Microcystins in Lakes Rotoiti and Rotoehu

A report for Environment Bay of Plenty on microcystin levels in water, trout and freshwater mussels in Lakes Rotoiti and Rotoehu during 2003/4.

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Cover Picture: Buoys on the net closing off part of Te Weta Bay (Lake Rotoiti). The photo was taken on a fine calm day and the cyanobacteria cells are clearly visible in the lake water. On this day, 18 March 2004, the highest cell counts of the enclosure experiment were recorded.

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1. Summary

Cyanobacteria in water bodies can produce cyanotoxins known to be hazardous to humans exposed to them through recreational activities or via ingestion of contaminated drinking water and food. Microcystins have been found in a number of the Rotorua Lakes, sometimes in concentrations potentially hazardous to humans. While it is unlikely that a lethal dose of microcystins would be ingested acutely, there is now sufficient evidence to show that there is a risk from chronic exposure, particularly if there is long-term frequent contact. A number of studies have demonstrated that microcystins can increase the risk of liver tumours and long-term liver damage.

Microcystins are known to accumulate in freshwater organisms. In this study we investigated the accumulation of microcystins in rainbow trout (*Oncorhynchus mykiss*) in Lakes Rotoehu and Rotoiti. The study involved using both trout that had access to the entire lake and hatchery trout added to an enclosure in Lake Rotoiti where microcystin levels in the water and cell counts could be closely monitored. Accumulation of microcystins in freshwater mussel (*Hydridella menziesi*) placed in the enclosure was also investigated. As filter feeders, freshwater mussels might have value as bio-indicators or early warning organisms for detecting low levels of microcystins in a water body. Microcystins in water samples, trout liver, trout muscle tissue and in the mussels were analysed using the ADDA-ELISA method.

Phytoplankton samples, when dominated by very high cell concentrations of *Microcystis* spp. were found to contain microcystins. A maximum level of 760 µg l^{-1} was recorded in Te Weta Bay, Lake Rotoiti, in March 2004. The ELISA results also confirmed presence of microcystins in the trout liver and muscle tissue, and in freshwater mussels.

The Total Daily Intake (TDI) for microcystins intake recommended by the World Health Organisation for human consumption is 0.04 µg kg^{-1} day^{-1}. A 70 kg human consuming 300 g of trout muscle tissue from Lakes Rotoiti and Rotoehu would have exceeded this level in 50% - 71% of the samples. Health problems could result if more than 300 g of trout muscle tissue was consumed on a regular basis over an extended period. The full human risk has not been accurately identified because some microcystins are likely to be covalently bound in trout muscle tissue and cannot be extracted using the methods used in this study.

This study also found that in general cell counts were a poor indicator of microcystin levels in the water samples, suggesting that cell counts alone are not a satisfactory way of identifying human health dangers in water bodies with cyanobacterial blooms. In addition, further work is needed to establish unambiguously which species of cyanobacteria are responsible for microcystin production.

2. Introduction

Cyanobacteria in water bodies can produce cyanotoxins, which can be hazardous to humans exposed to them through recreational activities, or via ingestion of contaminated drinking water and food.

Cyanotoxins include cyclic peptides (microcystin and nodularin) alkaloids (cylindrospermopsin, anatoxins and saxitoxins) and lipopolysaccharides (LPS). In humans these cyanotoxins have been known to cause allergic reactions (Carmichael and Falconer 1993; Pilotto *et al.* 1997), poisonings and in one case involving haemodialysis, death (Azevedo *et al.* 2002).

Microcystins are cyclic heptapeptides, which block protein phosphatase 1 and 2a in affected organisms (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 and Bartram 1999), most commonly species from the *Microcystis* genus.

During a survey (Wood, unpublished data) of toxic cyanobacteria in New Zealand, microcystins were found in water samples from Lakes Rotoiti and Rotoehu. At times the microcystin levels reached concentrations that could be hazardous to humans involved in recreational activity on or in the lakes (Wood 2003). In addition to microcystins, both anatoxin-a and saxitoxin have been found in the Rotorua lakes, however, they do not appear to commonly occur or reach high levels (Wood unpublished data).

The first objective of the present study was to investigate the variation in microcystin levels during a bloom event. Variation in microcystin levels over time has not previously been studied in New Zealand water bodies. Because Lakes Rotoiti and Rotoehu are known to contain microcystins and regularly experience cyanobacterial blooms they were chosen for the study. Microcystin levels, and the composition and abundance of cyanobacteria species were monitored weekly in the lakes. Resulting data allows correlations between microcystin levels and cell concentrations of certain species to be established, and the results used to assess periods of greatest human health risk.

Numerous studies overseas have shown that microcystins can accumulate in different organs of freshwater fish (Ernst *et al.* 2001; Magalhaes *et al.* 2003), freshwater (Eriksson *et al.* 1989) and marine mussels (Amorim and Vasconcelos 1999; Vasconcelos 1995), zooplankton 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 to be low. Current advice given in

the Bay of Plenty district to people eating fish from areas experiencing cyanobacterial blooms is to gut them. However, a recent study in Egypt detected levels of 102 µg kg^{-1} in the muscle of fish (Mohamed *et al.* 2003) indicating that the consumption of animals containing microcystins might adversely affect human health.

The maximum allowable concentration for microcystin in drinking water was established by Falconer et al. (1994) as 1 µg l^{-1} day^{-1}. A Daily Tolerable Intake (TDI) value of 0.04 µg kg^{-1} day^{-1} has been proposed as a provisional guideline value by the World Health Organisation (Chorus and Bartram 1999) for total microcystins. TDI is defined as the acceptable amount of a potentially toxic substance that can be consumed daily during a life period.

The rainbow trout (*Oncorhynchus mykiss*) is a species that is of particular importance to recreational fishers and to tourism in the Rotorua lakes. The second objective of this project was to assess whether microcystins accumulate in trout in the Rotorua lakes; which organs microcystins accumulate in, and if present whether or not, microcystins could reach levels that would be hazardous for human consumption.

Because of the variability in microcystin levels within the Rotorua lakes, trout were sampled in Lakes Rotoiti and Rotoehu that ranged over the entire lake, and from a group contained within an enclosure in Lake Rotoiti where microcystins were known to reach high concentrations. In the enclosure levels of microcystin and cyanobacteria cell counts could be closely monitored.

A third objective of this study was to establish whether significant levels of microcystins accumulate in the native freshwater mussel (*Hydridella menziei*), and if they accumulate, how the concentrations of microcystins changes with exposure time. Because of the variable distribution of cyanobacteria and their associated toxins, their concentrations in waterbodies can change very quickly with changing environmental conditions. Thus routine point sampling may not provide a true indication of the potential risks posed by cyanotoxins in any given area. *Hydridella menziesi* are filter feeders, and it seemed likely that this feeding process would lead to microcystin accumulation in them. If the accumulation built up and remained in the tissue over a period of time, freshwater mussels may have given a useful indication of the toxin levels at a particular site over a period of time. Additionally, if the mussels were to potentially accumulate higher levels of microcystins in their tissue than in the surrounding water, they could be used for early warning in water bodies with low levels of microcystins. For example, if levels of microcystins are low in the water body they might be near the limits of detection with the analytical procedures used. However levels in the mussels might be detectable. Such early

detection could allow proactive measures to be taken before microcystins reach levels that are deemed to be a health risk.

A British study (Hathaway 2001) found significantly higher levels of microcystins in mussels than in lake water suggesting that this was an area worthy of investigation in the present study.

3. Methods

3.1 Methods Field

3.1.1 Phytoplankton Sampling

Water samples (400 ml) were collected weekly as part of the Environment Bay of Plenty (ENVBOP) Rotorua Lakes cyanobacteria monitoring program. Collection began on the 14-11-2003 and finished on 02-05-2004. Three samples were collected from both Lake Rotoiti and Lake Rotoehu on each occasion. The sampling sites chosen were areas where cyanobacteria proliferation is known to occur and were spaced over a wide area on each lake. The sample sites were:

Lake Rotoiti - Te Weta Bay (2804295E 6346565N NZ Topographic map Grid Reference, Map U15), Okawa Bay (2803035E 6344690N, Map U15) and Hinehopu (2816215E 6345715N, Map V15) (Figure 1).

Lake Rotoehu – Kennedy Bay (2821450E 6347065N, NZ Topographic map Grid Reference, Map V15) Otautu Bay (2821675E 6348380N, Map V15) and Te Pohue Bay (2818190E 6345950N, Map V15) (Figure 1).

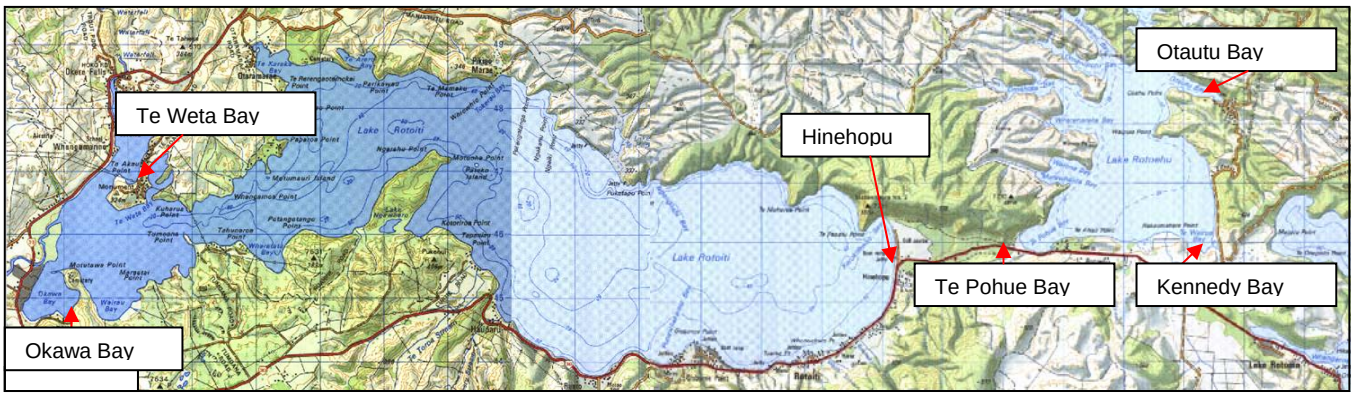


Figure 1. Map showing location of weekly sampling sites on Lakes Rotoiti and Rotoehu.

The water samples were well mixed and a 50 ml subsample was taken. It was preserved using Lugol's Iodine and stored in the dark and used later for species identification. The

remaining 350 ml was frozen and thawed twice, and then two 2 ml sub samples were taken and frozen at -20°C for later microcystin analysis.

Identification, enumeration and microcystin analysis was carried out on each sample as described in Section 3.2.1 below.

During the enclosure experiment, 400 ml samples were collected near the location of mussel cages at the centre of the enclosure bay. Samples were collected daily at 1000 h (surface only) and at 1500 h (surface, 1 m, 2 m and 3 m depths). The dissolved oxygen and water temperature were recorded at each sampling time and at each sampling depth. Dense blooms were observed at Te Akau Point (Figure 2) on several occasions. Samples of these were collected 15-03-2004 and 26-03-2004 and analysed for microcystins. A thick scum formed (26-03-2004) at the jetty area of the nearby Lake Rotoiti campground (Figure 2). Samples were collected to obtain an indication of the microcystin levels reached when scums form.

The water samples were collected, preserved and frozen in the same manner as described for the weekly samples (Section 3.1.1).

3.1.2 Free Roaming Rainbow Trout

From November 2003 to April 2004 up to five trout were caught monthly by local fishing guides in both Lakes Rotoiti and Rotoehu. The trout were frozen and transported in insulated containers by courier to Massey University, Wellington. Viscera and muscle tissue were removed as described in Section 3.2.3 and frozen at -80°C until microcystin analysis was carried out as described in Section 3.2.1.

3.1.3 Enclosure

A large scale mesocosm type enclosure was set up in an arm of Te Weta Bay, Lake Rotoiti. The location of the enclosure was 286460E 6346805N (NZ Topographic Map Grid Reference, Map U15) and is shown in Figure 2. The opening of the enclosed arm was approximately 100 m wide with a maximum water depth of 4 m. The length of enclosure was approximately 350 m. Depth profiles across the opening to the arm and along the length of the arm on the centre-line are shown in Figures 3 and 4. Resource consent was obtained from Environment Bay of Plenty to establish the enclosure.

A 100 m long net was used to section off the enclosure from the rest of Lake Rotoiti. The middle 70 m was a length of standard synthetic fishing net with a 20 x 20 mm mesh dimension. The 15 m long end pieces were made from synthetic garden shade cloth with a

4 x 3 mm aperture. The end pieces were sewn to the main section using synthetic cord. The net size chosen allowed water and phytoplankton to move freely into the area, creating an environment similar to other areas of the lake. The main section of fishing net was originally 10 m in depth. To avoid any excess net in the water it was rolled up so that the depth matched the profile of the bottom of the bay and the roll secured with plastic cable ties. To aid with buoyancy along the top of the net, cork floats were located every 1 m centres and five litre plastic containers were tied to the top edge 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 this together with the lead weights on the bottom of the fishing net assisted in weighing down any excess net and held the net to the bottom.

Figure 2. Map showing western end of Lake Rotoiti and location of enclosure experiment in Te Weta Bay. The red line shows the location of the net used to form the enclosure.

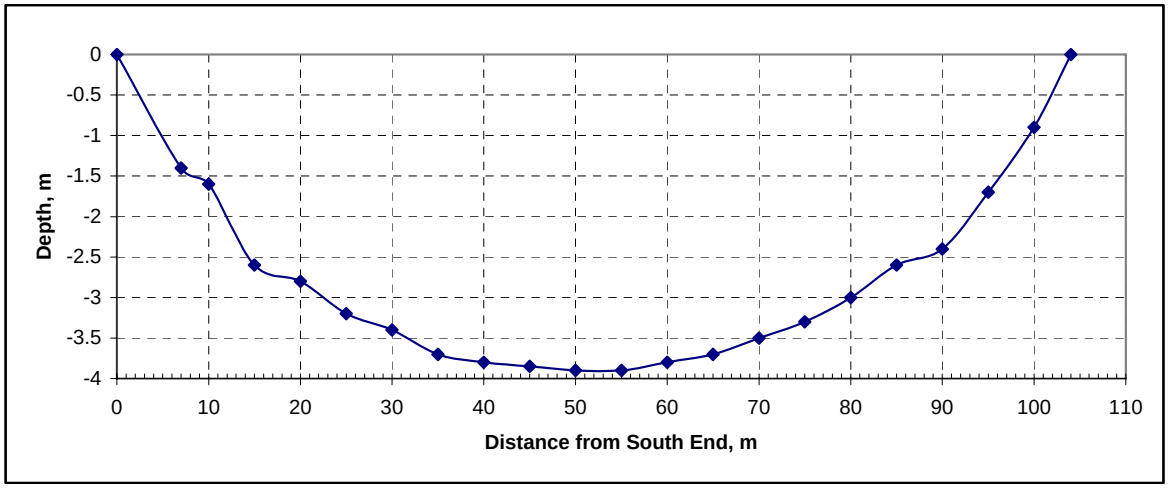


Figure 3. Cross-section profile of net at opening to enclosure.

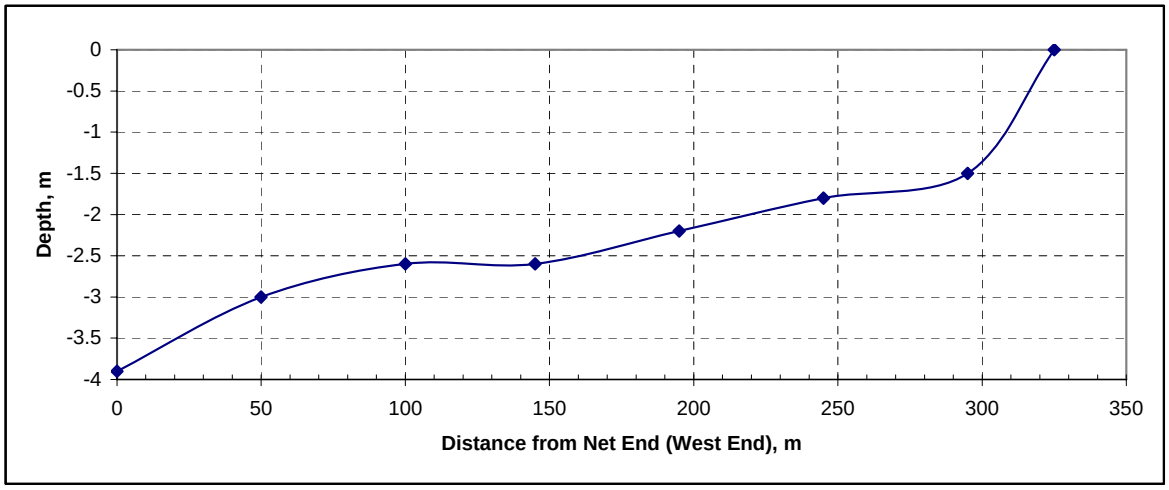


Figure 4. Longitudinal depth profile of embayment used for enclosure experiment.

The net was anchored to the bottom sediment of the lake using 1 m long U pins of 12 mm diameter deformed reinforcing rod. These were pushed into the soft bottom sediment by Scuba divers from ENVBOP and Rotorua District Council. The ends of the net were tied securely to trees at the north end and waratah stakes at the south end. Plates 1 and 2 show the main net and the end attachments. Plate 3 shows the net being placed and Plate 4 shows the net in place across the arm of the bay. Signs were erected at either side of the net to inform the public about the placement of the net and the study.

The net was installed on 07-03-2004 and removed on 27-03-2004. All material associated with the project was removed from the site.

3.1.4 Trout Enclosure Experiment

Eighty, two year old rainbow trout from the Fish and Game New Zealand, Rainbow Valley hatchery were released into the enclosure on 08-03-2004. Each trout was tagged by clipping one pectoral fin before release. Up to five of the released trout were caught every two to three days over a 21 day period. A total of 28 released trout were caught. In addition, three "wild" trout that were in the enclosure area prior to installing the net were captured.

The trout were caught by lure and by using two gill nets with a 50 x 50 mm mesh. Angling took place between 5 pm to 8 pm and 5 am to 8 am. Initially the nets were set and retrieved over these same periods. Careful monitoring was carried out to ensure that water birds did not become entangled in the nets. After the first 14 days, it was apparent that birds were identifying the nets and did not venture into the middle of the enclosed area during the hours of darkness. Following this observation, the nets were sometimes left in place overnight in the middle of the bay. Approximately half of the trout were caught by lure casting and the other half by netting.

Trout were killed immediately after capture by a heavy blow to the top of their heads. The trout were refrigerated shortly after catching and dissected within 12 hours.

Plate 1. Enclosure net, showing the main net and the synthetic garden shade cloth end attachments.

Plate 2. Enclosure net. 5-litre plastic containers were placed every 3 m along the headline of the net.

Plate 3. The enclosure net being installed in Te Weta Bay.

Plate 4. The enclosure net in place in Te Weta Bay, viewed from the enclosure side.

Viscera and muscle tissue were removed as described in Section 3.2.3 and frozen at -80°C until microcystin analysis was carried out as described in Section 3.2.1.

Three trout from the hatchery were used to determine if any baseline microcystin was present. These were killed and dissected at the time the batch was released into the enclosure, thus were not exposed to cyanobacteria in the lake water.

3.1.5 Mussel Cage Experiment

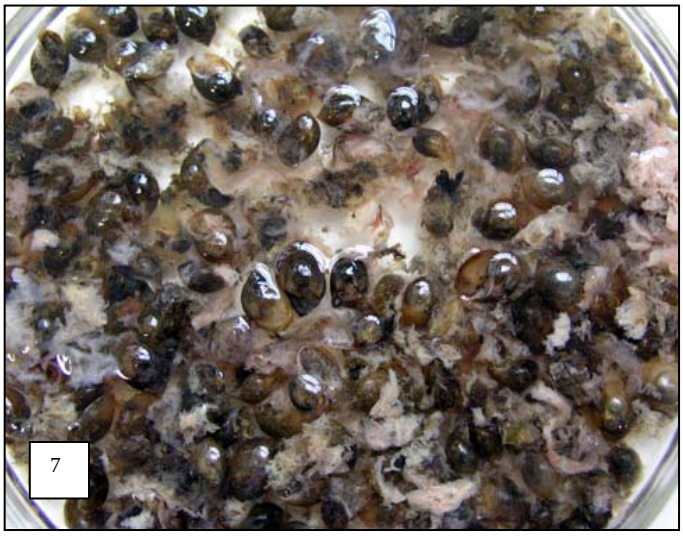
Approximately 130 freshwater mussels were collected on 06-03-2004 from the sediment in Lake Rotoma (free of microcystins) at approximately 10 m depth. Mussels were transported in buckets containing Lake Rotoma water to the study site in Lake Rotoiti.

