Changes in Concentrations of Microcystins in Rainbow Trout, Freshwater Mussels, and Cyanobacteria in Lakes Rotoiti and Rotoehu

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ABSTRACT: Microcystin concentrations in cyanobacteria and their accumulation in rainbow trout (Oncorhynchus mykiss) and freshwater mussels (Hyridella menziesi) in Lakes Rotoiti and Rotoehu (New Zealand) were investigated. Hatchery rainbow trout were added to an enclosure in Lake Rotoiti where concentrations of microcystins in the phytoplankton and cyanobacterial cell concentrations could be closely monitored. Rainbow trout that were free to roam in the entire area of each lake were also included in the study. Freshwater mussels were suspended subsurface in cages in the enclosure. Phytoplankton samples, rainbow trout liver and muscle tissue, and the tissues of mussels were analyzed for microcystins using the ADDA-ELISA method, and selected samples were analyzed using LC-MS. A maximum concentration of microcystins in the phytoplankton samples of 760 μg L⁻¹ was recorded in Te Weta Bay, Lake Rotoiti, in March 2004. ELISA results confirmed microcystin immunoreactivity in rainbow trout liver and muscle tissues and in freshwater mussels. The microcystin congeners LR, YR, RR, AR, FR, LA, and WR were detected by LC-MS in caged freshwater mussels in Lake Rotoiti but were not detected in either muscle or liver tissue of rainbow trout. The daily tolerable intake limit of microcystins for human consumption recommended by the World Health Organisation is 0.04 μg kg⁻¹ day⁻¹. Modeling was carried out for the human intake of microcystin compounds from rainbow trout muscle tissue, and the potential health risks were estimated, assuming the ADDA-ELISA was determining compounds of toxicity equivalent to microcystin-LR.

Keywords: microcystins; cyanobacteria; rainbow trout; freshwater mussels; Lake Rotoiti; Lake Rotoehu

INTRODUCTION

Microcystins are cyclic heptapeptides that block protein phosphatases 1 and 2a in affected organisms (MacKintosh et al., 1990). To date, more than 70 microcystins have been isolated and characterized (Zurawell et al., 2005). Microcystins are
produced by a variety of cyanobacterial species (Chorus and Bartram, 1999), most commonly species from the genus *Microcystis*. Ingestion of a lethal dose of microcystins by humans is unlikely; however, in Brazil in 1996 more than 50 dialysis patients died after receiving water contaminated with microcystins (Azevedo, 2002). Chronic effects (e.g., liver damage or increased likelihood of liver tumors) are probable if there is long-term frequent exposure (Falconer et al., 1988; Uno et al., 1996). The maximum allowable concentration of microcysts in drinking water was established by Falconer et al. (1994) as 1 $\mu g$ L$^{-1}$ day$^{-1}$. On the basis of this concentration, a daily tolerable intake (DTI) of total microcystins of 0.04 $\mu g$ kg$^{-1}$ day$^{-1}$ has been proposed as a provisional guideline by the World Health Organization (Chorus and Bartram, 1999).

Microcystins have been found to accumulate in different organs of freshwater fish (Ernst et al., 2001; Magalhaes et al., 2003), freshwater (Eriksson et al., 1989) and marine (Vasconcelos, 1995; Amorim and Vasconcelos, 1999) mussels, and invertebrates (Kotak et al., 1996; Vasconcelos, 1999). Traditionally, microcystins were thought to mostly accumulate in the liver of freshwater fish. Thus, the risks to humans consuming gutted fish were considered low. However, a recent study (Mohamed et al., 2003) detected microcystin concentrations of 102 $\mu g$ kg$^{-1}$ in the muscle tissue of fish, indicating that even the consumption of gutted fish might adversely affect human health.

Lakes Rotoiti and Rotoehu, in the central North Island of New Zealand, have intermittently experienced cyanobacterial blooms since the 1970s (Cassie, 1978). In the past decade the severity and extent of these blooms has increased, and microcystins have been detected, raising environmental and human health concerns (Wilding, 2000; Wood, 2005).

In this study concentrations of microcystins and the composition and abundance of cyanobacterial species in Lakes Rotoiti and Rotoehu were monitored weekly for a 6-month period. The resulting data allowed concentrations of microcystins and cell concentrations to be compared and the potential risk to human health to be assessed.

Rainbow trout (*Oncorhynchus mykiss*) is a species of particular importance to recreational fishers and to tourism in Lakes Rotoiti and Rotoehu. This study also aimed to identify whether microcystins accumulate in the liver and muscle tissues of rainbow trout, and if they did, whether the concentration in rainbow trout tissue could be hazardous to human health. Rainbow trout were sampled over the entire area of the lakes and from a group that had been contained in an enclosure for 3 weeks in Lake Rotoiti, where microcystins are known to reach high concentrations (Wood, 2005). In the enclosure, concentrations of microcystins and cyanobacterial cell concentrations were closely monitored.

