic acid
TFe	: Total iron
TN	: Total nitrogen
tMC	: Total microcystin

TN:TP : Ratio of total nitrogen to total phosphorus concentration
TP :Total phosphorus
v/v : Volume/volume

Potential publication arising from this thesis

Sinang, S.C., Reichwaldt, E.S., Ghadouani, A. Spatial and temporal variability in the relationship between cyanobacterial biomass and the occurrence of microcystin. Water Research, submitted December 2011 (**Chapter 3**)

Sinang, S.C., Reichwaldt, E.S., Ghadouani, A. Site-specific roles of environmental factors on cyanobacterial and microcystin dynamics: The influence of the local cyanobacterial community and nutrient concentration gradients. Applied and Environmental Microbiology, submitted June 2011 (**Chapter 4**)

Sinang, S.C., Reichwaldt, E.S., Ghadouani, A. Structure of phytoplankton community in response to changes in nutrient gradient implies the level of microcystin concentrations. Freshwater Biology, submitted June 2011 (**Chapter 5**)

Presentation arising from this thesis

Microcystin occurrences and sources of variability in water bodies- The 8th International Conference on Toxic Cyanobacteria (ICTC8), Istanbul, Turkey, 2010.

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Last but not the least, to the one above all of us, the omnipresent God, for answering my prayers and giving me the strength to continue until the end of this PhD journey, thank you so much.

Statement of candidate contribution

I hereby declare that all material presented in this thesis is original, except where due acknowledgement is given, and has not been accepted for the award of any other degree or diploma. The content of this thesis is the author's own work under the supervision of Associate Professor Anas Ghadouani and Dr Elke Reichwaldt. My personal contributions are outlined as the following:

Method development

I was responsible for the establishment of protocol for microcystin extraction and quantification in the Environment Research Laboratory, UWA together with postgraduate colleagues and supervisors. Many experiments and laboratory trials were carried out during the establishment of the protocol, as this research was among the first in my research group to deal with microcystin quantification. The works include the optimization of solid phase extraction of microcystin from cyanobacterial cells, and development of the analytical procedure for microcystin quantification in HPLC. These tasks were carried out and accomplished during my first year of candidature in 2008.

Samples collection and laboratory analyses

I performed all the sampling and laboratory procedures described in this thesis, except for the measurement of all nitrogen forms which was conducted by a private laboratory in Albany, Western Australia.

Statistical analyses

I performed all statistical analyses using the statistical package SPSS version 17 and R-language based on the knowledge I gained from statistical courses and assistance from the UWA Postgraduate Statistic Clinic, supervisors and Professor Pierre Legendre.

Publication

The main components of this thesis, chapters 3 to 5, consist of three journal papers which have been prepared for publication. Therefore, some repetition of literature review and methodology is necessary. The papers from chapter 3 to 5 have been submitted to Water Research, Applied and Environmental Microbiology, and Freshwater Biology, respectively. I was fully responsible for the preparation of the whole thesis and publication drafts, under the supervision of my supervisors, which are also the co-authors for all possible publications.

Student signature

Som Cit Si Nang

Coordinating supervisor signature

Associate Professor Anas Ghadouani

Co-supervisor signature

Dr. Elke S. Reichwaldt

Chapter 1

Introduction

1.1 Background

The world population had increased significantly in the last five decades from 3 billion in 1960 to 6.7 billion in 2009 (World Bank 2012). Based on the current population growth rate, the world's population is projected to increase to 7.8 billion by 2025 (Cai and Rosegrant 2002). The increasing world population will cause agricultural and non-agricultural water uses to grow rapidly (Cai and Rosegrant 2002). As shown in Table 1.1, total water withdrawal and the ratio of total water withdrawal to total renewal water resources are estimated to increase significantly at a global, regional and national level (Rosegrant and Cai 2002). At a global level, the total water withdrawals are projected to increase from 3,906 km^3 in 1995 to 4,794 km^3 in 2025. The total global water withdrawals in 2025 will represent 10% of the total renewable water resources. In addition, the total water withdrawals in South Asia, China, India, Western Asia and Northern Africa in 2025 will represent between 29 to 87% of the total renewal water resources. High ratios of the total water withdrawals to total renewal water resources in these countries and regions indicate a significant water stress in the future. Therefore, there is an urgent need to sustain the availability and quality of water resources to ensure an adequate water supply in the future.

Table 1.1: Total water withdrawal and ratio of total water withdrawal to total renewable water resources (%), estimated 1995 and projected to 2025 for selected countries and regions (Rosegrant and Cai 2002).

Countries/Regions	Total water withdrawal (km^3)		Ratio of total water withdrawal to total renewal water (%)	
	1995	2025	1995	2025
World	3906	4797	8	10
China	679	858	26	33
India	674	813	30	36
USA	497	533	24	26
South Asia	1027	1235	24	29
Southeast Asia	203	289	4	5
Western Asia and Northern Africa	236	297	69	87

Water availability and its quality will be one of the major challenges for the environment and society due to increased effects from anthropogenic disturbances and climate change. Changes in the hydrological cycle affect the function and operation of existing water infrastructures, thus leading to variability and risk in water supply availability (IPCC 2008, Gossling et al. 2012). Rising global temperatures (Elliott 2012, Zhang et al. 2012) and changes in the hydrological cycle, along with increase in human activities (Paerl et al. 2011a), has increased nutrient loading into water bodies, thus stimulating further eutrophication and leading to deterioration in the quality of the available water resources (IPCC 2008, El-Shehawy et al. 2011, Newcombe et al. 2011, Paerl et al. 2011a, Paerl and Paul 2011, Reichwaldt and Ghadouani 2011, Carey et al. 2012).

Eutrophication increases primary productivity in water bodies and results in an increased incidence of excessive proliferation of algal biomass, commonly known as bloom (Chorus and Bartram 1999). Excessive algae growth in eutrophic freshwater is often associated with the dominance of cyanobacterial biomass (Codd 2000). Cyanobacteria are photosynthetic prokaryotic organisms that are also known as blue-green algae (Chorus and Bartram 1999). An example of excessive cyanobacterial growth in eutrophic freshwater lake is shown in Fig. 1.1. High cyanobacterial biomass in freshwater systems is likely to affect the size structure of zooplankton community and change the stability of aquatic ecosystems (Rohrlack et al. 1999, Rohrlack et al. 2001, Ghadouani and Pinel-Alloul 2002, Ghadouani et al. 2004, Ghadouani et al. 2006). In addition, the issue of high cyanobacterial biomass is also affecting the treatment efficacy in wastewater treatment plant due to biomass accumulation in pipe and pumping delivery infrastructure (Barrington and Ghadouani 2008, Barrington et al. 2011, Martins et al. 2011). Moreover, the excessive growth of cyanobacterial biomass is

of additional concern due to toxin contamination, which can seriously deteriorate the quality of the water resources and present health hazards to human and animal (Codd 2000, Rohrlack et al. 2003, Rohrlack et al. 2005, Havens 2007, Djediat et al. 2011, Zamyadi et al. 2011, Romo et al. 2012). The issue of cyanobacterial blooms appears to be increasing worldwide, thus posing an additional threat to the global availability and quality of usable water resources. Shortage of portable water resources, in addition to increasing harm of toxic cyanobacterial blooms, will greatly impact human population sustainability, especially in developing and water scarce countries.