Ten mussels were killed immediately and kept frozen at -80°C until microcystin analysis was carried out as described in Section 3.2.1. These mussels were used to determine if there was any baseline microcystins present. The remaining 120 were placed in plastic mesh cages (Plate 5) suspended from buoys 300 mm below the surface in the centre of the enclosure bay about 20 m from the net (Plate 6).

Ten mussels were collected every 2-3 days over a period of 21 days, frozen shortly after collection and then the shells were opened and the flesh halved. They were then pooled into two sets each containing ten halves and kept frozen at -80°C until microcystin analysis was carried out on one of the pools as described in Section 3.2.1. The remaining pooled mussels were retained for individual analysis (not carried out in this project) or for use as backup samples.

Plate 5. Plastic mesh cages used to hold freshwater mussels during the enclosure experiment.

Plate 6. Location of mussel cage mooring buoys inside net buoys in Te Weta Bay.



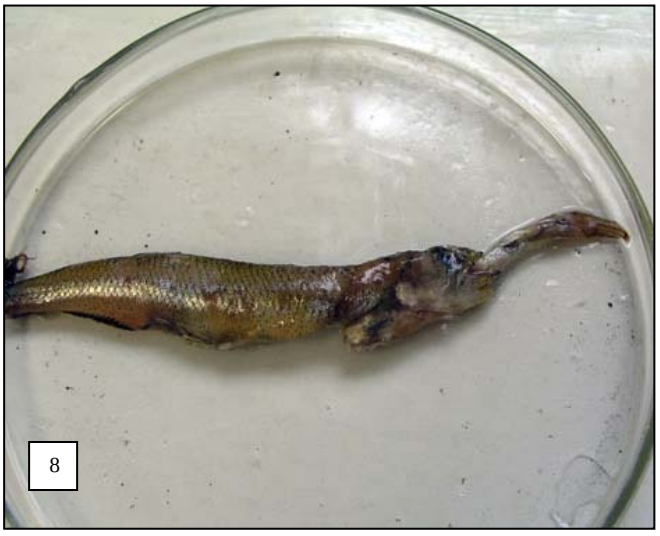


Plate 7. Trout stomach contents (April, Lake Rotoehu). This stomach contained approximately 130 of the gastropod snail *Physa acuta.*

Plate 8. Enclosure trout - Lake Rotoiti. Trout stomach contents - Smelt (*Retropinna retropinna*). The large smelt had a smaller fish partly ingested.

3.2 Laboratory Methods

3.2.1 Microcystin Analysis

The total microcystin content of the samples (phytoplankton and organisms) was analysed with a competitive indirect ELISA 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.

An ELISA plate (NUNC) was coated overnight with OVA-ADDA-hemiglutaryl, in 0.05M Na_2CO_3 buffer at pH 9.6 (100 µl, 20°C). After being washed twice with phosphate buffered saline (PBS), additional binding sites were blocked by incubation with Ovalbumin (Inovatech, BC, Canada) (1% in PBS, 200 µl, 1 h, 20°C). The plate was washed twice with PBS. The samples and Microcystin-LR standard (ALEXIS, 50 µl) were added to the wells together with antiserum (Cat-BSA-ADDA antiserum, AgResearch, Hamilton, New Zealand, 50 µl). After incubation at 20°C for 1.5 h, the wells were washed twice with PBS containing 0.05% Tween 20 (PBST) and twice with PBS. Anti-sheep secondary antibody (Anti-sheep IgG-HRP, Lifescience NZ Ltd, 100 µl) was then added to the wells and the plate incubated for 1.5 h. The wells were then washed twice with PBST and twice with PBS. BioFX (BioFX Laboratories, Maryland, United States) substrate solution (100 µl) was added and the plate incubated for 15 min. The reaction was stopped by addition of 2M H_2SO_4 (50 µl) and the absorbance at 450 nm was determined with a microplate spectrophotometer (VERSAmax, Molecular Devices, CA, United States).

For phytoplankton, 2 ml subsamples were freeze-thawed twice, centrifuged at 10 000 g for 2 min and the supernatant used for the ELISA assay. This gives a measure of the total microcystin present in the lake water. It does not distinguish between intra and extra cellular microcystin.

Pooled trout muscle tissue and liver, and freshwater mussels were homogenised in 75% methanol/water. The homogenate and solvent was centrifuged and the supernatant recovered and washed with hexane. The aqueous phase was retained and processed by solid phase extraction. The toxin containing fraction was then eluted with 80% methanol/water, diluted in assay buffer (PBS) and analysed by ELISA. For some samples it was necessary to dilute extracts 1:50 to overcome matrix effects.

To assess the effectiveness of the extraction procedure spiked recovery experiments were carried out on trout muscle tissue, trout liver and mussel samples. The samples were homogenised with 100 – 1000 µg l^{-1} of MC-LR (ALEXIS) for 1 h. The samples were then extracted and tested using the ELISA as described above.

All trout and mussel samples were analysed by the Toxinology Laboratory, AgResearch, Hamilton, New Zealand.

3.2.2 Cyanobacterial Identification and Enumeration

The preserved Lugol's subsamples were used for species identification and enumeration of cyanobacteria species. Enumeration and identification was carried out using an inverted Olympus microscope and Utermöhl settling chambers (Utermöhl 1958). Depending on the density of cyanobacteria, 2-10 ml samples were used for enumeration. When possible, cyanobacteria were identified to species level. The species identifications in this study were made primarily by reference to; Baker 1991, Baker 1992, Baker and Fabbro 2002, Komárek 1999, Komárek *et al*. 2002, McGregor and Fabbro 2001, Azevedo and Sant'Anna 2003. Cell counts and corresponding microcystin levels were compared using Pearson Correlation coefficient with Microsoft Excel Software (2003).

3.2.3 Trout Examination

All trout were weighed and the standard length measured. This allowed the Condition Factor (CF) to be calculated for each trout using the following formula (Fish & Game pers. com.);

$$Condition\ factor = 3612.8 \frac{Weight, g}{(Length, cm)^3}$$

Necropsy was performed and liver, kidney, gut and muscle tissue removed. Approximately 5 g of each trout muscle tissue was taken from each trout and combined into a pooled sample. A second 5 g of muscle tissue was taken from each trout and kept separately as back up samples or for individual muscle tissue sample analysis if required (not carried out in this project). Each liver was halved with one half combined in a pooled sample and the remaining half retained for individual analysis (not carried out in this project). Kidney samples were also retained for future analysis (not carried out in this project). Samples were stored at -80°C until extracted for ELISA analysis as described in Section 3.2.1.

An assessment of stomach contents was performed on each trout. The stomachs were opened and the contents were grouped into one of the following groups; plant material, molluscs, 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 then calculated for each trout. The fullness of the stomach contents was not taken into account. An average stomach contents for each pooled sample was then calculated.

4. Results

4.1 Weekly Phytoplankton Samples

4.1.1 Microcystin Levels

The variations in microcystin levels at the six weekly sampling locations on Lakes Rotoiti and Rotoehu are shown in Figure 5.

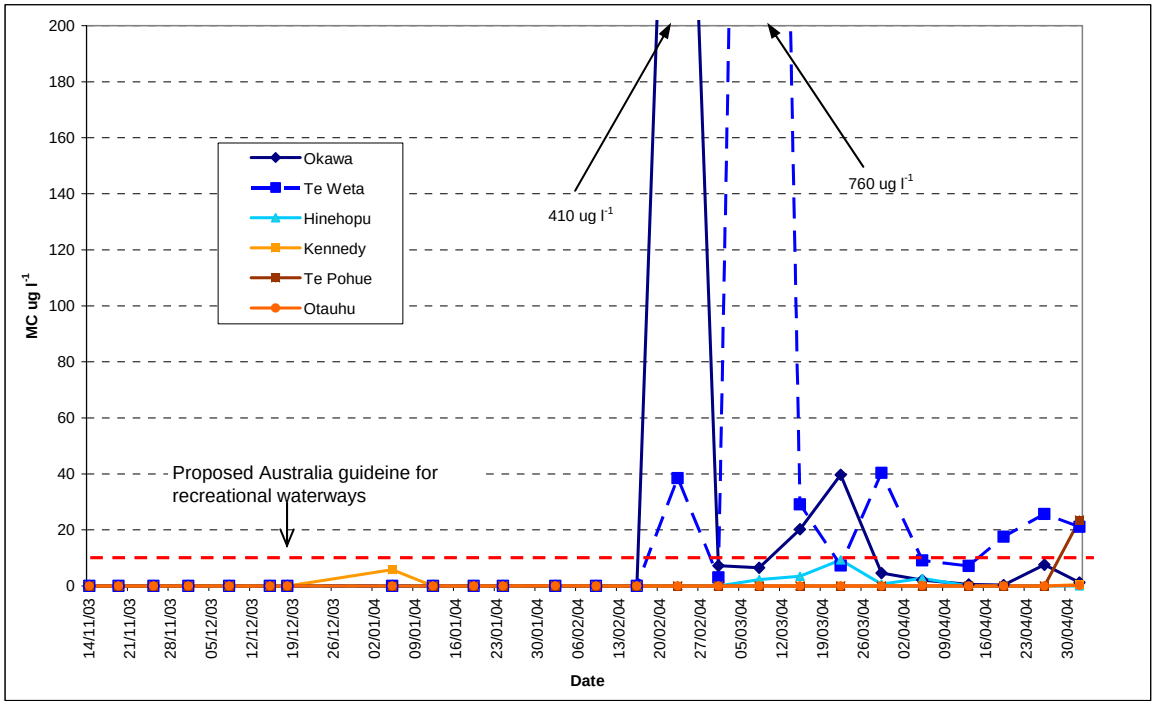


Figure 5. Microcystin levels in weekly samples - Lakes Rotoiti and Rotoehu.

Generally no or very low (<1 µg l^{-1}) microcystins levels were detected at the three sampling locations at Lake Rotoehu (Kennedy Bay, Te Pohue Bay and Otauhu Bay). This corresponded with low levels of cyanobacteria at these locations (see below). On the two occasions that significant levels of microcystins were detected (Kennedy Bay, 05-01-2004 – 5.8 µg l^{-1} and Te Pohue Bay, 02-05-2004 – 23.5 µg l^{-1}) these corresponded with an increase in the *Microcystis panniformis* cell counts.

Microcystins were absent from Lake Rotoiti samples until 23-02-2004 when they were detected in both the Te Weta Bay and Okawa Bay sites. Okawa Bay had very high levels of microcystins - 410 µg l^{-1}. This corresponded with a high cell count of *Microcystis aeruginosa* (940 000 cell ml^{-1} – Figure 6). Microcystins continued to be recorded at Okawa Bay until the conclusion of sampling. On two other occasions (15-03-2004 and 26-03-2004), the levels were above the proposed Australian recreational guideline (8 µg l^{-1}) for microcystins (National Health and Medical Research Council 2004). The sample from Te Weta Bay on 08-03-2004 recorded the highest levels of the study, 760 µg l^{-1}. This

corresponded with a level of 430 000 cells ml^{-1} of *Microcystis* spp. (Figure 7). Levels in Te Weta remained elevated during the entire study with samples regularly being above the proposed Australian guideline. Microcystin levels at Hinehopu remained generally low with only the sample from 22-03-2004 recording a level above the proposed Australian guideline (9.3 µg l^{-1}). This also corresponded with an increase in *Microcystis* spp.

4.1.2 Cyanobacteria Species Identification and Enumeration

A total of 29 cyanobacteria species were recorded during this study. A list of these is given in Appendix 1. Of the 29 species, four: *Aphanocapsula holsatica, Microcystis panniformis, Sphaerocavum brasiliensis* and *Pseudanabaena galeata* were recorded for the first time in New Zealand. The genus *Sphaerocavum* has not been recorded in New Zealand previously. Full descriptions of these species are recorded elsewhere (Wood and Crowe 2004).

The two cyanobacteria genera that dominate in Lakes Rotoiti and Rotoehu are *Anabaena* and *Microcystis.* During the study period, species from these genera usually accounted for over 90% of the cyanobacteria cells in the samples. Figures 6 - 11 show the total *Anabaena* spp. and *Microcystis* spp. and total cyanobacteria cell counts for each of the six sample sites. Trends at each site are discussed briefly below.

Okawa Bay: Figure 6 – Cyanobacteria counts were low in November with the first bloom occurring at this site in December. The bloom was composed of *Anabaena* spp. and the two dominant species were *A. circinalis* and *A. planktonica*. Although there were fluctuations in cell numbers, these two species continued to dominate until 16-02-2004 when there was a sudden decline in all cyanobacteria cell numbers. This was followed by a massive bloom of *M. aeruginosa* on the 23-02-2004. *Microcystis* species continued to dominate (although in lower numbers) until late March when *Anabaena* spp. once again became dominant.

Te Weta Bay: Figure 7 – Cyanobacteria cell numbers remained low in Te Weta Bay until the end of January when there was a bloom (46 000 cells ml^{-1}). The dominant species was *A. planktonica*. As with Okawa Bay, there was a large decrease in cyanobacteria numbers in mid February. This was followed by an increase in *Microcystis* spp. numbers, with a large peak (440 000 cells ml^{-1}) occurring on 08-03-2004. *Microcystis* spp. remained the dominant species in Te Weta Bay until the end of the study period.

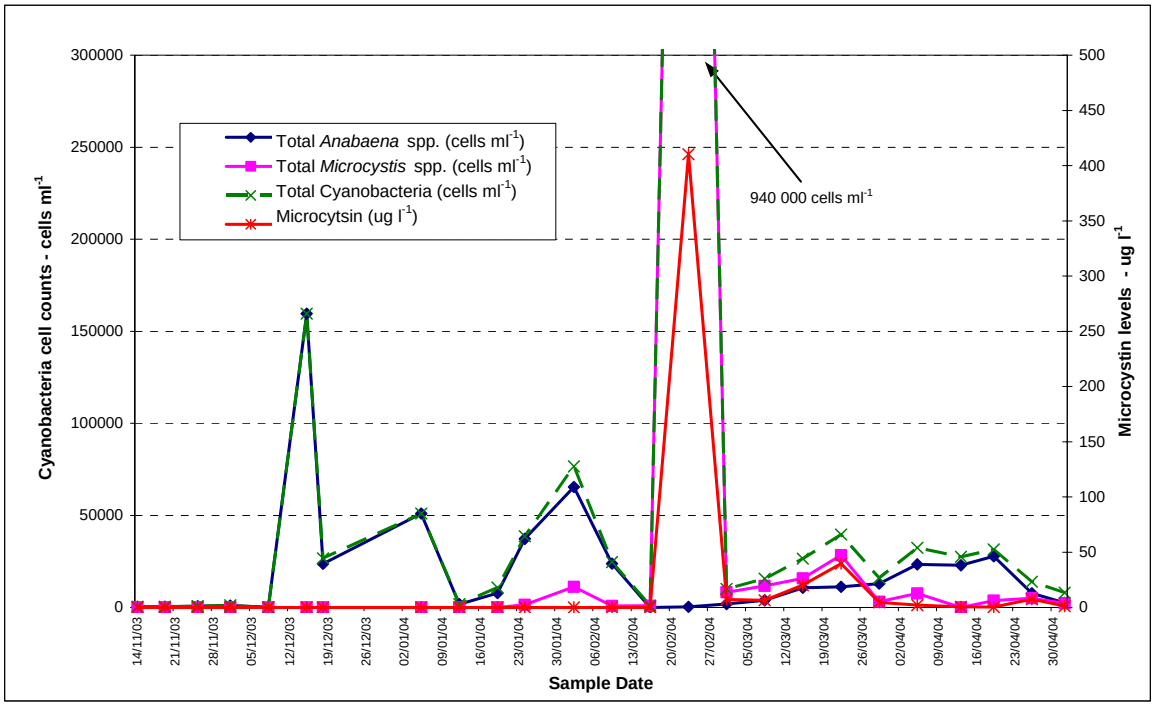


Figure 6. Cell counts and microcystin levels in Okawa Bay (Lake Rotoiti) water samples**.**

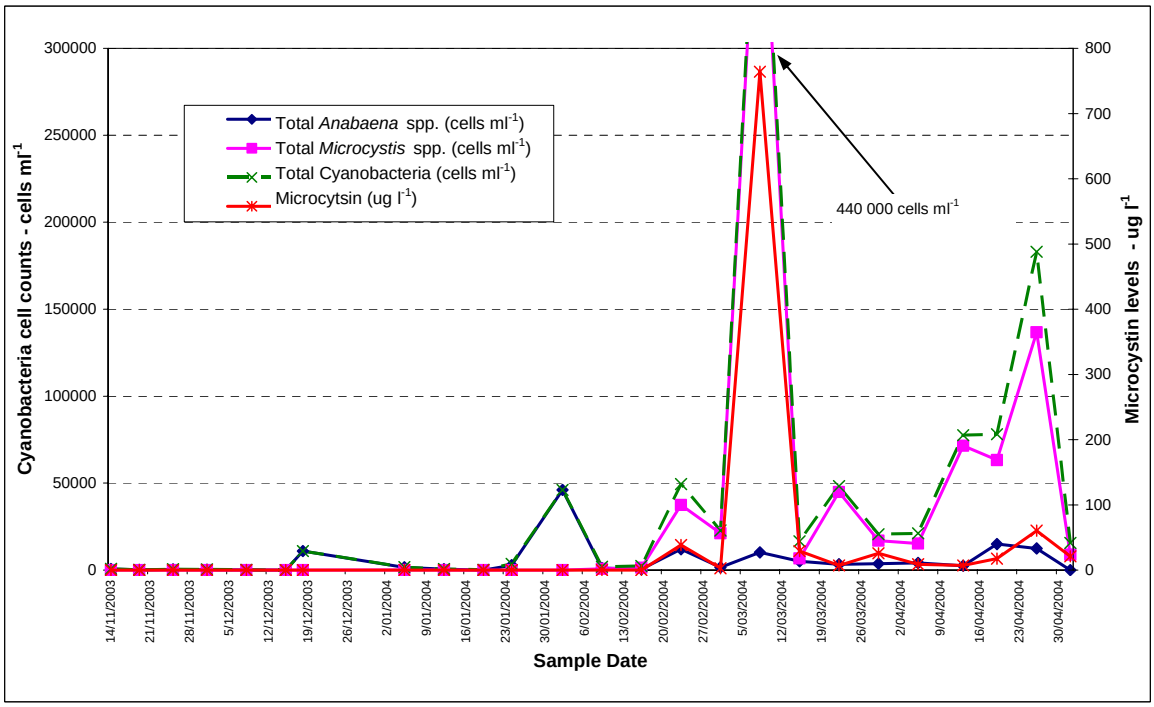


Figure 7. Cell counts and microcystin levels in Te Weta Bay (Lake Rotoiti) water samples.

Hinehopu: Figure 8 – This site experienced its first bloom (65 000 cells ml^{-1}) on 01-12-2003. The two dominant species in this bloom were *A. lemmermannii* and *A. circinalis.* On 24-01-2004 and 02-02-2004, Hinehopu experienced a further bloom with the dominant species being *A. planktonica.* Cyanobacteria cell numbers remained low from 02-02-2004 to 01-03-2004 when there was an increase in both *Anabaena* spp. and *Microcystis* spp which continued through until the end of April 2004.

Kennedy Bay: Figure 9 – *Anabaena* spp. were recorded in very low. *Microcystis* spp. were the main component of the cyanobacteria cell count. However, these did not exceed 5000 cells ml^{-1}. Until March *M. panniformis* was the dominant *Microcystis* species in the samples.

Te Pohue: Figure 10 - Cyanobacteria cell counts remained low (below 3 000 cells ml^{-1}), except for on of 02-05-2004 when cell counts peaked at 7 400 cells ml^{-1}. *Microcystis* spp. were always dominant with very low numbers of *Anabaena* spp. recorded. *M. panniformis* was the dominant *Microcystis* spp. in the samples.

Otauhu Bay: Figure 11 – *Microcystis* spp. dominate the cyanobacteria cell counts. Cell counts fluctuated but were always under 3 500 cells ml^{-1}. *Anabaen*a spp. comprised only a small proportion of the cyanobacteria species.