The variable distribution of cyanobacteria and cyanotoxins within a water body results in rapid changes in their concentrations as environmental conditions change (Codd et al., 1999). Routine point sampling therefore may not provide a true indication of the potential risks posed by cyanotoxins in any given area of a water body. If microcystins accumulate and remain in the tissue of *Hyridella menziesi* (New Zealand freshwater mussel) over a period of time, freshwater mussels may give a useful indication of the toxin concentrations at a particular site over a period of time. In addition, if the mussels accumulate higher concentrations of microcystins in their tissue than are present in the surrounding water, they could be used as an early-warning mechanism in water bodies with low concentrations of microcystins, when concentrations in the water might be below the limits of detection of analytical procedures. Pro-active measures could then be taken before microcystins reach concentrations deemed a health risk. In this study we compared concentrations of microcystins in phytoplankton samples taken twice daily with microcystins extracted from freshwater mussels suspended subsurface in the same enclosure as the rainbow trout.

**MATERIALS AND METHODS**

**Field Methods**

**Phytoplankton**

Between November 14, 2003, and May 2, 2004, surface phytoplankton samples (400 mL) were collected weekly from six locations in Lakes Rotoiti and Rotoehu (Fig. 1). The samples were collected by bottle without concentration. The phytoplankton samples were well mixed, and a 50 mL subsample was preserved using Lugol’s iodine. The subsamples were stored in the dark and used later for species identification and enumeration. The remaining 350 mL (unfiltered) was frozen at $-20^\circ$C for later analysis of microcystins.

During the 3-week enclosure experiment, 400 mL samples were collected at the center of the enclosure bay. These were preserved and frozen in the same manner as that described for the weekly samples. Samples were collected daily at 9 am (surface only) and at 2 pm (surface and 1, 2, and 3 m depths). Dissolved oxygen and water temperature were measured at each time and depth using a dissolved oxygen meter (YSI Environmental, Yellow Springs, OH, USA).

**Rainbow Trout Samples**

The free roaming rainbow trout were caught by local fishing guides, frozen, and transported in insulated containers to the Massey University ecotoxicology laboratory. Five rainbow trout (mean weight 1.4 kg) were obtained monthly from Lake Rotoiti between December 2003 and April 2004, except in February, when two were obtained. Five rainbow trout were obtained from Lake Rotoehu (mean weight...
0.8 kg) each month from November 2003 to April 2004, except in January, when only four were obtained.

Between March 3, 2004, and March 27, 2004, the southern arm of Te Weta Bay, Lake Rotoiti (Fig. 1), was enclosed by a net suspended across the entrance to the bay. The netted entrance was 100 m wide, with a maximum water depth of 4 m. The length of the enclosed arm was approximately 350 m. The middle 70 m section of net was standard synthetic fishing net with mesh 20 × 20 mm in size. The 15 m-long end pieces were synthetic garden shade cloth with an aperture 4 × 3 mm in size. This size mesh allowed water and phytoplankton to move freely into the area, creating an environment similar to other areas of the lake. To aid buoyancy along the top of the net, cork floats were located every 1 m, and 5 L plastic containers were tied to the headline of the net every 3 m. A rope with a lead core was attached along the middle and on the bottom of the net, and, together with lead weights on the bottom, this assisted in weighing down any excess net. The net was anchored to the bottom sediment of the lake with 1 m-long U pins of a deformed reinforcing rod 12 mm in diameter placed every 2 m.

Eighty 2-year-old rainbow trout from the Rainbow Valley hatchery of Fish and Game New Zealand were released into the enclosure on March 8, 2004. Each rainbow trout was tagged by clipping one pectoral fin before release. Up to five of the released rainbow trout were caught every 2–3 days over a 21-day period. Rainbow trout caught within a day of each other were pooled. The pooled sample size ranged from three to five rainbow trout. A total of 28 (mean weight 0.8 kg) released rainbow trout were caught. In addition, three "wild" rainbow trout that were in the enclosure area prior to installing the net were captured. The rainbow trout were caught by lure and by using gill nets whose mesh size was 50 × 50 mm. Rainbow trout were killed immediately after capture by a heavy blow to the top of the head. The rainbow trout were refrigerated shortly after catching and dissected within 12 h. Three rainbow trout from the hatchery were retained before the release and used to determine if any baseline microcystins were present.

**Freshwater Mussels**

Approximately 130 freshwater mussels (*Hyridella menziesi*) were collected on March 6, 2004, from sediment in Lake Rotoma (38°04'S, 176°35'E). Mussels were transported in buckets containing Lake Rotoma water to the study site in Lake Rotoiti.

Ten mussels were frozen immediately and kept at −20°C until analysis to establish baseline concentrations of microcystins. The remaining 120 were placed in plastic mesh cages suspended from buoys 300 mm below the surface in the center of the enclosed bay. Ten mussels were collected every 2–3 days over 21 days. The entire mussel was frozen at −20°C until analysis of microcystins was carried out.

**Laboratory Methods**

**Cyanobacterial Identification and Enumeration**

The preserved Lugol’s subsamples were used for species identification and enumeration using an inverted Olympus microscope (CKX41) and Utermöhl settling chambers (Utermöhl, 1958). When possible, cyanobacteria were identified to the species level. The species identifications
in this study were made primarily by reference to studies of Baker (1991, 1992), Baker and Fabbro (2002), Komárek and Anagnostidis (1999), Komárek et al. (2002), McGregor and Fabbro (2001), and Azevedo and Sant’Anna (2003).

