



CAWTHON

Cyanobacterial Biovolumes for the Rotorua Lakes

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Cyanobacterial Biovolumes for the Rotorua Lakes

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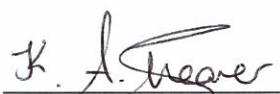


Prepared for
Environment Bay of Plenty



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EXECUTIVE SUMMARY

Cyanobacterial (blue-green algal) blooms have become a regular occurrence in the Rotorua lakes. Monitoring of cyanobacterial concentrations has been undertaken by Environment Bay of Plenty (ENVBOP) in conjunction with the Bay of Plenty District Health Board, since the early 1990s. Health warnings have traditionally been issued when cyanobacterial densities exceed a threshold of 15,000 cells mL⁻¹. In recent years, cell concentrations of pico-planktonic cyanobacteria (<2 µm) have become increasingly prevalent and at times exceeded this threshold, resulting in the unnecessary issuing of health warnings. Biovolume takes into account the variability in size of different species and is therefore a better indicator of potential health risk than cell concentrations. Calculation of biovolume requires time-consuming measurement of individual cells. A list of standardised volumes for cyanobacteria in the Rotorua lakes would greatly assist ENVBOP in incorporating biovolume thresholds into their current monitoring programme. Cawthon Institute and University of Waikato were asked by Environment Bay of Plenty to assemble a list of biovolumes for ten problematic cyanobacteria of the Rotorua lakes. Cell biovolumes were calculated for the following species; *Anabaena lemmermannii*, *A. planktonica*, *Aphanocapsa holsatica*, *Aphanizomenon gracile*, *Aphanothecce clathrata*, *Coelosphaerium kuetzingianum*, *Microcystis* sp. (small), *Microcystis* sp. (large), *M. wesenbergii* and *Snowella lacustris*. A database was developed in Microsoft Access to calculate biovolumes and assist in data management.

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1. INTRODUCTION

The Rotorua lakes, in the central North Island of New Zealand, comprise approximately 16 lakes. These lakes are highly utilised and are valued for recreation, culture, biodiversity and tourism. Many of these lakes have intermittently experienced cyanobacterial blooms since the 1970s (e.g. Cassie 1978). In the past decade the severity and extent of these blooms have increased and cyanotoxins have been detected, raising environmental and human health concerns (Wilding 2000; Wood *et al.* 2006).

During summer, Environment Bay of Plenty collects weekly samples from approximately 15 sites around the edges of Lakes Rotorua, Rotoiti, Rotoehu; and between 5-10 samples from other lakes in the region. Health warnings have traditionally been issued when cell concentrations exceeded 15,000 cells mL⁻¹. However, cell concentrations do not account for the variability in size of cyanobacteria. This is particularly relevant when there are high concentrations of cyanobacteria that are very small in size. Additionally, toxin concentration per cell is more closely related to cyanobacteria biovolume than to total cell number. Thus, simply relying on cell concentrations as an indicator of health risk may give biased results on the cyanobacterial taxa that are abundant in the water body. In the last three years there has been an increase in pico-cyanobacteria (<2 µm, e.g. *Aphanathece* sp. and *Aphanocapsa* sp.) in the Rotorua lakes. This has been particularly apparent in Lake Rotoiti where cell concentrations of *Aphanocapsa holistica* have exceeded 100,000 cells mL⁻¹. For example, cell concentrations of *Aph. holistica* above the threshold of 15,000 cells mL⁻¹ have been recorded at Te Weta Bay, (Lake Rotoiti) on multiple occasions (Figure 1). However, when converted to biovolumes, the thresholds given in the Australian Guidelines for Managing Risks in Recreational Water (NHMRC 2006) were never exceeded (Figure 2).

The use of biovolume thresholds in the Rotorua lakes would reduce the unnecessary issuing of health warnings (Figure 2). Guideline values in the recently released “Cyanobacteria and Algae in Freshwater” section of the 2006 “Australian Guidelines for Managing Risks in Recreational Water” (NHMRC 2006) are primarily based on biovolume and it is anticipated that future New Zealand guidelines will also utilise biovolumes. To more accurately target risks associated with cyanobacteria, Cawthon Institute and University of Waikato were asked by Environment Bay of Plenty to calculate volumes for ten problematic cyanobacterial species of the Rotorua lakes. A database was developed to convert cell concentrations to biovolumes and to manage weekly data collected by Environment Bay of Plenty.

