LakeScience Rotorua

A newsletter about research on the Rotorua Lakes

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Welcome to the eighth issue of our email newsletter for those involved in or interested in scientific or management work on the Rotorua Lakes. It is up to **you** to make this informal newsletter a success by providing it with copy – our Society is merely providing the vehicle. We email it free of charge to all those who attended the Rotorua Lakes 2001 Symposium and are on email, and also to anyone else who requests it. If you don't wish to receive future copies, please email us. We will snail mail it on request. The newsletters will also be posted on the Royal Society (Rotorua Branch) website at www.rotorua.rsnz.org . If you are interested in, or working on lakes, but not the Rotorua Lakes, we are still very happy to receive material from you and to send you newsletters.

The more copy we receive, the more frequently we will be able to send this newsletter out. Electronic copy is preferred but not essential. Only minimal editing is carried out. We hope to send another issue out in September 2003 – given copy.
Technical content of all contributions is essentially the responsibility of the authors

All material and correspondence relating to *LakeScience Rotorua* to Nick Miller, millern@wave.co.nz, 91 Te Akau Road, R D 4, ROTORUA.

CONTENTS

NEWS

Preparations for the Rotorua Lakes 2003 Symposium are well underway. The official title is *Rotorua Lakes 2003: Practical Management for Good Lake Water Quality: A Public Symposium.*

A number of proposals to present papers have been received. We expect to have three keynote speakers, two of them from the US. A call for papers was included in the previous issue of this newsletter. if you wish to present a paper and have not got a copy of the call for papers document, please contact Nick Miller (millern@wave.co.nz) to obtain one. Remember the closing date is 31 July 2003. There are only a few slots left for oral papers. Registration forms for the Symposium will be emailed to all recipients of this newsletter within a few days.

From John McIntosh, Environment BOP

John Gibbons-Davies, Environment Bay of Plenty's Environmental Scientist responsible for water quality monitoring has resigned his position in order to move to England to advance his career. An appointment for a replacement is pending.

John worked at Environment Bay of Plenty for eight years, after previously being employed at the Taupo Research Laboratory of the former DSIR. As a DSIR technician John worked exclusively on lake and lake inflow research projects and so gained an invaluable depth of experience in the methods that form the basis of Environment Bay of Plenty's monitoring techniques.

John developed the lake monitoring programme with Noel Burns' assistance and has reported annually on lake quality using the format of Noel's LakeWatch computer programme for the last few years. When Thomas Wilding (Freshwater Ecologist) left Environment Bay of Plenty to join NIWA, John liaised with the community over the blue-green algal monitoring until Matthew Bloxham (the new Freshwater Ecologist) gained the experience to assume this role.
Johns skills in water quality monitoring will be missed and a period of training will be put in place for his replacement so that the service to the community can continue. The water quality scientist is responsible for river, lake and estuarine monitoring including bathing and shellfish quality as well as undertaking specific projects. The demands of lake monitoring has increased over the 13 years of Environment Bay of Plenty's existence.

However, the relationship developed with the University of Waikato and the establishment of the Chair in Lake Management and Restoration has beefed up the capability for lake monitoring and expanded research on the Rotorua lakes immensely. So the demands for lake information are being met.

To remind readers just what we are facing, here is a photograph taken in Okawa Bay, Lake Rotoiti, on 30 June 2003. Like rust, 'cyanobacteria never sleep'.

Photo: Brentleigh Bond

On the same theme, our main contribution for this issue is from Susie Wood, who is studying cyanobacterial toxins throughout NZ, for her PhD, with financial assistance from the LakesWater Quality Society. Two photographs have been removed to reduce file size.

Microcystin and cyanobacteria species variations in samples from Lake Rotoiti

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1.0 Introduction

Cyanobacteria in waterbodies produce cyanotoxins, which can be hazardous to humans exposed to them through recreational activities and in drinking water.

