

A decision-making framework for ecological impacts associated
with accumulation of cyanotoxins
(cylindrospermopsin and microcystin)

Susan H. White¹, Leo. J. Duivenvoorden¹, Larelle. D. Fabbro¹

¹ Freshwater Ecology Group, Centre for Environmental Management, Central
Queensland University, Bruce Highway, Rockhampton Queensland 4702, Australia.

Corresponding author: Susan White
Biological and Environmental Sciences
Building 6, Central Queensland University
Bruce Highway, Rockhampton QLD
Australia 4702

Email: s.white@cqu.edu.au
Phone: (07) 4930 9647
Fax: (07) 4930 9209

Running title: Modelling cyanotoxin bioaccumulation and risk

Abstract

Toxins produced by cyanoprokaryotes are a key issue in aquatic management due to their potential to exert adverse effects on humans and aquatic biota. However, the information gap regarding bioaccumulation and biomagnification processes associated with cyanotoxins has resulted in inadequacies in the management and maintenance of biological diversity in lakes and reservoirs affected by toxic cyanoprokaryote blooms. This paper examines the potential for, and effects of, bioaccumulation of two common cyanotoxins, microcystin and cylindrospermopsin, in aquatic organisms. Factors influencing cyanotoxin bioavailability are discussed in context of the challenges associated with understanding and managing toxin accumulation. Based upon characteristics of cyanotoxin bioavailability, exposure and uptake routes, a theoretical predictive model for cyanotoxin bioaccumulation is proposed. Key concepts include monitoring changes in toxin availability throughout the progression of a toxic bloom, and the prediction of ecological effects based on internal tissue concentrations. The model explores the minimum requirements managers must undertake to order to properly assess bioaccumulation risk in terms of frequency of toxin testing, toxin fraction determination, and assessment of aquatic organisms.

Keywords: bioaccumulation, blue-green algae, cyanoprokaryotes, cylindrospermopsin, ecological risk assessment, microcystin, modelling.

Introduction

Reports of lakes and reservoirs affected by toxic cyanoprokaryote (blue-green algal) blooms are increasing internationally (e.g. Frank 2002; Bouvy *et al.* 2000; Chorus & Bartram 1999; Padisák 1997). Such blooms demand attention because of their toxicity to terrestrial and aquatic animals, and the possible health risks posed for humans in recreational and drinking-water storage bodies (Carmichael *et al.* 2001; Falconer 2001; Carmichael & Falconer 1993).

Two globally common freshwater toxins are cylindrospermopsin (CYN), produced by strains of *C. raciborskii*, *Umezakia natans*, *Aphanizomenon ovalisporum*, *Anabaena bergii* var *limnetica* and *Raphidiopsis curvata* (Li *et al.* 2001; Schembrii *et al.* 2001; Shaw *et al.* 1999); and microcystin (MC), produced by *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* (Rinehart *et al.* 1994; Botes *et al.* 1984). These toxins exert acute and chronic lethal and sublethal effects on a range of organisms: historically, Schwimmer & Scwhimmer (1968) gave accounts of deaths linked to blue-green algal intoxication in cattle and sheep, horses, dogs, cats, domestic fowl (geese, chickens, ducks), birds, squirrels, mice, turkeys and extensive fish kills. More recent work has also demonstrated a range of sublethal effects to other aquatic flora and fauna, including growth inhibition and reproductive effects in zooplankton (Ferrão-Filho & Azevedo 2003; Ferrão-Filho *et al.* 2002), macroinvertebrates and aquatic insects (Beattie *et al.* 2003; Hiripi *et al.* 1998; Delaney & Wilkins 1995), accumulation in crayfish (Saker & Eaglesham 1999; Lirås *et al.* 1998) and reduced growth in aquatic plants (Pflugmacher 2002; Romanowska-Duda & Tarczynska 2002; Kirpenko 1986). In humans, adverse health effects associated with MC and CYN include gastrointestinal complaints, skin allergies, and possibly, tumour promotion

and increased risk of primary liver cancer (Falconer 2001; Xu *et al.* 2000; Ueno *et al.* 1999). MCs were found responsible for the deaths of dialysis patients treated with water from the microcystin-containing Tabocas reservoir (Komarek *et al.* 2001), whilst CYN was implicated in the so-called Palm Island mystery disease, which resulted in the hospitalisation of over 100 children suffering acute gastroenteritis (Hawkins *et al.* 1985; Byth 1980).

This paper examines the potential for increased toxicity of MC and CYN due to bioaccumulation and biomagnification processes. Currently, lack of data and scientific understanding regarding bioaccumulation has contributed to inadequacies in management procedures addressing the ecological and human health hazards associated with exposure. Here, a new management approach is suggested, whereby a predictive model is used to enable managers of affected lakes and reservoirs to gauge periods of high bioaccumulation risk based on toxin availability, and to estimate adverse effects to aquatic biota based on tissue toxin concentrations.

Bioaccumulation of cyanotoxins: evidence and effects

The extensive range of effects associated with direct cyanotoxin exposure in aquatic animals, and evidence of their dose-dependency (Vasas *et al.* 2002; Zimba *et al.* 2001; Chong *et al.* 2000; Carmichael 1996) provides a compelling argument to study the bioaccumulation and biomagnification potential of cyanotoxins in aquatic organisms.

Several factors indicate that cyanotoxins may bioaccumulate and biomagnify similarly to other toxicants such as heavy metals and pesticides (Sivonen & Jones

1999). CYN and MC are both stable (Harada 1996; Ohtani *et al.* 1992), which is considered necessary for accumulation to occur (van der Oost *et al.* 2003). Both also have structural features conducive to accumulation processes: MC is generally hydrophobic (Vesterkvist & Meriluoto 2003), which increases the likelihood of partitioning into lipid tissues of aquatic organisms, and, although being a quite large compound (800 – 1100 Daltons) (Sivonen & Jones 1999) is possibly cell permeant (Duy *et al.* 2000). Though CYN is hydrophilic, it is a relatively small compound (Shaw *et al.* 2000; Sivonen & Jones 1999) and is usually represented by extracellular toxin fractions (Griffiths & Saker 2003; Hawkins *et al.* 2001). Thus, CYN accumulation may result from contact via gills and dermal surfaces, or from accidental ingestion by drinking.

The octanol-water partitioning coefficient (K_{OW}), a value often used to indicate bioaccumulation potential, also suggests likely accumulation. Values of $\log K_{OW} > 3$ are considered to have high potential (Franke *et al.* 1994): values for MC-LR, -LY, -LW and -LF were 2.16, 2.92, 3.46 and 3.56, respectively, when estimated using HPLC techniques (Ward & Codd 1999).

Lastly, the evolution of aquatic organisms alongside cyanoprokaryotes may have allowed the development of mechanisms to counter cyanotoxicity (Lirås *et al.* 1998), enabling organisms to continue to accumulate large amounts of toxins without side effects. Such organisms represent excellent vectors through which toxins can transfer up the food chain (Christoffersen 1996), thus enabling biomagnification.

To date, only two published works have examined bioaccumulation of CYN. Saker & Eaglesham (1999) detected CYN in the muscle, hepatopancreas and abdominal tissues of the crayfish *Cherax quadricarinatus*, at a maximum concentration of $4.3 \mu\text{g g}^{-1}$ freeze dried tissue. Toxin was also reported from the visceral tissue of rainbow fish *Melanotaenia eachamensis* ($1.2 \mu\text{g g}^{-1}$ freeze dried tissue⁻¹). Most recently, Saker *et al.* (2004) reported accumulation of CYN in the swan mussel *Anodonta cygnea*, with maximum levels of $61.5 \mu\text{g g}^{-1}$ (haemolymph); $5.9 \mu\text{g g}^{-1}$ (viscera) and $2.52 \mu\text{g g}^{-1}$ (whole body). In contrast, MC accumulation has been reported at ng to $\mu\text{g g}^{-1}$ levels in organisms representing all components of aquatic systems, including aquatic plants (Wiegand & Pflugmacher 2001), zooplankton (Ferrão-Filho *et al.* 2002b), snails (Ozawa *et al.* 2003; Zurawell 2001; Kotak *et al.* 1996), mussels and clams (Yokoyama & Park 2003;2002; Prepas *et al.* 1997; Williams *et al.* 1997; Eriksson *et al.* 1989), and higher animals including crayfish (Lirås *et al.* 1998; Vasconcelos 1995) and fish (Xie *et al.* 2004; Magalhães *et al.* 2001; Mohamed 2001).