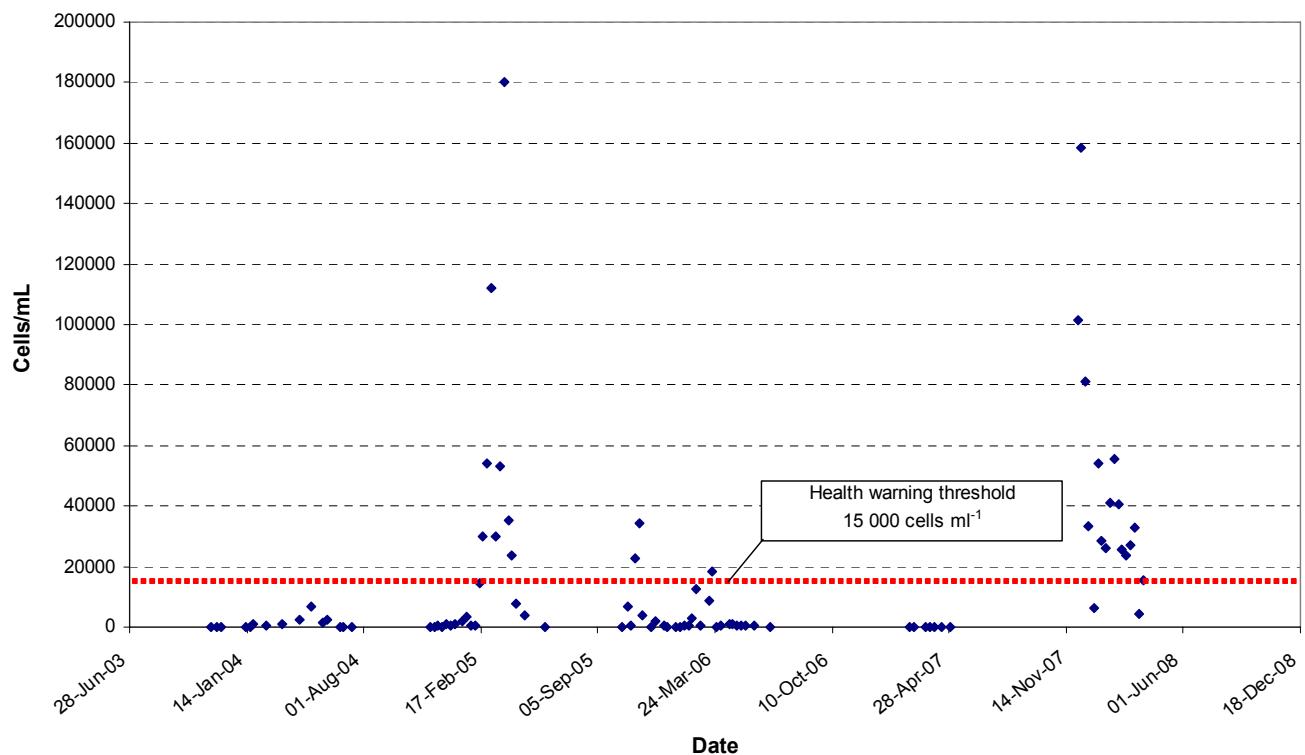


Figure 1. Cell concentrations of *Aphanocapsa holistica* in Te Weta Bay, Lake Rotoiti (2003–2008).
Data source: Environment Bay of Plenty.