Rainbow Trout Examination

Necropsy was performed. Half the liver and approximately 5 g of muscle tissue in the vicinity of the lateral line between the pectoral and pelvic fin were removed. Each

Fig. 2. *Anabaena* spp. and *Microcystis* spp. cell concentrations and I concentration of microcystins at the six sampling sites: (a) Okawa Bay in Lake Rotoiti, (b) Te Weta Bay in Lake Rotoiti, (c) Hinehopu in Lake Rotoiti, (d) Kennedy Bay in Lake Rotoehu, (e) Te Pohue Bay in Lake Rotoehu, (f) Otauhu Bay in Lake Rotoehu.
sample was pooled with samples collected at a similar time and stored at $-20^\circ$C until analysis of microcystins.

An assessment of stomach contents was performed on each rainbow trout. The stomach contents were grouped into one of the following: plant material, mollusks, insects, fish, pebbles, and unidentifiable organic material. The stomach contents were blotted dry, and the wet weight of each category was recorded and a percentage composition of stomach contents calculated for each rainbow trout. The fullness of the stomachs was recorded but not taken into
account in the percentage calculation. An average of the stomach contents for each pooled sample of rainbow trout was calculated.

**Microcystin Analysis**

The total concentration of microcystins in the phytoplankton samples was analyzed with a competitive indirect ELISA using the methods of Fischer et al. (2001). This ELISA uses antibodies raised against the ADDA moiety present in most (>80%) of the known toxic penta- and heptapeptide toxin congeners.

Two-milliliter phytoplankton subsamples were freeze-thawed twice to lyse the cyanobacteria, centrifuged (10,000 rpm, 2 min), and the supernatant used for the ELISA. Rainbow trout muscle tissue, rainbow trout liver, and freshwater mussels were separately pooled, and each pool was extracted and processed by solid-phase extraction as described by Magalhaes et al. (2001), except homogenization was carried out in 75% methanol/water and the fraction containing microcystins was eluted from the C18 cartridge with 80% methanol/water, diluted in assay buffer (PBS), and analyzed by ELISA. All samples were analyzed at a minimum dilution of 1:50 to overcome matrix effects. The assay was calibrated using dilution of a standard of microcystin-LR (ALEXIS), and all ELISA data are reported in micromolar equivalents for rainbow trout and mussels and phytoplankton samples, respectively.

To assess the effectiveness of the extraction procedure, spiked recovery tests were carried out on rainbow trout muscle tissue and liver and mussel tissues. The homogenized samples were spiked with 100–500 µg kg⁻¹ of MC-LR (ALEXIS). Samples were then extracted and analyzed using the ADDA-ELISA.

Selected samples were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) for the microcystin variants RR, dDMe-RR, dMe-RR, LR, YR, dMe-LR, FR, WR, AR, LA, LY, LW, and LF and for nodularin. These included one phytoplankton sample, four rainbow trout samples, one mussel sample, two spiked samples, and two control samples. A 5 mL subsample of the freeze-thawed phytoplankton sample was sonicated for 10 min with 5 mL of acetone/titrile and centrifuged (10,000 rpm, 10 min). An aliquot of the supernatant was taken for LC-MS analysis. LC-MS of microcystins in tissues was carried out in the same extracts as prepared for the ELISA. Microcystins were separated by LC (Alliance 2695, Waters Corp., MA) using a 150 × 2 mm Luna C18 5 µm column (Phenomenex, CA, USA) with a water/methanol/acetonitrile gradient containing 0.15% formic acid (0.2 mL min⁻¹, 10 µL injection). The Quattro Ultima TSQ mass spectrometer (Waters-Micromass, Manchester, UK) was operated in ESI⁺ with multiple reaction monitoring (MRM) using MS-MS channels set up for 13 microcystins and nodularin. The m/z 135 fragment from the protonated molecular cation was selected for each toxin (the doubly charged molecular species for MC-RR; the singly charged molecular species for all other toxins). The instrument was calibrated with authentic standards of MC-RR, MC-YR, and MC-LR and nodularin and gave highly linear calibration curves for concentrations in the range of 5–200 ng mL⁻¹. The response factors for MC-RR or MC-LR were applied to the other related toxins for which no pure analytical standards were available.

**RESULTS**

**Weekly Phytoplankton Samples**

Anabaena spp. and Microcystis spp. were dominant in phytoplankton samples collected from Lakes Rotoiti and Rotoehu (Fig. 2). During the study, species from these genera accounted for more than 90% of the cyanobacterial cells in the samples.

In Lake Rotoiti cyanobacterial cell concentrations were low in November, with the first blooms occurring in December. The bloom comprised Anabaena spp., dominated by A. circinalis, A. lemmermannii, and A. planktonica. Although cell numbers fluctuated, these three species continued to dominate until February 16, 2004, when there was a sudden decline in all cyanobacterial cell numbers. This was followed by blooms of M. aeruginosa and M. botrys on February 23, 2004, in Okawa Bay (940,000 cells mL⁻¹), and on March 8, 2004, in Te Weta Bay (440,000 cells mL⁻¹). Microcystis spp. continued to dominate (although in lower numbers) until late March when Anabaena spp. once again became dominant. In Te Weta Bay Sphaerocavum brasiliensis reached concentrations of 34,000 cell mL⁻¹ in late April.

