Benthic Cyanobacteria and Toxin Production in the Manawatu-Wanganui Region

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CAWTHRON

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RESEARCH BASED SOLUTIONS

Benthic Cyanobacteria and Toxin Production in the Manawatu-Wanganui Region

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EXECUTIVE SUMMARY

Benthic, mat-forming cyanobacteria are common throughout New Zealand rivers. The most widespread mat-forming genus is *Phormidium*. In New Zealand *Phormidium* is known to produce two neurotoxic compounds; anatoxin-a (ATX) and homoanatoxin-a (HTX). These toxins are a health threat to humans and animals when consumed or when there is contact with contaminated water. There have been numerous dog deaths and health warnings resulting from proliferations of toxic *Phormidium* in New Zealand's rivers.

Marked differences in the presence of *Phormidium* mats, and the presence and concentrations of toxins within mats, have been observed both within and between rivers. Until recently there has been limited knowledge on mechanisms leading to *Phormidium* proliferations and on why some mats contain toxins and others do not.

Phormidium prevalence in the Manawatu-Wanganui region has been notable over the past few years. This is of particular concern in rivers that are used as drinking water sources or at sites used for recreational activities. Prior to this study there was limited information on possible reasons for variability in *Phormidium* abundance among Manawatu-Wanganui Rivers and no toxins had been detected in *Phormidium* from this region, although only a limited number of samples had been analysed.

Phormidium abundance was surveyed (weekly or monthly) at fourteen sites from ten rivers. When mats were present, samples comprised of sub-samples pooled from ten *Phormidium* mats, were collected. The pooled samples were analysed for ATX, HTX and their degradation products using liquid chromatography-mass spectrometry. Water temperature was recorded and samples were collected monthly for analysis of nitrite (NO_2-N), nitrate (NO_3-N), ammonia (NH_4-N), total nitrogen (TN), dissolved reactive phosphorus (DRP) and total phosphorus (TP).

Phormidium mats were present in variable abundances at all 14 sampling sites. Five sites had *Phormidium* coverage at > 30% on at least one sampling occasion. Only three sites had extended periods of *Phormidium* coverage at > 20%; Makakahi at Hamua, Mangatainoka at State Highway 2 (SH2) and Tiraumea at Ngaturi. Anatoxin-a, HTX and their degradation products were detected in variable concentrations in samples from 11 sites. High concentrations of toxins were detected in two samples; Mangawhero at Pakahi Rd (26.6 mg/kg FDW, 12 April 2011), and Mangatainoka at SH2 (14.7 mg/kg FDW, 25 February 2011). Concentrations in most other samples were low (<5 mg/kg FDW). None of the mats from Tamaki at Stephensons, Oroua upstream of the sewerage treatment plant (STP) and Oroua downstream of STP contained detectable levels of toxins despite a relatively high percentage of *Phormidium* cover at the Oroua downstream of the STP site.

The World Health Organisation does not believe there is enough toxicological data available for ATX or HTX to develop drinking water guidelines for this toxin. Research by Fawell *et al.* (1999) suggested that a guideline value of 1 µg/L of ATX would be appropriate for drinking water and would provide a margin of safety of around three orders of magnitude. The New Zealand Ministry of Health has set provisional maximum acceptable values (PMAVs) in drinking water of 6 µg/L for ATX and 2

ug/L for HTX (New Zealand Ministry of Health, 2005). The toxins measured in this study were in *Phormidium* mats. It is not possible to relate these levels to the PMAVs, *i.e.,* the levels of toxins in the mats do not provide any information on likely concentrations in the water column. Until recently, little was known about the release of ATX and HTX from benthic cyanobacteria into the water column (*i.e* the extracellular component). Recent studies (Wood *et al.* 2010c) have shown that ATX and HTX are released into the water from cyanobacterial mats, either through active export from the cells or as a result of cell lysis. This result demonstrates that levels of ATX/HTX in river water can reach concentrations that may be hazardous to humans and animals, especially if the water is used for drinking.

The majority of 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). The large majority of positive samples in this study were less than 5 mg/kg FDW. Two samples (26.6 mg/kg FDW, Mangawhero at Pakahi Road, 12 April 2011and 14.7 mg/kg FDW, Mangatainoka at SH2, 25 February 2011), however, had total toxin concentrations that were higher than those measured in Wood *et al*. (2010b). The generally lower results 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.

There was no correlation between percentage coverage of *Phormidium* mats and temperature or the presence of toxins and water temperature. There was also no correlation between percentage coverage of *Phormidium* mats and the amount of total toxin measured. It is recommended 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. 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 knowledge of ATX/HTX concentrations and how these fluctuate at specific sites is recommended.

The four highest dissolved inorganic nitrogen (DIN): dissolved reactive phosphorus (DRP) and TN:TP ratios were observed at sites where *Phormidium* mat coverage exceeded 30% on at least one sampling occasion (Oroua downstream of STP, Mangatainoka at SH2, Mangatainoka upstream Tiraumea, Tiraumea at Ngaturi). This suggests that nitrogen, rather than phosphorus, may be limiting *Phormidium* growth. The Oroua upstream and downstream of the STP sites provide further evidence to support this hypothesis. The upstream site has low *Phormidium* coverage and a TN:TP ratio of 6. The STP discharge adds substantial amounts of nitrogen increasing the TN:TP ratio to 40. There is a corresponding increase in *Phormidium* coverage suggesting that upstream of the STP *Phormidium* proliferation is nitrogen-limited.

In summary this study has shown that:

- Toxic *Phormidium* mats occur in rivers in the Manawatu-Wanganui region.
- Percentage coverage of *Phormidium* mats, and the presence of toxins within these mats, is patchily distributed both spatially and temporally.
- There is no relationship between percentage coverage of *Phormidium* mats and the presence or levels of toxins.
- At some sites the frequency and intensity of flushing events could be used to predict the likelihood of *Phormidium* mats occurring. Longer term datasets are required to develop this.
- DIN:DRP and TN:TP ratios may be useful in predicting the likelihood of *Phormidium* proliferations at a site. Larger and longer-term datasets are required to explore these relationships further.
- Anatoxin-a and HTX may pose a risk to drinking water supplies that take water from affected rivers. Further studies are required.

A literature survey identified the following knowledge gaps that should be addressed in future studies:

- There is no information on whether ATX/HTX can accumulate in edible aquatic species in New Zealand *e.g.*, koura and trout.
- There is no data available on the effect of *Phormidium* mats (both toxic and non-toxic) on biodiversity and ecosystem health *e.g.*, macro-invertebrate abundance and composition.

We recommend that Horizons Regional Council:

- Continues to monitor cyanobacterial abundance using MfE and MoH (2009) protocols at the regular periphyton monitoring sites, along with any other sites that have high recreational use. Samples should be collected throughout the year.
- Continues to use cyanobacterial percentage cover to determine the recreational use alert level (as per the national guidelines).
- Undertakes a study to determine the risk *Phormidium* mats pose to drinking water supplies in the region. A possible site for an initial study is the Pahiatua water supply which is approximately four kilometres upstream of the Mangatainoka at SH2 site (where toxins were detected). This study should include site surveys, toxin testing of *Phormidium* mats and grab samples, and the use of SPATT.
- Continues to develop an early warning system based on river flow and temperature. For all sites this will require a longer-term dataset. Sites with high recreational use should be given priority for development of these systems. Site surveys should be undertaken at least weekly.
- Undertakes further analysis of the effect of nutrient concentrations and ratios on mat growth at selected sites. Nutrients that should be analysed include; NO_2-N, NO_3-N, NH_4-N, DIN, TN, DRP and TP. At these sites *Phormidium* coverage and a suite of physiochemical variables would need to be monitored weekly over six to twelve months.

- Undertakes a study on the effect of the Oroua STP discharge on *Phormidium* coverage. The data in this study suggests that the Oroua STP discharge appears to have a marked effect on the abundance of *Phormidium* mats. The Oroua sites (upstream and downstream of the STP) provide a unique opportunity to explore the influence of water chemistry and nutrient concentrations and ratios on *Phormidium* abundance.
- Undertakes studies to establish if ATX and HTX accumulate in edible species in New Zealand *e.g.*, koura and trout.
- Undertakes a study on the effect of *Phormidium* mats on biodiversity and ecosystem health *e.g.*, their effect on the abundance and species composition of macro-invertebrates.

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1. INTRODUCTION

Benthic, mat-forming cyanobacteria are common throughout New Zealand rivers (Biggs & Kilroy 2000). The most widespread mat-forming 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*. 2010, 2011). 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).

Until recently there has been limited knowledge on mechanisms leading to *Phormidium* proliferations. Heath *et al*. (2011) showed that river flow and water temperature could be used to predict *Phormidium* mat coverage in the Hutt River, Wellington. They also suggested that sites with high TN:TP ratios (above 20:1) were more likely to have extensive coverage of *Phormidium*. Toxin analysis of *Phormidium* mats from across New Zealand has shown that the occurrence of ATX and HTX is variable within and among rivers and that there is no correlation between the coverage of *Phormidium* mats and ATX/HTX concentrations (Wood *et al.* 2010b). Using multiple strains of cultured *Phormidium* sourced from rivers throughout New Zealand, Heath *et al*. (2010) showed that toxic and non-toxic genotypes co-occur in *Phormidium* mats. This co-occurrence may partially explain the variability in ATX/HTX concentrations reported for *Phormidium* mats. Variables regulating the abundance of each genotype within a mat are unknown, although Health *et al.* (2011) only detected toxins in mats when temperatures were above 13.4ºC.

Benthic cyanobacteria prevalence in the Manawatu-Wanganui region has been notable over the past few years (see Appendix 1 for photographic examples of *Phormidium* proliferation in this region). In some cases the presence of benthic cyanobacteria has lead to closure of swimming spots in accordance with the New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters (Ministry for the Environment and Ministry of Health 2009). Benthic cyanobacteria have also been recorded as prevalent in the vicinity of some public water supply intakes within the Manawatu-Wanganui Region. There is concern within the community that cyanobacteria may contaminate food sources from the river. Little is known about reasons for the prevalence of benthic cyanobacteria in the Manawatu-Wanganui region, nor if cyanobacteria in this region produce toxins. Prior to this study approximately 10 cyanobacterial mat samples collected from the Manawatu-Wanganui region had been tested and no toxins had been detected.

The specific aims of this study were to:

- Provide an overview of the existing knowledge of the risks of benthic cyanobacteria presence to water supplies, food sources, swimming and recreation, native and introduced fish populations and effects on aquatic biodiversity;
- Investigate spatial and temporal changes in ATX and HTX concentrations at 14 sites in ten Manawatu-Wanganui rivers;
- Improve knowledge on the environmental parameters regulating cyanobacterial mat formation at the investigated sites;
- Provide recommendations for future management and monitoring of benthic cyanobacteria in the Manawatu-Wanganui region.

2. METHODS

2.1. Sample locations

One or two sites at each of 10 rivers (Makotuku, Manawatu, Mangatainoka, Mangawhero Makakahi, Ohau, Oroua, Tamaki, Tiraumea, Tokomaru) were 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 ten sites and monthly at four sites (Figure 1) between 14 January 2011 and 6 May 2011.

2.2. Site surveys

All site surveys and samplings were undertaken by Horizons Regional Council staff. The transect method, outlined in the New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters (Ministry for the Environment and Ministry of Health 2009), was used to determine the percentage of the river substrate covered by cyanobacterial mats.

The surveys conducted in this study were based in stream/river runs and riffles. The length of the river surveyed varied from 40 metres to 120 metres. 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.

The size and percentage of cover of substrate types was assessed along the transects. An average of the different substrate types was calculated for each site.

Long-term median flows for each site are given in Appendix 2.

2.3. Cyanobacterial sample collection and preparation

At each site a single sample was collected by scraping mat material from ten rocks into a plastic container. These were transported, chilled and in the dark, to Horizons Regional Council where they were frozen (-20°C). Samples were later sent frozen to Cawthron.

