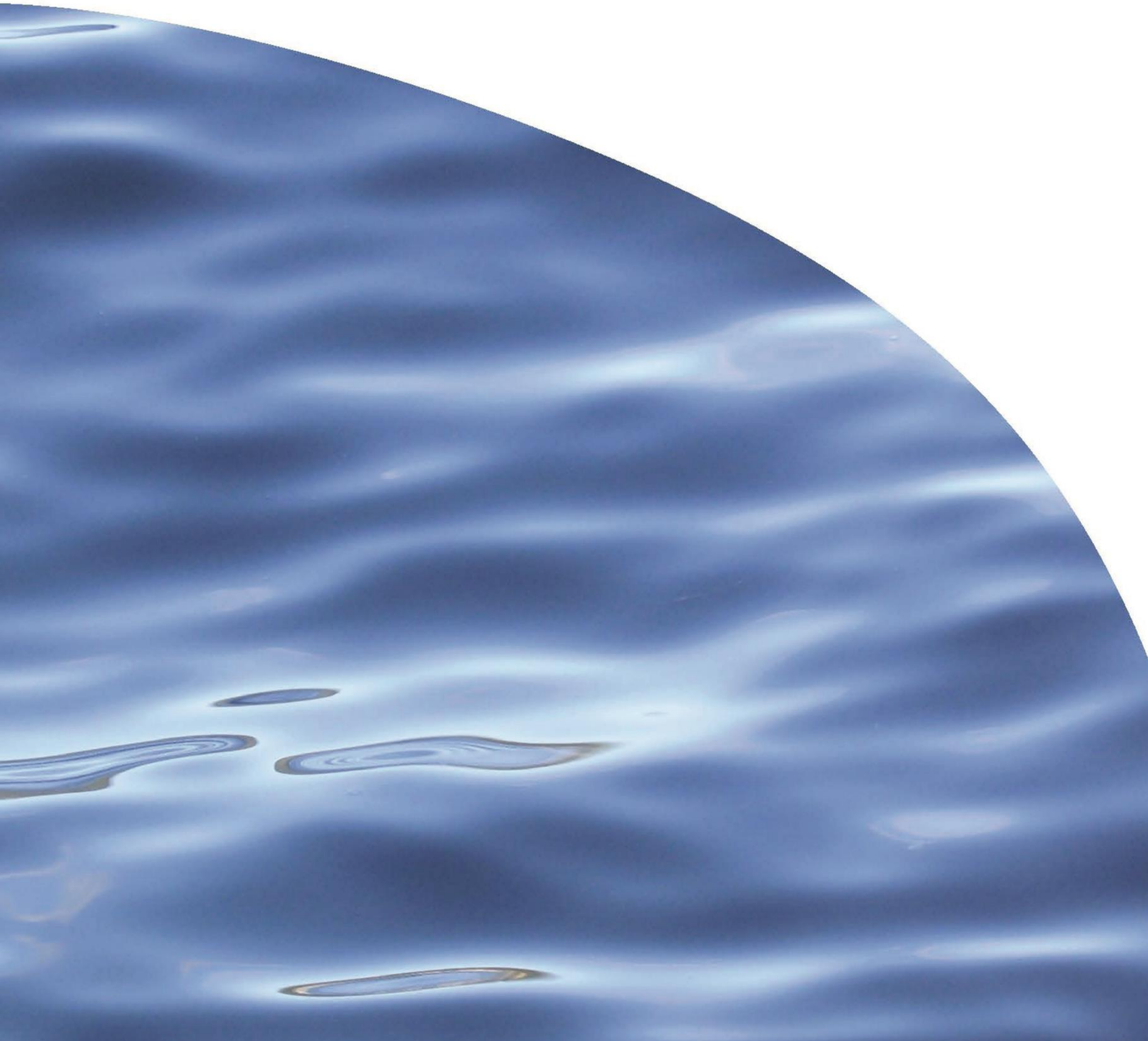
REPORT NO. 2752

ADVICE TO INFORM THE DEVELOPMENT OF A BENTHIC CYANOBACTERIA ATTRIBUTE



ADVICE TO INFORM THE DEVELOPMENT OF A BENTHIC CYANOBACTERIA ATTRIBUTE

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Prepared for the Ministry for the Environment

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EXECUTIVE SUMMARY

A review of national and international literature on benthic cyanobacteria and their associated toxins was undertaken. The key findings were:

Section 2[1] – Knowledge on toxin-producing cyanobacteria in New Zealand

- Six different benthic cyanobacterial taxa are known to produce cyanotoxins in New Zealand.
- The toxins they produce are: anatoxins (including anatoxin-a [ATX], homoanatoxin-a [HTX], dihydroanatoxin-a [dhATX], dihydrohomoanatoxin-a [dhHTX]), microcystins, nodularin and saxitoxins.
- The most abundant toxin-producing genus is Microcoleus (formerly *Phormidium*). Species from this genus can produce anatoxins (ATX, HTX, dhATX, dhHTX).
- *Microcoleus* blooms, defined as greater than 20% cover of a riverbed, have been identified in 103 rivers across New Zealand.
- It is the toxins, not the cyanobacteria that pose the health risk to humans. Anatoxins are the most prevalent toxin produced by benthic cyanobacteria in New Zealand. Concentrations can be extremely high in *Microcoleus*–dominated mats. We recommended that the literature review focuses on anatoxins and *Microcoleus*.

Section 3 –Toxicity and dose response of anatoxins

- There are no confirmed reports of human fatalities from anatoxins.
- Many animal poisonings and deaths have been attributed to anatoxins, however very few cases provide corresponding confirmatory anatoxin analyses.
- A limited number of studies using a small treatment/sample size have investigated ATX/HTX toxicity. These suggest:
  - An acute oral toxicity (mice) LD_{50} between 5 to 13.5 mg kg^{-1} and a maximum tolerable dose of 3 mg $kg^{-1}day^{-1}$ ATX.
  - An acute intraperitoneal (mice) LD_{50} between 0.25 to 0.32 mg kg^{-1} for ATX and HTX.
  - A No Observed Adverse Effect Level of 0.1 mg $kg^{-1}day^{-1}$ for ATX (based on a short-term mouse study [28-day]).
- There are very limited data indicating some reproductive/developmental toxicity; more research is required.
- There are no toxicological data available for dhATX and dhHTX.

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[1] Section 1 is a general introduction

- There are currently insufficient data to support derivation of a daily allowable intake for anatoxins.

Section 4 –Toxicity and dose response of skin contact with benthic cyanobacteria

- A study investigating lipopolysaccharides (LPS) in three *Microcoleus* strains found they were non-toxic. Caution is required when extrapolating these results to other *Microcoleus* as toxicity can vary markedly among species/strains.
- Studies on allergic responses caused by cyanobacteria (mostly planktonic species) suggest that a small proportion of the human population will experience reactions to cyanobacteria at low to moderate cell concentrations. These responses are usually mild and generally resolved within a short period (24-72 hours).

Section 5 –Toxin content of benthic mats

- Anatoxin data (n = 771) from 40 rivers were collated. Toxins were detected in 67% of samples.
- Total anatoxin concentration showed high spatial and temporal variability among and within rivers.
- The structural variants HTX, dhHTX and dhATX were predominant in samples analysed.
- The highest concentration of total anatoxin (712 mg kg^{-1} dried weight [dw]) was measured in a sample from the Oreti River (Southland).
- The median total anatoxin concentration for the majority of rivers was below 1 mg kg^{-1} dw.

Section 6 – Ingestion rate and likelihood of a child ingesting benthic cyanobacteria

- No studies were identified that investigated rate or likelihood of a child ingesting benthic freshwater cyanobacterial mats.
- Two approaches are proposed for estimating approximate ingestion rates:
    - Water ingestion rates based on primary and secondary recreational contact data. A Monte Carlo modelling approach is suggested to explore making quantitative predictions.
    - Soil ingestion rates.
- Factors identified as modifying the likelihood and rate of ingestion were susceptibility of user groups; and extrinsic and intrinsic bloom factors *i.e.* locality of river; and spatial and temporal variations in blooms.

Section 7 – Exposure rate and likelihood of skin exposure to benthic cyanobacteria

- No studies were found that investigated rate or likelihood of skin exposure to benthic freshwater cyanobacterial mats.

- Studies on planktonic and benthic marine cyanobacteria showed symptoms increase with duration of contact and cyanobacterial concentrations.
- Factors identified as modifying the likelihood of skin exposure are similar to those identified for ingestion (Section 6).

Section 8 – Relationships between abundance and environmental drivers

- *Microcoleus*-dominated mats require stable substrates on which to settle and grow, and a sufficient duration of non-scouring flow to accumulate to high biomass and cover.
- The abundance of *Microcoleus*-dominated mats in rivers is regulated by many of the same variables as periphyton. However once they form thick cohesive mat structures, they can develop attributes that isolate them from in-river conditions and need to be considered as functionally different, particularly with reference to nutrient dynamics, to other types of periphyton.
- *Microcoleus* blooms generally occur when water-column dissolved reactive phosphorus is low (approximately < 0.01 mg/L). Some observational studies suggest that some enrichment with dissolved inorganic nitrogen (DIN) favours growth, particularly during early colonisation. However blooms also occur in waters with low DIN concentrations. Further research is required to fully understand the relationship between DIN and bloom dynamics.
- Once established *Microcoleus*–dominated mats can trap sediment. Internal mat biogeochemistry may enable desorption of sediment-bound phosphorus which is then available for *Microcoleus* to utilise for growth.
- Re-setting primarily occurs due to shear stress and substrate disturbance. The magnitude of flow required to remove *Microcoleus*–dominated mats differs between growth stage and among rivers and as yet no universal predictor has been identified.
- Models to predict *Microcoleus* blooms are in their early stages, and currently revolve around statistical correlations between environmental drivers and the probability of a nuisance bloom (> 20% cover) developing.

Based on the literature review we recommend the following further work. Those marked with an asterisk (*) are considered high priority to assist with the current development of a benthic cyanobacteria attribute in New Zealand.

Section of review	Suggested further work required
2*	Systematic surveys of benthic cyanobacteria should be undertaken across New Zealand, especially at high recreational-use sites.
2	Further toxin testing should be undertaken on *Nostoc*, especially where it occurs in high densities at recreational use sites. The toxins produced by this genus (microcystins) can cause irreversible liver damage.

Section of review	Suggested further work required
3*	Further research is required on the toxicology of anatoxins. In particular there is a paucity of knowledge on HTX and dihydro variants. The toxicity of these variants should be tested in mice, relative to that of ATX, to gain some confirmation that the lower receptor binding of these variants carries through to lower *in vivo* toxicity.
3*	Once data from the Monte Carlo Modelling (6) are in place, repeated exposures of mice under 'worst case' exposure scenarios, being clearly aware of the limitations mouse bioassays have for human risk extrapolation, should be tested to establish whether exposures relatively close together in time cause any lowering of the apparent LD_{50}. It is possible that the irreversible binding of ATX to its receptor will cause delays in recovery and a mild build-up of effect over a day or two days of repeated exposures. The utility of an *in vitro* investigation of receptor recycling and ATX exposure should also be considered.
3*	Consult with international researchers to ascertain if there are any current toxicology studies underway or are planned on anatoxins. This may allow an opportunity to develop specific and well-designed toxicology studies with international researchers as a collaborative effort.
3*	Develop a cellular assay to assess ATX toxicity, specifically using human cell systems. This would facilitate easier testing of the intrinsic toxicity of any future ATX variants discovered. It would also provide preliminary toxicity estimates for those which there are insufficient information/material to allow animal testing or where animal testing could prove to be of limited value for extrapolation to humans.
3	There is indicative evidence to suggest the possibility of developmental and/or reproductive effects of ATX through interference with acetylcholine receptors. These might start being significant below the concentrations at which lethal effects are evident. Consideration should be given to using a zebra fish embryo assay to gain further insights into these processes.
4 and 7	Undertake an epidemiological study to investigate the health effects associated with recreational exposure to benthic cyanobacteria. This could follow a similar design to studies undertaken on planktonic cyanobacteria contact in Australia.
5	Anatoxin data should be collected from regions where it is lacking *e.g.* Taranaki, Northland, Bay of Plenty, to ensure that the current information is indicative of concentrations found nationally.
5*	Further data are required on the prevalence and concentrations of extracellular toxins and cyanobacterial filaments in the water column.

Section of review	Suggested further work required
5	Research should be undertaken on parameters that regulate toxin production and/or cause shifts in the relative abundance of toxic and non-toxic genotypes.
6*	The use of water and soil ingestion models to estimate rate and likelihood of benthic cyanobacteria ingestion should be explored further. A Monte Carlo modelling approach should be explored as a means of making quantitative predictions. Its usefulness will depends on the availability of dose-response data, and validity and method of inference from animal trials.
8*	An in-depth analysis of relationships between *Microcoleus* abundance and environmental drivers across a national dataset should be undertaken. This may identify common drivers of blooms and enable use of the River Environmental Classification to determine rivers susceptible to blooms.
8*	Undertake robust statistical analysis on existing extensive regional datasets (*i.e.* Manawatu, Canterbury) to improve knowledge on physiochemical parameters influencing *Microcoleus* bloom formation and distribution.
8*	Undertake further investigations into the role of groundwater in promoting blooms.
8	Conduct further research to increase knowledge on factors affecting colonisation, growth rates, autogenic detachment, and the impacts of grazers.

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1. INTRODUCTION

The National Policy Statement for Freshwater Management (NPS-FM) identifies 13 national values and uses for fresh water. Two of these are compulsory values which apply to all water bodies: ecosystem health and human health for recreation. Benthic cyanobacteria affect both of these compulsory values, however, the NPS-FM does not currently define attributes for them. The development of an attribute for benthic cyanobacteria for the human health value has been identified as a priority.

Benthic cyanobacteria grow attached to substrates and occur in all aquatic systems. When environmental and/or hydrological conditions are favourable, cells can multiply rapidly and benthic blooms may form. An increasing number of benthic cyanobacterial species have been identified as toxin producers. These natural toxins (cyanotoxins) pose a health risk to humans and animals through contact with, or ingestion of, cyanobacterial material or contaminated water.

The Cawthron Institute (Cawthron), University of Canterbury, NIWA, the Institute of Environmental Science and Research, and the University of Konstanz (Germany) were tasked by the Ministry of the Environment to undertake a review of national and international published literature on benthic cyanobacteria and their associated toxins. Advice was sought on 7 questions, and 21 sub-questions. The seven main areas to be addressed were:

1. Types and distribution of toxic benthic cyanobacteria in New Zealand
2. Toxicity and dose responses to anatoxins
3. Toxicity and dose response to skin contact with benthic cyanobacteria
4. Toxin content of benthic cyanobacteria in New Zealand
5. Likelihood of ingesting benthic cyanobacteria
6. Exposure rates and likelihood of skin exposure to benthic cyanobacteria
7. Relationships between *Microcoleus* (formerly *Phormidium*) and environmental drivers

Based on the findings and analysis of Question 1, this review focuses primarily on anatoxins, and *Microcoleus*-dominated mats, in New Zealand rivers.

2. WHAT ARE THE KNOWN TOXIN PRODUCING BENTHIC CYANOBACTERIA SPECIES IN NEW ZEALAND?

2.1. What types of toxins and at what levels do different species produce?

Most environmental benthic samples or mats consist of multiple species. Isolation of single filaments, culturing and toxin testing of pure uni-cyanobacterial strains is required to definitively identify toxin-producing species. This work has been undertaken for most of the known benthic toxin-producing species in New Zealand (Table 1).

Benthic cyanobacteria in New Zealand are known to produce congeners of anatoxin-a and homoanatoxin-a (ATX/HTX (hereafter referred to as anatoxins); also see Section 3), microcystins, nodularin and saxitoxins (Table 1). A brief description of each toxin-producing taxon follows. The congeners of ATX should not be confused with anatoxin-a(S) which has a completely different chemical structure and mode of toxicity.

Table 1. Summary of known toxin-producing benthic cyanobacterial species in New Zealand.

Genus/species	Cyanotoxin
Nostoc commune	Microcystins*1
Oscillatoria sp.	Anatoxin-a*2,microcystins*2
Microcoleus sp.	Anatoxin-a*3, homoanatoxin-a*3, dihydroanatoxin-a*3, dihydrohomoanatoxin-a*3
Planktothrix sp.	Microcystins*4
Scytonema cf. *crispum*	Saxitoxins*5
Unknown species	Nodularin*6

*This result was obtained from testing environmental samples. 1. Wood *et al.* 2006, 2. Hamill 2006, 3. Heath *et al.* 2010, 4. Wood *et al.* 2010, 5. Smith *et al.* 2011, 6. Wood *et al.* 2012a.

2.1.1. Nostoc commune

Thick gelatinous mats of *Nostoc commune* were observed on the eastern shore of Lake Taupo in 2003 (Figure 1). These accumulated along the shoreline following a storm event that dislodged colonies from rocks. Analysis using liquid chromatography -mass spectrometry (LC-MS) detected high concentrations of microcystins (708 mg kg^{-1} dry weight), predominantly the variants MC-RR (535 mg kg^{-1}) and DMeRR (142 mg kg^{-1}; Wood *et al.* 2006). Water samples collected close to the shoreline also contained microcystins, demonstrating toxins were being released.

Nostoc spp. are common in many New Zealand rivers and streams (Biggs & Kilroy 2000). They are well known from Lake Taupo (Hawes & Smith 1993, 1994), although there is little documentation of their distribution in other lakes and reservoirs. To our knowledge, no other *Nostoc* mats from New Zealand have been tested for cyanotoxins.

2.1.2. *Oscillatoria* sp.

Following rapid deaths of dogs at the Waikanae River (Wellington) in 1998, the toxicity of a benthic mat dominated by *Oscillatoria* sp. was investigated using a mouse bioassay and high performance liquid chromatography with fluorescence detection (HPLC-FLD). The presence of natural degradation products of anatoxin-a was confirmed (Hamill 2001). Further sudden deaths of dogs were reported at the Mataura River (Southland) in 1999 and 2000. Benthic *Oscillatoria* sp. mats were collected and mouse bioassays confirmed their high toxicity (death within 5 minutes; Hamill 2001). Detailed taxonomic identification of the causative species was not undertaken. Oscillatoriales are notoriously difficult to identify based on morphology alone, and it is likely that the *Oscillatoria* sp. documented in this study was *Microcoleus* (see Section 2.1.3).

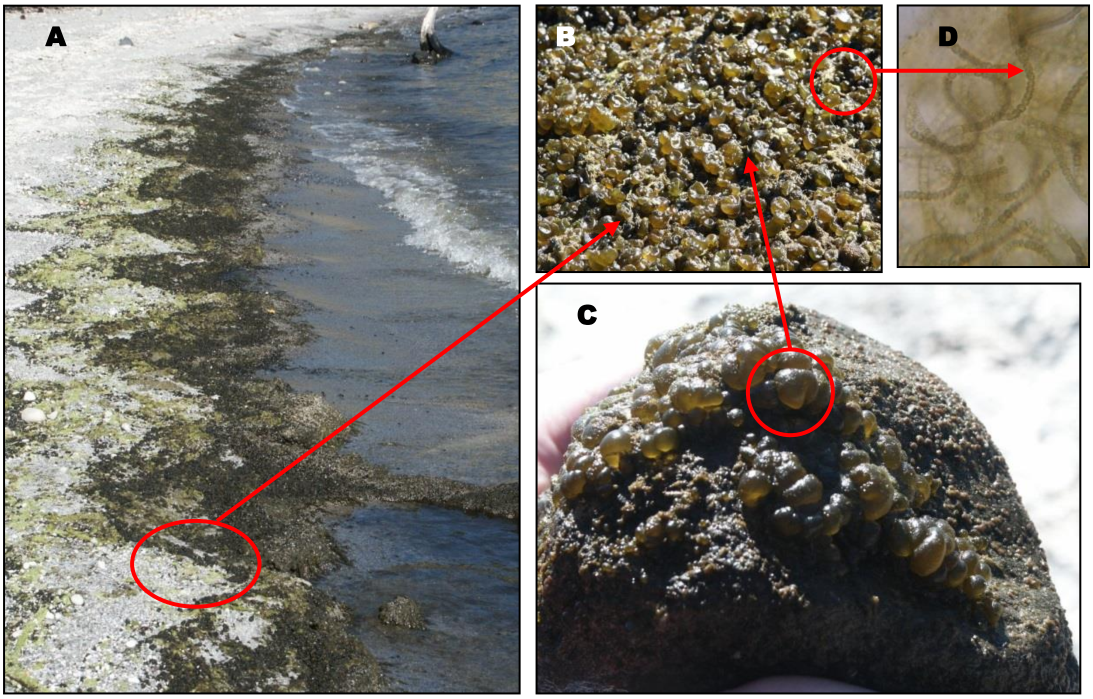

Figure 1. *Nostoc commune* mats along the shores of Lake Taupo. A-C, low lake levels and high winds caused *Nostoc commune* colonies to be dislodged from rocks in the lake and these accumulated along the shoreline; D *N. commune* filaments under the microscope.

2.1.3. *Microcoleus*

In the summer of 2005 to 2006 there were multiple dog deaths at the Hutt River (Wellington). Wood *et al.* (2007) used morphological and molecular techniques and identified the causative cyanobacterium as *Microcoleus autumnalis* (formerly *Phormidium autumnale*). Liquid chromatography-mass spectrometry of the *M. autumnalis* -dominated mats identified anatoxin-a, dihydroanatoxin-a, homoanatoxin-a and dihydrohomoanatoxin-a. Since this confirmation *M. autumnalis*-dominated mats have been identified across New Zealand (see Section 2.2). When conditions are optimal, *M. autumnalis* forms thick cohesive mats that may cover many kilometres of the riverbed (Figure 2). Heath *et al.* (2010) used a combined molecular and morphology approach to screen over 30 cultures isolated from *Microcoleus*-dominated mats collected across New Zealand. *Microcoleus autumnalis* was the only species found to produce anatoxins. Both toxic and non-toxic strains were identified. Non-toxic strains lacked the presence of genes involved in the biosynthesis of anatoxins (Wood *et al.* 2012b). *Microcoleus* species are challenging to distinguish based on morphology alone. Although detailed polyphasic analysis was undertaken in early studies (*i.e.* Wood *et al.* 2007; Heath *et al.* 2010), this is rarely done now and is not practical for routine monitoring. There are indications that *Microcoleus*-dominated mats may contain multiple *Microcoleus* species (Harland *et al.* 2014), therefore the term *Microcoleus*-dominated mats is used hereafter.

Routine testing and research on *Microcoleus*-dominated mats from around New Zealand has shown marked variations in the presence and concentrations of anatoxins and in the structural variants produced (see section 5.1.1). Wood *et al.* (2012b) demonstrated that this variability is partly due to differences in the relative abundance of toxic and non-toxic strains within a mat, and differences in the amounts of toxin each strain produces. The effect of environmental parameters *i.e.* temperature, is unclear and to date no relationships have been identified (Wood *et al.* 2014). Using cultures and laboratory-based studies Heath *et al.* (2014) and Smith *et al.* (2013) consistently identified an upregulation in toxin production early in the growth phase.

Microcoleus-dominated mats are commonly associated with cobble-bedded rivers in New Zealand; however, under stable flow conditions they also grow in rivers with fine substrate. Extensive mats have been identified on the bottom of lakes in the Rotorua region (those tested to date were non-toxic; Wood *et al.* 2012a). In 2014 ingestion of mats dislodged from the bottom of a small farm pond (Figure 2D), caused the death of a dog. Culturing, molecular and toxin analysis confirmed the causative species was also *M. autumnalis* (Wood unpubl. data).

2.1.4. *Planktothrix* sp.

In November 2008 a dog died soon after ingesting benthic ‘algal’ mat material from the Waitaki River (Canterbury). Based on morphology, the causative organism was putatively identified as *Microcoleus* sp. Subsequent molecular analysis of cultures demonstrated it was a benthic strain of *Planktothrix* (a common planktonic toxin-producing genus in the Northern Hemisphere). Using LC-MS, microcystin-LR, [D-Asp3, Dha7] microcystin-LR, [D-Asp3] microcystin-LR, and minor proportions of [D-Asp3, ADMAdda5] microcystin-LhR were identified (Wood *et al.* 2010). To our knowledge this is the only identification of this species in New Zealand, however, given its similar appearance to *Microcoleus* (Figure 3), it is probably commonly misidentified.

Figure 2. (A,B) *Microcoleus* mats in the Waipoua River (Wairarapa), (C) *Microcoleus*-dominated mats in Lake Rotoiti (Rotorua), and (D) detached *Microcoleus* mats in a small farm pond (Kaikoura). Photo C: Aleki Taumoepeau (NIWA).

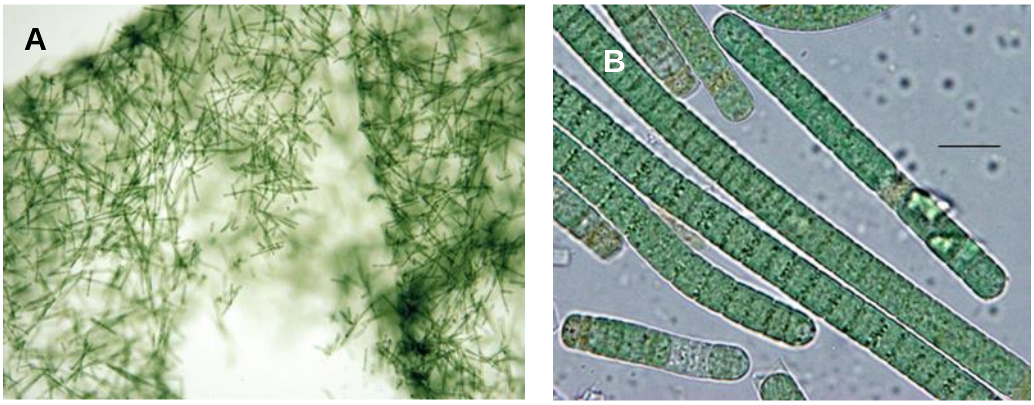

Figure 3. Light photomicrographs of *Planktothrix* sp. (VUW25). Scale bars B, 85 µm.

2.1.5. *Scytonema* cf. *crispum*

Samples collected from the metaphyton of a drinking-water supply's pre-treatment reservoir (Oamaru) and a small eutrophic lake (The Groynes, Christchurch) tested positive for saxitoxins using pre-column oxidation HPLC-FD (Smith *et al.* 2011). Cultures were established and morphological and molecular analysis identified the causative species as *Scytonema* cf. *crispum*. Initially saxitoxin was the only variant detected and this was found at concentrations of 65.6 mg kg^{-1} dry weight in the Groynes lake sample and 119.4 mg kg^{-1} in the culture (strain UCFS10). Subsequent surveys identified this species in additional lakes in the Canterbury region (see Section 2.2), and toxin analysis of environmental samples and cultures from these samples identified the variants: gonyautoxins (GTX 1–5), neosaxitoxin, decarbamoyl saxitoxin and decarbamoyl gonyautoxins (dcGTX2/3; Smith *et al.* 2012). Unlike the other toxic benthic cyanobacteria described in this section, *S*. cf. *crispum* does not attach to a substratum but is free-floating in the metaphyton amongst aquatic plants (Figure 4A), a habitat that is commonly overlooked in lake studies (Hawes and Smith 1994).

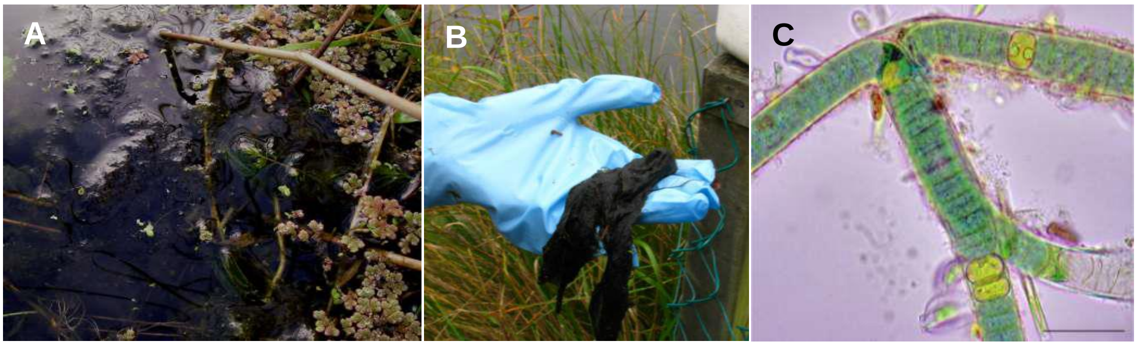

Figure 4. (A) *Scytonema* cf. *crispum* growing as metaphyton among aquatic plants, (B) dense filamentous mats, and, (C) light photomicrograph. Photos: Francine Harland.

2.1.6. Unknown nodularin producer

A survey of periphyton (depth 6–7.5 m) in Lake Tikitapu (Rotorua) revealed widespread thick spongy benthic mats dominated by a mixed assemblage of cyanobacterial species from the orders Oscillatoriales, Nostocales and Chroococcales (Wood *et al.* 2012a; Figure 5). All benthic mats tested contained nodularin (max. 0.61 mg kg^{-1} dry weight; Wood *et al.* 2012a). Genes involved in nodularin production were sequenced and had very low (≤ 89%) homology to those from other known nodularin producers, suggesting a new toxin-producing species. Attempts to identify the nodularin producer were unsuccessful (Wood *et al.* 2012a). Genetic analysis has identified the presence of the same species in cyanobacterial-dominated mats in alpine tarns near Nelson. Nodularin was also detected in these mats using LC-MS (Wood, unpubl. data).

Figure 5. Cyanobacterial-dominated mats in Lake Tikitapu (Rotorua). Photos: Aleki Taumoepeau (NIWA).

2.2. What are the distributions of different species in New Zealand?

No systematic surveys of toxic benthic cyanobacteria have been undertaken in New Zealand. However, there have been multiple studies of periphyton (including cyanobacteria, *e.g.*, Biggs *et al.* 1998; Biggs 1990; Biggs & Smith 2002). Regional councils undertake periphyton assessment (usually monthly) as part of their routine State of the Environment (SoE) programmes. The classification strategy used for macroscopic assessment of coverage (*i.e.* slimy filamentous, coarse filamentous, films, mats) makes determining the abundance of cyanobacteria using these data challenging. Samples are usually collected for microscopic assessment, and a wide range of cyanobacterial species are regularly identified (S Wood, pers. comm., Cawthron microalgae laboratory). The method of assessment (relative abundance) makes it impossible to undertake any comparative analysis using these data. A review of cyanobacterial species in SoE data or in previous research is beyond the scope of this report, and this section focuses on locations where blooms of toxic species have been identified, or where poisoning events have occurred.

Since the release of the New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters in 2009 (Ministry for the Environment and Ministry of Health, 2009), an increasing number of regional councils have assessed coverage of *Microcoleus*-dominated mats as part of their SoE monitoring and/or undertaken specific *Microcoleus* assessments at key recreational sites. We contacted freshwater scientists at all regional councils, the Nelson City Council and the Marlborough District Council. They were asked to provide names of (or data from) rivers in their region that had one (or more) sites that had experienced *Microcoleus* blooms (defined as greater than 20% coverage). Auckland Regional Council and Waikato Regional Council were the only regions who stated that they were not aware of any *Microcoleus* blooms, however, they also noted that they do not undertake any specific monitoring. Information from the other organisations identified *Microcoleus* blooms in 104 rivers spread across the entire country (Figure 6; North Island = 61 rivers, South Island = 42 rivers). *Microcoleus* blooms do not usually affect the entire river and are often confined to specific reaches, although in some rivers blooms may stretch for many kilometres. Regional council staff noted that in some rivers *Microcoleus* blooms occur annually during stable river flows, whereas other rivers only have sporadic blooms that may be related to events such as short-term sediment discharges.