In Lake Rotoehu Anabaena spp. was recorded at very low cell concentrations. Microcystis spp. was usually dominant, with M. panniformis particularly abundant. However, the highest cell concentration recorded during this study was only 7400 cells mL⁻¹ (Te Pohue Bay, May 2, 2004).

Microcystin were below detectable concentrations from Lake Rotoiti samples until February 23, 2004, when they were detected in Te Weta Bay (38 µg L⁻¹) and Okawa Bay (410 µg L⁻¹) [Fig. 2(a–c)], which corresponded with a high cell count of Microcystis spp. Microcystins continued to be detected at Okawa Bay until the conclusion of sampling. The sample from Te Weta Bay on March 8, 2004, recorded the highest concentration measured during this study, 760 µg L⁻¹, again corresponding to high cell concentrations of Microcystis spp. Concentrations of microcystins at Hinehopu remained generally low, with the highest concentration, 9 µg L⁻¹, on March 22, 2004, which also corresponded to increased concentrations of Microcystis spp.
Concentrations of microcystins detected at the three sampling locations in Lake Rotoehu [Kennedy Bay, Te Pohue Bay, and Otauhu Bay; Fig. 2(d–f)] ranged from 0 to 24 μg L⁻¹. Most concentrations were less than 1 μg L⁻¹, corresponding to low Microcystis spp. cell concentrations at these locations. When a significant concentration of microcystins was detected at Te Pohue Bay (24 μg L⁻¹; May 2, 2004), *M. panniformis* cell concentrations were also elevated (data not shown).

The phytoplankton sample from Te Weta Bay of Lake Rotoiti (March 8, 2004) was analyzed using LC-MS/MS and found to contain seven microcystin congeners [Fig. 3(a), Table I] and a total of 517 μg L⁻¹. MC-RR, MC-LR, MC-FR, MC-WR, and MC-LA were dominant.

Fig. 3. LC-MS/MS of microcystins in samples from Lake Rotoiti: (a) phytoplankton sampled March 8, 2004; (b) freshwater mussel tissue sampled March 18, 2004. Total ion current chromatograms are shown for 14 multiple-reaction monitoring channels. Inset: MRM channel 910.6 > 135.1, MC-LA retention time 17.3 min.
Spiked Recovery Tests

Approximately 70% of the microcystin-LR spiked in the rainbow trout muscle tissue and liver samples, and a freshwater mussel (Hyridella menziesi) sample using LC-MS and results from spiked recovery tests

Monthly Rainbow Trout Samples

Rainbow Trout Muscle and Liver Analysis

Microcystins (as detected by ELISA) were found in liver and muscle tissue in all monthly samples from November 2003 to April 2004. In Lake Rototomy the concentrations of microcystins in muscle tissue did not vary markedly (from 8 μg kg⁻¹ in March 2004 to 12 μg kg⁻¹ in February 2004). Concentrations of microcystins varied widely in liver samples (from 12 μg kg⁻¹ in March 2004 to 46 μg kg⁻¹ in February 2004). No microcystins were detected in weekly phytoplankton samples until February 2004. The highest concentration was recorded in March 2004 (63 μg L⁻¹; Fig. 4(a)). In Lake Rotome ito the highest concentrations of microcystins in liver were recorded in November 2003 (79 μg kg⁻¹) and in the rainbow trout muscle tissue in April 2004 (35 μg kg⁻¹). The amount of microcystins decreased between December 2003 and March 2004 before increasing again in April 2004. No microcystins were detected in the

Stomach Contents of Rainbow Trout

With the exception of December 2003, the majority of the stomach contents of the Lake Rototomy rainbow trout was common smelt (Retropinna retropinna) and bullies (Gobio morphas sp.) [Fig. 5(a)]. In December 2003 insects were in the stomach contents, but a large portion of the material

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could not be identified. Two rainbow trout also had empty stomachs in this month.

More than 40% of the stomach contents of Lake Rotoehu rainbow trout were fish, including common smelt and bullies [Fig. 5(b)]. Mollusks, mainly the gastropod snail *Physa acuta*, made up a large component of the rainbow trout diet in December 2003 and February and April 2004. The stomachs of two rainbow trout in February 2004 and of one in November 2003 were empty.

**Enclosure Experiment**

**Phytoplankton Composition, Enumeration, and Microcystin Concentration**

*Microcystis aeruginosa* and *A. planktonica* were dominant in almost all samples. *Sphaerocavum brasiliensis* was also present in high numbers in many samples. Average total cyanobacterial cell concentrations and average microcystin concentrations from the five samples (from the surface in the am and pm and from depths of 1, 2, and 3 m depths in the pm) collected each day are shown in Figure 6. The highest average total cell concentrations were recorded on March 18–19, 2004 (26,000 cells mL$^{-1}$). The highest average microcystin concentration occurred 7 days later, on March 26, 2004 (15 μg L$^{-1}$). There was no clear correlation between microcystin concentrations and cyanobacterial cell concentrations ($R^2 = 0.60$).