Fig.1.1: Satellite image (A) and photograph (B) of the excessive cyanobacterial growth in Yangebup Lake, South West Australia *(source of satellite image: www.nearmap.com; source of photograph: Nang, S.C.S., 2011).*

Against this background, it is important to protect available water resources from the increasing harm of cyanobacterial blooms. Therefore, this research aims to enhance our current understanding of the occurrence and the dynamics of cyanobacterial blooms and its associated toxin. This research seeks to identify the environmental drivers of the dynamics of cyanobacteria and microcystin. As microcystin production can be highly

dynamic on both spatial and temporal scales, this research also aims to assess how this affects the existing microcystin risk assessment strategy.

1.2 Historical descriptions of cyanobacterial blooms

The recognition and anecdotal evidence of massive cyanobacterial development were reported in the literature as early as in AD 77 (Codd et al. 1994). One of the earliest reports dates from the Han dynasty in China, where military troops were poisoned after crossing a "green coloured river" (Chorus and Bartram 1999). Later in the 12th century, an awareness of the poisonous nature of water bodies coloured with green scum, possibly cyanobacterial blooms, existed at the former Monasterium Virdis Stagni in South west Scotland (Codd 1996). In 1850, local Aboriginal hunting peoples at Milang, Lake Alexandrina, South Australia were also reported to have been aware of animal deaths associated with the formation of cyanobacterial scum (Anon 1878, as cited in Codd et al. 1994).

1.3 Scientific reports of toxic cyanobacterial blooms

The world's first scientific report of the toxicity of cyanobacterial blooms was published by George Francis in 1878. Francis described the rapid death of stock animals at Lake Alexandrina, a freshwater lake at the mouth of Murray River in South Australia (Francis 1878). Subsequent to the work of Francis, cyanobacterial blooms were reported at various latitudes and climatic zones (Chorus and Bartram 1999). As shown in Fig.1.2, field surveys in Europe, North America, South America, Australia, Asia and Africa, have shown that cyanobacterial blooms can occur under a range of environmental conditions and nearly in all parts of the world (Svrcek and Smith 2004, Zurawell et al. 2005, Haande et al. 2007).

Fig.1.2: Global occurrence of cyanobacterial blooms (modified after Zurawell et al. 2005)

The presence of high cyanobacterial biomass in water bodies, either used for drinking or recreational purposes, may pose serious health risks for human and animal populations. This is due to the fact that various cyanobacterial genera including *Microcystis*, *Anabaena*, *Anabaenopsis*, and *Planktothrix* are capable of producing a range of toxins, which can have lethal and sub-lethal effects in both humans and animals (Codd et al. 2005, Falconer 2005, Damkova et al. 2011, Froscio et al. 2011, Lance 2011, Nonga et al. 2011, Wood et al. 2012). Cyanobacterial toxins are categorised into two main types, namely cyclic peptides and alkaloids. Cyclic peptides are consist of microcystin and nodularin, while alkaloids include neurotoxins and cylindrospermopsin (Chorus and Bartram 1999, NHMRC 2011). Of all cyanobacterial toxins, microcystin is the most commonly encountered cyanobacterial toxin that is likely to pose a risk to the consumers of drinking and recreational water (Falconer and Humpage 2005). Furthermore, microcystin Microcystin is potent and previously described as "fast-death factor" (Bishop et al. 1959).

Humans may be exposed to cyanobacterial toxins through direct contact and ingestion of toxic cyanobacteria from drinking or recreational water (Chorus et al. 2000). Humans may also be exposed to cyanobacterial toxins through the consumption of contaminated plants (Chen et al. 2012), shellfish (Chorus et al. 2000) and intravenous exposures related to medical procedures (Azevedo et al. 2002). In the past, scientific studies on human illness due to cyanobacterial toxins were reported in the USA, Australia, Brazil, China, and England as early as 1931 (Chorus and Bartram 1999) (Table 1.2).

Table 1.2: Examples of early reports of human illness associated with the exposures to cyanobacterial blooms.

Year	Location	Routes of exposure	Signs and symptoms	Number affected	References
1931	Ohio and Potomac rivers, USA	Drinking water	Gastrointestinal illness	5000-8000	Tisdale 1931, as cited in Chorus et al. 2000
1979	Palm Island, Australia	Drinking water	Gastrointestinal illness	>100	Griffiths and Saker 2003
1989	England	Recreational water	Pneumonia	10	Van Apeldoorn et al. 2007
1993	China	Drinking water	Liver cancer	202	Yu 1995)
1996	Caruaru, Brazil	Intravenous exposure (dialysis)	Acute liver failure	100 (52 deaths)	Jochimsen 1998, Azevedo et al. 2002

In the USA, a massive *Microcystis* spp. bloom in the Ohio and Potomac Rivers caused gastrointestinal illness to between 5000 and 8000 people whose drinking water was sourced from these affected rivers (Tisdale 1931, as cited in Chorus et al. 2000). In 1979, a bloom of *Cylindrospermopsis raciborskii*, contaminated a drinking water reservoir in Palm Island, Australia, resulted in the serious illness of over 100 people from an Aboriginal community (Griffiths and Saker 2003). In 1989, cases of severe pneumonia were reported in England, when army recruits were exposed to *Microcystis*

aeruginosa blooms while swimming and canoeing (Van Apeldoorn et al. 2007). In 1993, incidences of liver cancer in China were correlated to people drinking from cyanobacterial infested surface water (Yu 1995). In 1996, the worst recorded incidence of human fatalities from cyanobacterial toxins was reported in Caruaru, Brazil, when 100 patients developed acute liver failure, which resulted in 52 deaths after being exposed to cyanobacterial toxins during renal dialysis (Jochimsen 1998, Azevedo et al. 2002). These human exposures to cyanobacterial blooms and toxins with associated health outcomes were reported to be related to microcystin poisoning (Codd et al. 2005, Huisman et al. 2005). Therefore, this research will specifically focus on microcystin, due to its potential harm if present in drinking and recreational water. With regards to the serious harm of toxic cyanobacterial blooms to the water safety, a better understanding of the ecological conditions leading to the intensification of cyanobacterial blooms and their toxicity in water bodies is needed.

Based on existing knowledge, occurrences of cyanobacterial blooms are not necessarily associated with the presence of toxins in the water column. For instance, Carrasco et al. (2006) has reported that only 45 to 70% of material in potentially toxic cyanobacterial blooms was found to contain toxins. Early studies have proposed that blooms toxicity is dependent on the presence of toxic cyanobacterial strains and the level of microcystin production. The proportion of cyanobacterial strains capable of producing microcystin can vary significantly even within a single population, and may range between 12% (Xu et al. 2010) to 60% (Kurmayer et al. 2011) and 80% (Briand et al. 2008b). Additionally, total microcystin content per cell within the same cyanobacterial population also vary on a spatial and temporal basis (Carrasco et al. 2006, Vasconcelos et al. 2011). Further, the formation of cyanobacterial blooms and microcystin production might be regulated by different ecological conditions. Accordingly, the ecology of cyanobacterial blooms

and microcystin production are discussed separately in the following sections. A conceptual model illustrating the ecology of cyanobacterial bloom and microcystin production is shown in Fig.1.3.

