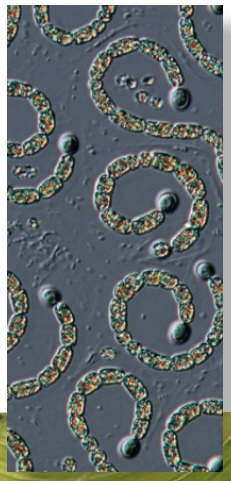
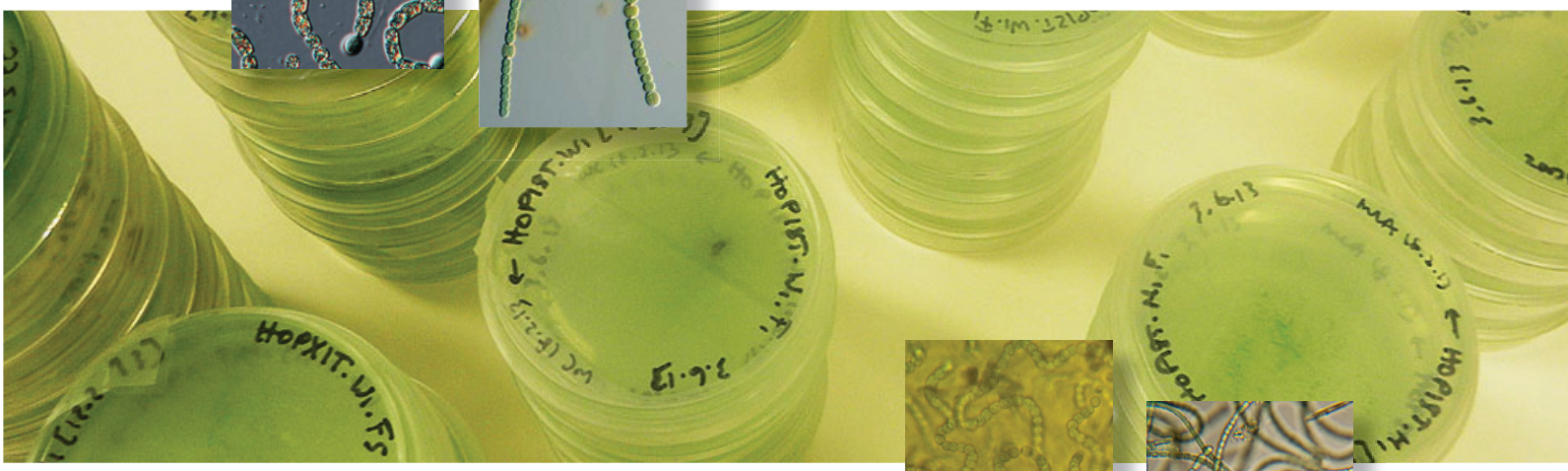
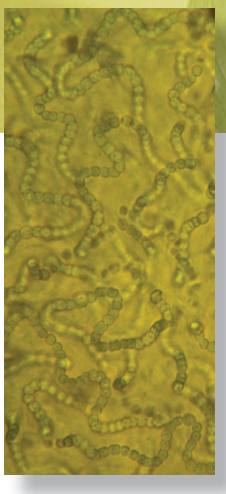
Benefits and application of research and technology for the management of cyanobacteria



Abstracts from
the Fourth National Cyanobacterial Workshop
22 - 24 September, 2014
Adelaide, South Australia





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Benefits and application of research and technology for the management of cyanobacteria

Abstracts from the Fourth National Cyanobacterial Workshop

22 – 24 September 2014, Adelaide South Australia

This National Cyanobacterial Workshop was hosted by the Australian Water Quality Centre, SA Water Corporation, The University of Adelaide and Water Research Australia Limited.

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

Organising of the workshop was led by Dr Gayle Newcombe, Manager Customer Value & Water Quality Research, Australian Water Quality Centre, SA Water, Adelaide

together with a committee comprising:

Dr Andrew Humpage – Australian Water Quality Centre, SA Water

Associate Prof Justin Brookes – Earth and Environmental Sciences, University of Adelaide

Dr Claudia Junge – Earth and Environmental Sciences, University of Adelaide

Dr Anna Rigosi – Earth and Environmental Sciences, University of Adelaide

Dr Virginie Gaget – Australian Water Quality Centre, SA Water

Ms Claire McInnes – Water Research Australia

Ms Angela Gackle – Water Research Australia

Sponsorship provided by the following organisations is gratefully acknowledged:

Water Research Australia Limited

Murray Darling Basin Authority

SA Health

SA Water Corporation

Cover photo of algal samples in petri dishes supplied by Dr Virginie Gaget

Workshop Program – Day 1

9:00 - 9:30		Registration	
9:30		Welcome	

Session 1 Overview presentations

09:40	Gayle Newcombe	Identifying and quantifying the benefits of cyanobacteria research for the Australian drinking water industry	4
10:00	Larelle Fabbro	Cyanobacterial Research in Central Queensland, - Past, Present and Future.	5
10:20	Yoshi Kobayashi	Control measures of freshwater cyanobacterial blooms: a mini review	6
10:40	Catherine Bernard-Pattinson	Preliminary review of the taxonomy of closely related taxa *Limnothrix* and *Geitlerinema*' (Pseudanabaenaceae, Oscillatoriales)	7

11:00 - 11:30 Coffee

Session 2 Research theme – Understanding

11:30	**Keynote**	**Rod Oliver - The Redfield Ratio under challenge - Paradigm loss or the unravelling of misconceptions?**	8
12:00	Anna Rigosi	Cyanobacterial development in warmer climate: does trophic state matter?	9
12:20	Susie Wood	"Toxic in crowds" - insights into microcystin regulation using mesocosms and surveys of natural lake populations	10
12:40	Jason Woodhouse	Temporal patterns of microbial and metabolic covariance within a freshwater cyanobacterial bloom	11

13:00 - 14:00 Lunch

Session 3 Research theme – Understanding

14:00	Michelle Burford	Environmental drivers of toxin production by the cyanobacterium *Cylindrospermopsis raciborskii*	12
14:20	Ming Su	MIB-producing cyanobacteria (*Planktothrix* sp.) in a drinking water reservoir: Distribution and odor producing potential	13
14:40	Anas Gadouani	Cyanobacterial blooms in waste stabilisation ponds: how can ecology help the water authorities in Australia	14
15:00	Yvette Gaweda	Biotic and abiotic factors influencing toxic cyanobacterial blooms in an urban and residential freshwater environment	15

15:20 - 15:50 Coffee

Session 4 Research theme – Understanding

15:50	Katie O'Neill	Saxitoxin at the Australian Drinking Water Guideline Level Alters Neuronal Differentiation of D3 Embryonic Stem Cells	16
16:10		Wrap-up of day 1 One minute poster presentations	

16:30 - 18:00 **Poster Session and socialising**
(Dinner options to be advised)

