

Benthic Cyanobacteria and Anatoxin-a and Homanatoxin-a Concentrations in Five Southland Rivers

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Reviewed by: _____ Approved for release by: _____
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Recommended citation:
Heath MW, Wood SA 2010. Benthic Cyanobacteria and Anatoxin-a and Homanatoixn-a Concentrations in Five Southland Rivers.
Cawthron Report No. 1841. 15pp plus appendices

1. INTRODUCTION

Benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers (Briggs & Kilroy 2000); the most common genus in New Zealand is *Phormidium*. During stable flow conditions, *Phormidium* can proliferate, forming expansive black/brown leathery mats across large areas of river substrate. Several *Phormidium* species are known to produce natural toxins, known as cyanotoxins. These toxins are a health threat to humans and animals when consumed or when there is contact with contaminated water.

The potential risks to human health and impacts to aquatic ecosystems in New Zealand from *Phormidium* are largely unknown. However, over the last decade more than 30 dog poisonings associated with benthic *Phormidium* have been reported (Hamill 2001, Wood *et al.* 2007, Heath *et al.* 2010a,b). In most instances the dog poisonings were linked with exposure to *Phormidium* mats containing the neurotoxins anatoxin-a (ATX) and homoanatoxin-a (HTX). Both ATX and HTX are powerful neuromuscular blocking agents that act through the nicotinic acetylcholine receptor. In affected animals ATX and HTX can cause convulsions, coma, rigors, cyanosis, limb twitching, hypersalivation and death (Carmichael 1994). Benthic cyanobacteria in New Zealand are also known to produce microcystins (heptatoxin) (Hamill 2001, Wood *et al.* 2010a), saxitoxins (neurotoxin) (Smith *et al.* 2010), cytotoxic compounds affecting mammalian cells (Wood, Froscio & Campbell, unpub data) and skin irritants.

Monitoring and research on *Phormidium* mats has shown that the occurrence of ATX/HTX is variable. Using multiple strains of cultured *Phormidium* sourced from rivers throughout New Zealand, Heath *et al.* (2010a) showed that toxic and non-toxic genotypes co-occur in *Phormidium* mats. This co-occurrence may at least partially explain the variability in ATX/HTX concentrations reported for *Phormidium* mats collected within and among rivers.

In 2009, New Zealand guidelines for managing cyanobacterial risk in water used for recreational purposes were released (Ministry for the Environment & Ministry of Health 2009). The aim of the guidelines was to help agencies responsible for managing cyanobacteria develop monitoring protocols appropriate for local conditions and circumstances, and to encourage the adoption of a nationally unified approach. The guidelines set out a monitoring framework for establishing the public health risk from cyanobacteria in lakes (mainly planktonic) and rivers (mainly benthic). A multi-tiered framework is used that incorporates a monitoring and management action sequence, which regulators can use for a graduated response to the onset and progress of a cyanobacterial bloom or benthic proliferation. The guidelines incorporate a specific section and alert level framework for benthic cyanobacteria. This section provides information on a transect system for monitoring the percentage cover of cyanobacterial mats at a sampling site, and includes a three-tier alert level framework that uses cyanobacterial abundance and the occurrence of mats visibly detaching from the substrate to determine the alert level status.

In 1998 benthic cyanobacteria were associated with the death of six dogs on the Mataura River in Southland (Hamill 2001). Since this incident samples have been collected sporadically and ATX/HTX have been detected (Environment Southland, unpub. data), underlining the need for further monitoring of cyanobacterial mats in Southland rivers.

The specific aims of this study were:

- To compare the site survey method given in the cyanobacterial guidelines to a visual assessment method.
- To investigate spatial and temporal changes in ATX/HTX concentrations at one site in each of five Southland rivers.
- To improve knowledge on the environmental parameters regulating cyanobacterial mat formation at the investigated sites.

2. METHODS

2.1. Sample locations

A single site at each of five rivers (Oreti, Makarewa, Waikaia, Mataura, Aparima) was selected for benthic cyanobacterial monitoring (Figure 1). Site selections were based on recreational use and history of cyanobacterial mat proliferations. Sampling and surveying was undertaken weekly at Sites 1 and 2 and monthly at Sites 3, 4 and 5.