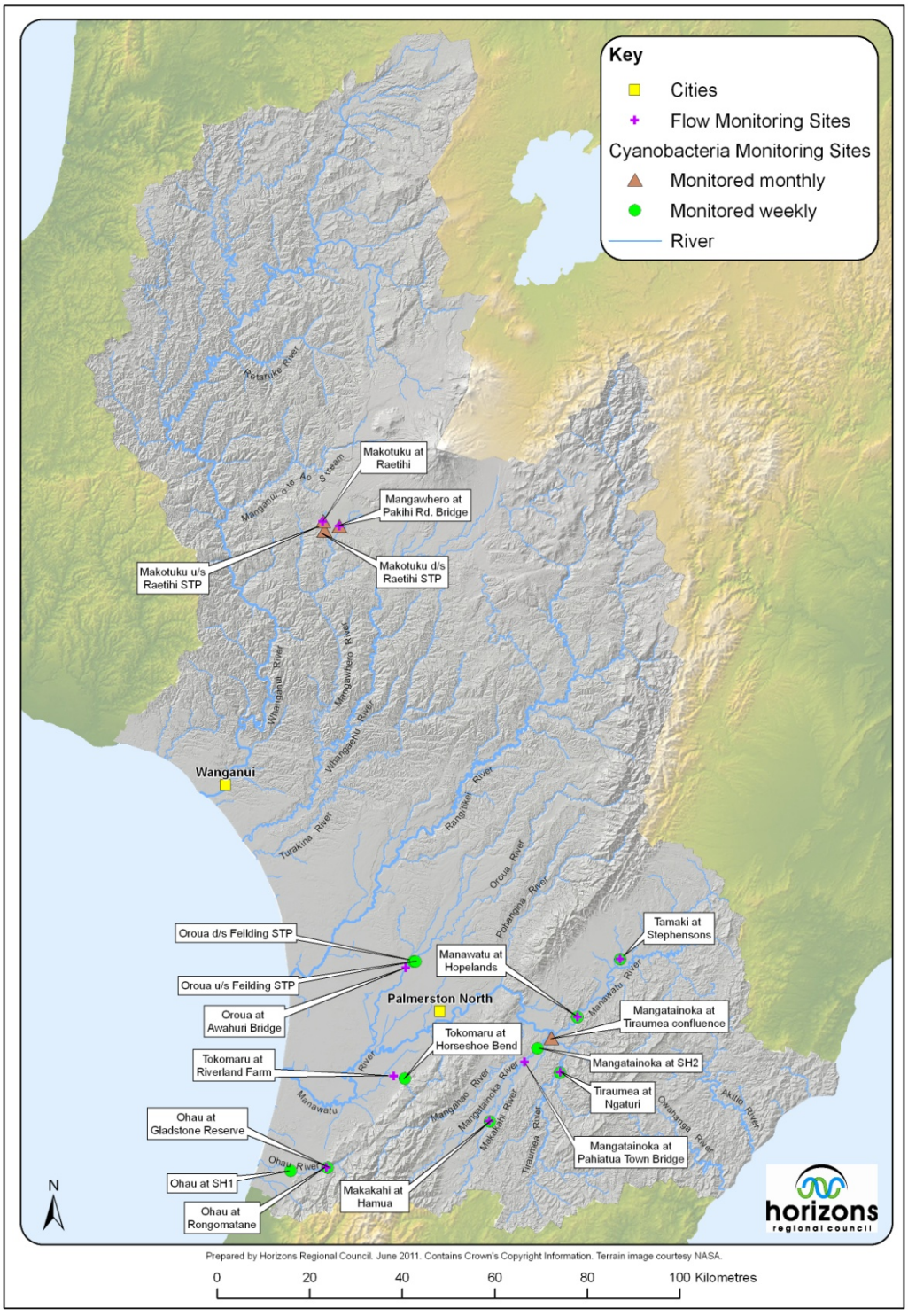

Figure 1. Location of sampling and flow monitoring sites used in this study, Manawatu-Wanganui Region, New Zealand. Oroua at Awahuri is a modelled flow site. STP = sewerage treatment plant, SH1/2 = state highway 1/2.

2.4. Toxin extraction and analysis

Cyanobacterial mat samples were thawed and the samples from each site were homogenised and lyophilized (freeze-dried).

Lypholized 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, NZ) for 20 minutes. Samples were centrifuged (3000 × *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.* (2010).

2.5. Water temperature, conductivity and river flow

Water temperature was measured at each collection using YSI Professional Plus handheld meters.

River flow was measured at State of the Environment flow recording sites and in accordance with Horizons ISO 1000- 1 and 1100-2 system (Roygard *et al.* 2011). The location of these loggers is shown in Figure 1. Daily mean flow data was used for Figures 2 to 11.

2.6. Nutrient data

Water samples were collected monthly at every site, with the exception of Ohau River at SH1 (where no samples were collected), to determine nitrite-N, nitrate-N, ammonia-N, total nitrogen, dissolved reactive phosphorus and total phosphorus. Water sampling was not always conducted on the same date as the closest of the weekly cyanobacterial sampling, but usually within two to four days. Samples were analysed by Watercare Laboratory Services. The small datasets prevented statistical analysis of temporal associations between water quality and *Phormidium* abundances. The available nutrient data (four or five records per site) was averaged for each site and used to explore relationships between nutrients and sites with less than or greater than 30% *Phormidium* mat coverage.

3. RESULTS

3.1. Substrate at each site

The dominant substrate size varied among sampling sites with a mixture of boulders, large and small cobbles, and gravels making up the bulk of the substrate at each site (Table 1)

Table 1. Average percentage coverage of different substrates on transects at each sampling sites. Values are average of four monthly measures.

Site name	Bedrock	Boulders (25 cm)	Large Cobbles (12-25 cm)	Small Cobbles (6-12 cm)	Gravels (0.2-6 cm)	Moss substrate	Sands (<0.2 cm)	Silt (fine, not gritty)
Manawatu at Hopelands	0	0	2.5	33.75	47.5	0	3.75	12.5
Makakahi at Hamua	0	0	27.5	37.5	26.25	0	3	5.75
Tamaki at Stephensons	1.25	0.5	16.25	40	37.5	0	3.5	1
Mangatainoka at SH2	1.25	3.75	27.5	36.25	26.25	0	2.5	2.5
Oroua downstream Feilding STP	0	0	11.25	32.5	43.75	0	3.75	8.75
Oroua upstream Feilding STP	0	0	5	28.75	53.75	0	5	7.5
Tiraumea at Ngaturi	0	0	18.75	41.25	25	0	6.25	8.75
Tokomaru at Horseshoe Bend	0	21.25	27.5	25	16.25	0	10	0
Ohau at SH2	0	4.5	16.25	35	34.25	0	10	0
Ohau at Gladstone Reserve	0	8.75	31.25	28.75	21.25	0	10	0
Makotuku upstream Raetihi STP	0	0	19.5	41.25	30	0.5	8.75	0
Makotuku downstream Raetihi STP	0	6.25	23.25	36.25	22.5	0	11.25	0.5
Mangatainoka upstream Tiraumea confluence	0	0	21.25	35	37.5	0	6.25	0
Mangawhero at Pakihi Rd Bridge	0	31.25	22.5	20	10	0	16.25	0

3.2. *Phormidium* mat coverage and anatoxin-a and homoanatoxin-a

Phormidium mats were present in variable abundances at all 14 sampling sites (Table 2; Figures 2 to 11). Five sites had *Phormidium* coverage at > 30% on at least one sampling occasion. Only three sites had extended periods of *Phormidium* coverage at > 20%; Makakahi at Hamua (Figure 3), Mangatainoka at State Highway 2 (SH2; Figure 5), and Tiraumea at Ngaturi (Figure 7).

To simplify visualisation of the toxin data, the concentrations of ATX, HTX and their degradation products detected in each sample were combined and a total toxin concentration given in Table 2 or graphed in Figures 2, 3, 5, 7 to 11. Although anatoxin degradation products are considered non-toxic (Smith & Lewis 1987), they are useful indicators of toxin-producing potential, previous toxic proliferations or nearby toxic mats. Full toxin results showing each variant are given in Appendix 3.

Anatoxin-a, HTX and their degradation products were detected in variable concentrations in the samples from 11 sites (Figures 2 to 11). There were temporal variations in the presence of toxins at the weekly sampled sites. There were only two sites (Ohau at Gladstone and Ohau at SH1) where all samples collected tested positive for toxins (Figure 9A, B). This may be an artefact of the small number of samples collected at these sites (three and two, respectively). None of the mats from Tamaki at Stephensons (Figure 4), Oroua upstream of the sewerage treatment plant (STP) and Oroua downstream of STP (Figure 6A, B) contained detectable levels of toxins, despite a relatively high percentage of mat cover at the Oroua downstream of the STP site.

Brief descriptions of temporal variations in *Phormidium* percentage coverage and toxin concentrations at each site are given below.

Table 2. Summary of number of samples, *Phormidium* mat coverage and toxins detected.

Site	No. of samples	% of samples with mat present	Max. cyanobacterial % coverage	% of mats with toxins	Max. anatoxin (mg kg^{-1})
Manawatu at Hopelands	17	29%	10	20%	0.06
Makakahi at Hamua	17	82%	70	50%	2.25
Tamaki at Stephensons	17	18%	3	0%	N/A
Mangatainoka at SH2	17	82%	50	86%	14.74
Oroua upstream of STP	17	41%	10	0%	N/A
Oroua downstream of STP	17	47%	65	0%	N/A
Tiraumea at Ngaturi	17	76%	60	54%	0.18
Tokomaru at Horseshoe Bend	16	69%	15	45%	1.42
Ohau at Gladstone Reserve	15	27%	5	50%	1.49
Makotuku at Raetihi	4	25%	2	100%	1.35
Makotuku downstream Raetihi STP	4	75%	2	100%	0.35
Mangatainoka upstream Tiraumea	4	75%	50	75%	1.68
Mangawhero at Pakahi Rd	4	100%	25	50%	26.60

3.2.1. Manawatu at Hopelands

The Manawatu at Hopelands site has a popular swimming spot just downstream and in previous years has had extensive *Phormidium* proliferations. *Phormidium* coverage was the highest (10%) on 14 January 2011 (Figure 2). Despite an extended period of relatively stable river flows mat coverage did not exceed 3% for the remainder of the sampling period (Figure 2). Toxins were only detected on one occasion (4 March 2011) from five samples and levels were low (0.06 mg/kg freeze dried weight, FDW; Figure 2).

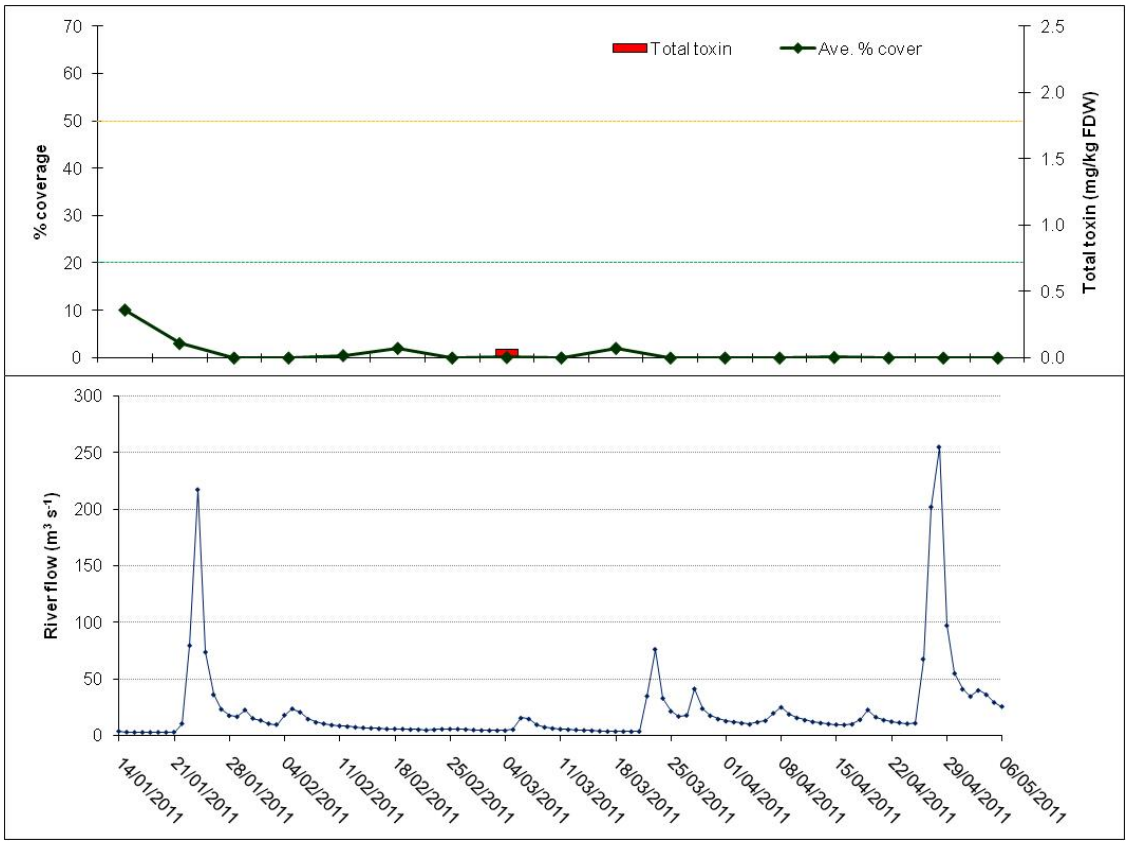

Figure 2. Toxin concentration, *Phormidium* mat cover and river flow for Manawatu at Hopelands. Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.2. Makakahi at Hamua

In February, during a period of low and stable flows, *Phormidium* coverage increased reaching a peak of 70% on 25 February 2011(Figure 3). *Phormidium* coverage was reduced to 10% by 4 March 2011. This decrease did not appear correlated with a significant flushing event. From 23 March 2011 to the final sampling, a series of flushing events maintained *Phormidium* coverage at less than 20% (Figure 3).

Toxins were found in seven out of the 14 mat samples analysed. The highest total concentration (2.25 mg/kg FDW) was recorded in the last sample collected (6 May 2011) when *Phormidium* coverage was only 3%.

