Recent Advances in Cyanobacterial Research

Abstracts from the third national meeting
23 & 24 August, 2012
Canberra, ACT

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Abstracts from the third National Cyanobacterial Workshop

23-24 August 2012, Canberra

This National Cyanobacterial Workshop was hosted by CSIRO's Water for a Health Country Flagship – Ecosystems and Contaminants Theme

Thanks to Dr Carol Couch, Ecosystems and Contaminants Theme Leader,for her generous support towards this workshop.

Attending the workshop were people involved in the management of public health, water resources and water supplies, and core cyanobacterial research.

The workshop was organised by **Dr Tim Malthus**, Research Program Leader, Environmental Earth Observation, CSIRO Land & Water

Together with the following individuals who participated on the organizing committee, reviewed abstracts and provided valuable advice:

Ms Janet Anstee – CSIRO

Dr Nagur Cherukuru – CSIRO

Dr Erin Hestir – CSIRO

Dr Arnold Dekker – CSIRO

Dr Barbara Robson – CSIRO

Dr Brad Sherman – CSIRO

Dr Lee Bowling – NSW Office of Water

Ms Vanora Mulvenna – Department of Health Victoria

Ms Rachael Poon – Department of Health Victoria

Ms Nicole Stals – CSIRO

*Cover photographs, of 2010 algal blooms on the Murray River, kindly supplied by **Vanora Mulvenna**, Department of Health, Victoria*

Book of abstracts compiled by Nicole Stals, CSIRO Land and Water, Canberra

3rd National Cyanobacterial Workshop Program

Day 1 – Thursday 23rd August					
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9:00	**9:20**	**Registration and coffee**			
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13:50	14:10	Incident management and case studies	Stuart Khan	Modelling the fate of cyanobacterial toxins in a drinking water reservoir	19
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15:50	16:10	Taxonomy/Ecology/ Scientific process and insight	Olivia Daniels, Larelle Fabbro, Sandrine Makiela and Catherine Bernard	Growth of the toxic cyanobacterium *Limnothrix* (strain AC0243) at various light intensities, temperatures and salinities	26
16:10	16:30	Taxonomy/Ecology/ Scientific process and insight	M. A. Burford, T.W. Davis, P. Muhid and M.J. Prentice	Nutrient utilization strategies for phytoplankton in stratified subtropical reservoirs	28
16:30	16:50	Taxonomy/Ecology/ Scientific process and insight	Fariba Moslih, Jenny Davis and Lien Sim	Allelopathic activity of a freshwater macroalga, *Chara australis*, and a submerged macrophyte, *Potamogeton crispus*, on microalgae	29
16:50	17:10	Taxonomy/Ecology/ Scientific process and insight	Elke S. Reichwaldt and Anas Ghadouani	Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: Between simplistic scenarios and complex dynamics	30
17:10	17:30	Taxonomy/Ecology/ Scientific process and insight	Susie Wood, Mark Heath, Roger Young and Ken Ryan	Toxic benthic cyanobacteria – an underestimated risk?	31
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9:40	10:00	Toxicity	Paul Whan	Identification of a novel *Limnothrix* sourced metabolite - Ph.D. University of Adelaide, 2012	33
10:00	10:20	Toxicity	Ian Stewart, Wasantha Wickramasinghe, Geoff Eaglesham, Anthony Carroll, Alan Seawright, Glenn McGregor, Glen Shaw	The cylindrospermopsin analogue deoxycylindrospermopsin: isolation, purification and acute toxicity in mice	34
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11:20	11:40	New methods in monitoring and management	Andrew Humpage, Melody Lau, Virginie Gaget, Barbara Sendall, Somprasong Laingam	CyanoSurvey – Comparison of Methods for the Detection of Cyanobacterial Toxins	40
11:40	12:00	New methods in monitoring and management	Glenn B. McGregor, Barbara C. Sendall	Cryptic toxicity: non-planktonic cyanobacteria represent a significant potential source of cyanotoxins in the freshwater environment	42
12:00	12:20	New methods in monitoring and management	Jonathan Yu, Kerry Taylor, Brad Sherman	Semantics-based approach for defining complex event processing events for real-time algal bloom detection	44
12:20	13:30	Lunch			
13:30	13:50	Wastewater treatment	Jennifer Dreyfus, Albane Barbero, Lionel Ho, David Dixon, Peter Scales, Werner Mobius, Jek Rozitis, Gayle Newcombe	Fate of intracellular geosmin and saxitoxins during simulated lagoon treatment of cyanobacterial sludge	45
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				Australia – an idea	
14:10	14:30	Forecasting and climate change	Friedrich Recknagel, Philip Orr and Hongqing Cao	Inductive reasoning and prediction of population dynamics of *Cylindrospermopsis* in the Wivenhoe Reservoir by means of evolutionary computation	48
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15:00	16:00	Discussion			
		Posters:	Stefania Sotora, Melody Lau, Somprasong Laingam, Andrew Humpage	Characterisation of a novel toxin from *Anabaena circinalis*	
			Milt Baker	Satellite Monitoring and Ground Sampling as a Best Management Practice for Water Quality Improvement Whole-Lake Satellite Monitoring for Total Phosphorus, Cyanobacteria and Chlorophyll-a	
			Tim Malthus	The case for a global inland water quality product using remote sensing	
			Jenny Skerratt	Modelling Lyngbya in coastal environments	
			Andrew Bradbury	Low Level Shellfish Toxin Detection by ULCMS-MS	
			Bianca Huider	Simply Unpredictable: The fickle Cyanobacteria of Lake Eppalock, Victoria	

Keynote Address: Examining 30 years of phytoplankton data for the Murray River, with particular emphasis on the factors determining the presence and abundance of cyanobacteria

Croome R[12], Wheaton L[1], Henderson B[3], Oliver R[3], Vilizzi L[1], Paul W[2] and McInerney P[1]

[1]Murray-Darling Freshwater Research Centre, [2]La Trobe University, [3]CSIRO Land and Water.

Weekly assessment of phytoplankton in the Murray and its major tributaries was initiated by the River Murray Commission in 1980. Continued now by the Murray-Darling Basin Authority, the database is an environmental resource of world standard.

Three MDBA databases (phytoplankton, water quality and hydrology) were used in an overarching statistical assessment of the phytoplankton for 1980-2008, relating their presence and abundance to ecological drivers identified in a Causal Model.

Plotting of the raw data showed the consistent presence of cyanobacteria system wide, for much of the time at >1,000 cells/mL, with *Anabaena* making a large contribution. Occurrences of cyanobacteria at >5,000 cells/mL were frequent for 2002-2008.