**Microcystins in Rainbow Trout in Enclosure**

No microcystins were found in the three rainbow trout from the hatchery (March 8, 2004) retained to check for background concentrations of microcystins. Microcystins were found in all muscle tissue and liver samples from rainbow trout caught during the 21-day enclosure experiment (Fig. 7). Muscle tissue microcystin concentrations increased between March 8 and March 13, 2004, to a maximum of 23 μg kg$^{-1}$. Concentrations then decreased and stayed between 7 and 12 μg kg$^{-1}$ for the remainder of the enclosure experiment.
From March 8 to March 18, 2004, the concentration of microcystins in the liver increased to a maximum concentration of 47 μg kg⁻¹. From March 19 to March 25, 2004, the concentrations decreased to 24 μg kg⁻¹, before increasing again to 45 μg kg⁻¹ in the final sample. Concentrations in the “wild” rainbow trout did not differ markedly from those found in enclosure rainbow trout caught on similar dates. Five of the seven muscle tissue samples were above the TDI for microcystins (Fig. 7). No microcystins were detected in the rainbow trout tissue sample (March 13, 2004) analyzed by LC-MS (Table I).

**Stomach Contents of Rainbow Trout**

From March 8 to March 19, 2004, of the 21-day enclosure experiment, a large portion of the diet of rainbow trout was made up of insects, predominately water boatman of the family Corixidae. After 16 days, the rainbow trout diet contained greater quantities (30%–60% by weight) of fish (smelt and bullies). Stomach contents of the three “wild” rainbow trout contained up to 58% fish [including goldfish (*Carassius auratus*), smelt, and bullies; Fig. 8].

**Concentrations of Microcystins in Mussels**

A smoothed microcystin concentration in surface phytoplankton [calculated by taking the average am and pm surface samples for each day and applying a smoothing formula; smoothed value = (microcystins on day)/2 + (microcystins previous day)/4 + (microcystins next day)/4], and microcystin concentrations measured in the mussels are shown in Figure 9. This smoothed graph highlights the
general trends by reducing the effects of measurement error and random variation in the data.

After 4 days in the enclosure, the mussels began to accumulate microcystins, which remained at a stable concentration of 5 µg kg⁻¹ for about 7 days. Over the next 2 days microcystins in the mussels increased dramatically, reaching a peak of 65 µg kg⁻¹. With the exception of the first 6 days, the increase and decrease in concentrations of microcystins in mussels approximately tracked the trend in surface phytoplankton of microcystins concentrations with a 2-day delay.

The LC-MS analysis of the March 18, 2004, mussel samples detected seven microcystin congeners, giving a total concentration of 97 µg L⁻¹ compared to a concentration of 65 µg L⁻¹ measured using the ADDA-ELISA method.

**Fig. 7.** Estimated daily intake of microcystins from enclosure experiment based on a 70 kg person ingesting a 300 g serving of rainbow trout (*Oncorhynchus mykiss*). The phytoplankton microcystin concentration is an average of 5 samples per day.
DISCUSSION

Cyanobacteria Species: Composition, Abundance, and Microcystin Concentrations

In Lake Rotoiti *Anabaena* spp. dominated cyanobacterial blooms until February, when *Microcystis* spp. occurred in increasing numbers. Of particular interest was the decline in cyanobacterial concentrations in mid-February. This was followed by large blooms of *Microcystis* spp. in Te Weta and Okawa Bays. The rapid appearance of this species possibly could be attributed to the exceptionally fast growth rate of *Microcystis* (Reynolds, 1997) or maybe to the result of recruitment of benthic *Microcystis* spp., as shown by Preston et al. (1980) and Latour and Giraudet (2004). Cyanobacterial cell concentrations remained relatively low in Lake Rotoehu and were dominated by *Microcystis* spp. This is in contrast to previous summers, when severe blooms of *Microcystis* spp. and *Anabaena* spp. were reported in this lake (Wilding, 2000). The concentration of cyanobacterial...
cells varied daily and at depth in the enclosure. The ability of some cyanobacteria to regulate their buoyancy and variations in environmental factors such as wind direction and strength are probable causes for these differences. This large daily variability illustrates problems with collecting and making management decisions about entire water bodies based on one-time point samples.

Oral administration of microcystin-LR to mice demonstrated a 24 h LD50 of between 5 (Fawell et al., 1999) and 10.9 (Yoshida et al., 1997) mg kg⁻¹. These studies led the World Health Organization (WHO) to develop drinking water guidelines for a maximum total microcystin concentration of 0.1 μg L⁻¹. Cyanotoxin guideline concentrations for recreational use of water bodies are variable and subject to ongoing review. For example, one German guideline recommends closure of water bodies at total microcystin concentrations above 100 μg L⁻¹ (Frank, 2002), and the recent Australian guideline recommended closure at a concentration greater than 10 μg L⁻¹ (National Health and Medical Research Council, 2004). Both guidelines acknowledge that cyanobacterial concentrations are likely to be used as a first indicator of potential risk at a site.