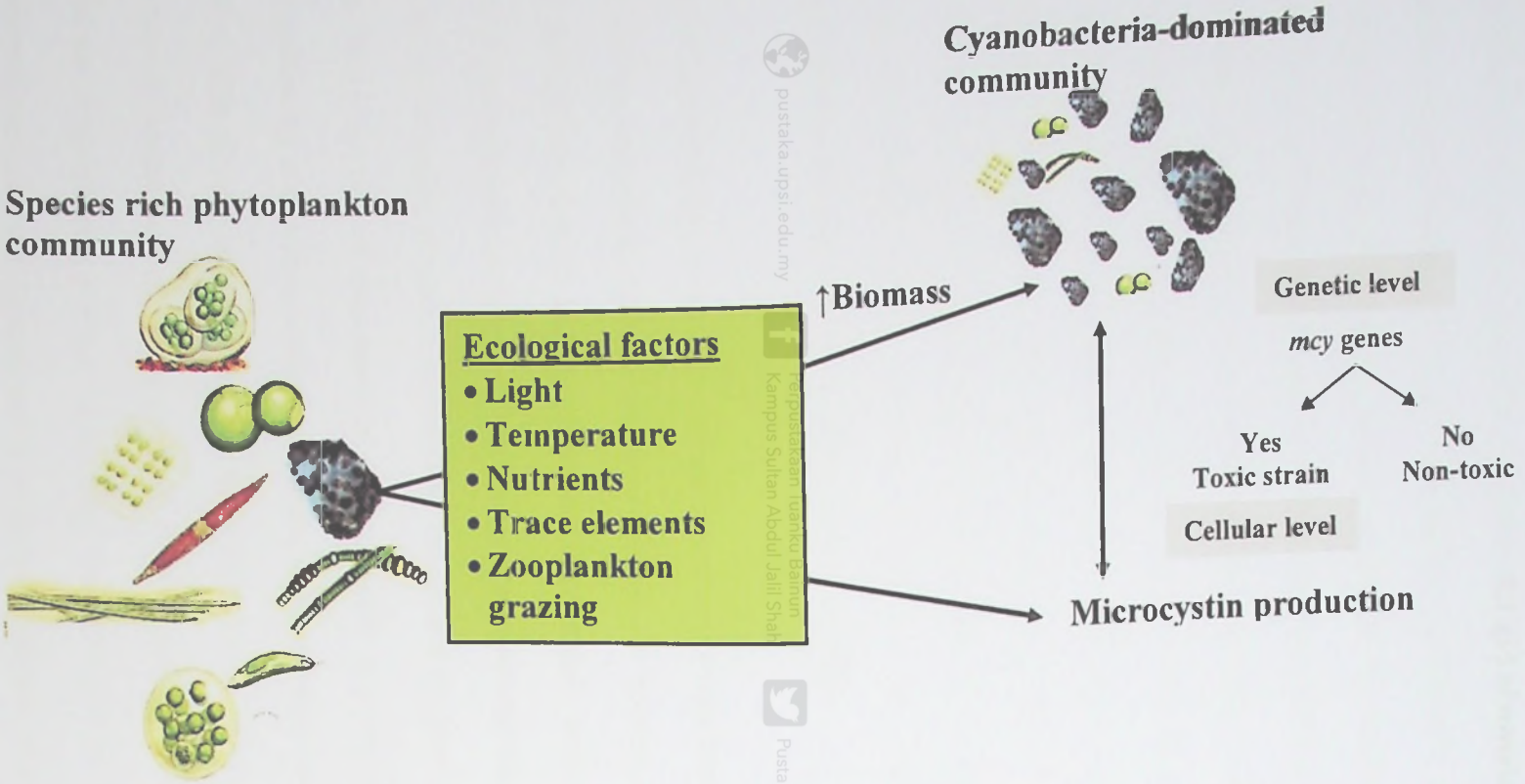

Fig.1.3. Conceptual model of the ecology of cyanobacterial bloom and microcystin production

1.4 Environmental factors affecting blooms

Current studies have proposed a range of physicochemical factors which trigger the occurrence of cyanobacterial blooms in freshwater ecosystems. Among other factors, excessive nutrient loading and increasing water temperatures are known as the basis for the presence of massive cyanobacterial biomass (Paerl and Huisman 2008). As massive cyanobacterial blooms usually occur in eutrophic water bodies, high phosphorus and nitrogen concentrations are assumed to be the primary triggers for their growth (Chorus and Bartram 1999, Paerl et al. 2011b). Additionally, rising water temperatures favour the formation of high cyanobacterial biomass through the migration of biomass from sediment into the water column (Schöne et al. 2010), increasing stratification and reducing vertical mixing (Paerl and Huisman 2008), and enhanced hypolimnetic phosphorus accumulation from sediment (Sondergaard et al. 2003).

1.4.1 Cyanobacterial ecostrategies

Cyanobacterial ecostrategies refer to specific behaviour and reactions to changes in environmental conditions (Chorus and Bartram 1999). Cyanobacterial ecostrategies determine the succession of cyanobacteria in dominating the phytoplankton community (Chorus and Bartram 1999, Dokulil and Teubner 2000). Cyanobacteria have a competitive advantage over other phytoplankton in environments that are nutrient limited, high water temperatures and low light availability.

Cyanobacteria are known to have a higher phosphorus uptake rate and lower half saturation constant than other phytoplankton (Amano et al. 2010). This means that cyanobacteria can out-compete other phytoplanktons under phosphorus limited conditions. Additionally, cyanobacteria have a higher internal phosphorus storage and can deplete phosphorus to much lower levels than other phytoplankton groups,

especially chlorophytes (Lang and Brown 1981). Therefore, it is possible that low phosphorus concentrations (< 0.1 mg L^{-1}) are sufficient to induce excessive cyanobacterial growth (Amano et al. 2010), and enable cyanobacteria to bloom in oligotrophic waters (Chorus and Bartram 1999, de Figueiredo et al. 2004).

Nitrogen fixation is another feature which allows certain cyanobacterial species from genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia* and *Nostoc* to gain a competitive advantage under nitrogen deficient conditions (Chorus and Bartram 1999). Although nitrogen fixation is costly from a physiological perspective, nitrogen fixation is advantageous when the concentrations of dissolved nitrate and ammonium are low (Huisman and Hulot 2005).

