Review

The rise of toxic benthic *Phormidium* proliferations: A review of their taxonomy, distribution, toxin content and factors regulating prevalence and increased severity

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A B S T R A C T

There has been a marked increase in the distribution, intensity and frequency of proliferations of some species of the benthic mat-forming, toxin-producing genus *Phormidium* in rivers globally over the last decade. This review summarises current knowledge on their taxonomy, distribution, toxin content, environmental drivers of proliferations, and monitoring and management strategies in New Zealand. Although toxic *Phormidium* proliferation occurs in rivers worldwide little is known about these factors in most countries. Proliferations, defined as >20% cover of a riverbed, have been identified in 103 rivers across New Zealand. Morphological and molecular data indicate the main species responsible is *Phormidium autumnale*. In New Zealand *Phormidium* produces anatoxins (anatoxin-a, homoanatoxin-a, dihydroanatoxin-a, and dihydrohomoanatoxin-a) and these were detected in 67% of 771 samples from 40 rivers. The highest concentration measured was 712 mg kg^{-1} dried weight (Oreti River, Southland), with considerable spatial and temporal variability in anatoxin concentrations between and within rivers. A synthesis of field based studies suggests that *Phormidium* proliferations are most likely when there is some enrichment of dissolved inorganic nitrogen but when water-column dissolved reactive phosphorus is less than 0.01 mg L^{-1}. Once established *Phormidium*-dominated mats trap sediment and internal mat biogeochemistry can mobilise sediment-bound phosphorus, which is then available for growth. Removal of *Phormidium*-dominated mats is primarily due to shear stress and substrate disturbance, although there is also evidence for autogenic detachment. A combination of factors including; changes to riparian margins, increased nitrate and fine sediment loads, and alterations in flow regimes are likely to have contributed to the rise in *Phormidium* proliferations.

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1. Introduction

The filamentous cyanobacterial genus *Phormidium* (Fig. 1A) is cosmopolitan and is found in diverse habitats including; intertidal marshes, alpine streams, thermal springs, and Arctic and Antarctic lakes (Broady and Kibblewhite, 1991; Quesada et al., 1999; Komárek and Anagnostidis, 2005). Under favourable hydrological and environmental conditions, *Phormidium* forms cohesive mats which can cover large areas of substrate (Fig. 1B–D). Expansive *Phormidium* mats have been recorded in both oligotrophic and eutrophic environments (Mez et al., 1998; Komárek, 1999; Wood et al., 2012a). Although dominated by *Phormidium*, these mats also contain a plethora of other organisms including bacteria, other cyanobacteria, and to a lesser extent eukaryotic algae (Hart et al., 2013; Brasell et al., 2015). These consortia of micro-organisms often benefit each other physiologically, and are bound together, and to the substrate, by extracellular polymeric substances (EPS). Hereafter these microbial communities are collectively referred as *Phormidium*-dominated mats. These mats also contain inorganic matter, including sediment (Fig. 1B; Wood et al., 2015b).

Species belonging to the genus *Phormidium* can produce a range of cyanotoxins (Gugger et al., 2005; Teneva et al., 2005; Wood et al., 2007; Borges et al., 2015). The most commonly produced toxins are the powerful neuromuscular-blocking anatoxin-a (ATX) and homoanatoxin-a (HTX) and their structural derivatives (Quiblier et al., 2013). Animal deaths linked to ingestion of microbial mats containing anatoxin producing *Phormidium* species have been reported in France, Netherlands, the United States of America, and New Zealand (Gugger et al., 2005; Wood et al., 2007; Puschner et al., 2008; Faassen et al., 2012). In France, ingestion of mats dominated by ATX-producing *Phormidium favosum* were implicated in the rapid death of two dogs at the Loue River (Gugger et al., 2005). A similar event occurred in Netherlands, where three dogs died at Lake IJmeer after ingesting mats containing *Phormidium* and high concentrations of ATX (Faassen et al., 2012).

Over the last decade there has been an apparent increase in the prevalence of benthic *Phormidium* proliferations (defined as >20% coverage of the benthos by *Phormidium*-dominated mats) in some New Zealand rivers (Heath, 2009). The first documented animal toxicosis event due to ingestion of benthic cyanobacteria occurred in 1998 in the Waikanae River (lower North Island), where five dogs died in three days (Hamill, 2001). Using a mouse bioassay and high-performance liquid chromatography with fluorescence detection (HPLC-FLD) the presence of natural degradation products of ATX was subsequently confirmed in a benthic mat dominated by *Oscillatoria*-like species (Hamill, 2001). Further sudden deaths of dogs were reported at the Mataura River (lower South Island) in 1999 and 2000. Benthic *Oscillatoria*-like mats were collected and their toxicity confirmed by mouse bioassay (Hamill, 2001).

In November 2005, at least five dogs died rapidly after visiting the Hutt River (lower North Island). Extensive benthic mats dominated by *Phormidium autumnale* were found in the river (Wood et al., 2007). Liquid chromatography–mass spectrometry (LC–MS) analysis of the mats and the stomach contents from a
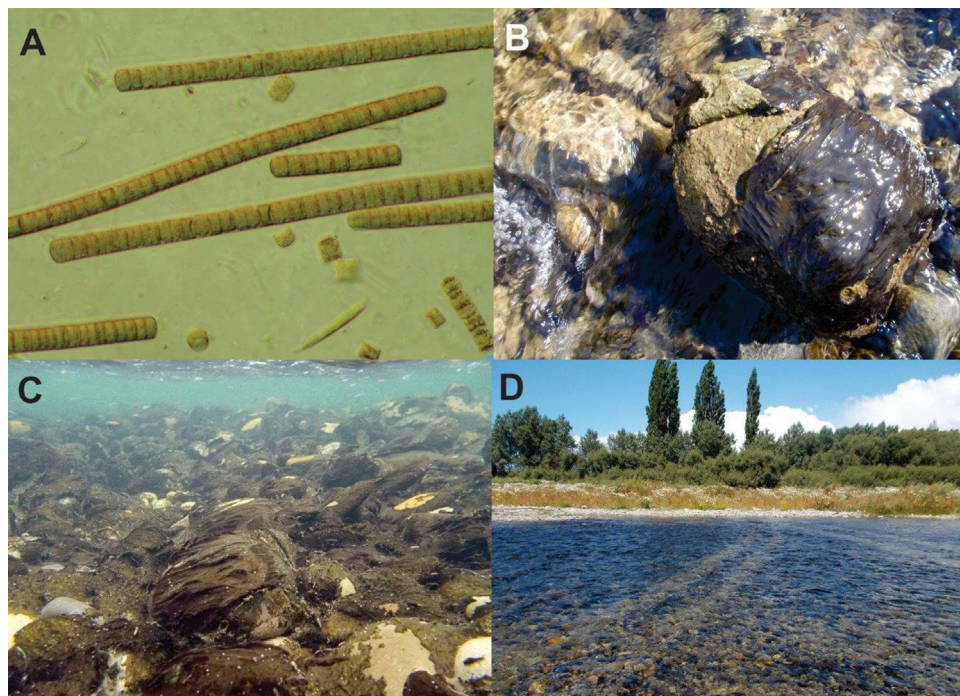
Fig. 1. (A) Example of typical *Phormidium* filament observed in *Phormidium*-dominated mats in New Zealand. This filament was from the study of Wood et al., 2007 (Hutt River, Wellington, New Zealand), (B) a thick *Phormidium*-dominated mat from the Selwyn River (New Zealand), the left-hand side of the mat has been peeled back to show the layer of fine sediment commonly observed at the mat-rock interface, and (C, D) *Phormidium* proliferations in the Opihi River (New Zealand).

deceased dog identified ATX, HTX and the variants dihydro-anatoxin-a (dhATX) and dihydro-homoanatoxin-a (dhHTX; Wood et al., 2007). Since this incident, ingestion of *Phormidium*-dominated mats has been linked to dog toxicosis events in other parts of New Zealand and anatoxins detected on multiple occasions (e.g., Heath et al., 2010; Wood et al., 2012b).

