CAWTHRON

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. Cawthron 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, Aphanothece clathrata, Coelosphaerium kuetzingianum, Microcystis* sp. (small), *Microcystis* sp. (large), *M. wesenbergeii* 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. Aphanothece* 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, Cawthron 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.

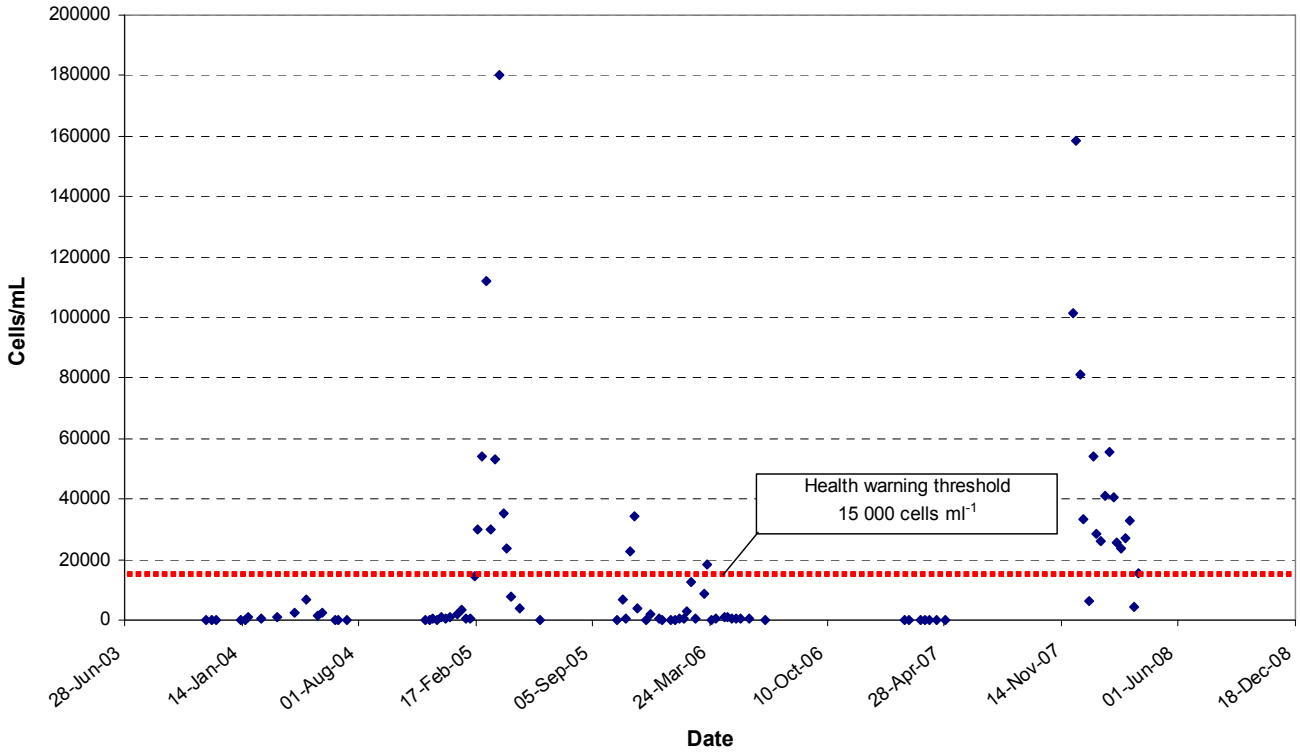


Figure 1. Cell concentrations of *Aphanocapsa holistica* in Te Weta Bay, Lake Rotoiti (2003-2008). Data source: Environment Bay of Plenty.