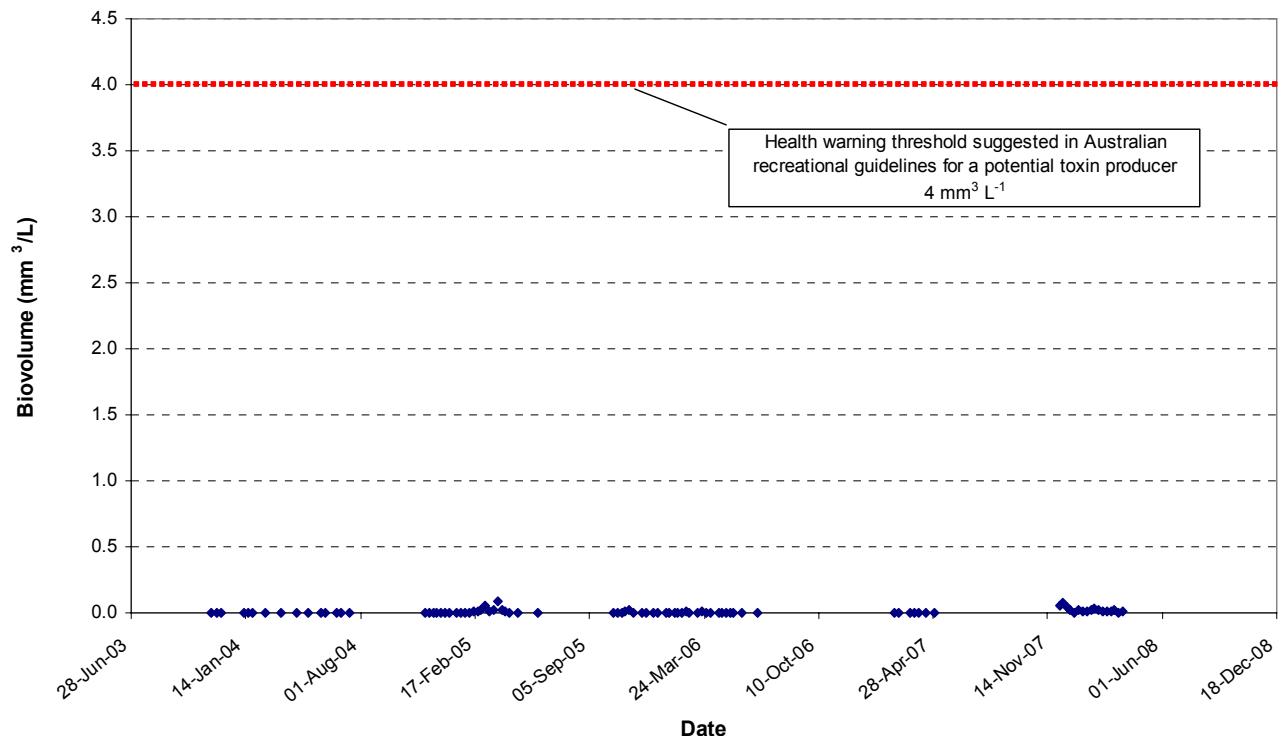


Figure 2. Biovolumes of *Aphanocapsa holistica* in Te Weta Bay, Lake Rotoiti (2003–2008).
Data source: Environment Bay of Plenty.

2. METHODS

2.1. Sample collection and storage

Surface samples (0.5 – 1L) were collected in March 2008 from six lakes in the Rotorua area using a plankton net (mesh size 20 µm). Samples were collected from Lakes Tarawera, Rotoiti, Rotoehu, Rotorua, Okaro, Okareka, and the Kaituna River. Sub-samples were preserved immediately in the field with Lugol's Iodine solution.

2.2. Identification and measurement

Sub-samples (1-5 mL) were settled in the laboratory using Utermöhl chambers (Utermöhl 1958). Morphological measurements were carried out using an inverted Olympus microscope (Ix71) at 640x magnification. Photomicrographs were taken with an Axiocam (HR) digital camera, using the bright field objective. Between 30 and 63 microscope measurements of vegetative cell lengths and widths were made. When colonial or filamentous species were measured no more than three cells per colony/filament were measured.

Measurements were made of the following ten species; *Anabaena lemmermannii*, *A. planktonica*, *Aphanocapsa holsatica*, *Aphanizomenon gracile*, *Aphanothecace clathrata*, *Coelosphaerium kuetzingianum*, *Microcystis* sp. (small), *Microcystis* sp. (large), *M. wesenbergii* and *Snowella lacustris*.

The species identifications were made primarily by reference to Baker (1991, 1992), Baker & Fabbro (2002), Cronberg & Annadotter (2006), Komárek & Anagnostidis (2000), McGregor & Fabbro (2001) and Wood *et al.* (2004).

2.3. Volume calculations

Volumes (V) for each species were calculated using the equations given in Table 1.

Table 1. Volume equations (US Environmental Protection Agency 2007).

Cell shape	Formula	Species
Ovoid (round)	$V=((4 / 3)*\pi * (\text{diam} / 2)^3)$	<i>Aphanocapsa holsatica</i> , <i>Microcystis</i> sp. (small), <i>Microcystis</i> sp. (large), <i>M. wesenbergii</i>
Ovoid	$V=(4 / 3) * \pi * (\text{width} / 2)^2 * (\text{length} / 2)$	<i>Anabaena lemmermannii</i> , <i>A. planktonica</i> <i>Coelosphaerium kuetzingianum</i> , <i>Snowella lacustris</i>
Cylinder	$V=(\pi * (\text{width} / 2)^2 * (\text{length}))$	<i>Aphanothecace clathrata</i> , <i>Aphanizomenon gracile</i> .

2.4. Database

A database was developed in Microsoft Access to convert cell concentrations to biovolumes and assist in data management. Historic data for the Te Weta Bay (Lake Rotoiti) sampling site was imported into Microsoft Access to demonstrate how the database could be utilised.

Volumes were not available for all species observed at this site. Published literature values (Olenina *et al.* 2006; Burch *et al.* 2003) or literature values from similar species were used in these instances. The source of the cell volume used can be viewed in the “Species Table” section of the database (Section 3.4).

3. RESULTS

3.1. Species, biovolumes and cell dimensions

Mean, maximum and minimum volumes and cell dimensions for each species are given in Table 2. Figure 3 shows a comparison of biovolumes for the ten species.

Table 2. Mean, maximum and minimum biovolumes and cell dimensions for ten cyanobacteria species from the Rotorua lakes.