Wavelet analysis was utilised to investigate cyanobacterial blooms over time, and indicated their frequency within the system as a whole did not change markedly over the 30 years of record, remaining within a one to four year range, with higher frequencies at the upstream sites and lower frequencies at the downstream sites. However, there was an increase in the duration of individual blooms observed at the upstream sites in the 2000s, and this accords with the ever increasing concentrations of *Anabaena, Aphanizomenon, Microcystis* and other cyanobacteria observed.

Multivariate analysis of the data as a whole indicated seasonality and a long-term trend in community composition, with discharge and water temperature being important, but not the whole explanation. An increase in cyanobacteria below Lake Hume was related to decreasing water levels within the storage.

Generalised Additive Models were used to describe changes over time and season along the main stem of the Murray, involving nine taxa for the period 1994-2008.

Substantial, consistent and statistically significant increases in phytoplankton counts were found across almost all sites and taxa, with the greatest changes occurring further up the river, particularly at Heywoods Bridge immediately downstream of Lake Hume.

Tailem Bend was different to other sites, and generally showed the least change, although it did exhibit substantial increases in *Anabaena* and *Aphanizomenon*. It exhibited low counts for both *Aulacoseira granulata* and *Aulacoseira distans* and also Total Cyanobacteria, other than a strong increase in the last few years.

Two sites (Heywoods and Swan Hill) were examined in more detail in order to consider the relationship between phytoplankton abundance and key environmental variables. While this part of the analysis was more exploratory, important associations and differences between sites were found, worthy of further investigation.

Notes

Of droughts and flooding rains – cyanobacterial presence in NSW over the past 5 summers

Lee Bowling
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The past 5 summers have seen some extremes in weather conditions across NSW, with the initial 3 years experiencing drought, and the past 2 summers with high rainfall and flooding in various parts of the state. The number and persistence of cyanobacterial blooms across NSW since July 2007 has been investigated to assess whether these climatic conditions may have impacted on bloom occurrence. Recreational alert level data accumulated by the NSW Office of Water was assessed. Despite annual differences in sampling effort and reporting by some agencies supplying data, these data provide a general overview of cyanobacterial presence across NSW.

Statewide, the number of locations reported with Red alerts increased during the last 3 years of the drought, then fell during the following 2 years of higher rainfall. The number of Amber and Green alerts however changed little. Bloom persistence, measured as the cumulative number of weeks all sites were on alert, showed only minor variation over the 5 year period.

Differences were more marked in different regional areas of NSW. Cyanobacterial presence in some coastal areas (e.g. Hunter) and northern and central inland (e.g. Barwon) regions showed less variation over the 5 year period. Many blooms in these regions of NSW are associated with reservoirs or small urban water bodies, especially in the Newcastle and Sydney areas, and riverine cyanobacterial blooms are less common. In comparison there was a reduction in cyanobacterial presence, and especially Red alerts after the breaking of the drought in the southern (e.g. Murray) and western (e.g. Far West) regions of inland NSW. There are far fewer standing water bodies in these parts of the state, and most cyanobacterial presence in dry years is associated with rivers. Higher rainfall in catchment areas meant higher flows in these rivers and a lower cyanobacterial presence.

Notes

Phytoplankton blooms in inland floodplain lakes

Tsuyoshi Kobayashi, Stephen J. Jacobs and Simon J. Hunter

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Inland floodplain lakes are often shallow, turbid, wind-exposed and high in nutrients. They may provide aquatic habitats that are conducive to phytoplankton blooms. During the summer of 2012 (January and February), we measured the productivity and biomass of phytoplankton and physico-chemical conditions of three floodplain lakes (Tala, Paika and Yanga, with approximate maximal depths of 2-8 m) in the lower Murrumbidgee River floodplain (143°40'E/34°35'S) where cyanobacteria including *Anabaena*, *Microcystis* and *Oscillatoria* were identified. The gross productivity of phytoplankton (GPP, mean±SE, n=4) ranged from 230.5±18.1 to 4995.3±47.1 µg C L^{-1} day^{-1} in the surface layer (>0.3 m depth) between the lakes. The biomass (as chlorophyll-*a*) of phytoplankton ranged from 31.5±12.0 to 147.8±1.9 µg L^{-1}. A preliminary correlation analysis of GPP with nutrient data indicates that relative supply (i.e. stoichiometry) of nitrogen and phosphorus may be more important than their concentration per se in controlling the GPP in the lakes.

Notes

Unusual Presence of Cyanobacteria: A Sydney Experience

Bala Vigneswaran

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Lake Nepean, located below a 320 square kilometre catchment, can retain 70,000 ML of water at its full supply level. The land within the catchment is predominantly either natural bushland or land used for grazing and cropping. Other landuses include forestry, intensive agriculture and mining. Prior to 2011, cyanobacteria have remained at the background levels, well below the alert threshold. However, spikes of *Microcystis aeruginosa* have occurred following large inflows in 2011 and 2012.

Following an inflow in March – April 2011, a *Microcystis aeruginosa* population of up to 6000 cell/mL and toxin content of around 1 µg/L microcystin-LR toxicity equivalent (peak value of 1.26 µg/L) were detected in Lake Nepean in May – June 2011. Such populations or toxin contents were never reported in the past in Lake Nepean. *Microcystis aeruginosa* was fairly evenly distributed horizontally across three monitoring sites from the dam wall towards the upper reaches, which suggested that the growth of *Microcystis aeruginosa* was not a result of localised conditions near the dam wall. The vertical variations in *Microcystis aeruginosa* population at multiple locations showed that the cell counts remained high, above the Minor Alert threshold, at depths to 30 metres. This phenomenon is unusual, as populations sustained over two months at those depths. The potential for diurnal migration of cells to cause large transient populations in deeper layers was also tested, but there was no evidence to indicate appreciable vertical migration.

Inflows in February - March 2012 brought significant quantities of water to Lake Nepean and caused a spill. The peak flow in 2012 was nearly thrice as much of the peak flow in 2011. Considering the nutrient levels and the timing of both events, *Microcystis aeruginosa* and toxin detections of similar magnitude were expected in 2012 as well. However, unlike the 2011 cyanobacteria growth, the population and population distribution were moderate in 2012 in Lake Nepean. Although a single sample revealed a toxin concentration of 1.9 µg/L in Lake Nepean in 2012, exceeding the Australian Drinking Water Guidelines (NHMRC 2011), high concentrations did not prevail.

This paper discusses the unusual presence of *Microcystis aeruginosa* population in 2011 and a preliminary comparative analysis of water quality and hydrodynamics data in 2011 and 2012, and shares the lessons learned during and following the inflow events with respect to cyanobacteria.