The location of the other toxic benthic cyanobacteria described in Section 2.1 is shown in Figure 7. This illustrates the extremely limited knowledge on the distribution of these taxa.

Biggs and Kilroy (2000) state that *Nostoc* is usually found in fast-flowing relatively clean water, upland streams and rivers, and grows attached to rocky substrates. They also note that *Nostoc* is abundant and is widespread in New Zealand. It is common around Lake Taupo (Hawes & Smith 1993, 1994), and to our knowledge the samples from Lake Taupo are the only ones that have been tested for toxins. Further analysis should be undertaken to assess whether multiple strains/species produce toxins and to establish if this genus poses a health risk.

We suggest that the *Oscillatoria* sp. mats involved in the dog deaths in 1998–2000 should be considered as *Microcoleus*. *Microcoleus* blooms have now been recorded in these rivers (Figure 6). As stated above, the microcystin-producing *Planktothrix* sp. identified in Wood *et al.* (2010) is only known from a single location in New Zealand.

Following the identification of saxitoxin-producing *S.* cf. *crispum* in two South Island water bodies, Environment Canterbury commissioned a survey of 34 high-use recreational lakes across Canterbury. *Scytonema* was observed in 10 of the lakes surveyed (Smith *et al.* 2012; Figure 7). Three morpho-species were identified: *S*. cf. *crispum*, *S*. cf. *chiastum* and *S*. cf. *fritschii*. Saxitoxins were only identified in samples containing *S*. cf. *crispum*. *Scytonema* has been reported in the North Island, New Zealand where it is common in the littoral zone in Lake Taupo (Hawes & Smith 1994), in algal mats in Lake Taharoa, and in the Waimangu geothermal lakes (Gaylarde *et al*. 2006). In the South Island it is reported from the benthos of Lake Coleridge (Hawes & Schwarz 1996). However, toxin testing of mats from these locations has not been undertaken.

Until the identification of the nodularin producer is confirmed, knowledge of its distribution remains limited. Detection of nodularin and nodularin synthesis gene sequences from two contrasting environments (*i.e.* an oligotrophic lowland lake and a shallow alpine tarn) suggest it may be relatively tolerant of a wide range of conditions. Further efforts to identify this organism should be a priority.

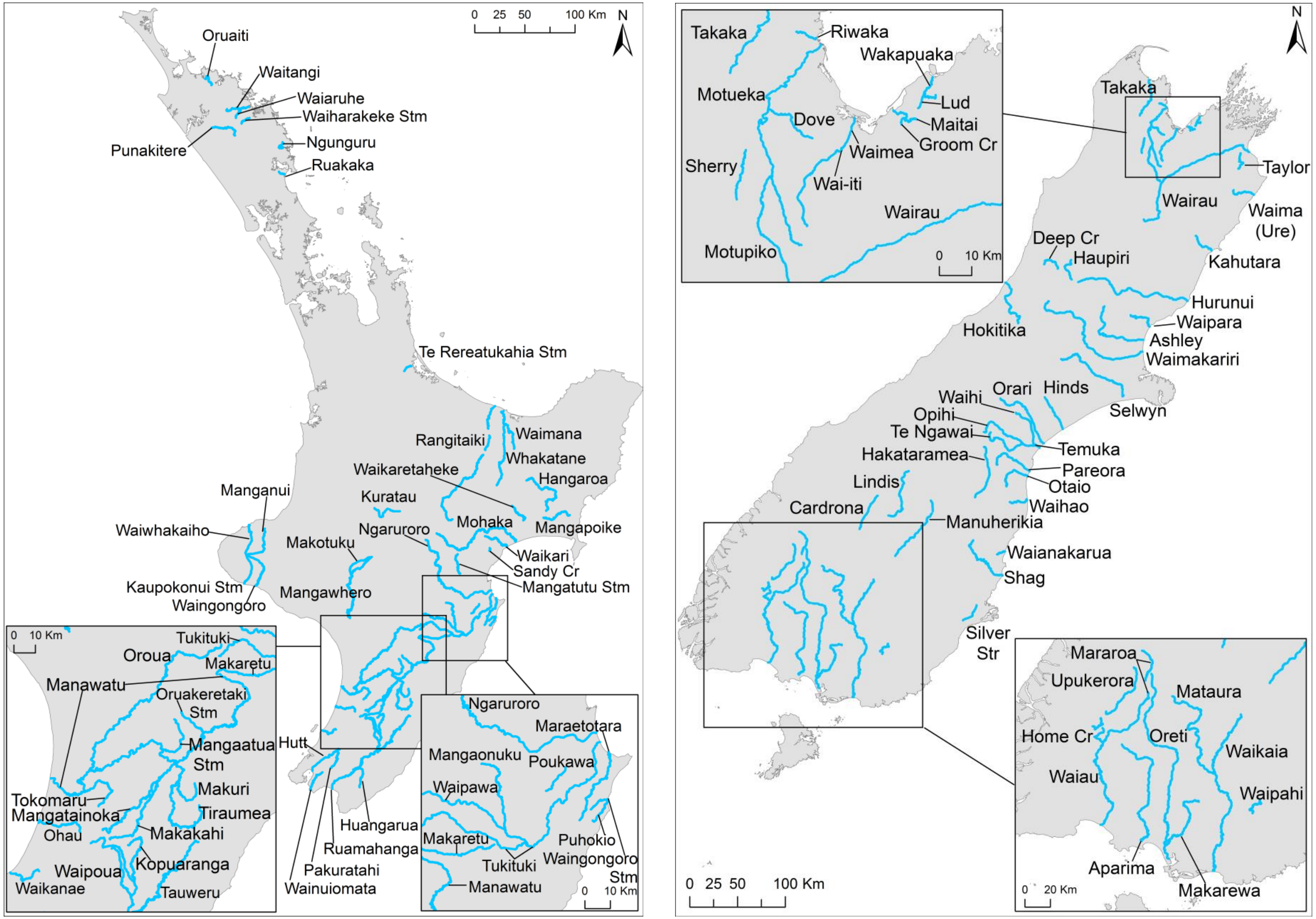

Figure 6. Rivers where one (or more) sites have experienced *Microcoleus* blooms (defined as greater than 20% coverage) on one (or more) occasions since 2009.

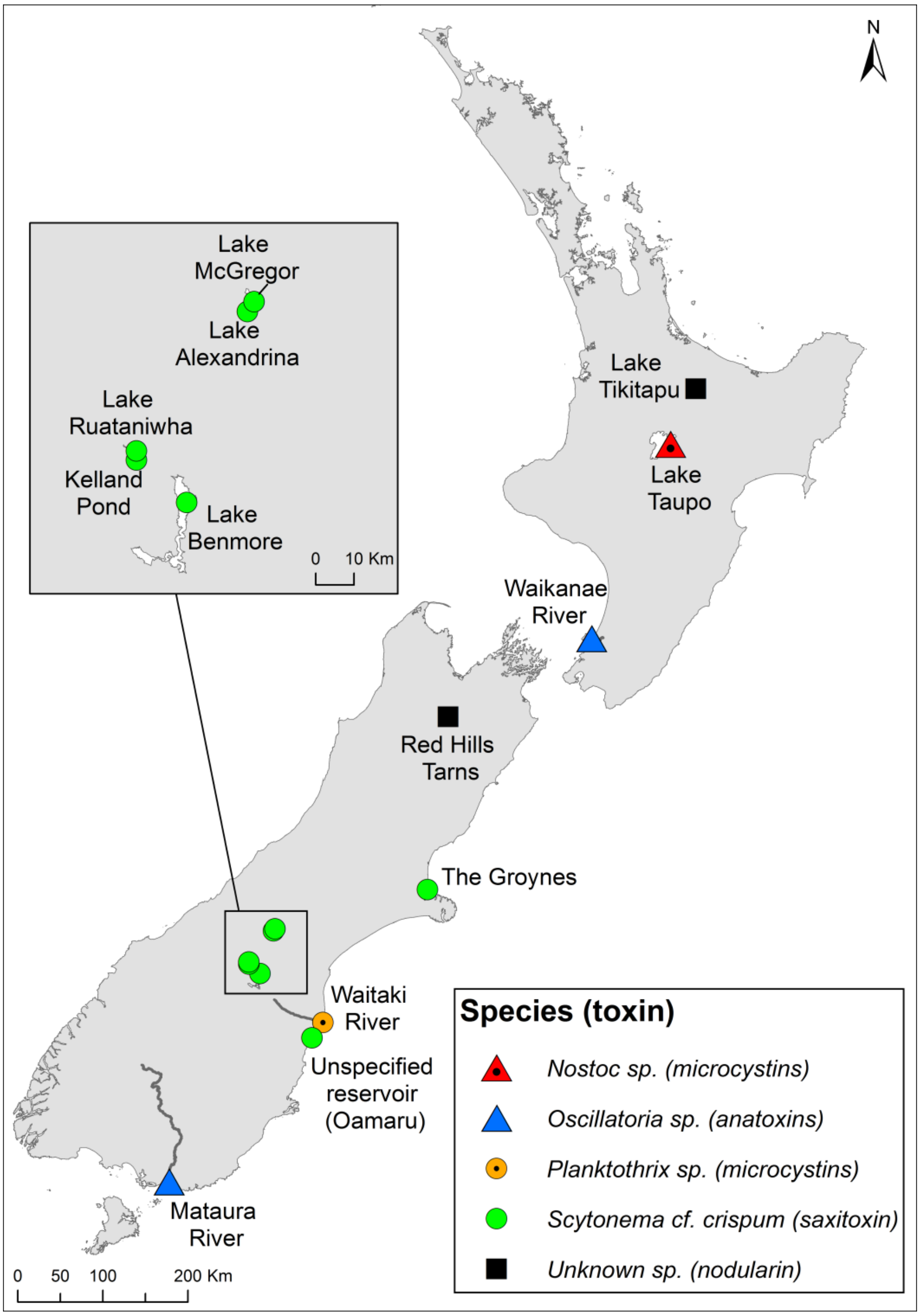

Figure 7. Known locations of toxic benthic cyanobacteria in New Zealand (excluding *Microcoleus* see Figure 6).

2.3. What are the benefits and limitations of focusing management on *Microcoleus* versus a range of species?

The Ministry for the Environment has identified benthic cyanobacteria as a possible attribute to use when setting objectives for secondary contact recreation under the National Objectives Framework.

In the last 10 years blooms dominated by *Microcoleus* appear to have increased in their prevalence and severity across New Zealand. Blooms dominated by *Microcoleus* occur nationally (Section 2.2). Many blooms contain anatoxins (see Section 5.1.1). The health risk posed by anatoxins has been demonstrated by the deaths of many dogs across New Zealand (estimated greater than 100 deaths since 2005). There are also anecdotal reports of human illness or skin irritation after contact with rivers containing *Microcoleus*-dominated mats.

Of the other toxin-producing species identified in New Zealand, both *S.* cf. *crispum* and *Nostoc* sp. clearly pose a potential health risk to humans involved in recreational activities. They can accumulate in high densities at the edges of lakes, including some of our most pristine lake environments, which are areas of high recreational contact. Data on *Planktothrix* sp. and the nodularin-producer are insufficient to determine if they pose a potential human health risk. There is a need to collect more information on the distribution and toxin content of other benthic cyanobacteria in New Zealand.

While other toxins may be present in benthic cyanobacterial mats in New Zealand, we believe that during the development of this attribute the focus should be on anatoxins—the most widespread toxins produced by benthic cyanobacteria in New Zealand. To date *Microcoleus* (specifically *M. autumnalis*) is the only known benthic anatoxin producer in New Zealand and the attribute should primarily be developed using data available on this genus. Although *Microcoleus* occurs in lakes, the focus of this attribute should be on rivers.

The reasons for focusing on anatoxins produced by *Microcoleus* in rivers are as follows.

- The high concentrations of anatoxins, accessibility of mats to humans (*i.e.* detached mats often wash up on the edge of rivers), and prevalence of blooms in areas used for recreation clearly demonstrate they pose a health risk to humans.
- On-going research has started to elucidate environmental variables that regulate *Microcoleus* blooms in rivers (Section 8). This information is required before informed management actions can be initiated.
- The methods used to assess the abundance of *Microcoleus*-dominated mats, to collect samples, and to undertake accurate anatoxin analysis, are well defined and tested.

- Although 'blooms' of *Microcoleus* occur in lakes and ponds, little is known about their distribution, and current methods for assessing coverage are not applicable. For this reason we suggest the attribute focuses only on *Microcoleus* in rivers.

Once developed the thresholds and statistics used in the attribute should be tested on other benthic cyanobacterial species. A similar approach was used for the New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters, whereby toxicology data for microcystin and toxin data for *Microcystis* were used to develop thresholds. These thresholds were then tested using data from an anatoxin-producing planktonic species, to ensure that the toxin concentrations were within a range deemed unlikely to have an adverse effect on human health. We suggest that the benthic cyanobacterial attribute is developed using anatoxin toxicology data and anatoxin data from *Microcoleus*. Once developed the attribute could be applied to all benthic cyanobacteria in rivers (*i.e.* in the same way that the planktonic cyanobacterial attribute is applied to all cyanobacteria in lakes).

3. WHAT IS THE TOXICITY AND DOSE RESPONSE OF INGESTING CYANOTOXINS IN HUMANS?

This section focuses only on anatoxins (see Section 2.3 for justification). The order of questions 3.3 and 3.4 is reversed from that given in the Request for Proposal to enable continuity in the literature review.

Anatoxin-a (ATX) has a semi-rigid bicyclic secondary amine structure, 2-acetyl-9-azbicyclo[4:2:1]non-2-ene, and a molecular weight of 165.26 (Figure 8). Six structural analogs have been described; homoanatoxin-a (HTX), 2,3-epoxy-anatoxin-a, 4-hydroxy- and 4-oxo-derivatives, dihydroanatoxin-a (dhATX), dihydrohomoanatoxin-a (dhHTX) and 11-carboxyanatoxin-a (Skulberg *et al.* 1992; Namikoshi *et al.* 2003; Selwood *et al.* 2007; Mann *et al.* 2012). *Microcoleus* strains producing high concentrations of ATX have been isolated in New Zealand (Heath *et al.* 2010). However, the majority of environmental samples collected in New Zealand from *Microcoleus*-dominated mats contain low concentrations of ATX, and are dominated by dhATX, HTX and dhHTX (Figure 8). This is an important consideration as dhATX appears to have an approximately 10-fold reduction in its affinity for nicotinic acetylcholine receptor binding sites in comparison to ATX (Wonnacott *et al.* 1991) which may be indicative of a reduction in its toxicity.

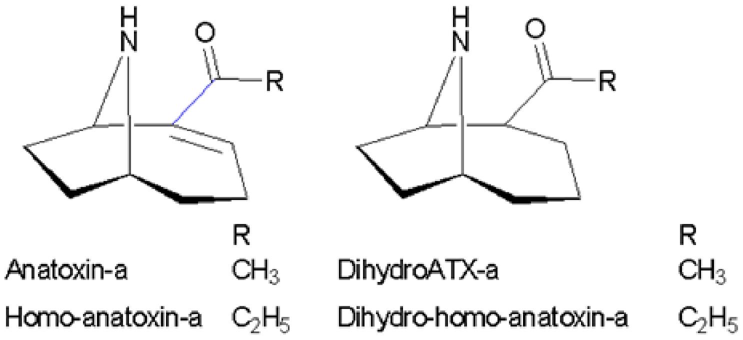

Figure 8. Structure of anatoxin-a, homoanatoxin-a and the dihydro derivatives.

Anatoxins mimic the action of acetylcholine at neuromuscular nicotinic receptors of the post-synaptic membrane at the neuromuscular junction (Aronstam & Witkop 1981; Carmichael *et al*. 1975, 1979; Swanson *et al*. 1986; Figure 9). During normal muscle activity, acetylcholine is released from vesicles of the motor neurons at the neuromuscular junction (axon terminal, Figure 9). It then binds to the acetylcholine-receptors on the postsynaptic muscle cell, opening sodium ion channels, allowing for sodium ion influx from the synaptic cleft and thereby triggering muscle cell contraction

(Figure 9). Extracellular acetylcholinesterases degrade acetylcholine thereby preventing overstimulation of the muscle cells and consequent muscle fatigue. Anatoxins are not degraded by cholinesterase and in its presence muscle cells continue to be stimulated, causing muscular twitching, fatigue, paralysis and respiratory arrest (Carmichael *et al.* 1977; Devlin *et al.* 1977; Stevens and Krieger 1991; Figure 9). MacPhail *et al*. (2007) used comparative behavioural studies (nicotine versus ATX) in rats to show that ATX does not act in quite the same way as nicotine. The rats developed a tolerance to nicotine, whereas this was not observed for ATX. This suggests that multiple exposures could potentially result in additive toxicity without appreciable regeneration.

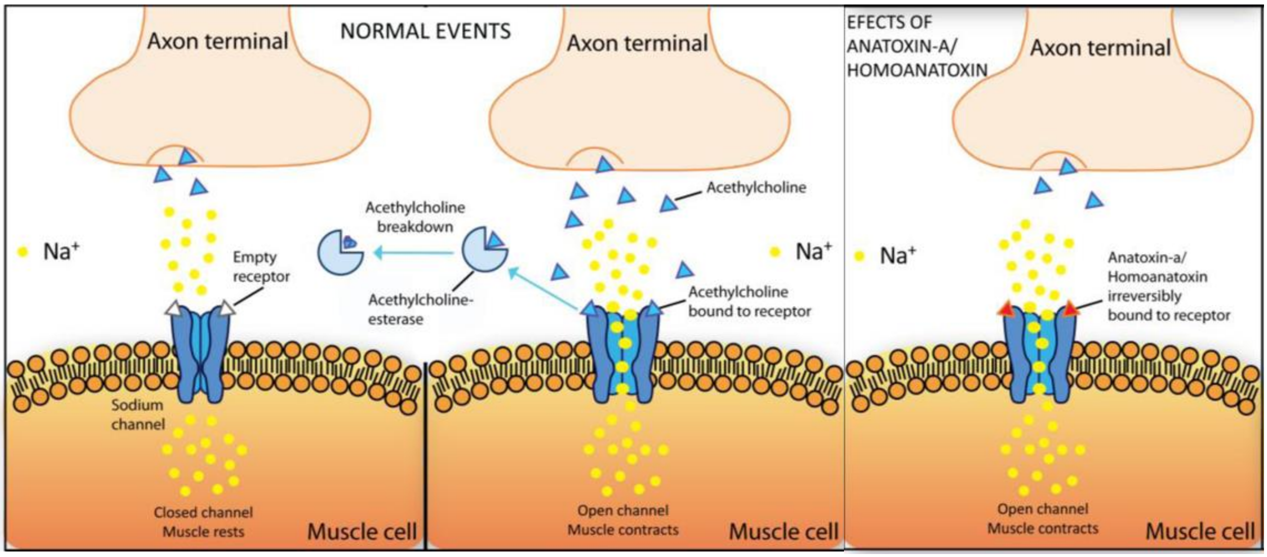

Figure 9. Schematic representation of the mode of action of anatoxin-a/homoanatoxin-a. From Valério *et al.* (2010).

3.1. What have studies that directly addressed this question found?

There is no information available on epidemiology studies or confirmed case reports of human poisoning from exposure to anatoxins.

Symptoms described in humans and other larger mammals for either acute nicotine poisoning or intoxications with acetylcholinesterase inhibitors such as organophosphate, pesticide intoxications (*e.g.* malathion, parathion), or chemical warfare agents (*e.g.* tabun, sarin and soman) include: nausea and vomiting, excessive salivation, abdominal pain, pallor, sweating, hypertension, tachycardia, ataxia, tremor, headache, dizziness, muscle fasciculations, and seizures (Haley & Kurt 1997; Leibon & Lifshitz 2008; Schep *et al.* 2009). Similar symptoms may be expected after exposure to anatoxins.

Anatoxin-a was implicated in the death of a 17-year-old boy in America who died two days after swallowing water while swimming in a pond containing a cyanobacterial bloom (Behm 2003). The teenager went into shock and suffered a seizure before dying from heart failure. Another teenage boy who swam in the pond experienced severe diarrhoea and abdominal pain but survived, and three others developed unspecified minor symptoms. Analysis of samples (water and human) failed to definitively identify the cause as anatoxin poisoning (Carmichael *et al.* 2004).

A similar case occurred in a fun park situated in the city of Wavre (Belgium). A 21-year-old male went swimming with colleagues despite signs warning of toxic cyanobacterial blooms. Of ten colleagues that went swimming five developed nausea, diarrhoea, itching and numbness of the extremities. The 21-year old, who also had the strongest acute symptoms, subsequently developed peripheral neuropathy that resulted in complete working disability two years subsequent to the initial event. The other swimmers with symptoms did not report residual effects. Unfortunately no samples were taken from the pond or patients, thus anatoxin poisoning could not be confirmed (D Dietrich, pers. obs. 2015).

In New Zealand there have been anecdotal reports of human illnesses associated with recreational activities in rivers containing *Microcoleus*-dominated mats. In one instance, a young child was taken to hospital with severe stomach pains after swimming in the Waipoua River (Wairarapa). *Microcoleus*-dominated mats were later tested and found to contain high concentrations of ATX and HTX. However, there was no conclusive evidence to prove that anatoxins caused the observed symptoms (Wood *et al.* 2011). During most New Zealand summers, Cawthron scientists receive phone calls from concerned river users who have experienced rashes or flu-like symptoms after partaking in recreational activities at rivers (S. Wood, pers. obs.). Often the river users do not immediately associate symptoms with river water contact. This connection usually occurs at a later date when animal deaths and local media raise awareness. It is almost impossible to retrospectively gather definitive data on symptoms and possible contact with cyanobacteria.

3.2. How can toxicology studies for other species be used to approximate toxicity to humans?

This section leverages a recent and extensive review undertaken by the U.S. Environmental Protection Agency (US EPA; U.S. Environmental Protection Agency 2015).

3.2.1. Case reports

Anatoxins have been associated with poisonings and deaths of livestock, dogs and ducks after ingestion of cyanobacterial material and/or exposure to water contaminated with cyanotoxins (Carmichael and Gorham 1978; Edwards *et al.* 1992; Gunn *et al.* 1992; Gugger *et al*. 2005; Puschner *et al.* 2008; Faassen *et al.* 2012; Backer *et al.* 2013). Quantitative exposure data are not provided but clinical signs are generally reported as neurologic and deaths due to respiratory paralysis.

An extensive analysis and post-mortem of a dead 20-month-old Labrador was undertaken following a series of dog deaths at the Hutt River (Wellington) in 2005 (Wood *et al.* 2007). Anatoxin-a and HTX, and *Microcoleus* filaments were detected in the stomach of the dead dog. These findings in the absence of other causes provided strong evidence that the neurotoxins had caused the sudden death. Histological examination of the dog's tissues was limited, but no underlying pre-existing health problems were found, nor any evidence of inflammation or infection. The lung changes of pulmonary oedema were consistent with agonal inspiratory gasping and shock (Dungworth 1993). The other organs, although autolytic, did not show any abnormality. Drowning was ruled out due to the time periods elapsing between swimming and death (1 hr) and the healthy appearance of the animal on the walk home. The dog was found dead, so clinical signs of the dying animal were not observed. However, other dogs that died (no autopsies undertaken) after contact with the Hutt River in November 2005 were reported to shake, convulse, froth at the mouth and collapse shortly before death. These are all symptoms commonly associated with anatoxin poisoning. Post-mortems and toxin testing of stomach contents are now rarely undertaken on deceased dogs in New Zealand. If the dog has had contact with a river and symptoms match those expected, anatoxin poisoning is assumed. Unfortunately no national database of animal poisonings associated with cyanotoxins is maintained in New Zealand, but based on reports to Cawthron staff we estimate that greater than 100 dogs have died due to anatoxin poisoning since 2005.

3.2.2. Acute toxicity - oral exposure

Three studies have investigated acute oral toxicity.

1. Stevens and Krieger (1991) used a single dose gavage in adult male Swiss Webster ND-4 mice and determined an LD_{50} of 16.2 mg kg^{-1} (Confidence Interval [CI] of 95%: 15.4–17.0) for synthetic (+)-ATX hydrochloride (> 98% pure commercial product), which is equivalent to 13.3 ATX kg^{-1} (95% CI: 12.8–14.1). They also used an extract from lyophilised *Dolichospermum (*previously known as *Anabaena*) *flos-aquae* (NRC-44-1) cells, and calculated an LD_{50} of 6.7 mg ATX^{-1} kg^{-1} (95% CI: 6.3–7.1). The LD_{50} values were determined using moving averages for four doses with six animals per dose. The (+) and (-) isomers of ATX have been reported to demonstrate up to a 20-fold difference in affinity for the nicotinic acetylcholine-receptor (Daly 2005). The racemic mixture was found to have a similar toxicity to the pure (+)-ATX isomer (MacPhail *et al.* 2007). Caution is required when comparing toxicity data obtained using synthetic versus ATX purified from cyanobacterial material.
2. A single dose gavage study in newly weaned CBA/BalbC mice of unspecified sex determined an LD_{50} of > 5 mg ATX^{-1} kg^{-1}. The authors used a 'suitably

purified' but an unspecified form of commercial product (Fitzgeorge *et al.* 1994). There is no information on whether the commercially available ATX consisted of a racemic mixture of synthetic ATX or of purified ATX from a cyanobacterial source. Deaths due to neurotoxicity, described as muscular twitching, loss of coordination and death by respiratory paralysis, occurred within two minutes of administration (Fitzgeorge *et al.* 1994).

3. Fawell and James (1994) undertook a 5-day gavage, range-finding study to determine the maximum tolerated dose for use in a 28-day study (Section 3.3). Doses of 1.5, 3, 7.5 or 15 mg kg^{-1} day^{-1} using aqueous (+)-ATX hydrochloride (commercial product, purity not reported; equivalent to 1.2, 2.5, 6.2 or 12.3 mg ATX^{-1} kg^{-1} day^{-1}) were administered to two male and two female Crl:CD-1(ICR)BR mice groups (no control group included). After 24 hours of administering the lower dose (1.2 mg kg^{-1} day^{-1}) the 6.2 and 12.3 mg kg^{-1} day^{-1} dosing started. After 5 days, the 2.5 mg kg^{-1} day^{-1} (intermediate level) dosing was administered. Evaluation of clinical signs, food consumption and body weight were undertaken and surviving animals necropsied. During the first four days, all mice in the high-dose group died (within five minutes of dosing), and one female mouse from the 6.2 mg kg^{-1} day^{-1} dose group died. The male mice in the 6.2 mg kg^{-1} day^{-1} group were hyperactive following the third dose. Surviving animals in this group did not express any abnormal clinical signs and no other signs of neurotoxicity were reported. The study was limited by the small number of animals tested (two mice per sex per dose), the lack of concurrent controls and by the extent and type of endpoints evaluated (clinical signs, body weight, food consumption and necropsy). A dose of 6.2 mg kg^{-1} day^{-1} was identified as the Frank Effect Level (a level of exposure that produces unmistakable and irreversible effects) based on the death of one of the two female mice. The maximum tolerated dose was established as 3 mg kg^{-1} day^{-1} ATX hydrochloride (2.5 mg kg^{-1} day^{-1} ATX).

In summary, the data generated from these studies need to be interpreted with caution due to inconsistencies in experimental designs. Similar studies have been undertaken in mice using intraperitoneal (i.p.) injection of mice with ATX (see below), thus bypassing the gastrointestinal tract. Comparisons of these data indicate that gastrointestinal absorption is critical for the toxicity of ATX. Based on the observed differences in oral LD_{50} between studies using the synthetic ATX and ATX purified from cyanobacteria extracts, it seem plausible that some constituents in the extract may facilitate gastrointestinal uptake of ATX.

3.2.3. Acute toxicity – intraperitoneal injections

The following studies have investigated i.p. toxicity of ATX or HTX.

1. Stevens and Krieger (1991) undertook a single dose i.p. study in mice and identified an LD_{50} of 0.25 mg kg^{-1} (95% CI: 0.24-0.28) for (+)-ATX hydrochloride (commercial product, > 98% pure) equivalent to 0.21 mg ATX^{-1} kg^{-1}.
2. Skulberg *et al.* (1992) demonstrated HTX has an LD_{50} in mice of 0.250 mg kg^{-1}. Toxicosis in the lethal dose range led to severe body paralysis, convulsions and death by respiratory arrest in 7–12 minutes.
3. Fitzgeorge *et al.* (1994) determined an i.p. LD_{50} of 0.375 mg kg^{-1} for commercial ATX (form and purity not reported).
4. Single i.p. injections of ATX hydrochloride (>95% pure) were administered in male BalbC mice (Valentine *et al.,* 1991). LD_{50} values were determined as 386 µg kg^{-1} (95% CI: 365-408) for ATX hydrochloride equivalent to 0.32 mg ATX^{-1} kg^{-1}.
5. A 2-day i.p. study in 18 female CD-1 mice was performed to determine a maximum dose to evaluate acute neurotoxicity (Rogers *et al.* 2005). Dosages of anatoxin-a (commercial product, >90% purity) in distilled water were 10, 100, 200, 250, 300 and 400 µg kg^{-1} (0.008, 0.08, 0.17, 0.21, 0.25 and 0.33 mg ATX^{-1} kg^{-1} day^{-1}). At the 400 µg kg^{-1} dose mice exhibited decreased motor activity, altered gait, difficulty breathing and convulsions within five minutes and all died after 10 minutes. Mice receiving 100 or 200 µg kg^{-1} survived, and received a second dose the following day. All mice survived after the second dose. Clinical signs of toxicity after 10 minutes of administering the lower doses included decreased activity level, altered gait and breathing irregularities. At the lower doses, mice did not have convulsions and recovery was observed 15 to 20 minutes after treatment (Rogers *et al.* 2005).
6. Adult male Long Evans rats were subcutaneously injected with (+)-ATX, the racemic mixture of ATX and with (-)-nicotine, once a week for up to four weeks (MacPhail *et al.* 2007). Exposed and control animals were tested daily in a photocell device, that recorded both horizontal and vertical motor activity during 30 minute sessions. Dose ranges were: (+)-ATX, 0.075–0.225 mg kg^{-1}; (+/−)-ATX, 0.2–0.95 mg kg^{-1}; and (−)-nicotine, 0.3–1.8 mg kg^{-1}. Nicotine initially decreased both horizontal activity and, to a greater extent, vertical activity. Tolerance developed to nicotine's effects with weekly administration. Both forms of ATX also initially decreased horizontal and vertical activity to approximately equivalent degrees. Neither form of ATX induced tolerance upon weekly administration.

Although i.p. and subcutaneous data are not comparable, the data by Rogers *et al.* (2005) and MacPhail *et al.* (2007) demonstrate that the values obtained with regard to neurotoxicological symptoms were largely comparable in mice and rats. The rapid distribution of the readily soluble ATX, whether via i.p. or subcutaneous administrations, would ensure that peripheral and central nervous system neurons would be reached within minutes of the initial application. Rogers *et al.* (2005) report a 'recovery' in mice exposed twice (every 24 hours) to low concentrations. In contrast no recovery was observed by MacPhail *et al.* (2007), despite the subcutaneous exposure intervals being decisively longer. Thus multiple exposures to ATX and HTX could result in functional neurotoxicity.

The above studies show an array of responses, namely the acute lethality of ATX and HTX as well as the neurotoxicity of acute and subacute ATX. All of the LD_{50} determinations gave approximately the same values (0.2–0.4 mg kg^{-1}). This suggests that the differences observed were most likely a result of variability in experimental design, purity of ATX and data evaluation. The acute toxicity of ATX and HTX from Stevens & Krieger (1991) and Skulberg *et al.* (1992) was comparable suggesting that ATX and HTX should be considered as equally toxic.