	Mean volume $\mu\text{m}^3/\text{cell}$	Max. volume $\mu\text{m}^3/\text{cell}$	Min. volume $\mu\text{m}^3/\text{cell}$	Mean length μm	Max. length μm	Min. length μm	Mean width μm	Max. width μm	Min. width μm	Mean dia. μm	Max. dia. μm	Min. dia. μm	Count n
<i>Anabaena lemmermannii</i>	107.37	216.52	26.19	5.57	8.52	3.08	5.96	8.51	4.03				39
<i>Anabaena planktonica</i>	367.25	723.41	97.52	6.58	10.15	3.85	10.28	13.34	7.31				63
<i>Aphanocapsa holsatica</i>	1.73	6.12	0.31							1.40	2.27	0.84	45
<i>Aphanizomenon gracile</i>	82.40	403.66	7.49	4.43	11.27	2.04	2.46	3.82	0.98				32
<i>Aphanothece clathrata</i>	6.46	14.63	2.38	2.30	3.21	1.75	1.05	1.44	0.71				30
<i>Coelosphaerium kuetzingianum</i>	8.89	21.24	3.07	2.03	2.93	1.18	2.74	4.04	2.01				32
<i>Microcystis</i> sp. (small)	18.20	38.52	6.88							3.21	4.19	2.36	54
<i>Microcystis</i> sp. (large)	93.06	208.75	34.78							5.54	7.36	4.05	54
<i>Microcystis wesenbergii</i>	190.33	434.89	49.65							7.04	9.40	4.56	51
<i>Snowella lacustris</i>	98.65	300.27	38.24	5.02	7.70	3.51	5.97	8.63	4.18				48

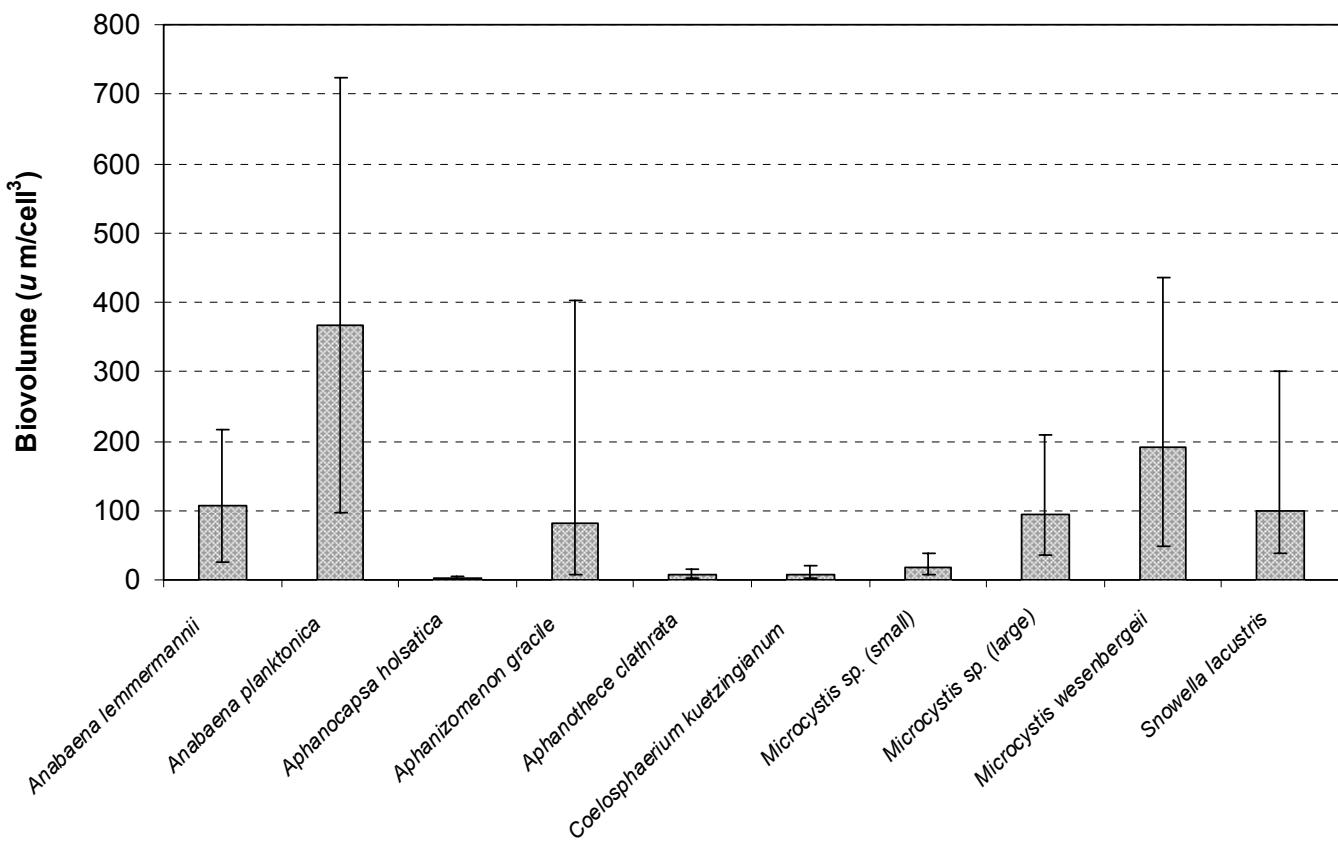


Figure 3. Mean biovolumes for ten cyanobacteria species from the Rotorua lakes. Error bars show maximum and minimum biovolumes.