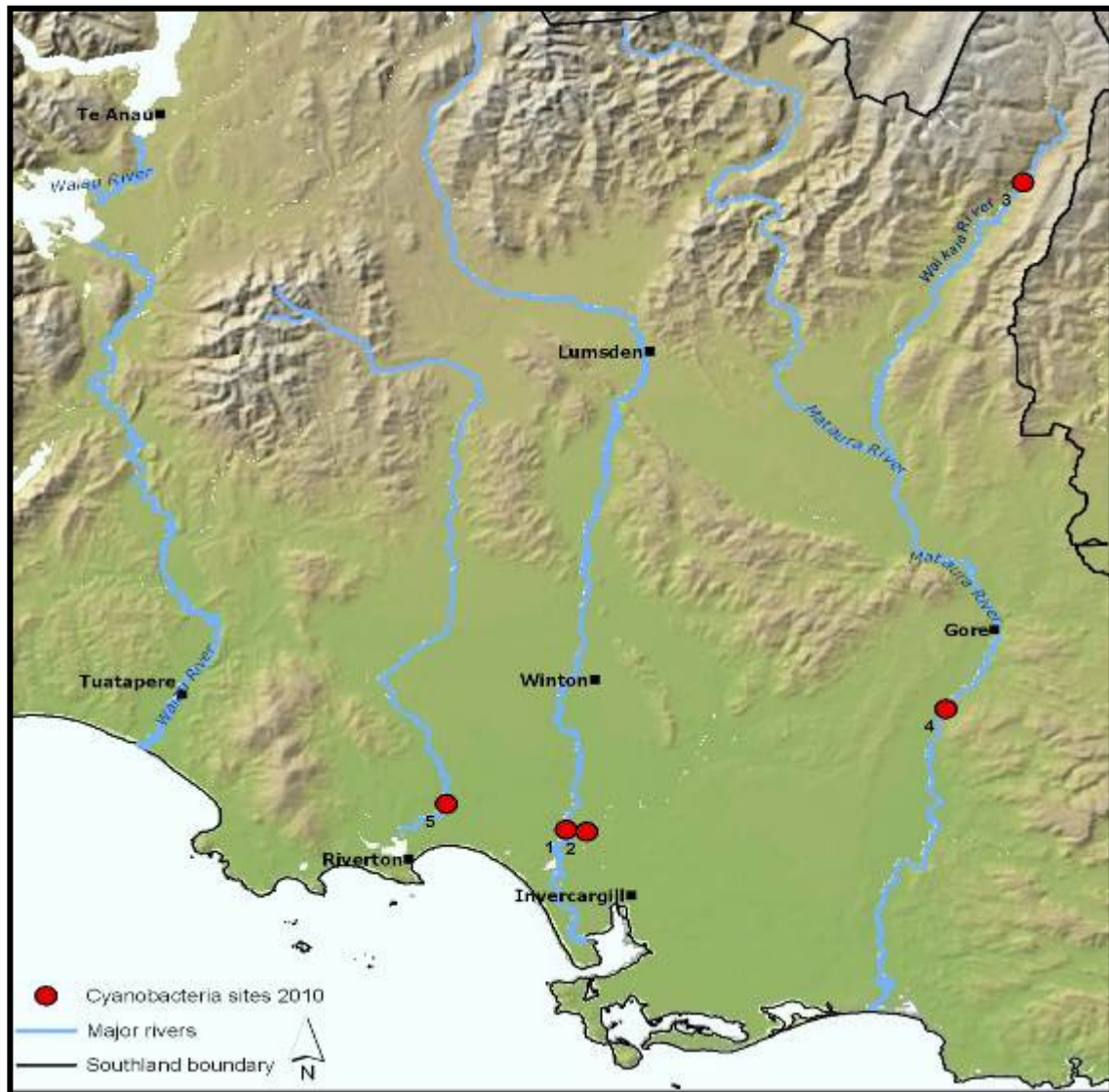


Figure 1 Location of sampling sites, Southland, New Zealand
 Site 1 – Oreti River, Site 2 – Makarewa River, Site 3 – Waikaia River, Site 4 – Mataura River,
 Site 5 – Aparima River.

Both Site 1, on the Oreti River, and Site 2, on the Makarewa River, were located in a densely populated areas and experience high recreational use. Site 3, on the Waikaia River, is also used extensively for recreational activities but is less populated. Site 4, located 200 metres downstream of Mataura Township on the Mataura River, has a long history of dense periphyton mats. Site 5, on the Aparima River near Thornbury, is also used for recreational activities and has previously experienced moderate cyanobacterial proliferations. Further in-depth site profiles are given in Appendix 1.

2.2. Site surveys

All site surveys and samplings were undertaken by Environment Southland staff. Two methods were used to determine the percentage of the river substrate covered by cyanobacterial mats.

Method 1. Visual Method

The surveyor wades through the river at the area where cyanobacterial mat cover is highest at each site and makes a visual prediction of percentage cover of cyanobacterial mats.

Method 2. Transect Method

This is the method outlined in the New Zealand guidelines for managing cyanobacteria in recreational fresh waters (Ministry for the Environment & Ministry of Health 2009). The surveys conducted in this study were primarily based in riffles, but also included some run and pool habitat. The length of the river surveyed varied from 26 metres (Site 2) to 100 metres (Site 1). At each site four transects at right angles to the water's edge and going out to a depth of 0.6 metres were surveyed. The cyanobacterial mat cover was assessed at five points along each transect using an underwater viewer. The 20 data points were averaged to obtain an overall cyanobacterial percentage mat cover at each site.

2.3. Sample collection and preparation

At each site 10 cyanobacterial mat samples were collected by scraping mat material from one rock into separate sterile plastic tubes. On arrival to the laboratory, samples were frozen (-20°C) until further analysis.

Samples were thawed and a sub-sample (0.25 g) from each of the 10 samples from each site were combined, homogenised and lyophilized (FreeZone6, Labconco, USA). The remaining material was pooled per site and preserved in Lugol's iodine solution for later morphological identification.

2.4. Morphological identification

The dominant cyanobacterium in each pooled sample was identified by microscopy (BX51, Olympus, Wellington, New Zealand).

2.5. Toxin extraction and analysis

Lyophilized material (100 mg) was resuspended in 10 mL of double distilled water (DDW) containing 0.1% formic acid and sonicated (Cole Parmer 8890, Biolab, Auckland, New Zealand) for 15 minutes. Samples were centrifuged ($4000 \times g$, 10 minutes) and the supernatants analysed for ATX, HTX and their degradation products dihydroanatoxin-a (dhATX) and dihydrohomoanatoxin-a (dhHTX), using liquid chromatography-mass spectrometry (LC-MS) as described in Heath *et al.* 2010a.

2.6. River flow and water temperature

Continuous river flow and water temperature data were measured at, or close to, each sampling site using data loggers. The data loggers were 10-15 kilometres upstream at Site 2, 2.5 kilometres downstream at Site 3 and 8 kilometres downstream at Site 4. Nutrient data was only available for six individual data points from four different sites. This lack of data meant that statistical analysis of associations between water quality and cyanobacterial abundance was not possible.

3. RESULTS

3.1. Comparison of site survey methods

At all five sites sampled Method 1 (visual) gave higher cyanobacterial abundances than Method 2 (transect; Figure 2). On average the percentage cover measured using Method 1 was 3.3 times higher than that measured using Method 2. On four occasions low cyanobacterial mat cover detected using Method 1 was not detected using Method 2. Of the 33 occasions where cyanobacterial mats were detected, 19 occasions had large enough discrepancies between the two assessment methods to result in different alert levels. Across all sites, Method 1 gave 15 green modes, 9 orange modes and 8 red modes whereas Method 2 resulted in 26 green modes, 3 orange modes and no red modes.