Homoanatoxin-a, dhATX and dhHTX are prevalent in *Microcoleus*–dominated environmental samples in New Zealand. N-methylation of ATX greatly reduces the acetylcholine-mimicking effect at nicotinic cholinergic receptors as shown in neuromuscular and neuronal assays of structure activity relationships (Aracava *et al.* 1987; Costa *et al.* 1990; Stevens & Krieger 1990; Swanson *et al.* 1989, 1991). Wonnacott *et al.* (1991) suggested the dihydro analogues have an approximately 10-fold reduction in their affinity for nicotinic acetylcholine binding sites in comparison to ATX. Although robust data to confirm the toxicity of these structural analogues are lacking, collectively these studies suggest that some of the analogues are likely to be less toxic than ATX or HTX. Uptake and metabolism may also affect toxicity. For example, the dihydro products may be taken up more readily through the gut than ATX, or could be metabolised to more (or less) toxic analogues. Neither scenario has been investigated for anatoxins.

3.3. Are there studies that have investigated the toxicity and/or effects from short term versus long term exposure and how do the effects differ?

Based on the preliminary data in Fawell & James (1994), a 28-day study was conducted in which four groups of 10 male and 10 female mice were dosed by gavage once a day for 28 days with 0, 0.12, 0.6 or 3 mg kg^{-1} day^{-1} (corresponding to 0.098, 0.49 and 2.46 mg ATX kg^{-1} day^{-1}; Fawell *et al.* 1999). Histological, microscopic tissue examinations and blood analysis were performed. Three deaths (one unrelated to the treatments) occurred within 2.5 hours of dosing. The only adverse clinical signs observed among the survivors, although not considered toxicologically significant, were a significant increase in mean cell haemoglobin concentration in males at > 0.1 mg kg^{-1} day^{-1} and in females at > 0.5 mg kg^{-1} day^{-1}, and an increase in serum sodium in females at > 0.5 mg kg^{-1} day^{-1}. Based on the deaths in the higher dose groups the authors suggest a No Observed Adverse Effect Level (NOAEL) of 0.1 mg^{-1} kg^{-1} day^{-1}. It is unfortunate that the authors were not able to determine whether the gavaging procedure caused the observed mortality. Because no cause of death was determined in the post-mortem examination of the mid-dose group, the authors indicated that the true NOAEL could have been 2.5 mg kg^{-1} day^{-1}.

One long-term subacute oral toxicity study has been undertaken. This involved a 7-week sub-chronic drinking water study in rats (Astrachan & Archer 1981; Astrachan *et al.* 1980). A NOAEL of 0.05 mg kg^{1} day^{-1} was identified and a Lowest Observed Adverse Effect Level (LOAEL) of 0.5 mg kg^{-1} based on an increase in white blood cell counts (30 to 50%) over the first 5 weeks. However, the study was limited by the use of only two dose levels (0.05 and 0.5 mg kg^{-1} day^{-1}), a lack of comprehensive examinations, especially haematology (two indices), blood chemistry (four serum enzymes) and histology (seven tissues), and inadequate reporting (composition of the extract). They also did not employ functional neurotoxicity endpoints (*i.e.* neuronal protein adduct levels, neuronal health and functionality), limiting the reliability of the NOAEL or LOAEL reported.

No data are available on the chronic oral toxicity of anatoxins. Because ATX appears to irreversibly bind to the nicotinic acetylcholine-receptors this suggests that chronic neurotoxicity is an important issue to investigate. Additionally there is no information on ATX carcinogenicity in humans or animals. However, based on the structure of ATX and its congeners as well as their most likely rapid kinetics, genotoxicity or genotoxic metabolites are not expected.

3.3.1. Reproductive/developmental toxicity

Fawell *et al.* (1999) gavage dosed (2.46 mg kg^{-1} day^{-1} ATX) timed-pregnant female Crl:CD-1(ICR)BR mice with ATX hydrochloride. On gavage day 18, the mice were sacrificed and live or dead foetuses were counted, weighed, sexed and observed for external abnormalities. The authors reported a lack of maternal toxicity and no treatment-related major foetal abnormalities. Unfortunately the authors did not ascertain whether the offspring had neuromuscular deficiencies or developmental neuropathies. This is now considered standard practise when investigating the toxicology of similar compounds applied during pregnancy or post-parturition *e.g.* the voltage-specific sodium channel inhibitors or organophosphate insecticides (Ricceri *et al.* 2006; Oulhote & Bouchard 2013).

Male mice were administered i.p. doses of 0 (control), 0.05, 0.1 and 0.15 mg kg^{-1} day^{-1} ATX for seven consecutive days (Yavasoglu *et al.* 2008). There were no significant changes in body weight gain or testes weights. However, there was a significant ($p < 0.01$) reduction in absolute and relative weights of cauda epididymis in the 0.1 and 0.15 mg kg^{-1} treatment groups, and a significant ($p < 0.01$) dose-dependent reduction in sperm count in the cauda epididymis in all treatment groups. Based on these data the author recommended a LOAEL of 50 µg kg^{-1} (Yavasoglu *et al.* 2008). These data need to be interpreted with caution. Examination of the results suggests that reduction in sperm counts occurs across all treatments and the degeneration of the seminiferous tubules could not be ascertained by a trained toxicological pathologist (D Dietrich, pers. obs.). Yavasoglu *et al.* (2008) do not provide any mechanistic explanation on how ATX could inhibit spermatogenesis or adversely affect Sertoli cells. It is likely that the reduced sperm counts are an artefact of preparation rather than an effect causally related to ATX exposure.

Of all the developmental toxicological studies undertaken with ATX in mice, the most comprehensive one is that of Rogers *et al.* (2005). They exposed time-pregnant mice to 125 or 200 µg kg^{-1} ATX by i.p. on gestation days (GD) 8–12 or 13–17. Pup viability and weight were monitored over a 6-day period. Maternal toxicity (decreased motor activity) was observed at 200 µg kg^{-1} in both treatment periods. There were no significant treatment-related effects on pup viability or weight on postnatal day (PND) 1 or 6. The GD 13–17 pups were evaluated on PND 6, 12 and 20 for standard markers of neurodevelopmental maturation (righting reflex, negative geotaxis and hanging grip time). No significant postnatal neurotoxicity was observed. *In vitro* developmental toxicity was evaluated in GD 8 CD-1 mouse embryos exposed to 0.002, 0.02, 0.2 and 0.51 µg mL^{-1} ATX (90% pure) for 26–28 hours. Perturbations in mouse yolk sac vasculature were noted from the 1.0 µm concentration in the absence of significant embryonic dysmorphology (abnormal tissue formation). This study demonstrated that no overt neurotoxicity can be observed following in-utero exposure of embryos to ATX, albeit functional and cognitive assessments were not carried out. Further research is required to establish long-term persistent neurotoxicity or the potential for ATX induced late stage neuropathies.

3.3.2. Summary

A recent toxicological review document by the US EPA (US EPA 2015) states that there are currently insufficient data to support derivation of a short-term oral reference dose (RfD) for ATX, a view which we support. There are very limited data available on the toxicity of other variants which are prevalent in *Microcoleus*-dominated mats in New Zealand (HTX, dhATX, dhHTX). These knowledge gaps need to be addressed before robust RfDs can be established.

Based on the comparison of all available data the difference in effects from short term versus long term exposure cannot be ascertained at the moment. This is primarily due to the fact that:

1. Only sub-chronic studies have been conducted and longer-term chronic studies are required.
2. The short-term (sub-chronic) studies, with the exception of Rogers *et al.* (2005), addressed parameters that do not allow the detection of neurotoxic effects.
3. None of the studies undertaken to date utilise parameters that allow detection of functional and cognitive neurotoxicity. They therefore cannot be used to determine long-term neurotoxicity or the potential for ATX to induce late stage

neuropathies *e.g.* via ATX-adducted-nicotinic acetylcholine receptors and/or reduced nicotinic acetylcholine-receptor recycling (Bruneau *et al.* 2005).

4. The acute ATX toxicity is nearly exclusively related to irreversible nicotinic acetylcholine-receptor binding which maintains sodium ion influx. It is thus conceivable that lower concentrations of ATX will also irreversibly bind to the nicotinic acetylcholine-receptors thereby incapacitating some nicotinic acetylcholine-receptors. Continuous nicotinic acetylcholine-receptors incapacitation may lead to neurological functionality, either by itself or through higher susceptibility to other neurotoxicants, *e.g.* pesticides, solvents, drugs.

3.4. How can toxicology studies for other similar toxins be used to approximate toxicity of anatoxins to humans?

As discussed in the previous section, the major effect of ATX in people is acute neurotoxicity, where non-lethal exposures may cause illness. Whether acute and/or non-lethal exposures have long term effects is currently unknown. Based on the available animal studies (rats, mice), repeated low level exposures do not appear to cause any cumulative effects (Rogers *et al*. 2005).

The nearest 'relative' of ATX exposure is nicotine exposure, and there is a long history of study of human exposure to nicotine through smoking. For smoking adults, exposure is voluntary and self-regulated to be at a neuroactive level, but at blood concentrations that do not induce symptoms of toxicity (nausea, vomiting, tremor). A recent review of the literature (Mayer 2014) suggests that an oral LD_{50} of 6.5–13 mg kg^{-1} might be lethal to an adult human, very similar to the acute toxicity of ATX. However, as indicated earlier, the fundamental difference between nicotine and ATX is that the nicotine and ATX interact with the nicotinic acetylcholine-receptor at different locations and the interaction of ATX appears irreversible, whereas the interaction of nicotine is reversible. This has not been observed for ATX in the animal assays undertaken to date.

There are numerous incidents of accidental nicotine poisoning, some lethal, through dermal exposure and ingestion *(e.g.* Woolf *et al.* 1996; Solinaro *et al.* 2010), where non-lethal exposures to nicotine are not thought to cause any long-lasting effect (Solinaro *et al.* 2010; Mayer 2014).

Numerous studies suggest that although tobacco smoke itself has long term effects on a smoker's health, very little of that effect is due to nicotine. Nicotine's effects are thought to include increases in heart rate and blood pressure, potentially leading to an increase in cardiovascular risk (reversible on smoking cessation). There is no evidence for any direct carcinogenic effect (Surgeon General's Report 2014). A further effect is, however, seen in the lung, where nicotine binds to acetylcholine receptors causing chronic inflammatory responses (*e.g.* Schweitzer *et al.* 2015). The literature on this is somewhat contradictory but the 2014 Surgeon General's Report states that the evidence is sufficient to infer immunomodulatory effects (both suppressive and excitatory) from nicotine itself, and that nicotine exposure during foetal development has lasting adverse consequences for brain development. These findings are important for the comparison with ATX exposure. Anatoxin-a exposure, as with nicotine, may not result in an overall visible adverse effect. However, it may lead to a higher susceptibility of those exposed to either neurotoxic insults by other compounds or infectious insults due to the immunomodulatory effects. Nicotine may also affect maternal and foetal health during pregnancy, thus contributing to multiple adverse outcomes such as preterm delivery and stillbirth. The current developmental toxicity data for ATX in mice do not suggest such effects occur, albeit these studies are very limited and preliminary.

There are key differences between nicotine exposure in smokers and ATX exposure from ingestion of water. The first is the route of exposure—nicotine is either inhaled or continuously absorbed via dermal applications and not ingested. The second is that nicotine binds reversibly, whereas ATX binding is irreversible, requiring receptor (or cell) replacement in order to remove the effect. Bruneau *et al.* (2005) showed that in mouse peripheral synapses, chronic synaptic activity blockade with the reversibly binding curare nearly completely prevented nicotinic acetylcholine recycling and resulted in delayed and low level *de novo* synthesis of nicotinic acetylcholine-receptors. As chronic ATX exposure could lead to at least partial synaptic activity blockade, the question remains whether this would prevent nicotinic acetylcholine-receptor recycling and whether *de novo* synthesis could compensate for the non-recycled and thus absent receptors. The third is the difference in exposure. Smokers are exposed to nicotine multiple times a day, to around 1/100th of the lethal concentration every day, over periods of years. Similarly, former smokers on a nicotine replacement regime are sub-chronically exposed to low concentrations of nicotine via dermal patches over periods of months. The latter are extreme chronic exposure regimes compared with currently likely exposures to ATX. Nevertheless this does reinforce the need to be vigilant for non-lethal toxicity evidence such as adverse outcomes on pregnancy and foetal development (Surgeon General's Report 2014). There is as yet no evidence that this occurs for ATX.

3.5. What studies could be done to gather data using modern methodologies?

Modern methods of investigating toxicological problems have been driven by the increasing realisation that it is not possible, practicable or affordable to study every toxin, every toxin variant, and every toxin combination in sufficient detail to obtain definitive toxicological data for each.

Thus several large scale initiatives have started up to apply omics[2] technologies in various forms to toxicological problems, with the aim of establishing critical links between *in vitro* effects of chemical exposures and their *in vivo* effects enabling a reduction in the amounts of classical toxicological work that is required (*e.g.* Krewski *et al.* 2010; Battacharya *et al.* 2011). These methods rely on establishing robust comparators that allow prediction of toxicity from more readily measurable output data. These are 'big data' projects that will take some years, possibly decades, to come to fruition and will also face major obstacles with regard to data interpretation, especially with regards to hazard and risk assessment.

In the interim there are two main approaches: one is hypothesis-free. It applies the same omics approaches mentioned above, but to a single problem. The toxin's effects *in vitro* are examined globally, and the data emerging are analysed for evidence of disturbance of biological pathways, by systematic comparison with the literature (pathways analysis, *e.g.* Young *et al.* 2009). This approach is becoming increasingly powerful as the supporting data build up. It is an accessible technology through the major universities in New Zealand and some crown research institutes.

A possible use of this approach, in this situation, would be to find the concentration of ATX at which a cell type expected to be susceptible (*i.e.* human nerve cells containing nicotinic receptors) showed essentially no perturbation in gene expression/proteome. This would essentially provide a cellular NOAEL. If the concentrations at which effects became evident mirrored those at which ATX binds to nicotinic receptors, this would provide supporting evidence as to whether there were pathways other than direct binding to nicotinic receptors that needed to be considered (for this particular cell type). If these effects are compared with those of a non-susceptible cell type (*i.e.* human liver cells) that would provide further supporting evidence, although not proof, that off-target effects of the toxin were unlikely to be of significance *in vivo*.

A variant of this approach is to use commercially available systems to look at perturbations in known toxicity pathways. This essentially involves investigating up/down-regulation of specific genes known to be affected by agents affecting these pathways (*e.g.* oxidative stress responses). Once baseline responses to ATX were determined it would be relatively simple to look at the analogues of ATX to find the relative concentrations which produce the same effects.

A further use of human neuronal cells, *e.g.* central and peripheral neuronal cells derived from human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSCs) would be to investigate the response of the nicotinic acetylcholine receptors to ATX, HTX, dhATX and dhHTX, and allow investigations of nicotinic acetylcholine

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[2] Omics informally refers to a field of study in biology ending in -omics, such as genomics, proteomics or metabolomics.

receptors recycling following single and multiple exposures. Although such studies have the caveat that they are an *in vitro* system, they have the advantage of not having to extrapolate from a non-human system. Several different cell types (*i.e.* glia and neuronal) as well as hepatocytes and renal cells could be assessed.

All *in vitro* approaches are necessarily comparative. They do not consider the body's defence mechanisms. They concentrate on the steps of binding of the toxin to an effector molecule, and, in the case of cellular assays, the cell's attempts to maintain its own homeostasis. As such they give useful information to aid decision making but there are key elements of information missing that can only come from studying effects of exposure on humans or from animal testing. However, if evidence emerges that a given toxicological effect were of concern, this can be specifically re-tested *in vivo*, allowing great saving in time and resources.

It is also possible to do very sophisticated toxicological work in other organisms (Perkins *et al.* 2013). Two examples that are available in New Zealand are:

1. Yeast chemical genetics, available at Victoria University Wellington (Dr Andrew Munkacsi). It is possible to undertake a screen, using minimal amounts of material, which can determine which gene products and biochemical pathways the toxin interacts with (Smith *et al.* 2009). The advantages of this approach are that it is hypothesis-free and very little material is required. Further, the ease of genetic manipulation in yeast makes subsequent investigations into mode of action far more practicable than when using other species. Yeast cells have ion channels, though their homology with mammalian ion channels is limited. Ion channel agonists do affect yeast pathway biochemistry (Dr D Maass, Victoria University, pers. comm.).

2. The zebrafish model, available at University of Auckland (Professor P Crozier). It is in studies of embryo development that this model is at its most powerful. Single embryos can be individually exposed and their subsequent development studied, with minimal toxin required (Jonas *et al.* 2015). Because the embryos are transparent, good images of internal organ development are obtained. Indices of normal/abnormal behaviour are also available. This approach would provide initial knowledge of the type of effects which might occur. For example, it might demonstrate that the irreversible binding of ATX to its receptor results in some limited build-up of effect, after repeated exposure, depending on the time taken to replace/repair the receptor in the body. Based on preliminary findings from yeast or in zebrafish, resulting hypotheses could be followed up with *in vitro* analysis using a well characterised cellular or biochemical system or, depending on circumstances, could be tested directly *in vivo* in a mammalian system.

4. WHAT IS THE TOXICITY AND DOSE RESPONSE TO SKIN CONTACT EXPOSURE FROM BENTHIC CYANOBACTERIA?

4.1. What have studies that directly addressed this question found?

Lipopolysaccharides (LPS) are an integral component of the cell wall of all gram-negative bacteria, including cyanobacteria. Found in the outer cell membrane, LPS form complexes with proteins and phospholipids (Chorus & Bartram, 1999). Lipopolysaccharides can elicit irritant and allergenic responses in humans and animals (Torokne *et al.* 2001; Pilotto *et al.* 1997). A detailed review of cyanobacterial LPS is provided in Stewart *et al.* (2006). They suggested that although cyanobacterial LPS are often cited as a putative toxin, most studies have found that cyanobacterial LPS are weakly toxic compared to LPS from Enterobacteriaceae (Stewart *et al.* 2006). Stewart *et al.* (2006) review the few toxicological investigations that have been undertaken on cyanobacterial LPS (Table 2). These studies are mostly limited to the end-points of lethality and the local Shwartzman reaction, in which sequential subcutaneous and intravenous injections of LPS produce a dermonecrotic lesion in rabbit skin.

Mikheyskaya *et al.* (1977) investigated LPS in three *Microcoleus* spp. They demonstrated that the serological properties of *Microcoleus* LPS are similar to those of bacterial endotoxins. However, unlike bacterial endotoxins, the *Microcoleus* LPS in the strains they tested were nontoxic (Table 2). Sharma and Rai (2008) tested the allergenic potency of crude extracts of *Phormidium fragile.* Crude extracts from the cyanobacteria were subjected to intra-dermal mouse allergy tests and leukocyte counts. The extracts produce allergenic responses in mice including changes in wheal diameter and redness of tested skin. Leukocyte counts revealed an increase in eosinophils and neutrophils. Caution is required when extrapolating these results to other *Microcoleus* species/strains as toxicity can vary markedly within genera and among species (as shown in Table 2).

4.2. Are the toxicity and/or effects different from short term versus long term exposure?

Despite many anecdotal reports of allergic reactions to cyanobacteria, few systematic studies exist, and to our knowledge long-term exposures have not been investigated. A number of laboratory studies have investigated the irritation effects of cyanobacteria following short-term exposure. These use planktonic species, however some of the genera studied are also found in benthic habitats *i.e. Microcoleus* and *Lyngbya*. With the caveat that responses are likely to vary between species and strains, these data provide some relevant information.

Table 2. Cyanobacterial lipopolysaccharides and lethality (adapted from Stewart et al. 2006).

Cyanobacterium	**Lethality**	**Reference**
Anacystis nidulans	non-toxic at 10 mg kg^{-1}**	Weise *et al.* 1972
A. nidulans KM	2.5 mg kg^{-1}† (= approx. 800-fold greater than *Salmonella minnesota* LPS)	Katz *et al.* 1977
Microcoleus spp. (x3)	all non-toxic at mean dose of 333 mg kg^{-1}	Mikheyskaya *et al.* 1977
Schizothrix calcicola	non-toxic at 200 mg kg^{-1}	Keleti *et al.* 1979
Anabaena flos-aquae UTEX 1444	non-toxic at 250 mg kg^{-1}	Keleti and Sykora 1970
Anabaena cylindrica UTEX 1611	LD_{50} 130 mg kg^{-1}	Keleti and Sykora 1970
Oscillatoria brevis	LD_{50} 190 mg kg^{-1}	Keleti and Sykora 1970
Microcystis aeruginosa 006	LD_{50} approx. 45 mg kg^{-1}**	Raziuddin *et al.* 1983
lipid A	LD_{50} 60 mg kg^{-1}**	Raziuddin *et al.* 1983
M. aeruginosa NRC-1	LD_{50} 40 mg kg^{-1}**	Raziuddin *et al.* 1983
lipid A	LD_{50} approx. 45 mg kg^{-1}**	Raziuddin *et al.* 1983
Spirulina platensis Lb 1475/4a	LD_{100} 425 mg kg^{-1}**	Tornabene *et al.* 1985
Microcystis sp. PCC 7806	1 of 3 mice* died at 50 µg kg^{-1}**	Scholtissek *et al*. 1991
M. aeruginosa (bloom sample)	no deaths at 70 mg kg^{-1}	eprint.uq.edu.au
Cylindrospermopsis raciborskii AWT 205	no deaths at 70 mg kg^{-1}	eprint.uq.edu.au

*galactosamine-sensitised (= TNF-α hypersensitised)
† adrenalectomised mice
** assumed weight of mice: 20 g

Mittal *et al.* (1979) investigated respiratory allergy to a range of planktonic cyanobacteria and algae (*Lyngbya, Microcoleus, Anabaena, Scytonema, Chlorella, Westiellopsis, Anabaenopsis, Oscillatoria, Nostoc* and *Chlorococcum*). Intradermal skin tests were undertaken on 400 people suffering from nasal-bronchial allergies (n=4000 tests) and on 30 healthy individuals (n = 300 tests). Positive skin reactions ranged from 25.7% for *Lyngbya* to 1.7% for *Oscillatoria* in allergic volunteers. In contrast, no positive skin reactions were reported in non-allergic volunteers. In patients with skin reactions positive results were obtained for the Prausnitz-Kustner (70.9%), provocation (50%) and conjunctival (48%) tests. No positive responses were observed in patients with negative skin reactions. Levels of total immunoglobulin E in patients with nasobronchial allergies ranged from 1225 to 1550 international units (IU)/mL, compared to 885 IU/mL in healthy volunteers.

Pilotto *et al.* (2004) investigated skin irritation effects in humans caused by toxic planktonic cyanobacteria cell suspensions and extracts. Samples were applied to the skin of 114 volunteers at varying cell concentrations using adhesive skin patches. Patches were removed after 24 hours and erythema assessed by a dermatologist. Atopic and non-atopic individuals (12%) reacted to the whole and lysed cells. The study did not identify any consistent dose-response relationship, nor did it find a difference in reaction between atopic and non-atopic individuals.

Stewart *et al.* 2006 used diagnostic skin patch testing of 20 hospital outpatients to investigate skin irritation effects from cyanobacteria. Patches containing aqueous suspensions of various cyanobacteria at three concentrations were applied for 48 hours. Dermatological assessment was made 48 hours and 96 hours after application. A single outpatient reacted to several cyanobacteria suspensions. This participant was the only one of the study group with a diagnosis of atopic dermatitis.

Collectively these studies suggest that a small proportion of the population will experience allergic reactions to cyanobacteria at low to moderate cell concentrations. Irritation in the affected individuals did not appear severe, and was generally resolved within a short period (24–72 hours).

5. WHAT IS THE TOXIN CONTENT OF A BENTHIC CYANOBACTERIA MAT?

5.1. What have studies that directly addressed this question found?

5.1.1. Anatoxins in Microcoleus-dominated mats

Following animal poisonings or during blooms events some regional councils, or concerned parties, collect and test cyanobacterial mats for anatoxins. This testing has primarily been undertaken using LC-MS at the Cawthron Institute. Whenever mat samples are tested by a commercial laboratory, results are reported as toxin concentration in the mat (*i.e.* an amount of toxin per kilogram of wet weight). *Microcoleus*-dominated mats can vary in the amount of water they contain, depending on the mat composition and desiccation status when sampled. This makes comparison of historical datasets of this type challenging. Consequently they have not been included in this review. To overcome the problems associated with varying quantities of water in mats, samples for research purposes are freeze dried prior to analysis. In this section we considered only these data.

Five studies have explored the anatoxin content of *Microcoleus*-dominated mats in New Zealand rivers. Wood *et al.* (2010) compared toxin concentrations in a survey of 7 rivers throughout New Zealand, Heath *et al.* (2011) studied toxin variability at 7 sites in the Hutt and Wainuiomata rivers over 12 months, Wood *et al.* (2014) investigated toxin production weekly at 10 sites in seven rivers in the Manawatu-Whanganui region, Wood *et al.* (2015) measured toxins in mats over 2 summers at 7 sites in the Maitai River (Nelson), and Heath and Wood (2005) compiled toxin data for 5 Southland rivers collected over a 4 month period in 2010. The Horizon Regional Council has been extremely proactive in investigating toxin content of mats in their region, and has analysed samples from any State of the Environment monitoring site observed to have *Microcoleus*-dominated mats over the last two years. All of these data, and additional unpublished data from S. Wood were compiled and analysed for this section. This comprised 771 data points from 40 rivers.

To simplify visualisation of toxin data, the concentrations of ATX, HTX, dhATX and dhHTX in each sample were combined and a total toxin concentration used for Figure 10. The number of samples per river, and the number of different time points the data are collected on, varies among rivers. Comparisons across rivers need to be interpreted cautiously, *i.e.* no toxins detected from a limited number of samples, does not necessarily mean no toxins occur in *Microcoleus*-dominated mats from the specified river. Anatoxins were detected in 520 of the samples. Total anatoxin concentrations showed high spatial and temporal variability among and within rivers. The highest concentrations were measured in the Oreti River (712 mg kg^{-1} dried weight (dw)). Single samples from the Whakatikei, Wainuiomata, Waikaia, Wai-iti, Mataura, Mangaroa, Maitai and Hutt rivers also contained high concentrations

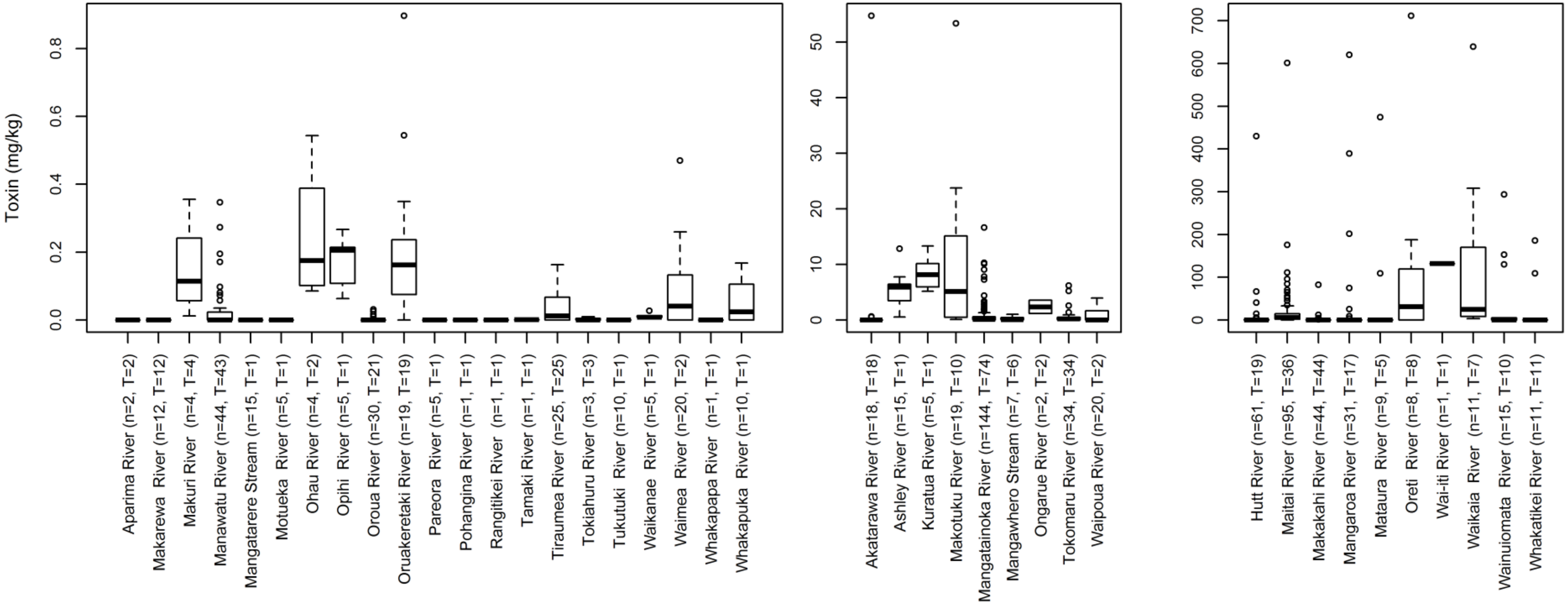

Figure 10. Total anatoxin concentrations in *Microcoleus*-dominated mats from 40 New Zealand rivers (n=771). Toxins are expressed as mg/kg of dried weight. Note different y-axis scales in each panel. T= number of different dates samples were collected on. Solid black line shows median, box shows 1st and 3rd quartiles, whiskers extend to the last data point within 1.5 times the inter-quartile range if there is data that far from it. Open circles are outliers beyond this range.

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(greater than 100 mg kg^{-1} dried weight (dw)) of toxins. The median value for the large majority of rivers was below 1 mg kg^{-1} dw (Figure 10).

Although not discernible from Figure 10, multiple studies (*e.g.* Heath *et al.* 2011, Wood *et al.* 2014, 2015) have observed marked changes in total toxin concentrations within a one week period. For example, Wood *et al.* (2014) noted an increase in toxin concentrations from 1.2 to 82.4 mg kg^{-1} dw in *Microcoleus*-dominated mats from the Makakahi River between 10 February 2012 and 17 February 2012.

There are several possible explanations for these large spatial and temporal variations. Research has shown that toxic and non-toxic genotypes co-occur in *Microcoleus*-dominated mats (Heath *et al*. 2010, Wood *et al*. 2010, 2012). The relative amount of each genotype affects the total toxin in each sample. Wood *et al*. (2012) isolated multiple single filaments from 1 cm^2 areas from three different mats collected in two rivers, and grew these into uni-cyanobacterial cultures. Molecular and chemical analysis showed both toxic and non-toxic genotypes co-exist, and that among toxic strains the concentration of toxin produced varies up to approximately 100-fold (Table 3). Consequently, not only the relative amount of toxic versus non-toxic genotypes is important, but also the abundance of toxic genotypes that produce higher anatoxin quotas (*i.e.* the amount of toxin produced per cell).

Variables that regulate the presence and abundance of each genotype within a mat are unknown. Heath *et al*. (2010) suggested that toxin-producing strains in the Hutt River 'out-competed' non-toxic *Microcoleus* strains at temperatures above 15˚C, but subsequent studies in other rivers have not observed this pattern (Wood *et al.* 2014).

A further possibility is that conditions (*e.g*. dissolved oxygen, pH) within the mat or surrounding water (*e.g*. nutrient concentrations) cause an upregulation in the amount of toxin produced per cell. Using culture-based studies Heath *et al*. (2014b) showed increased nitrogen and phosphorus concentrations resulted in higher anatoxin quotas. Heath *et al*. (2014b) and Harland *et al*. (2013) demonstrated that toxin quota peaked in the initial growth phase. To date analysis of field data has not identified any correlations among toxin production and measured parameters, and further research is required to explore possible relationships.

Microcoleus-dominated mats can contain a mixture of cyanobacteria, other algae and inorganic material (*i.e.* sediment). Differences in the relative amount of these in mats may also account for some of the variability. Current research is focused on using molecular methods to quantify *Microcoleus* cells (toxic and non-toxic) in each sample. This will allow 'normalisation' of toxin values and may provide insights into the observed variability. From a human exposure perspective, data on the actual toxin content of the mats as they occur in the environment are the most useful and relevant information.