This review summarises the current knowledge on *Phormidium*-dominated mat taxonomy, distribution, toxin content, environmental drivers of proliferations and monitoring and management strategies in New Zealand. Data on proliferation occurrence and toxin content have been collated from researchers and management authorities to provide a national overview. Although *Phormidium* mats have been identified in other environments in New Zealand, including lakes (Wood et al., 2012a), this review focuses only on rivers, and primarily cobble-bedded rivers, as these are the sites with the most pronounced and problematic proliferations. Previous literature of periphyton dynamics and more recent *Phormidium*-specific research was reviewed to provide an in-depth synthesis of potential factors causing proliferations. Finally, data are provided to support and speculate on reasons for the apparent increase in the distribution and extent of *Phormidium*-dominated proliferations and areas requiring further research are highlighted. The information collated in this review will assist other countries beginning to address the causes of, and risk associated with, toxic *Phormidium* proliferations (Quiblier et al., 2013; Borges et al., 2015; Bouma-Gregson, 2015; Fetscher et al., 2015).

2. Taxonomy

The genus of *Phormidium* is species-rich with approximately 170 described species (Komárek and Anagnostidis, 2005; Strunecký et al., 2013). It is characterised morphologically by curved trichomes which are usually contained in a colourless sheath. Trichomes contain cells (2.5–11 μm wide) which are roughly isodiametric and usually have a pointed or rounded apical cell sometimes with a calyptra (Fig. 1A; Komárek and Anagnostidis, 2005). There have been significant taxonomic challenges with this genus, with molecular approaches often contradicting morphological studies (Marquardt and Palinska, 2007; Palinska and Marquardt, 2008; Palinska et al., 2011; Strunecký et al., 2013). For example, Marquardt and Palinska (2007) characterised 30 strains belonging to the *Phormidium* genus, on the basis of morphology, using a polyphasic approach. They found that strains fell into different phylogenetic clusters, which also contained *Microcoleus*, *Leptolyngbya* and *Oscillatoria* ribotypes. Furthermore, two species that were morphologically assigned to *P. autumnale*, were found to be genetically dissimilar, grouping in different clusters. These results indicate that the genus *Phormidium* is not monophyletic and is likely to require significant further revision.

Studies in New Zealand have used polyphasic approaches to investigate species isolated from *Phormidium*-dominated mats (Heath et al., 2010; Wood et al., 2012a; Harland et al., 2014). Heath et al. (2010) isolated strains from *Phormidium*-dominated mats across New Zealand. They noted considerable morphological variability among strains, but based on an analysis of an approximately 650 base pair (bp) region of the 16S ribosomal RNA (16S rRNA) gene they consistently identified the dominant species in the mats as *Phormidium autumnale*. Heath et al. (2010) further concluded that *P. autumnale* mats are composed of multiple morphospecies and that only some strains have the ability to produce toxins. Using morphology, Harland et al. (2014) classified 15 strains isolated from South Island rivers as *Phormidium* cf. *uncinatum* but based on their phylogenetic analysis (16S ribosomal RNA, approximately 1330 bp; GenBank accession numbers JX08873-82, JX08895-99) these clustered with other *P. autumnale*

characterised using phenotypic and molecular methods (Comte et al., 2007; Palinska and Marquardt, 2008). Based on morphology Wood et al. (2012a) was unable to identify differences among 30 *Phormidium* strains isolated from four mats. Comparatively, 16S rRNA (1340 bp; GenBank accession numbers JX847017–036, JX088073–93) gene sequence comparisons showed a difference of at least 17 nucleotides among anatoxin and non-anatoxin-producing strains and these formed two separate sub-clades during phylogenetic analysis. All strains showed high sequence homology to *P. autumnale* (Comte et al., 2007; Palinska and Marquardt, 2008).

Considerable uncertainty regarding the classification of *Phormidium autumnale* and *Phormidium uncinatum* exists internationally. Komárek and Anagnostidis (2005) separated the two species based on cell morphometrics; *P. autumnale* is in Group VII of the *Phormidium* genus, having roughly isodiametric cells and having attenuation towards the apical cell, whereas, *P. unicinatum* is placed in Group VIII based on abrupt tapering of cylindrical trichomes and distinctly shorter cells. Whitton (2011) suggested that they should be amalgamated to form a single species (*P. autumnale*), as both species possess a calyptra and their trichome widths overlap. Recent research suggests that the genus *Microcoleus* should include several common *Phormidium* species, including *P. autumnale* (Strunecký et al., 2013). Phylogenies based on 16S rRNA and internally transcribed spacer (ITS) demonstrated that strains of *Microcoleus vaginatus* and *P. autumnale* are members of a highly supported monophyletic clade. Morphologically *M. vaginatus* and *P. autumnale* are very similar, particularly in trichome morphology, differing only in the formation of colonies and organisation of filaments. On-going research using a multi-gene approach may resolve these taxonomic uncertainties, however at present it is considered that the organism forming most of the riverine accumulations in New Zealand should be ascribed to *P. autumnale*.

Phormidium-dominated mats in New Zealand rivers have a distinct macroscopic appearance, comprising a several millimetre-thick tightly adhering darkly pigmented and cohesive mat that colonises river substrate by gradual spread. This ready field identification has resulted in a pragmatic approach being adopted for taxonomic identification during monitoring programmes or research aimed at investigating environmental drivers. Consequently, *Phormidium* is now rarely identified to species level in monitoring programmes, and instead is referred to as *Phormidium*-dominated mats.

3. The current distribution of *Phormidium*-dominated proliferations in New Zealand

Freshwater scientists from governmental organisations across New Zealand were asked to provide names of rivers in their regions that had experienced *Phormidium* proliferations. Information from these organisations identified that *Phormidium* proliferations had occurred at one or more sites in 103 rivers across New Zealand since 2009 (Fig. 2; North Island = 61 rivers, South Island = 42 rivers). While by no means an exhaustive national survey, the rivers where proliferations are recorded tend to be on the eastern side of the country, often in the rain shadow of prevailing westerly winds.