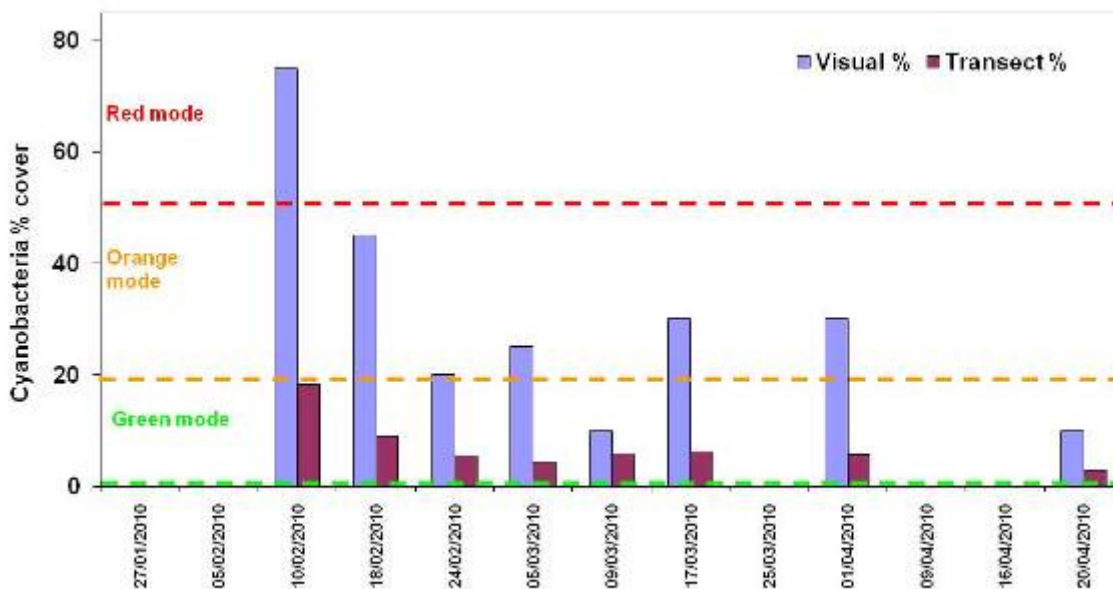


Figure 2 Histogram of data recorded at Site 2 (Makarewa River) showing the difference between Method 1 (visual) and Method 2 (transect) in assessing the overall percentage cyanobacterial mat cover at a site. The horizontal dashed lines correspond to the alert levels given in the New Zealand guidelines for cyanobacteria in recreational fresh waters.

3.2. Species identification, anatoxin-a and homantoxin-a concentrations and mat coverage

The dominant species in all mats was *Phormidium*.

Full toxin results are given in Appendix 2. Anatoxin-a, HTX and their degradation products were detected at Sites 1, 3 and 4 (Figures 3, 4 and 5). None of the mats from Site 2 (Makarewa River, sampled weekly) contained detectable levels of toxins despite a relatively high percentage cover of mats (Figure 2). No toxins were detected at Site 5.

At Site 1 (Oreti River) the highest cyanobacterial mat coverage was observed on 10 February 2010 (75% visual, 20% transect). However, the highest toxin concentrations were recorded on 1 April 2010 (690 µg/kg dhATX and 21 µg/kg HTX) when coverage was much lower (30% visual, 5% transect; Figure 3).

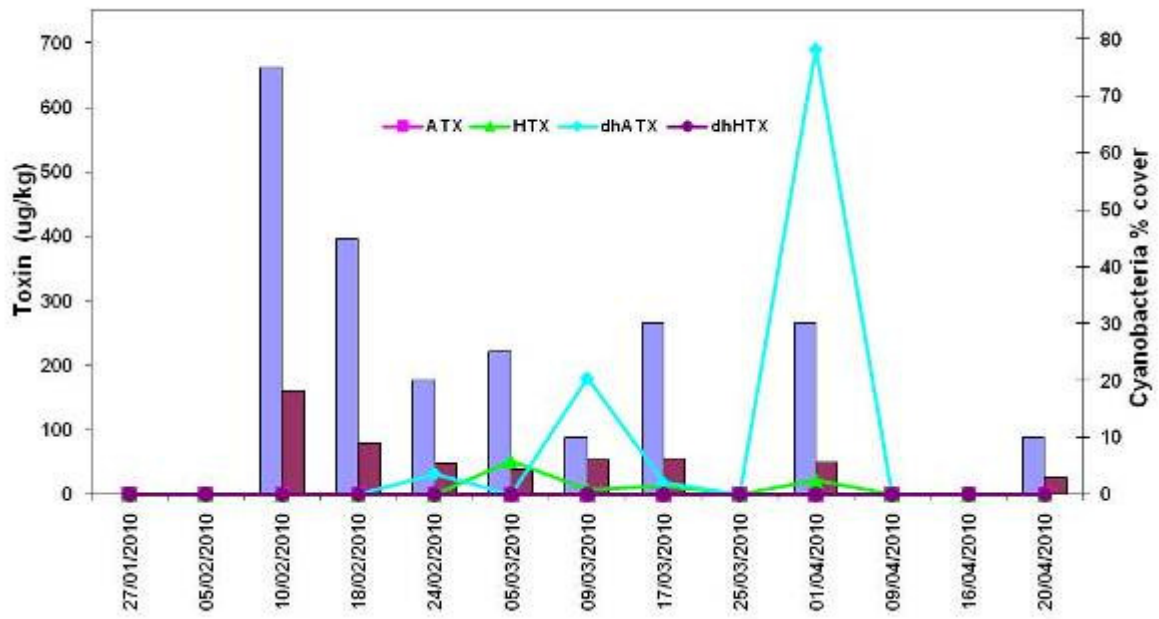


Figure 3 Toxin concentrations and cyanobacterial mat cover (Method 1 – blue bar; Method 2- purple bar) for Site 1 on the Oreti River. ATX – anatoxin-a, HTX – homoanatoxin-a, dhATX – dihydroanatoxin-a, dhHTX – dihydrohomoanatoxin-a.