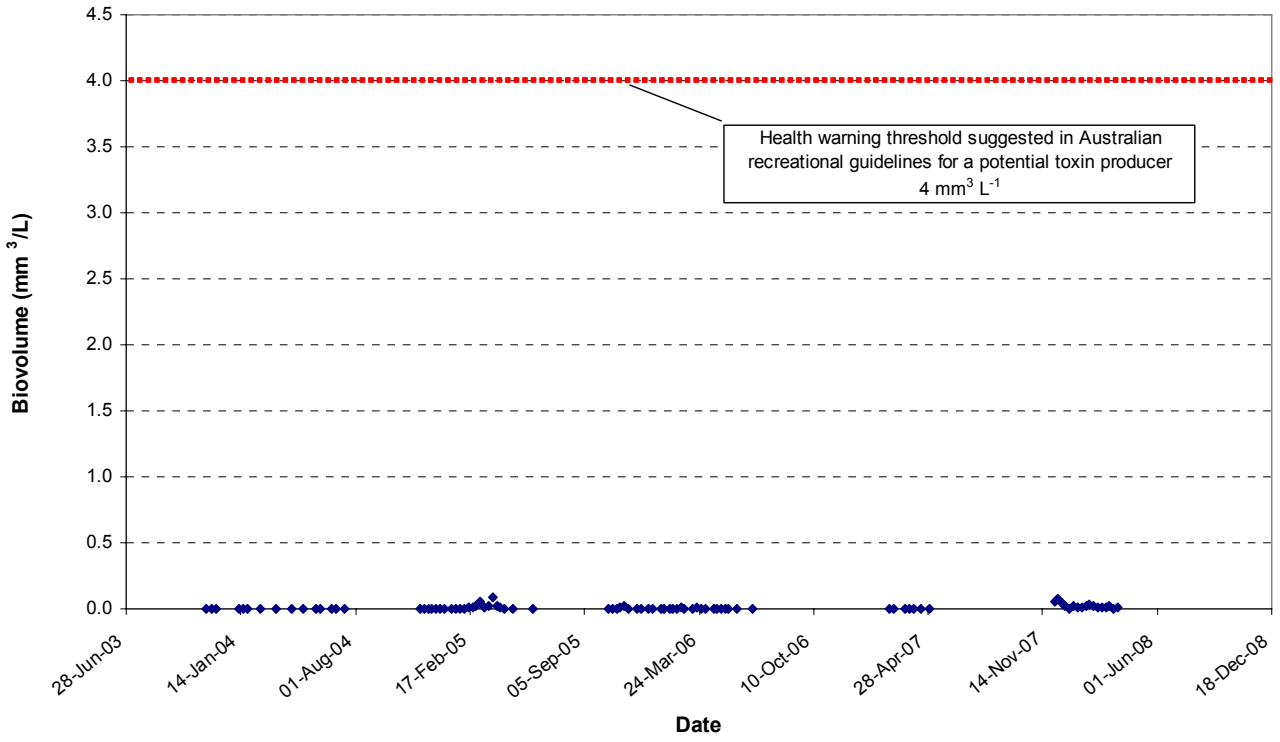


Figure 2. Biovolumes of *Aphanocapsa holistica* in Te Weta Bay, Lake Rotoiti (2003-2008). Data source: Environment Bay of Plenty.

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*, *Aphanothece clathrata*, *Coelosphaerium kuetzingianum*, *Microcystis* sp. (small), *Microcystis* sp. (large), *M. wesenbergeii* 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)	$V=((4 / 3)*\pi * (diam / 2)^3)$	*Aphanocapsa holsatica, Microcystis* sp. (small), *Microcystis* sp. (large), *M. wesenbergeii*
Ovoid	$V=(4 / 3) * \pi * (width / 2)^2 * (length / 2)$	*Anabaena lemmermannii, A. planktonica Coelosphaerium kuetzingianum*, *Snowella lacustris*
Cylinder	$V=(\pi * (width / 2)^2 * (length))$	*Aphanothece clathrata, Aphanizomenon gracile*.

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 µm³/cell	Max. volume µm³/cell	Min. volume µm³/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
Anabaena lemmermannii	107.37	216.52	26.19	5.57	8.52	3.08	5.96	8.51	4.03				39
Anabaena planktonica	367.25	723.41	97.52	6.58	10.15	3.85	10.28	13.34	7.31				63
Aphanocapsa holsatica	1.73	6.12	0.31							1.40	2.27	0.84	45
Aphanizomenon gracile	82.40	403.66	7.49	4.43	11.27	2.04	2.46	3.82	0.98				32
Aphanothece clathrata	6.46	14.63	2.38	2.30	3.21	1.75	1.05	1.44	0.71				30
Coelosphaerium kuetzingianum	8.89	21.24	3.07	2.03	2.93	1.18	2.74	4.04	2.01				32
Microcystis sp. (small)	18.20	38.52	6.88							3.21	4.19	2.36	54
Microcystis sp. (large)	93.06	208.75	34.78							5.54	7.36	4.05	54
Microcystis wesenbergeii	190.33	434.89	49.65							7.04	9.40	4.56	51
Snowella lacustris	98.65	300.27	38.24	5.02	7.70	3.51	5.97	8.63	4.18				48