The number of cyanobacteria species known to produce cyanotoxins is continually increasing and includes species from the following genera; *Anabaena, Anabaenopsis, Aphanizomenon, Cylindrospermopsis, Cylindrospermum, Hapalosiphon, Lyngba, Microcystis, Nodularia, Nostoc, Phormidium, Planktothrix, Pseudanabaena, Raphidiopsis, Schizothrix, Synechocystis* and *Umezakia* (Harada et al. 1994, Chorus & Bartram 1999, Baker et al. 2001, Li et al. 2001, Oudra et al. 2002).

All of these genera, except *Umezakia,* are known to occur in New Zealand (Broady 2000, Wood & Stirling 2003, Wood unpublished data).

Cyanotoxins include the cyclic peptides (microcystin and nodularin) the alkaloids (cylindrospermopsin, anatoxins and saxitoxins) and lipopolysaccharides (LPS). In humans these cyanotoxins have been known to cause allergic reactions, poisonings and in one case involving hemodialysis, death (Bourke et al. 1983, Carmichael & Falconer 1993, Pilotto et al. 1997, Falconer 1999, Kuiper-Goodman et al. 1999, Azevedo et al. 2002).

Recreational activities in or on water containing cyanobacteria can result in contact with the skin and facial region which may lead to accidental ingestion, inhalation, intranasal and ocular contact. Contaminated domestic water supplies used for washing or bathing may also result in exposure via these routes. There are a number of reports world-wide where illness has been associated with recreational contact in or on water contaminated with cyanobacteria. Summaries of these cases are given in (Ressom et al. 1994) and (Chorus 2001). Many of these instances involved exposure to hepatotoxic blooms involving *Microcystis* sp. and *Nodularia* sp..

During the summer of 2002/2003 there were several reports of people feeling unwell after having contact with water from Lake Rotoiti (Waikato Times 25 Feb 2003). Some of the symptoms experienced by these people such as "burning skin", flu like symptoms and an enlarged liver could have been a result of cyanobacterial poisoning. Some residents of Te Weta Bay use Lake Rotoiti water for bathing and have indicated the presence of irritant compounds in this water as demonstrated by the following quote "*It certainly seems to be producing irritants – we are showering in this water, and it is not pleasant!*" (Te Weta Bay Resident, *Pers. Comm.*)

As part of a larger study on toxic cyanobacteria in New Zealand, samples were collected from three locations in Lake Rotoiti on 7 April 2003, cyanobacteria species were identified and some analysis of cyanotoxins carried out. Results and implications are outlined in this report.

2.0 Methods

2.1 Sample Collection and Cyanobacterial Identification and Enumeration

2-litre samples were collected from Lake Rotoiti on the 7 April 2003 using a plankton net (22 µm). The three samples were collected at the following locations:

- Okawa Bay (NSMS 260 U15 031447)
- Te Weta Bay (NSMS 260 U15 044471)
- Western Basin (Mouth of Te Weta Bay) (NSMS 260 U15 044464)

Upon arrival at Massey University (approximately 24 hrs after sample collection) 20 ml of the concentrated sub-sample was preserved immediately with Lugol's solution for morphological measurements and enumeration of cyanobacteria species. Enumeration was carried out using an inverted Olympus microscope and an Utermöhl settling chamber (Utermöhl 1958).

2.2 Toxin Analysis

A 10 ml aliquot of the each sample was freeze-thawed twice and filtered through a glass fibre filter (Whatman GF/C, 25 mm dia). The sample was then analysed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) for anatoxin-a and homoanatoxin-a following derivatisation as described in the method of James et al. (1998).

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The total microcystin content of the samples was analysed with a competitive indirect ELISA (Enzyme-Linked Immunosorbent Assay) using the methods of Fischer et al. (2001). This ELISA uses antibodies raised to the Adda moiety that is present in most (>80%) of the known toxic penta- and heptapeptide toxin congeners. Three 2 ml sub-samples were freeze-thawed twice then centrifuged at 12 000 g for 10 min and the supernatant used for the ELISA assay. The remainder of the samples (approx. 1.97 litre) were lyophilised and the resulting powders weighed. Sub-samples of the lyophilised material were weighed out and re-suspended in 1 ml of distilled water. These were then shaken for 10 mins and allowed to stand for 30 mins before been centrifuged at 12 000 g for 10 mins and the supernatant used for the ELISA assay.