Notes

Application of cyanobacterial toxin health guideline values in the Gippsland Lakes

R. Poon[1], V. Mulvenna[1], K. Dale[2], B. Priestly[3], U. Mueller[4], A. Humpage[5], G. Shaw[6], G. Allinson[7], I. Falconer[8] and R. Dedman[1]

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Cyanobacterial blooms are a common occurrence in the Gippsland Lakes situated in south-east Victoria. Fish, eels and prawns are harvested commercially within the Gippsland Lakes alongside a significant recreation fishery for fish, mussels, prawns and crustaceans. An inshore prawn fishery also operates outside of Lakes Entrance where prawns are landed for commercial sale. *Nodularia spumigena* previously bloomed in the Gippsland Lakes in 2001. Nodularin toxin was produced and seafood sampling determined uptake of nodularin toxin in fin fish, mussels and prawns. The levels of nodularin detected in seafood samples led to restrictions in commercial seafood harvest and recreation fishing in the Gippsland Lakes. The 2001 health guideline levels for cyanobacterial toxin in seafood have recently been reviewed using a standard human health risk assessment approach. The latest nutritional survey information and seafood consumption rates were taken into consideration to inform the health guideline values. The updated health guideline values have been published and applied in the N. spumigena bloom that occurred in the Gippsland Lakes from September 2011 till April 2012. During the bloom seafood samples were collected from fish, mussels and prawns for toxin testing. Levels of nodularin toxin exceeded the health guideline levels in whole fish, mussels and prawns. Health advisories were issued based on seafood sampling results and health advisories were lifted when sampling detected nodularin below health guideline levels.

Notes

What we learnt from the Gippsland Lakes *Nodularia* bloom of 2011-2012

Daryl Holland[1], Ryan Woodland[2], John Beardall[1] and Perran Cook[2]

[1]Monash University School of Biological Sciences

[2]Monash University Water Studies Centre

A widespread and persistent toxic bloom of *Nodularia spumigena* occurred in the estuarine Gippsland Lakes between December 2011 and April 2012. Toxins were detected in fish, prawns and mussels, leading to restrictions on both commercial and recreational fishing. Warnings were also issued regarding water contact, leading to a general loss of amenity. These blooms were a common occurrence during the 1980s and 90s, but this was the first such bloom since 2002. The conditions leading up to this bloom mirrored those that occurred before similar blooms in the past: high winter-spring inflows, low salinity and low dissolved nitrogen.

Plant and algal growth in the Gippsland Lakes is typically nitrogen limited, especially during the summer. *Nodularia*, on the other hand, is typically phosphorus limited, as it has the ability to incorporate atmospheric nitrogen, which is abundant, into its cells. We investigated the relationship between *Nodularia* and nitrogen, and whether the new nitrogen that *Nodularia* introduced into the system was used by other organisms. By analysing the natural abundances of nitrogen isotopes, we found that the *Nodularia* were fixing large amounts of atmospheric nitrogen and that this nitrogen was then moving through the food web and into the zooplankton. Paradoxically, we also found that the *Nodularia*, were, by and large, not being eaten by the zooplankton, while other phytoplankton species, particularly diatoms, were heavily grazed. We conclude that the atmospherically derived nitrogen was being released by the *Nodularia* and then recycled through diatoms that were then consumed by zooplankton. The nitrogen that is fixed by *Nodularia* is therefore an important nutrient source for the wider ecosystem.

Notes

Adaptive Incident Management of the 2011/12 Toxic Blue-Green Algae Bloom in the Gippsland Lakes, Victoria

Dr Daniel Mainville, Program Manager – Water & Sustainable Landscapes

Department of Sustainability and Environment

Regional Services – Gippsland, Victoria

A toxic bloom of *Nodularia spumigena* broadly occurred in the estuarine areas of the Gippsland Lakes (Lakes) between December 2011 and April 2012. A secondary mixed bloom of *Anabaena circinalis, Microcystis aeruginosa,* and *Cylindrospermopsis raciborskii* also developed in Lake Wellington, a fresher part of the Lakes.

Advisories were issued regarding contact with affected waters in accordance with the Victorian Department of Health guidelines. Toxins were also detected in fish, prawns and mussels which led to the imposition of restrictions on commercial and recreational fishing.

A multi-agency Incident Management Team (IMT) was convened consistent with the Australian Interservice Incident Management System (AIIMS) to manage the response. The aim of the operation was to protect human and animal health whilst responding to the needs of the fishing and tourism sectors. An adaptive management approach dependent on evidence-based decision making enabled advisories to be imposed and lifted at various zones around the Lakes.

In addition to managing the logistics of water and seafood flesh sample collection and analysis, communication and stakeholder engagement became one of the key activities of the IMT. Through direct liaison, regional media, community meetings, signage, websites and fact sheets, the large range of stakeholders were kept up-to-date with all advisories. Public information combined with innovative traffic light maps enabled the community to easily locate safe areas for recreation. This approach was well received by the tourism industry.

In response to the feedback from the commercial fishing industry, sample testing methodologies were tailored to reflect adaptable fishing practices. A strict provision to gill and gut fish prior to dispatch to markets enabled the in-lake fishers to continue to operate throughout the bloom ensuring the long-term commercial viability of the industry.

Key strengths of the response was the commitment and teamwork from the relevant agencies, effective participation of the tourism and regional economic development sectors, ability to efficiently respond to local concerns, and constructive relationships with local media.

Recommendations from a debrief conducted following the end of the bloom will be adopted to further improve the effectiveness of the response to future blooms.

Notes

Cyanobacteria and Recreational Lake Management

Ian R. Falconer

Pharmacology, Faculty of Medical Sciences, University of Adelaide

During the last decade the major lake in Canberra, Lake Burley Griffin, has been plagued with extreme concentrations of cyanobacteria. These recur at peak holiday and recreational periods, December, January and February, sometimes continuing into March and April, as well as spring and autumn. The summer water blooms are *Microcystis aeruginosa*, and form the expected scums, which float into the swimming beaches. During autumn and spring the water blooms are *Anabaena circinalis* similarly forming scums, most recently at over 60,000,000 cells/mL, with a scenic turquoise film over the lake.

ACT has a set of legislated *Guidelines for Recreational Water Quality,* which covers the Canberra urban lakes and Lake Burley Griffin. These extreme concentrations of cyanobacteria cause the lakes to be closed for body-contact recreational use, with detailed warnings for secondary water users such as rowers on the lakes.

A recent report to the ACT Legislative Assembly sets out causes of cyanobacterial proliferation, and potential remedial actions. These will be discussed in the context of feasibility, cost and potential outcomes.

Notes

Modelling the fate of cyanobacterial toxins in a drinking water reservoir

Stuart Khan

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Phone: 02 93855070, Email: s.khan@unsw.edu.au.