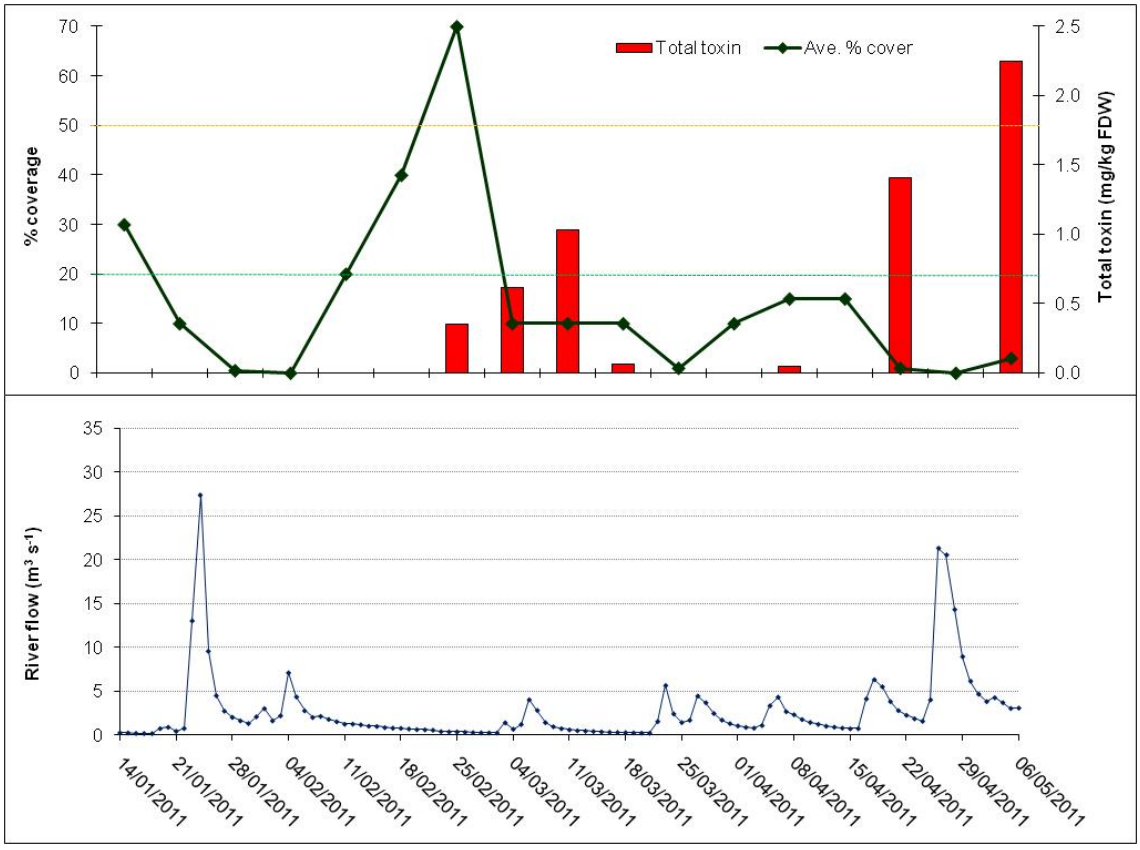

Figure 3. Toxin concentration, *Phormidium* mat cover and river flow for Makakahi at Hamua.
Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.3. Tamaki at Stephensons

This site is located between two large irrigation takes and has had significant growths of *Phormidium* in the past. Despite an extended period of relatively stable river flows, particularly in February 2011 (Figure 4), *Phormidium* mat coverage did not exceed 3% during the sampling period. Only three samples were collected for toxin analysis and all contained no toxins.

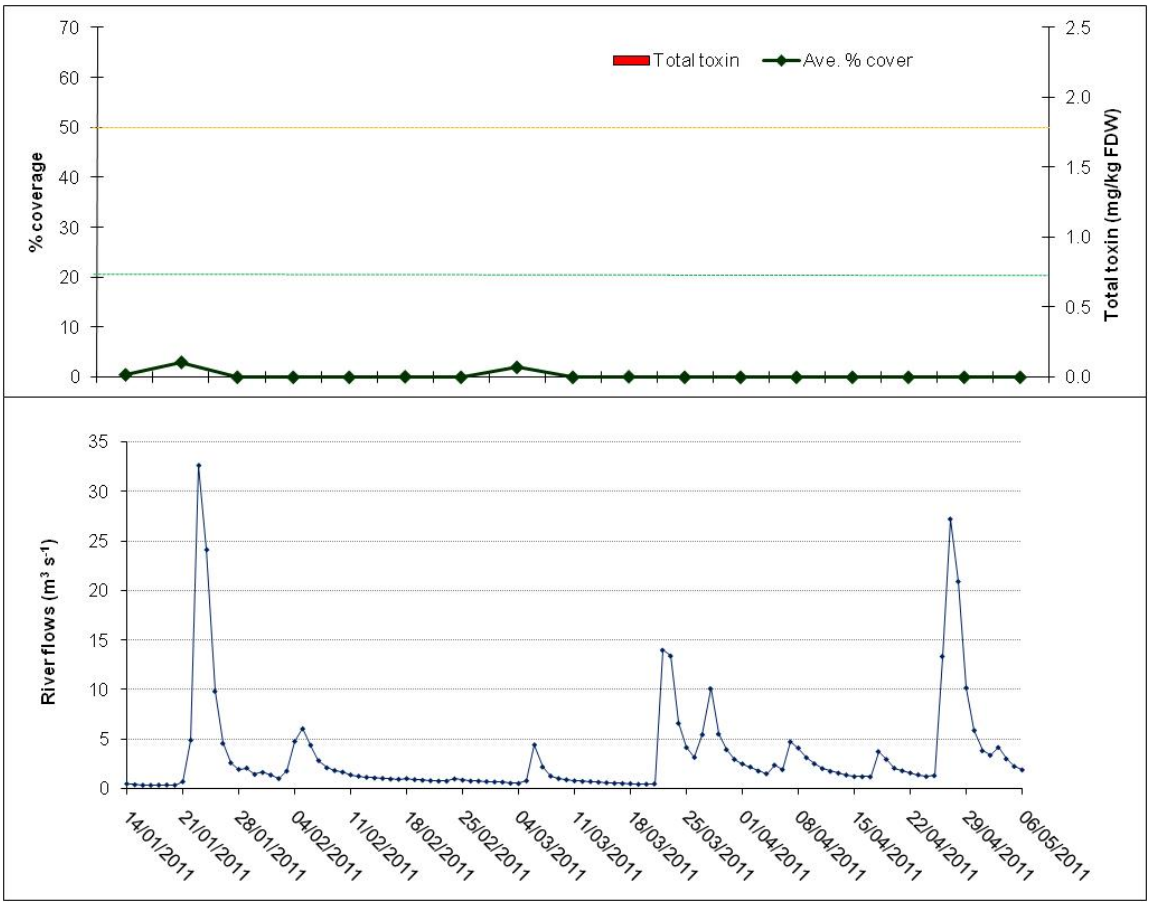

Figure 4. Toxin concentrations, *Phormidium* mat cover and river flow for Tamaki at Stephensons. Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.4. Mangatainoka at State Highway 2

The Mangatainoka is a popular swimming spot located about four kilometres downstream of Pahiatua's water supply take. In February 2011, during a period of low and stable flows, *Phormidium* coverage increased, reaching a maximum of 50% for at least one week between 18 February 2011 and 25 February 2011 (Figure 5). On 6 March 2011 a small flushing event (1.2 times median) may have contributed to the reduction in *Phormidium* coverage to 15% (1 April 2011). From 1 April 2011 to the final sampling, it is likely that a series of flushing events contributed to maintaining *Phormidium* coverage at less than 20% (Figure 5). A flushing event (8.4 times the median flow, 26 April 2011) removed all *Phormidium* mats.

Toxins were detected in 12 out of 14 of the mat samples analysed (Figure 5). The highest total concentration (14.7 mg/kg DFW) was recorded on 25 February 2011. Thereafter, toxin levels decreased but remain markedly higher than was recorded at other sites (Figure 5).

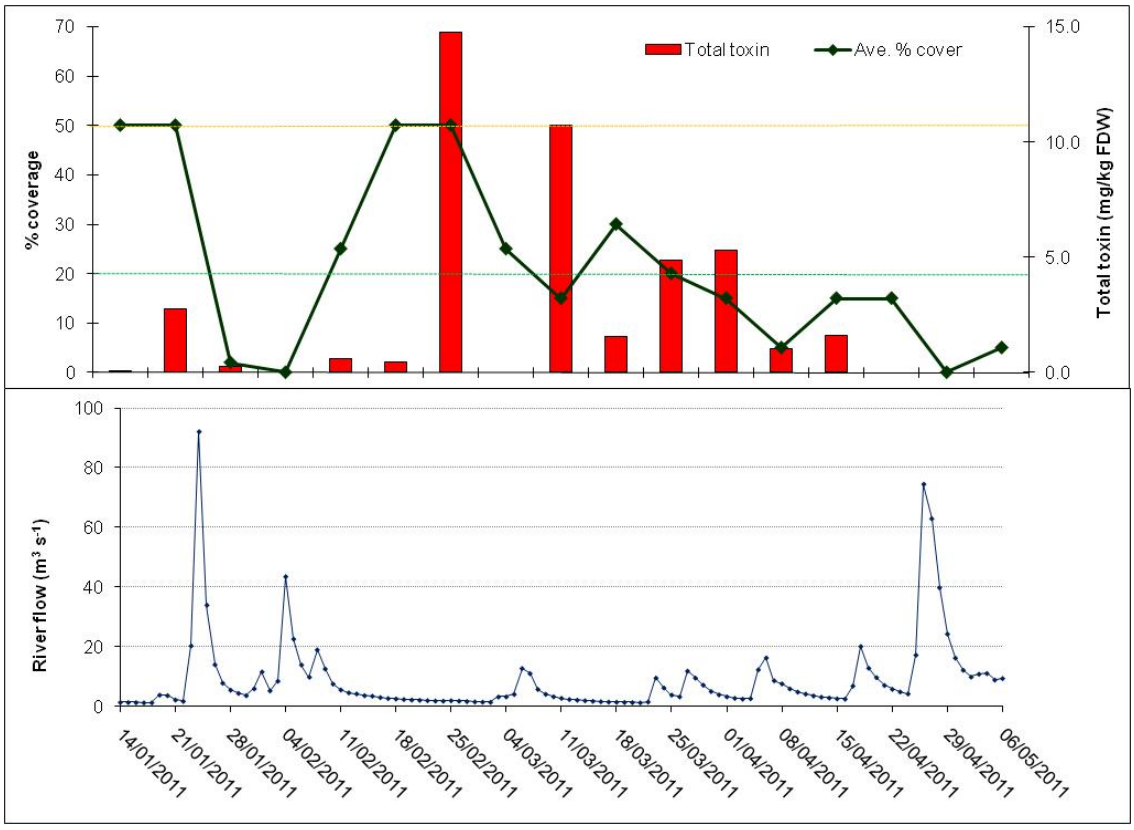

Figure 5. Toxin concentrations, *Phormidium* mat cover at Mangatainoka at State Highway 2 and river flow from Mangatainoka at Pahiatua town bridge. Note different secondary y-axis scale compared to other similar figures.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.5. Oroua upstream of Feilding Sewerage Treatment Plant

Phormidium coverage was the highest (10%) on 14 January 2011 (Figure 6A). Despite an extended period of relatively stable river flows in February 2011 (Figure 6C) mat coverage did not exceed 2% for the remainder of the sampling period. No toxins were detected from the five samples taken (Figure 6A).

3.2.6. Oroua downstream of Feilding Sewerage Treatment Plant

Phormidium coverage was high on 14 January 2011 and 21 January 2011 (40% and 65% respectively; Figure 6B). A flushing flow 10 times the median (24 January 2011) may have contributed to the removal of all mats (Figure 6C). With the exception of a flushing event (4.6 times median, 6 March 2011) the relatively stable flow conditions during February and March enabled a slow increase in mat coverage to a maximum of 20% (18 March 2011). No, or very low, levels of mat coverage were observed thereafter, most likely due to frequent flushing events (Figure 6C). No toxins were detected from the eight samples taken (Figure 6B).

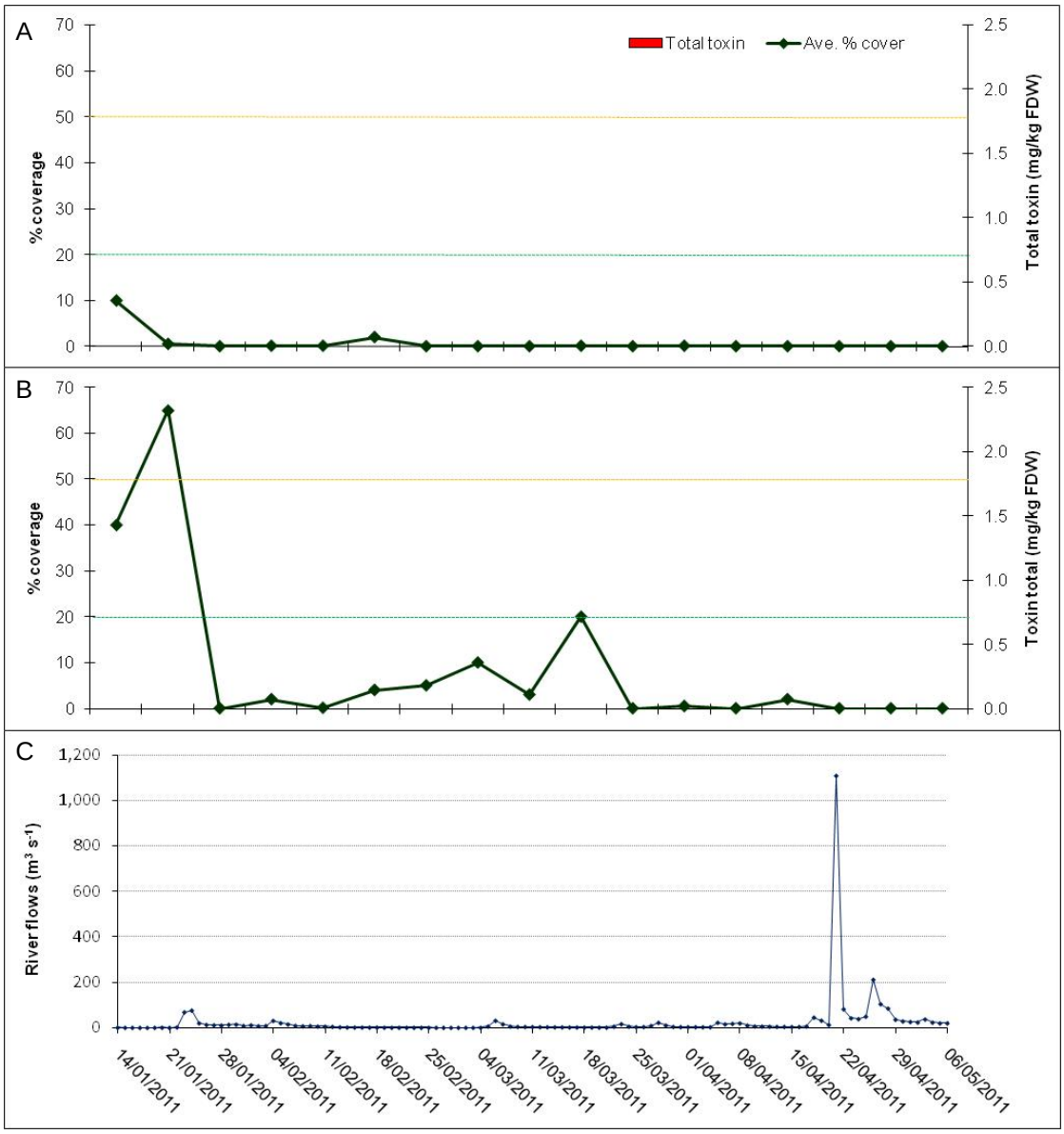

Figure 6. Toxin concentrations, *Phormidium* mat cover at (A) Oroua upstream of sewage treatment plant and, (B) Oroua downstream of sewage treatment plant, and (C) river flow at Oroua at Awahuri Bridge.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.7. Tiraumea at Ngaturi

Phormidium mat coverage was highest (60%) on 21 January 2011 (Figure 7). A significant flushing on 24 January 2011 may have contributed to the removal of all mats. A relatively stable period of river flow in February and March allowed mat coverage to increase with peak coverage of 30% on 25 February 2011 (Figure 7). Toxins were not detected until 25 February 2011 and remained low or undetectable until the end of the sampling period (Figure 7).