Site 3 (Waikaia River) was the only site where toxins were detected in all samples (Figure 4). Cyanobacterial abundance was low (<5% visual, <1% transect) at all sampling times (Figure 4). The highest toxin concentrations were detected on 16 March 2010 (19.94 $\mu\text{g}/\text{kg}$ ATX, 132.66 $\mu\text{g}/\text{kg}$ HTX, 456.8 $\mu\text{g}/\text{kg}$ dhATX, 29.86 $\mu\text{g}/\text{kg}$ dhATX) and correlated with the highest cyanobacterial abundance (5% visual, 1% transect) recorded at this site (Figure 4).

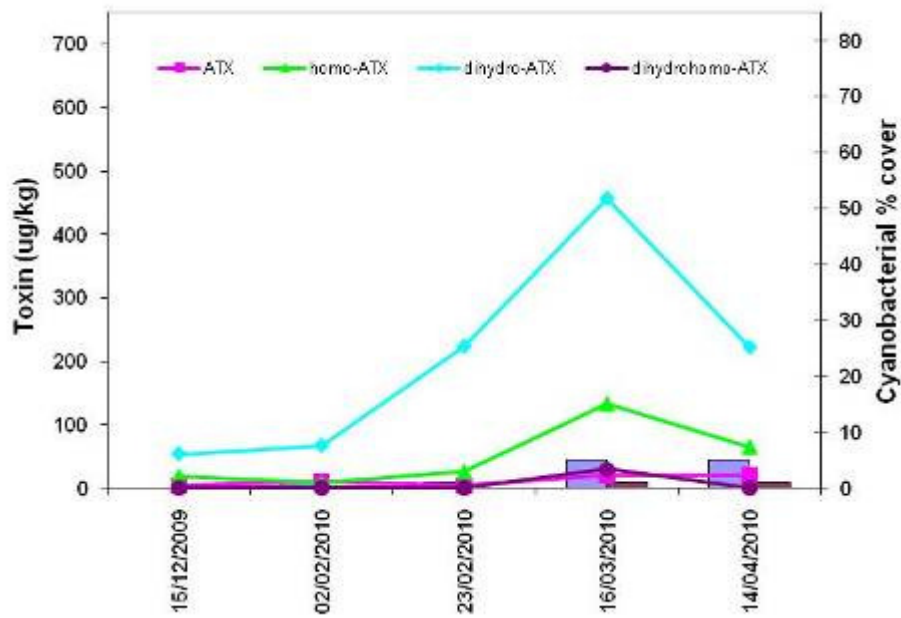


Figure 4 Toxin concentrations and cyanobacterial mat cover (Method 1 – blue bar; Method 2- purple bar) for Site 3 on the Waikaia River. ATX – anatoxin-a, HTX – homoanatoxin-a, dhATX – dihydroanatoxin-a, dhHTX – dihydrohomoanatoxin-a.

Toxins were detected in three of the four samples collected from Site 4 (Mataura River). The highest toxin concentrations were measured on 13 April 2010 (438.29 $\mu\text{g}/\text{kg}$ HTX, 36.24 $\mu\text{g}/\text{kg}$ dhATX). The highest cyanobacterial abundance was recorded on 18 February 2010 (30% visual, 20% transect; Figure 5).

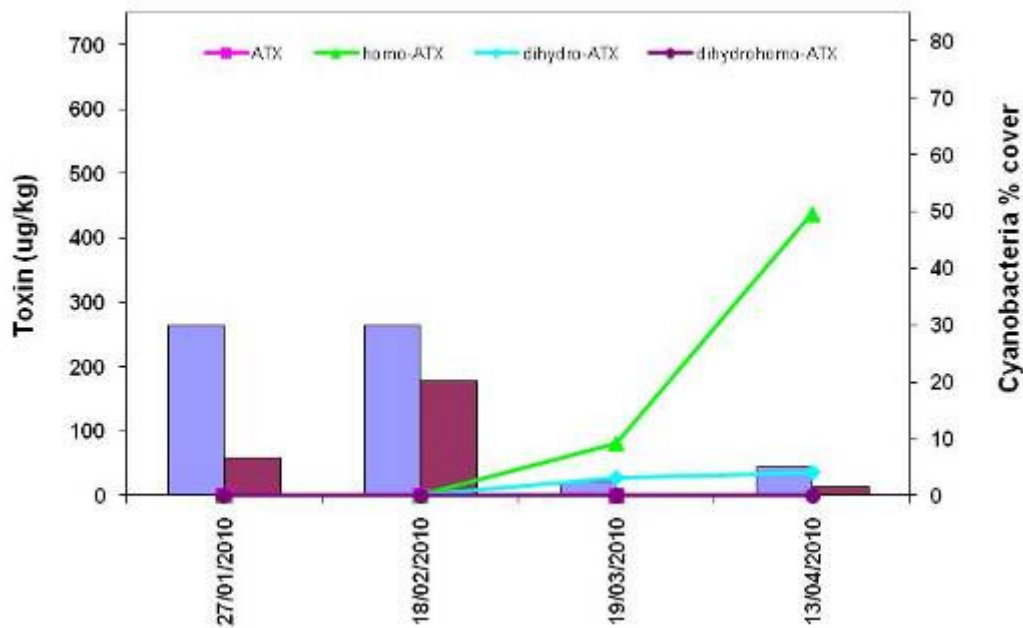


Figure 5 Toxin concentrations and cyanobacterial mat cover (Method 1 – blue bar; Method 2- purple bar) for Site 4 on the Mataura River. ATX – anatoxin-a, HTX – homoanatoxin-a, dhATX – dihydroanatoxin-a, dhHTX – dihydrohomoanatoxin-a.

3.3. River flow, water temperature, cyanobacterial mat abundance and toxin detection

River flow was negatively correlated with cyanobacterial mat cover (Figures 6 and 7a). Increases in river flow resulted in the decrease or total removal of cyanobacterial mats. For example at Site 1 (Oreti River) a decrease in cyanobacterial mat abundance from 75% (10 February 2010) to 40% (18 February 2010) followed an increase in river flow from half the median flow to a median flow. Likewise, cyanobacterial mat cover decreased from 30% (1 April 2010) to 0% (9 April 2010) following a river flow that peaked at over six times the median flow (Figure 6).