Cyanobacterial competitive advantages under nitrogen and phosphorus limitation suggest that low N:P ratio may infer cyanobacterial dominance, particularly of nitrogen-fixing species, over the other phytoplankton communities (Smith 1983, Bouvy 1999). Smith (1983) has shown that cyanobacterial blooms are likely to occur when the N:P ratio is below 29. However, low N:P ratios might not always determine cyanobacterial dominance as phytoplankton cells have the ability to store a certain amount of phosphorus (Davies et al. 2010).

Iron is an element with low biological availability and may become a limiting resource for the growth of phytoplankton (Boyer et al. 1987) including cyanobacteria (Molot et al. 2010). Cyanobacteria have a competitive advantage to dominate under low iron availability due to their ability to alter their cellular iron requirements and increase their ability to utilize iron at low concentration through the presence of siderophores (Boyer et al. 1987, Wilhelm 1995, Lee et al. 2011). For example, many cyanobacterial genera

including *Anabaena*, *Microcystis* and *Planktothrix* can produce siderophores to facilitate the uptake of ferric ions into cells under iron limited conditions (Wilhelm 1995, Nagai et al. 2007).

Temperatures exceeding 25°C are likely to provide optimum conditions for cyanobacteria to grow rapidly and dominate in water bodies, through regulation of cyanobacterial photosynthetic capacity, respiration and growth rate (Robarts and Zohary 1987). These optimum temperature values are higher than those for chlorophytes and diatoms (Chorus and Bartram 1999), indicating that global warming will favour cyanobacterial dominance in the phytoplankton community. However, there is also evidence that cyanobacteria can form blooms under ice (Vasas et al. 2010) and have active photosynthetic activity at low temperatures (Konopka and Brock 1978). Therefore, the effects of temperature on cyanobacterial dominance may be direct or indirect (Robarts and Zohary 1987, Hyenstrand et al. 1998). Temperature, for instance, might indirectly contribute to the dominance of cyanobacteria through its effect on water mixing and nutrient transport from sediment (Sondergaard et al. 2003, Paerl and Huisman 2008). On the other hand, direct effects of temperature are associated with its combined effects with other environmental factors to affect cyanobacterial dominance (Tilman et al. 1986, as cited in Robarts and Zohary 1987).

Cyanobacteria are able to absorb light efficiently due to the presence of two reaction centers, PS I and PS II, in their photosynthetic apparatus. This feature allows cyanobacteria to use effectively the light spectrum between the absorption peaks of chlorophyll-*a* and carotenoids (Ormerod 1992 , Chorus and Bartram 1999). Additionally, cyanobacteria are also known to have a lower specific maintenance rate than chlorophytes. Hence, cyanobacteria may require smaller amounts of energy to

sustain their cellular function and growth (Van Liere and Mur 1979, Fogg and Thake 1987). As a result, cyanobacteria are able to maintain a higher growth rate than other phytoplankton under low light intensity. Having a competitive advantage in relation to other phytoplankton under low light energy supply allows cyanobacteria to become dominant in turbid water (Hyenstrand et al. 1998, Chorus and Bartram 1999).

Many cyanobacteria including *Microcystis* spp. and *Aphanizomenon* spp. contain gas vacuoles, which give their cells a lower density than water (Chorus and Bartram 1999). With the presence of gas vacuoles, cyanobacterial cells are able to control their buoyancy and can migrate vertically along physical, chemical and light gradients to achieve optimum growth conditions (Chorus et al. 2000, Xiao et al. 2012).

1.4.2 The relationship between cyanobacterial biomass and environmental factors

In spite of the cyanobacterial ecostrategies described in the previous section, the environmental factors causing cyanobacterial blooms in water bodies remain the subject of a long-standing debate. This is due to the fact that eco-physiological differences between cyanobacterial groups in mixed blooms have led to non-homogenous behaviour and responses to the natural environment (Chorus and Bartram 1999). In the literature, the reported relationships between environmental factors and cyanobacterial biomass are contradictory. In terms of phosphorus, Izydorczyk et al. (2008) has suggested that high phosphorus concentrations promote high cyanobacterial biomass. On the other hand, de Figueiredo et al. (2006) has reported that low phosphorus concentrations favour high cyanobacterial biomass. Similarly, nitrogen was also reported to have either positive (Wilhelm et al. 2011, Srivastava et al. 2012) or no correlation (Babanazarova and Lyashenko 2007) with cyanobacterial biomass. The relationship between the ratio of total nitrogen to total phosphorus (TN:TP ratio) and

cyanobacteria also differs between studies. The hypothesis of low TN:TP ratio favouring the dominance of cyanobacteria (Smith 1983) has been challenged by studies which reported contrasting findings (Downing et al. 2001, Barros et al. 2010). The potential correlations between iron concentration in the water column and cyanobacterial biomass have been reported to be either positive (Jiang et al. 2008, Molot et al. 2010), negative (Sharma et al. 2009) 2009) or no clear correlation (Yan et al. 2004). Analogously, the correlations between temperature, light and cyanobacterial biomass are also contrasting (Hyenstrand et al. 1998, de Figueiredo et al. 2006, Wagner and Adrian 2009, Lehman 2011). The presence of high cyanobacterial biomass in water bodies is usually associated with warmer water temperature, as this positive relationship was previously observed in many studies (de Figueiredo et al. 2006, Izydorczyk et al. 2008, Wu et al. 2008, Markensten et al. 2010). However, there is also evidence that this is highly species specific and that the length of the warm period is more important that the absolute temperature (Galvao et al. 2008, Wagner and Adrian 2009). In terms of light, it has been suggested that low light availability in the water column is responsible to initiate the presence of high cyanobacterial biomass (Hyenstrand et al. 1998). In contrast, it has been found that low light conditions were not the trigger for the development of high cyanobacterial biomass, but rather caused by it (Presing et al. 1996).

The contrasting findings of the potential relationship between the environmental factors and cyanobacteria have limited our ability to understand the key environmental drivers of the occurrence of cyanobacterial blooms in water bodies. Hence, this research aims to identify the ecological factors which trigger the proliferation of massive cyanobacterial biomass and the dominance of these organisms in the water column.

1.5 Ecology of microcystin production

In addition to knowledge of the ecological conditions leading to the formation of cyanobacterial blooms in general, it is also crucial to take into account the regulatory factors of the blooms' toxicity. The ability of cyanobacteria to produce microcystin is firstly determined at the genetic level with the presence of microcystin synthesis genes known as *mcy* genes (Tillett et al. 2000, Tooming-Klunderud et al. 2007, Kehr et al. 2011). Microcystin is synthesized non-ribosomally by the thio-template functions of large multifunctional enzyme complexes. Gene clusters encoding the biosynthetic enzyme (*mcyS*) have been sequenced and characterized in *Microcystis* genera (Fig.1.4). In *Microcystis aeruginosa PCC7806*, the *mcyS* genomic locus spans 55kb and comprises 10 genes arranged in two divergently transcribed operons (*mcyA-C* and *mcyD-J*) (Tillett et al. 2000).