Workshop Program – Day 2

09:00	**Keynote**	**Susie Wood - Molecular techniques for cyanobacterial research and monitoring - current applications and future perspectives**	17

Session 1 Research theme – Measuring

09:30	Louise Baker	Rapid, multiplex-tandem PCR assay for automated detection and differentiation of toxigenic cyanobacterial blooms.	18
09:50	Leo Pinheiro	Development of CyanoDTec: A rapid molecular assay for the routine monitoring of toxic cyanobacterial blooms	19
10:10 - 10:40		**Coffee**	

Session 2 Research theme – Measuring

10:40	Lee Bowling	Evaluation of a hand-held spectrophotometer for the proximal remote sensing of cyanobacterial abundance in water bodies.	20
11:00	Tim Malthus	Advances in Earth Observation based technologies to assist algal management	21
11:20	Bala Vigneswaran	Profiling Cyanobacteria Risk in Drinking Water Supply Reservoirs	22
11:40	Elke Reichwaldt	Risk based analysis for cyanobacteria in waste stabilisation ponds	23
12:00	**13:00**	**Lunch**	

Session 3 Research theme – Controlling

13:00	Andrea Gonzalez Torres	Tailoring algal floc properties for more robust cyanobacteria removal during drinking water treatment	24
13:20	Arash Zamyadi	Management of toxic cyanobacteria in full scale water treatment plants	25
13:40	Ning Lu	Effect of bromide on treatment of algae-containing water by preozonation: cell Integrity and Br-DBPs.	26
14:00	Emma Sawade	Effect of water quality changes on biological filtration efficacy	27
14:20	**14:50**	**Coffee**	

Session 4 Research theme – Controlling

14:50	Peter Hobson	Hydrogen peroxide: A new way to control cyanobacteria	28
15:10	Petra Reeve	Management of treatment sludge impacted by cyanobacteria	29
15:30		Wrap up of day 2 Nominations for the 5th Cyanobacteria Workshop 2016 Planning for workshops on day 3	

7:00 pm Dinner at the German Club, 223 Flinders Street, Adelaide

Identifying and quantifying the benefits of cyanobacteria research for the Australian drinking water industry

Gayle Newcombe

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Abstract: The program of cyanobacterial research undertaken in Australia over the past twenty years has produced wide-ranging and significant benefits for the water industry. The outcomes of the research have been applied in areas such as the development of guidelines, procedures and processes for risk mitigation, the development and validation of analytical techniques, and the assessment and optimisation of control and treatment measures. In 2013 the Australian Water Quality Centre was contracted by Water Research Australia to produce a report that identified, described and, if possible, quantified, the impacts of that research program.

This presentation of the outcomes of the report will include:

- A timeline of the significant published events, internationally and in Australia, where human health has been impacted by cyanobacteria
- A case study describing major incidents in Australia involving *Anabaena circinalis*, and the consequent evolution of our knowledge and management practices
- A thematic model that was developed and used to describe the most important research themes (Understanding, Measuring and Controlling cyanobacteria) and the relationship between these themes and outcomes in the areas of Knowledge Foundation, Managing Risk, and Optimising Operations
- Specific examples illustrating the widespread uptake and implementation of research outcomes by the Australian water industry
- A semi-quantitative method utilised to assess the three research themes and various individual projects in each theme using a "Research Benefits Calculator" (RBC) spreadsheet.

Key words: cyanobacteria research, benefits, impacts, water industry

Cyanobacterial Research in Central Queensland, - Past, Present and Future.

Larelle D Fabbro

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Abstract: The Central Queensland Region houses Australia's second largest catchment, - the Fitzroy River system. This region has had a striking history of cyanobacterial diversity, blooms and toxicity. A history of the various research projects in this area and their contributions to the current knowledge of cyanobacteria and their management is presented. The first blooms noted by Captain Cook were of *Lyngbya* and *Trichodesmium* in Keppel Bay. Research on marine forms has covered the four watercourses draining into Keppel Bay as well as the *Lyngbya* blooms and purple prawns just north of Gladstone Harbour.

The green water coming from the taps of Rockhampton in the late 1980's was the trigger for in depth research into the quality of the municipal water supply. The Fitzroy River Barrage Impoundment, thought to originally contain only coiled *Anabaena* (*Dolichospermum*), green algae and diatoms was shown to be ideal habitat for most cyanobacterial species identified within Australian waters. By 1993, the incidence of blooms of *Dolichospermum circinale (Anabaena circinalis)*, coiled *Cylindrospermopsis raciborskii* and *Nostoc linkia* and the depths at which these species proliferate had been documented. Water treatment processes and intake depths were refined to cover the bloom periods where taste and odour were absent. Large collaborative projects between government agencies and universities then concentrated on refining models of bloom formation and studying the extent of algal blooms within the catchment. These enabled training of relevant personnel and gave water authorities some warning in relation to implementation of water treatment processes. One major benefit of this research was the identification of toxin producing organisms or toxins prior to generation of adverse human impacts. It also enabled the collation of data sets and photographs of these organisms so that identification guides for Australian material could be produced. Post 2000, laboratory studies were completed in relation to the bioaccumulation of cylindrospermopsin in selected plant and animal species, and the accompanying growth, behavioural and histological impacts of toxin in the tissues. The ACARP project investigated the morphology, genetics and toxicity of various cyanobacteria from the Fitzroy catchment. The aim of the project was to reduce the potential risk of a repeat of the Solomon Dam incident, - particularly for isolated mining communities. Recent discoveries have included the production of toxin by *Limnothrix* as a result of this research project and elements of neurotoxicity in tadpoles. Dominance of cyanobacterial species in the dry season and the precise water chemistry and accompanying catchment management elements have been analysed as part of the BMA Fitzroy Aqua-Eco Health Project. This will hopefully lead to improved catchment management processes and knowledge of those river sections where water quality may be compromised.

For the future, the focus is on the extent of *Limnothrix* proliferation in regions once dominated by C. *raciborskii* and reasons for such changes. Also, high on the agenda is ascertaining the adequate treatment of drinking water containing this organism, particularly where pre-chlorination is used to remove manganese and iron.

Key words: Fitzroy River, Central Queensland, Cylindrospermopsis, Limnothrix, cyanobacteria, tropical

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Control measures of freshwater cyanobacterial blooms: a mini review

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Abstract: There are many control measures of freshwater cyanobacterial blooms with varying degrees of effectiveness, longevity and resource requirements. Most of them are based on physical, chemical and biological principles to destroy or deter the cell growth directly or indirectly. They include artificial mixing, applications of algicides and chlorine, biomanipulation to enhance food-chain effects, and others. In this talk, I provide a mini review on the past and current applications of the freshwater cyanobacterial-bloom control measures based on those principles. A simple schematic guidance tree is provided in choosing and applying the control measures according to the type and scale of freshwater ecosystems that developed the blooms. I suggest that a whole-cycle model is important in choosing and applying any control measure to cyanobacterial blooms because of the potential ecological cascading effects that may not only modify but also transfer (or export) the bloom-associated problems that occurred within a specific ecosystem boundary.