3.2. Photographs of the cyanobacteria and their distribution

Photographs of the ten cyanobacterial species and their distribution as observed in this study are given below.

3.2.1. *Anabaena lemmermannii*



Distribution: Lake Okareka, Lake Rotoehu, Lake Rotoiti, Lake Rotorua, Lake Tarawera

3.2.2. *Anabaena planktonica*



Distribution: Lake Okaro, Lake Rotoiti, Lake Rotorua, Lake Tarawera, Kaituna River

3.2.3. *Aphanocapsa holsatica*



Distribution: Lake Okareka, Lake Okaro, Lake Rotoiti, Kaituna River

3.2.4. *Aphanizomenon gracile*



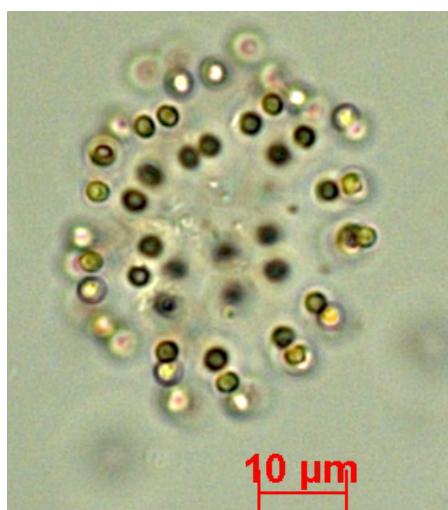
Distribution: Lake Rotoiti, Lake Tarawera

3.2.5. *Aphanothecce clathrata*



Distribution: Lake Okareka, Lake Okaro, Lake Tarawera

3.2.6. *Coelosphaerium kuetzingianum*



Distribution: Lake Rotoiti, Kaituna River

3.2.7. *Microcystis sp.* (small)



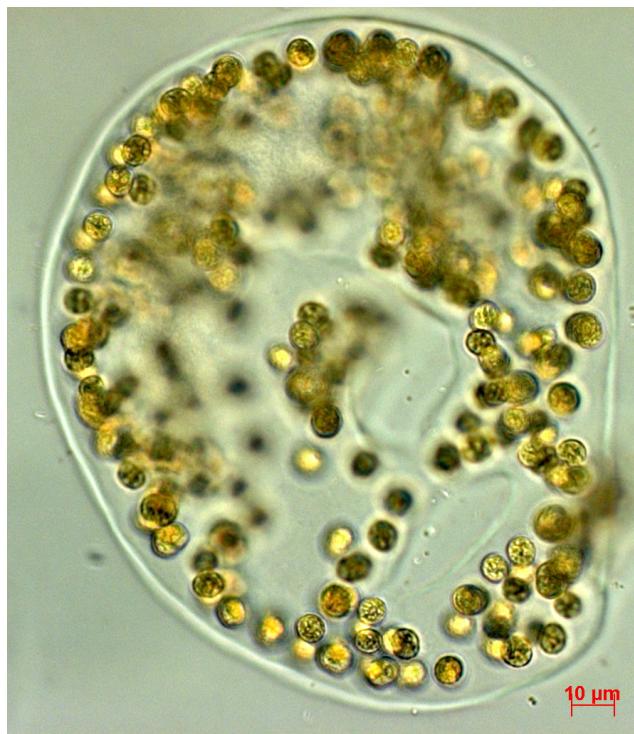
Distribution: Lake Okareka, Lake Okaro, Lake Rotoehu, Lake Rotoiti, Lake Tarawera

3.2.8. *Microcystis sp.* (large)



Distribution: Lake Rotoehu, Lake Rotoiti, Lake Rotorua, Lake Tarawera, Kaituna River

3.2.9. *Microcystis wesenbergii*



Distribution: Lake Rotoiti, Lake Rotorua, Kaituna River

3.2.10. *Snowella lacustris*



Distribution: Lake Rotoiti, Lake Rotorua

3.3. Additional species observed

A total of 13 additional cyanobacterial species were observed in the lake samples. These species were in low abundance and cell dimensions were not measured (Table 3).

Table 3. Additional species and their distribution.