Environment Bay of Plenty and Pacific Health, which are responsible for issuing health warnings about cyanobacterial concentrations in Lakes Rotoiti and Rotoehu, base those warnings on total cyanobacterial cell concentrations and currently do not use cyanotoxin concentrations. When total cyanobacterial concentrations exceed 15,000 cells mL⁻¹, the water is considered unsuitable for general-contact recreation, and warnings are issued.

During this study it was observed that high total cyanobacterial cell concentrations did not indicate high concentrations of microcystins. On several occasions in Lake Rotoiti, elevated microcystin concentrations were found with low cell concentrations. This demonstrates that relying on cell counts alone does not give an indication of concentrations of microcystins, and thus the potential health risk as toxins may persist for a time after cell lysis and death. Testing for microcystins should be incorporated into routine monitoring programs where possible.

It was not possible to establish with absolute certainty which species were responsible for microcystin production from the results of this study. However, a comparison of species composition and concentrations of microcystins demonstrated that when very high concentrations of Microcystis spp. (in particular, *M. aeruginosa* and *M. botrys* in Lake Rotoiti and *M. panniformis* in Lake Rotoehu) were found, there were higher concentrations of microcystins. *Anabaena* spp. were not associated with high concentrations of microcystins. Further work is required to establish which species in these lakes produce microcystins.

Given the current concentrations of microcystins in Lakes Rotoiti and Rotoehu, acute intoxication with microcystins during human recreational activity is unlikely. However, chronic liver damage from microcystins is a potential risk, especially from repeated exposure. Of particular concern is the use of Lake Rotoiti waters by some residents for bathing, showering, and drinking.

### Rainbow Trout Samples and Microcystins

Microcystins were detected in muscle and liver tissue of rainbow trout ranging through both lakes at times when no microcystins or microcystins at very low concentrations were present in the lake phytoplankton. This could indicate that phytoplankton samples were not a true indication of concentrations in the lake as a whole or, more likely, that there was accumulation of microcystins in rainbow trout and/or food sources of rainbow trout.

It was unexpected that microcystins were detected in rainbow trout muscle tissue from Lake Rotoiti in December 2003 and from Lake Rotoehu in November 2003, as cyanobacterial blooms had not been reported in the previous months (Environment Bay of Plenty, unpublished data). It is possible, however, that some *Microcystis* was over-wintering in the sediment, and because part of the rainbow trout diet comes from benthic grazers, this caused bioaccumulation of microcystins. Ihle et al. (2005) recently detected microcystins in samples containing benthic *Microcystis* spp.

In an investigation of the biochemistry of microcystins in rainbow trout, Tencalla et al. (1994) observed that the absorption of microcystin-LR from the gastrointestinal tract into the blood occurred rapidly. However, the intestinal tract represented an important barrier, and less than 5% of the applied microcystins reached the blood. Once in the blood, the microcystin-LR was quickly transported to the liver. In the enclosure study, microcystin concentrations in rainbow trout muscle tissue decreased after 4 days and in liver tissue after 12 days. Tencalla and Dietrich (1997) noted that the decrease of microcystins in the liver occurred more slowly than the uptake and that microcystins could still be detected in the liver 3 days after exposure. Soluble glutathione S-transferase enzymes are active in the detoxification of microcystins in organisms (Pflugmacher et al., 1998). Wiegand et al. (1999) in a study of early life stages of zebra fish (*Danio rerio*) found that their detoxification system reacted to microcystin-LR, indicating that they may have the ability to metabolize microcystin-LR to a less harmful compound. It is likely that at least some of the decrease with time in the concentrations of microcystins observed in the enclosure rainbow trout is a result of detoxification and depuration processes.

The differences in total concentrations of microcystins in the selected phytoplankton sample from Lake Rotoiti as measured by LC-MS and ELISA (517 versus 760 μg L⁻¹) were not unexpected, as several studies have shown there can be marked differences between these methodologies (e.g., Lawrence et al., 2001; Rapala et al., 2002; Mountford...
et al., 2004). It is probable that, because the LC-MS analysis used in this study only screened for 13 congeners, not all the microcystins in the sample were detected. The ELISA detects all ADDA-containing compounds even at very low concentrations, for example, below the detection limits of LC-MS.

Surprisingly, no microcystin congeners were detected in the rainbow trout samples by LC-MS analysis, despite their presence in both the phytoplankton and the freshwater mussel samples (see below for further discussion on mussel samples). Most studies using LC-MS or HPLC to detect microcystin congeners in fish tissue have either involved spiking the tissue with microcystins (e.g., Lawrence and Menard, 2001; Karlsson et al., 2005) or studying fish fed directly on cyanobacteria in artificial conditions (e.g., Moreno et al., 2005). A recent study (Xie et al., 2005) of the accumulation of microcystin congeners -LR and -RR in various organs of wild fish from four trophic levels detected using HPLC in one or both congeners in all fish. The sample preparation and extraction techniques used were different than those in the present study, and tissue extraction used butanol:methanol:water (1:4:15). This makes comparisons of the data difficult. The maximum concentration of microcystins in the muscle tissue in the carnivorous fish (expressed as a MC-LR equivalent) was significantly higher than those detected using the ADDA-ELISA in this study. Further LC-MS analysis of other samples in this study (e.g., rainbow trout from early in the enclosure experiment when microcystin concentrations were still rising) or controlled laboratory studies would be required to understand why microcystins were not detected using LC-MS.