Key words: Anabaena, blue-green algal toxins, life cycle, Microcystis, nutrients, photosynthesis, thermal stratification, water-retention time

Review of the current knowledge of the taxonomy and ecology of *Limnothrix* (Pseudanabaenaceae, Oscillatoriales)

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Abstract: Small filamentous cyanobacteria belonging to the *Pseudanabaenaceae* (Oscillatoriales) are often difficult to identify and their ecology is not well-known. The genus *Limnothrix* was coined in 1987 by Meffert, but its differentiation from close genera such as *Leptolyngbya* and *Geitlerinema* is still not clear. Some years ago, we isolated a strain of *Limnothrix* called AC0243 from Central Queensland (Bernard *et al*. 2011). AC0243 appeared to harbour a new type of toxin, limnothrixin, the effects of which were further studied (Humpage et al. 2012). Studies of the ecology of *Limnothrix* showed that it often cohabitates with another potentially toxic taxa, *Cylindrospermopsis raciborskii* and that its autoecology made it successful. This paper will describe the state of our knowledge of *Limnothrix* from both a taxonomical and an ecological aspect.

The Redfield Ratio under challenge- Paradigm loss or the unravelling of misconceptions?

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Abstract: Since the report by A. C. Redfield in 1958 on the similarity of the average atomic ratio of carbon to nitrogen to phosphorus (C:N:P) in phytoplankton, the "Redfield Ratio" has been embroiled in controversy. Despite this, it has become a major paradigm in phytoplankton ecology, underpinning a range of practical and experimental applications. It is used to identify the major nutrient likely to become limiting in aquatic systems, to assess the potential phytoplankton biomass supported, and the likelihood that nutrient conditions will lead to the growth of cyanobacteria. In this context it is used to assess water quality and to interpret the potential impact of nutrient loads in surface waters and ground waters derived from both natural and anthropogenically modified sources. The Redfield ratio is used to direct additions of nutrients to experimental lakes, to field mesocosm incubations, and to laboratory experiments investigating the influences of nutrient supplies and nutrient ratios on cellular growth, biochemistry and ecophysiology. The ratio has been used extensively in modelling at system scale, where relationships between nutrients and nutrient ratios are used to predict phytoplankton responses in surface waters. It has also been applied in models at the cellular scale, frequently being used to set a target for cellular composition in order to estimate the incorporation of nutrients into phytoplankton cells. Yet it has long been known that the composition of phytoplankton can vary widely from the Redfield ratio. This was identified in culture experiments, particularly with continuous cultures, but the lack of natural environmental variation in these experiments made comparisons with natural cellular compositions uncertain. Recently large data sets of field phytoplankton have been examined and these demonstrate that cellular composition varies greatly from the Redfield Ratio, both within and between species.

Does this mean the loss of the Redfield Ratio paradigm? If so, how can this ratio have been so successful in identifying nutrient conditions in the field, been critical to the interpretation of nutrient loading models, been almost universally adopted to estimate the likelihood of cyanobacterial dominance in freshwater systems, yet be an unreliable indicator of cellular composition? A crucial question is whether or not it is still appropriate to apply the concept of the Redfield Ratio, and if so in what situations? An extensive discussion of this issue is taking place in the literature and this information will be used to provide an overview of the current state of the debate in relation to global, regional and cellular interpretations of the Redfield ratio. This will form the basis of an analysis of the status and fate of the Redfield ratio, especially its role in defining conditions for the occurrence of cyanobacteria in inland waters.

Key words: Redfield ratio; global nutrient ratios; phytoplankton nutrient requirements; nutrients and cyanobacteria growth

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Cyanobacterial development in warmer climate: does trophic state matter?

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Abstract: Concerns about increasing cyanobacterial abundance and associated toxins under a changing climate have prompted considerable interest from lake managers, lake modellers and the public.

Changes in temperature and nutrients are considered the most important factors controlling phytoplankton composition and cyanobacterial abundance in freshwater lakes, although their relative importance and their interaction under at different trophic conditions are still unclear. In this study we applied a recently developed open source 1D ecological model GLM-FABM to two lakes with different trophic state (Mt Bold reservoir, AU; Lake Tarawera, NZ). After calibrating and validating the models, a matrix of 25 scenarios, combining temperature and nutrient changes during a period of two years was simulated for both lakes. Changes in physical and chemical variables affecting phytoplankton abundance and composition was analysed and the relative importance of temperature and nutrient and their interaction was evaluated.

Different sensitivity to cyanobacterial bloom development was identified for systems with different trophic status when analysing the combined effect of warmer climate and nutrient variation. Moreover, the competition between algal groups (e.g. chlorophytes and cyanobacteria) was identified as a significant factor controlling the development of the phytoplankton community and its response to the external drivers.

Model results indicate that an increase in cyanobacterial biomass is less likely to occur in oligotrophic than in eutrophic systems. The cyanobacterial response to increasing temperature in eutrophic system is ultimately determined by both nutrient availability and the interaction/competition of cyanobacteria with other phytoplankton groups. Finally, the effect of temperature by itself is not enough to sustain cyanobacterial growth, so reducing nutrient input will be beneficial to mitigate the global effect of climate change.

Key words: climate change, algal community composition, cyanobacterial growth, ecological modelling, nutrients

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"Toxic in crowds" - insights into microcystin regulation using mesocosms and surveys of natural lake populations

Susie Wood[1,2], Jonathan Puddick[1], Debin Meng[1], Hugo Borges[1,2], Daniel Dietrich[3], Ian Hawes[4], David Hamilton[1]

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Abstract: Microcystins are the most commonly detected freshwater cyanobacterial toxin worldwide and are usually associated with the bloom forming cyanobacteria *Microcystis*. Despite the global occurrence of toxic *Microcystis* blooms, the parameters that regulate microcystin production remain uncertain. Laboratory studies have shown correlations between microcystin quotas and a multitude of physiochemical variables including nutrients, temperature and pH. Whilst these laboratory-based experiments allow environmental conditions to be stringently controlled, studying cyanobacteria in these 'artificial' environments may alter or remove variables that regulate microcystin production. Over the past three years, our group has studied microcystin production in a small eutrophic lake (Lake Rotorua, Kaikoura, New Zealand), using a series of mesocosms experiments and surveys of natural populations. Manipulating *Microcystis* cell densities in mesocosms resulted in an increase of ca. 20-fold in intracellular microcystin over a 5-hour period. Recently we have undertaken two extensive profiles of the surface waters across the whole lake, and a further intense study focused on a small enclosed bay to explore whether this same phenomenon occurred naturally with spatial variation in cell density around the lake. Cyanobacterial density was assessed fluorometrically and microscopically, and samples were collected to determine microcystin (liquid chromatography-mass spectrometry) and nutrient concentrations, and to examine expression of genes involved in toxin production. A range of physiochemical variables including temperature, pH and dissolved oxygen were also measured. Initial analysis shows greater than 50-fold difference in microcystin quota, with varying correlation to physiochemical variables and cell densities. These data are being used to elucidate how interactions between abiotic and biotic variables influence microcystin synthesis and may ultimately help to predict parts of a lake or periods of greatest health risk.