Species	Distribution
<i>Anabaena</i> cf. <i>affinis</i>	Lake Rotoiti, Lake Okareka
<i>Anabaena</i> sp.	Lake Rotoehu, Lake Okareka
<i>Anabaena spiroides</i>	Lake Rotoiti, Lake Tarawera, Lake Okareka
<i>Anabaena</i> cf. <i>torulosa</i>	Lake Rotoehu
<i>Chrococcus</i> sp.	Lake Rotorua
<i>Cylindrospermum</i> sp.	Lake Okaro
<i>Lyngbya</i> sp.	Lake Okareka
<i>Oscillatoria</i> spp.	Kaituna River, Lake Rotoiti, Lake Okaro
<i>Planktolyngbya contorta</i>	Lake Tarawera
<i>Planktothrix</i> sp.	Lake Okaro, Kaituna River
<i>Pseudoanabaena limnetica</i>	Lake Okareka, Lake Rotoiti, Lake Rotoehu
<i>Sphaerotilus brasiliense</i>	Kaituna River
<i>Trichodesmium</i> sp.	Lake Okareka, Lake Okaro, Lake Rotoiti, Lake Tarawera

3.4. Database

A database to assist with biovolume conversions and data management was developed. Details on how to use this database are given below.

3.4.1. Using the Rotorua Lakes Cyanobacterial Database

Data Entry

1. Open database – Rotorua Lakes.mdb.
2. **Sampling date** - enter sampling date. The date must be entered in the following format DD/MM/YEAR.
3. **Sample Location** - Select sample location from drop down menu.
4. **Species** – Select cyanobacterial species from drop down menu.
5. **Cell Count** – Enter cell concentration for this species in cells mL⁻¹.
6. The biovolume for this species, total biovolume and total cell concentration will be automatically calculated.
7. Continue to enter species and cell concentrations on this page until all data for this date and site has been recorded.
8. Click on “Add New Record” to enter data for a new site and/or date.

Rotorua Lakes Cyanobacterial Database

Species	Cell Count	Biovolume
Anabaena planktonica	27384	10.05677

Sampling date: 29/01/1999 [Open Report by Date](#) [Biovolume Summary by date](#)

Sample location: Rotoiti - Te Weta Bay [Open Report by Location](#) 

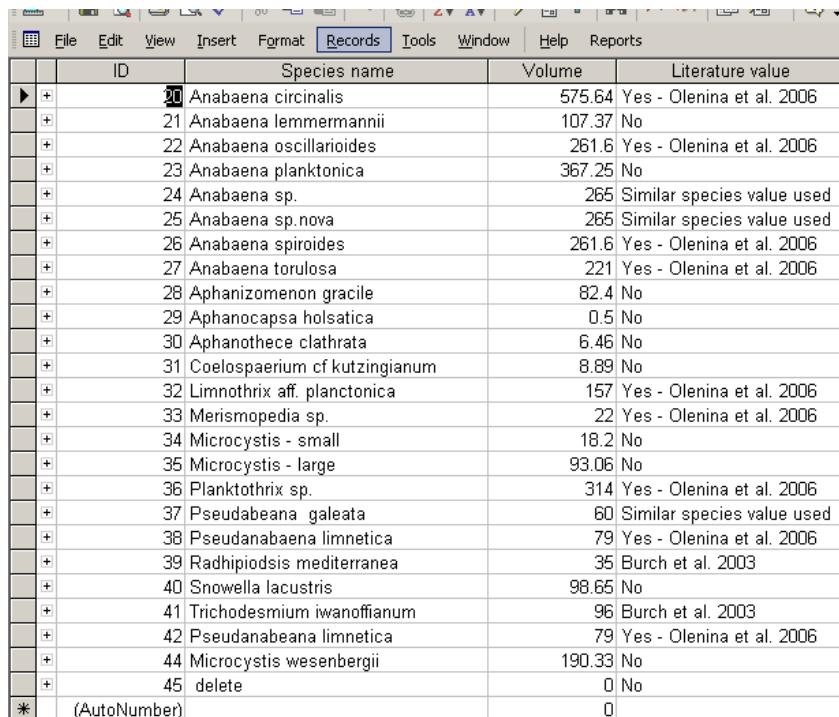
Total Biovolume: 10.05677 mm³/L Total Cell Concentration: 27384 cells/mL

Add New Record

Enter New Species

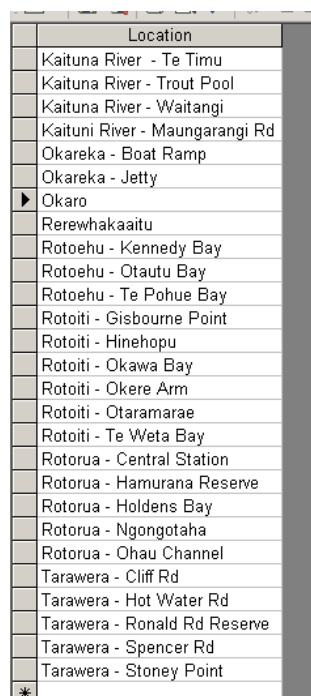
Enter New Location

9. New species can be added by clicking the “Enter New Species” button. This will open a “Species Table”. Enter the name of the species and the associated volume. In this table the method by which the volume was obtained can be included. For future reference it is recommended that for values taken from literature the source is included.