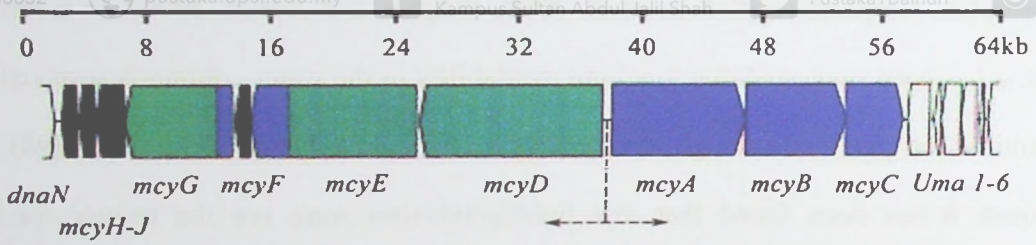

Fig.1.4: Microcystin biosynthesis gene cluster in *Microcystis aeruginosa*. The direction of transcription is shown by arrow (Tillett et al. 2000).

In nature, cyanobacterial populations may consist of single or mixed species, and a single species may be a mixture of toxic and non-toxic strains (Chorus and Bartram 1999, Lopes et al. 2012, Wood et al. 2012). Strains are specific genetic subspecies with slightly different traits (Chorus and Bartram 1999). As an example, 11 strains of *Microcystis aeruginosa* were isolated from a single cyanobacterial population (Rico et al. 2006). The proportions of cyanobacterial strains capable of producing microcystin

within a single population are highly variable, ranging from 12% (Xu et al. 2010, Srivastava et al. 2012) to 80% (Briand et al. 2008b). Therefore, the concentration of microcystin produced during a bloom will, to some extent, depend on variations in the proportion of strains containing *mcy* genes (Srivastava et al. 2012).

On the other hand, microcystin content in the individual cell can vary considerably in any given strain due to different levels of expression of genes which are involved in microcystin biosynthesis (Briand et al. 2008a). A range of environmental factors including temperature (Davis et al. 2009), light intensities (Kaebernick et al. 2000, Phelan and Downing 2011, Renaud et al. 2011), phosphorus (Rinta-Kanto et al. 2009), nitrogen (Long et al. 2001), TN:TP ratio (Vezie et al. 2002), iron (Sevilla et al. 2010) and the presence of other competing phytoplankton (Engström-Öst et al. 2011) have been suggested to correlate with the level of gene expression involved in microcystin biosynthesis. However, the influences of these environmental factors on microcystin production remain largely unclear and inconsistent between studies, as discussed in the following sections.

1.5.1 Physical and chemical factors

Temperature gradient has been reported to cause up to a three-fold difference in cellular microcystin content (Chorus and Bartram 1999). For example, microcystin production increased from 300 to 900 μg g^{-1} phytoplankton dry mass as water temperature increased from 25 to 29°C (Ame et al. 2003). On the other hand, there are also reports suggesting that microcystin production was reduced from 2 to 1 mg g^{-1} dry mass when the water temperatures increased from 25 to 30°C (Rapala et al. 1997).

High concentrations of nitrogen and phosphorus in the water column have also been suggested to have a positive correlation with microcystin production (Oh et al. 2000, Takahashi et al. 2007). This may be due to extra energy required for toxin biosynthesis in toxic cyanobacteria (Vezie et al. 2002). For example, higher nitrogen availability was found to be associated with higher microcystin concentrations in non-nitrogen fixing cyanobacteria such as *Microcystis aeruginosa* (Li et al. 2007). Nevertheless, there are also studies which reported that microcystin production in *Microcystis aeruginosa* is independent of nitrogen availability (Sevilla et al. 2010, Wilhelm et al. 2011). Similar to the role of nitrogen, the effect of phosphorus on microcystin production appears to differ between studies. Wang et al. (2002) and Rinta-Kanto et al. (2009) have shown that the cellular microcystin content in *Microcystis aeruginosa* increased with higher total phosphorus concentrations in the water column. In contrast, other studies suggests a negative correlation or no correlation between phosphorus and microcystin production (Wu et al. 2006, Srivastava et al. 2012). These conflicting findings are possibly due to non-linear effects of nutrients on microcystin production (Graham et al. 2004). Graham et al. (2004) has demonstrated that microcystin concentrations exceeding 2 $\mu g\ L^{-1}$ occurred when the total amount of nitrogen in the water column varied between 1 to 4 $mg\ L^{-1}$. In contrast, microcystin concentration decreased to below 1.5 $\mu g\ L^{-1}$ when total nitrogen exceeded 8 $mg\ L^{-1}$. Therefore, it is possible to speculate that nutrient regulation on microcystin production may depend on the range of nutrient present in the systems.

Iron availability has similarly been demonstrated to have a significant effect on microcystin production (Wilhelm 1995). *Microcystis aeruginosa* was found to produce 20-40% more microcystin when grown in media in the absence of, or at low iron concentrations ($<2.5\ \mu M \approx 0.14\ mg\ L^{-1}$) (Lukac and Aegerter 1993). It has been assumed that microcystin production occurs as a response to environmental stress

associated with iron deficient conditions (Alexova et al. 2011). On the other hand, contrasting findings relating to the role of iron in microcystin production argue that higher levels of iron (0.9 mg L^{-1}) will potentially enhance microcystin production (Jiang et al. 2007). In addition, results published in Yan et al. (2004) have shown that microcystin production was enhanced at higher iron concentrations, when cyanobacteria were grown at iron concentrations ranging between 0.5 to 10 mg L^{-1}. A possible explanation to support this result is related to the fact that iron is an essential element involved in photosynthesis and many other metabolic pathways.

The contrasting findings relating to the effects of physicochemical factors on microcystin production suggest there are gaps in knowledge in terms of understanding the environmental drivers of microcystin production during cyanobacterial blooms. A better understanding of the environmental conditions leading to high microcystin production is essential for the assessment of risk associated with cyanobacterial bloom in water bodies. Therefore, this research aims to identify the environmental factors which trigger microcystin production in natural blooms.

1.5.2 The structure of phytoplankton community

In addition to physical and chemical factors, microcystin production in cyanobacterial cells is potentially influenced by the interspecific interaction between cyanobacteria with other phytoplankton groups, or within the cyanobacterial community. It has been proposed, through the theory of allelopathic interaction, that cyanobacteria may increase microcystin production to outcompete their competitors in ecosystems (Huisman and Hulot 2005, Schatz et al. 2007, Engström-Öst et al. 2011). Under growth limiting conditions, the benefit of producing microcystin might outweigh the cost (Briand et al. 2008b). It was shown that the presence of microcystin in the water column can affect

the growth of co-existing organisms such as microalgae (Babica et al. 2006, Camacho 2008).