Table 3. Results of liquid chromatography-mass spectrometry (LC-MS) analysis of 30 strains of *Microcoleus* isolated from the Waimakariri (WR) and Hutt (HR) rivers. HR-1-1 = Site 1, mat 1, HR-1-2 = Site 1, mat 2, ND = Not detected, ATX = anatoxin-a, dhATX = dihydroanatoxin, HTX = homoanatoxin-a, dhHTX = dihydrohomoanatoxin. Modified from Wood *et al.* (2012).

Strain	**Location**	**LC-MS data (mg kg^{-1} dried weight)**				
		ATX	**dhATX**	**HTX**	**dhHTX**	**TOTAL**
CYN103	WR	5.92	205.92	ND	ND	211.83
CYN104	WR	5.79	156.07	ND	ND	161.86
CYN105	WR	0.28	40.80	ND	ND	41.08
CYN106	WR	0.47	56.39	ND	ND	56.86
CYN107	WR	0.93	9.13	ND	ND	10.06
CYN108	WR	ND	ND	ND	ND	ND
CYN109	WR	3.25	128.38	ND	ND	131.63
CYN110	WR	6.40	171.80	ND	ND	178.20
CYN111	WR	1.08	71.90	ND	ND	72.98
CYN112	WR	2.38	165.75	1.00	ND	169.13
CYN113	HR-1-1	0.04	66.91	ND	ND	66.95
CYN115	HR-1-1	0.25	28.57	ND	ND	28.83
CYN116	HR-1-1	0.04	31.29	ND	ND	31.33
CYN117	HR-1-1	0.12	152.16	ND	ND	152.28
CYN126	HR-1-2	0.19	115.23	ND	ND	115.42
CYN127	HR-1-2	0.63	79.92	ND	ND	80.55
CYN128	HR-1-2	0.12	32.58	ND	ND	32.70
CYN129	HR-1-2	ND	ND	ND	ND	ND
CYN130	HR-1-2	ND	ND	ND	ND	ND
CYN131	HR-1-2	ND	4.75	ND	ND	4.75
CYN132	HR-1-2	0.25	74.32	ND	ND	74.58
CYN133	HR-1-2	0.06	73.56	ND	ND	73.62

In *Microcoleus*-dominated mats, ATX, HTX, dhATX and dhHTX are almost always detected simultaneously; however, their relative concentrations vary (Figure 10). Dihydroanatoxin-a and dhHTX were the most prevalent variants in samples from the 30 rivers that contained anatoxins (Figure 11). Anatoxin-a was least commonly detected; it only occurred on a few occasions in the Hutt, Wai-iti, Waikaia and Wainuiomata rivers (Figure 11). A possible explanation for the low detection of ATX is that it degrades readily, especially in sunlight and at high pH; whereas the dihydro-compounds are more stable (Smith and Lewis 1987). This may partially explain the absence of ATX, although HTX is expected to be just as unstable as ATX.

It is likely that *Microcoleus* cells are continually dying in the mats. Additionally, under certain environmental conditions, the entire mat may die and detach from the substrate (see Section 8), potentially resulting in pulses of toxins being released into the water. Limitations in the use of ‘grab’ samples to detect extracellular toxins in rivers led to a study by Wood *et al.* (2011). They evaluated the potential of an *in situ* method known as solid phase adsorption toxin tracking (SPATT) for collecting and concentrating anatoxins in river water. A three-day field study in a river containing toxic *Microcoleus*-dominated mats was undertaken and toxins were detected in all SPATT bags. Surface grab samples were taken throughout the field study and toxins were only detected in one of the water samples, highlighting the limitations using this methodology to assess extracellular toxin concentrations. A subsample of the positive water sample was examined microscopically and no filaments were detected indicating that the toxins had been released into the water. Collectively these data show that low concentrations of anatoxins are likely to be present in river water when *Microcoleus*-dominated mats occur. More extensive studies over a wider range of coverages and differing stages of *Microcoleus*-dominated mat development are required to gain an in-depth understanding of variability in extracellular toxin concentrations in river water.

5.1.2. Other toxins

Although not the focus of this review (Section 2.3) we provide the limited data available on toxins in other benthic cyanobacterial mats in New Zealand for completeness.

***Nostoc* sp.**

To our knowledge, microcystins have only been detected in one environmental sample in New Zealand (collected Lake Taupo) which was dominated by *Nostoc commune* (Section 2.1.1). The microcystin concentrations are given in Table 4.

Table 4. Microcystin variants detected by liquid chromatography- mass spectrometry in an environmental sample collected from Lake Taupo and dominated by *Nostoc commune*.

Microcystin Variant	**Conc. (mg kg^{-1} dried weight)**
Microcystin-RR	535
Microcystin desmethyl- RR	142
Microcystin-LR	24
Microcystin desmethyl-LR	7
Total	**708**

***Planktothrix* sp.**

No quantification of microcystin variants in the actual environmental sample (Section 2.1.4) was undertaken. A culture (VUW25) isolated from the mat contained of 1,489 mg kg^{-1} dw [D-Asp^{3}] microcystin-LR, 86 mg kg^{-1} dw of [D-Asp^{3}, Dha^{7}] microcystin-LR and 32 mg kg^{-1} dw microcystin-LR (all calibrated from microcystin-LR), together with trace amounts of [D-Asp^{3}, ADMAdda^{5}] microcystin-LhR (not quantified; Wood *et al.* 2010).

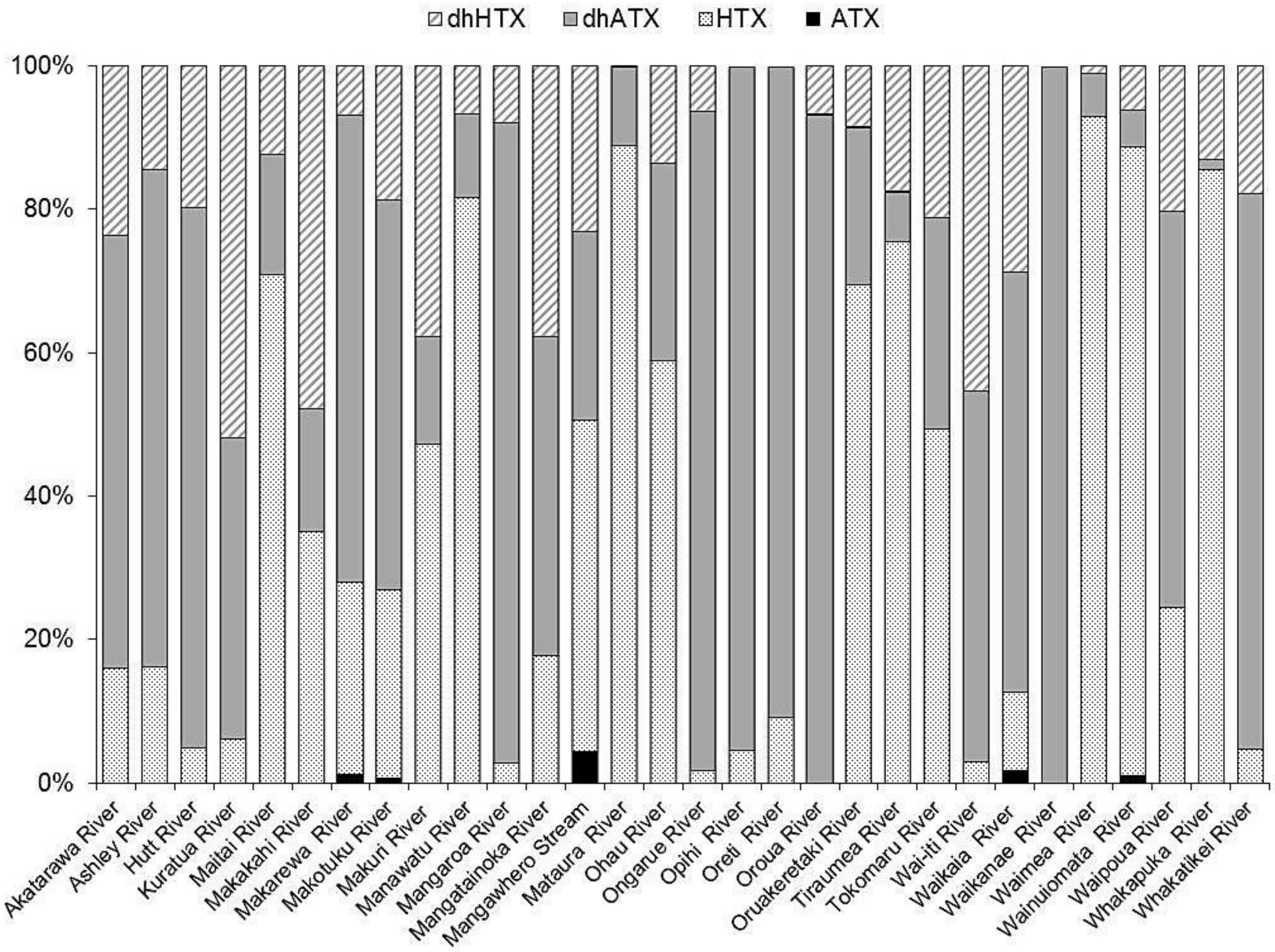

Figure 11. Stacked bar graph showing the percentage of each anatoxin variant present in *Microcoleus*-dominant mats in 30 rivers in positive samples. ATX = anatoxin-a, dhATX = dihydroanatoxin, HTX = homoanatoxin-a, dhHTX = dihydrohomoanatoxin.

Scytonema cf. *crispum*

Saxitoxins have been detected in *Scytonema* cf. *crispum* mats from multiple lakes in the Canterbury region (2.1.5). Toxin concentrations and the variants contained in each varied markedly (Smith *et al.* 2012; Table 5).

Table 5. Saxitoxin variants detected by high-performance liquid chromatography with fluorescent detection in environmental samples dominated by *Scytonema* cf. *crispum*. Results are presented in mg kg^{-1} of dried material. dcGTX = decarbamoyl gonyautoxins, dcSTX = decarbamoyl saxitoxins, GTX = gonyautoxins, neoSTX = neosaxitoxin, STX = saxitoxins, ND = not detected. Adapted from Smith *et al.* (2012).

Saxitoxin Variant	**The Groynes**	**Alexandrina**	**Benmore (Site 1)**	**Benmore (Site 3)**	**Ruataniwha (Site 1)**
dcGTX2/3	ND	0.10	0.25	0.94	0.22
dcSTX	ND	0.95	0.69	0.90	0.50
GTX1/4	ND	0.29	0.76	2.81	0.65
GTX2/3	ND	0.16	0.41	1.58	0.30
GTX5	ND	ND	1.34	11.76	ND
neoSTX	ND	ND	0.13	0.11	0.12
STX	65.5	2.07	0.37	0.77	1.85

Unknown Nodularin producer

Nodularin was detected in five benthic cyanobacterial mats collected from Lake Tikitapu (Section 2.1.6). Concentrations were; 0.32, 0.56, 0.14, 0.26 and 0.61 mg kg^{-1} dw.

5.2. How could studies for other cyanobacteria genera be used to approximate the toxin content of a benthic cyanobacteria mat?

Different species can produce markedly different concentrations of toxins and approximating these values from one species to another is not recommended. Different species can also be toxic or non-toxic, or even produce completely different toxins in various geographic locations. For example, the common bloom-forming planktonic species *Cylindrospermopsis raciborskii* produces cylindrospermopsins in Australia, saxitoxins in South America, and is largely non-toxic in New Zealand (Chiswell *et al.* 1997; Lagos *et al.* 1999; Wood *et al.* 2014).

As shown above, there can be huge variability in the amount of toxin production within strains of the same species (Table 3). This variability is further exacerbated when comparing different species. In view of the latter, making approximations regarding toxin concentrations based on other species is inappropriate and could lead to severe under-, or overestimation, of toxin concentrations, and thus would provide a flawed estimation of risk. Due to the fact that anatoxins are the most predominant toxin found in benthic cyanobacterial mats in New Zealand, and that these mats are primarily dominated by *Microcoleus*, the presently available extensive dataset provides a strong baseline for undertaking an initial estimate of risk.

Highly sensitive and accurate toxin testing is now readily available at a reasonable cost for anatoxins (and all cyanotoxins known to occur in New Zealand benthic mats). It is thus far more efficient and accurate to measure the actual content of benthic cyanobacterial mats if further data are required.

5.3. How does toxin content relate to different measures of abundance, including biomass (*i.e.*, chlorophyll-*a* or Ash-Free Dry Weight) and percent cover?

As stated above, *Microcoleus* co-occurs in the mats with other microbial taxa, such as bacteria, diatoms and green algae (Brasell *et al.* 2014). The relative proportion of these varies among mats and can change over time. *Microcoleus*-dominant mats also contain inorganic matter, *i.e.* sediment, which also varies spatially and temporally among mats.

Normalising the concentrations of toxin detected to either chlorophyll-*a* (a proxy for total algal biomass) or ash free dry weight (AFDW; *i.e.* the weight of organic material in the mat), may reduce some of variability in the toxin concentrations among samples. Currently there are no datasets available to assess this. Ph.D. student Tara McAllister (University of Canterbury—supervised by Professor Ian Hawes and Susie Wood) is currently obtaining data that may allow this assessment in the near future. Additionally, large sample sets (stored frozen from Horizons Regional Council) are available that could be readily analysed (at least for AFDW). As stated above, from a human exposure perspective, the actual toxin content of the mats as they occur in the environment is the most useful and relevant information for assessing human health risk.

Percent cover at a site is usually estimated following 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). Twenty views using an underwater viewer are taken on four transects and these data used to estimate the percent of the riverbed covered by *Microcoleus*-dominated mats. Corresponding percent cover data were not available for all toxin analysis data points given in Figure 10. However, this information has been collected for subsets of these data in multiple studies, *e.g.* Wood and Heath 2010, Heath *et al*. 2011, Wood *et al.* 2014, 2015. These studies found no correlation between the percentage coverage at a site and anatoxin concentrations. An example is given in Figure 12.

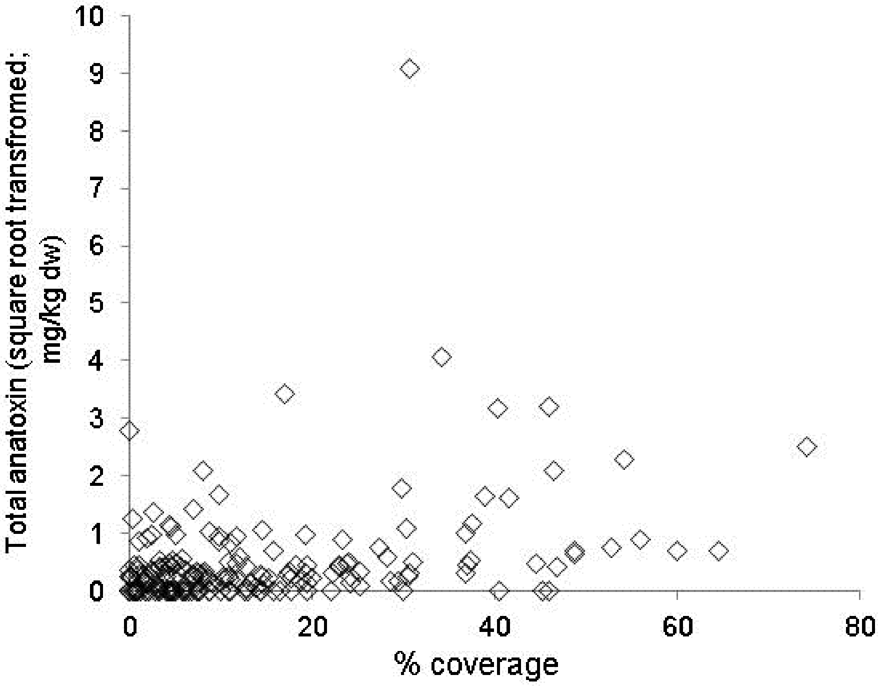

Figure 12. Total anatoxin toxin concentration (square root transformed; mg kg^{-1} of freeze-dried material) versus mean *Microcoleus* coverage per site for ten sites at seven rivers in the Manawatu region (modified from Wood e*t al.* 2014). N = 79.

6. WHAT IS THE INGESTION RATE AND LIKELIHOOD OF A CHILD INGESTING BENTHIC CYANOBACTERIA?

6.1. What have studies that directly addressed this question found?

To our knowledge, no studies have investigated rate and likelihood of a child ingesting benthic freshwater cyanobacterial mats. Examination of current literature has revealed a paucity of quantitative information on this topic. Literature examined included compilations of exposure studies (the 'Exposure Handbooks' of United States Environmental Protection Agency (2011) and enHealth (2012)), as well as relevant national and international recreational water quality guidelines (World Health Organisation 2003; National Health and Medical Research Council 2008).

6.2. How could estimates of ingestion rates for other materials (*e.g.*, soil) be used to approximate ingestion rates of benthic cyanobacteria?

6.2.1. Approach 1 – water ingestion rates

Despite the paucity of information on ingestion of benthic cyanobacterial mats there is some robust information from studies on water ingestion rates for primary contact (swimmers) and for water activities involving secondary contact.[3] That enables us to make some calculations about the most health-challenging exposure route for water users*, i.e.* ingestion of water containing mat fragments and associated toxins. At certain times during their lifecycle, or due to disturbance (See Section 8.1), benthic cyanobacterial mats detach from the substrate. These mats usually float, and can accumulate on obstructions (*i.e.* branches) or along the edges of lakes, rivers or ponds (Figure 13) increasing their availability for human contact. Low levels of toxins have been detected in river water when mats are present (Section 5.1), and low concentrations of cyanobacterial cells/filaments are commonly observed during algal analysis of river water (Cawthron microalgae laboratory, pers. comm.).

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[3] Under the National Objectives Framework *i.e.* the *E. coli* attribute table of MfE 2014, page 31, the former activity includes "… undertaking activities likely to involve full immersion" while the latter involves "… contact with water during activities with occasional immersion and some ingestion of water (such as wading and boating)". A more complete list for secondary contact may be stated as paddling, rowing, motor boating, fishing, wading, kayaking and canoeing (so long as there is only "occasional immersion").

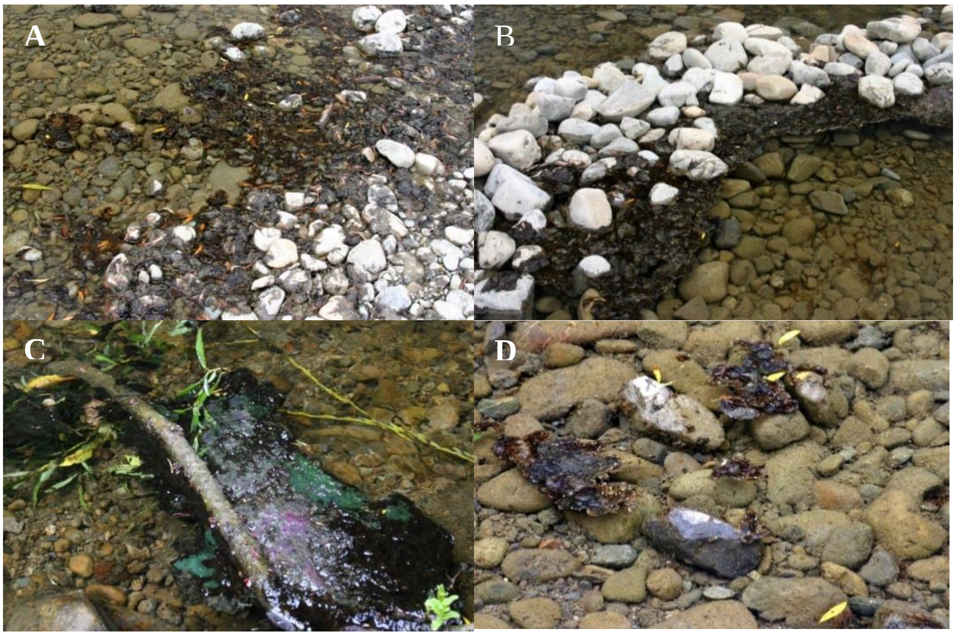

Figure 13. Detached *Microcoleus*-dominated mats in the Hutt river accumulating; (A) river edge, (B) a small 'manmade' dam in river, (C) a tree branch, and (D) free floating in river. Photos: Greater Wellington Regional Council.

Definitive studies have been carried out, for adults and for children, in swimming pool dosing studies for primary contact (Dufour *et al.* 2006), and for secondary contact (Dorevitch *et al.* 2011).

Primary contact

Dufour *et al.* (2006) calculated the volume of water ingested in a clinical trial observing 53 volunteers involved in recreational swimming in an outdoor community swimming pool. Swimmers were assumed to ingest similar amounts of water during swimming in pools or in freshwater due to similar behaviours in each (frequently immersing their heads under the surface and remaining in the water for long periods of time). Cyanuric acid was used to trace water ingestion because it is present in outdoor swimming pools (as a decomposition product of chlorine-stabilising chloroisocyanurate) and passes through the human body unmetabolised. For each swimmer, the volume of water ingested during active swimming events lasting at least 45 minutes was calculated from:

$$V_{\text{water\_ingested}} = (V_{\text{urine\_collected}})U/P$$

where: $V_{\text{urine\_collected}}$ is the volume of urine collected by the volunteer over the 24 hours following swimming; U is the concentration of cyanuric acid in the urine samples (U); and P is the concentration of cyanuric acid measured in the pool water samples before the trial.

The study found that children (≤ 18 years of age) ingested significantly more water (average = 37 mL, range = 0 to 154 mL, n = 41) than adults (average = 16 mL, range = 0 to 53 mL, n = 12).

Prior to publication of the data from Dufour *et al.* (2006), ingestion of water during recreational activities, as incorporated into quantitative microbial risk assessments, was not well characterised. Such studies have generally used single values (*i.e.* not a range) of ingestion volumes for a single event (*e.g.* daily) of 30–100 mL empirical distribution of ingested volumes. The widest and highest range (0 to 190 mL per dive) is given by Schijven & de Roda Husman (2006). This value, however, was specific to sport divers and the data based on questionnaires.

More recently, Schets *et al.* (2011) conducted an exposure assessment for swimmers in bathing waters and swimming pools that was based on approximately 10,000 questionnaires. Overall, men swallowed on average 27–34 mL per swimming event, women 18–23 mL, and children 31–51 mL, where each swimming event lasted between 45 to 90 minutes. Suppes *et al.* (2014) found that head submersion was not a good indicator of ingestion exposures in recreational swimmers but splashing and bobbing behaviours were. Overall swimmer ingestion rates were not highly varied from Dufour *et al.* (2006) with a mean of 13.7 mL swim^{-1}, standard deviation of 24 mL swim^{-1} and a range of 0 to105.5 mL event^{-1}. Children had higher exposure estimates with a mean of 25.7 mL event^{-1} and standard deviation of 29.2 mL swim^{-1}.

Secondary contact

Dorevitch *et al.* (2011) conducted studies on water ingestion rates for canoeing, fishing, kayaking, motor boating, and rowing. Two approaches were used. First, at Chicago area surface waters, survey research methods were used to characterise self-reported estimates of water ingestion during canoeing, kayaking, and fishing among 2,705 people. Second, at outdoor swimming pools, survey research methods and the analysis of cyanuric acid (as in the study of Dufour *et al.* 2006) were used to characterize water ingestion among 662 people who engaged in a variety of full-contact and limited-contact recreational activities. Data from the swimming study were used to derive translation factors that quantify the volume of self-reported estimates. The data from the surface waters study showed that less than 2% of canoers and kayakers reported swallowing a teaspoon or more, and 0.5% reported swallowing a mouthful or more. Swimmers in a pool were about 25 to 50 times more likely to report swallowing a teaspoon of water compared to those who participate in limited-contact recreational activities on surface waters. Mean and upper confidence estimates of water ingestion during limited-contact recreation on surface waters are about 3 to

4 mL and 10 to 15 mL, respectively. These estimates of water ingestion rates may be useful in modelling the health risks of water recreation.[4,5]

Implications

Because there are ranges of exposures and uncertainty about their limits, it would seem that a Monte Carlo modelling approach may be the best way to develop quantitative predictions (leading to appropriate numeric attributes). That will require obtaining the Dufour and Dorevitch volunteers' study data to fit appropriate statistical distributions. Graham McBride has strong collaborations with both researchers and anticipates that this will not be problematic. Information on the range of toxin concentrations in the ingested water and mats would also be required. This information is available for anatoxins in *Microcoleus*-dominated mats, however, data on 'free' toxins in the water column are limited (Section 5).

6.2.2. Approach 2 – soil ingestion rates

Aside from ingestion while swimming, a further exposure route may be from direct ingestion of cyanobacterial mat material, particularly by younger children playing at the water's edge. We have found no direct reference to this occurring or causing problems, and the best surrogate information as to potential exposures comes from estimates of soil ingestion by children. Estimates of such exposure are made by a variety of methods, and have been recently reviewed (Moya & Phillips 2014). The most reputable current estimate for daily ingestion of soil by children aged 1 to 6 comes from the USEPA (2011, Chapter 5), who suggest 200 mg day^{-1} as the upper percentile figure (3–6 years), and an average of 50 mg day^{-1} (1-6 years) for soil ingestion, with hand-to-mouth soil ingestion as the major exposure route. The extensive review of the literature by Moya and Phillips (2014) essentially confirmed these estimates. Some children (and adults) actively ingest non-food substances, including soil, and for this subgroup, ingestion was higher.

Analysis of different approaches demonstrates they can differ dramatically in their estimates of soil ingestion. The estimates of Calaberese and Stanek (1995), and Calabrese *et al.* (1996) are based on 2-week observation and measurement periods using a tracer-element mass balance approach with intertracer elements *e.g*. aluminium, silicon, yttrium and zirconium. Using this approach the estimated median value daily soil ingestion averaged over a year was 75 mg day^{-1}, while the 95th percentile was 1,751 mg day^{-1}. Ozkaynak *et al.* (2011) use an ingestion modelling approach. This uses a geometric mean (GM), or median ingestion rates for aluminium and silicon based on tracer ingestion studies (25–60 mg day^{-1}). Mean variances from the tracer studies were employed to derive a geometric standard deviation (GSD) in

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[4] This paragraph is based on the abstract presented by Dorevitch *et al*. (2011).

[5] Note in developing secondary contact ingestion rates McBride (2012) used the probabilistic results reported by Rijal *et al*. (2011). However, it is now considered that the study of Dorevitch *et al.* (2011) gives firmer evidence of these rates, especially as it involved direct ingestion measurements.

fitting a log-normal distribution. In addition a bootstrap methodology was employed to generate (GM, GDS) pairs to empirically describe the uncertainty surrounding the fitted soil ingestion rate variability distribution (Ozkaynak *et al.* 2011). Based on these analyses the hand-to-mouth soil ingestion for children aged 3 to 6 were 41 (mean), 5.2 (25th percentile), 15.3 (50th percentile), and 175.6 (95th percentile) mg day^{-1}. Modelled uncertainties ranged within a factor of 3 to 30, especially for the lower (< 20th percentile) and upper (> 95th percentile) distributions.

Soil ingestion estimate approaches generally assume 'random foraging' (*e.g.* Ferguson *et al.* 2006) by children. This implies that there is no visual cue which could specifically attract the child to a contaminant, and a random distribution of a contaminant in the soil is assumed. This approach may not be applicable to benthic cyanobacteria where mats accumulated on the river may provide a visual cue. Perhaps the evidence that is most applicable to the probability of children ingesting benthic mat material comes from activity pattern analysis. This methodology results in estimates of how often children put their hands or other dirty objects into their mouths, and for how long this contact occurs. Putting this information together with knowledge on duration and type of activity for children playing around water might allow for improvements in the data on which risk assessments will be based.

In a study of 23 children (aged 6 to 27 months) conducted indoors, the median hand-to-mouth frequency was 15.2 events hour^{-1}, and the median object-to-mouth frequency was 27.2 events hour^{-1} (Beamer *et al.* 2008). In a similar study of outdoor activity, 18 children aged 7–12 were videotaped. Median mouthing frequencies were 12.6 events hour^{-1} and 2.6 events hour^{-1} for hands and non-dietary objects, respectively (Beamer *et al.* 2012). A recently published study from Taiwan suggested somewhat lower estimates for young children in indoor settings, and concluded that mouthing behaviours may be different between different countries or populations with different ethnic or lifestyle characteristics (Tsou *et al.* 2015). We found no data on whether the closer parental supervision expected for younger children playing around water resulted in lower hand-to-mouth activity.

It is important to note that soil ingestion estimates are based on daily exposures, and were designed to provide data relevant to chronic and longer term exposures. A more likely scenario for benthic cyanobacteria is a single dose/acute *i.e*. one or several handful-to-mouth types of exposure in a very specific time-frame. There are no robust data to validate for this scenario. It may be appropriate to use a linear approach, correcting for age, using the 20th and 95th percentiles, as well as the uncertainty factors of 3–30 given in Ozkaynak *et al*. (2011). Using such an approach we estimate that a 3-year-old could ingest between approximately 300 to 10,560 mg wet weight[6],

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[6] For soil hand to mouth ingestion the 20th and 95th percentile is approximately 5 and 176 mg day^{-1} and the mean 41 mg day^{-1} for 3-5 year old children (Ozkaynak *et al.* 2011). We employed a 10-hour day calculation to result in approximately 0.5, 4.1 and 17.6 mg hour^{-1} for the 20th, 50th and 95th percentile of hand-to-mouth soil ingestion as dry matter. Employing the worst case scenario (underestimation) of a factor 30 uncertainty, this would result in 15, 123 and 528 mg hour^{-1} for the

while a 6-year old could ingest between approximately 600 to 21,120 mg wet weight of mat material within a determined period of exposure (approximately 30–90 minutes; Ozkaynak *et al.* 2011). Further analysis and validation of this approach is required.

6.3. What factors might modify the likelihood and rate of ingestion?

6.3.1. Susceptible groups

Certain users groups may be more likely to come in contact with or ingest benthic cyanobacteria than others, or the consequences may be more severe if they do. These groups include children, tourists or people from culturally and linguistically diverse backgrounds, and the elderly or those with existing medical conditions (NHMRC 2008). These groups are discussed below.

Children

Children usually spend more time in the water than adults and are therefore more likely to swallow water or benthic cyanobacterial mats, either intentionally or unintentionally (WHO 2003). The risk of ingestion is elevated when children are left unattended, therefore factors such as parental supervision at rivers will influence likelihood of ingestion. Between 10% and 30% of children aged 1 to 6, have an eating disorder known as pica. This is characterised by persistent and compulsive cravings (lasting 1 month or longer) to eat non-food items. Although we could find no information on this condition in relation to ingestion rates of algae or cyanobacteria, we suggest children with pica may be more prone to consuming benthic cyanobacterial mats.

Although there is currently little evidence to verify this, children may also be more susceptible to cyanotoxin poisoning than adults. For example, following a large-scale cyanotoxin poisoning event involving contaminated drinking water in Australia (Hawkins *et al.* 1985) considerably more children were affected than adults. However, the higher rate of intoxication observed may have been due to exposure rather than to age-related susceptibility (Azevedo *et al.* 2008).

Tourists and people from culturally and linguistically diverse backgrounds

Tourists and people from culturally and linguistically diverse backgrounds may be unaware of the risks posed by the benthic cyanobacterial mats, and may not understand warning signs.