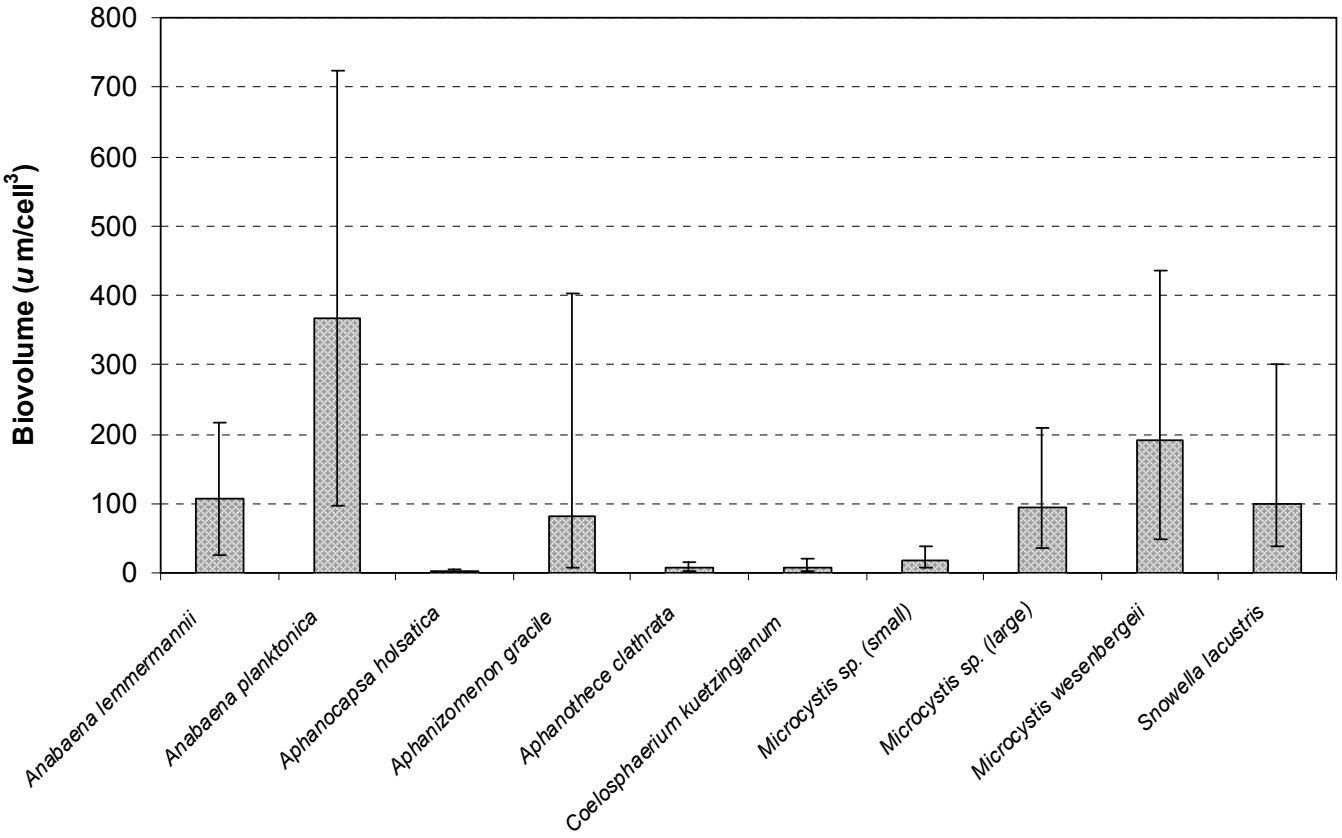


Figure 3. Mean biovolumes for ten cyanobacteria species from the Rotorua lakes. Error bars show maximum and minimum biovolumes.

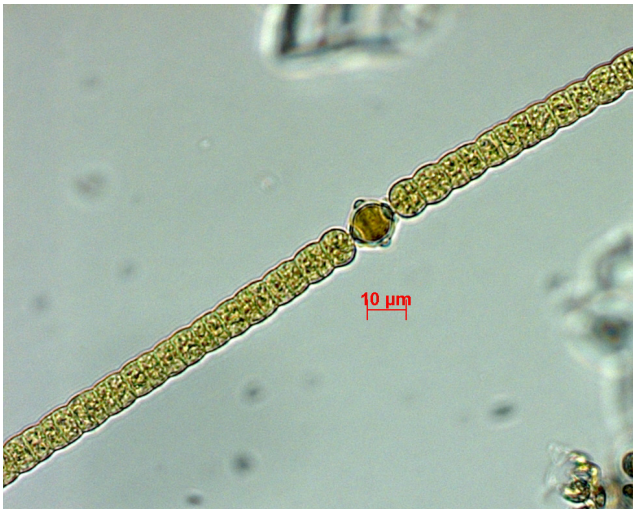
3.2. Photographs of the cyanobacteria and their distribution

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

3.2.1. Anabaena lemmermannii

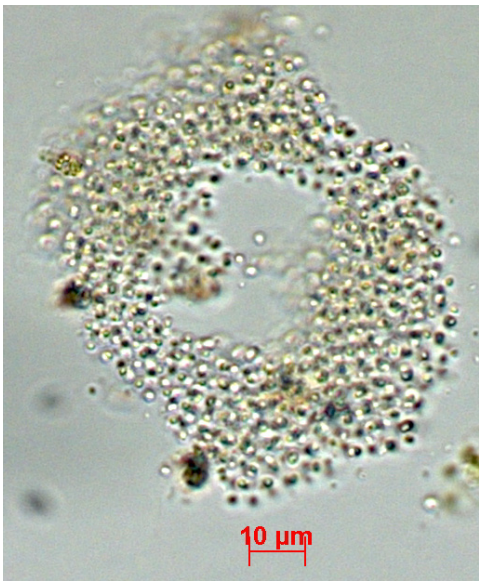
Distribution: Lake Okareka, Lake Rotoehu, Lake Rotoiti, Lake Rotorua, Lake Tarawera