A key issue surrounding bioaccumulation and biomagnification of cyanotoxins is the possibility for toxic blooms to exert greater toxicity, due to higher concentrations of internalised toxins being accrued in bodily tissues. Currently, there is a dearth of information regarding the sublethal effects of cyanotoxins, especially in aquatic organisms. Most studies have focussed on effects at the lowest levels of biological organisation, thus ecosystem-level effects remain the least well understood of all possible impacts, especially the long-term implications for higher-level consumers (Landsberg 2002). What new effects may be possible due to increased tissue toxin levels and exposure?

At the organ level, effects of MC exposure include necrosis and haemorrhaging of liver tissue, hepatic malfunction and heart stress in laboratory rats and fish (Best *et al.* 2001; Fischer *et al.* 2000; Heinze 1999; Dawson 1998; Falconer *et al.* 1981). In aquatic plants, MC exposure reduces growth rates, seed germination and alters photosynthetic pigmentation (Pflugmacher 2002; Romanowska-Duda & Tarczynska 2002; McElhiney *et al.* 2001; Casanova *et al.* 1999). Reduced embryo survival, developmental abnormalities and population changes have also been reported in fish (Liu *et al.* 2002; Oberemm 2001; Oberemm *et al.* 1999). These 'developmental' effects may have significant implications, as prolonged or recurrent toxic blooms which cover entire or successive breeding seasons (e.g. Baker & Humpage 1994), may prevent the development of larval and young stages of organisms. Other effects include reduced species diversity (MacKintosh *et al.* 1990), altered predator-prey interactions and other behavioural changes (Oberemm 2001).

Little is known regarding MC transfer in food webs. The sensitivity of aquatic organisms to MC exposure is known to vary with trophic level: fish, crustaceans and rotifers appear most tolerant ($0.5 - 20 \mu\text{g L}^{-1}$), followed by the phytoplankton ($0.05 - 1 \mu\text{g mL}^{-1}$) and macrophytes ($0.001 - 0.05 \mu\text{g mL}^{-1}$) (Christoffersen 1996). Kotak *et al.* (1996) examined MC-LR bioaccumulation in different trophic levels but found detectable MC in grazers only, with a strong possibility of MC-LR transfer to terrestrial food webs. Ferrão-Filho *et al.* (2002b) concluded that zooplankton were more efficient at MC transfer to higher trophic organisms than were other organisms such as molluscs. Such food chain transfers represent potential human risk in consumed organisms such as crayfish, mussels and fish (Saker & Eaglesham 1999). Moreover, they have implications for biomanipulation management approaches, as

consumers may remove toxic phytoplankton from the water (Boon *et al.* 1994; Matveev *et al.* 1994), only to accumulate it into other food web compartments.

The effects of CYN exposure are critically understudied. CYN is associated with severe hepatic necrosis (Terao *et al.* 1994), which results from inhibition of protein synthesis (Shaw *et al.* 2000) and possibly, DNA disruption (Shen *et al.* 2002). In mammals, CYN toxicity is associated with hepatic and kidney malfunction (Duy *et al.* 2000; Falconer *et al.* 1999). CYN has also been found to exert effects on molluscan neurones and neurotransmitter receptors (Kiss *et al.* 2002), and causes mortality in insects and cattle (Saker *et al.* 1999; Hiripi *et al.* 1998). Studies have not been conducted on chronic CYN exposure in any species (Carson 2000).

Current Algal Management Approaches

In 2001, the World Health Organisation (WHO) released an internationally accepted guide to the monitoring, assessment and management of toxic algal blooms (Chorus 2001). However, these guidelines have a definite focus on human health consequences rather than environmental risk. The nominated safe levels for drinking water are generally aimed at protecting human users; although accumulation of toxins into aquatic biota is mentioned as a potentially hazardous for consumers, no strategies for minimising ecological risks are proposed. In Australia, a global hotspot for toxic blooms, monitoring and management of toxic blooms occurs at both national and state levels (Jones *et al.* (2002); QDNR 2003). Risk levels are assigned based on cell and toxin concentration data, however, again, these data are not interpreted with respect to bioaccumulation and/or biomagnification.

The effects exerted by MC and CYN (summarised above) clearly indicate that consideration of ecological risk is required for a total approach to toxic bloom management. Guidelines which rely on cell concentration data for calculation of human risk are not adequate for this purpose. Similarly, whilst being critical for management of drinking water bodies, reactive management strategies such as flocculation, sedimentation and chlorination (Hoeger *et al.* 2004) are inadequate for ecological risk management, as these are aimed at removing algal cells and toxins already present in the system. Predictive modelling approaches, from which likely periods of bioaccumulation and biomagnification can be calculated, are best equipped to minimise the toxic effects of blooms regarding both human and ecological risk.

Challenges in modelling cyanotoxin accumulation

Modelling the bioaccumulation processes of cyanotoxins is likely to be highly complex as cyanotoxins, unlike most other toxicants, are produced within living cells. For most (synthetic) toxicants, the likelihood of bioaccumulation depends on bioavailability, and other species-specific factors including feeding, metabolism, growth dilution, and uptake efficiencies (McCarty & Mackay 1993). However, with cyanotoxins, bioavailability itself is governed by further factors including the type and concentration of available toxins, their spatial and temporal variability, and their uptake routes. Understanding and inclusion of these factors is critical for effective bioaccumulation modelling.

Many factors influence toxin production by cyanoprokaryote cells, though the significance of each remains poorly understood. Toxin content of blooms may be variable depending on the number and physiological status of algal cells (Orr & Jones

1998), and on various environmental conditions (Saker & Griffiths 2000; Utkilen & Gjølme 1995). Whilst a single species (or strain) can be identified as being toxigenic, this does not necessarily mean toxin production occurs (Chorus *et al.* 2001). Moreover, increased cell densities of cyanoprokaryotes do not necessarily correspond with increased toxicity (Bickel *et al.* 2000; Jacoby *et al.* 2000). Many studies have examined likely ‘triggers’ for toxin production and conditions under which maximal toxin production occurs. These include relationships between cell division rates and the toxin content of cells (e.g. Bickel *et al.* 2000; Orr & Jones 1998) and environmental parameters, such as nutrient, temperature and light conditions, (Long *et al.* 2001; Saker & Neilan 2001; Saker & Griffiths 2000). Toxin decomposition processes, which also play a role in toxin content, also depend on factors including time, pH, temperature, light, water quality and microbial activity (e.g. Chiswell *et al.* 1999; Lahti *et al.* 1997b).

Variability in toxin production is also related to the toxin composition of a given bloom, even for monospecific occurrences. For example, over 65 variants of microcystins have been identified (Carmichael 2001), though a natural bloom rarely contains more than ten (Geoff Eaglesham, National Centre for Environmental Toxicology, pers. comm.). Similarly, *Cylindrospermopsis raciborskii* may produce more than one variant of CYN (Norris *et al.* 2002; Falconer 1999; Hawkins *et al.* 1997). Additive or synergistic effects may also result from the presence of more than one toxin or toxin variant. The implication for bioaccumulation modelling here is that predictions of toxicity based on identification and cell counts of toxigenic species are not sufficient, as toxin production is highly variable within these parameters.