4. Anatoxins in *Phormidium*-dominated mats

The primary toxins identified in *Phormidium*-dominated mats in New Zealand are; ATX, HTX, dhATX, and dhHTX (Wood et al., 2007, 2014a, 2015a). Anatoxins are low molecular weight alkaloids, which mimic the action of acetylcholine at neuromuscular nicotinic receptors of the post-synaptic membrane at the
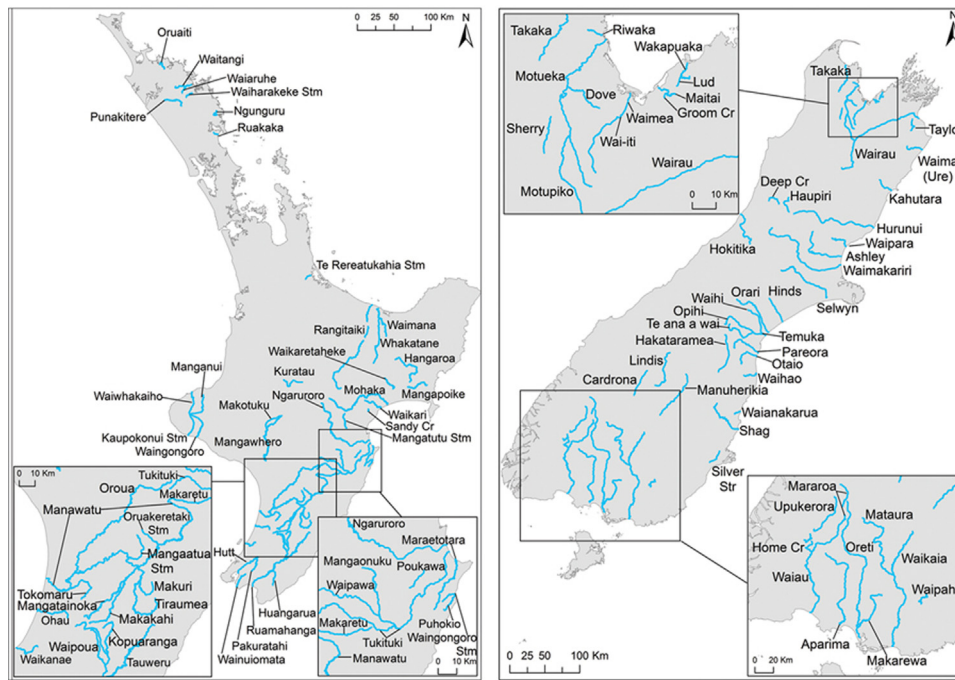
Fig. 2. New Zealand Rivers where one (or more) sites have experienced *Phormidium* proliferations (defined as greater than 20% coverage) on one (or more) occasions since 2009.
Adapted from Wood et al. (2015c).

neuromuscular junction (Carmichael et al., 1975; Aronstam and Witkop, 1981; Carmichael, 1997). Anatoxins are not degraded by cholinesterase and consequently continually stimulate muscle cells, causing muscular twitching, fatigue, paralysis and eventually respiratory arrest (Carmichael et al., 1977; Devlin et al., 1977; Stevens and Krieger, 1991).

In this review, published (Heath and Wood, 2010; Wood et al., 2010, 2014a, 2015a; Heath et al., 2011) and unpublished data on anatoxin concentrations detected in *Phormidium*-dominated mats has been compiled. This dataset comprised of 771 samples 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 presented (Fig. 3). Sampling effort varied among rivers, and some rivers were sampled multiple times on the same day to assess spatial variability in anatoxin concentrations (Fig. 3; see *x*-axis).

Anatoxins were detected in 30 (75%) rivers and in 520 (67%) of the samples and showed high spatial and temporal variability among and within rivers (Fig. 3). The highest concentration was 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 (>100 mg kg^{-1}dw) of toxins. The median anatoxin concentration for the majority of rivers was below 1 mg kg^{-1} dw
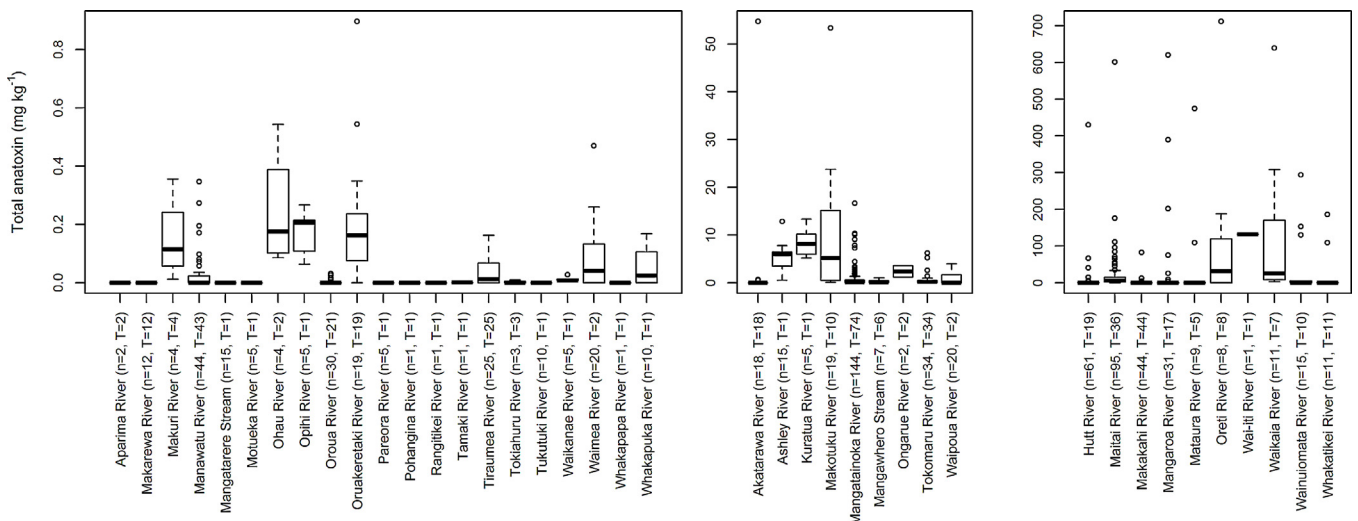

Fig. 3. Total anatoxin concentrations in *Phormidium*-dominated mats from 40 New Zealand rivers (*n* = 771). Toxins are expressed as mg kg^{-1} of dried weight. Note different *y*-axis scales in each panel to accommodate different ranges of concentration. T = number of different dates samples were collected. 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.
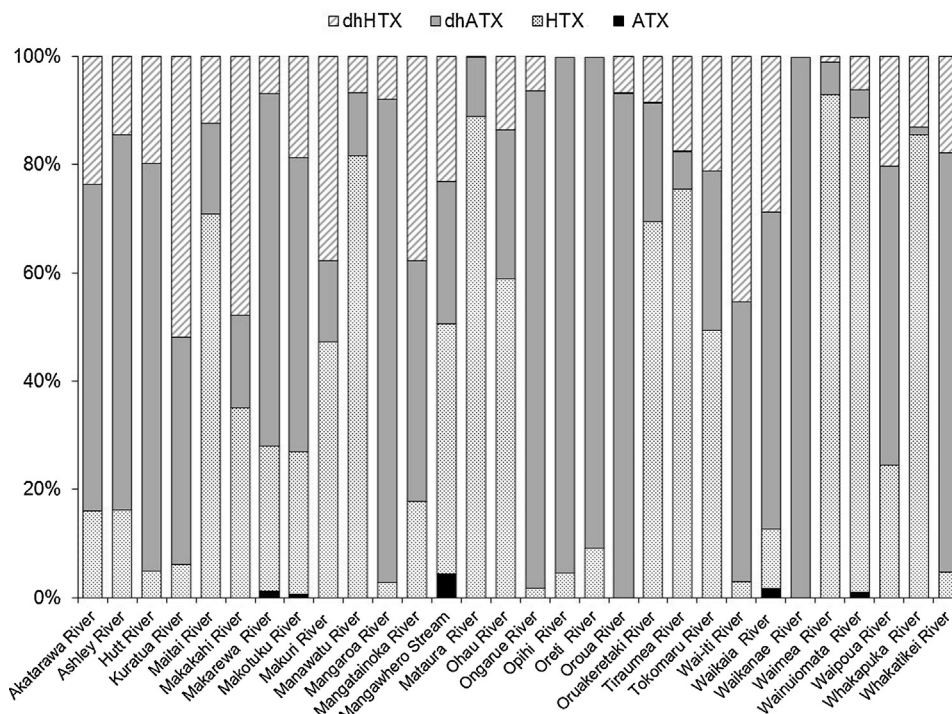Adapted from Wood et al. (2015c).