BACKGROUND:

Lake Burrogorang (also known as 'Warragamba Dam') is the principle raw drinking water supply for the city of Sydney, NSW, Australia. Located about 65 kilometres west of Sydney in a narrow gorge on the Warragamba River, Lake Burrogorang is one of the largest domestic water supply dams in the world. The reservoir has a total storage capacity of around 2 million megalitres and supplies an average of around 1000 megalitres of raw drinking water per day. A cyanobacterial bloom of historical proportion appeared in Lake Burrogorang in late winter/early spring of 2007. Following that bloom, a number of research projects were established to improve the understanding and management of cyanobacteria and their toxins in Sydney's drinking water catchment.

ABSTRACT:

A fugacity-based fate model was developed to describe and assess the overall fate of selected cyanobacterial exudates in the Gorge area of Lake Burrogorang. This fate model was based in the previously described Quantitative Water Air Sediment Interaction (QWASI) Model, but some important additional details were added including consideration of temperature stratification in the lake and the use of probabilistic analysis for stochastic variables.

The model was used to incorporate various experimentally determined half-lives for biodegradative and photolytic decay of cyanobacterial exudates, along with literature-acquired data describing partitioning to sediment and volatilisation to air.

In most cases, biodegradation and photolysis were shown to be the key processes governing the fate of the cyanbacterial exudates. However, extraction from the lake via the Warragamba pipelines was also significant for some chemicals. Volatilisation was identified as a minor process contributing to the fate of MIB, while adsorption to sediment was not shown to be significant for any of the investigated chemicals.

A diverse range of modelled results and insights were gleaned from close assessment and scenario testing of the model. Improved refinement of the input data (particularly for variability in biodegradation and photolysis) will lead directly to improvements in modelling capabilities and prediction.

ACKNOWLEDGMENT:

This work was funded by the Sydney Catchment Authority.

Notes

A new modified clay for removal of dissolved phosphorus from aquatic systems

Dr Grant Douglas

Senior Principal Research Scientist, CSIRO Land and Water, Floreat, WA

Phoslock™, a lanthanum-modified clay developed, patented and commercialised by CSIRO in the 1990's, and now used in over 25 countries internationally, offers one of the few methods to effectively remove dissolved phosphorus from eutrophic aquatic systems. In the past few years, however, the research focus has shifted to alternative methods of modification and beneficiation of clay minerals for dissolved phosphorus uptake. One such development is the synthesis of a hydrotalcite-clay nanohybrid with a substantial phosphorus uptake capacity. Hydrotalcites are a class of Mg, Al-rich layered double hydroxides endowed with a substantial anion exchange capacity. The basis of the hydrotalcite-clay nanohybrid synthesis is the selective leaching of Mg and Al from suitable clay precursors to provide the feedstock for hydrotalcite formation. The clay residuum then forms a substrate on which the hydrotalcite nanocrystals precipitate. An increased hydrotalcite:clay ratio may also be achieved by addition of supplementary Mg and Al during synthesis. Depending on the precursor clays and mode of formation, the hydrotalcite-clay nanohybrids may be synthesized with phosphorus uptake capacities of ~0.5 – 4.0% by mass. Importantly, the hydrotalcite-clay nanohybrids can be used in both fresh and saline waters, and thus have the potential to be applied across a variety of eutrophic aquatic systems. Extensive laboratory-based experiments have similarly demonstrated the efficacy of dissolved phosphorus removal in natural waters with a wide range of salinities and dissolved organic carbon concentrations. Over the coming year, small-scale field trials will be undertaken with research partners in a range of aquatic systems to both further investigate the efficacy of the hydrotalcite-clay nanohybrids, and to refine methods of synthesis at a pre-commercial scale.

Notes

Environmental conditions that influence toxin biosynthesis in cyanobacteria

Muenchhoff J and Neilan BA

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Over the past 15 years, the genetic basis for production of many cyanobacterial bioactive compounds has been described. This knowledge has enabled investigations into the environmental factors that regulate the production of cyanobacterial toxins at the molecular level. Such molecular or systems level studies are also likely to reveal the physiological role of the toxin and contribute to effective water resource management. We review the environmental regulation of some of the cyanotoxins most relevant to Australian fresh water resources, namely the microcystins, nodularin, cylindrospermopsin and saxitoxins. The most important factors influencing the production of these toxins are the availability of nutrients, especially nitrogen, iron and phosphorus availability. Other factors such as light intensity, temperature and the growth phase of cells might also play a role. Interestingly, the production of the toxins investigated here seem to respond to several of these factors simultaneously, rather than to a single one only. So far studies have mainly focussed on the effect of a single isolated factor, e.g. the limitation of a single nutrient in batch culture, but the findings here suggest such an approach may not yield results that are relevant in an environmental setting. Therefore, further studies are needed to investigate the combined effects of these factors on toxin production.

Notes

Cyanotoxins as indicators of major environmental shifts in aquatic systems: Towards a comprehensive framework for the management, risk assessment and mitigation of toxic cyanobacteria

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The first step in managing any hazard is a thorough risk assessment. A framework has been developed to assess the risk of cyanobacterial blooms in a variety of freshwater reservoirs. This can be used to determine the optimum cyanobacterial monitoring regime which will reduce the risk of toxic blooms harming human and ecological communities. The assessment also allows agencies to determine the risk a bloom poses once it exists, dependent upon its physical characteristics and the potential for the infected water to interact with humans and the environment. The completion of the assessment then leads to the determination of the action required to manage the bloom.

In this presentation, a comprehensive framework for the management, risk assessment and mitigation of cyanobacterial toxins will be presented. Many procedures for managing cyanobacterial blooms have been investigated in the past, yielding mixed results. The use of hydrogen peroxide (H2O2) for cyanobacterial and cyanobacterial toxin (cyanotoxin) removal has been suggested as an environmentally benign mitigation method for the management of toxic blooms. Past studies have mainly investigated its use under laboratory conditions, where it has been relatively unsuccessful as an algicide. However, the algicidal action of H2O2 suggests it will be more efficient under environmental conditions than on batch cultures and purified cyanotoxins in the laboratory.

Notes

Effect of Iron Availability on Growth and Toxicity of Microcystis Species Grown under Batch and Continuous Culture Conditions

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Increasing evidence exists that oxidative stress conditions induced by low nutrient availability and high light result in preferential growth of Microcystis species containing the toxin microcystin, possibly because this compound is effective at scavenging reactive oxygen species generated under such conditions (Alexova et al., 2011). In view of the apparent importance of availability of both major and minor nutrients to the physiology and toxicity of cyanobacteria, we have undertaken extensive studies of factors controlling the availability of iron to Microcystis species. Results of studies using the well-defined iron chelator EDTA have shown that both ligand concentration and light are critical to the bioavailability and rate of uptake of this trace nutrient (Fujii et al., 2010, 2011) with mathematical models describing the coupling of iron speciation to iron uptake in batch cultures extendable to continuous culture conditions where steady state cell densities are determined by dilution rate (Dang et al., 2012). In this presentation, results of studies of iron uptake under conditions where iron availability is controlled by complexation by natural organic matter (NOM) are presented with examination of both the effect of NOM concentration and light.