Furthermore, cyanotoxins may be represented by three different toxin fractions. Firstly, intracellular toxins are those held within algal cells. Secondly, extracellular are those lysed from cells into the surrounding water. Lastly, incorporated toxins are INC or EXC toxins which, as a result of uptake and/or adsorption processes, become part of or attached to the cells of aquatic organisms. Incorporated toxins may be reversibly bound to protein phosphatases or other cellular enzymes, but do not include toxins lying in the alimentary canal of algal grazers, which may later pass out of the organism. Blooms of different cyanotoxins are comprised of different toxin fractions: for example, MC is usually intracellular (Long *et al.* 2001; Lahti *et al.* 1997b; Watanabe *et al.* 1992; Berg *et al.* 1987) though Wiedner *et al.* (2003) found extracellular concentrations to be 20% higher in cultures with higher light irradiance. In contrast, CYN is mostly extracellular (Carson 2000; Chiswell *et al.* 1999). Some evidence suggests variation in toxin fractions may be controlled even at the species level, according to environmental conditions (Hawkins *et al.* 2001).

The availability of the different toxin fractions is critical to bioaccumulation modelling because of their influence on uptake route. Intracellular toxins may pose significant risk to organisms which ingest toxin-laden cells, either through grazing or accidental ingestion. Contrarily, aquatic plants may be at greater risk from extracellular toxins, if dermal uptake is possible. Incorporated toxins may represent risk for consumers not exposed to toxins directly (e.g. terrestrial birds). Consequently, the availability of particular toxin fractions will be significant in influencing bioaccumulation in a given set of organisms (e.g., intracellular toxins for grazers).

The temporal and spatial variability of toxins also impacts on the availability of toxins to aquatic biota. Temporal variation in each of the toxin fractions occurs as a result of production, decomposition and uptake processes during the progression of toxic blooms, which results in some fractions being more abundant at certain times. For example, these dynamics can be mapped for a *Microcystis* bloom (Figure 1): early stages of a toxic bloom are associated with mostly intracellular toxin, consistent with production and storage within cyanoprokaryote cells. However, as the bloom ages, some cells die and lyse, releasing small amounts of extracellular toxins into the water. Meanwhile, newly made cells continue to add to intracellular toxin levels. In the latter stages of the bloom, the situation becomes reversed: more cells lyse, the production of intracellular toxin is slowed, and extracellular toxin becomes more abundant. Furthermore, incorporated toxins may now be present in aquatic organisms capable of accumulation. Finally, during and after the bloom collapse, extracellular toxins may either persist or be rapidly degraded (Lahti *et al.* 1997a), whilst incorporated toxins remain until depuration and/or clearance occurs (Beattie *et al.* 2003; Yokoyama & Park 2003; Zurawell 2001; 1997; Falconer 1993).

Though Christoffersen (1996) suggested peak toxicity to occur in the later stages of toxic blooms, when cells lyse and release large amounts of toxins into the water, Figure 1 shows this may not necessarily be true for bioaccumulation effects. Rather, as each toxin fraction becomes dominant, certain exposure routes vary in importance, and organisms with those uptake routes become susceptible to toxin bioaccumulation and its effects. For example, plants cannot ingest algal cells and therefore are not at risk from intracellular toxins; hence, plants may not be predisposed to bioaccumulation early in bloom formation. Conversely, algal grazers

could be considered at higher risk during this time. The dynamics of toxin fraction availability is therefore a key issue to identifying peak bioaccumulation risk times in different aquatic organisms.

Spatial change in toxin fractions may also impact on bioavailability to aquatic organisms. Intracellular toxins are held within living cells, however the distribution of these cells is not homogenous in a system. As a result, concentrations of intracellular toxin may occur at certain depths, due to gas-vacuolate cells regulating buoyancy according to light preferences. Wind-induced cell (and therefore, intracellular toxin) accumulations may occur in surface species such as *Microcystis* (Webster 1990), whilst other subsurface species (e.g. *C. raciborskii*) may impact pelagic fauna. Furthermore, sinking, senescing cells contribute to an abundance of extracellular toxins in benthic habitats. Accordingly, the habitats of aquatic animals may predispose them to exposure to certain toxin fractions—for example, benthic animals and sinking, senescing cells releasing extracellular toxin.

A final factor influencing bioaccumulation likelihood is the individual capability of each aquatic animal to respond to cyanotoxins. Some organisms may be able to minimise or avoid exposure to cyanotoxins, so decreasing their likelihood for bioaccumulation. For example, Zurawell (2001) surmised that sessile filter feeders may experience greatest exposure as they are unable to move away, and have limited alternatives for food. However, coexistence of such animals with toxic algae over evolutionary time scales may also have given them the opportunity to develop tolerance to the toxins. Internal physiology may also be significant: in some organisms, interactions between toxins and internal proteins may occur (Kankaanpää

et al. 2002), whilst others may convert toxins to a different form or compound, particularly for CYN (Runnegar *et al.* 1994). These kinds of processes may limit or prevent bioaccumulation of cyanotoxins and their effects.

Clearly, the range of factors influencing cyanotoxin bioavailability, and thus bioaccumulation potential, makes constructing a predictive model for bioaccumulation difficult. However, the ecological effects associated with cyanotoxin bioaccumulation makes a clear case for developing such a model.

A model for predicting toxin bioaccumulation and its risks

Based on current knowledge of general bioaccumulation processes and of the specific nature of cyanotoxins, three factors are critical in enabling bioaccumulation of cyanotoxins: (1) that toxin is in a form available for uptake by a given organism (fraction availability); (2) that toxin is available in sufficiently high concentrations that bioaccumulation occurs (toxin threshold); and (3) that toxin is present for a sufficient period of time to allow accumulation into organismal tissues (exposure). The first of these, fraction availability, has already been discussed in the context of influencing bioaccumulation, through associations with different uptake routes.

The second requirement for bioaccumulation is suitable toxin concentration. Presumably, a threshold exists below which toxin accumulation does not occur, as the metabolic capabilities of aquatic organisms will regulate or prevent accumulation into tissues. Moreover, these are likely to be different between species, according to the metabolic capabilities of each (Figure 2). The first animal ('non regulator') has no metabolic capability to process (depurate, detoxify or metabolise) toxin, so, once

exposure occurs, toxin accumulates into the tissues. The second animal ('regulator'), though exposed to the same toxin concentration, is capable of processing small amounts ($< 4 \mu\text{g L}^{-1}$), and therefore prevents bioaccumulation from low-level exposure. However, this capability fails at higher toxin levels, and eventually, a threshold is reached ($12 \mu\text{g L}^{-1}$) at which toxins accumulate. It is difficult to estimate likely thresholds for microcystin and cylindrospermopsin accumulation from current literature, however this knowledge is critical to bioaccumulation management.

The third requirement for accumulation, minimum exposure time, may vary depending on uptake route(s). For example, organisms accumulating toxins from dermal exposure may need only short periods of time, whilst bioaccumulation from orally-ingested cells may depend on feeding activity and metabolism, particularly as these change according to reproductive status and season (e.g. Yokoyama & Park 2002; Vasconcelos *et al.* 2001).

Once bioaccumulation risk has been predicted based on fraction availability, concentration and exposure time, determining the risk of ecological effects will depend on internal tissue concentrations. Basing ecological risk assessment (ERA) and management strategies on internal concentrations is considered more reliable than doing so using water concentrations, because the issues of uptake and metabolism have already been resolved (Sijm & Hermens 2000). In fact, use of internal tissue concentrations is now highly recommended over other methods (Connell *et al.* 1999). However, for this to succeed, it is crucial that problems such as preferential storage of toxins into some organs have been identified, to avoid underestimation of toxin levels. A strong knowledge base is required to predict likely sublethal/lethal effects

corresponding to internal toxin levels; unfortunately, next to no data are currently available regarding tissue toxin levels and sublethal/lethal toxicities for cyanotoxins.