Fig. 4. Stacked bar graph showing the percentage of each anatoxin variant present in *Phormidium*-dominated mat samples from 30 rivers. dhATX = dihydroanatoxin, dhHTX = dihydrohomoanatoxin, HTX = homoanatoxin-a, ATX = anatoxin-a.
Adapted from Wood et al. (2015c).

(Fig. 3). Multiple studies have observed marked changes in total anatoxin concentrations within a one week period (Heath et al., 2011; Wood et al., 2014a, 2015a). For example, Wood et al. (2014a) noted an increase in anatoxin concentrations from 1.2 to 82.4 mg kg^{-1} dw in *Phormidium*-dominated mats from the Maka-kahi River between 10 February 2012 and 17 February 2012. Total anatoxin concentrations can also be highly spatially variable. For example, Wood et al. (2010) analysed 15 samples from 10×10 grids in seven rivers. Of the seven sites sampled, there was only one site where all 15 samples contained toxins.

Differences in the relative abundance of co-occurring organisms or inorganic material in *Phormidium*-dominated mats could account for some of the observed spatial and temporal variability in weight-specific total toxin concentrations. Additionally, toxic and non-toxic genotypes co-occur in *Phormidium*-dominated mats, often in close proximity (Heath et al., 2010; Wood et al., 2010, 2012b), and their relative abundance may also affect the total anatoxin concentrations. Wood et al. (2012b) isolated and cultured multiple *Phormidium* strains from 1 cm^2 areas taken from four different mats collected in two rivers. Molecular and chemical analysis showed both toxic and non-toxic genotypes co-existed, and that among toxic strains the concentration of toxin produced per unit biomass varied approximately 100-fold. Consequently the relative amount of toxic versus non-toxic genotypes is not only important, but also the abundance of toxic genotypes that produce higher anatoxin quotas (amount of toxin produced per cell).

Variables that regulate the presence and abundance of each genotype within *Phormidium*-dominated mats are unknown. Heath et al. (2010) suggested that toxin-producing strains in the Hutt River 'out-competed' non-toxic *Phormidium* strains at temperatures above 15 °C, but subsequent studies in other rivers have not observed this pattern (Wood et al., 2014a). To date analysis of field data has not identified correlations between toxin concentrations and any environmental parameters, but further research is required to elucidate possible environmental drivers of toxicity. Using culture-based studies, (Heath et al., 2014) showed

increased nitrogen and phosphorus concentrations resulted in a significant decrease in HTX quotas. Multiple studies have demonstrated that toxin quota peaked in the initial growth phase (Harland et al., 2013; Heath et al., 2014, 2016).

Analysis of the toxin data collated in this review showed that, ATX, HTX, dhATX and dhHTX are usually detected simultaneously although their relative concentrations vary (Fig. 4). Dihydro-anatoxin-a and dhHTX were the most prevalent variants (Fig. 4). Anatoxin-a was least commonly detected; only occurring on a few occasions in the Hutt, Wai-iti, Waikaia and Wainuiomata rivers (Fig. 4). 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 thought to be just as unstable as ATX.

Limitations in the use of 'grab' samples to detect low concentrations of extracellular toxins in rivers led to a study by Wood et al. (2011) to evaluated the potential of an *in situ* method known as solid phase adsorption toxin tracking (SPATT) for detecting anatoxins in river water. A three-day field study in a river containing toxic *Phormidium*-dominated mats was undertaken and toxins were detected in all SPATT bags, even when grab samples failed to show detectable concentrations. These data suggest that low concentrations of anatoxins are likely to be present in river water when *Phormidium*-dominated mats occur. This may be of particular concern when river water is used by drinking supplies.

5. Environmental drivers of proliferations

The concept of accrual cycles is fundamental to understanding periphyton dynamics (Biggs et al., 1998). The accrual cycle describes colonisation and initiation of a periphyton community, its subsequent growth, and the process that removes it and allows the cycle to restart (Fig. 5; Biggs, 1996). At its simplest level, the accrual concept describes a cycle whereby following colonisation, periphyton biomass and cover increases, at a rate determined by
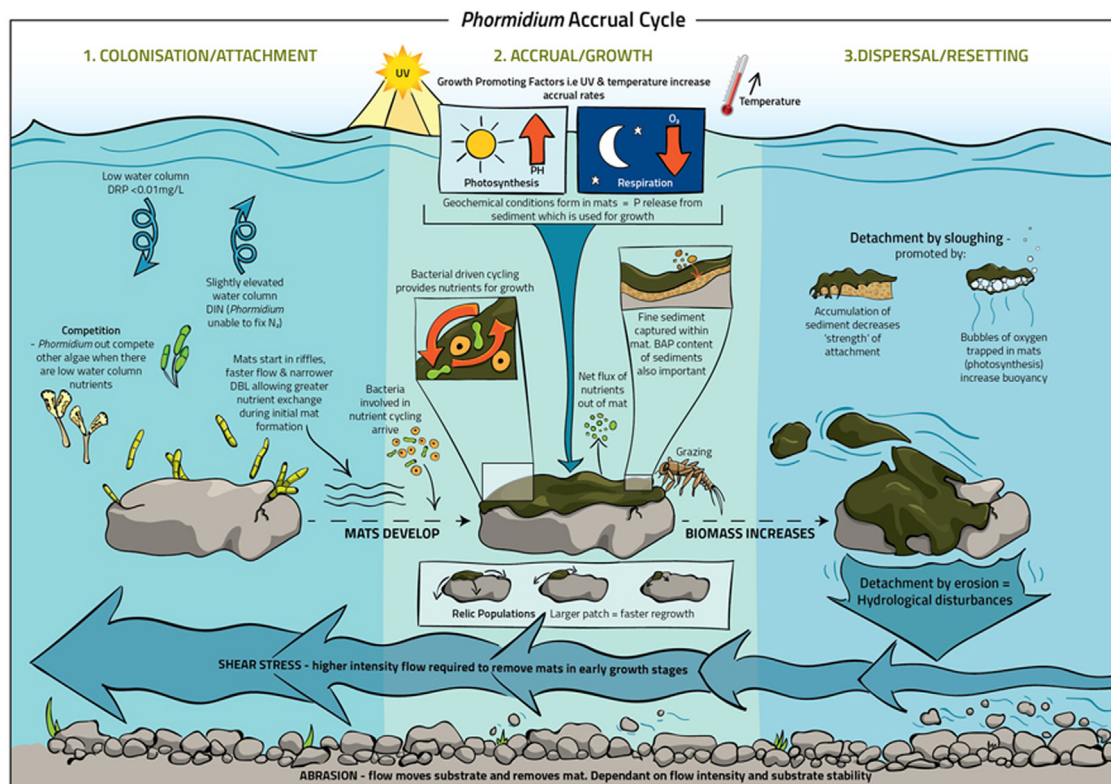
Fig. 5. Schematic diagram of accrual cycle for *Phormidium*-dominated mats in New Zealand rivers. DRP = dissolved reactive phosphorus, P = phosphorus, BAP = biologically available phosphorus, DIN = dissolved inorganic nitrogen, DBL = diffuse boundary layer.
Adapted from Wood et al. (2015c).

the balance of growth-promoting and growth-inhibiting factors, until removed and reset by full or partial disturbance (Fig. 5). Typically growth-controlling factors include water-column nutrients, light and temperature, grazing and ablation.

Mats dominated by *Phormidium* differ from other periphyton types, like filamentous greens and diatoms, due to their thick, cohesive mat growth form. Mats can be many millimetres thick and the environment inside the mat can be very different to that in the river itself (Wood et al., 2015b). Steep biogeochemical gradients can occur within such systems, which can promote nutrient fixation and recycling, that can make them to some degree independent of nutrient fluxes from overlying water (Stal, 2012; Wood et al., 2015b).