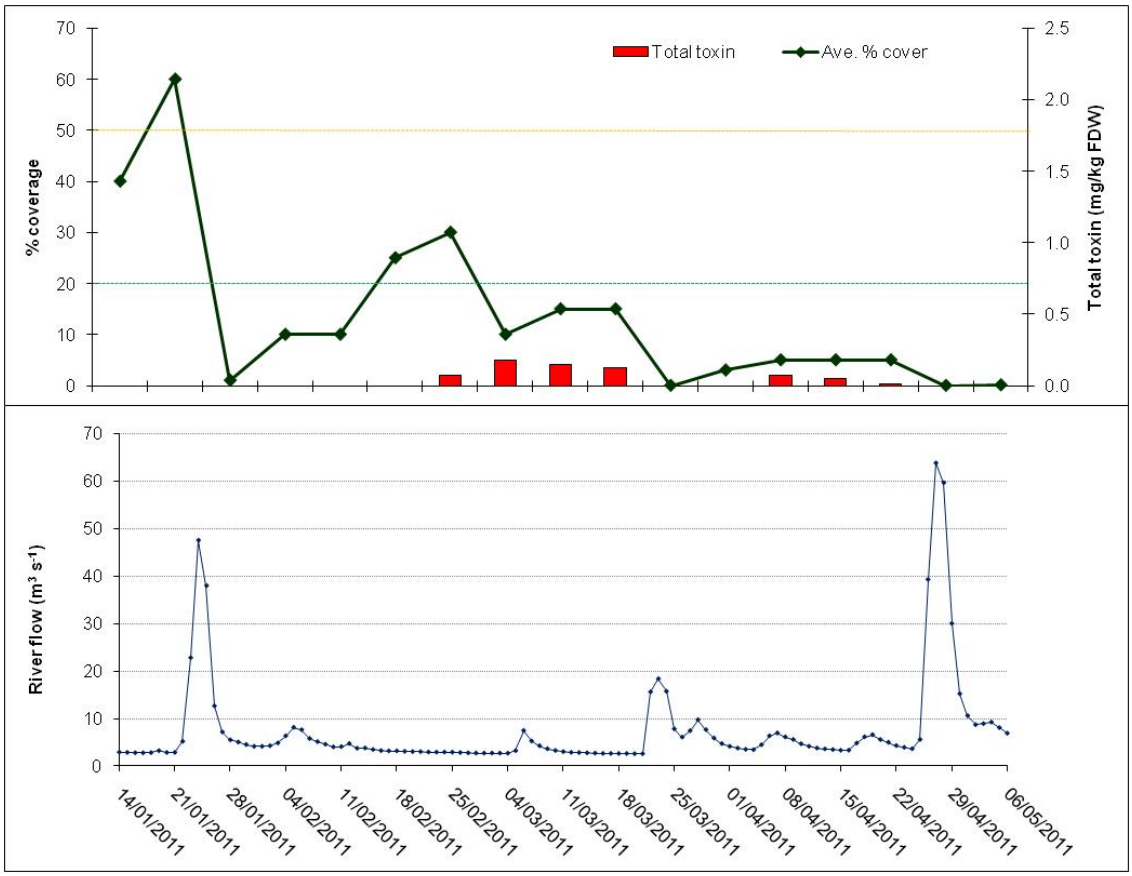

Figure 7. Toxin concentrations, *Phormidium* mat cover and river flow at Tiraumea at Ngaturi.
Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.8. Tokomaru at Horsehoe Bend

Horseshoe Bend is a popular swimming spot, just upstream of Tokomaru township's water supply take from the Tokomaru River. This site experienced numerous flushing events throughout the sampling period (Figure 8). During relatively stable periods of flow in February and March *Phormidium* coverage peaked at 15% (18 March 2011 and 1 April 2011). Low levels of toxins were detected in five of 11 of the mat samples analysed (Figure 8).

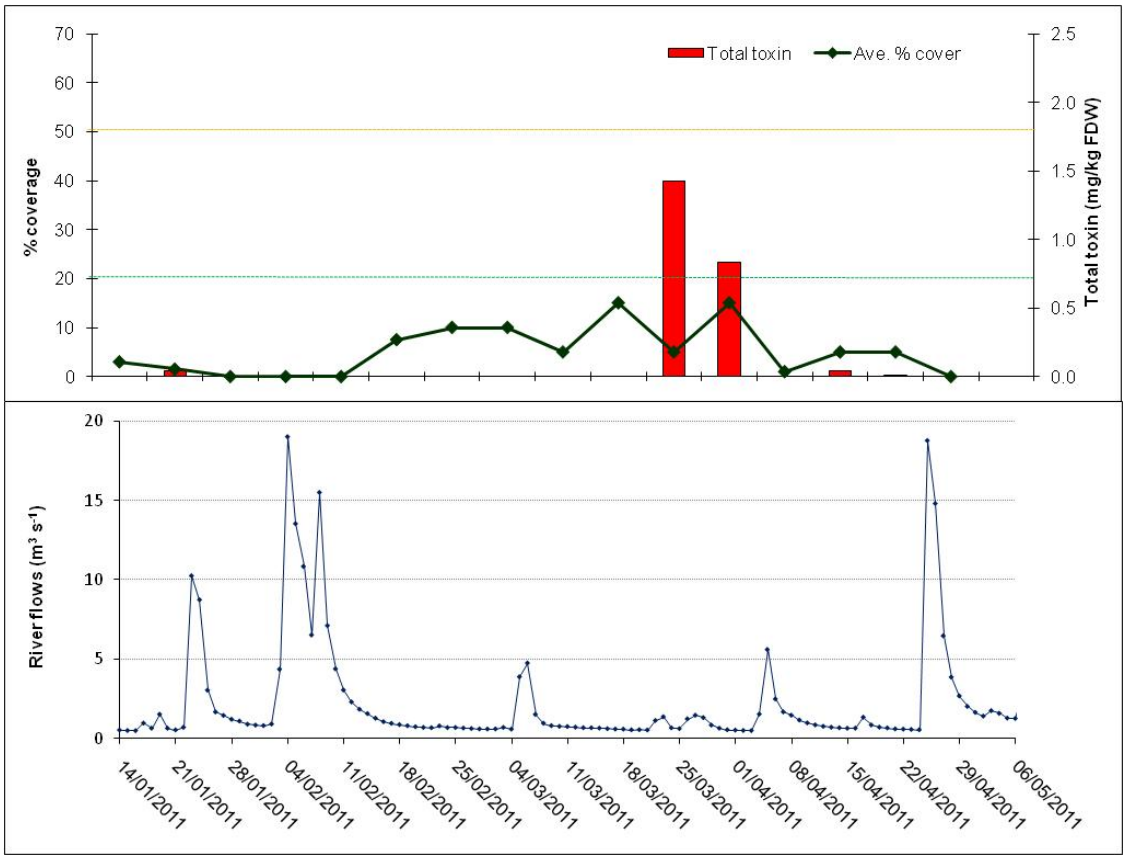

Figure 8. Toxin concentrations and *Phormidium* mat cover at Tokomaru at Horseshoe Bend and river flow at Tokomaru Riverland farm.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.9. Ohau at Gladstone Reserve

Gladstone Reserve is a popular swimming spot just upstream of Levin's water take from the Ohau River. *Phormidium* mat coverage did not exceed 7% during the sampling period (Figure 9A). A possible reason for this is the relatively frequent flushing events in this river (Figure 9C). All three samples collected contained low levels of toxins (Figure 9A).

3.2.10. Ohau at SH1

Phormidium mat coverage did not exceed 5% during the sampling period (Figure 9B). A possible reason for this is the relatively frequent flushing events in this river (Figure 9C). The sample collected on 18 March 2011 had higher levels of toxins than the sample collected on 28 January 2011 (Figure 9B).

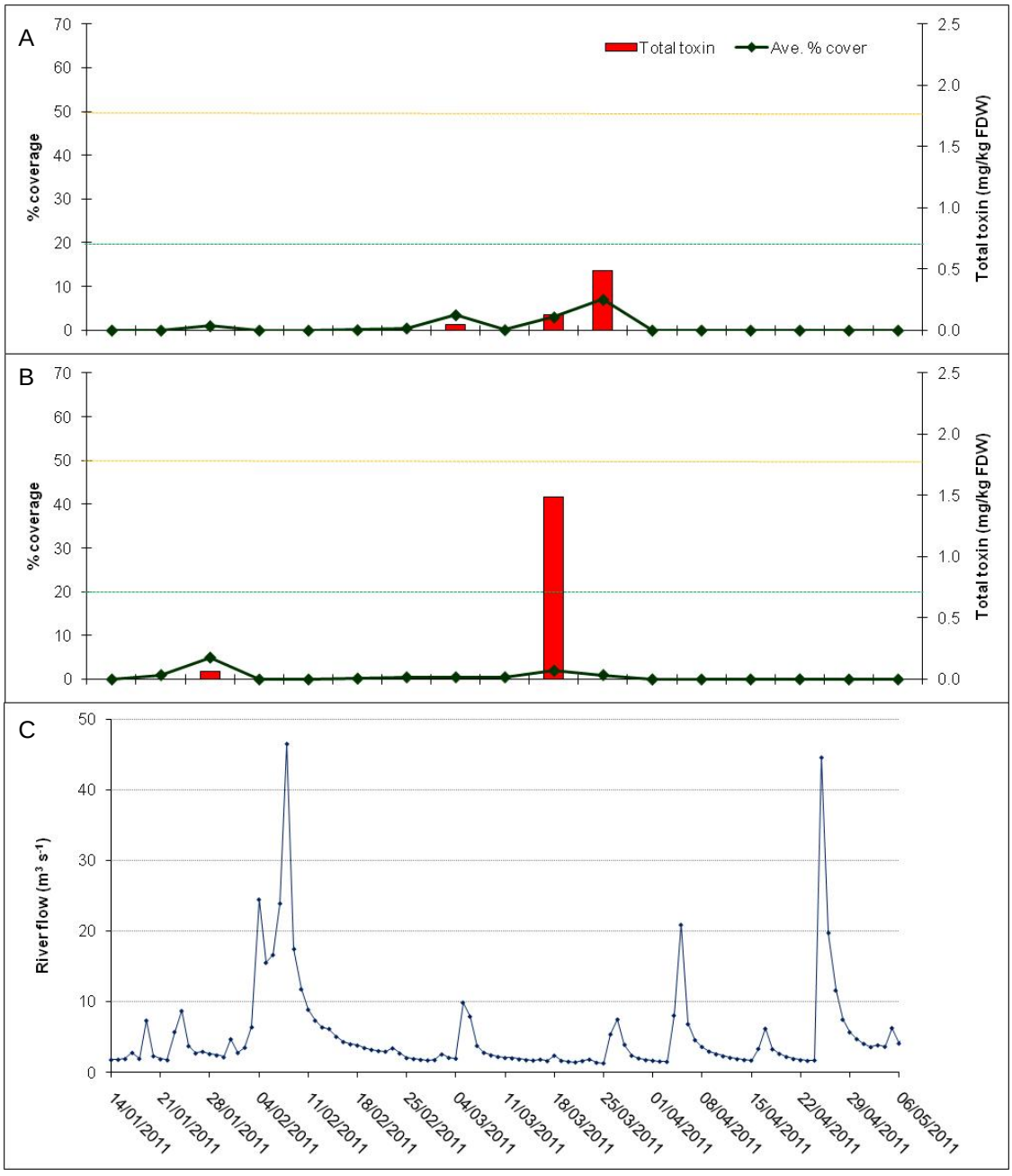

Figure 9. Toxin concentrations, cyanobacterial mat cover at (A) Ohau at Gladstone Reserve and, (B) Ohau at State Highway 1and, (C) river flow at Ohau at Rongomatane.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.11. Makotuku upstream of Raetihi sewerage treatment plant

Despite an extended period of relatively stable river flows, particularly in February and March (Figure 10C) *Phormidium* mats (2% coverage) were only recorded on one of four sampling dates (23 February 2011; Figure 10A). The monthly sampling regime at this site may have missed times of higher mat coverage. Low levels of toxins (1.38 mg/kg FDW) were detected in the one sample collected at this site (Figure 10A).