Clearly, a useful and accurate management model will need to address the following four principal elements regarding the prediction of cyanotoxin bioaccumulation and its ecological effects in aquatic organisms:

1. knowledge of toxin concentrations in the water, including concentrations of each toxin fraction;
2. understanding of the likely toxin uptake routes for principal aquatic organisms of concern,
3. knowledge of the minimum concentration thresholds and exposure times required for bioaccumulation to occur, and
4. data linking internal tissue toxin concentrations with expected sublethal and lethal effects.

The model proposed below (Figure 3) combines these elements into a decision framework, utilizing a predictive modelling approach to toxic algal bloom management specifically regarding bioaccumulation. A worked example is provided at the end of this discussion.

The step-wise process (Figure 3) begins with monitoring of water bodies likely to be affected by toxic cyanoprokaryote blooms, based on prior history and/or current conditions (1). Algal sampling is conducted (2), and samples examined for potentially toxigenic species (3). Where these are present at concerning levels (Chorus 2001; Chorus & Bartram 1999), toxin testing is initiated (4). At this stage, all

procedures are already accepted algal monitoring protocols. However, this model further requires separate quantification of the intracellular and extracellular toxin fractions (5), as these are critical in determining bioaccumulation potential.

Evaluation of sampling results is then made by referring to a table of threshold values (6) for aquatic organisms, calculated from data of laboratory-executed and field-validated ecotoxicity experiments. As no such data are yet available, an exemplar table for microcystin, using arbitrary values, is provided in Table 1. The table works by identifying a species of concern and its likely toxin uptake route(s), then provides the specific threshold value(s) above which bioaccumulation is deemed likely.

Species with multiple uptake routes can still be evaluated by this method: it matters not how many routes are available, as long as each one of the thresholds is not exceeded. However, these require the use of caution factors (step 7); examples of which have also been included in Table 1. Caution values have been used in other ecotoxicological applications, often to account for the differences between laboratory measured lethal effects and the expected sublethal effects occurring in the field, or short versus long term experimental data (Markich & Camilleri 1996). Here, however, their role is to ensure that two sub-threshold fraction values are not interpreted as being risk-free. The caution values act to decrease the singular thresholds, after which the risk from each fraction should be re-evaluated using the new (reduced) threshold value. Using such factors provides ‘an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms’ (Stephan *et al.* 1985).

Returning to the model, the likelihood of bioaccumulation is determined (8), by calculating if any thresholds have been exceeded, before or after application of caution factors. If so, tissue testing is initiated for those organisms considered at risk of bioaccumulation (9). The results of these tests can then be compared against known sublethal and lethal toxicities for similar internal concentrations in order to assess the likely ecological and human health effects of bioaccumulation (10).

A worked example

The following demonstrates the decision pathway for managers of a reservoir stocked with *Cherax* crayfish. Early in the bloom, algal sampling and enumeration identifies the predominance of potentially toxic *Microcystis*, prompting the need for toxin analysis. The first toxin analysis is performed; results obtained are 3 $\mu\text{g L}^{-1}$ intracellular (IC) toxin and 2 $\mu\text{g L}^{-1}$ extracellular (EXC) toxin. According to Table 1, the risk evaluation values for *Cherax* are 4 $\mu\text{g L}^{-1}$ for the intracellular fraction and 3 $\mu\text{g L}^{-1}$ for the extracellular fraction. Based on individual toxin fractions, no risk thresholds have been exceeded for *Cherax*. However, as *Cherax* has multiple uptake routes, caution factors must be introduced. As more than two sub-threshold values are present, each individual threshold value must be reduced by half. As a result, both thresholds are exceeded, requiring tissue toxin testing to be initiated, and the results used to determine possible ecological effects of bioaccumulation.

Model evaluation

This model is unique in addressing the challenges posed by predictive modelling of cyanotoxin bioaccumulation values. Inter-specific variability in toxin

production is addressed by regular toxin testing, based on the species composition of the bloom. Temporal and spatial change in toxin fraction variability is accounted for because the toxin tests separately quantify intracellular and extracellular fractions, and each organism has likely uptake routes (and hence likely exposure risk) nominated. Lastly, the responses of aquatic organisms to cyanotoxin exposure (uptake, metabolism and adverse effects) are each considered, by dividing up the risk evaluation table according to species.

The decision making process followed in this model may need to be repeated several times in the event of a particularly long toxic bloom, especially where early toxin sampling indicates levels below threshold values. In this event, monitoring should be continued in order to ensure levels do not rise above risk concentrations at a later date. Incorporating exposure times into this model is difficult, because of the need to introduce almost continuous toxin testing to document fluctuations in toxin concentrations between tests. It may therefore be useful to initiate toxin testing at critical stages of a bloom, for example, 'middle', 'late' and 'collapse' stages as shown in Figure 1, possibly using predictions from cell count data.

The model does not attempt to model the processes of uptake and depuration rates, nor the possibility of metabolic transformation or conjugation of toxins, although these have been studied in some organisms (Yokoyama & Park 2003; Pflugmacher 2002; Zurawell 2001). The model also does not include the possibility of incorporated toxins as a further uptake route (toxin source) for some animals (i.e., biomagnification). This is because incorporating the complexities of these may risk

underestimation of the bioaccumulation hazard, especially if all components are not properly accounted for (Franke *et al.* 1994).

This model could be easily integrated into current algal management approaches; especially as field officers would already be familiar with required sampling methods. Furthermore, there are no requirements for extra testing or data collection methods other than those used at present—except for performing toxin analyses on intracellular and extracellular, rather than total, fractions. The model also simplifies the rather complex concepts regarding algal bloom periodicity and toxicity.

Drawbacks to the model include potential doubling of toxin analyses costs by requiring the cell-bound and non-cell bound fractions to be determined separately, and for toxin analyses to be repeated in the event of long bloom periods, or in blooms where bioaccumulation effects persist past the bloom collapse. One downfall of using the risk threshold-approach (Table 1) is that it presumes uptake will always occur in the presence of toxin fractions. This may be likely for intracellular toxin: oral consumption of MC appears to correspond well with toxin uptake in gastropods (Yokoyama & Park 2002; Kotak *et al.* 1996), however this situation is uncertain for incorporated and extracellular toxins. The model also has a certain degree of error: results of toxin analysis may not reflect exactly the ratios of toxin fractions in the sampled water body, due to variability in sampling (for example, sample collection, transport and storage) and toxin detection methods. In particular, lysing of cells and/or degradation of dissolved fractions in storage may underestimate actual toxin concentrations (Nicholson & Burch 2001). Evaluating these levels will give only

gross indicator of the bioaccumulation risk, nonetheless, the model will still act as a valuable screening tool to minimise costly tissue testing.

Future improvements to this model could be the inclusion of exposure thresholds for time, and the impact of environmental parameters (pH, temperature) in affecting uptake and bioaccumulation processes. As research data become available, the model can be expanded to include more species. Possibly, this could include species not specified in the risk evaluation table by examining their trophic level and food preferences and hence likely modes of exposure (e.g. oral ingestion). The model may also be used with respect to human health risk assessment, for example, for decision-making regarding the suitability of aquatic organisms for consumption.

A number of challenges still exist with respect to determining the ecological effects (and human health risks) of cyanotoxin bioaccumulation. Through lack of studies, the range of sublethal effects associated with direct toxicity for many cyanotoxins remains unknown, let alone bioaccumulation effects. The inability to accurately detect incorporated MC and CYN also represents a considerable barrier for linking internal tissue concentrations with toxicity. This model represents an important step in toxic algae management, by considering the ecological effects of bioaccumulation and biomagnification of cyanotoxins in aquatic organisms.

Acknowledgements

This work was supported by an Australian Postgraduate Award and a support grant from Nebo Shire Council.