Differences in the functioning of *Phormidium*-dominated mats from other periphyton types, and differences at successional stages, mean that the environmental control of *Phormidium*-dominated biomass expansion can be complex, nuanced and at times contradictory. Factors that are important to a thin film or trailing filaments may be less so to a thick mat, making simple relationships difficult to extract. Key phases of the accrual cycle for *Phormidium*-dominated mats are discussed below.

5.1. Colonisation and attachment

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. Stable substrates, such as bedrock, boulder and cobble, tend to be preferred sites for *Phormidium* growth (Heath et al., 2015). The presence of certain bacteria may also enhance colonisation. Brasell et al. (2015) used morphological and molecular approaches to track microbial succession following a disturbance event that cleared the substrate in the Hutt River

(Wellington). They identified three distinct and concordant phases of bacteria assemblages as *Phormidium*-dominated mats developed, although they acknowledge that further research is required to fully understand the functional roles of bacteria at each stage.

Substrate heterogeneity, including particle clusters, cracks, crevices and projections, may be important in facilitating *Phormidium* colonisation. Heterogeneous substrate provides 'refuges' during flushing flows (Francoeur et al., 1998; Bergey, 2005; Murdock and Dodds, 2007). Rapid recovery of *Phormidium* cover has been observed after partial ablation in Canterbury rivers (Thiesen, 2015), suggesting that relic populations may facilitate subsequent proliferation (Fig. 5).

Several studies (Wood et al., 2014a, 2015a) suggest that water-column nutrient concentrations during the initial colonisation phase strongly influence whether *Phormidium* can establish and subsequently form mats. This may be 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 (Fig. 5; Nausch et al., 2004).

5.2. The transition from biofilm to mat

Several studies and numerous field observations by the authors have shown that *Phormidium*-dominated mats are at least initially, largely confined to riffles (high velocity, turbulent areas) in rivers (Heath, 2009; Heath et al., 2015). Flow requirements for successful accrual have, however, received less attention than those required for removal. A dynamic relationship between flow and accrual exists that has different influences 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 nutrient poor water may be enhanced by flow, as it increases the flux of nutrients from river to periphyton by reducing the thickness of the diffusive boundary layer separating the substrate from river water and through which solutes must pass by diffusion (Larned et al., 2004). This may partially explain why *Phormidium*-dominated mats begin their growth phase predominately in riffles (Heath, 2009). In later stages of growth, the subsidy-stress concept suggests that stress increases as high velocity increases the probability of detachment of thicker periphyton communities.

5.3. Accrual/growth

The duration of an accrual cycle is particularly important in determining the biomass and cover that can be accumulated (Biggs, 2000). Within this overall constraint, growth promoting variables affect biomass dynamics across all accrual phases, and these can relate to the supply of resources or factors that affect the rate of specific biological processes. For river periphyton in general, Francoeur et al. (1999) found using artificial substrate experiments, that temperature was the most important variable affecting accrual rate. Heath et al. (2011) also suggested that water temperature is an important factor in determining whether *Phormidium* proliferations were present or absent, based on field observations of greater proliferation during warm, summer months. Conversely, subsequent studies with larger datasets have shown no relationship between temperature and the occurrence of *Phormidium* proliferations (McAllister, 2014; Wood et al., 2014a, 2015a). Biggs (2000) concluded that, when the effects of accrual time were allowed for, the best predictors of overall periphyton biomass were inorganic nitrogen and phosphorus concentrations.

More recently, targeted research on *Phormidium* proliferations has gathered data to test the nutrient requirements for their formation. Wood et al. (2014a) investigated the nutrient concentrations that favour *Phormidium* proliferations through weekly sampling of 10 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 as nutrients are progressively depleted from the water column as biomass accrues. To allow for this time-integration effect, Wood et al. (2014a) used a mean of the DIN and DRP concentrations over the cumulative accrual period, as the mean nutrient concentrations since *Phormidium* coverage was zero. Their data suggested that *Phormidium* proliferations were most likely when DRP was less than 0.01 mg L^{-1}, but they did not identify a lower limit (Fig. 6A; Wood et al., 2014a). Most proliferations occurred at a dissolved inorganic nitrogen (DIN – mostly nitrate) of >0.2 mg L^{-1}. Exceptions were identified, in particular downstream of sewage treatment plants, and these still require further investigation (Fig. 6A). Data analysed from twenty four Canterbury rivers suggest a similar upper threshold of 0.01 mg L^{-1} DRP for high *Phormidium* cover, but again identify no lower limit (Fig. 6B) and again that proliferations were rare below a DIN concentration of 0.2 mg L^{-1} (Fig. 6B).

Analysis of two summers of monitoring data from seven sites in the Maitai River (Nelson) also shows the capacity of proliferations to occur at low DRP concentrations (<0.01 mg L^{-1}) though here, as in Canterbury, proliferations were present when DIN was below the apparent Manawatu-Whanganui lower threshold of 0.2 mg L^{-1} (Fig. 6A; Wood et al., 2014a, 2015a). Analysis of the Maitai River data showed that a reduction in DIN was not associated with a reduction in *Phormidium* cover (Wood et al., 2015a). Additionally, despite prolonged stable flows following a detachment event, *Phormidium*-dominated mats did not re-establish at sites where proliferations previously persisted. The authors suggested that monitoring may not have started early enough to capture elevated DIN concentrations in the river during the initial stages of mat establishment. Furthermore, established *Phormidium*-dominated mats are likely to be able to persist under lower than predicted DIN concentrations due to nutrient cycling occurring, aided by nitrogen fixing bacteria. The synthesis of these field-based studies suggests that *Phormidium* proliferations 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 and accumulate and retain nutrients as they grow is a well described, microbial mat trait (Bonilla et al., 2005; Quesada et al., 2008).

A common feature of most *Phormidium*-dominated mats is a thin layer of fine sediment at the substrate/mat interface (Fig. 1B). 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). Filaments of *Phormidium* 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. Wood et al. (2015b) showed that biogeochemical conditions inside natural *Phormidium*-dominated mats can be very different to the outside water column, including the development of high pH (>9)
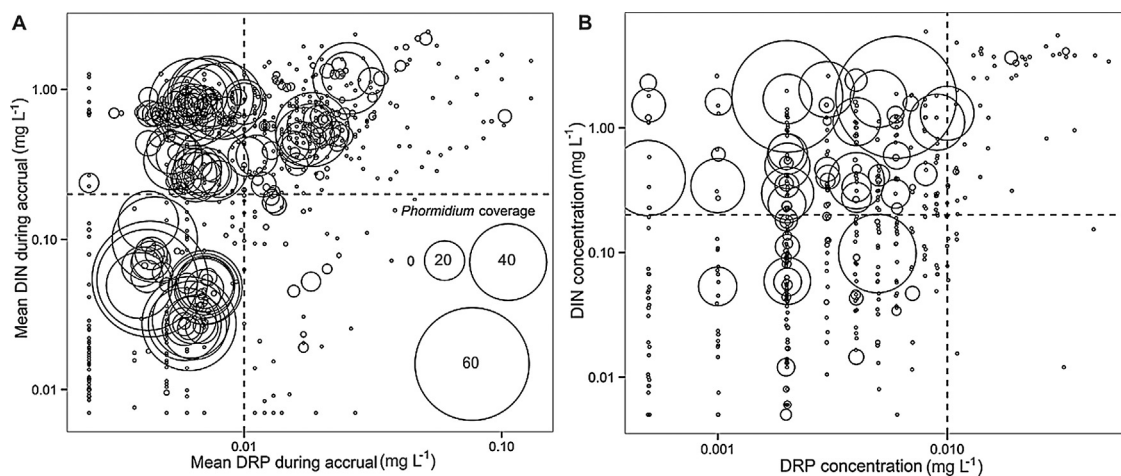