3.2.12. Makotuku downstream of Raetihi sewerage treatment plant

The downstream site showed a similar trend to the upstream site, with only low *Phormidium* coverage on three of four sampling occasions (Figure 10B). Low levels of toxins were detected in all three samples (Figure 10B).

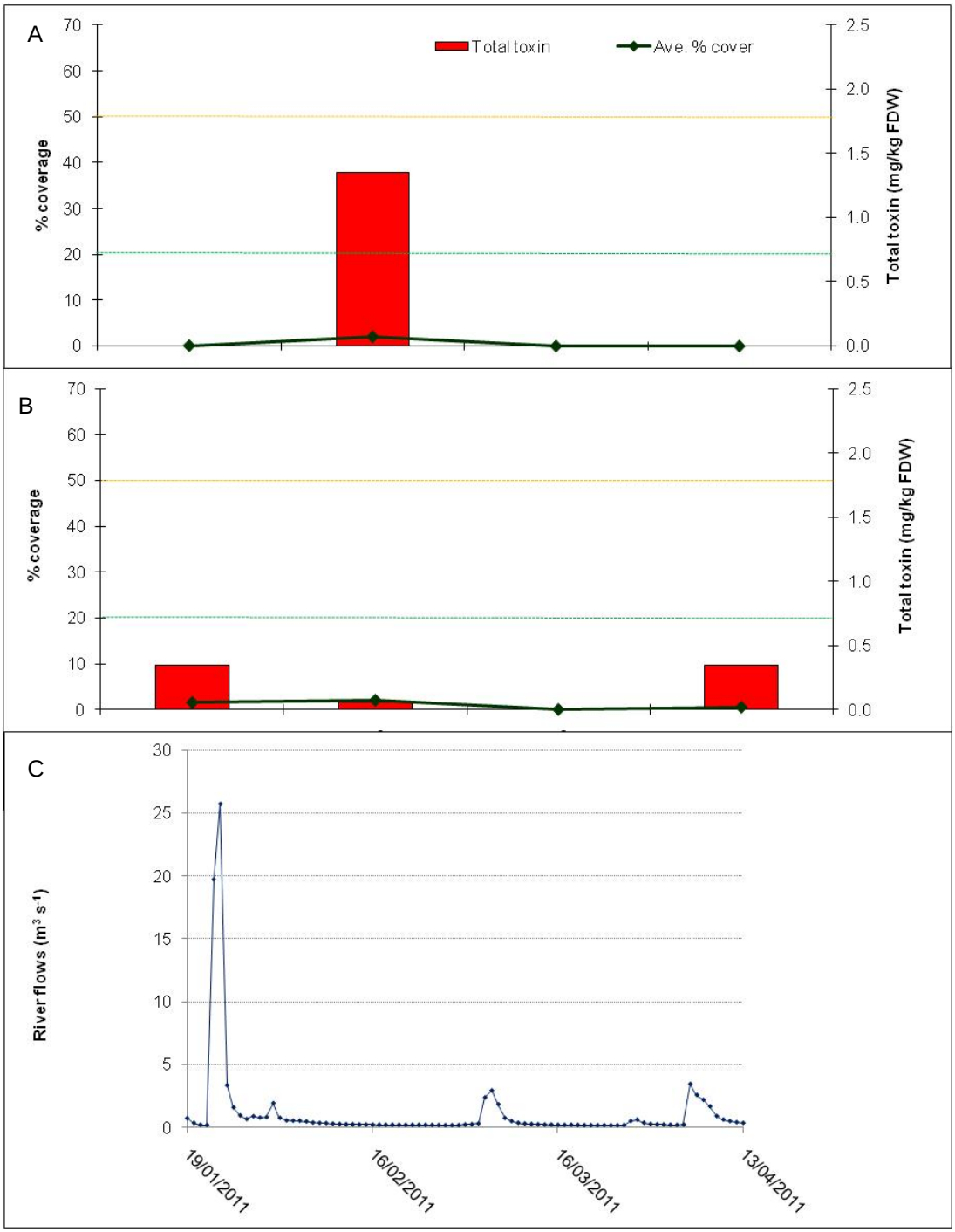

Figure 10. Toxin concentrations, *Phormidium* mat cover at (A) Makotuku upstream of Raetihi sewerage treatment plant, and (B) Makotuku downstream of Raetihi sewerage treatment plant, and (C) river flow at Makotuku upstream at Raetihi.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.13. Mangawhero at Pakahi Road

In March after 14 days of stable flow the *Phormidium* abundance peaked at 25% (Figure 11). Toxins were only detected in two of the five samples collected. The levels in the 12 April 2011 sample were the highest recorded in this study (26.6 mg/kg FDW).

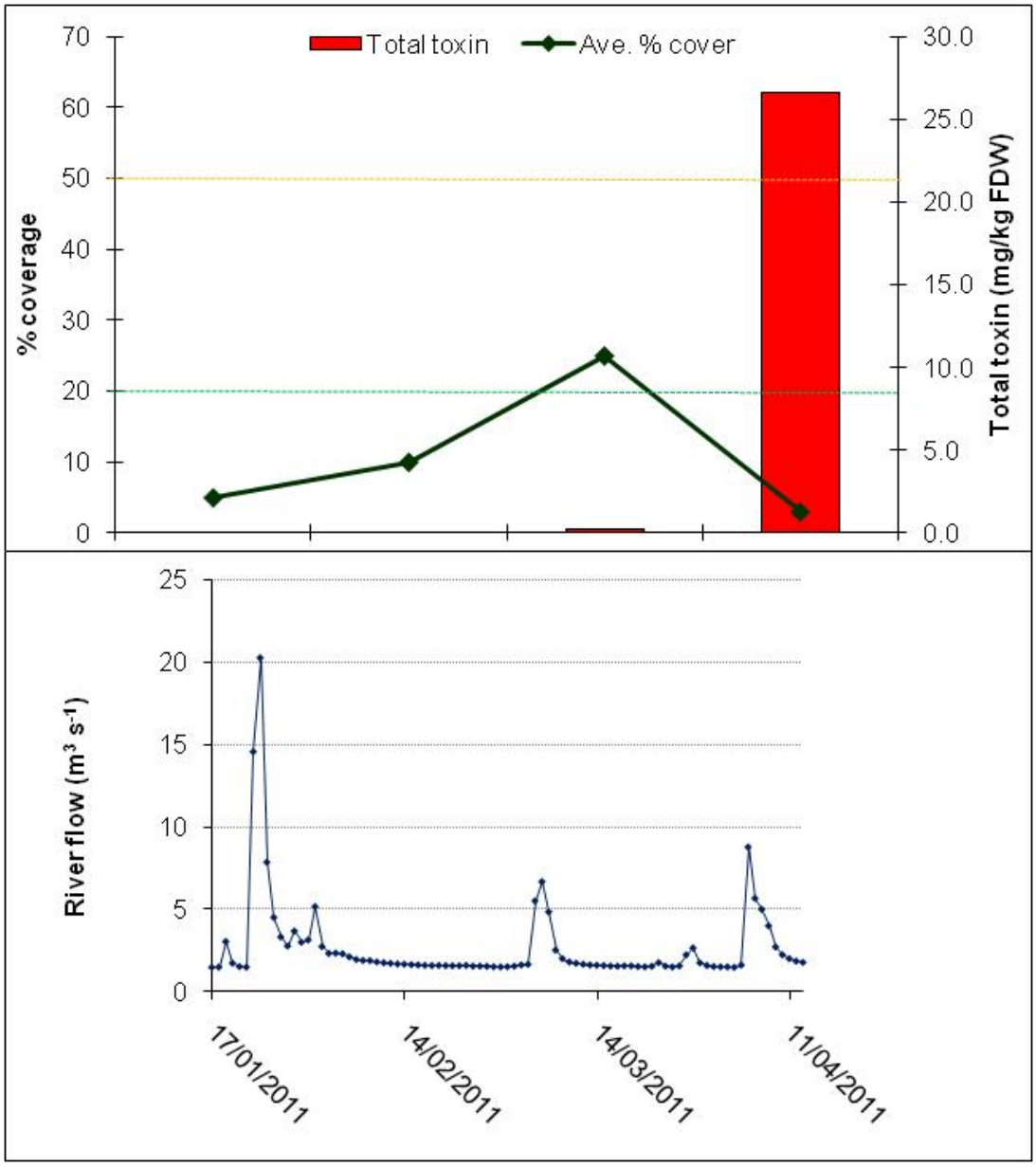

Figure 11. Toxin concentrations, *Phormidium* mat cover and river flow at Mangawhero at Pakihi road bridge. Note different secondary y-axis scale compared to other similar figures.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.2.14. Mangatainoka upstream of Tiraumea

Phormidium mats were present at all four sampling dates (Figure 12). The highest coverage (50%) was recorded on 21 January 2011 prior to flushing flows in late January. Low levels of toxin were detected in all three samples analysed (Figure 12).

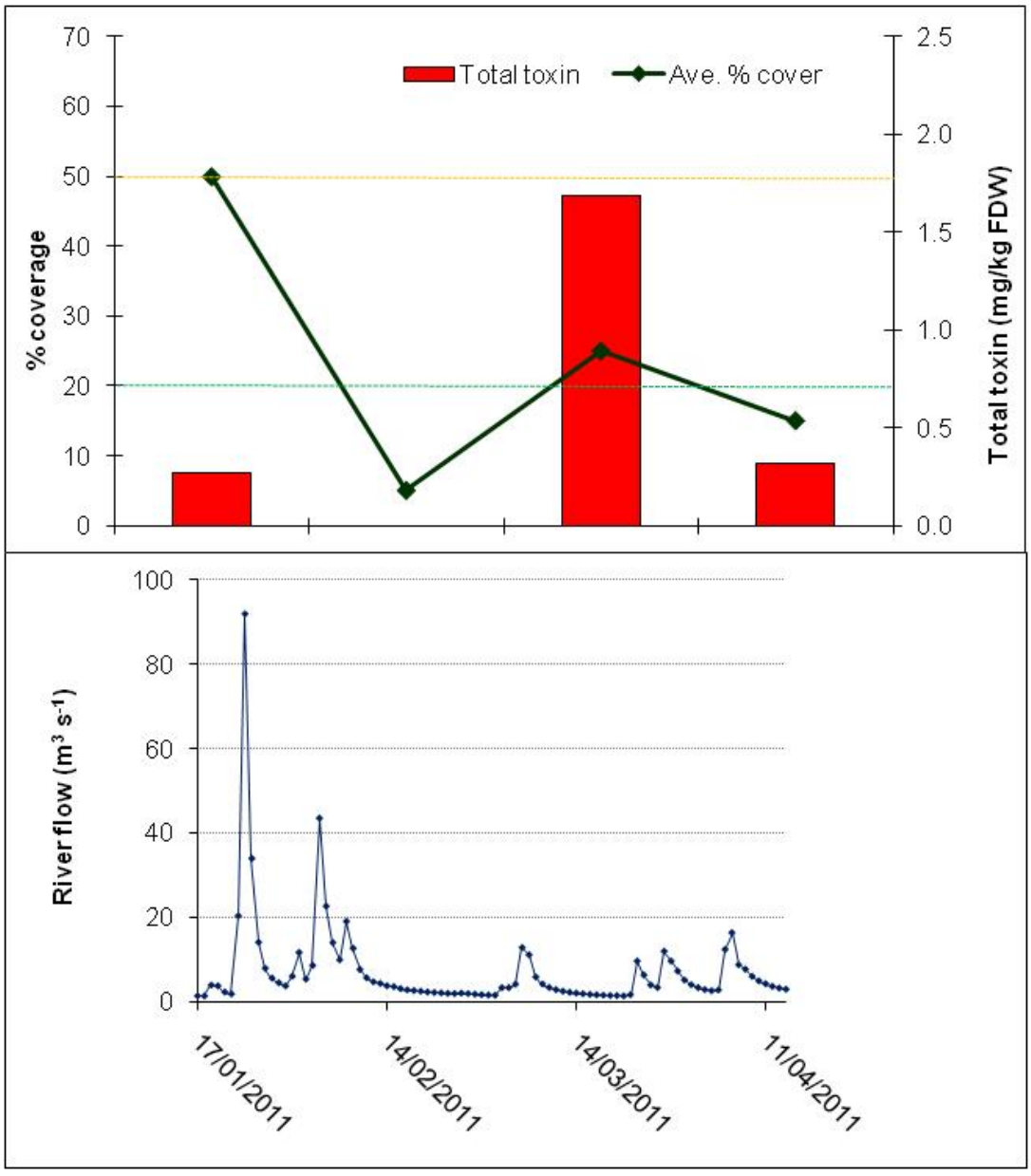

Figure 12. Toxin concentrations, *Phormidium* mat cover and river flow at Mangatainoka upstream of Tiraumea.

Dashed lines indicate MfE percentage coverage thresholds; surveillance (green mode) – up to 20%, alert (amber mode) – 20−50%, action (red mode) > 50% or up to 50% visibly detaching. FDW = freeze dried weight. See Appendix 3 for detailed toxin data.

3.3. Temperature, *Phormidium* mat coverage and toxin production

Phormidium mats were present in a wide range of water temperatures (10˚C to 26.7˚C). There was no correlation between percentage coverage of *Phormidium* mats and temperature (Figure 13A). However, *Phormidium* mat coverage of >30% did not occur until water temperatures were greater than 15.5˚C (Figure 13A).