At this stage, there is insufficient evidence to support the theory that microcystin is produced by cyanobacteria in order to provide a competitive advantage in the phytoplankton community. This is due to the limited number of studies which have focused on the effects of the dynamics of the phytoplankton community and cyanobacterial composition on microcystin production. This issue has limited our ability to predict, especially under highly dynamic environmental conditions, the potential toxicity of cyanobacterial blooms from the structure of phytoplankton community and cyanobacterial composition. The ability to predict microcystin production from the dynamics of phytoplankton community structures in response to nutrient will improve our ability to assess the bloom toxicity in water bodies. It is beneficial if the level of microcystin production could be estimated from the commonly tested parameters included in water quality monitoring, such as the fraction of cyanobacterial biomass in the total phytoplankton community, fraction of the biomass of a certain cyanobacterial genera in cyanobacterial community and nutrient concentration. To address this knowledge gap, this research seeks to investigate how changes in the structure of phytoplankton community and cyanobacterial composition, in response to nutrient concentration, can affect the dynamics of microcystin production.

1.6 Variations in the correlations between the physicochemical factors and cyanobacterial and microcystin dynamics

As described in previous sections, both laboratory and field studies reveal inconsistent findings in the relationship between environmental factors and cyanobacterial and microcystin dynamics. Inconsistencies regarding the roles of physicochemical factors

on cyanobacterial biomass and microcystin in laboratory studies could be due to variations in experimental setup, including the use of different cyanobacterial strains and the range of the tested physicochemical factors. Inconsistent findings between laboratory and field studies could be explained by the fact that laboratory conditions can be controlled, and the effects of single parameters can be tested, while several physicochemical factors may vary simultaneously in field studies (Kardinaal and Visser 2005). Furthermore, in field studies, inconsistent reports on the relationship between environmental factors and cyanobacterial biomass or microcystin may be related to the roles and characteristics of the environmental factors that are subjected to spatial heterogeneity.

The questions on the extent to which the cyanobacterial biomass and microcystin are subjected to site-specific physicochemical factors remain unclear. As suggested by Sabart et al. (2010), the effect of local environmental conditions could add to the difficulty of managing toxic cyanobacterial blooms. For example, the remedial or prevention method, which has been proven to be a successful management strategy in certain water bodies, might not give a similar result when used in different locations. In addition, the site specificity of cyanobacterial blooms and toxin dynamics could add to the difficulty of managing and predicting the potential toxicity of cyanobacterial blooms.

This is a crucial issue which requires further research. Integrating the effect of site specificity to a list of significant environmental causation factors will provide a more accurate understanding of the potential reasons behind the inconsistent outcomes of adopted cyanobacterial bloom management strategies. As a step forward, this research will focus on the understanding of how the physicochemical environmental factors

correlate with cyanobacterial blooms and microcystin dynamics under local environmental conditions. To achieve this aim, it is essential to compare the physicochemical factors responsible for cyanobacterial blooms and microcystin dynamics on a spatial basis.

1.7 Microcystin dynamics

Microcystin concentrations in the natural environment are highly dynamic on both spatial and temporal scales. From the results published in the literature (Table 1.2), total microcystin concentrations in water may range over five orders of magnitude, from 5 μg L^{-1} to 37 mg L^{-1}. Similarly, when expressed per unit of algal dry mass, the reported microcystin concentrations also ranged within the same orders of magnitude, from 9 μg g^{-1} to 20 mg g^{-1}. Additionally, there are possibilities that the microcystin concentrations in water bodies could range in more than five orders of magnitude as not all extreme occurrences of microcystin in water bodies are reported in the literature. The variability of microcystin concentration reported in the literature, suggest a variation in the level of microcystin production per cyanobacterial cell (Vasconcelos et al. 2011). Hence, the highly dynamic microcystin production may result in unexpected microcystin concentrations with small notable changes in cyanobacterial biomass.

Table 1.3: A comparison of the highest total (cellular and extracellular) microcystin concentrations reported in studies conducted throughout the world. Concentrations are presented in μg L^{-1}, or else in μg g^{-1} dry mass as indicated.

Range (μg L^{-1} or μg g^{-1})	The highest total microcystin concentration detected (μg g^{-1} dry mass or μg L^{-1})	Country	References
<10	5	USA	Graham et al. 2004
	7	France	Briand et al. 2005
	9 μg g^{-1}	Spain	Aboal and Puig 2005
10 - 1000	14	Singapore	Te and Gin 2011
	37	Czech Republic	Blahova et al. 2007
	87	Portugal	Vasconcelos et al. 2011
	120	Spain	Romo et al. 2012
	415 μg g^{-1}	Africa	Wicks and Thiel 1990
	590 μg g^{-1}	China	Liu et al. 2011
>1000	1295 μg g^{-1}	Philippines	Baldia et al. 2003
	1385 μg g^{-1}	USA	Jean et al. 2000
	2400 μg g^{-1}	Argentina	Ame et al. 2003
	2500	Australia	White et al. 2003
	3284 μg g^{-1}	Japan	Ozawa et al. 2005
	6171 μg g^{-1}	Czech Republic	Blaha et al. 2010
	19800 μg g^{-1}	Kenya	Ballot et al. 2004
	29163	Algeria	Nasri et al. 2004
	36500	New Zealand	Wood 2006

1.8 Microcystin risk assessment methods

In relation to public health protection, a provisional guideline value of 1 and 20 μg L^{-1} microcystin are set for drinking and recreational water, respectively (WHO 1998,2003). The guideline values are based upon Tolerable Daily intake (TDI) derived from animal study (No Observed Adverse Effects Level, NOAEL), with the application of appropriate uncertainty factors (NHMRC 2011). However, in many countries including Australia, the current alert level framework for potentially toxic cyanobacterial blooms in public water is based on cyanobacterial biomass (ANZECC 2000, NHMRC 2011), which are used as an indicator to indirectly estimate the risk of microcystin contamination. Certain levels are set as thresholds which trigger action by water authorities during water quality monitoring (Watzin et al. 2006). As an example, 2 000 and 20 000 cyanobacterial cells mL^{-1}, or 1 and 10 μg L^{-1} chlorophyll-a has been set as the safe level of potential microcystin concentration in drinking and recreational water,

respectively (ANZECC 2000, WHO 2003). This indirect microcystin risk assessment is based on the assumption of a linear relationship between cyanobacterial biomass and microcystin concentrations, and assuming a constant microcystin production for a given strain or genotype (WHO 2003). As an example, microcystin content per cyanobacterial cell is estimated as 0.2 and 0.4 pg $cell^{-1}$ for *Microcystis* spp. and *Planktothrix* spp., respectively.

1.9 The effects of the variation in microcystin production on the indirect microcystin risk assessment

At this stage, it is unclear as to how the dynamics of cyanobacterial biomass and microcystin production affect the indirect microcystin risk assessment. Current studies have proposed that small variations in microcystin concentrations can be explained by the dynamics of potentially microcystin producing cyanobacteria, and total microcystin content per cell can vary significantly between toxic cells (Briand et al. 2008b). As published by WHO (2003), an average toxin content of 0.2 pg per *Microcystis* spp. cell is used to estimate the potential microcystin concentration that is likely to occur in cyanobacterial blooms consisting of *Microcystis* spp. On the other hand, Downing et al. (2005) has reported significantly higher microcystin content per *Microcystis* cell of 1.7 pg $cell^{-1}$. To illustrate the risks of underestimating microcystin concentration in indirect microcystin risk assessment, high microcystin production is calculated based on microcystin content published in Downing et al. (2005), while the average microcystin production is calculated based on microcystin content published in WHO (2003) (Fig.1.5). In the case of high microcystin production, it is clear that 2 000 and 20 000 cyanobacterial cells/mL could result in much higher microcystin concentrations than the recommended WHO guideline value of 1 and 20 μg L^{-1}. Under high microcystin

production of 1.7 pg cell^{-1}, 2 000 and 20 000 cells mL^{-1} could result in microcystin concentrations exceeding 3 and 30 µg L^{-1}, respectively.