	ID	Species name	Volume	Literature value
►	20	Anabaena circinalis	575.64	Yes - Olenina et al. 2006
+	21	Anabaena lemmermannii	107.37	No
+	22	Anabaena oscillarioides	261.6	Yes - Olenina et al. 2006
+	23	Anabaena planktonica	367.25	No
+	24	Anabaena sp.	265	Similar species value used
+	25	Anabaena sp.nova	265	Similar species value used
+	26	Anabaena spiroides	261.6	Yes - Olenina et al. 2006
+	27	Anabaena torulosa	221	Yes - Olenina et al. 2006
+	28	Aphanizomenon gracile	82.4	No
+	29	Aphanocapsa holsatica	0.5	No
+	30	Aphanothecce clathrata	6.46	No
+	31	Coelosphaerium cf kutzingianum	8.89	No
+	32	Limnothrix aff. planctonica	157	Yes - Olenina et al. 2006
+	33	Merismopedia sp.	22	Yes - Olenina et al. 2006
+	34	Microcystis - small	18.2	No
+	35	Microcystis - large	93.06	No
+	36	Planktothrix sp.	314	Yes - Olenina et al. 2006
+	37	Pseudabeana galeata	60	Similar species value used
+	38	Pseudanabaena limnetica	79	Yes - Olenina et al. 2006
+	39	Radhiopidsis mediterranea	35	Burch et al. 2003
+	40	Snowella lacustris	98.65	No
+	41	Trichodesmium iwanoffianum	96	Burch et al. 2003
+	42	Pseudanabeana limnetica	79	Yes - Olenina et al. 2006
+	44	Microcystis wesenbergii	190.33	No
+	45	delete	0	No
*	(AutoNumber)		0	

10. A new sampling location can be added by clicking the “Enter new Location” button. This will open a “Sampling Location Table”.



Location
Kaituna River - Te Timu
Kaituna River - Trout Pool
Kaituna River - Waitangi
Kaituni River - Maungarangi Rd
Okareka - Boat Ramp
Okareka - Jetty
► Okaro
Rerewhakaaitu
Rotoehu - Kennedy Bay
Rotoehu - Otautu Bay
Rotoehu - Te Pohue Bay
Rotoiti - Gisbourne Point
Rotoiti - Hinehopu
Rotoiti - Okawa Bay
Rotoiti - Okere Arm
Rotoiti - Otaramarae
Rotoiti - Te Weta Bay
Rotorua - Central Station
Rotorua - Hamurana Reserve
Rotorua - Holdens Bay
Rotorua - Ngongotaha
Rotorua - Ohau Channel
Tarawera - Cliff Rd
Tarawera - Hot Water Rd
Tarawera - Ronald Rd Reserve
Tarawera - Spencer Rd
Tarawera - Stoney Point
*

11. To delete a record select “delete” from the species drop down menu. The record will be removed when the database is closed.

Rotorua Lakes Cyanobacterial Database

Sampling date:	05/08/2008	Open Report by Date	Biovolume Summary by date
Sample location:	Kaituna River - Trout Pool	Open Report by Location	
Species	Cell Count	Biovolume	
<i>delete</i>	125	0.07196	Enter New Species
			Enter New Location

Summary Reports

Three reports are available for viewing and printing. To view these reports click on the hyperlinks position to the right of the sampling data and sampling location boxes.

- 1. Results by date.** The report shows data from the current date in the form. Species present at each site and associated cell concentrations and biovolumes are displayed. The total biovolume and cell concentration for each site are given.

<i>Results by date</i>						
Date	Location	Species	Cell conc.	Biovolume	Tot. biovolume	Tot. cell conc.
16/04/2007	Rototiti - Te Weta Bay	<i>Aphanocapsa holsatica</i>	59	0.00003	0.04876	165
		<i>Microcystis</i> - large	22	0.00205		
		<i>Anabaena circinalis</i>	76	0.04375		
		<i>Anabaena planktonica</i>	8	0.00294		

- 2. Report by location.** The report shows data from the current sampling location in the form. Species present at each site and their associated cell concentrations and biovolumes are displayed. The total biovolume and cell concentration for each site are given.

<i>Report by Location</i>						
Date	Location	Species	Cell conc.	Biovolume	Tot. Biovolume	Tot. cell conc.
29/01/1999	Rototiti - Te Weta Bay	<i>Anabaena planktonica</i>	27384	10.05677	10.05677	27384
03/02/1999		<i>Anabaena planktonica</i>	11033	4.05187	4.05187	11033
17/02/1999		<i>Anabaena lemmermannii</i>	8	0.00086	0.42162	1152
		<i>Anabaena circinalis</i>	3	0.00173		
22/02/1999		<i>Anabaena planktonica</i>	1141	0.41903		
		<i>Anabaena circinalis</i>	14	0.00806	0.15349	410
		<i>Anabaena planktonica</i>	396	0.14543		
00/00/0000		<i>Anabaena circinalis</i>	11	0.00404	0.00404	11