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3.0 Results

3.1 Cyanobacterial Identification

Cyanobacteria species observed in the samples and concentrations are shown in Tables 1 to 3. Only cyanobacteria species were counted. Figures 1 and 2 visually show the difference between cyanobacteria species concentration at the Te Weta Bay and Okawa Bay sites.

Table 1. *Cyanobacteria species and concentrations in Okawa Bay sample. Counting error ± 30%.*

Species	Cells/ml *(2s.f.)*
Microcystis spp.	37 000
Anabaena sp. (cf. planktonica)*	320 000
Anabaena sp. *1 (Coiled species)*	3 000
Anabaena sp. *2 (Coiled species)*	50
TOTAL CELLS	**360 000**

***** *no akinetes seen in this sample or any samples taken Lake Rotoiti by author. For this reason the identification of this species is ambiguous.*

Table 2. *Cyanobacteria species and concentrations in Te Weta Bay sample. Counting error ± 30%.*

Species	**Cells/ml** *(2s.f.)*
Microcystis spp.	160 000
Anabaena sp. (cf. planktonica)*	99 000
Anabaena sp. *1 (Coiled species)*	18 000
Pseudanabaena sp.	260
*Unidentified cyanobacteria species***	58 000
TOTAL CELLS	**340 000**

** no akinetes seen in this sample or any samples taken from Lake Rotoiti by author. For this reason the identification of this species is ambiguous.*

*** species resembles microcystis but cell diameters much smaller (approx. 1 um)*

Table 3. *Cyanobacteria species and concentrations in Western Basin sample. Counting error ± 30%.*

Species	**Cells/ml** *(2s.f.)*
Microcystis spp.	86 000
Anabaena sp. *(cf. planktonica)**	67 000
Anabaena sp. *1 (Coiled species)*	6 700
Anabaena sp. *2 (Coiled species)*	1 900
*Unidentified cyanobacteria species***	21 000
TOTAL CELLS	**180 000**

***** *no akinetes seen in this sample or any samples taken from Lake Rotoiti by author. For this reason the identification of this species is ambiguous.*

*** species resembles microcystis but cell diameters much smaller (approx. 1 um)*

3.2 Toxin Analysis
No evidence of anatoxin-a or homoanatoxin-a was detected by HPLC-FLD in any of the Lake Rotoiti samples.

Table 3 lists the levels of total microcystins from the three sampling locations.

Table 3. *Microcystin concentrations at the three sampling locations*

Sample Location	Microcystins (µg/L) in concentrated samples	Microcystins µg per gram of FDW
Okawa Bay	44	14
Te Weta Bay	350	160
Western Basin	330	115

4.0 Discussion
4.1 Microcystins
Microcystins are cyclic heptapeptides, which block protein phospatase 1 and 2a (MacKintosh et al. 1990). To date more than 60 microcystins have been isolated and characterised (Chu 2000). Microcystins are produced by a variety of cyanobacteria species (Chorus & Bartram 1999) most commonly *Microcystis* sp..

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There are numerous documented cases of toxicity and fatality in wild and domestic animals from *Microcsytis* blooms in their drinking water supplies. (Ressomm et al. 1994). One area of considerable concern is that long term exposure to microcystins (even at low levels) may pose a cancer risk to humans. There is some epidemiological evidence to support this (Yu 1995, Ueno et al. 1996) and results from animal studies demonstrating the potential for promotion of tumour growth (Falconer & Humpage 1996). Microcystins may also cause progressive active hepatic injury (Falconer, et al. 1988).

Microcystins occur primarily intracellularly, however they are released into the waterbody when cyanobacteria cells are decaying. Once released into the waterbody the toxins can persist for a few days to a few weeks (Welker et al. 2001). Thus, even after a bloom has subsided, microcystins may still be present.

Microcystins can accumulated in different organs of freshwater fish. A recent study in Egypt detected levels of 102 ng/g in the muscle of fish (Mohamed et al. 2003). The microcystin in the bloom in the Egypt study was recorded at 1.12 mg/g dry weight compared to the lower levels 0.16 mg/g recorded in Lake Rotoiti. However, the risk of microcystin accumulation in fish in New Zealand is unknown as it is possible that such accumulation will be species dependent.