Toxins were detected in mats in a wide range of water temperatures (10˚C to 21˚C). There was no correlation between the presence of toxins and water temperature (Figure 13A). Interestingly, no toxins were detected when water temperatures were greater than 21˚C (Figure 13A).

Water temperature did not appear to influence the total amount of toxin produced. The two highest toxin concentrations were recorded at contrasting water temperatures; 26.6 mg/kg FDW at 11.2˚C (Mangawhero at Pakahi Rd), and 14.7 mg/kg FDW at 20.9˚C (Mangatainoka at SH2; Figure 13B).

There was no correlation between percentage coverage of *Phormidium* mats and the amount of total toxin measured. The highest toxin concentration recorded (26.6 mg/kg FDW) corresponded to a *Phormidium* percentage coverage of only 3% (Figure 13C). Conversely, when *Phormidium* percentage coverage was greatest (70%), toxin concentrations were relatively low (0.35 mg/kg FDW; Figure 13C).

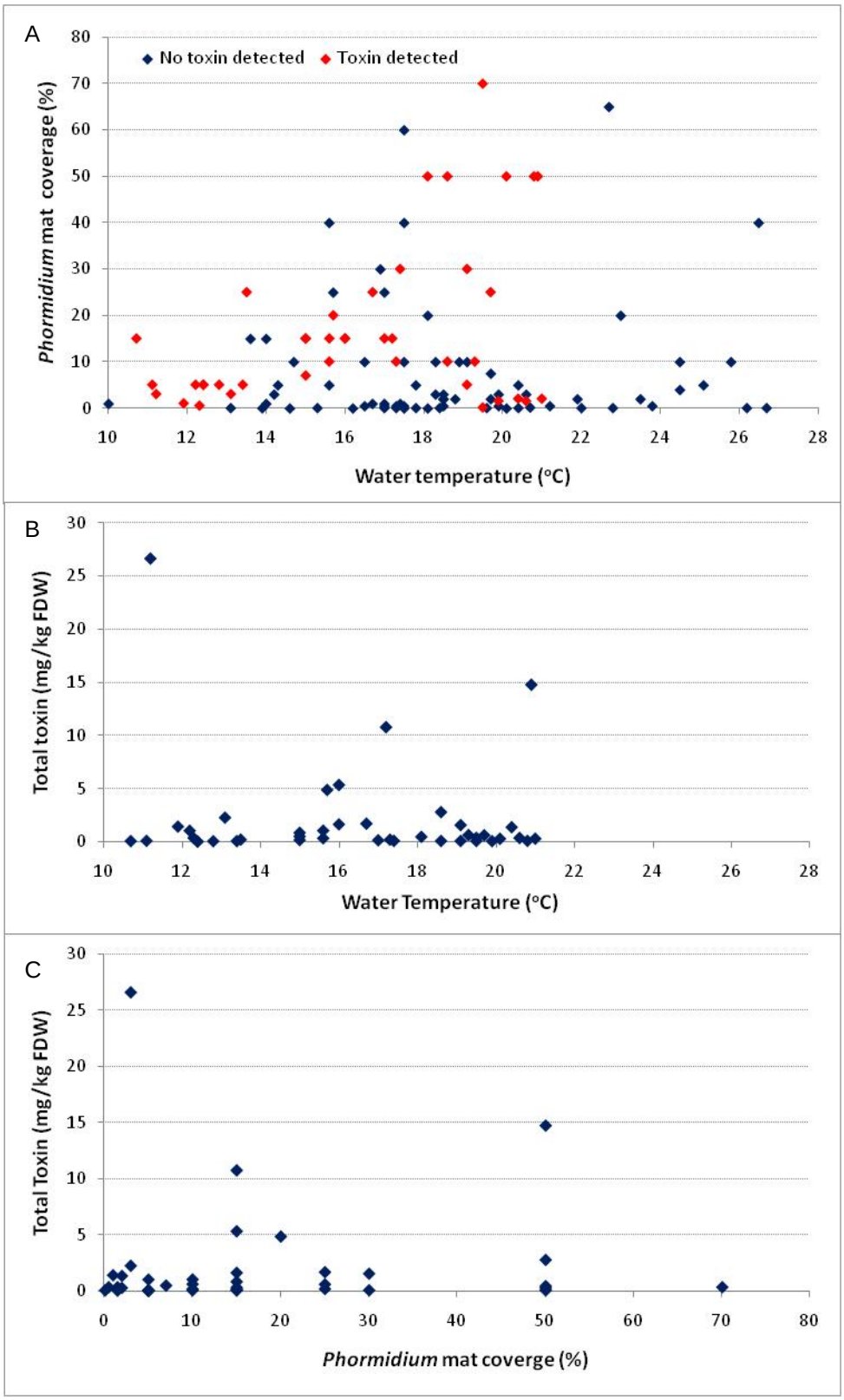

Figure 13. (A) *Phormidium* mat coverage versus water temperature showing the presence/absence of toxins. (B) Water temperature versus total toxin concentration. (C) *Phormidium* mat coverage versus total toxin concentration.

For all graphs only samples where toxins were present are plotted. FDW = freeze dried weight.

3.4. Nutrients and *Phormidium* mat coverage

The Oroua downstream of the STP site had markedly higher concentrations of nitrite (NO_2-N), nitrate (NO_3-N), ammonium (NH_4-N), total dissolved inorganic nitrogen (DIN) and total nitrogen (TN) than all other sites (Figure 14A-E). Four of the sites, where *Phormidium* mat coverage exceeded 30% on at least one sampling occasion, had higher NO_3-N, DIN and TN than all other sites (Oroua upstream of STP, Oroua downstream of STP, Mangatainoka at SH2 Mangatainoka upstream Tiraumea; Figure 14B,D,E).

The site with the highest dissolved reactive phosphorous (DRP) and total phosphorous (TP) was Makotuku downstream of the Raetihi STP (Figure 15A, B). There were no notable differences in DRP and TP between sites where *Phormidium* mat coverage exceeded 30% and other sites (Figure 15A, B).

The four highest DIN:DRP and TN:TP ratios were observed at sites where *Phormidium* mat coverage exceeded 30% on at least one sampling occasion (Tiraumea at Ngaturi, Oroua downstream of STP, Mangatainoka at SH2 Mangatainoka upstream Tiraumea; Figure 15C, D).

The Oroua downstream and upstream of the STP sites provide valuable comparative data. The two are in very close proximity to one another (Figure 1) and are exposed to the same flow regimes (Figure 6C). They also have similar substrate characteristics (Table 1). The downstream site has much greater *Phormidium* coverage than the upstream site (Figure 6A, B). TP concentrations only increase slightly between the upstream and downstream sites (Figure 15A, B), however there is a large increases in NO_2-N, NO_3-N, NH_4-N, DIN and TN between sites (Figure 14A-E). These changes result in markedly different DIN:DRP (6 upstream, 80 downstream) and TN:TP (6 upstream, 40 downstream) ratios between sites (Figure 15C, D).

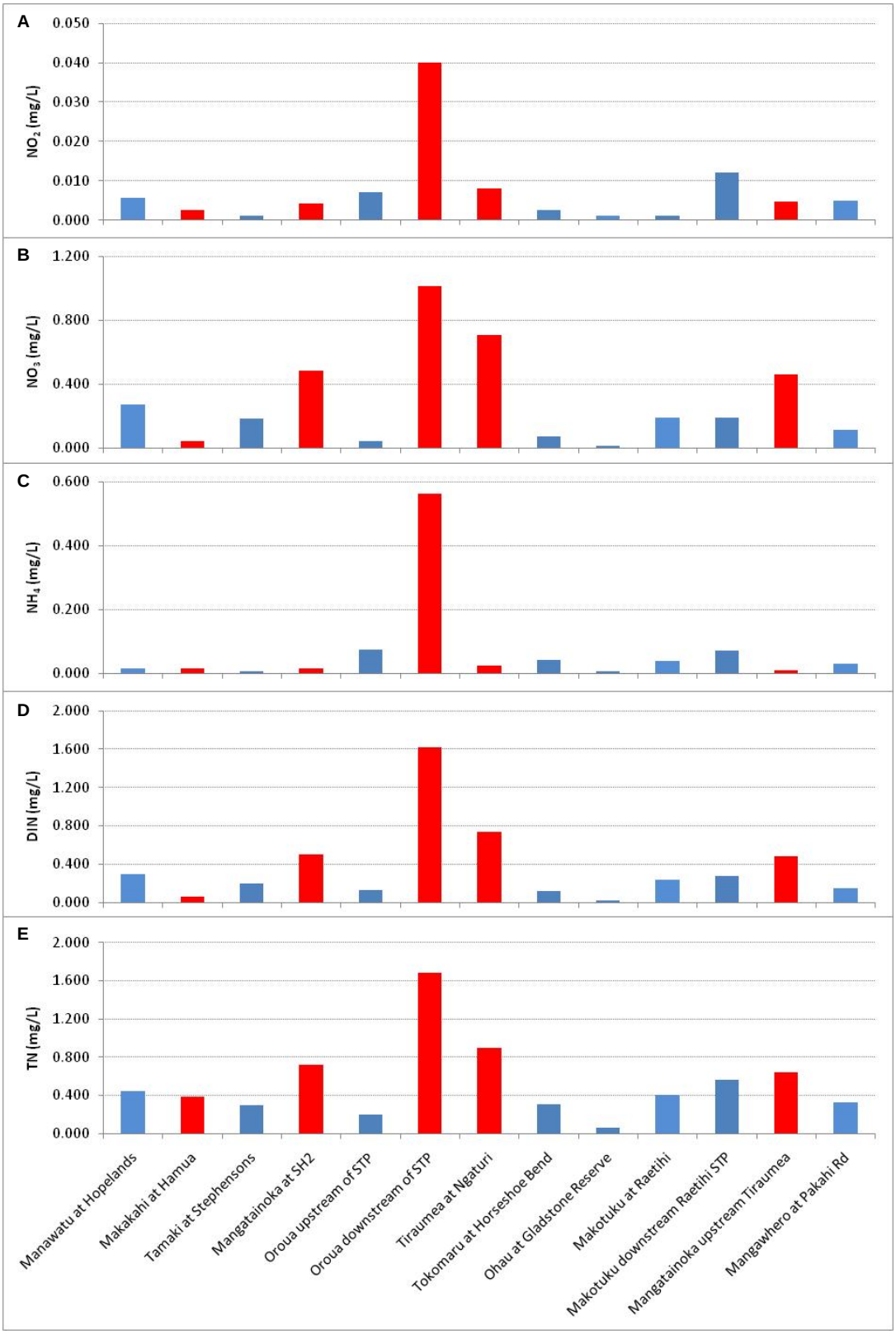

Figure 14. Concentrations of (A) nitrite (NO_2), (B), nitrate (NO_3), (C) ammonium (NH_4), (D) dissolved inorganic nitrogen and (E) total nitrogen.

Results are averages from four or five monthly samples from each site. Sites where *Phormidium* mat coverage exceeded 30% on at least one sampling occasion are shown in red. SH = state highway, STP = sewerage treatment plant.

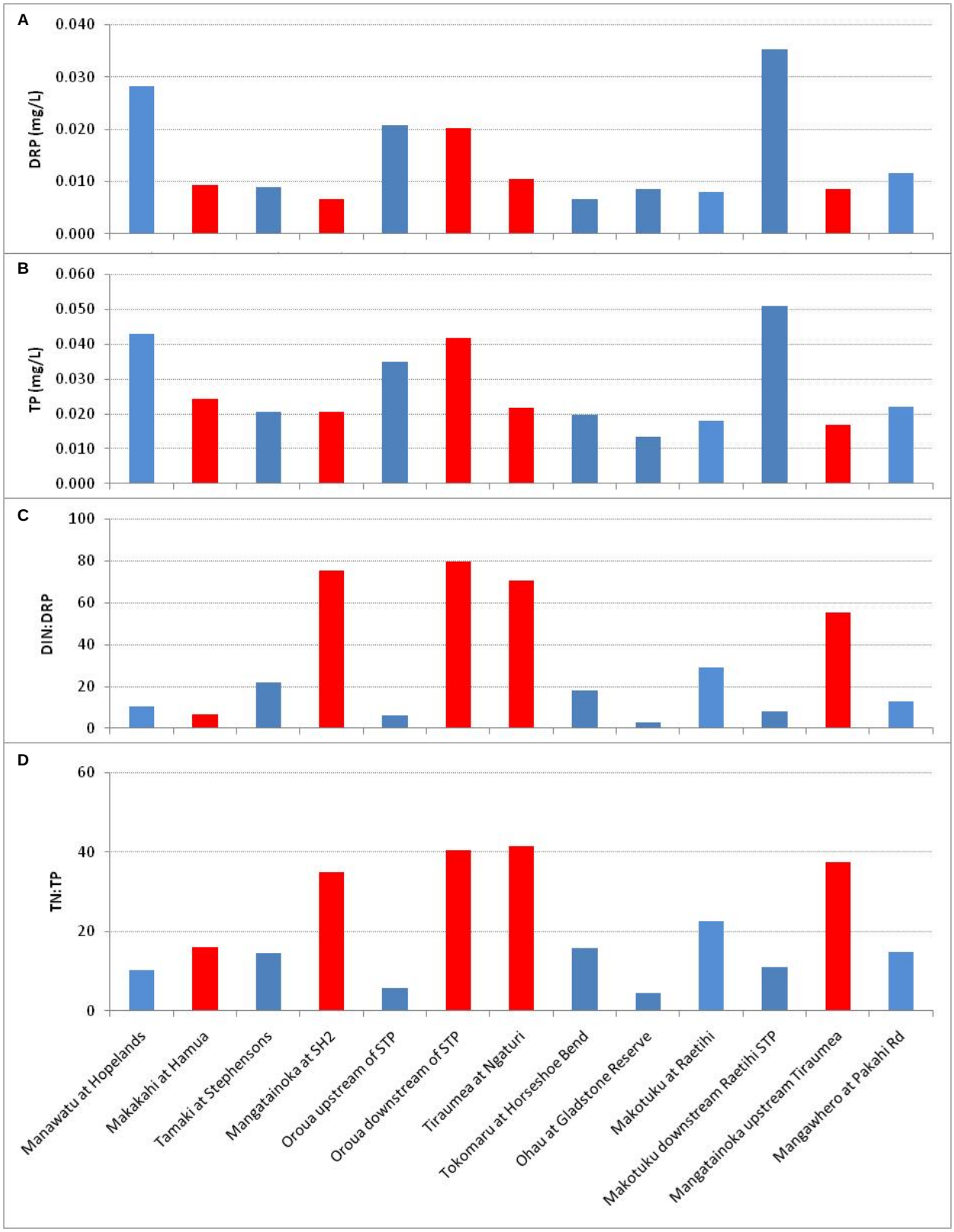

Figure 15. Concentrations of (A) dissolved reactive phosphorus (DRP), (B), total phosphorus (TP), (C) ratio of dissolved inorganic nitrogen (DIN) to DRP, and (D) ratio of total nitrogen (TN) to TP.