Key words: mesocosm, in-lake studies, microcystin, Microcystis

Temporal patterns of microbial and metabolic covariance within a freshwater cyanobacterial bloom

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Abstract: Freshwater cyanobacterial blooms are typified by the rapid proliferation of one or more cyanobacterial species. Their proliferation is associated with the production of toxic secondary metabolites, or cyanotoxins, that exhibit potent activity against a range of biological targets. The occurrence of these blooms and the production of toxic metabolites has been the focus of many studies in the context of abiotic influences. Increasing evidence is emerging that these organisms and the fate of their metabolites is dependent largely on biotic rather than abiotic factors. These include, but are not limited to, nutrient cycling, predation, signalling, cellular attachment and catabolism of nitrogenous metabolites.

We have applied systems biology approaches, including correlation (network) analysis to determine associations between; ecophysiological parameters, cyanobacterial cell counts, bacterial and fungal OTUs and secondary metabolites across a cyanobacterial bloom. A cyanobacterial bloom, in Yanga Lake, NSW, Australia was monitored at five sites across a 6-month period. Microbiome profiling of bacterial and fungal communities was obtained using the MiSeqTM platform, and secondary metabolome profiling was performed on methanol extracts using UPLC-HRMS.

Over the bloom period 22 cyanobacterial species were observed, with the highly dynamic cyanobacterial community transitioning from a state dominated by the saxitoxin-producing, diazotrophic Anabaena circinalis to one dominated by the microcystin-producing Microcystis, before dispersing. The microbial community mirrored this trend, with the occurrence of cyanobacteria positively influencing the microbial diversity and richness observed. The secondary metabolite profile was less defined across a temporal scale, reflective of the many abiotic, organismal and genetic factors that contribute to the production of these molecules. Pearson correlation coefficients between variables were visualised as a network to identify ecophysiological factors, microbial OTUs and metabolites that coincided with bloom stages.

It is becoming increasingly apparent that a holistic view of cyanobacterial blooms, incorporating systems biology approaches is necessary to understand the fundamental processes that occur. This study provides a glimpse into how the freshwater microbial communities respond to the rapid proliferation of cyanobacteria and the ecological mechanisms of the vast array of toxic metabolites present.

Key Words: Systems Biology, Next-Generation Sequencing, Microbial Ecology, Metabolomics, Metagenomics

Environmental drivers of toxin production by the cyanobacterium *Cylindrospermopsis raciborskii*

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Abstract: The toxic cyanobacterium *Cylindrospermopsis raciborskii* blooms in water supplies throughout Australia. Significant resources are spent by water authorities to monitor cell densities and toxin concentrations to ensure that water supplies are safe for drinking and recreation. Toxin concentrations are not always well correlated with cell densities such that densities cannot be used as a proxy for toxin concentrations. A recently completed ARC Linkage project ($1.2M), which was a collaboration between Griffith University, Monash University, University of NSW and the water authority, Seqwater, tackled this issue by examining the factors affecting toxin production by C. *raciborskii*. Laboratory studies of strains of C. *raciborskii* which examined the toxin gene, toxin gene expression, toxin cell quotas and growth cycle showed that cylindrospermopsin is constitutively produced with higher cell densities resulting in higher cell concentrations, irrespective of growth conditions. Additionally, extracellular toxin concentrations only became significant late in the growth phase, suggesting that cell lysis is primarily responsible for extracellular toxin concentrations. The studies also identified that strains vary considerably in their cylindrospermopsin cell quotas under the same environmental conditions. This was irrespective of whether the strains were from the same or different water sources. Therefore strain dominance appears to be an important factor in determining toxin concentrations. This was substantiated in mesocosm experiments which examined toxin producing strains of C. *raciborskii* within a mixed algal population in a drinking water reservoir. Studies also showed that environmental factors, such as nutrients, light intensity and CO2 levels affected toxin production, and strains varied considerably in their response to these factors. This variability in response provides a mechanism for C. *raciborskii* to thrive and dominate under a range of environmental conditions, which may be an important driver in its success worldwide.

Key words: nutrients, reservoirs, cylindrospermopsin, algal toxins

MIB-producing cyanobacteria (*Planktothrix* sp.) in a drinking water reservoir: Distribution and odor producing potential

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Abstract: The production behaviours of odorant 2-methylisoborneol (MIB) in water bodies by *Planktothrix* sp. have not been understood very well. Through four-year investigation in Miyun Reservoir, a huge mesotrophic drinking water reservoir for Beijing known to have the MIB episodes, we found that the *Planktothrix* sp. bloomed with MIB in this reservoir together during September and October. The spatial distribution of MIB was investigated with a large sample size to trace the main living areas of the deep living MIB-producing cyanobacteria. The results indicated that the north shallow region is the major habitat for *Planktothrix* sp. due to the relatively high concentrations of nutrients. Quantile regression analysis between *Planktothrix* biomass and MIB concentration shows that the risk of MIB exceeding the odour threshold (15 ng L^{-1}) in water was as high as 90 % when the *Planktothrix* density was more than 5.0×10^5 cells L^{-1}, while the risk was reduced to 10 % when the *Planktothrix* density was below 1.6×10^4 cells L^{-1}. This study will allow better understanding of the environmental behaviours of *Planktothrix* sp., and can provide useful information for better management of drinking water lakes/reservoirs experiencing the T&O problems caused by deep living cyanobacteria species.

Key words: Planktothrix, 2-Methylisoborneol, Taste and odour, Drinking water, Deep living cyanobacteria

Cyanobacterial blooms in waste stabilisation ponds: how can ecology help the water authorities in Australia

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Abstract: There is evidence, that wastewater can be used as a fertile testing ground for a range of fundamental ecological questions. Wastewater stabilisation ponds (WSPs) are highly productive systems designed to treat wastewater using only natural biological and chemical processes, but they are known to operate at different levels of efficiency, with some developing extensive cyanobacterial blooms. Phytoplankton, microbial communities and hydraulics play important roles for ecosystem functionality of these pond systems. Although WSPs have been used for many decades, they are still considered as 'black box' systems as very little is known about the fundamental ecological processes which occur within them. However, a better understanding of how these highly productive ecosystems function is important, as treated wastewater is commonly discharged into streams, rivers, and oceans, and subject to strict water quality guidelines.