Fig. 6. Relationships between mean *Phormidium* coverage and dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP) for (A) ten rivers monitored weekly for two years in the Manawatu region. All points in the top right corner with *Phormidium* coverage are downstream of sewerage treatment plants (modified from Wood et al. (2014a) and (B) 24 rivers monitored monthly for two years in the Canterbury region. For (A) DIN and DRP are a cumulative mean over the accrual period, and (B) shows the DIN and DRP concentrations on the day of sampling. Dashed lines show suggested DIN and DRP thresholds that favour *Phormidium* proliferations.

during the day (due to photosynthetic depletion of bicarbonate) and low oxygen (<4 mg L^{-1}) concentrations at night (due to respiration). Such conditions are conducive to the release of DRP loosely bound to sediments trapped in the mat matrix, and this may be an additional source of DRP and one of the reasons why *Phormidium*-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, with higher concentrations within than outside the mat the net diffusive flux of DRP will be out of the mats (Fig. 5), and water-column nutrient concentrations may be of little relevance to biomass accrual.

Wood et al. (2015a) and Wood et al. (2015b) used sediment traps to investigate whether there was a relationship between increased sedimentation rates and the prevalence of *Phormidium*-dominated mats. Deposition of fine sediment (<63 μm, the dominant sediment size observed within *Phormidium* mats) was higher at sites with proliferations. Sequential extraction of phosphorus from trapped sediment found that biologically available phosphorus concentrations were higher at sites with proliferations (Wood et al., 2015b). Collectively these studies suggest suspended fine sediment, provided it contains biologically available phosphorus, is an important factor in promoting *Phormidium* proliferations once the mat stage in the accrual cycle is reached.

To date, in-depth investigations into nitrogen dynamics within *Phormidium*-dominated mats have not been undertaken. Molecular techniques have shown that cultures of *Phormidium*, isolated from New Zealand rivers, do not contain key enzymes required to fix nitrogen (Heath, 2014). Preliminary data, however, indicate that nitrogen fixing bacteria are present within mats (McAllister et al., unpub. data) and may contribute to nitrogen cycling.

Analysis of a limited number of river water samples for essential elements from two separate studies found no correlation between proliferation and any single element, but suggested a relationship between the sodium:potassium ratio (McAllister, 2014; Wood et al., 2015a). Using cultures (Harland et al., 2013) showed that low (40 μg L^{-1}) and high (4000 μg L^{-1}) iron concentrations inhibited *Phormidium* growth. Additionally, at high iron concentrations the cultures no longer adhered to the culture container walls. No correlation existed in field data though between proliferation and iron concentration.

Grazing is a final consideration in periphyton accrual dynamics. Large losses in biomass can result from consumption by grazers, with the extent dependent on herbivore type and density, and periphyton type and successional stage (Steinman, 1996; Holomuzki et al., 2010). To our knowledge, no studies have specifically investigated the impact of invertebrate grazing on *Phormidium*-dominated mats. The mud snail, *Potamopyrgus antipodarum* is known to significantly reduce algal biomass and has been shown to have a dominant presence in *Phormidium* mats (Hart et al., 2013). Wood et al. (2014b) showed that *Deleatidium*, *Pycnocentrodes*, *Polypedilum*/Orthocladiinae and the fly larvae *Maoridiamesa* were abundant within *Phormidium*-dominated mats in four New Zealand rivers. These taxa all have the potential to influence algal growth through grazing (Chadderton, 1988; Quinn and Hickey, 1990; Lester et al., 1994; Jaarsma et al., 1998). The presence and abundance of these macroinvertebrate taxa varied among study rivers and further research is required to determine their impact on *Phormidium* biomass. Toxins produced by *Phormidium* may act as a chemical defence reducing the impact of grazers. Recent studies in New Zealand indicate high toxicity of aqueous *Phormidium* extracts to the mayfly *Deleatidium* (Bridge, 2013). Collectively these studies suggest grazers may facilitate and/or inhibit *Phormidium*

colonisation and growth, and this likely varies with habitat, geographic location or successional stage.

5.4. Resetting/dispersal

Re-setting primarily occurs due to shear stress exceeding the attachment tenacity of periphyton, or through substrate disturbance (Fig. 5). Both velocity and the concentration of entrained suspended sediment can impact the degree of abrasion. For tightly adhering growth forms, such as *Phormidium* 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 three times the median both tended to reduce long term average periphyton biomass. These observations, however, were not species resolved and it has been shown that *Phormidium* mats often tolerate higher flows than other periphyton classes (Hart et al., 2013). Heath et al. (2015) undertook an intensive field survey and then used habitat suitability curves to show that *Phormidium* had a large tolerance to velocity, depth and substrate type.

More recently, studies have specifically focussed on flows that reset *Phormidium* 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 regions (Wood et al., 2014a) have consistently shown that the magnitude of flow increase required to remove *Phormidium* mats is river specific, and not a uniform three times the median flow. Wood et al. (2014a) used a quantile regression approach and defined the '*Phormidium* flushing flow' for each river as the multiple of median flow at which 90% of measurements of cover were predicted to be below 20%. Their analysis highlighted marked differences in '*Phormidium* flushing flows' among rivers.

It has also been reported that loss of *Phormidium*-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 (Fig. 5; Biggs and Thomsen, 1995; Boulêtreau et al., 2006; Quiblier et al., 2013). 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 (Hawes et al., 2014). Boundary layers limit both influx and efflux of materials, and are thicker under slow, laminar flows (Larned et al., 2004). Thus *Phormidium* may be expected to perform best under a moderate flow regime, 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) suggested that different strength flows may be required to remove mats at various stages of the accrual cycle (Fig. 5). During the initial attachment and growth phase *Phormidium* filaments adhere 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 become increasingly vulnerable to abrasion by flows. This shift in vulnerability to high and low flows over time further complicates relationships between flow and *Phormidium* proliferations.

6. Management and mitigation

New Zealand guidelines for managing cyanobacterial risk in water used for recreational purposes were developed in 2009 (Wood et al., 2009). These guidelines include a section specifically

on benthic cyanobacteria. They include a three-tier alert level framework that uses benthic cyanobacterial abundance and the occurrence of mats visibly detaching from the substrate to determine alert level status. Abundance is determined by assessing the percentage coverage of benthic cyanobacteria at five points on four transects set out perpendicular to the river edge. The threshold levels currently defined are: surveillance – up to 20% coverage, alert – 20–50% coverage and action – greater than 50% coverage. Monitoring and management actions are associated with each threshold, some of which involve changing the frequency of monitoring and issuing of health warnings. Detached mats that accumulate along river edges are deemed a high risk to human and animal health due to enhanced accessibility. Therefore the presence of detaching mats, regardless of percentage coverage, automatically raises the site to highest alert level status (red mode). The protocols and guidelines given in the 2009 document are now used by most governmental agencies involved in Phormidium monitoring.

The current guidelines for drinking-water quality management for New Zealand suggest that river intakes should be inspected for benthic cyanobacterial mats (Ministry of Health, 2015a). Methodologies are consistent with the recreational guidelines, however only a small number of drinking water supplies currently undertake regular inspections using this methodology. Given the detection of anatoxins in river water (Wood et al., 2011), and the relatively high number of water supplies in New Zealand with river intakes (Ministry of Health, 2015b) more knowledge on their abundance and potential risk is required.