Cyanobacterial mats were detected at all sites across a wide range of water temperatures with no obvious differences in mat abundances (Figure 7a). For example, at Site 1 (Oreti River) cyanobacteria abundance was 20% on 24 February 2010 with a water temperature of 18°C. On 1 April 2010 water temperature had dropped to 12.5°C, however cyanobacterial mat cover was 30% (Figure 6).

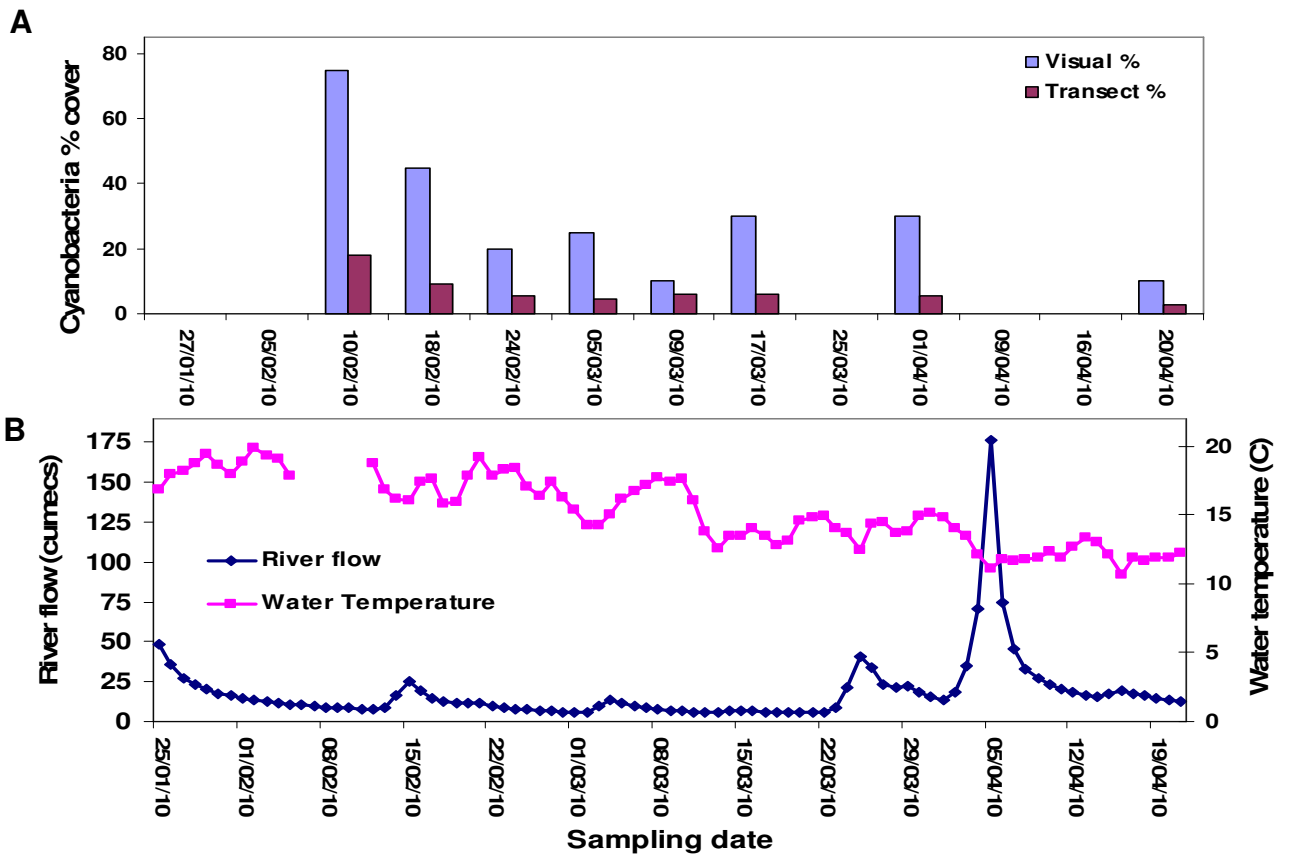


Figure 6 Site 1 (Oreti River). (A) Cyanobacterial mat percentage coverage using Method 1 (visual) and Method 2 (transect). (B) River flow and water temperature.

On only one occasion ATX/HTX was detected at river flows below the median (Figure 7b). Anatoxin-a and HTX were detected in mats that were present at a wide range of water temperatures between 17°C and 8°C (Figure 7b).

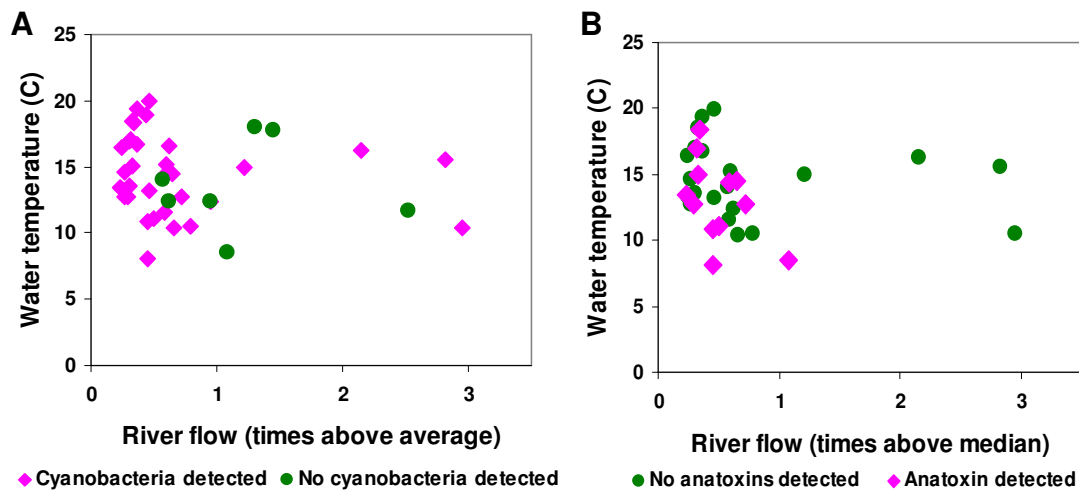


Figure 7 (A) Water temperature versus river flow showing the presence/absence of cyanobacterial mats. (B) Water temperature versus river flow showing the presence/absence of anatoxin-a and homanatoxin-a at sites where cyanobacterial mats were detected.