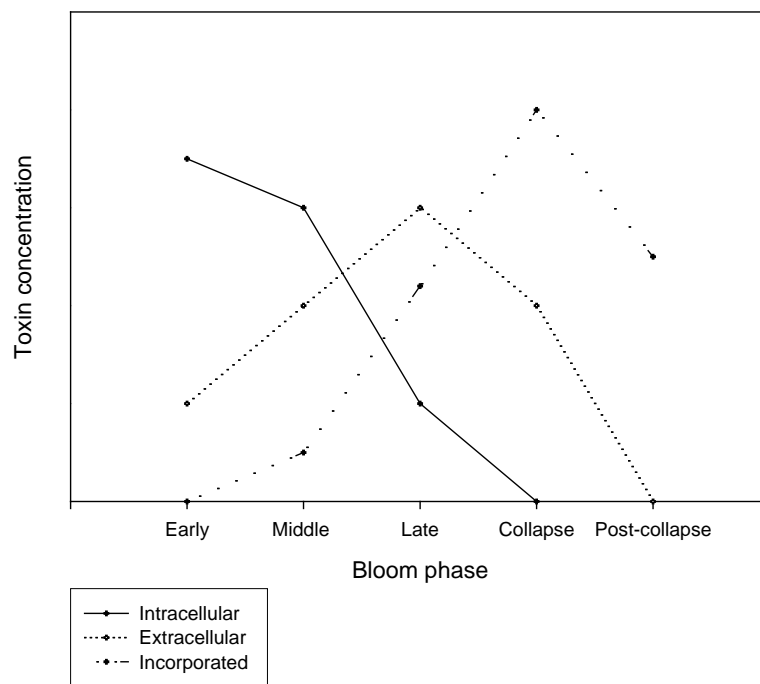
Figure 1. Speculated change in microcystin toxin fractions with *Microcystis* bloom progression

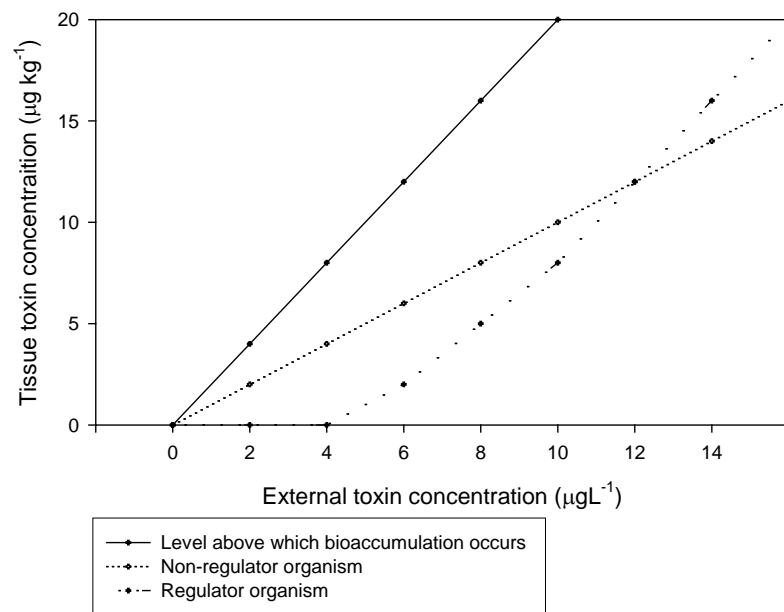
Figure 2. Bioaccumulation thresholds in animals of different metabolic capabilities.

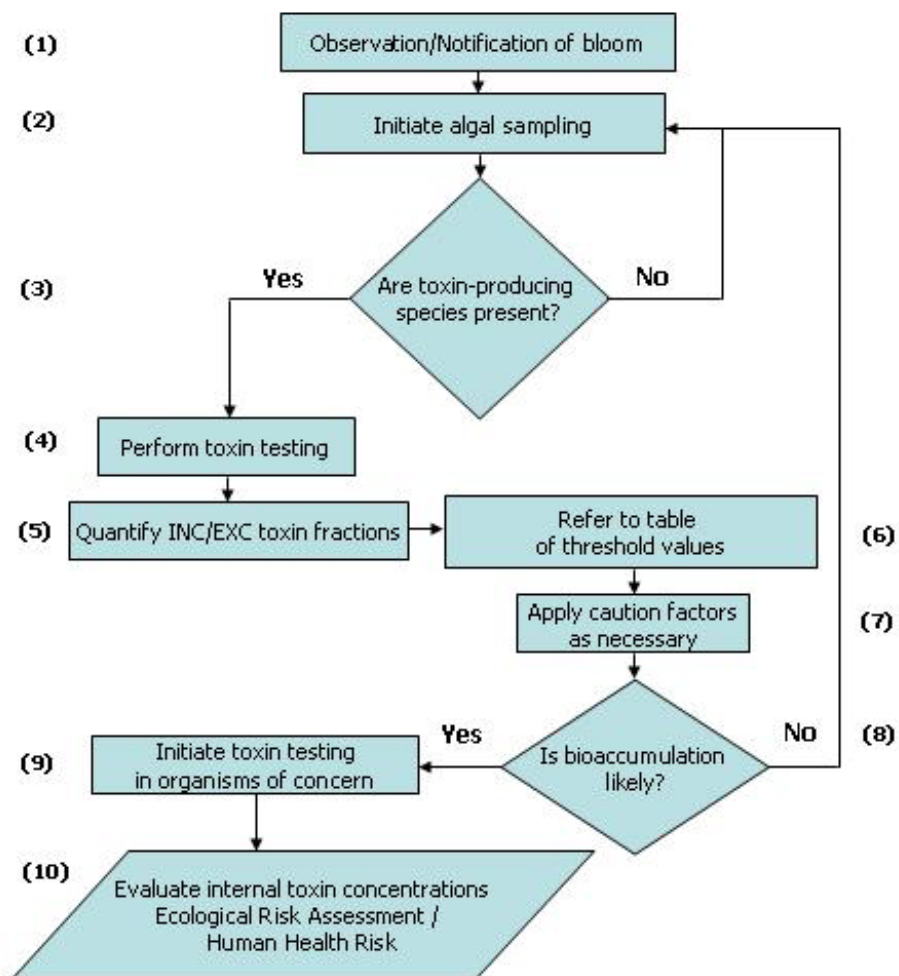
Figure 3. Proposed step-wise model for monitoring and management of cyanotoxin bioaccumulation and its ecological effects. INC: intracellular; EXC: extracellular.

Table 1. Bioaccumulation risk threshold values for microcystin (example only). NA: not applicable; NR: not required (only one uptake route is considered to be of significance). †Possible route, unlikely to be major contributor (hence caution factors not required)

Species	Primary exposure route(s)	Intracellular threshold	Extracellular threshold	Caution Factor(s)
Aquatic plant <i>Lemna</i> sp.	Dermal	NA	1 µg L ⁻¹	NR
Macroinvertebrates				NR
<i>Tubifex</i> sp. (Tube worm)	Dermal; oral	2µg g ⁻¹	1 µg L ⁻¹ †	
<i>Thiara</i> sp. (herbivorous snail)	Oral	0.5 µg g ⁻¹	6 µg L ⁻¹ †	NR
<i>Eusthenia</i> sp.(carnivorous stonefly)	Incorporated (in prey)	NA (do not graze on algae)	4 µg L ⁻¹ †	NR
Higher animals				
<i>Cherax</i> sp. (omnivorous crayfish)	Oral; dermal	4 µg g ⁻¹	3 µg L ⁻¹	Reduce threshold values by 1/2 in presence of 2 sub-threshold values.
<i>Bidyanus</i> sp. (silver perch)	Oral (incorporated; dermal; drinking)	10 µg g ⁻¹	4 µg L ⁻¹	Reduce threshold values by 1/2 in presence of 2 sub-threshold values.