Biotic and abiotic factors influencing toxic cyanobacterial blooms in an urban and residential freshwater environment

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Cyanobacterial blooms exhibit a number of potential threats to the ecosystem and human health. In addition to strong aesthetic impacts, including dense surface scums and the production of the odourous chemicals, the production of secondary metabolites by toxic cyanobacterial species jeopardise the health of livestock, birds, aquatic species and humans. While cyanobacterial blooms can occur in marine environments, blooms that occur in fresh waters are of most concern due to the risk of exposure through consumption or recreational use. Many studies have focused on an analysis of abiotic factors that influence the presence of toxic cyanobacterial blooms. A thorough comprehension of biotic factors influencing toxin bloom formation is yet to be achieved.

Situated just over 3 km from Central Station, Centennial Park is located in the heart of Sydney. A 7 month monitoring program spanning October 2013 to May 2014 was undertaken at two sites within Centennial Park. During this period, a combination of *in situ* YSI probe measurements, cell enumerations, cyanobacterial toxin and toxin gene quantification were conducted. The weekly frequency and long duration of sampling period allowed for in-depth of the abiotic and biotic influences on toxin quantity at the two sites under pre-bloom, bloom, and post-bloom conditions. The comparison of a new qPCR method for the detection of cyanobacterial toxin genes with toxin quantification approaches could prove beneficial in providing a proactive solution to bloom management.

There exists a lack of understanding of the abiotic and biotic factors influencing toxin production and regulation under true environmental conditions. This study, in taking an in-situ approach to the study of ecological influences on toxin production allows for an insight into the complex networks governing toxic blooms in an urban and residential system.

Key Words: Urban, Residential, YSI, qPCR, Microbial Ecology, Toxin Quantification.

Saxitoxin at the Australian Drinking Water Guideline Level Alters Neuronal Differentiation of D3 Embryonic Stem Cells

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Abstract: The neurotoxin Saxitoxin (STX) is part of a large group of structurally related analogues produced in both marine and freshwater environments which are most commonly known for their role in paralytic shellfish poisoning. Its production by cyanobacteria in freshwater from which some Australian drinking water is sourced makes STX a potential public health concern. STX acts by blocking voltage-gated sodium channels that are vital for the function of nerves. At high doses seen in shellfish this can lead to paralysis and death by respiratory depression. Acute exposure from marine sources has been well researched and strict guidelines preventing exposure exist. To date there have been no poisonings from freshwater sources and a drinking water guideline, derived from human data on acute exposure through shellfish ingestion, does exist. However, there are no guidelines for chronic low dose exposure despite this being a more likely scenario in drinking water.

Previously we have shown adverse morphological effects of model neuronal cells exposed to STX at or below the guideline level (3µg/L) for 7 days[1]. This together with the known involvement of voltage-gated sodium channels in neurite outgrowth and development lead us to hypothesise that chronic low dose exposure to STX could affect developing neurons.

We therefore used mouse embryonic stem cells to determine if STX has an adverse effect on their differentiation into a neural lineage. D3 stem cells were differentiated into a neural lineage using retinoic acid in the presence or absence of STX at the ADWG guideline level (3µg/L) following the 4-/4+ protocol[2]. This protocol includes 4 days without retinoic acid or treatments (4-) to allow for aggregation of cells followed by 4 days in the presence of retinoic acid and treatments (4+). This protocol has been shown to successfully induce stem cells to express neuronal morphology. Cells were assessed by scoring the development of morphological neuronal features and expression of 3 genetic markers of neuronal differentiation (*oct4, mixL1* and *nestin)*. Preliminary morphology results showed a statistically significant decrease in neuronal scores in STX treated cells (21% one way ANOVA $p<0.05$), suggesting STX reduced their neural differentiation compared to controls. If results from the gene analysis confirm that differentiation is inhibited then further investigation into this pattern of exposure would be warranted, as this has potential implications for the safety of STX-affected drinking water.

Key words: Saxitoxin (STX), Chronic exposure, Neuronal development

[1]O'Neill et al., Chronic Low Dose Exposure to Saxitoxin Inhibits Neurite Formation in Model Neuronal Cells 3rd National Cyanobacteria Workshop 2012, Oral Presentation
[2]Bain, G., et al., Embryonic stem cells express neuronal properties in vitro. *Developmental Biology*, 1995. **168**(2): p. 342-57.

Molecular techniques for cyanobacterial research and monitoring - current applications and future perspectives

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Abstract: The intensity and regularity of cyanobacterial blooms is escalating globally. There is a corresponding need for rapid, reliable and high-throughput methods to identify, characterise, and understand how these microorganisms and their toxins function in complex environmental samples. Recent advances in molecular technologies provide many opportunities to advance knowledge and provide innovative diagnostic tools. This presentation will use examples from New Zealand to demonstrate how molecular genetic techniques have been used to investigate research questions, and how they are being applied to improve baseline knowledge and routine monitoring programmes.

Research case studies will show how we used to molecular techniques to investigate; (1) if Australians were responsible for the arrival of *Cylindrospermopsis* in New Zealand, (2) to explore if microcystins are continuously produced during a *Microcystis* bloom, and (3) how bacterial communities are involved with facilitating bloom formation in benthic cyanobacteria. In a more applied example, I will show how end-point PCR and Next-Generation Sequencing were used to obtain a 'snapshot' of cyanobacterial diversity and toxin production in a survey of planktonic cyanobacteria from 150 lakes across New Zealand.

Despite research and validation demonstrating their potential, the application of molecular techniques by monitoring agencies has been limited. Legislative requirements, costs, and a reluctance to change methodologies are the most likely reasons for this and I will discuss how, or if, these limitations can be overcome as technology advances.

Key words: end-point PCR, sanger sequencing, quantitative PCR, Next-Generation Sequencing.

Rapid, multiplex-tandem PCR assay for automated detection and differentiation of toxigenic cyanobacterial blooms.

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Abstract: Cyanobacterial blooms are a major water quality issue and potential public health risk among freshwater, marine and estuarine ecosystems globally because of their potential to produce cyanotoxins (primarily microcystins, paralytic shellfish toxins (e.g., saxitoxin), cylindrospermopsins and/or nodularins). To date a significant challenge to the effective management of cyanobacterial blooms has been the inability of classical microscopy-based approaches to consistently and reliably detect and differentiate toxic from non-toxic blooms. The potential to produce cyanobacterial toxins has been linked directly to the presence of specific biosynthetic gene clusters. Here, we describe the development of a robotic PCR-based assay for the semi-automated and simultaneous detection of genes of each of the major toxin biosynthetic genes characterized to date for the production of microcystins, nodularins, cylindrospermopsins and saxitoxins. We demonstrate the sensitivity and specificity of each assay using well-characterised, cultured isolates and establish its utility as a quantitative PCR using DNA, clone and cell-based dilution series. In addition, we used 206 field-collected samples and 100 known negative controls to compare the performance of each assay against conventional PCR as well as direct toxin detection. We report a diagnostic specificity of 100% and a sensitivity of 97.7% or greater for each assay.