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Notes

Growth of the toxic cyanobacterium *Limnothrix* (strain AC0243) at various light intensities, temperatures and salinities

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In 2009, a *Limnothrix* strain (AC0243), was found to produce a novel toxin. *Limnothrix* is generally portrayed as a freshwater genus found in temperate regions. In the Fitzroy River system, Central Queensland, *Limnothrix* is often recovered in the metalimnion but can also form surface blooms. Our strain AC0243 has been kept in monospecific cultures since 2007. In this study, we are presenting the first data on the growth conditions of *Limnothrix* (AC0243).

The growth of *Limnothrix* (AC0243) was studied in a range of selected light intensities, temperatures and sodium chloride (NaCl) concentrations. The cultures can grow in light intensities ranging between 0 μE m^2 s^{-1} and 560 μE m^2 s^{-1}. Growth in the dark occurred only when an alternative carbon source (glucose dehydrogenase) was added, suggesting a facultative heterotrophic metabolism. The strain grew well at 160 μE m^2 s^{-1} indicating that this species shows a preference for low light intensities. Temperature trials determined that it was able to grow at temperatures ranging from 7 ^{o}C to 55 ^{o}C. Growth was faster in temperatures above 25 ^{o}C, with optimal growth occurring at 35 ^{o}C.

Limnothrix (strain AC0243) was able to grow in ASM-1 media with NaCl concentrations ranging from 0 to 40 gL^{-1}. Statistical analysis showed concentrations of 0.25 gL^{-1} had no significant effect on *Limnothrix* (strain AC0243) growth when compared to the control. The maximum growth occurred in 1 gL^{-1} concentrations of NaCl with the majority of the growth occurring on the day 14 of the trial, suggesting *Limnothrix* (strain AC0243) may undergo an acclimatisation period, where growth is minimal. Correlation analysis demonstrated a weak but significant negative correlation between NaCl concentrations and *Limnothrix* (strain AC0243) growth. With the exception of 1 gL^{-1} treatments, greater NaCl concentrations promoted less growth. 40 gL^{-1} treatments yielded significantly less growth than all other treatments.

The key findings of the research suggest that although *Limnothrix* (strain AC0243) is generally recovered from the metalimnion of water bodies, this strain may not be limited to any particular niche. *Limnothrix* (strain AC0243) appears able to adapt to a wide range environments and has the potential to colonise in areas such as pipelines, hot springs and estuaries. Such characteristics enhance the probability of this strain becoming a successful invader of a range of new habitats and a serious concern for future water management.

Notes

Nutrient utilization strategies for phytoplankton in stratified subtropical reservoirs

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Stratification of subtropical reservoirs in summer months results in low and variable availability for phytoplankton growth in surface waters. Our studies have shown that phytoplankton in these systems have developed a range of strategies to deal with this including: utilization of organic nutrient sources; active uptake mechanisms at low phosphate concentrations; and flexibility in inorganic nitrogen uptake vs. nitrogen fixation as nitrogen availability fluctuates. The result is that phytoplankton may be less limited by nutrients than may appear based on nutrient concentrations. This is substantiated by use of micro- and mesocosm scale experiments which show a consistent phytoplankton response to nitrogen plus phosphorus addition, but no increase in phytoplankton biomass with phosphorus alone, and periodic responses to nitrogen. This study highlights the need to understand the complexity of nutrient utilization processes by phytoplankton in order to manage and mitigate future nitrogen and phosphorus inputs.

Notes

Allelopathic activity of a freshwater macroalga, *Chara australis*, and a submerged macrophyte, *Potamogeton crispus*, on microalgae

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Biology Honours Research Project, April 2012

Eutrophication (and consequent algal blooms) is a major management issue in shallow lake systems worldwide. The role of allelopathy, in which aquatic macrophytes can produce chemicals that inhibit the growth of phytoplankton, has received increasing attention as a means of controlling algal blooms. Macrophytes with allelopathic potential may play a central role in the restoration of eutrophic lakes. The main objective of this project was to investigate the allelopathic effect of the freshwater macroalga, *Chara australis*, and the submerged macrophyte, *Potamogeton crispus*, on microalgae. To detect possible allelopathic interactions, the growth of a cyanobacterial species, *Anabaena variabilis*, was assessed in the presence of macrophyte exudates and live material, compared with controls. A test for species-specificity of macrophyte allelochemicals was accomplished by comparing the effect of macrophyte extracts on algae from two different groups, the cyanobacterium, *A. variabilis* and the green alga, *Scenedesmus quadricauda.* This study found a significant negative effect of macrophyte extracts, live material and exudates on *A. variabilis.* The strongest effect was produced by the macroalga *C. australis*. In contrast, no effect of macroalgal or macrophyte extracts was evident on the growth of *S. quadricauda*. This absence of effect suggested that macrophyte allelochemicals are specific to particular target organisms. These findings support the use of these macrophytes as biological control agents to mitigate phytoplankton blooms in systems dominated by *Anabaena* and perhaps other cyanobacteria. However, allelopathic interactions should be tested on a range of target microalgal species prior to establishing a restoration plan.

Notes

Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: Between simplistic scenarios and complex dynamics

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Toxic cyanobacterial blooms represent a serious hazard to environmental and human health, and the management and restoration of affected waterbodies can be challenging. While cyanobacterial blooms are already a frequent occurrence, in the future their incidence and severity are predicted to increase due to climate change. Climate change is predicted to lead to increased temperature and changes in rainfall patterns, which will both have a significant impact on inland water resources. While many studies indicate that a higher temperature will favour cyanobacterial bloom occurrences, the impact of changed rainfall patterns is widely under-researched and therefore less understood. To be able to assess and mediate the significant threat cyanobacterial blooms pose to our water resources, more effort is needed to understand the relationship between rainfall patterns and cyanobacterial bloom dynamics, and in particular toxin production.

In this presentation, we synthesize the predicted changes in rainfall patterns and their potential impact on inland waterbodies, and identify mechanisms that influence the occurrence and severity of toxic cyanobacterial blooms. We investigate the correlations between the intensity of rainfall events or the length of the dry period on waterbody conditions that could lead to cyanobacterial blooms. We will also present a framework that explores the future trend of the toxicity of blooms. We will conclude this presentation with the identification of knowledge gaps that need further investigation to enable a better understanding of the impact of changed rainfall patterns on cyanobacterial biomass and toxicity.