Elderly and those with medical conditions

Although the likelihood of ingestion is not elevated in this group, the consequence if they do consume cyanobacteria material may be more severe. For example, irritation

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20th, 50th and 95th percentile of hand-to-mouth soil ingestion as dry matter, respectively. 95% water content was used to calculate wet weight of the algal biomass: 15 mg/hour: 5% of weight x 100 = 300 mg wet weight/hour.

caused by exposure to air–borne toxins may be greater in people with underlying respiratory disease (NHMRC 2008), and in regards to anatoxin, people with pre-existing peripheral or central nervous neuropathies or predispositions for neuropathies (*e.g.* familial ALS) may have a higher susceptibility than healthy individuals. However, the present literature on neurotoxicity offers little information as to the effect of additional intoxications on top of a higher susceptibility scenario.

6.3.2. Geographical and environmental factors

Extrinsic factors such as the attractiveness or suitability of a site (*e.g.* availability of swimming holes, picnic areas, ease of access to water) and locality to residential areas impact the frequency of visitors and therefore likelihood of human contact with benthic cyanobacterial mats. The Hutt River (Wellington) provides evidence to substantiate this. Multiple dog deaths due to ingestion of *Microcoleus*-dominant mats have been reported from this river. The middle and lower reaches of the Hutt River run beside the heavily populated urban centres of Upper Hutt and Lower Hutt and are used extensively for recreational activities with over 1 million visits per year. The riverside is also a popular dog exercising area.

Recreation activity is greatest in New Zealand rivers during summer—the period when benthic cyanobacterial blooms are most prolific (Section 8).

Intrinsic bloom factors such as spatial and temporal variability and bloom extent are also important considerations in regard to likelihood of ingestion. In some rivers blooms are confined to a few sites, and only occur for several weeks (Section 8), and therefore contact with benthic mats would be less likely. In contrast, in some rivers, *i.e.* Opihi (Canterbury), blooms can be present from spring to autumn and can cover many kilometres of the river substrate. The likelihood of benthic mat ingestion by humans engaging in select recreational activities *e.g.* swimming, at these sites would be high.

Small sections of well-developed mats continually slough off rocks, and these can accumulate on the river’s edge (Figure 13). Additionally, simultaneous mass autogenic detachment of *Microcoleus*-dominated mats can occur (see Section 8, with a specific example from the Maitai River: Figure 20). During these events large sections of mats detach and float down river, often accumulating on the river’s edge (Figure 13). Detachment events are thought to be partly due to entrapment of oxygen within the mats caused by photosynthesis (Section 8), and therefore primarily occur during daylight. The likelihood of contact and therefore ingestion of *Microcoleus*-dominated mats will be escalated during mass detachment events.

7. WHAT IS THE EXPOSURE RATE AND LIKELIHOOD OF SKIN EXPOSURE TO BENTHIC CYANOBACTERIA MAT

7.1. What have studies that directly addressed this question found?

To our knowledge, no studies have investigated exposure rate and likelihood of skin exposure to benthic freshwater cyanobacterial mats. Severe dermatitis has been reported in Florida following recreational activities in waters with extensive benthic/metaphytic mats of *Lyngbya wollei* in Florida's freshwater springs (J Burns, I Stewart, G. Shaw, unpublished data, as cited in Azevedo *et al.* 2008). No further information on these incidents could be obtained. Two extensive studies on recreational exposure to freshwater planktonic cyanobacteria provide some relevant information that demonstrates an increased response with rising biomass and exposure time. Acute adverse health effects in humans are well described from contact with water or biomass containing the benthic marine cyanobacterium *Lyngbya majuscula* (Osborne *et al.* 2001, 2007, 2008).

Pilotto *et al.* (1997) investigated the health effects after recreational exposure to planktonic cyanobacteria in Australia through an epidemiological study of 852 participants, 777 of whom had water contact. Symptoms observed included; vomiting or diarrhoea, cold and flu symptoms, mouth ulcers, eye irritation, ear irritation, skin rash and fever. No significant differences in symptoms were found between the unexposed and the exposed subjects after 2 days. At 7 days, there was a significant ($P = 0.03$) increase in symptom occurrence with duration of exposure and with increasing cell concentration ($P = 0.04$). The authors conclude that symptom occurrence was associated with duration of contact (> 60 minutes) and cyanobacterial concentrations greater than 5,000 cells/mL.

Stewart *et al.* (2006) studied 1331 individuals engaged in various water recreation activities at freshwater and brackish lakes and reservoirs in New South Wales and northeast and central Florida. The study concluded that individuals exposed to waters with high cyanobacterial cell concentration (where total cyanobacterial surface areas exceeded 12 mm^2/mL), were more likely to report symptoms after exposure than those exposed to waters with low cell concentrations (total surface areas < 2.4 mm^2 /mL). Mild respiratory symptoms were the predominant symptom category. The data from Stewart *et al.* (2006) were used during the development of New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters in 2009, (Ministry for the Environment and Ministry of Health 2009).

The marine benthic cyanobacterium *Lyngbya majuscula*, commonly known as 'fireweed', has been implicated in acute adverse health effects with symptoms including dermatitis (involving itching, rashes, burning blisters and deep desquamation and pain), respiratory irritation and burning of the upper gastrointestinal tract on ingestion. Osborne *et al.* (2007) surveyed 1,350 residents in an area subject to annual toxic *L. majuscula* blooms in Queensland (Australia). They showed that participants with greater exposures/contact with the water were more likely to have skin and eye symptoms than less exposed groups. The authors concluded that high numbers of participants reported symptoms after exposure to waters subject to *L. majuscula* blooms, but only a small number appeared to be serious in nature, suggesting limited exposure to toxins. The main exposure routes are reviewed in Osborne *et al.* (2001). Grauer and Arnold (1961) documented contact through water-based activities, noting that symptoms occurred on exposed areas as well as areas covered by swimsuits, leading the authors to question whether exposure to the toxin occurs through contact with toxic lipophilic exudates released by the cyanobacterium, rather than with the cyanobacterium itself. Symptoms after skin contact (Dennison & Abal 1999) and exposure to aerosolised *L. majuscula* (Hashimoto 1979; Izumi & Moore 1987) have also been documented.

7.2. What factors might modify the likelihood and rate of exposure?

We suggest symptoms after exposure through recreational activities to freshwater planktonic and benthic species would be similar. However, the key difference is the exposure routes. The main exposure routes to cyanobacteria through recreation contact include direct contact with cell, oral (from accidental or deliberate ingestion of recreational water), and via inhalation. Stewart *et al.* (2006) state that they believe the sole natural exposure route that might explain aquatic freshwater cyanobacterial LPS-related illness is via inhalation of aerosolised cells or fragments.

When freshwater benthic cyanobacteria are healthy they grow attached to substrates *e.g.* rocks, lake beds. Human contact would be largely limited to the soles of feet. Such activities will disturb and detach mats which may then float and some limited contact may occur. Additionally, at certain points during the lifecycle of benthic mats they can become positively buoyant and naturally detach forming floating rafts. These rafts can accumulate along the edges of lakes and rivers (Figure 13). However, in contrast to planktonic cyanobacteria, where humans may be submersed in a relatively homogeneous mixture of cyanobacteria, actual skin contact with benthic cyanobacteria is likely to be limited. Although illness linked to aerosolised cells or fragments has been suggested from benthic *L. majuscula* in the marine environment, we suggest this is currently unlikely in freshwaters in New Zealand. The scale of mat accumulation is relatively small and short-lived, compared to the thousands of tonnes of *L. majuscula* that accumulates on beaches where problems associated with aerosols have been observed (Osborne *et al.* 2007).

The other factors discussed in Section 6.3, *i.e.* susceptible groups and geographical and environmental factors, are also relevant to this question and could increase the rate and likelihood of skin exposure to benthic cyanobacteria.

8. WHAT ARE THE RELATIONSHIPS BETWEEN *MICROCOLEUS* ABUNDANCE AND ENVIRONMENTAL DRIVERS?

8.1. What have studies that directly addressed this question found?

8.1.1. Introduction

This section focuses on relationships between environmental drivers and the abundance of *Microcoleus*-dominated mats in New Zealand rivers (see Section 2.3 for justification). Existing research on the dynamics of *Microcoleus*–dominated mats is described by combining evidence obtained on their role as a component of river periphyton, and as a microbial mat (Figure 14). We describe how different environmental drivers may exert varying degrees of control on biomass dynamics through an accrual cycle (Figure 15).

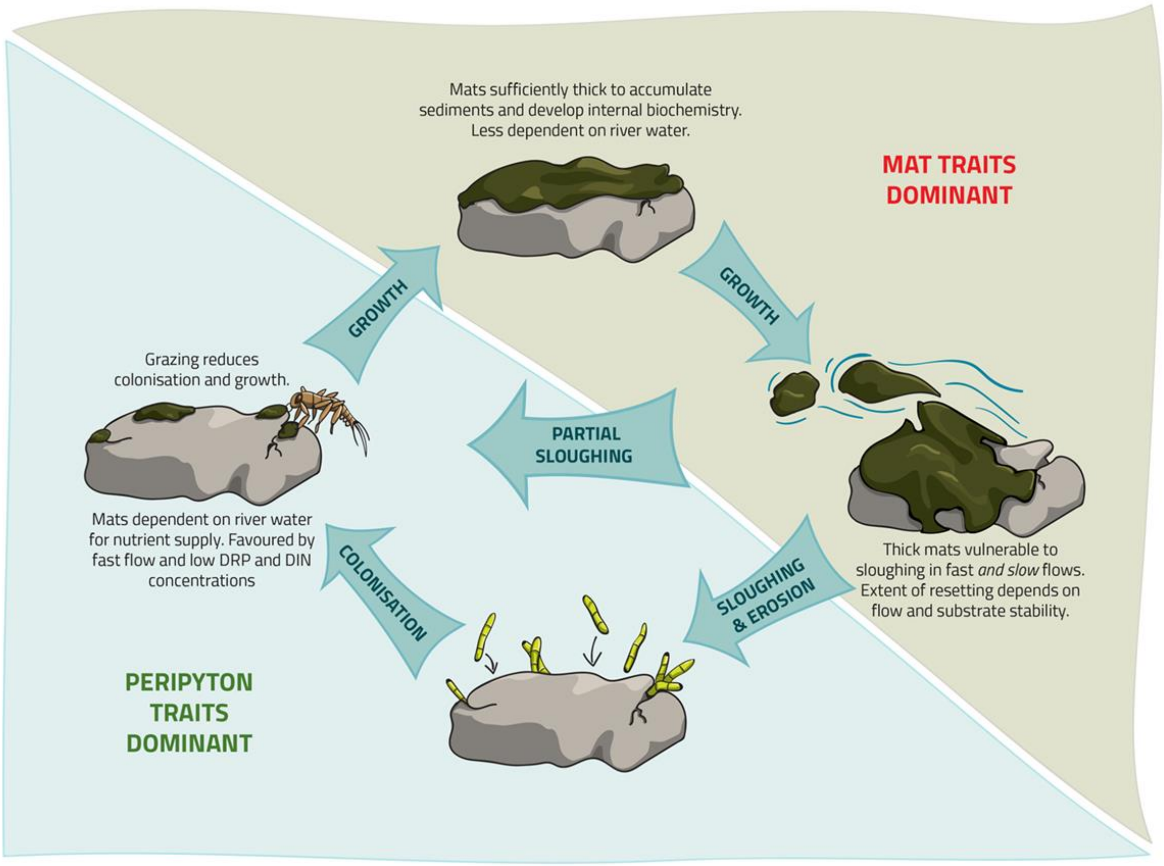

Figure 14. Systematic diagram of accrual cycle for *Microcoleus*-dominated mats in rivers and classification of stages as periphyton or mat traits.

Microcoleus-dominated mats occupy in the same habitat in New Zealand rivers as other epilithic periphyton, *i.e.* filamentous greens and diatoms that grow attached to rocks, and to a large extent are regulated by many of the same variables, such as flow dynamics and nutrient concentrations. However, the thick, cohesive mat growth-forms seen in dense *Microcoleus* proliferations can be many millimetres thick and the environment inside the mat can be very different to that in the river itself. Once developed, the mats may need to be considered as functionally different to other types of periphyton. Although dominated by *Microcoleus,* these mats also contain abundance of other organisms including bacteria, other cyanobacteria, and to a lesser extent green algae and diatoms (Hart *et al.* 2013; Brasell *et al.* 2014; Figure 15). These consortia of organisms often benefit physiologically from each other, and are bound together and to the substrate, by extracellular polymeric substances (EPS) that immobilise water within a mucilaginous matrix (Gerdes 2010). The development of the mucilaginous matrix essentially allows a volume of water to be isolated from the overlying river, and this can result in localised changes to the immediate growth environment. The development of steep biogeochemical gradients *e.g.* oxygen, is well described in cyanobacteria-dominant microbial mats (Stal 2012). Conditions that favour rapid nutrient fixation, release and recycling can occur within such systems making them independent of nutrient flux from overlying water (Stal 2012).

Differences in the functioning of *Microcoleus*-dominated mats from other periphyton types, and differences at successional stages, mean that the environmental control of *Microcoleus* biomass expansion can be complex, nuanced and at times contradictory. What is important to a thin film may be less so to a thick mat, making simple relationships difficult to extract. To date this aspect of *Microcoleus* ecology has been poorly addressed, and through this section we attempt to identify where such conflicts occur.

8.1.2. Accrual cycles

The concept of accrual cycles is fundamental to our understanding of periphyton dynamics in New Zealand rivers (Biggs *et al.* 1998). The accrual cycle essentially describes the process of colonisation and initiation of a periphyton community, its subsequent growth, and the process that removes the periphyton and allows the cycle to restart (Figure 14 and Figure 15). At its most basic level, the accrual concept argues that following colonisation, periphyton biomass and cover increases at a rate determined by growth-promoting factors, until removed by full or partial disturbance (Figure 14 and Figure 15). Key phases of this cycle, with particular focus on *Microcoleus,* are discussed below.

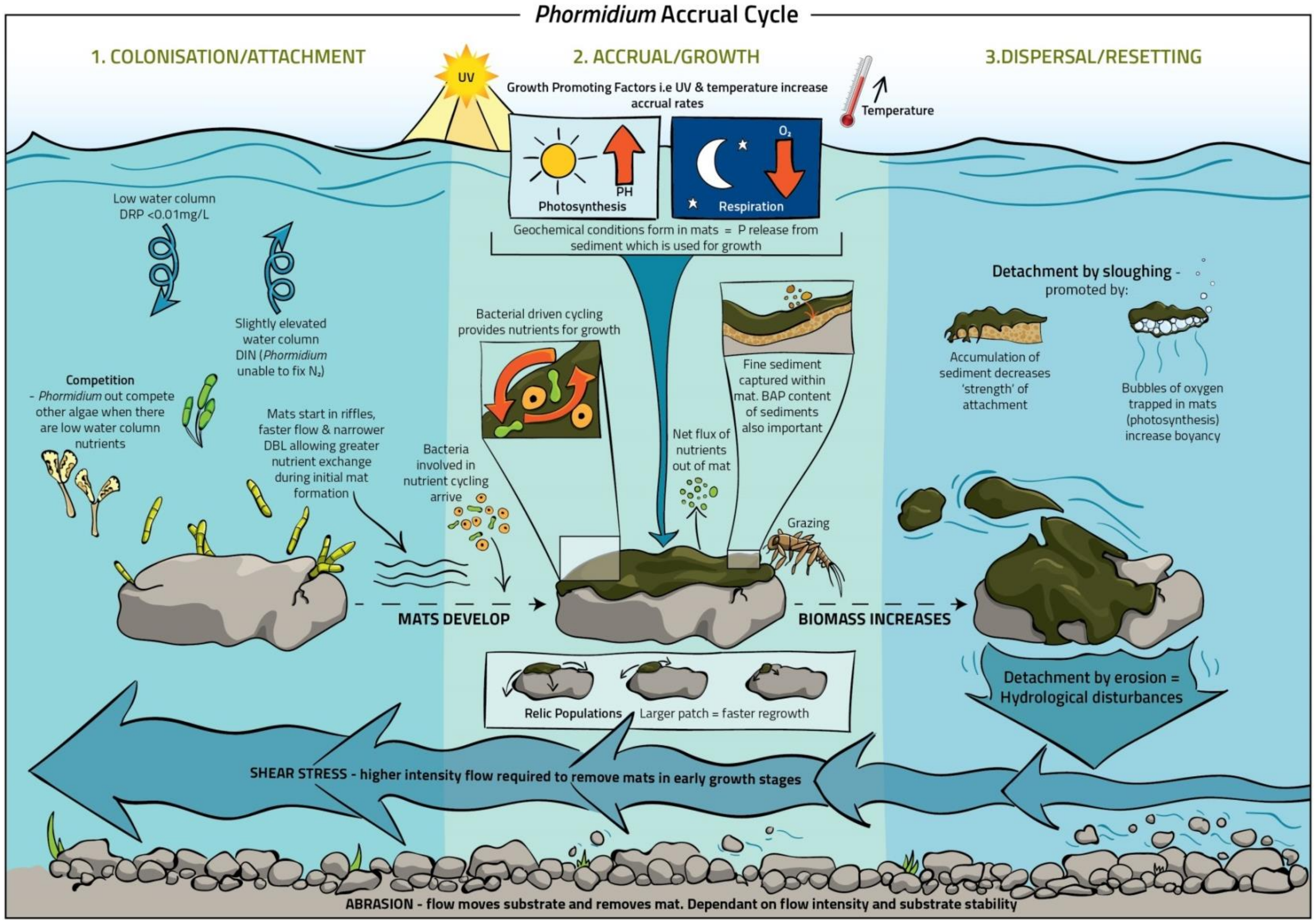

Figure 15. Systematic of accrual cycle for *Microcoleus*-dominated mats in New Zealand rivers. DRP = dissolved reactive phosphorus, P = phosphorus, BAP = biologically available phosphorus, DIN = dissolved inorganic nitrogen, DBL = diffuse boundary layer.

Colonisation and attachment

The initiation of an accrual cycle may involve growth from new colonists, or from residual populations left after the previous cycle has been completed. The first of these is still poorly understood in the context of river periphyton, although the widespread economic importance of biofouling means that the general topic continues to attract attention (see review by Gerbersdorf & Wieprecht 2014). Stable substrates, *e.g.* bedrock, boulder and cobble, tend to be preferred sites for *Microcoleus* growth (Heath *et al.* 2015). Adhesion of propagules to such substrates tends to be enhanced by surface roughness (Schneck *et al.* 2011), which reduces exposure of settling organisms to shear forces and increases near-bed turbulence to reduce the thickness of laminar boundary layers and bring propagules close to surfaces. There is also some evidence for the requirement of a preconditioning coating of organic materials to facilitate adhesion, though this is not definitive (Characklis & Cooksey 1983). In natural rivers it is reasonable to expect most substrates to have some degree of pre-coating with organic molecules, particularly if they have been previously coated with periphyton. Preconditioning may also be bacterially driven and there may be aspects of succession involved with *Microcoleus* development. Brasell *et al.* (2014) used morphological and molecular approaches to track microbial succession following a rainfall event that cleared the substrate in the Hutt River (Wellington). They identified three distinct and concordant phases of bacteria assemblages as *Microcoleus*-dominated mats developed. Further work is required to fully understand the functional roles of bacteria at each phase.

Little attention has been paid to the significance of partial abrasion, biomass accrual, and residual populations, though this can be an important consideration for periphyton recovery from disturbance. Francoeur *et al.* (1998) showed how 'microform bed clusters', riverbed structural elements that combine small and large cobbles and stones in ways that resist entrainment during floods, provided refuge for some periphyton (their study did not include *Microcoleus*), which translated to higher biomass during the subsequent accrual cycle. Substrate heterogeneity (*e.g.* cracks and crevices, projections) may also be important in facilitating *Microcoleus* colonisation. Heterogeneous substrate provides 'refuges' during flushing flows (Bergey 2005; Murdock & Dodds 2007). We suspect that residual populations may be particularly important for rapid *Microcoleus* regrowth. Our observations indicate regrowth often initiates near crevices (S Wood, pers. obs.), which are obvious refuge sites. In addition, rapid recovery of *Microcoleus* cover has been observed after partial ablation in Canterbury rivers (Thiesen 2015), suggesting that a relic population (*i.e.* the amount of *Microcoleus* remaining on substrate after a disturbance event; Figure 15) may facilitate rapid recovery.

Nutrients and their association with later stages of periphyton accrual and development of *Microcoleus* blooms are discussed below, but nutrients may also play a role during early establishment. Several studies (Wood *et al.* 2014, 2015) suggest that water nutrient concentrations during the initial colonisation phase strongly influence whether *Microcoleus* can establish and subsequently form mats. It is likely that this is integrally linked with competition with other periphyton components, for example, some cyanobacteria are known to be very adept at luxury uptake of phosphorus, storing it in their cells for later cell division (Nausch *et al*. 2004). It is possible that *Microcoleus* has the ability to do this, arriving with sufficient internal nutrients to give them a competitive advantage over other periphyton (such as diatoms or greens) during the initial stage of mat formation.

The transition from biofilm to mat

Several studies and numerous field observations by the authors have shown that *Microcoleus*-dominant mats are at least initially, largely confined to riffles (fast, turbulent areas) in rivers (Heath 2010; Thiesen 2015). Flow requirements for successful accrual have, however, received less research than those for removal. It has been argued that a dynamic relationship exists between flow and accrual that has different solutions at different growth stages (Quiblier *et al.* 2013), though there are only limited data to support this. Biggs *et al.* (1998) developed a general stress-subsidy model for periphyton to describe this relationship. The stress-subsidy concept suggests that during the initial accrual stages growth in low-nutrient water may be enhanced by flow (the subsidy element) since it increases the flux of nutrients by reducing the thickness of the diffusive boundary layer separating substrate from river water and through which nutrients must pass by diffusion (Larned *et al.* 2004). This may partly explain why *Microcoleus*-dominant mats may begin their growth phase predominately in riffles (Heath *et al.* 2015). In later stages of growth, the stress-subsidy concept suggests that subsidy decreases and stress increases as high velocity enhances the probability of detachment of thicker periphyton communities (discussed in more detail below).

Accrual/Growth

The duration of an accrual cycle is particularly important in determining the biomass and cover that can be accumulated (Biggs 2000). However, within this overall constraint, growth promoting variables affect biomass dynamics across all accrual cycle phases, and these can relate to the supply of resources or factors that affect the rate of biological processes (Figure 15). For river periphyton in general, Francoeur and Biggs (1999) found in artificial substrate experiments[7] that temperature was the most important variable affecting accrual rate. Heath *et al.* (2011) identified water temperature as an important factor in determining whether *Microcoleus* blooms were present or absent. However, subsequent studies with larger datasets have shown no relationship between temperature and the occurrence of *Microcoleus* blooms and they were not confined to periods of warm temperature (McAlister 2014; Wood *et al.* 2014, 2015). An effect of temperature on accrual rate is not inconsistent with blooms occurring at both low and high temperature, though to date the influence of

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[7] Artificial substrates involve placing surfaces for growth, which can be with or without nutrient enrichments, into rivers and following the accrual of biomass. They measure a mix of colonisation and net growth, and often develop communities that do not mimic the natural community. Interpretation requires care.

temperature on actual accrual rate of *Microcoleus* has not been investigated. These issues do however highlight the potential interaction between growth rate and accrual duration in determining the biomass at any stage in the cycle.

The role of nutrients

Biggs (2000) concluded that, when the effects of accrual time were allowed for, the best predictors of periphyton biomass were inorganic nitrogen and phosphorus concentrations (Figure 16). However, this effect was not strong for *Microcoleus*, and the overall pattern reported by that study was biased by the positive response of some filamentous green and yellow-green (*Vaucheria* sp.) algae to high nutrient concentrations. The selective effect of nutrients on the specific type of periphyton complicates nutrient-biomass relationships. Evaluation of the data in Biggs (2000) suggests that a hump-back curve rather than a straight line may best describe the relationships between filamentous cyanobacteria (such as *Microcoleus*) and nutrients (Figure 16), with optimal concentrations of soluble inorganic nitrogen (SIN=DIN) and soluble reactive phosphorus (SRP=DRP) of approximately 0.1 and 0.01 mg L^{-1} respectively.

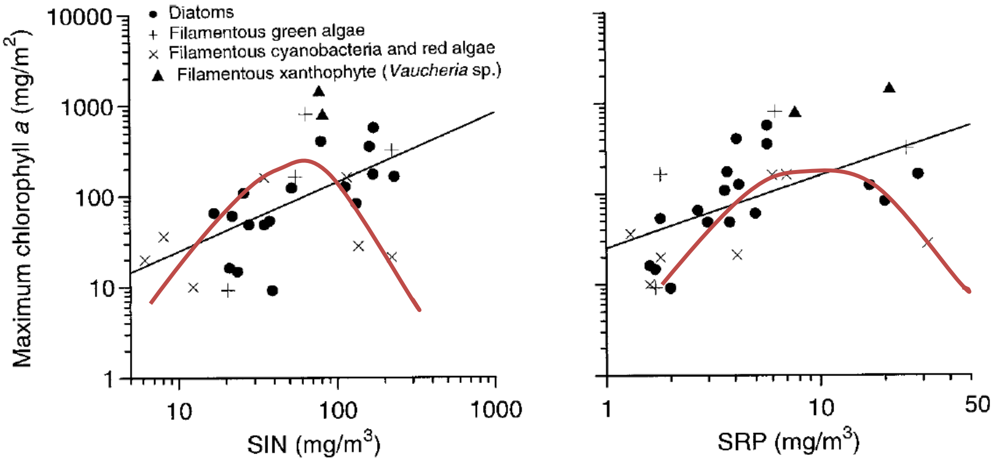

FIG. 4. Maximum benthic chlorophyll *a* concentration at 30 stream sites as a function of mean monthly soluble inorganic N (SIN) and mean monthly soluble reactive P (SRP) concentration. Best-fit regression lines are given. See Table 3 for regression statistics.

Figure 16. Figure 4 from Biggs (2000) annotated with the red curve to approximate a fit to the data points for periphyton likely to include *Microcoleus* (×).

Based on many years of accumulated research, Biggs *et al.* (1998) placed cyanobacterial mats (both *Schizothrix* and *Microcoleus*) in a category of periphyton that for accrual requires only moderate nutrient supplies, but which develops slowly and is thus favoured by low frequency disturbance. More recently, targeted research on *Microcoleus* blooms (defined as greater than 20% coverage) has gathered evidence to test the nutrient requirements for bloom formation. These data tend to confirm the tendency for *Microcoleus* to perform well at low-moderate nutrient concentrations, and perversely to be less prevalent at high nutrient concentrations, *i.e.* the hump-back curve.

Wood *et al.* (2014) investigated the nutrient concentrations that favour *Microcoleus* blooms by sampling 12 rivers in the Manawatu-Whanganui region over a two year period. They based their analysis on the suggestion of Biggs and Close (1989) that simple point-by-point correlations between nutrient concentrations and periphyton biomass do not provide a true indication of the historic nutrient loading on the community. This is because nutrients are progressively depleted from the water column as biomass accrues—and this may be particularly the case for mat-forming periphyton as discussed above. To allow for this time-integration effect, Wood *et al.* (2014) used a cumulative mean of the DIN and DRP concentrations over the accrual period for each time-point (*i.e.* the mean nutrient concentrations since the previous sampling point where *Microcoleus* coverage was zero). Their data suggested that *Microcoleus* blooms were most likely when DRP was less than 0.01 mg L^{-1}, but they did not identify a lower limit (Wood *et al.* 2014; Figure 17). Exceptions were identified, in particular downstream of sewerage treatment plants, and these still require further investigation (Figure 17).

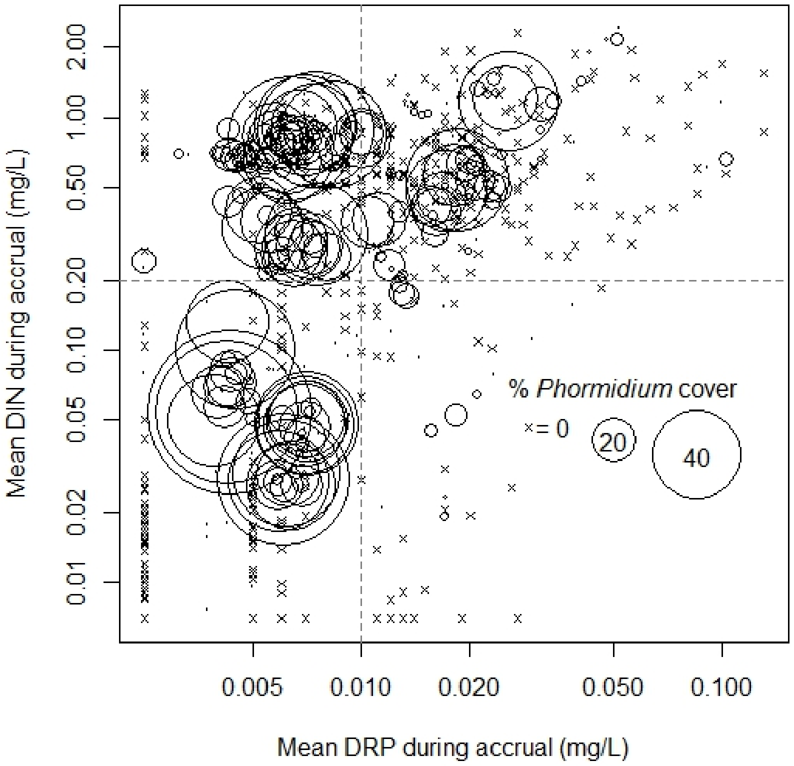

Figure 17. Relationship between mean *Microcoleus* coverage and dissolved reactive phosphorus (DRP) and dissolved inorganic nitrogen (DIN) for ten rivers monitored weekly for two years in the Manawatu region. Dissolved inorganic nitrogen and DRP are a cumulative mean over the accrual period. Dashed lines show suggested DRP and DIN thresholds that favour *Microcoleus* blooms. All points in the top right corner with *Microcoleus* coverage are downstream of sewerage treatment plants (modified from Wood *et al.* 2014).

We have extended the analysis from Wood *et al.* (2014), using data from 60 periphyton monitoring sites in the Manawatu region, sampled approximately monthly since 2011 (when differentiation between *Microcoleus*–dominated mats and other periphyton began in this region). The annual mean DIN and DRP is compared to the maximum *Microcoleus* coverage at each site over the corresponding hydrological calendar year, *i.e.* July 1 to June 30. This analysis showed a similar pattern to that observed by Wood *et al.* (2014) with blooms most commonly seen when DRP was below 0.01 mg L^{-1}. Examination of Figures 17 and 18 together suggest that a lower limit of 0.005 mg L^{-1} for DRP to support bloom formation may also apply, though care needs to be taken as this approaches the detection limits of many routine DRP analytical techniques.