4.1.3 Cell Counts Versus Microcystin Levels

The correlation between cyanobacteria cell counts and microcystin levels in all samples is shown in Figure 12. Figure 13 is a modified version of Figure 12 and shows these data without the two extremely high levels of microcystin, as including these tended to skew the data by not allowing trends at lower levels to be observed. Figure 13 shows only a very weak correlation (R^2 = 0.20) between cell numbers and microcystin levels. Occasionally there are high cell counts with low or no microcystin levels detected. On some occasions cyanobacteria cell counts are low and microcystin levels are elevated, as highlighted by the red circle in Figure 13. To assess whether microcystin levels correlated with cell counts from certain genera the *Microcystis* spp. and *Anabaena* spp. were plotted separately against the microcystin levels for the data from the enclosure experiment. This is shown in Figures 14 and 15. There was no correlation between cell numbers of these genera and microcystin levels (*Anabaena* spp. R^2 = 0.001, *Microcystis* spp. R^2 = 0.01).

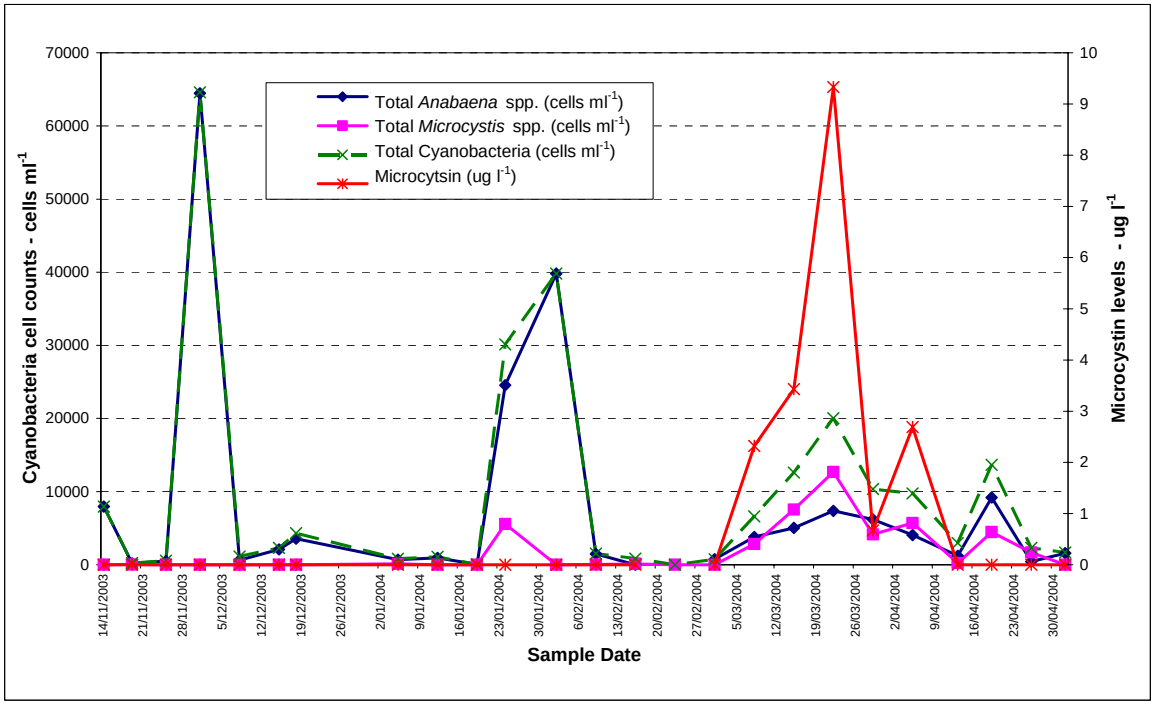


Figure 8. Cell counts and microcystin levels in Hinehopu (Lake Rotoiti) water samples.

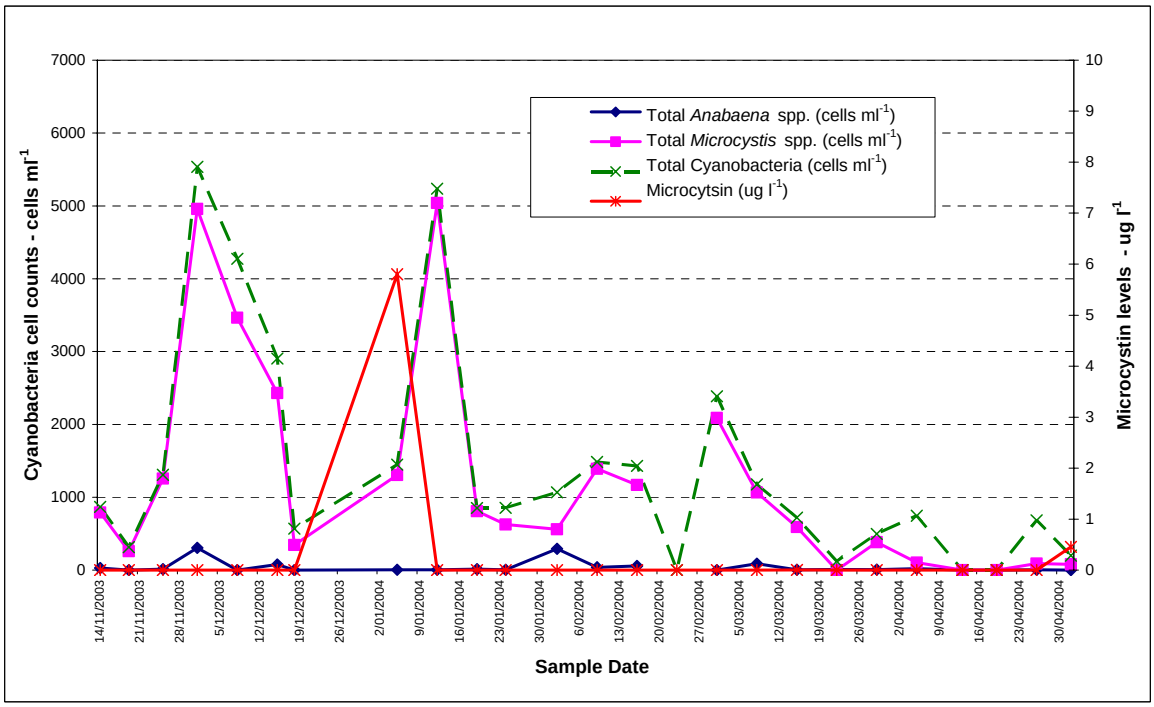


Figure 9. Cell counts and microcystin levels in Kennedy Bay (Lake Rotoehu) water samples.

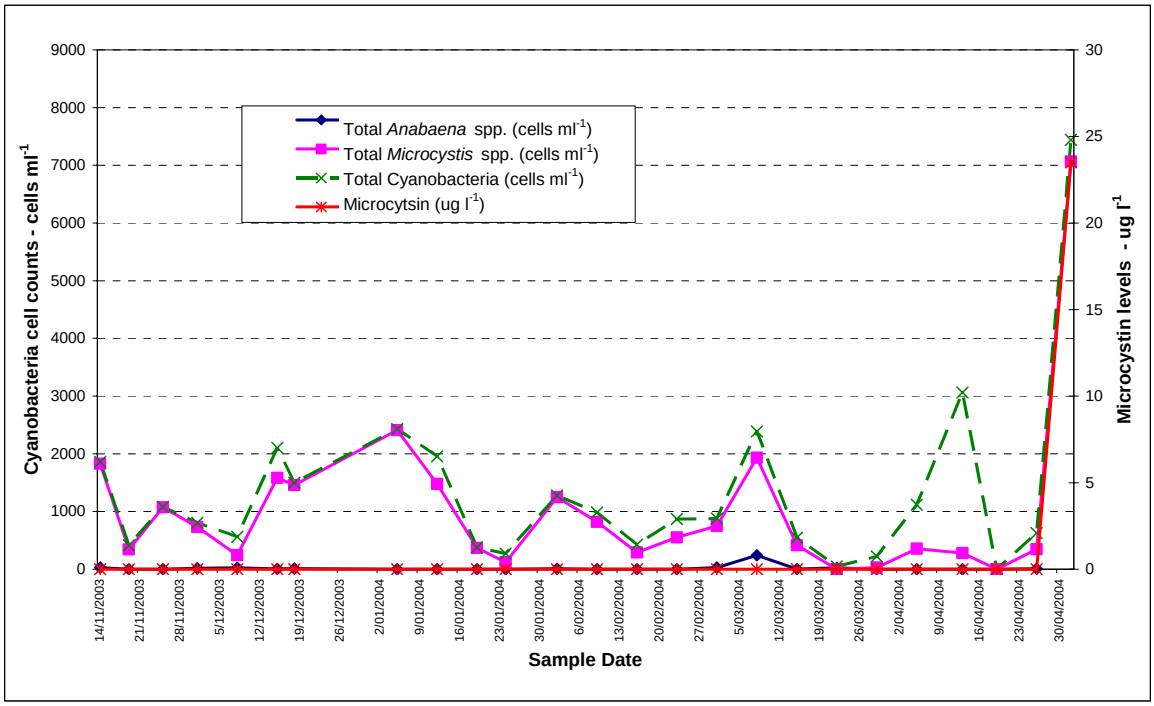


Figure 10. Cell counts and microcystin levels in Te Pohue Bay (Lake Rotoehu) water samples.

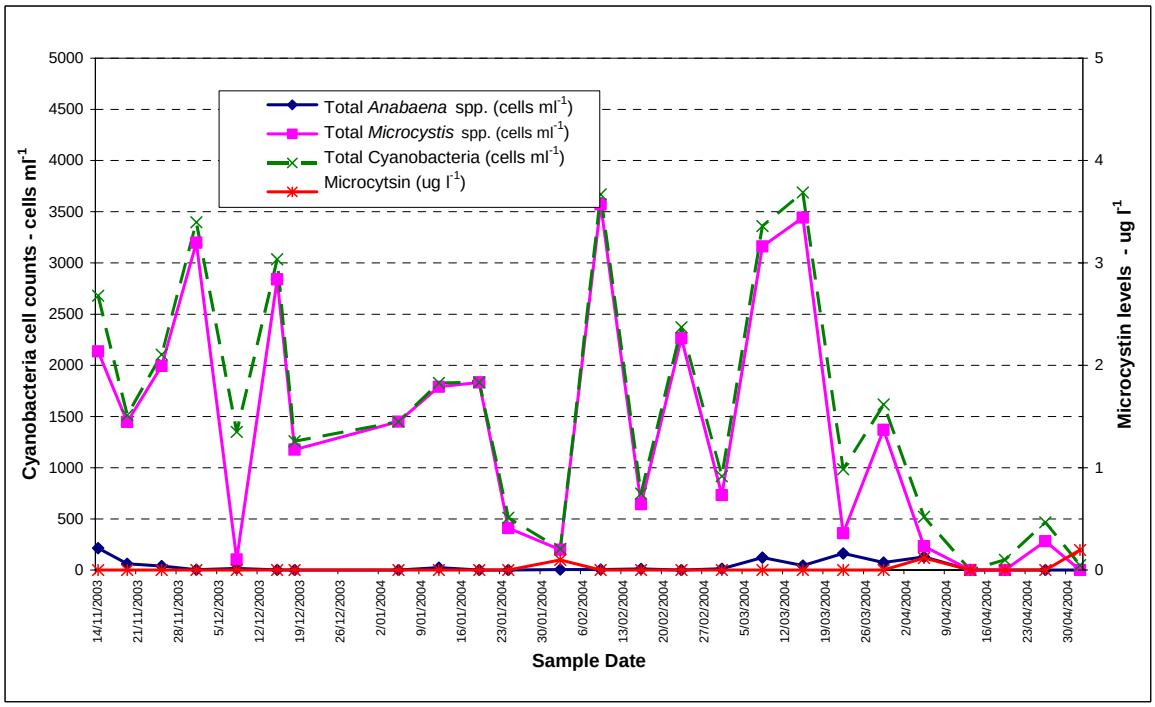


Figure 11. Cell counts and microcystin levels in Otauhu Bay (Lake Rotoehu) water samples.

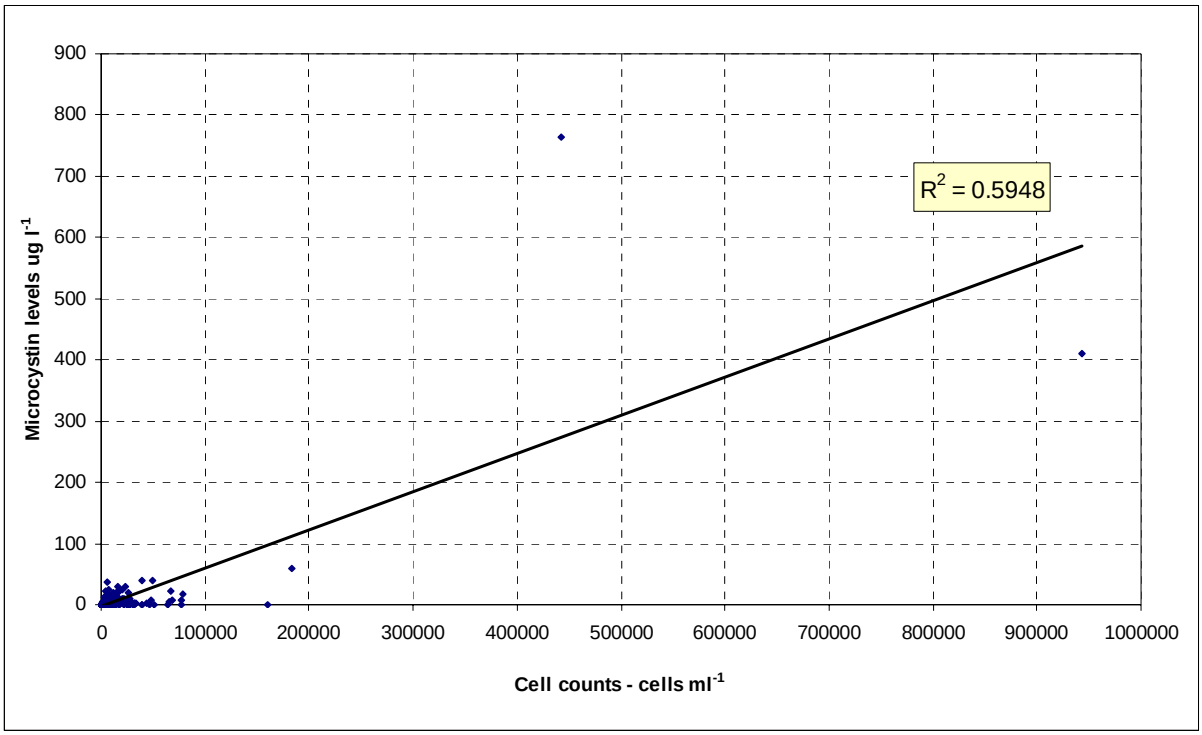


Figure 12. Cyanobacteria cell counts versus microcystin levels in all water samples.

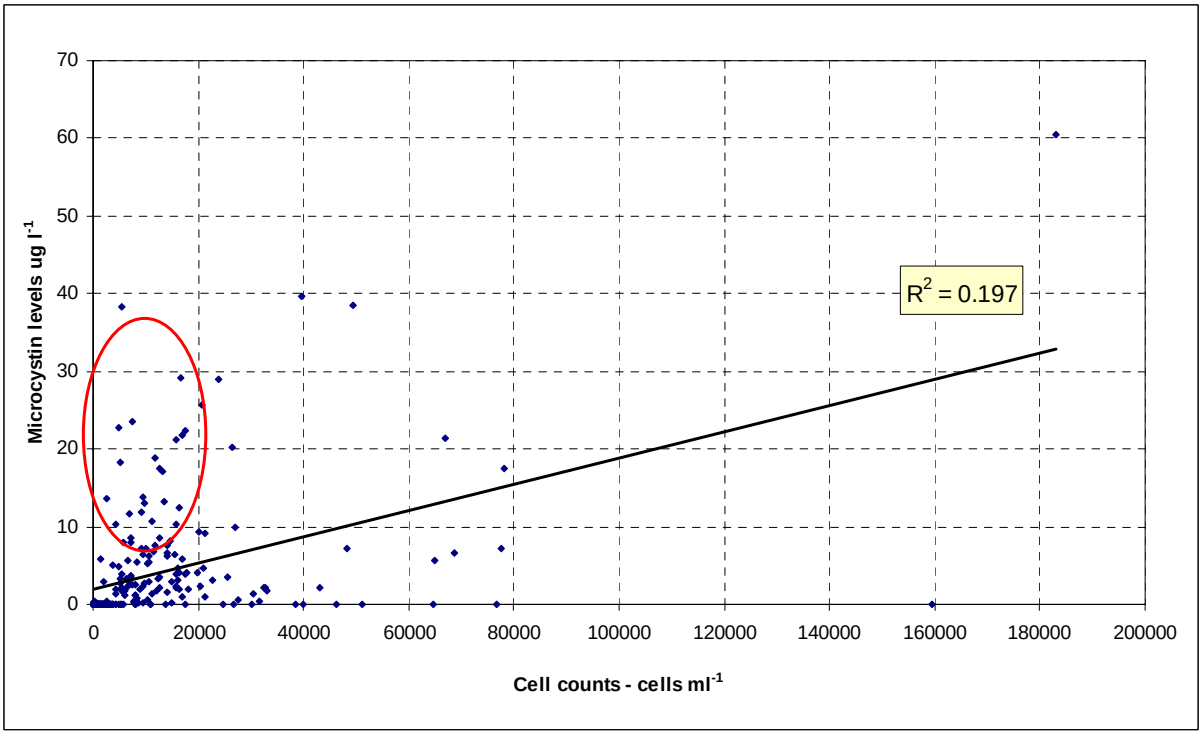


Figure 13. Cyanobacteria cell counts versus microcystin levels in all water samples. Two extremely high microcystin values removed. Red circle shows data where elevated microcystin levels corresponded with low cell counts.

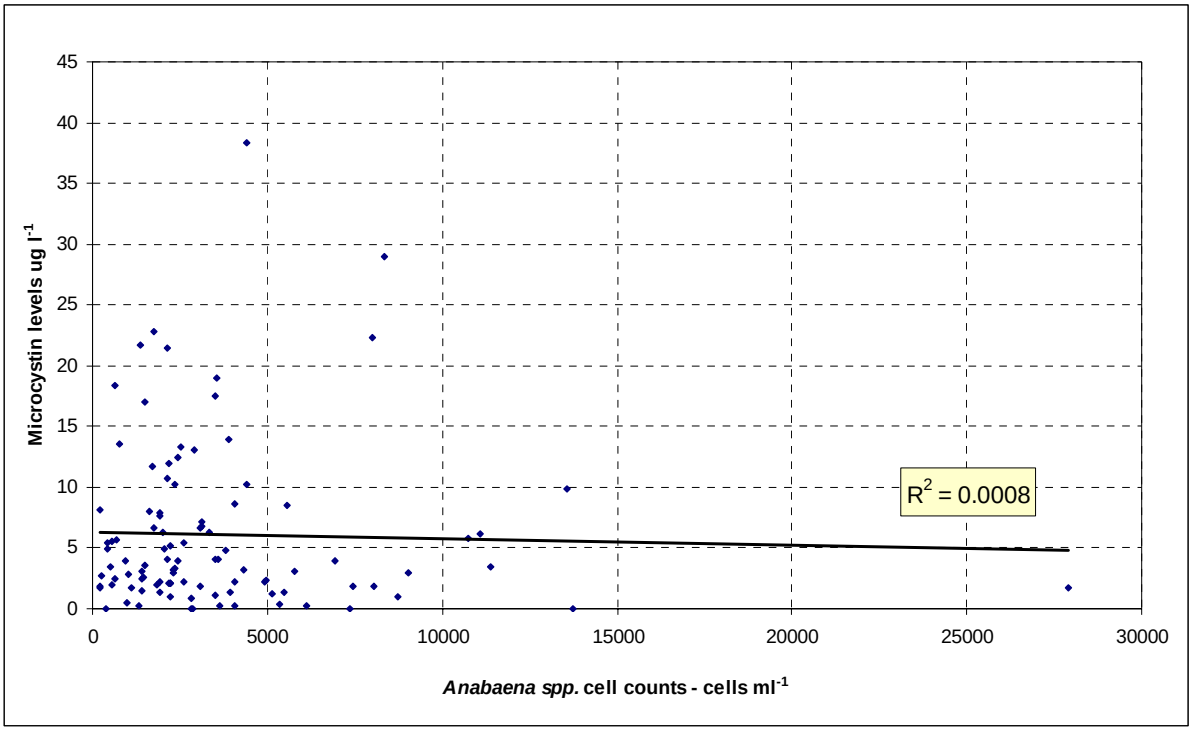


Figure 14. Total *Anabaena* spp. cell counts versus microcystin levels in water samples from the Te Weta Bay enclosure, Lake Rotoiti.