Notes

Toxic benthic cyanobacteria – an underestimated risk?

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Until recently cyanotoxin production has primarily been associated with planktonic cyanobacteria. Benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers and are found in a wide range of water quality conditions, including relatively pristine waters used for recreational activities and as drinking-water supplies. Although benthic taxa have received less attention than their planktonic counterparts, they have been found to produce potent cyanotoxins. In New Zealand, reports of dog poisonings linked to benthic cyanobacteria have increased markedly in the last decade and drinking water supplies and processing plants have been closed due to toxic benthic proliferations. Most problems have been associated with *Phormidium* and the toxins anatoxin-a and homoanatoxin-a, although more recently saxitoxins and microcystins have also been identified in benthic cyanobacteria in New Zealand. Our recent research has focused on identifying toxin-producing species, investigating spatial and temporal variations in benthic cyanobacteria and associated toxin production, identifying environmental parameters linked to bloom formation and developing new sampling and management methodologies. A summary of our recent research will be presented.

Notes

Chronic low dose exposure to saxitoxin inhibits neurite formation in model neuronal cells

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Saxitoxin (STX) is a neurotoxin known to be produced in both marine and freshwater environments. Its production by freshwater cyanobacteria make saxitoxin a potential hazard to drinking water supplies. STX causes toxicity by binding to voltage gated sodium channels, halting the inflow of sodium ions required for the generation of action potentials.

Guidelines for acute exposure through drinking water exist, derived from guidelines established for acute exposure through shellfish ingestion; yet chronic low dose exposure has not been investigated. Due to the implication of voltage gated sodium channels in neurite outgrowth and development we hypothesise that this pattern of exposure to STX could affect developing neurons. To test this hypothesis the effect of long term exposure to STX have been measured using model neuronal cells.

PC12 Ordway and SHSY5Y cells were grown on poly-L-lysine coated coverslips and exposed to a range of concentrations (0.25, 0.5, 0.7, 1, 2, 3µg/L) for seven days. Concentrations were chosen based on Australian drinking water guidelines suggesting a maximum of 3µg/L. Following the exposure period, cells were stained with TRITC-phalloidin and imaged using a fluorescence microscope. Using ImageJ the number and length of neurites present were measured.

Control cells developed a neuronal habit over the exposure period, developing from a circular to elongated or pyramidal cell body with long axonal like extensions (neurites). Cells exposed to toxin remained in a circular habit, developing numerous short filipodia instead. Both cells lines were affected similarly in a concentration dependent manner. The results suggest that long term exposure to low concentrations of STX is having an effect on neuronal development. This has important implications for the safety of drinking water and the appropriateness of a guideline based on acute toxicity. Further work is underway with sub-chronic and episodic patterns of exposure and research needs to be extended to include other toxin analogues, namely Gonyautoxins and the C-toxins.

Notes

Identification of a novel *Limnothrix* sourced metabolite - Ph.D. University of Adelaide, 2012

Paul Whan

Cyanobacterial metabolites are a consistent and recurring problem in the pursuit of providing safe potable drinking water. The discovery of a new, toxic metabolite from the common *Limnothrix* cyanobacteria presents a challenge to this goal. The metabolite has been shown to have novel toxicity in bioassay experiments. Identifying, characterising and risk-assessing this toxic metabolite (or metabolites) is the primary purpose of this project.

Comparative LC/MS experiments have determined that the metabolite is unique; it is not one of the families of commonly occurring cyanobacterial metabolites. Using a protein synthesis inhibition assay in conjunction with appropriate solid phase extraction (SPE) and HPLC a method to concentrate the metabolite has been developed. The chemical properties of the toxin and thus, the implications for toxin removal from water sources are currently being investigated. The *Limnothrix* toxic metabolite is highly water soluble and pH stable. It is non-volatile and temperature stable within the operating range of source-water. Therefore, when present, this compound could pose a risk for drinking water quality that will need to be managed.

Notes

The cylindrospermopsin analogue deoxycylindrospermopsin: isolation, purification and acute toxicity in mice

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The cylindrospermopsin analogue deoxy-cylindrospermopsin (d-CYN) was described as non-toxic to mice when it was first discovered in 1999. Since then, two independent groups in the USA and Australia have reported that d-CYN acts as a protein synthesis inhibitor *in vitro*, with a similar potency to that of cylindrospermopsin (CYN) *in vitro*.

We extracted d-CYN from field-harvested *Lyngbya wollei*, known from earlier work to produce high concentrations of the compound. Lyophilised, powdered biomass was sonicated, extracted in aqueous methanol, further concentrated by solid-phase extraction, and separated by preparative HPLC. Chromatographic separation of d-CYN from smaller quantities of CYN produced by *L. wollei* is unequivocally achievable. Purity was confirmed by ^{1}H NMR. Purified d-CYN was shown to be a stable compound with the uracil moiety in the keto form. D-CYN was quantified by HPLC-MS/MS against a validated analytical standard which we had previously prepared.

Separate groups of female Balb/c mice were injected i.p. with 2mg/kg and 5mg/kg doses of d-CYN; no signs of acute toxicity were observed or measured over the following six days. D-CYN was recovered in the urine of dosed mice. Five mice injected with CYN at 2mg/kg CYN (as a positive control) displayed a range of acute toxicity signs, including sustained core hypothermia – employed here as a non-lethal endpoints model of acute toxicity. Tissue histology of d-CYN-dosed mice was unremarkable; CYN-dosed mice showed a range of pathological changes typical of acute cylindrospermopsin intoxication.

Notes

Performance comparison of three phylogenetic markers used for cyanobacterial identification and classification

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A selection of cyanobacterial isolates (n=28), obtained from twelve WA freshwater systems, was used to compare the performance of three phylogenetic markers commonly used for identification and classification of members of this phylum. Phylogenetic analysis was carried out using degenerate primers targeting the small subunit rRNA gene (16SrDNA), the γ-subunit of the DNA-dependent RNA polymerase (*rpo*C1) locus, and the phycocyanin (*cpc*) operon.

For the three markers, amplification efficiency was: 92.9%, 46.4%, and 71.4% respectively, with amplicon lengths, for each locus, ranging between 318-482, 548-663, and 484-746 bp, respectively. BLAST-searches identified the isolates as belonging to chroococcales (n=7), oscillatoriales (n=6), and nostocales (n=14), while identification of one isolate was dubious, and varied depending on the marker used.