REFERENCES

- Baker P.D. & Humpage A.R. (1994). Toxicity Associated with Commonly Occurring Cyanobacteria in Surface Waters of the Murray-Darling Basin, Australia. *Australian Journal of Marine and Freshwater Research* 45, 773-86.
- Beattie K.A., Ressler J., Wiegand C., Krause E., Codd G.A., Steinberg C. & Pflugmacher S. (2003). Comparative effects and metabolism of two microcystins and nodularin in the brine shrimp *Artemia salina*. *Aquatic Toxicology* 62, 219-226.
- Berg K., Skulberg O.M. & Skulberg R. (1987). Effects of decaying toxic blue-green algae on water quality - a laboratory study. *Arch. Hydrobiol* 108(4), 549-563.
- Best J.H., Eddy F.B. & Codd G.A. (2001). Effects of purified microcystin-LR and cell extracts of *Microcystis* strains PCC 7813 and CYA 43 on cardiac function in brown trout (*Salmo trutta*) alevins. *Fish Physiology and Biochemistry* 24, 171-178.
- Bickel H., Lyck S. & Utkilen H. (2000). Energy state and toxin content - experiments on *Microcystis aeruginosa* (Chroococcales, Cyanophyta). *Phycologia* 39(3), 212-218.
- Boon P.I., Bunn S.E., Green J.D. & Shiel R.J. (1994). Consumption of Cyanobacteria by Freshwater Zooplankton: Implications for the Success of 'Top-down' Control of Cyanobacterial Blooms in Australia. *Australian Journal of Marine and Freshwater Research* 45, 875-887.
- Botes D.P., Tuiman A.A., Wessels P.L., Viljoen C.C., Kruger H., Williams D.H., Santikarn S., Smith R.J. & Hammond S.J. (1984). The structure of cyanoginosin-LA, a cyclic heptapeptide toxin from the cyanobacterium *Microcystis aeruginosa*. *J Chem Soc Perkin Trans 1*, 2311-2318.
- Bouvy M., Falcão D., Marinho M., Pagano M. & Moura A. (2000). Occurrence of *Cylindrospermopsis* (Cyanobacteria) in 39 Brazilian tropical reservoirs during the 1998 drought. *Aquatic Microbial Ecology* 23, 13-27.
- Byth S. (1980). Palm Island Mystery Disease. *Medical Journal of Australia* 2, 40-42.
- Carmichael W.W. (1996). Toxic *Microcystis* and the environment. In: Watanabe, Harada, Carmichael and Fujiki. *Toxic Microcystis*. Boca Raton, CRC Press: 1-12.
- Carmichael W.W. (2001). Health effects of Toxin-Producing Cyanobacteria: 'The CyanoHABs'. *Human and Ecological Risk Assessment* 7(5), 1393-1407.
- Carmichael W.W., Azevedo S.M., Molica R., Jochimsen E.M., Lau S., Rinehart K.L., Shaw G. & Eaglesham G. (2001). Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environmental Health Perspectives* 109(7), 663-668.

- Carmichael W.W. & Falconer I.R. (1993). Diseases related to freshwater blue-green algal toxins, and control measures. In: Falconer. Algal toxins in seafood and drinking water. Sydney, Academic Press.
- Carson B. 2000. Cylindrospermopsin - A review of toxicological literature. North Carolina, USA, National Institute of Environmental Health Services.
- Casanova M.T., Burch M.D., Brock M.A. & Bond P.M. (1999). Does toxic *Microcystis aeruginosa* affect aquatic plant establishment? *Environmental Toxicology* 14, 97-109.
- Chiswell R.K., Shaw G.R., Eaglesham G., Smith M.J., Norris K.R., Seawright A.A. & Moore M.R. (1999). Stability of cylindrospermopsin, the toxin from the cyanobacterium, *Cylindrospermopsis raciborskii*: Effect of pH, temperature and sunlight on decomposition. *Environmental Toxicology* 14, 155-161.
- Chong M.W.K., Gu K.D., Lam P.K.S., Yang M. & Fong W.F. (2000). Study on the cytotoxicity of microcystin-LR on cultured cells. *Chemosphere* 41, 143-147.
- Chorus I., Ed. (2001). Cyanotoxins Occurrence, Causes, Consequences. Springer-Verlag, Berlin.
- Chorus I. & Bartram J., Eds. (1999). Toxic cyanobacteria in water:: A guide to their public health consequences, monitoring and management. Published on behalf of the World Health Organisation by E & FN Spon, London.
- Chorus I., Neisel V., Fastner J., Wiedner C., Nixdorf B. & Lindenschmidt K. (2001). Environmental Factors and Microcystin Levels in Waterbodies. In: Chorus. Cyanotoxins Occurrence, Causes and Consequences. Berlin, Springer-Verlag.
- Christoffersen K. (1996). Ecological implications of cyanobacterial toxins in aquatic food webs. *Phycologia* 35(6 Supplement), 42-50.
- Connell D.W., Chaisuksant Y. & Yu J. (1999). Importance of internal biotic concentrations in risk evaluations with aquatic systems. *Marine Pollution Bulletin* 39(1-12), 54-61.
- Dawson R.M. (1998). The Toxicology of Microcystins. *Toxicon* 37(7), 953-962.
- Delaney J.M. & Wilkins R.M. (1995). Toxicity of Microcystin-LR, isolated from *Microcystis aeruginosa*, against various insect species. *Toxicon* 33(6), 771-778.
- Duy T.N., Lam P.K.S., Shaw G.R. & Connell D.W. (2000). Toxicology and Risk Assessment of Freshwater Cyanobacterial (Blue-Green Algal) Toxins in Water. *Review of Environmental Contamination and Toxicology* 163, 113-186.

- Eriksson J.E., Meriluoto J. & Lindholm T. (1989). Accumulation of a peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anodonta cygnea*. *Hydrobiologia* 183, 211-216.
- Falconer I.C., Jackson A.R., Langley J. & Runnegar M. (1981). Liver pathology in mice in poisoning by the blue-green algae *Microcystis aeruginosa*. *Australian Journal of Biological Sciences* 34, 179-87.
- Falconer I.R. (1993). Mechanism of toxicity of cyclic peptide toxins from blue-green algae. In: Falconer. Algal toxins in seafood and drinking water. Sydney, Academic Press.
- Falconer I.R. (1999). An overview of problems caused by toxic blue-green algae (Cyanobacteria) in drinking and recreational water. *Environmental Toxicology* 14, 5-11.
- Falconer I.R. (2001). Toxic cyanobacterial bloom problems in Australian waters: Risks and impacts on human health. *Phycologia* 40(3), 228-233.
- Falconer I.R., Hardy S.J., Humpage A.R., Froscio S.M., Tozer G.J. & Hawkins P.R. (1999). Hepatic and renal toxicity of the blue-green alga (Cyanobacterium) *Cylindrospermopsis raciborskii* in Male Swiss Albino Mice. *Environmental Toxicology* 14, 143-150.
- Ferrão-Filho A. & Azevedo S.M.F.O. (2003). Effects of unicellular and colonial forms of toxic *Microcystis aeruginosa* from laboratory cultures and natural populations on tropical cladocerans. *Aquatic Ecology* 37, 23-35.
- Ferrão-Filho A., Domingos P. & Azevedo M.T.P. (2002). Influences of a *Microcystis aeruginosa* Kützinger bloom on zooplankton populations in Jacarepaguá Lagoon (Rio de Janeiro, Brazil). *Limnologia* 32, 295-308.
- Ferrão-Filho A., Kozłowsky-Suzuki B. & Azevedo S.M.F.O. (2002b). Accumulation of microcystins by a tropical zooplankton community. *Aquatic Toxicology* 59, 201-208.
- Fischer W.J., Hitzfield B.C., Tencalla F., Eriksson J.E., Mihailov A. & Dietrich D.R. (2000). Microcystin-LR toxicodynamics, induced pathology, and immunohistochemical localisation in livers of blue-green algae exposed rainbow trout (*Onorhynchus mykiss*). *Toxicological Sciences* 54, 365-373.
- Frank C. (2002). Microcystin-producing cyanobacteria in recreational waters in southwestern Germany. *Environmental Toxicology* 17(4), 361-366.
- Franke C., Studinger G., Berger G., Böhling S., Bruckman U., Cohors-Fresenborg D. & Jöhncke U. (1994). The assessment of bioaccumulation. *Chemosphere* 29(7), 1501-1514.