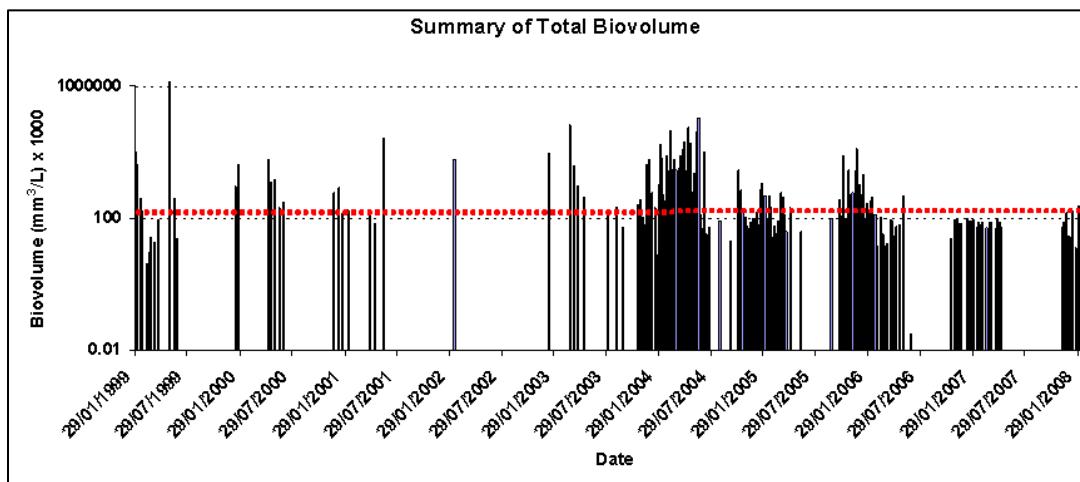
- 3. Biovolume and cell concentration summary by date.** The report shows the total biovolumes and cell concentrations for the current date in the form. It is designed for quick viewing of which sites exceed prior defined thresholds.

Biovolume and cell conc. summary by date

Date	Location	Total biovolume	Total cell conc.
16/04/2007	Rototiti - Te Weta Bay	0.04876	166

Summary Graph

A graph showing the total biovolumes at the site selected in the form is available for viewing and printing by clicking on the graph button. This graph shows long-term trends at sampling locations. The red dashed line shows the approximate location of the biovolume threshold used in the “Cyanobacteria and Algae in Freshwater” section of the 2005 “Australian Guidelines for Managing Risks in Recreational Water” (NHMRC 2006).



4. DISCUSSION

Biovolume takes into account the variability in size of cyanobacteria in a sample. Using biovolume in routine cyanobacterial monitoring programmes may reduce unnecessary issuing of health warnings. Guideline values in the recently released “Cyanobacteria and Algae in Freshwater” section of the 2006 “Australian Guidelines for Managing Risks in Recreational Water” (NHMRC 2006) are primarily based on biovolume. Biovolumes of **>4 mm³/L** (for the combined total of all cyanobacteria where a known toxin producer is dominant), or **>10 mm³/L** (for total biovolume of all cyanobacterial material where known toxins are not present), are used as thresholds for issuing health warnings. It is anticipated that future New Zealand guidelines will also utilise similar biovolume thresholds.

It is time-consuming and impractical to measure and calculate a biovolume for every individual in routine counting, thus it is recommended that the standardised species lists with fixed biovolumes are used. Where possible these should be specific to the waterbodies being monitored. The current project has established volumes for ten of the most problematic species in the Rotorua lakes. Low abundance or absence of other species prevented full characterisation of all problematic species. We recommend that further measurements are made as samples become available or new species are recorded.

There are several caveats that need to be considered when using biovolumes;

1. In taxa that contain specialised cells such as akinetes and heterocysts volume measurements are of vegetative cells only. Specialised cells usually make up a very small proportion of all cells and this is unlikely to have a significant effect on biovolume.
2. Hawkins *et al.* (2005) showed that preservation of samples with Lugol’s Iodine (the preservative used by Environment Bay of Plenty) causes shrinkage rates of up to 40% dependant on Lugol’s concentration, species and the length of time in Lugol’s Iodine. Using a low concentration of Lugol’s Iodine and analysing samples within 24-48 hours of collection will minimise shrinkage. The cell biovolumes produced in this report were obtained on Lugol’s-preserved samples that had been stored for several months.

Further customisation of the database developed in this project is recommended but is beyond the scope of this work. Additional features that could be added include; the ability to convert microscopic counts to cell concentrations and improved graphing functions. Importing all historic data into the database is recommended. This would enable tracking of changes in cyanobacterial concentrations, biovolumes and species composition.

5. ACKNOWLEDGEMENTS

We thank Matthew Bloxham (Environment Bay of Plenty) for sample collection. SAW acknowledges the New Zealand Foundation for Research, Science and Technology (FRST) for post-doctoral fellowship funding (CAWX0501). The authors also acknowledge FRST through the Outcome Based Investment in Lake Biodiversity (UOWX0505).

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