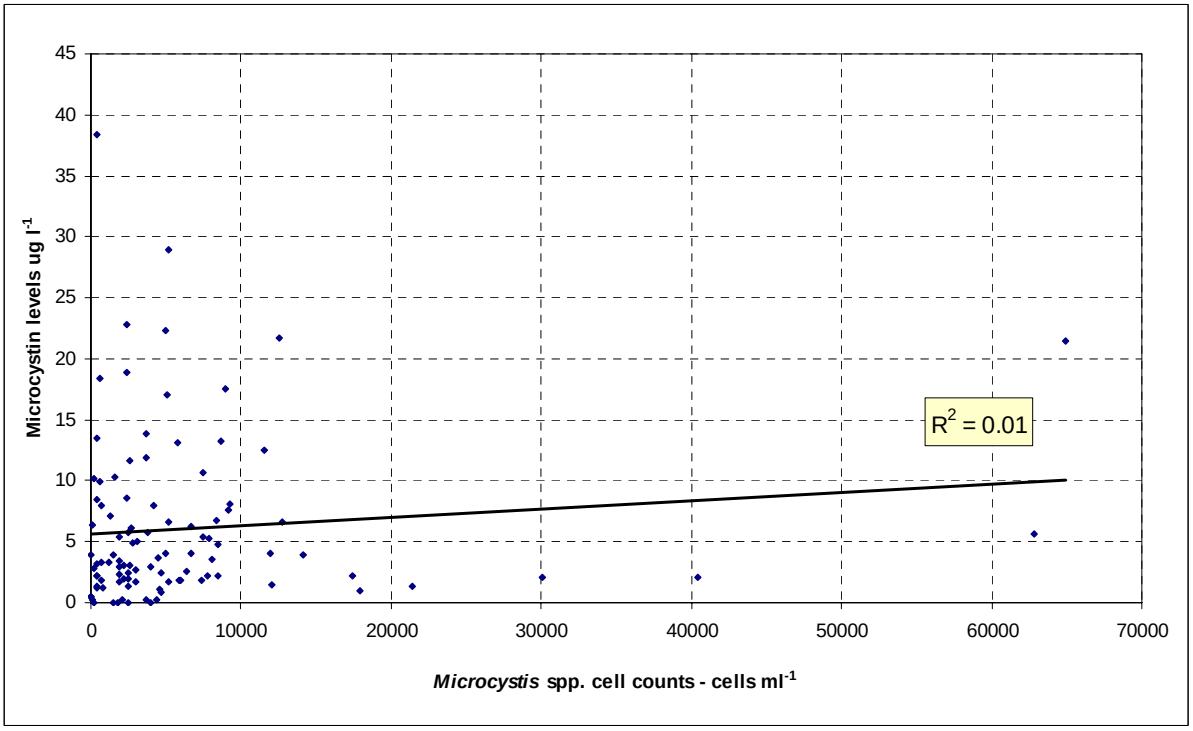


Figure 15. Total *Microcystis* spp. cell counts versus microcystin levels in water samples from the Te Weta Bay enclosure, Lake Rotoiti.

4.2 Spiked Recovery Experiments

The mussels, trout muscle tissue and trout liver were spiked with microcystin-LR at levels ranging from 0.1 to 1 µg kg^{-1} and recoveries ranged from 69 - 84% (average 78%).

4.3 Monthly Rainbow Trout Samples

4.3.1 Trout Muscle and Liver Analysis - Lake Rotoehu

Table 1 shows the number of trout that were received each month from Lake Rotoehu (caught by local fishing guides).

Month	Number of Trout Received
November	5
December	5
January	4
February	5
March	5
April	5

Table 1. Number of rainbow trout *(Oncorhynchus mykiss)* received for analysis each month from Lake Rotoehu, November 2003 - April 2004.

Microcystins were found in liver and muscle tissue samples in all monthly samples from November 2003 to April 2004 (Figure 16). Microcystin levels were always higher in liver than in muscle tissue. Highest levels of 27.57 µg kg^{-1} for muscle tissue and 78.92 µg kg^{-1} for liver were recorded in November 2003. The amount of microcystin decreased between December 2003 to March 2004 before increasing again in April 2004. These values are the minimum figures and do not consider the possible 20 - 30% not extractable using the method developed in this study.

The average weekly water microcystin levels from the three sampling sites is plotted for each month in Figure 16. Microcystin levels were zero or very low in the water during the study period. Thus there is no apparent correlation between microcystin levels in the water and microcystin levels in the trout samples.

An estimated daily intake (EDI) was calculated using an adult human weight of 70 kg and ingestion of 300 g of trout muscle tissue. The EDI values for monthly samples from Lake Rotoehu are shown in Figure 17. The TDI of 0.04 µg kg^{-1} day^{-1} value suggested by the WHO (Chorus and Bartram 1999) also is also shown on the graph. The EDI in the muscle

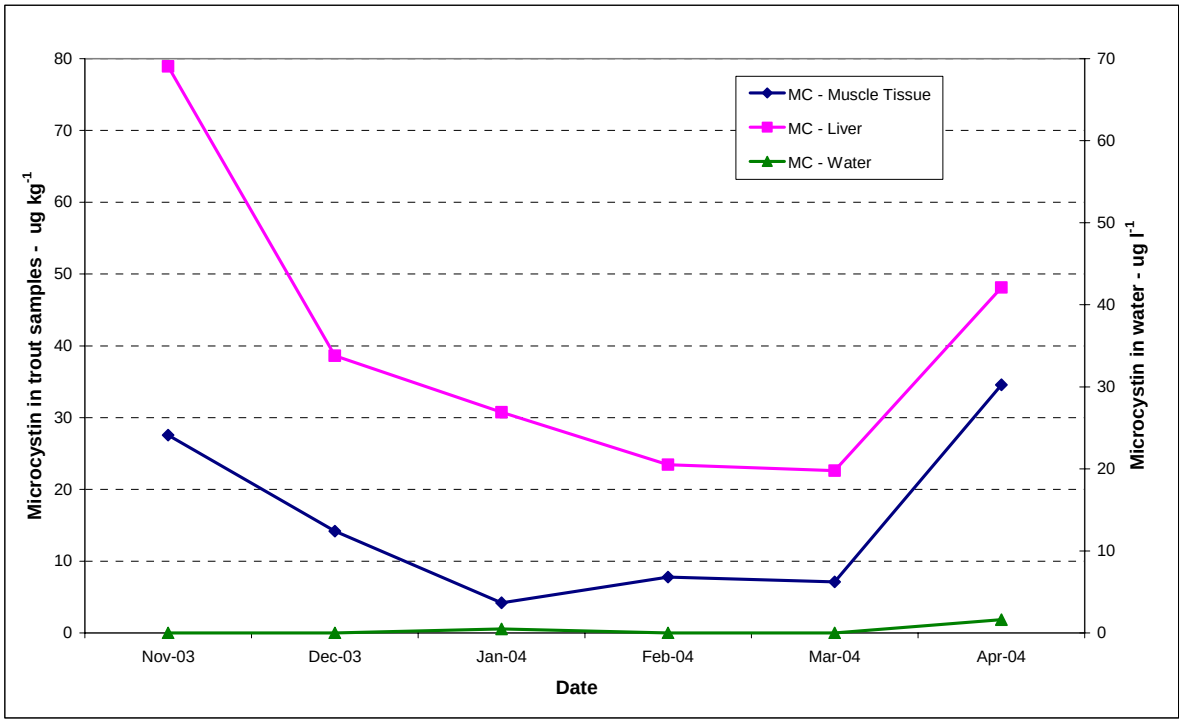


Figure 16. Microcystin levels in rainbow trout (*Oncorhynchus mykiss)* muscle tissue and liver, and in the monthly water samples from Lake Rotoehu. The water microcystin level is an average of weekly samples at three locations in lake**.**

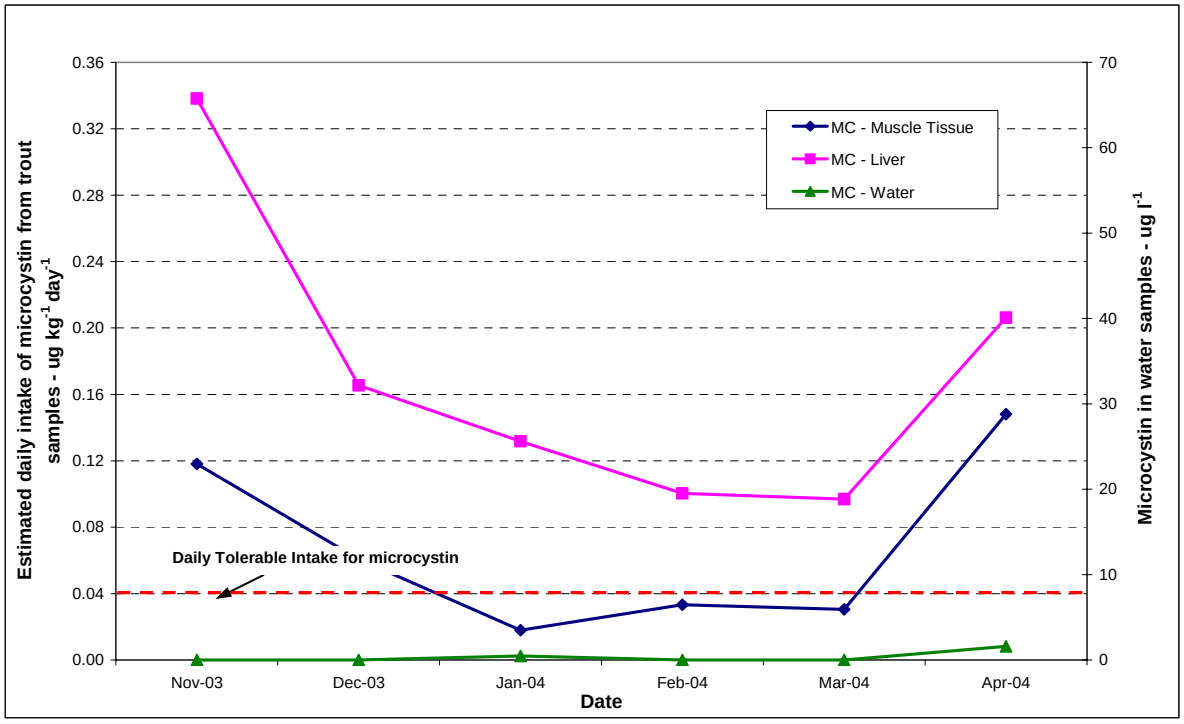


Figure 17. Estimated human daily intake of microcystin from Lake Rotoehu based on a 70 kg person ingesting a 300 g serving of rainbow trout *(Oncorhynchus mykiss)*. The water microcystin level is an average of weekly samples at three locations in Lake Rotoehu**.**

tissue exceeds this guideline in the samples from November and December 2003 and April 2004. The TDI value is not relevant to liver samples as trout liver is not usually consumed.

The condition factor (CF) for the Trout from Lake Rotoehu is shown in Figure 18. The CF increased slightly from November 2003 to March 2004 before decreasing again in April 2004. This decrease in CF is possibly related to the increase in microcystin levels during the same period. The weights and lengths of the November sample were not recorded thus a CF could not be calculated for these trout.

4.3.2 Stomach Contents of Lake Rotoehu Trout

Stomach contents of Lake Rotoehu trout were varied (Figure 19). However, in all monthly samples over 40% of the diet was fish. The majority of these fish were common smelt (*Retropinna retropinna*) (Plate 8), with bullies (*Gobiomorphus* sp.) identified in some samples. Molluscs, mainly the gastropod snail, *Physa acuta*, made up a large component of trout diet in December 2003, February and April 2004. One stomach in the April 2004 sample contained over 130 *P. acuta* (Plate 8). The stomachs of two trout in February 2004, and one in November 2003 were empty.

4.3.3 Trout Muscle and Liver Analysis - Lake Rotoiti

Table 2 lists the number of trout that were received each month from Lake Rotoiti.

Month	Number of Trout Received
November	0
December	5
January	5
February	2
March	5
April	5

Table 2. Number of rainbow trout (*Oncorhynchus mykiss)* received for analysis each month from Lake Rotoiti, November 2003 – April 2004.

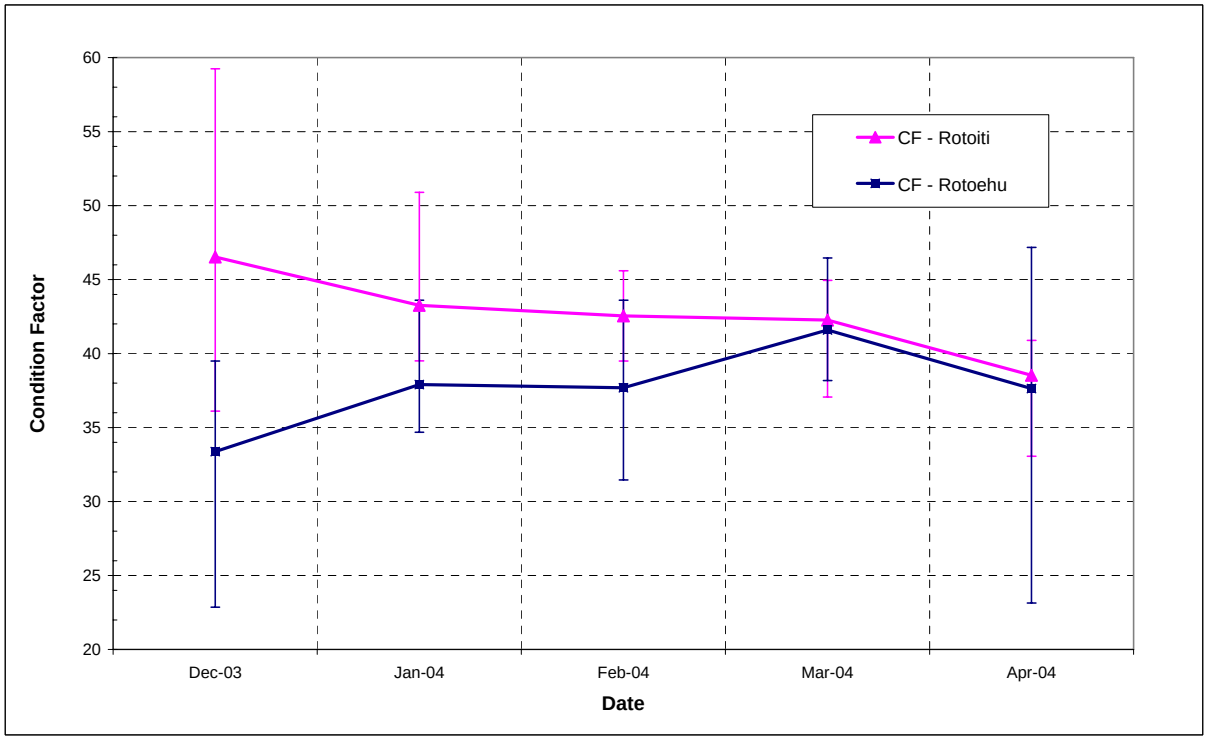


Figure 18. Condition factor of rainbow trout (*Oncorhynchus mykiss)* received monthly from Lake Rotoehu and Rotoiti. Error bars show maximum and minimum values for each month.

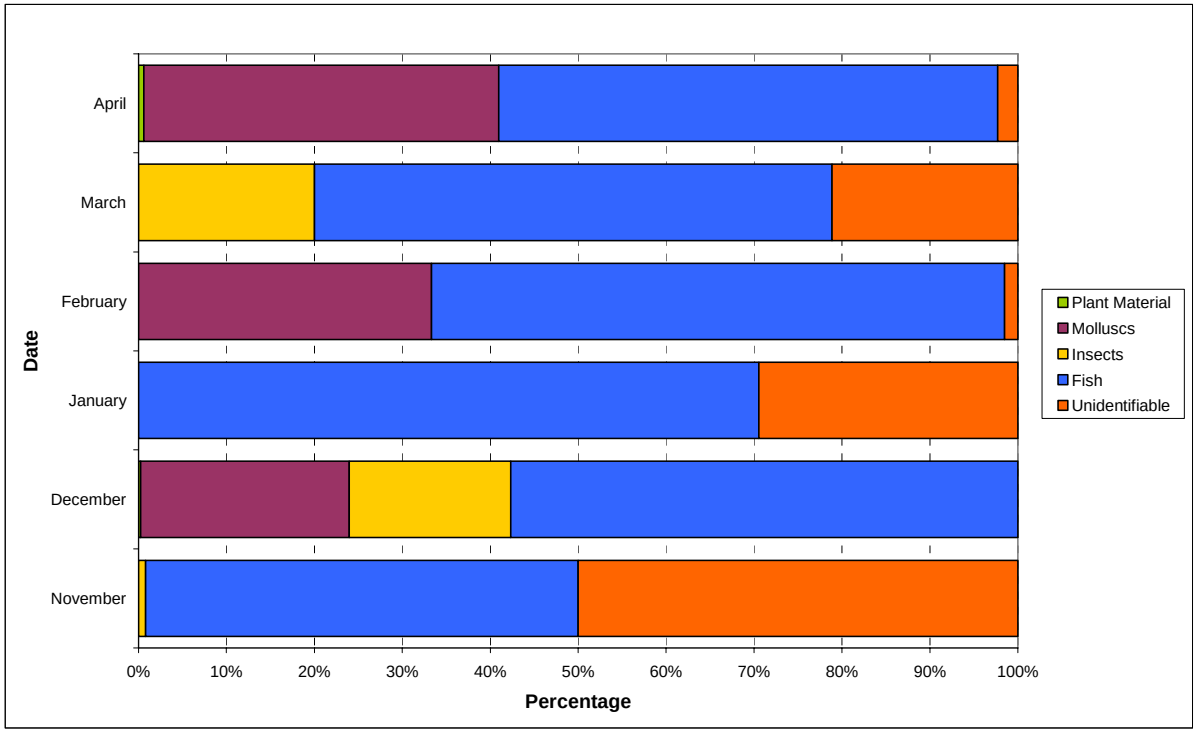


Figure 19. Percentage composition by weight of organic material in stomach contents of Lake Rotoehu rainbow trout (*Oncorhynchus mykiss*).

Microcystins were detected in all trout muscle tissue and liver samples from the lake Rotoiti monthly samples (Figure 20). Levels in the muscle tissue samples did not vary greatly (8.0 µg kg^{-1} (March, 2004) to 12.3 µg kg^{-1} (February, 2004)).

Microcystin levels varied more widely in liver samples with peaks recorded in December 2003 (44.5 µg kg^{-1}) and February 2004 (46.4 µg kg^{-1}).

No microcystins were recorded in weekly water samples until February 2004 (Figure 20). The highest average was recorded in March 2004 (62.5 µg l^{-1}). Water microcystin levels do not appear to correlate with an increase or decrease in trout microcystin levels.

Figure 21 shows the EDI for Lake Rotoiti trout. On three occasions (December 2003, February and April 2004) microcystin levels in muscle tissue were just above the TDI as recommended by the WHO.

The CF's for the Lake Rotoiti trout are shown in Figure 18. During the five month study period the CF of trout gradually decreased from 46.5 (December 2003) to 38.5 (April 2004). The CF's of trout caught during this study were compared to data for trout caught in Lake Rotoiti during the equivalent six month study period (R Pitkethley, Fish and Game, unpublished data). Using a student t-test a significant difference was found between the mean CF of the two samples ($n_1 = 58$, $n_2 = 21$, $t = 4.64$, $p < 0.05$). This result may reflect differences in measurement methods and small sample sizes. It could also be due to the diversity of habitats and feeding sources available in Lake Rotoiti.

4.3.4 Stomach Contents of Trout from Lake Rotoiti

With the exception of December 2003 the majority of the stomach contents of the Lake Rotoiti trout were fish (Figure 22). In January 2004, both smelt (*Retropinna retropinna*) and bully (*Gobiomorphus* spp.) were identified. In all other months sampled only smelt could be identified. In December 2003 insects were present in the stomach contents, however a large portion of the material could not be identified. Two trout also had empty stomachs in this month. In contrast to Lake Rotoehu, Molluscs were not identified in any of the Rotoiti trout stomachs.