Oral administration of microcystin-LR has demonstrated a 24hr LD_{50} of between 5 mg/kg (Fawell et al. 1999) and 10.9 mg/kg (Yoshida et al. 1997). Drinking water guidelines for the protection of human health have been developed by the World Health Organisation (WHO). The guideline value for total microcystin has been set at 1 ug/L. Recreational guidelines are currently being developed for New Zealand. In Germany, guidelines state that if cyanobacteria are found at a site, users of the waterbody must be warned and receive information on cyanobacteria. Microcystin analysis can be used as an option for narrowing down the health risk. The guidelines German recommend closure at total microcystin concentrations above 100 ug/L (Frank 2002).

In Germany, authorities have also recognised that on-site information is important and that recreational users are best protected if they learn to recognise these risks. One message that was well received by the public was, *"if you see scums, or if you carefully wade up to your knees into the water without stirring up sediment, and you cannot see you feet because of a greenish discoloration, don't swim at this locality!"* Chorus and Fastner (2001).

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4.2 How representative are the Lake Rotoiti samples?
The samples from Lake Rotoiti were not collected for the specific purpose of testing and quantifying microcystins, however after examining the samples and identifying potential microcystin producing species analyses were carried out.

The samples were collected with a plankton net which concentrates the cyanobacteria, therefore the toxin levels may be higher than those expected if quantitative samples were collected. However, cyanobacteria concentration can change rapidly and the results obtained may indicate levels expected during wind concentration of cyanobacterial. Furthermore, the person carrying out the sampling indicated that while a

plankton net was used to collect the samples, the concentration factor was minimal (of the order of two times) and that cell concentrations were already high in Lake Rotoiti (Nick Miller, Pers. comm.).

Also the total cyanobacteria cell count results were at concentration levels that on occasion are recorded in the regular Environment Bay of Plenty monitoring programme. For example, in March 2003, a total cell count of 490 000 cells/ml was recorded at Te Weta Bay (ENBOP, unpublished data) over 100 000 cell/ml higher than recorded in the Te Weta Bay sample in the present study. Cell counts from Okawa Bay also reached in access of 200 000 cells/ml on several occasions during the 2003 summer (ENBOP, unpublished data).

4.3 Potential Health Risk from Recreational Exposure in Lake Rotoiti

Are the microcystins concentrations in Lake Rotoiti sufficient to threaten human life?
The risk can be estimated based on a worst-case scenario from toxicological data and on the levels of microcystin from Lake Rotoiti. The following assumptions need to be made:

- The microcystin in Lake Rotoiti was all microcystin-LR.
- Humans are as sensitive to microcystins as mice (Sensitive individuals are likely to show lethal responses at lower concentrations). Thus the LD_{50} = 5 mg/kg.

A summary of the findings of this risk assessment is:

- For a 10 kg toddler a dose of 50 mg could be lethal dose.
- At Te Weta Bay the microcystin levels = 0.35 mg/L
- Therefore a 10 kg toddler would have to consume 140 L to receive a lethal dose.

While consumption of this amount of water is very unlikely, the major risk is more likely from repeated or chronic exposure to microcystins at sub-lethal concentrations.

How much bloom material from Te Weta Bay would need to be consumed to possibly cause health impairment in a small child? Results using a calculation method adapted from Chorus & Fastner (2001);

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Microcystin concentration at Te Weta Bay = 350 ug/L = 0.3 ug/ml

	kg^{-1}	For 10 kg child
TDI*: 0.04ug/kg bw	0.11 ml	1.14 ml

* tolerable daily intake, calculated by applying uncertainty factors to the no observed or lowest observed effect levels in several week exposure experiments. bw = body weight.

Ingestion of the above amounts of water is very likely during recreational activities or during bathing.

The microcystin results from all three locations are well above the WHO drinking water guideline of 1 ug/L indicating that the water from Lake Rotoiti should not be used for drinking. Boiling of water does not destroy microcystins.

4.4 Toxin Testing

Microcystin levels were much higher in the Te Weta Bay and Western Basin than in Okawa Bay. Reasons for these differences are discussed in Section 4.5.