Rainbow trout ingest small amounts of lake water (which may contain some microcystins). However, the major source of microcystin intake is via consumption of prey (Tencalla et al., 1994). On the basis of the absence of microcystins in gut samples of some fish, Xie et al. (2005) suggested that routes other than the gastrointestinal tract are important for some fish species. The form in which microcystins occur in the prey of rainbow trout in Lakes Rotoiti and Rotoehu is unknown, and it is likely that in at least some (e.g., fish and aquatic invertebrates) the microcystins will have already been at least partly metabolised or detoxified. The toxicity of microcystin metabolites to humans is unknown. Recent studies (Barford and Keller, 1994; Goldberg et al., 1995) have shown that the ADDA region of the molecule is critical for interactions with protein phosphatase molecules, and therefore it seems likely that it has a significant role in the toxicity of microcystins. Because of its ability to detect all ADDA-containing compounds, the ADDA-ELISA method may prove to be the most appropriate method of screening for microcystins in tissue samples if ADDA-containing metabolites are confirmed to be toxic.

The present study investigated and reported concentrations of only free ADDA-containing microcystins in phytoplankton and mussels as determined by LC-MS. It is likely these will also contain glutathione and cysteine conjugates of microcystins that are produced during detoxification (Pflugmacher et al., 1998) and covalently bound microcystins. Covalent binding of microcystins can occur within hours (Williams, 1997a). It involves the formation of a covalent linkage between the microcystins and PP-1 and PP-2A enzymes, the result of a secondary in vitro interaction (MacKintosh et al., 1995; Craig et al., 1996; Runnegar et al., 1999). Few studies have addressed the issue of covalently bound microcystins in freshwater organisms. In zebra mussels, Dreissena polymorpha (Pires et al., 2004) only small amounts of covalently bound microcystins could be detected in the first week of exposure. In the second week, covalently bound microcystins increased dramatically and accounted for approximately 62% of total microcystins. Similarly, Williams et al. (1997b) found that in the marine mussel Mytilus edulis almost all the microcystins existed as a covalent complex after 3 days. These results may explain why concentrations of microcystins in the enclosure rainbow trout decreased after 5 days in muscle tissue and 12 days in liver tissue. Covalently bound microcystins may have lower cross reactivity with the antibodies, but the ADDA-ELISA used in this study should detect covalently bound microcystins, as they would still contain the ADDA molecule. It is also possible that a considerable portion of the covalently bound microcystins was not extracted by the procedure followed, and over time, more microcystins may have become covalently bound and not able to be extracted and detected. It is recommended that future studies assess concentrations of covalently bound microcystins in Lakes Rotoiti and Rotoehu rainbow trout and attempt to establish whether the total microcystins (or ADDA-containing compounds) in the rainbow trout are a potential health risk to human consumers.

Effect of Diet on Concentrations of Microcystins in Rainbow Trout

Laboratory studies (Tencalla et al., 1994) indicated that the main route of uptake for microcystins in rainbow trout is the gastrointestinal tract and that only very small amounts are taken up through skin or gills. Thus, to understand how microcystins might be bioaccumulating in Lakes Rotoiti and Rotoehu rainbow trout, an attempt was made to assess dietary composition. Studies have shown that gastric emptying in rainbow trout takes between 6 and 18 h (Grove et al., 1978). Thus, stomach content analysis gives some indication of diet just prior to capture.

Hatchery rainbow trout were fed formulated feed pellets, and how long it took them to adapt to feeding in the lake environment prior to the enclosure experiment was unknown. Stomach analysis showed that hatchery rainbow trout released into the enclosure began feeding within 2
days. However, for the first 16 days, the diet of released rainbow trout was quite different from that of free-roaming rainbow trout in Lake Rotoiti. A large portion of the initial diet of the enclosure rainbow trout was insects, predominately water boatmen of the family Corixidae. Most of the diet of the Lake Rotoiti rainbow trout was smelt and bullies. The results of the stomach analysis suggest that microcystins from phytoplankton accumulate in many aquatic organisms. The different feeding modes of these organisms may be one reason why microcystins were detected even when cyanobacterial blooms were absent.

**Freshwater Mussels and Microcystins**

*Hyridella menziesi* accumulated microcystins at a slow rate for the first 6 days of the enclosure experiment. This is in contrast to other studies that have shown that freshwater and marine mussels accumulate microcystins rapidly (Amorim and Vasconcelos, 1999; Yokoyama and Park, 2003). The mussels used in this experiment were from Lake Rotoma sediment and were transferred to Lake Rotoiti within 12 h. The initial low accumulation rate suggests mussels required approximately 7 days to acclimatize to a new environment and begin feeding normally. After 7 days the mussels appeared to commence normal feeding and the concentrations of microcystin in their tissue approximately followed the trend of the phytoplankton microcystin concentrations with about a 2-day delay. During this study the entire mussel was homogenized before microcystin analysis. It is plausible that a portion of the microcystins detected in the mussels had not been accumulated but were contained in phytoplankton in the gut contents.