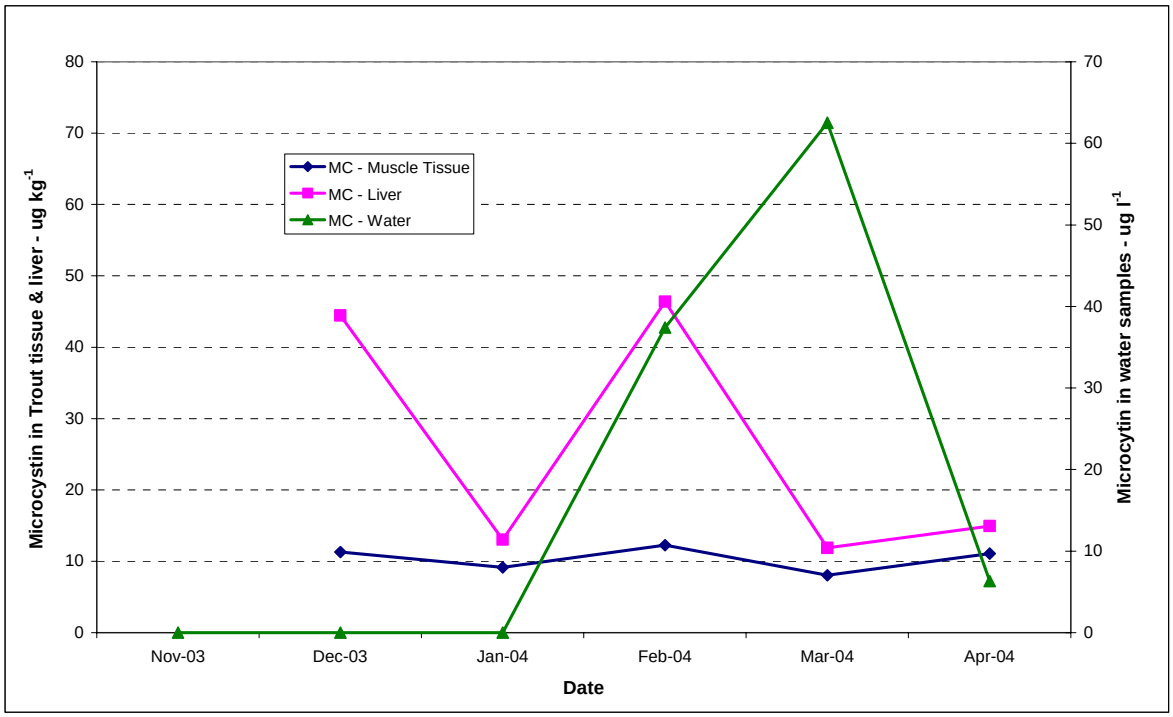


Figure 20. Microcystin levels in rainbow trout (*Oncorhynchus mykiss)* muscle tissue and liver, and in the monthly water samples from Lake Rotoiti. The water microcystin level is an average of weekly samples at three locations in lake**.**

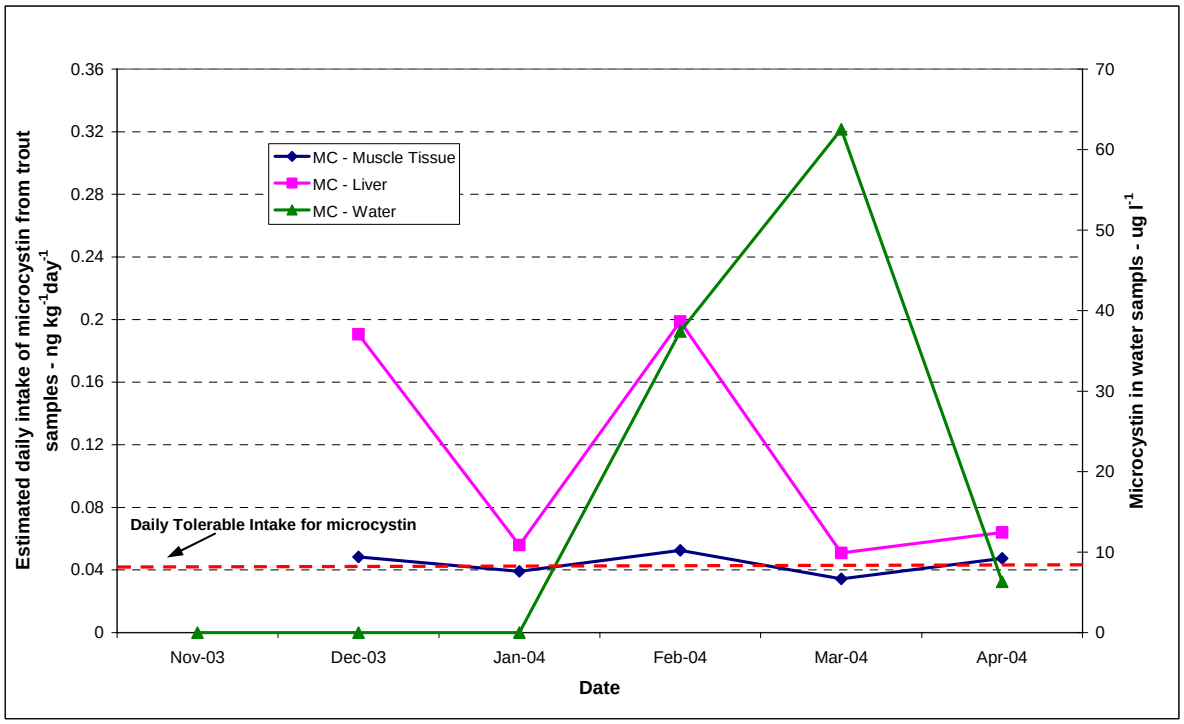


Figure 21. Estimated human daily intake of microcystin from Lake Rotoiti based on a 70 kg person ingesting a 300 g serving of rainbow trout *(Oncorhynchus mykiss)*. The water microcystin level is an average of weekly samples at three locations in Lake Rotoiti

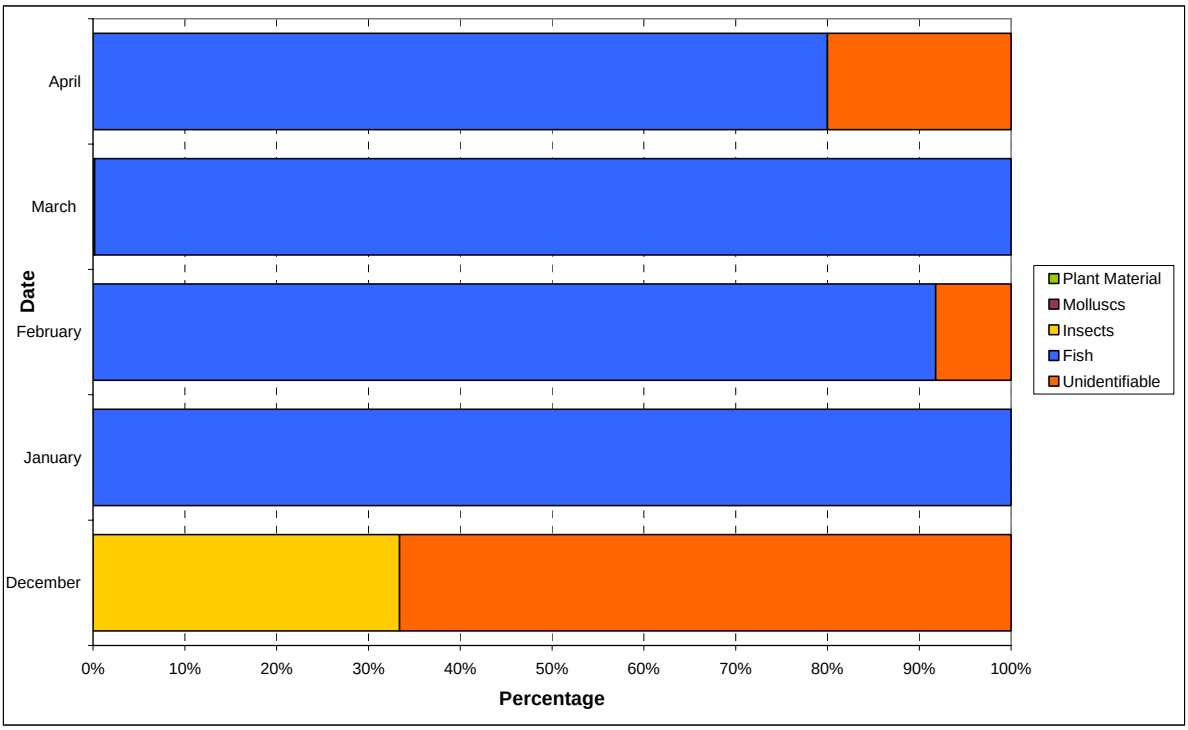


Figure 22. Percentage composition by weight of organic material in stomach contents of Lake Rotoiti rainbow trout (*Oncorhynchus mykiss*).

4.4. Enclosure Experiment

4.4.1 Water Microcystin Levels and Cell Counts

Microcystin levels in the enclosure water varied between 0 µg l^{-1} and 38.3 µg l^{-1} as shown in Figure 23. The depth at which the highest microcystin levels were recorded varied from day to day - for example, on 13-03-2004 the highest microcystin levels were recorded at the surface, however on 26-03-2004 the highest microcystin levels were recorded at 1 m depth.

Average microcystin levels in upper samples - surface morning and afternoon and 1 m depth (afternoon only) and in the lower samples - 2 and 3 m depths were calculated. A smoothed microcystin level was calculated for the upper and lower samples using the following formula; smoothed value = (microcystin on day)/2 + (microcystin previous day)/4 + (microcystin next day)/4. These smoothed values are plotted in Figure 24 and are useful in estimating general trends.

Between 07-03-2004 to 18-03-2004 the microcystins were always higher in upper samples. Between the 19-03-2004 to 28-03-2004 microcystin levels were higher in lower samples and from the 26-03-2004 to the completion of the study levels were higher in upper samples**.** On 18-03-2004 a high cell count was recorded at 2 m depth and on 19-03-2004 at 3 m depth (Figure 25).

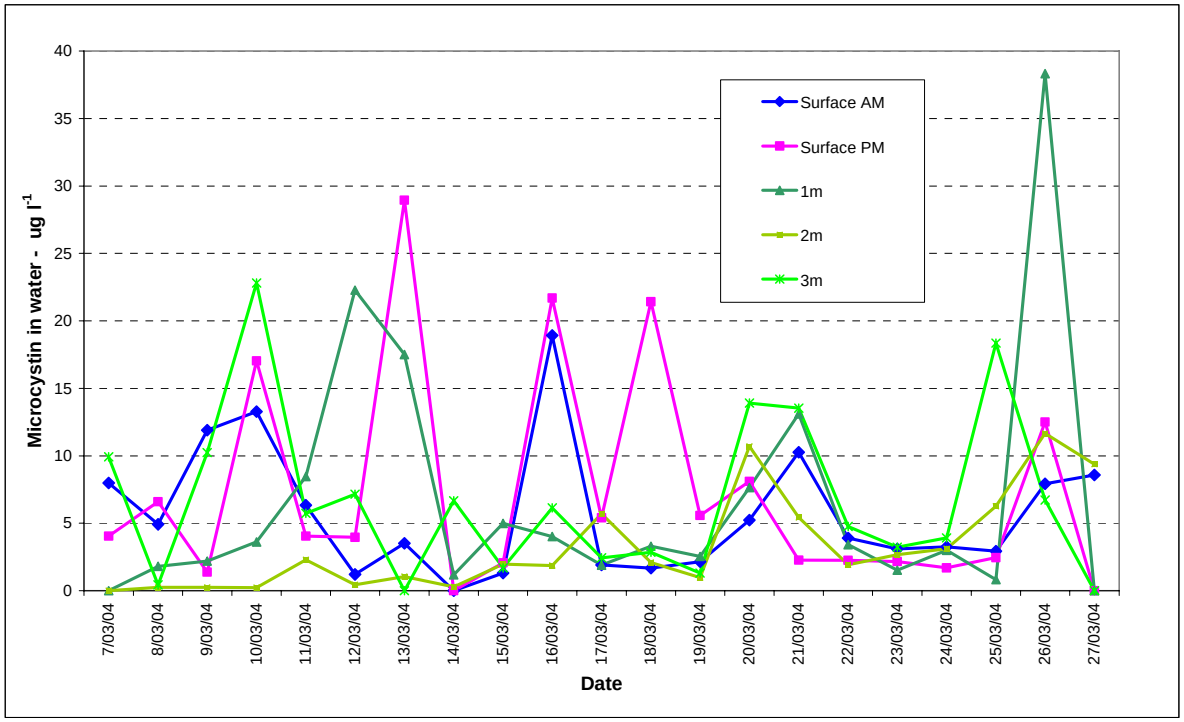


Figure 23. Microcystin levels in water samples - enclosure experiment (Lake Rotoiti).

Cyanobacteria cell count data for each sample taken during the enclosure experiment are listed in Appendix 2. Figure 25 shows the variation in total cell count for each sample. Highest cell counts (69 000 cell ml^{-1}) were recorded in the surface sample from the 08-03-2004 (afternoon). *Microcystis aeruginosa* and *Anabaena planktonica* were the two cyanobacteria species present in almost all samples and made up the majority of cells in counts. The species *Sphaerocavum brasiliensis* was also present in high numbers in many samples.

Average microcystin levels in the five samples from each day were calculated. Figure 26 plots this average together with the average of the total cyanobacteria cell counts per day. The proposed recreational Australian guideline of 8 µg l^{-1} (National Health and Medical Research Council 2004) is shown as a red dotted line on the graph and the ENVBOP guideline for issuing a health alert (15 000 cell ml^{-1}) is shown as a blue dotted line. On the 18-03-2004 and 19-03-2004 the number of cells ml^{-1} exceeded the ENVBOP health alert but on these days microcystin levels were below the proposed Australian guideline. On six days, the microcystin level exceeded the proposed Australian guideline but on these occasions cell counts were below the ENVBOP health alert. There was no clear correlation between microcystin level and cell counts.

Samples collected from Te Akau Point on (15-03-2004 and 26-03-2004) had microcystin levels of 414 and 214 µg l^{-1}. The campground sample (26-03-2004) contained 50 µg l^{-1}.

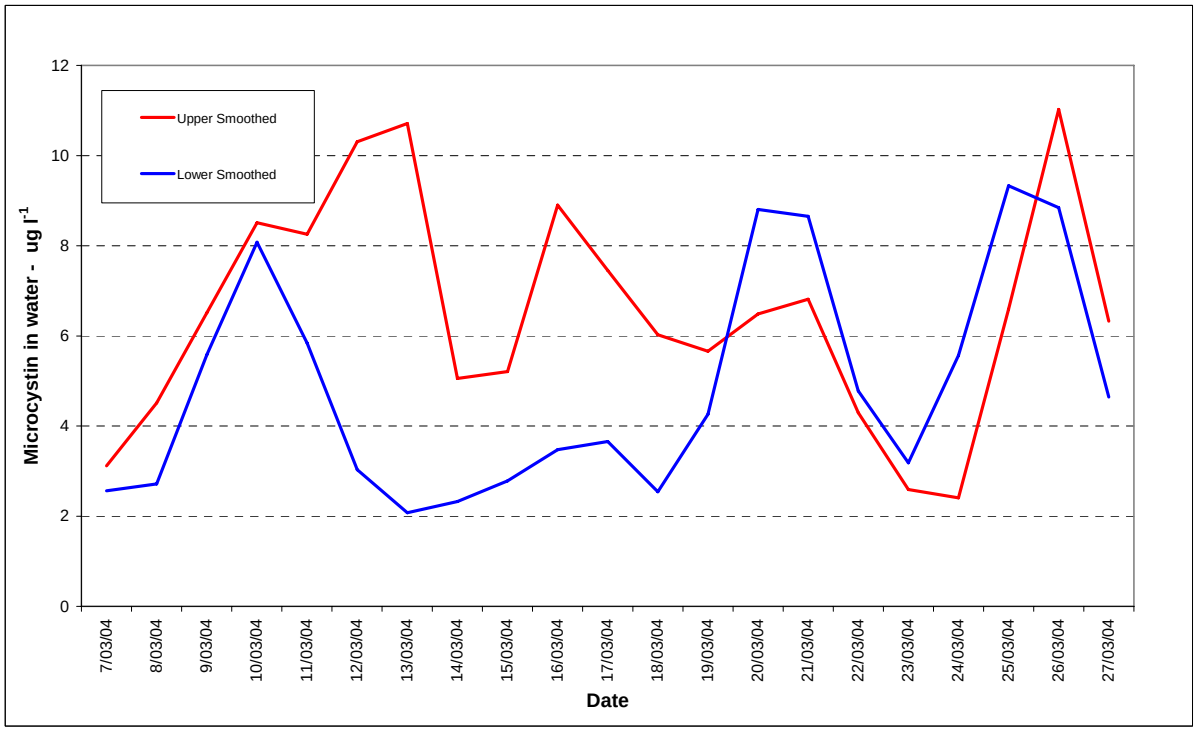


Figure 24. Microcystin levels in upper (surface and 1 m) and lower (2 and 3 m) levels during enclosure experiment.

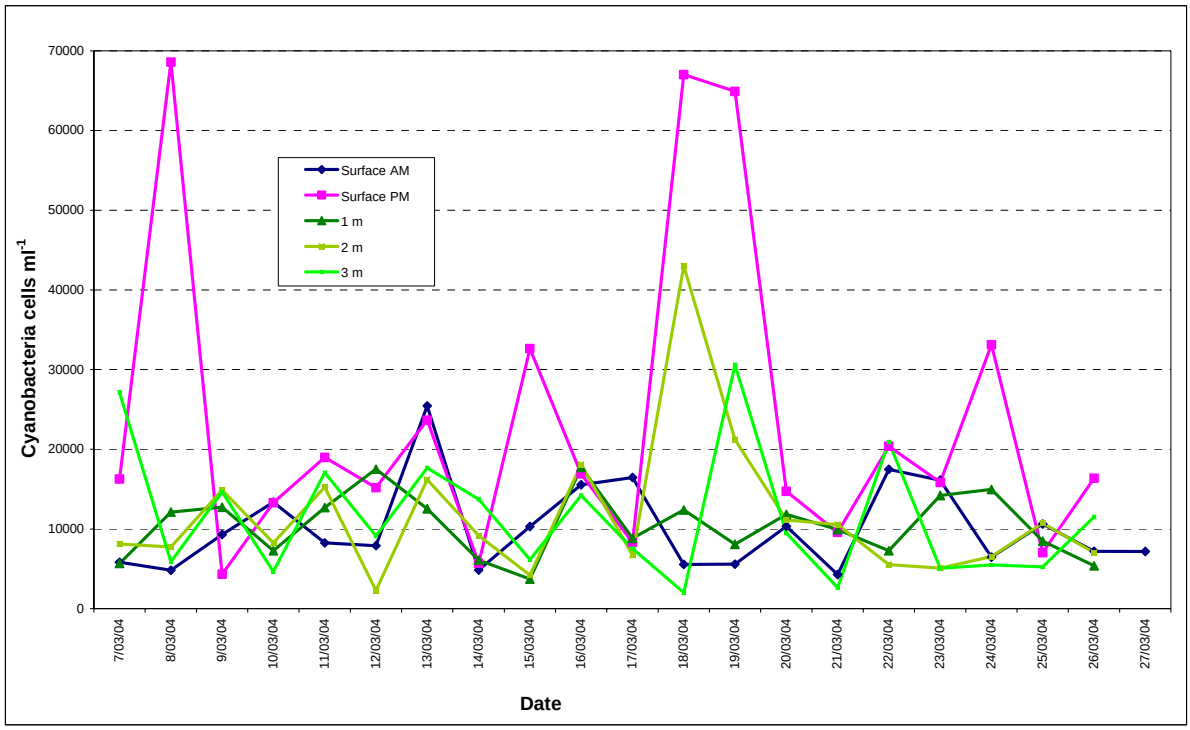


Figure 25. Total cyanobacteria cell counts for each sample during enclosure experiment (Lake Rotoiti).

4.4.2 Microcystin Levels in Rainbow Trout Samples with the Enclosure

Of the 80 trout released into the enclosure 28 were recaptured. Three "wild" trout were also caught in this area. The number of trout caught on each date is listed in Table 3. The composition of the pooled samples used for analysis is also indicated. On occasion where only one or two trout were caught these were pooled with the trout caught on the preceding or following day, with the aim of obtaining a pooled sample of approximately 5 trout.

Catch Date	Number of Trout Caught
08-03-04	3 (Control – Fish & Game Hatchery)
10-03-04	5
13-03-04	3
16-03-04	4
19-03-04	3
19-03-04	1 "wild" trout
20-03-04	1 "wild" trout
23-03-04	1 (pooled with trout caught on 24-03-04)
24-03-04	4
25-03-04	3
25-03-04	1 "wild" trout
26-03-04	2 (pooled with non "wild" trout caught on 25-03-04)
27-03-04	3

Table 3. Capture date and number of rainbow trout (*Oncorhynchus mykiss*) caught during enclosure experiment.