Key words: cyanobacteria, toxigenic, real-time quantitative PCR, robotic detection, high resolution melt

Development of CyanoDTec: A rapid molecular assay for the routine monitoring of toxic cyanobacterial blooms

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Abstract: Routine monitoring of water bodies for the presence of toxic cyanobacterial species is standard practice for all water management authorities. The standard approach still involves species identifications and cell count enumerations by a trained phycologist/microbiologist to identify potentially toxic species. The identification of potentially toxic species usually results in the restricted use of water bodies, with the implementation of analytical detection using UV-HPLC avoided due to technical and cost limitations. A significant caveat of this approach is that it makes use of large assumptions regarding the distribution of toxic genes amongst cyanobacteria and ignores competition between toxic and non-toxic strains.

The identification of cyanotoxin biosynthesis pathways within the genomes of numerous freshwater cyanobacteria has enabled molecular probes targeting these genes to be developed. Four sets of highly specific TaqMan® probes were developed targeting cyanobacteria 16S rRNA (*16S*), microcystin and nodularin synthetase (*mcy/nda*), cylindrospermopsin synthetase (*cyr*) and saxitoxin synthase (*sxt*) genes. Here we describe the development and validation of CyanoDTec, a rapid "off-the-shelf" molecular assay for the identification and quantification of toxic and non-toxic cyanobacterial species. Reference material comprising of plasmid DNA containing defined copy number of target sequences for *16S, mcy/nda, cyr* and *sxt* gene was designed and characterised at NMI's laboratories using droplet digital polymerase chain reaction (ddPCR) technology. This reference material was used for the production of standard solutions suitable for constructing calibration curves to be incorporated into the CyanoDTec DNA test kits.

The CyanoDTec DNA test kit and NMI developed reference materials were validated across three independent bloom events, including in the Great Lakes, USA, Centennial Parklands, Sydney and Yanga Lake, NSW. This work describes how the implementation of data derived from the CyanoDTec DNA test kit and NMI developed reference materials can inform water management authorities and readily form part of routine monitoring activities.

Key words: Microbial Ecology, Cyanobacteria, Molecular assays, DNA test kit, DNA reference materials.

Evaluation of a hand-held spectrophotometer for the proximal remote sensing of cyanobacterial abundance in water bodies.

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Abstract: A hand-held Water Insight BV WISP-3 spectrophotometer was assessed as a potential instrument for proximal remote sensing of cyanobacterial abundance in 3 small urban lakes in eastern Sydney, NSW during spring, summer and autumn of 2013-14. The instrument provides measurements of chlorophyll, phycocyanin and total suspended material (TSM) concentrations within the water column, and of the downwelling vertical attenuation coefficient (K_d). The instrument was assessed against laboratory measurements of water samples collected at the same time of the field measurements for chlorophyll, TSM, and total cyanobacterial biovolume. Chlorophyll, phycocyanin, and turbidity measurements were also made in-situ using a Yellow Springs Instruments (YSI) EXO2 water quality sonde equipped with fluorometric sensors, and K_d was measured in-situ with a Licor quanta meter and underwater quanta sensor.

Up to 20 replicate measurements were made with the WISP-3 per lake on each sampling visit. There was generally little variability between measurements on fine sunny days, but sometimes (but not always) considerable variability on overcast and windy days. Data with high variability were deleted from subsequent analysis.

Generally the chlorophyll measurements made by the WISP-3 were in good agreement with the laboratory measurements, albeit slightly lower. Some data points found to have been measured on cloudy days nevertheless appeared anomalous, even when between-replicate variation was small. Exclusion of all data measured on days with 5/8 cloud cover or more considerably improved the fit (R^2) between the WISP-3and laboratory chlorophyll data. YSI chlorophyll measurements were mostly lower than the WISP-3 and laboratory measurements, although the WISP-3 and YSI data correlated positively.

Phycocyanin measurements made with the WISP-3 (cloudy days excluded) changed roughly in unison with the laboratory total cyanobacterial biovolume estimates, however the relationship between the two measures was weak. A similar relationship was found between YSI phycocyanin measurements and total cyanobacterial biovolume. A strong positive correlation was found between WISP-3 and YSI phycocyanin measurements, although WISP-3 measurements were approximately 50 times greater than the YSI.

The WISP-3 measurements of TSM and K_d were poorly correlated with the laboratory and field measurements of these, respectively.

The WISP-3 appears to perform well in terms of remote sensing chlorophyll in these lakes, provided the weather is fine and there is little cloud cover. WISP-3 phycocyanin measurements may provide a rapid field based surrogate measure for total cyanobacterial biovolume, but we believe that further adjustment of the WISP-3 algorithms is needed to improve the relationship between the two. There remains uncertainty over the units of phycocyanin measurements made by the WISP-3 and YSI instruments.

Key words: Remote sensing, phycocyanin, chlorophyll, total cyanobacterial biovolume, fluorometry

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Advances in Earth Observation based technologies to assist algal management

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Abstract: Water quality is a critical component of global fresh water security and ecosystem health, yet existing data are scarce and declining, have poor geographic and temporal coverage, and may be of questionable accuracy. The value of remote sensing to improving understanding of water quality is recognised[1]. These methodologies may provide a complementary data stream in the water quality monitoring toolkit, complementing and adding value to existing monitoring technologies, including detection of the formation of algal blooms.

Whilst the underlying physics of optical inland water quality is the same as that for ocean colour, remote sensing of inland waters is complicated by greater variability in optical properties. Inversion algorithms are sufficiently mature to cope with the variability of optical properties in inland waters, but they are primarily limited by reduced knowledge of the bio-optical properties of inland waters.

CSIRO has continued its strategic investment in Australian inland water quality remote sensing to address knowledge gaps to achieve these goals. These include studies of the detailed optical properties of selected Australian inland waters, the testing of spectral inversion algorithms (developed originally for coastal waters) on in situ reflectance and satellite data in inland waters, and algorithm improvement and for improved prediction of inland water quality.

The presentation will illustrate the key findings of these studies, which include: 1) water quality from high resolution satellite imagery reveal spatial patterns and variability under bloom forming conditions; 2) the accuracy of chlorophyll-a retrieval from remotely sensed data significantly improves with improved knowledge of the optical properties of inland waters; 3) biogeochemical contribution to absorption differs significantly by site and season; 4) optical complexity in Australian inland waters varies significantly with latitude and region; 5) new satellite systems will significantly enhance our ability to monitor water quality at the medium scale. The implications of these findings will also be presented.