4. DISCUSSION

4.1. Comparison of site survey methods

The two site survey methods assessed differed markedly in their estimate of cyanobacterial mat coverage. The visual method (Method 1) gave considerably higher coverage estimates than the transect method (Method 2) in every instance in this study. The transect method possibly provides a more representative indication of the cover over an entire site, however may miss “pockets” of high cyanobacterial abundance. Given that high abundance areas may pose the highest human and animal health risk we recommend that in addition to the transect method dense patches of coverage are recorded and that discretion is used when determining the alert level status of a site. If doubt exists, the higher alert level should be used. For example, if the transect method indicates a green mode but several dense patches of cyanobacterial mat are present, an orange or red mode should be issued. Additionally, flexibility in the selection of transect locations between sampling periods may be necessary to ensure that at least some transects are positioned in areas with high mat abundance. At sites where high water velocity prevents transects the visual approach may be necessary.

4.2. Variability in anatoxin-a and homanatoxin-a concentrations

Toxin concentrations varied between rivers and among sampling times within rivers. This result is consistent with several recent studies. During research on *Phormidium* mats in the Hutt River (Lower Hutt) ATX and HTX concentrations varied markedly among sampling sites and over short time frames, *e.g.*, a week (Heath *et al.* 2010b). In a second study Wood *et al.* (2010b) sampled seven rivers in New Zealand and showed fine-scale spatial variability of ATX and HTX within 10 × 10 metre grids. Of the seven sites sampled, there was only one site where all samples contained detectable levels of ATX and HTX. At three sites, both toxic and non-toxic samples co-occurred and mats less than 1 metre apart varied in ATX and HTX content. This finding has led to the suggestion that at least ten samples are collected to determine the approximate ATX/HTX concentrations at a site. Although no toxins were detected at two sites in this study we recommend that toxin testing be continued at these sites, or that mats at these sites are regarded as potentially toxic. Our recent research has shown that the presence of toxins at a site can vary from one year to the next (Wood, unpub. data).

The results of this study showed that there was no correlation between the percentage cover of *Phormidium* mats and presence/absence of ATX and HTX or the concentrations of these toxins. These results are consistent with other recent studies (Heath *et al.* 2010b, Wood *et al.* 2010b).

The recent New Zealand cyanobacterial guidelines (Ministry for the Environment & Ministry of Health 2009) uses a three-tier alert level framework that uses cyanobacterial abundance and the occurrence of mats visibly detaching from the substrate to determine the alert level status. Anatoxin-a and HTX detection is not currently included as part of the rationale for determining alert level. Our recent research suggests the presence of cytotoxic compounds affecting mammalian cells in multiple *Phormidium* species collected around New Zealand (Wood, Froscio & Campbell, unpublished data). Therefore, we recommend that health warnings should not rely solely on the presence of known cyanotoxins and that the percentage cover of benthic mats within a river should be used as a predictor of human health risk. Additionally, under certain environmental conditions *e.g.*, prolonged periods of low and stable flow, or as mats become thicker (and bubbles of oxygen become entrapped within the mats), cyanobacteria detach from the substrate and may accumulate along river edges. Cyanobacterial accumulations along river edges result in higher risk to human and animal health due to the increased probability of river users coming into contact with cyanobacterial material. If protecting animal health, *i.e.*, dogs, is an important consideration then some monitoring of ATX/HTX concentrations is recommended.

The toxin concentrations in this study were relatively low compared to those from a survey of seven rivers throughout New Zealand (Wood *et al.* 2010b). Wood *et al.* (2010b) measured a maximum total anatoxin concentration of 12 800 µg/kg compared with 640 µg/kg in this study. The lower result in this study may be because 10 samples were pooled at each site; for example, if five samples from a site contained no toxins, the pooled average would be reduced. In Wood *et al.* (2010b) all samples were analysed individually.

4.3. River flow, water temperature, cyanobacterial mat abundance and toxin detection

Previous investigations of benthic cyanobacterial proliferations in New Zealand have shown that proliferations generally occur in the summer months when water temperatures are elevated and river flows are low (Biggs 1990, Wood *et al.* 2007, Heath *et al.* 2010a, b). In this study winter data was not collected therefore it was not possible to determine if this trend also occurs in Southland. However, all five sites surveyed had high abundances of *Phormidium* mat cover for at least several weeks during the summer.

River flow had a major influence on the cyanobacterial percentage cover at all five sites. Heath *et al.* (2010b) showed that in the Hutt River (Wellington) mats were present predominantly when river flows were below the yearly mean. This is consistent with the current study where cyanobacterial mats were found in the most part below the median flow. The ability of river flow to flush *Phormidium* sp. mats has led the Wellington Regional Council to designate river flow as one of the factors used to predict *Phormidium* mat abundance (Milne & Watts 2006). Two weeks without a river flow of three times the median is used as an early warning indicator of the strong likelihood of benthic *Phormidium* mat proliferation. A similar system may prove useful for Environment Southland. Further information on substrate stability and an in-depth analysis of hydrographs would be required for each river.

In this study *Phormidium* mats were detected at water temperatures between 7.5°C and 20°C, consistent with the findings of Heath *et al.* (2010b) who reported cyanobacterial mats between 8°C and 21°C. *Phormidium* mats are generally assumed to grow fastest at higher temperatures. For example, following a flushing flow, mats will grow back faster in 20°C water as opposed to 10°C water. Further studies are required to determine these temperature thresholds, however, water temperature effects on growth of cyanobacterial mats should be considered when developing early warning or management strategies.