No microcystins were found in the three trout from the hatchery retained to check for background levels of microcystins. Microcystins were found in all muscle tissue and liver samples from trout caught during the enclosure experiment. Microcystin levels in trout samples are plotted together with the average smoothed microcystin levels in the water samples (Figure 27). Water microcystin levels shown are the average of the five daily samples smoothed using the following formula given in Section 4.4.1. Microcystin levels were also analysed for two of the three "wild" trout caught in the enclosure experiment (Figure 27). Levels in the "wild" trout do not differ markedly from those found in enclosure trout caught on similar dates.

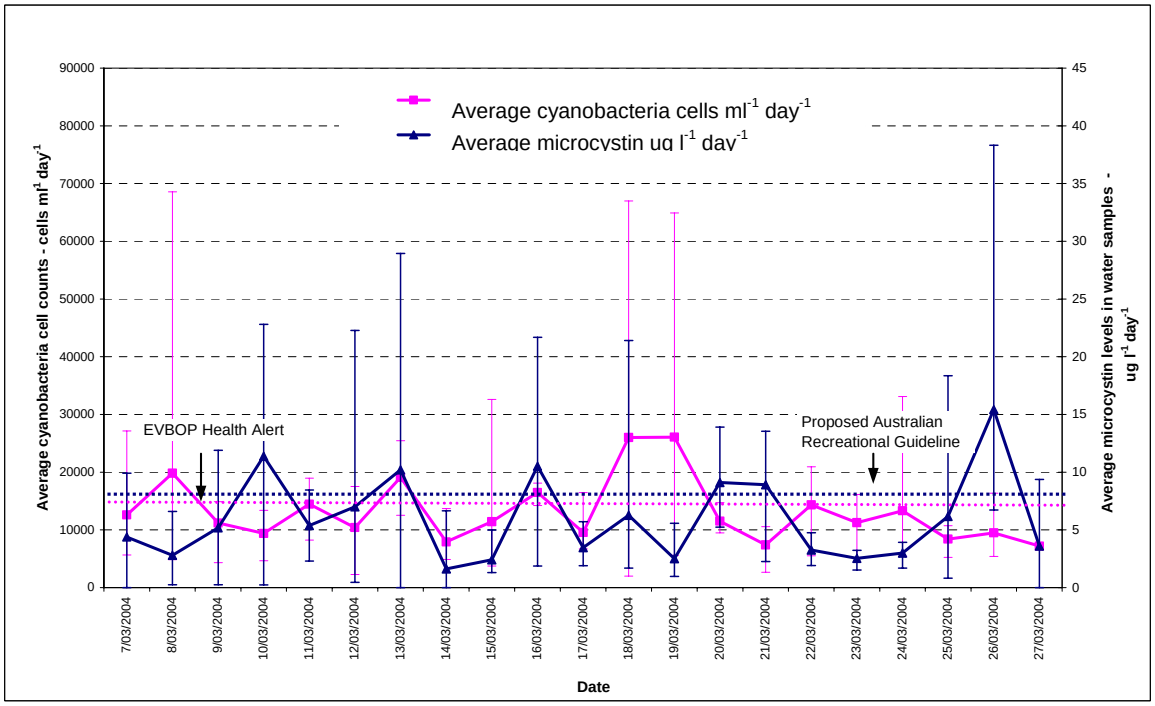


Figure 26. Average cyanobacteria cell counts per day and average microcystin levels per day during enclosure experiment. Error bars show maximum and minimum values for each day.

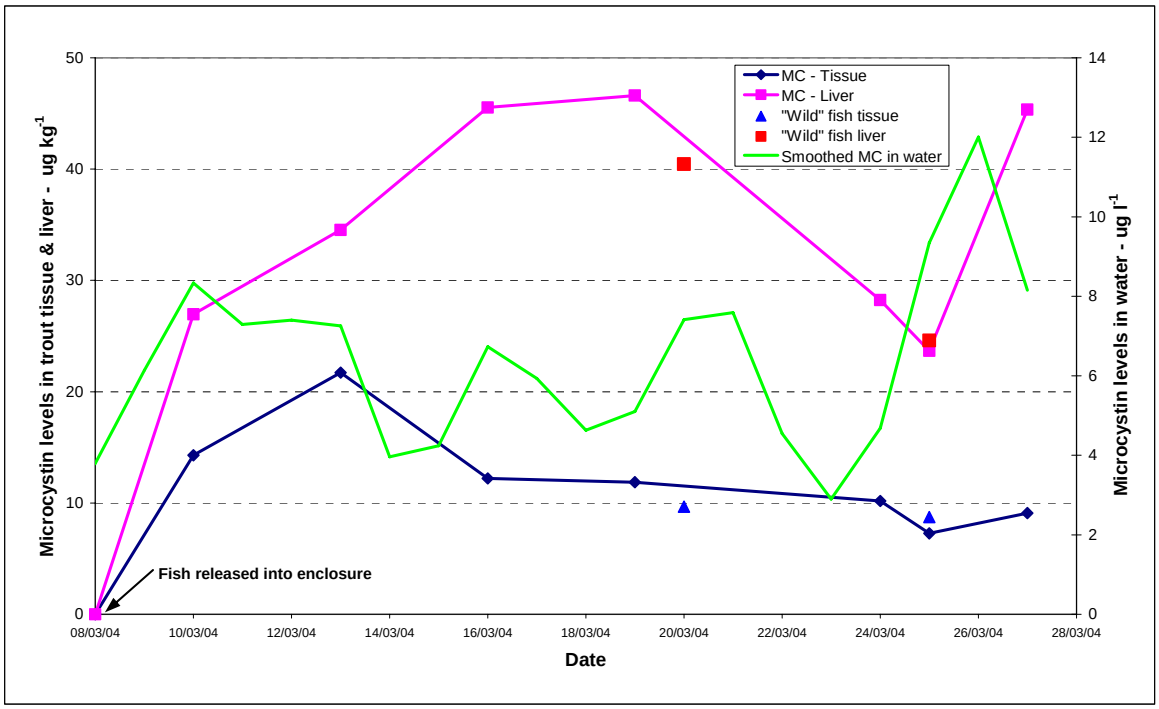


Figure 27. Microcystin levels in rainbow trout (*Oncorhynchus mykiss*) muscle tissue and liver samples and in water samples from enclosure experiment. Water microcystin levels are an average of five samples per day. Only two of the three "wild" trout were analysed for microcystins.

Liver microcystin levels were always higher than those recorded in muscle tissue samples. From day 1 to 11 the microcystin levels increased to a maximum value of 46.6 µg kg^{-1}. From day 12 to18 the levels decreased to 23.7 µg kg^{-1}, before increasing again to 45.4 µg kg^{-1} in the final sample (day 21). Muscle tissue microcystin levels increased from days 1 to 5 to a maximum of 22.7 µg kg^{-1}. Levels then decreased and remained between 7.3 µg kg^{-1} and 12.2 µg kg^{-1} for the duration of the enclosure experiment.

There was no clear correlation between microcystin levels in the water and those in the trout muscle tissue and liver samples. However, lower microcystin levels in the water and the sudden rise between 23-03-2004 to 26-03-2004 was possibly the cause of the fall and rise in liver microcystin values over the period 23-03-2004 to27-03-2004.

Figure 28 shows the EDI for enclosure trout. Five of the seven muscle tissue samples were above the TDI for microcystins.

4.4.3 Condition Factors of Rainbow Trout

The CF of released trout increased slightly to 45.6 during the first two days of the enclosure experiment and then gradually decreased to 37.1 on 25-03-2004 (Figure 29). Plotted values are averages for the pooled groups of trout. Maximum and minimum values are shown for each sample and indicate a wide scatter in the data. This scatter reduces the significance of any general trend.

4.4.4 Stomach Contents of Rainbow Trout

The percentage composition by weight of stomach contents of the trout from the enclosure experiment is shown in Figure 30. For days 1 to 11 of the enclosure a large portion of the trouts' diet was made up of insects. The dominant insect was the water boatman (*Corixidae* sp.). Smelt and bullies were absent or only present as a small proportion of the stomach contents. After 16 days, the trout diet contained large quantities (30 - 60%) of fish. Both smelt (*Retropinna retropinna*) and bully (*Gobiomorphus* spp.) were identified. Stomach contents of 3 "wild" trout are also shown in Figure 30. Fish (including goldfish, smelt and bullies) made up 58% of the stomach content of these trout.

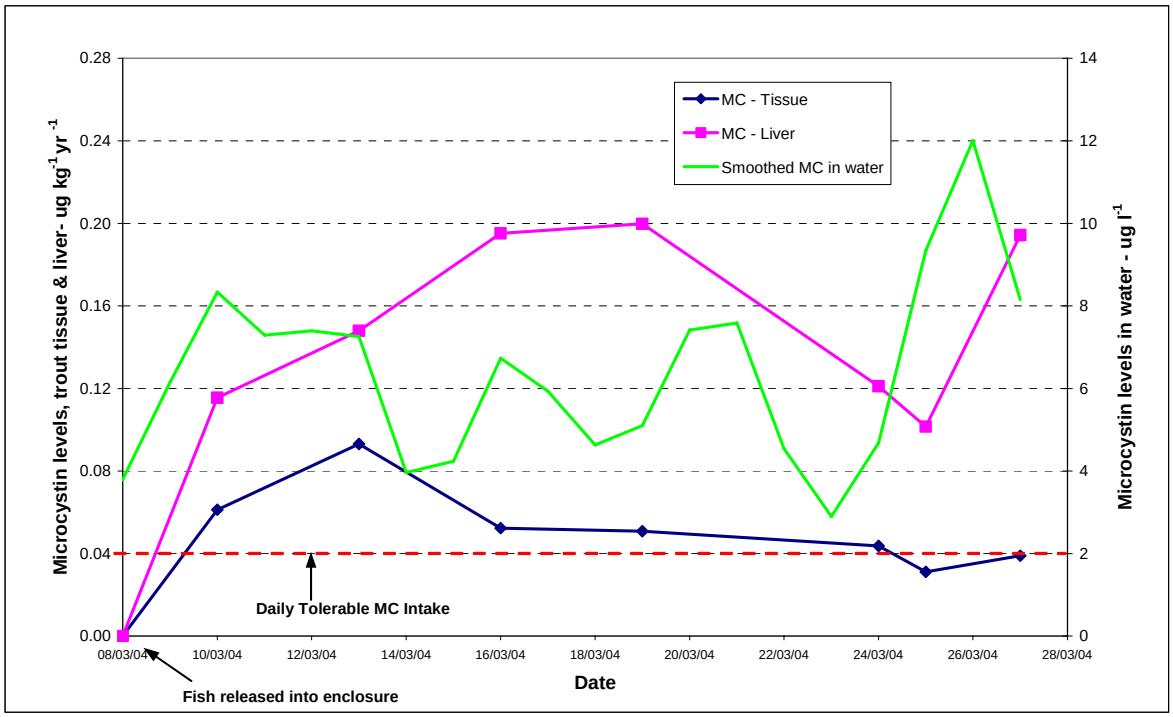


Figure 28. Estimated human daily intake of microcystin from enclosure experiment based on a 70 kg person ingesting a 300 g serving of rainbow trout *(Oncorhynchus mykiss)*. The water microcystin level is an average of 5 samples per day.

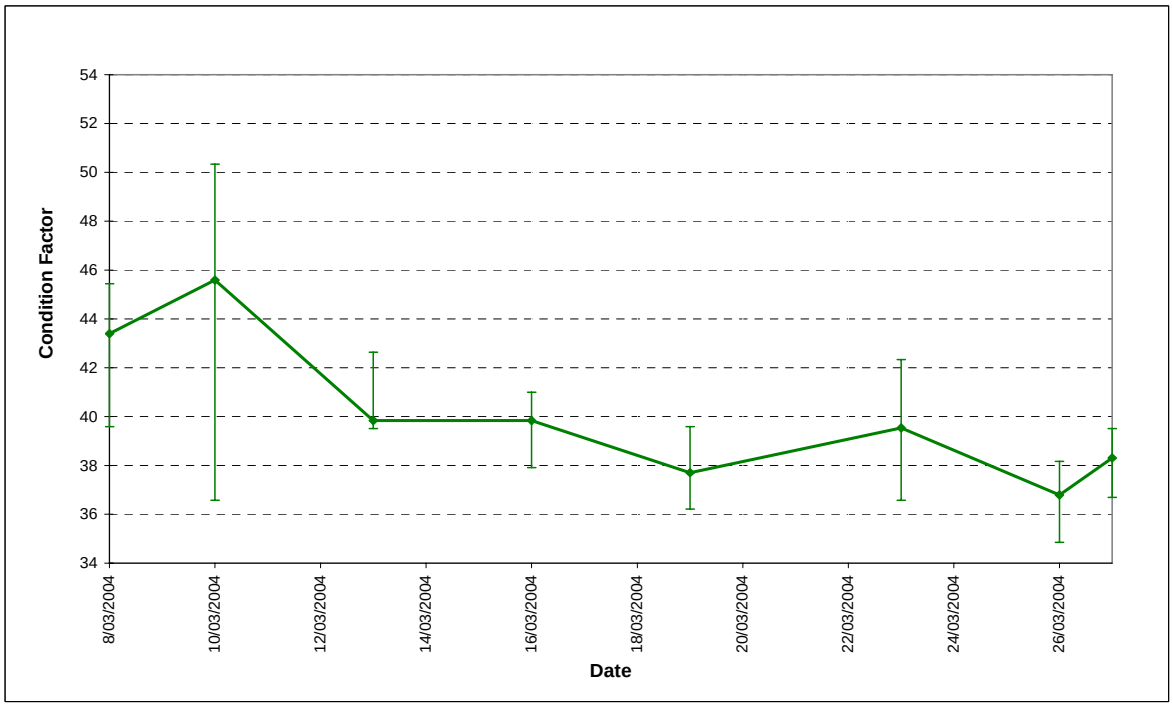


Figure 29. Condition Factor of rainbow trout *(Oncorhynchus mykiss)* caught during enclosure experiment. The data on the 08-03-2004 is from the trout retained to check background levels of microcystin. Error bars show the maximum and minimum values for each pooled sample.

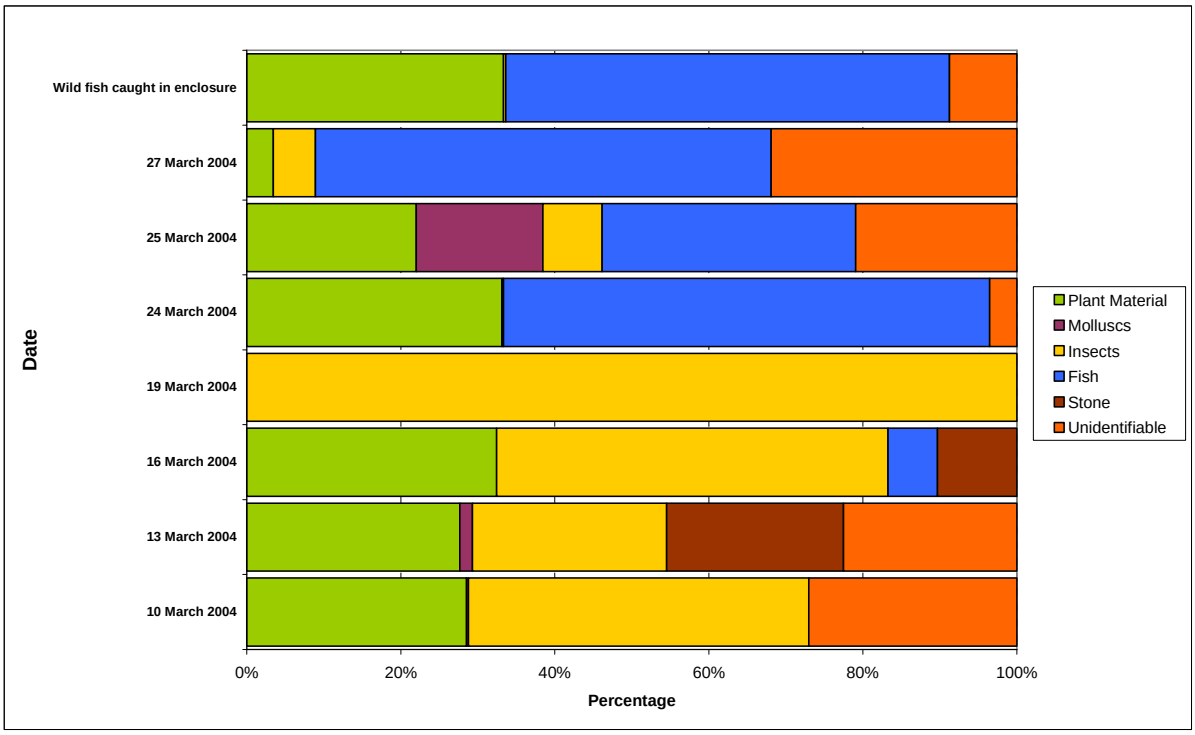


Figure 30. Percentage composition by weight of stomach content of enclosure and "wild" rainbow trout *(Oncorhynchus mykiss).*

4.4.5 Microcystin Levels in Mussels

Microcystin levels measured in the enclosure mussels are plotted in Figure 31 together with a smoothed surface water microcystin level. The smoothed surface water microcystin level was calculated by taking the average am and pm surface samples for each day and applying the smoothing formulae given in Section 4.4.1. Only surface samples were used as the mussels were positioned just below the surface.

After four days in the enclosure the mussels began to accumulate microcystins. Initially this accumulation was gradual with only 4.5 µg kg^{-1} recorded after five days and 4.6 µg kg^{-1} after seven days. Over the next two days microcystin levels in the mussels increased dramatically to reach a peak of 65.1 µg kg^{-1}. Microcystin levels in the water were much lower than those in mussels. With the exception of the first six days, the increase and decrease in microcystin levels in mussels tracked a similar trend in surface microcystin water levels with a two day delay.

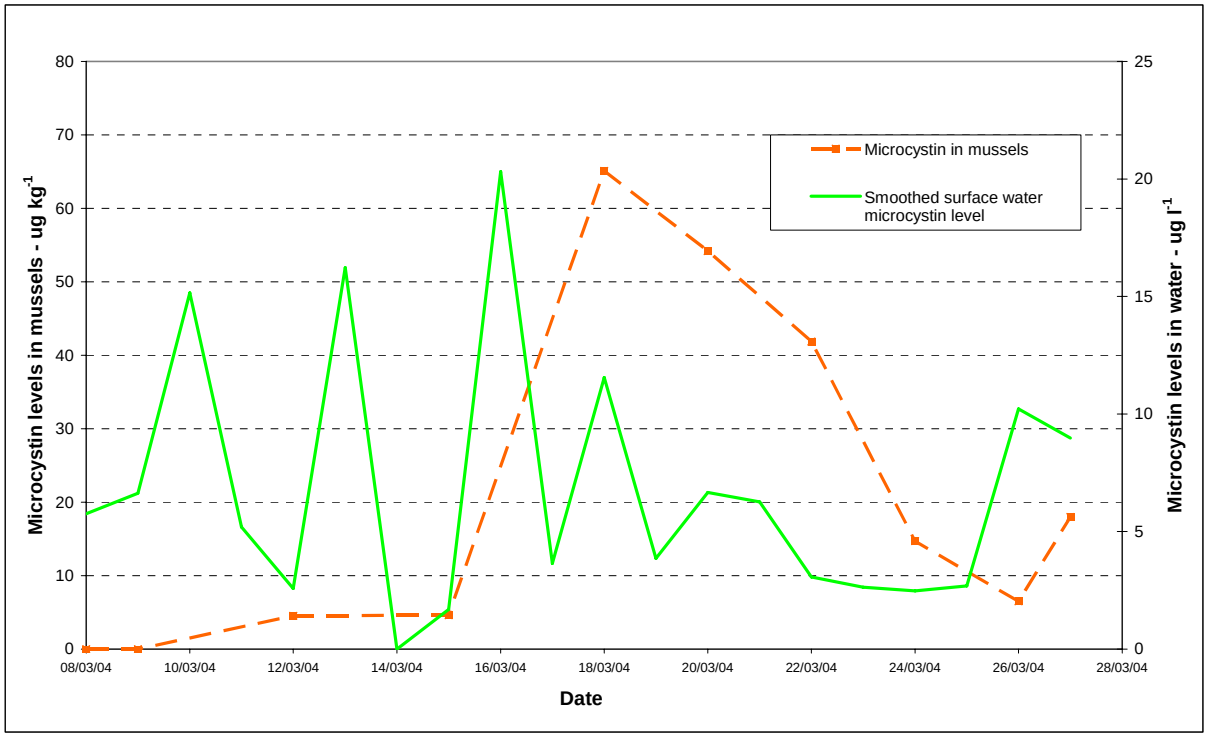


Figure 31. Microcystin levels in freshwater mussels *(Hydridella menziesi)* and water during enclosure experiment.