All the microcystin congeners that were detected by LC-MS in the phytoplankton sample were also detected in the mussel samples. Of interest was the ratio between the different congeners in the phytoplankton and mussel samples. Surprising the more hydrophobic microcystins for example, MC-AR and MC-LA, were accumulated in the mussels at lower proportions than the more polar congeners, MC-RR, MC-YR, and MC-RR. Hydrophobic microcystins should more readily penetrate the lipid membranes of cells. The phytoplankton sample tested by LC-MS was collected several weeks after the mussel sample, and further testing of samples collected in parallel would be required to explore this further. Few studies (e.g., Watanabe et al., 1997) have investigated the accumulation of individual microcystins in freshwater mussels, and none has assessed how the ratios of microcystins congeners in the mussels and surrounding water differ. Unlike the rainbow trout, it appears that the microcystins in *H. menziesi* have not been metabolized into other microcystins or ADDA-containing compounds. A major difference between the freshwater mussels and the rainbow trout in this study is that the mussels are feeding directly on the phytoplankton (not on a combination of organisms higher up a food chain), and therefore no previous metabolism of microcystins has taken place. Amorim and Vasconcelos (1999) showed that a high proportion of the microcystins fed to the mussels were accounted for in their feces and in the surrounding water (presumably excreted in urine). Further laboratory studies are needed to establish the dynamics of microcystins in *H. menziesi*.

Laboratory studies on concentrations of microcystins in the marine mussel *Mytilus galloprovincialis* (Vasconcelos, 1999) found that when they were placed in an environment free of microcystins, there was a 50% decrease in the amount of detectable toxin in the mussels within 2 days. Freshwater mussels are poor indicators of the accumulated concentrations of microcystins at any one location over an extended period because of their rapid depuration of microcystins from their tissue. However, mussel microcystin concentrations were higher than in lake phytoplankton samples taken at the same time, indicating they may be useful as an early-warning organism.

**Human Health Risk from Rainbow Trout Consumption**

Using the ELISA results from this study and assuming the compounds detected are of equivalent toxicity to MC-LR, a 70 kg person can safely eat a 300 g serving of rainbow trout every 3.6 days. This calculation used a muscle tissue concentration of 35 μg kg⁻¹ (the highest concentration recorded, Lake Rotoehu, April 2004), a TDI of 0.04 μg kg⁻¹ day⁻¹ (WHO), a 70 kg human, and a rainbow trout meal size of 300 g. If 70 kg humans eat rainbow trout at less than this rate or in smaller portions, they will consume concentrations of microcystins below the WHO guidelines, and there is low risk of adverse health effects. The TDI is a recommended limit for a healthy adult. Children, the elderly, and sensitive individuals may be at a higher risk. The concentrations of microcystins are significantly higher in rainbow trout liver, but current advice given by Fish and Game New Zealand and local district health boards to gut and thoroughly wash the fish before eating would substantially reduce any potential health risks.

**CONCLUSIONS**

In Lakes Rotoiti and Rotoehu it was demonstrated that microcystins can accumulate in rainbow trout (*Oncorhynchus mykiss*) tissue to concentrations that, although nontoxic to them, are above the daily tolerable intake limit for human consumption (assuming all microcystins detected have a toxicity to equal that of MC-LR) recommended by the World Health Organization. It is unlikely that eating these rainbow trout as part of a regular diet (several meals per week) would have any adverse health effects. However,
caution should be taken until further work assesses the concentrations, bioavailability, and toxicity of covalently bound microcystins that may not have been extracted and measured in this study. Unconjugated microcystins were not detected in the rainbow trout samples by LC-MS. This raises questions about the chemical nature of the microcystins measured by the ELISA, and the proposal that ADDA-containing compounds play a significant role in toxicity needs to be confirmed so that the relative health risks that these compounds pose to humans can be established.

Microcystins in the tissue of the freshwater mussel (Hyridella menziesi) were rapidly accumulated and removed, as their concentrations varied in the surrounding water. The LC-MS detected a profile of the microcystin congeners in the mussels similar to those found in the lake phytoplankton. Microcystin concentrations in the tissue were markedly higher than those in the surrounding phytoplankton, and mussels therefore have a potential use as an early-warning organism in lakes. Because this organism is seldom eaten in New Zealand, the health risk from its consumption is considered minimal.

Cyanobacterial species abundance and composition can change rapidly in Lakes Rotoiti and Rotoehu. Within 2 weeks the bloom in Lake Rotoiti changed from a nontoxic Anabaena spp. bloom to a Microcystis spp. bloom containing cell concentrations of microcystins. Cyanobacterial cell concentrations were not always a good indication of microcystin concentrations, and of most concern was the situation when there were elevated concentrations of microcystins, whereas cell concentrations were below the threshold for issuing health alerts. These lakes are highly valued for recreation, cultural significance, and tourism. Therefore, future regular monitoring programs should incorporate analyses for microcystins.

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