The relationship between water temperature and ATX/HTX presence is variable. In a year-long study of the Hutt River ATX/HTX-containing mats were found primarily in waters above 15°C (Heath *et al.* 2010b). In contrast, ATX/HTX-containing mats in this study were usually detected in temperatures below 15°C. This difference may indicate that the strain(s) of *Phormidium* present in Southland rivers differ from those found in the Hutt River. Heath *et al.* (2010b) suggested that the toxin-producing strains in the Hutt River “outcompeted” non-toxic *Phormidium* strains at temperatures above 15°C. The strain(s) present in Southland rivers appear to dominate at lower temperatures.

5. RECOMMENDATIONS

We recommend that Environment Southland:

- Continue to monitor the five sites sampled in this study, along with any other sites that have high recreational use, using a combination of the transect and visual methods.
- Continue to use cyanobacterial percentage cover to determine the recreational use alert level (as per the national guidelines). This study has shown that ATX and HTX concentrations can vary rapidly and it is safest to presume that all *Phormidium* mats are potentially toxic.
- Develop an early warning system based on river flow and possibly temperature. This would require further analysis of hydrological data and may need to be specific for each river.
- Undertake further analysis of the effect of nutrients, *e.g.*, nitrogen and phosphorus, on mat growth at selected sites.

6. ACKNOWLEDGEMENTS

Michael Boundy (Cawthron) for assistance with LC-MS analysis.

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APPENDIX 1. IN-DEPTH SITE DESCRIPTIONS

These site notes and descriptions were prepared by Environment Southland staff.

Site 1. Oreti River at Wallacetown

This site was sampled on nine occasions over the 13 weeks. There were inconsistencies in transect position due to the discovery of large areas of cyanobacteria outside of the original reach after sampling began. The first two surveys were carried out in the downstream riffle used for the annual biomonitoring survey, but only one of these included toxin samples. Surveys and samples carried out from 10 February 2010 onwards covered a larger area, from under the bridge to the original downstream riffle. Spacing of these transects was irregular given the depths and inherent dangers of the site. The transect cover estimates cannot be relied upon to be representative of the area. This site is not appropriate for the national guideline technique.

There is a hydrological station at this site, and water quality data is sampled adjacent to transect 4, immediately upstream of the bridge. From 1 December 2009 to 1 April 2010 weekly water sampling is also carried out in the pool above transect 2, to ensure safe water quality for recreation.

Sampled:	Weekly
Median flow:	28.182 m ³ /s
Reach length:	~100 m
Reach width:	~60 m
Pressures:	Site is located at the bottom of a very large and intensively farmed catchment, with treated sewage discharges and gravel extraction.
Transect 1 (d/s):	In the centre of the downstream riffle in TL channel. This entire riffle had all four transects for the first two surveys. The site had only black cyanobacteria. The entire channel could be surveyed at all levels.
Transect 2:	Surveyed from TL bank. Rapid on TR of transect, riffle on TL. Could not get across channel even at low flows. This was at the base of a very large and deep pool. This transect had only black cyanobacteria.
Transect 3:	Very rough shallow rapid. Could only get half way across channel due to high velocities. The site had both black and green cyanobacteria.
Transect 4 (u/s):	Run/head of rapid in line with upstream edge of bridge. Could get right across and has both black and green cyanobacteria.



Figure 8 Oreti River at Wallacetown. Downstream, transect 1 (left), and upstream from transect 1 (right).

Site 2. Makarewa River at Wallacetown

Transect positions did not change throughout the survey period. Sampling was performed successfully for 13 weeks, with only one sample batch being reduced in size due to lack of cover. Cyanobacteria at this site was black.

Hydrological data for this site has been taken from Makarewa River at Counsell Road, approximately 10-15 kilometres upstream of the site. Water quality data was sampled approximately 200 metres downstream, on the other side of the bridge.

Sampled: Weekly
 Median flow: 7.723 m³/s
 Reach length: 26 metres
 Reach width: 18-27 metres
 Pressures: Site is located at the bottom of an intensively farmed catchment. There is also a treated sewage discharge upstream of this site.

Transect 1 (d/s): Pool on right bank and slight run on left. Pool commonly had other stagnant type algae that prevented cyanobacteria habitation. Transect too deep to get across.

Transect 2: Bottom end of riffle. The riffle increased across transect in lower flows. Riffle is diagonal to channel. Can wade all the way across, in all flows.

Transect 3: Middle/top edge of riffle, depending on flow. At low flow it becomes middle, and at higher flows it is top edge of riffle. Can wade all the way across in low flows only.

Transect 4 (u/s): Riffle head, or run above riffle at higher flows. Can wade all the way across.



Figure 9 Makarewa River at Wallacetown, looking downstream from just above transect 4 (left) and upstream from just below transect 1 (right).

Site 3. Waikaia River up stream of Piano Flat

This site had safe and had easy access. Transect positions did not change throughout the survey period. Despite supporting abundant cyanobacteria the previous summer, this year cover was very sparse. However sampling was performed successfully for 5 months, with the first sample and 2 vials of the second sample taken 200 m downstream, due to lack of cover. Cyanobacteria at this site was brown.

Hydrological data for this site has been taken from Waikaia River at Piano Flat, approximately 2.5 kilometres downstream from the site. Water quality data was sampled at the cyanobacteria site.

Sampled	Monthly
Median flow:	8.596 m ³ /s
Reach length:	40 m
Reach width:	30 m
Pressures:	Site is in the upper reaches of the Waikaia River, and is fed by a pristine native bush catchment. Low impact/reference site.

Transect 1 (d/s):	Run. Complete crossing.
Transect 2:	Run, and riffle at low flow. Complete crossing.
Transect 3:	Middle of riffle. Complete crossing.
Transect 4 (u/s):	TL of transect is run, TR is head of riffle. Complete crossing.



Figure 10 Waikaia River upstream of Piano Flat looking downstream (left), and upstream (right). Sampler (left) is approximate position of transect 2.