Phylogenetic reconstructions were generated using different approaches, consisting of combinations of methods, models and parameters. Results were generally in agreement, with isolates showing similar grouping patterns, regardless of the locus considered. The 16S rDNA and *rpo*C1 loci, in particular, appeared to consistently provide coherent identifications and classifications. Two isolates from the oscillatoriales subgroup, however, displayed a significantly different clustering pattern when the highly variable *cpc* locus was used. This result was consistent even when different phylogenetic reconstruction approaches were adopted for this locus.

This study confirms the potential for different phylogenetic markers to affect proper identification and classification of cyanobacteria, and clearly advocates the implementation of complementary strategies based on the analysis of DNA and morphology. The selection of phylogenetic markers should be carefully considered to obtain reliable information. In fact, at least for some data-sets, this selection appears more critical than the phylogenetic reconstruction approach adopted.

Notes

Diagnostics for saxitoxins using saxiphilins

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The saxitoxins are a globally distributed, naturally occurring contaminant which can cause fatal neurotoxicity if ingested by humans and other animals. They occur in freshwater cyanobacteria and marine microalgae, contaminating the water column and moving through the food chain as they are ingested and bioaccumulated.

Saxiphilins are a protein found in the circulatory fluid of an increasing number of vertebrates and invertebrates. It is a transferrin, proteins known more for their iron binding and sequestration properties. Saxitoxins have only been found in the marine and freshwater environment, yet many terrestrial organisms possess this unusual saxitoxin binding protein in their blood, providing a mystery as to its biological role with a role in bioaccumulation or chemical defense being possible. Specifically, animals found to date to possess saxiphilin include lizards, amphibians, fish, spiders, scorpions, insects, crabs, centipedes, molluscs and onycophorans, most of which do not harbour or bioaccumulate saxitoxins.

To satisfy the increased demand for rapid toxin tests, saxiphilin has been used to develop rapid, microtitre plate assays for the saxitoxins, and in a bench-top biosensor. This latter aspect was enabled by the biotinylation of saxitoxin creating a novel bifunctional analogue of these toxins. The original saxiphilin, that from the North American bullfrog, has also been expressed in a yeast vector with a His-tag and an expression signal enabling the large scale production, purification and coating of inert surfaces for biodetection.

Many test samples contain a multitude of saxitoxin analogues with very different potencies and understanding how toxin mixtures behave in these diagnostic systems is critical to their wider deployment. A mathematical model that explains the behaviour of very complex saxitoxin mixtures has been developed and validated and may be extended to other bioactive chemicals. Each isoform of saxiphilin has a different pattern of sensitivity to the different saxitoxins and this is a potentially powerful property upon which to develop tests with a very broad coverage of all of the saxitoxins. By combining different isoforms with divergent sensitivities to the various classes of the saxitoxins, the spread of toxin binding strength against these isoforms may provide an indication of the classes of toxins present.

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Notes

CyanoSurvey – Comparison of Methods for the Detection of Cyanobacterial Toxins

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The CyanoSurvey Project began in late 2009 and is now reaching completion. The primary aim of the project was to update our risk assessment of the toxic cyanobacteria that occur in Australia. One of the key questions underlying this objective is "How do we determine that a species or sample is toxic, and how do we quantify the risk that toxicity represents?" There is a range of methods available encompassing surrogate methods like counting the cells of known toxin producers and molecular detection of toxin genes, direct detection of the known toxins via their chemical/structural attributes (Dip-sticks, ELISA's, HPLC, LC/MS), and detection of the effects of the toxins on specific biological processes (bioanalytical methods). These methods all have advantages and disadvantages, but they are rarely compared side-by-side to gauge the relative effects these characteristics may have on assay outcomes from the same sample.

Collection of a large number of raw cyanobacterial samples for the CyanoSurvey project provided an opportunity to do this. Twenty-nine samples were sent for cell identification and counting by the NATA-accredited AWQC Biology Services Laboratory. Either raw or filtered samples (when cell counts were too low for toxin detection in the raw water) were also analysed by ELISA, a toxin-specific toxicity assay, a non-specific toxicity assay, and toxin gene detection. Selected samples were analysed by the appropriate NATA-accredited HPLC-based method.

An overall conclusion is that, when toxins were present in significant amounts (at or above ADWG guideline levels), all of the methods proved useful for detection of the toxins. However, as might be expected, they did not agree on the quantity of toxin present. None of the methods proved completely reliable when lower amounts of toxin were present. For microcystins, there was good concordance between the ELISA and the protein phosphatase inhibition (PPase) assay. HPLC-PDA analysis produced one false negative. Toxin gene detections were fairly reliable for microcystin producers (but less so for producers of other toxins). It was not quantitative by the method employed. Interestingly, most of the samples with the highest amounts of microcystin were dominated by what was typed morphologically as *Microcystis flos-aquae*. Furthermore, microcystins were detected by both the ELISA and PPase assay in almost all filtered samples. This suggests that a microcystin producer (probably a *Microcystis* species) is ubiquitous in freshwaters across Australia. Results for the other toxins will also be reported, and the implications for value-for-money risk assessment discussed.

Notes

Cryptic toxicity: non-planktonic cyanobacteria represent a significant potential source of cyanotoxins in the freshwater environment

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The production of cyanotoxins by planktonic cyanobacteria has been well documented for eutrophic water bodies around the world. However, cyanobacteria also occupy a variety of other niches in aquatic environments including periphytic, metaphytic, and epipelic habitats in lotic and lentic systems. Cyanotoxin occurrence in these habitats is not as well known. In recent years, there have been increasing reports of cyanotoxins detected in cyanobacterial mats from rivers, and the littoral areas in lakes, reservoirs and wetlands. In a number of cases the ingestion of such mats has been linked to animal poisonings. In Australia, these habitats have been poorly studied in general, and to date, no systematic evaluation of their potential as a source of cyanotoxins has been undertaken.

To evaluate this potential, we screened for cyanotoxins in samples from a variety of lake and riverine habitats from twelve sites throughout Queensland, Australia. The presence of genes involved in the synthesis of cyanotoxins from the four major toxin groups known to occur in Australia was assessed using multiplex tandem real-time PCR. Toxin genes were detected in three of these samples. An epipelic sample from a Brisbane lake was positive for both the *ndaF* gene for nodularin production, and the *sxtI* gene for PST-production. In another metaphytic lake sample the *cyrC* gene for cylindrospermopsin production, and the *mcyE* gene for production of microcystins were also detected. The *sxtI* gene was also found in an epipelic river sample.

Using the phylogenetic and toxicological characterisation of the freshwater benthic cyanobacterium *Lyngbya wollei* from Queensland, we provide a further example of the toxigenic potential of non-planktonic cyanobacteria. We hypothesise that potential freshwater HAB events involving non-planktonic cyanobacteria may go undetected because subsurface mats are easily missed by conventional sampling and monitoring methods. For this reason, it is critical that sampling be conducted at scales appropriate to resolve cyanobacterial biomass from these habitats.