To our knowledge mitigation strategies to remove/reduce Phormidium-dominated mats have not been implemented in New Zealand. Flushing flows have been used, in flow regulated rivers, to remove other nuisance periphyton growth (Measures and Kilroy, 2013). Most rivers with Phormidium-dominated mats are not flow regulated and therefore there is limited scope to utilise this technique. As knowledge on environmental variables that regulate benthic proliferations improves it may be viable to develop and implemented catchment wide or in-river solution to remove or reduce Phormidium-dominated mats.

7. What variables have changed that could account for the increase in intensity and extent of proliferations in New Zealand rivers

Surveying of general periphyton has been undertaken for many decades in New Zealand. The classification strategy commonly used involves coarse macroscopic assessment of benthic coverage, and classification of periphyton into groups such as, slimy filamentous, coarse filamentous, films and mats, and types of 'mats' have not been further distinguished. This makes hindcasting the occurrence and extent of Phormidium proliferations challenging. Since 2009, however, when most monitoring agencies started to differentiate Phormidium from other mats, records have increased and anecdotal evidence suggests that Phormidium proliferations were not present to the same extent in many of the rivers where they now occur.

The rivers with observed Phormidium issues are primarily non-alpine rivers on the lower-lying parts of the dry, eastern side of New Zealand (Fig. 2). These are also often areas with shallow aquifers that are part of an increasingly allocated water supply, often used to support intensive agriculture. Based on our current understanding of drivers of Phormidium proliferations, the most likely processes that may have changed in last decade are: flow regimes (due to water abstraction or climate change), run-off of nutrients, sediment and other contaminants, and habitat modification.

7.1. Flow regimes

Demand for water 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 exacerbate low flow conditions. Long term records of flow in lowland rivers that are frequent sites of Phormidium proliferations are limited. To further investigate the possible association between change in flows and Phormidium abundance two rivers were selected, the Opihi (Canterbury region) and Hutt (Wellington region), where Phormidium proliferations have increased in the last decade and where long-term (15–30 year) flow and nutrient data was available. Generalised Additive Models (GAMs; Wood, 2006) were used to individually model non-linear trends in nutrients and frequency of three times the median flow (3FRE) events during summer, defined as November to March, for each river and to detect periods of change. Frequency of 3FRE events were modelled using poisson distributed errors and nutrient data was modelled using gaussian distributed errors.

Analysis of the frequency of 3FRE events during summer, showed large inter-annual variability (Fig. 7; Hutt River: mean = 11.95, range = 1–30 and Opihi River: mean = 11.76, range = 0–50). The GAM models for both rivers were significant,
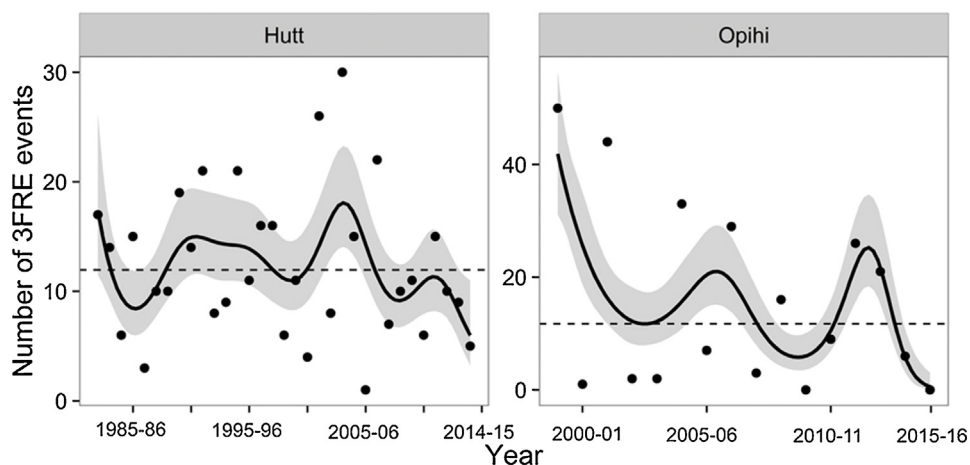

Fig. 7. Number of three times the median flow (3FRE) events per summer (November to March) in the Hutt and Opihi rivers. The black line is a cubic regression spline (±95% confidence intervals, grey shading) fitted using a generalised additive model (GAM). The dotted lines illustrate model intercepts for each river, representing the overall mean number of 3FRE events (baseline conditions). Note different y-axis scales.

indicating temporal changes in the frequency of 3FRE events in the Hutt and Opihi rivers ($p < 0.01$, $p < 0.001$ respectively). Significant increases in the frequency of 3FRE events occurred in the Hutt River during 2003/2004 and 2004/2005 and a significant decrease of events occurred between 2012/2013 and 2014/2015. Comparatively, 1999/2000, 2006/2007, 2011/2012 and 2012/2013 were identified as periods with a higher frequency of 3FRE events in the Opihi River and 2013/2014 and 2014/2015 had less 3FRE events compared to baseline conditions. This analysis suggests that there has not been a consistent decrease in the frequency of 3FRE for the Hutt and Opihi rivers and demonstrates there is marked inter-annual variability. An in-depth analysis, potentially including other flow metrics, including the amount of time between 3FRE events, across a wider number of rivers may be required to reveal national scale change. This analysis suggests that changes in flow regimes, while likely to be important in explaining temporal variability within a river, are not the only factor contributing to an increase in *Phormidium* proliferations nationally.

7.2. Nutrients, sediment and contaminant run off

The data collated in this review indicated that only slightly elevated water-column DIN concentrations are required for *Phormidium* growth, and that proliferation occurs when water-column DRP is low. A recent national analysis of New Zealand water quality in rivers showed that between 1994 and 2013 DRP concentrations decreased at the sites monitored by local authorities, with 48% of the 132 monitored sites showing statistically significant decreases (Ministry for the Environment and Statistics New Zealand, 2015). Conversely, nitrate-nitrogen concentrations increased. These trends would be consistent with current knowledge on the nutrient regimes that might promote *Phormidium* proliferations.

Generalised additive modelling of the annual mean DIN and DRP concentrations for the Hutt and Opihi rivers illustrated contrasting temporal trends (Fig. 8). The Hutt River had a relatively lower mean DIN concentration of 0.24 mg L^{-1} compared to the Opihi River, which had a mean of 0.48 mg L^{-1}. The DIN model for the Hutt River was significant ($p < 0.001$) whereas the model for the Opihi River was only marginally significant ($p = 0.04$). Dissolved inorganic nitrogen concentrations in the Hutt River were relatively stable between 1989 and 2005 (with the exception of in 1994 and 1997), however from 2006 onwards there was a decreasing trend in DIN concentrations in this river (Fig. 8). Comparatively, the model indicated that DIN concentrations in the Opihi River had increased between 1989 to 2010, after which concentrations stabilised until 2013 and then decreased in 2014 (Fig. 8).