- Griffiths D.J. & Saker M. (2003). The Palm Island Mystery Disease 20 Years on: A review of research on the cyanotoxin cylindrospermopsin. *Environmental Toxicology* 18(2), 78-93.
- Harada K.I. (1996). Chemistry and detection of microcystins. In: Watanabe, Harada, Carmichael and Fujiki. *Toxic Microcystis*. Boca Raton, CRC Press: 103-148.
- Hawkins P.R., Chandrasena N.R., Jones G.J., Humpage A.R. & Falconer I.R. (1997). Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon* 35, 341-346.
- Hawkins P.R., Putt E., Falconer I.R. & Humpage A.R. (2001). Phenotypical variation in a toxic strain of the phytoplankter, *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) during batch culture. *Environmental Toxicology* 16(6), 460-467.
- Hawkins P.R., Runnegar M.T.C., Jackson A.R.B. & Falconer I.R. (1985). Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Applied and Environmental Microbiology* 50(5), 1292-1295.
- Heinze R. (1999). Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with drinking water. *Environmental Toxicology* 14, 57-60.
- Hiripi L., Nagy L., Kalmar T., Kovacs A. & Voros L. (1998). Insect (*Locusta Migratoria Migratorioides*) test monitoring the toxicity of cyanobacteria. *Neurotoxicology* 19(4-5), 605-608.
- Hoeger S.J., Shaw G.R., Hitzfield B.C. & Dietrich D.R. (2004). Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants. *Toxicon* 43, 639-649.
- Jacoby J.M., Collier D.C., Welch E.B., Hardy F.J. & Crayton M. (2000). Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. *Canadian Journal of Fisheries and Aquatic Sciences* 57(1), 231-240.
- Jones G., Baker P.D., Burch M.D. & Harvey F.L. (2002). National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Waters. Draft V5.0 for consideration LWBC, July 2002.
- Kirpenko N.I. (1986). Phytopathic properties of blue-green algae toxin. *Hydrobiological Journal* 22(1), 44-47.
- Kiss T., Vehovsky Á., Hiripi L., Kovács M. & Vörös L. (2002). Membrane effects of toxins isolated from a cyanobacterium, *Cylindrospermopsis raciborskii*, on identified molluscan neurones. *Comparative Biochemistry and Physiology Part C* 131, 167-176.

- Komarek J., Azevedo S., Domingos P., Komarkova J. & Tichy M. (2001). Background of the Caruaru tragedy; a case taxonomic study of toxic cyanobacteria. *Algological studies* 103, 9-29.
- Kotak B.G., Zurawell R., Prepas E. & Holmes C.F. (1996). Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status. *Canadian Journal of Fisheries and Aquatic Sciences* 53, 1974-1985.
- Lahti K., Niemi M., Rapala J. & K. S. (1997a). Biodegradation of cyanobacterial hepatotoxins - characterisation of toxin degrading bacteria. In: Fernandez and Watt. Harmful Algae Algas Nocivas, VIII International Conference on Harmful Algae. Grafisant, Spain.
- Lahti K., Rapala J., Färdig M., Niemelä M. & Sivonen K. (1997b). Persistence of cyanobacterial hepatotoxin, Microcystin-LR in particulate material and dissolved in lake water. *Water Research* 31(5), 1005-1012.
- Landsberg J.H. (2002). The effects of harmful algal blooms on aquatic organisms. *Reviews in Fisheries Science* 10(2), 113-390.
- Li R., Carmichael W., Brittain S., Eaglesham G., Shaw G., Liu Y. & Watanabe M. (2001). First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (Cyanobacteria). *Journal of Phycology* 37(7).
- Lirås V., Lindberg M., Nystrom P., Annadotter H., Lawton L. & Graf B. (1998). Can ingested cyanobacteria be harmful to the signal crayfish (*Pacifastacus leniusculus*)? *Freshwater Biology* 39, 233-242.
- Liu Y., Song L., Li X. & Liu T. (2002). The toxic effects of microcystin-LR on embryo-larval and juvenile development of loach, *Misguruns mizolepis* Gunthe. *Toxicon* 40, 395-399.
- Long B.M., Jones G.J. & Orr P.T. (2001). Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. *Applied and Environmental Microbiology* 67(1), 278-283.
- MacKintosh C., Beattie K.A., Klumpp S., Cohen P. & Codd G.A. (1990). Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. *FEBS Letters* 264(2), 187-192.
- Magalhães V.F., Soares R. & Azevedo S. (2001). Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. *Toxicon* 39, 1077-1085.
- Markich S.J. & Camilleri C. (1996). Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian Water Quality Guidelines. Internal Report 223. Office of the Supervising Scientist of the Alligator Rivers Region, Jabiru. 75

- Matveev V., Matveeva L. & Jones G.J. (1994). Study of the ability of *Daphnia carinata* King to control phytoplankton and resist cyanobacterial toxicity: Implications for biomanipulation in Australia. *Australian Journal of Marine and Freshwater Research* 45, 899-904.
- McCarty L.S. & Mackay D. (1993). Enhancing ecotoxicological modelling and assessment. *Environmental Science and Technology* 27(9), 1719-1728.
- McElhiney J., Lawton L. & Leifert C. (2001). Investigations into the inhibitory effects of microcystins in plant growth, and the toxicity of plant tissues following exposure. *Toxicon* 39, 1411-1420.
- Mohamed Z.A. (2001). Accumulation of cyanobacterial heptatotoxins by *Daphnia* in some Egyptian irrigation channels. *Ecotoxicology and Environmental Safety* 50, 4-8.
- Nicholson B.C. & Burch M.D. (2001). Evaluation of Analytical Methods for Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines. Cooperative Research Centre for Water Quality and Treatment; Prepared for the National Health and Medical Research Council of Australia., Canberra.
- Norris K.R., Seawright A.A., Shaw G., Senogles P., Eaglesham G., Smith M.J., Chiswell R.K. & Moore M.R. (2002). Hepatic xenobiotic metabolism of cylindrospermopsin *in vivo* in the mouse. *Toxicon* 40, 471-476.
- Oberemm A. (2001). Effects of cyanotoxins on early life stages of fish and amphibians. In: Chorus. Cyanotoxins: Occurrence, Causes, Consequences. Berlin, Springer-Verlag.
- Oberemm A., Becker J., Codd G.A. & Steinberg C. (1999). Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. *Environmental Toxicology* 14, 77-88.
- Ohtani I., Moore R.E. & Runnegar M.T.C. (1992). Cylindrospermopsin: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *Journal of the American Chemical Society* 114,, 7941-7942.
- Orr P. & Jones G. (1998). Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnology and Oceanography* 43(7), 1604-1614.
- Ozawa K., Yokoyama A., Ishikawa K., Kumagi M., Watanabe M. & Park H.-D. (2003). Accumulation and depuration of microcystin produced by the cyanobacterium *Microcystis* in a freshwater snail. *Limnology* 4, 131-138.
- Padisák J. (1997). *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding highly adaptive cyanobacterium: worldwide distribution