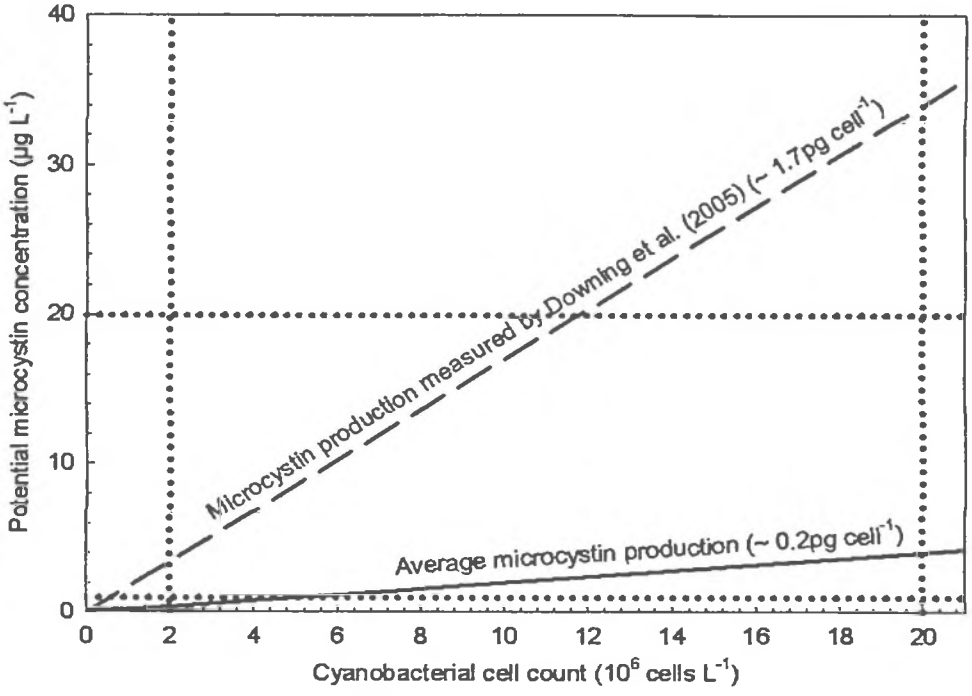

Fig.1.5: The estimation of microcystin concentration from cyanobacterial biomass based on the average microcystin production (solid line: microcystin content ~ 0.2 pg cell^{-1}, WHO 2003) and microcystin production measured by Downing et al. (2005) (long dashed line: microcystin content ~ 1.7 pg cell^{-1}). Two vertical dotted lines represent the ANZECC cyanobacterial cell count threshold value of 2 000 and 20 000 cells mL^{-1}, and two horizontal dotted lines indicate the recommended WHO guideline value of 1 and 20 µg L^{-1}.

In addition, the correlative evidence for a link between cyanobacterial biomass and microcystin concentration remains contentious. A review of results from field studies shows no consistent empirical relationship between cyanobacterial biomass and microcystin concentration (Table 1.4). The reasons for why this may be the case remain unclear, as no trend has been observed with regards to the sampling frequency used in these studies, the range of microcystin concentrations detected, the dominant cyanobacterial genera during blooms, and the units of measurement used to express

both cyanobacterial biomass and microcystin concentrations. These contrasting results illustrate that the presence of low cyanobacterial biomass is not necessarily connected to the presence of a low microcystin concentration, or vice versa. Therefore, indirect microcystin risk assessment from cyanobacterial biomass might not be a satisfactory method to evaluate the safety of water resources affected by cyanobacterial blooms.

The present state of knowledge shows that it is becoming crucial to assess the correlation between the cyanobacterial biomass and microcystin concentration, in order to fulfil the need for reliable microcystin risk assessment in public waters. Future climate change is likely to increase the incidence of toxic cyanobacterial blooms and the presence of elevated microcystin concentrations in water bodies. The presence of toxic cyanobacterial genotypes may intensify with higher nutrients and warmer temperatures (Davies et al. 2010). Furthermore, the changing physicochemical factors may cause up to 50 fold changes to microcystin production (Chorus and Bartram 1999). As a result, indirect microcystin risk assessment may underestimate the blooms' toxicity and as a consequence pose harm to public health.

Despite the number of studies focusing on the empirical relationship between cyanobacterial biomass and microcystin, only few attempts have been made to compare this relationship on spatial and temporal scales. A greater understanding of the variability of the relationship between the cyanobacterial biomass and microcystin is needed, as this information is necessary to be generalized, if the existing microcystin risk assessment strategy was to be reliable for protecting public health from the occurrence of highly dynamic microcystin production. Therefore, this research aims to examine the variability of cyanobacterial biomass-microcystin relationship on spatial and temporal scales.

Table 1.4: The comparison of the empirical relationship between cyanobacterial biomass and microcystin concentration reported in studies conducted throughout the world. Concentrations are presented in μg L^{-1}, or else in μg g^{-1} dry mass as indicated.

Biomass-toxin correlation	Biomass indicator	Dominant species	Highest microcystin concentration (μg L^{-1}, mg g^{-1} dry mass)	Sampling frequency	References
Positive	Cell numbers (cells mL^{-1})	*Microcystis* spp.	87.0	Not reported	Vasconcelos et al. 2011
Positive	Cell numbers (cells mL^{-1})	*Planktothrix* spp.	7.4	Twice per month	Briand et al. 2008b
Positive	Cell numbers (cells mL^{-1})	*Planktothrix* spp.	6.7	Once or twice per month	Briand et al. 2005
Positive	Cell numbers (cells mL^{-1})	*Microcystis* spp.	10.0	Monthly	Okello et al. 2010
Positive	Biovolume (mm^3 L^{-1})	*Microcystis* spp.	34.0 mg L^{-1}	Twice per month to weekly	Naselli-Flores et al. 2007
Positive	Biovolume (μm^3 L^{-1})	*Oscillatoria* spp.	5.0	< Once per month	Graham et al. 2004
Positive	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	28.0	Monthly	Nasri et al. 2007
Positive	Chlorophyll-a (μg L^{-1})	Mixed population	1.1 mg g^{-1}	Not reported	Frank 2002
Positive	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	2.6	Weekly	Hotto et al. 2008
Positive	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	4.5	Not reported	Wu et al. 2008
Positive	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	125.0	Daily	Znachor et al. 2006
Negative	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	34.6	Not reported	Al-Shehri 2010
No correlation	Cell numbers (cells mL^{-1})	*Microcystis* spp.	1.1 mg g^{-1}	< Once per month	Pavlova et al. 2006
No correlation	Cell numbers (cells mL^{-1})	Mixed population	2.4	Twice per month to weekly	Watzin et al. 2006
No correlation	Cell numbers (cells mL^{-1})	*Microcystis* spp.	17.3	Monthly	Yang et al. 2006
No correlation	Biovolume (mm^3 L^{-1})	*Microcystis* spp.	81.0	Monthly	Jayatissa et al. 2006
No correlation	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	0.9 mg g^{-1}	Weekly	Baldia et al. 2003
No correlation	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	19.8 mg g^{-1}	< once per month	Ballot et al. 2003
No correlation	Chlorophyll-a (μg L^{-1})	*Microcystis* spp.	70.0	Monthly to weekly	Carrasco et al. 2006
No correlation	Chlorophyll-a (μg L^{-1})	*Anabaena* spp.	0.7	Monthly	Chen et al. 2009