3.2.2. Anabaena planktonica



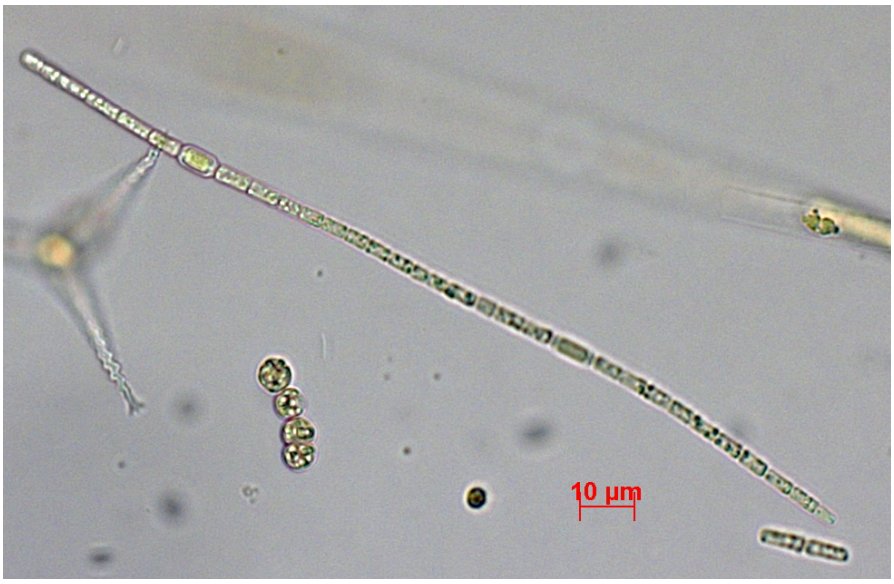
Distribution: Lake Okaro, Lake Rotoiti, Lake Rotorua, Lake Tarawera, Kaituna River

3.2.3. *Aphanocapsa holsatica*



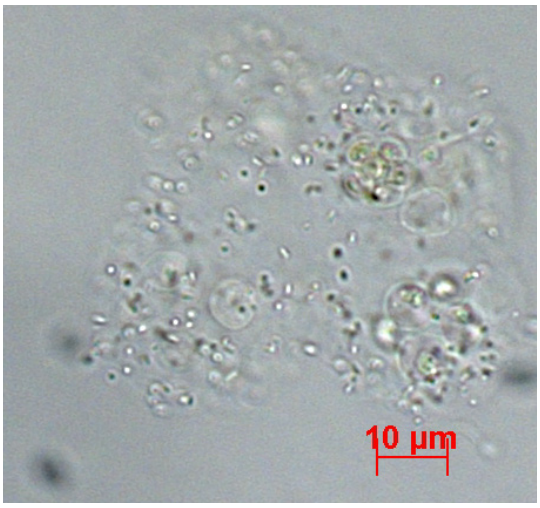
Distribution: Lake Okareka, Lake Okaro, Lake Rotoiti, Kaituna River

3.2.4. *Aphanizomenon gracile*



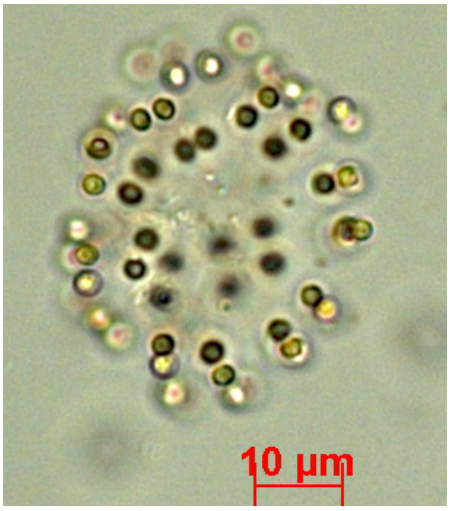
Distribution: Lake Rotoiti, Lake Tarawera