Results are averages from data from four or five monthly samples from each site. Sites where *Phormidium* mat coverage exceeded 30% on at least one sampling occasion are shown in red. SH = state highway, STP = sewerage treatment plant.

4. DISCUSSION

4.1. Risks posed by anatoxin-a and homoanatoxin-a producing benthic cyanobacteria – literature review

Benthic cyanobacteria in New Zealand are also known to produce microcystins (heptatoxins) (Hamill 2001, Wood *et al.* 2010a), nodularins (heptatoxins) (Wood *et al*. 2011a), saxitoxins (neurotoxins) (Smith *et al*. 2010), cytotoxic compounds affecting mammalian cells (Wood, Froscio & Campbell, unpub data) and skin irritants. This review focuses on ATX and HTX-producing *Phormidium*; the most prevalent cyanotoxin-producer in New Zealand rivers. As knowledge on toxin production by other benthic species increases consideration will need to be given to the risks these pose. For example, in 2009 a dog died in the Canterbury region after consuming a benthic mat comprised of a microcystin-producing *Planktothrix* sp. in the Waitaki River (Wood *et al*. 2010a).

Anatoxin-a and HTX are also produced by multiple planktonic cyanobacteria species (Sivonen & Jones, 1999). Much of the research discussed below was undertaken using planktonic species, or toxins extracted from these species. The health effects and risks from the toxins are identical regardless of habitat; however the benthic mats may also have ecological impacts (discussed in 4.1.2).

4.1.1. Human and animal health risks

Anatoxin-a and homoanatoxin-a

Anatoxin-a and its homologue HTX are neurotoxic poisons that interfere with transmission of nervous impulses. In affected animals the most common cause of death is by respiratory arrest due to over-stimulation of muscles. Degradation of ATX and HTX in the environment can lead to formation of non-toxic keto, dihydro and epoxy derivatives (James *et al*. 1998). Anatoxin-a and HTX have intra-peritoneal mouse toxicities of 200−250 µg/kg body weight (Devlin *et al.* 1977, Skulberg *et al.* 1992).

Animal and human poisonings and health risks

Anatoxin-a and HTX have been linked to multiple animal deaths worldwide including; dogs (Edwards *et al.* 1992, Gugger *et al*. 2005), cows (Gorham *et al*.1964) and Lesser Flamingos (Krienitz, *et al*. 2003). In New Zealand, dog deaths associated with consumption of ATX- and HTX-producing *Phormidium* have become increasingly common (Hamill 2001, Wood *et al.* 2007). Examination of stomach contents from dead dogs has revealed copious amounts of 'algal' material, suggesting that the dogs ingest the *Phormidium* mats rather than being exposed directly to toxins that are free in the water column (Wood *et al.* 2007). It is unknown whether dogs are more susceptible to ATX/HTX poisoning than other organisms.

There have been no reported human fatalities from ATX and HTX. In New Zealand there have been anecdotal reports of human illnesses associated with recreational activities in rivers containing *Phormidium* mats. In one instance, a young child was taken to hospital with severe

stomach pains after swimming in the Waipoua River (Wairarapa). *Phormidium* mats were later tested and found to contain high levels of ATX and HTX. There was no conclusive evidence to prove that the *Phormidium* had caused the observed symptoms.

The World Health Organisation does not believe there is enough toxicological data available for ATX or HTX to develop a drinking water guidelines for this toxin (they have recommended a guideline for microcystin-LR of 1 ug/L). Research by Fawell *et al.* (1999) suggested that a guideline value of 1 µg/L of ATX would be appropriate for drinking water and would provide a margin of safety of around three orders of magnitude. The New Zealand Ministry of Health has set provisional maximum acceptable values (PMAVs) in drinking water of 6 µg/L for ATX and 2 ug/L for HTX (Ministry of Health of New Zealand, 2005). It is unclear how these values were determined and why there is a difference between the two variants. The toxins measured in this study were in *Phormidium* mats. It is not possible to relate these levels to the PMAVs, *i.e.,* the levels of toxins in the mats do not provide any information on likely concentrations in the water column.

Until recently, little was known about the release of ATX and HTX from benthic cyanobacteria into the water column (*i.e.*, the extracellular component). This information is vital to the management of toxic benthic cyanobacteria particularly for drinking water supplies. For example, toxic cyanobacteria may grow on the substrate of the river and, while healthy, they may pose little risk as the majority of cyanotoxins are likely to be intracellular and thus not being released into the water. Under certain environmental conditions these mats may die or detach from the substrate, potentially releasing massive pulses of cyanotoxins into the water. Currently sampling techniques *e.g.*, taking grab samples of water near the intake, would miss these pulses and thus significantly underestimate the risk posed by benthic cyanobacteria. Wood *et al.* (2010c, 2011b) recently developed an *in situ* methodology known as solid phase adsorption toxin tracking technology (SPATT) specifically for detecting ATX and HTX in rivers. SPATT involves suspending in the water column small bags containing adsorption substrates which accumulate toxins. The toxins can then be extracted and measured, providing information on the toxins released from the mats over an extended period. A field trial using this technique was undertaken in the Waipoua River (Wairarapa) in 2010 (Wood *et al.* 2010c). Toxins were detected in all SPATT bags demonstrating that ATX and HTX are released into the water from cyanobacterial mats, either through active export from the cells or as a result of cell lysis. During the trial toxin levels of 21.7 ug/L (total of HTX, dhHTX and dhATX) were also detected in a grab sample. This was the first time ATX and HTX had been detected in straight water samples. This result demonstrates that levels of ATX/HTX in river water can reach concentrations that may be hazardous to humans and animals, especially if the water is used for drinking.

No studies have been undertaken in New Zealand to determine if aquatic organisms found within close vicinity to toxin-producing benthic mats bio-accumulate ATX and HTX. Because of the high instability of ATX and HTX (Stevens & Krieger, 1991) and the lack of human casualties, it is considered of less concern than other cyanotoxins. However, two recent studies have detected ATX in aquatic organisms. Osswald *et al.* (2007a) placed juvenile carp

(*Cyprinus carpio*) in water contaminated with extracts of an ATX-producing strain. After 96 hours of exposure, minor levels (0.005- 0.073 µg/g) were found to have accumulated in the fish.

In a similar experiment, blue mussels (*Mytilus galloprovincialis*) were exposed to water contaminated with extracts of an ATX-producing strain and accumulation and depuration monitored (Osswald *et al*. 2008). Anatoxin-a was detected in the digestive tract, muscles and foot. One day after beginning the depuration, toxins could not be detected, suggesting it is actively detoxified (Osswald *et al.* 2008).

Given the low levels detected in these studies, and instability of ATX/HTX, it is unlikely that contaminated food sources pose a major risk to humans. Studies on New Zealand species are required to establish if ATX and HTX accumulate in edible species in New Zealand.

Effects on aquatic organisms and plants

There is increasing evidence to suggest that ATX and HTX can have negative effects on a variety of aquatic organisms. However, the effect of ATX and HTX on New Zealand species has not been investigated. Below is a summary of available international data:

- Gilbert (1994) demonstrated that fertility and survival of rotifers decreased when exposed to ATX. This toxicity was enhanced when water temperature was increased and food availability decreased (Gilbert, 1996a, b).
- Toad (*Bufo arenarum*) embryos at certain stages of development experienced dose-dependent transient necrosis (cell death), edema (swelling) and loss of equilibrium when exposed to ATX. At the highest dose (30 mg/L) there were no survivors (Rogers *et al.* 2005).
- A variety of effects on different developmental stages of fish have been demonstrated. Oberemm *et al*. (1999) demonstrated that ATX concentrations of 400 µg/L caused the heart rate of zebrafish to be temporarily altered. Osswald *et al.* (2007) exposed juvenile carp (*C. carpio*) to water contaminated with extracts from ATX-producing strains and noted that swimming was altered. Rymuszka and Sieroslawska (2010) showed that ATX is an inducer of apoptosis (programmed cell death) in fish immune cells.
- Mitrovic *et al.* (2004) provided evidence that ATX has negative effects on the aquatic plants *Lemna minor* and *Chladophora fracta*. Exposure of the plants to 25 µg/mL increased detoxification processes and resulted in the formation of reactive oxygen species indicating oxidative stress.

4.1.2. Effect of Phormidium mats on river health and biodiversity

There is evidence to indicate that the abundance of *Phormidium* mats (toxic and non-toxic) is increasing in New Zealand rivers. To our knowledge no studies have been undertaken globally, nor in New Zealand, to investigate how the presence of toxic and non-toxic *Phormidium* mats impacts river health or aquatic biodiversity.

Phormidium mats trap sediment which collects as a fine layer underneath the mats. This layer is often anoxic. This anoxia combined with the smothering effect of the *Phormidium* mats may cause changes in aquatic biodiversity. Studies on other mat-forming periphyton have shown significant effects on abundances and composition of aquatic species. For example, *Didymosphenia geminata* proliferations have resulted in increased invertebrate abundance and diversity, and caused a shift in the relative assemblages of Ephemeroptera, Plecoptera and Trichoptera taxa to a predominance of crustaceans (Larned *et al*. 2007).

Phormidium mats grow quickly with changes in coverage in short time frames *e.g.*, 0 to 50% in two weeks at the Mangatainoka at SH2 site. This extremely fast growth may be a factor contributing to the large diurnal fluctuations in dissolved oxygen that have been observed at some sites within the Manawatu-Wanganui region (Young & Clapcott 2010). High rates of photosynthesis will release a large amount of oxygen into the water during the day. Associated respiration will consume a large amount of oxygen at night, potentially reducing oxygen concentrations below life-supporting levels, *i.e.,* <6 mg/L (ANZECC 2000).

A useful initial experiment could involve using sites with and without mats, and with and without toxins, to investigate the influence of these factors on macroinvertebrate densities and composition.

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

In this study ATX and HTX concentrations varied among rivers and among sampling times within rivers. This result is consistent with recent studies. During research on *Phormidium* mats in the Hutt River (Lower Hutt) and in five Southland rivers, ATX and HTX concentrations varied markedly among sampling sites and over short time frames *e.g.*, a week; (Heath & Wood 2010, Heath *et al*. 2011). Wood *et al.* (2010b) sampled seven rivers in New Zealand and showed fine-scale spatial variability of ATX and HTX within 10 × 10 m 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 (the protocol that was followed in this study). The most likely reason for this variability is that toxic and non-toxic genotypes co-occur in *Phormidium* mats (Heath *et al*. 2010). The relative amount of each genotype will affect the total toxin in each sample. Variables that regulate the presence and abundance of each genotype within a mat are unknown. Heath *et al.* (2010b) suggested that the toxin-producing strains in the Hutt River "out-competed" non-toxic *Phormidium* strains at temperatures above 15˚C. In this study toxins were detected in a wide range of temperatures suggesting that temperature is unlikely to regulate the abundance of toxic/non-toxic strains. Recently the putative gene cluster involved in ATX production was characterised (Méjean *et al*. 2009). This will enable molecular tools to be used to study the abundance of toxic and non-toxic genotypes in a mat. Our on-going research aims to develop a quantitative molecular-tool which will enable toxic and non-toxic strains to be differentiated within a mat. This may allow us to determine which variables,

or at what thresholds, these variables are required to trigger the dominance of toxic over non-toxic strains or vice versa.

The majority of 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 12.8 mg/kg FDW. The large majority of positive samples in this study were less than 5 mg/kg FDW. Two samples (26.6 mg/kg FDW, Mangawhero at Pakahi Road, 12 April 2011and 14.7 mg/kg FDW, Mangatainoka at SH2, 25 February 2011), however, had total toxin concentrations that were higher than those measured in Wood *et al*. (2010b). The generally lower results 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.

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 (Wood *et al.* 2010b, Heath & Wood 2010, Heath *et al*. 2011). In this study there were a number of samples with elevated toxin concentrations which occurred when mat abundance was low. In recent laboratory-based experiments we have observed that the amount of toxin produced per cell is greatly enhanced during the initial colonisation phase (Wood unpub. data). During this period biomass is low (*i.e.,* total toxin load at a site will be low). Additionally, filaments are likely to be firmly attached to the substrate and thus less available to humans and animals that are more likely to intentionally and accidentally consume detached mats.