1.10 Research aims

Given the complexity and dynamics of toxic cyanobacterial blooms in freshwater ecosystems, this research investigates the causes and effects of cyanobacterial and microcystin spatiotemporal dynamics in freshwater systems. More specifically, this research examines how cyanobacterial and microcystin spatiotemporal dynamics affect the indirect microcystin risk assessment in public waters. This research aims to explore the environmental factors which significantly correlate with cyanobacterial and microcystin dynamics, and determine the site specificity of environmental drivers. This research also aims to investigate how changes in the structure of phytoplankton and cyanobacterial community, in response to nutrient concentration, can affect the dynamics of microcystin concentration.

The originality of this research is manifold. Firstly, this research compares the variability in the dynamics of toxic cyanobacterial blooms in a gradient of shallow lakes that vary in their physicochemical characteristics. Secondly, this research also incorporates new approaches, including a critical assessment of the cyanobacterial biomass-microcystin relationship by comparing the variability of the correlation on spatial and temporal scales; integrating site-specific effects of environmental factors to explain divergences in highly debated relationships between the environment and cyanobacterial biomass, and the environment and cyanobacterial microcystin content; and incorporating the community structure of phytoplankton and cyanobacteria with nutrient factors to infer the level of cyanobacterial microcystin content. Consequently, this research will improve the overall ability to manage toxic cyanobacterial blooms.

1.11 Thesis outline

The body of work presented in this thesis is comprised of three chapters (3 to 5). Each of these chapters is a paper written for journal publication. Each of these chapters has its own literature review, discussion and conclusion section. Table 1.5 provides an outline of the thesis structure, including the research aims pursued in each section. The connection between chapters in this thesis is illustrated in Fig.1.6.

Table 1.5: Thesis structure

	Research focus
Chapter 1 *Introduction*	
Chapter 2 *Research design and study sites*	
Chapter 3 *Spatial and temporal variability in the relationship between cyanobacterial biomass and the occurrence of microcystin*	Field study in eight shallow lakes on Swan Coastal Plain (2008-2009) • Quantify the dynamics of cyanobacterial biomass and microcystin concentration in eight lakes on Swan Coastal Plain. • Evaluate the empirical biomass-toxin relationship which is traditionally used as an indirect microcystin risk assessment. • Identify the source of divergence between the dynamics cyanobacterial biomass and microcystin.
Chapter 4 *Site-specific environmental drivers of cyanobacterial and microcystin dynamics: The influence of the local cyanobacterial community and nutrient concentration gradients*	Field study in three shallow lakes on Swan Coastal Plain (2010) • Identify the environmental drivers which correlated significantly with cyanobacterial and microcystin dynamics. • Investigate the site specificity of significant environmental drivers by comparing the environmental drivers of cyanobacterial and microcystin dynamics between lakes.
Chapter 5 *Structure of phytoplankton community in response to changes in nutrient gradient implies the level of microcystin concentrations*	Field study in three shallow lakes on Swan Coastal Plain (2010) • Quantify the effects of the changes in the structure of phytoplankton and cyanobacterial community on cyanobacterial microcystin content. • Identify the effects of changes in nutrient concentration on the structure of phytoplankton community. • Investigate the nutrient factors which correlated to the succession of different cyanobacterial genera within a population.
Chapter 6 *General conclusions*	

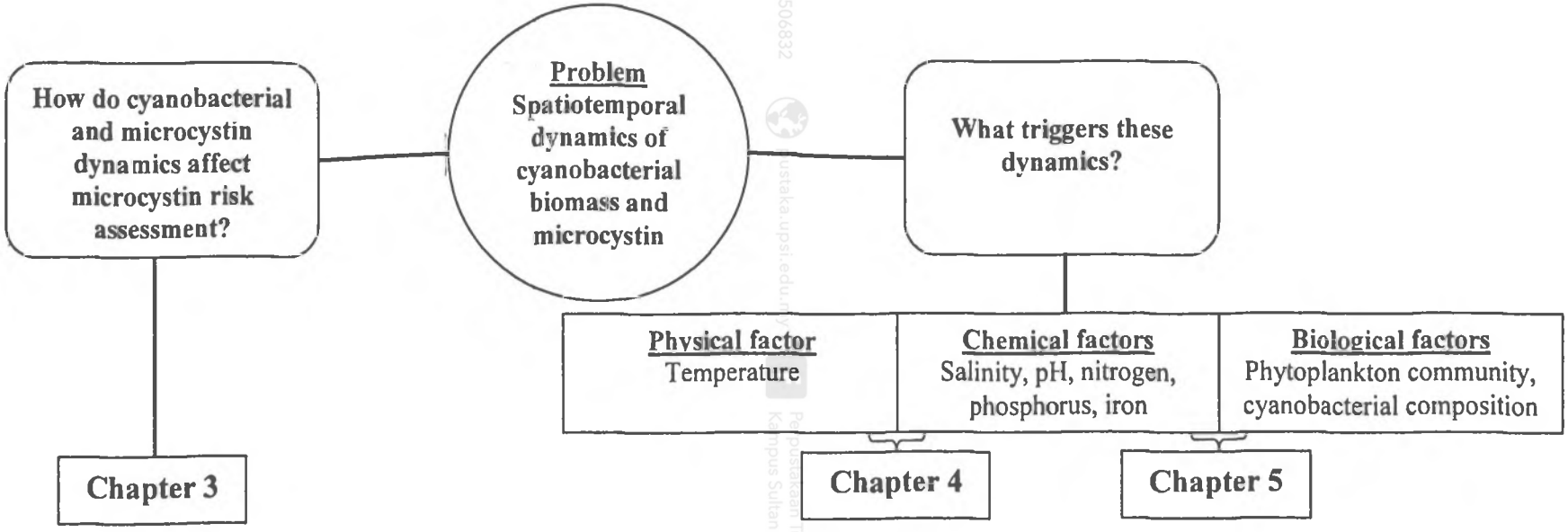

Fig.1.6: Framework showing the structure and connection between bodies of work discussed in the main chapters in this thesis.

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