Site 4. Mataura River 200 m downstream of Mataura

This site unpleasant and difficult to sample due to the slipperiness and the deep rapid in the centre of the channel. The original survey was carried out in the TL channel as it was the only area of riffle that was accessible. Unfortunately this channel dried up so transects were relocated directly across into the main stem on 18 February 2010. At its lowest flow (19 March 2010) transects 1 and 2 were very shallow and had a lot of exposed rock. Full samples were taken with all four surveys. No transects were able to cross the full channel. Cyanobacteria at this site was initially an olive colour but was black later in the season.

Hydrological data for this site has been taken from Mataura River at Tuturau, approximately 8 kilometres downstream from the site. Water quality data was sampled at this site.

Sampled	Monthly
Median flow:	55.754 m ³ /s
Reach length:	40 m
Reach width:	40-50 m
Pressures:	Site is located in the mid-lower reaches of a very large and intensively farmed catchment. It has major industrial discharges (<i>i.e.</i> Alliance Meat Works) immediately upstream, along with treated sewage inputs.

Transect 1 (d/s):	Riffle at low flow, beginning to pool when higher.
Transect 2:	Mid-riffle, TR of transect a raised rocky area before deep unsafe rapid.
Transect 3:	Top edge of riffle area.
Transect 4 (u/s):	Run, or TR of transect a riffle in low flows.



Figure 11 Mataura River 200 m downstream. Mataura, looking upstream from below the bottom transect (left), and downstream from above the top transect (right).

Site 5. Aparima River at Thornbury

This site has a bed of loose gravel that is easily disturbed; hence cyanobacteria mats were often easily flushed away. Four surveys were done, but only two yielded enough material for toxin samples. Cyanobacteria at this site was black.

There is a hydrological station beneath the bridge at this site, and water quality data is sampled approximately 200 m downstream, on the other side of the bridge.

Sampled: Monthly
Median flow: 15.658 m³/s
Reach length: 60 m
Reach width: 35 m
Pressures: This site is at the bottom of a large farmed catchment, with treated sewage discharges.

Transect 1 (d/s): Head of pool, too deep to cross.
Transect 2: TL is top of riffle, TR is mid-riffle. Willows on TR make it too deep to cross.
Transect 3: Head of riffle on TR, and end of run on TL.
Transect 4 (u/s): Base of run.



Figure 12 Aparima River at Thornbury, looking upstream with bucket level with transect 1 (left) and looking downstream (right).

APPENDIX 2. TOXIN DATA

	Date	% coverage		Toxin concentrations (µg/kg dw)				TOTAL
		Visual %	Transect	ATX	dhATX	HTX	dhHTX	
Site 1 - Oreti River	27/01/2010	0	0	NS	NS	NS	NS	
	05/02/2010	<1	0	NS	NS	NS	NS	
	10/02/2010	75	18.15	0	0	0	0	0
	18/02/2010	45	9.05	0	0	0	0	0
	24/02/2010	20	5.4	0	31.9	0	0	31.9
	05/03/2010	25	4.45	0	0	51.02	0	51.02
	09/03/2010	10	5.94	0	180.12	7.52	0	187.64
	17/03/2010	30	6.2	0	17.66	12.69	0	30.35
	25/03/2010	0	0	NS	NS	NS	NS	
	01/04/2010	30	5.6	0	690.74	21.05	0	711.79
	09/04/2010	0	0	NS	NS	NS	NS	
	16/04/2010	0	0	NS	NS	NS	NS	
	20/04/2010	10	2.85	0	0	0	0	0
Site 2 - Makarewa River	27/01/2010	>5	1	0.0	0.0	0.0	0.0	0.0
	05/02/2010	5	2.4	0.0	0.0	0.0	0.0	0.0
	10/02/2010	20	6.35	0.0	0.0	0.0	0.0	0.0
	18/02/2010	15	7.35	0.0	0.0	0.0	0.0	0.0
	24/02/2010	50	12.35	0.0	0.0	0.0	0.0	0.0
	05/03/2010	60	19.55	0.0	0.0	0.0	0.0	0.0
	09/03/2010	80	28	0.0	0.0	0.0	0.0	0.0
	17/03/2010	60	16.25	0.0	0.0	0.0	0.0	0.0
	25/03/2010	45	16	0.0	0.0	0.0	0.0	0.0
	30/03/2010	50	20.25	0.0	0.0	0.0	0.0	0.0
	09/04/2010	>1	0	NS	NS	NS	NS	
	13/04/2010	10	2.6	0.0	0.0	0.0	0.0	0.0
	20/04/2010	10	4.4	0.0	0.0	0.0	0.0	0.0

	Date	% coverage		Toxin concentrations ($\mu\text{g}/\text{kg dw}$)				TOTAL
		Visual %	Transect	ATX	dhATX	HTX	dhHTX	
Site 3 - Waikaia River	15/12/2009	0	0	3.5	53.7	18.9	0.0	76.1
	02/02/2010	<1	0.05	10.2	66.9	8.9	0.0	86.0
	23/02/2010	<1	0	4.4	223.3	26.5	0.0	254.2
	16/03/2010	5	0.9	19.9	456.8	132.7	29.9	639.3
	14/04/2010	<5	0.9	21.1	221.9	65.4	0.0	308.4
Site 4 - Maitai River	27/01/2010	30	6.75	0.0	0.0	0.0	0.0	0.0
	18/02/2010	30	20.25	0.0	0.0	0.0	0.0	0.0
	19/03/2010	2.5	0.25	0.0	27.6	81.5	0.0	109.1
	13/04/2010	5	1.6	0.0	36.2	438.3	0.0	474.5
Site 5 - Aparima River	27/01/2010	0	0	NS	NS	NS	NS	
	23/02/2010	70	17.4	0.0	0.0	0.0	0.0	0.0
	17/03/2010	50	17.75	0.0	0.0	0.0	0.0	0.0
	13/04/2010	0	0	NS	NS	NS	NS	

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Total Editing Time: 2 Minutes
Last Printed On: 4/04/2011 4:13:00 p.m.
As of Last Complete Printing
Number of Pages: 24
Number of Words: 5,073 (approx.)
Number of Characters: 28,921 (approx.)