Key words: remote sensing; in situ reflectance, algorithm, algal blooms

[1] The Group on Earth Observations (GEO) Inland and Near-Coastal Water Quality Remote Sensing Working Group

Profiling Cyanobacteria Risk in Drinking Water Supply Reservoirs

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Abstract: The Cyanobacteria Risk Profile, a key component of the Sydney Catchment Authority (SCA) Cyanobacteria Management Strategy, provides a long term view of cyanobacteria risk status in the Sydney raw water supply system and forms the basis for a meaningful risk management approach. Further, the Risk Profile informs ongoing research gaps specific to SCA's operating needs. The Cyanobacteria Risk Profile brings together demonstrated knowledge in the field of algae and cyanobacteria and analysis of nutrient and algae trends in each of the SCA reservoirs. The presentation highlights the SCA experience with cyanobacteria risk profiling. Risk matrices that draw on trophic indices and the historical record are presented. SCA reservoirs are grouped according to their potential for toxic cyanobacterial blooms and trophic status and trajectory. These groups range from storages with high trophic status/trajectory and a history of potentially toxic blooms to those with low trophic status and no history of blooms. Novel quantitative methods are presented, including a probabilistic seasonal risk method and a parametric trend model. The trend model is a significant advancement on non-parametric tests (e.g. Mann-Kendall), handling both linear and non-linear trends. Significance of non-linear trends is determined by finite difference. Challenges in stochastic modelling of long-term nutrient and cyanobacteria data will be discussed and quantitative methods for capturing seasonal and long term trends presented. The presentation highlights the recent experiences of SCA with the occurrence of cyanobacteria, and draws on conclusions from a number of SCA research projects, compares and contrasts the susceptibility of different reservoirs, and facilitates the development of an annual algal risk forecasting capability. Finally the presentation sets out how the Risk Profile informs the SCA's Cyanobacteria Management Strategy which prioritises the short-, medium- and long-term knowledge and management actions, and objectively allocates resources to deliver those requirements.

Key words: (12 maximum) Research / Knowledge / Trend / Risk Profile / Trophic Index / Cyanobacteria / Management Strategy

Risk based analysis for cyanobacteria in waste stabilisation ponds

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Abstract: Waste stabilisation ponds (WSPs) are engineered water bodies designed to treat sewage using only natural processes. Many of them frequently develop potentially toxic cyanobacterial blooms. The potential for releasing effluent from WSPs that contain both, cyanobacterial biomass and cyanotoxins represents a significant risk that requires close monitoring, especially where treated effluent is reused. We developed a framework, based on a risk assessment approach, to calculate the optimum monitoring frequency for WSPs in Western Australia. The framework, adapted from medical diagnosis approaches, is based on the definition of hazard (i.e. cyanobacterial biomass, cyanotoxin concentration), an accurate measurement of hazard frequency, and the tolerance chosen that a hazard will go undetected during periods when monitoring is not undertaken. The developed framework allows determining flexible and site-specific optimized monitoring regimes for water bodies that develop cyanobacterial blooms.

Tailoring algal floc properties for more robust cyanobacteria removal during drinking water treatment

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Abstract: The presence of cyanobacterial blooms in drinking water sources represents a challenge to water utilities as it creates operational problems including increasing coagulant demand and cell carry-over to downstream processes. A key barrier to cyanobacteria in the water treatment plant is the use of separation processes, such as sedimentation and dissolved air flotation (DAF), preceded by coagulation and flocculation (C-F). However, C-F is difficult to optimise as its effectiveness is governed by variables including operational parameters, such as coagulant type, coagulant dose, pH and raw water character, such as cell species and concentration and the composition and concentration of algogenic organic matter (AOM). The coagulation conditions in turn drive floc properties which impact separation efficiency. The aim of this research was to investigate how adjustment of coagulation conditions (type, dose and pH) impact *Microcystis aeruginosa* (*M. aeruginosa*) floc properties and therefore provide information on how the coagulation process could be optimised dependent on the downstream separation process. The properties of flocs produced by coagulating *M. aeruginosa* with aluminium sulphate and ferric chloride at different doses and pH values were evaluated, including floc size, growth rate, capacity to resist breakage on exposure to different shear rates and regrowth potential. Floc properties varied depending on the coagulation mechanism, for example, charge neutralisation (CN), sweep flocculation (SF) or a combination of these. Overall, it was demonstrated that it was possible to tailor coagulation conditions by manipulating the coagulation conditions. The optimal scenario for effective sedimentation was to apply ferric as a coagulant under SF mechanisms as flocs are large, compact and strong, which is beneficial in the settling process. In contrast, alum was reported to produce smaller flocs thus are favoured in DAF as small flocs are effectively floated. Furthermore, the impact of floc exposure to high turbulence in this process is less important. A decision flow sheet was developed to be used to assist in determining operating conditions for coagulation during cyanobacterial blooms depending on the separation process employed.

Key words: coagulation and flocculation, cyanobacteria, aluminium sulphate, ferric chloride, drinking water

Management of toxic cyanobacteria in full scale water treatment plants

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Abstract: The detection of cyanobacteria and their associated toxins has increased in recent years in water sources, within water treatment plants (WTP) and in potable water across the world. The objectives of this project were to: (1) study in detail the concentration patterns of cyanobacterial cells in full scale processes including clarification, filtration and oxidation, (2) map the location of cyanobacterial cells in the sedimentation tank and over the filter, and (3) propose operational solutions to manage and if possible prevent these accumulations.

Five cyanobacterial bloom events were closely monitored in three full scale WTPs with samples taken from raw water, after the addition of coagulant and powdered activated carbon, after clarification, within the clarifier sludge bed, over the filter, after the sand-anthracite filter and after chlorination. Cyanobacterial taxonomic enumeration and cyanotoxins analysis were completed on water and sludge samples, along with detailed treatment operation data. *In vivo* phycocyanin fluorescence probes were used to monitor cyanobacteria presence in raw water, clarified water, filtered water and treated water. These probes were also used to measure the spatial distribution of cyanobacterial cells in the water over the lamella plates in sedimentation tank and over the filter media.

Varying species dominated the blooms including *Anabaena* sp., *Aphanizomenon* sp. and *Microcystis* sp. A maximum total cell number of 38.7×10^6 cells/mL was recorded. Clarification was identified as the major accumulation site within the WTPs. However, coagulation of *Aphanizomenon* sp. cells was challenging and resulted in high numbers of cells at the surface of the clarifier (21.6×10^6 cells/mL) and breakthrough to the surface of the filter (14.6×10^6 cells/mL). *Aphanizomenon* sp. cells were also observed in filtered water and caused turbidity breakthrough from the usual levels of 0.06NTU to 1.4NTU, leading to a six weeks drinking water advisory. *In vivo* phycocyanin profiles showed clear accumulation of cells at the surface of the sedimentation tank and a quasi-homogeneous distribution over the filter media. Toxic *Microcystis* sp. cells were dominant species in the clarifier sludge. A maximum cyanobacterial concentration of 3700 cells/mL, dominated by *Aphanizomenon* sp., was also measured in chlorinated drinking water.

This work demonstrates that transient elevated concentrations of cyanobacteria can enter plants and disrupt conventional treatment process resulting in the breakthrough of cyanobacteria and the loss of disinfection credits, even with excellent overall removals of cells (>2.6Log). Results also show selective removal of the cyanobacteria species by coagulation. The results of this study demonstrate the need for on-line monitoring of cyanobacteria to insure appropriate and on time treatment adjustment. The new ARC Linkage Project, conducted at the UNSW Australia in collaboration with AWQC is aimed to develop a protocol for the use of *in vivo* fluorescence probes to predict coagulation dose and powdered activated carbon application in removal of cyanobacteria and their harmful metabolites.