3.2.5. *Aphanothece clathrata*



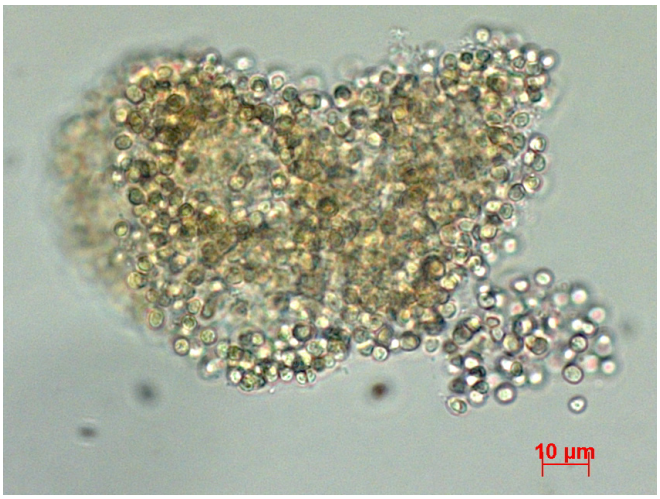
Distribution: Lake Okareka, Lake Okaro, Lake Tarawera

3.2.6. *Coelosphaerium kuetzingianum*



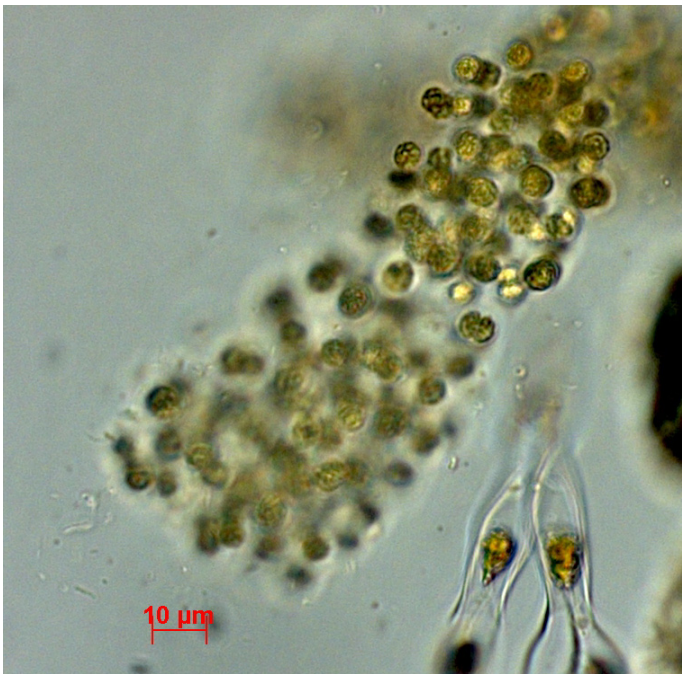
Distribution: Lake Rotoiti, Kaituna River

3.2.7. *Microcystis sp. (small)*



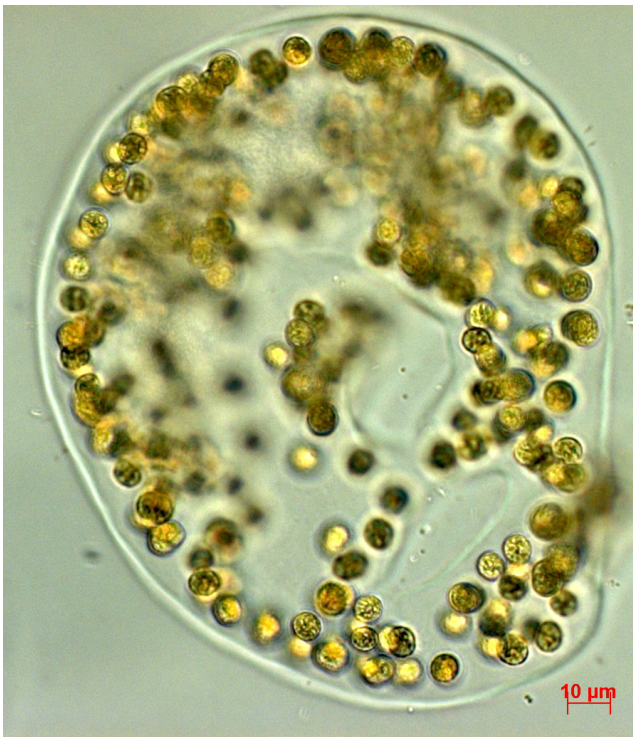
Distribution: Lake Okareka, Lake Okaro, Lake Rotoehu, Lake Rotoiti, Lake Tarawera

3.2.8. *Microcystis sp. (large)*



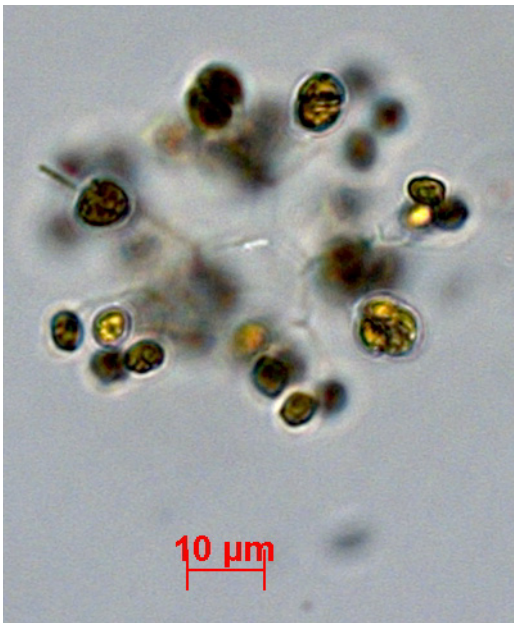
Distribution: Lake Rotoehu, Lake Rotoiti, Lake Rotorua, Lake Tarawera, Kaituna River