The recent New Zealand cyanobacterial guidelines (Ministry for the Environment and Ministry of Health 2009) uses a three-tier alert level framework based on 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. The rationale for this is that many cyanobacteria produce lipopolysaccharides which can cause skin and other irritations. These reactions are likely to be more severe as biomass increases. Additionally, 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 used as a predictor of human health risk. 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 knowledge of ATX/HTX concentrations and how these fluctuate at specific sites is recommended.

4.3. River flow, water temperature and *Phormidium* coverage

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 Manawatu-Wanganui region. However, we understand that benthic cyanobacteria proliferations have been observed previously in the Manawatu-Wanganui region during winter (Carol Nicholson, pers. comm.).

At selected sites the frequency of flushes appeared to have a major influence on the *Phormidium* percentage coverage. For example, at Mangatainoka at State Highway 2 site, frequent flushing events in the second half of the sampling period appeared to reduce *Phormidium* percentage coverage). Heath *et al.* (2010b) showed that in the Hutt River, Wellington, mats were present predominantly when river flows were below the yearly mean. In the current study flushing events did not appear to be the only variable that regulated mat coverage. For example, at the Makakahi at Hamua site between 18 and 25 February there were no flushing flows above the median yet *Phormidium* percentage coverage reduced from 50% to 25%. The ability of river flow to flush *Phormidium* mats has led the Greater Wellington Regional Council to use river flow as one predictor of *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 Horizons Regional Council but further long-term datasets incorporating weekly samples are required before this system could be established in the Manawatu-Wanganui region.

Phormidium mats are generally assumed to grow fastest at higher temperatures, however, laboratory-based experiments are required to confirm this. For example, following a flushing flow, mats will grow back faster in 20˚C water as opposed to 10˚C water. In this study *Phormidium* mats were detected at water temperatures between 10˚C and 26.7˚C. This is consistent with the findings of Heath *et al.* (2010b) who reported cyanobacterial mats between 8˚C and 21˚C, and Heath and Wood (2010) where mats occurred between 8˚C and 17˚C. Further studies are required to determine these temperature thresholds. In the future, models could be developed that take water temperature into consideration when predicting risk of *Phormidium* mat proliferation.

4.4. Nutrients and *Phormidium* coverage

In general, the sites with the highest *Phormidium* coverage were found to have high TN:TP ratios and DIN:DRP ratios providing evidence that nitrogen, rather than phosphorus may be limiting *Phormidium* growth. Waters with high TN:TP ratios (>15:1) are indicative of phosphorus limitation, while low TN:TP ratios (<7:1) are indicative of nitrogen limitation. The Oroua upstream and downstream of the STP sites provide further evidence to support this hypothesis. The upstream site has low *Phormidium* coverage and a TN:TP ratio of 6.

The STP discharge adds substantial amounts of nitrogen increasing the TN:TP ratio to 40. There is a corresponding increase in *Phormidium* coverage suggesting that upstream of the STP *Phormidium* proliferation is nitrogen limited. Analysis of a larger dataset across a wider gradient of water qualities, and the inclusion of sites with no *Phormidium* would assist in establishing these relationships.

The Horizons Regional Council standard 48 periphyton monitoring sites could provide a data set for determining the relationship between nutrients and *Phormidium.* Although cyanobacteria are now distinguished from other periphyton this has not always been done historically, preventing analysis of longer-term datasets. Evaluating how temporal changes in nutrients affect *Phormidium* coverage at each site would be valuable. This was not possible in the current study due to the limited nutrient data available for each site. At selected sites, it would be useful to collect nutrient data weekly in relation to *Phormidium* cover.

5. RECOMMENDATIONS FOR FUTURE MANAGEMENT AND MONITORING

We recommend that Horizons Regional Council:

- Continues to monitor cyanobacterial abundance using MfE and MoH (2009) protocols at the regular periphyton monitoring sites, along with any other sites that have high recreational use. Samples should be collected throughout the year. When there is doubt about identification, samples should be collected, preserved with Lugol's Iodine and the identification confirmed using microscopy.
- Continues 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.
- Undertakes a study to determine the risk *Phormidium* mats pose to drinking water supplies in the region. A possible site for an initial study is the Pahiatua water supply which is approximately 4 kilometres upstream of the Mangatainoka at SH2 site (where toxins were detected). This study should include site surveys, toxin testing of *Phormidium* mats and grab samples, and the use of SPATT.
- Continues to develop an early warning system based on river flow and temperature. For all sites this will require a longer-term dataset. Sites with high recreational use should be given priority for development of these systems. Site surveys should be undertaken at least weekly.
- Undertakes further analysis of the effect of nutrient concentrations and ratios on mat growth at selected sites. Nutrients that should be analysed include; NO_2-N, NO_3-N, NH_4-N, DIN, TN, DRP and TP. At these sites *Phormidium* coverage and a suite of physiochemical variables would need to be monitored weekly over 6-12 months.

Sites with and without *Phormidium* are required for this study. Based on the results of this study potential sites could include; Oroua upstream of STP, Oroua downstream of STP, Makakahi at Hamua, Tamaki at Stephensons and Mangatainoka at SH2.

- Undertakes a study on the effect of the Oroua STP discharge on *Phormidium* coverage. The data in this study suggests that the Oroua STP discharge appears to have a marked effect on the abundance of *Phormidium* mats. The Oroua sites (upstream and downstream of the STP) provide a unique opportunity to explore the influence of water chemistry and nutrient concentrations and ratios on *Phormidium* abundance. In addition to the commonly analysed nutrients, samples from upstream, downstream and the discharge could be analysed for a range of elements known to influence cyanobacterial growth *e.g.*, iron (Li *et al.* 2009, Paerl *et al.* 1994). Initially a full suite of elements could be analysed in one or several samples from upstream, downstream and the discharge. Based on these data selected elements could be chosen for more regular long-term analysis.
- Undertakes studies to establish if ATX and HTX accumulate in edible species in New Zealand *e.g.,* koura and trout.
- Undertakes a study on the effect of *Phormidium* mats on biodiversity and ecosystem health *e.g.,* their effect on the abundance and species composition of macro-invertebrates. Samples would be required from sites with and without *Phormidium* and from sites with *Phormidium* but with and without toxins. Macro-invertebrate samples could be collected at different stages of *Phormidium* growth using Surber samplers. Macro-invertebrate species would need to be identified and enumerated. Site surveys and toxin testing of mats would be required. Additionally, nothing is known about effects of ATX and HTX on larger aquatic organisms *i.e.*, native fish. Multiple toxin-producing strains are maintained in the Cawthron micro-algal culture collection, which would enable laboratory studies to be undertaken.

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APPENDICES

Appendix 1. Photographs of *Phormidium* sp. mats at sampling sites.

Manawatu at Hopelands January 2011

Manawatu at Hopelands January 2009

Makakahi at Hamua January 2011

Tamaki at Stephensons February 2011

Cyanobacteria mats at Mangatainoka SH2 January 2011

Oroua Upstream Feilding STP March 2011

Oroua Downstream Feilding STP March 2011

Tiraumea at Ngaturi March 2011

Tokomaru at Horseshoe Bend January 2011

Ohau at State Highway 1 February 2011

Ohau at Gladstone Reserve February 2011

Makotuku Upstream of Raetihi STP Feb. 2011

Makotuku Upstream of Raetihi STP Feb. 2011

Mangawhero at Pakahi Road January 2011

Mangatainoka U/S of Tiraumea Cnfl. Jan. 2011

Appendix 2. Long term median flows for sampling sites. Refer to Figure 1 for location of sites.

Site	Long term median flow ($m^3 s^{-1}$)
Manawatu at Hopelands	15.70
Makakahi at Hamua	3.18
Tamaki at Stephensons	2.45
Mangatainoka at Pahiatua Town Bridge	8.90
Oroua at Awahuri	7.82
Tiraumea at Ngaturi	7.21
Tokomaru at Riverland Farm	1.25
Ohau at Rongomatane	3.82
Makotuku at Raetihi	0.70
Mangawhero at Pakahi Rd	3.17

Appendix 3. Toxin data (mg/kg freeze dried weight) for each sample.
Positive results in bold text. ATX = anatoxin-a, HTX = homoanatoxin-a, dhATX = dihydroanatoxin-a, dhHTX = dihydrohomoanatoxin-a.

Date	ATX	HTX	dhATX	dhHTX
Manawatu at Hopelands				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	0.00	0.00	0.00
11/02/11	0.00	0.00	0.00	0.00
18/02/11	0.00	0.00	0.00	0.00
4/03/11	0.00	**0.06**	0.00	0.00
Makakahi at Hamua				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	0.00	0.00	0.00
28/01/11	0.00	0.00	0.00	0.00
11/02/11	0.00	0.00	0.00	0.00
18/02/11	0.00	0.00	0.00	0.00
25/02/11	0.00	**0.30**	**0.05**	0.00
4/03/11	0.00	**0.38**	**0.07**	0.16
11/03/11	0.00	**0.37**	**0.43**	**0.24**
18/03/11	0.00	**0.06**	0.00	0.00
1/04/11	0.00	0.00	0.00	0.00
8/04/11	0.00	**0.05**	0.00	0.00
15/04/11	0.00	0.00	0.00	0.00
21/04/11	0.00	**0.20**	**1.01**	**0.20**
6/05/11	0.00	**0.32**	**1.36**	**0.57**
Tamaki at Stephensons				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	0.00	0.00	0.00
4/03/11	0.00	0.00	0.00	0.00
Mangatainoka at SH2				
14/01/11	0.00	0.00	**0.07**	0.00
21/01/11	0.00	**0.35**	**1.98**	**0.44**
28/01/11	0.00	**0.09**	**0.19**	0.00
11/02/11	0.00	**0.20**	**0.33**	**0.07**
18/02/11	0.00	**0.11**	**0.29**	**0.05**
25/02/11	0.00	**1.76**	**7.35**	**5.64**
11/03/11	0.00	**1.54**	**5.63**	**3.59**
18/03/11	0.00	**0.36**	**0.99**	**0.20**
25/03/11	0.00	**0.65**	**3.24**	**0.97**
1/04/11	0.00	**0.52**	**3.66**	**1.14**
8/04/11	0.00	**0.22**	**0.61**	**0.19**
15/04/11	0.00	**0.31**	**1.07**	**0.23**
21/04/11	0.00	0.00	0.00	0.00
6/05/11	0.00	0.00	0.00	0.00
Oroua upstream of STP				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	0.00	0.00	0.00
4/02/11	0.00	0.00	0.00	0.00
11/02/11	0.00	0.00	0.00	0.00
18/02/11	0.00	0.00	0.00	0.00
Oroua downstream of STP				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	0.00	0.00	0.00
4/02/11	0.00	0.00	0.00	0.00
11/02/11	0.00	0.00	0.00	0.00
18/02/11	0.00	0.00	0.00	0.00
25/02/11	0.00	0.00	0.00	0.00
11/03/11	0.00	0.00	0.00	0.00
18/03/11	0.00	0.00	0.00	0.00

Date	ATX	HTX	dhATX	dhHTX
Tiraumea at Ngaturi				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	0.00	0.00	0.00
28/01/11	0.00	0.00	0.00	0.00
4/02/11	0.00	0.00	0.00	0.00
11/02/11	0.00	0.00	0.00	0.00
18/02/11	0.00	0.00	0.00	0.00
25/02/11	0.00	**0.07**	0.00	0.00
4/03/11	0.00	**0.12**	0.00	**0.05**
11/03/11	0.00	**0.09**	0.00	**0.05**
18/03/11	0.00	**0.13**	0.00	0.00
8/04/11	0.00	**0.07**	0.00	0.00
14/05/11	0.00	**0.05**	0.00	0.00
21/04/11	0.00	**0.01**	0.00	0.00
Tokomaru at Horsehoe Bend				
14/01/11	0.00	0.00	0.00	0.00
21/01/11	0.00	**0.04**	0.00	0.00
18/02/11	0.00	0.00	0.00	0.00
25/02/11	0.00	0.00	0.00	0.00
4/03/11	0.00	0.00	0.00	0.00
11/03/11	0.00	0.00	0.00	0.00
18/03/11	0.00	0.00	0.00	0.00
25/03/11	0.00	**0.89**	**0.37**	**0.16**
1/04/11	0.00	**0.23**	**0.58**	**0.03**
14/05/11	0.00	**0.04**	0.00	0.00
21/04/11	0.00	**0.01**	0.00	0.00
Ohau at SH1				
28/01/11	0.00	**0.06**	0.00	0.00
18/03/11	0.00	**1.23**	**0.11**	**0.15**
Ohau at Gladstone Reserve				
4/03/11	0.00	**0.05**	0.00	0.00
18/03/11	0.00	**0.13**	0.00	0.00
25/03/11	0.00	**0.43**	0.00	**0.06**
Makotuku @ Raetihi				
23/02/11	0.00	**1.35**	0.00	0.00
Makotuku d/s Raetihi STP				
19/01/11	0.00	**0.35**	0.00	0.00
23/02/11	0.00	**0.06**	0.00	0.00
12/04/11	0.00	**0.30**	0.00	**0.04**
Mangawhero @ Pakahi Rd				
17/01/11	0.00	0.00	0.00	0.00
23/02/11	0.00	0.00	0.00	0.00
22/03/11	0.00	**0.14**	**0.05**	0.00
12/04/11	**0.07**	**7.91**	**6.99**	**11.62**
Mangatainoka u/s Tiraumea				
21/01/11	0.00	**0.05**	**0.21**	0.00
8/03/11	0.00	**0.40**	**1.08**	**0.20**
5/04/11	0.00	**0.06**	**0.21**	**0.05**

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