4.4.6 Water Temperature

Afternoon surface water temperatures were always higher than the morning surface temperature (Figure 32). In the afternoon, when samples were also taken at depth, the temperatures always decreased with depth. At times differences between surface and 3 m depth was as much as 3^{o}C. The temperatures increased over the first three days of the enclosure experiment and then generally decreased over the remaining 18 days to the end of the experiment. The surface temperatures on 18-03-2004 were above the general trend and this peak was attributed to a fine calm day following cooler cloudy weather. Surface temperature appeared to be quite sensitive to the cloud cover (or solar radiation) and wind speed. A significant drop in temperatures between the 22-03-04 and 25-03-04 was related to the moderate to fresh south west wind blowing over this period. Water temperature did not generally appear to have a significant effect on cell numbers with the exception of the 18-03-04 when high cell counts did correspond with an increase in water temperature. Wind also had an effect on cell counts with calm days tending to have higher cell counts. This is probably due to the wind causing the buoyant cyanobacteria cells to accumulate on the leeward side of the bay away from the mid-bay sampling location.

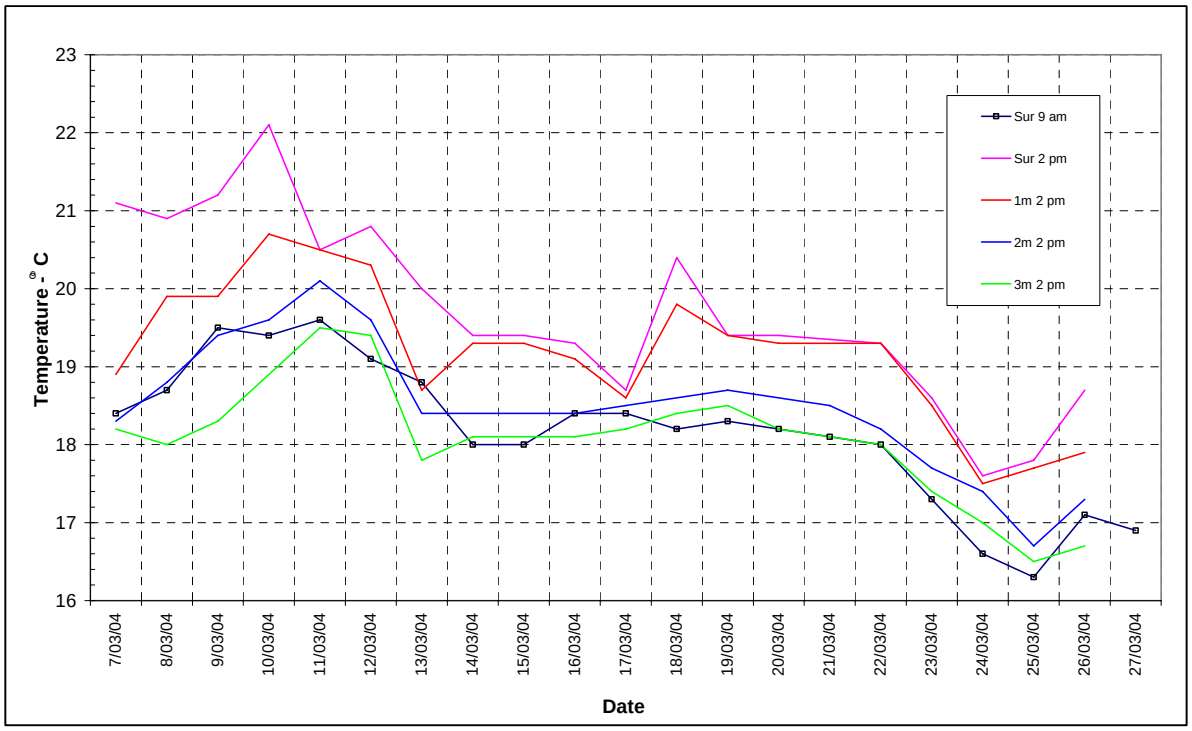


Figure 32. Water Temperatures: 9:00 am and 3:00 pm during enclosure experiment, Te Weta Bay, Lake Rotoiti.

4.4.7 Dissolved Oxygen

Dissolved oxygen (DO) levels in water recorded at the time of water sampling are shown in Figure 33. At surface and 1 m and 2 m depths, DO levels did not vary greatly during the duration of the enclosure experiment with the levels always higher in the afternoon (pm) than in the morning (am). Dissolved oxygen levels at the 3 m depth changed more significantly on a daily basis, sometimes changing by 2.3 mg l^{-1} from day to day. Readings at this depth may have been influenced to some extent by benthic weed. Recorded DO levels do not indicate oxygen depletion at this site in Lake Rotoiti.

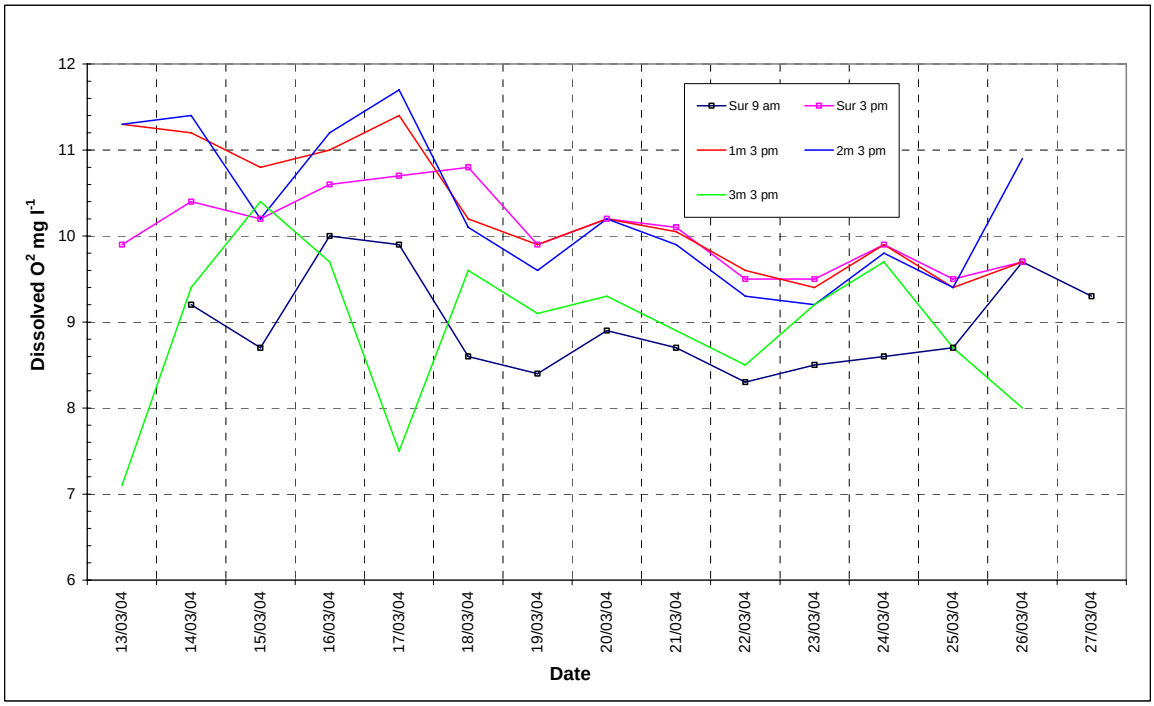


Figure 33. Dissolved Oxygen: 9:00 am and 3:00 pm in Te Weta Bay enclosure, Lake Rotoiti.

5. Discussion

5.1 Microcystins in Phytoplankton

Oral administration of microcystin-LR to mice has demonstrated a 24hr LD_{50} of between 5 mg kg^{-1} (Fawell *et al.* 1999) and 10.9 mg kg^{-1} (Yoshida *et al.* 1997). These studies have led the World Health Organisation (WHO) to developed drinking water guidelines for total microcystins. The guideline value for has been set at 1 µg l^{-1}. In Germany, recreational guidelines state that if cyanobacteria are found at a site, users of the water body must be warned and receive information on cyanobacteria. The German guidelines recommend closure at a total microcystin concentration above 100 µg l^{-1} (Frank 2002). Recreational guidelines are currently being developed for New Zealand.

The National Health and Medical Research Council of Australia have recently released the document Guidelines for the Managing Risks in Recreational Water (National Health and Medical Research Council 2004), which includes a section on cyanobacteria. These guidelines reason that any toxin guidelines should be based primarily upon risk of exposure to known toxins via ingestion. This is because potential adverse health effects from dermal exposure are not as significant as from ingestion of toxic cells. Only guideline limits for microcystins of 8 µg l^{-1} are given in the Australian document, as there is insufficient toxicology data for the other cyanotoxins.

The Australian recreational guideline values are based on the lowest observed effect levels for microcystin-LR of 100 µg kg^{-1} body weight/day derived from a 44-day study in pigs (Kuiper-Goodman *et al.* 1999). The Guideline value is 8 µg l^{-1} of water and is calculated on the accidental swallowing of approximately 100 ml of lake water per day. The Guideline also acknowledges that because toxin testing is not widely available, cell counts are likely to be used as a first measure, and this will be followed by other measures such as toxin testing.

Environment Bay of Plenty and Pacific Health, which are responsible for the issuing of heath warnings with regard to cyanobacteria concentrations in the Rotorua Lakes, base their warnings on cell counts and currently do not use toxin levels. When concentrations reach >15,000 cells ml^{-1} the water is considered unsuitable for general contact recreation, and warnings are issued.

Microcystins reached high levels in both Te Weta Bay and Okawa Bay from 23 February 2004 to the end of the study. Before this date, microcystins were not detected at significant levels. Figure 5 shows that Okawa Bay was above the Australian recreational microcystin guideline on three sampling occasions, Te Weta Bay on seven, and Hinehopu on one sampling occasion. However, Okawa Bay was above the ENVBOP cell

concentration guideline on 14 sampling occasions, Te Weta Bay for 12, and Hinehopu on three sampling occasions. This demonstrates that relying on cell counts alone does not necessarily give an indication of microcystin levels.

Only one sample from Lake Rotoehu (Te Pohue Bay – 2 May 2004) contained microcystin levels (23.5 μg l^{-1}) above the proposed Australian recreational guideline. On no occasion did cell counts exceed the ENVBOP cell concentration guideline.

The high microcystin levels in the scum samples collected at Te Akau point and in the water near the Lake Rotoiti campground illustrate that potentially dangerous levels of microcystins can rapidly occur due to the formation of wind accumulated scum.

It is not possible to establish unambiguously which species are responsible for toxin production without culturing individual strains or using sensitive detection methods with single cell/filament picking. However, a comparison of species composition and microcystin levels demonstrated that when very high levels of *Microcystis* spp. (in particular *M. aeruginosa* in Rotoiti and *M. panniformis* in Rotoehu) are found, there are higher levels of microcystin. *Anabaena* spp. are not associated with high microcystin levels.

Comparison of all cell count and microcystin data showed that there is usually no correlation between microcystin levels and cell count data, even when cell count data are compared at a genus level. This could be due to different species within the genus being responsible for toxin production or to genetically variability within the species. That is, some strains have the ability to produce toxin and others don't. Unknown environmental triggers, such as increase in temperature (Rapala 1998), may increase the production of microcystin. Additionally microcystins may be produced only at specific stages during the life cycles of some species. The lack of correlation could also be explained by continual death of cells, which subsequently lyse and release toxin into the lake, but death will not necessarily be at a constant rate. This may explain why elevated microcystin levels can coincide with low cell numbers. The data suggest that cell counts alone are not a reliable method for assessing potential health risk from microcystins. Testing for microcystins should be incorporated into routine monitoring programmes where possible.

Linking microcystin levels to specific species (in particular *Microcystis* sp) may prove useful. However, the identification of *Microcystis* species can be very problematic as separate species have similar morphology at varying stages in their lifecycle. A number of *Microcystis* species were observed in the study, including *Microcystis aeruginosa, M. panniformis, M. protocystis and M. flos-aquae*. Several other species from different genera with morphological similarities to *Microcystis e.g. Aphanocapsula* spp. *and*

Sphaerocavum brasiliensis were also identified. The unequivocal identification of *Microcystis* sp. to species level can be difficult and time consuming. However, identification of potentially toxic genera does not require such a high skill level. Further work is required to establish which species in these lakes produce microcystins. Once this knowledge is gained further assessments correlating cell counts and microcystin levels could be made.

The presence of cyanobacteria species known to produce cyanotoxins other than microcystins in Lakes Rotoiti and Rotoehu means that tests for other cyanotoxins and/or toxicity should not be ignored until it is known which species are responsible for toxin production in the Rotorua Lakes (and New Zealand). Cyanobacteria species composition and toxin types/levels can change during a cyanobacterial bloom (Baker *et al.* 2002) suggesting the need for regular monitoring.

Microcystins occur primarily intracellularly with the majority of microcystins released into the water body when cyanobacteria cells are decaying. Once released into the water body 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. Monitoring should continue for a number of weeks following the subsidence of a bloom.

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

5.2 Phytoplankton

In Lake Rotoiti the cyanobacteria species abundance and composition varied during the six month study period. In the Te Weta Bay and Okawa Bay sites *Anabaena* spp. dominated cyanobacteria blooms until February when *Microcystis* spp. occurred in increasing numbers. A similar trend (although with lower cell numbers) was also seen at the Hinehopu site. Of particular interest was the decline in cyanobacteria cell numbers in mid-February. This was followed by large blooms of *Microcystis* spp in Te Weta Bay and Okawa Bay.

Reasons for the switch in species dominance were not investigated in this study. However, the change is likely to be due to variations in environmental and limnological variables. For example, *Anabaena* species fix nitrogen and *Microcystis* species do not. Therefore variations in nutrient levels may influence species abundances. Other factors such as temperature tolerances, growth rates and light tolerances are also likely to have an

effect. The reason for the decline in all cyanobacteria in mid-February is unknown. Because of the rapid increase in cell numbers in samples in the following weeks, it seems unlikely that the cyanobacteria blooms died. It is more likely that they moved to lower levels in the water column.

In a study in Grangent Reservoir (Loire, France) Latour and Giraudet 2004, noted that colonies of *Microcystis aeruginosa* remain on the sediment as vegetative cells during winter and constituted an inoculum for the following year. Thus benthic *M. aeruginosa*, play an important role in the cycle of this species. It is possible that a similar situation occurs in Lake Rotoiti and that an unknown environmental trigger results in this species again becoming dominant in the water column. It is also interesting to note that Sirenko (1972) found that fewer colonies survive in oxygenated shallow water and Brunberg (1995) noted that anoxic conditions found in deeper water seem to prolong the survival of *Microcystis* in the sediment.

There were also variations in cell counts between locations within the lake. For example, cell counts were often lower at the Hinehopu site. This can be explained by the limnology and geographic and environmental variables. Both Te Weta Bay and Okawa Bay are sheltered, shallow, nearly enclosed bays, whereas Hinehopu is an exposed site at the eastern end of the lake. Factors such as wind direction are more likely to affect cell counts at the Hinehopu site. Cyanobacteria accumulation can be a result of the cyanobacteria positive buoyancy and wind driven hydrodynamics, with downwind (leeward) shorelines and embayments being likely sites for scum formation (Hutchinson and Webster 1994).

Phytoplankton samples were collected in the enclosure daily at 10 am and 3 pm. Highest cell counts were usually recorded at 3 pm at the surface, but high cell counts were sometimes recorded at lower depths. These results are not unexpected as cyanobacteria are able to regulate their buoyancy. This is achieved by using mechanisms that collapse gas vacuoles or result is gas vesicle synthesis (Oliver and Gnaf 2000). However the most frequent method of buoyancy regulation is by adjusting cell density. Usually cyanobacteria will accumulate at the surface early in the morning and evening (Wallace *et al.* 2000). During daylight, through photosynthesis the cyanobacteria accumulate carbohydrates which increase the cell density, thus the cells sink. As this carbohydrate store is used up they will often rise to the surface again. It is possible therefore that the sampling times in this study lay outside the times of highest surface density and this may explain high cell counts recorded at other depths. To obtain a true reflection of the concentration of cyanobacteria in the water column, depth integrated samples should be used in regular sampling programmes.

Cyanobacteria cell numbers remained relatively low in Lake Rotoehu during the study period. This is in contrast to previous summers when severe cyanobacterial blooms were reported in Lake Rotoehu (Wilding 2000). Wilding (2000) noted that in Lake Rotoehu between 1995 and 1999 a gradual increase in cell concentrations was recorded. The reason for the low cell concentrations in Lake Rotoehu in 2003/4, might be related to nutrient levels or environmental factors, but this was not investigated in this study. *Microcystis* spp. dominated cyanobacteria populations in Lake Rotoehu. Large numbers of *Microcystis* spp. were first noted in January 1997 and became the autumn dominant algae in 1998. Prior to this *Anabaena* spp. had dominated (Wilding 2000).

Several of the species found in Lakes Rotoiti and Rotoehu were recorded for the first time in New Zealand. The identification of *Microcystis panniformis* is interesting as this species was originally described as a tropical species and had previously been reported only from Brazil (Komárek *et al.* 2002) and tropical Australia (White *et al.* 2003). This species has since been identified in other lakes from the Waikato region and it is highly likely that *M. panniformis* has previously been wrongly identified as *M. aeruginosa* or *M. flos-aquae*. *M. panniformis* was identified based on the morphology described in Komárek (2002).

5.3 Trout Samples and Microcystins

The levels of microcystin in the trout liver samples were higher than the levels detected in the muscle tissue samples on all occasions. This result is consistent with other studies (Mohamed *et al.* 2003) and is expected, as the liver is the target organ for microcystins.

Microcystins were recorded in the samples from trout that were ranging the entire lakes, at times when no, or very low levels, of microcystins were present in the lake water. This could indicate that the water samples were not indicative of circumstances in the lake as a whole, or more likely that there is a persistence of microcystins in the food sources of trout (See Section 5.2.2).

Detection of microcystin in trout muscle tissue in Lake Rotoehu in November 2003 and December 2003 from Rotoiti was not expected as cyanobacteria blooms had not being reported in the previous months. It is possible that some *Microcystis* was over-wintering in the sediment and because part of the trout diet comes from benthic grazers this caused bioaccumulation of microcystins.

In an investigation of the biochemistry of microcystin in rainbow trout Tencalla and Dietrich (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 that 5% of the applied microcystin reached the blood. Once in the blood, the microcystin-LR was quickly transported to the liver.

In our enclosure study, the levels of the microcystin in the trout muscle tissue decreased after five days and in the liver after 12. Tencalla (1997) noted that the decrease of microcystin concentrations in the liver occurred more slowly than uptake, and that microcystin could still be detected in the liver three days after exposure. Microcystins are known to be metabolised by Zebra Fish (*Danio rerio*) (Wiegand *et al.* 1999). This occurs via detoxication enzymes known as soluble glutathione S-transferase (Pflugmacher *et al.* 1998). It is likely that at least some of the decrease in the microcystin levels in the enclosure trout is due to this detoxication process.

Further investigation into the variation in microcystin in individual trout rather than the pooled samples used in this study could prove useful. This information could be used to assess the sample size required to obtain a true reflection of microcystin levels in trout populations. The decrease in the microcystin levels in the muscle tissue and liver might be related to condition factor and stomach contents and this requires the study of individual samples.

5.4 Human Health Risk from Trout Consumption

Our results confirm that microcystins do accumulate and persist in the muscle tissue and liver of rainbow trout in Lakes Rotoiti and Rotoehu. The results illustrate that there is a potential human health risk from the consumption of trout muscle tissue, as 50% (Rotoehu), 60% (Rotoiti) and 71% (enclosure) of the muscle tissue samples were above the Total Daily Intake (TDI) of 0.04 µg $kg^{-1}day^{-1}$ recommended by the WHO (calculations based on a 70 kg person eating 300 g per day). Using the levels found in the muscle tissue of the trout sampled in this study and ignoring the issue of covalently bound microcystins (described in Section 5.4.0) the health risk to humans from microcystins consumed while eating trout muscle tissue from Lakes Rotoiti and Rotoehu is very low because trout is not usually consumed every day.