- and review of its ecology. *Archiv fuer Hydrobiologie. Supplement 107 (Monographic Studies)* 4, 563-593.
- Pflugmacher S. (2002). Possible allelopathic effects of cyanotoxins, with reference to Microcystin-LR, in Aquatic Ecosystems. *Environmental Toxicology* 17, 407-413.
- Prepas E.E., Kotak B.G., Campbell L.M., Evans J.C., Hrudey S.E. & Holmes C.F. (1997). Accumulation and elimination of cyanobacterial heptatotoxins by the freshwater clam *Anodonta grandis simpsonia*. *Canadian Journal of Fisheries and Aquatic Sciences* 54(1), 41-46.
- QDNR (2003). Queensland Harmful Algal Bloom Operational Procedures. Harmful Algal Blooms Steering Committee, Queensland Department of Natural Resources and Mines., Rocklea.
- Rinehart K.L., Namikoshi M. & Choi B.W. (1994). Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *Journal of Applied Phycology* 6, 159-176.
- Romanowska-Duda Z. & Tarczyska M. (2002). The influences of Microcystin-LR and Hepatotoxic Cyanobacterial Extract on the Water Plant *Spirodela oligorrhiza*. *Environmental Toxicology* 17, 434-440.
- Runnegar M.T., Kong S.M., Zhong Y.Z., Ge J.L. & Lu S.C. (1994). The role of glutathione in the toxicity of a novel cyanobacterial alkaloid cylindrospermopsin in cultured rat hepatocytes. *Biochemical and Biophysical Research Communications* 201(1), 235-241.
- Saker M. & Neilan B.A. (2001). Varied diazotrophies, morphologies and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from Northern Australia. *Applied and Environmental Microbiology* 67(4), 1839-1845.
- Saker M., Thomas A.D. & Norton J.H. (1999). Cattle mortality attributed to the toxic cyanobacterium *Cylindrospermopsis raciborskii* in an outback region of North Queensland. *Environmental Toxicology* 14, 179-182.
- Saker M.L. & Eaglesham G.K. (1999). The accumulation of cylindrospermopsin from the cyanobacterium *Cylindrospermopsis raciborskii* in tissues of the Redclaw crayfish *Cherax quadricarinatus*. *Toxicon* 37, 1065-1077.
- Saker M.L. & Griffiths D.J. (2000). The effect of temperature on growth and cylindrospermopsin content of seven isolates of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju from water bodies in northern Australia. *Phycologia* 39(4), 349-354.
- Saker M.L., Metcalf J.S., Codd G.A. & Vasconcelos V.M. (2004). Accumulation and depuration of the cyanobacterial toxin cylindrospermopsin in the freshwater mussel *Anodonta cygnea*. *Toxicon* 43, 185-194.

- Schembri M.A., Neilan B.A. & Saint C.P. (2001). Identification of genes implicates in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. *Environmental Toxicology* 16, 413-421.
- Shaw G., Seawright A.A., Moore M.R. & Lam P.K.S. (2000). Cylindrospermopsin, A Cyanobacterial Alkaloid: Evaluation of Its Toxicologic Activity. *Therapeutic Drug Monitoring* 22, 89-92.
- Shaw G., Sufenik A., Livne A., Chiswell R.K., Smith M.J., Seawright A.A., Norris K.R., Eaglesham G. & Moore M.R. (1999). Blooms of the cylindrospermopsin containing cyanobacterium, *Aphanizomenon ovalisporum* (Forti) in newly constructed lakes, Queensland, Australia. *Environmental Toxicology* 14, 167-177.
- Shen X., Lam P.K.S., Shaw G.R. & Wiickramasinghe W. (2002). Gentoxicity investigation of a cyanobacterial toxin, cylindrospermopsin. *Toxicon* 40, 1499-1501.
- Sijm D.T.H. & Hermens J.L. (2000). Internal effect concentration: link between bioaccumulation and ecotoxicity for organic chemicals. In: Beek. Bioaccumulation New Aspects and Developments. Berlin, Springer: 167-233.
- Sivonen K. & Jones G. (1999). Cyanobacterial toxins. In: Chorus and Bartram. Toxic cyanobacteria in water. London, E & FN Spoon.
- Tencalla F. & Deitrich D. (1997). Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*). *Toxicon* 35(4), 583-595.
- Terao K., Ohmori S., Igarshi K., Ohtani I., Watanabe M.F., Harada K.I., Ito E. & Watanabe M. (1994). Electron microscope studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*. *Toxicon* 32(7), 833-843.
- Ueno Y., Makita Y., Nagata S., Tsutsumi T., Yoshida F., Tamura S. & al e. (1999). No chronic oral toxicity of a low dose of microcystin-LR, a cyanobacterial hepatotoxin, in female BALB/c mice. *Environmental Toxicology* 14, 45-55.
- Utkilen H. & Gjølme N. (1995). Iron-stimulated Toxin Production in *Microcystis aeruginosa*. *Applied and Environmental Microbiology* 61(2), 797-800.
- van der Oost R., Beyer J. & Vermeulen N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13, 57-149.
- Vasas G., Gáspár A., Surányi G., Batta G., Gyémánt G., M-Hamvas M., Máthé C., I. G., Molnár E. & Borbély G. (2002). Capillary Electrophoretic Assay and Purification of Cylindrospermopsin, A cyanobacterial toxin from *Aphanizomenon ovalisporum*, by plant test (Blue-Green *Sinapis* Test). *Analytical Biochemistry* 302, 95-103.

- Vasconcelos V.M. (1995). Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytilus galloprovincialis*. *Aquatic Toxicology* 32(2), 227-237.
- Vasconcelos V.M., Oliveira S. & Teles F.O. (2001). Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. *Toxicon* 39, 1461-1470.
- Vesterkvist P.S.M. & Meriluoto J.A.O. (2003). Interaction between microcystins of different hydrophobicities and monolayers. *Toxicon* 41, 349-355.
- Ward C.J. & Codd G.A. (1999). Comparative toxicity of four microcystins of different hydrophobicities to the protozoan, *Tetrahymena pyriformis*. *Journal of Applied Microbiology* 86, 874-882.
- Watanabe M.F., Tsuji K., Watanabe Y., Harada K.I. & Suzuki M. (1992). Release of a heptapeptide toxin (microcystin) during the decomposition process of *Microcystis aeruginosa*. *Natural toxins* 1, 48-53.
- Webster I. (1990). Effect of wind on the distribution of phytoplankton cells in lakes. *Limnology and Oceanography* 35(5), 989-1001.
- Wiedner C., Visser P.M., Fastner J., Metcalf J.S., Codd G.A. & Mur L.R. (2003). Effects of light on the microcystin content of *Microcystis* strain PCC 7806. *Applied and Environmental Microbiology* 69(3), 1475-1481.
- Wiegand C. & Pflugmacher S. (2001). Uptake, Enzyme Effects and Metabolism of MC-LR in Aquatic Organisms. In: Chorus. Cyanotoxins Occurrence, Causes, Consequences. Berlin, Springer-Verlag.
- Williams D.E., Dawe S.C., Kent M.L., Andersen R.J., Craig M. & Holmes C.F. (1997). Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus edulis*, and *in vivo* evidence for covalently bound microcystins in mussel tissues. *Toxicon* 35(11), 1617-1625.
- Xie L., Xie P., Ozawa K., Honma T., Yokoyama A. & Park H.-D. (2004). Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. *Environmental Pollution* 127, 431-439.
- Xu L.H., Lam P.K.S., Chen J.P., Xu J.M., Wong B.S.F., Zhang Y.Y., Wu R.S.S. & Harada K.I. (2000). Use of protein phosphatase inhibition assay to detect microcystins in Donghu Lake and a fish pond in China. *Chemosphere* 41, 53-58.
- Yokoyama A. & Park H. (2002). Mechanism and prediction for contamination of freshwater bivalves (Unionidae) with the cyanobacterial toxin microcystin in hypereutrophic Lake Suwa, Japan. *Environmental Toxicology* 17(5), 424-433.

- Yokoyama A. & Park H. (2003). Depuration kinetics and persistence of the cyanobacterial toxin microcystin-LR in the freshwater bivalves *Unio douglasiae*. *Environmental Toxicology* 18(1).
- Zimba P.V., Gaunt P.S., Brittain S. & Carmichael W. (2001). Confirmation of catfish, *Ictalurus punctatus* (Rafinesque) mortality from *Microcystis* toxins. *Journal of Fish Diseases* 24, 41-47.
- Zurawell R. (2001). Occurrence and toxicity of microcystins in the freshwater pulmonate snail *Lymnaea stagnalis*. Ph D thesis, University of Alberta, Department of Biological Sciences. 242 p.