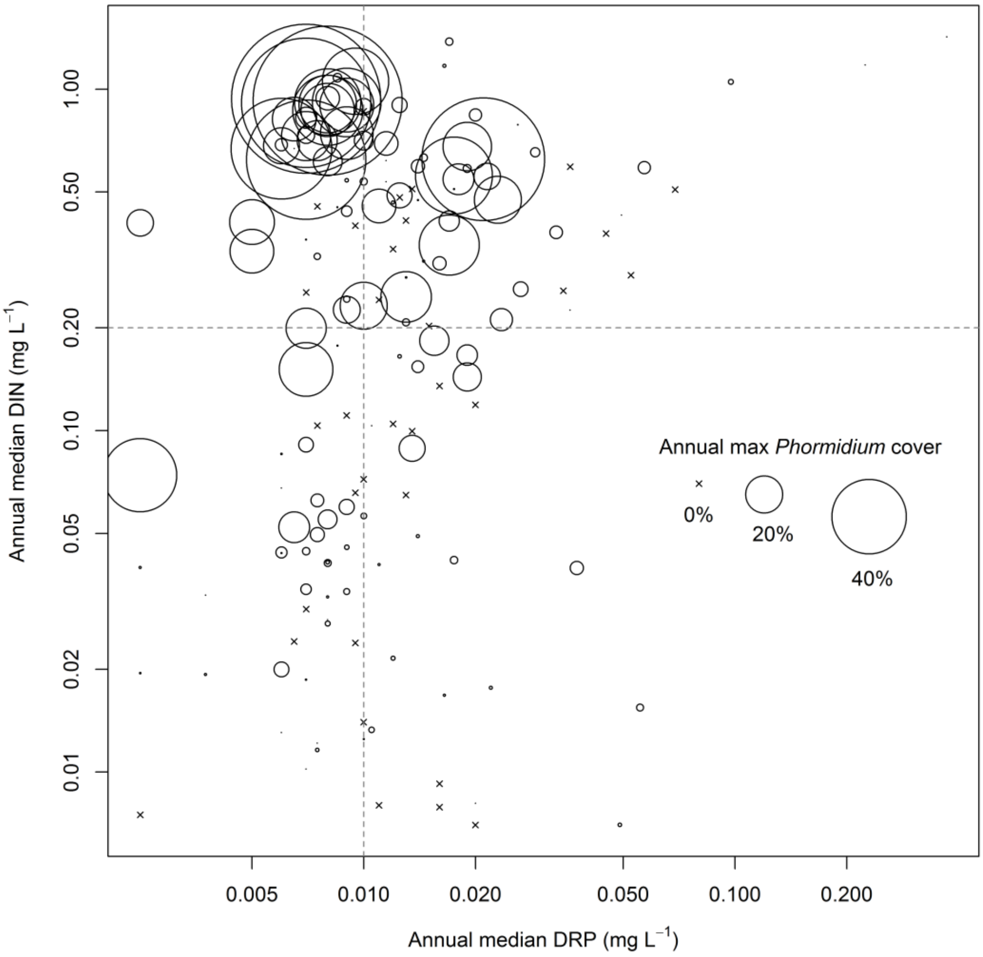

Figure 18. Relationship between maximum *Microcoleus* coverage and mean dissolved reactive phosphorus (DRP) and dissolved inorganic nitrogen (DIN) at 60 sites over a 4-year period (2011–2015) in the Manawatu region. Each of the sites had data to calculate maximums or medians for 1 to 3 hydrological years. Dashed lines show suggested DRP and DIN thresholds that favour *Microcoleus* blooms.

Data analysed from twenty-four Canterbury rivers suggest a similar upper threshold of 0.01 mg L^{-1} DRP for high biomass, but no lower limit (McAllister 2014; Figure 19). Canterbury rivers show an upper threshold for DIN (mostly nitrate) concentrations of 2.0 mg L^{-1}, above which blooms are not seen, though no lower limit is evident (Figure 19). Wood *et al.* (2014) did not describe an upper limit for DIN, though inspection of their data and our analysis of the 60 sites in the Manawatu region suggest that blooms were rare above 1 mg L^{-1}. Wood *et al.* (2014) identify a lower limit for DIN of greater than 0.2 mg L^{-1} for bloom formation (with one exception; Figure 17) a trend also apparent in the 60-site analysis (Figure 18), but not in the Canterbury data.

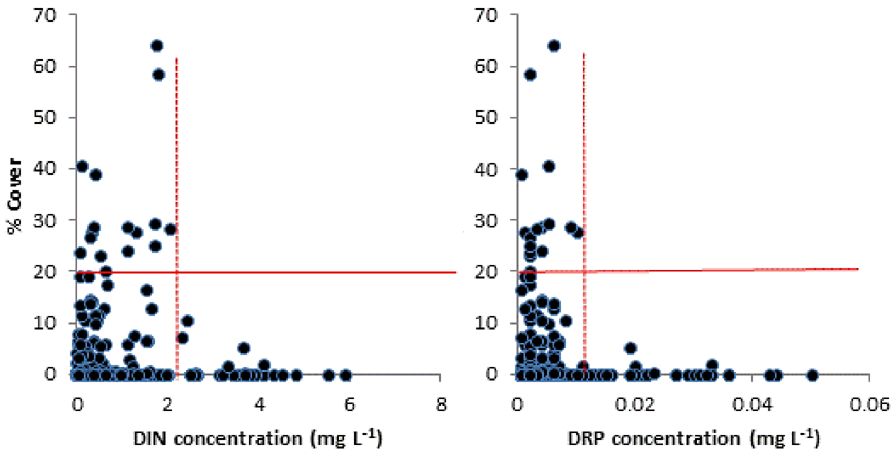

Figure 19. Relationships between dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP) concentrations and percent cover of *Microcoleus* in approximately 24 samples from each of 24 Canterbury rivers. The horizontal red line indicates the bloom threshold of 20% cover, and the dotted vertical line is the threshold value above which blooms do not occur. Data are courtesy of Environment Canterbury, and plot synchronous measurements of nutrients and cover rather than nutrient concentration over the accrual period prior.

Analysis of two summers of monitoring data from seven sites in the Maitai River (Nelson) again shows the capacity of blooms to occur at low DRP (< 0.01 mg L^{-1}). However, in common with the Canterbury data, blooms were present when DIN was below the apparent Manawatu-Whanganui lower threshold of 0.2 mg L^{-1} (Wood *et al.* 2014, 2015; Figure 20). Analysis of the Maitai River showed that a reduction in DIN was not associated with a reduction in *Microcoleus* cover. Additionally, despite prolonged stable flows following a detachment event, *Microcoleus*-dominated mats did not re-establish at sites where blooms previously persisted (Figure 20). The authors suggest that monitoring did not start early enough to capture elevated DIN concentrations in the river during the initial stages of mat establishment, and once established within-mat nutrient cycling occurs, and thus the mats can persist in lower than predicted DIN concentrations.

The synthesis of these field-based studies suggests that *Microcoleus* blooms can occur, or at least initiate, at low ambient concentrations of both DRP and DIN. The ability to accumulate biomass at low ambient nutrient concentrations is a mat-like trait, in that mats tend to accumulate nutrients as they grow, and retain them within the mat matrix (Bonilla *et al.* 2005; Quesada *et al.* 2008). For *Microcoleus* mats, as they achieve size and thickness, the probability increases that mat-like traits will emerge, that river concentrations of nutrients will be increasingly different to those inside the mats (Wood *et al.* 2015), and that independence from river supply may increase. In such cases as shown in Figure 20, it may be possible that initiation of mat growth during the later accrual periods may be prevented by insufficient nutrient flux, where mature mats may have been able to persist and grow.

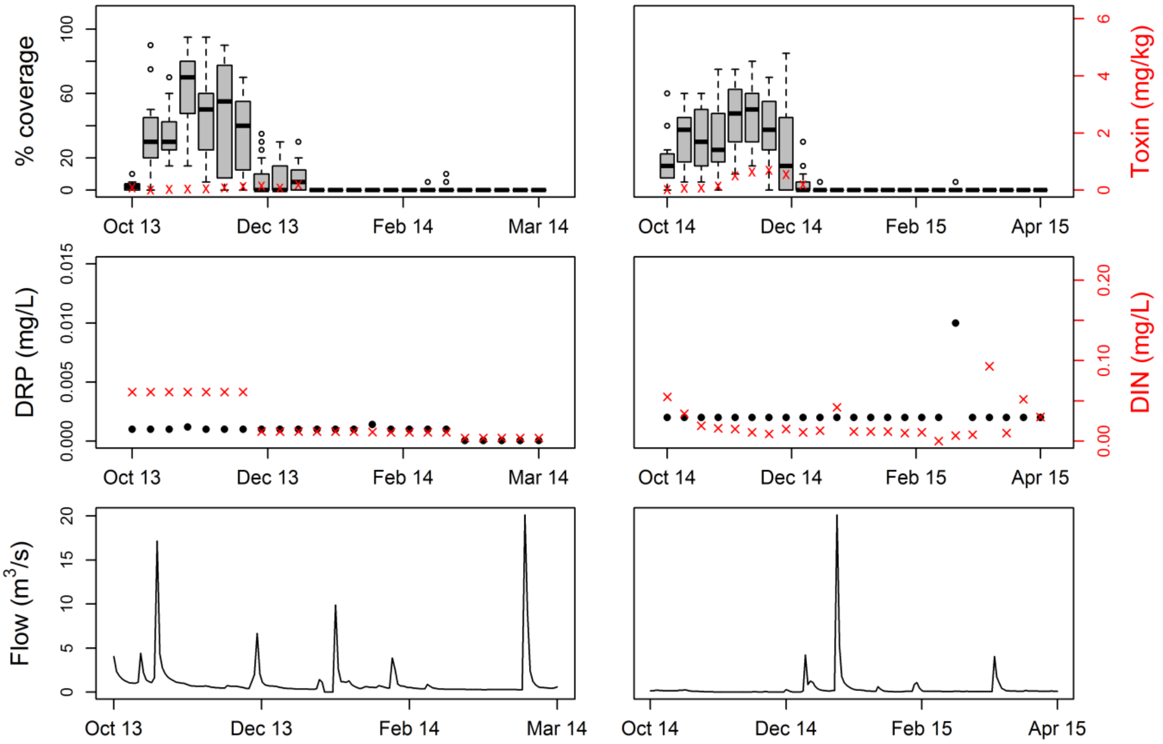

Figure 20. Relationships between *Microcoleus* abundance, dissolved inorganic nitrogen (DIN) and flow at the site downstream of Dennes Hole (Maitai River, Nelson). Top panel = *Microcoleus* percentage cover and total anatoxin concentration. Solid black line shows median, box shows 1st and 3rd quartiles, whiskers extend to the last data point within 1.5 times the inter-quartile range. Open circles are outliers beyond this range. Middle panel = dissolved reactive phosphorus (DRP), and dissolved inorganic nitrogen (= nitrite + nitrate + ammonium; DIN). In 2013/14 DRP was only sampled monthly and this value used for each weekly sampling point. Bottom panel = daily mean river flow. Reproduced from Wood *et al*. (2015).

A role for fine sediments?

A feature of most *Microcoleus*-dominated mats is a thin layer of fine sediment at the substrate/mat interface (Figure 21). Fine-grained sediment particles that are washed across the mat surface stick to the EPS and are incorporated into the mat matrix (Frantz *et al.* 2015). *Microcoleus* filaments are very motile, and likely use this to stay above the trapped particles and thus fine sediments are gradually moved to the lower mat matrix (Figure 15). Recent research has shown that biogeochemical conditions inside natural *Microcoleus*-dominated mats can be very different to the outside water column, including the development of high pH (> 9) during the day (due to photosynthetic depletion of bicarbonate) and low oxygen (< 4 mg L^{-1}) concentrations at night (due to respiration; Wood *et al.* 2015). Such conditions are conducive to the release of DRP loosely bound to sediments trapped in the mat matrix, and these authors suggest that an internal source of DRP is one of the reasons *Microcoleus*-dominated mats can reach high biomass when water column DRP is low. They provide further support for this suggestion through the detection of 320-fold higher DRP concentrations in water trapped within the mucilaginous mat matrix compared to overlying river water. At this stage of the accrual cycle it is likely that the net flux of DRP is out of the mats (Figure 15), and water column nutrient concentrations may be of little relevance to biomass accrual.

Figure 21. Photograph showing the layer of sediment at the *Microcoleus*-dominant mat/substrate interface. Photo: T. McAllister (University of Canterbury).

If sediment provides a source of phosphorus for *Microcoleus*-dominated mats a relationship between increased sedimentation rates and their prevalence might be expected. Wood *et al.* (2015) used sediment traps to investigate this at three sites on the Mangatainoka River (Manawatu-Whanganui) with and without *Microcoleus* proliferations. Deposition of fine sediment (< 63 µm, the dominant sediment size observed within *Microcoleus* mats) was similar among sites. However, sequential extraction of phosphorus from trapped sediment found that biological available phosphorus concentrations were two- to four-fold higher at sites with proliferations. Metallic oxide-bound phosphorus, which can be released by high pH, was the most abundant form of phosphorus. Wood *et al.* (2015) used a similar approach on the Maitai River, and found both higher sedimentation rates and biologically available phosphorus at sites with blooms compared to a control site in the upper catchment. Collectively these studies suggest suspended fine sediment, provided it contains biologically available phosphorus, is an important factor in promoting *Microcoleus* blooms once the mat stage is reached.

To date, in-depth investigations into nitrogen dynamics within *Microcoleus*-dominated mats have not been undertaken in New Zealand. Molecular techniques have shown that cultures of *Microcoleus* isolated from New Zealand rivers do not contain key enzymes required to fix nitrogen (Heath 2014), however, preliminary data indicate that nitrogen-fixing bacteria are present within mats (Wood unpubl. data; ongoing research). These may contribute to nitrogen cycling within the mat matrix, as has been demonstrated in other locations where mats prevail (Pinckney & Pearl 1997).

Grazing

Grazing is a final consideration in periphyton accrual dynamics. Large losses in biomass can result from consumption by grazers, with extent dependent on herbivore type and density, and periphyton type and successional stage (Steinman 1996; Holomuzki *et al*. 2010).

Holomuzki and Biggs (2006) and Holomuzki *et al*. (2006) studied three common New Zealand algivores (*Potamopyrgus antipodarum* [mud snail], *Deleatidium* spp. [leptophlebiid mayfly], *and Pycnocentrodes aeris* [caddisfly]). Although their studies do not specifically mention *Microcoleus*, they show stark differences between grazed and ungrazed treatments, varying with growth phase and dominant algal taxa. To our knowledge, no studies have specifically investigated the impact of invertebrate grazing on *Microcoleus*-dominated mats. However, *P. antipodarum,* known to significantly reduce algal biomass, has been shown to have a dominant presence in *Microcoleus* mats (Hart *et al*. 2013). Wood *et al.* (2014) investigated macroinvertebrates associated with *Microcoleus* mats in four rivers: Ashley (Christchurch), Maitai (Nelson), Pukuratahi and Hutt (Wellington). We compared their data with information on functional feeding types for New Zealand macroinvertebrates (Winterbourn *et al.* 1984; Chadderton 1988; Quinn & Hickey 1990; Lester *et al.* 1994; Jaarsma *et al.* 1998). *Deleatidium*, *Pycnocentrodes*, *Polypedilum/*Orthocladiinae and the fly larvae *Maoridiamesa* were abundant within the *Microcoleus*-dominated mats and these all have the potential to influence algal growth through their grazing or browsing. The presence and abundance of these varied among study rivers and further research is required to determine their impact on *Microcoleus* biomass.

Grazing macroinvertebrates may also have indirect effects on *Microcoleus* growth. The long filamentous morphology of *Microcoleus* may make it less palatable than diatoms or green algae (Scott & Marcarelli 2012). Preferential consumption of diatoms or green algae may open up new space for *Microcoleus* colonisation. Conversely, Hart (1985) describes how a caddisfly larva (*Leucotrichia pictipes*) removed, but did not ingest, filaments of *Microcoleus* (a cyanobacterium with a very similar morphology to *Microcoleus*), which in turn facilitated growth of preferred food items.

Toxins produced by *Microcoleus* (see Section 5), may act as a chemical defence reducing the impact of grazers. Recent studies in New Zealand indicate high toxicity of aqueous *Microcoleus* extracts to the mayfly *Deleatidium* (Bridge 2013; Champeau *et al.* 2013), in apparent contradiction to observations described above of this taxon occurring in mats. Hepatotoxic and neurotoxic effects on mayflies, stoneflies and caddisflies were also observed during palatability/toxicity tests with benthic cyanobacteria sourced from Spanish streams (Aboal *et al.* 2000, 2002). In contrast to these findings, purified saxitoxins and extracts from the saxitoxin-producing benthic cyanobacterium *Lyngbya wollei*, have been shown to stimulate feeding in the omnivorous amphipod, *Hyalella azteca* (Camacho & Thacker 2006).

Collectively these studies suggest grazers may facilitate and/or inhibit *Microcoleus* colonisation and growth, and this likely varies with habitat, geographic location or successional stage. Further research is clearly required.

Other parameters identified as possibly influencing *Microcoleus* accrual

Analysis of a relatively limited number of samples for elemental compositions from two separate studies suggested a relationship between sodium and potassium, and the propensity of a site to experience *Microcoleus* blooms (Wood *et al.* 2015; McAllister 2015). However, the concentrations of no specific elements from a wide suite of macro and trace elements by Inductively Coupled Plasma – Mass Spectroscopy were correlated with *Microcoleus* biomass (Table 6). Of the individual elements in Table 6, iron is the closest to achieving significance and, using a culture-based approach, Smith *et al.* (2013) showed that low (40 µg L^{-1}) and high (4000 µg L^{-1}) iron concentrations inhibited *Microcoleus* growth. Additionally at high iron concentrations the cultures no longer adhered to the culture container walls.

Table 6. Summary of the results of linear regression between various elements/ratios and the maximum *Microcoleus* cover at each of eight Canterbury rivers. * indicates a statistically significant correlation ($P \leq 0.05$).

Element or ratio	**R^2**	**P-value**
Sodium:Potassium	0.51	0.05*
Iron	0.4	0.09
Magnesium	0.29	0.16
Sodium	0.28	0.17
Calcium	0.28	0.17
Copper	0.1	0.44
Dissolved inorganic carbon	0.09	0.48
Zinc	0.07	0.51
Chromium	0.05	0.60
Cobalt	0.04	0.62
Arsenic	0.04	0.64
Potassium	0.031	0.67
Nickel	0.02	0.75
Aluminium	0.018	0.75
Manganese	0.01	0.80
Vanadium	0.01	0.80
Lead	0.004	0.87

8.1.3. Resetting/dispersal

Re-setting primarily occurs due to shear stress exceeding the attachment tenacity of periphyton, or through substrate disturbance (Figure 15). Both flow velocity and the concentration of entrained suspended sediment can impact the degree of abrasion, though the incremental effect of suspended sediment varies between periphyton types. For tightly adhering growth forms, such as *Microcoleus* mats, Francoeur and Biggs (2006) found that flow velocity alone was important, and that adding turbidity to flow had little further impact. Field observations have been used to relate the flood frequency in New Zealand streams to periphyton biomass, and Clausen and Biggs (1997) found that high median flows and high frequency of flows greater than 3× median both tended to reduce long term average periphyton biomass[8]. These observations were, however, not species resolved, and it has been shown that *Microcoleus* mats often tolerate higher flows than other periphyton classes (Hart *et al.* 2013). Heath *et al.* (2015) showed from an intensive field survey that the best predictor for *Microcoleus* presence/absence was stable substrates unlikely to be

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[8] It has become something of a New Zealand myth, derived from this study, that a 3x median flow is a 'resetting flow'. What Clausen and Biggs (1997) actually showed was a statistical correlation between the frequency of floods of greater than 3x median flow at a location and a long-term geometric mean and maximum, of periphyton biomass as chlorophyll-*a* and ash-free dry mass, also species richness and species diversity.

disturbed by flood flows. Heath *et al*. (2010) went further to show that, whilst mats tended to initiate in riffles, with their faster flows, once established they can spread to cover all available substrate across a wide range of velocities.

More recently, studies have specifically focussed on flows that reset *Microcoleus* mats, and a nuanced picture emerges. Differences in the effect of flow on detachment appear to exist between rivers and among floods in the same river. Multiple river studies in both Canterbury (McAllister 2014) and Manawatu-Whanganui region (Wood *et al.* 2014) have consistently shown that the magnitude of flow increase required to remove *Microcoleus* mats is different between rivers, and not a uniform 3× median. Wood *et al.* (2014) used a quantile regression approach to model a '*Microcoleus* flushing flow' for each river as the multiple of median flow. This approach predicts the median flow (at a given percentile) at which measurements of cover will be below 20%. Their analysis highlighted marked differences in *Microcoleus* flushing flows among rivers. For example, the Mangatainoka River at the State Highway 2 sites required a *Microcoleus* flushing flow event 5.36 times the median flow to reduce *Microcoleus* cover below 20% (using a 90th percentile). In contrast a flow of 16.5 times median flow was predicted to be required to reduce *Microcoleus* cover below 20% at Tokomaru River at the Horseshoe Bend site (85th percentile; Figure 22). This empirical approach may be useful in establishing re-setting flow requirements for specific rivers, and if data are assembled from many rivers it is possible that a generalisable pattern will emerge.

It has also been reported that loss of *Microcoleus*-dominated mats can occur when flows decline (McAllister 2015). This may be partly related to gas bubble formation within mats, due to local oxygen supersaturation during rapid photosynthesis creating buoyancy and increasing the risk of exfoliation (Biggs & Thomsen 1995, Boulêtreau *et al.* 2006, Quiblier *et al.* 2013; Figure 15). Gas bubble formation within the mat matrix is more likely under low flow regimes, since diffusion of oxygen out of the mat will be slowed by the existence of a thick boundary layer (cf. Hawes *et al.* 2014). Boundary layers limit both influx and efflux of materials, and are thicker under slow, laminar flows. Thus *Microcoleus* may be expected to perform best in a moderate flow regime, another example of a hump-back curve, creating flows that are sufficient to enhance nutrient and gas flux during growth in oligotrophic waters, yet insufficient to cause exfoliation of mature mats.

Wood and Young (2012) also suggest that different strength flows may be required to remove mats at various stages of the accrual cycle (Figure 15). During the initial attachment and growth phase *Microcoleus* filaments are adhered relatively tightly to the substrate, and thus a greater flow may be required to remove the mats. Conversely as mats increase in size and drag increases, they may be increasingly vulnerable to abrasion by flows.

This shift in vulnerability to high and low flows over time complicates relationships between flow and bloom formation, but can be accommodated with the arguments developed by Biggs *et al.* (1998), as a stress-subsidy model of the effect of flow on periphyton growth. Their studies did not address *Microcoleus* specifically, but they found that the subsidy effect, and boundary layer flux limitation, was dominant in well-attached, conforming communities (such as early stage *Microcoleus*), and the stress effect most prevalent for thicker and trailing filamentous ones (also see Hart *et al.* 2014).

8.2. What existing models describe these relationships?

8.2.1. Introduction

This section focuses on how relationships between environmental drivers and the abundance of periphyton, and *Microcoleus*-dominated mats in particular, have been used to develop predictive models. For New Zealand rivers this work is in its early stages, and revolves around statistical correlations that are developing as the body of work on which they are based increases. In this section we show the potential for approaches that use the River Environmental Classification (REC, Ministry of the Environment 2010) scheme to produce broad scale models of the susceptibility of rivers to *Microcoleus* growth. Finally we introduce the current status of mechanistic models that provide more specific predictions of periphyton dynamics—but require much more input information and have not yet been developed for *Microcoleus*.

8.2.2. Statistical approaches and river classification

Models describing the relationships between *Microcoleus*-dominated mat development and environmental drivers have mostly been statistical. This refers to the correlation of information on environmental conditions (independent variables) and *Microcoleus* cover (dependent variable) in rivers. Such relationships are correlations, and not necessarily causative relationships, and thus need to be interpreted with care. To acquire a degree of robustness, such statistical models need to be developed from training sets, then applied to independent test data sets before they can become generally accepted. Ideally, experimental testing of putative causal relationships is then carried out, though to date this level of evidence is not available. Once a robust relationship is established, the advantage is that interpolations, and cautious extrapolations, can be made to wherever suitable independent variable data are available or can be assembled.

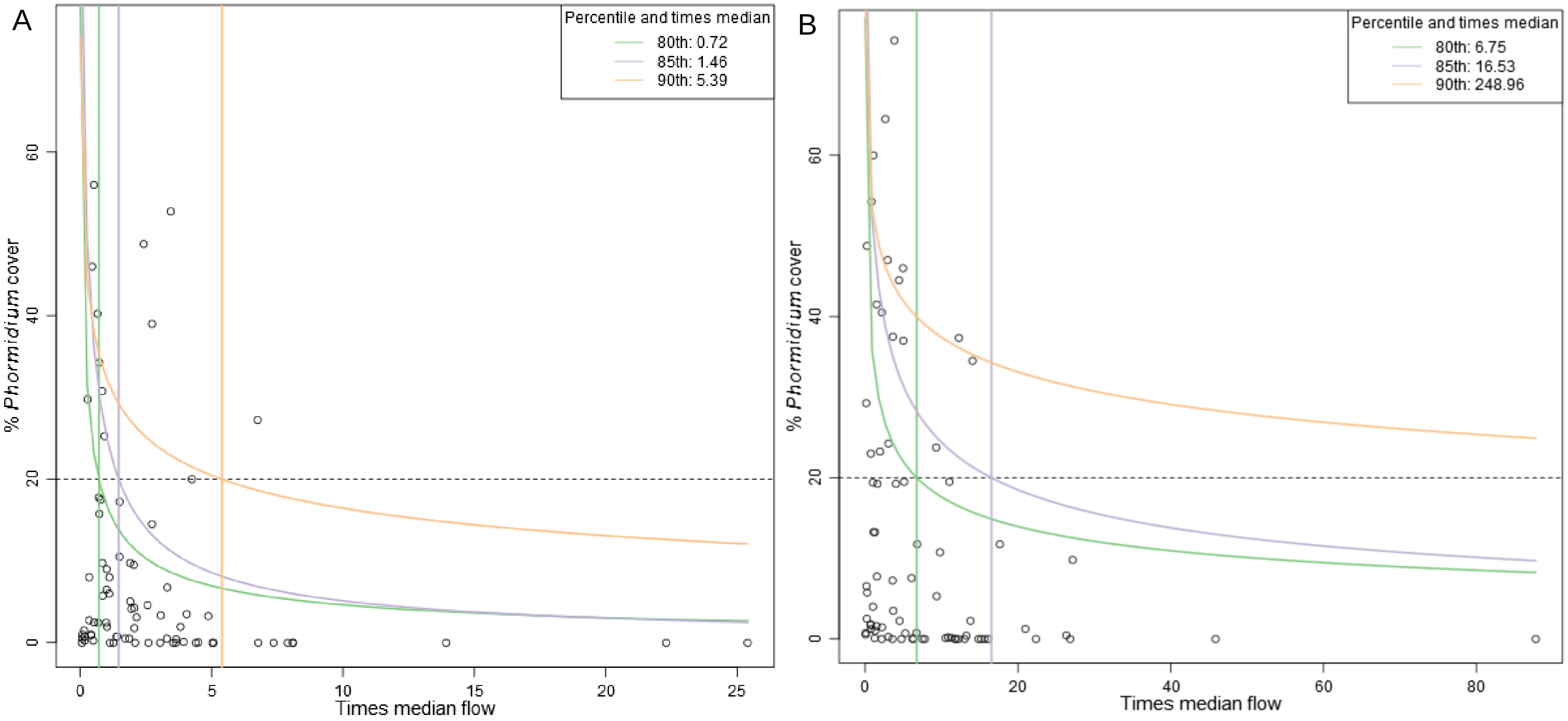

Figure 22. Mean *Microcoleus* mat cover and magnitude of maximum daily flow above long-term median river flow on the day of sampling or during the six days prior derived by 0.80, 0.85 or 0.90 quantile regression for (A) Mangatainoka River at State Highway Two, and (B) Tokomaru River at Horseshoe Bend (modified from Wood *et al.* 2014)

The statistical basis for modelling of *Microcoleus* blooms is based on the types of relationship described in Section 8.1. These have developed thresholds above or below which a bloom may be possible or likely. Based on the information summarised in 8.1, we would expect habitat suitable for *Microcoleus* blooms to be characterised as being of low to moderate nutrient enrichment, with stable substrate, moderate flow, and low to moderate levels of disturbance. It is possible that the degree of resolution could be improved by a national initiative to collate all available data (which is often of very mixed scope, collected in different ways and hard to combine without considerable manipulation).

Heath *et al.* (2015) attempted to develop a more discriminatory habitat-preference model based on the relationships between *Microcoleus* mat development and flow, substrate and depth in the Hutt River. This approach, while still statistical in nature, involved developing a habitat suitability curve for *Microcoleus*, which relates a metric of occurrence to specific habitat features. More than 650 data points were collected from seven sites. A clear preference for blooms to initiate and develop on stable substrates was identified, and upper velocity limits to high growth were proposed that were substrate-specific, that is the upper velocity limit increased with increasing substrate stability. No suitability curve could be found for depth, and there was a very wide tolerance of velocity. Data were limited to one time point (following a stable period when growth conditions were optimal), which limits wide-scale application of this model (Heath *et al.* 2015).

New Zealand is well suited to the geographic extrapolation of robust statistical models because of the existence of the River Environments Classification (REC). The REC is a Geographic Information System (GIS)-based tool that uses a hierarchy of environmental factors to generate groupings of river types, specifically targeting factors that can be expressed as geographic overlays, and which are believed to be predominant influences on ecological properties of rivers (Snelder & Hughey 2005). An example of how statistical models of periphyton performance can be linked to the REC to provide broad-scale predictions is given by Snelder *et al.* (2004). The REC was able to produce a statistically significant prediction of the flood frequency of rivers based on climate and topography. The statistical relationships between maximum periphyton biomass, accrual time and nutrient concentration described in Section 8.1 above (see Biggs 2000) allowed target upper nutrient concentrations for each of a series of river classes to be developed that should prevent specific periphyton biomass targets being exceeded.

Statistical/REC modelling has both enormous potential and serious drawbacks. Problems include:

- the amount of input data required. In the application of Snelder *et al.* (2004), the limited volume of data meant that all data were used for training the model. This left no independent dataset to determine how robust the statistical relationships were.

- uncertainties in the relationships between both REC class and flood frequency, and between nutrient concentration and periphyton biomass, which tend to be lost in this type of analysis.

Because of these issues, while a general picture of the ecology of river classes can be built up, statistical/REC models are never able to provide site-specific resolution. By incorporating uncertainty, however, this type of relationship can be used to develop an indicator of risk rather than an indication of central tendency.

To date no attempt has been made, to our knowledge, to specifically identify environmental drivers of *Microcoleus* blooms within the REC to develop a risk framework. Given that there appears to be a link between blooms and substrate stability, temperature, flow, accrual time and nutrient thresholds, such an exercise does offer some potential to determine the river classes which have the greatest risk of *Microcoleus* blooms. As discussed above, simple single-variable linear relationships are unlikely, due to the shifts associated with stage of mat development and non-linear responses to flow and nutrient concentrations. Possible non-linear relationships that emerged in Section 8.1 may be applicable to modelling risk of bloom formation, for example, the apparent lower/upper thresholds for DIN and DRP. A more in-depth analysis across a national dataset of requirements including critical upper and lower flows and flow constancy may also yield usable relationships.

8.2.3. Mechanistic models

In the field of planktonic algal blooms, the ongoing development of ecosystem models of lakes promises opportunities for an improved prediction capacity (Oliver *et al.* 2012). The goal with such models is to develop a predictive capacity that is based on underlying mechanisms rather than statistical relationships. Rivers are more difficult to model than lakes, due to open boundaries and greater structural complexity, but some mechanistic models do exist that may help predict *Microcoleus* blooms.

A recent example of a mechanistic river model is provided by Graba *et al.* (2014) and validated using the Garonne River (France). The model was made by modifying a series of other models to build up an increasing complexity (Uehlinger *et al.* 1996; Boulêtreau *et al.* 2006), and this iteration is designed to capture complete accrual/detachment cycles. It incorporates:

- a growth component that includes temperature, irradiance, a minimum biomass for recovery, and an asymptotic maximum mat thickness. No nutrient terms are explicit in growth, which is scaled to the river under study
- a flow-related detachment term, in two parts, the chronic and catastrophic detachment

- autogenic detachment, the self-generated detachment that occurs when mats become too loosely attached and buoyant
- a grazing component.

A comprehensive model that can reproduce accrual cycles in a river presently requires an empirical fitting approach to derive river-specific parameters for each sub-component of the model. It thus requires a large amount of baseline data on the river, after which the model can reproduce measured cycles, while migration to other rivers requires a similar mass of data. At present the prospect of a mechanistic model that can be fitted to rivers without a substantial research input, and therefore be used for broad-scale bloom prediction, is distant. However, the ability of the Garonne model to reproduce observed data implies that the mechanisms incorporated in the model do capture most of the important aspects of the accrual cycle. They thus point towards those areas of *Microcoleus* ecology that will be most fruitful for research in New Zealand targeting broad-scale assessments. These include a better understanding of hydraulics and bed movement, growth rates, non-catastrophic and autogenic detachment, and the impacts of grazers.