There have been significant changes in DRP concentrations in both the Hutt and Opihi rivers ($p < 0.001$, $p < 0.01$ respectively). Dissolved reactive phosphorus concentrations in the Hutt River showed a similar pattern to DIN concentrations; after a period of relative stability from 1990 to 2002, concentrations decreased substantially and remained below 0.005 mg L^{-1} until 2015. In the Opihi River, DRP concentrations were more variable temporally and decreased significantly between 2004 and 2007. The increasing DIN and low DRP in the Opihi River is consistent with the notion that shifts in nutrient concentrations may be contributing to the severity of *Phormidium* proliferation. However, the
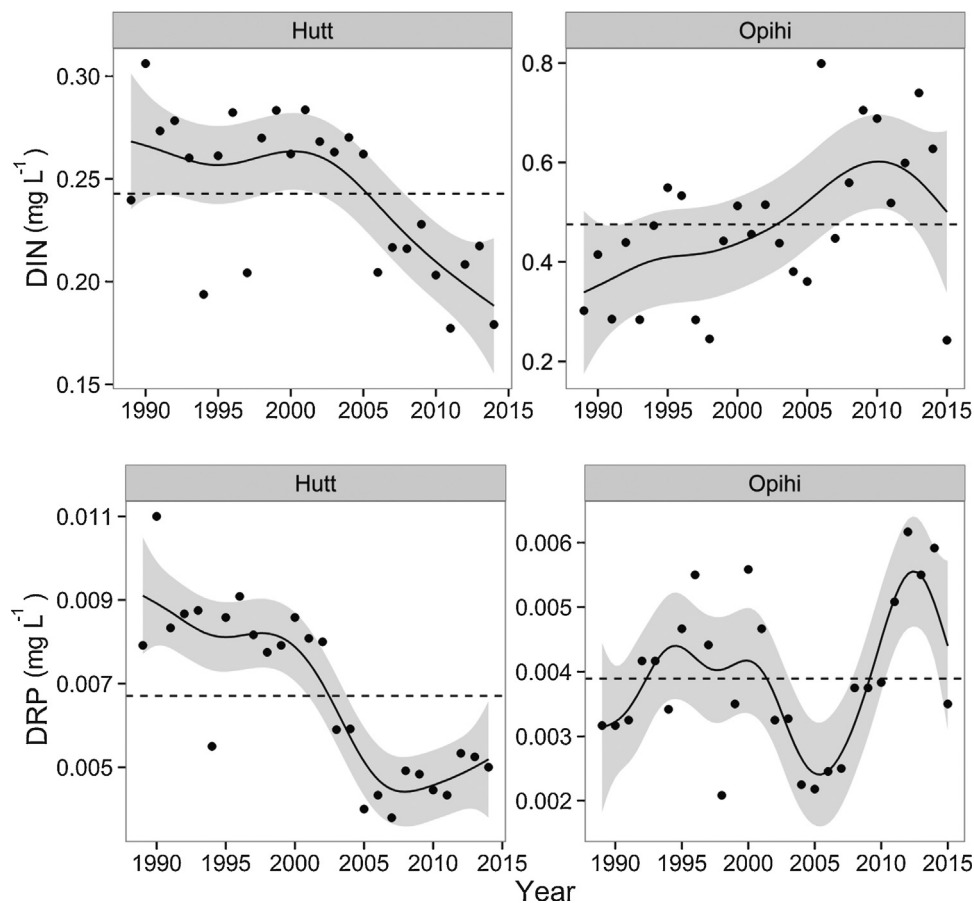

Fig. 8. Annual average DIN and DRP time series for the Hutt and Opihi rivers. The black line is a cubic regression spline (±95% confidence intervals, grey shading) fitted using generalised additive models (GAMs). The dotted lines show each GAM model intercept, representing the overall mean nutrient concentrations for the time period (baseline conditions). Note different *y*-axis scales to accommodate different concentrations.

decreasing DIN in the Hutt River does not support this and suggests that identifying and relating nation-wide shift to *Phormidium* dominance may be challenging, and that regional or catchment scale studies will be required.

A further important consideration related to nutrients is the role of groundwater. Our observations suggest that *Phormidium* proliferations commonly occur where there are groundwater seeps or in gaining reaches. In New Zealand nitrate concentrations are often elevated in groundwater, particularly in shallow well-oxygenated sources. Nationally approximately 20% of monitored groundwater sites have experienced significant increases in nitrate concentrations between 1995 and 2008 (Daughney and Randall, 2009). Further research is required to determine whether *Phormidium* proliferations are more frequent in rivers where groundwater supplements flows.

Phormidium proliferations tend to occur in areas where the land use is predominantly agriculture or forestry. There are links between agricultural land use, especially pasture-based dairying, and increased sediment-bound phosphorus run-off into waterways (McDowell et al., 2009). The extent of agricultural land has not changed significantly since 1996, however, it has become more intensive in some regions and forestry has increased by 11.5% (Ministry for the Environment and Statistics New Zealand, 2015). While quantitative data on sedimentation rates in rivers is lacking at a national scale, increasing land use intensification and forestry are likely to result in increased sediment in rivers, which may be partly responsible for observed rise in *Phormidium* proliferations.

Continued urbanization, 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 of these compounds than other algae (Lürling and Roessink, 2006; Forlani et al., 2008; Villeneuve et al., 2011; Drury et al., 2013). This highlights the possibility that cyanobacteria gain a competitive edge over other less tolerant taxa. Direct evidence linking increases in *Phormidium*-dominated mats to pesticides, herbicides and ECs is currently lacking. In the Manawatu region *Phormidium*-dominated mats are common downstream of sewerage treatment plants where ECs are expected to be at high concentrations (Carmichael et al., 1975; Hamill, 2013; Wood et al., 2014a,b).

7.3. Habitat modification

Habitat modification of New Zealand lowland rivers has occurred for many decades, and published evidence that it has increased in recent years could not be found, however on-going modification practices may synergistically contribute to proliferations. 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 and increases velocity and bed shear stress which may favour *Phormidium* proliferations (Hart et al., 2013; Wood et al., 2015a). Practices that reduce the efficacy of riparian zones may also directly or indirectly enhance *Phormidium* abundance. Intact riparian zones provide a buffer from hillslope sediment input, reduce water temperatures (which might limit *Phormidium* growth rates) and increase shade (Osborne and Kovacic, 1993; Parkyn et al., 2003). They also prevent access of livestock, thereby reducing sediment and nutrient inputs into rivers, currently quantitative data to explore the relationship between *Phormidium* proliferation and these practises is limited, although anecdotal evidence suggests that gravel extraction and associated sediment mobilization promotes proliferations in the Hutt River and that *Phormidium* is more prevalent at sites with no shading (Wood, unpub. data).

8. Conclusions and research gaps

There has been an increase in the distribution and extent of *Phormidium*-dominated proliferations in New Zealand's rivers over the last decade. The data collated in this review identified proliferations in 103 rivers and anatoxins in 67% of samples tested. Evidence is mounting to support the proposition that rivers most susceptible to proliferations have slightly elevated DIN (>0.1 mg L^{-1}) and low water-column DRP (<0.01 mg L^{-1}) and higher loads of fine sediment enriched with biologically available phosphorus. To date data primarily comes from correlative studies and there is a need for more manipulative experimental research, using stream channels or mesocosms, to explore the causative relationships of these and other variables. In well-developed *Phormidium*-dominated mats, within-mat water is largely isolated from the overlying water. In-depth investigations into nutrient cycling within *Phormidium*-dominated mats would provide valuable information on the role that bacteria and geochemistry play in creating growth promoting/inhibiting conditions. Studies to date suggest grazers both facilitate and inhibit *Phormidium* colonisation and growth, and further research is clearly required on this aspect. Further studies are needed to improve knowledge on the long-term relationships between river flow and *Phormidium* accrual cycles and this may assist water managers in predicting periods of highest risk. To date most studies have been regionally focused and investigations at a national scale, or in ways that will allow broad-scale inference, might provide the opportunity to develop enhanced, regionally weighted approaches for predicting *Phormidium* proliferations. Toxic and non-toxic genotypes co-exist within mats, however, knowledge on how the relative abundance of genotypes and environmental factors affect mat-scale anatoxin concentrations are lacking. Molecular techniques, such as quantitative PCR, could be used to address this knowledge gap. Finally increasing knowledge on the potential effects of *Phormidium*-dominated mats on aquatic organisms, both through habitat modification and toxicity, should be a priority.

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