Notes

Semantics-based approach for defining complex event processing events for real-time algal bloom detection

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Continuous in-stream water-quality monitors such as sensors networks are important tools for real-time assessments of water resources. Such real-time observational data can be used for early warning of imminent threats and decision support in the conservation of water systems, aquatic habitats, and management of water storages, for example, a potential algal bloom event within a reservoir. Events of interest for monitoring may involve several measurements of parameters, like pH, water temperature, turbidity, dissolved solids. Furthermore, events of interest may span across multiple monitoring stations, adding complexity to the task of defining and monitoring these events. However, defining a set of complex events to monitor on a sensor network, may require specialist expertise. Also, sensor data formats and interfaces are heterogeneous, which makes it difficult to aggregate the data from disparate sources as well as accommodate for the availability of additional sensors. In this work we seek to provide the means for combining, enriching and integrating various data sources and provide timely notifications based on user defined events. We propose a tool aimed at facilitating non-technical users to be able to compose complex events of interest from the available sensors and receive notifications of matching events e.g. a set of conditions that favour harmful algal blooms to facilitate appropriate responses ahead of the actual algal blooms. Ontologies are used to capture semantics of the various system components such as a sensor's set of observable parameters and enables users to compose a complex event based using rules defined against the ontologies and the availability of sensors. As a test case, we replay a historical dataset from algal bloom events from a Chaffey dam study carried out in the 1990s as input sensor observations to our complex event processing system.

Notes

Fate of intracellular geosmin and saxitoxins during simulated lagoon treatment of cyanobacterial sludge

Jennifer Dreyfus, Albane Barbero, Lionel Ho, David Dixon, Peter Scales, Werner Mobius, Jek Rozitis, Gayle Newcombe

The major water quality implication of the proliferation of cyanobacteria blooms for the drinking water industry is the production of secondary metabolites, in particular taste and odour compounds and cyanotoxins. In Australia one of the cyanobacteria species of most concern to drinking water authorities is *Anabaena circinalis*. The main metabolites produced by these cyanobacteria are the saxitoxins (nerve toxins) and geosmin (earthy odour).

The most important process in the management of cyanobacterial metabolites is the removal of cyanobacteria cells, intact and without damage. When *A. circinalis* cells are effectively removed during the normal particulate removal process the majority of the cyanobacterial metabolites are also removed. If the cells become damaged or stressed they may release toxins or taste and odour compounds into the extracellular, or dissolved form, which requires more advanced treatments such as oxidation or activated carbon. Previous studies on cultures of *Microcystis aeruginosa* have shown that the cells become damaged within several hours of capture in an alum floc. This can result in the release of high concentrations of dissolved toxins in the sludge. Therefore there is a risk of cell lysis and subsequent release of the dissolved compounds into the waste supernatant. The rate and mechanism of cell damage during sludge formation and treatment, the release of metabolites into the sludge supernatant during treatment, the process of degradation of these metabolites in treatment lagoons, and the impacts on water quality of the return of the sludge supernatants to the head of the plant, have not been studied previously. In this paper we describe an investigation into the kinetics of release of geosmin and saxitoxins from a natural bloom of *Anabaena circinalis* after coagulation with alum and sedimentation. During subsequent simulation of lagoon treatment the rate of biological degradation of the metabolites by indigenous bacteria is also quantified. These rates are then utilised in an empirical model that describes the removal, release and degradation of the metabolites through conventional treatment processes, powdered activated carbon application and chlorination. The model also takes into account the removal of the sludge from the sedimentation basins, lagoon treatment, and the return of the supernatant to the head of the plant. The model is used to estimate the metabolite concentration that can be expected in the finished water given a particular cell concentration, and the maximum cell concentration that can be treated before the supernatant can no longer be returned to the head of the plant.

Notes

Hindcasts and Prediction of algal blooms in continental Australia – an idea

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Australian freshwaters like other freshwater systems in the world are susceptible to the occurrence of possibly harmful cyanobacterial blooms. Climate change and changes in land use are modifying these systems and it is expected that in future there will be a higher risk in the development of harmful algal blooms in freshwater systems due to changes in nutrient availability, increase in water temperature and stability against water mixing, and the invasion of non-native species. Having possibly negative effects on animal but also on human health it is desirable to have a system to predict such blooms for any water body given known meteorological, hydraulic, and biogeochemical conditions.

It is the intention of this presentation to layout a basic overview and framework of a possible system to predict algal blooms in freshwater systems, i.e. lakes, reservoirs, streams, using biological-hydrodynamic coupled numerical modelling, supported by data on cyanobacteria physiology, forced by gridded meteorological fields over Australia, combined with remote sensing for model calibration. Such a system, once calibrated for a large range of situations and over different geo-climatic regions using historic water quality and remote sensing data, could be used to predict the development of algal blooms in lakes and rivers of Australia on a short time scale.

Notes

Inductive reasoning and prediction of population dynamics of *Cylindrospermopsis* in the Wivenhoe Reservoir by means of evolutionary computation

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Limnological time series from 1999 to 2011 of the dam wall site of Wivenhoe Reservoir are modelled by the hybrid evolutionary algorithm HEA to: (1) reveal ecological relationships, thresholds and time lags that drive population dynamics of *Cylindrospermopsis*, and (2) predict timing and magnitude of bloom events of *Cylindrospermopsis*.

In a first experiment key physical, chemical and biological data monitored over the 13 years period are used as inputs for cyanobacteria modelling. In a second experiment model ensembles are developed solely based on electronically-measurable input variables in order to test models' suitability for real-time forecasting.

Both experiments resulted in models for 7-days-ahead forecasting with r^2 (coefficient of determination) > 0.7 suggesting good predictability of *Cylindrospermopsis* dynamics. IF-THAN-ELSE rules of the models revealed a water temperature of 25.5 °C as threshold above which fast growth of *Cylindrospermopsis* is triggered. Input sensitivity analysis for all models displayed complex relationships with physical, chemical and biological input variables revealing inhibitory and excitatory conditions for *Cylindrospermopsis* growth. Time lags specific to input variables have been identified and considered for improved forecasting validity.

Model ensembles solely based on electronically-measurable input variables achieved good results for 7-days-ahead forecasting, and hold out the prospect of implementing the models for real time forecasting and early warning.

Key words: Cylindrospermopsis, Wivenhoe Reservoir, 7-days-ahead forecasting, ecological relationships, ecological thresholds, ecological time lags, evolutionary computation, sensitivity analysis

Notes