Key words: Toxic cyanobacteria, drinking water, treatment process, clarification, filtration, oxidation, accumulation, in vivo monitoring

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Effect of bromide on treatment of algae-containing water by preozonation: cell Integrity and Br-DBPs.

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Abstract: High level of bromide ion (>200 μg/L) occurred as the result of saline water intrusion in an estuarial reservoir for drinking water resource. Cell lysis rate in algae solutions with bromide was almost same as that without bromide during the first 10 min. However, the cell lysis was prolonged in bromide-containing solutions, due to the formation of free bromine during ozonation. Furthermore, DBPsFP experiments with free bromine disinfection were conducted using aqueous suspensions of *Microcystis aeruginosa*, to evaluate the formation of trihalomethanes (THMs) and haloacetonitriles (HANs) and their cytotoxicity. Brominated disinfection by-products were the major products when free bromine was applied. The total THMs formed after bromination was much as that formed after chlorination, whereas HANs were elevated after bromination. Dibromoacetonitrile ($C_2H_2NBr_2$) and bromoform ($CHBr_3$) were the only detected species during free bromine disinfection. The production of $C_2H_2NBr_2$ and $CHBr_3$ increased with disinfectant dosage but decreased with dosing ammonia. $CHBr_3$ increased with the pH changing from 5 to 9. However, $C_2H_2NBr_2$ achieved the highest production at neutral pH, which was due to a joint effect of variation in hydrolysis rate and free bromine reactivity. Finally, estimation of cytotoxicity of the disinfected algae solutions showed that HANs formation was responsible for the majority of toxicity. Considering its highest toxicity among the measured disinfection by-products, the elevated $C_2H_2NBr_2$ should be considered when using bromine-related algaecide.

Key words: Bromide; Preozonation; Cell integrity; Br-DBPs; Drinking water

Effect of water quality changes on biological filtration efficacy

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Abstract: A major concern for water utilities worldwide is the aesthetic drinking water quality impact associated with cyanobacterial blooms, in particular the taste and odour compounds 2-methylisoborneol (MIB) and geosmin. Previous pilot and laboratory studies have demonstrated that biofiltration is extremely effective for the removal of these taste and odour compounds. Biological removals of geosmin and MIB have also been demonstrated at full scale during conventional dual media filtration and granular activated carbon filtration at a number of Australian plants. However, the specific operating conditions that favour effective biological removal of a range of cyanobacterial metabolites have not yet been identified. Examples of operational changes that are known to have an impact on biological removal at the full scale include – disinfectant residual in the backwash water, optimised coagulation for turbidity removal and pre-chlorination. However, the cause of instances of reduced biological removal at full scale remains unexplained. One factor that all examples have in common is that the effect on the biofiltration process was an unintended consequence of an operational change for some other water quality goal.

Current knowledge indicates that the presence of cyanobacterial metabolites and the presence, or lack, of degrading organisms impacts the removal rates of biological filters. Three scenarios were investigated to determine if seeding with indigenous organisms would increase biological removal of MIB and geosmin and whether the presence of these compounds increases the removal efficiency: (1) intermittent spiking of metabolites, (2) seeding with indigenous bacteria, (3) no bacteria in feed water source followed by continuous spiking of metabolites. The objective was to assess potential effects of changes in water quality on the efficacy of biological filtration at full scale. The scenarios demonstrated that it is possible to encourage biological degradation by acclimation and seeding; however the conditions required for the establishment and maintenance of a degrading biofilm may not always be maintained. Overall, in the absence of disinfection all filter media will sustain a biofilm; however the ability of the biofilm to remove cyanobacterial metabolites will depend on the number and the composition of the colonising bacteria.

Key words: biological filtration, cyanobacteria, metabolites, MIB, geosmin, water quality.

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Hydrogen peroxide: A new way to control cyanobacteria

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Water utilities around the world and in Australia have perennial problems with the control of growth of cyanobacteria in source water leading to downstream drinking water challenges. Currently SA Water uses copper sulphate as an algaecide to control cyanobacteria but this chemical is not regarded as the long-term preferred option due to awareness of its adverse environmental impacts on the aquatic ecosystem. In addition there are issues with sourcing chemical of suitable quality to meet registration requirements due to the nature of the commodity chemical market which adds another business risk to long term usage. These issues have driven the need and an extensive research program to develop an alternative control method. Laboratory based research undertaken at SA Water identified stabilised hydrogen peroxide (SHP) as a viable option to control cyanobacterial growth. However, before it can be used in reservoirs it needs to undergo a thorough assessment on its efficacy in an open water body and its impact on other aquatic organisms. Results from SHP treatment using large scale on-site open tanks filled with reservoir water containing cyanobacteria will be presented. Laboratory based eco-toxicological studies investigating the impact of SHP on various aquatic organisms will also be discussed.

Key words: Cyanobacteria, Source Water Management

Management of treatment sludge impacted by cyanobacteria

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Abstract:
The fate of cyanobacteria in drinking water treatment sludge has historically gained little attention in the scientific literature. The limited information available suggests that cyanobacteria, once incorporated into a floc during coagulation, rapidly lose viability and release metabolites such as the cyanotoxins and taste and odour compounds (T&O) MIB and geosmin. However, recent research has suggested that cyanobacterial cells remain intact after aluminium sulphate (alum) coagulation and as a result cyanobacterial metabolites can accumulate within the sludge. Therefore the sludge is recognised as a potential source of concentrated toxins and/or T&O that could affect water quality if supernatant from the sludge treatment facility is recycled to the head of the plant, or if sludge is retained for longer than several hours in the clarifier. Therefore, the key aims of the project were to (i) investigate the rate of biological degradation of dissolved metabolites, (ii) determine the increase in metabolite concentration in the sludge and supernatant, and (iii) monitor the proliferation of cyanobacterial species in treatment sludge. In order to determine whether cyanobacteria can proliferate in sludge as well as determine the fate of the cyanobacterial metabolites, experiments were conducted using alum coagulated water samples spiked with cyanobacteria (cultured and environmental samples) with monitoring of cell growth and metabolite concentrations in the sludge and supernatant over an extended period. The results indicated that the cyanobacteria present in the alum sludge continued proliferating and remained intact for up to 12 days, and releasing metabolites for up to 30 days following sludge formation. These findings suggest that the recycling of sludge supernatant to the head of a water treatment plant during a cyanobacterial bloom event may result in dissolved metabolites being reintroduced. This is of major concern to water utilities using conventional treatment (coagulation, flocculation, sedimentation and disinfection), as these processes do not effectively remove cyanotoxins and/or T&O.

Key words: Sludge, cyanobacteria, metabolites, cyanotoxins, MIB, geosmin, biological degradation

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