3.2.9. *Microcystis wesenbergeii*



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
Anabaena cf. *affinis*	Lake Rotoiti, Lake Okareka
Anabaena sp.	Lake Rotoehu, Lake Okareka
Anabaena spiroides	Lake Rotoiti, Lake Tarawera, Lake Okareka
Anabaena cf. *torulosa*	Lake Rotoehu
Chrococcus sp.	Lake Rotorua
Cylindrospermum sp.	Lake Okaro
Lyngbya sp.	Lake Okareka
Oscillatoria spp.	Kaituna River, Lake Rotoiti, Lake Okaro
Planktolyngbya contorta	Lake Tarawera
Planktothrix sp.	Lake Okaro, Kaituna River
Pseudoanabaena limnetica	Lake Okareka, Lake Rotoiti, Lake Rotoehu
Sphaerocavum brasiliense	Kaituna River
Trichodesmium sp.	Lake Okareka, Lake Okaro, Lake Rotoiti, Lake Tarawera

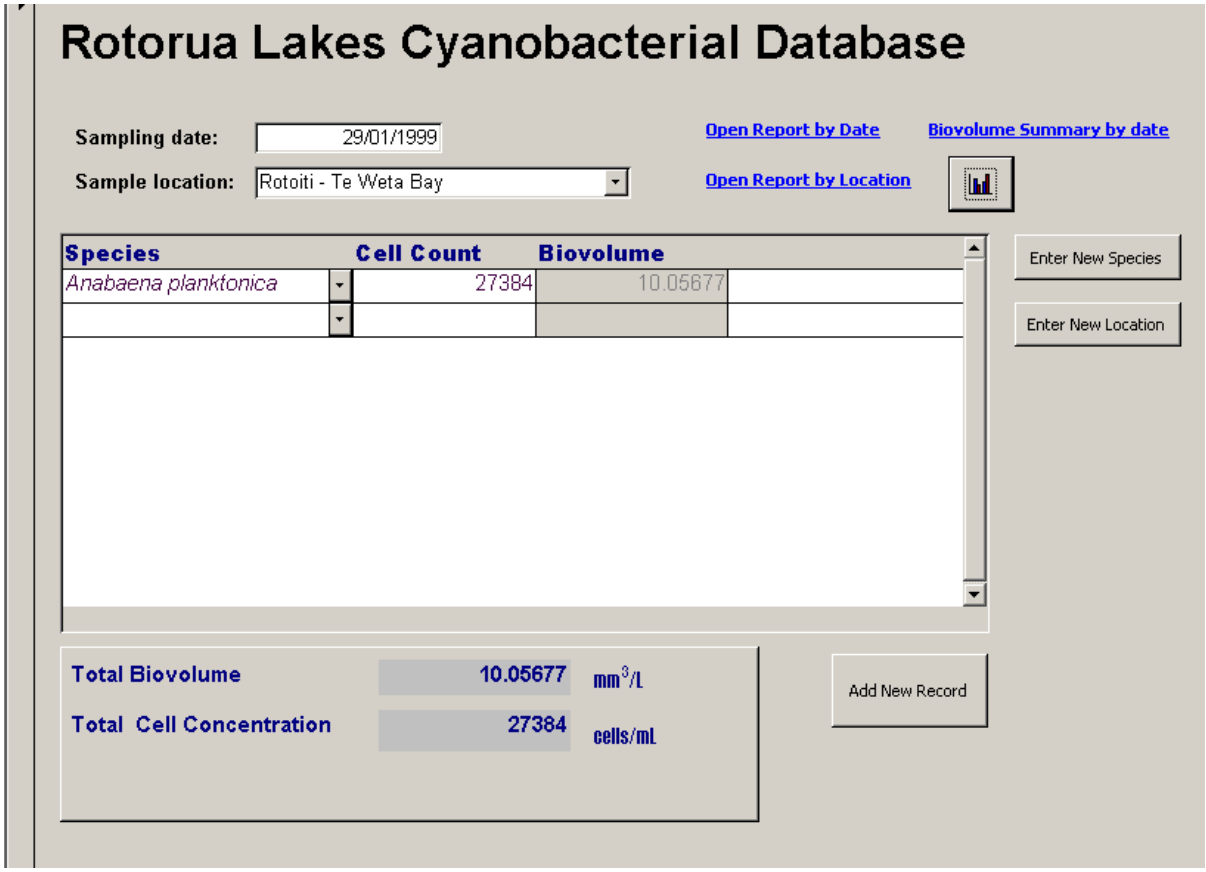
3.4. Database

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.