Using the results of this study a 70 kg person can safely eat a 300 g serving of trout every 3.6 days. This calculation used a muscle tissue concentration of 34.5 µg kg^{-1}, (which was the highest value recorded in the muscle tissue during this study – Rotoehu, April 2004), the TDI of 0.04 µg kg^{-1} day^{-1} (WHO), a 70 kg human and a trout meal size of 300 g. If a 70 kg human eat trout at less than this rate, they will consume microcystin levels below the WHO standards and there is probably no need for concern. The TDI is a recommended limit for a healthy adult. Children, elderly and sensitive individuals may be at a higher risk from the ingestion of microcystins. The microcystin levels are

significantly higher in the trout liver but the 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. Microcystins are heat stable and therefore would not be denatured by cooking (Harada *et al.* 1996).

5.5 Covalently Bound Microcystins

In our study we have only investigated and reported levels of free ADDA-containing microcystins in trout and mussels. It is likely that our samples will also contain conjugates of microcystins (these are a produced during the detoxication of microcystins (Pflugmacher *et al.* 1998)) and covalently bound microcystins. Covalent binding of microcystins can occur within a matter of hours (Williams 1997). It involves the formation of a covalent linkage between the microcystins and PP-1 and PP-2A enzymes this is the result of a secondary in *vitro* interaction (Craig *et al.* 1996; MacKintosh *et al.* 1990; Runnegar *et al.* 1995). The presence of conjugates was not tested in this study, although it is likely that they would be detected using the ADDA-ELISA and therefore included in our results. Chemical methods such as LCMS are required to distinguish conjugates from other microcystins.

Few studies have addressed the issue of covalently bound microcystins in freshwater organisms. The levels of covalently bound microcystin (Table 4) vary dramatically and depend on the study species, types of toxin used, methodology used to test for covalent microcystins, the method of administration to the organism and the length of time following administration that the microcystin was measured.

Species (Organ)	**Reference**	**Percentage of Total Microcystin Detected That is Covalently Bound**
Atlantic Salmon (Liver)	Williams *et al.* (1997)	76%
Mussel - *Mytilus edulis*	Williams *et al.* (1997)	99.9%
Rainbow trout - (*Oncorhynchus mykiss*)	Tencalla & Dietrich (1997)	63%
Cypress Island Dungeness Crab Larvae	Williams *et al.* (1997)	Covalently bound microcystin 10,000 x higher than free microcystin
Zebra mussel - *Dreissena polymorpha*	Pires *et al.* (2004)	38%

Table 4. Summary of published studies recording levels of covalently bound microcystins in freshwater organisms.

In a study on Zebra mussels (Pires *et al.* 2004) that investigated both free and covalently bound microcystins found that in the first week of exposure only low amounts of covalently bound microcystins could be detected. In the second week the amounts of covalently bound microcystin increased dramatically and accounted for approximately 62% of the total microcystin burden. Similarly, Williams *et al.* (1997) found that in the marine mussel (*Mytilus edulis*) almost all the microcystins present existed as a covalent complex after three days. These results may explain why the microcystin levels in the enclosure trout decreased after five days in muscle tissue and 12 days in the liver. Over time more of the microcystins may have became covalently bound and we were not able to extract and detect them.

There is very little published information on the toxicity and bioavailability of covalently bound microcystins complexes. There is also a lack of information on whether there are enzymes capable of reversing the covalent linkage and "freeing" the toxic microcystins.

We strongly recommend that future studies assess covalently bound microcystins in Lakes Rotoiti and Rotoehu rainbow trout and attempt to establish whether the total microcystin burden in the trout is a potential health risk to human consumers. If covalently bound microcystin are found in large quantities there is also a need to investigate the toxicity and bioavailability of such microcystins. The setting of guideline quantities of trout that can safely be consumed will not be fully relevant until these data are obtained.

5.6 Effect of Diet on Microcystin Levels in Trout

Results from laboratory studies (Tencalla *et al.* 1994) indicated that the main route of uptake for microcystins in rainbow trout is the gastro-intestinal tract and that only very small amounts are taken up through the skin or gills. Thus to understand how the microcystin might be bio-accumulating or bio-magnifying in the Lake Rotoiti and Rotoehu trout an attempt was made to assess dietary composition.

Studies have shown that the gastric emptying in rainbow trout is between 6-18hrs (Grove *et al.* 1978) thus the stomach content analysis gives us some indication of diet of prior to capture.

Hatchery trout were fed on formulated feed pellets. Prior to this experiment the length of time taken for them to adapt to feeding in the lake environment was uncertain. Stomach analysis showed that the hatchery trout released into the enclosure began feeding within two days. However, for the first 16 days the diet of the released trout was quite different to that of free roaming trout in Lake Rotoiti. A large portion of their diet was made up of insects, mostly water boatmen (*Corixidae* sp.). However, despite feeding on insects the

trout still accumulated microcystins. It is likely that the water boatmen have themselves accumulated microcystin as they feed on algae and minute aquatic organisms. As the trout adapted to the new environment and began feeding on smelt and bullies it was anticipated toxin levels would increase rather than the observed decrease. It is possible that different organisms accumulate different quantities of toxin and the bioavailability of the microcystin may determine how much is transferred to the trout. For example, as water boatmen feed directly on the phytoplankton they may accumulate higher levels of microcystins than the smelt which feed on zooplankton.

The results of the stomach analysis infer that microcystin from the phytoplankton accumulate in many different aquatic organisms. The different feeding modes of these organisms may be one reason why microcystins were detected even when cyanobacterial blooms were not present. Benthic grazers might accumulate microcystins from the sediment during winter while cyanobacteria are over-wintering in the sediment. Further research investigating microcystins and their transfer through the Rotorua lakes food webs would throw further light on this issue. Chrstoffersen (Christoffersen and Burns 2000) found that microcystins were toxic to some indigenous zooplankton, indicating that it is not just the transfer of microcystin through the food web that is important but also the effect of these toxins on aquatic life.

5.7 Mussels and Microcystins

Mussels accumulated microcystins at a slow rate for the first four days of the enclosure experiment. This is in contrast to other studies which have shown that freshwater and marine mussels accumulate microcystins rapidly (Yokoyama and Park 2003, Amorim and Vasconcelos 1999). The mussels used in this experiment were harvested from the sediment in Lake Rotoma, and placed in cages and suspended near the surface in the enclosure at Lake Rotoiti within 12 hours. The initial low accumulation rate infer mussels required several days to acclimatise to this new environment and to begin feeding normally. After seven days the mussels appeared to commence normal feeding and there was a rapid increase in microcystins in their tissue. This followed the trend of the water microcystin levels which increased two days previously (see Figure 31). Laboratory studies on toxin levels in marine mussel *Mytilus galloprovincialis* (Vasconcelos 1999) found that when they where placed in an environment free of microcystins there was a 50% decrease in the amount of detectable toxin in the mussels within two days. Yokoyama and Park (2003) investigated the depuration of microcystin-LR in the freshwater bivalve *Unio douglasiae* and demonstrated that microcystin is removed faster at higher temperatures. Because of the change in the microcystin levels in the water in the enclosure the degree to which the rapid change in microcystin level in mussels was due to

the increase in water microcystin levels or to the resumption of normal feeding habits is not clear.

Because of the rapid depuration of microcystins from mussel tissue, freshwater mussels will be poor indicators of the accumulated microcystin levels at any one location over an extended period of time. However, the enclosure results show that it may be possible to correlate the toxin levels in mussels with levels in the water approximately two days prior to the collection.

Because mussel microcystin concentrations were higher than levels in the lake water *Hydridella menziesi* mussels may be useful as early warning organisms. Their higher levels of microcystin would be easier to detect than in water samples with low levels of microcystin which could be near the limit of quantification of some detection methods. Further studies in controlled laboratory-type situations where the mussels were removed from high microcystin environments and placed in environments free of microcystins would provide valuable information on depuration rates in *H. menziesi.* Controlled experimental work may also assist in developing a method involving *H. menziesi* as early warnings organisms in freshwater systems.

6. Conclusions and Recommendations

6.1 Cell Counts, Species and Toxins

The microcystin levels in Lake Rotoiti have at times reached levels that could be hazardous to humans participating in recreational activities on or in the lake. When *Microcystis* species cell concentrations are very high, microcystin levels are also likely to be high. However, because total cyanobacteria cell counts were found to be a poor indicator of microcystin levels in the water samples testing for microcystins as part of the regular phytoplankton monitoring programme is suggested.

Recommendations

- *Monitor microcystin levels and the species composition of blooms in Lakes Rotoiti and Rotoehu.*
- *Inform the public of potential dangers.*
- *Test for microcystin as part of the regular phytoplankton monitoring programme.*
- *Establish unambiguously which species are responsible for the synthesis and release of microcystins.*
- *Investigate the fate of microcystins in water column and sediment.*

6.2 Accumulation of Microcystins in Trout

Our results confirm that microcystins accumulate and persist in both muscle tissue and liver of rainbow trout in Lakes Rotoiti and Rotoehu. In the enclosure microcystins appeared to accumulate readily in these tissues following the initial ingestion of contaminated food sources. However, our results showed that levels in both liver and muscle tissue reached a peak after 5 - 10 days and then levelled off, or dropped slightly, with more prolonged exposure. The microcystin levels in monthly muscle tissue samples from trout in Lake Rotoiti were reasonably constant over a six month period and were at about the same level as in the enclosure trout after the initial 10 days in the enclosure. In Lake Rotoehu, the monthly levels in the muscle tissue were more variable but the average level was similar to the Rotoiti trout in spite of the microcystin level in the water being very low over the six month period. These results suggested that levels in muscle tissue were not strongly affected by the presence of the observed bloom or short periods of high microcystin levels in the water.

Monthly levels of microcystin in the liver of the trout in the enclosure and from Lake Rotoiti were more variable than in the muscle tissue, and results appeared to mirror, the changes in the water microcystin levels. However, there was no indication of this trend in the monthly samples from Lake Rotoehu where the liver levels were high at times when water microcystin levels were low. For these samples, a decrease and increase of levels in

liver followed a similar trend to those in flesh levels indicating that the food source (presumably contaminated) maybe a more relevant factor than the water microcystin levels.

Presence of microcystins in liver and muscle tissue when cyanobacterial levels in the water are low may indicate concentration of microcystins in food web. This study showed that accumulation and variation of the microcystin levels in the trout (both liver and muscle tissue) and may depend on a complex interaction between levels in the water and food web. A need for further monitoring of microcystins in trout muscle tissue and in the food web is indicated.

Recommendations

- *Monitor trout over time to track microcystin levels in muscle tissue during periods of both cyanobacterial bloom and low cell counts.*
- *Investigate microcystin levels and feeding patterns of organisms ingested by trout.*

6.3 Human Health Risk from Trout Consumption

Assuming a 70 kg person consumes 300 g of trout muscle tissue, the ingested microcystins would exceed the Total Daily Intake (TDI) recommended by the World Health Organisation (WHO) (0.04 µg $kg^{-1}day^{-1}$) for 50 and 60% of the samples from Lakes Rotoiti and Rotoehu respectively. Health problems might result if this quantity of trout tissue was consumed on a regular basis. For example, if the muscle tissue from the samples with the highest levels recorded (Lake Rotoehu) were consumed at a rate of more than 300 g within 3.6 days over a continuing period of time, the TDI limit would be continually exceeded. If the consumption rate is less than this quantity the WHO guidelines indicate that there is probably no need for concern.

Recommendation

- *Provide clear, unambiguous health information to enable the public to make informed decisions regarding consumption of trout (the quantities that can be safely eaten should be given) based on the best information available at the time the health information is issued.*

6.4 Covalently Bound Microcystin

The microcystin levels given in this study are levels extracted from the tissue samples using the methods applied here. Based on the results of other studies it seems likely that further covalently bound microcystins would be present in all the tissue samples, thus increasing the total microcystin burden in the tissue significantly.

Recommendations

- *Develop methods of analysing for covalently bound microcystins and to assess their toxicity and bioavailability. Until this work is completed guidelines for safe levels of trout consumption cannot be stated precisely.*

6.5 Freshwater Mussels

Hydridella menziesi accumulated microcystins rapidly and levels in the mussels were always higher than those in the water. However, because mussels rapidly remove the microcystins from their tissue these organisms will be poor indicators of microcystin levels at any one location over an extended period of time. They may however be useful as early warning organisms when low levels of microcystins occur in a water body. *H. menziesi is* not commonly consumed by most New Zealanders but for some Maori they are a traditional food source.

Recommendations

- *Issue warnings concerning consumption of freshwater mussels.*
- *Conduct controlled studies investigating the value of H. menziesi in monitoring programmes.*

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Appendix 1.

Table A1. List of cyanobacteria species observed in Lakes Rotoiti and Rotoehu samples during this study.

Choococcales
Aphanocapsula cf. delicatissima
Aphanocapsula holsatica
Chroococcus cf. minutes
Coelosphaerium aff. cf. natans
Coelosphaerium cf. confertum
Coelosphaerium cf. kutzingianum
Microcystis aeruginosa
Microcystis panniformis
Microcystis protocystis
Microcytsis flos-aquae
Snowella cf. lacustris
Sphaerocavum brasiliensis
Oscillatoriales
Geitlerinema splendidium
Limnothrix aff. planctonica
Lyngba sp.
Oscillitoria sp.
Planktothrix aff. planctonica
Planktothrix peromata
Planktothrix sp.
Pseudanabaena galeata
Pseudanabaena limnetica
Trichodesmium iwanoffianum
Nostocales
cf Radhipiodsis mediterranea
Anabaena lemmermannii
*Anabaena cf. flos-aquae**
*Anabaena cf. lemmermannii **
*Anabaena cf.torulosa**
Anabaena cf. affinis
Anabaena circinalis
*Anabaena planktonica**
Anabaena sp. A
Anabaena sp. B
*Anabaena spiroides**
Aphanizomenon gracile
Gloetrichia sp.

* no akinetes seen in sample
Anabaena sp. A - straight species - no akinetes/heterocytes seen in sample.
Anabaena sp. B - coiled species - no akinetes/heterocytes seen in sample.

Appendix 2.

Table A2. Cyanobacteria cell counts for each sample during enclosure experiment.

Date	Depth	*Aphanocapsa holsatica*	*Anabaena sp.*	*Anabaena planktonica*	*Anabaena lemmermannii*	*Anabaena torsulsoa*	*Coelosphaerium cf kutzingianum*	*Geitlerinema splendidium*	*Limnothrix aff. planctonica*	*Microcystis aeruginosa*	*Microcystis protocystis*	*Planktothrix aff. planctonica*	*Pseudanabaena galeata*	*Sphaerocavum sp.*	TOTAL cell ml^{-1}
07/03/04	Surface AM			1637						744			74	3385	5840
	Surface PM			112	2009					5022				9114	16256
	1 m			2809						1748			186	930	5673
	2 m			7347						223			558		8128
	3 m	1860	37	13526						558				11160	27141
08/03/04	Surface AM			1183	856					2790					4829
	Surface PM			1774						12722				54089	68586
	1 m			8035						744				3348	12127
	2 m			4085						3646					7730
	3 m	558		5357				1116				595			7626
09/03/04	Surface AM			2176						3720				3422	9319
	Surface PM			1934						372			223	1786	4315
	1 m			4843	74					7812					12730
	2 m			6108						4390				4390	14887
	3 m			4390					1116	223				10044	15773
10/03/04	Surface AM			2522						8630				2232	13385
	Surface PM			1481						5059				6770	13310
	1 m			1481						4464				1339	7284
	2 m			1339			446			2083				4836	8705
	3 m			1741					372	2381				521	5015
11/03/04	Surface AM			3125	223		1190			149			74	4687	9449
	Surface PM			2753	744		744			6696				8779	19716
	1 m			4352	1190					446				6696	12685
	2 m			3757	1190		595			1190	670		112	8333	15847
	3 m			7031	3720					3125	670		283	2232	17060
12/03/04	Surface AM			5096	56					707	74			1972	7905
	Surface PM			4966	1972							558		8258	15754
	1 m	1488		7998						4464	521			3050	17521
	2 m			1004					223				149	1116	2492
	3 m			3117						1339			223	4464	9144
13/03/04	Surface AM			11398						7440	670			5952	25460
	Surface PM			8333			298			4910	298			10118	23957
	1 m			3527						8928	74				12529
	2 m	1488		8749			298		223	3497	1042	372		1414	17082
	3 m			13719						3720	223				17663

Table A2 continued.

Date	Depth	*Aphanocapsa holsatica*	*Anabaena sp.*	*Anabaena planktonica*	*Anabaena lemmermannii*	*Anabaena torsulsoa*	*Coelosphaerium cf kutzingianum*	*Geitlerinema splendidium*	*Limnothrix aff. planctonica*	*Microcystis aeruginosa*	*Microcystis protocystis*	*Planktothrix aff. planctonica*	*Pseudanabaena galeata*	*Sphaerocavum sp.*	TOTAL cell ml^{-1}
14/03/04	Surface AM			372						1510				2976	4858
	Surface PM			2344	521					2083	372		149	223	5692
	1 m			2969	521					298	74			2232	6093
	2 m			3646			298			149			149	5208	9449
	3 m			3073						5134		372	298	5208	14084
15/03/04	Surface AM			5468			818			2158	298			2381	11123
	Surface PM			2083	112					28644	1414			372	32624
	1 m			409	37					3125				149	3720
	2 m			565						2530				1116	4211
	3 m			201						5134	74			744	6153
16/03/04	Surface AM			3541						1860	521			5729	11651
	Surface PM			1354						11755	744			3050	16904
	1 m			3594						10639	1339			2158	17730
	2 m			3073						4538	1339			9151	18102
	3 m			11093					446	2678					14218
17/03/04	Surface AM			7447						7142	223			1637	16450
	Surface PM			417						6696	818			372	8303
	1 m			201			446			5134	818			2232	8831
	2 m			677						2083	446			3497	6703
	3 m			625						4464	223			2232	7544
18/03/04	Surface AM			1094						1637	223			2604	5558
	Surface PM			2135						62645	2232				67012
	1 m			1823	521						744			9300	12388
	2 m			2239						40399				372	43011
	3 m			30	1004						223			744	2001
19/03/04	Surface AM	744		2239							372			2232	5587
	Surface PM			543						61008	1860			1488	64899
	1 m			1458						5654	744			223	8080
	2 m			2239						17633	298			1042	21211
	3 m			3958						21204	223			5208	30593
20/03/04	Surface AM			2187	30					6919	967			223	10327
	Surface PM			223						8333	967			5208	14731
	1 m			1927						6919	2232			744	11822
	2 m			2135						6696	744			1562	11138
	3 m			3906						3497	223			1860	9486
21/03/04	Surface AM			2344						1190	372			372	4278
	Surface PM			4062			446	223		372				4464	9568
	1 m			2916						5357	446			1190	9910
	2 m			2604						1562	372			6026	10565
	3 m			781						372				1488	2641

Table A2 continued.

Date	Depth	Aphanocapsa holsatica	Anabaena sp.	Anabaena planktonica	Anabaena lemmermannii	Anabaena torsulsoa	Coelosphaerium cf kutzingianum	Geitlerinema splendidium	Limnothrix aff. planctonica	Microcystis aeruginosa	Microcystis protocystis	Planktothrix aff. planctonica	Pseudanabaena galeata	Sphaerocavum sp.	TOTAL cell ml-1
22/03/04	Surface AM			2448			149			13392	744			744	17477
	Surface PM			1927						16368	1042			1042	20378
	1 m			506						1488	446			4836	7276
	2 m			1823						2158			74	1488	5543
	3 m	2232		3802			10			7738	744		446	5952	20924
23/03/04	Surface AM			5781			744			2158				7440	16122
	Surface PM			2604						7738	744			4762	15847
	1 m			1406						10416	1637			744	14203
	2 m			260						2976				1860	5096
	3 m			4323						372				372	5067
24/03/04	Surface AM			2292						967	223			2976	6458
	Surface PM			27915						2232	744			2232	33123
	1 m			8802	223				149	2753	1190			1860	14977
	2 m			1406			298			670	1934			2232	6540
	3 m			937					372	1116	372		74	2604	5476
25/03/04	Surface AM			2292			446			1488	372			6026	10624
	Surface PM	1302		1406						2232	223			1860	7023
	1 m			2812						3422	1265			967	8467
	2 m			2031						5803	893			2009	10736
	3 m			625			298			595				3720	5238
26/03/04	Surface AM			1927						3348	818			1116	7209
	Surface PM			2448						10342	1190		149	2232	16361
	1 m			4427						446			149	372	5394
	2 m			1719			298			2604			409	2009	7038
	3 m			3125						7440	893		52		11510
27/03/03	Surface AM			4062						1934	446			744	7187