With the correct sample collection and preparation methods, the microcystin ELSIA used in this study is a reliable and effective way of screening for total microcystins in a sample. The ELISA can quantify the level of total microcystins in the sample and results should be able to be obtained within 24 hrs of receiving a sample. Commercials testing of samples using this ELSIA are available from AgReasearch – Hamilton.

The presence of cyanobacteria species in Lake Rotoiti known to produce cyanotoxins other than microcystins means that tests for other cyanotoxins and/or toxicity should not be ignored until at least our knowledge of which species are responsible for toxin production in New Zealand improves. Both cyanobacteria species composition and toxin types/levels can change during a cyanobacterial bloom (Baker et al. 2002) indicating that regular monitoring should be carried out.

4.5 Cyanobacteria Species

The abundance of individual cyanobacteria species varied between sampling locations. In Okawa Bay *Anabaena* sp. *(cf. planktonica)* dominated the bloom. However in Te Weta and Western Basin *Microcystis* species dominated. Several *Microcystis* species were observed in the study. One was *Microcystis aeruginosa* (known to commonly produce microcystins) *Microcystis flos-aquae* was also observed. The correct identification of *Microcystis* sp. to species level can be difficult and time consuming and for the purpose of this study they were all grouped together. Likewise, without seeing both akinetes and heterocytes confirmation of *Anabaena* species is difficult or impossible.

The presence of the unidentified cyanobacteria species in the Te Weta Bay and Western Basin samples is of interest. The author has not seen this species in other samples. The small cell size may indicate that it is a picocyanobacteria – these are also known to produce cyanotoxins (Domingos et al. 1999).

Without the culturing of individual strains it is not possible to establish unambiguously which species are responsible for toxin production. However, a comparison of species composition and microcystin levels demonstrate that the higher the levels of *Microcsytis* sp. the higher the levels of microcystin. For this reason, cell counts of *Microcystis* sp. should be carried out carefully.

Correct identification of cyanobacteria species can be time consuming and often difficult, however identification of potentially toxic genera does not require such a high skill level. This is important if cell counts are too be used as an indicator of potential health risk. The results from the present study also indicate that the composition of individual cyanobacteria species/genera should be taken into account when conducting cell counts. For example, the presence of *Microcystis* sp. might represent a higher health risk.

5.0 Work in progress

- Further work on the Lake Rotoiti samples to identify the presence of other cyanotoxins is currently underway.
- Attempts are been made to culture individual cyanobacterial species from Lake Rotoiti – this may help lead to the unambiguous confirmation of which species are responsible for the production of microcystin.
- Culturing may also help in confirmation of the taxonomy of cyanobacteria species present in the Lake Rotoiti.
- Samples from other lakes in the Rotorua area are currently being analysed for cyanobacteria species identification and the presence of cyanotoxins.

6.0 Conclusions

- Acute intoxication with microcystins during recreational activity is unlikely given the current levels of microcystins in Lake Rotoiti.

- Liver damage from microcystins is a potential risk, especially when exposure occurs repeatedly. Of particular concern is the use of Lake Rotoiti waters by Te Weta Bay residents for bathing/showering.
- Microcystin levels and species composition of blooms in Lake Rotoiti should be monitored and the public made aware of potential dangers.
- When *Microcystis* sp. cell concentrations are high, microcystin levels are also likely to be high.
- Correct identification of potentially toxic cyanobacteria species/genera is very important if cell counts alone are going to be used to indicate potential health risks. Current taxonomic literature must be used.
- No anatoxins were found in the samples. The presence of other cyanotoxins (apart from microcystins) including lipopolysaccharides is unknown for Lake Rotoiti.
- These results only apply to three locations within Lake Rotoiti and the samples were all taken on one day. A more extensive survey/monitoring programmme would be required to assess how toxin levels and species composition changes in Lake Rotoiti.

6.0 Acknowledgements

- Toxinology Group (AgResearch - Ruakura) for materials and technical assistance used in ELISA methods. For further information on these assays contact Neale Towers – neale.towers@agresearch.co.nz
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