8.3. How does *Microcoleus* abundance change in response to catchment management and resultant changes to environmental drivers?

This section focuses on the extent to which we can identify how activities in catchments cause environmental effects that enhance *Microcoleus* blooms. Research on environmental drivers of *Microcoleus* proliferations is ongoing, and substantial knowledge gaps exist (Section 8.1). The failure to identify key factors that promote blooms, despite some considerable research, is not unusual in periphyton science where systems are responding to multiple dynamic variables. It does, however, make it challenging to definitively associate changes in catchment management and land use with *Microcoleus* proliferations.

When considering the consequences of environmental change on a system property, in the absence of perfect understanding of the driving relationships between them, Sutherland (2006) suggests reversing our thinking from traditional mechanistic approaches to ‘outcome-driven’ assessment. For example, while it may be difficult to determine ‘how does a specific environmental change lead to ecosystem responses’, it may be more fruitful to consider whether it is possible that an observed ecosystem response may be related to a known environmental change. Given our current level of understanding, a combination of these two approaches may be suitable for *Microcoleus*.

The rivers with observed *Microcoleus* issues are primarily non-alpine rivers on the lower-lying parts of the dry, eastern side of New Zealand (Figure 23). These are also often areas with shallow aquifers that are part of an increasingly allocated water supply, often used to support intensive agriculture. Outcome-driven thinking would require that we consider possible ways in which these common features could promote *Microcoleus* growth. Based on our current understanding, the most likely processes are:

- water abstraction (both direct and indirect via groundwater abstraction) and flow modification. These can affect median flow, velocity and flood frequency
- run-off of nutrients, fine sediments and other contaminants (*i.e.* herbicides, hormones, pesticides) from intensive agriculture, and to a lesser extent forestry and urban development
- habitat modification including; changes in riparian zones, and channel modification through removal of gravel for construction or flood protection.

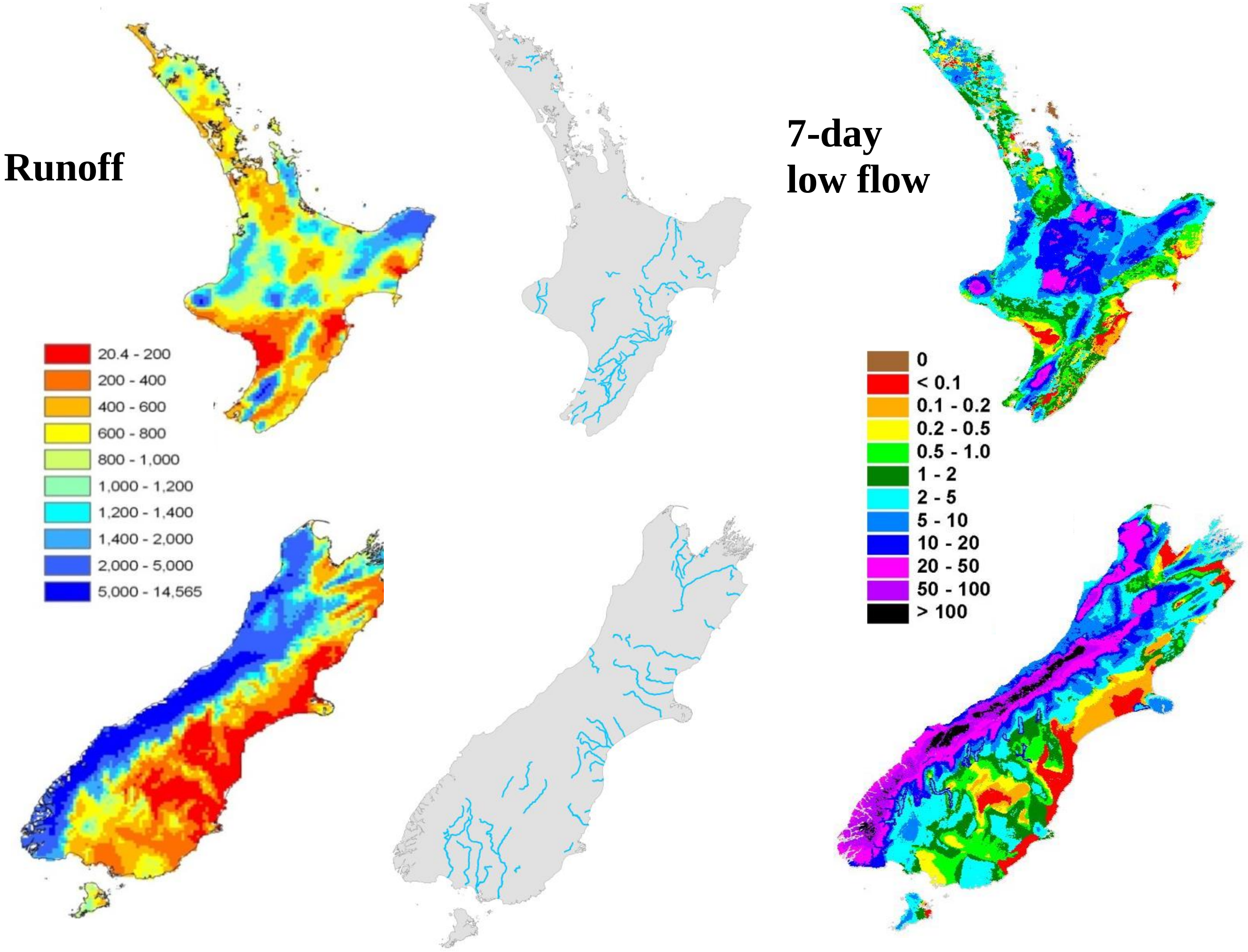

Figure 23. Comparison of the location of mean annual runoff (left hand colour images, scale is in mm year^{-1}), known *Microcoleus*-prone rivers (centre image, modified from Figure 6), and mean seven day low flow (right hand colour image, scale is in L s^{-1} km^{-2}). Runoff and low flow data are from NIWA.

8.3.1. Water abstraction and flow modification

Demand for water in eastern New Zealand is increasing, particularly during summer. Direct removal of surface water reduces river flows, and abstraction of groundwater may affect the interaction between river flow and groundwater storage which may further reduce flows. Long term records of flow in lowland rivers that are frequent sites of *Microcoleus* blooms are limited, though in Canterbury there is clear evidence of a gradual decline in median summer flows in both the Hurunui and lower Selwyn rivers (Figure 24). This pattern is not repeated in all Canterbury lowland rivers though for most rivers relatively short flow records are available that make trend recognition difficult. Another Canterbury river, the Ashley River, does have a similarly long record, though there the picture of change is different, with a downwards trend in median flows which is not statistically significantly (data not shown). There is however a significant downwards trend in 90th percentile flows, suggesting that the intensity of flood flows is declining. No decline in 90th percentile flows was evident in the rivers shown in Figure 24.

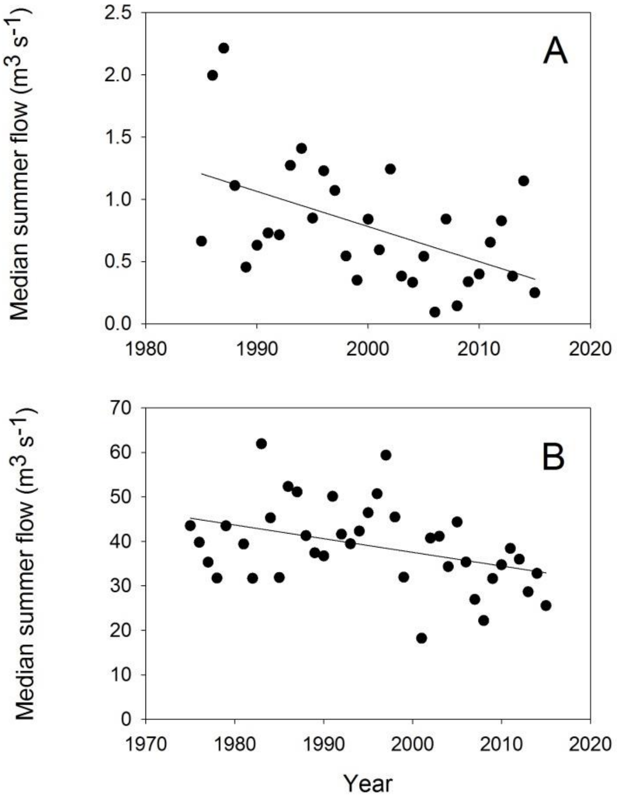

Figure 24. Median summer (December-April) flow over time in (A) the Selwyn River (at Coe's Ford) and, (B) the Hurunui River (at State Highway 1). In each case the linear regression line is significant at $p < 0.05$. Data from Environment Canterbury.

Flows in the Selwyn River were analysed in detail by McKerchar and Schmidt (2007) who concluded that, while recent years of limited rainfall have contributed to low flows, irrigation withdrawals have exacerbated the effect. Elsewhere, low rainfall over the east of New Zealand in recent decades have had a greater contribution to reductions in river flows than abstraction, and climate variability appears to have reduced high flows, for reasons as yet unknown (McKerchar *et al.* 2010). Could increased *Microcoleus* growth be in response to a general reduction in median and high flows in East Coast rivers? The relationship with reduced flow and *Microcoleus* blooms is imperfectly understood. Heath *et al.* (2015) showed that *Microcoleus* has a wide tolerance of flow, and Hart *et al.* (2013) showed it tolerates higher stream velocities than other periphyton forms (see Section 8.1.3). However, this review has shown how *Microcoleus* does not have a unimodal response to flow variables, with different phases of the accrual cycle responding differently (Section 8.1). It may be that any tendency towards an accumulation of a greater numbers of refuge populations within rivers, through extended accrual periods and reduced disturbance, may contribute to enhanced accrual in following cycles. Further research is required to improve knowledge on the long-term relationships between river flow and *Microcoleus* accrual cycles.

Flow-regulated rivers have some form of control structure such as a dam, weir or a flow-controlling lake. These rivers tend to have relatively stable flow regimes, providing longer accrual periods which generally promote periphyton proliferations (Section 8.1.1). Flow regulated rivers have been linked to blooms of some benthic algae, such as *Didymosphenia geminata* (Kirkwood *et al.* 2009). However, while not yet formally validated, it appears there is no causative link between flow-regulated rivers and enhanced *Microcoleus* abundance, with most blooms occurring in unregulated rivers during prolonged low flows.

At present linkages between flow dynamics, managed or natural, and proliferations of *Microcoleus* have not been examined in a national context or in ways that will allow broad-scale inference, for example via the REC systems suggested in Section 8.2. Establishment of clear links could, however, provide the opportunity to develop an enhanced, regionally weighted mechanism for predicting *Microcoleus* blooms, and therefore managing risk.

8.3.2. Nutrients, sediment and contaminant run off

Nutrient enrichment is commonly associated with periphyton reaching nuisance proportions (see Section 8.1; Biggs 2000), and the attributes and threshold values developed for managing periphyton in New Zealand rivers reflect this issue. Intensification of agriculture can result in increased flux of nutrients to waterways (Abel *et al.* 2011) and historically there has been a tendency to suggest that nutrient enrichment leads to periphyton proliferation—and to include *Microcoleus* blooms in this generalisation. However, information assembled in this report appears to suggest that neither elevated nitrogen nor phosphorus play a major role in promoting *Microcoleus* blooms, though they may assist during the early colonisation period (Section 8.1). The current working hypothesis is that only slightly elevated water column DIN concentrations are required for *Microcoleus* growth, and that proliferation requires little, if any, increase in dissolved phosphorus (Section 8.1).

While the catchments of many areas identified as supporting *Microcoleus* (Figure 23) contain intensive agriculture with its associated high nutrient run-off, blooms also occur in areas dominated by forestry and in urban situations. Cooper and Thompsen (1988) compared nitrogen flux from pastoral, pine and native forest catchments, and found that on an areal basis, pasture catchments exported about 3 and 10 times more nitrogen than native and pine catchments respectively. The observation that blooms can occur in low nutrient-yielding forested catchments supports the view that high nutrient export may not be a prerequisite. Of significance here is the potential for mat morphologies to harbour nitrogen cycling and fixation pathways (Section 8.1), and for a gradual accumulation of nitrogen from river water passing over the mat surface. A nationwide analysis of the relationship between catchment land use, river nutrient status and prevalence of *Microcoleus* blooms is lacking, but if undertaken could begin to address the question of the relative importance of nutrients, and thus whether a reduction in blooms could be achieved by managing river nutrients.

A role for groundwater?

A feature of Figure 23 is that many of the rivers where *Microcoleus* is a problem are in areas with shallow groundwater aquifers. Interactions between groundwater and river run-off may thus play an important role. Rivers can either gain or lose to groundwater, depending on the relationship between river level and the groundwater table, and this balance can vary along the length of a river. Canterbury rivers, such as the Selwyn River, are often losing to groundwater in their upper reaches, but in the lower catchment the groundwater table rises towards the land surface , resulting in groundwater recharging river flows. Our observations suggest that *Microcoleus* blooms commonly occur where there are groundwater seeps or in gaining reaches. In New Zealand nitrate concentrations are commonly elevated in groundwater, particularly in shallow well-oxygenated sources, and discussion of groundwater interactions with natural waterbodies often focus on nitrate enrichment. Nationally about 20% of monitored groundwater sites have experienced significant increases in nitrate levels between 1995 and 2008 (Daughney & Randall 2009). However, any conclusion that nitrate in groundwater is important in driving *Microcoleus* blooms may be premature, and it may be that another component of groundwater, or the presence of a supplemental water source itself in these otherwise arid catchments, is important. Further research is required to determine whether *Microcoleus* blooms are more frequent in rivers where groundwater supplements flows.

Phosphorus and sediment inputs

As well as low DIN requirements, the current understanding of the relationship between DRP and *Microcoleus* indicates that rivers with low concentrations (< 0.01 mg L^{-1}) are susceptible to blooms, and even as phosphorus approaches limits of analytical detection, blooms are still possible (Section 8.1). This again suggests that enhanced nutrient flux as a result of agriculture may have a limited role in *Microcoleus* blooms, though this may relate to the ability of *Microcoleus* mats to access sediment-bound phosphorus (Section 8.1.4). As discussed above, *Microcoleus* blooms tend to occur in areas where the land use is agricultural or forestry, and there are links between agricultural land use, especially pasture-based dairying, and increased sediment-bound phosphorus run-off into waterways (McDowell *et al.* 2009). Fine sediment generally enters rivers during rainfall events via surface runoff (Sharpley *et al.* 2008). Runoff from forests and non-cultivated soils usually carries little sediment, whereas cultivation and stock-related erosion of agricultural land greatly increases erosion, and thus sediment input to waterways (McDowell & Wilcock 2007). Clear-felling of plantation forests also results in high amounts of sediment input to rivers in the years following harvest. The amount of suspended sediment in a river is probably of relevance to regulating *Microcoleus* blooms, although a nationwide analysis of this is lacking. Biologically-available phosphorus in sediment varies and appears integrally linked to bloom propensity, *i.e.* sites with high biological available phosphorus are more likely to have blooms irrespective of sedimentation rates (Wood *et al.* 2015 and Section 8.1.4). The proportion of biologically available phosphorus in sediment is partially associated with catchment geology, but anthropogenic modification of sediments in developed areas through the use of phosphate fertilisers is common.

Organic contaminants

Continued urbanisation, and intensified agriculture and horticulture in catchments can result in augmented run-off or leaching of pesticides, herbicides and other emerging contaminants (ECs). Research suggests cyanobacteria may be more resistant to many such compounds than other algae (Lürling & Roessink 2006, Villeneuve *et al.* 2011), raising the possibility that they gain a competitive edge over other, less tolerant, groups. For example, unlike many other algae, some cyanobacteria not only tolerate glyphosate (*e.g*. Roundup®) at high concentrations, but also can use it as their sole phosphorus source (Forlani *et al.* 2008).

Studies on the impact of other emerging contaminants, such as personal care products (PCPs) and antimicrobials on benthic communities are limited but also suggest significant impacts that favour cyanobacteria in some situations. Drury *et al.* (2013) investigated triclosan (TCS), a broad-spectrum antimicrobial compound found in many everyday PCPs (*e.g.* cleansers, detergents and deodorants). They showed the presence of TCS increased benthic cyanobacteria but caused a die-off of eukaryotic algae. Direct evidence linking increases in *Microcoleus*-dominated mats to pesticides, herbicides and ECs is, however, lacking at present. In the Manawatu region *Microcoleus*-dominated mats are common downstream of sewerage treatment plants where ECs can be expected to be at high concentrations (Hamill 2013; Wood *et al.* 2014). Further research on the impacts of such contaminants on sustaining competitive success of cyanobacteria over other types of algae may be warranted.

8.3.3. Habitat modification

Cobble-bedded rivers, particularly those close to urban areas, are used widely as sources of aggregate material for building and roading. Gravel extraction results in the mobilisation of fine sediment which can occur directly during extraction and from vehicle crossings, or indirectly via vegetation clearance, beach raking and bar skimming. Extraction during low flows and from dry beaches can also have substantial impacts, as these practices result in deposition of large quantities of fine sediment on river margins which are mobilized into the wetted channel following rainfall events (Death *et al.* 2012). Increases in fine sediment loads in rivers may favour *Microcoleus* blooms (Section 8.1.4). While this is not a new practice, and not confined to rivers with *Microcoleus* blooms, it may contribute to the suite of land-use practices that increase sediment loads in rivers.

River engineers may also need to manage gravel extraction to maintain the flood capacity of channels (Kelly *et al.* 2005). In addition to mobilising sediment (discussed above) this may affect *Microcoleus* abundance though increasing flow velocity and bed shear stress. Hart *et al.* (2013) showed *Microcoleus* was dominant over other algae at higher velocities. Channelisation may also expedite flood recession, and a faster recession period may increase accrual periods for periphyton and be partly responsible for apparent increase in *Microcoleus* blooms in some rivers, though evidence to support this is lacking. Analysis of recession characteristics in the Hutt River, which is subjected to on-going flood remediation, showed these had not changed over the period of record (1979 to 2015; Hutt at Taita Gorge site; Greater Wellington Regional Council, unpubl. data), though there has been an apparent increase in *Microcoleus* blooms in the lower river over the last decade.

Practices that reduce the efficacy of riparian zones may directly or indirectly enhance *Microcoleus* abundance. Intact riparian zones:

- exclude livestock and allow tree roots to stabilise river banks, both actions reduce localised sediment inputs (see above regarding role of sediment)
- provide a long-term buffer from hillslope sediment inputs (see above regarding role of sediment)
- reduce water temperatures if sufficient lengths of upstream shade exist. Although *Microcoleus* blooms occur across a wide range of temperature (Section 8.1), lower temperature should reduce growth rates
- increase shade. *Microcoleus* can contain high concentrations of UV-protecting compounds such as mycosporine-like amino acid and scytonemin (George *et al.* 2001). This may confer a competitive advantage at unshaded sites. Preliminary

analysis of a four-year 60 site dataset from the Manawatu region using boosted-regression tree analysis identified shade as the third highest predictor variable (% deviance explained;12.9%) – with unshaded sites favouring *Microcoleus* (Wood & Wagenhoff, unpubl. data).

With present knowledge it is difficult to identify how land use practices and catchment management have led to the perceived proliferation of *Microcoleus* blooms. Natural climate variability may have played a part in this increase, compounding the reduction of flows due to abstraction of water, and increases in DIN, sediments and organic contaminants associated with agricultural, forestry and urban activities. Compilation of national datasets (as suggested in Section 8.2) may provide an opportunity to use the REC, and other spatially resolved tools, to investigate potentially complex relationships among land-use, catchment characteristics and *Microcoleus* abundance. Additionally, larger regional datasets such as the above-mentioned 60 sites in the Manawatu region and a recent 30 week study of eight Canterbury rivers (Ph.D. student Tara McAllister, University of Canterbury) provide an opportunity to apply more sophisticating modeling/predictive approaches, *e.g.* Clapcott & Goodwin (2014). In addition to observational studies that generate hypotheses on causative factors, experimental approaches are needed that mirror pioneering work done on periphyton dynamics in New Zealand in the 1980-90s, but with a clear focus on *Microcoleus* These methods may assist in improving knowledge on relationships between *Microcoleus* abundance, and potential responses to catchment management.

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10. REFERENCES

Abell M, Özkundakci D, Hamilton D, Miller SD 2011. Relationships between land use and nitrogen and phosphorus in New Zealand lakes. Marine and Freshwater Research 62: 162-175.

Aboal M, Puig MA, Ríos H, López-Jiménez E 2000. Relationship between macroinvertebrate diversity and toxicity of Cyanophyceae (Cyanobacteria) in some streams from eastern Spain. Verhandlungen des Internationalen Verein Limnologie 27: 555-559.

Aboal M, Puig MA, Mateo P, Perona E 2002. Implications of cyanophyte toxicity on biological monitoring of calcareous streams in north-east Spain. Journal of Applied Phycology 14: 49-56.

Aracava Y, Swanson KL, Rapoport H, Aronstam RS, Albuquerque EX 1987. Anatoxin-a analog: Loss of nicotinic agonism and gain of antagonism at the acetylcholine-activated channels. Federation Proceedings 46: 861.

Aronstam RS, Witkop B 1981. Anatoxin-a interactions with cholinergic synaptic molecules. Proceedings of the National Academy of Sciences 78: 4639-4643.

Astrachan NB, Archer BG 1981. Simplified monitoring of anatoxin-a by reverse-phase high performance liquid chromatography and the sub-acute effects of anatoxin-a in rats. In: W. W. Carmichael, (ed). The water environment: algal toxins and health. Plenum Press, New York, NY: pp 437-446.

Astrachan NB, Archer BG, Hilbelink DR 1980. Evaluation of the subacute toxicity and teratogenicity of anatoxin-a. Toxicon 18: 684-688.

Azevedo SM, Chernoff N, Falconer IR, Gage M, Hilborn ED, Hooth MJ, Jensen K, MacPhail R, Rogers E, Shaw GR, Stewart I, Fournie JW 2008. Human Health Effects Workgroup report. Advances in Experimental Medical Biology 619:579-606.

Backer L, Landsberg J, Miller M, Keel K, Taylor T 2013. Canine cyanotoxin poisonings in the United States (1920-2012): review of suspected and confirmed cases from three data sources. Toxins 5: 1597-1628.

Beamer P, Key ME, Ferguson AC, Canales RA, Auyeung W, Leckie JO 2008 Quantified activity pattern data from 6 to 27-month-old farmworker children for use in exposure assessment. Environmental Research 108: 239-46.

Beamer PI, Luik CE, Canales RA, Leckie JO 2012. Quantified outdoor micro-activity data for children aged 7-12-years old. Journal of Exposure Analysis and Environmental Epidemiology. 22: 82-92.

Behm D. 2003. Coroner cites algae in teen’s death. Milwaukee Journal Sentinel. September 6 2003.

Bergey EA 2005 How protective are refuges? Quantifying algal protection in rock crevices. Freshwater Biology 50: 1163-1177.

Bhattacharya S, Zhang Q, Carmichael PL, Boekelheide K, Andersen ME 2011. Toxicity testing in the 21st century: defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. PLoS One. 6(6):e20887.

Biggs BJB, Close ME 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biology 22: 209–231.

Biggs BJF 1990. Periphyton communities and their environments in New Zealand rivers. New Zealand Journal of Marine and Freshwater Research 24: 367-386.

Biggs BJF, Thomsen HA 1995. Disturbance of stream periphyton by perturbations in shear stress: time to structural failure and differences in community resistance. Journal of Phycology 32: 233–241.

Biggs BJF, Goring DG, Nikora VI 1998. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology 34: 598–607.

Biggs BJF 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19: 17-31.

Biggs BJF, Kilroy C 2000. Stream periphyton monitoring manual. NIWA: Christchurch. 245 pp.

Biggs B, Smith RA 2002. Taxonomic richness of stream benthic algae: effects of flood disturbance and nutrients. Limnology and Oceanography 47: 1175–1186.

Bonilla S, Villeneuve V, Vincent WF 2005. Benthic and planktonic algal communities in a high arctic lake: pigment structure and contrasting responses to nutrient enrichment. Journal of Phycology 41: 1120–1130.

Boulêtreau S, Garabetian F, Sauvage S, Sánchez-Pérez JM 2006 Assessing the importance of a self-generated detachment process in river biofilm models. Freshwater Biology 51: 901–912.

Brasell K, Heath M, Ryan K, Wood S 2015. Successional change in microbial communities of benthic *Phormidium*-dominated biofilms. Microbial Ecology 69: 254-266.

Bridge B 2013. Establishing toxic thresholds of the cyanobacteria *Phormidium* spp. to the mayfly *Deleatidium* spp. Unpublished Report, University of Birmingham and Cawthron Institute.

Bruneau E, Sutter D, Hume RI, Akaaboune M 2005 Identification of nicotinic acetylcholine receptor recycling and its role in maintaining receptor density at the neuromuscular junction *in vivo*. Journal of Neuroscience 25: 9949-9959.

Calabrese EJ, Stanek EJ 1995 Resolving intertracer inconsistencies in soil ingestion estimation. Environmental Health Perspectives 103: 454-457.

Calabrese EJ, Stanek EJ, Barnes R, Burmaster DE, Callahan BG, Heath JS, Paustenbach, D, Abraham J, Gephart LA 1996. Methodology to estimate the amount and particle size of soil ingested by children: implications for exposure assessment at waste sites. Regulatory Toxicology and Pharmacology 24: 264-268.

Camacho FA, Thacker RW 2006. Amphipod herbivory on the freshwater cyanobacterium *Lyngbya wollei*: chemical stimulants and morphological defences. Limnology and Oceanography 51: 1870-1875.

Carmichael WW, Biggs DF, Gorham PR 1975. Toxicology and pharmacological action of *Anabaena flos-aquae* toxin. Science 187: 542-544.

Carmichael WW, Gorham PR 1978. Anatoxins from clones of *Anabaena flos-aquae* isolated from lakes of western Canada. Mitteilungen Internationale Vereinigung für Theoretische und Angewandte Limnologie 21: 285-295.

Carmichael WW, Gorham PR, Biggs DF 1977. Two laboratory case studies on the oral toxicity to calves of the freshwater cyanophyte (blue-green alga) *Anabaena flos-aquae* NRC-44-1. Canadian Veterinary Journal 18: 71-75.

Carmichael WW, Biggs DF, Peterson MA 1979. Pharmacology of anatoxin-a, produced by the freshwater cyanophyte *Anabaena flos-aquae* NRC-44-1. Toxicon 17: 229-236.

Carmichael WW, Yuan M, Friday CF 2004. Human mortality from accidental ingestion of toxic cyanobacteria – a case re-examined. Poster presentation. 6th International Conference on Toxic Cyanobacteria, Bergen, Norway. June 21-25.

Chadderton, WL 1988. Faunal and chemical characteristics of some Stewart Island streams. New Zealand Natural Sciences 15: 43-50.

Champeau O, Bridge B, Tremblay LA, Wood S 2013. Toxicity of benthic cyanobacteria mats on *Deleatidium* spp. Poster presented at the Society of Environmental Toxicology and Chemistry (SETAC) Conference, Melbourne, Australia.

Chiswell RK, Smith MJ, Norris RL, Eaglesham GK, Shaw GR, Seawright AA, Moore MR 1997. The cyanobacterium, *Cylindrospermopsis raciborskii*, and its related toxin, cylindrospermopsin. Australian Journal of Ecotoxicology. 3: 7-23.

Chorus I, Bartram J. 1999. Toxic cyanobacteria in water: a guide to their public health consequences, Monitoring and Management. London, E & F Spon.

Clapcott J, Goodwin E 2014. Relationships between macroinvertebrate community index and environmental drivers. Prepared for the Ministry of the Environment. Cawthron Report 2507. 23 p.

Clausen B, Biggs B 1997. Relationships between benthic biota and hydrological indices in New Zealand streams. Freshwater Biology 38: 327-342.

Cooksey KE, Wigglesworth-Cooksey B 1983. Adhesion of bacteria and diatoms to surfaces in the sea: a review. Aquatic Microbial Ecology 9: 87-96.

Cooper AB, Thompsen CE 1988. Nitrogen and phosphorus in stream waters from adjacent pasture, pine, and native forest catchments. New Zealand Journal of Marine and Freshwater Research 22: 279-291.

Costa ACS, Swanson KL, Aracava Y, Aronstam RS, Albuquerque EX 1990. Molecular effects of dimethylanatoxin on the peripheral nicotinic acetylcholine receptor. Journal of Pharmacology and Experimental Therapeutics 252: 507-516.

Daly JW 2005. Nicotinic agonists, antagonists, and modulators from natural sources. Cellular and Molecular Neurobiology 25: 513–552

Daughney C, Randall M 2009. National groundwater quality indicators update: state and trends 1995-2008, GNS Science Consultancy Report 2009/145. Prepared for Ministry for the Environment, Wellington, New Zealand. 60 p.

Death AM, Death RG, Death F, Fuller I, Paine S. 2012. Keeping gravel extraction out of the water may not be the best solution for instream ecology. Proceedings of the Inaugural International Rivers Conference. France. http://www.graie.org/ISRivers/actes/pdf2012/2B314-097DEA.pdf

Dennison W, Abal E 1999. Moreton Bay Study: a scientific basis for the healthy waterways campaign. South East Queensland Regional Water Quality Management, Brisbane.

Devlin JP, Edwards OE, Gorham PR, Hunter NR, Pike RK, Stavric B. 1977. Anatoxin-a, a toxic alkaloid from *Anabaena flos-aquae* NRC-44h. Canadian Journal of Chemistry 55: 1367-1371.

Dorevitch S, Panthi S, Huang Y, Li H, Michalek AM, Pratap P, Wroblewski M, Liu L, Scheff PA, Li A 2011. Water ingestion during water recreation. Water Research 45: 2020–2028.

Drury B, Scott J, Rosi-Marshall EJ, Kelly JJ 2013. Triclosan exposure increases triclosan resistance and influences taxonomic composition of benthic bacterial communities. Environmental Science & Technology 47: 8923-8930.

Dufour AP, Evans O, Behymer TD, Cantú R 2006. Water ingestion during swimming activities in a pool: a pilot study. Journal of Water and Health 4: 425–430.

Dungworth DL 1993. The respiratory system. In: Jubb, K.V.F., Kennedy, P.C., Palmer N. (Eds.), Pathology of Domestic Animals, 4th ed., vol. 2, Academic Press, New York, USA, pp. 626-628.

Edwards C, Beattie KA, Scrimgeour CM, Codd GA 1992. Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. Toxicon 30: 1165-1175.

enHealth 2012 Australian Exposure Factor Guidance. Department of Health and Ageing, Canberra, Australia. http://www.health.gov.au/internet/main/publishing.nsf/Content/A12B57E41EC9F326CA257BF0001F9E7D/$File/doha-aefg-120910.pdf eprint.uq.edu Recreational exposure to freshwater cyanobacteria: epidemiology, dermal toxicity and biological activity of cyanobacterial lipopolysaccharides [http://eprint.uq.edu.au/archive/ 00001883/] 25 July 2015

Faassen EJ, Harkema L, Begeman L, Lurling M 2012. First report of (homo)anatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands. Toxicon 60: 378-384.

Fawell JF, James HA 1994. Toxins from blue-green algae: toxicological assessment of anatoxin-a and a method for its determination in reservoir water. FWR Report No. FR0492/DOE372.

Fawell JK, Mitchell RE, Hill RE, Everett DJ 1999. The toxicity of cyanobacterial toxins in the mouse: II Anatoxin-a. Human and Experimental Toxicology 18: 168-173.