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.

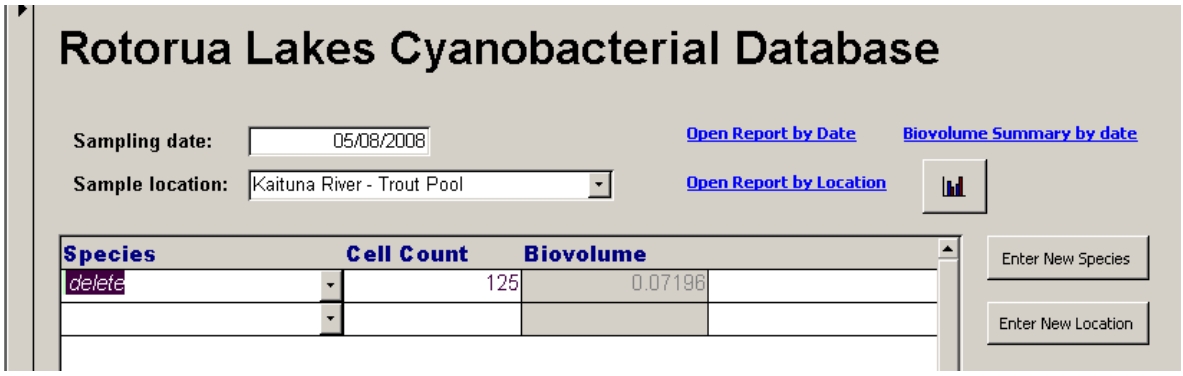
File Edit View Insert Format Records Tools Window Help Reports

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	Aphanothece clathrata	6.46	No
31	Coelospaerium 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	Radhipiodsis 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

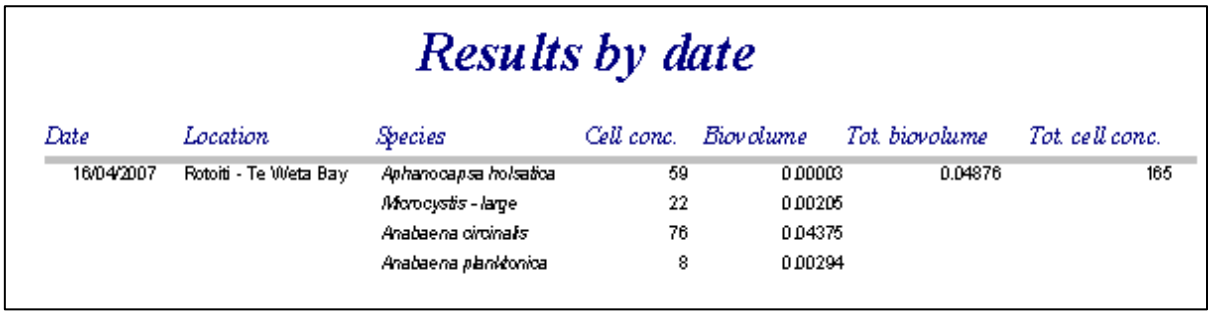
11. To delete a record select "delete" from the species drop down menu. The record will be removed when the database is closed.



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.

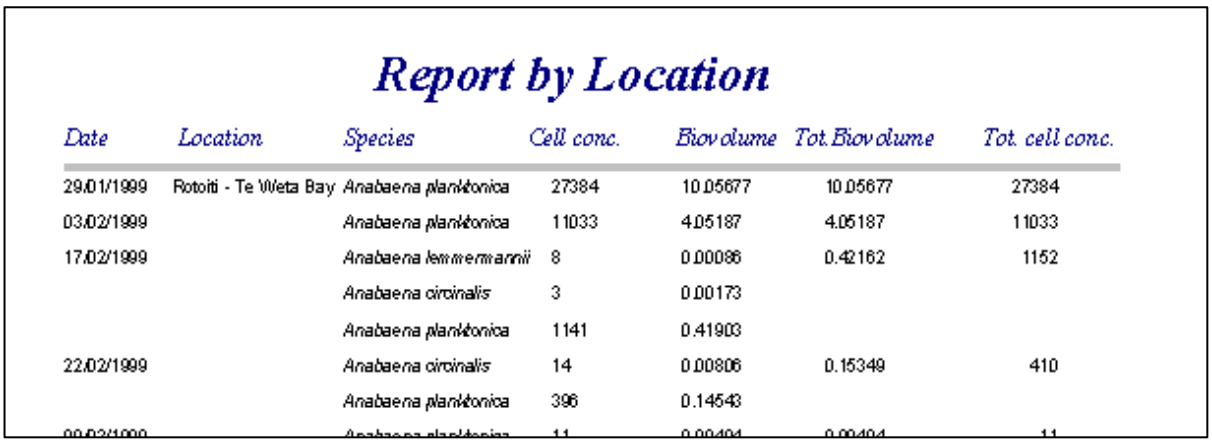
1. **Results by date.** The report shows data from the current date in the form. Species present at each site and associated cell concentrations and biovolumes are displayed. The total biovolume and cell concentration for each site are given.