Ferguson AC, Canales RA, Beamer P, Auyeung W, Key M, Munninghoff A, Lee KT, Robertson A, Leckie JO 2006. Video methods in the quantification of children's exposures. Journal of Exposure Science and Environmental Epidemiology 16: 287-298.

Fitzgeorge NLM, Clark SA, Kelvin CW 1994. Routes of intoxication. In: G. A. Codd, T. M. Jeffreies, C. W. Kelvin and E. Potter, (Eds.). Detection methods for cyanobacterial (blue-green algal) toxins and first international symposium on detection methods for cyanobacterial (blue-green algal) toxins. Royal Society of Chemistry, Cambridge, U.K. p. 69-74. (As cited in Kuiper-Goodman *et al.* 1999 and WHO 1999)

Francoeur SN, Biggs BJF, Smith RA, Lowe RL 1999. Nutrient limitation of algal biomass accrual in streams: seasonal patterns and a comparison of methods. Journal of the North American Benthological Society 18: 242-260.

Francoeur SN, Biggs BJF 2006 Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. Hydrobiologia 561: 59–69.

Frantz CM, Petryshyn VA, Corsetti FA 2015. Grain trapping by filamentous cyanobacterial and algal mats: implications for stromatolite microfabrics through time. Geobiology. doi: 10.1111/gbi.12145.

Gaylarde PM, Jungblut A-D, Gaylarde CC, Neilan BA 2006. Endolithic phototrophs from an active geothermal region in New Zealand. Journal of Geomicrobiology 23: 579 - 587.

George AL, Murray AW, Montiel PO 2001 Tolerance of Antarctic cyanobacterial mats to enhanced UV radiation. FEMS Microbiology Ecology 37: 91–101.

Gerbersdorf SU, Wieprecht S 2015. Biostabilization of cohesive sediments: revisiting the role of abiotic conditions, physiology and diversity of microbes, polymeric secretion, and biofilm architecture. Geobiology, 13: 68–97.

Gerdes G. What are microbial mats? In: Seckbach J, Oren A., (eds). Microbial mats: modern and ancient microorganisms in stratified systems. Springer; Dordrecht: 2010. pp. 3-25.

Graba M, Sauvage S, Majdi N, Mialet B, Moulin FY, Urrea G, Buffan-Dubau E, Tackx M, Sabater S, Sanchez-Pierre J-M 2014. Modelling epilithic biofilms combining hydrodynamics, invertebrate grazing and algal traits. Freshwater Biology 56: 1213-1228.

Grauer FH, Arnold HL 1961. Seaweed dermatitis: first report of dermatitis-producing marine algae. Archives of Dermatology: 84: 720–732

Gugger M, Lenoir S, Berger C, Ledreux A, Druart J-C, Humbert J-F, Guette C, Bernard C 2005. First report in a river in France of the benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis. Toxicon 45:919-928.

Gunn GJ, Rafferty AG, Rafferty GC, Cockburn N, Edwards C, Beattie KA, Codd GA 1992. Fatal canine neurotoxicosis attributed to blue-green algae (cyanobacteria). Veterinary Record 130: 301-302.

Haley R, Kurt T 1997. Self-reported exposure to neurotoxic chemical combinations in the Gulf War: a cross-sectional epidemiologic study. Journal of the American Medical Association 277**:** 231–237.

Hamill KD. 2001. Toxicity in benthic freshwater cyanobacteria (blue-green algae): first observations in New Zealand. New Zealand Journal of Marine and Freshwater Research 35: 1057−1059.

Hamill KD 2013. Processes driving periphyton growth in the Manawatu River and implication for wastewater treatment. Prepared for Horizons Regional Council. RiverLake report wk-1103. 94 p.

Harland F, Wood SA, Broady P, Williamson W, Gaw S 2014. Polyphasic Studies of benthic freshwater cyanobacteria in Canterbury, New Zealand. New Zealand Journal of Botany 52: 116-135.

Hart DD 1985. Grazing insects mediate algal interactions in a stream benthic community. Oikos 44:40-46.

Hart DD, Biggs BJF, Nikora VI, Flinders CA 2013 Flow effects on periphyton patches and their ecological consequences in a New Zealand river. Freshwater Biology 58: 1588–1602.

Hashimoto Y 1979. Marine toxins and other bioactive marine metabolites. Japan Scientific Societies Press, Tokyo.

Hawes I, Smith RA 1993. Effects of localised nutrient enrichment on the shallow epilithic periphyton of oligotrophic Lake Taupo, New Zealand. New Zealand Journal of Marine and Freshwater Research 27:365-372.

Hawes I, Smith R 1994. Seasonal dynamics of epilithic periphyton in oligotrophic Lake Taupo, New Zealand. New Zealand Journal of Marine and Freshwater Research 28: 1-12.

Hawes I, Giles H, Doran PT 2014. Estimating photosynthetic activity in microbial mats in an ice-covered Antarctic lake using automated oxygen microelectrode profiling and variable chlorophyll fluorescence. Limnology and Oceanography 59: 674–688.

Hawes I, Schwarz A-M 1996. Epiphytes from a deep-water characean meadow in an oligotrophic New Zealand lake: species composition, biomass and photosynthesis. Freshwater Biology 36: 297-313.

Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR 1985. Severe hepatotoxicity caused by the tropical cyanobacterium *Cylindrospermopsis raciborskii* (Woloszyska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Applied and Environmental Microbiology 50: 1292-1295.

Heath M, Wood SA, Ryan K 2011. Spatial and temporal variability in *Phormidium* sp. abundance and toxin production in the Hutt River, New Zealand. Aquatic Environmental Microbiology 64: 69-79.

Heath M. 2010. Investigation of toxic benthic cyanobacteria and ecotoxicity in New Zealand. Unpublished MSc Thesis. Victoria University.

Heath M, Wood SA, Ryan K. 2010. Polyphasic assessment of fresh-water benthic mat forming cyanobacteria isolated from New Zealand. FEMS Microbiology 73: 95-109.

Heath MH, Wood SA, Barbieri RF, Young RG, Ryan KG 2014. Effects of nitrogen and phosphorus on anatoxin-a, homoanatoxin-a, dihyro-anatoxin -a and dihydro-homoanatoxin-a production by *Phormidium autumnale*. Toxicon 92: 179-185.

Heath MW 2015. Environmental drivers of *Phormidium* blooms in New Zealand rivers. PhD thesis. Victoria University.

Heath MW, Wood SA, Brasell K, Young R, Ryan KG 2015. Development of habitat suitability criteria and in-stream habitat assessment for the benthic cyanobacteria *Phormidium*. Rivers Research and Applications 31: 98-108.

Holomuzki JR, Biggs JF 2006. Food limitation affects algivory and grazer performance for New Zealand stream macroinvertebrates. Developments in Hydrobiology 185: 83-94.

Holomuzki JR, Feminella JW, Power ME 2010. Biotic interactions in freshwater benthic habitats. Journal of the North American Benthological Society 29: 220-244.

Holomuzki JR, Lowe RL, Ress JA 2006. Comparing herbivory effects of stream macroinvertebrates on microalgal patch structure and recovery. New Zealand Journal of Marine and Freshwater Research 40: 357-367.

Izumi AK, Moore RE1987. Seaweed (*Lyngbya majuscula*) dermatitis. Clinics in Dermatology 5: 92–100.

Jaarsma NG, de Boer SM, Townsend CR, Thompson RM, Edwards ED 1998. Characterising food-webs in two New Zealand streams. New Zealand Journal of Marine and Freshwater Research 32: 271-286.

Jonas A, Scholz S, Fetter E, Sychrova E, Novakova K, Ortmann J, Benisek M, Adamovsky O, Giesy JP, Hilscherova K 2015. Endocrine, teratogenic and neurotoxic effects of cyanobacteria detected by cellular in vitro and zebrafish embryos assays. Chemosphere 120: 321-327.

Katz A, Weckesser J, Drews G, Mayer H 1977. Chemical and biological studies on the lipopolysaccharide (O-antigen) of *Anacystis nidulans*. Archives of Microbiology 113: 247-256.

Keleti G, Sykora JL, Lippy EC, Shapiro MA 1979. Composition and biological properties of lipopolysaccharides isolated from *Schizothrix calcicol*a (Ag.) Gomont (cyanobacteria). Applied and Environmental Microbiology 38: 471-477.

Keleti G, Sykora JL1982. Production and properties of cyanobacterial endotoxins. Applied and Environmental Microbiology 43: 104-109.

Kelly D, McKerchar A, Hicks M 2005. Making concrete: ecological implications of gravel extraction in New Zealand rivers. Water & Atmosphere 13: 20-21.

Kirkwood AE, Jackson LJ, McCauley E 2009. Are dams hotspots for *Didymosphenia geminata* blooms? Freshwater Biology 54: 1856-1863.

Krewski D, Westphal M, Al-Zoughool M, Croteau MC, Andersen ME 2010. New directions in toxicity testing. Annual Review of Public Health 32:161-78.

Lagos N, Onodera H, Zagatto PA, Andrinolo D, Azevedo S, Oshima Y 1999. The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. Toxicon 37: 1359-1373.

Larned ST, Nikora VI, Biggs BJF 2004. Mass-transfer controlled nitrogen and phosphorus uptake by stream periphyton: a conceptual model and experimental evidence. Limnology and Oceanography 49: 1992–2000.

Leibson T, Lifshitz M 2008. Organophosphate and carbamate poisoning: review of the current literature and summary of clinical and laboratory experience in Southern Israel. Journal of Toxicology 10: 767–7704.

Lester PJ, Mitchell SF, Scott D 1994. Effects of riparian willow trees (*Salix fragilis*) on macroinvertebrate densities in two small Central Otago, New Zealand streams. New Zealand Journal of Marine and Freshwater Research 28: 267-276.

Lürling M, Roessink I 2006. On the way to cyanobacterial blooms: impact of the herbicide metribuzin on the competition between a green alga (*Scenedesmus*) and a cyanobacterium (*Microcystis*). Chemosphere 65: 618-626.

MacPhail RC, Farmer JD, Jarema KA 2007. Effects of acute and weekly episodic exposures to anatoxin-a on the motor activity of rats: comparison with nicotine. Toxicology 234: 83-89.

Mann S, Cohen M, Chapuis-Hugon F, Pichon V, Mazmouz R, Méjean A, Ploux O 2012. Synthesis, configuration assignment, and simultaneous quantification by liquid chromatography coupled to tandem mass spectrometry, of dihydroanatoxin-a and dihydrohomoanatoxin-a together with the parent toxins, in axenic cyanobacterial strains and in environmental samples. Toxicon 60: 1404-1414.

Mayer B 2014. How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. Archives of Toxicology 88: 5–7.

McAllister T 2014. Environmental factors that promote *Phormidium* blooms in Canterbury rivers. Waterways Centre for Freshwater Management Report No. 2014-001.

McAllister T 2015. Identifying environmental parameters that promote *Phormidium* blooms in Canterbury rivers. Waterways Centre for Freshwater Management Report No. WCFM Report 2015-001.

McBride GB 2012. Issues in setting secondary contact recreation guidelines for New Zealand freshwaters. Report to the Ministry for the Environment, Wellington, 9 September 2012., 12 p. http://www.mfe.govt.nz/publications/fresh-water/issues-setting-secondary-contact-recreation-guidelines-new-zealand?_ga=1.205114071.650948968.1421035452

McDowell RW, Wilcock RJ 2007. Sources of sediment and phosphorus in stream flow of a highly productive dairy farmed catchment. Journal of Environmental Quality 36: 540-548.

McDowell RW, Larned ST, Houlbrooke DJ 2009. Nitrogen and phosphorus in New Zealand streams and rivers: control and impact of eutrophication and the influence of land management. New Zealand Journal of Marine and Freshwater Research 43: 985-995.

McKerchar A, Schmidt J 2007. Decreases in low flows in the lower Selwyn River? Journal of Hydrology (New Zealand) 46: 63-72.

McKerchar A, Renwick J, Schmidt J 2010. Diminishing streamflows on the East Coast of the South Island New Zealand and linkage to climate variability and change. Journal of Hydrology (New Zealand) 49: 1-14.

Mikheyskaya LV, Ovodova RG, Ovodov YS 1977. Isolation and characterization of lipopolysaccharides from cell walls of blue green algae of the genus *Phormidium*. Journal of Bacteriology 130: 1-3.

Ministry for the Environment 2010. New Zealand river environment classification user guide. Ministry for the Environment PO Box 10-362, Wellington, New Zealand. http://www.mfe.govt.nz/sites/default/files/environmental-reporting/about-environmental-reporting/classification-systems/rec-user-guide-2010.pdf

Ministry for the Environment 2014. National policy statement for freshwater management 2014. Ministry for the Environment, Wellington. http://www.mfe.govt.nz/publications/fresh-water/national-policy-statement-freshwater-management-2014?_ga=1.163843704.650948968.1421035452

Ministry for the Environment and Ministry of Health. 2009. New Zealand guidelines for managing cyanobacteria in recreational fresh waters – interim guidelines. Prepared for the Ministry for the Environment and the Ministry of Health by SA Wood, DP Hamilton, WJ Paul, KA Safi, WM Williamson. Wellington: Ministry for the Environment. 89 p.

Mittal A, Agarwal M, Schivpuri D 1979. Respiratory allergy to algae: clinical aspects. Annals of Allergy 42: 253–265.

Moya J, Phillips L 2014. A review of soil and dust ingestion studies for children. Journal of Exposure Science and Environmental Epidemiology 24: 545-554.

Murdock JN, Dodds WK 2007. Linking benthic algal biomass to stream substratum topography. Journal of Phycology 43: 449-460.

Namikoshi M, Murakamia T, Watanabe MF, Yamada TOJ, Tsujimura S, Nagaia H, Oishie S 2003. Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. Toxicon 42: 533–538.

National Health and Medical Research Council 2008. Guidelines for managing risks in recreational water. Australian Government National Health and Medical Research Council. https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh38.pdf

Nausch M, Nausch G, Wasmund N 2004. Phosphorus dynamics during the transition from nitrogen to phosphate limitation in the central Baltic Sea. Marine Ecology Progress Series 266:15-25.

Oliver RL, Hamilton DP, Brookes JD, Ganf GG 2012. Physiology, blooms and prediction of planktonic cyanobacteria. In Whitton BA (Ed), The ecology of cyanobacteria II: their diversity in space and time. Springer 155-194

Osborne NJT, Webb PM, Shaw GR 2001. The toxins of *Lyngbya majuscula* and their human and ecological health effects. Environment International 27: 381–392.

Osborne NJT, Webb PM, Shaw GR 2007. Health effects of recreational exposure to Moreton Bay, Australia waters during a *Lyngbya majuscula* bloom. Environment International 33: 309-314.

Osborne NJT, Seawright A, Shaw GR 2008. Dermal toxicology of *Lyngbya majuscula*, from Moreton Bay, Queensland, Australia. Harmful Algae 7: 584-589.

Oulhote Y, Bouchard MF 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. Environmental Health Perspectives121:1378-1384.

Ozkaynak H, Xue J, Zartarian VG, Glen G, Smith L 2011. Modelled estimates of soil and dust ingestion rates for children. Risk Analysis 4: 592-608

Perkins EJ, Ankley GT, Crofton KM, Garcia-Reyero N, LaLone CA, Johnson MS, Tietge JE, Villeneuve DL 2013. Current perspectives on the use of alternative species in human health and ecological hazard assessments. Environmental Health Perspectives 121: 1002-1010.

Pilotto LS, Douglas RM, Burch MD, Cameron S, Beers M, Rouch GR, Robinson P, Kirk M, Cowie CT, Hardiman S, Moore C, Attewell RG 1997. Health effects of exposure to cyanobacteria (blue-green algae) due to recreational water-related activities. Australian and New Zealand Journal of Public Health 21: 562–566.

Pilotto LS, Hobson P, Burch MD, Ranmuthugala G, Attewell RG, Weightman W 2004. Acute skin irritant effects of cyanobacteria (blue-green algae) in healthy volunteers. Australian and New Zealand Journal of Public Health 28: 220−224.

Pinckney JL, Paerl HW 1997. Anoxygenic photosynthesis and N2 fixation by a microbial mat community in a Bahamian hypersaline lagoon. Applied and Environmental Microbiology 63: 420-426.

Puschner B, Hoff B, Tor ER 2008. Diagnosis of anatoxin-a poisoning in dogs from North America. Journal of Veterinary Diagnostic Investigation 20: 89-92.

Quesada A, Fernandez-Valiente E, Hawes I, Howard-Williams C. 2008. Benthic primary production in polar lakes and rivers. In Vincent WF and Laybourne-Parry, J (eds) Polar lakes and rivers. Oxford University Press, Oxford, UK. Pp 179-196.

Quiblier C, Wood SA, Echenique I, Heath M, Villeneuve A, Humbert JF 2013. A review of current knowledge on toxic benthic freshwater cyanobacteria – ecology, toxin production and risk management. Water Research 47: 5464-5479.

Quinn JM, Hickey CW 1990. Characterisation and classification of benthic invertebrate communities in 88 New Zealand rivers in relation to environmental factors. New Zealand Journal of Marine and Freshwater Research 24: 387-409.

Raziuddin S, Siegelman HW, Tornabene TG 1983. Lipopolysaccharides of the cyanobacterium *Microcystis aeruginosa*. European Journal of Biochemistry 137: 333-336.

Ressom R, Soong FS, Fitzgerald DJ, Turczynowicz L, Saadi OE, Roder D, Maynard T, Falconer I 1994. Health Effects of Toxic Cyanobacteria (Blue-green Algae*).* Canberra: National Health and Medical Research Council.

Ricceri L, Venerosi A, Capone F, Cometa MF, Lorenzini P, Fortuna S, Calamandrei G. 2006. Developmental neurotoxicity of organophosphorous pesticides: fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. Toxicology Science 93:105–113.

Rijal G, Tolson J, Petroulou C, Granato T, Glymph T, Gerba C, DeFlaun M, O'Connor C, Kollias L, Lanyon R 2011. Microbial risk assessment for recreational use of the Chicago area waterway system. Journal of Water Health 9: 169–186.

Rogers EH, Hunter III ES, Moser VC, Herkovits J, Muñoz L, Hall LL, Chernoff N 2005. Potential developmental toxicity of anatoxin-a, a cyanobacterial toxin. Journal of Applied Toxicology 25: 527-534.

Schep LJ, Slaughter RJ, Beasley DM 2009. Nicotinic plant poisoning. Clinical Toxicology 8: 771-781.

Schets FM, Schijven JF, de Roda Husman AM 2011. Exposure assessment for swimmers in bathing waters and swimming pools. Water Research 45: 2392-2400.

Schijven J, de Roda Husman AM 2006. A survey of diving behaviour and accidental water ingestion among Dutch occupational and sport divers to assess the risk of infection with waterborne pathogenic microorganisms. Environmental Health Perspectives 114: 712-717.

Schneck F, Schwarzbold A, Melo AS 2011.Substrate roughness affects stream benthic algal diversity, assemblage composition, and nestedness Journal of the North American Benthological Society 30: 1049-1056.

Scholtissek B, Jürgens UJ, Weckesser J 1991. Purification and electrophoretic pattern of the lipopolysaccharide of *Microcystis* sp. PCC 7806. Archives of Hydrobiology, Supplement, Algological Studies 64: 361-368.

Schweitzer KS, Chen SX, Law S, Van Demark MJ, Poirier C, Justice MJ, Hubbard WC, Kim ES, Lai X, Wang M, Kranz WD, Carroll CJ, Ray RD, Bittman R, Goodpaster J, Petrach I 2015. Endothelial disruptive pro-inflammatory effects of nicotine and e-cigarette vapor exposures. American Journal of Physiology - Lung Cellular and Molecular Physiology. DOI: 10.1152/ajplung.00411.2014

Scott JT, Marcarelli AM 2012. Cyanobacteria in freshwater benthic environments. In Whitton BA (ed) The ecology of cyanobacteria II: their diversity in space and time. Springer, pp 271-289.

Selwood A, Holland PT, Wood SA, Smith K, McNabb P 2007. Production of anatoxin-a and a novel biosynthetic precursor by the cyanobacterium *Aphanizomenon issatschenkoi*. Environmental Science & Technology. 41: 506-510.

Sharma NK, Rai AK 2008. Allergenicity of airborne cyanobacteria *Phormidium fragile* and *Nostoc muscorum*. Ecotoxicology and Environmental Safety 69: 158-162.

Sharpley AN, Kleinman PJA, Heathwaite AL, Gburek WJ, Folmar GJ, Schmidt JP 2008. Phosphorus loss from an agricultural watershed as a function of storm size. Journal of Environmental Quality 37: 362-368.

Skulberg OM, Carmichael WW, Anderson RA, Matsunaga S, Moore RE, Skulberg R 1992. Investigations of a neurotoxic Oscillatorialean strain (cyanophyceae) and its toxin. Isolation and characterization of homoanatoxin-a. Environmental Toxicology and Chemistry 11: 321-329.

Smith AM, Heisler LE, Mellor J, Kaper F, Thompson MJ, Chee M, Roth FP, Giaever G, Nislow C 2009. Quantitative phenotyping via deep barcode sequencing. Genome Research 19: 1836-1842.

Smith F, Wood SA, Ginkel Rv, Broady P, Gaw S 2011. First report of saxitoxin production by a species of the freshwater benthic cyanobacterium, *Scytonema* Agardh. Toxicon 57: 566–573.

Smith F, Wood SA, Wilks T, Kelly D, Broady P, Gaw S 2012. Survey of *Scytonema* (Cyanobacteria) and associated saxitoxins in the littoral zone of recreational lakes in Canterbury (New Zealand). Phycologia 51: 542–551.

Smith F, Wood SA, Moltchanova E, Broady P, Williamson W, Gaw S 2013. *Phormidium autumnale* growth and anatoxin-a production under iron and copper stress. Toxins 5: 2504-2521.

Smith RA, Lewis D 1987. A rapid analysis of water for anatoxin-a. The unstable toxic alkaloid from *Anabaena flos-aquae*, the stable non-toxic alkaloids left after bioreduction and a related amine which may be nature's precursor to anatoxin-a. Veterinary and Human Toxicology 29:153-154.

Snelder TH, Weatherhead M, Biggs BJF 2004. Nutrient concentration criteria and characterization of patterns in trophic state for rivers in heterogeneous landscape. Journal of the American Water Resources Association 40: 1-13.

Snelder TH, Hughey KFD 2005. On the use of an ecological classification to improve water resource planning in New Zealand. Environmental Management 36: 741-756.

Solarino B, Rosenbaum F, Riesselmann B, Buschmann C, Tsokos M. 2010. Death due to ingestion of nicotine-containing solution: case report and review of the literature. Forensic Science International 195: 19–22.

Stal LJ 1995. Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytologist 131: 1-32.

Stal LJ 2012. Cyanobacterial mats and Sstromatolites. In Whitton BA (ed). The Ecology of Cyanobacteria II: their diversity in space and time. Springer, 65-125.

Steinman AD 1996. Effects of grazers on benthic freshwater algae. In: Algal ecology—freshwater benthic ecosystems. Stevenson RJ, . Bothwell ML, Lowe RL (eds), pp. 341–373. Academic Press, San Diego, CA.

Stevens DK, Krieger RI 1990. N-Methylation of anatoxin-a abolishes nicotinic cholinergic activity Toxicon 28: 133-134.

Stevens DK, Krieger RI 1991. Effect of route of exposure and repeated doses on the acute toxicity in mice of the cyanobacterial nicotinic alkaloid anatoxin-a. Toxicon 29: 134-138.

Stewart I, Robertson IM, Webb PM, Schluter PJ, Shaw GR 2006.Cutaneous hypersensitivity reactions to freshwater cyanobacteria--human volunteer studies. BMC Dermatology 6:6.

Stewart I, Schluter PJ, Shaw GR 2006. Cyanobacterial lipopolysaccharides and human health: a review. Environmental Health: A Global Access Science Source 5: 7.

Stewart I, Webb PM, Schluter PJ, Fleming LE, Burns JW, Gantar M, Backer L, Shaw GR 2006. Epidemiology of recreational exposure to freshwater cyanobacteria – an international prospective cohort study. BMC Public Health, 6, 93. doi:10.1186/1471-2458-6-93

Suppes LM, Abrell L, Dufour AP, Reynolds KA 2014. Assessment of swimmer behaviors on pool water ingestion. Journal of Water Health 12: 269-279.

Surgeon General's Report 2014. The health consequences of smoking—50 years of progress: a report of the Surgeon General. US Department of Health and Human Services. Public Health Service. Office of the Surgeon General, Rockville, MD.

Sutherland WJ 2006. Predicting the ecological consequences of environmental change: a review of methods. Journal of Applied Ecology 43: 599-616.

Swanson KL, Allen CN, Aronstam RS, Rapoport H, Albuquerque EX 1986 Molecular mechanisms of the potent and stereospecific nicotinic receptor agonist (+)-anatoxin-a. Molecular Pharmacology 29: 250-257.

Swanson KL, Aracava Y, Sardina FJ, Rapoport H, Aronstam RS, Albuquerque EX 1989. N-methylanatoxinol isomers: derivatives of the agonist (+)-anatoxin-a block the nicotinic acetylcholine receptor ion channel. Molecular Pharmacology 35: 223-231.

Swanson KL, Aronstam RS, Wonnacott S, Rapoport H, Albuquerque EX 1991. Nicotinic pharmacology of anatoxin analogs. Side chain structure-activity relationships at peripheral agonist and noncompetitive antagonist sites. Journal of Pharmacology and Experimental Therapeutics 259: 377-386.

Thiesen R 2015. Patch dynamics of *Phormidium* in Canterbury rivers. Waterways Centre for Freshwater Management. WCFM Report 2015-002. Wellington.

Tornabene TG, Bourne TF, Raziuddin S, Ben-Amotz A 1985.Lipid and lipopolysaccharide constituents of cyanobacterium *Spirulina platensis* (Cyanophyceae, Nostocales). Marine Ecology Progress Series 22: 121-125.

Torokne A, Palovics A, Bankine M 2001. Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria-experimental evidence. Environmental Toxicology 16: 512-516.

Tsou MC, Özkaynak H, Beamer P, Dang W, Hsi HC, Jiang CB, Chien LC 2015. Mouthing activity data for children aged 7 to 35 months in Taiwan. Journal of Exposure Analysis and Environmental Epidemiology 25: 388-398.

U.S. Environmental Protection Agency 2006. DRAFT. Toxicological reviews of cyanobacterial toxins: anatoxin-a. NCEA-C-1743. ttp://cfpub.epa.gov/si/si_public_file_download.cfm?p_download.

U.S. Environmental Protection Agency 2015. Health effects support document for the cyanobacterial toxin anatoxin-a. EPA Document Number: 820R15104. http://www2.epa.gov/sites/production/files/2015-06/documents/anatoxin-a-report-2015.pdf

Uehlinger U, Buhrer H, Reichert P 1996. Periphyton dynamics in a floodprone pre-alpine river: evaluation of significant processes by modeling. Freshwater Biology 36: 249-263.

United States Environmental Protection Agency (USEPA) 2011. Exposure factors handbook 2011 edition (final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F.

Valentine WM, Schaeffer DJ, Beasley VR 1991. Electromyographic assessment of the neuromuscular blockade produced *in vivo* by anatoxin-a in the rat. Toxicon 29: 347-357.

Valério E, Chaves S, Tenreiro R. 2010 Diversity and impact of prokaryotic toxins on aquatic environments: a review. Toxins 2: 2359-2410.

Villeneuve A, Larroudé S, Humbert JF 2011. Herbicide contamination of freshwater ecosystems: impact on microbial communities. Chapter 15 in: Pesticides: formulations, effects, fate. InTech Open Access Publishers. pp: 285-312.

Weise G, Drews G, Jann B, Jann K 1970. Identification and analysis of a lipopolysaccharide in cell walls of the blue-green alga *Anacystis nidulans*. Archives of Microbiology 71: 89-98.

Winterbourn MJ, Cowie B, Rounick JS 1984. Food resources and ingestion patterns of insects along a West Coast, South Island, river system. New Zealand Journal of Marine and Freshwater Research 18: 43-51.

Wonnacott S, Jackman S, Swanson KL, Rapoport H, Albuquerque EX 1991. Nicotinic pharmacology of anatoxin analogs. II. Side chain structure-activity relationships at neuronal nicotinic ligand binding sites. Journal of Pharmacology and Experimental Therapeutics 259: 387-391.

Wood SA, Depree C, Brown L, McAllister T, Hawes I 2015. Entrapped sediments as a source of phosphorus in epilithic cyanobacterial proliferations in low nutrient rivers. PloS One. In press.

Wood SA, Heath M, McGregor G, Holland PT, Munday R, Ryan K. 2010. Identification of a benthic microcystin producing filamentous cyanobacterium (Oscillatoriales) associated with a dog poisoning in New Zealand. Toxicon 55: 987-903.

Wood SA, Holland PT, MacKenzie, L.A 2011. Development of solid phase adsorption toxin tracking (SPATT) for monitoring anatoxin-a and homoanatoxin-a in river water. Chemosphere 82: 888-894.

Wood SA, Kuhajek J, de Winton M, Phillips NR 2012a. Species composition and cyanotoxin production in periphyton mats from three lakes of varying trophic status. FEMS Microbiology Ecology 79: 312-326.

Wood SA, Pochon X, Luttringer-Plu L, Vant BN, Hamilton DP 2014. Recent invader or indicator of environmental change? A phylogenetic and ecological study of *Cylindrospermopsis raciborskii* in New Zealand. Harmful Algae 39: 64–74.

Wood SA, Selwood AI, Rueckert A, Holland PT, Milne JR, Smith KF, Smits B, Watts LF, Cary CS 2007. First report of homoanatoxin-a and associated dog neurotoxicosis in New Zealand. Toxicon 50: 292-301.

Wood SA, Shearer K, Clapcott J 2014. Advice on a monitoring programme to assess the ecological effects of *Phormidium* on macroinvertebrate communities. Prepared for Horizons Regional Council. Cawthron Report No. 2624.

Wood SA, Smith FMJ, Heath MW, Palfroy T, Gaw S, Young Y, Ryan KG 2012b. Within-mat variability in anatoxin-a and homoanatoxin a production among benthic *Phormidium* (Cyanobacteria) strains. Toxins 4: 900-912.

Wood SA, Stirling DJ, Briggs LR, Sprosen J, Holland PT, Ruck JG, Wear RG 2006. Survey of cyanotoxins in New Zealand waterbodies between 2001 and 2004. New Zealand Journal of Marine & Freshwater Research 40: 585-595.

Wood SA, Wagenhoff A, Kelly D 2015. *Phormidium* blooms—relationships with flow, nutrients and fine sediment in the Maitai River. Prepared for Nelson City Council. Cawthron Report No. 2723. 44 p. plus appendices.

Wood SA, Wagenhoff A, Young R 2014. The effect of flow and nutrients on *Phormidium* abundance and toxin production in rivers in the Manawatu-Whanganui region. Prepared for Horizons Regional Council. Cawthron Report No. 2575. 41 p. plus appendices.

Wood SA, Young RG 2012. Review of benthic cyanobacteria monitoring programme 2012. Prepared for Horizons Regional Council. Cawthron Report No. 2217. 29 p. plus appendices

Woolf A, Burkhart K, Caraccio T, Litovitz T 1996. Self-poisoning among adults using multiple transdermal nicotine patches. Clinical Toxicology 34: 691–698.

World Health Organization (WHO) 2003. Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. WHO. 253 p.

Yavasoglu A, Karaaslan MA, Uyanikgil Y, Sayim F, Ates U 2008. Toxic effects of anatoxin-a on testes and sperm counts of male mice. Experimental and Toxicologic Pathology 60: 391-396.

Young C, Truman P, Boucher M, Keyzers RA, Northcote PT, Jordan TW 2009. The algal metabolite yessotoxin affects heterogeneous nuclear ribonucleoproteins in HepG2 cells. Proteomics 9: 2529-2542.