Results by date

Date	Location	Species	Cell conc.	Biovolume	Tot. biovolume	Tot. cell conc.
16/04/2007	Rotoiti - Te Weta Bay	*Aphanocapsa holsatica*	59	0.00003	0.04876	165
		Microcystis - large	22	0.00205		
		Anabaena circinalis	76	0.04375		
		Anabaena planktonica	8	0.00294		

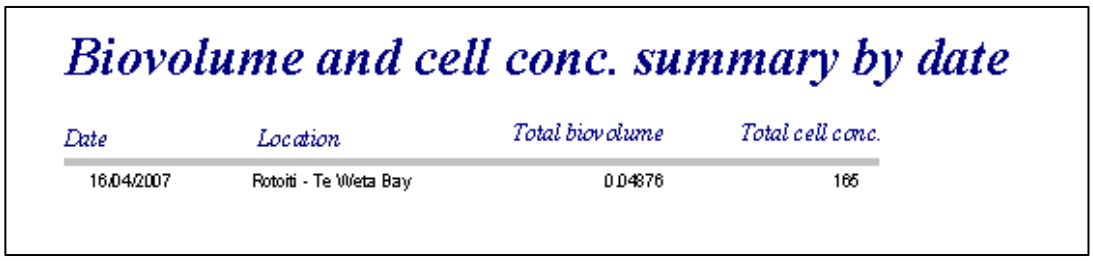
2. **Report by location.** The report shows data from the current sampling location in the form. Species present at each site and their associated cell concentrations and biovolumes are displayed. The total biovolume and cell concentration for each site are given.



Report by Location

Date	Location	Species	Cell conc.	Biovolume	Tot.Biovolume	Tot. cell conc.
29/01/1999	Rotoiti - Te Weta Bay	*Anabaena planktonica*	27384	10.05677	10.05677	27384
03/02/1999		*Anabaena planktonica*	11033	4.05187	4.05187	11033
17/02/1999		*Anabaena lemmermannii*	8	0.00086	0.42162	1152
		Anabaena circinalis	3	0.00173		
		Anabaena planktonica	1141	0.41903		
22/02/1999		*Anabaena circinalis*	14	0.00806	0.15349	410
		Anabaena planktonica	396	0.14543		

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.

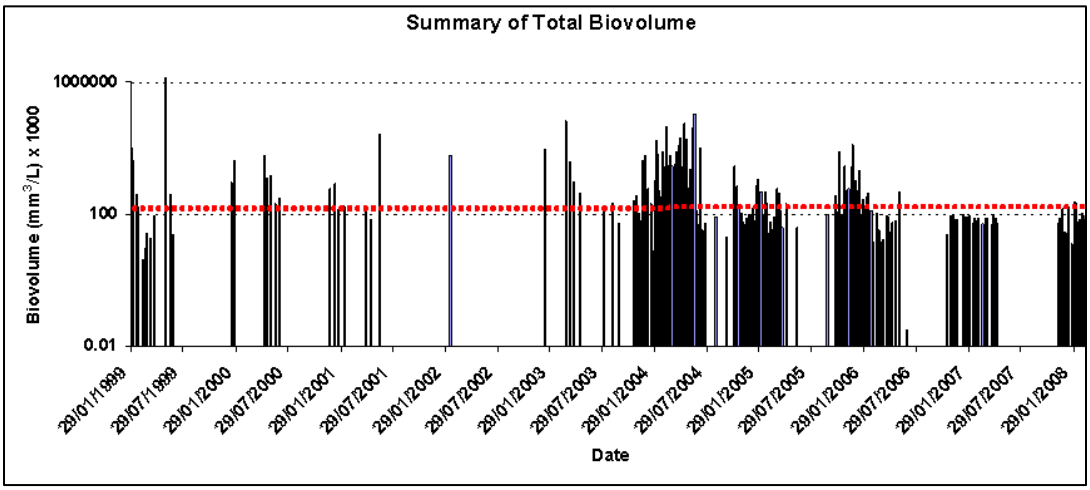


Biovolume and cell conc. summary by date

Date	Location	Total biovolume	Total cell conc.
16/04/2007	Rotoiti - Te Weta Bay	0.04876	165

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 mm^3/L** (for the combined total of all cyanobacteria where a known toxin producer is dominant), or >**10 mm^3/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 heterocytes 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.

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